

Verotoxin-Producing *Escherichia coli* O157:H7 in Health and Diarrheic Cattle

Yahya Tahamtan⁺, Mohammad Mehdi Namavari and Masoumeh Hayati

Razi Vaccine and Serum Research Institute Shiraz-Iran

Abstract. The present study was investigated the frequency of *E. coli* O157 in cattle in south of Iran. The RAMS samples from cattle were enriched and continued by direct culture and PCR assay. We found RAMS sample in cattle is the main site for *E. coli* O157 localization. Of 892 *E. coli* isolates from 502 both healthy and diarrheic animals were analyzed, by screening for the presence of Shiga toxin-producing (VT 1 and VT 2) and intimin (*eae*) genes. The frequency of VT2 gene was found to be more frequent than VT1. The animal was kept in pen was more localized than tethered. Both diarrheic and healthy animals were shed *E. coli* O157:H7 in their feces.

Keyword: *E. coli* O157, PCR, Cattle, Iran

1. Introduction

Because the toxin is virtually identical to that produced by another bacteria known as *Shigella dysenteriae* type 1 [1], the bacteria that make these toxins are called “Shiga toxin-producing” *E. coli* (STEC) [2,3]. Of the numerous STEC serotypes identified, O157:H7 continue to be the dominant causes of illness in humans [3,4]. Most of what we know about *E. coli* O157:H7 comes from during an investigation into an outbreak of hemorrhagic colitis (HC) (bloody diarrhea) in the North-West USA in 1982 associated with consumption of contaminated hamburgers. It causes so called hamburger disease [5]. This serotype had only been isolated once before, from a sick patient in 1975 in Argentine [6]. They have a very low infectious dose, less than 50, are needed to cause infection [7]. The important complication of *E. coli* O157:H7 infections are hemolytic-uremic syndrome (HUS), HC and thrombotic thrombocytopenic purpura (TTP) [8,9] (Bielaszewska *et al* 2000, Garcia-Aljaro *et al* 2004). *E. coli* O157:H7 live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk [10,11], but cattle is the major source for human illnesses [3,12].

Accordingly, reduction of infection requires preventive measures that either reduces the number of animals that carry *E. coli* O157:H7 or the elimination of *E. coli* O157:H7 [13,14]. This phenomenon will effect a reduction in the rate of disease in humans [15,16]. To this end, a great deal of research has focused on describing the ecology and epidemiology of *E. coli* O157:H7 in cattle, with the hope of identifying interventions to reduce its prevalence in those animals [17,18]. The O157:H7 strain is distinguished microbiologically from other *E. coli* by its inability to ferment sorbitol and, most importantly, by its production of either one, or both, of the two phage encoded toxins (VT1 and VT2) [19]. Another virulence factors known to be *eae* gene, coding for intimin, a 94–97 Kda outer membrane protein [14] which is involved in the formation of attaching-and-effacing (A/E) lesions in the intestine of the host [18].

The present study applied by novel type of sample, obtained recto-anal mucosal swab (RAMS) rather than fecal material. RAMS samples were obtained from cattle with both selective enrichment and direct culture techniques. We described the isolation rate of *E. coli* O157:H7 strains in healthy and diarrheic cattle

⁺ Corresponding author. Tel.: (0098 711 524 0331); fax: (0098 711 624 0201).
E mail address: (yahyatahamtan@yahoo.com)

together from different farms in Fars province, and compared the serotypes and virulence markers of the strains with those previously reported.

2. Material and Methods

2.1. Bacterial strains

E. coli O157:H7 EDL933 which harbor stx1 and stx2 was used as a positive control (Kindly from Professor David Gally, University of Edinburgh, UK). *E. coli* O157 T-Shiraz 1387 (local collection obtained from field animal disease), which produces neither stx1 nor stx2, was used as negative control. The samples from cattle were collected during the two year periods in 2009 to 2010. Samples were then put in to the sterile container and transferred to the laboratory as soon as possible.

2.2. Isolation of O157 STEC

RAMS sample pre-enriched in TSB and incubated at 37°C for four h. Pre-enriched swab was streaked on Sorbitol-Mac Conkey agar supplemented with cefeximine (0.05 mg/L) and potassium tellurite (2.5 mg/L) (CT-SMAC). Sorbitol-nonfermenting colonies on SMAC agar were selected for testing by O157 and H7 monoclonal antibodies.

2.3. DNA extraction

DNP Cina-Gene kit was used for DNA extraction as described by the manufacturer. Briefly, the colony were grown overnight at 37°C with agitation (100 rpm) in TSB, centrifuged at 3000 g for 10 min. The extraction was completed by following the steps as indicated in the kit. DNA extracts were stored at -20 degree C until required.

2.4. Nucleotide sequence

The nucleotide sequences and predicted product sizes of the primers are shown in table 1.

2.5. PCR procedure

10X PCR buffer, 2.5 mmol/L MgCl₂, 200 micro mol/L dATPs, 50 pmol/L of each primer (variant), DNA template (1µl, 100ng), and Taq DNA polymerase (0.25µl) for a final volume of 25 µl by added distilled water. PCR was performed. The PCR reaction mixtures were processed in the gradients thermal cycler (Eppendorf, England) as followed by primary denaturation step, 5 min at 94 °C, the 35 cycles program consisted of 30 s at 94 °C for second denaturation, 30 s at 56 °C for annealing and 72 °C for primary extension. Final extension was carried out by 7 min at 72 °C to ensure complete strand extension. For all PCR reactions, standard positive and negative control strains were used. Seven micro liters of PCR products were run on a 1.5% agarose gel (Sigma) and visualized under UV-light gel doc (Kodak, logic gel logic 200) with ethidium bromide staining.

3. Results

Totally 502 cattle were examined for presence of *E. coli* O157:H7. Of them 892 *E. coli* isolates obtained from and were analyzed by multiplex PCR with VT1 and VT2 (variants), eae specific gene primers. More details were shown in table 2 and 3. The isolation rate of *E. coli* O157 was observed in 52(10.35%) of cattle were colonized with O157, while 7(13.46%) of these animals were healthy. There is no difference significant on the *E. coli* O157 isolation rate was observed between healthy and diarrheic cattle (just adult cattle, no calves). Altogether, 87 (9.75%) *E. coli* O157:H7 was isolated from 892 isolates (table 2). Therefore, one isolate was recovered per ten cattle and *E. coli* O157 was recovered from one in ten isolates. The main dominant of VT positive isolates was observed among the young cattle. There was observed significant differences on the occurrence of VT among the age of cattle ($P < 0.05$). Cattle originating from warm region seems to be more often infected than cold area where, no significant differences was observed between geographic region and seasonal variation in the prevalence of *E. coli* O157 ($P > 0.05$) (Figure 1).

4. Discussion

It is a major principle accepted STEC O157:H7 that has been isolated mainly from cattle is an important zoonotic agent [20]. Totally 9.75% of STEC cattle isolates were recovered *E. coli* O157:H7. These rates are higher than what have been found in some countries but lower than others [21,22]. *E. coli* O157 is world wide and studies performed in the Japan, China, Taiwan, European countries and USA was demonstrated that 0 to 100% of cattle have been contaminated [2,11]. Animals kept in pens will have more faecal-oral contact than tethered animals, and therefore probably maintain a higher level of stx in the intestine [21,23].

According to table 3, VT2 gene was found to be more frequent than VT1. Some studies were analysed in Japan [24], France [25], England [26] and Belgium [27] documented VT genes were detected in *E. coli* O157 ranged between 28.57 to 71.4%. They showed VT2 in isolates was more frequent than VT1 genes. These finding were parallel with results in the present study which revealed 54.02% versus 26.43%.

The relationship between the carriage of the *eae* gene and the potency of *E. coli* O157 strains to cause severe human disease, was proved [28,29]. Although some studies revealed that *E. coli* O157 strains isolated from HUS patients posses *eaeA* gene [30], but several *E. coli* O157 strains involved in severe human illnesses have lacked this gene or did not express intimin function [31,32]. Therefore, expressing additional adherence factors attributed *E. coli* O157 strains can colonize the small intestine [33]. There were significant seasonal differences in the levels of shedding of bacteria in the cattle observed. The prevalence of *E. coli* O157:H7 in the feces of cattle has been demonstrated to be higher during the warm months [34, 35] and parallel with the timing of most human illness outbreaks [36,37, 38]. During the winter, *E. coli* O157:H7 reservoirs would likely be reduced or absent [5]. The high diversity of *E. coli* serotypes which may carry the VT1 and VT2 genes may explain such a difference [39].

It is important to know the image of high-level fecal shedding has major definitions not only for epidemiological studies in public health but also for the control of *E. coli* O157 in the abattoir and on the farm [20]. Decrease *E. coli* O157 shedding in feces [40] and stop colonized animals [5] is the best ways to protect the food chain.

Boerling et al. revealed that *E. coli* O157:H7 isolated from humans different from those found in cattle reservoir [41]. Therefore, more investigation is needed to determine if animal and human strains belonged to the same clone.

5. Acknowledgments

We thank Dr M.H. Hosseini and the Immunology Laboratories for their excellent assistance and Professor D. Gally from the University of Edinburgh for providing the STEC O157:H7 strain EDL 933 used in this study.

6. References

- [1]. P. M. Griffin and R. V. Tauxe. The epidemiology of infections caused by *Escherichia coli* O157:H7., other enterhemorrhagic *E. coli.*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 1991, **13**: 60–98.
- [2]. M. Blanco, J. E. Blanco, A. Mora, G. Dahbi, M. P. Alonso, E. A. Gonzalez, M. I. Bernardez, and J. Blanco. Serotypes, Virulence Genes., and Intimin Types of Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Isolates from Cattle in Spain and Identification of a New Intimin Variant Gene (*eae*). *J. Clin. Microbiol.* 2004, **42**: 645-651.
- [3]. T. R. Callaway, M. A. Carr, T. S. Edrington, R.C Anderson and D. J. Nisbet. Diet, *Escherichia coli* O157:H7, and Cattle: Curr. Issues Mol. Biol. 11: 67-80. A Review After 10 Years. *Molecular Biology.* 2009, **11**: 67-80.
- [4]. G. L. Armstrong, J. Hollingsworth and J. G. Morris. *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 1996, **18**: 29-51.
- [5]. D. H. Rice, H. Q. Q Sheng, S. A. Wynia and C. J. Hovde. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157: H7-colonized cattle and those transiently shedding the same organism. *J. Clin. Microbiol.* 2003, **41**: 4924-4929.
- [6]. L. W. Riley, R. S. Remis, S. D. Helgerson, H. B. McGee, J. B. R. D. Wells, R. Hebert, E. Olcott, L. Johnson, N. Hargrett, P. Blake and M. Cohen. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Eng. J. Med.* 1983, **308**: 681–685.
- [7]. J. P. Nataro and J. B. Kaper. Diarrheagenic *Escherichia coli.* *Clin. Microbiol. Rev.* 1998, **11**: 142-201.

- [8]. M. Bielaszewska, H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschape, A. Cizek, J. Janda, K. Blahova and H. Karch. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H strains and a source of human diseases. *J. Clin. Microbiol.* 2000, **38**: 3470-3473.
- [9]. C. García-Aljaro, M. Muniesa, J. Jofre and A.R. Blanch. Prevalence of the stx2 Gene in Coliform Populations from Aquatic Environments. *Appl. Environ. Microbiol.* 2004, **70**: 3535–3540.
- [10]. K.A. Feldman, J.C. Mohle-Boetani, J. Ward, K. Furst, S. L. Abbott, D. V. Ferrero, A. Olsen, and S. B. Werner. A cluster of *Escherichia coli* O157: nonmotile infections associated with recreational exposure to lake water. *Public Health Report.* 2002, **117**: 380-385.
- [11]. H. S Hussein and T. Sakuma. Invited Review: Prevalence of Shiga Toxin-Producing *Escherichia coli* in Dairy Cattle and Their Products. *J. Dairy Sci.* 2005, **88**: 450-465.
- [12]. G. Conedera, P. A. Chapman S. Marangon, E. Tisato, P. Dalvit and A. Zuin. A field survey of *Escherichia coli* O157 ecology on a cattle farm in Italy. *Int. J. Food Microbiol.* 2001, **66**: 85-93.
- [13]. I. T. Kudva, S. Jelacic, P. I. Tarr, P. Youderian and C.J. Hovde. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Environ. Microbiol.* 1999, **65**: 3767–3773.
- [14]. A. Yilmaz, H. Gun, M. Ugur, N. Turan and H. Yilmaz. 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.* 2006, **106**: 213 – 217.
- [15]. B. A. Magnuson, M. Davis, H. Subele, P.R. Austin, I. T. Kudva, C.J. Williams, C.W. Hunt and C.J. Hovde . Ruminant gastrointestinal cell proliferation and clearance of *Escherichia coli* O157:H7. *Infect. Immun.* 2000, **68**: 3808–3814.
- [16]. P. McGee, D. J. Bolton, J. J. Sheridan, B. Earley, G. Kelly and N. Leonard. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. *J. Appl. Microbiol.* 2002, **93**: 706–713.
- [17]. J. L. Holland, L. Louie, A.E. Simor and M. Louie. PCR detection of *Escherichia coli* O157:H7 directly from stools: Evaluation of commercial extraction methods for purifying fecal DNA. *J. Clin. Microbiol.* 2000, **38**: 4108-4113.
- [18]. D. Law, A review: virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. *J. Appl. Microbiol.* 2000, **88**: 729-745.
- [19]. N. Pradel, V. Livrelli, CD. Champs, J. B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot and B. C. F. Joly. Prevalence and Characterization of Shiga Toxin-Producing *Escherichia coli* Isolated from Cattle., Food., and Children during a One-Year Prospective Study in France. *J. Clin. Microbiol.* 2000, **38**: 1023–1031
- [20]. J. C. Low, I. J. McKendrick, C. McKechnie, D. Fenlon, S. W. Naylor and C. Currie. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Appl. Environ. Microbiol.* 2005, **71**: 93-97.
- [21]. C. O. Gill and T. Jones. Microbiological sampling of carcasses by excision or swab. *J. Food Protect.* 2000, **63**: 167– 173.
- [22]. S. Park, R. W. Worobo and R. A. L. Durst. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. *Critical Rev. Food Sci. and Nut.* 1999, **39**: 481– 502.
- [23]. A. M. Urdahl, L. Beutin, E. Skjerve, S. Zimmermann, and Y. Wasteson. Animal host associated differences in Shiga toxin-producing *Escherichia coli* isolated from sheep and cattle on the same farm. *J. Appl. Microbiol.* 2003, **95**: 92–101.
- [24]. J. Sekiya. *Escherichia coli* O157:H7 in livestock in Japan. Review Science Technology-Off. *Int. Epizootol.* 1997, **16**: 391– 394.
- [25]. R. Guyon, F. Dorey, J. P. Malas, F. Grimont, J. Foret, B. Rouviere and J. F. Collobert. Superficial contamination of bovine carcasses by *Escherichia coli* O157:H7 in an slaughterhouse in Normandy (France). *Meat Sci.* 2001, **58**: 329– 331.
- [26]. P. A. Chapman, A. T. Cerdan Malo, M. Ellin, R. Ashton and M. A. Harkin. *Escherichia coli* O157 in cattle and sheep at slaughter., on beef and lamb carcasses and in raw beef in raw beef and lamb products in South Yorkshire., UK. *Int. J. Food Microbiol.* 2001, **64**: 139–150.

- [27]. A. V. TuteneL, D. Pierard, J. Van Hoof, M. Cornelis and L. D. Zutter. Isolation and molecular characterization of *Escherichia coli* O157 isolated from cattle., pigs and chickens at slaughter. *Int. J. Food Microbiol.* 2003, **84**: 63– 69.
- [28]. G. Johnsen, W. Yngvild, E. Heir, O.I. Berget and H. Herikstad. *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.* 2001, **65**:193-200.
- [29]. F. Omisakin, M. Macrae, I.D. Ogden and N.J. Strachan. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl. Environ. Microbiol.* 2003, **69**: 2444-2447.
- [30]. L. Beutin, S. Aleksic, S. Zimmermann and K. Gleier. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. *Med. J. Microbiol. Immun.* 1994, **183**: 13-21.
- [31]. T. M. I. Vaz, K. Irino, M. A. M. F. Kato, A. M. G. Dias, T. A. T. Gomes, M. I. C. Medeiros, M.M.M. Rocha and B.E.C. Guth. Virulence properties and characteristics of Shiga toxin-producing *Escherichia coli* in Sao Paulo., Brazil., from 1976 through 1999. *J. Clin. Microbiol.* 2004, **42**: 903-905.
- [32]. S. M. Farah, L. R. Silva, L. Castilhos, M. A. M. F. Kato, I.I. Ramos, T.M.I Vaz and K. Irino. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle., Parana., Brazil. In: Fifth International Symposium on Shiga toxin (Verocytotoxin)- producing *Escherichia coli* infections, VTEC, Edinburgh, Scotland. 2003, P: 186.
- [33]. H. S. H. Sakuma. Invited Review: Prevalence of Shiga Toxin-Producing *Escherichia coli* in Dairy Cattle and Their Products. *J. Dairy Sci.* 2005 **88**: 450-465.
- [34]. J. T. LeJeune, T. E. Besser, D. D. Hancock. Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl. Environ. Microbiol.* 2001, **67**: 3053-3057.
- [35]. D. G. Renter, S. L. Checkley, J. Campbell and R. King. Shiga toxin-producing *Escherichia coli* in the feces of Alberta feedlot cattle. *Canadian J. Veterinary Res.* 2004, **68**: 150-153.
- [36]. USDA-APHIS-VS. An update: *Escherichia coli* O157:H7 in humans and cattle. USDA Animal and Plant Health Inspection Service., Veterinary Service., Centers for Epidemiology and Animal Health., Fort Collins., CO. In., City. 1997.
- [37]. USDA: ERS. Estimated annual costs due to selected foodborne pathogens. In: <http://www.ers.usda.gov/Emphases/SafeFood/features.htm#start>. (Ed.). City. 2001.
- [38]. R. O. Elder, J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koochmaraie and W.W. Laegreid. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in faeces., hides., and carcasses of beef cattle during processing. *Proceeding National Academic Sci.* USA. 2000, **97**: 2999-3003.
- [39]. S. H. Hussein, H. Brandolyn, A. G. Tand Hudson. Verotoxin-Producing *Escherichia coli* in Sheep Grazing an Irrigated Pasture or Arid Rangeland Forages. VTEC in Sheep. 2003, **4**: 358-364.
- [40]. D. Jordan, S. A. McEwen, A. M. Lammerding, W. B. McNab and J. B. Wilson. Pre-slaughter control of *Escherichia coli* O157 in beef cattle: a simulation study. *Prevent. Vet. Med.* 1999, **41**: 55-74.
- [41]. P. Boerling, S. A. McEwen, J. B. Wilson, R. P. Johnson and C. L. Gyles. Association between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J. Clin. Microbiol.* 1999, **37**: 497- 503.

Table 1: Primers used in multiplex PCR for amplification of VT1, VT2, and eaeA genes

Primers	Oligonucleotide sequence(5'-3')	Product size (bp)	References
VT2-F VT2-R	CCA TGA CAA CGG ACA GCA GTT CCT GTC AAC TGA GCA CTT TG	779	(Fagan et al., 1999)
VT1-F VT1-R	ACA CTG GAT GAT CTC AGT GG CTG AAT CCC CCT CCA TTA TG	614	(Fagan et al., 1999)
eaeA-F eaeA-R	AAG CGA CTG AGG TCA CT ACG CTG CTC ACT AGA TGT	450	(Yilmaz et al., 2006)

Table 2: Frequency and characteristics of RAMS *E. coli* O157 isolates from Iranian cattle.

Virulence factor	Frequency
Vt1	23(26.43)
Vt2	47(54.02)
eae	24(27.58)
Vt1+vt2	17(19.54)
Vt1+eae	11(12.64)
Vt2+eae	19(21.83)
Vt1+vt2+eae	9(10.34)
Total	87

Table 3: Frequency and distribution of positive isolates, vt positive isolates and virulence markers encoding genes of RAMS *E. coli* O157 isolates from Iranian cattle

Cattle	Health	Ill	Total
No of cattle	102(20.31)	400(79.68)	502
No of cattle VT+	7(13.46)	45(86.53)	52(10.35)
No of isolates	98(10.98)	794(89.01)	892
No of VT+ isolates	19(21.89)	68(78.16)	87(9.75)

Fig. 1: Prevalence rate of *E. coli* O157:H7 in cattle and sheep according to seasonal area

