Verocytotoxic *E. coli* (VTEC) in Milk and Milk Products of Kanpur and Mathura, U.P., India

P. Pandey, B. Bist*, U. Jain and J.K. Yadav

Department of Veterinary Public Health, College of Veterinary Science and AH, DUVASU, Mathura 281001, India

(Received 27.07.2015; accepted 01.10.2015)

**ABSTRACT**

The aim of the present study was to know the occurrence of verocytotoxic *E. coli* (VTEC) by PCR in 87 milk samples (57 raw and 30 pasteurized) and 150 milk products (30 each of *burfi*, *paneer*, curd, *rasugulla* and milk powder) collected from Kanpur and Mathura, U.P. These isolates were further serotyped and then characterized for *stx* genes by PCR. Per cent positivity of VTEC in raw milk was found to be 7.01 (4/57). Pasteurized milk samples were free from VTEC strains. Milk products like *peda* and *paneer* showed per cent positivity of 6.66 (2/30) and 3.33 (1/30) for VTEC, respectively. Over all 2% (3/150) milk products were found positive for VTEC and the 7 isolates belonged to O60, O22, O55 and O102 serotypes and harboured *stx*2 gene.

**Keywords:** Milk, milk products, serotype, *stx*, verocytotoxic *E. coli*

**Introduction**

Several pathotypes of diarrhoegenic *Escherichia coli* are now recognized on the basis of production of virulence factors, distinct O:H serogroups, distinct diarrhoeal syndromes and differences in epidemiology viz., enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) or Verocytotoxigenic *E. coli* (VTEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Nataro and Kaper, 1998).

Verocytotoxin producing *E. coli* also referred as shiga toxin producing *E. coli* (STEC) are the one most important emerging foodborne pathogens (Couturier et al., 2011; Louri et al., 2011; Amezquita-Lopaz et al., 2014). VTEC strains were first identified as foodborne human pathogenic bacteria in 1982 (Riley et al., 1983). VTEC produces potent cytoxins that are responsible for watery diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome and thrombocytopenic purpura (Bhong et al., 2008).

Cattle act as a main reservoir for human infection (Krause et al., 2005). These animals may harbour and shed VTEC while remaining healthy or exhibiting only mild signs of infection. VTEC has been established as emerging pathogen in foods of animal origin like undercooked beef, raw milk and dairy products (like curd, cheese from raw milk) (Lior, 1994; Morgan et al., 1998; Allerberger et al., 2003; Pontello et al., 2003; Kiranmayi et al., 2010). Approximately 52% of recorded human disease outbreaks have been associated with bovine products (Griffin and Tauxe, 1991). In VTEC infection, about 10% of infections occur as outbreaks, the rest are sporadic. Thus, considering its importance as a foodborne pathogen, the current investigation was made to study the occurrence, molecular characterization and serotypes of VTEC in milk and milk products in Mathura and Kanpur region of U.P.

**Materials and Methods**

A total of 237 samples comprising of 57 raw milk, 30 pasteurized milk and 150 milk products (Table 1) were collected for isolation of *E. coli*. Raw milk samples were collected from organized DDD farm, DUVASU and local shops of Mathura, while milk products (like curd, *rasugulla*, *peda*, *paneer* and milk powder) were collected from local shops of Mathura and Kanpur. Milk samples were collected in sterile screw capped MacCartney bottles and milk products were collected in sterile polythene bags (UV treated) in chilled condition and processed within 3 h. All the samples were enriched with modified tryptase soya broth supplemented with 10 mg/L acriflavin to reduce the growth of Gram positive organism for primary isolation of *E. coli*. All the samples were incubated at 42°C for 24 h (OIE, 2004). MacConkey
agar (MCA) and eosin methylene blue agar media were used for differential and selective plating, respectively. Presumed E. coli isolates were identified using biochemical test kit (Hi-Media). All confirmed isolates of E. coli were sent to Central Research Institute, Kasauli for serotyping.

For detection of virulent genes (stx1 and stx2), PCR was carried out as per the method by Osek (1999) and Rahman (2002). For preparation of DNA template, the bacterial cells from overnight cultures were incubated at 37°C and suspended in 200 µl of sterilized distilled water and boiled at 100°C for 10 min. The boiled cultures were immediately cooled on ice bath. Details of primers used for the amplification of the different genes are given in Table 2.

PCR was carried out in a final reaction volume of 50 µl containing 10 µl of 2x Red Dye Master mix (Merck Specialities Private Limited, containing Taq DNA polymerase, dNTPs, reaction buffer with 1.5 mM magnesium chloride), 3 µl of DNA template, 1 µl of each of the primers (forward and reverse) and rest of DNase free water. The PCR tubes with all the components were transferred to thermal cycler (Eppendorf, Germany). The condition of PCR were 94°C for 5 min for initial denaturation of DNA within the sample, followed by 30 cycles of 94°C for 2 min (denaturation), 58°C for 2 min (annealing) and 72°C for 2 min (DNA synthesis). Agarose gel (1.5%) was prepared by boiling agarose (Bangalore Genei) in 1x TAE buffer.

Results and Discussion

Dairy cattle are the known major reservoir of O157:H7 as well as non-O157 VTEC (Wells et al., 1991) for human infection (Mainil., 1999). Milk gets contaminated during milking and becomes one of the important sources of VTEC infection.

In the present study, per cent positivity of VTEC in raw milk samples collected from Mathura was 7.01 (4/57). They belonged to serotypes O60 (2), O22 and O55 and VTEC could not be detected in any of the pasteurized milk sample. Das et al. (2005) and Antonio et al. (2009) reported somewhat similar prevalence of VTEC in milk samples i.e. 4.5% and 5.7%, respectively. Lower estimates than the present study on the occurrence of VTEC in raw milk ranging from 0 to 3.6% have also been reported (Steele et al., 1997; Stephan and Kuhn; 1999; Hinig et al., 2005). In contrast, investigations by Ray et al. (2006) have shown a higher detection rate of 18.5% and 10.8%. Fatch et al. (2001) reported a further high prevalence rate of 21.5% for strains of VTEC in raw milk. Raw milk collected from local shops of Mathura had more isolation rate of VTEC (11.76%) in comparison of that collected from organized farms of Mathura (5%) suggesting poor hygienic conditions prevailing at local shops. The presence of VTEC in raw milk suggests that milk is produced under unhygienic conditions, as a result of faecal/environmental contamination and that awareness of the risks associated with drinking raw milk and eating raw milk products is essential for public health protection.

On the basis of PCR, the overall per cent positivity of VTEC in milk product samples of Mathura and Kanpur was 2% (3/150). The per cent positivity of VTEC in peda samples was found to be 6.66% (2/30) and the isolates belonged to serotype O55 and O102. Only 1 paneer sample was positive for VTEC showing 3.33% (1/30) prevalence, whereas no VTEC could be detected in other milk product (curd, rasugulla and milk powder). In accordance to present study, low occurrence of VTEC in milk and milk products has been reported by McKee et al. (2003) and Barua et al. (2007). It is interesting to note that, in contrast to present study, Barua et al. (2007) have shown no VTEC in peda and paneer. In concordance to our study, Stephan et al. (2008) detected 4.6% isolation of VTEC in cheese samples. In contrast to our study, higher isolation rates (13-55%) of VTEC in cheese were also reported (Pradel et al., 2000; Fatch et al., 2001; Vernozy-Rozand et al., 2005). In contrary to negative result of VTEC in curd, 3.9% VTEC has been reported in curd by Rey et al. (2006). In milk products, presence of VTEC indicated inadequate heating of milk, contamination during processing and recontamination after heat treatment from environment.

Fig. 1. Agarose Gel showing PCR amplified product (584bp) for stx2 gene in VTEC isolates from milk and milk products. Lane 1- 100bp DNA marker, Lane 2 - Positive sample of peda, Lane 4- Positive sample of raw milk, Lane 3, 5, 6- Negative samples.
In the present investigation 3 different VTEC serotypes were reported from raw milk viz., O60 (2), O22 and O55 (1 each) and from milk products 3 different serotypes viz., O20, O55 and O102 (1 each) were revealed, all harbouring stx2 gene. Similar to present study, serotype O55 harbouring stx2 gene have also been reported by Kobori et al. (2004). From raw milk Fatch et al. (2001) and from raw milk cheese Vernozy-Rozand et al. (2005) reported serotype O22 carrying stx2 gene. Same serogroups as revealed in the present study have been reported previously (Blanco et al., 1997; Blanco et al., 2003 and Hussain et al., 2003). This result suggests that milk and milk product gets the faecal contamination and that milk borne infection is one of the routes of transmission of VTEC. Other workers have also reported milk as vehicle of transmission of VTEC (Samadpour et al., 1994).

Serotypes O22 and O55, which were isolated in present study were also reported by Dirk et al. (2008) in HUS cases of human beings which signifying the public health significance of these serotypes.

### References


<table>
<thead>
<tr>
<th>Source</th>
<th>Place of collection</th>
<th>No. of samples tested</th>
<th>No. of samples positive for VTEC</th>
<th>Percentage of VTEC (%)</th>
<th>Serotypes of VTEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>DDD Farm Mathura</td>
<td>40</td>
<td>2</td>
<td>5%</td>
<td>O22 (1), O55 (1)</td>
</tr>
<tr>
<td></td>
<td>Local shops Mathura</td>
<td>17</td>
<td>2</td>
<td>11.76%</td>
<td>O20 (2)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>Local shops Mathura</td>
<td>30</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Curd</td>
<td>Local Shops, Mathura</td>
<td>30</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Rasgulla</td>
<td>Local Shops, Kanpur</td>
<td>10</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local Shops, Mathura</td>
<td>20</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Peda</td>
<td>Local Shops, Kanpur</td>
<td>15</td>
<td>2</td>
<td>13.33%</td>
<td>O55 (1), O102 (1)</td>
</tr>
<tr>
<td>Paneer</td>
<td>Local Shops, Kanpur</td>
<td>10</td>
<td>1</td>
<td>10.00%</td>
<td>O20 (1)</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>Local Shops, Mathura</td>
<td>20</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local Shops, Kanpur</td>
<td>10</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Details of primers used for PCR reaction.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences (5'- 3')</th>
<th>Target Gene</th>
<th>Size of Amplified product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stx1 F</td>
<td>cac tta atg tcg tgg cga agg</td>
<td>Stx1</td>
<td>348</td>
<td>Rahman (2002)</td>
</tr>
<tr>
<td>Stx1 R</td>
<td>cac cag aca atg taa cag atg</td>
<td>Stx1</td>
<td>584</td>
<td></td>
</tr>
<tr>
<td>Stx2 F</td>
<td>atc cta ttc ccc gga gtt tac g</td>
<td>Stx2</td>
<td>348</td>
<td></td>
</tr>
<tr>
<td>Stx2 R</td>
<td>gcg tca tct tat acg cag gac c</td>
<td>Stx2</td>
<td>584</td>
<td></td>
</tr>
</tbody>
</table>


