http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (1): 104-108, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.104.108
© 2014 Asian Network for Scientific Information

Prevalence of O157: H7 and Non-O157 *E. coli* in Iranian Domestic Sheep

Yahya Tahamtan and Mehdi Namavari
Razi Vaccine and Serum Institute-Shiraz, Iran

Abstract: The aim of the present study was the isolation of both *E. coli* O157 and non-O157 in sheep. Verotoxins (VT) 1, 2 and *eae* genes were tested for this propose. Sheep faces are an important source of Shiga toxin-producing *Escherichia coli* (STEC). *Escherichia coli* O157:H7 is a highly virulent food-borne pathogen and threat to public health. Rectal swab samples from sheep were collected during 2009-2010. Conventional plating and Polymerase Chain Reaction (PCR) were carried out according to virulence factors (*Stx*1, *Stx*2 and *eae*A).There significant differences between prevalence of STEC and session were observed. It was at highest in spring and late summer. Six (3.92%) sheep carcasses were contaminated by *E. coli* O157:H7.Only six samples were positive by PCR specific for the VT2 gene and produced verocytotoxin VT2, whereas all isolates were negative for the presence of VT1 and *eae* virulence genes considered. Geographical variations and season may be influenced in the prevalence rate. The composition of the gastrointestinal flora may be changed by different diet and, therefore O157 STEC rate in sheep and lamb was different. Iranian sheep indicated as a natural host of *E. coli* O157 strains therefore, may be potentially pathogenic for humans. This is the first report of *E. coli* O157 detection from sheep in Iran.

Key word: *E. coli* O157, *E. coli* non-o15, sheep, Iran

INTRODUCTION

Escherichia coli O157:H7 is belonging to Entrohemorrhagic *E. coli* (EHEC) a highly virulent food-borne pathogen with an infective dose of near 100 cells in humans (Raya *et al*., 2006). *Escherichia coli* O157:H7 and O157:H⁻ (non-motile) can be the main causes of disease in humans (Boyce *et al*., 1995). The Shiga toxin Producing *E. coli* (STEC) strains most frequently associated with clinical disease is serotype O157:H7 (Nataro and Kaper, 1998; Mead *et al*., 1999). However, in some countries, other serotypes are associated with sever disease outbreaks (Nataro and Kaper, 1998; Acheson, 2000). Since cattle and sheep, are recognized the most important reservoirs of STEC in the absence of clinical signs (Heuvelink *et al*., 1998; Johnson *et al*., 1999). STEC are widely distributed in sheep feces prior to slaughter (Fegan and Desmarchelier, 1999) and contamination of meat with both O157 and non-O157 STEC occurs during slaughter or meat processing (Armstrong *et al*., 1996) but consumption of contaminated foods and direct contact by infected animals or humans are the main route of transmission (Griffin and Tauxe, 1991; Boyce *et al*., 1995). The STEC spread, is complex and unknown (Stacey *et al*., 2007) but those are carried in the gastrointestinal tract of healthy animals and do not cause disease in mature ruminants even when administered at very high doses (Cornick *et al*., 2007). The virulence factor for disease caused by STEC is not understood. However, Shiga toxin is a major factor responsible for pathogenesis (Acheson, 2000). They have cytotoxic effects on African Green Monkey kidney (Vero) cells in cultures (Konowalchuk *et al*., 1977). Shiga toxin 1 (*stx*) homologous with *Shigella dysenteriae* type 1 and *Stx*2 that antigenically distinct from *stx*1 (Paton and Paton, 1998). They caused Hemorrhagic Colitis (HC) and Hemolytic-uremic Syndrome (HUS) (Heuvelink *et al*., 1998). Some studies indicated that strains with only *stx*1 or both *stx*1 and *stx2* less virulent and caused less frequently disease than strains harboring *stx*2 (Tesh *et al*., 1993; Nataro and Kaper, 1998; Dorn *et al*., 1989). Intimin encoded by *eae* gene is responsible for attachment of STEC to the intestinal epithelial cells (Nataro and Kaper, 1998; Acheson, 2000). The present study describes the isolation and virulence factors characterization of O157 and non-O157 STEC strains on Iranian sheep during one year period.

MATERIALS AND METHODS

Sampling: During one year period from September 2009 to August 2010, rectal swab samples from sheep were collected before slathering practice at several slaughterhouses located at different geographic sites in Fars province-Iran. Conventional plating and Polymerase

Corresponding Author: Yahya Tahamtan, Razi Vaccine and Serum Institute-Shiraz, Iran

Chain Reaction (PCR) were carried out on samples for the presence of STEC (both O157 and non-O157). Three main virulence factors (*Stx*1, *Stx*2 and *eae* A) were tested for this proposes. A lot by means of sheep from the same source defined as a group. Fifteen percent of each lot was sampled during each month. Immediately after slaughter, the samples were collected aseptically and were placed into transfer media and kept in ice-pack container. Animals were randomly selected and no information on the farms of origin was recorded. The microbiological examination was started the day after sample collection.

Bacterial Isolation by selective media: The transfer media which described above contain modified trypticase-soy broth (Merck VM61475930) supplemented with 0.05 mg L^{-1} ceffexime (Daru Pakhsh-Irn-32) and 2.5 mg L^{-1} potassium tellurate (Scharlau PO0380) (mTSB). The samples were homogenized and incubated overnight at 37°C on a rotary shaker (100 rpm). One loop full of enrichment culture was spread plated onto sorbitol MacConkey agar (Hi Media M298), supplemented with cefixime and potassium tellurite (SMAC-CT) as well. All plates were incubated over night at 37°C. Ten to eleven sorbitol and non-sorbitol fermenting colonies were selected for further experiments. The sorbitol negative and positive colonies were picked and further identification was performed using biochemical test such as indol, rhamnose, urease activity and motility.

Antiserum test: Every colony was assayed for expression of the O157 antigen by latex agglutination with the Wellcolex™ *E. coli* O157:H7 Latex Test (Mast, UK). O157 positive and negative colonies were selected for PCR assay.

DNA extraction: All samples that were previously positive and negative were screened for presence of *stx*1, *stx*2 and *eae*A using multiplex PCR. One mililitter overnight culture was used for DNA extraction by DNTP kit (Cina-gene) and 2 μL of elution results was used as template DNA in a 25 μL reaction PCR.

PCR and reaction: Twenty five micro litter reactions mixture containing 0.2 μM of each primers (Table 1) (Holland *et al*., 2000; Yilmaz *et al*., 2006; Urdahl *et al*., 2003) I-F_1/IIR_2. The mixture continued by 0.2 μM of each deoxynucleotide triphosphates (Roche, Germany Company), 1X PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.001% gelatin), 1.5 mM $MgCl_2$. Finally, 2.5 U of Taq DNA polymerase (Roche Molecular system) was use and then 2 μL DNA templates were added. The reactions were carried out with a Thermocycler (Eppendorf, Germany) and cycling condition with some modification was as follows: 94°C for 2 min, 30 cycle of 94°C for 30 sec, 57.5°C for 45 sec and 72°C for 30 sec and a final extension at 72°C for 10 min. Ten μL of the amplification PCR product were analyzed on 1% agarose (Merck) gels containing 0.4 μg mL^{-1} ethidium bromide, visualized under UV illumination, saved and photographed. Each agarose gel electrophoresis run included DNA molecular size standards (100 bp Ladder).

RESULTS

Thirteen samples were shown at least one sorbitol negative colonies on CT-SMAC identified susceptible as an O157. Forty nine (32.02%) colonies with sorbitol positive were considered as a non-O157 STEC. Out of 62 (40.52%) STEC from 153 animas, 7 (4.57%) and 45 (29.41%0 recognized O157 and non-O157 by PCR respectively (Table 2). For all animals, the location of slaughter was not linked to the location of origin of the animals. Any sorbitol positive *E. coli* isolate harboring at least one shiga toxin was considered as a non-O157 STEC.

The monthly prevalence of ETEC in sheep was obtained 2-40% and was at its highest in spring and late summer (Fig. 1). As is noted, the prevalence rate of non-O157 is more than O157. There was observed significant differences between prevalence rate in spring and summer with O157 and non-O157.

The PCR assay revealed that some isolates possessive *stx*1, *stx*2 and both of them and also *eae* genes. PCR results indicated that of 153 isolate, 98 (64.05%) had *stx*2 and 53 (34.64%) possessive *stx*1. Both

Table 1: Primers used in PCR for amplification of *Stx*1, *Stx*2 and *eae* genes

Product size (bp)	Oligonucleotide sequence (5'3')	Primers
614	ACA CTG GAT GAT CTC AGT GG	*Stx*1-f
	CTG AAT CCC CCT CCA TTA TG	*Stx*1-r
779	CCA TGA CAA CGG ACA GCA GTT	*Stx*2-f
	CCT GTC AAC TGA GCA CTT TG	*Stx*2-r
450	AAG CGA CTG AGG TCA CT	*eae*-f
	ACG CTG CTC ACT AGA TGT	*eae*-r

Table 2: STEC isolation rate from sheep slaughterhouse, Shiraz-Iran 2009-2010

		Sorbitol (%)				PCR⁺ (%)	
Animals	No.	+ (Non-O157)	- (O157)	STEC (%)	O157 antisera⁺ (%)	Non-O157	O157
Ewes	115	38 (33.04)	10 (8.69)	48 (41.73)	8 (6.95)	35 (30.43)	6 (5.21)
Lambs	38	11 (28.97)	3 (7.89)	14 (36.84)	2 (5.26)	9 (23.63)	1 (2.63)
Total	153	49 (32.02)	13 (8.49)	62 (40.52)	10 (6.53)	45 (29.41)	7 (4.57)

Table 3: Distribution of virulence factor associated with *E.coli* O157

STEC virulence	*Stx*1	*Stx*2	*eae*	*Stx*1, *Stx*2	*Stx*1, *eae*	*Stx*2, *eae*	*Stx*1, *Stx*2, *eae*	Total
No. of isolates (%)	53 (34.64)	98 (64.05)	34 (22.23)	44 (28.75)	36 (23.53)	59 (38.56)	21 (13.72)	153

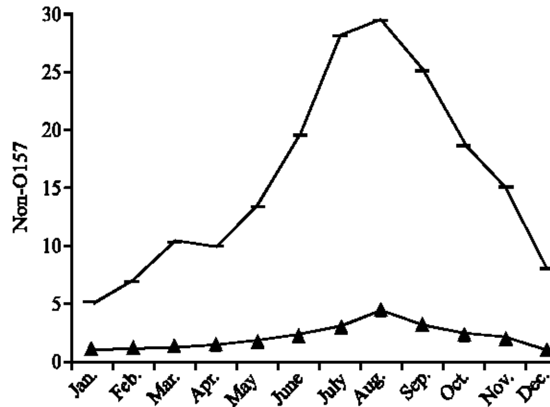

Fig. 1: Monthly prevalence of *E. coli* O157 and Non-O157 from sheep in Fars-Iran

*stx*1 and *stx*2 were harboring at 44 (28.75%) of STEC isolates. Except five percent, all isolates fermented sorbitol on SMAC agar. Thirty four (22.23%) isolates produced intimin encoded by eae. Nineteen isolates (12.58%) possessed the combination of virulence factors (eae and at least one shiga toxin gene) that has been associated with severe disease in humans (Table 3).

DISCUSSION

In the previous study similar variations in incidence rates of disease was found. The seasonal prevalence of disease in sheep parallel the disease in cattle and sheep did not differ a great deal from previous studies. Although at significantly lower incidence of sheep according to data was observed (Tahamtan *et al*., 2010).

Sheep, the second most commonly reared species of ruminant food animals, appears to have a role similar to that of cattle as a natural reservoir of STEC (Heuvelink *et al*., 1998). STEC shedding by sheep peaks during the summer (Hancock *et al*., 1994; Kudva *et al*., 1997), parallel to the human infections (Griffin and Tauxe, 1991; Boyce *et al*., 1995). Different diet change the composition of the gastrointestinal flora and differences in STEC isolate from sheep and lambs. In the other hand, young lambs are directly sold and transported from fattening herds to slaughterhouse but adult sheep are at several risk of coming into contact with infected animals. These data are difficult to compare with those of other studies in the world. Because they may be greatly influenced by factors such as the target population, the sampling strategy, the methodology of screening and isolation and also by possible seasonal variation in prevalence (Callaway *et al*., 2009; Hancock *et al*., 2001). In the present study, the PCR technique for identification of STEC, a method which is regarded as one of the most sensitive isolation methods for STEC was used (Johnsen *et al*., 2001) and then compare with conventional methods. In general, lambs are more susceptible to disease than sheep shown more sever clinical signs. The data indicated that Iranian sheep is natural hosts of *E. coli* O157 strains and Non-O157as well, therefore there is a potential risk for contamination of carcasses and entry of STEC into the food chain and considered for humans public health threat. Contamination of the carcasses and environment with *E. coli* O157:H7 from intestinal contents of sheep during slaughtering practice is one of the most important risk factor in transmission to human (Mead and Griffin, 1998; Philips, 1999). Carcasses become contaminate at the time of slaughter and processing may introduce the organism when performed in no hygienic conditions (Chapman *et al*., 1993). It is difficult to directly compare results from different studies due to varying sampling and testing regimes. Therefore, critical points must be determined in the abattoir to prevent the contamination by *E. coli* O157:H7 (Gun *et al*., 2003).

CONCLUSION

This investigation was indicated the epidemiological survey on the occurrence of STEC in healthy sheep in Iran. This confirms that in Iran, as well as in other countries, sheep represent and also important reservoir of EHEC. The prevalence of the bacterium appear to be similar to those to the other parts of the world (Cornick *et al*., 2007; Johnsen *et al*., 2001). Despite the increasing significance of EHEC strains in the etiology of disease in Iran, the epidemiology of infections caused by these strains is poorly understood (Orth *et al*., 2006).

ACKNOWLEDGMENTS

We thank Dr M.H. Hosseini and the Immunology Laboratories for their excellent assistance and Professor David Gally of the University of Edinburgh for providing the STEC O157:H7 strain EDL 933 used in this study. This study was supported by the Project Fund (grant 2-84-18-68008).

REFERENCES

Acheson, D.W., 2000. How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically. J. Food Prot., 63: 819-821.

Armstrong, G.L., J. Hollingsworth and J.G. Morris, 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol. Rev., 18: 29-51.

Boyce, T.G., D.L. Swerdlow and P.M. Griffin, 1995. *Escherichia coli* O157: H7 and the hemolytic-uremic syndrome. N. Engl. J. Med., 333: 364-368.

Callaway, T.R., M.A. Carr, T.S. Edrington, R.C. Anderson and D.J. Nisbet, 2009. Diet, *Escherichia coli* O157:H7 and cattle: A review after 10 years. Curr. Issues Mol. Biol., 11: 67-79.

Chapman, P.A., D.J. Wright and R. Higgins, 1993. Untreated milk as a source of verotoxigenic *E. coli* O157:H7. Vet. Rec., 133: 171-172.

Cornick, N.A., A.F. Helgerson and V. Sharma, 2007. Shiga toxin and Shiga toxin-encoding phage do not facilitate *Escherichia coli* O157:H7 colonization in sheep. Applied Environ. Microbiol., 73: 344-346.

Dorn, C.R., S.M. Scotland, H.R. Smith, G.A. Willshaw and B. Rowe, 1989. Properties of vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7. Epidemiol. Infect., 103: 83-95.

Fegan, N. and P. Desmarchelier, 1999. Shiga toxin-producing *Escherichia coli* in sheep and pre-slaughter lambs in eastern Australia. Lett. Applied Microbiol., 28: 335-339.

Griffin, P.M. and R.V. Tauxe, 1991. The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. Epidemiol. Rev., 13: 60-98.

Gun, H., A. Yilmaz, S. Turker, A. Tanlasi and H. Yilmaz, 2003. Contamination of bovine carcasses and abattoir environment by *Escherichia coli* O157: H7 in Istanbul. Int. J. Food Microbiol., 84: 339-344.

Hancock, D., T. Besser, J. Lejeune, M. Davis and D. Rice, 2001. The control of VTEC in the animal reservoir. Int. J. Food Microbiol., 66: 71-78.

Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I. Tarr, D.H Rice and M.G. Paros, 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect., 113: 199-207.

Heuvelink, A.E., F.L.A.M. van den Biggelaar, E.D.E. Boer, R.G. Herbes, W.J.G. Melchers, J.H.J.H. In't Veld and L.A.H. Monnens, 1998. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J. Clin. Microbiol., 36: 878-882.

Holland, J.L., L. Louie, A.E. Simor and M. Louie, 2000. PCR detection of *Escherichia coli* O157: H7 directly from stools: E valuation of commercial extraction methods for purifying fecal DNA. J. Clin. Microbiol., 38: 4108-4113.

Johnsen, G., Y. Wasteson, E. Heir, O.I. Berget and H. Herikstad, 2001. *Escherichia coli* O157: H7 in faeces from cattle, sheep and pigs in the Southwest part of Norway during 1998 and 1999. Int. J. Food Microbiol., 10: 193-200.

Johnson, R., J. Wilson, P. Michel, K. Rahn, S. Renwick, C. Gyles and J. Spika, 1999. Human Infection with Verocytotoxigenic *Escherichia coli* associated with Exposure to Farms and Rural Environments. In: *Escherichia coli* O157 in Farm Animals, Stewart, C.S. and H.J. Flint (Eds.). CABI Publishing, Wallingford, UK., pp: 147-168.

Konowalchuk, J., J.I. Speirs and S. Stavric, 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun., 18: 775-779.

Kudva, I.T., P.G. Hatfield and C.J. Hovde, 1997. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* serotypes isolated from sheep. J. Clin. Microbiol., 35: 892-899.

Mead, P.S. and P.M. Griffin, 1998. *Escherichia coli* O157:H7. Lancet, 352: 1207-1212.

Mead, P.S., L. S lutsker, V. Dietz, L.F. McCaig and J.S. Bresee *et al*., 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.

Orth, D., K. Grif, M.P. Dierich and R. Wurzner, 2006. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157: Indications for an animal reservoir. Epidemiol. Infect., 134: 719-723.

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

Philips, C.A., 1999. The epidemiology, detection and control of *Escherichia coli* O157. J. Sci. Food Agric., 79: 1367-1381.

Raya, R.R., P. Varey, R.A. Oot, M.R. Dyen and T.R. Callaway *et al*., 2006. Isolation and characterization of a new T-even bacteriophage, CEV1 and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. Applied Environ. Microbiol., 72: 6405-6410.

Stacey, K.F., D.J. Parsons, K.H. Christiansen and C.H. Burton, 2007. Assessing the effect of interventions on the risk of cattle and sheep carrying *Escherichia coli* O157:H7 to the abattoir using a stochastic model. Preventive Vet. Med., 79: 32-45.

Tahamtan, Y., M. Hayati and M.M. Namavari, 2010. Prevalence and distribution of the stx1, stx2 genes in Shiga toxin producing *E. coli* (STEC) isolates from cattle. Iran. J. Microbiol., 2: 9-14.

Tesh, V.L., J.A. Burris, J.W. Owens, V.M. Gordon, E.A. Wadolkowski, A.D. O`Brien and J.E. Samuel, 1993. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect. Immun., 61: 3392-3402.

Urdahl, A.M., L. Beutin, E. Skjerve, S. Zimmermann and Y. Wasteson, 2003. Animal host associated differences in Shiga toxin-producing *Escherichia coli* isolated from sheep and cattle on the same farm. J. Applied Microbiol., 95: 92-101.

Yilmaz, A., H. Gun, M. Ugur, N. Turan and H. Yilmaz, 2006. Detection and frequency of VT1, VT2 and eaeA genes in *Escherichia coli* O157 and O157: H7 strains isolated from cattle, cattle carcasses and abattoir environment in Istanbul. Int. J. Food Microbiol., 106: 213-217.