Virulence Repertoire and Antimicrobial Resistance Profile of Shiga Toxin-Producing E.coli Isolated from Sheep and Goat Farms from Al-buhayra Egypt

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ABSTRACT

Shiga toxin-producing E. coli (STEC) evokes a paramount concern from the public health point of view. Many reports dealt with the characterization of STEC from large ruminants. This study aimed to investigate the presence of STEC in sheep and goats, distribution of stx1, stx2, eaeA, and hlyA genes encoding Shiga toxins, intimin, enterohemolysins, and the antimicrobial resistance index (MAR). A total of 170 samples collected from (diarrheic, apparently healthy, and milk samples) from sheep, goats, and bedding (136, 27 and 7) respectively. E. coli was detected at a rate of 71 (41.7%) distributed as 62 (44%) and 9 (31%) from sheep and goat, respectively. The prevalent serotypes were O111: H2, O26: H11, O103:H2, O55: H7, O86, O121: H7, O125: H21, and O124. The frequency of stx1 gene was 13/15 (86.7%), stx2 was 14/15 (93.3%), the eaeA gene was 8/15 (53.3%), and hlyA gene was 10/15 (66.7%). The most effective antimicrobials were Chloramphenicol, Doxycycline, and Cephradine. It was clear that 6/15 (40%) of the obtained serotypes exhibited MAR index <0.5 while 9/15 (60%) gave MAR index >0.5 with a significant difference between them (P<0.05). Hence, genotyping and antimicrobial resistance are pivotal epidemiological tools promoting felicitous control strategies against STEC serotypes.

INTRODUCTION

Recently the E.coli classification is based upon pathotype detection. The pathotype that produces Shiga toxins (STEC) is characterised by its capacity to contain one of the famous Shiga toxin genes (stx1 or stx2), or both of them. The enterohemorrhagic E. coli (EHEC), which is a subset of STEC causes two prominent syndromes the hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Ferreira et al., 2015; Castro et al., 2017; Cundon et al., 2018). The non-O157 STEC E. coli in small ruminants may lead to diarrhea and gastrointestinal troubles while O157 can cause mucoid or bloody diarrhea could progress to septicemia and meningoencephalitis. The characteristic post mortem findings are inflamed mucosa of the large intestine accompanied with a fibrinohemorrhagic exudate (ISUCFSPH, 2016). While signs in humans ranges from simple diarrhea to hemorrhagic colitis, there could be progression to HUS, which is distinguished by the famous triad of thrombocytopenia, thrombotic microangiopathy, and hemolytic anemia in addition to grave acute renal failure (Mayer et al., 2012; Momtaz et al., 2013). The samples of ovine meat are highly contaminated with STEC types of E. coli O157 and non O157 and are sources of human infections (Momtaz et al., 2013). The significant role of sheep and goats for spreading of STEC to human was suggested by many reports (Ferens and Hovde, 2011). The most notorious STEC serotypes encountered in human disease are E. coli O26, O45, O103, O111, O121, O145, and O157 (Scallan et al., 2011). A huge arsenal of virulence factors are essential for the pathogenicity of STEC. From them the stx1 toxin imparted similarity of the amino acid sequence with clear antigenic differences to Shigella dysenteriae serotype 1 cytotoxin (Beutin et al., 1997; Djordjevic et al., 2002). Based upon the previous epidemiological data the stx2 exceeds stx1 in its association with severe diseases and
HUS syndrome (Orth et al., 2007; Kawano et al., 2008). The Shiga toxin genes that encode for stx1 and stx2 which causes cell death after inhibiting the cellular protein synthesis. Recently, there are at least three stx1 subtypes (stx1a, c, and d) and seven stx2 subtypes (stx 2a, b, c, d, e, f, and g) were recognized (Baylis, 2009).

The intimin encoded by eaeA gene, is an outer membrane essential for STEC attachment to intestinal cells. Furthermore, the enterohemolysins are encoded by hlyA or ehxA genes and that enhance the effect of Shiga toxins (Croxen et al., 2013). In the last few years, there is an increased pathogenicity and resistance to most antimicrobials conferred by E. coli isolates that lower the selection chances of effective antimicrobials to control human infections (Saei et al., 2012).

This research was aimed to isolate and identify STEC serotypes from adult and neonate sheep and goats either (healthy or showing diarrhea) and their bedding to carry out genotyping and detection of the vital virulence genes of the obtained isolates. In addition, the antimicrobial susceptibility testing was applied to screen for the resistance patterns of the obtained serotypes.

MATERIALS AND METHODS

Sampling: The collection of 170 samples was performed from sheep, goats, and bedding (136, 27 and 7) respectively. Samples were distributed as 136 fecal samples (34 diarrheic and 102 apparently healthy sheep and goats), 29 milk samples (25 sheep and 4 goats) and 7 bedding samples (5 sheep and 2 goats). The sample collection was carried out in a period from November 2015 to March 2016 from different sheep and goat farms at Badr City in Al-Buheria Governorate.

E. coli isolation: The fecal samples were pre-enriched firstly in nutrient broth and kept for 24 hrs at 37°C, once growth confirmed a loopful of broth was streaked on MacConkey agar plates (MAC) and then aerobic incubation for 24 hrs at 37°C. The pink Lactose positive colonies were selected and cultivated on Eosin Methylene Blue (EMB) then incubated for 24 hrs at 37°C, typical E. coli colonies appeared metallic green. Positive colonies were stabbed into the semisolid medium and incubated for 24 hrs at 37°C, then, kept in the refrigerator at 4°C for preservation and for further morphological, biochemical, serological, and molecular characterization. The isolated E. coli strains upon specific media were determined at the statistical significance of P<0.05.

Antimicrobial susceptibility patterns: Cultivation of serotypes was performed onto Muller-Hinton (Oxoid) broth for 18 hrs at 37°C, until the bacterial count was adjusted to 1x10⁸/ml, using spectrophotometer. Once adjusted, then spreading on Mueller-Hinton agar (Oxoid) plates, then the disc diffusion method was performed using these antimicrobial disks: Amikacin (Ak 30), Amoxicillin /Clavulanic acid (AMC 30), Ampicillin (AM 10), Cephotaxime (CTX 30), Cephradine (CE 30), Chloramphenicol (C 30), Ciprofloxacin (CIP 5), Doxycycline (DO 30), Gentamycin (CN 10), Nalidixic acid (NA 30), Norocillin (Nor 10), Penicillin (P 10), Streptomycin (S 10), Trimethoprim/Sulphamethoxazole (SXT 25) (Oxoid). After incubation for 24 hrs at 37°C, the inhibition zones were measured and the results recorded as sensitive, intermediate, and resistant (Clinical Laboratory Standards Institute, 2013).

Statistical analysis: The STEC E. coli rates of isolation, the prevalence of the obtained serotypes and the sensitivity and resistance of isolates to antimicrobials were presented as percentages (%). The Multiple Antibiotic Resistance (MAR) index was displayed as a percentage of effective antimicrobials to the total used types. The significance of difference between STEC rates from sheep and goats, and between the serotypes exhibited MAR index ≤0.5 and MAR index >0.5 were determined using the Fisher’s exact test and the Z-test in R statistical software at the statistical significance of P<0.05.

Table 1: Oligonucleotide Primers used for amplification of specific genes

| Primer | Oligonucleotide sequence (S-3') | Product size (bp) | References |
|--------|--------------------------------|------------------|------------|
| stx1 (F) | AAGCTTAGATGATCTCACTGG | 614 | (Fagan et al., 1999; Osman et al., 2012) |
| stx1 (R) | CTGAATCCCCCTCCATTAG | | |
| stx2 (F) | CATGACAACAGGCAGCAGTTT | 779 | (Fagan et al., 1999; Osman et al., 2012) |
| stx2 (R) | CCTGCAACTGACGACACTTTGG | | |
| eaeA (F) | GGTGGCGAATCTGGCGAGACT | 890 | (Fagan et al., 1999; Osman et al., 2012) |
| eaeA (R) | CCCCATTCTTTTTCACCCTGC | | |
| hlyA (F) | AGCATGTGGTTATTTGGA | 165 | (Fagan et al., 1999; Osman et al., 2012) |
| hlyA (R) | CTTCACGTGACCATACATAT | | |
RESULTS

Prevalence of *E. coli* among examined samples: A total of 170 samples distributed as 141 from sheep comprising 111, 25, and 5 were fecal, milk and bedding samples, respectively. In addition to that, 29 goat samples comprising 23, 4, and 2 were fecal, milk and bedding samples respectively. All of them were subjected to isolation and biochemical identification using specific media. *E. coli* was detected at a rate of 71 (41.7%) which was distributed as 62 (44%) and 9 (31%) from sheep and goat samples, respectively, with no significant difference between these percentages (P=0.1961) (Table 2).

Detection of prevalent *E. coli* serotypes, virulence markers and antimicrobial susceptibility of serotyped isolates: From the obtained 71 *E. coli* isolates, 15 isolates were randomly selected for serotyping. The most prevalent serotypes were O111:H2, O26: H11, O103:H2, O55: H7, O86, O121:H7, O125:H21, and O124. The serotype O111:H2 (EHEC) was the most prevalent from one sheep fecal sample, 2 goat fecal samples and one sample from sheep bedding with a rate of 4 (26.7%). The O111:H2 harbored stx1, stx2, eaeA, and hlyA encoding virulence markers and exhibited Multiple Resistance Index (MAR) of 0.64 with resistance to AK30, AM30, AM10, CIP5, CN10, CTX30, P10, SXT25, and S10. Furthermore, O26: H11 (EHEC) was isolated from 3 sheep fecal samples with a rate of 3 (20%) it contained stx1, stx2, eaeA, and hlyA virulence genes. The O26: H11 gave MAR of 0.21 with pronounced resistance to AM10, CTX30, and SXT25. Additionally, O103:H2 (EHEC) gained from one sheep fecal sample with a rate of 1 (6.7%) and marked by the existence of stx1, stx2, and hlyA genes. It gave MAR of 0.35 with clear resistance to AMC30, AM10, CN10, NA30, and P10. The serotype O55:H7 (EHEC) represented a rate of 2 (13.3%) one from the sheep fecal and one from sheep milk sample. It showed the highest MAR (0.71) of the isolated serotypes with resistance to AK30, AMC30, AM10, CE30, CIP5, CN10, CTX30, D030, S10, and P10. Both the O86 (EHEC) and O121: H7 serotypes were isolated with a similar rate of 1 (6.7%) from sheep fecal samples. Although, O86 contained stx2 the serotype O121: H7 displayed stx1 and stx2 virulence genes. The serotype O86 gave MAR of 0.42 with resistance to CIP5, CN10, NA30, Nor10, S10, and SXT25. While, the serotype O121: H7 produced MAR of 0.50 with resistance to AK30, CIP5, CN10, CTX30, NA30, NOR10, and SXT25. The serotype O125: H21 (EHEC) was isolated with a rate of 2 (13.3%) representing 2 isolates from sheep fecal samples moreover; it owned stx1 and stx2 virulence genes. It gave MAR of 0.86 with resistance to AK30, AM30, AM10, NA30, C30, CIP5, CN10, CTX30, SXT25, NOR10, P10, and S10. The last detected serotype was O124 that was isolated from one goat fecal sample with a rate of 1 (6.7%). It contained the eaeA gene and exhibited MAR of 0.86 with resistance to AK30, AM30, AM10, CIP5, CN10, CTX30, D030, NA30, P10, S10, and SXT25. It was lucid that all virulence genes were equally distributed in both the serotypes O111:H2 and O26:H11. Besides both O103:H2 and O55:H7 serotypes equally harbored the same virulence markers. The two serotypes O121:H7 and O125:H21 similarly gained two virulence markers, whereas O86 and O124 exhibited the lowest distribution pattern of virulence genes. The prevalence of stx1 gene was 13/15 (86.7%), stx2 was 14/15 (93.3%), the eaeA gene was 8/15 (53.3%), and hlyA gene was 10/15 (66.7%). The most effective antimicrobials were Chloramphenicol, Doxycycline and Cefpodoxime with sensitivity rates of 73.4%, 73.4%, and 73.3% respectively. Both the serotypes O125: H21 and O124 gave the highest MAR index 0.73.3% respectively. While O55:H7 showed MAR indices of 0.71, 0.64, 0.50, 0.42, 0.35, and 0.21 in a descending manner respectively. It was clear that 6/15 (40%) of the obtained serotypes exhibited MAR index ≤0.5 while 9/15 (60%) gave MAR index >0.5 with a significant difference between them P<0.05 using Fisher Exact Probability Test (Table 2 and 3).

Table 2: Prevalence of *E. coli* among examined samples

| Species | No. | Breed | Management/efficiency | Types of samples | Isolated E. coli |
|---------|-----|-------|-----------------------|------------------|-----------------|
|         |     |       |                       | Fecal            |                 |
|         |     |       |                       | Diarrheic        | Healthy         |
| Sheep   | 141 | local cross | Extensive/fattening | 34 (24.11%) | 77 (54.6%) |
| Goats   | 29  | local cross | Extensive/fattening | -                | 23 (16.3%) |
| Total   | 170 |       |                       | 134              | 27              | 71 (41.7) |

Table 3: Antimicrobial susceptibility pattern of 15 *E. coli* serotypes isolated from examined sheep and goats

| Antimicrobial agents | Resistance | Intermediate | Sensitive |
|---------------------|------------|--------------|-----------|
| No. | % | No. | % | No. | % | No. | % |
| Amikacin (AK30) | 9 | 60.0 | 4 | 26.7 | 2 | 13.3 | |
| Amoxicillin/Clavulanic acid (AMC 30) | 7 | 46.6 | 0 | 0 | 8 | 53.4 | |
| Ampicillin (AM10) | 10 | 66.7 | 0 | 0 | 5 | 33.3 | |
| Cefoxime (CTX30) | 13 | 86.7 | 2 | 13.3 | 0 | 0 | |
| Cefpodoxime (CE30) | 4 | 26.7 | 0 | 0 | 11 | 73.3 | |
| Chloramphenicol (C30) | 2 | 13.3 | 2 | 13.3 | 11 | 73.4 | |
| Ciprofloxacin (CIP5) | 8 | 53.4 | 5 | 33.3 | 2 | 13.3 | |
| Doxycycline (DO30) | 3 | 20.0 | 1 | 6.6 | 11 | 73.4 | |
| Gentamycin (CN10) | 10 | 66.7 | 1 | 6.6 | 4 | 26.7 | |
| Nalidixic acid (NA30) | 8 | 53.4 | 4 | 26.7 | 3 | 20.0 | |
| Norfloxacin (Nor10) | 7 | 46.6 | 4 | 26.7 | 4 | 26.7 | |
| Penicillin (P10) | 9 | 60.0 | 0 | 0 | 6 | 40.0 | |
| Streptomycin (S10) | 8 | 53.4 | 2 | 13.3 | 5 | 33.3 | |
| Trimethoprim / Sulphamethoxazole (SXT25) | 11 | 73.3 | 0 | 0 | 4 | 26.7 | |
Table 4: Distribution of E. coli serotypes, virulence markers and antimicrobial susceptibility among obtained isolates from sheep and goats

| Serotypes | Strain characterization | Type of sample | Sheep | Goat | Total | Virulence gene distribution patterns | Antimicrobial resistance patterns | Multiple Antibiotic Resistance (MAR) index |
|-----------|------------------------|----------------|-------|------|-------|-------------------------------------|----------------------------------|----------------------------------------|
| O111:H2   | EHEC                   | Fecal sheep (1) | 2(16.7%) | 2(16.7%) | 4(26.7%) | + + +                                | AK30, AMC30, AM10, CIPS, CN10, CTX30, P10, SXT25, S10 | 0.64                                  |
|           |                        | Fecal goat (2)  |       |      |       |                                     |                                  |                                        |
|           |                        | Bedding sheep (1) |            |      |       |                                     |                                  |                                        |
| O26:H11   | EHEC                   | Fecal sheep (3) | 3(25%) |      | 3(20%) | + + +                                | AM10, CTX30, SXT25               | 0.21                                  |
|           |                        | Fecal sheep (1) | 1(8.3%) |      | 1(6.7%) | + + -                                | AM10, CIPS, AM10, CN10, NA30, P10 | 0.35                                  |
| O103:H2   | EHEC                   | Fecal sheep (1) | 2(16.7%) |      | 2(13.3%) | + + -                                | AK30, AM30, AM10, CE30, CIPS, CN10, CTX30, DO30, S10, P10 | 0.71                                  |
|           |                        | Milk sheep (1)  |       |      |       |                                     |                                  |                                        |
| O55:H7    | EHEC                   | Fecal sheep (1) | 1(8.3%) |      | 1(6.7%) | - + -                                | CIPS, CN10, NA30, Nor10, S10, SXT25 | 0.42                                  |
|           |                        | Fecal sheep (1) | 1(8.3%) |      | 1(6.7%) | - + -                                | AK30, CIPS, CN10, CTX30, NA30, NOR10, SXT25 | 0.50                                  |
| O64       | EHEC                   | Fecal sheep (1) | 1(8.3%) |      | 1(6.7%) | - + -                                | AK30, AM10, AM30, NA30, CIPS, CN10, CTX30, SXT25, NOR10, P10, S10 | 0.86                                  |
| O121:H7   | EHEC                   | Fecal sheep (1) | 1(8.3%) |      | 1(6.7%) | - + -                                | AK30, AM10, AM10, CE30, CIPS, CN10, CTX30, DO30, S10, P10, S10 | 0.86                                  |
| O125:H21  | EPEC                   | Fecal sheep (2) | 2(16.7%) |      | 2(13.3%) | + + -                                | AK30, AM30, AM10, CE30, CIPS, CN10, CTX30, DO30, S10, P10, S10 | 0.86                                  |
| O124      | EHEC                   | Fecal goat (1)  | _      | 1(33.3%) | 1(6.7%) | - + +                                | AK30, AM30, AM10, CE30, CIPS, CN10, CTX30, DO30, S10, S10, SXT25 | 0.86                                  |

Amikacin (AK30), Amoxicillin/Clavulanic acid (AMC30), Ampicillin (AM10), Cephapredine (CE30), Ciprofloxacin (CIPS), Gentamycin (CN10), Cefotaxime (CTX30), Doxycycline (DO30), Nalidixic acid (NA30), Norocillin (NOR10), Penicillin (P10), Streptomycin (S10), Trimethoprim/Sulphanethoxazoلة (SXT25).

Ethical considerations: This study agrees with the U.S. Government rules for the employment and nursing of animals intended for experimental, training, and research objectives. The study design was accepted by the Faculty Committee for Animal Care and Use, University of Sadat City.

DISCUSSION

In Egypt, sheep and goat exhibit socioeconomic impact for meat, wool, and hide production, these species constituted about 5.6 and 4.13 million heads respectively. The wide field of investment and insurance in sheep and goat farming because of the adaptation to harsh conditions, high productivity, increased fertility, and short generation interval (Khalil et al., 2013). E. coli is the most notorious bacterial agent causes drastic losses in sheep and goat farms. E. coli was detected at a rate of 71/170 (41.7%) which was distributed as 62/141 (44%) and 9/29 (31%) from sheep and goat samples, respectively, with no significant difference (P=0.1961). The highest trend of isolation was from sheep which comes in contradiction with Osman et al. (2013) who proved the vice versa. This high isolation rate was confirmed by Zaki et al. (2010) and Akhila et al. (2013).

The STEC isolates were serotyped and the most prevalent serotypes were O111:H2, O26:H11, O103:H2, O55:H7, O86, O121:H7, O125:H21, and O124. These results were similar to that reported by Koch et al. (2001), Cookson et al. (2006), Mousa et al. (2010), and EL Malt et al. (2017). Most of these serotypes of public health classified as verotoxigenic serotypes associated with bloody diarrhea or hemolytic uremic syndrome in human (Johnson et al., 1996; Delannoy et al., 2012). The pathogenicity of STEC mainly ambuscades in a huge arsenal of virulence factors, including Shiga toxins (stx1 and stx2, and its subtypes), adhesion protein intimin (eae), and enterohemolysin (Ehly) (Law, 2000; Delannoy et al., 2012). The eaeA gene is present on chromosome at the pathogenicity island; it is an essential factor for strong attachment to cells of the host intestinal mucosa. The attaching and effacing (A/E) lesions are a result of interaction between intimin and the bacterial “translocated intimin receptor” (Tir), this lesion is lacking in mutants which represent a public health problem. This result elucidated the high resistance pattern of the STEC isolates from sheep and goat with eminent focus on these isolates which represent a public health problem. This result confirmed by Mukherjee et al. (2017) who observed that there is a high frequency of antimicrobial drug resistance in O157 and non-O157 Shiga toxin-producing E. coli. Furthermore, the most effective antimicrobials were Chloramphenicol, Doxycycline, and Cephapredine with sensitivity rates of 73.4, 73.4 and 73.3%, respectively. Sheep and goat represent a possible reservoir of STEC to other pathogens. The monitoring of STEC antimicrobial resistance is pivotal due to the likelihood horizontal transmission of resistance genes from notorious STEC to other pathogens. The monitoring
process will aid in unraveling new treatment approaches and help in developing effective control strategies that help in stopping the spread of resistance (Islam et al., 2008).

**Conclusions:** Our findings proved that sheep and goats call for special concern. Because both species represented a vital reservoir of Shiga-toxin producing E. coli, most of the obtained serotypes harbored many virulence genes and proved to be multiresistant to most used antimicrobials that considered crucial for public health. Hence, the molecular typing and contentious monitoring of antimicrobial resistance could be helpful for developing efficacious control strategies against STEC and for the formulation of new antimicrobials with lowered liability for antimicrobial resistance.

**Authors contribution:** MSAEE was the leader of this study, who planned, monitored and evaluated the research steps. He helped also in the sampling, the isolation, the genotyping, the antimicrobial susceptibility testing, the writing, the revision of the manuscript and the data analysis. AM helped in the sampling, the isolation, and most of the genotyping. AA and RT helped in the conceptualization of the study, gave some technical advice, and data analysis.

**REFERENCES**

Aklilu M, Sisay T, Tefera G, et al., 2013. Identification and biotyping of E.coli from diarrheic lambs in and around Debre Birhan town. Ethiop. J Environ Anal Toxicol 3:6.

Bai X, Hu B, Xu Y, et al., 2016. Molecular and phylogenetic characterization of Non-O157 Shiga toxin-producing E.coli strains in China. Front Cell Infect Microbiol 6:143.

Baylis CL, 2009. Raw milk and raw milk cheeses as vehicles for infection facilitated by Shiga toxin-producing E. coli. J Dairy Res 76:501-21.

Beutin L, Geier D, Zimmermann S, et al., 1997. Epidemiological relatedness and clonal types of naturally populations of E.coli producing Shiga toxsis in separate populations of cattle and sheep. Appl Environ Microb 63:2175-80.

Castro VS, Carvalho RCT, Conte-Junior CA, et al., 2017. Shiga-toxin producing E.coli: pathogenicity, superhedging, diagnostic methods, occurrence, and foodborne outbreaks. Comp Rev Food Sci Food Saf 16:1269-80.

Clinical and Laboratory Standards Institute, 2013. Performance standards for antimicrobial disk and dilution susceptibility testing of bacteria isolated from animals approved 6 standard; 4th Edition. CSLI document Vet 01-A4. Wayne, USA 33.

Cookson A, Taylor S and Attwood G, 2006. The prevalence of shiga toxin-producing E.coli in cattle and sheep in the lower North Island, New Zealand. N Z Vet J 54:28-33.

Croxen MA, Law RJ, Scholz R, et al., 2013. Recent advances in understanding enteric pathogenic E.coli. Clin Microbiol Rev 26:822-80.

Cundon C, Carbonari CC, Zolezzi G, et al., 2018. Putative virulence factors and clonal relationship of O174 Shiga toxin producing E.coli isolated from human, food and animal sources. Vet Microbiol 215:29-34.

Delannoy S, Beutin L and Faeh P, 2012. Use of clustered regularly interspaced short palindromic repeat sequence polymorphisms for specific detection of enterohemorrhagic E.coli strains of serotypes O26:H11, O45:H2, O103:H2, O111:H8, O121:H9, O145:H28, and O157:H7 by real-time PCR. J Clin Microbiol 50:4035-40.

Djordjevic SP, Hornitsky MA, Bailey G, et al., 2000. Virulence properties and serotypes of Shiga toxin-producing E.coli from healthy Australian slaughter-age sheep virulence properties and serotypes of Shiga toxin-producing Escherichia coli from healthy Australian slaughter-age sheep. J Clin Microbiol 39:2017-21.

EL Malt LM, Abd El Wanis SA, El-Zamkan M, et al., 2017. Molecular characterization of E.coli isolated from raw sheep and goat milk. Int J Agric Sci Vet Med 5:127-38.

Fagan PK, Hornitsky MA, Betterlehi KA, et al., 1999. Detection of Shiga- like Toxin (stx1 and stx2), intimin (eaeA), and Enterohemorrhagic E. coli (EHEC) hemolysin (EHEC-1 Mma) genes in animal feces by multiplex PCR. Appl Environ Microbiol 65:868-72.

Farfana MJ and Torres AG, 2012. Molecular mechanisms that Mediate colonization of shiga toxin-producing E.coli strains. Infect Immun 80:903-13.

Ferens WA and Hovde CJ, 2011. E. coli O157:H7: animal reservoir and sources of human infection. Foodborne Pathog Dis 8:465-87.

Ferreira MRA, Silva TS, Stella AE, et al., 2015. Detection of virulence factors and antimicrobial resistance patterns in shiga toxin-producing E.coli isolates from sheep. Pesq Vet Bras 35:775-80.

Iowa State University Center for Food Security and Public Health (USCSPH), 2016. E. coli Stx and other E.coli causing hemolytic uremic syndrome. The Food Security and Public Health Technical Factsheets 6:1-15.

Islam MA, Mondal AS, de Boer E, et al., 2008. Prevalence and genetic characterization of Shiga Toxin-producing E.coli isolates from slaughtered animals in Bangladesh. Appl Environ Microbiol 74:5414-21.

Johnson RP, Clarke RC, Wilson JB, et al., 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic E. coli. J Food Prot 59:1122-26.

Kawano K, Okada M, Haga T, et al., 2008. Relationship between pathogenicity for humans and stx genotype in Shiga toxin-producing E.coli serotype O157. Eur J Clin Microbiol 25:272-32.

Khalil MA, Sammour HB and Egdardani MA, 2013. Socio-economic and technical evaluation of sheep and goat farms in North-West coast of Egypt. Egypt J Sheep and Goat Sci 8:59-62.

Koch C, Hertwig S, Lurz R, et al., 2001. Isolation of a lysogenic bacteriophage carrying the stx1OX3 gene, which is closely associated with shiga toxin-producing E.coli strains from sheep and humans. J Clin Microbiol 39:3992-8.

Law D, 2000. Virulence factors of E.coli O157 and other Shiga toxin-producing E. coli. J Appl Microbiol 88:729-45.

Lorenz SC, Son I, Maunouenuen-Laasri A, et al., 2013. Prevalence of hemolysin genes and comparison of exha subtype patterns in Shiga toxin-producing E.coli (STEC) and Non-STEC strains from cattle, food, and animal sources. Appl Environ Microbiol 79:6301-11.

Mayer CL, Leibowitz CS, Kurosawa S, et al., 2012. Shiga toxins and the pathophysiology of hemolytic uremic syndrome in humans and animals. Toxins 4:1261-87.

Montez H, Dehkordi FS, Rahimi E, et al., 2013. Incidence of Shiga toxin-producing E.coli serogroups in ruminate’s meat. Meat Sci 95:381-8.

Mousa MS, Akeila MA, Khalil SA, et al., 2010. Virulence factors of E.coli isolated from diarrheic sheep and goats. Alex J Vet Sci 30:137-47.

Mukherjee S, Mosci RE, Anderson CM, et al., 2017. Antimicrobial drug-resistant Shiga toxin-producing E. coli infections, Michigan, USA. Emerg Infect Diseases 23:1609-11.

Oorth D, Grif K, Khan AB, et al., 2007. The Shiga toxin genotype rather than the amount of Shiga toxin or the cytotoxicity of Shiga toxin in vitro correlate with the appearance of the hemolytic uremic syndrome. Diagn Microbiol Infect Dis 59:235-242.

Osman KM, Mustafa AM, Aly HAK, et al., 2012. Serotypes, virulence genes, and intimin types of Shiga toxin producing E.coli and enteropathogenic Escherichia coli isolated from mastitic milk relevant to human health in Egypt. Vector Borne Zoonotic Dis 12:297-305.

Osman KM, Mustafa AM, Elhariri M, et al., 2013. The distribution of E. coli serovars, virulence genes, gene association and combinations and virulence genes encoding serotypes in pathogenic E. coli recovered from diarrhoeic calves, sheep and goat. Transbound Emerg Dis 60:69-78.

Quinn P and Markey BK, 2003. Enterobacteriaceae I and 2 In: Concise Review of Veterinary Microbiology. Blackwell Publishing Ltd, Oxford, UK:38-41.

Saei DH, Ahmadi E, Kazemnia A, et al., 2012. Molecular identification and antibiotic susceptibility patterns of E. coli isolates from sheep faeces samples. Comp Clin Pathol 21:467-73.

Scallen E, Hoekstra RM, Angulo FJ, et al., 2011. Foodborne illness associated with the United States-major pathogens. Emerg Infect Dis 17:7-15.

Zaki MS, Ata NS, Shalaby SI, et al., 2010. Diarrhoea in neonatal baraki kids-goats. Life Sci J 7:129-32.