Enterohemorrhagic *Escherichia coli* O26:H11/H−: A New Virulent Clone Emerges in Europe

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**Background.** Enterohemorrhagic *Escherichia coli* (EHEC) O26 causes diarrhea and hemolytic uremic syndrome (HUS). Strains harboring the stx1a gene prevail, but strains with stx2a as the sole Shiga toxin–encoding gene are now emerging. The traits and virulence of the latter set of strains are unknown. We correlated stx genotypes of 272 EHEC O26 strains isolated in 7 European countries between 1996 and 2012 with disease phenotypes. We determined phylogeny, clonal structure, and plasmid gene profiles of the isolates and portrayed geographic and temporal distribution of the different subgroups.

**Methods.** The stx genotypes and plasmid genes were identified using polymerase chain reaction, phylogeny was assigned using multilocus sequence typing, and clonal relatedness was established using pulsed-field gel electrophoresis.

**Results.** Of the 272 EHEC O26 isolates, 107 (39.3%), 139 (51.1%), and 26 (9.6%) possessed stx1a, stx2a, or both genes, respectively. Strains harboring stx2a only were significantly associated with HUS (odds ratio, 14.2; 95% confidence interval, 7.9–25.6; P < .001) compared to other stx genotypes. The stx2a-harboring strains consist of 2 phylogenetically distinct groups defined by sequence type (ST) 21 and ST29. The ST29 strains are highly conserved and correspond by plasmid genes to the new virulent clone of EHEC O26 that emerged in Germany in the 1990s. This new clone occurred in 6 of the 7 countries and represented approximately 50% of all stx2a-harboring EHEC O26 strains isolated between 1996 and 2012.

**Conclusions.** A new highly virulent clone of EHEC O26 has emerged in Europe. Its reservoirs and sources warrant identification.

**Keywords.** enterohemorrhagic *Escherichia coli* O26; new clone; Shiga toxin; hemolytic uremic syndrome.

Enterohemorrhagic *Escherichia coli* (EHEC) causes diarrhea, bloody diarrhea, and the hemolytic uremic syndrome (HUS) [1]. HUS, consisting of hemolytic anemia, thrombocytopenia, and acute renal insufficiency [1], complicates approximately 15% of childhood EHEC O157:H7 infections and is the most common cause of childhood acute renal failure [2].

Although *E. coli* O157:H7 is the predominant cause of HUS worldwide [1], EHEC of serotype O26:H11/H− (nonmotile) has emerged as the most common non-O157 EHEC strain causing human diseases in Europe [3–10] and the United States [11–13]. It has also been increasingly isolated from patients in South America [14], Asia [15], and Australia [16]. EHEC O26 can cause disease that is as severe as that caused by EHEC O157:H7 [3, 17, 18], and there appears to be

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no significant difference in the long-term outcome of HUS caused by non-O157 EHEC (including EHEC O26) and EHEC O157 [19]. Children suffering from HUS caused by EHEC O26 and other non-O157 EHEC strains are younger than those with HUS caused by EHEC O157:H7 [3, 10].

Thrombotic microangiopathy caused by Shiga toxins (Stx), the major virulence factors of EHEC, forms the histopathological basis of HUS [1]. EHEC O26:H11/H7 produces, singly or together, Stx1a and Stx2a encoded by stx1a and stx2a genes, respectively [20]. Most EHEC O26 strains isolated from patients harbor stx1a only [9, 11, 12, 14, 15, 21], but strains possessing stx2a as the sole stx gene, which were first identified in Germany in the mid-1990s [22], have been increasingly detected throughout Europe [5, 8, 18, 23–25] and the New World [11, 14]. However, characteristics of such isolates and their potential to cause HUS compared to that of EHEC O26 with other stx genotypes have not been systematically studied. Here we correlated clinical outcomes of the infection with stx genotypes of EHEC O26 isolates, determined the isolates’ phylogeny and clonal structure, and portray geographic and temporal distribution of the different subgroups in different European countries.

METHODS

Strains and Patients
Two hundred seventy-two EHEC O26:H11/H7 strains were isolated between January 1996 and September 2012 from stools of epidemiologically unrelated patients with HUS (n = 127), bloody diarrhea (n = 18), or nonbloody diarrhea (n = 127) in Germany (n = 182), Austria (n = 38), Italy (n = 24), the Czech Republic (n = 18), Belgium (n = 7), the United Kingdom (n = 2), and Slovakia (n = 1). Demographic characteristics of the patients are provided in Table 1.

Case Definitions
Diarrhea was defined as 3 or more liquid stools without visible blood per day, and as bloody if blood was visible by the naked eye. The information about the number of stools per day, stool consistency, presence of mucus and/or visible blood, and duration of diarrhea was obtained from treating physicians or patients’ parents or guardians using standardized questionnaires. The questionnaires were reviewed nonanonymously by laboratory staff. HUS was defined by a hematocrit level of <30%, with smear evidence of intravascular hemolysis, thrombocytopenia (platelet count <150 000/mm3), and renal insufficiency (serum creatinine concentration greater than the upper limit of the normal range for age) [1]. Clinical diagnoses based on the case definitions were provided for all patients by treating physicians.

Genotypic and Phenotypic Characterization of EHEC O26 Isolates
Isolates were verified as E. coli (API 20 E; bioMérieux, Marcy l’Étoile, Lyon, France) and O:H serotyped [26]. H types were confirmed using fliC genotyping [7]; all strains, both motile and nonmotile, possessed the fliC gene encoding the H11 antigen. All stx genotypes, presence of eae (encoding adhesin intimin), and plasmid genes were determined using polymerase chain reaction (PCR) [20, 22]. Stx production was confirmed by Vero cell assay [26].

Multilocus Sequence Typing
Multilocus sequence typing (MLST) was built on sequences of 7 housekeeping genes [7], and sequence types (STs) were assigned (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Pulsed-Field Gel Electrophoresis
Pulsed-field gel electrophoresis (PFGE) was performed using the standardized PulseNet protocol [27] with a subset of geographically and temporally representative strains. Restriction patterns were analyzed and the cluster analysis was performed with BioNumerics software, version 6.5 (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The dendrogram was generated using the band-based Dice similarity coefficient (1% band position and 1% optimization tolerance) and the unweighted pair group method with arithmetic means.

Antimicrobial Susceptibility Testing
Susceptibility to ampicillin, cefuroxime, cefotaxime, cefpodoxime, ceftazidime, piperacillin/tazobactam, tigecycline, meropenem, gentamicin, amikacin, trimethoprim/sulfamethoxazole, ciprofloxacin, fosfomycin, and nitrofurantoin was tested using the disk diffusion method according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints [28] and standard recommendations [29].

Statistical Analysis
Statistical analysis was performed using χ2, Fisher exact (EpiInfo version 7.1.0.6) or Mann-Whitney U (IBM SPSS Statistics 20.0) tests. Two-tailed P values <.05 were considered significant.

Table 1. Demographic Characteristics of 272 Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sexa</td>
<td>141/272 (51.8%)</td>
</tr>
<tr>
<td>Female sexa</td>
<td>115/272 (42.3%)</td>
</tr>
<tr>
<td>Children</td>
<td>261/272 (96.0%)</td>
</tr>
<tr>
<td>Adults</td>
<td>11/272 (4.0%)</td>
</tr>
<tr>
<td>Age, all patients, median (range)</td>
<td>23 mo (2 mo–63 y)</td>
</tr>
<tr>
<td>Age, children, median (range) (n = 261)</td>
<td>21 mo (2 mo–14 y)</td>
</tr>
<tr>
<td>Age, adults, median (range) (n = 11)</td>
<td>35 y (26–63 y)</td>
</tr>
</tbody>
</table>

* Sex was unknown in 16 patients.
Among the 272 EHEC O26:H11/H− isolates, 107 (39.3%) possessed stx1a, 139 (51.1%) stx2a, and 26 (9.6%) both stx1a and stx2a (Table 2). Of the 107 stx1a-containing strains, 9 (8.4%) were isolated from patients with HUS and 98 (91.6%) from patients with diarrhea without HUS, demonstrating a significant association of the stx1a genotype with uncomplicated diarrhea (odds ratio [OR], 27.3; 95% confidence interval [CI], 12.8–58.6; P < .001) compared to HUS (OR, 0.04; 95% CI, 0.02–0.8; P < .001) (Table 2). In contrast, 104 of the 139 stx2a-harboring isolates (74.8%) originated from patients with HUS, and 35 (25.2%) from patients with diarrhea without HUS, demonstrating the statistically significant association of the stx2a genotype with HUS (OR, 14.2; 95% CI, 7.9–25.6; P < .001) compared to uncomplicated diarrhea (OR, 0.07; 95% CI, 0.04–0.13; P < .001) (Table 2). Strains harboring both stx1a and stx2a had equal potential to cause HUS and diarrhea (Table 2). Children were significantly more frequently infected with strains harboring stx2a only (138 of 261 [52.9%]) than adults (1 of 11 [9.1%]) (OR, 11.2; 95% CI, 1.1–88.9; P = .004). Accordingly, in children the infection had a tendency to progress to HUS (124 of 261 [47.5%]) (OR, 2.4; 95% CI, 1.6–9.3; P = .228; Supplementary Table 1).

Phylogeny of EHEC O26:H11/H− With Different stx Genotypes

Two major STs (21 and 29), which share 6 of the 7 MLST loci, and 5 single locus variants (SLVs) of these 2 STs were identified (Table 3, Supplementary Table 2). Almost all strains harboring stx1a only (105 of 107 [98.1%]), and all strains harboring stx1a and stx2a, belonged to ST21 (Table 3); the remaining 2 strains of the stx1a genotype belonged to ST1565 and ST1705, respectively (Table 3), which are SLVs of ST21 (Supplementary Table 2). In contrast, strains containing stx2a but not stx1a were equally distributed between ST21 (67 of 139 [48.2%]) and ST29 (69 of 139 [49.6%]) (Table 3); 3 strains belonged to ST396, ST591, and ST1566 (1 each) (Table 3), which are SLVs of ST29 (ST396 and ST1566) and ST21 (ST591) (Supplementary Table 2). Thus, EHEC O26:H11/H− strains harboring stx1a alone or in combination with stx2a are phylogenetically positioned in the same genetic background (as defined by MLST core genome genes), whereas strains with stx2a but not stx1a consist of 2 distinct but closely related phylogenetic groups. Of these 2 groups characterized by ST21 and ST29, ST29 and its 2 SLVs (ST396 and ST1566) and ST591 (SLV of ST21) are unique for such strains (Table 3).

Plasmid Gene Profiles

We plasmid genotyped stx2a-harboring EHEC O26:H11/H− strains belonging to different STs to provide deeper characterization, with emphasis on the presence of the unique plasmid gene combination found previously in the new stx2a-harboring EHEC O26 German clone [22]. Almost all (65 of 69 [94.2%]) ST29 strains possessed the plasmid gene combination typical for the new clone (EHEC-hlyA+/katP+/espP+/etpD−); in contrast, this combination was found in no other STs (Table 4). Of the 4 different plasmid gene combinations in ST21, the most frequent (52 of 67 strains [77.6%]) was EHEC-hlyA+/katP+/espP+/etpD− (Table 4). This combination was also found in 2 strains of ST29 and single strains of STs 396 and 591 (Table 4). Two strains of ST29 and the single ST1566
strain had none of the plasmid genes sought (Table 4). Thus, based on the phylogeny and plasmid gene profiles, stx2a-harboring EHEC O26 consists of 2 major subgroups: (i) strains of ST29, which typically contain EHEC-hlyA and etpD, but not espP and katP, and belong to the new EHEC O26 German clone, and (ii) strains of ST21, which differ phylogenetically and by plasmid gene profiles from strains of ST29. Both groups showed a significant risk of progression of the infection to HUS (ST21: OR, 3.4; 95% CI, 1.9–6.0; P < .001; ST29: OR, 9.0; 95% CI, 4.5–17.9; P < .001; Table 2). Although ST29 had a 2.6-fold higher OR in terms of HUS association than ST21, there was no statistically significant difference between these 2 STs in their association with HUS (OR, 2.2; 95% CI, 1.0–4.9; P = .089; Table 2). Also, ages of children who developed HUS after infection with strains of ST29 (median, 24 months [range, 8–156 months]) and ST21 (median, 20 months [range, 2–84 months]) did not significantly differ (P = .053, Mann-Whitney U test), and sex was equally distributed among the 2 groups (P = .88, Mann-Whitney U test).

### Clonal Structure of stx2a-harboring EHEC O26:H11/H−

All but 2 strains (ST396 and ST1566) belonged to 3 PFGE-defined clusters (Figure 1). Cluster A was comprised of all 16 ST21 strains and the single ST591 isolate, with a 78% total similarity (Figure 1). ST29 strains belonged to clusters B (n = 28) and C (n = 13). The similarity between and within clusters B and C was 80%, and 84% and 86%, respectively (Figure 1). Thus, strains of ST29 (the new clone) were more closely related to each other than were strains of the other STs. Intriguingly, within each of clusters B and C there were strains with closely related (similarity >95%) or even identical PFGE patterns despite their recovery in different years and/or in different countries (Figure 1). The PFGE data suggest the clonal origin of ST29 strains and demonstrate that the new

### Table 3. Phylogeny of Enterohemorrhagic Escherichia coli O26:H11/H− Strains With Different stx Genotypes Based on Multilocus Sequence Typing

<table>
<thead>
<tr>
<th>stx Genotype</th>
<th>ST21</th>
<th>ST29</th>
<th>ST396</th>
<th>ST591</th>
<th>ST1565</th>
<th>ST1566</th>
<th>ST1705</th>
<th>Strains Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1a</td>
<td>105</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>107</td>
</tr>
<tr>
<td>stx2a</td>
<td>67</td>
<td>69</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>139</td>
</tr>
<tr>
<td>stx1a + stx2a</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Strains total</td>
<td>198</td>
<td>69</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>272</td>
</tr>
</tbody>
</table>

Abbreviations: ST, sequence type; stx, Shiga toxin–encoding gene.

a Allelic profiles of the different STs are shown in Supplementary Table 2.

b Single locus variant of ST29.

c Single locus variant of ST21.

### Table 4. Plasmid Gene Profiles of Enterohemorrhagic Escherichia coli O26:H11/H− of the stx2a Genotype Belonging to Different Sequence Types

<table>
<thead>
<tr>
<th>Plasmid Gene Combination (Gene Order EHEC-hlyA/katP/espP/etpD)</th>
<th>ST21</th>
<th>ST29</th>
<th>ST396</th>
<th>ST591</th>
<th>ST1566</th>
<th>Strains Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+/+/+ +/+/+/+</td>
<td>0</td>
<td>52</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>+/+/+/-</td>
<td>65</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>+/-/+/-</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>+/+/-/+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>+/+/-/-</td>
<td>0</td>
<td>56</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>139</td>
</tr>
</tbody>
</table>

Abbreviations: +, gene present; –, gene absent; EHEC, enterohemorrhagic Escherichia coli; ST, sequence type.

a Presence of the plasmid genes EHEC-hlyA, katP, espP, and etpD (encoding EHEC hemolysin, catalase-peroxidase, serine protease EspP, and type II secretion system, respectively) was determined using polymerase chain reaction.

b Plasmid gene profile typical for the new EHEC O26 German clone [22].
Figure 1. Dendrogram of XbaI pulsed-field gel electrophoresis (PFGE) band patterns of 60 enterohemorrhagic Escherichia coli O26 isolates harboring stx2a only and belonging to different sequence types (STs). The isolates are representatives of strains from all 7 countries and the entire study period 1996 to 2012. ST21 and ST29 strains share 6 of 7 multilocus sequence typing loci; the other STs are single locus variants of either ST21 or ST29 (Supplementary Table 2). The dendrogram was created by unweighted pair group method with arithmetic means in BioNumerics software 6.5 (Dice similarity coefficient; positional tolerance settings were as follows: optimization 1%; tolerance 1%; H>0%; S>0%); to design patterns, any discernible band difference was considered according to the criteria of PulseNet. The assigned clusters (A, B, and C) are indicated by brackets. The degree of similarity (%) is shown on the scale at the top left of the figure. The following strains had identical PFGE patterns (country and year of isolation in parentheses): E-10-202 (Germany, 2010) and ED411/99 (Italy, 1999); 1530/99, 2160/99 (Germany, 1999) and EH281/03 (Austria, 2003); 2983/00 and 05-02232 (Germany, 2000 and 2005, respectively); 5244/00-1 (Germany, 2000) and ED733/10 (Italy, 2010); LB235794/12 and LB234952-2/11 (Germany, 2012 and 2011, respectively). Abbreviations: CR, Czech Republic; G, Germany; PFGE, pulsed-field gel electrophoresis; ST, sequence type.
EHEC O26 clone is highly conserved, in accordance with its recent emergence [22].

**Geographic and Temporal Distribution of EHEC O26 ST29 Strains**

EHEC O26 ST29 strains were recovered in 6 of the 7 countries (Table 5), and in all but 1 study years (Figure 2). There were considerable interannual variations in the relative proportion of ST29 strains among all stx2a-harboring EHEC O26 (Figure 2A) and in spatial distribution of such isolates (Figure 2B). Whereas in Germany ST29 strains were isolated during the entire study period (except 2001), their isolation in the other countries was intermittent (Figure 2B).

**Antibiotic Susceptibilities of stx2a-harboring EHEC O26 of Different STs**

Twenty-three ST29 strains and 11 strains of the other STs selected to span the entire study region and period were tested for their susceptibility to 14 antimicrobials. Twenty ST29, 7 ST21, and 1 each ST396, ST591, and ST1566 strains were susceptible to all tested antimicrobials. One ST21 strain was resistant to ampicillin, cefuroxime, cefpodoxime, tazobactam/piperacillin, and trimethoprim/sulfamethoxazole and had intermediate susceptibility to cefotaxime. The 3 remaining ST29 strains were resistant to ampicillin (n = 2; one of them had intermediate susceptibility to tigecycline and ciprofloxacin) or to amikacin (n = 1). Hence, there was no correlation between STs and antimicrobial resistance patterns.

**DISCUSSION**

EHEC O26:H11/H" are globally emerging human pathogens. They accounted for 5%–7% of all human EHEC isolates and for 15%–19% of HUS-associated EHEC isolates reported to the European Surveillance System of the European Centre for Disease Prevention and Control between 2008 and 2010, following in frequency EHEC O157 [30, 31]. In the countries participating in this study, EHEC O26 accounted for 16%–55% of EHEC isolates from HUS patients between 2008 and 2012, being in some countries and years the most common cause of pediatric HUS [32] (authors’ unpublished data). We now demonstrate that EHEC O26 strains harboring stx2a as the sole stx have a significant potential to cause HUS, and those harboring both stx1a and stx2a have equal potential to cause HUS and diarrhea without HUS, based on analysis of the available clinical isolates. The strong association of the stx2a genotype in the EHEC O26 infecting strains with the progression of the infection to HUS is in accordance with data from other EHEC series [3, 5, 8, 11].

This study extends our current knowledge about this serotype by demonstrating that stx2a-harboring EHEC O26 are heterogeneous, consisting of 2 major phylogenetically distinct subgroups (ST21 and ST29), each of which is associated with a particular plasmid gene profile. ST29 strains represent a highly virulent clone of EHEC O26:H11/H" which emerged in Germany in the mid-1990s [22], and appear to have spread throughout Europe. However, we do acknowledge that this analysis depends on strain recovery practices, and cannot be considered to be a population-based survey. We wish to note that stx2a-harboring EHEC O26 of ST21 and ST29 do not substantially differ in their association with HUS (Table 2). Thus, the presence of stx2a rather than ST of the EHEC O26 isolate is a predictor for HUS development in infected individuals.

Why is stx2a-harboring EHEC O26 a successful clone? We speculate that the ability of EHEC O26 of the new clone to lose the stx2a-harboring bacteriophage [33] might contribute to its evolutionary success. The lysogenic state is potentially suicidal because it makes the bacterium prone to lysis by stimuli present in its environment, including the human digestive system. Therefore, the absence of the stx-phage might not only increase the adaptability of the pathogens outside the host, but also enable them to avoid lysis in the human gut. Such stx-negative EHEC O26 variants then represent suitable targets for lysogenization by stx-harboring phages released from other EHEC during infection, which could facilitate emergence of highly virulent EHEC O26. Thus, interconversion between EHEC O26 and their stx-negative variants, mediated by recycling stx2a genes via loss and gain of bacteriophages [33, 34], might abet the emergence of highly pathogenic new clones of EHEC O26. Although a similar scenario has been proposed for the emergence of the EHEC O104:H4 2011 outbreak strain [35], the new EHEC O26 clone and the O104:H4 outbreak strain are clearly distinct pathogens, which share the ability to cause HUS, albeit in different age groups (as shown in the current study) [35].
The distinct virulence potentials of EHEC O26 harboring stx1a and stx2a, respectively, offer clinical opportunities. In particular, rapid stx genotyping of EHEC O26 isolates from patients with diarrhea might define risk of progression of the disease to HUS and provide a rationale for further management of patients infected with stx2a-harboring EHEC O26 similarly, as is recommended for patients infected with EHEC O157:H7 [1, 36, 37]. However, because EHEC O26 harboring stx1a or stx1a and stx2a can also cause HUS (Table 2) [5, 7, 17, 22, 25], individual analysis of each particular case based on clinical parameters is necessary for a final decision about the therapeutic approach. Also, the possibility exists that ex vivo or in-host loss of the stx2a gene [33, 34] could inappropriately cause an isolate to be categorized as low risk. In this context we wish to note that although we analyzed typical stx-harboring EHEC O26, this fact is unlikely to bias our results toward an increased significance of the new clone, because the stx2a loss, which we observed in our previous studies [33, 34] occurred in EHEC O26 of ST29. Hence, the emerging role of the new clone in HUS might be rather underestimated in our

Figure 2. Temporal and geographic distribution of stx2a-harboring enterohemorrhagic Escherichia coli (EHEC) O26 belonging to sequence type (ST) 29 (new clone) and other STs (ST21, ST398, ST591, ST1566) in 7 European countries during 1996 to 2012. A, Relative proportions of ST29 strains among all stx2a-harboring EHEC O26 isolates from the 7 countries in different years. B, Numbers of ST29 strains (new clone) per year and country. Abbreviation: ST, sequence type.
EHEC O26 harboring stx (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit infection prevention practices.

Although therapeutic strategies should primarily rely on the presence of the stx2 genotype as a general risk factor for HUS development, the identification of an EHEC O26 isolate as a member of the new clone is urgent for infection control measures. For this purpose, MLST to determine ST29 is required, in addition to stx subtyping. To accelerate the diagnosis of EHEC O26 ST29, we are developing a real-time multiplex PCR for a rapid and specific detection of this pathogen. Importantly, because ST29 strains have no typical antibiotic resistance pattern, this characteristic cannot be used to identify the new clone. It should be kept in mind that although most EHEC O26 isolates in this study were susceptible to a broad spectrum of antimicrobials, these agents are generally not recommended for treatment of patients with confirmed or suspected EHEC infections because there is no credible evidence that antimicrobials avert HUS, and there are considerable data in support of these agents increasing the risk of this adverse outcome [37].

Interannual variation in recovery of strains of the new clone might reflect varying exposure of the population to a source/reservoir of these organisms. Cattle, a major reservoir of EHEC, are also reservoirs for EHEC O26 [25, 38, 39], but EHEC O26 harboring stx2a only and/or belonging to ST29 have only rarely been isolated from cattle [23, 25, 39]. Moreover, such strains have only rarely been identified in food [23], making the risk analysis for acquiring infections caused by stx2a-harboring EHEC O26 difficult, although some limited data do suggest a bovine source [23]. Improved knowledge about the source of these infections, requiring a close collaboration between public health agencies, veterinary microbiologists, and food safety authorities should facilitate specific infection prevention practices.

In conclusion, in this largest study analyzing the role of EHEC O26 as a human pathogen, we identified the new EHEC O26 clone first found in Germany in the 1990s as an emerging cause of HUS in Europe. These data should prompt efforts to identify reservoirs and sources of these pathogens and to implement rapid diagnostic approaches for their detection.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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