JOURNAL OF CLINICAL MICROBIOLOGY, June 2001, p. 2043–2049 Vol. 39, No. 6
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.6.2043–2049.2001

MINIREVIEW

Sorbitol-Fermenting Shiga Toxin-Producing *Escherichia coli* O157:H$^-$ Strains: Epidemiology, Phenotypic and Molecular Characteristics, and Microbiological Diagnosis

HELGE KARCH[1]* AND MARTINA BIELASZEWSKA[1,2]

Institute for Hygiene and Microbiology, University of Würzburg, 97080 Würzburg, Germany,[1] and Institute for Medical Microbiology, The 2nd Medical Faculty, Charles University, 150 06 Prague, Czech Republic[2]

The significance of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H7 as the major cause of hemorrhagic colitis and the hemolytic-uremic syndrome (HUS) worldwide has been well established (for reviews, see references 21, 31, 41, and 60). The recognition of this pathogen has been facilitated by the availability of classical microbiological diagnostic procedures that are based on the characteristic phenotypic feature of this pathogen, in particular, its inability to ferment sorbitol after overnight incubation (40). However, in addition to *E. coli* O157:H7, STEC strains of serotype O157:H$^-$ (nonmotile) which do ferment sorbitol rapidly have emerged as important causes of human diseases in continental Europe during the past decade (7, 8, 9–12, 16, 22, 27, 35, 39). Such strains are missed by diagnostic procedures recommended for the detection of *E. coli* O157:H7, and their significance in other parts of the world might thus be underestimated. This review summarizes the current knowledge on the significance of sorbitol-fermenting (SF) STEC O157:H$^-$ strains as causes of human diseases, the epidemiology of the infection, phenotypic and molecular characteristics of these pathogens, and strategies that are available for their microbiological diagnosis.

SF STEC O157:H$^-$ STRAINS AS HUMAN PATHOGENS

SF STEC O157:H$^-$ was first recognized in 1988 during an outbreak of HUS in Bavaria, Germany (26). During this outbreak, *E. coli* O157 strains that harbored the stx_2 gene but, in contrast to STEC O157:H7, were nonmotile and fermented sorbitol within 24 h of incubation were isolated from stools of two of six affected children by using molecular methods (26). The finding of these atypical STEC O157 pathogens in HUS patients resulted in further efforts to determine their significance as causes of human diseases.

In a 3-year (1988 to 1991) prospective controlled study that investigated the role of SF STEC O157:H$^-$ in the etiology of sporadic cases of pediatric HUS and diarrhea in Germany, these pathogens were isolated from 14 (13.5%) of 104 HUS patients and from 3 (0.45%) of 668 hospitalized patients with diarrhea (22). Several studies performed during ensuing years (10–12, 29; H. Karch, unpublished data) identified SF STEC O157:H$^-$ strains in 3.2 to 17.7% of HUS patients and in 0.4 to 1.5% of patients with diarrhea (Table 1). The relative frequency of SF STEC O157:H$^-$ isolates among all STEC O157 strains isolated in these studies ranged from 13.3 to 40.5% in HUS patients and from 7.4 to 25% in patients with diarrhea (Table 1).

In the winter of 1995 to 1996, a second, and to date the largest, outbreak of infection caused by SF STEC O157:H$^-$ occurred in Bavaria, Germany (4). Twenty-eight HUS cases in children, three of them fatal, were attributable to this organism. The total number of affected persons could not be determined, but an additional 300 to 600 undetected cases of diarrhea were estimated (4) because HUS develops in only 5 to 10% of persons with symptomatic STEC O157 infection (21, 41).

SF STEC O157:H$^-$ strains were first isolated outside of Germany in 1995 (7). Both of the pediatric HUS patients from which these strains were recovered lived in northern Bohemia, in a region of the Czech Republic that borders Germany. The lack of an epidemiological association of the Czech HUS patients with Germany (7) made domestic origin of the infection very likely and suggested the ability of SF STEC O157:H$^-$ strains to spread. Accordingly, additional reports of the isolation of such strains from patients with diarrhea or HUS followed during the next few years from Hungary (16), Finland (35), another region of the Czech Republic (8), and Austria (3). However, no reports of the isolation of SF STEC O157:H$^-$ outside continental Europe have been published to date.

EPIDEMIOLOGY OF INFECTIONS CAUSED BY SF STEC O157:H$^-$

Despite the increasing significance of SF STEC O157:H$^-$ in the etiology of HUS and diarrhea in Europe, the epidemiology of this infection is poorly understood. However, the limited data available till now suggest that the epidemiology of infections caused by SF STEC O157:H$^-$ differs in some aspects from the epidemiology of infections caused by STEC O157:H7 (Table 2). In particular, the predominance of SF STEC O157:H$^-$ infections during cold months (4, 7, 8, 26) and in children younger than 3 years (67; Karch, unpublished) may point to differences in the reservoir(s) and/or the vehicle(s) of these infections in comparison to those caused by STEC O157:H7.

* Corresponding author. Present address: Institute for Hygiene, University of Münster, Robert-Koch-Strasse 41, D-48149 Münster, Germany. Phone: 49 251 8355361. Fax: 49 251 8355341. E-mail: hkarch@uni-muenster.de.

TABLE 1. Prevalence of SF STEC O157:H$^-$ isolates in patients with sporadic cases of HUS and sporadic cases of diarrhea in Germany from 1994 to 2000

Year[a]	Patients with HUS[b]				Patients with diarrhea[e]				Reference or source
	No. of patients	No. of STEC O157 isolates		Isolation rate of SF STEC O157 from patients[d]	No. of patients	No. of STEC O157 isolates		Isolation rate of SF STEC O157 from patients[d]	
		Total	SF (%)[c]			Total	SF (%)[c]		
1994–1995	347	69	11 (15.9)	11/347 (3.2)	943	19	4 (21.0)	4/943 (0.4)	10, 29
1996	96	42	17 (40.5)	17/96 (17.7)	NA[f]	33	7 (21.2)	NA	11
1997	101	45	6 (13.3)	6/101 (5.9)	NA	27	2 (7.4)	NA	12
1998	91	30	6 (20.0)	6/91 (6.6)	389	12	2 (16.7)	2/389 (0.5)	Karch, unpublished
1999	82	27	5 (18.5)	5/82 (6.1)	202	12	3 (25.0)	3/202 (1.5)	Karch, unpublished
2000	140	34	5 (14.7)	5/140 (3.6)	347	9	2 (22.2)	2/347 (0.6)	Karch, unpublished

[a] The data from 1994 to 1997 are from studies performed in collaboration by the Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany, the National Reference Center for *Salmonellae* and Other Bacterial Enteric Pathogens, Hamburg, Germany, and the Robert Koch Institute, Wernigerode, Germany. The data from 1998 to 2000 were obtained from the Institute for Hygiene and Microbiology, University of Würzburg.
[b] Children under 15 years of age hospitalized for HUS at different pediatric centers throughout Germany.
[c] The percentage values in parentheses represent the relative frequencies of SF STEC O157:H$^-$ isolates among all STEC O157 isolates from the respective years.
[d] Number of SF STEC O157:H$^-$ isolates per number of patients (%).
[e] The majority of patients with diarrhea were hospitalized children under 16 years of age who suffered from painful nonbloody diarrhea.
[f] NA, data not available.

Indeed, whereas cattle have been well established as a major reservoir of STEC O157:H7 (21, 31, 41, 69), a single SF STEC O157:H$^-$ strain has been isolated to date from a cow (8), although more than 1,300 samples of bovine feces have been investigated for these pathogens in Germany and the Czech Republic (27; M. Bielaszewska, unpublished data). Moreover, except for a single isolation of a SF STEC O157:H$^-$ strain from a pony (54), SF STEC O157:H$^-$ could not be isolated from other domestic or wild animals, including sheep, goats, and deer (Bielaszewska, unpublished), which have been identified as reservoirs for STEC O157:H7 (6, 34, 37) (Table 2). While these observations suggest that cattle and perhaps other animals can be reservoirs of SF STEC O157:H$^-$ strains, the rare isolation of these pathogens from animals led to the assumption that SF STEC O157:H$^-$ strains might be adapted to the human intestine and that humans could be their major reservoirs (32), similar to the situation known for enteropathogenic *E. coli* (38). Further investigation is necessary to confirm this hypothesis.

Vehicles and routes of transmission of SF STEC O157:H$^-$ infection remain unknown in most cases. However, two of the three principal routes of transmission of STEC O157:H7 infection have been also identified for SF STEC O157:H$^-$ (Table 2). The large German HUS outbreak in 1995 to 1996 probably had a food-borne origin; two sausages, including mortadella and teewurst, which contains raw beef (4), were identified in a case control study as probable sources of SF STEC O157:H$^-$ infection (4). Direct contact with animals, including a cow (8) and a pony (54) that shed SF STEC O157:H$^-$ strains in their feces, was the most likely route of transmission of the infection to patients in sporadic cases of HUS and diarrhea reported from the Czech Republic (8) and Germany (54). Although the transmission through direct animal contact suggests that the infectious dose for SF STEC O157:H$^-$ might be very low, similar to that for STEC O157:H7 (64) (Table 2), this remains to be determined. Also, the role of person-to-person transmission, which is assumed to be the major route of spreading STEC O157:H$^-$ infection provided that humans are reservoirs of these pathogens, needs to be established.

PHENOTYPIC AND VIRULENCE CHARACTERISTICS OF SF STEC O157:H$^-$ STRAINS

Since SF STEC O157 strains are nonmotile, their H antigens cannot be assessed by serotyping. Therefore, analysis of the flagellin subunit-encoding (*fliC*) genes of representative German and Czech SF STEC O157:H$^-$ isolates was conducted (8) using the restriction fragment length polymorphism (RFLP) method described by Fields et al. (19). This approach demonstrated that all investigated SF STEC O157:H$^-$ strains pos-

TABLE 2. Comparison of the epidemiology of infections caused by SF STEC O157:H$^-$ and STEC O157:H7

Epidemiological characteristic	SF STEC O157:H$^-$[a]	STEC O157:H7[b]
Geographic distribution	Continental Europe	Worldwide
Seasonal prevalence	September to April	June to August
Age distribution (median age of HUS patients)	Children of <3 yr (25.5 mo)	Children of >3 yr (47 mo)
Reservoirs	Cattle (a single report); pony (a single report); humans?	Cattle (well established); other domestic and wild animals (goats, sheep, horses, pigs, dogs, deers, seagulls)
Transmission	Contaminated food; animal contact; person-to-person?	Contaminated food and water; person-to-person; animal contact
Infectious dose	Not known	Very low (<50 organisms)

[a] Data on the epidemiology of SF STEC O157:H$^-$ infection are based on references 4, 7, 8, 9, 26, 32, 54, and 67, and on unpublished data of Bielaszewska and Karch.
[b] Data on the epidemiology of STEC O157:H7 infection are based on references 6, 21, 24, 31, 34, 37, 41, 50, 51, 53, 64, 65, and 67 to 69.

TABLE 3. Phenotypic and virulence characteristics of SF STEC O157:H$^-$ and STEC O157:H7

Serotype	Phenotypic characteristics[c]					Chromosomal virulence loci			Large plasmid (ca. 90 kb)	Plasmid genes[i]				
	SF/GUD	PT	Stx	EHEC Hly	Tellurite resistance[f]	*stx*	*eae*	TAI[h]		EHEC *hlyA*	*katP*	*espP*	*etp*	*sfp*
O157:H$^-$[a]	+/+	88, 23	Stx2 only	− (+)[e]	No	stx_2 only	γ	−	Yes	+	−	−	+	+
O157:H7[b]	−/−	Various, never 88 or 23	Stx1, Stx2, Stx2c[d]	+	Yes	stx_1, stx_2, stx_{2c}[g]	γ	+	Yes	+	+	+	+	−

[a] The data on phenotypic and virulence characteristics of SF STEC O157:H$^-$ are based on references 2, 4, 7, 8, 11, 15, 15a, 22, 27, 28, 35, 39, and 61.
[b] The data on phenotypic and virulence characteristics of STEC O157:H7 are based on references 1, 11 to 14, 20, 23, 25, 28, 30, 36, 39, 44, 45, 55, 57, 58, 61 to 63, 66, and 72.
[c] SF/GUD, sorbitol fermentation/β-D-glucuronidase activity; PT, phage type; Stx, Shiga toxin phenotype; EHEC Hly, production of enterohemolysin; +, positive result; −, negative result.
[d] Single toxins or combinations of them are produced by STEC O157:H7.
[e] Only 12% of SF STEC O157:H$^-$ strains isolated at the Institute for Hygiene and Microbiology, University of Würzburg, from 1996 to 2000 displayed the enterohemolytic phenotype although all these isolates contained the EHEC *hlyA* gene (Karch, unpublished).
[f] As measured by the ability to grow on cefixime-tellurite SMAC.
[g] Single *stx* genes or combinations of them are present in STEC O157:H7.
[h] +, presence of TAI; −, absence of TAI.
[i] The plasmid genes encode the following: enterohemorrhagic *E. coli* hemolysin (EHEC *hlyA*), catalase-peroxidase (*katP*), serine protease (*espP*), type II secretion pathway system (*etp*), fimbrial gene cluster (*sfp*). +, presence of the gene; −, absence of the gene.

sessed the *fliC* gene encoding H7 antigen (8). A nucleotide sequence analysis of the *fliC* gene of a SF STEC O157:H$^-$ strain performed in another study (48) showed that the SF STEC O157 *fliC* gene accumulated multiple mutations, presumably as a result of silencing of flagellin expression (48). Moreover, two insertions have been identified in the 5′ conserved region of the SF STEC O157 *fliC* gene that produce a shift in the reading frame, thus introducing a premature stop codon (48); this probably forms the molecular basis of the nonmotility of such strains.

The SF STEC O157:H$^-$ strains investigated to date have identical phenotypic and virulence characteristics, which are compared with those of STEC O157:H7 in Table 3. In contrast to STEC O157:H7 strains, SF STEC O157:H$^-$ strains ferment sorbitol within 24 h of incubation and exhibit β-D-glucuronidase activity (2–4, 7, 8, 22, 35). Phage typing using the *E. coli* O157:H7 phage typing scheme (1, 36) demonstrated that SF STEC O157:H$^-$ strains belong predominantly to phage type 88 (4, 7, 8, 39) and, to a lesser extent, to closely related phage type 23 (39), which have not been found among STEC O157:H7 strains (39).

Although their virulence characteristics are similar overall to those of STEC O157:H7 strains, SF STEC O157:H$^-$ strains possess a specific combination of virulence traits. This includes stx_2 as the sole *stx* gene, *eae* encoding γ-intimin, and a large, ca. 90-kb plasmid that contains the enterohemorrhagic *E. coli* (EHEC) *hlyA* and *etp*, but not *espP* and *katP*, accessory virulence genes (Table 3). stx_2 genes in SF STEC O157:H$^-$ strains were shown to be carried by *stx*-converting bacteriophages (27), as has been demonstrated for stx_1 and stx_2 of STEC O157:H7 (42, 43, 52, 71). Unlike STEC O157:H7 strains that were recently shown to harbor a pathogenicity island termed TAI (tellurite resistance- and adherence-conferring island), which carries genes encoding a novel adherence-conferring protein and tellurite resistance (61), SF STEC O157:H$^-$ strains do not contain this pathogenicity island (61) (Table 3). The absence of TAI from the genome of SF STEC O157:H$^-$ (61) provides a genetic explanation for the tellurite susceptibility of such strains (28), compared to the tellurite resistance of STEC O157:H7 (28, 72). The differences between large plasmids of SF STEC O157:H$^-$ and STEC O157:H7 include, in addition to the absence of *katP* and *espP* from the former pathogens, different expression of the EHEC *hlyA* gene. This gene is regularly expressed in STEC O157:H7 (5, 11), giving rise to a typical enterohemolytic phenotype on blood agar plates containing washed red blood cells and Ca^{2+} ions (enterohemolysin agar) (5, 57). However, the EHEC *hlyA* gene is not expressed in the majority of SF STEC O157:H$^-$ strains that are thus usually nonhemolytic on enterohemolysin agar (4, 7, 8, 11, 35; Karch, unpublished) (Table 3). Moreover, a new gene cluster, *sfp* (sorbitol-fermenting EHEC O157 fimbriae, plasmid encoded), which mediates mannose-resistant hemagglutination and the expression of a novel type of fimbriae, has been recently identified on the large plasmid of SF STEC O157:H$^-$ (15a) (Table 3); *sfp* is a unique characteristic of SF STEC O157:H$^-$ that was not found in STEC O157:H7, other STEC strains, or other members of the family *Enterobacteriaceae* (15a).

CLONAL ORIGIN AND EVOLUTIONARY ASPECTS OF SF STEC O157:H$^-$

To investigate clonal relationships between SF STEC O157:H$^-$ strains from the 1988 to 1991 prospective German study (22), 21 isolates were analyzed using pulsed-field gel electrophoresis (PFGE) (27). All 21 SF STEC O157:H$^-$ strains had identical or closely related *Xba*I patterns that differed markedly from those of STEC O157:H7, non-sorbitol-fermenting (NSF) STEC O157:H$^-$, and SF Stx-negative *E. coli* O157:H45 strains (27). These PFGE results, combined with the specific phenotypic features and virulence profiles of such strains (22, 27) (Table 3), led to the conclusion that SF STEC O157:H$^-$ strains represent a new, distinct clone within the *E. coli* O157 serogroup (27). This was confirmed in a recent study in Germany (39) in which clonal relationships of 210 STEC O157 isolates including 40 SF STEC O157:H$^-$ strains isolated during 1988 to 1998 were investigated by PFGE and *P* gene typing (number and genomic position of lambdoid bacteriophages) (17). This analysis demonstrated (39) that in contrast to the genomic diversity observed among STEC O157:H7 and

NSF STEC O157:H$^-$ strains, all 40 SF STEC O157:H$^-$ isolates have a unique PFGE pattern, which was not seen among NSF STEC O157 strains, and two closely related *P* gene profiles. Moreover, PFGE analysis of SF STEC O157:H$^-$ strains isolated in the Czech Republic from patients (7, 8) and a cow (8) and comparison of them with representative SF STEC O157:H$^-$ strains isolated in Germany demonstrated that all Czech and German isolates had identical or closely related PFGE patterns and were located in the same cluster (8). This suggests that the SF STEC O157:H$^-$ strains isolated in the Czech Republic belong to the SF STEC O157:H$^-$ clone that is widespread in Germany.

Recently, the evolutionary relationship of the SF STEC O157:H$^-$ clone to the STEC O157:H7 clone complex has been investigated. Feng et al. (18) used multilocus enzyme electrophoresis to assess the genetic relatedness of a variety of STEC O157 strains, including German SF STEC O157:H$^-$ isolates. Their analysis demonstrated that the STEC O157 strains comprise a cluster of five closely related electrophoretic types (ET) that differ from one another by only one or two enzyme alleles. The SF STEC O157:H$^-$ strains belonged to ET4 and were the most divergent lineage of the O157:H7 complex, differing by two enzyme alleles from the common *E. coli* O157:H7 ET (18). In a stepwise evolution model of STEC O157 proposed by Feng et al. (18), both STEC O157:H7 and SF STEC O157:H$^-$ were derived from a common EPEC-like O55:H7 ancestor that carried the pathogenicity island LEE (locus of enterocyte effacement) and acquired during the evolution the stx_2 gene, a large plasmid, and the *rfb* region encoding O157 antigen (18). In the model, the SF STEC O157:H$^-$ clone, however, evolved from an early diverging branch of the O157:H7 clone complex, along which the bacteria lost motility but retained the ancestral ability to ferment sorbitol and to express β-D-glucuronidase activity (18). In accordance with the findings by Feng et al. (18), analysis of a variety of STEC O157 and non-O157 strains from different origins based on multilocus enzyme electrophoresis (70) placed SF STEC O157:H$^-$, STEC O157:H7, and *E. coli* O55:H7 in the same clonal group (EHEC 1), which was only distantly related to other STEC strains. These results are also consistent with a molecular phylogenetic analysis derived from multilocus sequencing of seven housekeeping genes (49).

MICROBIOLOGICAL DETECTION OF SF STEC O157:H$^-$ STRAINS

SF STEC O157:H$^-$ strains cannot be distinguished from commensal *E. coli* on sorbitol MacConkey agar (SMAC) (40) and are thus missed in laboratories that use SMAC as the only procedure for the detection of STEC O157. To screen for and isolate SF STEC O157:H$^-$ strains in addition to STEC O157:H7, culture on SMAC must be combined with approaches which target two important characteristics shared by SF STEC O157:H$^-$ strains, namely, the stx_2 gene and/or Stx2 production and the O157 lipopolysaccharide (LPS). Accordingly, the diagnostic protocol that has been successfully used to detect SF STEC O157:H$^-$ in patients in Germany includes a selective stool enrichment by the immunomagnetic separation (IMS) procedure followed by plating magnetic beads with attached O157 bacteria on SMAC (28). The primary stool cultures are then screened for the presence of stx_2-containing bacteria by PCR (22). To identify SF STEC O157 strains in PCR-positive stool cultures, colony hybridization of 100 to 200 well-separated colonies is performed using a digoxigenin-labeled stx_2 probe (56). Alternatively, Stx2-producing colonies can be identified in the mixed cultures by colony immunoblot using a specific antibody (31). The SF colonies that contain stx_2 and/or produce Stx2 need to be confirmed as *E. coli* O157 using standard biochemical tests (2) and agglutination with anti-O157 serum (22). A diagnostic approach used to detect SF STEC O157 infection in the Czech Republic (7, 8) combines a direct culture or the IMS-enriched culture on SMAC (28) with the screening for the O157 antigen in stool using a commercial enzyme-linked immunosorbent assay (46). If the latter test suggests *E. coli* O157 infection but no NSF colonies are present on SMAC, detection of SF STEC O157 colonies is performed by a slide agglutination assay with O157 antiserum followed by the detection of Stx2 using a commercial latex agglutination test (33).

A substantial limitation in microbiological diagnosis of SF STEC O157:H$^-$ infection results from the fact that these strains are susceptible to tellurite (28) and cannot be isolated on cefixime-tellurite SMAC agar (28), which is an appropriate selective medium for STEC O157:H7 (28, 72). Moreover, the absence of the enterohemolytic phenotype from most SF STEC O157:H$^-$ strains (4, 7, 8, 11, 35) (Table 3) makes impossible the detection of such strains on enterohemolysin agar. Thus, microbiological diagnosis of SF STEC O157:H$^-$ infection is difficult at present and requires laborious and time-consuming methods. This, combined with the emergence of these pathogens as important causes of serious human diseases in continental Europe, accentuates the need to develop a selective medium for the isolation of SF STEC O157 strains. Before such a medium is available, increased diagnostic efforts to isolate SF STEC O157 strains optimally by combining the IMS selective enrichment with methods detecting stx_2 and/or Stx2 are warranted in patients with HUS and patients with diarrhea who have evidence of *E. coli* O157 infection (e.g., the presence of the O157 antigen in stool and/or the presence of immunoglobulin M [IgM] anti-O157 LPS antibodies [9] but have no NSF colonies in their stool cultures.

SHIGA TOXIN-NEGATIVE SF *E. COLI* O157:H$^-$ STRAINS ISOLATED FROM PATIENTS

Recently, SF *E. coli* strains of serotype O157:H$^-$ that did not contain *stx* genes were reported from our laboratory (59). These isolates originated from five epidemiologically unrelated patients who suffered from HUS ($n = 2$) or from diarrhea ($n = 3$). Similar to SF STEC O157:H$^-$ strains, all *stx*-negative isolates harbored the *fliC* gene encoding H7 antigen, the *eae* gene encoding γ-intimin, and plasmid genes including the EHEC *hlyA* and *etp* genes. Random amplified polymorphic DNA-PCR analysis demonstrated that all *stx*-negative SF *E. coli* O157:H$^-$ isolates belonged to the same genetic cluster and were closely related to SF STEC O157:H$^-$ strains. Both HUS patients had anti-O157 IgM antibodies supporting the etiological role of the isolates in the underlying disease. However, one of the HUS patients was coinfected with STEC O103:H2 (59). Between 1994 and 2000, a total of nine *stx*-negative SF *E. coli* O157:H$^-$ strains were isolated in our laboratory, five of them

from HUS patients and four from patients with diarrhea (Karch, unpublished). During this 7-year period, *stx*-negative SF *E. coli* O157:H$^-$ accounted for 9.1 and 16.7% of SF *E. coli* O157:H$^-$ isolates from patients with HUS and patients with diarrhea, respectively, and for 2 and 3.4% of all *E. coli* O157 isolates from the respective patients (Karch, unpublished).

Moreover, an additional six *stx*-negative SF *E. coli* O157:H$^-$ strains were isolated during a family outbreak in Austria (3). Five of these isolates were undistinguishable by their PFGE patterns, phage types, and *P* gene profiles from the SF STEC O157:H$^-$ clone that causes human disease in Germany. Two of the family members from whom these strains were isolated, including children aged 10 months and 2 years, suffered from severe watery diarrhea for 30 and 10 days, respectively; three adults were asymptomatic. Stools from all family members were negative for obligatory bacterial enteric pathogens, rotaviruses, and parasites. The only serum sample tested during this outbreak was obtained from one of the asymptomatic persons and contained IgM antibodies against O157 LPS (3), as observed previously in German HUS patients infected with *stx*-negative SF *E. coli* O157:H$^-$ strains (59).

The origin of *stx*-negative SF *E. coli* O157:H$^-$ strains, their role in human disease, and their pathogenic mechanism(s) are not understood at present. Such strains could arise from original infecting SF STEC O157:H$^-$ organisms by the loss of their *stx* genes during infection, isolation, or subculture. Alternatively, the *stx*-negative SF *E. coli* O157:H$^-$ strains might be progenitors of SF STEC O157:H$^-$ that could, in the future, become STEC by transduction with *stx*-converting bacteriophages. If the strains isolated in Germany and Austria were inherently *stx* negative and indeed caused the underlying diseases including HUS, they might possess an additional, as-yet-unidentified, virulence factor(s) that could contribute to the pathogenesis of such diseases. Moreover, the role of intimin in the development of watery diarrhea upon infection with *stx*-negative but *eae*-positive SF *E. coli* O157:H$^-$ strains has been proposed (3). Important from the diagnostic point of view is the fact that the *stx*-negative SF *E. coli* O157:H$^-$ strains would be overlooked in patients' stools not only on SMAC but also when diagnostic protocols that rely on the detection of *stx* genes or Stx production were used. Their isolation requires *stx*-independent recovery techniques such as the detection of the *eae* gene (59) and/or the detection of the O157 antigen (3).

FUTURE PERSPECTIVES

As a prerequisite for investigating the significance of SF STEC O157:H$^-$ infection in human diseases worldwide and for a better understanding of the epidemiology of this infection, microbiological detection of these pathogens must be improved. Optimally, a combination of a selective medium for the isolation of SF STEC O157:H$^-$ and the IMS enrichment could result in a highly sensitive and specific diagnostic procedure that would allow a maximum isolation rate of these pathogens from clinical and environmental samples to be achieved. The application of such an optimized diagnostic protocol in clinical and epidemiological studies worldwide should provide information about whether SF STEC O157:H$^-$ strains are indeed limited to continental Europe or whether they are also distributed in other parts of the world where they are currently underdetected. Moreover, such a diagnostic approach would also enable the role of cattle and other animals versus humans as reservoirs of SF STEC O157:H$^-$ to be further evaluated and additional routes of transmission of this infection to be assessed. This would provide a basis for implementation of effective measures for the prevention of the human diseases caused by these pathogens.

To gain deeper insight into the clonal structure of SF STEC O157:H$^-$ strains that are closely related based on their PFGE profiles, and to subtype such strains in epidemiological studies, a typing method which could provide additional strain discrimination within the SF STEC O157:H$^-$ clonal group needs to be established.

Recently, the sequence of the whole genome of STEC O157:H7 strain EDL 933 was published (47), and the genomic sequencing of the STEC O157:H7 strain from the Japanese Sakai outbreak is in progress [K. Makino, K. Yokoyama, T. Hayashi, M. Ohnishi, M. Hattori, T. Yasunaga, K. Kurokawa, T. Honda, T. Iida, C. Sasakawa, and H. Shinagawa, Abstr. 4th Int. Symp. Workshop "Shiga toxin (Verocytotoxin)-producing *Escherichia coli* infections" 2000, Kyoto, Japan, abstr. P5-1, p. 57, 2000]. A complete sequence analysis of the SF STEC O157:H$^-$ genome, which is under way in our laboratory, will allow genomic differences between SF STEC O157:H$^-$ and STEC O157:H7 to be defined, a full spectrum of virulence characteristics of SF STEC O157:H$^-$ strains to be identified, a comparison of these virulence characteristics with those of STEC O157:H7 to be made, and the genetic, evolutionary, and phylogenetic relationships between both STEC O157 pathogens to be further elucidated.

Finally, the origin of *stx*-negative SF *E. coli* O157:H$^-$ strains which are being increasingly isolated from patients with HUS or diarrhea, their etiological role in human disease, and their pathogenic mechanism(s) need to be clarified.

ACKNOWLEDGMENTS

We thank Mohamed A. Karmali (Health Canada, Guelph, Ontario), Phillip I. Tarr (Children's Hospital and Regional Medical Center, Seattle, Wash.), Thomas S. Whittam (University of Michigan), and Werner Brunder and Herbert Schmidt (University of Würzburg) for their continuous collaborative work and helpful discussions during preparation of the manuscript.

The research on SF STEC O157 in the laboratory of Helge Karch has been supported by grants SFB 479 and Ka 717/3-1 from the Deutsche Forschungsgemeinschaft and by the Bundesministerium für Bildung und Forschung Verbundprojekt, Forschungsnetzwerk "Emerging Foodborne Pathogens in Germany," grant 01KI9903. The research of Martina Bielaszewska on SF STEC O157 strains was supported by grants IGA 2063-3 and IGA 4563-3 from the Ministry of Health of the Czech Republic.

REFERENCES

1. **Ahmed, R., C. Bopp, A. Borczyk, and S. Kasatiya.** 1987. Phage-typing scheme for *Escherichia coli* O157:H7. J. Infect. Dis. **155:**806–809.
2. **Aleksic, S., H. Karch, and J. Bockemühl.** 1992. A biotyping scheme for Shiga-like (Vero) toxin-producing *Escherichia coli* O157 and a list of serological cross-reactions between O157 and other Gram-negative bacteria. Zentbl. Bakteriol. **276:**221–230.
3. **Allerberger, F., M. P. Dierich, U. Gruber-Moesenbacher, A. Liesegang, R. Prager, G. Hartmann, W. Rabsch, H. Tschäpe, and H. Karch.** 2000. Nontoxigenic sorbitol-fermenting *Escherichia coli* O157:H$^-$ associated with a family outbreak of diarrhea. Wien. Klin. Wochenschr. **112:**846–850.
4. **Ammon, A., L. R. Peterson, and H. Karch.** 1999. A large outbreak of hemolytic uremic syndrome caused by an unusual sorbitol-fermenting strain *E. coli* O157:H$^-$. J. Infect. Dis. **179:**1274–1277.
5. **Beutin, L., M. A. Montenegro, I. Orskov, F. Orskov, J. Prada, S. Zimmer-**

mann, and R. Stephan. 1989. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J. Clin. Microbiol. **27:**2559–2564.
6. **Bielaszewska, M., J. Janda, K. Blahova, H. Minarikova, E. Jilkova, M. A. Karmali, J. Laubova, J. Sikulova, M. A. Preston, R. Khakhria, H. Karch, H. Klazarova, and O. Nyc.** 1997. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. Epidemiol. Infect. **119:**299–305.
7. **Bielaszewska, M., H. Schmidt, M. A. Karmali, R. Khakhria, J. Janda, K. Blahova, and H. Karch.** 1998. Isolation and characterization of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains in the Czech Republic. J. Clin. Microbiol. **36:**2135–2137.
8. **Bielaszewska, M., H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschäpe, A. Cizek, J. Janda, K. Blahova, and H. Karch.** 2000. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains and a source of human diseases. J. Clin. Microbiol. **38:** 3470–3473.
9. **Bitzan, M., K. Ludwig, M. Klemt, H. König, J. Buren, and D. E. Müller-Wiefel.** 1993. The role of *Escherichia coli* O157 infections in the classical (enteropathic) haemolytic uraemic syndrome: results of a Central European, multicentre study. Epidemiol. Infect. **110:**183–196.
10. **Bockemühl, J., and H. Karch.** 1996. Zur aktuellen Bedeutung der enterohämorrhagischen *Escherichia coli* (EHEC) in Deutschland (1994–1995). Bundesgesundhblatt **8:**290–296.
11. **Bockemühl, J., H. Karch, and H. Tschäpe.** 1997. Infektionen des Menschen durch enterohämorrhagische *Escherichia coli* (EHEC) in Deutschland, 1996. Bundesgesundhblatt **6:**194–197.
12. **Bockemühl, J., H. Karch, and H. Tschäpe.** 1998. Zur Situation der Infektionen des Menschen durch enterohämorrhagische *Escherichia coli* (EHEC) in Deutschland, 1997. Bundesgesundhblatt Suppl. (October):2–5.
13. **Brunder, W., H. Schmidt, and H. Karch.** 1996. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology **142:**3305–3315.
14. **Brunder, W., H. Schmidt, and H. Karch.** 1997. EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. Mol. Microbiol. **24:**767–778.
15. **Brunder, W., H. Schmidt, M. Frosch, and H. Karch.** 1999. The large plasmids of Shiga toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. Microbiology **145:**1005–1014.

15a. **Brunder, W., A. Salam Khan, J. Hacker, and H. Karch.** Novel type of fimbriae encoded by the large plasmid of sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H$^-$. Infect. Immun., in press.

16. **Caprioli, A., and A. E. Tozzi.** 1998. Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe, p. 38–48. *In* J. B. Kaper and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. American Society for Microbiology, Washington, D.C.
17. **Datz, M., C. Janetzki-Mittmann, S. Franke, F. Gunzer, H. Schmidt, and H. Karch.** 1996. Analysis of the enterohemorrhagic *Escherichia coli* O157 DNA region containing lambdoid phage gene *p* and Shiga-like toxin structural genes. Appl. Environ. Microbiol. **62:**791–797.
18. **Feng, P., K. A. Lampel, H. Karch, and T. S. Whittam.** 1998. Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. J. Infect. Dis. **177:**1750–1753.
19. **Fields, P. I., K. Blom, H. J. Hughes, L. O. Helsel, P. Feng, and B. Swaminathan.** 1997. Molecular characterization of the gene encoding H antigen in *Escherichia coli* and development of a PCR-restriction fragment length polymorphism test for identification of *E. coli* O157:H7 and O157:NM. J. Clin. Microbiol. **35:**1066–1070.
20. **Fürst, S., J. Scheef, M. Bielaszewska, H. Rüssmann, H. Schmidt, and H. Karch.** 2000. Identification and characterisation of *Escherichia coli* strains of O157 and non-O157 serogroups containing three distinct Shiga toxin genes. J. Med. Microbiol. **49:**383–386.
21. **Griffin, P. M., and R. V. Tauxe.** 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. **13:**60–98.
22. **Gunzer, F., H. Böhm, H. Rüssmann, M. Bitzan, S. Aleksic, and H. Karch.** 1992. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J. Clin. Microbiol. **30:**1807–1810.
23. **Hii, J. H., C. Gyles, T. Morooka, M. A. Karmali, R. Clarke, S. De Grandis, and J. L. Brunton.** 1991. Development of verotoxin 2- and verotoxin 2 variant (VT2v)-specific oligonucleotide probes on the basis of the nucleotide sequence of the B cistron of VT2v from *Escherichia coli* E32511 and B2F1. J. Clin. Microbiol. **29:**2704–2709.
24. **Jones, I. G., and M. Roworth.** 1996. An outbreak of *Escherichia coli* O157 and campylobacteriosis associated with contamination of a drinking water supply. Public Health **110:**277–282.
25. **Karch, H., J. Heesemann, R. Laufs, A. D. O'Brien, C. O. Tacket, and M. M. Levine.** 1987. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect. Immun. **55:**455–461.
26. **Karch, H., R. Wiss, H. Glonning, P. Emmrich, S. Aleksic, and J. Bockemühl.** 1990. Hämolytisch-urämisches Syndrom bei Kleinkindern durch Verotoxin-produzierende *Escherichia coli*. Dtsch. Med. Wochenschr. **115:**485–495.
27. **Karch, H., H. Böhm, H. Schmidt, F. Gunzer, S. Aleksic, and J. Heesemann.** 1993. Clonal structure and pathogenicity of Shiga-like toxin-producing, sorbitol-fermenting *Escherichia coli* O157:H$^-$. J. Clin. Microbiol. **31:**1200–1205.
28. **Karch, H., C. Janetzki-Mittmann, S. Aleksic, and M. Datz.** 1996. Isolation of enterohemorrhagic *Escherichia coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. J. Clin. Microbiol. **34:**516–519.
29. **Karch, H., H.-I. Huppertz, J. Bockemühl, H. Schmidt, A. Schwarzkopf, and R. Lissner.** 1997. Shiga toxin-producing *Escherichia coli* infections in Germany. J. Food Prot. **11:**1454–1457.
30. **Karch, H., H. Schmidt, and W. Brunder.** 1998. Plasmid-encoded determinants of *Escherichia coli* O157:H7, p. 183–194. *In* J. B. Kaper and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. American Society for Microbiology, Washington, D. C.
31. **Karch, H., M. Bielaszewska, M. Bitzan, and H. Schmidt.** 1999. Epidemiology and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Diagn. Microbiol. Infect. Dis. **34:**229–243.
32. **Karch, H., and B. Köhler.** 1999. Neue Erkenntnisse zur Molekularbiologie der enterohämorrhagischen *Escherichia coli* (EHEC) O157. Gesundheitswesen **61**(Suppl. 1)**:**S46–S51.
33. **Karmali, M. A., M. Petric, and M. Bielaszewska.** 1998. Evaluation of a microplate latex agglutination method (Verotox-F) for detecting and characterizing verotoxins (Shiga toxins) in *Escherichia coli*. J. Clin. Microbiol. **37:**396–399.
34. **Keene, W. E., E. Sazie, J. Kok, D. H. Rice, D. D. Hancock, V. K. Balan, T. Zhao, and M. P. Doyle.** 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. JAMA **277:**1229–1231.
35. **Keskimäki, M., M. Saari, T. Heiskanen, and A. Siitonen.** 1998. Shiga toxin-producing *Escherichia coli* in Finland from 1990 through 1997: prevalence and characteristics of isolates. J. Clin. Microbiol. **36:**3641–3646.
36. **Khakhria, R., D. Duck, and H. Lior.** 1990. Extended phage-typing scheme for *Escherichia coli* O157:H7. Epidemiol. Infect. **105:**511–520.
37. **Kudva, I. T., P. G. Hatfield, and C. J. Hovde.** 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. J. Clin. Microbiol. **34:**431–433.
38. **Levine, M. M, and R. Edelman.** 1984. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol. Rev. **6:**31–51.
39. **Liesegang, A., U. Sachse, R. Prager, H. Claus, H. Steinrück, S. Aleksic, W. Rabsch, W. Voigt, A. Fruth, H. Karch, J. Bockemühl, and H. Tschäpe.** 2000. Clonal diversity of Shiga toxin-producing *Escherichia coli* O157:H7/H$^-$ in Germany—a ten-year study. Int. J. Med. Microbiol. **290:**269–278.
40. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869–872.
41. **Mead, P. S., and P. M. Griffin.** 1998. *Escherichia coli* O157:H7. Lancet **352:**1207–1212.
42. **Newland, J. W., and R. J. Neill.** 1988. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J. Clin. Microbiol. **33:**1602–1605.
43. **O'Brien, A. D., J. W. Newland, S. F. Miller, R. K. Holmes, S. W. Smith, and S. B. Formal.** 1984. Shiga-like toxin converting bacteriophages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **226:**694–696.
44. **Ostroff, S. M., P. I. Tarr, M. A. Neill, J. H. Lewis, N. Hargrett-Bean, and J. M. Kobayashi.** 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J. Infect. Dis. **160:**994–998.
45. **Oswald, E., H. Schmidt, S. Morabito, H. Karch, O. Marches, and A. Caprioli.** 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. Infect. Immun. **68:**64–71.
46. **Park, C. H., N. M. Vandel, and D. L. Hixon.** 1996. Rapid immunoassay for detection of *Escherichia coli* O157 directly from stool specimens. J. Clin. Microbiol. **34:**988–990.
47. **Perna, N. T., G. Plunket III, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529–533.
48. **Reid, S. D., R. K. Selander, and T. S. Whittam.** 1999. Sequence diversity of flagellin (*fliC*) alleles in pathogenic *Escherichia coli*. J. Bacteriol. **181:**153–169.
49. **Reid, S. D., C. J. Herbelin, A. C. Bumbaugh, R. K. Selander, and T. S. Whittam.** 2000. Parallel evolution of virulence in pathogenic *Escherichia coli*. Nature **406:**64–67.
50. **Reida, P., M. Wolff, H. W. Pohls, W. Kuhlmann, A. Lehmacher, S. Aleksic, H. Karch, and J. Bockemühl.** 1994. An outbreak due to enterohaemorrhagic *Escherichia coli* O157:H7 in a children day care centre characterized by person-to-person transmission and environmental contamination. Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. **281:**534–543.

51. **Renwick, S. A., J. B. Wilson, R. C. Clarke, H. Lior, A. A. Borczyk, J. Spika, K. Rahn, K. McFadden, A. Brouwer, A. Copps, N. G. Anderson, D. Alves, and M. A. Karmali.** 1993. Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and a human. J. Infect. Dis. **168:**792–793.
52. **Rietra, P. J. G. M., G. A. Willshaw, H. R. Smith, A. M. Field, S. M. Scotland, and B. Rowe.** 1989. Comparison of Vero-cytotoxin-encoding phages from *Escherichia coli* of human and bovine origin. J. Gen. Microbiol. **135:**2307–2318.
53. **Rios, M., V. Prado, M. Trucksis, C. Arellano, C. Borie, M. Alexandre, A. Fica, and M. M. Levine.** 1999. Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, and food products. J. Clin. Microbiol. **37:**778–781.
54. **Robert Koch-Institut.** 1999. Fallbericht: Inkomplettes HUS bei EHEC-Enteritis, übertragen durch Minipferde. Epidemiol. Bull. **37:**276.
55. **Rüssmann, H., H. Schmidt, J. Heesemann, A. Caprioli, and H. Karch.** 1994. Variants of Shiga-like toxin II constitute a major toxin component in *Escherichia coli* O157 strains from patients with haemolytic uraemic syndrome. J. Med. Microbiol. **40:**338–343.
56. **Schmidt, H., H. Rüssmann, A. Schwarzkopf, S. Aleksic, J. Heesemann, and H. Karch.** 1994. Prevalence of attaching and effacing *Escherichia coli* in stool samples from patients and controls. Zentbl. Bakteriol. **281:**201–213.
57. **Schmidt, H., L. Beutin, and H. Karch.** 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. **63:**1055–1061.
58. **Schmidt, H., B. Henkel, and H. Karch.** 1997. A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohaemorrhagic *Escherichia coli* O157 strains. FEMS Microbiol. Lett. **148:**265–272.
59. **Schmidt, H., J. Scheef, H. I. Huppertz, M. Frosch, and H. Karch.** 1999. *Escherichia coli* O157:H7 and O157:H$^-$ strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. J. Clin. Microbiol. **37:**3491–3496.
60. **Tarr, P. I.** 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin. Infect. Dis. **20:**1–8.
61. **Tarr, P. I., S. S. Bilge, J. C. Vary, S. Jelacic, R. L. Habeeb, T. R. Ward, M. R. Baylor, and T. E. Besser.** 2000. Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect. Immun. **68:**1400–1407.
62. **Thomas, A., H. R. Smith, and B. Rowe.** 1993. Use of digoxigenin-labelled oligonucleotide DNA probes for VT2 and VT2 human variant genes to differentiate Vero cytotoxin-producing *Escherichia coli* strains of serogroup O157. J. Clin. Microbiol. **31:**1700–1703.
63. **Thompson, J. S., D. S. Hodge, and A. A. Borczyk.** 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J. Clin. Microbiol. **28:**2165–2168.
64. **Tilden, J., Jr., W. Young, A. M. McNamara, C. Custer, B. Boesel, M. A. Lambert Fair, J. Majkowski, D. Vugia, S. B. Werner, J. Hollingsworth, and J. G. Morris, Jr.** 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. Am. J. Public Health **86:**1142–1145.
65. **Trevena, W. B., R. S. Hooper, C. Wray, G. A. Willshaw, T. Cheasty, and G. Domingue.** 1996. Vero cytotoxin-producing *Escherichia coli* O157 associated with companion animals. Vet. Rec. **138:**400.
66. **Tzipori, S., H. Karch, K. I. Wachsmuth, R. M. Robins-Browne, A. D. O'Brien, H. Lior, M. L. Cohen, J. Smithers, and M. M. Levine.** 1987. Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infection caused by enterohemorrhagic *Escherichia coli* O157:H7 in gnotobiotic piglets. Infect. Immun. **55:**3117–3125.
67. **Verweyen, H. M., H. Karch, F. Allerberger, and L. B. Zimmerhackl.** 1999. Enterohemorrhagic *Escherichia coli* (EHEC) in pediatric hemolytic uremic syndrome: a prospective study in Germany and Austria. Infection **27:**1–7.
68. **Wallace, J. S., T. Cheasty, and B. Rowe.** 1997. Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. **82:**399–404.
69. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
70. **Whittam, T.** 1998. Evolution of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains, p. 195–209. *In* J. B. Kaper and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. American Society for Microbiology, Washington, D.C.
71. **Willshaw, G. A., H. R. Smith, S. M. Scotland, A. M. Field, and B. Rowe.** 1987. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J. Gen. Microbiol. **133:**1309–1317.
72. **Zadik, P. M., P. A. Chapman, and C. A. Siddons.** 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. **39:**155–158.