# PREVALENCE OF VEROCYTO TOXIN PRODUCING *ESCHERICHIA COLI* O157 IN BEEF – A SRI LANKAN PERSPECTIVE

**SUMATHY PUVANENDIRAN**

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**For the degree of MASTER OF PHILOSOPHY**

**UNIVERSITY OF KELANIYA**

**Department of Microbiology**

**Faculty of Science**

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**ABSTRACT**

*E. coli* O157 has emerged as an important food borne pathogen and is of considerable significance in public health aspect, because of the severity of infection with a low infectious dose. This study investigated the presence of *E . coli* O157 in beef obtained from two major slaughterhouses in Kandy and Colombo in Sri Lanka. ISO method 16654 was successfully followed to isolate *E. coli* O157 from a total number of 150 raw beef samples. All samples were enriched in modified Tryptone Soy Broth with novobiocin, concentrated by immuno magnetic separation and plated on Sorbitol MacConkey agar supplemented with cefixime and tellurite and Sorbitol MacConkey agar -5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Pale colonies on both media that were oxidase negative and indole positive were subjected to *E. coli* O157 latex agglutination test as well as to *E. coli* H7 latex agglutination test, along with other biochemical tests. Out of hundred-and fifty samples tested, *E. coli* O157 was present in 20 samples (13.3%). Percentage of positive samples was 11.8% (13/110) from Kandy and 17.5% (7/40) from Colombo. Out of twenty isolates, 16 isolates were (80%) *E. coli* O157:H7 and 4 isolates were *E. coli* O157: H⁻. All the isolates showed same biochemical properties except two isolates, which did not ferment dulcitol. All the isolates were susceptible to antibiotics amoxycillin, amoxicillin-clavulonic acid, chloramphenicol, streptomycin, kanamycin, nalidixic acid, sulpha-trimethoprim, cephalothin and ciprofloxacin and resistant to tetracycline to which all isolates were resistant. Moreover, all twenty isolates produced enterohemolysin, which was examined on washed sheep erythrocytes agar.

Production of Verocytotoxin by isolates was detected by verotoxin producing *E.coli*-reverse passive latex agglutination assay. All isolates were found to be

positive for vero toxin 2. Titre of vero toxin 2 varied from 8-128. Since all isolates produced vero toxin 2, 10 isolates were used to study on development of an animal model in Swiss Albino mice. A dose range of $1x10^7$ – $2x10^8$ CFU/ml was injected to Swiss Albino mice subcutaneously in the neck area. All the mice died within 3 days of inoculation. Histopathology revealed that congestion of blood vessels, nervous cell necrosis and brain edema with hemorrhages and tubular necrosis in the kidney, epithelial cell necrosis of intestine and focal degenerative changes of hepatocytes in the liver were present. Kidney lesions were very prominent. Genotypical characterization showed that all the isolates possess *eaeA* and *vt*2 genes. *eae*A gene encodes intimin, which is necessary for attaching and effacing lesions and vt2 gene encodes vero toxin 2 toxin. Immuno magnetic separation - Polymerase chain reaction was applied to detect *E. coli* O157 directly from beef. The minimum detection limit was 10CFU/g of beef at the time of inoculation. Growth and survival pattern in beef at room temperature and refrigeration temperature indicated that *E. coli* O157 could survive at these temperatures without significant growth. Although, number *E. coli* O157 were significanthy reduced with time, they were able to survive for a month in the freezer.