Incidence of *E. coli* with Special Reference to VTEC in Faeces of Dairy Cattle,
Milk and Milk Products in Mathura and Vrindavan Region, India

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**ABSTRACT**

A study was undertaken to assess the prevalence, serotypes, and virulence genes (*stx* and *stx* through PCR) of *E. coli*. A total of 405 samples comprising 155 faecal samples, 100 milk samples and 150 milk product samples were screened for *E. coli*. Out of 405 samples processed, 147 *E. coli* isolates were obtained. The highest occurrence was observed in faecal sample (60%) followed by milk (22%) and milk products (21.33%). Serotyping results showed that out of 147 *E. coli* isolates, 18 isolates were rough, 19 isolates were untypable and 110 isolates belonged to 24 different ‘O’ serogroups. Serogroups O55 and O60 were obtained from all the three sources. A total of 110 *E. coli* isolates (faeces-70, milk-20, milk products-20) were screened by PCR to detect virulence genes *stx* and *stx*. Out of 110 isolates tested, 1 isolate (O55) from faeces of diarrhoeic cow was positive for *stx* gene whereas, 1 isolate (O60) from faeces of non-diarrhoeic cow revealed the presence of *stx* gene, while 1 isolate (O172) from faeces of diarrhoeic calf was found to be positive for both *stx* and *stx* gene. None of the samples from milk and milk products were found to be positive for the above mentioned virulence genes. Thus on the basis of PCR the prevalence of VTEC in faecal sample was found to be 4.28% (3 out of 70). However, no VTEC was detected from milk and milk products. Thus the overall occurrence of VTEC in the present study was found to be 2.72% (3 out of 110 isolates).

**Keywords:** *E. coli*, faeces, milk, milk products, PCR, VTEC

**Introduction**

*E. coli* are normal inhabitants of gastrointestinal tract of animals and humans of which some strains have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases. Pathogenic *E. coli* are considered to be one of the most important groups of bacteria causing diarrhoea and extra-intestinal infections in humans and animals (Levine, 1987). These bacteria include toxin producing strains of enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC) or verotoxic *E. coli* (VTEC), enteroaggregative *E. coli* (EAEC) and non toxin producing strains like enteropathogenic *E. coli* (EPEC) and entero-invasive *E. coli* (EIAC) as reported by Nataro and Kaper (1998).

Among the diarrhoea causing *E. coli*, VTEC have emerged as important foodborne pathogens and are responsible for hemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) in humans (Hiruta et al., 2001).

An important characteristic of verotoxic *E. coli* O157:H7, is its very low infective dose i.e. as few as 50-100 bacteria, which is much lower than for most of the other foodborne pathogens (Armstrong et al., 1996). VTEC have been isolated from the faeces of...
variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens and wild birds (Montengro et al., 1990). Consumption of food or beverage that becomes contaminated by animals (especially cattle) manure can result in contracting the disease. Foods that have been source of VTEC infection include ground beef, venision, sausages, dried salami, unpasteurised milk, cheese, milk products, vegetables, unpasteurised apple juice, radish sprouts and water.

Keeping in view the importance of this organism, the present study was planned to reveal the occurrence of E. coli with special reference to VTEC in faecal sample of dairy cattle, milk and milk products because very few studies have been done on this microorganism.

**Material and Methods**

A total of 405 samples (155 faeces, 100 milk and 150 milk products) were collected for primary isolation of E. coli. Samples were collected from various organized and unorganized farms, Gaushalas, retail shops of Mathura and Vrindavan region of India.

Milk samples were collected in sterile McCartney bottles, milk products were collected in sterile polythene bags and faecal samples were taken from rectum, or from freshly voided faeces on the farm or from intestinal contents after slaughter. Sterile cotton swabs were used for collection of faecal samples from rectum. The collected samples were brought to the laboratory aseptically in chilled state and processed immediately.

Samples were enriched with modified trypticase soya broth (mTSB) supplemented with 10 mg/lit. acriflavin to reduce the growth of Gram-positive organism for primary isolation of E. coli. For faecal sample the optimum incubation time-temperature used to minimize the over growth by other organism was 6 h at 37°C and for milk and milk products 42°C for 24 h (OIE, 2004).

MacConkey’s agar (MCA) was used as differential media and eosin methylene blue (EMB) agar was used for selective plating.

Suspected E. coli strains were subjected to morphological, cultural and biochemical characterization as per method described by Edwards and Ewing (1972). Isolates were sent to National Salmonella and E. coli Centre, Kasauli for serotyping.

PCR detection for the virulence genes stx1 and stx2 were carried out as per the method described by Ösek et al. (1999b) and Rahman (2002).

Bacterial cells from overnight cultures incubated at 37°C were suspended in 200 µl of sterilized distilled water and boiled at 100°C for 10 mins. The boiled cultures were immediately cooled on ice bath and used as DNA template for detection of specific genes using specific primers by PCR.

Multiplex PCR was used for detection of stx1 and stx2 genes. Details of the primers and PCR conditions used for the amplification of the different genes are given in the Table 1.

The PCR mixture (25 µl) included 12.5 µl of master mix (Fermentas, U.S.A. containing 2.5 units of Taq DNA polymerase, 200 µM each of dATP, dTTP, dGTP, and dCTP, 1.5 µM of MgCl2, 2 µl of template DNA and 0.5 µl of each primer (20 pmole). Distilled water was used to make up the total volume of 25 µl. After the amplification, amplicons were separated in 1.5% agarose gel in tris acetate EDTA (TAE) buffer at 60 volt for 80 min. The electrophoresed gel was stained with 0.5% ethidium bromide solution, visualized in gel documentation system and photographed (Fig. 1).

**Results and Discussion**

Out of 405 samples, 147 E. coli isolates were obtained and were sent to National Salmonella and E. coli Centre Kasauli (Himachal Pradesh) for serotyping. Of the 147 isolates, 18 isolates were rough, 19 isolates were untypable and 110 isolates belonged to 24 different ‘O’ serogroups namely O4 (3 isolates), O13 (3 isolates), O17 (1 isolate), O22 (9 isolates), O26 (2 isolates), O36 (2 isolates), O43 (1 isolate), O55 (13 isolates), O56 (2 isolates), O60 (12 isolates), O68 (8 isolates), O69 (2 isolates), O80 (5 isolates), O84 (9 isolates), O85 (2 isolates),
Table 1. Primer sequences and PCR conditions for detection of virulence genes of *E. coli* isolates.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence</th>
<th>PCR condition for Amplified</th>
<th>Reference</th>
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<tr>
<td></td>
<td></td>
<td>25 cycles (°C/min) product</td>
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<tr>
<td></td>
<td></td>
<td>Denaturation Annealing Extension</td>
<td></td>
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<tr>
<td>stx1</td>
<td>5'-cag tta atg tcg tgg cga agg-3' 5'-cac cag aca atg taa ccg atg-3'</td>
<td>94/5 58/2 72/2 348</td>
<td>Rahman (2002)</td>
</tr>
<tr>
<td>stx2</td>
<td>5'- atc cta ttc ccg gga gtt tac g-3' 5'-gcg tca tcg tat aca cag gag c-3'</td>
<td>94/5 58/2 72/2 584</td>
<td>Rahman (1992)</td>
</tr>
</tbody>
</table>

Fig. 1. PCR amplification of *stx1* and *stx2* genes of *E. coli*.
Lane 1 =100 bp DNA marker; Lane 3 = Positive sample from faeces of dairy cow (*stx*1 gene); Lane 7 = Positive sample from faeces of non diarrhoeic cow (*stx*2 gene); Lane 2,4,5,6,8,9 and 10 = Negative samples O88 (5 isolates), O98 (2 isolates), O102 (3 isolates), O111 (6 isolates), O116 (2 isolates), O119 (3 isolates), O168 (2 isolates), O170 (2 isolates), and O172 (10 isolates).

In the present study, serogroups O55 and O60 were observed in all the three sources of sample. Thus, this clearly explains the faecal contamination of milk and milk products by these serogroups.

Overall incidence of *E. coli* from various sources was 36.29%. Occurrence of *E. coli* in faecal samples was found to be 60%. Previous workers have also reported somewhat similar findings on occurrence of *E. coli* from faecal sample such as 43% by Wani *et al.* (2005). However, Sharma *et al.* (2004) reported somewhat lower findings such as 30.82%.

In the present study, incidence of *E. coli* in milk samples was found to be 22%. Among the milk samples highest prevalence (30%) was found in raw cow milk. Other workers have also reported the prevalence of *E. coli* in milk samples (Arimi *et al.*, 2006; Rey *et al.*, 2006; Guh *et al.*, 2008). In the current study the prevalence of *E. coli* in pasteurized milk was found to be 8.57% (3 out of 35 milk
samples), which is of public health concern. Based on U.S.A. and European regulation pasteurized milk must be *E. coli* negative (Potter and Hotchkiss, 1995). In milk products the incidence of *E. coli* was found to be 21.33% and highest prevalence was found in curd 60% (9 out of total 15 samples). Soomro et al. (2002) also reported higher incidence from various milk products showing prevalence rate of 51.6% (31 out of 60 sample). Similarly, high prevalence of *E. coli* in ready to eat milk products was also reported by Barua et al. (2007).

A total of 110 *E. coli* isolates (70 faecal sample, 20 milk samples and 20 milk product samples) were screened for the presence of stx genes and 3 isolates were found to be positive for the stx genes. Among the isolates, 1 isolate (O55) from faecal sample of diarrhoeic cow was found to be positive for stx1 gene and 1 isolate (O60) from faeces of non-diarrhoeic cow was found to be positive for stx2 gene, while 1 isolate (O172) from faeces of diarrhoeic calf was found to be positive for both stx1 and stx2 gene. However, no stx gene was detected from milk and milk product isolates.

On the basis of PCR the incidence of VTEC in faeces in current study was found to be 4.28% (3 out of 70 samples). Somewhat similar findings were reported by Chattopadhyay et al. (2003). However, higher incidence of VTEC (7.4% to 18.47%) was reported by several workers (Cobbold et al., 2004, Rogere et al., 2001, Rabin, 1999). The prevalence of VTEC in faeces of diarrhoeic calves was 5% (1 out of 20 isolate) in the current study. Similar results have been reported by previous workers with a prevalence rate of 6.02% (Chattopadhyaya et al., 2001) and 7.14% (Sharma et al., 2004) from faeces of diarrhoeic calves.

In the current study no VTEC was detected in milk samples through PCR. But VTEC have been reported by previous workers from milk samples such as 10.8 % (Rey et al., 2006) and 2.2 % (Kang’ethe et al., 2007).

Thus, the prevalence of VTEC in the faeces of domesticated cattle as revealed by this study presents a public health risk. Constant monitoring and surveillance programme to keep a record of the prevalence from time to time is needed and proper hygienic measure may reduce the chances of infection.

**References**


Incidence of *E. coli* in faeces of dairy cattle, milk and milk products


