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Molecular serotyping of Shiga toxin-producing *Escherichia coli* (STEC) of animal origin in Iran reveals the presence of important non-O157 seropathotypes

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Abstract

The present study reported the first serotyping (O:H typing) data documented in Shiga toxin-producing *Escherichia coli* (STEC) strains of animal origin in Iran in isolates recovered between 2008 to 2016. A total number of 75 STEC strains previously isolated from fecal samples of cattle, sheep, goats, pigeons, humans, and deer were assessed by different polymerase chain reaction (PCR) assays detecting the major virulence genes of STEC and phylogroups. Then, the strains were tested for the 16 important O-groups by PCR. Finally, twenty strains were selected for H-genotyping by PCR plus sequencing. The predominant serogroup was O113 which was detected in nine isolates (five cattle, 55.50%; two goats, 22.20%; two red deer, 22.20%) followed by O26 (3/3, 100%) in cattle, O111 (3/3, 100%) in cattle, O5 (3/3, 100%) in sheep, O63 (1/1, 100%) in pigeon, O75 (2/2, 100%) in pigeons, and O128 in goats (2/3, 66.60%) and pigeon (1/3, 33.30%). The most important recognized serotypes were O113:H21 in cattle (2/3) and goat (1/3), O113:H4 in red deer (1/1), O111:H8 in calves (2/2), O26:H11 in calve (1/1), O128:H2 in goats (2/3) and pigeon (1/3), and O5:H19 in sheep (3/3). One cattle strain carrying *stx*1, *stx*2, *eae*, and *Ehly* genes belonged to O26:H29 serotype. Most strains with determined O-groups were from the bovine source that highlighted the importance of cattle as reservoirs of potentially pathogenic serovars. The present study suggested that the top seven non-O157 serogroups should be assessed along with O157 in all future research and clinical diagnostics of STEC in Iran.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains have been implicated as major human pathogens in gastro-intestinal infections and foodborne outbreaks worldwide.[1] These infections range from mild diarrhea to serious diseases, such as hemorrhagic colitis (HC) or hemolytic uremic syndrome (HUS).[1,2] Progressive renal failure, acute hemolytic anemia and thrombocytopenia are the main symptoms of HUS which are associated with acute kidney injury in humans, especially in infants and children.[3]

The importance of STEC strains has been well documented worldwide and also in developing countries such as Iran.[4,5] Transmission of these strains occurs through the consumption of undercooked ground beef, unpasteurized dairy products, contaminated drinking water and contact with infected animals.[6] In the previous reports in Iran and other countries cattle were highlighted as the main reservoirs of HUS-associated *E. coli* (HUSEC).[7-9]

Among virulence factors of STEC, Vero toxins (VT), also called Shiga toxins (Stx), are the key virulence factors which are classified into two main types: *stx*1 and *stx*2, responsible for inhibiting protein synthesis in eukaryotic cells.[4] STECs producing only *stx*2 toxin are posing a high risk to the development of severe human infections including HUS.[10,11] Another virulence factor associated with HUS is the production of enterohemolysin encoded by *Ehly* (*ehxA*) which has a cytolytic effect.[4] Reports from previous studies have shown a significant number of *Ehly* positive isolates in STEC strains contributing to human HUS.[12,13] Intimin is another virulence-associated factor encoded by the *eae* gene accountable for intimate attachment of STEC to intestinal epithelial cells.[4] The STEC strains which are positive for intimin are closely linked with cases of HC and HUS.[14]

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The STEC strains causing human infections belong to a large number of O:H serotypes which have been classified into five seropathotypes A to E.[4,15] Seropathotype A consists of O157:H7 and O157:NM (nonmotile) which are considered to be the most important serotypes in human HUS outbreaks. Seropathotype B comprises O26:H11, O103:H2, O111:NM, O121:H19 and O145:NM which are also associated with HUS, however, are more associated with smaller outbreaks. Seropathotype C is composed of serotypes O91:H21 and O113:H21 which are related to sporadic cases of HUS. Seropathotype D is associated with individual cases of diarrhea and seropathotype E has not been involved in human infections.[4]

Although the O157:H7 is the most frequently involved serotype in large outbreaks, other serotypes can also be important in sporadic cases of HUS and life-threatening health conditions.[4,15] For decades in Iran, a large number of studies on STEC was aimed at O157:H7 detection.[15,16] Recent studies are increasingly reporting the importance of non-O157 STEC strains in both animal and human hosts from different regions, however, documented data about these serotypes are still limited.[17-19]

Lack of the availability of O:H serotyping data in most developing countries has been a public health challenge to track outbreaks and monitor the possible sources in different geographical regions. To this end, here we reported for the first time the distribution of major STEC serotypes (O:H types) in a collection of STEC strains isolated from different provinces and a variety of sources in Iran.

Materials and Methods

STEC isolates. During eight years of fecal sampling (2008 to 2016), a total number of 75 non-duplicate STEC strains isolated in previous studies were selected. Samples were obtained from different provinces and a variety of animal hosts in Iran: 35 isolates were originated from cattle, 22 from sheep and goats, 14 from pigeons and four were obtained from other sources (two human isolates and two isolates from red deer) which were archived as cryopreserved stocks at – 70.00 ˚C. The study was carried out in accordance with Iran National Committee for Ethics in Biomedical Research. Accordingly, a written or verbal informed consent was obtained from all participants for human experimentations and verbal informed consent was obtained from the owners of the companion animals. The research committee reviewed and approved that all the study protocols were conducted in accordance with the related guidelines and regulations (IR.1396.1236). For recovery, each isolate was aseptically transferred to brain heart infusion broth (Merck, Darmstadt, Germany) and incubated for 24 hr at 37.00 ˚C. Then, the cultures were streaked on MacConkey agar and subjected to DNA extraction after 24 hr incubation at 37.00 ˚C.

DNA extraction. All 75 confirmed STEC isolates on MacConkey agar were cultured on Luria Bertani (LB) agar and incubated for 24 hr at 37.00 ˚C. After overnight culture on LB agar, total genomic DNA was extracted by boiling method as described previously.[20]

Multiplex Polymerase chain reaction (PCR) for *stx*1, *stx*2, *stx*2*f*, *Ehly*, *eae*. The virulence genes of STEC isolates were evaluated by multiplex PCR as described previously.[21,22] The PCR was carried out in 25.00 µL using 3.00 µL template DNA, 1.00-unit *Taq* DNA polymerase (Ampliqon A/S, Odense, Denmark), 0.30 µM of each primer, 2.00 mM $MgCl_2$ (Ampliqon A/S) and 200 µM dNTP mix in 1x buffer (Ampliqon A/S) (Table 1). *E. coli* O157:H7 strain (ATCC 35218) was used as positive control. For detection of *stx* in pigeon isolates, a pair of primers were used that amplifed the *stx*2*f* according to Schmidt's *et al*. study (Table 1). [22]

Molecular determination of serogroups (O-serogroups). The 16 important O-groups of STEC isolates were studied by different PCR assays via targeting serogroup-associated antigen genes (Table 1) as described previously.[23-28] The first PCR panel included the top eight STEC serogroups (O26, O45, O103, O111, O113, O121, O145, and O157) as described by DebRoy *et al*.[23] Other panels included Panel 2: O91, O118, O55 and Panel 3: O5, O104, O128. The remaining serogroups were tested using uniplex assays including O63 and O75. For final confirmation, PCRs were repeated separately for the positive serogroups. *E. coli* O157:H7 (295 EC-TMU) and Ferdowsi University of Mashhad collection strains were used as control for O26 (Strain code: 162s2 EC) and O111 (Strain code: 11s EC-2008).

Amplification and sequencing of *fliC* gene (H-typing). A PCR assay was applied to amplify the variable portion of the *fliC* gene based on the method presented by Machado *et al*.[29] Then, 20 strains with a known O-types (except two important strains with unknown O-types) were selected from different animals for identification of H-genotypes by sequencing of the *fliC* gene in both reverse and forward directions with the same primer used for the *fliC* gene amplification (Sinaclon, Tehran, Iran), (Table 1). The obtained sequences were blasted in NCBI databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Only the strains in which both forward and reverse directions designated a particular H-types were kept for data analyses.

Phylogenetic group analysis. All confirmed O:H serotyped strains were subjected to determination of phylogenetic groups based on the updated Clermont's PCR based method.[30] Amplification was performed at a final volume of 25.00 µL containing 50.00 ng template DNA, 1-unit *Taq* DNA polymerase (Ampliqon A/S), 1.00 µM of each primer, 1.50 mM $MgCl_2$ (Ampliqon A/S) and 200.00 µM dNTP mix in 1x PCR buffer (Ampliqon A/S). Strains were assigned into one of the eight phylogenetic groups (A, B1, B2, C, D, E, F and Clade I) based on the

possession or absence of four genetic markers including *arpA*, *yjaA, chuA*, and *tspE* (Table 1). The complementary PCR tests applied on the isolates were not typed in the first step as recommended.[30]

Table 1. Primers used for identification of virulence genes, serogroups, H-types, and phylogenetic groups in this study.

Name	Sequence (5′ to 3′)	Target (bp)	Condition		References
			Annealing temperature (˚C)	Time (sec)	
stx1-F	ATAAATCGCCATTCGTTGACTAC	*stx1* (180)	58.00 - 65.00*	30 - 60*	21
stx1-R	AGAACGCCCACTGAGATCATC				
stx2-F	GGCACTGTCTCTCTGAAACTGCTCC	*stx2* (255)	58.00 - 65.00*	30 - 60*	-
stx2-R	TCGCCAGTTATCTGACATTCTG				
stx2f-1	AGATTGGGCGTCATTCACTGGTTG	*stx2f* (428)	56.00	60	22
stx2f-2	TACTTTAATGGCCGCCCTGTCTCC				
Ehly-F	GCATCATCAAGCGTACGTTCC	*Ehly* (534)	58.00 - 65.00*	30 - 60*	21
Ehly-R	AATGAGCCAAGCTGGTTA AGCT				
eae-F	GACCCGGCACAAGCATAAGC	*eae* (384)	58.00 - 65.00*	30 - 60*	-
eae-R	CCACCTGCAGCAACAAGAGG				
O26-F	CAATGGGCGGAAATTTTAGA	O26 (155)	56.00	60	23
O26-R	ATAATTTTCTCTGCCGTCGC				
O45-F	TGCAGTAACCTGCACGGGCG	O45 (238)	56.00	60	-
O45-R	AGCAGGCACAACAGCCACTACT				
O103-F	TTGGAGCGTTAACTGGACCT	O103 (321)	56.00	60	-
O103-R	GCTCCCGAGCACGTATAAAG				
O111-F	TGTTTCTTCGATGTTGCGAG	O111 (438)	56.00	60	-
O111-R	GCAAGGGACATAAGAAGCCA				
O113-F	TGCCATAATTCAGAGGGTGAC	O113 (514)	56.00	60	-
O113-R	AACAAAGCTAATTGTGGCCG				
O121-F	TCCAACAATTGGTCGTGAAA	O121 (628)	56.00	60	-
O121-R	AGAAAGTGTGAAATGCCCGT				
O145-F	TTCATTGTTTTGCTTGCTCG	O145 (750)	56.00	60	-
O145-R	GGCAAGCTTTGGAAATGAAA				
O157-F	TCGAGGTACCTGAATCTTTCCTTCTGT	O157 (894)	56.00	60	-
O157-R	ACCAGTCTTGGTGCTGCTCTGACA				
O118-F	TGCAAGAGATGGTATTGAGCTGGG	O118 (517)	54.00	60	24
O118-R	TCCTGAGCCAATTTCTGTAGGTCG				
O55-F	TCCTTATTTGTGTCGGGGG	O55 (207)	54.00	60	25
O55-R	CCAGGAAAGCTGCCAATTATC				
O63-F	ATTCGGTGCTGCTGGAATTA	O63 (995)	54.00	60	23
O63-R	TGAACATTATGCCACCGATG				
O91-F	TTGCATCTGGCGCAATAAACACGG	O91 (616)	54.00	60	24
O91-R	ACACCATCCCAAATACCTGCTTGC				
O128-F	ATGATTTCTTACGGAGTGC	O128 (782)	50.00	50	26
O128-R	CTCTAACCTAATCCCTCCC				
O104-F	TGAACTGATTTTTAGGATGG	O104 (351)	50.00	50	27
O104-R	AGAACCTCACTCAAATTATG				
O5-F	CTTATCCGATTAATGGCTTC	O5 (144)	50.00	50	28
O5-R	TAGTCGATTTGCTTTTATGG				
O75-F	GAGATATACATGGGGAGGTAGGCT	O75 (511)	54.00	60	29
O75-R	ACCCGATAATCATATTCTTCCCAAC				
fliC-F	CAAGTCATTAATAC(A/C)AACAGCC	*fliC* (Variable)	56.00	70	30
fliC-R	GACAT(A/G)TT(A/G)GA(G/A/C)ACTTC(G/C)GT				
chuA.1b	ATGGTACCGGACGAACCAAC	*chuA* (288)	59.00	20	-
chuA.2	TGCCGCCAGTACCAAAGACA				
yjaA.1b	CAAACGTGAAGTGTCAGGAG	*yjaA* (211)	59.00	20	-
yjaA.2b	AATGCGTTCCTCAACCTGTG				
TspE4C2.1b	CACTATTCGTAAGGTCATCC	*TspE4.C2* (152)	59.00	20	-
TspE4C2.2b	AGTTTATCGCTGCGGGTCGC				
AceK.f	AACGCTATTCGCCAGCTTGC	arpA (400)	59.00	20	-
ArpA1.r	TCTCCCCATACCGTACGCTA				

*According to a touchdown PCR protocol as described previously.[21]

Results

Multiplex PCR (*stx*1, *stx*2, *stx*2*f*, *Ehly*, *eae*). In 35 cattle isolates, 6 (17.10%) carried only stx1, 25 (71.40%) only *stx*2 and 4 (11.40%) both *stx*1 and *stx*2 genes. All 35 cattle isolates (100%) harbored *Ehly* gene and 11 isolates (31.40%) were also positive for *eae* gene. In 22 sheep and goats' isolates, 11 (50.00%) carried *stx*1, 3 (13.60%) harbored *stx*2 and 8 (36.30%) both *stx*1 and s*tx*2 genes. Sixteen isolates (72.70%) harbored *Ehly* gene and all isolates (100%) were *eae*-negative. All 14 pigeons' isolates (100%) carried only *stx*2*f* and 12 isolates (85.70%) were also positive for *eae* gene. All isolates (100%) were negative for *stx*1 and *Ehly* genes. All human isolates (2/2, 100%) were positive for *stx*1 and *Ehly* genes and all of them (100%) were negative for *stx*2 and *eae* genes. All red deer isolates (2/2, 100%) carried *stx*2 and *Ehly* and all of them (100%) were negative for *stx*1 and *eae* genes. Figure 1 shows the distribution of STEC major virulence genes in cattle, sheep/goats and pigeons isolates.

O-serogroups. The predominant serogroup was O113 as it was detected in nine isolates from different sources including cattle (five isolates, 55.50%), goats (two isolates, 22.20%) and red deer (two isolates, 22.20%). O26 (3/3, 100%) and O111 (3/3, 100%) were found only in cattle isolates. O5 (3/3, 100%) was only detected in sheep, O63 (1/1, 100%) and O75 (2/2, 100%) were only found in pigeons and O128 was detected in goats (2/3, 66.60%) as well as pigeon (1/3, 33.30%). All strains were negative for O157, O45, O121, O145, O55, O91, O103, O104 and O118 serogroups (Table 2).

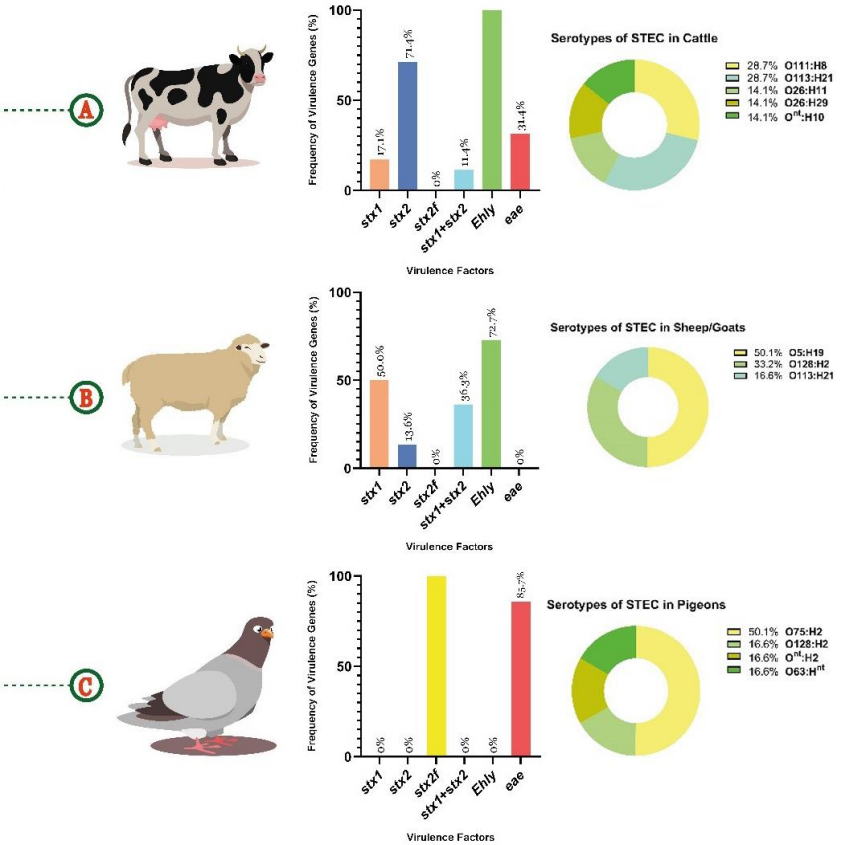

Fig. 1. Distribution of virulence genes among 71 STEC isolates and serotypes of 20 STEC isolates with respect to hosts: Cattle **(A)**, sheep/goats **(B)** and pigeons **(C)** in Iran.

Table 2. Distribution of the top eight and the other important STEC serogroups in 75 *E. coli* isolates in Iran.

Serogroup	Cattle (n = 35)	Sheep and Goats (n = 22)	Pigeons (n = 14)	Others (n = 4)	Total (n = 75)
O26	3	-	-	-	3 (4.00%)
O111	3	-	-	-	3 (4.00%)
O113	5	2[G]	-	2[R]	9 (12.00%)
O5	-	3[S]	-	-	3 (4.00%)
O63	-	-	1	-	1 (1.30%)
O75	-	-	2	-	2 (2.60%)
O128	-	2[G]	1	-	3 (4.00%)

G: Goats, R: Red deer, and S: Sheep.

Molecular H-types. Of 20 strains selected for identification of H-genotypes, H2 was the predominant H-types obtained from goats (two isolates) and pigeons (five isolates), followed by other H-types including H21 from cattle (two isolates) and goat (one isolate), H19 from sheep (three isolates), H8 from calves (two isolates), H11 from calve (one isolate), H29 from calve (one isolate), H10 from cattle (one isolate) and H4 from red deer (one isolate). The H-type for one isolate from pigeon was not typeable (Table 3).

Molecular serotypes (O:H types). Among twenty serotyped strains in this study (Fig. 1), the main recognized serotypes were O113:H21 (3/20, 15.00%) in cattle (2/3, 66.60%) and goat (1/3, 33.30%), O113:H4 (1/20, 5.00%) in red deer (1/1, 100%), O111:H8 (2/20, 10.00%) in calves (2/2, 100%), O26:H11 (1/20, 5.00%) in calve (1/1, 100%), O128:H2 (3/20, 15.00%) in goats (2/3, 66.60%) and pigeon (1/3, 33.30%), and O5:H19 (3/20, 15.00%) in sheep (3/3, 100%). One strain (1/20, 5.00%) from cattle carrying *stx*1, *stx*2, *Ehly* and *eae* was belonged to O26:H29 serotype (Table 3).

Phylogenetic groups of serotyped strains. Phylogenetic analysis of serotyped strains revealed B1 as the predominant phylotype (17/20, 85.00%) followed by other types including A (2/20, 10.00%) and B2 (1/20, 5.00%). Five STEC isolates from pigeons had a similar phylogroup (B1) and harbored *stx*2*f/eae* genes. Two isolates from calves yielded the same virulence profile (*stx*1*/Ehly/eae*) and phylotype (B1), (Table 3).

Discussion

The main virulence factor of STEC is *stx* with two major types *stx*1 and *stx*2. As shown in our results, out of 75 STEC isolates, 30 (40.00%) harbored only *stx*2-encoding genes which was the predominant *stx* genotype, especially in cattle isolates (25/35, 71.40%). Similar to our study, Gutema *et al*. and Hoyle *et al*. reported that among 17 and 1948 (over the 110 sampled herds) STEC was identified in cattle fecal samples, 82.30% and 70.20% of isolates were positive for *stx*2 as the sole toxin type, respectively.[31,32] Importantly, in the study of Scheutz, it has been shown that the HUS-associated *E. coli* (HUSEC) carrying *stx*2 genotypes had a higher risk and were more pathogenic than those strains producing *stx*1 alone or both *stx*1 and *stx*2.[33] Moreover, Karmali in Canada and Scotland *et al*. in the United Kingdom indicated that STEC producing only *stx*2 had a higher prevalence of infection among HUS patients.[34,35] As a result, it seems that STEC strains that possess only *stx*2 are associated with the highest rates of HUS, accordingly, *stx* typing may provide worthy information about the virulence of STEC strains.[36] Of particular interest in this study was the presence of plasmid-borne gene *Ehly* in all (35/35, 100%) of the bovine STEC isolate. In accordance with our study, Fremaux *et al*. found *Ehly* gene in 92.00% of 118 STEC strains isolated from environment and fecal samples of dairy cattle farms in France.[37] In addition, in the study of Mercado *et al*., 80.00% of 15 STEC isolates were positive for *Ehly* gene isolated from diarrhoeic calves in different farms of Argentina.[38] Besides, the studies of Welinder-Olsson *et al*. and Brunder *et al*. indicated that most isolates from patients with severe infections such as HUS carried the *Ehly* gene and this might be due to the role of *Ehly* in stimulating the growth of STEC in the gut by providing the iron source.[12,13,39] Therefore, if we consider only the major toxins of STEC, isolates from cattle may pose higher risk for development of severe infections in humans.

In research projects conducted in Iran and other countries, acute diarrhea has been associated with STEC O157 and non-O157 serogroups with animal origins.[15,17-19,40-43] In our study, the predominant serogroup was O113 as it was detected in nine isolates from different sources (five cattle, two goats and two red deer) and importantly O157 was not present in any sources. In agreement with the present study, the O113 serogroup was also common among STEC strains reported in dairy cattle in Brazil and our previous study conducted in cattle in Iran.[44,17]

Table 3. Characteristics of twenty serotyped STEC strains in Iran.

No.	Serotype (number)	Source	Province (year)	Virulence Genotype	Phylotype
1	O26:H11	Calve	Tehran (2009)	*stx2/Ehly*	B1
2	O26:H29	Calve	Tehran (2010)	*stx1/stx2/Ehly/eae*	B1
3	O111:H8 (2)	Calve [a]	Tehran (2008)	*stx1/Ehly/eae*	B1
4	O113:H21 (2)	Cattle	Golestan (2012)	*stx2/Ehly*	B1
5	O*:H10 [b]	Cattle	Golestan (2012)	*stx2/Ehly*	A
6	O113:H4	Red deer	Tehran (2014)	*stx1/stx2/Ehly*	A
7	O5:H19 (3)	Sheep	Semnan (2012)	*stx1/stx2/Ehly*	B1
8	O128:H2 (2)	Goat	Fars (2013)	*stx1*	B1
9	O113:H21	Goat	Fars (2013)	*stx2/Ehly*	B1
10	O128:H2	Pigeon	Mazandaran (2012)	*stx2f/eae*	B1
11	O75:H2 (3)	Pigeon	Tehran (2011)	*stx2f/eae*	B1
12	O*:H2 [c]	Pigeon	Tehran (2011)	*stx2f/eae*	B1
13	O63:H*	Pigeon	Mazandaran (2012)	*stx2f/eae*	B2

a: Diarrheic calves, b: Hybrid pathogenic strain (Enteroaggregative Hemorrhagic *E. coli*: EAHEC), c: T5b-Ir isolate (Accession number: KJ397538), and *: Non-typeable.

The role of the O113 serogroup in human HUS has been well demonstrated in some previous studies reported from Australia, Argentina and Germany.[45,46] Similar to our results, Koochakzadeh *et al*., also did not detect any O157 isolates among STEC strains obtained from fecal samples of 180 clinically healthy cattle in Iran.[18] Also, in the study of Aslani and Bouzari , of 29 STEC strains isolated from diarrheal and asymptomatic persons during three years in Iran, none of the isolates were belonged to O157.[19] In addition to O157 serogroup importance, the similarity between our results and mentioned studies revealed that non-O157 serogroups were particularly important as a cause of HUS and might be involved in both small outbreaks and sporadic cases in Iran.[4,16] Another important point in the current study was the host specificity of O26 and O111 serogroups which were among the top eight STEC O-groups. Accordingly, O26 and O111 were identified only in cattle isolates. Other studies similarly reported this in cattle isolates in the UK, France and our previous study in Iran.[17,32,37] O26 and O111 account for a significant proportion of causing diarrhea in children based on studies conducted in Iran.[17] Remarkably, Fukushima *et al*., demonstrated that O26 and O111 could survive for a long time (1 to 8 weeks at 15.00 ˚C) in bovine feces indicating cattle were potential reservoirs for transmitting these serogroups to foodstuff and environment.[47]

Based on the results of the present study, the main recognized serotypes were O113:H21 (cattle and goats), O113:H4 (red deer), O111:H8 (calves), O26:H11 (calve), O128:H2 (goats and pigeons), and O5:H19 (sheep). Despite the fact that there were no O157:H7 found in the studied isolates, the reported serotypes are known to be associated with human gastrointestinal illness and sporadic HUS cases as we are going to briefly discuss.[15,16] O113:H21 is considered as one of the non-O157 HUS-causing STEC serotypes in the studies of Newton *et al*. and Mellmann *et al*.[41,48] Moreover, O113:H21 was initially reported in clinical cases of HUS patients in Australia.[15] O113:H4 is an emerging serotype of human clinical significance linking to human gastrointestinal illness as demonstrated by Monaghan *et al*. in Ireland.[42] As shown in the study of Karch *et al*., O111:H8 and O26:H11 are also among serotypes isolated from patients with HUS in Germany.[49] O128:H2 was recognized worldwidly to be a non-O157 STEC isolated from HUS patients reported by Domingue *et al*. in the UK.[43] In order to control the spread of these dangerous serotypes, it is indispensable to exert practical strategies through a national surveillance network to trace, identify and report the top important serotypes circulating in the animals, environment and diarrheic cases in Iran.

Regarding other recognized serotypes not associated with severe infections, similar to our study, O5:H19 has been isolated from sheep in China as reported by Liu *et al*.[50] Although O5:H19 were not related to human HUS, O5 serogroups were associated with human gastrointestinal symptoms.[16] It is worthwhile to note that all ovine serotyped isolates in the current study were belonged to O5:H19 serotype suggesting the host specificity of this serotype, whereas, pigeon isolates were belonged to four serotypes expressing a high degree of serotype diversity. Due to the limited number of other serotypes, it is difficult to conclude host specificity or diversity for other serotypes. Among our calve isolates, strain O26:H29 carried *stx*1, *stx*2, *Ehly* and *eae*. We believed that this was the first report of O26:H29 serotype which carried two main types of *stx* along with *Ehly*, and *eae* virulence genes. To our knowledge, this O26:H29 serotype has not yet been reported elsewhere andO26 serogroups have emerged as highly virulent clones causing human diseases and spread throughout Europe after its emergence in Germany in the mid-1990s.[15,16] Resultantly, we suggested O26:H29 to be considered a potentially highly pathogenic clone in Iran.

In conclusion, this study provided the first serotyping (O:H typing) data documented in STEC strains of animal origin in Iran. Although most studies have been focused on O157:H7, the results of this study demonstrated that O157:H7 might not be a predominant serotype in Iran. Additionally, most strains with determined O-groups were from the bovine source that highlighted the importance of cattle as reservoirs of potentially pathogenic sero-pathotypes. Besides, O26, O111, and O113 should be included in all future serotyping studies of STEC in humans and animals in Iran. Finally, the recognized O26:H29 strain in this study carried the essential facility for development of severe infections in humans that needs further investigations as a possible emerging strain.

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None to declare.

Conflict of interest

None to declare.

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