Research Journal of Dairy Sciences 5 (1-4): 9-14, 2011
ISSN: 1993-5277

Detection and Antimicrobial Resistance of *Escherichia coli* O157 Isolated From Traditional Cheese, Ice Cream and Yoghurt in Iran, Tehran

Sepehr Shekarchian Chaleshtori and Amin Jazayeri
Department of Veterinary Medicine, Faculty of Veterinary Medicine,
University of Shahrekord, Shahrekord, Iran

Abstract: Verotoxin-producing of *Escherichia coli* O157 is an increasingly common cause of severe gastrointestinal illness, enlisted among the most important emerging pathogens. The present study was conducted to investigate the presence of *E. coli* O157 and *E. coli* O157:H7 strains and to detect the presence of the stx1, stx2, eae and ehxA insolates derived from 290 samples (120 samples from traditional fresh cheese, 120 samples from traditional ice cream and 50 samples from yoghurt). The samples were purchased from the Tehran province in Iran, over a period 6 months from August, 2010 to February, 2011. Standard cultural method and polymerase chain reaction were applied for these analyses. *E. coli* O157 was detected in nine of the 290 (3.1%) samples tested (5 isolated from traditional cheese and 4 isolated from traditional ice cream samples) whereas *E. coli* O157:H7 was not detected in any samples. The genes *stx1* and *stx2* were detected in three *E. coli* isolated obtained from traditional cheese samples none of the stx1, stx2, eae and ehxA was detected in the *E. coli* isolates obtained from traditional ice cream samples. Susceptibilities of nine *E. coli* O157 isolates were determined for ten antimicrobial drugs using the disk diffusion assay. Resistance to ampicillin and gentamycin was the most common finding (44.4%) followed by resistance to erythromycin (33.3%), amoxicillin (11.1%), tetracycline (11.1%) and nalidixic acid (11.1%). All *E. coli* O157 isolates were susceptible to chloramphenicol, cefuroximeand streptomycin. Thus, traditional cheese and ice cream manufactured from unpasteurized milk have appositional risk as a result of *E. coli* O157 existence.

Key words: *Escherichia coli* O157, cheese, ice cream, yoghurt, pathogens, Iran

INTRODUCTION

Shiga Toxin (Stx)-producing *Escherichia coli* (STEC) were first recognized as a human pathogen in 1982 in the USA when strains of the serotype O157:H7 caused two outbreaks of hemorrhagic colitis (Riley *et al*., 1983; Wells *et al*., 1983). In 1983, the association of *E. coli* O157:H7 and of several other STEC serotypes with sporadic cases of the classical Hemolytic Uremic Syndrome (HUS) was first de-Hemolytic Ureic Syndrome (HUS) was first described and subsequently confirmed in a prospective study (Karmali *et al*., 1985). Since, then epidemiological studies from diriment parts of the world established STEC as the major cause of bloody diarrhea and HUS in temperate climates and as an important cause of uncomplicated watery diarrhea in some geographic areas (Spika, 1998). Cattle are the reservoir of the pathogen (Chapman *et al*., 1993) and consumption of undercooked meat (Riley *et al*., 1983; Dontorou *et al*., 2003) and raw milk (Chapman *et al*., 1993; Oksuz *et al*., 2004; Solomakos *et al*., 2009) of bovine origin are considered to be the main cause of several outbreaks of *E. coli* O157:H7. Nevertheless, a variety of other foods have also been implicated in causing outbreaks such as unpasteurized goat's milk and cheese (Bielaszewska *et al*., 1997; Solomakos *et al*., 2009), deer's meat (Keen *et al*., 1997), meat sandwiches (McDonnell *et al*., 1997), lettuce (Mermin *et al*., 1997) and unpasteurized apple cider and apple juice (Besser *et al*., 1993).

Ruminants seem to constitute a reservoir of *E. coli* O157 in nature (Rey *et al*., 2003; Oporto *et al*., 2008). Contaminated unpasteurized dairy products such as raw milk and raw milk cheese have been incriminated in recent foodborne STEC outbreaks (Deschenes *et al*., 1996; Honish *et al*., 2005; CDC, 2007). Fermented dairy products manufactured using raw milk contaminated with *E. coli* O157:H7 can pose a threat to human health as it has been shown that if present in raw milk, the pathogen can survive during the manufacturing and ripening stages of selected fermented dairy products that do not undergo a sufficient heating step or are contaminated after the heat treatment. The ability of the pathogen to survive in raw goat milk lactic (soft) cheeses (Vernozy-Rozand *et al*., 2005) aged cheddar cheese made from unpasteurized milk (Schlesser *et al*., 2006), feta cheese (Govaris *et al*., 2002a) and even yogurt (Morgan *et al*., 1993; Govaris *et al*.,

Corresponding Author: Sepehr Shekarchian Chaleshtori, Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran

2002b) has been well documented. Regarding the prevalence of *E. coli* O157 in the raw milk supply in Iran, to the knowledge, there have been only a few published surveys (Mansouri-Najand and Khalili, 2007). In this study, the researchers assayed raw milk cheeses for the presence of shiga-like toxigenic *E. coli*. The tested raw milk cheeses samples originated from the province of Kerman (Southern part of Iran).

The researchers reported the isolation of the pathogen in one sample of raw ovine milk. Besides the study of Mansouri-Najand and Khalili (2007) no other study has looked into the prevalence of *E. coli* O157 in ice cream and yoghurt in Iran. Therefore, the purpose of the present study were to estimate the prevalence of *E. coli* O157 in the traditional cheese, ice cream and yoghurt supply in Iran to assess the frequency in the isolated strains of four genes that encode for known STEC virulence factors, namely stx1, stx2, eae and ehxA and to determine the antibiotic resistance of the isolates. The antimicrobial agents tested in this study are widely used to treat infections in people and in food animals in Iran.

MATERIALS AND METHODS

Samples: Traditional sampling cheese (n = 120), ice cream (n = 120) and yogurt (n = 50) samples were obtained from different supermarkets and retailer shops from Tehran, provinces in Iran, over a 6 months period (August, 2010 to February, 2011). Samples (0.5 kg each in sterile glass containers) were transported to the laboratory at 4°C within a maximum of 6-12 h after sampling.

Microbiological analyses: About 25 g of each sample were homogenized in 225 mL trypton soya broth supplemented with novobiocin (20 mg L^{-1}) and incubated at 37°C for 18-24 h.

Then the enrichment samples were streak onto levine eosin methylene blue agar and sorbitol McConkey agar plates supplemented with cefexime (0.5 mg L^{-1}) and potassium tellurite (2.5 mg L^{-1}) and incubated as above. Suspected colonies were confirmed by TSI agar and Indole, Methyl red, Voges-Proskauer, Citrate (IMViC) tests.

Detection of *E. coli* O157:H7 and virulence genes by PCR: Sorbitol negative colonies were confirmed as *E. coli* O157:H7 with PCR assay by using the O-antigen encoding region of *O157* gene (Paton and Paton, 1998) and flagellar H7 gene (*fli C*) generic primers as described previously (Gannon *et al*., 1997). The primer sequences of virulence genes used were VT1-A and VT1-B for gene *stx1* (Rey *et al*., 2003); VT2-A and VT2-B for gene *stx2* (Rey *et al*., 2006); Hly A1 and HlyA4 for gene *ehxA* (Schmidt *et al*., 1995) and EAE-1 and EAE-2 for gene *eae*.

All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). Purification of DNA was achieved using a Genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instruction and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001). DNA amplification was performed in a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany). The amplification conditions and reagents for the PCR assays were those described by Rey *et al*. (2003). PCR products were analyzed by agarose gel electrophoresis and the specific DNA bands were visualized using ethidium bromide staining under UV illumination.

Antimicrobial susceptibility testing: One strain from each *E. coli* O157-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood according to the Clinical Laboratory Standards Institute (Wikler and CLSI, 2006). The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used; Nalidixic acid (30 μg), cefuroxime (30 μg), erythromycin (15 μg), tetracycline (15 μg), streptomycin (30 μg), gentamicin (10 μg), amoxicillin (30 μg), ampicillin (10 μg) and chloramphenicol (30 μg). After incubation at 42°C for 48 h in a microaerophilic atmosphere, the susceptibility of the *Campylobacter* sp., to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by Wikler and CLSI (2006). *Staphylococcus aureus* and *Escherichia coli* were used as quality control organisms in antimicrobial susceptibility determination.

Statistical analysis: Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a Pearson Chi-square (χ^2) test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $p<0.05$.

RESULTS

Analysis results of the traditional cheese, ice cream and yoghurt samples are shown in Table 1. A total of 9 *E. coli* O157 strains were isolated from nine (3.1%)

Table 1: Prevalence of *E. coli* O157 from traditional cheese, ice cream and yoghurt in Iran

Samples	No. of samples examined	No. of positive samples (%)	Virulence genes			
			Stx1	*Stx2*	*eae*	*ehxA*
Cheese	120	5 (4.2)	1	2	0	0
Ice cream	120	4 (3.3)	0	0	0	0
Yoghurt	50	0 (0.0)	0	0	0	0
Total	290	9 (3.1)	1	2	0	0

traditional cheese and ice cream sources out of 290 samples tested, *E. coli* O157:H7 was not detected in any sample. Five *E. coli* O157 strain was isolated from the traditional cheese samples and the other four were from the traditional ice cream samples. *Stx1* and *stx2* genes were detected in the three *E. coli* O157 isolates obtained from the three traditional cheese samples. None of these genes were detected in the *E. coli* O157 isolates isolated from the traditional ice cream samples. Overall, 8 of 9 *E. coli* O157 isolates (88.9%) were resistant to one or more antimicrobial agent. Two strains (22.2%) were resistant to single antibiotic and 5 strains (55.6%) showed resistance to two antimicrobial agents. Multiresistance which was defined as resistance to three or more of drug tested was found in 22.2% of *E. coli* O157 strains. Resistance to ampicillin and gentamycin was the most common finding (44.4%) followed by resistance to erythromycin (33.3%), amoxicillin (11.1%), tetracycline (11.1%) and nalidixic acid (11.1%). All *E. coli* O157 isolates were susceptible to chloramphenicol, cefuroximeand streptomycin.

DISCUSSION

E. coli O157 and causes severe disease and death in humans (Elder *et al*., 2000; Su and Brandt, 1995). Human infections of *E. coli* O157 have been mostly attributed or linked to food products from animals (Riley *et al*., 1983; Kim *et al*., 1998; Elder *et al*., 2000). Since, there was no available data regarding the prevalence of *E. coli* O157 in Iran, the aim of this study was to determine the occurrence of *E. coli* O157 in traditional cheese, ice cream and yoghurt produced in Iran.

The study showed that nine of the 240 traditional cheese and ice cream (3.75%) from Tehran were contaminated with *E. coli* O157. These *E. coli* were found to be positive for the two target genes *stx1* and *stx2* genes. No significant differences in the prevalence rates were observed between traditional cheese and ice cream samples isolated in Tehran. There are a number of studies from different countries concerning the incidence of *E. coli* O157 and *E. coli* O157:H7 isolation on a variety of foods (Abdul-Raouf *et al*., 1996; Ansay and Kaspar, 1997; Coia *et al*., 2001; Caro *et al*., 2006; Cizek *et al*., 2007; Abongo and Momba, 2009; Solomakos *et al*., 2009). Abdul-Raouf *et al*. (1996) reported that 6% of raw cow's milk samples examined in Egypt were contaminated with *E. coli* O157:H7. Allerberger and Dierich (1997) reported 3% of the milk samples tested in Austria to be positive for *E. coli* O157:H7 and Klie *et al*. (1997) found that only 0.3% of the milk analyzed in Germany was contaminated with this serotype. Similar studies on raw cow's milk performed in the USA analyzing 42 samples (Ansay and Kaspar, 1997) and in the Netherlands analyzing 1011 samples (Heuvelink *et al*., 1998) resulted in no *E. coli* O157:H7 isolation. In the study, no *E. coli* O157:H7 strain was isolated from the samples tested. In another study conducted in Egypt, 2% of Kareish cheese samples were positive for *E. coli* O157 by a biochemically and serologically assay (Abd El-Hady *et al*., 1995). Similar results of cheese samples were reported by Aksu *et al*. (1999), Abd El-Hady *et al*. (1995) and El-Kosi (2001) reported higher values. On the other hand, Ansay and Kaspar (1997) and Ibrahim and Sobeih (2006) failed to isolate *E. coli* O157 from cheese samples. The presence of *E. coli* O157 in traditional cheese and ice cream samples could be attributed to the fact that it is usually made from raw milk, in addition to the primitive way of processing, handling and selling. In the present study, no *E. coli* O157 isolate was detected in yogurt samples. Survival of *E. coli* O157 in foods depends on the sample acidity; the bacteria disappear when the pH falls to 3.5. Furthermore, the absence of *E. coli* O157 in yogurt samples in this study could possibly be accounted for by the acidity of these products, however it could also be due to the boiling stage performed during the processing of these products. The genes encoding for verotoxins (*stx1* and *stx2* genes) that determine the virulence potential of the organism which are essential in the establishment of the disease (Schmidt *et al*., 2001) were detected in the three *E. coli* O157 isolates from traditional cheese samples. These findings are supported by several studies (Vivegnis *et al*., 1999; Pradel *et al*., 2000; Caro *et al*., 2006; Mansouri-Najand and Khalili, 2007).

The results of antimicrobial susceptibility testing in the present study indicate that there is a high resistance of *E. coli* O157 to ampicillin, gentamycinand erythromycin. These results are comparable to those reported by other investigators (Lira *et al*., 2004; Picozzi *et al*., 2005; Caro *et al*., 2006; Cizek *et al*., 2007; Solomakos *et al*., 2009; Abongo and Momba, 2009; Ngwai *et al*., 2010). The results of antimicrobial resistance found in this study are correlated to antibiotics that are being used to treat infection in food animals in Iran. Also, high percentage of *E. coli* O157 isolates was found to be resistant to ampicillin, an antibiotic used in human medicine for the treatment of coliform infections. Due to

the high number of antimicrobial-resistant isolates, researchers recommend that *in vitro* antimicrobial susceptibility testing of *E. coli* be performed and appropriate treatment be instituted, especially for those cases of food borne *E. coli* with sever or prolonged symptoms or in immunocompromised patients.

CONCLUSION

From the present data, it can be said that the traditional cheese and ice cream represents a potential hazard for consumers due to the potential presence of *E. coli* O157 as well as there is neglected sanitary measures adopted during manufacturing, handling and distribution of such fresh foods. Consequently, food manufacturers and specialists should design comprehensive programs as Good Manufacturing Practices (GMP) and implementation of HACCP system to ensure the freedom of such foods from these pathogens. In addition, effective heat treatment for foods, provision of information to food handlers and consumers as well as application of strict hygienic measures during manufacturing, storage and selling of these products to improve its quality and safeguard the consumers against infections of such organisms.

REFERENCES

Abd El-Hady, H.M., M.A. Halawa and S.H. El-Shenawy, 1995. Surveillance of enterohemorrhagic *Escherichia coli* (*E. coli* O157:H7) in milks and kareish cheese. Assiut. Vet. Med. J., 33: 110-113.

Abdul-Raouf, U.M., M.S. Ammar and L.R. Beuchat, 1996. KIsolation of *Escherichia coli* O157:H7 from some Egyptian foods. Int. J. Food Microbiol., 29: 423-426.

Abongo, B.O. and M.N.B. Momba, 2009. Prevalence and characterization of *Escherichia coli* O157:H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa. Int. J. Food Microbiol., 26: 173-176.

Aksu, H., A. Aydin and M. Ugur, 1999. *Escherichia coli* O157:H7 nin hayvansal kokenli gida maddelerinde varligi. Pendik Veteriner Mikrob. Dergisi., 30: 77-81.

Allerberger, F. and M.P. Dierich, 1997. Enterohemorrhagic *Ecsherichia coli* in Austria. Proceedings of the 3rd International Symposium and Workshop on Shiga toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, June 22-26, 1997, Baltimore, MD., USA.

Ansay, S.E. and C.W. Kaspar, 1997. Survey of retail cheeses, dairy processing environments and raw milk for *Escherichia coli* O157:H7. Lett. Applied Microbiol., 25: 131-134.

Besser, R.E., S.M. Lett, J.T. Weber, M.P. Doyle, T.J. Barrett, J.G. Wells and P.M. Griffin, 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA, 269: 2217-2220.

Bielaszewska, M., J. Janda, K. Blahova, H. Minarikova, E. Jikova, M.A. Karmali, J. Laubovia and J. Sikulova *et al*., 1997. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. J. Epidemiol. Infect., 119: 299-305.

Caro, I., V.M. Fernandez-Barata, A. Alonso-Liamazares and M.R. Garcia-Armesto, 2006. Detection, occurrence and characterization of *Escherichia coli* O157:H7 from raw ewe's milk in Spain. J. Food Prot., 69: 920-924.

CDC, 2007. Escherichia coli O157: H7 infection associated with drinking raw milk-Washington and Oregon, November-December 2005. Morb. Mortal. Weekly Rep., 56: 165-167.

Chapman, P.A., D.J. Wright and R. Higgins, 1993. Untreated milk as a source of verotoxigenec *Escherichia coli* O157. Vet. Rec., 133: 171-172.

Cizek, A., M. Dolejska, R. Novotna, D. Haas and M. Vyskocil, 2007. Survey of Shigatoxigenic *Escherichia coli* O157 and drug-resistant coliform bacterform bacteria from in-line milk filters on dairy farms in the czech republic. J. Applied Microbiol., 104: 82-860.

Coia, J.E., Y. Johnston, N.J. Steers and M.F. Hanson, 2001. A survey of thd prevalence of *Escherichia coli* O157 in raw meats, raw cow's milk and raw-milk cheeses in south-est Scotland. Int. J. Food Microbiol., 66: 63-69.

Deschenes, G., C. Casenave, F. Grimont, J.C. Desenclos and S. Benoit *et al*., 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised chees. Pediatr. Nephrol., 10: 203-205.

Dontorou, C., C. Papadopoulou, G. Filioussis, V. Economou and I. Apostolou *et al*., 2003. Isolation of *Escherichia coli* O157:H7 from foods in Greece. Int. J. Food Microbiol., 82: 273-279.

El-Kosi, O.H.R., 2001. Occurrence of some enteric pathogens and their indicators in some Egyptian raw milk products. Assiut Vet. Med. J., 45: 48-62.

Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmaraie and W.W. Laegreid., 2000. Correlation of enterohemorrhagic *Escherichia colii* O157:H7 prevalence in feces, hides and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci., 7: 2999-3003.

Gannon, V.P.J., S. D'Souza, T. Graham, R.K. King, K. Rahn and S. Read, 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J. Clin. Microbiol., 35: 665-662.

Govaris, A., P. Koidis and K. Papatheodorou, 2002a. Behavior of *Escherichia coli* O157:H7 during the manufacture and ripening of Feta and Telemes cheese. J. Food Prot., 65: 609-615.

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

Heuvelink, A.E., B. Bleumink, F.L.V.D. Biggelaar, M.C. Te Giffel, R.R. Beumer and E.D. Boer, 1998. Occurrence and survival of verocytotoxin producing Escherichia coli O157: H7 in raw cow's milk in the Netherlandx. J. Food Prot., 61: 1597-1601.

Honish, L., G. Predy, N. Hislop, L. Chui and K. Kowalewska-Grochowska *et al*., 2005. An outbreak of Escherichia coli O157: H7 hemorrhagic colitis associated with unpasteurized Gouda cheese. Can. J. Public Health, 96: 182-184.

Ibrahim, E.M.A. and A.M.K. Sobeih, 2006. Diarrhoeagenic *E.coli* in kareish cheese manufactured by different methods with special reference to *Escherichia coli* O157: H7. Minufiya Vet. J., 4: 169-178.

Karmali, M.A., M. Petric, C. Lim, P.C. Fleming, G.S. Arbus and H. Lior, 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis., 151: 775-782.

Keen, W.E., E. Sazie, J. Kok, D.H. Ric and D.D. Hancook *et al*., 1997. An outbreak of Escherichia coli O157: H7 infections traced to jerky made from deer meat. J. Am. Med. Associat., 277: 1229-1231.

Kim, Y.B., J. Okuda, C. Matsumoto, T. Morigaki and N. Asai *et al*., 1998. Isolation of an *Escherichia coli* O157: H7 strain producing shiga toxin 1 but not shiga toxin 2 from patient with hemolytic uremic syndrome in Korea. FEMS Microbiol. Lett., 166: 43-48.

Klie, H., M. Timm, H. Richter, P. Gallien, K.W. Perlberg and H. Steinruck, 1997. Detection and occurrence of verotoxin-forming and shigatoxin producing *Escherichia coli* (VTEC and/of STEC) in milk. Berl. Much. Tierarzrl. Wochenschr., 110: 337-341.

Lira, W.M., C. Macedo and J.M. Marin, 2004. The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. J. Applied Microbiol., 97: 861-866.

Mansouri-Najand, L. and M. Khalili, 2007. Detection of shiga-like toxigenic *Escherichia coli* from raw milk cheeses produced in Kerman-Iran. Vet. Arhiv., 77: 515-522.

McDonnell, R.J., A. Rampling, S. Crook, P.M. Cockcroft and G.A. Willshaw *et al*., 1997. An outbreak of *Verocytotoxin* producing *Escherichia coli* O157 infection associated eith takeaway sandciches. CDR Wkly, 7: 201-205.

Mermin, J., E. Hilborn, A. Voetsch, M. Swartz and M.A. Laambert-Fair *et al*., 1997. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating mesclun mix lettuce. Proceedings of the 3rd International Symposium and Workshop on Shiga toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, June 22-26, 1997, Baltimore, MD, USA.

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

Ngwai, Y.B., M.O. Akpotu, R.E. Obidake, A.A. Sounyo, A. Onanuga and S.O. Origbo, 2010. Antimicrobial susceptibility of *Escherichia coli* and other coliforms isolated from urine of asymptomatic students in Bayelsa State, Nigeria. Afr. J. Microbiol. Res., 5: 184-191.

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

Oporto, B., J.I. Esteban, G. Aduriz, R.A. Juste and A. Hurtado, 2008. *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in northern. Zoonoses Public Health, 55: 73-81.

Paton, A.W. and J.C. Paton, 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, rfbO157. J. Clin. Microbiol., 36: 598-602.

Picozzi, C., R. Foschiion, A. Heuvelink and R. Beumer, 2005. Phenotypic and genotypic characterization of sorbitol-negative of slow-fermenting (suspected O157) *Escherichia coli* isolated from milk samples in Lombardy region. Lett. Appl. Microbiol., 40: 941-496.

Pradel, N.V., C. Livrelli, J.B. Champs, A. Palcoux, F. Reynaud, J. Scheutz and B. Siro*et al*., 2000. Prevalence and characterization of Shiga Toxinproducing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol., 38: 1023-1031.

Rey, J., J.E. Blanco, M. Blanco, A. Mora, G. Dahbi, J.M. Alonso and M. Hermoso *et al*., 2003. Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep in Spain. Vet. Microbiol., 94: 47-56.

Rey, J., S. Sachez, M. Mendoza, A. Garcia, C. Gil and N. Tejero *et al*., 2006. Prevalence, serotypes and virulence genes of Shiga tocxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. Int. J. Food Microbiol., 107: 212-217.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee and B.K. Wells *et al*., 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med., 308: 681-685.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, ISBN-13: 9780879695774, Pages: 99.

Schlesser, J.E., R. Gerdes, S. Ravishankar, K. Madsen and J. Mowbray, 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. J. Food Prot., 69: 990-998.

Schmidt, H., L. Beutin and H. Karch, 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun., 66: 1055-1061.

Schmidt, H., H. Karch and M. Bitzan, 2001. Pathogenic Aspects of Stec Infection in Humans. Duffy, G., P. Garvey and D. McDowell, (Eds.), Verocytotoxigenic E. coli. Food and Nutrition Press, INC, Trumbell, CT, USA pp. 241-262.

Solomakos, N., A. Govaris, A.S. Angelidis, S. Pournaras, A.R. Burriel and S.K. Kritas *et al*., 2009. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. J. Food Microbiol., 26: 865-871.

Spika, J.S., 1998. Shiga tocin-producing *E.coli* Infections in Canada. In: In: *Escherichia coli* O157:H7 and other Shiga Toxin-Producing *E. coli* Strains. (Eds.)., Kaper, J.B. and A.D. O'Brien, American Society for Microbiology, Washington, pp: 23-29.

Su, C. and L.J. Brandt, 1995. *Escherichia coli* O157:H7 infection in humans. Ann. Internal Med., 123: 698-707.

Vernozy-Rozand, C., C. Mazuuy-Cruchaudet, C. Bavai, M.P. Montet, V. Bonin, A. Dernburg and Y. Richard, 2005. Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. Int. J. Food Microbiol., 105: 83-88.

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

Wells, J.G., B.R. Davis, I.K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow and G.K. Morris, 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol., 18: 512-520.

Wikler, M.A. and CLSI, 2006. Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement, 16th Edn. Vol. 26, Clinical and Laboratory Standards Institute, USA., ISBN-13: 9781562385880, Pages: 183.