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Detection of the Emerging Shiga Toxin-Producing *Escherichia coli* O26:H11/H$^-$ Sequence Type 29 (ST29) Clone in Human Patients and Healthy Cattle in Switzerland

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Shiga toxin-producing *Escherichia coli* O26:H11/H$^-$ strains showing the characteristics of the emerging human-pathogenic ST29 clone (*stx*$_{2a}$$^+$ only, *eae*$^+$, plasmid gene profile *hlyA*$^+$ *etpD*$^+$) were detected from human patients and healthy cattle, indicating a possible reservoir. Sheep also appear to shed strains related to the new pathogenic clone O26:H11/H$^-$ (ST29, *stx*$_{1a}$$^+$ only, *eae*$^+$, plasmid gene profile *hlyA*$^+$ *etpD*$^+$).

Shiga toxin-producing *Escherichia coli* (STEC) strains of serotype O26:H11/H$^-$ (nonmotile) have emerged as the most common non-O157 STEC strains causing human diseases in many countries (1). STEC O26 can cause gastrointestinal illnesses (diarrhea and bloody diarrhea), but conditions may be complicated by neurological and renal sequelae, including the life-threatening hemolytic-uremic syndrome (HUS) (2, 3, 4). STEC O26 represents a highly dynamic group that rapidly generates new pathogenic clones (5, 6). This is exemplified by the emergence of a highly virulent clone of STEC O26:H11/H$^-$ in Germany in the mid-1990s that harbored *stx*$_{2a}$ as the only Shiga toxin gene (5).

A recent study investigating STEC O26 strains isolated from human patients in seven European countries between 1996 and 2012 showed that STEC O26:H11/H$^-$ strains harboring *stx*$_{2a}$ as the sole Shiga toxin gene consist of two major subgroups, which both have a strong association with progression of infection to HUS (1). These two subgroups comprise (i) strains of sequence type 29 (ST29), which typically harbor plasmid genes *hlyA* and *etpD* (but not *espP* and *katP*) and belong to the emerging STEC O26 German clone, and (ii) strains of ST21, which differ phylogenetically and by plasmid gene profiles from ST29 strains. However, the environmental reservoirs and sources of STEC O26:H11/H$^-$ strains belonging to the emerging ST29 clone are widely unknown. Cattle and sheep, major reservoirs of STEC, are also reservoirs for STEC O26 (7, 8, 9, 10), but STEC O26 strains harboring *stx*$_{2a}$ only and belonging to ST29 have to our knowledge not yet been described. In this study, we determined the phylogeny and clonal structure, *stx* genotypes (Shiga toxins), and plasmid gene profiles of STEC O26:H11/H$^-$ strains from human patients and of *E. coli* O26:H11/H$^-$ from healthy cattle and sheep in Switzerland.

The 27 human STEC O26:H11/H$^-$ strains were isolated between 2000 and 2009 from fecal samples of 27 patients with reasonable clinical suspicion of infection with STEC in Switzerland (11). The 12 *E. coli* O26:H11/H$^-$ strains originated from fecal samples of healthy cattle and sheep at slaughter in Switzerland. The majority of strains were isolated during 2011 from cattle aged between 3 and 24 months (10). One bovine strain from 2005 and one ovine strain from 2004 were additionally included (12, 13).

To provide a clonal framework for studying the phylogeny of the strains, multilocus sequence typing (MLST) was performed. Internal fragments of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were sequenced (14), and alleles and sequence types (STs) were assigned in accordance with the *E. coli* MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli). For further characterization, strains were examined for *stx*$_1$, *stx*$_2$, and their subtypes; the presence of *eae* (adhesion factor intimin); and plasmid gene profiles (*hlyA*, *katP*, *espP*, and *etpD*) using PCR (5, 15).

MLST identified two STs (21 and 29) sharing six of the seven MLST loci among the 27 STEC O26:H11/H$^-$ strains from human patients (Table 1) and the 12 *E. coli* O26:H11/H$^-$ strains from domestic ruminants (Table 2).

STEC O26:H11/H$^-$ strains of ST29 represent a highly virulent clone, which has spread throughout Europe and the New World after its emergence in Germany in the mid-1990s (1, 5). Of the 27 human STEC O26:H11/H$^-$ strains, 11 (40.7%) belonged to ST29 (Table 1). Since the year 2000, ST29 strains have regularly been isolated from human patients in Switzerland. HUS developed in eight patients, and bloody diarrhea and nonbloody diarrhea were noted in each case for four patients. All STEC O26:H11/H$^-$ strains of ST29 harbored *stx*$_{2a}$ and *eae*, and two of them additionally possessed *stx*$_{1a}$. In the recent study by Bielaszewska et al. (1), ST29 strains from human patients harbored only *stx*$_{2a}$ and represented 28% of all STEC O26:H11/H$^-$ strains and 50% of *stx*$_{2a}$-harboring strains isolated between 1996 and 2012. Moreover, all our human ST29 strains possessed the plasmid gene profile typical for the emerging clone (*hlyA*$^+$ *etpD*$^+$) (1, 5).

ST29 was also identified among three *E. coli* O26:H11/H$^-$ strains isolated from healthy domestic ruminants in Switzerland, namely, two cattle and one sheep (Table 2). One bovine ST29 strain (isolated in 2011) harbored *stx*$_{2a}$, *eae*, and the plasmid gene combination *hlyA*$^+$ *etpD*$^+$. Hence, this bovine O26 STEC strain of ST29 showed the characteristics of the emerging human-pathogenic clone, which has so far not been detected or investigated in domestic ruminants. In a study from Scotland, ST29 was also identified in bovine STEC O26, but no strains harboring *stx*$_{2a}$ only

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TABLE 1 Characteristics of STEC O26:H11/H$^-$ strains isolated from human patients in Switzerland between 2000 and 2009[a]

Sequence type	Strain	Yr of isolation	stx_{1a}	stx_{2a}	*eae*	Plasmid gene combination				Disease(s)
						hlyA	*katP*	*espP*	*etpD*	
ST21	1953	2001	+	−	+	+	+	+	−	HUS
	2163	2001	+	−	+	+	+	+	−	BD
	2164	2001	+	−	+	+	+	+	−	BD
	1183	2002	+	+	+	+	+	+	−	D, HUS
	1543	2002	+	−	+	+	+	+	−	BD
	1895	2003	+	+	+	+	+	+	−	BD
	596	2004	+	−	+	+	+	+	−	ND
	708	2005	+	−	+	+	+	+	−	BD
	901	2005	+	−	+	+	+	+	−	BD
	1591	2005	+	−	+	+	+	+	−	D, HUS
	1138	2007	+	−	+	+	+	+	−	BD
	1828	2007	+	−	+	+	+	+	−	BD
	1614	2008	+	+	+	+	+	+	−	BD, HUS
	1972	2008	+	+	+	+	+	+	−	HUS
	1974	2008	−	+	+	+	+	+	−	D, HUS
	1319	2009	+	−	+	+	+	+	−	BD
ST29	3528	2000	−	+	+	+	−	−	+	HUS
	2000	2001	−	+	+	+	−	−	+	HUS
	1103	2002	−	+	+	+	−	−	+	HUS
	1995	2002	−	+	+	+	−	−	+	BD
	2109	2002	−	+	+	+	−	−	+	BD
	964	2003	−	+	+	+	−	−	+	BD, HUS
	968	2003	+	+	+	+	−	−	+	BD
	781	2004	−	+	+	+	−	−	+	D, HUS
	2393	2004	+	+	+	+	−	−	+	D, HUS
	299	2007	−	+	+	+	−	−	+	D, HUS
	1621	2008	−	+	+	+	−	−	+	D, HUS

[a] STEC, Shiga toxin-producing *Escherichia coli*; ST, sequence type; *stx*, Shiga toxin gene; *eae*, intimin gene; *hlyA*, *katP*, *espP*, and *etpD*, genes encoding hemolysin, catalase-peroxidase, serine protease EtpD, and type II secretion system, respectively; BD, bloody diarrhea; D, nonbloody diarrhea; HUS, hemolytic-uremic syndrome.

were found (9). The two other ST29 strains showed different characteristics: (i) the ovine ST29 strain (isolated in 2004) possessed the plasmid gene profile of the emerging clone but harbored stx_{1a} and (ii) the second bovine ST29 strain lacked *stx* and the tested plasmid genes.

The other strains examined in this study, 16 human STEC O26:H11/H$^-$ strains and nine bovine *E. coli* O26:H11/H$^-$ strains, belonged to ST21 (Tables 1 and 2). ST21 strains were regularly isolated from human patients in Switzerland during the study period. HUS developed in six patients, and bloody diarrhea was noted for 10 patients and nonbloody diarrhea was noted for three patients. Clinical STEC O26:H11/H$^-$ strains of ST21 produce, alone or in combination, Stx1a and Stx2a (1, 5). The majority (11/16) of the human ST21 strains harbored only stx_{1a}, four strains possessed

TABLE 2 Characteristics of *Escherichia coli* O26:H11/H$^-$ isolated from healthy cattle and sheep at slaughter in Switzerland[a]

Sequence type	Strain	Yr of isolation	Origin	stx_1	stx_2	*eae*	Plasmid gene combination			
							hlyA	*katP*	*espP*	*etpD*
ST21	36	2011	Cattle	−	−	+	+	+	+	−
	45	2011	Cattle	−	−	+	+	−	+	−
	224	2011	Cattle	−	−	+	+	+	+	−
	245	2011	Cattle	−	−	+	+	+	+	−
	263	2011	Cattle	−	−	+	+	−	+	−
	268	2011	Cattle	−	−	+	+	+	+	−
	276	2011	Cattle	−	−	+	+	+	+	−
	710	2011	Cattle	−	−	+	+	+	+	−
	738	2011	Cattle	−	−	+	+	+	+	−
ST29	5	2011	Cattle	−	−	+	−	−	−	−
	1200	2011	Cattle	−	stx_{2a}	+	+	−	−	+
	43	2004	Sheep	stx_{1a}	−	+	+	−	−	+

[a] ST, sequence type; *stx*, Shiga toxin gene; *eae*, intimin gene; *hlyA*, *katP*, *espP*, and *etpD*, genes encoding hemolysin, catalase-peroxidase, serine protease EtpD, and type II secretion system, respectively.

stx$_{1a}$ and *stx*$_{2a}$, and one strain harbored only *stx*$_{2a}$ (Table 1). The plasmid gene combination was *hlyA*$^+$ *katP*$^+$ *espP*$^+$ in all human ST21 strains, a combination frequently found in clinical ST21 strains (1). With regard to bovine *E. coli* O26:H11/H$^-$ (Table 2), ST21 strains (*eae*$^+$) showed plasmid gene profiles described for human STEC O26 strains of ST21 (1), whereas Shiga toxin genes were lacking. However, ST21 is also frequently found in STEC O26 strains from cattle (7, 9). Such *E. coli* O26 strains can probably undergo transition via loss and gain of Stx-encoding phages, as has been shown for human *E. coli* O26 (6, 16). Interconversion between STEC O26 strains and their *stx*-negative variants might support the emergence of new clones with pathogenic potential for humans. Thus, cattle constitute a potential reservoir and source of new STEC O26 pathotypes. In addition, it must be considered that the absence of *stx*-harboring phages in *E. coli* O26 might increase their adaptability outside the host and enable adaptation to stress conditions encountered in the gastrointestinal tract (16).

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