SHORT COMMUNICATION

Genotypic Study of Verocytotoxic E. coli in Cattle by Multiplex Polymerase Chain Reaction

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ABSTRACT

E. coli is the most commonly observed gastrointestinal flora of animals and environmental contaminant considered as important food borne pathogen causing serious complications in man and animals. The study was undertaken to detection of virulence gene using polymerase chain reaction (PCR) in cattle. In present study, a total of 160 samples were processed for isolation of verocytotoxic E. coli (VTEC). All samples were positive for Escherichia coli. Out of 160 Escherichia coli, 14 samples were found positive for VTEC. Out of 14 VTEC, 6 were found to be positive for stx1 gene (180 bp), 5 were stx2 (255 bp) and 3 were for stx1, stx2 and hlyA gene.

Keywords: Fecal sample, Verocytotoxic E. coli, PCR

In human beings diarrhoea is one of the most common and multifactorial disease mainly caused by E. coli (Kumar et al., 2013). E. coli is the most commonly observed gastrointestinal flora of animals and environmental contaminant considered as important food borne pathogen causing serious complications in man and animals (Mailk et al., 2013; Dhama et al., 2013; Anita et al., 2014). VTEC is also termed as shiga-like toxin producing E. coli (SLTEC) or shiga toxin producing E. coli or STEC. Acronym STEC is derived from the fact that the toxins are shiga like that is similar to those produced by Shigella dysenteriae type1 (Brien et al., 1987). The adherence factors (intimin) enables the organism to attach to and colonize intestinal mucosal cells (Hiruta et al., 2001).

Among VTEC, serotype O157:H7 has been closely associated with the sporadic and clinical outbreaks of hemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in human beings (Croxen and Finlay, 2010; Gyles and Fairbrother, 2010; Sanchez et al., 2013). Healthy domestic ruminants are recognised as the main natural reservoir of STEC and large game animal may be healthy carriers of STEC (Diaz et al., 2011; Sanchez et al., 2010).

MATERIALS AND METHODS

Sampling and isolation of E. coli

A total 160 randomly selected fecal samples of cattle was taken for the detection of virulent genes of VTEC. The samples were collected aseptically in UV sterile polythene bags and immediately transported to the laboratory under chilled conditions for microbiological analysis. For primary isolation of E. coli (VTEC), 10gm of faecal sample were enriched in 90 ml modified trypticase soya broth (mTSB) containing acriflavine (10 mg/l) to reduce the growth of gram positive organism. The method used for collection of materials, and isolation and identification
techniques were made as per the lines suggested by World organization for Animal Health (OIE, 2014). These samples were incubated at 37°C for 6 h. MacConkey’s Agar (MCA) was used as differential media, while eosin methylene blue (EMB) agar (Hi-Media, India) was used as selective media. Suspected E. coli strains were subjected to morphological, cultural and biochemical characterization as per the standard method (Ewing, 1986).

**Molecular characterization**

Multiplex Polymerase chain reaction (PCR) was used for detection of virulent genes (stx₁, stx₂, eaeA and hlyA) of VTEC. All the E. coli isolates were subjected to genomic DNA isolation. The bacterial growth in TSB broth (HiMedia) was centrifuged at 3000 rpm for 15 min to make the pellet of bacterial cells. These cells were washed twice with PBS (pH 7.4) to remove any impurity of broth media. Bacterial DNA was extracted by using DNA extraction kit (Genei, Bangalore) as per the manufacturer’s protocol. For the PCR reaction, PCR Master Mix solution (Genei, Bangalore) was used. To amplify DNA targeted to virulent genes (stx₁, stx₂, eaeA and hlyA) of VTEC by using primers on 3µl of DNA template in 25µl reaction mixture (Paton and Paton, 1998). After an initial denaturation step at 95 °C for 4 min, 30 amplification cycles were performed, each consisting of 94°C for 2 min, 65°C for 2 min. and 72°C for 1.5 min and followed by a final extension step at 72°C for 2.5 min. After the amplification, amplicons were separated in 1.5% gel in tris acetate EDTA (TAE) buffer at 60 volt for 80 min, stained with 0.5% ethidium bromide solution and visualized under ultraviolet light.

**RESULTS AND DISCUSSION**

Out of 160 fecal sample, a total of 160 E. coli strains were obtained. All the strains of E. coli were screened to detect the presence of VTEC genes using multiplex PCR (Fig. 1). An overall prevalence of VTEC in Cattle fecal sample was found to be 8.75% (14/160). Out of 14 VTEC, 6 was found to be positive for stx₁ gene (180 bp), 5 was stx₂ (255 bp) and 3 was for stx₁, stx₂, and hlyA gene (255 bp and 534 bp).

In present study prevalence of VTEC was 8.75% which showed similar finding to previous study, the prevalence of VTEC in cattle were reported as 8.9% (Eriksson et al., 2005). However, prevalence of VTEC in higher level was reported by previous workers as prevalence of VTEC was 16.66% (Parul et al., 2014), 18% (Rogerie et al., 2001) and 18.47% (Rabin, 1994). In contrast, investigations have shown a higher detection rate of 46% (Kobayashi et al., 2001) in fecal samples of cattle. Lower isolation rate of VTEC in cattle i.e. 9% (Blanco et al., 1993). Prevalence rate as low as 1.0% has also been reported (Khurana and Kumar, 2005). VTEC possessed the stx₁ gene, either alone (22 of 32 isolates, 68.8 percent) or with stx₂ (eight isolates, 25.0%) (Wieczorek et al., 2011).

The close association with ruminants, low infective dose and unusual acid tolerance, have made VTEC a serious global zoonotic problem of great public health significance. VTEC can be present in the intestinal tract of a wide range of domestic and wild ruminants (cattle, buffalo, sheep, goats and deer) (Beutin et al., 1995; Johnson et al., 1996; Schouten et al., 2005; Oporto et al., 2008).

**CONCLUSION**

The presence of VTEC genes in feces of a large population of cattle in different rearing places, public health awareness including safe and hygienic practices while handling the cattle would be paramount importance in reducing the VTEC infections in humans.

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