

Isolation and Identification of Enterohaemorrhagic *E. Coli* in Raw Beef

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Abstract- An investigation on occurrence of Enterohaemorrhagic *E. coli* in beef was carried out to determine the prevalence rate in Thrissur. A total of 175 fresh beef samples were collected from four retail outlets and two slaughter houses located at Thrissur. Hundred gram of the material was taken from each sample. The isolation and identification was carried out by FDA method with necessary modification. Five samples were positive for Enterohaemorrhagic *E. coli* and virulence gene *stx 1* was obtained from two isolates using polymerase chain reaction. None of the isolates was positive for *stx 2* and *eae A* genes.

Index Terms- Enterohaemorrhagic, *E. coli*, virulence gene, *stx1*, *stx 2*, *eae A*

I. INTRODUCTION

Enterohaemorrhagic *E. coli* (EHEC) is an emerging foodborne pathogen of public health importance. It is becoming one of the greatest microbiological challenges which hit the food industry over the last two decades. The main source of infection is through ingestion of contaminated and undercooked meat and meat products with EHEC organisms. The food can be contaminated at any point in production, processing, transportation and distribution.

According to WHO (2008) diarrhoeal diseases alone, which are foodborne, can bring about mortality of 1.5 million children every year worldwide. Although most of these diarrhoeal deaths occur in poor countries, foodborne diseases are neither limited to developing countries nor to children. It is estimated that in the United States only, foodborne diseases result in 37.2 million illnesses, 228,744 hospitalizations, and 2,612 deaths each year. Among the EHEC strains, *E. coli* O157: H7 has recognized as an emerging cause of food-borne epidemics. The infection is associated with severe devastating or life - threatening systemic manifestations like Haemorrhagic Colitis (HC) and Haemorrhagic Uraemic Syndrome (HUS) in humans (Amstrong *et al.*, 1996)

The formation of World Trade Organization (WTO) has significantly increased trade in foods of animal origin and live animals between different countries. But emergence and re-emergence of diseases due to [pathogenic bacteria](#) are the key issue of the new pattern of meat food trades. Currently, India ranks as the third largest exporter of beef in the world. The current study was focused on determining prevalence of EHEC in beef in Thrissur, since the data on the occurrence rate are lacking from our state.

II. MATERIALS AND METHODS

For the isolation and identification of EHEC organisms, the method described by Meng *et al.* (2001) was used with some modifications.

One hundred and seventy five fresh beef samples of 100 g each were collected from four retail outlets namely Mannuthy, Kuriachira, Sakthan Nagar and Chettupuzha and two slaughter house one at Kuriachira (SH 1) and at Thrissur (SH 2). The samples were taken from rump and back regions were found to be contaminated more through contact with intestinal contents and hide. The samples were collected aseptically in sterilized polythene bags and transported to the laboratory under chilled condition. The samples were processed upon arrival on the laboratory on the same day of collection.

The samples were homogenized in a stomacher for three minutes. From the homogenized sample 25 g was weighed and added to 225 ml of Trypticase Soya Broth (TSB) supplemented with Novobocin (20 mg/l) for pre enrichment and incubated for 24 h. at 37°C. After incubation 0.1 ml of the primary enriched broth were transferred to 10 ml of selective enrichment EC O157: H7 broth at 37 °C for 24 h. After incubation a loopful of the inoculum was plated on Cefixime Tellurite- Sorbitol Mac Conkey (CT- SMAC) agar and 4-methylumbelliferyl -beta- D-glucuronide (MUG EC O157) agar (Fujisawa *et al.* 2000) and incubated at 37 °C for 24 h. The colonies showing characteristics neutral grey green colonies with smoky centers on CT- SMAC agar were then subjected to primary and secondary biochemical identification test. Then the identification of virulence genes (*stx 1*, *stx 2* and *eae A* genes) was carried out using polymerase chain reaction (Louie *et al.*, 1994).

III. RESULTS

Of the 175 samples screened, five had colonies characteristics of EHEC organisms on CT- SMAC agar. The colonies on CT- SMAC agar appeared as neutral grey with smoky center and having a diameter of one to two centimeters. On further plating on MUC EC agar, colonies appeared non fluorescent under UV illumination which was the typical character of *E. coli* O 157: H7 strains (March and Ratnam, 1986). Out of five positive samples, two were from Kuriachira. One sample each from Mannuthy, Sakthan Nagar and Chettupuzha possessed EHEC organisms out of 31, 40 and 24 samples respectively. All the samples collected from two slaughter houses were negative for EHEC organisms.

The isolates obtained were Gram negative, short rods, catalase positive, oxidase negative, sorbitol non fermentative and IMViC +++-.

The results of polymerase chain reaction revealed that two isolates from five positive samples possessed *stx 1* gene where as none of the isolates were carrying *stx 2* and *eae A* gene.

IV. DISCUSSION

Of the 175 fresh beef samples screened, the prevalence rate of EHEC was 2.86 per cent.

The present study revealed that the occurrence of EHEC in beef was 2.86 per cent which is in accordance with the results of Fantelli and Stephan (2001) who had isolated EHEC from 2.37 per cent beef sample and Baumgartner and Grand (1995) who had reported 2.4 per cent occurrence in beef samples. Desmarchelier and Grau (1997) reported slightly higher occurrence rate of Enterohaemorrhagic *E. coli* (3.7 per cent) from raw beef samples. Suthienkul *et al.* (1990) could not isolate any *E. coli* O157: H7 from retail meat. A high occurrence rate (6.36 per cent) was reported by Voravuthikunchai *et al.* (2002).

In the present study, 5.71 per cent positive beef samples were obtained from Kuriachira, followed by Chettupuzha (4.17 per cent), Mannuthy (3.23 per cent) and Sakthan Nagar (2.5 per cent). No positive samples were obtained from any of the two slaughter houses from which beef samples were collected. This is in agreement with the study results of Hiko *et al.* (2008) in which they have reported that the rate of occurrence of *E. coli* O 157: H7 was higher from butcher shops (7.8 per cent) compared to municipality and export abattoirs. The results of recent study also resemble with the report by Stampi *et al.* (2004) that all the strains of *E. coli* O157 recovered in the study were from meat samples collected from small retailers.

On statistical analysis, no significant difference ($p < 0.05$) was noticed between any two sources in occurrence of EHEC in beef samples.

Strategies to reduce EHEC in foods will depend much on hygienic and sanitary production and processing practices. This is to reduce the colonisation, transmission and cross contamination of EHEC in foods and the environment. An effective control measure for this pathogen has to target the farm, processing plants and the environments. At all these stages, strict adherence to standard operating measures must be practiced. The study also suggested the need for stringent food safety measures through the implementation of hygienic practices at all levels from the production to the consumption.

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