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Prevalence and Genetic Characterization of Shiga Toxin-Producing *Escherichia coli* Isolates from Slaughtered Animals in Bangladesh[▽]

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To determine the prevalence of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) in slaughter animals in Dhaka, Bangladesh, we collected rectal contents immediately after animals were slaughtered. Of the samples collected from buffalo ($n = 174$), cows ($n = 139$), and goats ($n = 110$), 82.2%, 72.7%, and 11.8% tested positive for stx_1 and/or stx_2, respectively. STEC could be isolated from 37.9%, 20.1%, and 10.0% of the buffalo, cows, and goats, respectively. STEC O157 samples were isolated from 14.4% of the buffalo, 7.2% of the cows, and 9.1% of the goats. More than 93% ($n = 42$) of the STEC O157 isolates were positive for the stx_2, *eae*, *katP*, *etpD*, and enterohemorrhagic *E. coli hly* (hly_{EHEC}) virulence genes. STEC O157 isolates were characterized by seven recognized phage types, of which types 14 (24.4%) and 31 (24.4%) were predominant. Subtyping of the 45 STEC O157 isolates by pulsed-field gel electrophoresis showed 37 distinct restriction patterns, suggesting a heterogeneous clonal diversity. In addition to STEC O157, 71 STEC non-O157 strains were isolated from 60 *stx*-positive samples from 23.6% of the buffalo, 12.9% of the cows, and 0.9% of the goats. The STEC non-O157 isolates belonged to 36 different O groups and 52 O:H serotypes. Unlike STEC O157, most of the STEC non-O157 isolates (78.9%) were positive for stx_1. Only 7.0% ($n = 5$) of the isolates were positive for hly_{EHEC}, and none was positive for *eae*, *katP*, and *etpD*. None of the isolates was positive for the *iha*, *toxB*, and *efa1* putative adhesion genes. However, 35.2% ($n = 25$), 11.3% ($n = 8$), 12.7% ($n = 9$), and 12.7% ($n = 9$) of the isolates were positive for the lpf_{O113}, *saa*, $lpfA_{O157/01-141}$, and $lpfA_{O157/OI-154}$ genes, respectively. The results of this study provide the first evidence that slaughtered animals like buffalo, cows, and goats in Bangladesh are reservoirs for STEC, including the potentially virulent STEC strain O157.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) organisms, also called verocytotoxin (VT)-producing *E. coli* (VTEC), are one of the most important groups of foodborne pathogens (2, 32). Infection can cause gastroenteritis that may be complicated by hemorrhagic colitis or the hemolytic-uremic syndrome (HUS), which is the main cause of acute renal failure in children. STEC strains causing human infections belong to a large, still-increasing number of O:H serotypes. Most outbreaks and sporadic cases of hemorrhagic colitis and HUS have been attributed to the STEC O157 strains (39). However, infections caused by some non-O157 serotypes have also been frequently associated with severe illness in humans. In some geographic areas, STEC non-O157 strains are more commonly isolated from persons with diarrhea or HUS than STEC O157 strains (33).

STEC are defined by the production of one or more types of Shiga toxin (Stx1 or Stx2 or their variants), which inhibit the protein synthesis of host cells, leading to cell death. Stx1 and Stx2 are encoded by alleles in the genome of temperate, lambdoid bacteriophages that are integrated in the *E. coli* chromosome (38). Besides the *stx* gene(s), human pathogenic STEC strains often carry the *eae* gene, encoding the adherence factor intimin, which is an outer membrane protein (32). The *eae* gene is carried by a pathogenicity island in the chromosome called the locus of enterocyte effacement (LEE), which is required for intimate attachment to the host intestinal mucosa (32). Furthermore, human pathogenic STEC strains often harbor a large plasmid encoding possible additional virulence traits such as the enterohemorrhagic *E. coli* (EHEC) hemolysin (hly_{EHEC}) gene, which acts as a pore-forming cytolysin on eukaryotic cells (36); the bifunctional catalase peroxidase (*katP*) gene (7); a secreted serine protease (*espP*) gene, which can cleave human coagulation factor V (8); and the *etpD* gene cluster, which probably encodes a type II secretion pathway (37). With more LEE-negative STEC strains being reported, investigations of adhesins encoded outside of the LEE have been carried out (40). Several proteins were proposed to be novel adhesion factors; these include ToxB (a protein identified from the large, 93-kb plasmid pO157 and required for full expression of adherence of O157:H7 strain Sakai), Saa (an autoagglutinating adhesin identified in the LEE-negative strains), Iha (an adherence-conferring protein similar to the *Vibrio cholerae* IrgA protein), Efa1 (an EHEC factor for adherence), and LPF (the long polar fimbriae protein, closely related to LPF of *Salmonella enterica* serovar Typhimurium) (40).

Domestic ruminants, mainly cattle, sheep, and goats, have been established as major natural reservoirs for STEC and

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play a significant role in the epidemiology of human infections (16). During the processing of the carcasses, fecal contamination or transfer of bacteria from the animal's hide to the carcass can facilitate transmission of pathogenic *E. coli* to the meat (15).

In most of the developing and underdeveloped countries, hygienic conditions are severely compromised, and living with domestic animals within the same premises is a common practice in both rural and urban areas. Although the socioeconomic status and living style of the people in developing countries support the prevalence of STEC infection, surprisingly, until recently, only a few studies have been done in this part of the world. Human infections associated with STEC strains have been described in Latin America, India, and other developing countries (24, 26). In Bangladesh, the predominant group of *E. coli* associated with childhood diarrhea is enterotoxigenic *E. coli*, accounting for approximately 20% of all diarrheal cases (34). Recently, we determined the prevalence of STEC and the organisms' characteristics among hospitalized diarrheal patients and children with diarrhea in the urban slum community of Dhaka, Bangladesh. The prevalence of STEC was 0.5% among the hospitalized patients, which was 1.9% of the cases of community patients. No STEC strain O157 organisms were isolated in the study (23). This result is comparable with data from other developing countries. The low prevalence of STEC in diarrheal patients can be explained by different facts, the first of which is the absence of any potential reservoir for this organism. Limited studies of the ecology of STEC have been reported, particularly from developing countries. In Bangladesh, no study of the occurrence of STEC in animals had been done. Therefore, the primary objective of the present study was to determine the prevalence of STEC in feces collected from buffalo, cows, and goats slaughtered for meat production.

MATERIALS AND METHODS

Sampling. In the period from 1 January to 1 May 2006, we collected fecal samples from 174 buffalo, 139 cows, and 110 goats at a slaughterhouse in Dhaka of the Mohakhali area in Bangladesh. The animals sampled were randomly selected. The origin of the animals could not be recorded due to the lack of information. However, according to local information, animals were received from different places across the country. Immediately after they were slaughtered, a piece of large intestine containing fecal material (~5 cm), 1 to 1.5 cm away from the rectum, was excised aseptically and kept at 4 to 8°C. The samples were transported immediately to the laboratory, where the microbiological examination was started within 8 h.

Isolation of STEC O157. From each sample, a 25-g portion of feces was collected from a piece of intestine (~5 cm), which was aseptically excised from the animal immediately after slaughter and added to 225 ml of modified tryptone soy broth (Oxoid). After samples were homogenized in a stomacher for 1 min, they were incubated for 18 to 20 h at 37°C. STEC O157 was isolated as described previously (22), by using the immunomagnetic separation technique, and presumptive isolates were confirmed by an agglutination test using an *E. coli* O157 latex agglutination kit (Murex Biotech Ltd., Dartford, Kent, United Kingdom) and a PCR for the *rfb* (O-antigen-encoding) region of *E. coli* O157 ($rfbE_{O157}$) (32).

Isolation of STEC non-O157 strains. The overnight enrichment cultures were screened by PCR for the presence of the stx_1 and stx_2 genes. DNA was extracted from 1 ml of broth culture by thermal cell lysis, using Chelex-100 resin (Bio-Rad Laboratories Ltd., Richmond, CA), and 5 μl of the DNA extract was used in the PCR. Multiplex PCR for stx_1 and stx_2 was carried out in a PTC-200 Peltier thermal cycler (Bio-Rad) as described previously (23). Broth cultures that yielded positive PCR results for stx_1 and/or stx_2 were streaked onto tryptone bile x-glucuronide (TBX) agar (Oxoid) supplemented with cefixime (20 μg/liter), cefsulodin (3 mg/liter), and vancomycin (30 mg/liter) (CCV-TBX). STEC colonies were identified by a colony patch technique, described previously (23).

Serotyping. All isolates were sent to the National Institute of Public Health and the Environment (RIVM) in Bilthoven, The Netherlands, for O and H typing.

Phage typing. Isolates confirmed to be STEC O157 were phage typed at the laboratory of Enteric Pathogens, Central Public Health Laboratory, London, United Kingdom.

PCR detection of putative virulence and adhesin genes. Isolates were tested for both chromosomally carried (e.g., stx_1, stx_2, and *eae*) and plasmid-encoded (e.g., hly_{EHEC}, *katP*, and *etpD*) virulence genes by PCR according to the procedure described previously (23). The presence of eight putative adhesin genes in STEC non-O157 strains (*iha*, *toxB*, *saa*, *efa1*, $lpfA_{O113}$, $lpfA_{O157/OI-141}$, and $lpfA_{O157/OI-154}$) was determined by using the primer sets and reaction conditions described by Toma et al. (40).

Pulsed-field gel electrophoresis. All STEC O157 and STEC non-O157 isolates that belonged to the same serotype were selected to be analyzed by pulsed-field gel electrophoresis (PFGE). PFGE was performed following the standardized protocol developed by PulseNet for *E. coli* O157:H7 (35). Analysis of the tagged image file format (TIFF) images was carried out with BioNumerics software (Applied Maths, Belgium) using the dice coefficient and unweighted-pair group method using average linkages to generate dendrograms with 1.0% tolerance values.

Statistical methods. The chi-square test with Yates' correction or, if necessary, Fisher's exact test was used as indicated to compare the test results. Statistical testing was performed using Epi Info version 3.3.2 on a standard personal computer. Statistically, P values of <0.05 were considered significant for comparisons.

RESULTS

Prevalence of STEC. Of the fecal samples collected from buffalo ($n = 174$), cows ($n = 139$), and goats ($n = 110$), 82.2% ($n = 143$), 72.7% ($n = 101$), and 11.8% ($n = 13$) tested positive for stx_1 and/or stx_2, respectively. Higher numbers of samples from buffalo (66.7%; $n = 116$) and cows (45.3%; $n = 101$) were positive for both the stx_1 and the stx_2 genes than were positive for only the stx_1 or the stx_2 gene ($P < 0.001$). Only stx_1 was positively identified in 8.6% ($n = 15$) of buffalo, 12.9% ($n = 18$) of cow, and 3.6% ($n = 4$) of goat samples. Only stx_2 was positively identified in 6.9% ($n = 12$), 14.4% ($n = 20$), and 7.3% ($n = 8$) of buffalo, cow, and goat samples, respectively. STEC could be isolated from 37.9% ($n = 66$), 20.1% ($n = 28$), and 10.0% ($n = 11$) of the buffalo, cow, and goat samples, respectively. Significantly higher numbers of buffalo samples were positive for STEC than cow and goat samples ($P < 0.001$). STEC O157 strains were isolated from 14.4% ($n = 25$) of the buffalo, 7.2% ($n = 10$) of the cows, and 9.1% ($n = 10$) of the goats. STEC non-O157 strain was isolated from 23.6% ($n = 41$) of the buffalo, 12.9% ($n = 18$) of the cows, and 0.9% ($n = 1$) of the goats. Sometimes more than one serotype was isolated from a single sample, so the number of samples with STEC non-O157 isolates was 41 (49 different isolates from 41 *stx*-positive samples) for buffalo and 18 (21 different isolates from 18 *stx*-positive samples) for cows.

Characterization of STEC O157. The results of the PCR assays for different virulence genes are presented in Table 1. Among 45 STEC O157 isolates, seven phage types (PT) were identified. Ten isolates from buffalo could not be characterized by any recognized PT, 5 of which were identified as untypeable, and the remaining were reacts but not confirmed. Digestion of genomic DNA from the 45 isolates with XbaI and analysis by contour-clamped homogeneous electric field (CHEF)-PFGE revealed 37 distinct restriction profiles (Fig. 1).

TABLE 1. Virulence and (putative) adhesin gene typing of STEC O157 and non-O157 isolates from fecal samples of slaughter animals

Source (*n*)	No. of isolates positive for (%)[a]:										
	Virulence genes							Adhesin genes			
	stx$_1$	*stx*$_2$	*stx*$_1$ and *stx*$_2$	*eae*	*katP*	*etpD*	*hly*$_{EHEC}$	*lpf*$_{O113}$	*saa*	*lpfA*$_{O157/01-141}$	*lpfA*$_{O157/OI-154}$
STEC O157 isolates											
Buffalo (25)	3 (12)	22 (88)	0 (0)	23 (92)	22 (88)	22 (88)	22 (88)	ND	ND	ND	ND
Cow (10)	0 (0)	9 (90)	1 (10)	10 (100)	10 (100)	10 (100)	10 (100)	ND	ND	ND	ND
Goat (10)	0 (0)	10 (100)	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)	ND	ND	ND	ND
Total (45)	3 (6.7)	41 (91.1)	1 (2.2)	43 (95.5)	42 (93.3)	42 (93.3)	42 (93.3)	ND	ND	ND	ND
STEC non-O157 isolates											
Buffalo (49)	41 (83.7)	4 (8.2)	4 (8.2)	0	0	0	3 (6.1)	15 (30.6)	4 (8.1)	7 (14.3)	7 (14.3)
Cow (21)	15 (71.4)	4 (19.0)	2 (9.5)	0	0	0	2 (9.5)	10 (47.6)	4 (19.0)	2 (9.5)	2 (9.5)
Goat (1)	0 (0)	1 (100)	0 (0)	0	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (71)	56 (78.9)	9 (12.7)	6 (8.4)	0	0	0	5 (7.0)	25 (35.2)	8 (11.3)	9 (12.7)	9 (12.7)

[a] ND, not done.

Characterization of STEC non-O157. Results of the PCR assays for different virulence and (putative) adhesin genes are presented in Table 1. None of the isolates was positive for putative virulence and adhesion genes, including *eae*, *katP*, *etpD*, *iha*, *toxB*, and *efa1* (results not shown). More than 30% ($n = 15$) of the isolates from buffalo and 47% ($n = 10$) of the isolates from cows were positive for the *lpf*$_{O113}$ gene. The *saa* gene was present in 8.1% ($n = 4$) and 19.0% ($n = 4$) of the isolates from buffalo and cows, respectively. Seven isolates from buffalo and two isolates from cows were positive for both the *lpfA*$_{O157/01-141}$ and *lpfA*$_{O157/OI-154}$ genes. The goat isolate was negative for all adhesin genes.

Serotyping of the STEC non-O157 isolates revealed that the isolates belonged to 36 different O groups (grouping isolates with an untypeable O serogroup, as serogroup ONT) and 52 O:H serotypes (Table 2). Isolates of different serotypes were found in the same sample; at the same time, isolates of the same serotype were found in different samples of the same/different origins (Table 2). Isolates belonging to the same serotype isolated from same/different samples were analyzed by PFGE, which showed a clonal relationship among different isolates of the same serotype (results not shown). However, polymorphism was also observed between isolates of the same serotype. Three isolates of serotype O80:H19 and two isolates of serotype O125:H19 isolated from both cow and buffalo feces showed identical PFGE patterns. Likewise, three isolates of serotype O149:H8 and two isolates of serotype O141:H21 isolated from buffalo feces generated identical patterns.

DISCUSSION

This study provides the first evidence that slaughtered animals in Bangladesh are reservoirs for STEC, including STEC O157. Around 37.9% of buffalo, 20.1% of cows, and 10.0% of goats were positive for STEC. STEC O157 strains were isolated from 14.4% of buffalo, 7.2% of cows, and 9.1% of goats. The prevalence of STEC, including STEC O157, was significantly higher in buffalo feces than in cow or goat feces ($P < 0.05$). Within similar geographical locations, a few studies of the occurrence of STEC have been done, and most of the studies were done in India and Thailand (26, 28). Recently, a study was done in central Vietnam that found a prevalence of STEC of 27% in buffaloes, 23% in cattle, and 38.5% in goats. Although a high number of isolates were recovered and characterized based on the virulence determinants, no STEC O157 could be isolated, and little information was provided on the serotypic diversity of the isolates (41). In India, Manna et al. reported that the prevalence of STEC O157 in fecal samples from slaughtered cattle and diarrheic calves was 2.0% and 7.6%, respectively (28). STEC O157 has also been isolated in India from foods of cattle origin, namely, raw minced beef samples (9%; $n = 22$) (14), beef surface swabs (3.7%; $n = 27$), and milk samples (2.4%; $n = 81$) (28). In China, STEC O157:H7 was isolated from 10 to 20% of the animals in the villages, including pigs, cattle, goats, and chickens (43). The results of the investigations of the prevalence of STEC O157 in cattle are clearly influenced by the sampling and detection methods adopted. As a matter of fact, the use of specific immunoconcentration procedures for STEC O157 (21, 22) strongly enhances the sensitivity of the isolation methods. Hence, studies based on such procedures reported prevalence rates for STEC O157 that were much higher than those reported with the use of conventional methods (9). It is therefore difficult to determine whether the results reported reflect true differences in isolation rates or are the consequence of the different methodologies adopted.

When buffalo samples were tested for the presence of *stx* genes by PCR, around 80% of the samples were positive. Of the cows and goats, around 73% and 12%, respectively, were positive. STEC non-O157 strains could be isolated from 23.6%, 12.9%, and 0.9% of the buffalo, cow, and goat samples, respectively. Failure to isolate the STEC non-O157 strains from PCR-positive fecal samples is a common problem (3). In India, viable STEC were recovered from 24 to 40% of PCR-positive samples, depending on the origin of the sample. The

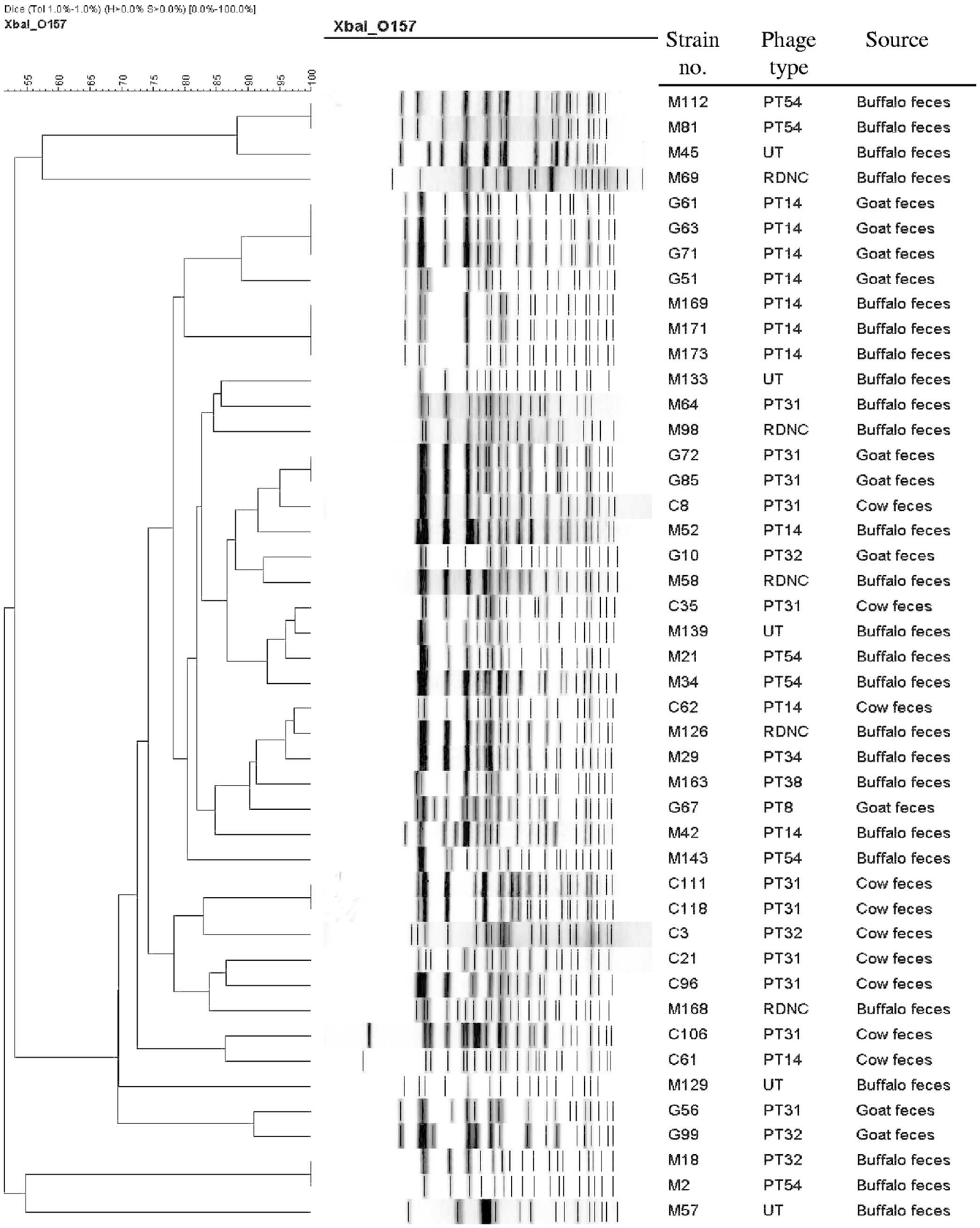

FIG. 1. Dendrogram generated by BioNumerics software, showing the distance calculated by the dice similarity index of PFGE XbaI profiles for 45 STEC O157 isolates isolated from fecal samples of slaughter animals. The degree of similarity (%) is shown on the scale.

TABLE 2. Characteristics of STEC non-O157 isolates from fecal samples of slaughter animals in Bangladesh

Isolate	Origin	Presence of putative virulence and adhesin gene(s)	Serotype	Previous source of isolation[a]	Reference(s) or source
C12	Cow	stx_1	ONT:H8	Human (D), cattle, meat, milk, sheep	3, 4
C14(1)	Cow	stx_1, $lpfA_{O113}$	O89:H−	Human (D)	27
C14(2)	Cow	stx_1, $lpfA_{O113}$	O89:H38		This study
C20	Cow	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O80:H19		This study
C22	Cow	stx_1	ONT:H−	Human (D, HUS) cattle, sheep, milk, beef, pigs	3, 4
C25	Cow	stx_1, $lpfA_{O113}$	O18:H−	Pigeon	13
C26	Cow	stx_1	O146:H21	Human (H, D, BD), cattle, sheep	18
C31	Cow	stx_2, $lpfA_{O113}$	O8:H19	Human (HUS, D), beef, cattle, pork	1
C33	Cow	stx_2, $lpfA_{O113}$	O103:H21	Human (BD, D, HUS)	1
C59	Cow	stx_1	O110:H2	Cattle	20
C64	Cow	stx_1	O110:H2	Cattle	20
C91	Cow	stx_1	O91:H21	Human (D, HUS, BD), milk, beef, ham, cheese, pork, cattle, sheep, sausage	1
C94	Cow	stx_1	O57:H21		This study
C100(1)	Cow	stx_1	O103:H16	Cattle	19
C100(2)	Cow	stx_1, stx_2, hly_{EHEC}, *saa*, $lpfA_{O113}$	O174:H−	Human (D, HUS, H), cattle	20
C100(3)	Cow	stx_1, stx_2, hly_{EHEC}, *saa*, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O130:H9	Cattle	19
C102	Cow	stx_2, *saa*, $lpfA_{O113}$	ONT:H16	Human (D), cattle, sheep, mutton	20
C103	Cow	stx_2, *saa*	O74:H−	Cattle, goat	20
C109	Cow	stx_1	ONT:H8	Human (D), cattle, meat, milk, sheep	3, 4
C124	Cow	stx_1, $lpfA_{O113}$	O125:H19	Human (D), cattle	2, 4
C138	Cow	stx_1, $lpfA_{O113}$	O52:H−		This study
M5	Buffalo	stx_1	ONT:H41	Human (D), bovine	20
M7	Buffalo	stx_2, $lpfA_{O113}$	ONT:H41	Human (D), bovine	20
M10	Buffalo	stx_1	O65:H−	Cattle	11
M12	Buffalo	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O121:H15		This study
M15	Buffalo	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O132:H49		This study
M16	Buffalo	stx_1	O121:H15		This study
M17	Buffalo	stx_1	ONT:H7	Human (D, H), cattle, meat, beef	20
M23(1)	Buffalo	stx_1	O103:H28	This study	
M23(2)	Buffalo	stx_1, $lpfA_{O113}$	ONT:H8	Human (D), cattle, meat, milk, sheep	3, 4
M35	Buffalo	stx_1	O80:H12		This study
M37	Buffalo	stx_1	O75:H10		This study
M41	Buffalo	stx_1	O175:H15		This study
M45	Buffalo	stx_1	ONT:H?	Human (D), cattle, meat, water, sheep, beef	3, 4
M55	Buffalo	stx_1, $lpfA_{O113}$	ONT:H49	Bovine	20
M63	Buffalo	stx_1	O158:H?		This study
M66(1)	Buffalo	stx_1	ONT:H19	Human (D), cattle, beef, pork, meat, milk	20
M66(2)	Buffalo	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O35:H31		This study
M70	Buffalo	stx_1, $lpfA_{O113}$	ONT:H−	Human (D, HUS) cattle, sheep, milk, beef, pigs	3, 4
M80	Buffalo	stx_1	O112:H2	Cattle, beef	20
M82	Buffalo	stx_1, $lpfA_{O113}$	ONT:H16	Human (D), cattle, sheep, mutton	20
M84	Buffalo	stx_1	O149:H8	Cattle	11
M85(1)	Buffalo	stx_1	O149:H8	Cattle	11
M85(2)	Buffalo	stx_1, $lpfA_{O113}$	O149:H8	Cattle	11
M88	Buffalo	stx_1	ONT:H2	Cattle	4
M99	Buffalo	stx_1	ONT:H41	Human (D), cattle	20
M100(1)	Buffalo	stx_1	ONT:H14	Human (D), cattle	20
M100(2)	Buffalo	stx_1, $lpfA_{O113}$	O8:H30	Beef, sheep	1
M101(1)	Buffalo	stx_1	O19:H−		This study
M101(2)	Buffalo	stx_1	O116:H9	Cattle	12
M110	Buffalo	stx_1	O116:H9	Cattle	12
M111(1)	Buffalo	stx_1	O80:H19		This study
M111(2)	Buffalo	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O80:H19		This study
M117(1)	Buffalo	stx_1, hly_{EHEC}, *saa*, $lpfA_{O113}$	O141:H21		This study
M117(2)	Buffalo	stx_1, hly_{EHEC}, *saa*	O141:H21		This study
M117(3)	Buffalo	stx_2, $lpfA_{O113}$	ONT:H16	Human (D), cattle, sheep, mutton	20
M118	Buffalo	stx_2, $lpfA_{O113}$	O73:H18	Human (D)	2
M122	Buffalo	stx_1	07:H6	Human (D), bovine	3, 4
M130	Buffalo	stx_1, $lpfA_{O113}$	O8:H7		This study
M135	Buffalo	stx_1, stx_2, hly_{EHEC}, *saa*, $lpfA_{O113}$	O76:H21	Cattle	20
M137	Buffalo	stx_1	O26:H32	Cattle	1
M140	Buffalo	stx_1	O38:H21	Human (HUS), bovine	10, 5
M141	Buffalo	stx_1, stx_2	O41:H−		This study
M142	Buffalo	stx_1, stx_2, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O35:H32		This study
M145	Buffalo	stx_1, stx_2, *saa*, $lpfA_{O113}$	076:H21	Cattle	20
M148	Buffalo	stx_2	ONT:H−	Human (D, HUS) cattle, sheep, milk, beef, pigs	3
M149	Buffalo	stx_1	O39:H21	Bovine	4
M152	Buffalo	stx_1, $lpfA_{O113}$	O125:H19	Human (D), cattle	2, 4
M153	Buffalo	stx_1, $lpfA_{O113}$	O51:H2		This study
M159	Buffalo	stx_1, $lpfA_{O157/OI-141}$, $lpfA_{O157/OI-154}$	O26:H32	Cattle	1
G19	Goat	stx_2	O32:H25	Human (D)	23

[a] HUS, hemolytic uremic syndrome; BD, bloody diarrhea; D, nonbloody diarrhea; H, healthy human.

nonisolation of viable STEC from PCR-positive samples might be due to the presence of very low numbers of bacteria, the presence of free *stx* phage in the sample, and the loss of *stx* genes upon subcultivation of strains (23). Under routine diagnostic conditions, there is no definitive biochemical characteristic, like sorbitol fermentation in the case of serogroup O157, which can identify STEC irrespective of the serotypes from commensal flora.

Most of the STEC O157 isolates (91.1%) were positive for the stx_2 gene only. In humans, epidemiologic data suggest that *E. coli* O157 strains that express Stx2 are more important than Stx1 in the development of HUS (17) and that strains that express Stx2 alone are more likely to be associated with the progression to HUS than strains that produce both Stx1 and Stx2 (16). When the presence of other virulence genes was analyzed, most of the strains were found to be positive for the *eae* (95.5%), hly_{EHEC} (93.3%), *katP* (93.3%), and *etpD* (93.3%) genes (Table 1). Thus, STEC O157 isolates in this study were found to be carrying additional virulence factors, clearly associated with increased human pathogenicity. In our previous study of patients in Bangladesh, we found the prevalence of STEC in diarrheal patients was relatively low compared to that of other enteric pathogens, including *V. cholerae* and *Shigella* spp. (23). STEC O157 was not found in any of the patients. The higher prevalence of STEC in cattle, in tandem with a low isolation rate in diarrheal patients in Bangladesh, can be explained by the presence of the high prevalence of antibodies in the population, which prevent infection and/or clinical manifestations of STEC infection. However, further studies are needed to establish this explanation.

At least 90 PT have been reported for STEC O157 (25), but only 7 of these (PT2, PT4, PT8, PT14, PT21/28, PT32, and PT54) account for the majority (>75%) of the human strains isolated in Europe and Canada (30). More than 77% ($n = 35$) of the STEC O157 isolates in this study were characterized by seven recognized PT. PT14 (24.4%), -31 (24.4%), and -32 (8.9%) were commonly found in all three types of animals, with differences in prevalence. Other PT that were found in this study were PT8 for goat isolates and PT34, PT38, and PT54 for buffalo isolates. PT14 has commonly been found among STEC O157 strains from different sources, including cattle feces, meat, and human patients (25). Furthermore, a significant association has been detected between PT14 and the presence of acute pathologies (30). PT8 is one of the PT most frequently found among STEC O157:H7 strains from humans and bovines in many European countries, including Spain, Belgium, Finland, Germany, Italy, England, and Scotland (25). PT34 and PT54 have also been found among strains from both human and animal origin but appeared to be more frequent among strains from animal origins (30). PT32 was detected in STEC O157 strains associated with several large outbreaks, including a multistate outbreak in the United States (6) and a recent one in Ireland (29). PT31 was found in both human and non-human sources (meat and slaughter houses) in Canada, suggesting a food-borne transmission in human cases (25).

A high degree of polymorphism was observed for STEC O157 isolates analyzed by PFGE. A total of 37 distinct profiles were obtained among 45 isolates. Strains which had identical PFGE patterns belonged to the same type of animal (Fig. 1). No PFGE profiles were found to be commonly generated by isolates from different types of animals (Fig. 1).

STEC non-O157 isolates have been shown to be important pathogens, despite being severely underreported, because in many laboratories, the facilities to isolate, identify, and characterize them do not exist. Where facilities do exist, STEC non-O157 isolates are found (1). In the present study, we have characterized 71 STEC non-O157 isolates. These isolates belonged to 36 O serogroups and 52 O:H serotypes (Table 2), of which 32 were isolated from buffalo only, 14 from cow only, 1 from goat only, and 5 from both cow and buffalo. All non-O157 serotypes in the study were compared with the serotypes isolated in previous studies from different sources. The majority of STEC isolates belonged to serotypes (42.3%; $n = 22$) previously found among STEC of human origin, of which 31.8% ($n = 7$) were of serotypes associated with severe infections, including bloody diarrhea and HUS (Table 2). In addition, 17 serotypes found in this study were found previously among STEC strains isolated from multiple origins, including those of human patients, cattle, and milk/meat (Table 2). Interestingly, 18 serotypes in this study could not be matched with the recognized STEC serotypes reported so far (Table 2).

When virulence genes were analyzed, 78.9% of the isolates were found to be positive for stx_1 only, 12.7% were positive for stx_2 only, and 8.4% were positive for both stx_1 and stx_2. Higher prevalence rates of stx_1 have been reported in strains from animal origin in previous studies (42), which corroborates the results of the present study. Only 7% of the isolates were found to be positive for the hly_{EHEC} gene (Table 1), and no isolates were positive for other virulence genes, including *eae*, *katP*, and *etpD*. STEC strains, which are positive for *eae* that is harbored by the large pathogenicity island LEE are considered to be highly virulent in humans (40). The LEE appears to confer enhanced virulence; however, the presence of the LEE is not essential for pathogenesis, since a number of cases of severe STEC disease, including HUS, as well as occasional outbreaks, were caused by the LEE-negative strains (31). It has been suggested that additional virulence factors, including adhesins encoded outside of the LEE, may play a role in STEC pathogenesis (40). Since all non-O157 isolates in this study were negative for *eae*, the isolates were tested for the presence of several novel adhesin genes. About 30% ($n = 15$) of the buffalo and 47% ($n = 10$) of the cow isolates were positive for the lpf_{O113} gene (Table 1). This is in accordance with a previous study where lpf_{O113} was found to be one of the most prevalent adhesin genes among STEC non-O157 strains (40). More than 8% ($n = 4$) of the buffalo and 19% ($n = 4$) of the cow isolates possessed the *saa* gene. In the study previously referred to, it was shown that there is a correlation between the presence of *saa* and hly_{EHEC} for certain serotypes (40). In the present study, similar results were found; among eight isolates positive for *saa*, five (62.5%) were positive for hly_{EHEC}.

Although a high degree of serotypic diversity was found among STEC non-O157 isolates in this study, isolates of the same serotype were found in different samples from different animal types. In addition, more than one isolate belonging to the same serotype with different virulence profiles was isolated from the same sample. PFGE analysis was applied to find out if there was any genetic relationship among isolates of the same serotype. Identical PFGE patterns were found among isolates belonging to the same serotype but isolated from different samples, and at the same time, different PFGE patterns were found in the case of some serotypes.

In our previous study, we found a low prevalence of STEC in diarrheal patients in Bangladesh, with no cases of STEC O157 infection (23). In this study, we found a significantly higher prevalence of both STEC O157 and non-O157 serotypes in the animal population. Therefore, there must be a limiting factor

existing in the transmission route of this pathogen from the reservoir to the human population or there might be natural protection against STEC infection among the general population in Bangladesh. Further studies are being carried out to confirm these assumptions.

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