Research Article

CrossMark
← click for updates

Detection of Virulent Genes in *E. coli* O157:H7 Isolated from Puppies and Adult Dogs by Polymerase Chain Reaction

Mustafa Salah Hasan[1], Afaf Abdulrahman Yousif[1*], Mohammad Jweid Alwan[2]

[1]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine; [2]Department of Pathology and Poultry Disease, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract | *E. coli* O157:H7 is considered as an important pathogen of diarrhea in adult dogs and puppies. The presented study aimed to isolate *E. coli* O157:H7 serotypes from dogs and puppies and to detection of the *stx1*, *stx2* and *eaeA* virulence genes in these strains using conventional PCR assay. One hundred and four fecal swab samples were collected from dogs and puppies with different ages, breeds and sex. After primary cultivation of the collected samples onto MacConkey's and EMB agar media, the suspected *E. coli* colonies were subcultured onto CHROM and CT-SMAC agar media. The non sorbitol fermenter *E. coli* were serotyped using latex agglutination test for detection of O157 and H7 antigens. The results of latex test revealed 18 *E. coli* O157:H7 isolates. From 16 diarrheic animals, 11 (68.75%) were positive for *E. coli* O157:H7; 8 (72.72%) from puppies and 3 (60%) from adults. Meanwhile, 7 *E. coli* O157:H7 isolates were recovered from 88 non diarrheic animals (7.95 %); 4 (9.52%) from puppies and 3 (6.52%) from adults. The 18 isolates of *E. coli* O157:H7 were conducted to PCR assay, the results showed that 6 (33.33%) and 1(5.55%) *E. coli* isolates had *stx1* and *eaeA* genes, respectively. The results also showed that three isolates (16.66%) had all the investigated genes, three isolates (16.66%) had *Stx1* and *eaeA* genes, one isolate (5.55%) had *Stx2* and *eaeA* genes, while four (22.22%) isolates were negative for these virulence genes. In conclusion, we may suggest that dogs and puppies can be regarded as important reservoirs for *E. coli* O157:H7 which is one of the main causes of diarrhea and other diseases in human.

Keywords | Diarrhea, *E. coli* O157:H7, PCR, *Stx1*, *Stx2* and *eaeA*

INTRODUCTION

Enterohaemorrhagic *Escherichia coli* (EHEC) is the main cause of the recent outbreaks of diarrhea, hemolytic-uremic syndrome (HUS), and hemorrhagic colitis worldwide (Kwon and Cho, 2015). *E. coli* O157:H7 serotype are worldwide zoonotic and major foodborne pathogens responsible for the majority of severe cases of human enterohemorrhagic *Escherichia coli* (EHEC) diseases (Dulo, 2014). *Escherichia coli* O157:H7 infection has been documented in dogs (Jay-Russell et al., 2014). Dogs as other animals like cattle appear to be acting as a reservoir for *E. coli* O157 (Heuvelink et al., 2002). Shiga toxin (*stx*), a potent cytotoxin, is the major virulence factor linked to the presentation of both HC and HUS (Mohawk and O'Brien, 2011).

Blanco et al. (2004) recorded that intimin is required for intimate bacterial adhesion to epithelial cells, inducing a characteristic histopathological lesion defined as "attaching and effacing" (A/E). This lesion is governed by a large pathogenicity island named the locus of enterocyte effacement (LEE). The products of LEE are a type III secretion system, intimin and its translocated intimin receptor, and other secreted proteins. The secretion system is a molecular syringe for which secreted proteins are transferred into host cell cytoplasm. Intimin is encoded by *eaeA* gene that presents heterogeneity in their 3' end, involved in binding to the enterocytes.

The aim of this study was to isolate and identify *E. coli* O157:H7 serotypes in dogs and puppies with determining their virulence genes by conventional PCR assay.

MATERIALS AND METHODS

BACTERIOLOGICAL EXAMINATION

One hundred and four dogs aged up to 7 years`, from different breed and both sexes found in different places from Baghdad Province. One hundred and four faecal swabs with transport media were collected from 16 diarrheic dogs and 88 non diarrheic dogs.

Samples were cultured onto macConkey's and eosin methylene blue agar media (EMB) and incubated aerobically at 37°C for 24- 48 hours. the growing colonies were examined by naked eye concerning their shape, size and colour. A film from the suspected colonies were stained with Gram's stain and examined microscopically for morphological characteristics of bacterial isolates. Biochemical identifications were carried out according to Koneman (1997) by using indole, methyl red, Voges-Proskauer and citrate tests and cultivation onto triple sugar iron medium.

The suspected *E. coli* were subcultured on two specific media, CHROM O157 agar [The Pioneer of Chromogenic Media/Paris] and Cefixime Tellurite - Sorbitol MacConkey agar (CT-SMAC) [LABM™ (England)] supplemented with potassium tellurite (2.5 mg/L) and cifixime (0.05 mg/L) for differentiation of *E. coli* O157:H7 from other type of *E. coli*. (Tahamtan et al. 2011; Khanjar and Alwan, 2014).

Serological identification was done by using a latex agglutination test for *E. coli* O157:H7 from (Wellcolex *E. coli* O157:H7, Remel) to detect both the somatic antigen O157 and the flagellar antigen H7 according to (Ewing, 1986).

DNA EXTRACTION

All isolates of *E. coli* O157:H7 which identified bacterialiologically and serologically were conducted to the DNA extraction by using genomic DNA extraction Kit from (Presto™ Mini g DNA Bacteria Kit Geneaid. USA).

DETECTION OF VIRULENCE GENES (STX1, STX2 AND EAEA) USING POLYMERASE CHAIN REACTION (PCR)

PCR assay was performed in the laboratories of internal and preventive Veterinary Department, College of Vet. Medicine, University of Baghdad.

a- Primers: Three primers (Bioneer, Korea) were used to detect *E. coli* O157:H7 virulence genes *Stx1*, *Stx2* (Gannon et al., 1992) and intimin (*eaeA*) gene (Paton and Paton, 1998) (Table 1).

The purity and concentration of extracting DNA were measured using a Nanodrop spectrophotometer (NanoDrop ND-2000 Thermo Fisher scientific, USA). Then gel electrophoresis was used to check the extracted DNA by loading the DNA in 1% agarose gel.

Table 1: The primers with their sequences and product size

Target gene		Primer sequence (5' to 3')	Product size (bp)
Stx1	F	ACA CTG GAT GAT CTC AGT GG	614
	R	CTG AAT CCC CCT CCA TTA TG	
Stx2	F	CCA TGA CAOA CGG ACA GCA GTT	779
	R	CCT GTC AAC TGA GCA CTT TG	
eaeA	R	CCA CCT GCA GCA ACA AGA GG	384

b- PCR mixture components for s*tx1*, *stx2* and *eaeA* genes: The reaction for stx1 and stx2 were included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 2 µL templet DNA, 12.5 µL PCR master mix, 2 µL of each primer, 6.5 µL PCR water. The reaction for *eaeA* was included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 2 µL templet DNA, 12.5 µL PCR master mix, 2 µL of each primer, 6.5 µL PCR water.

c- Thermo- cycler program: The program of thermo-cycle for detection of stx1 and stx2 was performed as follows. One cycle for 3 minutes at 94°C to denaturate template. It was continued by 35 cycles, each cycle including denaturation 60 seconds at 94°C, annealing 30 seconds at 53°C, and extension 60 seconds at 72°C. Final extention was done 7 minutes at 72°C (Osek, 2004; Pradel et al 2000).

The program of thermo-cycle for detection of *eaeA* gene was performed as follows.

Initial denaturation started by 1 cycle at 94°C for 5 minutes. Followed by 35 cycles, each cycle including denaturation, annealing and extention at 94°C for 60 seconds, 57°C for 30 seconds, 72°C for 60 seconds. The 35 cycles Followed by final extention for 10 minutes at 72°C

d- PCR product Analysis (Agarose Gel Electrophoresis): This step was used to analyze the PCR product by 2% agarose gel electrophoresis, stained with 0.5 µg/mL ethedium bromide, The PCR products (bands) were visualized by using a UV transilluminator [Cleaver Scientific (U.K)] and photographed by using digital camera.

ETHICALLY APPROVED

This study was approved by the ethical and research commity of Veterinary Medicine of College, University of Baghdad, Ministry of High Education and Scientific Research.

RESULTS

RATE OF *E. COLI* O157:H7 IN DOGS WITH AND WITHOUT DIARRHEA

The results showed 18 isolates of *E. coli* O157:H7 from 87 *E. coli* isolates. *Escherichia coli* O157:H7 was isolated

Table 2: Rate of infection of *E. coli* O157:H7 in puppies and adult dogs

Signs	No. of Animals	Number of samples	No & % of positive samples to *E. coli* O157:H7
Animals with diarrhea	Puppies (11)	11	8 (72.72%)
	Adult (5)	5	3 (60.0%)
Animals without diarrhea (88)	Puppies (42)	42	4 (9.52%)
	Adult (46)	46	3 (6.52%)
Total	104	104	18(17.3%)

from puppies and adult dogs with diarrhea at a percentage (68.75%) and from puppies and adults without diarrhea at a percentage (7.95%). The overall percentage of both with and without diarrhea was 17.3% (Table 2).

ESHERICHIA COLI O157:H7 ISOLATION

The bacterial cultivation revealed red/pink colonies onto MacConky's agar and metallic sheen colonies onto eosin methylene blue agar. Isolated bacteria were appeared as gram negative rod under light microscope. The biochemical tests showed that *E. coli* were mostly motile, gave positive reactions on catalase, indole and methyl red tests and –ve reactions on voges – proskaure, oxidase and citrate tests., TSI agar medium gave yellow colour in both the slant and bottom of with ability for gas production.

Bacterial colonies were detected by a characteristic mauve color on Chrom agar, and as smooth colorless with smoky center On CT- SMAC, thus indicating that this bacterium colony belongs to *E. coli* O157:H7.

SEROTYPING TEST (WELLCOLEX *E. COLI* O157:H7, REMEL)

The smooth colourless colonies with smoky center appeared on CT- SMAC was tested for distinguishing both O157 and H7 antigens by latex agglutination test. The positive isolates for the O157 antigen were sub-cultured for overnight on blood agar for the detection of H7 flagellar antigen. Our result of latex test showed that 18 isolates were positive for O157 and H7 antigens.
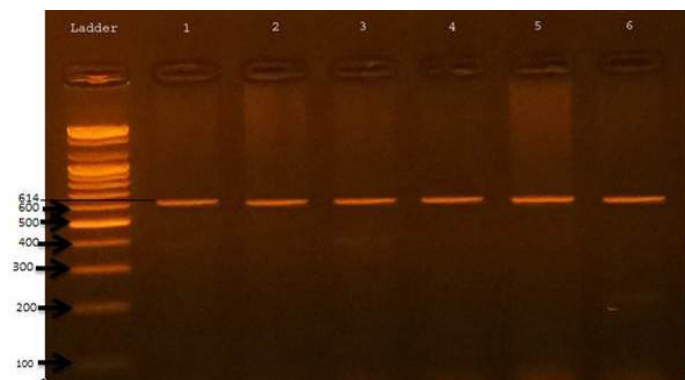

Figure 1: Agarose gel (2%) electrophoresis showed amplification of 614 bp fragments of *Stx1* gene
Lanes 1, 2, 3, 4, 5, 6 showed positive amplification of *stx1* in *E.coli O157:H7*; Lane M shows PCR marker.

MOLECULAR DETECTION OF *stx1*, *stx2* AND *eaeA* VIRULENCE GENES AMONG *E. COLI* O157:H7 ISOLATES

The results showed that the amplified PCR product were 614 bp, 779 bp, 384 bp for *Stx1*, *Stx2* and *eaeA* bp. for *E. coli* O157:H7 isolates, respectively (Figure 1, 2 and 3).
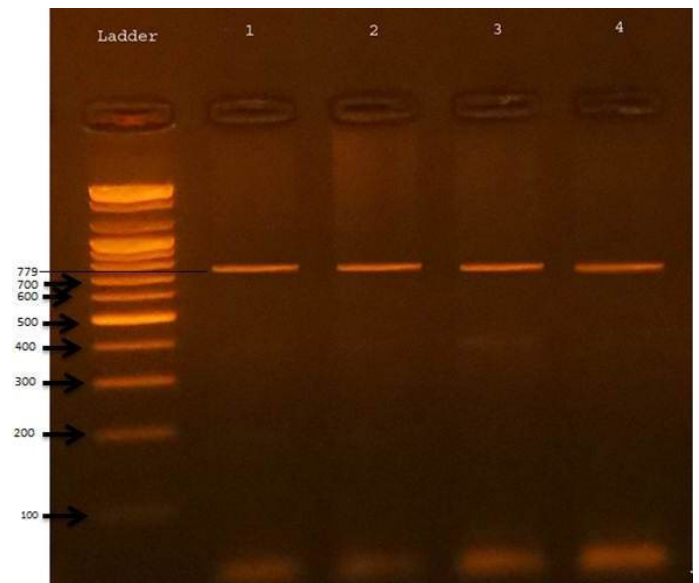

Figure 2: Agarose gel (2%) electrophoresis showes amplification of 779 bp fragments of *Stx2* gene of *e.coli 0157:H7*
Lanes 1, 2, 3, 4, showed positive amplification of stx2 in *E.coli O157:H7*; Lane M shows PCR marker.
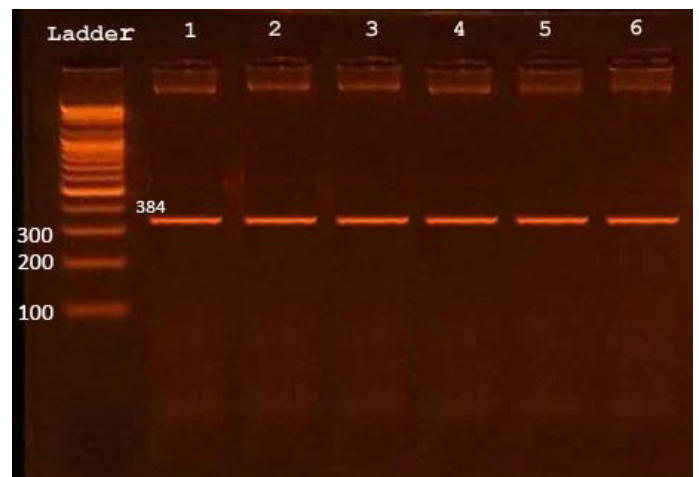

Figure 3: Agarose gel (2%) electrophoresis shows amplification of 384 bp fragments of *eaeA* genes.
Lanes 1, 2, 3, 4, 5, 6 showed positive amplification of *eaeA* in *E.coli 0157:H7*; Lane M shows PCR marker.

The results of PCR assay on 18 isolates of *E.coli* O157 from total number of 104 samples revealed that 1 isolate (5.55%) had s*tx1* gene, 6 (33.33%) isolates had *eaeA* gene, 3 isolates (16.66%) possessed two genes had s*tx1* and *eaeA* genes also one isolates (5.55%) had s*tx2* and *eaeA* genes. Only three isolates (16.66%) possessed three genes (s*tx1*, s*tx2* and *eaeA)*, while four (22.22%) isolates were negative for these three virulence genes (Table 3).

Table 3: Number and percentage of *E.coli* O157:H7 isolates that possessed one or mixed virulence genes (*stx1, stx2* and *eaeA*)

No of genes in each isolate	Gene(s)	No of *E.coli* O157 possessed gene(s)	%
one gene	Stx1	1	(5.55%)
	eaeA	6	(33.33%)
Two genes	*Stx1+eaeA*	3	(16.66%)
	Stx2+eaeA	1	(5.55%)
Three genes	*Stx1+Stx-2+eaeA*	3	(16.66%)
No one of the 3 genes	-	4	(22.22%)
		18	

DISCUSSION

Dogs and cats called as companion animals have a closely relationship with humans. For that reason, a companion animal carrying with EHEC may become a human health threat (Murinda et al., 2004; Sancak et al., 2004).

We described the rate of *E. coli* O157:H7 isolated from dogs and puppies in Baghdad, Iraq with detecting of major virulence genes of *E. coli* O157:H7. Our study shown extremely high rates of *E. coli* O157:H7 in puppies and adult dogs with diarrhoea (68.75%) while non diarrheic animals showed low rates (7.95%) and percentage of both diarrheic and non diarrheic animals (17.3%). According to previous studies, it is difficult to detect EHEC, especially *E. coli* O157:H7 in the companion animals as recorded by (Khakhria et al., 1990; Bentancor et al., 2007).

Our study was compatible with Beutin (1999) who found the rate of STEC bacteria was ranged between 3.2-12.3% in healthy dogs while the results disagree with the results of Ojo et al. (2014) who found that the percentage of *E. coli* O157:H7 in non diarrheic dogs was 26.9% .

The percentage was very high in diarrheic dogs which disagreed with many researcher, Beutin (1999) reported that the percentage of positive samples for STEC in diarrhoeic dogs was 8.9%. Similarly, Hammermueller et al. (1995) reported that 17.2% of STEC in diarrhoeic dogs and Sancak et al. (2004) detected STEC in the faeces of 28.1% of dogs. Bentancor et al. (2007) reported that STEC isolates showed 1.1% of the dogs (5/450) and the 0.16% in Japan (Kataoka et al., 2010). While our results were showed that the rate of infection was 68.75% of diarrheic animals.

The higher prevalence of STEC O157:H7 as observed in the present study might be due to the possibly higher exposure of the dogs examined to the organism. It has been suggested that dogs may acquire STEC O157:H7 from foods which might be contaminated with *E. coli* O157:H7 or from ruminants which are considered to be the principal reservoirs of the organism (Hogg et al., 2009; Ojo et al., 2010). Besides, dogs might wander from home and have direct access to slaughter-houses and meat markets where they scavenge for remnants of fresh meat (Adeyemo, 2002).

Our results showed that Chrome agar aids in diagnosis of *E. coli* O157, this result is in agreement with Tavakoli et al. (2008) who recorded that the use of chrome agar allowed presumptive identification of *E. coli* O157 from the primary isolation plate (EMB, MacConkey) and differentiation from other organisms. A similar study by Yousif and Al-Taii (2014) and Yousif and Hussein (2015) reported that the Chrome agar is useful for diagnosis of *Escherichia coli* O157. *E. coli* O157:H7 (CT-SMAC) appear as smooth colorless colonies with smoky center, this result is compatible with Garcia et al. (2010) who found that typical *E. coli* O157:H7.

Latex agglutination test gave a good results and higher sensitivity and specifity for the diagnosis of *E. coli* O157:H7, this result is compatible with a result of (Yousif and Al-Taii, 2014; Al-Dawmy and Yousif, 2013) the used this test for detection of *E.coli* O157:H7. Also Karmali et al. (1999) describe latex test for detection of *E.coli* O157:H7 was a rapid, reliable, easy to perform and interpret, and it should allow testing for ST to become more widely performed.

Our study showed that the percentage of *Stx1* were higher than *Stx2*, these results agree with a result of (Ojo et al., 2014). Sancak et al. (2004) conclude that *eaeA* gene and the *Stx1* gene were often found together than *Stx2* and *eaeA*, This result was incompatible with our result. The *eaeA* which was a necessary gene for attaching and effacing activity (Kaper et al., 1998). Many investigators have underlined the strong association between carrying *eaeA* and the capacity of STEC to cause severe human disease, especially HUS (Oswald et al., 2000). In the present study, this important virulence gene was detected in most isolates. A similar results of the intimin (*eaeA*) gene has also been found in other studies (Blanco et al., 2001; Blanco et al., 2004).

CONFLICT OF INTEREST

Authors declares no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors contributed equally in all details of this manuscript.

REFERENCES

- Adeyoma O (2002). Unhygienic operation of a city abattoir in South Western Nigeria: Environmental implication. African J. Environ. Assess. Manag. 4: 23-28.
- Al–Dawmy F, Yousif A (2013). Prevalence of *E. coli* O157:H7 in intestinal and urinary tract infection in children. Int. J. Adv. Res. 1(8): 111-120.
- Beutin L (1999). *Escherichia coli* as a pathogen in dogs and cats. Vet. Res. 30: 285-298.
- Blanco J, Blanco M, Blanco JE, Mora A, Alonso MP, Gonzalez EA, Bernardez MI (2001). Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants. In Duffy, G.; Garvy, P.; McDowell, D.A. eds. Verocytotoxigenic *E. coli* Trumbull, Connecticut: Food & Nutrition Press, Inc., pp. 113-148.
- Blanco M, Blanco JE, Mora A, Dahbi G, Alonso MP, Gonzlez EA (2004). 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 (*eaeA*-xi). J. Clin. Microbiol. 42: 645-651. http://dx.doi.org/10.1128/JCM.42.2.645-651.2004
- Bentancor A, Rumi M, Gentilini M, Sardoy C, Irino K, Agostini A, Cataldi A (2007). Shiga toxin-producing and attaching and effacing *Escherichia coli* in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. FEMS Microbiol. Lett. 267: 251-256. http://dx.doi.org/10.1111/j.1574-6968.2006.00569.x
- Dulo F (2014). Prevalence and antimicrobial resistance profile of *Escherichia coli* O157:H7 in goat slaughtered in dire dawa municipal abattoir as well as food safety knowledge , attitude and hygiene practice assessment among slaughter staff, Ethiopia. MSc., Thesis, Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Microbiology, Immunology and Veterinary Public Health.
- Ewing E (1986). Genus Esophagenica. In: Ewing, W. H. (Ed.), Edwards and Ewing's identification of Enterobacteriac *eaeA*, 4th ed. Elsevier Science publishers, New York. Pp. 96-134.
- Gannon VP, King RK, Thomas EJ (1992). Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol. 58(12): 3809–3815.
- Garcia A, Fox J, Besser T (2010). Zoonotic enterohemorrhagic *Escherichia coli*: A One Health perspective. ILAR J. 51(3): 221-232. http://dx.doi.org/10.1093/ilar.51.3.221
- Hammermuller J, Kruth S, Prescott J, Gyles C (1995). Detection of toxin genes in *Escherichia coli* isolated from normal dogs and dogs with diarrhea. Can. J. Vet. Res. 59: 265-270.
- Heuvelink AE, Van Heerwaarden C, Zwartkruis-Nahuis JTM, Van Oosterom R, Edink K, Van Duynhoven YT, de Boer

E (2002). *Escherichia coli* O157 infection associated with a petting zoo. Epidemiol. Infect. 1292): 295–302.
- Hogg RA, Holmes JP, Ghebrehewet S, Elders K, Hart J, Whiteside C, Willshaw GA, Cheasty T, Kay A, Lynch K, Pritchard GC (2009). Probable zoonotic transmission of verocytotoxigenic *Escherichia coli* O157 by dogs. Vet. Rec. 164: 304-305. http://dx.doi.org/10.1136/vr.164.10.304
- Jay-Russell M, Hake AF, Bengson Y, Thiptara A, Nguyen T (2014). Prevalence and characterization of *Escherichia coli* and salmonella strains isolated from stray dog and coyote feces in a major leafy greens production region at the United States-Mexico Border. PLOS ONE. 9(11): 1-14. http://dx.doi.org/10.1371/journal.pone.0113433
- Kaper JB, Elliott S, Sperandio V, Perna NT, Mayhew G (1998). Attaching-and-effacing intestinal histopathology and the locus of enterocyte effacement. In *Escherichia coli* O157:H7 and Other Shiga-Toxin-Producing *E. coli* Strains ed Kaper, J.B. and O'Brien, A.D. pp.163^182.Washington, DC: American Society for Microbiology.
- Karmali MA, Petric M, Bielaszewska M (1999). Evaluation of a microplate latex agglutination method (Verotox-F Assay) for detecting and characterizing verotoxins(Shiga Toxins) in *Escherichia coli*. J. Clin. Microbiol. 37: 396–399.
- Kataoka Y, Irie Y, Sawada T, Nakazawa M (2010). A 3-year epidemiological surveillance of *Escherichia coli* O157:H7 in dogs and cats in Japan. J. Vet. Med. Sci. 72(6): 791–794. http://dx.doi.org/10.1292/jvms.09-0439
- Khakhria R, Duck D, Lior H (1990). Extended phage- typing for E. coli O157: H7. Epidemiol. Infect. 105: 511–520. http://dx.doi.org/10.1017/S0950268800048135
- Khanjar AF, Alwan MJ (2014). Genotypic study of *Escherichia coli* O157:H7 isolated from stool samples of humans and cattle. Int. J. Adv. Res. 2(6): 204-212.
- Koneman E, Allen D, Janda W, Schreckenberger P, Winn W (1997). Color Atlas and Textbook of Diagnostic Microbiology, 5th edn. Philadelphia: Lippincott.
- Kwon T, Cho S (2015). Draft genome sequence of enterohemorrhagic *Escherichia coli* O157 NCCP15739, isolated in the Republic of Korea. Genome Announc. 3(3): e00522-15. http://dx.doi.org/10.1128/genomeA.00522-15
- Markey B, Leonard F, Archambault M, Cullinane A, Maguire D (2013). Clinical veterinary microbiology. Second edition, Mosby, Elsevier. 246-249.
- Mohawk K, O'Brien A (2011). Mouse models of *Escherichia coli* O157:H7 infection and shiga toxin injection. J. Biomed. Biotechnol. 2011: 17. http://dx.doi.org/10.1155/2011/258185
- Murinda SE, Nguyen LT, Landers TL, Draughon FA, Mathew AG, Hogan JS, Smith KL, Hancock DD, Oliver SP (2004). Comparison of *Escherichia coli* isolates from humans, food, and farm and companion animals for presence of Shiga toxin-producing *E. coli* virulence markers. Food borne Pathog. Dis. 1: 178–184. http://dx.doi.org/10.1089/1535314042158161
- Ojo O, Ajwape A, Otesile E, Owoadi A, Oyekunel M, Adetosoye A (2010). Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. Intl. J. Food Microbiol. 142: 214-221. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.06.030
- Ojo O, Bello A, Amosun E, Ajadi R (2014). Multidrug resistant verocytotoxin-producing *Escherichia coli* O157:H7 in the faeces of diarrhoeic and non-diarrhoeic dogs in Abeokuta, Nigeria. Veterinarski. arhiv. 84(1): 63-73.

• Osek J (2004). Phenotypic and genotypic characterization of *Escherichia coli* O157 strains isolated from humans, cattle and pigs. Vet. Med. Czech. 49 (9): 317–326.

• Oswald E, Schmidt H, Morabito S, Karch H, Marches O, Caprioli A (2000). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. Infect. Immun. 68: 64–71. http://dx.doi.org/10.1128/IAI.68.1.64-71.2000

• Paton AW, Paton JC (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*A, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. J. Clin. Microbiol. 36(2): 598-602.

• Pradel N, Livrelli V, De Champs C, Palcoux J-B, Reynaud A, Scheutz F, Sirot J, Joly B, Forestier C (2000) 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. 38: 1023–1031.

• Sancak A, Rutgers H, Hart C, Batt R (2004). Prevalence of enteropathic *Escherichia coli* in dogs with acute and chronic diarrhoea. Vet. Rec. 154: 101-106. http://dx.doi.org/10.1136/vr.154.4.101

• Tahamtan Y, Pourbakhsh, Hayati SA, Namdar M, Shams N, Namvari ZMM (2011). Prevalence and molecular characterization of verotoxin-producing *Escherichia coli* O157:H7 in cattle and sheep in Shiraz-Iran. Archiv. Razi Instit. 66 (1): 29-36.

• Tavakoli H, Bayat M, Kousha A, Panahi P (2008). The application of chromogenic culture media for rapid detection of food and water borne pathogen. American-Eurasian J. Agri. Environ. Sci. 4(6): 693-698.

• Tille P (2014). Bailey & Scott's Diagnostic Microbiology. 13th ed. , Mosby: 210-230.

• Yousif A, Al-Taii D (2014). Isolation and characterization of *E. coli* O157:H7 from human and animals. Mirror Res.Vet. Sci. Anim. 3(2): 11-18.

• Yousif A, Hussein M (2015). Prevalence and molecular detection of intimin (*eaeA*A) virulence gene in *E. coli* O157:H7 in calves. Res. J. Vet. Pract. 3(3): 47. http://dx.doi.org/10.14737/journal.rjvp/2015/3.3.47.52