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Are Non-O157 Shiga Toxin-producing *Escherichia coli* Imposing Their Predominance Over O157 in Farm Animals and Human?

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Abstract: Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic pathogen that causes diarrheal disease in humans which may progress to serious complications and death. The aim of this study was to investigate the occurrences of STEC in farm animals and human in Egypt. A total of 461 faecal samples were collected from buffalo, dairy cattle, feedlot (beef) cattle, sheep and goats as 40, 86, 67, 192 and 76 samples, respectively. Additionally, 93 stool samples were obtained from human, living in urban (42 samples) and rural (51 samples) areas and all had gastro-intestinal complaints. Samples were screened withenzyme-linked immunosorbent assay to detect shiga toxins (stx) and then positive samples were cultured onto selective chromogenic media for STEC. Afterwards, positive isolates were subjected to biochemical tests to be proved as *E. coli* and confirmed molecularly by the polymerase chain reaction for the presence of stx-encoding genes. The occurrence of STEC in faeces of animals were 45.0%, 26.7%, 4.5%, 2.6% and 2.6% for buffalos, dairy cattle, feedlot (beef) cattle, sheep and goats, respectively, with 11.1% as an overall occurrencerate of STEC in sampled animals. No STEC was detected in stool samples of human living in urban areas, while 5samples from those living in rural areas tested positive for the presence of STEC. The overall occurrence rate of STEC in human samples was 5.4%. It is noteworthy that all of the obtained STEC isolates, according to O157 latex agglutination test, proved to be non-O157 STEC. This study demonstrated the predominance of non-O157 STEC, highlighted the role of buffalo as an overlooked reservoir for STEC and showed the higher risk of STEC infection to human in rural areas.

Key words: Shiga toxin-producing *Escherichia coli* · Non-O157 STEC · Farm animals · Human

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), also referred to as verocytotoxin-producing *E. coli* (VTEC), are pathogenic bacteria characterized by the production of shiga (like) toxins (stx1 and/or stx2 or their variants). They are considered an important group of zoonotic human pathogens, with the notorious *E. coli* O157:H7 being the best known and most studied serotype [1]. Because of the importance of serotype O157:H7, it is common to consider STEC serotypes in 2 major categories, O157 and non-O157 serogroups.

Diseases caused by *E. coli* O157:H7 has been reported from more than 30 countries on six continents [2]. STEC-caused illnesses cost the American economy more than $1 billion each year in direct and indirect costs from more than 175,000 human illnesses [3, 4]. Furthermore, the largest food safety and economic impacts on the cattle industry has been the emergence of STEC bacteria [5].

STEC can be found in the faeces of numerous animal species, but domesticated ruminants have been identified as the major reservoir. The most important source of human infections with STEC is cattle [6] and, to a lesser extent, sheep and goats [7]. Transmission of STEC to human occurs mainly through the consumption of undercooked meat [8], unpasteurized milk and dairy products [9], vegetables [10] and drinking water contaminated with animal faeces or manure [11].

Contact with animals and their environment is considered to be a risk factor for acquiring STEC infections and it has been associated with episodes of infection with STEC [12-15].

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Prevalence studies have focused primarily on *E. coli* O157:H7, because of its initial predominance in human clinical infection. Culture and molecular methods for the detection of STEC have thus been developed and optimized for that serotype, with little attention to and resultant underestimation of the risks posed by the non-O157 serogroup [16].

Non-O157 STEC has been associated with disease outbreaks worldwide. Due to clinical screening limitations in many countries, the true worldwide incidence of STEC is not known. Current methods used in researches and clinical diagnosis rely on classical culture-based techniques using sorbitol MacConkey plates to identify only O157 sorbitol non-fermenting STEC. Molecular diagnostic assays, such as PCR, screen for the genetic determinants that define STEC rather than rely on phenotypic traits, but the labor-intensive samples preparation and the increase of the costs may limit its use [17].

In this study we aimed to investigate the occurrence of both O157 and non-O157 STEC in farm animals (buffalo, cattle, sheep and goat) and human (urban and rural).

MATERIALS AND METHODS

Samples Collection: Ethical clearances to use human subjects for this research were got from the designated health facilities after official correspondences being submitted. Human enrolled for this research were patients in 2 hospitals and a clinic and they gave their consent after the purpose of the study was explained to them. All animals and human samples were collected within the Great Cairo Metropolitan area.

A total of 461 faecal samples were collected from 40 buffaloes, 86 dairy cattle, 67 feedlot beef cattle, 192 sheep and 76 goats. Additionally, 93 human samples were collected from individuals suffering from gastro-intestinal problems, 42 of them were living in the city and 51 came from rural areas for treatment. Faecal samples were collected in sterile containers. All samples were kept inside ice box containing ice and/or gel ice packs and taken to the laboratory for processing within 2 to 6 hours after collection.

Enrichment and Screening: In the laboratory, all samples were enriched in modified tryptone soya broth (mTSB) supplemented with mitomycin C (RIDA Enrichment Broth, r-biopharm, Germany). As instructed by the manufacturer, 50 to 100 milligrams of each faecal samplewere put in tube containing 4 ml of broth and incubated at 37°C for 18 to 24 hours. All enriched faecal samples were screened with enzyme-linked immuno-sorbent assay (ELISA) targeting the shiga toxins (RIDASCREEN Verotoxin, r-biopharm, Germany) and the ELISA-positive enriched samples were cultured onto chromogenic agar media (CHROMagar STEC, CHROMagar, France) and incubated at 37 ° C for 18 to 24 hours.

Cultural and Biochemical Identification: All isolates from positively screened samples were confirmed as *E. coli* after beingsubjected to series of cultural and biochemical tests. These tests includedsorbitol fermentation, oxidase, catalase, triple sugar iron agar, nitrate reduction, urease, indole, methyl red and citrate tests. All tests were applied according to the manufacturers' recommendations and as described in the Manual of Clinical Microbiology [18, 19].

Serogrouping: All isolates were then examined by *E. coli* O157 latex agglutination test (Oxoid, UK) and consequently categorized as O157 or non-O157 serogroup.

Molecular Confirmation

DNA Extraction: The isolates were cultured in tryptone soya broth and then subjected to the DNA extraction process with the use of QIAamp DNA Mini Kit (QIAGEN, Germany), as instructed by the manufacturer.

Polymerase Chain Reaction (PCR): Two ready to use conventional PCR kits were used to detect the stx1 and stx2-encoding genes (Genekam Biotechnology AG, Germany).Two PCR mixtures were made from both kits for each sample, according to the manufacturer's instruction.

The thermo-cycling machine was programmed, per instructions, as follows;

- Five cycles of (95 ° C for 30 seconds, then 72 ° C for 60 seconds).
- Twenty cycles of (95 ° C for 30 seconds, then 63 ° C for 30 seconds and then 72 ° C for 30 seconds).
- Five minutes at 72 ° C.

Electrophoresis and UV Illumination: After running gel electrophoresis for 40 minutes at 100 volts, UV illuminator was used to visualize the bands. If present, *stx1* gene should be detected at 121 bp and *stx2* gene at 102 bp levels (Figure1).

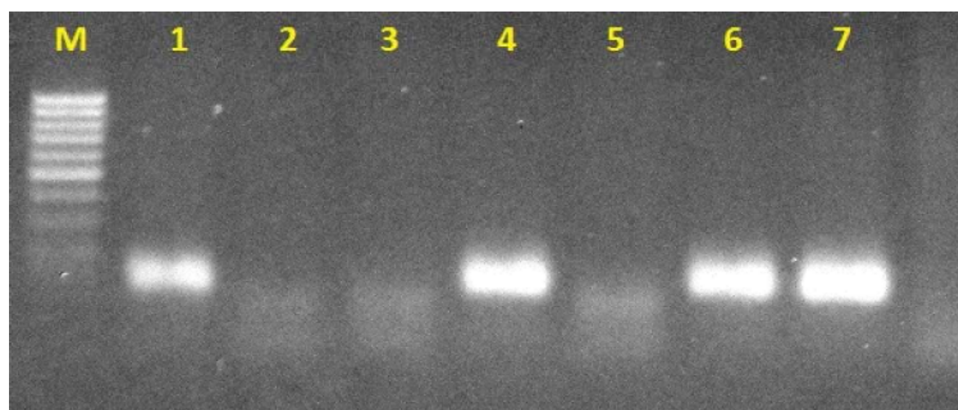

Fig. 1: PCR results demonstrated as DNA bands on electrophoresis gel.
Keys: M=100 base pairDNA ladder (Marker); Lane 1= Positive control; lane 2: Negative control; Lanes 4, 6 and 7= Positive isolates for stx1-encoding gene showed specific bands at 121 bp; Lanes 3 and 5= Negative isolates.

RESULTS

STEC isolates were recovered from 51 faecal samples of animals (11.1%), among which 18 were recovered from buffalo (45.0%), 23 from dairy cattle (26.7%), 3 from feedlot (beef) cattle (4.5%), 5 from sheep (2.6%) and 2 from goats (2.6%). The overall occurrence rate of STEC in faeces of cattle was 17.0% (26/153). Details are shown in Table (1);

Out of the 93 human samples, 5 (5.4%) were found STEC-positive. All five positive samples were from the individuals living in rural areas (9.8%). No STEC was detected in the samples of the individuals living in the city. Details are shown in Table (2);

Table 1: Occurrence of STEC in faecal samples from animal.

	No. of examined animals	No. of positive	Percentage
Buffalos	40	18	45.0%
Dairy Cattle	86	23	26.7%
Feedlot cattle	67	3	4.5%
Sheep	192	5	2.6%
Goats	76	2	2.6%
Total	461	51	11.1%

Table 2: Occurrence of STEC in stool samples ofhuman

Area of residency	No. of collected samples	No. of positive samples	Percentage
Urban	42	0	0.0%
Rural	51	5	9.8%
Total	93	5	5.4%

In total, 56 STEC isolates were recovered; only one was non-sorbitol fermenter (NSF). *E. coli* O157 agglutination test revealed that all recovered isolates were non-O157 STEC. Furthermore, PCR detected the presence of stx1-encoding gene in 7 isolates. No stx2-encoding gene was detected (Figure 1).

DISCUSSION

In this study, the occurrence rates of STEC in faeces of buffalo, dairy cattle, beef cattle, sheep and goat were found as 45.0%, 26.7%, 4.5%, 2.6% and 2.6%respectively. The overalloccurrence rate of STEC in faeces of cattle was 17.0% (26/153) and in all animals was 11.1% (51/461). Additionally, 5.4% (5/93) of tested human were STEC-positive, with zero percent (0/42) of individuals living in the city and 9.8% (5/51) of those living in rural areas.

Many prevalence studies were conducted in different regions of Egypt. To the best of our knowledge, no published study in Egypt addressed the significant role of buffalos as STEC reservoir hosts and the occurrences of STEC in the faeces of feedlot beef cattle and goat.

El-Alfy *et al.* [20] reported much less STEC prevalence rates in faeces of cattle and human, 3.8% (2/53) and 0.9% (1/107), respectively. They also reported 11.1% (1/9) in sheep, which is higher than 2.6% reported in our study but this may be due to low number of examined animals in their study.

Abd Al-Azeem *et al.* [21] reported a much less STEC (O157) prevalence rate of 1.4% (1/70) in faeces of cattle and a higher rate of 13.3% (4/30) instool of human.

Galal *et al.* [22] reported the rate of 2.9% (1/35) in faeces of cattle. In total, they collected six STEC isolates; all of them were non-O157.

Zidan and El-Sify [23] reported a higher rate of 7.0% (15/214) of STEC in faeces of sheep and a lesser 3.4% (7/209) in human stool samples. Out of the 22 isolates, 7 were O157 and 15 were non-O157 STEC.

Allam *et al*. [24] recovered 91 STEC isolates from different types of samples which included cattle faeces; non-O157 STEC represented 93.5% of them.

Globally and because buffalo are not domesticated in most American and European countries, studies of STEC occurrence in faeces of buffalo were mostly coming from some Asian countries like India, Bangladesh and Vietnam.

A study in India reported less STEC occurrence rates of 6.9% (3/40) in faeces of buffalo and 15.0% (9/60) in cattle [25]. Another study in India reported 24 STEC isolates out of 363 *E.* coli recovered from 165 buffalo faecal samples [26].

Yaghobzadeh *et al.* [27] claimed that their study is the first evidence that buffalo are reservoirs for STEC in Iran. They reported a less occurrence rate of 7.2% (26/360) in faeces of buffalo, with 25 out of the 26 isolates belonged to non-O157 STEC. Also a study in Vietnam [28] reported 27.0%, 23.0% and 38.5% occurrence rates of STEC in faeces of buffalo, cattle and goat, respectively. A total of 568 faecal samples were collected.

In Bangladesh, Islam *et al.* [29] with the use of PCR, detected stx genes in 82.2% (143/174), 72.7% (101/139) and 11.8% (13/110) in faeces of buffalo, cattle and goat, respectively, which were much higher than our rates. Isolation rates of STEC from samples were 37.9%, 20.1% and 10.0%, respectively. Out of 116 recovered isolates, 71 were non-O157.

Consistent with our records of feedlot cattle being with much less STEC occurrence rate than dairy cattle, Cobbold *et al*. [30], in a study in USA, reported prevalence rates of stx-encoding genes as 6% and 20% in faeces of feedlot and dairy herds cattle, respectively. STEC isolation rates were 3% and 9%, respectively.

Occurrence rates of STEC in faeces of feedlot cattle were reported higher in studies from Canada [31], Australia [32] and Norway [33], who reported 42.6% of 1247 samples, 35.4% of 949 samples and 20.0% of 365 samples, respectively.

In this study, no isolate was recovered from 42 individuals living in the city, while 5 isolates were recovered from 51 individuals (9.8%) living in rural areas. In Egypt, residents of rural areas rely on farming and raising animals for living and as sources of income. Direct contact with animals and their contaminated environment [14] might explain the higher incidence of human STEC infection in rural as opposed to urban areas and the consistent spatial association between STEC incidence in human and measures of livestock density. These findings were consistent with case-control studies reported from New Zealand [34], Argentina [35] and Canada [36, 37], but contradicted the finding of Lathrop *et al.* [38] who reported higher number of STEC infections in urban counties (93 cases) than in rural counties (18 cases) between 2004-2007 in New Mexico, USA.

The PCR results indicated that out of 56 STEC isolates, stx-encoding genes were detected in only 7 isolates, all of which were stx1-encoding genes. This could be attributed to, but not confirmed, the loss of stx-encoding genes during repeated subcultures which was reported in many other studies from all over the world [39-49].

CONCLUSION

This study and many recent STEC prevalence studies are showing an increase in the rates of non-O157 STEC detection, which can be due to the tendency of more non-O157 *E. coli* to become pathogenic by acquiring stx-encoding genes and /or may be due to the continuous improvement of research and laboratory media and kits that can efficiently detect the non-O157 STEC. Further studies are needed to investigate these concepts.

Buffalo represents a significant reservoir host for STEC and should be considered as a risk factor and a major source of infection of human with STEC in Egypt and other places where buffalo are considered as farm animals. Feedlot beef cattle proved to be less significant than dairy cattle which may be attributed to the differences in management systems and movement restriction.Sheep and goatare less important reservoir, but shouldn't be ignored because they can still act as sources of STEC infections.

Individuals living in rural areas are more susceptible to STEC infections due to the high density of livestock in those areas and the close encounter between human and animals.

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