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SHORT REPORT
Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157: indications for an animal reservoir

D. ORTH, K. GRIF*, M. P. DIERICH AND R. WÜRZNER

Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University and Austrian Reference Laboratory for EHEC/STEC/VTEC, Innsbruck, Austria

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SUMMARY

This study investigates a sorbitol-fermenting enterohaemorrhagic *Escherichia coli* (SF EHEC) O157 infection in a farmer's family in the Austrian province of Salzburg. The investigation commenced after a 10-month-old boy was admitted to hospital with the clinical diagnosis of a haemolytic–uraemic syndrome (HUS) and his stool specimen grew SF EHEC O157:H$^-$. In a subsequent environmental survey, a stool specimen of the 2-year-old brother and faecal samples of two cattle from the family's farm were also found to be positive for SF EHEC O157:H$^-$. All four isolates had indistinguishable phenotypic and molecular characteristics and were identical to the first strain detected in Bavaria in 1988. Despite identical isolates being demonstrated in Bavaria after 1988, and until this report, increased surveillance in neighbouring Austria had not found this organism. We propose that the strain may have recently spread from Bavaria to Austria. Although SF EHEC O157:H$^-$ strains are still rare, they may represent a considerable health threat as they can spread from farm animals to humans and between humans.

Infections with enterohaemorrhagic *Escherichia coli* (EHEC) are the major cause of haemolytic–uraemic syndrome (HUS), the most common cause of acute renal failure in childhood. The increasing number of reported cases of disease and resulting deaths, the limitations in therapy, and the rates of chronic renal sequelae emphasize the danger of this pathogen for public health. Outbreaks of EHEC infections have been reported worldwide and *E. coli* O157:H7 has been the predominant bacterial strain identified [1]. The recognition of this pathogen has been facilitated by the availability of classical microbiological diagnostic procedures, which are based on the characteristic phenotypical feature of this pathogen, namely, its inability to ferment sorbitol [2].

* Author for correspondence: Dr K. Grif, Department of Hygiene, Microbiology and Social Medicine, Schoepfstrasse 41, A-6020 Innsbruck, Austria.
(Email: Katharina.Grif@i-med.ac.at)

EHEC strains of serotype O157:H$^-$ (non-motile) which do ferment sorbitol and exhibit β-glucuronidase activity have, however, also been recognized as causes of HUS; the first isolation of such strains was reported during an outbreak of HUS in Bavaria, Germany in 1988 [3].

In contrast to non-sorbitol-fermenting (NSF) EHEC O157:H7 strains, where cattle are the major reservoir, the transmission routes of sorbitol-fermenting (SF) EHEC O157:H$^-$ are still unknown. Until now, only a single SF EHEC O157:H$^-$ strain, which was associated with human disease, has in addition been isolated from a cow in the Czech Republic [4].

Here we report on a SF EHEC O157 infection in a farmer's family in the Austrian province of Salzburg in June 2003. A 10-month-old boy was admitted to the University Children's Hospital, Salzburg, with a 5-day history of fever, diarrhoea and vomiting. With acute renal failure, haemolysis and thrombocytopenia

Table. *Phenotypic and genotypic characteristics of Austrian SF EHEC O157:H7 isolates compared to the Bavarian outbreak strain 493/89*

Strain no.	Source	Serotype	Motility	Sorbitol fermentation	EHEC-Hly	stx_1	stx_2*	*eaeγ*	EHEC-*hlyA*	*etpD*	*espP*	*sfpA*	*Efa13*†	*cdt-V* ‡	CDT titre (CHO)§
58/03	Patient	O157:H$^-$	−	+	−	−	+	+	+	+	−	+	+	+	1:4
109/03	Cow	O157:H$^-$	−	+	−	−	+	+	+	+	−	+	+	+	1:4
111/03	Bull	O157:H$^-$	−	+	−	−	+	+	+	+	−	+	+	+	1:2
119/03	Brother	O157:H$^-$	−	+	−	−	+	+	+	+	−	+	+	+	1:4
493/89	Bavarian isolate	O157:H$^-$	−	+	−	−	+	+	+	+	−	+	+	+	1:4

Genes encode the following putative virulence factors: *stx*, Shiga toxin; *eae γ*, intimin *γ*; EHEC-*hlyA*, EHEC haemolysin (EHEC-Hly); *etpD*, putative type II secretion system; *espP*, serine protease; *sfpA*, plasmid-encoded fimbriae of SF EHEC O157:H$^-$; *efa*1 EHEC factor for adherence (Efa1); *cdt-V*, cytolethal distending toxin (CDT)-V.

* All strains possess the stx_2 (but not stx_{2c}) gene as determined by the *Hae*III restriction of the GK3–GK4 amplification product.

† A complete *efa1* gene was present in all strains.

‡ All three open reading frames encoding *cdt-V* (*cdtA*, *cdtB*, and *cdtC*) were identified.

§ CHO, Chinese hamster ovary cells; the CDT titres are the highest dilutions of culture filtrates of the strains that caused a typical distension in 50% of CHO cells after 5 days.

being present, a diagnosis of HUS was then made. The boy recovered and was discharged after 3 weeks with normal renal function. As part of a routine case-control study conducted at the National Reference Laboratory to identify risk factors associated with HUS and possible EHEC infection, his parents were interviewed and requested to complete a standardized questionnaire. Information regarding the clinical illness of the child, his potential exposure during the 6 days prior to the onset of diarrhoea, and demographic issues were obtained.

His parents denied consumption of potentially contaminated food by the patient. However, they stated that the 2-year-old brother regularly frequented the barn, and there was a high probability that he had direct contact with cattle. Stool samples were also collected from each of the parents and the 2-year-old brother. In addition, a 27-year-old uncle and 62-year-old grandmother who had contact with this family in the preceding 3 weeks, and who reported suffering from non-bloody diarrhoea were also screened.

Three stool specimens of the patient, taken within 2 weeks of admission, and one from the asymptomatic brother, yielded shiga toxin-positive *E. coli*, confirmed by enzyme immunoassay (Premier EHEC; Meridian, Milano, Italy [5]). These specimens were negative for other intestinal pathogens, including salmonella, campylobacter, shigella, yersinia, aeromonas, plesiomonas, rotavirus and parasites. The family residence was their cattle farm and faecal samples were collected from all 42 healthy cattle within 3 days of interview. Two specimens here yielded shiga toxin-positive *E. coli* (one bull and one calf). All isolates were *E. coli* (identified by API 20E; bioMérieux, Marcy-l'Etoile, France), O157:H$^-$ isolates (typed by latex agglutination; Wellcolex, Remel, Dartford, UK) that fermented sorbitol after overnight incubation on sorbitol MacConkey agar, and all were *β*-glucuronidase positive (tested on specific agar plates; Diagnostica Merck, Darmstadt, Germany), non-motile (using a culture in a 0·3% semi-solid agar). Using polymerase chain reaction and restriction fragment length polymorphism (performed as described previously [5], with primers detailed elsewhere [6–12]), several putative virulence genes were identified, including the flagellin subunit-encoding *fliC*, Shiga toxin 2 (stx_2), intimin *γ* (*eae γ*), a putative adhesin Efa1 (*efa1*), cytolethal distending toxin-V (*cdt-VA, cdt-VB, cdt-VC*; biological activity was investigated on Chinese hamster ovary (CHO) cells

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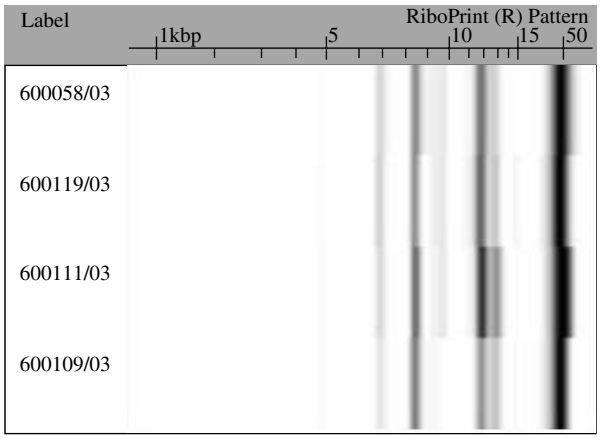

Fig. Ribotyping patterns of SF EHEC O157:H$^-$ isolates (source of strains: 600058/03 from index case; 600119/03 from brother; 600109/03 from calf; 600111/03 from bull, 600492/89 Bavarian strain).

[12]), EHEC haemolysin (EHEC-*hly*A), a serine protease (*espP*), a putative type II secretion system (*etpD*) and Sfp fimbriae (*sfpA*). The EHEC-*hlyA* gene was not expressed in any of the isolates investigated (phenotypically detected on enterohaemolysin agar [13]. Details of these virulence factors are shown in the Table.

The putative virulence characteristics of the Austrian human and cattle isolates isolated here were identical to the Bavarian outbreak prototype (493/89) of 1988 [3]. Furthermore, all were indistinguishable by pulsed-field gel electrophoresis by the CDC standardized protocol (PulseNet, CDC, Atlanta, GA, USA, using the restriction enzyme *Xba*I) and automated ribotyping (RiboPrinter® microbial characterization system; Qualicon Europe Ltd, Warwick, UK), using a mixture of restriction endonucleases *Pst*I and *Sph*I [14] (Fig.).

Since the Bavaria outbreak, several SF O157:H$^-$ isolates have been identified as the cause of sporadic cases of HUS and diarrhoea not only in Bavaria but also throughout Europe, including the Czech Republic [15], Hungary [8], Finland [13] and the United Kingdom [16]. Further, these isolates were identified as the cause of two recent large outbreaks of HUS and diarrhoea in Germany [13]. Outside Europe, only one report from Australia detailing isolation of SF EHEC O157:H$^-$ has been published [17].

SF EHEC O157:H$^-$ strains share a specific combination of their phenotypic and virulence features, which differentiates them from NSF EHEC O157:H$^-$. In addition to their ability to rapidly ferment sorbitol, SF EHEC O157:H$^-$ produce β-D-glucuronidase, and uniformly possess stx_2 as their sole *stx* gene [13]. In contrast to *E. coli* O157:H7, SF EHEC O157:H$^-$ strains possess the complete EHEC factor for adherence (*efa1*) open reading frame that is only rudimentarily present in EHEC O157:H7 [11], and most contain the gene cluster encoding a new member of the CDT family, CDT-V, which is rarely found in EHEC O157:H7 [12]. Differences are also observed between the large plasmid in NSF EHEC O157 and that in SF EHEC O157:H$^-$ strains. The large plasmid of SF EHEC O157:H$^-$ typically contains the EHEC-*hlyA* gene encoding EHEC haemolysin and the *etp* gene cluster that encodes a putative type II secretion system. However, *espP* and *katP*, which encode a serine protease and a catalase-peroxidase respectively, in EHEC O157:H7, are absent from SF EHEC O157:H$^-$. Instead of these loci, the plasmid contains a *sfp* gene cluster encoding novel pili and a haemagglutinin [10, 13].

All these characteristics were found in the two human and two cattle isolates in this study, demonstrating that they belong to the same SF EHEC O157:H$^-$ clone, widespread in Europe. Moreover, both human and cattle isolates produced high levels of Stx2, in contrast to some stx_2 variant-harbouring *E. coli* strains which express no detectable Stx, although they contain structurally intact stx_2 genes [18]. In Austria, only one case of SF EHEC O157:H$^-$ has been detected in the period from 1996 to 2002. In this case, the patient affected was a German child on vacation in the Austrian province of Salzburg in 2002.

Here we report the first documented SF EHEC O157:H$^-$ infection originating in Austria and identify cattle as a reservoir for SF EHEC O157. Despite the increasing significance of SF EHEC O157:H$^-$ strains in the aetiology of HUS in Europe [5, 13], the epidemiology of infections caused by these strains is poorly understood. Although it has been well established that cattle present a major reservoir of EHEC O157:H7 [19], only one bovine faecal sample yielded a SF EHEC O157:H$^-$ strain, out of more than 1300 samples investigated in Germany and the Czech Republic [13]. Moreover, except for a single isolation of a SF EHEC O157:H$^-$ strain from a pony [13], SF EHEC O157:H$^-$ has not been isolated from any other domestic or wild animals, including sheep, goats and deer [13]. As there are only these

two reports on the isolation of SF EHEC O157:H$^-$ strains from animals, it was hypothesized that these pathogens might be adapted to the human intestine and that humans may represent their major reservoir [13].

The large outbreak of HUS caused by SF EHEC O157:H$^-$ during 1995–1996 in Germany probably had a foodborne origin; two types of sausages, namely mortadella and teewurst, which contain raw beef, were identified in a case-control study as probable sources of SF EHEC O157:H$^-$ infection [13].

By demonstrating the clonal relatedness between the SF EHEC O157:H$^-$ strains isolated from two cattle as well as both patients described in this study, we provide additional evidence that cattle can represent a reservoir of SF EHEC O157:H$^-$ strains and be a source of infection for humans.

Although the family affected by this strain lived on a cattle farm, the patient had not had any direct contact with the farm animals. His 2-year-old brother, however, regularly frequented the barn. As the consumption of raw farm products was excluded for both children, we propose that the brother of the patient was probably infected via direct contact with farm animals and was a carrier who subsequently infected his younger brother via person-to-person contact.

The SF EHEC O157:H$^-$ infection, described here, occurred in the Austrian province of Salzburg, which borders Bavaria. All phenotypic and genotypic characteristics investigated for the strains described in the present report are identical to the strain identified in the Bavarian outbreak of SF EHEC O157:H$^-$ in 1988. Thereafter, several identical isolates were found in Bavaria, but not in neighbouring Austria, despite increased vigilance, we propose that the SF EHEC O157:H$^-$ strain recently spread from Bavaria to Austria. Similarly, the first SF EHEC O157:H$^-$ strain detected outside Germany (in the Czech Republic) was also isolated from a region bordering Bavaria [20].

In conclusion, although SF EHEC O157:H$^-$ strains are still rare, they may represent a considerable health threat to humans as they can spread both from farm animals to humans and from human to human, potentially causing life-threatening illnesses such as HUS. Furthermore, this case report highlights the need for screening for shiga toxins rather than for sorbitol-non fermenting bacteria for the optimum public health surveillance process.

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DECLARATION OF INTEREST

None.

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