

Evidence of verocytotoxigenic *Escherichia coli* (VTEC) in livestock of Tamil Nadu—an explorative study

R GOPINATHAN¹, GAGANDEEP KANG², ASOKAN G V³, ARUNAGIRINATHAN N⁴ and GEORGE VASANTHANA⁵

Vellore Institute of Technology, Vellore, Tamil Nadu 632 004 India

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ABSTRACT

There is a paucity of data about the prevalence, burden of disease and complications associated with verocytotoxigenic *Escherichia coli* (VTEC) in animals in India. Cattle and other livestock harbour VTEC strains associated with haemorrhagic colitis (HC) and haemorrhagic ureamic syndrome (HUS). Therefore this explorative study analysed 115 livestock including healthy and diarrhoic animals for the excretion of VTEC.

Isolates (12) belonging to non-sorbitol fermenting *Escherichia coli* (10.4%) were obtained out of 115 samples screened to tellurite-cefixime-sorbitol MacConkey agar (TC-SMAC) that produced CPE on verocell monolayer. Culture filtrate of 4 out of these 12 *E. coli* isolates extracted after treatment with 10% (w/v) polymyxin B showed neutralization with antishiga toxin-1 suggesting them to be true VTEC strains. This study marks the first evidence of the isolation of VTEC from livestock in Tamil Nadu and underscores the need for large-scale epidemiological and bacteriological investigation to discern the mode of transmission and prevalent serotypes of VTEC in India.

Key words: *Escherichia coli*, HUS, Haemorrhagic colitis, Haemorrhagic ureamic syndrome, Verocytotoxigenic *E. coli*, VTEC

Verocytotoxigenic *Escherichia coli* (VTEC) causes a diarrhoeal illness, particularly in children (Rowe *et al.* 1998). Whipp *et al.* (1995) demonstrated that livestock, particularly cattle, are a reservoir of infection. VTEC strains have been associated with cases of haemorrhagic colitis (HC) and haemolytic ureamic syndrome (HUS) (Karmali *et al.* 1985). Both HC and HUS have been associated with high morbidity and mortality. The observation made by Karmali *et al.* (1985), that patients with HUS had prior enteric infection with VTEC, and VT was found in faecal filtrates along with rising antibody titre to VT, indicate the direct role of VT in the genesis of HUS.

Human infection with these pathogens are often caused by consumption of contaminated meat and unpasteurized milk (Dorn 1988). Children are more susceptible to VTEC (Parry *et al.* 1995) and UHS is the commonest cause of acute renal failure among Indian children (Ragupathy *et al.* 1981, Srivastava 1987). Serological evidence of VTEC infection

with serotype O157, O11 and O26+ was shown in Indian patients suffering from HUS, though no organism could be isolated from them perhaps due to their arrival to the hospital later during the course of illness (Kishore *et al.* 1992).

VTEC strains have been isolated from calves with diarrhoea and pigs with edema disease. In diarrhoic calves, 13 (4.3%) strains were isolated from 306 animals. Serogroups O6 and O111 isolated from 5 calves in this study have also been found associated with VTEC recovered from diarrhoic humans (Sherwood *et al.* 1985). Hence, it is possible that calves may harbour VTEC capable of producing enteric infection in man (Chapman *et al.* 1993).

Dutta *et al.* (2000) and Chattopadhyay *et al.* (2001) revealed VTEC O157 : H7 in 6.02% diarrhoic cattle, 3.12% diarrhoic food handlers, and 1.78% raw beef samples. However, there are no reports available from other parts of the country and the magnitude of VTEC prevalence remains to be addressed. Therefore this explorative study sought evidence for excretion of VTEC among livestock of Kanchipuram, Tamil Nadu.

MATERIALS AND METHODS

Since there is no prevalence estimate of the disease associated with VTEC from India, this explorative study was carried out to assess the burden of VTEC among livestock of Tamil Nadu. Various farm management factors are linked

Present address: ¹Department of Microbiology, Ahdiparasakthi College of Science, GB Nagar, Kalavai, Vellore district, Tamil Nadu 632 506.

²Department of Gastrointestinal Sciences, Wellcome Research Unit, Christian Medical College and Hospital, Vellore 632 004.

³Institute of Veterinary Preventive Medicine, Ranipet, Vellore district, Tamil Nadu.

⁵Department Biotechnology.

to the reservoir state of VTEC in livestock. Therefore, a pretested questionnaire was administered to record such practices as per Shere *et al.* (1998).

Rectal swab samples were collected from 55 cows, 34 heifers, 6 bulls, 11 calves, and 9 lambs. Midstream milk samples were obtained from 32 out of 55 cows. Samples were collected from healthy animals from household and from diarrhoeic animals attending the district veterinary hospital, Kanchipuram, Tamil Nadu, following prior consent from animal owners and the hospital authorities. Out of this 115 animals a batch of samples was collected from 29 animals (that included 10 healthy heifers and 9 healthy lambs) of a well-managed farm of an agricultural college.

Culture of samples on sorbitol MacConkey agar with tellurite and cefixime (TC-SMAC)

Rectal swab samples were collected as per Chapman *et al.* (1993), and milk samples as per Machie *et al.* (1997). The samples were transported in ice box to the laboratory on the same day of collection and cultured according to Chapman *et al.* (1993).

VT detection from *E. coli* isolates using verocell monolayer

All non-sorbitol fermenting and sorbitol-fermenting gram negative bacilli that were identified as *E. coli* based on biochemical tests were screened for the production of verocytotoxin (VT) following minor modifications of 2 methods as per Karmali *et al.* (1985) and Wilson *et al.* (1992). VT production from these isolates was detected at a reference microbiology laboratory of the Department of Gastrointestinal sciences, Christian Medical College and Hospital, Vellore.

Briefly, 50 µl of either brain heart infusion broth culture supernatant or the extract of these *E. coli* isolates pelleted from the broth subsequent to treatment with 10% polymyxin B (0.1g/ml of PBS) was added to duplicate wells in a 96 well flat bottomed tissue culture plate. *E. coli* O157:H7 strain 84-01 (VTEC) was used as a positive control and *E. coli* ATCC-25922, as a negative control respectively. 50µl of sterile Eagle's minimal essential medium (MEM) with 5% FCS and 100 µl of verocells (ATCC #76) was added to the same wells. The plate was incubated at 37°C in a 5% CO₂ incubator and the wells were examined periodically for the

development of cytopathic effect for up to 3 days with an inverted microscope. Scoring of wells showing CPE was done according to Wilson *et al.* (1992).

Neutralization of VT produced with anti-shiga toxin (SAST)

Each 20 µl of VT positive *E. coli* culture supernatant or polymyxin B extract showing >3+CPE was added into 2 set of duplicate wells of a 96 well flat bottomed microtitre plate. 20 µl of 1:1000 dilution of antishiga toxin to *Shigella dysenteriae* type 1 (Reference Microbiology Laboratory, Welcome Research Unit, Christian Medical College and Hospital, Vellore) was added into these wells, mixed well and incubated at 37°C for 24 hr. *E. coli* O157:H7 (strain 84-01) was used as control for VT assay as well as neutralization with AST. The plates were read at 24 hr interval up to 3 days. Test samples were considered neutralized if no cytopathic effect could be detected as compared to control (Wilson *et al.* 1992).

RESULTS AND DISCUSSION

A total of 12 (10.43%) non-sorbitol fermenting (NSF) and 6 (5.24%) sorbitol fermenting (SF) *E. coli* isolates were obtained out of 115 rectal swab samples grown on TC-SMAC. Apart from these *E. coli* isolates, 77 other isolates of gram negative bacilli also were obtained that were presumptively identified as *Proteus* sp., *Aeromonas* sp., and *Shigella* sp., based on the biochemical reactions. Neither non-sorbitol fermenting nor sorbitol fermenting *E. coli* was isolated from milk samples.

All those 12 NSF (100%) *E. coli* and 2 (33.3%) SF *E. coli* out of 6 isolates, showed CPE on verocell monolayer. Nevertheless, only 4 NSF *E. coli* that showed CPE on verocell monolayer subsequent to incubation with AST did not produce any CPE suggesting that only these 4 NSF (33.3%) *E. coli* out of the 12 could be VTEC. The remaining 8 NSF (66.6%) and 2 (33.3%) SF *E. coli* strains though showed CPE did not get neutralized (Table 1).

Out of these 4 NSF *E. coli* isolates suggestive of VTEC, the rate of isolation was 3 (3.6%) out of 55 healthy cows, and 1 (3.2%) out of 31 diarrhoeic adult cows screened. Taken together these 4 NSF *E. coli* isolates (4.7%) suggestive of VTEC were obtained from 86 domestic livestock screened,

Table 1. *In vitro* detection of verotoxin (VT) from *E. coli* isolates and toxin neutralization done with antishiga toxin to *Shigella dysenteriae* type 1 toxin on verocell monolayer

Type of <i>E. coli</i> isolates obtained on TC-SMAC (n=18)	Screening <i>E. coli</i> isolates for toxin production on verocell culture and neutralization with antishiga toxin to <i>Shigella dysenteriae</i> type-1 toxin (AST)			
	Test for *CPE on vero cells		Test for neutralization	
	CPE positive	CPE negative	Neutralised	Not neutralised
NSF** <i>E. coli</i> (n=12)	12 (100%)	0	4(33.3%)	8(66.6%)
SF*** <i>E. coli</i> (n=6)	2 (33.3%)	4(66.6%)	0	2(33.3%)

while no isolates were obtained from 29 animals screened from the well-maintained farm. Out of the 115 animals screened the overall prevalence of VTEC was 3.5% (4 animal) in livestock of Kanchipuram, Tamil Nadu.

Our observation that though 12 NSF isolates produced CPE on verocell monolayer only 4 were neutralised does not correlate with the previous report that all NSF *E. coli* isoates on TC-SMAC to be definite producers of VT (Gray 1995). Therefore it highlights the need for inoculating TC-SMAC as a routine screening medium in diagnostic laboratories in Indian, so that VTEC strains would not be missed out from diarrhoeic stool samples particularly those obtained from children. Besides serotyping is suggested for not missing out *E. coli* O157:H7, which is a non-sorbitol fermenter and a definite producer of VT. As some of the SF *E. coli* isolated on TC-SMAC could be VT producers as well, such as *E. coli* serotype O118:H16 and its variant O118:H (Wieler *et al.* 2000) serotyping is also suggested for such SF *E. coli* isolates apart from testing for VT production (Zadik *et al.* 1993).

Farm management practices seem to reduce the excretion of VTEC as there were no isolates obtained from 29 farm animals screened. Also the rate of excretion of VTEC in livestock of Tamil Nadu suggests that cattle and other livestock could be healthy carriers. The overall prevalence of VTEC in livestock of Tamil Nadu is 3.5% compared to the global prevalence of 10% (Beutin *et al.* 1993). However, all these observations can be verified and the real magnitude can be revealed only if large number of animals could be screened this being a pilot study. Besides, molecular typing of VT genes of all the isolates suggestive of VTEC through toxin assay and serotyping needs to be carried out.

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