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CORRESPONDENCE

Isolation of Shiga toxin-producing *Escherichia coli* including O157:H7 strains from dairy cattle and beef samples marketed in Calcutta, India

Shiga toxin-producing *Escherichia coli* (STEC), particularly O157:H7, an emerging foodborne pathogen, has been associated clinically with a range of presentation extending from asymptomatic infection to severe bloody diarrhoea or haemorrhagic colitis, which can lead to life-threatening sequelae such as haemolytic uraemic syndrome (HUS). STEC has been implicated as an aetiological agent of in individual cases and outbreaks in the developed nations [1]. In developing countries the situation is different. Although an outbreak of bloody diarrhoea due to STEC has been reported recently in Cameroon [2], currently it is not recognised as a significant cause of human disease in Bangladesh and India [3, 4]. A few STEC of serogroup O157 have been isolated in India from sporadic cases of diarrhoea but the strains have not been characterised [5]. The reservoir of STEC in the intestinal tract of cattle and possibly other domestic animals usually transmitted by a number of food items, ground beef being the major vehicle of infection [6]. Extensive efforts have been made to isolate STEC from cattle and meat in various geographical regions across the world, but there has been no report of STEC in Indian cattle and beef marketed in India; one study has documented the presence of O157:H7 STEC in beef marketed in Malaysia, originally imported from India [7].

Here we report the surveillance of dairy farms conducted between Nov. 1997 and Sept. 1998. Samples were collected from state-owned dairy and livestock farms and some private dairy farms in the vicinity of Calcutta. Both adult cattle and buffaloes as well as calves are maintained in all these farms. Raw meat samples (beef, pork) were collected from Calcutta Municipal Corporation Slaughter Houses. Visits were made twice a week during the entire period of study.

Faecal samples were collected from 120 animals, 61 were diarrhoeic and 59 were apparently healthy animals. Both adults and calves were included in the study. Faecal samples were collected per rectum by finger wearing sterile gloves or aseptically by rectal swabs into Cary-Blair medium. A total of 85 samples were also collected from animal handlers: 45 faecal samples and 40 hand rinses. Any history of diarrhoea was noted. For the collection of hand rinses, the handlers were asked to rub their finger tips and hands in a sterile bowl containing 20 ml of 1% peptone water. The washings were then collected in a sterile container. Fifty food samples were collected from various study areas, 22 were raw beef, 15 raw pork and 13 raw pooled milk. Freshly butchered minced meat (10 g) (beef, pork) was collected in sterile McCartney bottles containing 3 ml of Ringer's lactate solution. Raw milk samples (10 ml) were drawn with sterile syringes after shaking the milk cans and were stored in sterile containers. All samples were labelled serially and kept in containers with ice packs, assuring a temperature below 4°C, and were transported to the laboratory for further processing.

From Jan. to June 1999 stool specimens were obtained from 360 children with acute bloody diarrhoea admitted to B. C. Roy Children's Hospital, Calcutta. Rectal swabs were collected from patients if stool samples could not be obtained. Stool specimens or rectal swabs were transported in Cary-Blair medium within 1 h of collection and were examined for STEC and other enteric pathogens within 1 h of arrival at the laboratory.

In the laboratory, all samples collected from the study area were homogenised for 1 min in peptone water, allowed to grow at 37°C for 4–6 h and screened by PCR for the presence of *stx* genes with published primers VTcom-u [5′ GAGCGAAATAATTTATATGTG 3′] and VTcom-d [5′ TGATGATGGCAATTCAGTAT 3′] [8]. The amplified band at 518 bp was visualised on agarose 1.5% gels after electrophoresis and staining with ethidium bromide. *E. coli* strains VTEC3 (positive for stx_1 and stx_2), VTEC2 (positive for stx_2) and VTEC1 (positive for stx_1) were used as positive controls; sterile distilled water was used as the negative control in each batch of the PCR assay.

Each enriched sample that yielded a positive PCR was serially diluted 10-fold in PBS and then spread plated on sorbitol MacConkey (SMAC) agar supplemented with cefixime 0.05 mg/ml for isolated colonies [6]. After overnight incubation both the sorbitol fermenting and non-fermenting colonies were tested for the presence of *stx1* and *stx2* genes by colony hybridisation with stx1 (pJN37-19) and stx2 (pNN 110-18) probes [9]. The DNA probes consist of 1142-bp *Bam*H1 fragment cloned into pUC 19 for stx1 and 842-bp *Sma*1-*Pst*1 fragment cloned into pUC18 for stx2 probe. Signals were visualised by a non-radioactive direct nucleic acid labelling and detection system (ECL; Amersham Life Science). Bead ELISA was performed as described previously [8] to determine the production of Stx_1, Stx_2, or both, by the identified STEC.

The presence of genes encoding accessory virulence factors such as intimin (encoded by *eaeA*) and the

plasmid-encoded enterohaemolysin encoded by enterohaemorrhagic *E. coli* (EHEC) *hlyA* was also investigated in the isolated STEC strains by multiplex PCR. The oligonucleotide primers selected were eaeAF [5′ CAGGTCGTCGTGTCTGCTAAA 3′] and eaeAR [5′ TCAGCGTGGTTGGATCAACCT 3′] for *eaeA* and hlyAF [5′ GCATCATCAAGCGTACGTTCC 3′] and hlyAR [5′ AATGAGCCAAGCTGGTTAAGCT 3′] for *hlyA* gene [10]. Strain EDL933 was used as positive control and reagents without template DNA was used as negative control.

A total of 255 samples from animals, foodstuffs and animal handlers was screened for the presence of STEC. Of the 61 samples from diarrhoeic cattle, four (6.5%) were positive for STEC, whereas only one sample (1.7%) from the 59 healthy cattle was positive. All five positive samples were from calves below 6 months of age. In food samples, only two (9%) of 22 raw minced beef samples showed the presence of STEC. None of the samples from animal handlers, raw pork or pooled raw milk yielded STEC. All 360 bloody diarrhoea samples from the B. C. Roy Children's Hospital, Calcutta were negative for STEC. The important sole aetiological agents of bloody diarrhoea were found to be *Shigella* spp. (15%), *Salmonella* spp. (7%), *Giardia lamblia* (5.1%) and *Entamoeba histolytica* (4.2%).

Table 1 shows the characteristics of the 10 STEC strains isolated during the study period. All O157:H7 strains possessed important virulence attributes such as production of Shiga toxin and presence of *eaeA* and *hlyA* genes. All STEC strains were uniformly susceptible to amikacin, gentamicin, augmentin, chloramphenicol, nalidixic acid, ciprofloxacin and norfloxacin. Only 60% of the strains were sensitive to furazolidone and co-trimoxazole. Two non-O157:H7 strains (A15 and A34) were from diarrhoeic calves, one belonged to a private dairy farm in Singur and one to the West Bengal State livestock farm in Kanchrapara. Another (A24) was isolated from a healthy calf that originated from the National Dairy Research Institute, Kalyani. The O157:H7 isolates (A52 and A53) were also from diarrhoeic calves that originated from the West Bengal government-owned dairy farm, Haringhata the largest milk supplier to the city, and from the State livestock farm, Kanchrapara (A85 and A86). Other O157:H7 strains (M16, M17, M44) were isolated from minced meat obtained from slaughterhouses in the New Market area of Central Calcutta and the Tangra area of East Calcutta.

This study provides the first evidence that dairy cattle in Calcutta are a potential reservoir for virulent O157:H7 strains. It also identified beef, marketed in Calcutta, as a source of O157:H7 strains, indicating that unhygienic practices prevailed in slaughterhouses in Calcutta. Although the mere presence of non-O157 STEC in meat may not be significant [6], the isolation of *eaeA*- and *hlyA*-positive O157:H7 from meat samples reflects its importance as a foodborne pathogen in Calcutta. Radu *et al.* [7] isolated O157:H7 from 36% of samples of beef marketed in Malaysia, originally imported from India. Of 12 (O157:H7) isolates seven produced both Stx_1 and Stx_2 and the remaining five were capable of producing Stx_2 only. In the present study, five of seven strains of O157:H7 produced only Stx_1.

In the present study STEC was not isolated from any of the diarrhoea cases. The reason for low prevalence of STEC-associated diarrhoea in India is not well understood, but may be due to cooking practices that effectively eliminate the STEC and the relatively small proportion of the population that consumes beef products. Nevertheless, the study indicated for the first time the presence of STEC, including virulent O157:H7 strains, in dairy cattle and beef marketed in Calcutta. Clearly, more intense surveys including a

Table 1. Characteristics of STEC strains isolated in Calcutta, India

			Stx* by bead ELISA				Presence of		
Sample no.	Origin	Serotype	Culture supernate	Sonic lysate	Sorbitol fermentation	Antimicrobial resistance	*stx*†	*eaeA*	*hlyA*
A15	Calf (d)	O96:H19	1,2	1,2	+	Cp, Ery, Tet, Cx	1,2	−	+
A24	Calf (h)	ONT:H16	1,2	1,2	−	Cp, Ery, Tet, Fx, Q, Cx	1,2	−	−
A34	Calf (d)	ONT:H19	1,2	1,2	+	Cp, Ery, Tet, Fx, Q, Cx	1,2	−	+
A52	Calf (d)	O157:H7	1	1	−	Cp, Ery, Tet, Cx	1	+	+
A53	Calf (d)	O157:H7	1	1	−	Am, Cp, ERy, Tet, Cx	1	+	+
A85	Calf (d)	O157:H7	2	2	−	Cp, Ery, Tet, Fx, Q, Cx	2	+	+
A86	Calf (d)	O157:H7	2	2	−	Am, Cp, Ery, Tet, Fx, Q, Cx	2	+	+
M16	Raw beef	O157:H7	1	1	−	Cp, Ery, Tet, Cx	1	+	+
M17	Raw beef	O157:H7	1	1	−	Am, Cp, Ery, Cx	1	+	+
M44	Raw beef	O157:H7	1	1	−	Cp, Ery, Tet, Cx	1	+	+

d, diarrhoeic; h, healthy; NT, not typable; Am, amoxycillin; Cp, cephalexin; Cx, cefotaxime; Ery, erythronyicin; Fx, furazolidon; Q, co-trimoxazole; Tet, tetracycline.
*Stx, Shiga toxin production; 1, Stx_1; 2, Stx_2.
†1, carriage of stx_1 gene; 2, carriage of stx_2 gene; 1,2 carriage of both genes; −, absent; +, present.

large number of food samples and clinical materials are required in different parts of the country. The surveillance system would help to clarify the magnitude of the public health problem posed by STEC in India.

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