DETECTION OF *VT1* AND *VT2* GENES IN *E. COLI* O157:H7 ISOLATED FROM SOFT CHEESE IN BASRAH, IRAQ USING DUPLEX PCR

BASIL ABDULZAHRA ABBAS, MOHAMMED HASAN KHUDOR AND OSAMA ISMAEL ABID SMEASEM
College of Veterinary Medicine, Basrah University
(Accepted for publication: June 9, 2013)

ABSTRACT

During the period extended from November 2010 to March 2011, One hundred fifty soft cheese samples were collected from three different markets in Basrah city. All samples were cultured in selective media to detect the presence of non-sorbitol fermenting colonies (NSF). 94 (62.66%) isolates were found non-sorbitol fermenting *E. coli*. Latex agglutination test was used to detect serotype O157:H7 in non- sorbitol fermenting isolates for 15 (33.34%) isolates. Duplex PCR were done to all *E. coli* O157:H7 isolates and the result showed 5/150 (3.34%) were positive to this test. All the *E. coli* O157:H7 isolates were positive to *vt1* gene which was observed in 100% of isolates but none of the isolates were detected to having a *vt2* gene.

KEYWORDS: *E. coli, duplex PCR, VT genes, Basrah*.

INTRODUCTION

Milk is a highly nutritious medium for growth and transmission of several types of microorganisms especially *E.coli* O157:H7, therefore; contamination occurs from different sources. Such contaminants may render the milk and its products unsafe to use and expose the consumers to risk of infection (Robert, 2008). Besides cattle, other domestic animals used to produce milk, such as goats and sheep, also harbor these bacteria in feces has been reported by (Sidjabat-Tambunan and Bensink 1997; Fagan *et al*. 1999). In recent years, Since the identification of *E. coli* O157:H7 as a human pathogen in 1982 in Oregon and Michigan, others are now commercially available for the major VTEC serogroups (O157, O26, O111, O103 and O145) previously identified as being commonly associated with human disease and have become a very important milk-borne pathogen which constitute a public health hazard (Fratamico and Smith, 2006).

Escherichia coli O157:H7 was first recognized as a human pathogen in 1982 by the center of disease control and prevention of two outbreaks of hemorrhagic colitis associated with eating hamburgers from a particular fast-food restaurant chain in Oregon and Michigan (Riley *et al*., 1983). Since that, this microorganism has been associated with many outbreaks in the United States and other countries around the world (Nataro and Kaper, 1998). *E. coli* O157 infections have a relatively low incidence compared to those of *Salmonella* (15.19 per 100,000), *Campylobacter* (13.02), *Shigella* (3.99) and *Cryptosporidium* 2.86 (CDC, 2010). More infection with *E.coli* O157:H7 is reported in developing countries (Dontorou *et al.*, 2003; Ihekweazu *et al.*, 2006 and Mashood *et al*., 2006). *E. coli* O157:H7 is a member of a group of pathogenic *E coli* strains, entrohaemorrhagic *E. coli* (EHEC), verotoxin producing *E. coli* (VTEC), shigatoxigenic *E. coli* (STEC) when it was isolated from individuals who developed bloody diarrhea and severe abdominal cramps (Yoon and Hovde, 2008) . In contrast to other *E. coli* strains, VTEC O157:H7 strains cannot rapidly ferment sorbitol referred to as non-sorbitol fermenting (NSF) *E. coli* O157, However, some strains can ferment sorbitol rapidly these are referred to as sorbitol fermenting (SF) *E. coli* O157 within 24 h. VTEC do not produce ß-glucuronidase, which making unable to hydrolyze 4-methylumbelliferyl-Dglucuronide [MUG] and resistant to the ant microbiological agent tellurite. These are all the features that can be used to indentify VTEC O157:H7 strains. (Meng, *et al*., 2007; Karch and Bielaszewska, 2001).These organisms are Inability to grow at temperatures above 44.5^{o}C in *E. coli* broth medium (Doyle and Beuchat, 2007). Other than its pathogenicity, it was also shown to be much more acid resistant than other *E. coli* (Diez-Gonzalez and Russell, 1997). *E. coli* O157:H7, produce Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2), also referred to as Verotoxin 1 (vt1) and Verotoxin 2 (vt2). Shiga toxin associate with hemorrhagic colitis (HC) and caused by EHEC strains and is presumed to be acquired through a bacteriophage from *Shigella* (Buchanan and Doyle, 1997). The two

key features of *E. coli* O157 that significantly increase its virulence are shiga toxin production , presence of pathogenic island termed the Locus of Enterocyte Effacement (LEE) and the lesser-known putative factors are usually controlled via the plasmid O157(pO157) which is capable of replicating independently of chromosomal DNA (Lim *et al*., 2010).

The aim of this study is to detect the presence of *vt1*and *vt2* toxin genes in studied *E. coli* O157:H7 by using duplex PCR technique.

MATERIALS AND METHODS

A total of one hundred fifty random locally made soft cheese samples were collected from local retail markets of three different markets of Basrah city which were (Al-Ashaar, Al-Hadi, and Al-jumhoorea). Fifty samples from each market were collected through the period from November 2010 to March 2011. Ten-gm of cheese sample added to 90 ml of (2%) sodium citrate which sterilized by autoclave at 121°C for 15 min, then homogenized by using sterile stomacher and incubated at 37 °C for 18-24h, finally examined microbiologically according to (Maher *et al*., 2000) and then was enriched in brilliant – green broth or tryptone soy broth supplemented with(4 mg/L) vancomysin and incubated at 37 °C for 18-24h. A loop full from perenrichment broth was transferred and streaked on the surface of sorbitol MacConkey agar which composed of 1% sorbitol instead of lactose in standard MacConkey agar. Sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (TC – SMAC) was used as selective medium for non-sorbitol fermenting *E.coli* (NSFEC) and incubated at 37 °C for 24h. Cefixime and potassium tellurite were used at the concentrations 0.05mg/L and 2.5mg/L respectively. Five of non sorbitol fermenting colonies (grow colorless) were picked with sterile tooth bick sticks and transferred onto eosin methlene blue (EMB), MacConkey agar incubated for additional overnight to identify lactose fermentation and metallic sheen green colored .Typical colonies of NSFEC grow on TC–SMAC are small, circular and colorless with smoky center (1-2) mm in diameter (Muehlherr *et al*., 2003). All suspected colonies were streak on the surface of pre-dried nutrient agar plates, in a manner which allowed well isolated colonies to develop. Plates were incubated at 37C° for 24 hrs. Thus the pure culture obtained was used for primary identification including classical biochemical tests (McFadden, 2000).

Latex agglutination Test for *E. coli*O157:H7 was used for more specific identification of *E. coli* O157:H7 by using commercial kit (Wellcolex *E. coli* O157:H7, Remel) to detect the somatic antigen O157 and flagella antigen H7.

Molecular Detection of verotoxine gene ((VT1 and VT2) gene by using Multiplex PCR technique was done by using commercially available DNA extraction and purification kit (Geneaid, USA). The purified DNA was detected by electrophoresis in 1% agarose gel with addition of ethedium bromide. Bromophenol blue stain added to the DNA sample and visualizes the DNA by U.V. light.

VT1a: GAAGAGTCCGTGGGATTACG 130 bp (Pollard *et al.*, 1990)
VT1b: AGCGATGCAGCTATTAATAA
VT2a: TTAACCACACCCACGGCAGT 346 bp (Pollard *et al*., 1990)
VT2b: GCTCTGGATGCATCTCTGGT

Protocol of (Pollared *et al*., 1990) was used to study the verotoxine genes. This was done by using customize primers shown above. The PCR reaction mixture contains 5 µl of green master mix, 5 µl of purified bacterial DNA, 1 µl of each forward and reverse primers, then the volume completed to 20 µl by deionized water.. The PCR tubes were transferred to the thermalcycler (after centrifuged for 10 seconds) to start the amplification reaction according to specific program for each gene. The results of the PCR were performed in post amplification process.10 *µ*l from amplified sample was directly loaded in a 2% agarose gel containing 0.5 *µ*l /25ml ethidium bromide with the addition of loading buffer and DNA size. Marker as standard in electrophoresis and the gel was run at 75 V. at 1 hr, then the products were visualized by UV transilluminator (Sambrook *et. al.,* 1989). The results were analyzed statistically by chi-square test (SPSS, 11). (Niazi, 2000).

RESULTS

This study includes detection of the prevalence of verotoxin- producing *Escherichia coli* O157:H7 (VTEC O157:H7) in collected samples from locally made soft cheese after isolation on selective media and identification by biochemical tests, latex agglutination test, and by duplex PCR technique. All samples were cultured in selective media to detect the presence of non-sorbitol fermenting colonies (NSF) in

sorbitol MacConkey agar supplemented with Cefixime and potassium tellurite (CT-SMAC) to increase selectivity, 94 (62.66%) isolates from soft cheese samples were found non-sorbitol fermenting *E. coli* isolates and 47.87% were found positive to biochemical tests. Latex agglutination test was used to detect serotype O157:H7 in non- sorbitol fermenting isolate. 15 (33.34%) isolates were found O157:H7 (table 1).

Table (1): Biochemical and serological test of isolated strains

Examined No.	Nonsorbitol fermenter	(IMViC, TSI & cellobiose) +ve	*E. coli*	O157 H7	O157:H7
150	94(62.66%)	45(47.87%)	45	18 (40%)	15(33.34%) 15(33.34%)

IMViC= Indole Test, Methyl Red Test, Voges- proskaur Test, Simmon's Citrate Test; TSI= Triple Sugar Iron

Duplex PCR were done to all *E.coli* O157:H7 isolates and the result showed 5/150 (3.34%) from soft cheese were positive to this test (table 2).

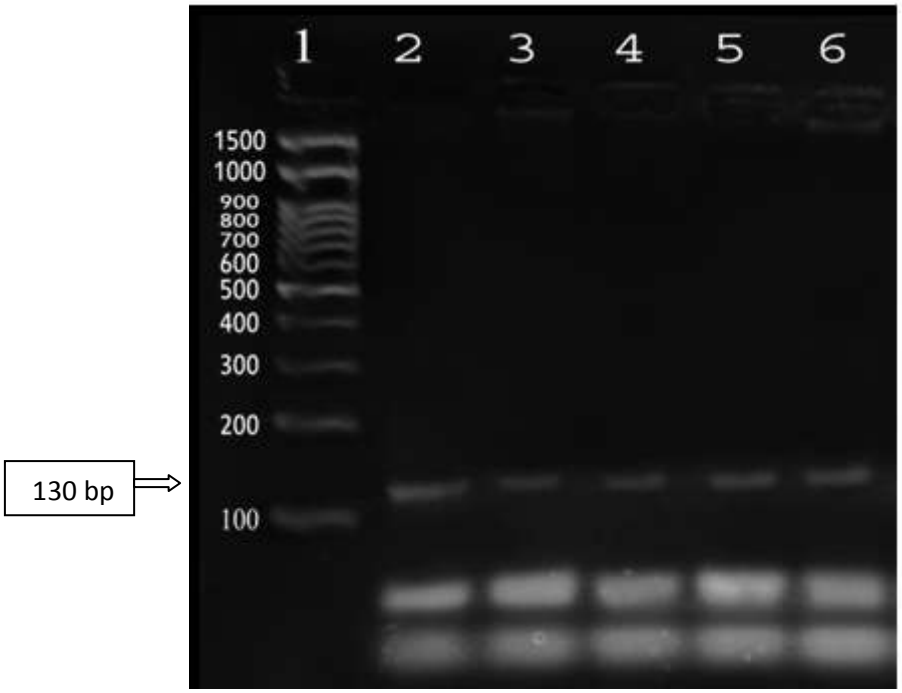
Table (2): Distribution of PCR positive E coli O157 and O157:H7 in tested strains.

Total		*E.coli* O157 isolates		O157:H7	
Exam No.	+ve PCR	Exam No.	+ve PCR	Exam No.	+ve PCR
150	5 (3.34%)	18	5(27.78%)	15	5(33.34%)

All the duplex PCR positive *E. coli* O157:H7 isolates were positive to *VT1* gene which was observed in 100%. None of isolates was observed as positive to both (*VT1*and *VT2*) genes from 5 isolated strains (table 3; fig. 1).

Table (3): Distribution *VT1* and *VT2* genes in *E.coli* O157:H7 isolates from soft cheese samples.

PCR +ve VTEC	vt1	vt2	vt1+vt2
5	5(100%)	0(0%)	0(0%)



Figure(1): PCR amplification of *VT1* gene (130 bp) Lane 2,3,4,5,6 (positive result). Lane 1(100bp ladder).

The estimation of *E. coli* O157:H7 distribution in soft cheese samples according to periods found the highest rate was observed in March ; 18.42% followed by December ;12% (table 4) .

Table (4): The Distribution of O157:H7 in Cheese Samples According to the Period (month) of Collection.

Month	No. of sampled	No. of positive O157:H7	%
November / 2010	30	2	6.6
December / 2010	25	2	8
January / 2011	20	1	5
February / 2011	35	3	8.5
March / 2011	40	5	12.5
Total	150	7	4.67
X^2 =1.248	*(p > 0.05)*		

The obtained results indicate that the distribution of VTEC O157:H7 isolates in soft cheese samples the high rate was observed in Al-Hadi which was 3(42.85%) followed by Al-Jumhoorea which was 1(33.34%) and Al-Ashaar 1(20%) (table 5).

Table (5): Distribution of VTEC O157:H7 in soft cheese isolates among to the different regions of the study.

Region	Soft cheese		
	No. of samples	No.of *E.coli* O157:H7	No.of VTEC (%)
Al-Hadi	50	7	3 (42.85)
Al-jumhoorea	50	3	1(33.34)
Al-Ashaar	50	5	1 (20)
Total	150	15	5(33.34)
	X X^2= 1.778 P>0.05		

DISCUSSION

Foodborne outbreaks of *Escherichia coli* O157:H7 infection has been associated with a wide range of food products, including raw and pasteurized milk and milk products such as cheese (Goh *et al*., 2002 and Strachan *et al*., 2005. VTEC is now a major cause of food – borne disease, mostly in the United states, Canada, Japan and Europe (Griffin andTauxe, 1991; Nataro and Kaper, 1998). In order to study the role of NSF *E. coli* in soft cheese, it is logical to consider its occurrence and frequency of NSF isolates at the same time. The frequency of NSF *E. coli* isolated in the present study was 94 out of 150 soft cheese samples (62.66%).

The frequency of NSF isolates from soft cheese samples in the present study were 62.66% which were lower than the results reported by (Abed Al-jaleel, 2007), Espie *et al*., (2006) who showed that the occurrence rates 73.63%, 88.9%, respectively. These differences of isolation rates between this study and other studies are due to contaminated environmental conditions and unhygienic measures in which the local soft cheese entirely passes in starting with the process of obtaining the required milk for manufactured process in which contamination occured during manual milking or collection and transport process (kosikowski and mistry.,1997 and Al-Azawi, 2006).

The polymerase chain reaction diagnostic techniques is rapid, easy, inexpensive protocol becoming the most widely used of all molecular genetics methods for detecting important toxin genes and identifying the bacteria in clinical material and contaminated food (Pollared *et al*.,1990). Its high sensitivity, specificity methods for detect specific nucleic acid

sequence found in the genome of pathogens, (Chotar *et al.*, 2006). Multiplex PCR, a more specific and more rapid method employing multiple sets of primers specific for the target genes, has been employed by Fratamico *et al*., (1995) who used four pairs of primers specific for vt_1,vt_2, *eaeA* and EHEC- *hlyA*. (Pollared *et al*., 1990). Paton and Paton (1998) used 2 pairs of primers, each specific for a particular *vt*, in PCR base test that efficiently identified verotoxigenic *E.coli* (VTEC) in food samples and in stool samples, respectively. In the present study, the frequency of isolates from soft cheese which confirmed positive as VTEC O157:H7 by detection of both vt_1 and vt_2 genes was 3.34%.

The high rate recorded in the present study can be attributed to the use of primers designed to target genes vt_1 and vt_2 genes which encoded for *Vt1* , *Vt2* toxines respectively and these two sets of oligonucleotide primer were used in multiplex PCR assay for the detection of *Vt* genes or as a means to increase sensitivity and specificity of this technique than other assays for the detection of VTEC O157:H7 in raw milk and soft cheese (Aslam *et al*., 2003). The detection of VTEC strains in the foods of animal origin including raw milk and its products have been implicated a health risk to consumers or as important vehicles for VTEC infections in humans (Ropnarine *et al*, 2007).

The rate of PCR positive for Vt_1 of *E.coli* O157:H7 isolates of soft cheese was 3.34% which is consistent with the results reported by Hassan and Elmalt, (2008) who cannot detected Vt_1 in their study on cheese isolates. Vivegnis *et al*., (1999) and Coia *et al*., (2001) they reported that the occurrence of VTEC in raw cow's milk and cheese range from 0 to 11.1%. On the other hand the isolation rate in this study was lower than that (13%) reported by Vernozy-Rozand *et al.* (2005) and (6%) which reported by Paneto *et al*., (2007).

In the present study, the highest isolation rate of *E. coli* O157:H7 from soft cheese samples was obtained in March (18.42%) followed by December (12%), November (10%), and February (8%). These results agree with the results obtained by Murinda *et al*., (2002), Spano *et al*., (2003), Rahimi *et al*., (2008) and Al-Aidi & Najim, (2009) who found that the highest isolation rate percentage. This is an increase in viability and survival of *E.coli* O157:H7 during the warm months of the year which was nearly from optimum temperature for growth than cold months. In contrast, a study in Scotland (Ogden *et al*., 2004) stated that the highest isolation rate was obtained in winter.

On other hand, according to the regions of study the highest rate was reported in Al-Hadi (42.85%) followed by Al-jumhoorea and Al-ashaar which was 33.34% and 20%, respectively. The results showed that season, geographical or locational variation of the farmer, contaminated environmental conditions and unhygienic measures had an effect on the isolation percentage of *E.coli* O157:H7 (Spano *et al*., 2003).

REFERENCES

Al-Aidi, S. R. A. and Najim, N. H. (2009). Prevalence of Enterohaemorrhagic *E. coli* O157:H7 that causes human bloody diarrhea in milk and it products in wassit. 9th International Vet. Med. Conference, Pp: 135- 145.

Aslam, M.; Hogan, J. and Smith, K.L. (2003). Development of a PCR-based assay to detect Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* in milk. Food Microbiol. 20: 345-350.

Buchanan, R.L. and M.P. Doyle. (1997). Foodborne Disease Significance of *Escherichia coli* O157:H7 and other Enterohemorrhagic *E. coli*. Food Technol. 51: 69-76.

Centers for Disease Control and prevention (2010). Preliminary Food Net data on the incidence of infection with pathogens transmitted commonly through food-10 states, Morb. and Mortal. Wkly. Rep. (59) 14: 418-422.

Chotar, M.; Vidova, B. and Godany, A. (2006). Development of specific and rapid detection of bacterial pathogens in dairy products by PCR. Folia Microbiol. 51: 639-646.

Coia, J.; Johnston, Y.; Steers, N. and Hanson, M. (2001). A survey of the prevalence of *Escherichia coli* O157:H7 in raw meats, raw cow's milk and raw-milk cheeses in south-east Scotland. International. J. Food Microbiol. 66: 63-69.

Diez-Gonzalez, F. and Russell, J. B. (1997). The ability of *Escherichia coli* 0157:H7 to decrease its intracellular pH and resist the toxicity of acetic acid. Microbiol. 143: 1175-1180.

Dontorou, C.; Papadopoulou, C. and Filioussis, G. (2003). Isolation of *Esherichia coli* O157:H7 from foods in Greece. International. J. Food Microbiol. 82: 273–279.

Doyle, P. M. and Beuchat, L. R. (2007). Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington D.C. Pp. 249-269.

Fagan, P.; Hornitzky, M. A.; Bettelheim, K. A. and Djordjevic, S. P. (1999). Detection of Shiga-like toxin (stx1 and stx2), intimin (eaeA) and enterohemorrhagic *Escherichia coli* (EHEC)

hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR. Appli. and Enviro. Microbiol. 65: 868–872.

Fratamico, P. M. and Smith, J. L. (2006). *Escherichia coli* infections. In: Riemann, H. P. and Cliver, D. O., Foodborne infections and intoxications. 3rd ed., Florida, Academic Press, an imprint of Elsevier. Pp. 205-208.

Fratamico, P. M.; Sackitey, S. K.; Wiedmann, M. and Deng, M. Y.(1995). Detection of *Escherichia coli* O157:H7 by multiplex PCR. J. Clin. Microbiol. 33: 2188- 2191.

Hassan, S. A. and Elmalt, L. M. (2008). Informally raw milk and kareish cheese investigation on the occurrence of toxigenic *Escherichia coli* in Qena city,Egypt with emphasis on molecular characterization. Assiut University. Bull.Environ.Res.11 (2): 35-42.

Ihekweazu, C.; Barlow, M.; Christensen, S. H.; Guttridge, B. and Lewis, D. (2006). Outbreak of *E. coli* O157 infection in the south west of the UK: risks from streams crossing seaside beaches. Euro. Surveill. 11(4): 128–130.

Karch, H. and Bielaszewska, M. (2001). Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. J. Clinic. Microbiol. 39 (6): 2043-9.

Lim, J. Y.; Yoon, J. W. and Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J. of Microbiol. Biotechnol. 20 (1):1-10.

Maher, M.M. and Murphy,P.M. (2000). Microbiological changes that occur during the ripening of two Irish, smear ripened cheese produced from raw milk. Irish J. Food Res. 39:107-121.

Mashood, A. R.; Minga, U. and Machangu ,R. (2006). Current epidemiological status of enterohaemorrhagic *Escherichia coli* O157:H7 in Africa. J. Chin. Med. 119 (3): 217–222.

McFadden, J. F. (2000). Biochmical tests .for identification of medical bacteria (3ed), Lippincott Williams and wilkins, USA Microbiol. Rev. 2: 15-38.

Meng, J.; Doyle, M. P.; Zhao, T. and Zhao, S. (2007). Enterohemorrhagic *Escherichia coli*. in Food Microbiology Fundamentals and Frontiers, 3rd ed. Doyle, M. P. and Beuchat, L. R. ASM Press, Washington D.C. Pp. 249-269.

Muehlherr, J. E.; Zweifel, C.; Corti, S.; Blanco, J. E. and Stephan, R. (2003). Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. J. Dairy sci. 86: 3849-3856.

Murinda, S. E.; Nguyen, L. T.; Ivey, S. J.; Gillespie, B. E.; Almeida, R. A.; Draughon, F. A. and Oliver, S. P. (2002). Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: a 12-month survey of dairy farms in east Tennessee. J. of Food Protec. 65: 752–759.

Nataro, J. P., Kaper, J. B. (1998). Diarrheagenic *Escherichia coli* published erratum in Clin Microbiol Rev. Clin. Microbiol. Rev. 11: 142-201.

Niazi, A. D. (2000). Statistical Analysis in Medical Research. Republic of Iraq. Al-Nehrien University. P. 148.

Ogden, I. D.; MacRae, M. and Strachan, N. J. C. (2004). Is the prevalence and shedding concentration of *E.coli* O157:H7 in beef cattle in Scotland seasonal? F.E.M.S. Microbiol. Lett. 233: 297 – 300.

Paneto, B. R.; Schocken-Iturrino, R. P.; Macedo, C.; Santo, E. and Marin, J. M. (2007). Occurrence of toxigenic *Escherichia coli* in raw milk cheese in Brazil. Arq. Bras. Med. Vet. Zootec. 59: 508-512.

Paton, J. C. and Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. 11 (3): 450-479.

Pollard, D. R.; Johnson, W. M.; Lior, H.; Tyler, S. D. and Rozee, K. R. (1990). Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J. Clinic. Microbiol. 28: 540-545.

Rahimi, E.; Momtaz, H. and Hemmatzadeh, F.(2008). The prevalence of *Escherichia coli* O157:H7,*Listeria monocytogenes* and *Compylobacter* SPP. on bovine carcasses in Isfahan, Iran. Iranian, J.Vet. Res., Shiraz University 4, 9 (25): 365- 369.

Riley, L. W.; Remis, R. S.; Helgerson, S. D.; McGee, H. B.; Wells, J.G. and Davis, B. R.(1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 308:681-685.

Robert, M. D. (2008). Serotypes and virutypes of enteropathogenic and enterohaemorrhagic *E.coli* strins from stool samples of children with diarrhea in Germany . J. Appl. Microbiol. 10: 5320-325.

Ropnarine, R. R.; Ammons, D.; Rampersad, J. and Adesiyun, A. A. (2007). Occurance and characterization of verocytotoxigenic *Escherichia coli*(VTEC) strains from dairy farms in Trindad. J. compilation, Zoonoses Public Health Blackwell verlag, Berlin. 54: 78-85.

Sambrook, J.; Fritsch, E. F. and Maniatis. (1989). Molecular cloning 2nd ed. Cold spring Harbor Laboratory Press, N.Y. 1746-1753.

Sidjabat-Tambunan, H. and Bensink, J. C. (1997). Verotoxin-producing *Escherichia coli* from the faeces of sheep, calves and pigs. Aust. Vet. J. 75: 292–293.

Spano, G.; Goffredo, E.; Beneduce, L.; Tarantino, D.; Dupuy, A. and Massa, S. (2003). Fate of *Escherichia coli* O157:H7 during the manufacture of mozzarella cheese. 36:73- 76.

Spano, G.; Goffredo, E.; Beneduce, L.; Tarantino, D.; Dupuy, A. and Massa, S. (2003). Fate of *Escherichia coli* O157:H7 during the manufacture of mozzarella cheese. 36:73- 76.

Vernozy-Rozand, C.; Montet, M. P.; Beradin, M.; Bavai, C. and Beutin, L. (2005). Isolation and characterization of Shiga toxin-producing *Escherichia coli* strains from raw milk cheeses in France. Lett. Appl. Microbiol. 41: 235-241.

Vivegnis, J.; Lioui, M.; Leclercq, A. B.; Decallonne, L. J. (1999). Detection of Shiga-like toxin producing *Escherichia coli* from raw milk cheeses produced in Wallonia. Biotechnol. Agron. Soc. Environ. 3: 159-164.

Yoon, J. W.; . Hovde, C. J. (2008). All blood, no stool: enterohemorrhagi *Escherichia coli* O157:H7 infection. J. Vet. Sci. 9: 219-231.

التحري عن الذيفانات vt1 و vt2 في الاشيريشيا القولونية O157:H7 المعزوله من الجبن الطري في مدينة البصرة – العراق بطريقه تفاعل تسلسل البلمرة الثنائي

الخلاصه

تم جمه 150عينه من الجبن الطري من ثلاث اسواق في مدينة البصرة وللفترة من تشرين الثاني 2010 لغاية أذار 2011 . تم زرع العينات على الاوساط الانتقائية للتحري عن الجراثيم غير المخمرة للسوربيتول. ووجد ان 94 عزله من E. coli وبنسبة (62.66%) كانت غير مخمرة للسوربيتول. تم استخدام اختبار التلازن المصلي للتحري عن النمط المصلي O157:H7 في العزلات ووجد بنسبة (3.34%) 5/150. وتم استخدام طريقة تفاعل تسلسل البلمرة الثنائي وكانت خمس عزلات موجبة لهذا الاختبار بنسبة (3.34%). سجل تواجد الجين vt1 في جميع عزلات الاشيريشيا القولونية O157:H7 بنسبة 100% و لم يؤشر تواجد الجين vt2 في اي من العزلات.