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Water Buffaloes (*Bubalus bubalis*) Identified as an Important Reservoir of Shiga Toxin-Producing *Escherichia coli* in Brazil[▽]

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The presence of Shiga toxin-producing *Escherichia coli* (STEC) in water buffaloes is reported for the first time in South America. The prevalence of STEC ranged from 0 to 64% depending on the farm. STEC isolates exhibiting the genetic profiles *stx*$_1$*stx*$_2$*ehxA iha saa* and *stx*$_2$*ehxA iha saa* predominated. Of the 20 distinct serotypes identified, more than 50% corresponded to serotypes associated with human diseases.

Shiga toxin-producing *Escherichia coli* (STEC) represents an important emerging group of food-borne pathogens. Domestic animals, particularly bovine and ovine, have been incriminated as natural reservoirs of STEC all over the world (2, 7). Although O157:H7 is known to be the most important STEC serotype in many industrialized countries, hundreds of distinct STEC serotypes have been isolated from human diseases in many geographic areas, including Brazil (3, 14, 16, 18, 29).

The presence of STEC in bovines and ovines in Brazil has been described previously (5, 17, 19, 31); however, the prevalence of STEC in Brazilian water buffaloes remains unknown, and all over the world very few data on the prevalence of STEC in water buffaloes are available (7, 10, 21). The aim of this study was to estimate the prevalence and the characteristics of STEC in healthy dairy water buffaloes in Brazil, where they are intensively reared and represent the most important herd in the Americas (11).

A total of 100 healthy dairy water buffaloes from nine farms located in the central area of Minas Gerais State, Brazil, were studied. These animals were randomly selected and represented at least 10% of the herd on each farm. Fecal samples that were collected with sterile swabs and dipped into 8 ml Cary Blair transport medium were directly inoculated into 5 ml of buffered peptone water and incubated overnight at 37°C. Cultures were streaked onto MacConkey sorbitol agar and incubated as described above. A total of 655 presumptively (24) identified *E. coli* colonies (sorbitol positive and negative) were investigated for the presence of the *stx*$_1$,*stx*$_2$, *eae*, and *ehxA* genes using colony hybridization assays and specific DNA probes (13).

STEC isolates were analyzed for the presence of genes coding for Saa, EfaI, ToxB, and Iha using colony hybridization assays and specific DNA probes (22, 23, 26, 27, 28). Cytotoxicity assays were performed as described by Gentry and Dalrymple (12), and the hemolytic activity was determined as described by Beutin et al. (1). *stx*$_1$ and *stx*$_2$ subtypes were determined for 56 strains representing one serotype from each animal. The genetic variant *stx*$_{1c}$ was determined as described by Zhang et al., (32), and differentiation of the *stx*$_2$ variants was carried out as described previously (30).

The antimicrobial susceptibility pattern (6) was determined for 56 strains representing one serotype from each animal. The following antimicrobial agents were tested: ampicillin, amikacin, cefoxitin, chloramphenicol, streptomycin, gentamicin, kanamycin, nalidixic acid, tetracycline, tobramycin, trimethoprim-sulfamethoxazole, cefotaxime, ceftazidime, cefepime, piperacillin-tazobactam, amoxicillin-clavulanic acid, aztreonam, imipenem, and ceftriaxone.

O/H serotypes were identified by using standard methods (9) and O (O1 to O181) and H (H1 to H56) antisera.

In accordance with worldwide data (15), the prevalence of STEC in the present study ranged from 0 to 64.3% depending on the farm (Table 1). STEC strains were not detected in animals from two farms. However, more than 60% of the animals from two farms carried STEC strains. These remarkable differences in the prevalence of STEC depending on the farm probably could be associated with the on-farm management practices, such as manure handling, housing conditions, diet, etc.

More than 70% of the STEC strains were typeable, and the strains belonged to 20 distinct serotypes. More than 50% of the serotypes and untypeable strains associated with H2, H7, H8, H14, H16, H18, and H21 antigens identified in this study had previously been described as serotypes and strains associated with human illness (www.microbionet.com.au/vtectable.htm). Seven serotypes, O23:H7, O74:H25, O77:H18, O82:H-, O93:H16, O141:H49, and O176:H2, accounted for 47.7% of the isolates. Serotype O74:H25 was the most frequent and was recovered from animals reared on four of seven farms, suggesting that there was widespread dissemination of certain serotypes among distinct farms. Other serotypes, such as O77:H18, O23:H7, and O141:H49, were recovered mostly from water buffaloes reared on one farm. Although in the area studied water buffaloes and other dairy and beef cattle are usually reared together, serotypes O23:H7, O49:H21, O59:H8,

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TABLE 1. Distribution of serotypes and genetic profiles of STEC strains isolated from healthy dairy water buffaloes on different farms of Minas Gerais, Brazil

Farm[a]	No. of animals studied	No. of STEC-positive animals (%)	Animal	Serotype[b]	No. of isolates	Genetic profile
A	16	2 (12.5)	29	**O137:H41**	1	*stx*$_1$ *ehxA iha saa*
				O74:H25	1	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
			32	**ONT:H7**	1	*stx*$_2$ *ehxA iha saa*
B	6	2 (33.3)	52	O159:H21	1	*stx*$_1$ *stx*$_{2vhb}$ *ehxA iha saa*
			54	O41:HNT[c]	1	*stx*$_{1c}$ *ehxA iha saa*
C	14	6 (42.8)	3	**O77:H18**	8	*stx*$_1$ *stx*$_{2vhb}$ *ehxA iha saa*
				ONT:H16[c]	1	*stx*$_{2d}$ *ehxA iha*
			6	**ONT:H21**	2	*stx*$_1$ *stx*$_2$ *ehxA iha saa*
			61	**ONT:H21**	1	*stx*$_1$ *stx*$_2$ *ehxA iha saa*
			65	**O88:H25**[c]	3	*stx*$_1$
			67	**O116:H21**	1	*stx*$_2$ *ehxA iha saa*
			70	**O77:H18**	3	*stx*$_1$ *stx*$_{2/2vhb}$ *ehxA iha saa*
D	14	9 (64.3)	14	O141:H49	6	*stx*$_{2vha}$ *ehxA iha saa*
			15	**O178:H19**	1	*stx*$_{2vhb}$ *ehxA iha saa*
			71	**O23:H7**	6	*stx*$_2$ *ehxA iha saa*
			73	O82:H8	2	*stx*$_2$ *ehxA iha saa*
				ONT:H7	1	*stx*$_2$ *ehxA iha saa*
			74	**ONT:H18**	2	*stx*$_1$ *stx*$_2$ *ehxA iha saa*
			75	ONT:HNT[c]	1	*stx*$_1$
				O93:H19	2	*stx*$_1$ *ehxA iha saa*
			76	**O116:H21**	1	*stx*$_2$ *ehxA iha saa*
				O74:H25	1	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
				ONT:H18	4	*stx*$_1$ *stx*$_2$ *ehxA iha saa*
			79	O74:H25	4	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
			80	ONT:H7	1	*stx*$_2$ *ehxA iha saa*
E	10	4 (40.0)	81	**O178:H19**	2	*stx*$_{2vhb}$ *ehxA iha saa*
				O77:H18	1	*stx*$_{2vhb}$ *ehxA iha saa*
			83	O59:H8	1	*stx*$_{2vha}$ *ehxA iha saa*
			85	O74:H25	2	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
			89	**O82:H-**	4	*stx*$_2$ *ehxA iha saa*
F	10	3 (30.0)	100	ONT:H42	1	*stx*$_1$ *stx*$_{2vhb}$ *ehxA iha saa*
			103	**O113:H21**	1	*stx*$_1$ *stx*$_{2vha}$ *iha*
			105	**ONT:H7**	1	*stx*$_1$ *ehxA iha saa*
G	18	11 (61.1)	110	**O93:H19**	1	*stx*$_2$ *ehxA*
				O156:H21	2	*stx*$_1$ *stx*$_{2vha}$ *iha*
				ONT:H21	5	*stx*$_{2unt}$ *ehxA iha saa*
			111	O156:H21	1	*stx*$_1$ *stx*$_{2vha}$ *iha*
			112	O74:H25[c]	3	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
			114	O74:H25[c]	4	*stx*$_1$ *stx*$_{2vha}$ *ehxA iha saa*
				O22:H16	1	*stx*$_1$ *stx*$_2$ *ehxA iha saa*
				ONT:H2	1	*stx*$_{2vhb}$ *ehxA iha saa*
			115	O82:H8	1	*stx*$_2$ *ehxA iha saa*
			117	O49:HNT[c]	1	*stx*$_{1c}$
				O49:H21[c]	1	*stx*$_{1c}$
			118	**O77:H41**	1	*stx*$_1$ *ehxA iha saa*
			119	O176:H2	1	*stx*$_{2vhb}$ *ehxA iha saa*
				O49:H21[c]	1	*stx*$_{1c}$
				ONT:H38[c]	2	*stx*$_{1c}$
				ONT:H14[c]	1	*stx*$_{1c}$
			121	O93:H16 (H-)	6	*stx*$_1$ *iha saa*
				ONT:HNT	1	*stx*$_{1c}$
			123	O79:H14	1	*stx*$_1$ *stx*$_{2vhb}$ *ehxA iha saa*
				ONT:H8	1	*stx*$_{1c}$
			126	O176:H2	3	*stx*$_{2vhb}$ *ehxA iha saa*
				ONT:H2	1	*stx*$_{2vhb}$ *ehxA iha saa*
Total	100	37 (37)			109	

[a] STEC was not detected on two farms (farms H and I).
[b] Bold type indicates serotypes associated with human diseases.
[c] Negative for the cytotoxicity assay with Vero cells.

O74:H25, O77:H41, O93:H16, O93:H19, O137:H41, and O176:H2, which accounted for 45% of the serotypes found in water buffaloes, were not found among STEC strains from dairy and beef cattle (data not shown).

Of the 109 STEC isolates, 42 (38.5%) carried stx_2, 43 (39.5%) carried stx_1 and stx_2 sequences, and only 24 (22%) harbored the stx_1 sequence. The majority of STEC strains belonging to serotypes not previously reported to be serotypes associated with human illness and carrying the stx_1 sequence alone carried the stx_{1c} subtype. These strains were devoid of additional virulence factors and were negative for the cytotoxicity assay with Vero cells. In contrast, STEC serotypes associated with human diseases harbored stx_2 or stx_1 and stx_2 sequences and belonged to the stx_2, stx_{2vha}, or stx_{2vhb} subtype; one strain belonged to the stx_{2d} subtype, and one strain was untypeable with the method currently used. STEC isolates exhibiting the genetic profiles stx_1 stx_2 *ehxA iha saa* and stx_2 *ehxA iha saa* accounted for more than 70% of the isolates.

All STEC isolates were devoid of the *eae* gene. This result can be related to the serotypes found in these animals. According to Sandhu et al. (25), the presence of the *eae* gene is associated with certain O groups, such as O26, O103, O111, O145, and O157, none of which was identified in the STEC in the present study. The *saa* and *iha* gene sequences were detected alone or in association in 83.5% of the STEC strains. This wide distribution of Saa among *eae*-negative strains is in agreement with the findings of Paton et al. (23). With the lack of the *eae* gene, distinct adhesins other than intimin can have an important role in adherence to the intestinal epithelium and colonization of the gut. The *ehxA* gene sequence was detected in 86 of the 109 STEC strains (78.9%), and all of the strains expressed hemolytic activity after 18 to 24 h of incubation.

The majority of STEC strains were susceptible to all antimicrobials tested. Resistance to one drug (nalidixic acid, streptomycin, or ampicillin) and resistance to two antimicrobials (ampicillin plus streptomycin and nalidixic acid plus ampicillin) were found in 10 (17.8%) and 2 (3.6%) of the strains, respectively.

The prevalence of STEC strains having the genetic profile stx_1 stx_2 *ehxA iha saa* or stx_2 *ehxA iha saa* deserves great attention as STEC strains carrying the stx_2 gene are commonly associated with more severe disease (4, 8). Moreover, these strains carry other virulence genes, such as *ehxA*, *iha*, and *saa*, which can enhance their virulence.

This is the first report of the presence of STEC in water buffaloes in South America. Unpasteurized water buffalo milk may represent a potential risk to public health since it is used for homemade mozzarella production due to its high fat and casein content (20).

Because water buffalo farming is increasing as an important economic activity, control measures for hygienic practice, particularly in milking, surveillance, and legislation have to be improved in order to prevent fecal contamination of milk and dairy products.

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