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OCCURRENCE OF ENTEROHAEMORRHAGIC *Escherichia coli* IN BUFFALO MEAT

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ABSTRACT

Enterohaemorrhagic *Escherichia coli* (EHEC) is a pathogenic *E.coli* which causes diarrhea or haemorrhagic colitis in human beings, which may occasionally progress to hemolytic uremic syndrome (HUS). A total of 100 buffalo meat samples (buffen) were processed for finding out the presence of enterohaemorrhagic *E. coli* (EHEC). Among them, thirty five samples were found positive for *E. coli* (35%), which yielded 37 isolates belonging to 17 different serogroups. However, one strain remained untypable. Further, out of 37 *E. coli*, nine were confirmed as EHEC and belonged to four different serogroups viz., O10, O68, O111 and O172. All the 9 EHEC strains were cytotoxicogenic to Vero-cells. Cytotoxic changes usually appeared at 12 hours post inoculation (hpi) and their intensity increased up to 72 hpi. The important cellular changes recorded were rounding and degeneration of cells followed by detachment of cell sheet and subsequently formation of homogenized cellular masses. Most predominant serotype of EHEC was O111 having 5 isolates (55.6%) and none of the *E. coli* isolate belonged to serotype O157. The prevalence of a wide range of EHEC serogroups in buffalo meat may be due to contamination during or after slaughter and may serve as major source of human infections.

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1 Introduction

Over the last few decades, enterohaemorrhagic *Escherichia coli* (EHEC) have emerged as an important food-borne enteropathogen of humans, apart from others like *Salmonella*, *Campylobacter*, *Listeria monocytogenes*; *Aerobacter* (Dhama et al., 2013a; Dhama et al., 2013b; Dhama et al., 2015). Various serotypes have been reported to cause serious episodes of haemorrhagic colitis (HC) leading to bloody diarrhoea in man, in which meat and meat products served as the vehicle (Cameron et al., 1995; Dhama et al., 2013b; Sethulekshmi et al., 2016). The most notorious serotype of EHEC responsible for 85-95% cases of haemorrhagic colitis, hemolytic uremic syndrome (HUS) and other human illnesses is *E. coli* O157 (Fernandez, 2008; Kiranmayi & Krishnaiah, 2010; Thomas & Elliott, 2013; Flamy et al., 2014). The other non-O157 serotypes that cause remaining 5-15% human diseases are: O10, O22, O26, O28, O39, O55, O68, O103, O111, O128, O145 and O172 (Gyles, 1994).

Bovines are perceived to be their natural reservoirs. Transmission generally occurs through soiling of carcass with animal faeces. Illness in humans is primarily caused by consumption of contaminated undercooked food and meat products, and lead to significant economical losses worldwide (Welinder-Olsson & Kaijser, 2005; Scharff, 2012; Sethulekshmi et al., 2016). The infection had occurred worldwide and serotypes may vary in different geographies (Strachan et al., 2015). Cattle, sheep, sometimes goats are major reservoirs of EHEC, but remain asymptomatic (Sethulekshmi et al., 2016). It may be found in bison, deer and occasionally in pigs, camels, horses, cats, dogs, bear, rats, raccoons, opossums (Navarro-Gonzalez et al., 2015). It may also be detected from several wild and domesticated birds. Present communication describes the prevalence of enterohaemorrhagic *E. coli* in meat of slaughtered buffaloes.

2 Materials and Methods

One hundred samples of buffalo-meat (buffen) were collected aseptically from the MCD slaughter house, Delhi, India. These meat samples included mainly inner thigh or neck muscles and were collected aseptically in approximately 25g amount in sterile plastic cups. The samples were then transported to laboratory on dry ice and kept in refrigerator for overnight before processing.

Preliminary isolation of *E. coli* from buffalo-meat samples was done according to the method described by Bennett et al. (1995) using modified tryptone soya broth (mTSB) as enrichment medium and cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) as selective medium. For this, Meat sample were homogenized in 25ml of mTSB and transferred to 200ml of mTSB and incubated at 37°C for 18-24 h. The enriched culture was further inoculated on CT-SMAC agar plates and incubated at 37°C for 18-24 h. The plates were examined for presence of various types of colonies such as circular colonies with either of uniform purple color, purple color with dark centre, light pink colony or colorless colony of 3-5 mm in diameter. Two to three representative colonies of

each type were picked up and considered as presumptive *E. coli*. The picked up colonies were then purified by sub culturing thrice on CT-SMAC agar plates. The purified cultures of *E. coli* were maintained on TSB agar slants for further identification and characterization.

Presumptive *E. coli* isolates were identified as per methods described by Edwards & Ewing (1972). To identify the enterohaemorrhagic *E. coli* (EHEC) O157 organisms or non-O157 EHEC, these cultures were further subjected to various types of tests viz., urease reaction, motility, hemolysin production and β -glucuronidase reaction by growing on modified sorbitol macConkey agar supplemented with 4-methyl umbelliferyl- β -D-glucuronide (MUG) substrate (Fujisawa et al., 2000). Their cytotoxicigenic potential on vero cell was also assayed as per the method described by Konowalchuk et al. (1977) keeping standard strain of *E. coli* O157 as a positive control. To examine the verocytotoxigenicity, cell-free culture filtrate (CFCF) was prepared as described by Konowalchuk et al. (1977) by growing the organism in brain heart infusion broth for 18 h at 37°C on a rotary shaker (200 rpm). Subsequently the broth culture was centrifuged at 10,000 rpm for 30 min at 4°C (Remi, India) and supernatant was filtered through membrane filter (0.45 μ m). After checking sterility, sterile filtrate (CFCF) were stored at -20°C and tested for cytotoxicity within a week on monolayers of Vero cells grown in 24 well tissue culture plate in minimum essential medium (MEM) supplemented with 8% foetal calf serum. All the identified cultures of *E. coli* were sent to the National *Salmonella* and *Escherichia coli* centre, Central Research Institute, Kasauli (Himachal Pradesh, India) for serotyping.

3 Results and Discussion

Among the studied 100 buffalo-meat samples, 35 samples were found positive for *E. coli* (35%), which yielded 37 isolates belonging to 16 different serogroups. All these 37 isolates were Gram negative motile rods and were positive for catalase, indole and methyl red tests but negative for oxidase, Voges-Proskauer and citrate tests. The isolates fermented lactose, sucrose and sorbitol but gave variable results for decarboxylation of lysine, arginine and ornithine. On Eosin methylene blue (EMB) agar, all the isolates exhibited metallic green sheen. All 37 isolates of *E. coli* were negative for urease reaction and positive for motility and β -glucuronidase reaction. Only 9 *E. coli* isolates were positive for hemolysin production. The results of sugar fermentation and other biochemical reactions are in agreement with the findings of Ali et al. (1998) and Collins & Boitumelo (2014).

Based on O antigen, out of 37 isolates, 34 were typed into 16 serogroups, while 2 were rough strains and one remained untypable. The serogroups to which these *E. coli* isolates belonged were: O8, O9, O10, O25, O27, O43, O48, O60, O68, O70, O73, O78, O100, O109, O111, O172 and rough (Table 1). Most predominant serogroup among typable isolates was O111 (13.5%) which is considered one of the important virulent serogroup as described by Baljer & Wieler 1999.

Table 1 Distribution of different serogroups of *E. coli* isolates in buffalo-meat

Sr.No.	Serogroups	No. of serotypes	No. of isolates in each serotype (%)	Total no of isolates
1	O111	1	5 (13.51)	5
2	O60	1	4 (10.81)	4
3	O8,O70,O78	3	3 (8.11)	9
4	O9,O25,O48,O172, O109, rough	6	2 (5.40)	12
5	O10, O27, O43, O68, O73,O100 Untypable	6	1 (2.70)	7
	Total	17	-	37

During Vero cell cytotoxicity assay, the uninoculated control Vero cells did not show any appreciable change (Figure 1). However, Vero cells inoculated with CFCF of tested *E. coli* isolates showed a variable degree of cytopathic changes. Cytotoxic changes usually appeared at 12 hpi and their intensity increased up to 72 hpi. The important cellular changes recorded were rounding and degeneration of cells followed by detachment of cell sheet and subsequently formation of homogenized cellular masses. The CFCFs capable of producing rounding and clumping of Vero cells involving about 25% of cell sheet within 72 hrs were designated moderately cytotoxic (Figure 2) while the rounding of Vero cells along with cellular degeneration and detachment forming large gaps in cell sheet within this period were considered as highly cytotoxic (Figure 3). Among 37 isolates, 17 *E. coli* isolates were positive for verocytotoxinigenicity and rest were negative. Out of these 17 verocytotoxinigenic *E. coli*, eight isolates belonging to serogroups O25, O73, O78 and rough strain were found moderately verocytotoxinigenic while nine isolates of serogroup O10, O68, O111 and O172 as highly verocytotoxinigenic.

Based on these findings, 9 isolates (24.3%) belonging to four different serogroups viz. O10, O68, O111 and O172 were categorized as enterohaemorrhagic *E. coli* (EHEC). Predominant serogroup of EHEC was O111 representing 5 isolates (55.6%). Two isolates belonged to serogroup O172 while 1 each to O68 and O10 (Table 2). None of the isolates belonged to EHEC O157 and

all the 37 isolates were negative for urease and positive for motility and β -glucuronidase reaction.

In this study, out of 37 obtained *E. coli* isolates, 9 (24.3%) were identified as non-O157 EHEC. Earlier, the epidemiological studies on worldwide occurrence of EHEC in beef, revealed the detection rate up to 36.4% in Southern Ontario, Canada (Clarke et al., 1994), 23-25% in Seattle, Washington (Samadpour et al., 1994), 17% in London (Willshaw et al., 1993) and, 14.1% in Netherlands (Heuvelink et al., 1996). Further, Suthienkul et al. (1990) isolated EHEC isolates from 9% market beef and 8-28% from fresh beef specimens in a slaughterhouse. Similarly, Sethulekshmi et al., (2016) had reported 15.68% prevalence rate of EHEC from beef samples. Thus the results of the above epidemiological investigations further strengthen the finding of present study. However, Manandhar et al. (1997) found only 3% EHEC in raw beef in Australia. The lower occurrence of EHEC in meat as recorded in Australia may be due to variation in geographical conditions. Similarly, Rahimi et al. (2012) found 4.7% of 295 meat samples positive for *E. coli* O157, and highest prevalence was found in beef samples (8.2%), which was followed by water buffalo (5.3%), sheep (4.8%), camel (2.0%) and goat (1.7%). Jure et al. (2015) have isolated and characterized seven *E. coli* O157 isolates from cattle and meat products with a prevalence of 1.4% from Tucuman Province of Argentina.

Figure 1 Vero cells inoculated with sterile BHI broth showing no appreciable changes at 48 hpi on staining with crystal violet (cv) x 40

Table 2 Distribution of EHEC serotypes in buffalo meat

Sr. No.	Sero groups	No. of meat samples positive	No. of isolates in each serotype (%)
1	O111	5	5 (55.56)
2	O172	2	2 (22.22)
3	O68	1	1 (11.11)
4	O10	1	1 (11.11)

The findings of present study are quite comparable to the previous findings, as *E. coli* in raw meat of buffalo has been earlier reported to be 43.75% (Banerjee et al., 2000). In another study, *E. coli* in buffalo-meat revealed 24.83% (Hazarika, 2002). However, Rathore (2000) reported only 9.4% occurrence in raw buffalo-meat. The prevalence of a wide range of EHEC serogroups in meat may be due to contamination during or after slaughter and may serve as major source of human infections.

Most predominant serogroup was O111 (13.5%) and it was followed by O60 (10.81%) and O8, O70, O78 (8.11% each). None of the *E. coli* isolate belonged to serogroup O157. During an investigation of food associated outbreak of HUS in South Australia, Cameron et al. (1995) recorded the isolation of *E. coli* O111 serotype from fermented meat which further strengthen the present findings. Isolation of EHEC of serogroups of O26, O111 and O157 has been reported from beef meat in Chile and Australia (Borie et al., 1997; Desmarchelier, 1997). Similarly, Banerjee et al. (2000) reported the isolation of verotoxigenic *E. coli* including O8,

O68, O84 and O88 serotypes from buffalo-beef but not a single strain belonging to serotype O157. Similar observations were made by earlier workers regarding the absence of O157 from meat and their products (Smith et al., 1998; Sethulekshmi et al., 2016). However, Doyle & Schoeni (1987) reported the isolation of O157 from 1.5-3.7% samples of ground beef. Further, Hazarika (2002) reported the occurrence of O8, O10, O25, O73 and O109 along with rough serotypes from meat and meat products of buffalo and cattle. Similarly, detection of O8, O43, O172 and rough strains of *E. coli* has been recorded from beef by Schurman et al. (2000).

During the present study the higher incidence of EHEC in raw buffalo meat may be as a result of soiling of the carcass with faecal material due to lack of hygienic conditions during slaughter. Modern techniques for reduction of microbial contamination during slaughter and processing of meat may reduce the risk (Saeedi et al., 2017). Meat should be properly cooked at proper temperature to destroy *E.coli*. Good hygienic practices by consumers to prevent contamination are of great importance.

Figure 2 Vero cells showing rounding up to 25-50% of cell sheet and clumping of cells (++) on exposure to CFCS of *E. Coli* O111 at 24 hpi on staining with crystal violet (cv) x 40

Figure 3 Vero cells showing detachment, degeneration and cellular masses (+ + +) on exposure to CFCS of *E. Coli* 0111 at 48 hpi on staining with crystal violet (cv) x 40

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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