Molecular based prevalence of shigatoxigenic *Escherichia coli* in rectal swab of apparently healthy cattle in Mymensingh district, Bangladesh

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**ABSTRACT**

**Objective:** Cattle are regarded as the principle reservoir of O157 and non-O157 shigatoxigenic *Escherichia coli* (STEC). Spreading of the STEC to human is primarily happens through contaminated meat, milk and their byproducts. The present study was aimed to explore the occurrence of STEC in the rectal swab of apparently healthy cattle.

**Materials and methods:** A total of 60 *E. coli* isolates that were previously isolated from the rectal swab of cattle were used in this study. DNA were extracted from the isolates and screened by PCR to detect *E. coli stx* (*stx*¹, *stx*²), *ebxA* and *rfb*O157 genes. Representative amplicons of the PCR products were sequenced. The prevalence of the STEC was determined based on the detection of STEC specific *stx* genes. The prevalence data were further analyzed by SPSS to elucidate any difference among different demographic groups of the study population.

**Results:** Overall, 43.33% (n=26/60) of the isolates were found carrying *stx* genes. Based on the presence of *stx* and *ebxA* genes, 6 different types of STEC were identified, of which 20% (n=12/60) were carrying both *stx*¹ and *stx*² genes. None of the isolates was positive for *rft*O157. The PCR amplicons were sequenced, and the nucleotide sequences were deposited in GenBank (accession: KM596779-KM596784).

**Conclusion:** In this study, non-O157 STEC were found highly prevalent in the local cattle. This study suggests that the apparently healthy cattle may act as a potential source of STEC infection for humans.

**KEYWORDS**

*ebxA*; non-O157; Rectal swab; STEC; Prevalence

INTRODUCTION

Shiga toxigenic *Escherichia coli* (STEC), has become an increasing public health concern since its first identification in 1982 (Mainil and Daube, 2005). STEC becoming major concern for their association with hemolytic uremic syndrome and hemorrhagic colitis in human. Along with O157:H7 STEC infection, outbreaks and isolation of no-O157:H7 are increasing from different sources with time, and from 1983 to 2002 STEC infection with non-O157:H7 was recorded as approximately 70% (Brooks et al., 2005).

Stx is the major virulence property of STEC resulting host cell death by inhibiting protein synthesis. STEC produces one or more heterogeneous and immunologically non-cross reactive Stxs (stx1, stx2 or variants). Though stx1 is identical to shigatoxin of *Shigella dysenteriae*, stx2 shares only ~56% identity with stx1 (Islam et al., 2008). In addition, some potential virulence genes viz., *ehx*, *ehx*, *katP*, *esp* and type II secreting system (espD) has been reported in a ~90kb plasmid present in certain STEC strains (Farooq et al., 2009). Vast majority of enterohaemorrhagic *E. coli* (EHEC) associated with HUS is identical to shigatoxin (Shigella dysenteriae) and has become an important pathogen. EHEC-Hly, a cytolysin, belongs to the RTX family (Schmidt et al., 1996; Bielaszewska et al., 2007). EHEC-Hly has the ability to injure microvascular endothelial cells (Aldick et al., 2007).

STEC (O157 and non-O157) has been reported in the intestinal tract and droppings of different animal and birds including its major reservoir as the cattle and sheep (Griffin and Tauxe, 1991; Hazarika et al., 2007; Jomezaden et al., 2009). Although ruminants harbor STEC in their intestine, they are not affected by shigatoxins, due to the lack of specific receptors for shigatoxins on their cell surface. There are reports suggesting that ruminants could shed and spread STEC to humans through fecal contamination of meat and milk (Elder et al., 2000; Asakura et al., 2001; Naidu et al., 2007). Additionally, person to person contact is also well documented as a mode of transmission of STEC (Rodolfo and Marin, 2007).

Prevalence of STEC in various sources have been reported worldwide including Bangladesh (Chapman et al., 1994; Fratamico et al., 2004; Cookson et al., 2006; Islam et al., 2007, 2008; Kesava et al., 2011; Islam et al., 2014; Hamza et al., 2017). However, no work on the prevalence of STEC in the rectal swab of apparently healthy cattle has yet been reported in Mymensingh, Bangladesh. The present study is describing the prevalence of non-O157 STEC in the rectal swab of apparently healthy cattle based on PCR and sequencing.

MATERIALS AND METHODS

Ethical statement: Not applicable.

Bacterial strains and cultural conditions: Previously, we isolated *E. coli* from rectal swab of apparently healthy cattle in Mymensingh (Hassan et al., 2014). Sixty *E. coli* strains (n=60) from that previous study were selected randomly and used in this study for detection of STEC.

DNA extraction and detection of virulence genes: DNA from the pure isolates was extracted by boiling (Hassan et al., 2014). The presence of virulent genes (*e.g., stx1, stx2, ehx*, and *rfbO157*) was detected using specific primers listed in Table 1. PCR reaction mixtures were adjusted to 25 µL with PCR master mix (Promega, USA) and 10 pmol of each primer. PCR was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles of amplification [denaturation: 94°C for 1 min; annealing: 1 min at varying temperature depending on the target genes (*i.e., 58°C for E. coli* 16S rRNA gene, 61°C for *stx1*, 59°C for *stx2*, 49°C for *ehx*, and 48°C for *rfbO157*); extension: 72°C for 2 min] with a final extension for 5 min at 72°C. PCR products were separated in 2.0% agarose and DNA was visualized in UV solo TS Imaging System (Biometra, Germany).

Table 1. Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer name</th>
<th>Sequence (5' - 3')</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>stx1F</td>
<td>CACAATCAGGCAGTCGCGACGCAGTGTGCT</td>
<td>606</td>
<td>Talukdar et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>stx1R</td>
<td>TGGTGAGAGATGACATGCTGTCGACGGGATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stx2</td>
<td>stx2F</td>
<td>CCAGACACGCTCTGATTACACACC</td>
<td>372</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stx2R</td>
<td>GACAAGCTGCTGGGATGCTCTGACGGGGATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ehx</td>
<td>ehx</td>
<td>GAGCGAGCTAAGGAGCTTG</td>
<td>889</td>
<td>Wiel et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>ehx</td>
<td>GCTGCTGCAGAAATAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rfbO157</td>
<td>rfbO157F</td>
<td>AAGATTCGCAAGGAGCTGT</td>
<td>497</td>
<td>Sánchez et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>rfbO157R</td>
<td>CATTGGCATCGTGACGAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sequencing of the PCR amplicons: Randomly selected PCR products of stx1, stx2, and ehxA were subjected to commercial sequencing from 1st BASE Laboratories-SdnBhd, Malaysia. The qualities of the obtained sequence were checked and processed using Chromas 2.23 and SeqMan II (DNASTAR). BLAST search was carried out to determine the identity of the nucleotide sequences using the NCBI, BLAST server. The sequences were deposited to GenBank.

Data analysis: Data collected during sampling were entered and statistically analyzed using SPSS version 17 (Chicago: SPSS Inc.). We applied Chi-square (χ²) test to find out the significant relationship between two interrelated qualitative variables. Fisher’s exact test was used for the contingency table whose cell frequency was less than 5. The associations between interrelated variables were measured by calculating the contingency coefficient and odds ratio (OR). P values ≤0.05 was considered as significant.

RESULTS

Prevalence of STEC and their virulence genes

The prevalence of the STEC was determined based on the PCR amplification of stx (stx1, stx2) genes. Among the 60 isolates, 43.33% (n=26/60) were found to be positive for stx genes (Figure 1-2). The primers targeting hemolysin i.e., ehxA and E. coli O157 specific O antigen i.e., rfbO157 were also used. Among the 60 isolates, 10% (n=6/60) were found to be positive for ehxA (Figure 3). None of the isolates was positive to rfbO157. Prevalence analysis based on the demographic factors revealed higher prevalence of non-O157 STEC in local cattle aged above 3 years that were maintained under unorganized farming (management) systems (Table 2).

Distribution of stx1, stx2 and ehxA among the STEC

Among the 60 isolates screened, STEC harboring 6 different combinations of target genes were identified among which isolates bearing both stx1 and stx2 are predominant (Table 3). The PCR amplicons of the target genes were sequenced. Upon alignment, all the stx1 gene sequences were found to be identical and 3 stx2 and 2 ehxA gene sequences were found dissimilar at different level. One (1) stx1 gene sequence, 3 of the stx2 gene sequences and 2 of the ehxA gene sequences were deposited to GenBank (accession: KM596779, KM596780, KM596781, KM596782, KM596783, KM596784).

DISCUSSION

The prevalence of non-O157 STEC in the rectal swab of apparently healthy cattle was found to be 43.33% as revealed by PCR based approach. Comparing the other studies performed in Bangladesh or abroad (Islam et al., 2008; Cookson et al., 2006; Menrath et al., 2010; Islam et al., 2014) the prevalence of STEC obtained in the present study is higher. This variation might be related with...
The prevalence of STEC has earlier been identified from cattle (Cookson et al. 2007). This study also revealed 6 sub-groups of STEC based on the presence of different genes screened, where the prevalence of stx1 or stx2 alone was lower than the finding of Kesava et al. (2011). The prevalence of stx1 alone was lower but occurrence of stx2 alone or in combination with stx1 was higher than that of Cookson et al. (2006). This variation might be due to differences in study population and location.

Prevalence of non-O157 STEC was found significantly higher in local cattle over 3 years of age maintained under unorganized farming system compared to organized farm which might be resulted from recurrent exposure of the animals to STEC contaminated feed materials. However, it’s difficult to make certain inference on the potential risk group of animals and minimize the spread of STEC to human beings.

**CONCLUSION**

Cattle are regarded as the major reservoir of STEC for human infection. About 43.33% of the rectal swab collected from the apparently healthy cattle was found to be positive for STEC in Mymensingh, Bangladesh. All the STEC isolates revealed in this study belongs to non-O157. In addition to the presence of stx encoding genes, hemolysin encoding genes have also been detected in the STEC isolates. The occurrence of STEC in the rectal swab of apparently healthy cattle signifies that these cattle could be the potential source for pathogenic E. coli to human.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declared that they had no conflict of interests.
AUTHORS’ CONTRIBUTION

JH, MSP and TK conducted main experiments. JH prepared the first draft of this manuscript. KHMMHN and MTR corrected and approved the manuscript for publication. All the authors read and approved the final version of the manuscript.

REFERENCES


