Pediatr Nephrol (2000) 14:73–83

OCCASIONAL SURVEY

Hege M. Verweyen · Helge Karch · Matthias Brandis
Lothar B. Zimmerhackl

Enterohemorrhagic *Escherichia coli* infections: following transmission routes

Received: 11 September 1997 / Revised: 4 January 1999 / Accepted: 15 February 1999

Abstract Infections with enterohemorrhagic *Escherichia coli* (EHEC) are the major cause of hemolytic-uremic syndrome (HUS), the most-common cause of acute renal failure in childhood. The mortality rate of HUS (0%–5% in most recent series and 10%–30% in individual reports) and residual chronic renal sequelae (in up to 50% of patients in long-term follow-up studies) emphasize the seriousness of HUS for public health. Several studies have described possible sources of EHEC infection. However, in the majority of cases the pathogen cannot be identified in food or animals and the routes of transmission remain unclear. In this review article the hypothesized routes of transmission are summarized. The medical data bases "Medline" and "Current contents" were screened for the years January 1966 through November 1998. The difficulties in following the chain of EHEC infection are discussed. A precise evaluation of the environmental aspects of the patient is a precondition for further analysis.

Key words Enterohemorrhagic *Escherichia coli* · Hemolytic uremic syndrome · Transmission routes · Epidemiology

Introduction

Cases of infection associated with enterohemorrhagic *Escherichia coli* (EHEC) have been increasingly reported worldwide. Clinicians fear the severe manifestations of EHEC infection, such as hemorrhagic colitis (HC), hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) [1]. The incidence of typical HUS in childhood varies from country to country and over the years. Incidences have been reported to be 0.2–4.0 per 100,000 children in Scotland from 1987 to 1991 [2] and 0.2 per 100,000 in Italy from 1988 [3]. In the United States the average incidence is 0.2–3.4 per 100,000. The highest incidence was found in Argentina, with 22 per 100,000 children under 5 years in Buenos Aires [4].

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. Epidemics of EHEC infection have been reported worldwide. Serotype O157:H7 was the predominant bacterial strain identified. More than 100 other EHEC subtypes have also been isolated [5].

The reservoir and the transmission routes of EHEC have been investigated ever since 1983, when undercooked beef was found to be the source of infection [6]. Several routes of transmission of EHEC have been delineated. In this review the published data concerning reservoir and routes of transmission of EHEC infections during outbreaks or in sporadic cases are summarized. Although in several cases contaminated food has been considered the source of EHEC infection, the pathogen has rarely been isolated from food. In most cases of EHEC infection the routes of transmission remain unclear. This leads to the following questions: (1) what are the difficulties in analyzing the routes of transmission of EHEC and (2) how can research be optimized? An epidemiological research program that co-ordinates systematic clinical and laboratory evaluations in selected European countries will be discussed: the *Biomed 2 Programme PL-950970*.

Methods

A literature search was performed in the medical databases "Medline" and "Current contents" for the years January 1966 through November 1998. Using special search strategies, articles on the following topics were identified: EHEC, verocytotoxin, Shiga-like

H.M. Verweyen · M. Brandis · L.B. Zimmerhackl (✉)
Kinderklinik, Universität Freiburg, Mathildenstrasse 1,
D-79106 Freiburg, Germany
e-mail: verweyen@kkl200.ukl.uni-freiburg.de
Tel.: +49-761-270-4309, Fax: +49-761-270-4407

H. Karch
Institut für Hygienie und Mikrobiologie, Universität Würzburg,
Würzburg, Germany

toxin, O157:H7, non-O157:H7, HUS, mechanisms of toxicity, epidemiology, food-borne infection, animal reservoirs, routes of transmission. In addition, representative articles were carefully studied, reference lists were scanned, and corresponding articles reviewed for additional information.

Causative organisms

E. coli is the most-frequent facultative anaerobe bacterium present in the normal flora of the large intestine of healthy individuals. In the past 3 decades several pathogenic groups of *E. coli* have been identified by new tests based on the molecular identification of virulence factors. *E. coli* serotype O157:H7 was recognized as a novel pathogen causing intestinal and renal illness in the early 1980s [6, 7]. Since then, *E. coli* O157:H7 has been isolated during several outbreaks and from sporadic cases of severe gastrointestinal illness and HUS worldwide. The nomenclature of EHEC is not uniform. This is a consequence of parallel paths of investigation. The term EHEC was originally used for Shiga toxin (Stx)-producing *E. coli* isolates from patients with HC or HUS. In addition, EHEC express the *eae* gene and contain a large plasmid of approximately 90 kilodaltons (kDa) in size [8]. The definition has been expanded to include non-O157:H7 serotypes that have the same virulence properties as *E. coli* O157:H7. In addition, the terms Shiga-like toxin or verotoxin-producing *E. coli* are used to characterize bacteria strains that express cytotoxin irrespective of pathogenicity. More than 100 serotypes have been isolated; many may cause different symptoms and disease, i.e., some have not been shown to cause HC or HUS [5, 9]. In the group of so-called non-O157:H7 *E. coli* serotypes, O157:H-, O111:H-, O26:H11, and O103:H2 were predominantly isolated [3, 10–12]. To date no common distinctive biochemical features are associated with the great majority of EHEC serotypes. For the identification of EHEC strains three different types of methods are used.

Isolation of the pathogen from stool specimen

The agar plates most commonly used to screen for O157:H7 are sorbitol MacConkey and cefiximetellurite sorbitol MacConkey. However, sorbitol-fermenting *E. coli* O157:H- or non-O157 strains cannot be isolated on these agar plates, since they were shown to be resistant to tellurite [13]. The isolation rates for EHEC O157 have been improved by the introduction of a selective enrichment procedure using specific anti-O157 antibodies attached to paramagnetic particles, named immunomagnetic separation [14].

Detection of Stx-producing bacteria or fecal Stx

Tissue culture cytotoxicity assays with vero cells have played an important role in the detection of Stx-producing bacteria. Konowalchuk et al. [15] were the first to report that Stx induces cell damage in vero cells. The vero cell cytotoxicity assay has been the commonly used method to detect Stx-producing bacteria. Immunological assays are also useful for screening for the presence of Stx-producing bacteria. For the direct detection of Stxs in stool cultures a number of enzyme-linked immunosorbent assays (ELISAs) have been established. Immobilized monoclonal or affinity purified polyclonal antibodies bind to the toxins that are detected by enzyme-labelled second antibodies. Additional information can be gained when gene probes and polymerase chain reaction (PCR) are used. The primary targets for PCR detection are phage-encoded *stx* genes. Several other genes have been used as indicators: chromosomal-encoded *eae* (intimin), plasmid-encoded *E-hly* (hemolysin), *uidA* (beta-glucuronidase), *fli C* (H7 flagellin), and plasmid-encoded *KatP* (catalase-peroxidase) [16]. Numerous procedures have been established for investigation of strain origin, clonal relatedness among strains, and epidemiology. Variations in phage content and chromosomal insertion sites of O157:H7 can lead to strain differences. Arbitrarily primed PCR has been successfully used to discriminate among O157:H7 strains [17, 18]. It involves low-stringency PCR amplification with arbitrarily chosen oligonucleotide primers that initiate DNA synthesis from sites to which the primer is randomly and usually only partially matched. The results are arrays of DNA fragments, often called random amplified polymorphic DNAs, that can serve as strain-specific fingerprints. Pulsefield gel electrophoresis (PFGE) is still considered the “gold standard” to analyze the pattern of *E. coli* strains. The Center for Disease Control and Prevention is establishing a national electronic database of PFGE subtypes to improve the surveillance of EHEC-associated outbreaks, the “PULSENET” [19]. For sensitive interstrain differentiation of pathogens isolated during outbreaks, restriction fragment length polymorphism (RFLP) using Stxs has been developed [20]. This method is based on the use of nucleic acid probes from structural genes of Stxs to generate RFLP patterns of Stx-producing *E. coli* strains.

Detection of antibodies against O157 antigen or other EHEC antigens in serum

As the pathogen might remain undetectable in stool cultures due to low concentration and late presentation of the patient, detection of serum antibodies is useful for the diagnosis of EHEC-induced infection, as antibodies persist at least 2 weeks after the acute phase. Antibodies against Stx or lipopolysaccharide (LPS) have been proposed [21]. The overall incidence of EHEC and the spreading of EHEC serotypes is difficult to estimate, due to a lack of standard methodology and the fact that stool culture is not commonly performed for EHEC infections [22].

Mechanisms of toxicity

Stx is the most-studied pathogenic factor of *E. coli* O157:H7 and a defining characteristic of EHEC. However, the mechanisms of pathogenesis and the complex interaction of the pathogen and the bacterial toxins with the host system are poorly understood. The infectious dose for some EHEC strains appears to be very low, probably less then 100 organisms [23]. Orally ingested Stx-producing bacteria colonize the mucosal site of the intestine and are then are transferred to the circulation. Important mechanims during colonization of the gut are acid resistance of bacteria [24] and adherence to epithelial cells. The best-characterized adherence phenotype is the so-called attaching and effacing lesion (A/E-lesion), hallmark of infections due to enteropathogenic *E. coli* [25]. The attachment of bacteria to the epithelial cells of the gut and destruction of the microvillus structure cause diarrhea and dehydration, which has been described for EHEC in animal models [26] and cell culture systems [27]. Histopathological features of destruction of enterocytes are edema, mucosal irritation, and ulcers [28]. As the bacteria probably do not invade the gut [29], uptake of Stx into the circulation may involve translocation of the toxin from the gut to the underlying tissue and the bloodstream [21].

Stx belongs to a "family" of compound toxins (the holotoxin is approximately 70 kDa), comprising an enzymatically active A unit (32 kDa) and a multimeric B unit (75 kDa), which is involved in the binding of the toxin to specific glycolipid receptors on the surface of target cells [30]. The binding of the toxin to its receptor, called Gb_3 (globotriasylceramide), is thought to be an important step in the pathogenesis. Several studies have examined the distribution of receptors on cell surfaces of different tissues, as well as the biochemical interaction between Stx and receptor [21]. In the cortex of human kidneys, the main site of renal lesions in HUS, Gb_3 is found in high levels [31]. Furthermore, in vitro studies have demonstrated toxic effects of Stx on cells or organ systems in animals. In a rabbit model, intravenous infusion of Stx1 or Stx2 produces vascular lesions in the intestine and central nervous system (CNS), organs in which high concentrations of Gb_3 were found [32].

The receptor-bound toxin is thought to be internalized by endocytosis [33]. The internalized toxin can inhibit protein biosynthesis, followed by cell death [34]. A direct cytotoxic effect of Stx has been demonstrated for human renal endothelial cells in vitro [35]. The typical human renal histopathology shows swollen glomerular endothelial cells and deposition of platelets and fibrin within the glomerulus [36]. The endothelial cell damage is probably caused by Stx and/or interaction with LPS and hormonal activators [i.e., tumor necrosis factor-α, interleukin (IL)-8, IL-1β, platelet-activating factor] [21]. Leukocyte activation also participates in endothelial cell damage. This is supported by studies with human umbilical vein endothelial cells (HUVEC), showing a dose-dependent increase of adherent leukocytes after administration of Stx1 [37]. In addition, an increase of von Willebrand factor and von Willebrand factor multimers are proposed to cause reduction in renal blood flow that leads to reduction of glomerular filtration [38]. Acute renal failure with oligo-/anuria might result. However, the mechanism that leads to acute renal failure is not clear. Recent studies have demonstrated a direct toxic effect of Stx on renal epithelial cells in culture [39–41]. In these studies Stx induced apoptosis in cultured renal cell lines. The mechanism transducing the apoptotic signal mediated by Stx binding is not understood [39, 42]. In the pathogenesis of Stx-induced HUS several questions remain unanswered: how does the endothelial injury occur, is the platelet involvement primary or secondary [43]; what is the role of renal tubular cell damage, does it initiate or contribute to a cascade of events leading to endothelial cell damage [42]?

Additional putative virulence factors of EHEC (i.e., enterohemolysin, serine protease and heat-stable enterotoxin) will not be further discussed here [21]. It should be emphasized that the role of the virulence factors in pathogenesis remains unclear.

EHEC infections

The spectrum of illness due to Stx-producing *E. coli* includes milder forms of diarrhea, HC, and classical HUS. Cases of asymptomatic *E. coli* infection have occasionally been detected in outbreaks [44–46]. The true incidence of EHEC infection is unknown, since in milder cases of diarrheal illness stool specimens are often not obtained. Severe illness is accompanied by bloody diarrhea and can lead to HC and the systemic manifestations of HUS and TTP [47]. TTP is rare and mainly occurs in adults [48]. HUS is more frequent in young children, although it is also seen in older children and adults. The so-called classical HUS mainly occurs after a prodrome of gastrointestinal illness, frequently including bloody diarrhea, followed by hemolytic anemia with red cell fragmentation, thrombocytopenia, and acute renal failure. Extrarenal involvement includes the pancreas, liver, gallbladder, CNS, heart, and lung [49]. In a quarter of patients the CNS is affected [50], with somnolence and seizures as the main features [51]. Almost all cases of death show CNS involvement [51]. The mortality rate of HUS is between 0% and 30% in individual reports [50, 52]. The risk of developing HUS after EHEC infection is reported to be 2%–7% [1]. Apart from the classical, diarrheal HUS there are variants designated as "atypical" HUS without diarrhea in the prodrome. Other HUS variants include a childhood form that is inherited, and adult forms associated with pregnancy, cancer and/or mitomycin treatment, cyclosporine A use, human immunodeficiency virus, and various chronic illnesses [53]. Recurrent HUS is rare and occurs mainly in the so-called atypical form. In 1993 Siegler [54] described a case of recurrent HUS, typical of the form associated with diarrhea. EHEC O157:H7 was only identified in

the relapse, but the authors had some evidence that both events were due to EHEC. HUS can be followed by chronic renal sequelae: hypertension, proteinuria, and chronic renal failure. In a long-term follow-up study in Utah, 51% of survivors showed chronic renal sequelae [50]. Studies from the years 1971–1988 in Germany showed renal symptoms in 45% of the patients, ranging from potentially harmless sequelae (10%) to variable degrees of renal insufficiency (26%), end-stage renal disease (8%), or deaths [55].

The risk of acquiring EHEC infection is influenced by several factors. The human being, as part of the chain of transmission, may incorporate bacteria (see routes of transmission). The mechanisms that trigger the disease are unknown. Young children and elderly patients seem to be at special risk and are affected predominantly. Risk factors for sporadic *E. coli* O157:H7 infection have been evaluated in epidemiological studies [56–58]. In most studies dietary risk factors, such as consumption of foods of bovine origin, particulary unpasteurized milk and undercooked ground beef, have been emphasized. Other food, person-to-person transmission, and contact with contaminated water (see routes of transmission) have been shown to be sources of EHEC infection. The association with certain foods occurred in less than 25% and therefore additional factors have to be considered. Characteristics of the host itself might be of importance: for example, history of viral or other infections prior to the occurrence of HUS. To date there are no data corresponding directly with the immune response to *E. coli* or its products. The amount of inoculated bacteria might also play a role in onset and disease course [23].

The use of antibiotics is controversial. Certain antibiotics, e.g, fosfomycin, are speculated to increase the amount of toxin released in the intestine [59, 60]. Some uncontrolled studies suggest that HUS might be more likely to develop in patients with antibiotic treatment [61, 62]. In a randomized controlled trial the intake of antibiotics did not affect the duration of illness or the risk of progression to HUS [63]. Other reports described a prolonged course of disease or an increase of secondary cases due to antibiotic treatment after the onset of HUS [64, 65].

In conclusion, there is a definite need for prospective studies using rapid diagnostic methods to permit early randomization [63]. Furthermore, some antidiarrheal agents may worsen the clinical course of infectious enteritis, which has been suggested in previous reports for infection with other pathogens [66].

Routes of transmission

Infected bovine species are the main EHEC reservoir today. Consumption of unpasteurized milk and undercooked beef is primary cause of infection. Several other foods have also been reported as sources of EHEC infection. However, it is not clear in which way the bacteria are transmitted. EHEC-infected persons can transmit the bacteria to other people. The known routes of transmission are discussed below.

Reservoir in animals

E. coli *in bovines*

Colonization rates of Stx-producing *E. coli* in the feces of bovines are high in many countries [67]. In Germany 50% of bovines are suggested to excrete Stx-producing *E. coli* [68]. However, full-grown animals, in particular, do not exhibit disease. Young animals may exhibit watery diarrhea. The isolation rates of O157:H7 are much lower than those of non-O157:H7 serotypes. Surveys of dairy and beef cattle in the United States have found *E. coli* O157:H7 in 0%–2.8%, with the highest isolation rates in younger animals [69]. The use of various detection methods for EHEC in animals hinder comparison of the study results, and prevalence data are not available. It is proposed that the prevalence of EHEC in bovines is high. In colostra of cows in Bavaria, Pirro et al. [70] found 84%–90% to be positive for Stx-neutralizing antibodies (neutralization assay of antibody titer). The results show that Stx-producing EHEC are more frequent than epidemiological studies would suggest.

E. coli *in sheep*

Some studies have found EHEC O157:H7 [71, 72] and non-O157 Stx-producing *E. coli* [73, 74] in sheep. Taken together, the prevalence data are lower in sheep than in bovines [67].

Other animals

Domestic animals (cats, dogs, rabbits), poultry, and birds (ravens, doves, gulls) have been examined and the results show prevalence rates from 0% to 5.2% [75]. In north Italy some cases of HUS during an outbreak of EHEC infections have been associated with chicken egg shells [76]. Although prevalence data for non-bovine animals are lower than in bovines, this mode of transmission has to be considered.

E. coli in food

In the United States and Canada the meat mainly associated with EHEC infections is beef [77–80]. Other foods of bovine origin, including roast beef and unpasteurized milk, and other types of meat from porcine, avian, and sheep sources, have also been directly linked to outbreaks [77, 81]. The spectrum of vehicles associated with EHEC infection is expanding: the consumption of mayonnaise, unpasteurized apple juice, and vegetables have been associated with cases of infection.

Table 1 Enterohemorrhagic *Escherichia coli* (EHEC) in meat products – association with cases of disease (*x* number unknown, ▲ O157:H7, *HUS* hemolytic uremic syndrome)

Site	No. cases total/HUS/deaths		Source	EHEC identified		Reference
				Stool	Meat	
Australia	x/22/1		Minced beef	▲ O111:H-	O111:H-	[10, 109]
Canada						
Ontario	73/12/19	Nursing home	Sandwich meal [a]	–	–	[65]
Alberta	1,488/8/11			–	–	[110]
Germany						
Bavaria	x/32/4		Sausage suggested	O157:H-	–	[111]
Italy	2/1/0	Two sisters	Ground beef	▲	–	[3]
United Kingdom						
Scotland	505/35/13		Hamburger, poultry	–	–	[2]
Lothian 1990	16/x/x	Restaurant	Cross-contaminated foodstuff	–	–	[112]
Lothian 1991	x/18/x	Residential home	Butcher's meat	–	–	[113]
Wales	8/0/0			▲	▲	[114]
Wishaw	490/x/18		Butcher's meat	▲	▲	[115]
USA						
Conneticut	23/0/0		Hamburger	▲	▲	[116]
Minnesota	54/0/0	School outbreak	Precooked roast beef	–	–	[117]
Nevada	34/1/4	Nursing home	Hamburger	▲	–	[31]
North Dakota	65/2/0	Community outbreak	Roast beef	–	–	[118]
Sunderland	14/4/x	Farm	Precooked meat	–	–	[119]
Oregon	≥47/x/x	Fast-food restaurant	Sandwich	▲	▲	[120]
Utah	51/8/4	Custodial institutions	Ground beef	–	–	[102]
Washington	37/4/2	Nursing home	Ground beef/ranch-dressing	▲	–	[32]
Wisconsin	61/0/0	Banquet		▲	▲	[120]

[a] Secondary person-to-person transmission

The mode of entry into the food chain is unclear. Primarily, ingestion of undercooked meat was shown to be involved [6, 77]. In undercooked food, bacteria may survive and retain pathogenicity. Mead et al. [82] analyzed knowledge, attitudes, and practices of food preparers in case and control households. They found an estimated population-attributable risk for inadequate handwashing in 34%. They concluded that transmission occurred more commonly when the hands of food preparers, contaminated by handling raw ground beef, were allowed to cross-contaminate other ingredients or utensils. It is possible that food can be cross-contaminated as a result of improper handling procedures during manufacturing, storage, and marketing, and even in the household itself. In order to reduce the concentration of bacteria in food, decontamination strategies (e.g., irradiation techniques) have been proposed [83].

The microbiological analysis of food includes enrichment procedures followed by screening using PCR or ELISA [84]. The determination of genotype characteristics (i.e., *eae* gene), as well as phage typing, are helpful for epidemiological analysis. Only the identification of pathogens with the same phage type in food and the infected patient allow a conclusion on the chain of infection. Data on analysis of food are difficult to compare, as different methods have been used for detecting the pathogens [67, 85–87]. Presently the main problem of analysis of food is the lack of standardized methodology and interdisciplinary co-operation of clinicians, public health authorities, and veterinarians. A precise history of the food ingestion prior to infection is a precondition for further critical analysis of food (see history of environmental aspects). Food analysis is much more likely in outbreaks than in sporadic cases.

Studies worldwide that have described cases or outbreaks of EHEC infections associated with consumption of contaminated foods are summarized in Tables 1–3: meat products (Table 1), unpasteurized milk and milk products (Table 2), and other food (Table 3). Identification of the causative organism throughout the whole chain of infection was possible only in some cases. Conclusions drawn from anamnestic data alone should be handled carefully, as shown during the great outbreak in Japan in 1996 [88–90]. The following example from our children's hospital, in 1995, shows how difficult it is to confirm the route of transmission in patients. The history of a 4-year-old boy, suffering from HUS, showed that he had been drinking unpasteurized milk prior to infection. The stool sample was positive for Stx1- and Stx2-producing EHEC. The isolated strain was O69:H-. The patient's serum showed elevated antibody levels against O157 LPS. The raw milk samples were positive for Stx2-producing *E. coli*. The isolated strain in the milk sample was O8:H21. The samples of the patient and the milk did not contain the same serotype. Thus, although there was a positive history for drinking unpasteurized

Table 2 EHEC in milk or milk products – association with cases of disease (▲ O157:H7, ■ Shiga toxin-producing EHEC)

Site	No. cases total/HUS/deaths	Source	EHEC identified			Reference
			Stool	Stool (bovine)	Food	
Argentina	91/x/x	Unpasteurized milk	–	–	–	[121]
Canada						
Ontario	48/3/0	Milk/milk filters	▲	▲	–	[122, 123]
Egypt	÷	Unpasteurized milk	–	–	■	[124]
France	x/4/x	Cheese from unpasteurized milk	■	■	■	[125]
United Kingdom						
Scotland 1991	>100/9/x	Contaminated milk pipes	▲	–	–	[126]
Scotland 1994	16/5/x	Yoghurt	–	–	–	[127]
Scheffield	÷	Unpasteurized milk	–	–	▲	[128]
USA						
No information	x/2/0	Unpasteurized milk	▲	▲	–	[129]
Wisconsin	52/x/x	Unpasteurized milk	–	–	–	[130]

Table 3 EHEC in non-animal-derived foodstuff – association with cases of disease (■ Shiga toxin-producing EHEC, ● *Citrobacter freundii*, ▲ O157:H7)

Site	No. cases total/HUS/deaths	Source	EHEC identified			Reference
			Stool	Stool (bovine)	Food	
Germany	x/9/1	Green butter	■●	–	●	[131]
Japan						
Sakai City	>400/101/2 School 9,000/x/9 lunch	Radish sprouts	▲	–	–	[132] [87–89]
United Kingdom						
Norwich	49/x/x	Potatoes	▲	–	–	[133]
USA						
Oregon	40–50/x/x	Salad dressing with mayonnaise	–	–	–	[134]
Massachusetts	18/x/x	Unpasteurized apple cider	–	–	–	[135]
Maine	4/1/1	Vegetables from a manured garden		–	–	[136]
No information	No information	Cantaloupe (melon)	–	▲	–	[137]

milk, the route of transmission of EHEC still remained unclear.

E. coli in drinking water and in public baths

Water-borne infections have been associated with outbreaks. In Missouri in 1990 an outbreak of EHEC infection that affected 243 people, including 4 deaths, was associated with drinking water. The outbreak was due to an improperly repaired water system, which allowed unchlorinated water to be widely distributed [91]. Other reports concerning EHEC infections associated with contaminated drinking water come from Scotland [92], South Africa [93] and Japan [94]. In the water-borne outbreak in Scotland the water supply came from a source that resembled a field-drain system and may have been contaminated by cattle slurry. In Africa the water was probably contaminated by cattle carcasses and dung washed into rivers and dams by heavy rains. EHEC infections have also been associated with bathing in contaminated water. Most of these reports come from the United States [95–97]. The Netherlands, with a relatively low rate of recorded EHEC infections, reported four cases of HUS associated with paddling in a lake. However, the pathogen was not isolated from the water [98].

Person-to-person transmission

EHEC infections have been reported to result from person-to-person contact in several cases. However, secondary infections occur at a rate lower than 10% [67]. Person-to-person transmission occurs predominantly in nursing homes. An example of a secondary transmission is the outbreak in Minnesota, in 1988, linked to undercooked meat patties at a junior high school. A total of 32 cases were reported, including a 12-year-old boy whose mother worked in a day-care center. About 1 week after the boy's illness, two toddlers attending the nursery de-

veloped HC, and *E. coli* O157:H7 was isolated from the stool sample [99]. Further studies reported outbreaks in nursing homes and from a facility for mentally retarded people [100–102].

History of environmental aspects

A careful evaluation of environmental aspects of the patients prior to infection is necessary for epidemiological studies on routes of transmission of EHEC. It is not known to what extent an accurate documented history improves epidemiological case-control studies. Epidemiological evaluations of EHEC infections should involve a precise investigation of proposed routes of transmission. Several countries have established programs to evaluate the epidemiology of EHEC infections: in Canada the Canadian Pediatric Kidney Disease Research Centre has found an evidence of 77% O157:H7 infection in a study of the years 1991–1994 [103]. In Belgium, Piérard et al. [104] are conducting a case-control study on so-called Epi-Info: cases of EHEC infection and controls were interviewed about contact with persons with diarrhea or animals, recent travel, outdoor activities, eating in a restaurant or in a fast-food establishment, consumption of untreated water and of various foods, including unpasteurized milk or undercooked beef. They found several different factors to be associated with an increased risk of becoming infected. In Europe, a national multicenter study (*Biomed 2 Programme PL-950970*) prospectively evaluates epidemiology, frequency, and clinical course of EHEC-associated HUS in childhood [105]; Internet page: *http://www.iss.it/centri/vtec/vtec.htm* (IVC news 10, 1998). The history of environmental aspects is evaluated on the day of admission to hospital. The epidemiological part of the questionnaire covers the following areas: (1) contact with persons with diarrhea up to 2 weeks prior to infection of the child (household members and non-household members); (2) history of consumption of food up to 2 weeks prior to infection, including unpasteurized milk products, yoghurt, undercooked meat meals, raw vegetables, mayonnaise, apple juice; (3) animal contact and which species; (3) contact with farming or agriculture; (5) drinking water supported by central water supply or by other sources (e.g.,well water); (6) bathing in outdoor water (probably cross-contaminated).

In order to prove the routes of transmission microbiological analysis of feces and/or sera of animals and persons with close contact with the patient, food, and bathing or drinking water is performed as soon as there is a diagnosis of EHEC infection. In Germany investigation of the environmental aspects is performed by the public health authorities. As shown in a report on laboratory investigations of bovine feces, it takes at least 2 weeks from the onset of disease to first epidemiological investigations in the animals. In the majority of cases investigations of the environment are performed more than 3 weeks later [106]. It is necessary to better co-ordinate the exchange of information between clinicians and public health authorities.

Discussion

Infections with EHEC are increasing worldwide. The reason for this increase remains unclear. HUS is a severe complication of EHEC infection. Evidence of EHEC infection in HUS has been found in over 80% of cases in Germany [107] and the United States [50]. Most of the studies show a predominance of the classical serotype O157:H7. The overall incidence of non-O157:H7 in EHEC-associated disease is not known. Monitoring of EHEC infections primarily needs laboratories to be aware of EHEC (O157:H7 and non-O157:H7) in the diagnosis of diarrheal infections. In 1996 Karch et al. [22] called for a network of laboratories and clinical centers to effectively monitor EHEC infections. Diagnosis of EHEC infection and associated diseases must be followed by registration. Registration is a pre-condition for the estimation of rates for incidence and prevalence. In Germany EHEC infection has been notifiable since November 1998.

The routes of transmission of EHEC remain unclear in the majority of cases. If bovine species are the main reservoir for EHEC how do the bacteria enter the food chain of the human? Improper handling techniques during manufacturing might play an important role in the contamination of food. However, this has not been adequately studied to date. For household food preparation, insufficient handwashing is a possible risk factor for infection [82]. It has been demonstrated that EHEC O157 can survive food preservation procedures (drying, cold storage, and acidification) [108]. This suggests that these organisms may survive in nature and be more widespread than has been suspected [22].

To follow a potential route of transmission a precise history of the environmental aspects of the patient needs to be evaluated. A history of animal contact or ingestion of certain food may lead to a possible source of infection. It is clear that the history of environmental aspects should be obtained as early as possible, since it might be difficult to remember the food ingested prior to infection later. Public health authorities in Germany advise performing microbiological analysis of animal feces and food if there are hints on potential sources of infection. A better co-ordination of this network of institutions is clearly warranted [22]. Taken together, early identification of EHEC infection is necessary to prevent continued transmission and to confirm prompt epidemiological investigations. The identification of the source of infection is the most useful to prevent further transmission. It remains unclear which mechanisms lead to infection and which host factors protect the human against the bacteria.

Conclusions

As long as there is no countrywide law for registration, rates for incidence and prevalence of EHEC infections will only partly tell the truth or will be limited to region-

al evaluations. Cases of EHEC infection should be reported to the public health authorities as soon as they are diagnosed. The history of environmental aspects should be evaluated early and should be included in the report to the public health services when EHEC infection is proven. Only an acceleration of the gathering of information will improve the microbiological investigations. The improvement of the information flow must be accompanied by efforts to improve laboratory analysis. Laboratories should include detection methods for EHEC in the diagnostic program for diarrheal infections, at least for younger children (≤4 years) and the elderly, i.e., samples should be examined systematically for EHEC O157:H7 and non-O157:H- strains (so-called streamlined care). Laboratories and clinical centers should be co-ordinated into a network to effectively monitor EHEC infections, e.g., PULSENET, and for HUS patients, HUSMED [22]. Prevention of EHEC infection should include public health warnings of the risks of consumption of unpasteurized milk or undercooked meat and should emphasize the importance of handwashing when handling raw meat products.

References

1. Siegler RL (1995) The hemolytic uremic syndrome. Pediatr Nephrol 42:1505–1529
2. Waters JR, Sharp JCM, Vicram JD (1994) Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987–1991. Clin Infect Dis 19: 834–843
3. Caprioli A, Luzzi I, Rosmini F, Rest C, Edefonti A, Perfumo F, Farina C, Goglio A, Gianviti A, Rizzoni G (1994) Communitywide outbreak of hemolytic-uremic-syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. J Infect Dis 169:208–211
4. López EL, Contrini MM, Devoto S, De Rosa MF, Graña MG, Aversa L, Gomez HF, Genero MH, Cleary TG (1995) Incomplete hemolytic-uremic syndrome in Argentinean children with bloody diarrhea. J Pediatr 127:364–367
5. World Health Organization (1994) Report of WHO Working Group Meeting on Shiga like Toxin-Producing Escherichia coli (SLTEC), with special emphasis on zoonotic aspects, Bergamo, 1 July 1994. WHO/CDS/VPH/94.136, Geneva
6. Riley LW, Robert SR, Steven DH, Mc Gee HB, Wells JG, Betty RD, Richard JH, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML (1983) Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 308: 681–685
7. Karmali MA, Steele BT, Petric M, Lim C (1983) Sporadic cases of hemolytic uremic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet I:619–620
8. Bockemühl J, Karch H (1996) Zur aktuellen Bedeutung der enterohämorrhagischen *Escherichia coli* (EHEC) in Deutschland (1994–1996). Bundesgesundheitsblatt 41:290–296
9. Karmali MA (1989) Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 2:15–38
10. Cameron AS, Beers MY, Walker CC (1995) Community outbreak of hemolytic-uremic syndrome attributable to *Escherichia coli* O111:NM-South Australia. MMWR Morb Mortal Wkly Rep 44:550–558
11. Law D (1994) Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*. Clin Microbiol Rev 7:152–173
12. Mariani-Kurdijan P, Denamur E, Milon A, Picard B, Cave H, Lambert Zechovsky N, Loirat C, Goullet P, Sansonetti PJ, Elion J (1996) Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic-uremic syndrome in France. J Clin Microbiol 31:296–301
13. Karch H, Schmidt H, Schwarzkopf A, Böhler O, Aleksić S, Rowe B (1997) Molecular characterization and epidemiology of sorbitol-fermenting *Escherichia coli* O157:H-(abstract). Fortschr Med 115:46
14. Karch H, Janetzki-Mittmann C, Aleksić S, Datz M (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
15. Konowalchuk J, Speirs J, Stavric S (1977) Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 18:775–779
16. Nataro JP, Kaper JB (1998) Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11:142–201
17. Heuvelink AE, Van de Kar NCAJ, Meis JFGM, Monnens LAH, Melchers WJG (1995) Characterization of verocytotoxin-producing *Escherichia coli* O157 isolates from patients with haemolytic uraemic syndrome in Western Europe. Epidemiol Infect 115:1–14
18. Madico BE, Griffin PM, Mead PS, Tauxe RV (1997) Arbitrarily primed PCR DNA fingerprinting of *Escherichia coli* O157:H7 strains by using templates from boiled cultures. J Clin Microbiol 33:1543–1536
19. Barrett TJ, Hunter SB, Swaminathan B (1997) A national network for molecular subtyping of *Escherichia coli* O157:H7 (abstract). Abstracts of the 3rd International Symposium on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, 22–26 June 1997, Baltimore, p 111
20. Samadpour M (1995) Molecular Epidemiology of *Escherichia coli* O157:H7 by restriction fragment length polymorphism using Shiga-like toxin genes. J Clin Microbiol 33:2150–2154
21. Paton JC, Paton AW (1998) Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin Microbiol Rev 11:450–479
22. Karch H (1996) Control of enterohemorrhagic *Escherichia coli* infection: the need for network involving microbiological laboratories and clinical and public health institutions. Eur J Clin Microbiol Infect Dis 15:276–280
23. Paton AW, Rarcliff R, Doyle RM, Seymour-Murrray J, Davos D, Lanser JA, Paton JC (1996) Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. J Clin Microbiol 34: 1622–1627
24. Goodson M, Rowbury RJ (1989) Habituation to normally lethal acidity by prior growth of *Escherichia coli* at a sub-lethal acid pH value. Lett Appl Microbiol 8:77–79
25. Staley TE, Jones EW, Corley ED (1969) Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. Am J Pathol 56:371–392
26. Tzipori S, Gibson R, Montanaro J (1989) Nature and distribution of mucosal lesionsassociated with enteropathogenic and enterohemorrhagic *Escherichia coli* in piglets and the role of plasmid-mediated factors. Infect Immun 57:1142–1150
27. Knutton S, Baldwin T, Williams PH, Mc Neish AS (1987) Actin accumulation at sites ofbacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic *Escherichia coli* to HEp-2-cells. Infect Immun 55:78–85
28. Griffin PM, Olmstead LC, Petras RE (1990) *Escherichia coli* O157:H7-associated colitis. Gastroenterology 99:142–149
29. Tesh VL, O'Brien AD (1992) Adherence and colonization mechanisms of enteropathogenic and enterhemorrhagic Escherich coli. Microb Pathog 12:245–254
30. O'Brien AD, Holmes RK (1987) Shiga and Shiga-like toxins. Microbiol Rev 51:206–220
31. Boyd B, Lingwood CA (1989) Verotoxin receptor glycolipid in human renal tissue. Nephron 51:207–210
32. Richardson SE, Rotman TA, Jay V, Smith CR, Becker LE, Petric M, Olivieri NF, Karmali MA (1992) Experimental verocytotoxicaemia in rabbits. Infect Immun 60:4154–4167

33. Lingwood CA (1993) Verotoxins and their glycolipid receptors. Adv Lipid Res 25:189-211
34. Sandvig K, Van Deurs B (1996) Endocytosis, intracellular transport, and cytotoxic action of Shiga toxin and ricin. Physiol Rev 76:949–966
35. Louise CB Obrig TG (1991) Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cell. J Infect Dis 172:1397–1401
36. Moake JL (1994) Hemolytic-uraemic syndrome: basic science. Lancet 343:393–397
37. Morigi M, Micheletti G, Figliuzzi M (1995) Verotoxin-1 promotes leukocyte adhesion to cultured endothelial cells under physiological flow conditions Blood 86:4553–4558
38. Remuzzi G, Galbusera M, Salvadori M, Rizzoni G, Paris S, Ruggenenti P (1996) Bilateral nephrectomy stopped disease progression in plasma resistant hemolytic uremic syndrome with neurological signs and coma. Kidney Int 49:282–286
39. Zimmerhackl LB, Garz C, Böhm N, Brandis M (1996) Das enteropathische hämolytisch-urämische Syndrom in der Pädiatrie: Ätiologie und Pathogenese. In: Ökosystem Darm VII. Springer, Berlin Heidelberg New York, p 31–37
40. Garz C, Wiegele G, Joos S, Brandis M, Karch H, Zimmerhackl LB (1997) Shigatoxin (Stx) I, II and lipopolysaccharide (LPS) from *E. coli* induce apoptosis in LLC-PKI cells (abstract). J Am Soc Nephrol 8:A2801
41. Kiyokawa N, Taguchi T, Mori T, Uchida H, Sato N, Takeda T, Fujimoto J (1998) Induction of apoptosis in normal human renal tubular epithelial cells by *Escherichia coli* Shiga toxins 1 and 2. J Infect Dis 178:178–184
42. Hughes AK, Stricklett PK, Kohan DE (1998) Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells Kidney Int 54:426–437
43. Neild GH (1998) Hemolytic uremic syndrome/thrombotic thrombo-cytopenic purpura: pathophysiology and treatment. Kidney Int 53 [Suppl 64]:45–49
44. Duncan L, Mai V, Carter A, Carlson JA, Borczyk AA, Karmali MA (1987) Outbreak of gastrointestinal disease – Ontario. Can Dis Wkly Rep 13-2:15–18
45. Laboratory Center of Disease Control (1983) Hemorrhagic colitis in a home for the aged – Ontario. Can Dis Wkly Rep 9: 29–32
46. Ryan CA, Tauxe RV, Hosek GW, Wells JG, Stoesz Pa, McFadden HW, Smith PW, Wright GF, Blake P (1986) *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis 154:631–638
47. Ruggenenti P, Remuzzi G (1996) The pathophysiology and management of thrombotic thrombocytopenic purpura. Eur J Haematol 56:191–207
48. Ostroff SM, Griffin PM, Tauxe RV (1990) A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am J Epidemiol 132:239–247
49. Siegler R (1994) Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. J Pediatr 125:511–518
50. Siegler RL, Pavia AT, Christofferson RD (1994) A 20 year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatrics 94:35–40
51. Robson WLM, Leung AKC, Montgomery MD (1991) Causes of death in hemolytic-uremic syndrome. Child Nephrol Urol 11: 228–233
52. Rahman M, Hossain M (1993) The bimodal mortality pattern of acute renal failure in children. Ren Fail 15:55–59
53. Remuzzi G, Ruggenenti P (1995) The hemolytic uremic syndrome. Kidney Int 47:2–19
54. Siegler RL (1993) Recurrent hemolytic uremic syndrome secondary to *Escherichia coli* O157:H7 infection. Pediatrics 91: 666–668
55. Tönshoff B, Sammet A, Sanden I, Mehls O, Waldherr R, Schärer K (1994) Outcome and prognostic determinants in the hemolytic uremic syndrome of children. Nephron 68:63–70
56. Bryant HE, Athar MA, Pai CH (1989) Risk factors for *Escherichia coli* O157:H7 infection in an urban community. J Infect Dis 160:858–864
57. MacDonald KL, O'Leary MJ, Cohen ML (1988) *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen: results of a one-year, prospective, population-based study. JAMA 259: 3567–3570
58. Le Saux N, Spika JS, Friesen B (1993) Ground beef consumption in noncommercial settings is a risk factor for sporadic *Escherichia coli* O157:H7 infection in Canada. J Infect Dis 167: 500–502
59. Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG (1992) Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. Infection 20:25–29
60. Yoh M, Honda T (1997) The stimulation effect of fosfomycin, an antibiotic in common use in Japan, on the production/release of verotoxin-1 from enterohaemorrhagic *Escherichia coli* O157:H7 in vitro. Epidemiol Infect 119:101–103
61. Pavia AT, Nichols CR, Green DP (1990) Hemolytic uremic syndrome during an outbreak of *Escherichia coli* O57:H7 in institutions for mentally retarded persons: clinical and epidemiologic observations. J Pediatr 116:544–551
62. Al-Quawari S, Fontaine RE, Al-Qahtani MS (1995) An outbreak of hemolytic uremic syndrome associated with antibiotic treatment of hospital inpatients for dysentery. Emerg Infect Dis 1:138–140
63. Proulx F, Turgeon JP, Delage G, Lafleur L, Chicoine L (1992) Randomized, controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. J Pediatr 121:299–303
64. Kohsaka T, Tagawa M, Suzuki T, Ito H (1997) The old and new problems of the antibiotics treatment in hemorrhagic enterocolitis and hemolytic-uremic syndrome, according to epidemiological studies. Nippon Rinsho 55:706–714
65. Carter AO, Borczyk AA, Carlson JAK (1987) A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. N Engl J Med 317:1496–1500
66. Cimolai N, Carter JE, Morrison BJ, Anderson JD (1990) Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. Clin Lab Observ 116: 589–592
67. Armstrong GL, Hollingsworth J, Morris JG (1996) Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiol Rev 18:29–51
68. Richter H, Klie H, Timm M, Gallien P, Steinrück H, Perlberg KW, Protz D (1997) Verotoxin-bildende *E. coli* (VTEC) im Kot von Schlachtrindern aus Deutschland. Berl Münch Tierärztl Wochenschr 110:121–127
69. Wells JG, Shipman LD, Greene KD, Sowers EG, Greene JH, Cameron DN, Downes FP, Martin ML, Griffin PM, Ostroff SM, Potter ME, Tauxe RV, Wachsmuth IK (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. Pirro F, Wieler LH, Failing K, Bauerfeind R, Baljer G (1995) Neutralizing antibodies against Shiga-like toxins from *Escherichia coli* in colostra and sera of cattle. Vet Microbiol 43:131–141
71. Kudva IT, Hatfield PG, Hovde CJ (1996) *Escherichia coli* O157:H7 in microbial flora of sheep. J Clin Microbiol 34: 431–433
72. Chapman PA, Siddons CA, Harkin MA (1996) Sheep as a potential source of verocytotoxin-producing *Escherichia coli* O157 (letter). Vet Res 138:23–24
73. Beutin L, Geier D, Steinruck H (1993) Prevalence and some properties of verotoxin (Shiga-like-toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol 31:2483–2488
74. Wray C, Mc Laren IM, Carroll PJ (1993) *Escherichia coli* isolated from farm animals in England and Wales between 1986 and 1991. Vet Rec 133:439–442
75. Baljer G (1997) EHEC Animal reservoirs. Fortschr Med 115:84
76. Tozzi AE, Niccolini A, Caprioli A (1994) A community outbreak of haemolytic-uraemic syndrome in children occurring in a large area of northern Italy over a period of several months. Epidemiol Infect 113:209–219

77. Griffin PM, Tauxe RV(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
78. Bell BP, Goldoft M, Griffin PM (1994) A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. JAMA 272: 1349–1353
79. CDC (1993) *Escherichia coli* O157:H7 outbreak linked to home-cooked hamburger – California. July 1993. MMWR Morb Mortal Wkly Rep 43:213–215
80. CDC (1993) Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – western United States, 1992–1993. MMWR Morb Mortal Wkly Rep 42:258–263
81. Griffin PM (1995) *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL (eds) Infections of the gastrointestinal tract. Raven, New York, pp 739–761
82. Mead PS, Finelli L, Lambert-Fair MA, Champ D, Townes J, Hutwagner L, Barrett T, Spitalny K, Mintz E (1997) Risk factors for sporadic infection with *Escherichia coli* O157:H7. Arch Intern Med 157:204–208
83. Corry JE, James C, James SJ, Hinton M (1995) *Salmonella*, *Campylobacter* and *Escherichia coli* O157:H7 decontamination techniques for the future. Int J Food Microbiol 28: 187–196
84. Timm M von, Klie H, Richter H, Gallien P, Perlberg KW, Lehmann S, Protz D (1998) Verfahren zum qualitativen Nachweis von Verotoxin-produzierenden *Escherichia coli* (VTEC) in Lebensmitteln und Fäzes. Bundesgesundheitsblatt (Sonderheft) 10:20–21
85. Willshaw GA, Smith HR, Roberts DM, Thirlwell J, Cheasty T, Rowe B (1993) Examination of raw beef products for the presence of verocytotoxin producing *Escherichia coli*, particularly those of serogroup O157. J Appl Bacteriol 75:420–426
86. Bennett AR, MacPhee S, Betts RP (1994) Evaluation of methods for the isolation and detection of *Escherichia coli* O157 in minced beef. Lett Appl Microbiol 20:375–379
87. Wright DJ, Chapman PA, Siddons CA (1994) Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. Epidemiol Infect 113:31–39
88. Swinbanks D (1997) Blood scandal and *E. coli* raise questions in Japan. Nature 385:9
89. Swinbanks D (1996) Outbreak of *E. coli* infection in Japan renews concern. Nature 382:290
90. Swinbanks D (1996) Japan shuns radishes after 'possible link' to *E. coli*. Nature 382:567
91. Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell D, Geldreich E, Payne BJ, Meyer A, Wells JG, Greene KD, Bright M, Bean NH, Blake PA (1992) A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann Intern Med 117: 812–819
92. Dev VJ, Main M, Gould I (1991) Waterborne outbreak of *Escherichia coli* (letter). Lancet 337:1412
93. Isaacson M, Canter PH, Effler P (1993) Haemorrhagic colitis epidemic in Africa (letter). Lancet 341:961
94. Akashi S, Joh K, Tsujii A (1994) A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. Eur J Pediatr 153:650–655
95. Keene WE, McAnulty JM, Hjoesly FC, Williams LP, Hedberg K, Oxman GL, Barrett TJH, Pfaller MA, Fleming DW (1994) A swimming associated outbreak of hemorrhagic colitis caused by *E. coli* O157:H7 and *Shigella sonnei*. N Engl J Med 331:579–584
96. Ackman D, Birkhefad G, Root T (1994) Swimming-associated hemorrhagic colitis due to *Escherichia coli* O157:H7 infection (abstract). Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, Sept 17–20, 1995. American Society for Microbiology, Washington, DC, p 35
97. Brewster DH, Brown MI, Robertson D (1994) An outbreak of *Escherichia coli* O157 associated with a children's paddling pool. Epidemiol Infect 112:441–447
98. Cransberg K, Kerkhof JHCT van den, Bänffer JRJ, Stijnen C, Wernears K, Kar NCAJ van de, Nauta J, Wolff ED (1996) Four cases of hemolytic-uremic syndrome – source contaminated swimming water? Clin Nephrol 46:45–49
99. Belongia EA, Osterholm MT, Soler JT (1993) Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day care facilities. JAMA 269:883–888
100. Reida P, Wolff M, Pohls HW (1994) An outbreak due to enterohemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. Int J Med Microbiol Virol Parasitol Infect Dis 281:534–543
101. Spika JS, Parsons JE, Nordenberg D (1986) Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. J Pediatr 109:287–291
102. Pavia AT, Nichols CR, Green DP (1990) Hemolytic uremic syndrome during an outbreak of *Escherichia coli* O157:H7 in institutions for mentally retarded persons: clinical and epidemiologic observations. J Pediatr 116:544–551
103. Rowe PC, Orrbine E, Wells GA, Lior H, McLaine PN and the CPKDRC-co-investigators (1997) Risk of hemolytic uremic syndrome (HUS) following sporadic *E. coli* O157 infection: results of a Canadian collaborative study. Proceedings of the 3rd International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, 22–26 June 1997, Baltimore, p V116/I
104. Piérard D, Van Loock F, De Bock S, Potters D, Crabbe G, Crowcroft N, Lauwers S (1997) A case-control study of sporadic cases of O157 and non-O157 VTEC infection. Proceedings of the 3rd International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections, 22–26 June 1997, Baltimore, p V136/I
105. Zimmerhackl LB (1997) HUS surveillance in Germany. Notiziario dell' Istituto Superiore di Sanità 10:4
106. Weber A, Klie H, Richter H, Gallien P, Timm M, Perlberg KW (1997) Über die derzeitigen Probleme zum Auffinden von Infektionsquellen und Infektionsketten bei Enterohämorrhagischen *E. coli* (EHEC). Berl Münch Tierärztl Wochenschr 110:211–213
107. Bitzan M, Ludwig K, Klemt M, König H, Büren J, Müller-Wiefel DE (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
108. Feng P (1995) *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. Emerg Infect Dis 1:47–52
109. Cameron S, Walker C, Beers M (1995) Enterohemorrhagic *Escherichia coli* outbreak in South Australia associated with the consumption of Mettwurst. Commun Dis Intell 19:70–71
110. Waters JR, Sharp JCM, Dev VJ (1994) Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987–1991. Clin Infect Dis 19: 834–843
111. Ammon A, Petereson LR, Karch H (1999) The large outbreak of hemolytic uremic syndrome caused by unusual sorbitol-fermenting strain of *Escherichia coli* O157:H-. J Infect Dis 197:174–177
112. Marsh J, MacLeod AF, Hanson MF, Emmanuel FX, Frost JA, Thomas A (1992) A restaurant-associated outbreak of *E. coli* O157 infection. J Public Health Med 14:78–83
113. Bisset JG, Brown MI, Bimson J (1992) An outbreak of haemorrhaigc colitis due to *E. coli* O157 in two residential homes for the elderly in the Borders. Commun Dis Environ Hlth Scot Wkly Rep 26:5–6
114. Willshaw G, Thirlwell J, Jones AP, Parry S, Salmon RL, Hickey M (1994) Verocytotoxin-producing *Escherichia coli* O157:H7 in beefburgers linked to an outbreak of diarrhea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. Lett Appl Microbiol 19:304–307

115. Cowden JM (1997) From Scotland. Notiziario dell' Istituto Superiore di Sanità 10 [Suppl 1]
116. Roberts DL, Mshar PA, Cartter ML, Hadler JL, Sosin DM, Hayes PS, Barrett TJ (1995) The role of heightened surveillance in an outbreak of *Escherichia coli* O157:H7. Epidemiol Infect 115:447–454
117. Belongia EA, Mac Donald KL, Parham GL (1991) An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J Infect Dis 164: 338–343
118. MMWR (1993) Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – western United States, 1992–1993. MMWR Morb Mortal Wkly Rep 42: 258–263
119. Stevenson J, Hanson S (1996) Outbreak of *Escherichia coli* O157 phage type 2 infection associated with eating precooked meats. Commun Dis Rep CDR Rev 6:R116–1183
120. Rodrigue DC, Mast EE, Greene KD, Davis JP, Hutchinson MA, Wells JG, Barrett TJ, Griffin PM (1995) A universitiy outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. J Infect Dis 172:1122–1125
121. Perez N, Rahman R, Zalba J, Bibiloni N, Lasarte P, Spizzirri F (1994) Haemolytic uraemic syndrome and unpasteurized milk (letter). Acta Paediatr 83:142
122. Borczyk AA, Karmali MA, Li or K, Duncan LMC (1987) Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet I:98
123. Clarke RC, McEwen SA, Gannon VP (1989) Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in south-western Ontario. Epidemiol Infect 122:253–260
124. Abdul-Raouf UM, Ammar MS, Beuchat LR (1996) Isolation of *Escherichia coli* O157:H7 from some Egyptian foods. Int J Food Microbiol 29:423–426
125. Dechênes G, Casenave C, Grimont F, Desenclos JC, Benoit S, Collin M, Baron S, Mariani P, Grimont PA-D, Nivet H (1996) Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. Pediatr Nephrol 10:203–205
126. Upton P, Coia JE (1994) Outbreak of *Escherichia coli* O157 infection associated with pasteurized milk supply (letter). Lancet 344:1015
127. Morgan GM, Newman C, Palmer SR (1988) First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. Epidemiol Infect 101:83–91
128. Chapman PA, Wright DJ (1993) Untreated milk as a source of verotoxigenic *E. coli* 0157 (letter). Vet Rec 14:173
129. Martin ML, Shipman LD, Wells JG et al. (1986) Isolation of *Escherichia coli* O157:H7 from dairy cattle asssociated with two cases of haemolytic uraemic syndrome (letter). Lancet II:1043
130. Dorn C (1988) Hemorrhagic colitis and hemolytic uremic syndrome caused by *Escherichia coli* in people consuming undercooked beef and unpasteurized milk (letter). J Am Vet Med Assoc 193:1360–1361
131. Tschäpe H, Prager R, Streckel W, Fruth A, Tietze E, Böhme G (1995) Verotoxinogenic *Citrobacter freundii* associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. Epidemiol Infect 114:441–450
132. Anonymous (1996) Food safety: enterohaemorragic *Escherichia coli* infection. Wkly Epidemiol Rec 35:267
133. Morgan D, Newman CP, Hutchinson DN, Walker AM, Rowe B, Majid F (1993) Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiol Infect 111:181–187
134. Keene WE, McAnulty JM, Williams LP (1993) A two-restaurant outbreak of *Escherichia coli* O157:H7 enteritis associated with the consumption of mayonnaise (abstract). Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Louisiana, Oct 17–20, 1993. American Society for Microbiology, Washington DC, p 354
135. Besser RE, Lett SM, Weber JT (1993) An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 269:2217–2220
136. Cieslak PR, Barrett TJ, Griffin PM, Gensheimer KF, Beckett G, Buffington J, Smith MG(1993) *Escherichia coli* O157:H7 infection from a manured garden (letter). Lancet 342:367
137. Anonymous (1993) Yet another outbreak of hemorrhagic colitis – Corvallis. Center for Disease Prevention and Epidemiology, Oregon Health Division, Portland. Commun Dis Summary 42:1–2

LITERATURE ABSTRACT

R.B. Isaacs · S.L. Nock · C.E. Spencer · A.F. Connors, Jr. X.Q. Wang · R. Sawyer · P.I. Lobo

Racial disparities in renal transplant outcomes

Am J Kidney Dis (1999) 34:706–712

The purpose of our study was to evaluate the association of race and ethnicity with outcomes in the living related donor (LRD) renal transplant population, using multivariable adjustment for potential confounding variables. We prospectively analyzed 14,617 patients from the UNOS Renal Transplant Registry who underwent LRD renal transplantations in the United States between January 1, 1988 and December 31, 1996 using the Cox proportional hazards model. This model adjusts for the effects of potential genetic, social, and demographic confounding variables that may be associated with race or ethnicity long-term graft survival. Blacks were 1.8 times as likely as whites ($P<0.01$, RR=1.77) to suffer graft failure during the 9-year study period, which decreased minimally to 1.7 ($P<0.01$, RR=1.65) after controlling for potential confounding variables. Neither genotypic nor phenotypic HLA matching improved outcomes in blacks. Black renal transplant recipients had lower graft survival even after adjustment for matching and rejection, suggesting that non-HLA or socioeconomic mechanisms may contribute to racial differences in transplantation outcomes.