Enterohemorrhagic *Escherichia coli*

James B Kaper

Enterohemorrhagic *Escherichia coli* has been responsible for an increasing number of large food-borne outbreaks of bloody diarrhea and hemolytic uremic syndrome. Recent developments in our understanding of the pathogenesis of disease due to enterhemorrhagic *E. coli* include the description of a pathogenicity island, a type III secretion system and potential plasmid-encoded virulence factors. Recent developments in our understanding of the epidemiology include a recognition of a widening spectrum of vehicles.

Addresses
Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St., Baltimore, Maryland, 21201-1509, USA; e-mail: jkaper@umabnet.ab.umd.edu

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Abbreviations

EHEC	enterohemorrhagic *E. coli*
EPEC	enteropathogenic *E. coli*
HUS	hemolytic uremic syndrome
Stx	Shiga toxin
REPEC	rabbit enteropathogenic *E. coli*

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) is an emerging pathogen that has stimulated worldwide interest in several large food-borne outbreaks. EHEC can cause nonbloody diarrhea, bloody diarrhea, and hemolytic uremic syndrome (HUS) in all age groups, but the young and the elderly are most susceptible. The most notorious *E. coli* serotype associated with EHEC is O157:H7, which has been the cause of several large outbreaks of disease in North America, Europe, and Japan.

The most important virulence factor of EHEC is a potent cytotoxin known as Shiga toxin or verocytoxin (VT). This toxin was also previously known as Shiga-like toxin (SLT) but an increasing number of reports employ the Shiga toxin nomenclature. In addition to the toxin, a number of other potential virulence factors have recently been described for this pathogen. This review will focus on new studies of the pathogenesis of EHEC-associated disease and will also briefly review recent clinical and epidemiologic observations. A detailed discussion of the detection and diagnosis of EHEC infections can be found in a current review [1•].

Pathogenesis

Shiga toxin

The most important virulence factor in the pathogenesis of disease due to EHEC is Shiga toxin (Stx) (reviewed in [2]). The Shiga toxin family contains two major, immunologically non-cross-reactive groups called Stx1 and Stx2; there are multiple subtypes of Stx2 (Stx2c, Stx2v, Stx2e etc.). This phage-encoded toxin consists of one A subunit and five identical B subunits. The B subunit pentamer binds to a specific cell surface glycolipid receptor, globotriaosyceramide or Gb_3; one variant, Stx2e, binds to Gb_4 (reviewed in [3•]). Shiga toxin enters host cells via clathrin-coated pits and is transported via the trans-Golgi network to the endoplasmic reticulum (reviewed in [4]). The A subunit inhibits host cell protein synthesis by an *N*-glycosidase activity that removes an adenine residue from the 28S rRNA. A novel therapy for preventing HUS has been proposed which uses a Gb_3 receptor analogue to bind Stx in infected patients [5]. This compound, called Synsorb PK, is currently being studied in clinical trials in Canada.

Stx is presumed to translocate from the intestine to the bloodstream, although the toxin has never been detected in the blood of patients. Acheson *et al.* [6•] report that, in a tissue culture model using polarized intestinal epithelial cells, toxin translocation requires energy and the toxin moves across epithelial cells without apparent cellular damage, probably through a transcellular pathway. Stx is cytotoxic to human renal endothelial cells and the typical renal histopathology consists of swollen glomerular endothelial cells and deposition of platelets and fibrin within the glomeruli. The decreased glomerular filtration rate leads to the acute renal failure seen with HUS. Stx is believed to directly damage the glomerular endothelial cells although there are also data supporting a role for cytokines in the disease process. Increased levels of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 are expressed by murine peritoneal macrophages in response to oral treatment with purified Stx [7]. Several cytokines, including TNF-α, IL-1, and TNF-β, as well as bacterial lipopolysaccharide (LPS) have been shown to induce expression of Gb_3 and increase binding of Stx to human endothelial cells [8–10]. Recent studies using LPS-responder and LPS-nonresponder mice demonstrated the importance of both LPS and Stx in the pathogenesis of disease [11•]. The relative contributions of direct toxin action, cytokine production, and other bacterial factors in the renal damage seen in HUS are very active areas of research.

The various forms of Shiga toxin do not all behave identically *in vitro* or *in vivo*. The Stx2e variant is associated almost exclusively with pig edema disease rather than human disease. There are epidemiological data suggesting that strains expressing Stx2 are more often associated with progression to HUS than strains producing Stx1 alone [12] as well as *in vitro* data with cultured

human renal endothelial cells suggesting that Stx2 is more cytotoxic to these cells than is Stx1 [13]. Melton-Celsa *et al.* [14••] showed that one variant of Stx2, Stx2vhb, showed increased cytotoxic activity when incubated with intestinal mucus whereas other Stx forms were not affected after incubation with mucus. Strains expressing this Stx variant require a lower dose for disease in mice than other EHEC strains and the authors suggest that activation of this one toxin form by intestinal mucus could account for the lower LD_{50} values (dose at which 50% of the mice died) seen with these strains.

Signal transduction

In addition to signals induced by Shiga toxins, other signal transduction responses follow adhesion of EHEC to epithelial cells. EHEC have been shown to produce in cell culture and animal models the attaching and effacing (A/E) histopathology that is characteristic of infection with enteropathogenic *E. coli* (EPEC). EPEC is a major cause of nonbloody diarrhea in infants. A crucial distinction between EPEC and EHEC is that the former do not produce Shiga toxin. The A/E lesion is characterized by effacement of the intestinal epithelial microvilli and intimate adherence between the bacterium and the epithelial cell membrane. Directly beneath the adherent bacteria, marked cytoskeletal changes are seen, including accumulation of polymerized actin. The cellular changes associated with the A/E lesion have been best studied with EPEC and include increases in inositol phosphates and intracellular calcium, activation of protein kinase C, phosphorylation of myosin light chain, and tyrosine phosphorylation of a 90 kDa protein inserted into the epithelial cell membrane called Hp90 (reviewed in [15]). Ismaili *et al.* [16] examined signal transduction responses following adhesion of EHEC to human epithelial cells and found elevated levels of inositol 1,4,5-triphosphate (IP_3) and intracellular free calcium, but not did not see tyrosine phosphorylation activity. An inflammatory response is also induced in response to EHEC adherence, apparently associated with the A/E histopathology. Increased levels of IL-8 have been reported in cultured human colon epithelial cells infected with EHEC [17]. Studies in rabbits show that the addition of anti-CD18 antibody blocks the influx of polymorphonuclear leukocytes in response to *E. coli* O157:H7 infection [18]. Inhibition of this inflammatory response led to a reduction, but not elimination, of diarrhea in this model.

Intimin

Intimin is a 94–97 kDa outer membrane protein that is the only intestinal colonization factor yet identified for EHEC. Intimin is encoded by the *eae* gene [19] and is expressed by both EPEC and EHEC as well as by the mouse pathogen *Citrobacter rodentium* and the rabbit pathogen REPEC; the latter two pathogens also produce A/E histopathology in their respective hosts (reviewed in [1•]). The importance of intimin in colonization is shown by studies in piglets using *eae* mutants of O157:H7 [20,21] and in volunteer studies using an *eae* mutant of EPEC [22]. Intimins expressed by EPEC, EHEC, REPEC, and *C. rodentium* share remarkably high homology (>95% identity) throughout most of their sequence but can vary considerably in sequence in the carboxy-terminal 250 amino acid residues [23•]. Intimin from different A/E pathogens can be grouped into at least three different classes based on the carboxy-terminal sequence [23•]. Both human and animal strains can be found in the individual classes and no specific sequence has been exclusively associated with a human or animal strain.

There have been two different receptors identified for intimin from EPEC, although studies addressing the receptor for intimin from EHEC have not been reported. Rosenshine *et al.* [24] report that intimin binds to a 90 kDa protein inserted into the epithelial cell membrane that is tyrosine phosphorylated in response to EPEC infection. The tyrosine phosphorylation activates the receptor binding activity. Frankel *et al.* [25] report that intimin binds to β_1 intergrins, specifically $\alpha_4\beta_1$ and $\alpha_5\beta_1$. The binding activity to integrins has been localized to the carboxy-terminal 280 residues and incubation with an RGD-containing peptide could block the adhesion. These two reports are not necessarily mutually exclusive and it is possible that different portions of intimin can bind to different receptors.

A type III secretion system

A type III secretion system has recently been reported for EHEC [26•]. The genes in this system, designated *sep* [27] and *esc* (S Elliott *et al.*, unpublished data), encode a number of protein products which share homology with a number of type III secretion system proteins from *Yersinia*, *Salmonella*, and *Shigella* spp. The proteins secreted by this pathway are called Esps (*E. coli* secreted proteins) and include EspA (24–25 kDa), EspB (37 kDa) and EspD (39 kDa) [26•,28], although the latter has been reported only for EPEC; the *espD* gene is present in *E. coli* O157:H7 but detection of the EspD protein has not yet been reported. A larger protein of 100–110 kDa is also secreted extracellularly by EHEC, although not via the type III pathway [26•]. This larger protein is the plasmid-encoded EspP described below. The secreted proteins have been reported not only for EHEC of the O157:H7 serotype but also for strains of O26:H11 and O111:H-serotypes [28]. In EPEC, mutation of the *espA*, *espB*, and *espD* genes inactivates the signal transduction responses induced by EPEC and prevents formation of the A/E lesion [29–31]. The same effect is presumed to occur in EHEC, although the phenotypes of these mutations in EHEC have not yet been reported. The secreted proteins also engender a strong antibody response in HUS patients [26•].

The LEE pathogenicity island

Genes for intimin, the type III secretion system, and the EspA, B, and D secreted proteins are encoded on a 35 kilobase pathogenicity island called the LEE, for

locus of enterocyte effacement ([32]; S Elliott *et al.*, unpublished data). The LEE is found in EPEC, *C. rodentium*, REPEC, and all EHEC that contain the *eae* gene. Cloning of the entire EPEC LEE into *E. coli* strain K-12 confers the ability to produce the A/E lesion on this strain [33•]. In EPEC strain E2348/69 and EHEC O157:H7, the LEE is inserted at minute 82 on the *E. coli* chromosome, at the same site (*selC*) where an unrelated pathogenicity island for uropathogenic *E. coli* is inserted. To determine whether the the LEE is inserted at the same site in all EPEC and EHEC strains, Wieler *et al.* [34] examined a set of EPEC and EHEC strains that had previously been studied by multilocus enzyme electrophoresis analysis of housekeeping proteins. The results of this investigation indicated that the LEE can insert into sites other than *selC* and that the insertion site is correlated with the evolutionary lineage predicted by multilocus enzyme electrophoresis. Thus, the LEE has apparently been introduced into *E. coli* at multiple times during the evolution of EPEC and EHEC, although the possibility of an initial insertion into *selC* and a subsequent intrastrain deletion and reinsertion at a different site cannot be excluded. Some strains of EPEC and EHEC have arisen from one evolutionary branch (called EPEC1 and EHEC1) with the LEE inserted at *selC* and others have arisen from a different branch (EPEC2 and EHEC2) with insertion at a different site. A likely evolutionary scenario is that in each of these branches, ancestor strains contained only the LEE inserted at the same site. These strains then evolved into EPEC and EHEC by acquisition of the EAF plasmid (for EPEC) or the pO157 plasmid (for EHEC, see below), with a separate introduction of the bacteriophage encoding Stx providing the terminal differentiation into EHEC.

Other potential virulence factors

E. coli O157:H7 and most other EHEC associated with human disease possess a ≈90 kilobase plasmid called pO157 [35]. The role of this plasmid in pathogenesis is not certain since the majority of animal studies performed with EHEC strains lacking this plasmid show no difference compared with the plasmid-containing parent strain. This plasmid encodes a serine protease called EspP which cleaves human coagulation factor V [36]. This 104 kDa protein shares homology with a family of proteins termed autotransporters which includes the IgA1 protease of *Neisseria gonorrhoeae*, VacA of *Helicobacter pylori*, and pertactin of *Bordetella pertussis*, among other members. A potential role in virulence was proposed whereby degradation of factor V could contribute to the mucosal hemorrhage seen in hemorrhagic colitis [36]. A type II secretion system is also encoded on this plasmid but the secreted targets of this system have not been identified [37]. This plasmid also encodes a hemolysin of the RTX family [38,39]. In a collection of O111:H- EHEC strains from Germany, this hemolysin was expressed in 88% of strains isolated from patients with HUS but in only 22% of patients with diarrhea but without HUS [40]. Hemoglobin and heme released from lysed erythrocytes could serve as iron sources and a 69 kDa outer membrane protein (encoded on the chromosome) called ChuA has recently been discovered in O157:H7 that is an iron-regulated heme-transport protein [41].

Non-O157:H7 serotypes

Over 200 different *E. coli* serotypes have been shown to produce Shiga toxin [42••]. By far the greatest attention has been paid to the O157:H7 serotype because it is the most prevalent EHEC serotype in the United States, Canada, United Kingdom, and Japan. In many countries of Europe as well as in Australia and Argentina, however, other serotypes are more prevalent than O157:H7. The difficulty arises in assessing the importance of these other serotypes because the majority of these serotypes are assumed to be nonpathogenic for humans [1•]. Unlike O157:H7, which can be detected on the inexpensive sorbitol MacConkey (SMAC) agar as a consequence of its sorbitol-negative phenotype, detecting the non-O157:H7 EHEC serotypes requires the use of expensive Stx-based assays. The issue of non-O157:H7 EHEC serotypes has been specifically reviewed in two recent publications [42••,43•].

The O157:H7 serotype has the complete range of proven and potential EHEC virulence factors including those encoded on the LEE pathogenicity island and the pO157 plasmid. Two of the most important non-O157:H7 serotypes, O26:H11 and O111:H-, also usually contain the LEE and pO157. But many other EHEC serotypes only express Stx without any other known virulence factors. It is thought that Stx alone is not sufficient to cause disease. Support for this idea is provided by Samadpour *et al.* [44], who reported the presence of Stx-producing *E. coli* of non-O157:H7 serotypes in 23% of beef, 48% of lamb, and 63% of veal samples from grocery stores in Seattle. It is assumed that there are additional virulence factors that are expressed by some but not all of these serotypes that enable them to cause disease.

Recent epidemiology

Reports of outbreaks and sporadic cases of EHEC infection have been increasing in recent years, in part due to better reporting and in part due to a genuine increase in infections. Despite the increasing awareness of EHEC infections on the part of public health officials and the public in general, the outbreak of EHEC disease in Japan in the summer of 1996 was a great surprise because the size of the outbreak far exceeded any previously reported outbreaks. Over 9000 cases were reported, which exceeded the largest previously reported outbreak by an order of magnitude. Molecular epidemiologic techniques, specifically pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), showed that this large outbreak actually consisted of multiple outbreaks with the largest cluster of cases (>6000 cases) showing PFGE and RAPD-

PCR patterns different from those found in isolates epidemiologically unrelated to this cluster [45]. PFGE has also been used for routine surveillance to identify otherwise undetected outbreaks [46•] and is being adapted to establish national databases for rapid strain comparison.

The largest cluster of cases in Japan was linked to the consumption of radish sprouts [47], which illustrates an emerging trend for the epidemiology of EHEC infections. Transmission of EHEC had initially been associated with consumption of beef products and subsequently with water-borne outbreaks and person-to-person transmission. The spectrum of vehicles has widened to now include uncooked vegetables and fruits such as lettuce and apple juice. Many of these uncooked vehicles are contaminated by animal feces during growth or processing but this is not always the case. Investigation of a recent outbreak of infection in the United States associated with eating alfalfa sprouts implicated contaminated alfalfa seeds which were shipped to two states where they were cultivated, processed, and consumed [48].

The spectrum of animal reservoirs is also increasing. In addition to cows, Stx-producing *E. coli* have been isolated from a number of animal species including sheep, goats, pigs, cats, dogs, poultry, and deer. A recent report from the United Kingdom shows that wild birds, particularly sea gulls, were also found to harbor O157:H7 [49]. In this study, 0.9% of fecal samples of birds feeding at an urban landfill and 2.9% of samples on intertidal sediments were positive for O157:H7. The widespread distribution of seagulls and the low infectious dose necessary to cause disease offers additional possibilities for transmission of disease due to EHEC.

A recent clinical report describes a potentially troublesome development in EHEC disease. Tarr *et al.* [50] describe HUS in a six-year-old girl after a urinary tract infection with an EHEC of serotype O103:H2. The development of HUS after this infection suggests that the human uroepithelium, like the gastrointestinal epithelium, might permit the absorption of Stx. The strain possesses the *eae* and stx_1 gene but the question of whether it may also have additional virulence factors that allow it to colonize the urinary tract in addition to the gastrointestinal tract is unanswered.

Conclusions

Infections due to enterohemorrhagic *E. coli* are receiving increasing attention from public health officials, infectious disease specialists, cell biologists, and microbiologists. The widening spectrum of vehicles, particularly foods that are normally consumed raw, complicates efforts to control transmission. Most of the research on pathogenesis has focused on the Shiga toxin but there have been recent insights into intestinal adherence factors and other potential virulence factors of EHEC. Further characterization of these and other as-yet-undiscovered virulence factors will be active areas of research in the future.

Note added in proof

Djafari *et al.* [51] have recently reported the cloning of a gene encoding a serine protease produced by Shiga toxin-producing *E. coli* (STEC). The authors call this gene *pssA* for protease secreted by STEC. Examination of the *pssA* gene sequence shows that it is identical to the *espP* gene previously published by Brunder *et al.* [36]. The article by Brunder *et al.* was published while the article by Djafari *et al.* [51] was in press.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Nataro JP, Kaper JB: **Diarrheagenic *Escherichia coli*.** *Clin Microbiol Rev* 1998, **11**:in press.

This comprehensive review covers all aspects of pathogenesis, epidemiology, detection, and treatment for all six classes of diarrheagenic *E. coli*. Many primary references that cannot be included in the present review due to space limitation will be found in this review.

2. O'Brien AD, Tesh VL, Donohue-Rolfe A, Jackson MP, Olsnes S, Sandvig K, Lindberg AA, Keusch GT: **Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis.** *Curr Top Microbiol Immunol* 1992, **180**:65-94.

3. • Lingwood CA: **Role of verotoxin receptors in pathogenesis.** *Trends Microbiol* 1996, **4**:147-153.

An extensive review of the glycolipid receptors for Shiga toxin that includes the structure of the receptor, the receptor-binding site of Shiga toxin, intracellular transport of the toxin, and signal transduction induced by the toxin.

4. Sandvig K, Van Deurs B: **Endocytosis and intracellular sorting of ricin and Shiga toxin.** *FEBS Lett* 1994, **346**:99-102.

5. Armstrong GD, Rowe PC, Goodyer P, Orrbine E, Klassen TP, Wells G, Mackenzie A, Lior H, Blanchard C, Auclair F *et al.*: **A phase I study of chemically synthesized verotoxin (Shiga-like toxin) Pk-trisaccharide receptors attached to chromosorb for preventing hemolytic-uremic syndrome.** *J Infect Dis* 1995, **171**:1042-1045.

6. • Acheson DWK, Moore R, Debreucker S, Lincicome LL, Jacewicz M, Skutelsky E, Keusch GT: **Translocation of Shiga toxin across polarized intestinal cells in tissue culture.** *Infect Immun* 1996, **64**:3294-3300.

This paper examines an aspect of pathogenesis that has received little attention, namely, how Shiga toxin (Stx) translocates from the intestine to the bloodstream. The authors show that biologically active Stx1 can move across intact polarized intestinal epithelial cells without apparent cellular damage, probably via a transcellular pathway, perhaps through nonspecific endocytosis. Much more research is needed on this topic but these authors make a good start in studying this process.

7. Tesh VL, Ramegowda B, Samuel JE: **Purified Shiga-like toxins induce expression of proinflammatory cytokines from murine peritoneal macrophages.** *Infect Immun* 1994, **62**:5085-5094.

8. Louise CB, Obrig TG: **Shiga toxin-associated hemolytic uremic syndrome: combined cytotoxic effects of Shiga toxin, interleukin-1β, and tumor necrosis factor alpha on human vascular endothelial cell *in vivo*.** *Infect Immun* 1991, **59**:4173-4179.

9. Kaye SA, Louise CB, Boyd B, Lingwood CA, Obrig TG: **Shiga toxin-associated hemolytic uremic syndrome: interleukin-1β enhancement of Shiga toxin cytotoxicity toward human vascular endothelial cells *in vitro*.** *Infect Immun* 1993, **61**:3886-3891.

10. Van de Kar NCAJ, Monnens LAH, Karmali MA, Hinsbergh VWM: **Tumor necrosis factor and interleukin-1 induce expression of the verotoxin receptor globotriaosylceramide on human endothelial cells: implications for the pathogenesis of the hemolytic uremic syndrome.** *Blood* 1992, **80**:2755-2764.

11. • Karpman D, Connell H, Svensson M, Scheutz F, Alm P, Svanborg C: **The role of lipopolysaccharide and Shiga-like toxin in a mouse model of *Escherichia coli* O157:H7 infection.** *J Infect Dis* 1997, **175**:611-620.
This work is notable because it reproduces nearly all symptoms of the disease, including gastrointestinal (although not bloody diarrhea), neurological, and glomerular symptoms in a single, orally inoculated convenient animal model, a feat which other animal models have had difficulty achieving. This model also produced bacteremia in many animals, in contrast to the usual absence of bacteremia in human disease. This work uses genetically defined mouse strains to show that lipopolysaccharide is important for disease development.

12. Griffin PM: ***Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*.** In *Infections of the Gastrointestinal Tract.* Edited by Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL. New York: Raven Press; 1995:739-761.

13 Louise CB, Obrig TG: **Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cells.** *J Infect Dis* 1995, **172**:1397-1401.

14. •• Melton-Celsa AR, Darnell SC, O'Brien AD: **Activation of Shiga-like toxins by mouse and human intestinal mucus correlates with virulence of enterohemorrhagic *Escherichia coli* O91:H21 isolates in orally infected, streptomycin-treated mice.** *Infect Immun* 1996, **64**:1569-1576.
The authors show that one variant of Shiga toxin is activated by the presence of intestinal mucus which results in a lower LD_{50} in mice. This result is particularly noteworthy because it is seen with a non-O157:H7 strain that lacks the LEE pathogenicity island and the intimin intestinal colonization factor. The activatable toxin may help explain why such strains can cause disease, i.e. the more active toxin may help compensate for the lack of the LEE and intimin. Another lesson to be learned from this study is the importance of keeping the actual *in vivo* disease conditions in mind when studying pathogenesis, i.e. the activity of the toxin should be examined in the presence of intestinal mucus rather than only as a highly purifed preparation in isolation.

15. Donnenberg MS, Kaper JB, Finlay BB: **Interactions between enteropathogenic *Escherichia coli* and host epithelial cells.** *Trends Microbiol* 1997, **5**:109-114.

16. Ismaili A, Philpott DJ, Dytoc MT, Sherman PM: **Signal transduction responses following adhesion of verocytotoxin-producing *Escherichia coli*.**. *Infect Immun* 1995, **63**:3316-3326.

17. Jung HC, Eckmann L, Yang S, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF: **A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion.** *J Clin Invest* 1995, **95**:55-65.

18. Elliott E, Li Z, Bell C, Stiel D, Buret A, Wallace J, Brzuszczak I, O'Loughlin E: **Modulation of host response to *Escherichia coli* O157:H7 infection by anti-CD18 antibody in rabbits.** *Gastroenterology* 1994, **106**:1554-1561.

19. Jerse AE, Yu J, Tall BD, Kaper JB: **A gentic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells.** *Proc Natl Acad Sci USA* 1990, **87**:7839-7843.

20. Tzipori S, Gunzer F, Donnenberg MS, Demontigny L, Kaper JB, Donohue-Rolfe A: **The role of the *eaeA* gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic *Escherichia coli* infection.** *Infect Immun* 1995, **63**:3621-3627.

21. Donnenberg MS, Tacket CO, James SP, Losonsky G, Nataro JP. Wasserman SS, Kaper JB, Levine MM: **Role of the *eaeA* gene in experimental enteropathogenic *Escherichia coli* infection.** *J Clin Invest* 1993, **92**:1412-1417.

22. Mckee ML, Melton-Celsa AR, Moxley RA, Francis DH, O'Brien AD: **Enterohemorrhagic *Escherichia coli* O157:H7 requires intimin to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells.** *Infect Immun* 1995, **63**:3739-3744.

23. • Agin TS, Wolf MK: **Identification of a family of intimins common to *Escherichia coli* causing attaching-effacing lesions in rabbits, humans, and swine.** *Infect Immun* 1997, **65**:320-326.
This study combines previously published sequences along with new data to show the existence of a family of intimin proteins with at least three different groups or subfamilies. Interestingly, sequences from some human isolates are closer to intimins from animal isolates than to other human isolates.

24. Rosenshine I, Ruschkowski S, Stein M, Reinscheid D, Mills SD, Finlay BB: **A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation.** *EMBO J* 1996, **15**:2613-2624.

25. Frankel G, Lider O, Hershkoviz R, Mould AP, Kachalsky SG, Candy DCA, Cahalon L, Humphries MJ, Dougan G: **The cell-binding domain of intimins from enteropathogenic *Escherichia coli* binds to β_1 integrins.** *J Biol Chem* 1996, **271**:20359-20364.

26. • Jarvis KG, Kaper JB: **Secretion of extracellular proteins by enterohemorrhagic *Escherichia coli* via a putative type III secretion system.** *Infect Immun* 1996, **64**:4826-2829.
The existence of a type III secretion system for enterohemorrhagic *E. coli* of the O157:H7 and O26:H11 serotypes is demonstrated in this paper. Type III secretion systems have been shown to be crucial in presenting virulence factors to host cells in a variety of Gram-negative pathogens. This study also shows that proteins secreted by the type III system are expressed *in vivo* as strong antibody responses against them are seen in serum from patients with hemolytic uremic syndrome.

27. Jarvis KG, Girón JA, Jerse AE, McDaniel TK, Donnenberg MS, Kaper JB: **Enteropathogenic *Escherichia coli* contains a specialized secretion system necessary for the export of proteins involved in attaching and effacing lesion formation.** *Proc Natl Acad Sci USA* 1995, **92**:7996-8000.

28. Ebel F, Deibel C, Kresse AU, Guzman CA, Chakraborty T: **Temperature- and medium-dependent secretion of proteins by Shiga toxin – producing *Escherichia coli*.** *Infect Immun* 1996, **64**:4472-4479.

29. Kenny B, Lai L, Finlay BB, Donnenberg MS: **EspA, a protein secreted by enteropathogenic *Escherichia coli*, is required to induce signals in epithelial cells.** *Mol Microbiol* 1996, **20**:313-324.

30. Foubister V, Rosenshine I, Donnenberg MS, Finlay BB: **The *eaeB* gene of enteropathogenic *Escherichia coli* (EPEC) is necessary for signal transduction in epithelial cells.** *Infect Immun* 1994, **62**:3038-3040.

31. Lai L-C, Wainwright LA, Stone KD, Donnenberg MS: **A third secreted protein that is encoded by the enteropathogenic *Escherichia coli* pathogenicity island is required for transduction of signals and for attaching and effacing activities in host cells.** *Infect Immun* 1997, **65**:2211-2217.

32. McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB: **A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens.** *Proc Natl Acad Sci USA* 1995, **92**:1664-1668.

33. • McDaniel TK, Kaper JB: **A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K-12.** *Mol Microbiol* 1997, **23**:399-407.
This work demonstrates that the LEE pathogenicity island is not only necessary for producing the attaching and effacing histopathology due to enteropathogenic *E. coli*, it is sufficient when cloned into K-12 or normal flora *E. coli.* This is the first example of a pathogenicity island from an enteric pathogen that can reproduce the pathognomic histopathology when cloned into *E. coli* K-12.

34. Wieler LH, McDaniel TK, Whittam TS, Kaper JB: **Insertion site of the locus of enterocyte effacement in enteropathogenic and enterohemorrhagic *Escherichia coli* differs in relation to the clonal phylogeny of the strains.** *FEMS Microbiol Lett* 1997, in press.

35. Schmidt H, Kernbach C, Karch H: **Analysis of the EHEC *hly* operon and its location in the physical map of the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7.** *Microbiology* 1996, **142**:907-914.

36. Brunder W, Schmidt H, Karch H: **EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V.** *Mol Microbiol* 1997, **24**:767-778.

37. Schmidt H, Henkel B, Karch H: **A gene cluster closely related to type II secretion pathway operons of Gram-negative bacteria is located on a large plasmid of enterohemorrhagic *Escherichia coli* O157 strains.** *FEMS Microbiol Lett* 1997, **148**:265-272.

38. Schmidt H, Beutin L, Karch H: **Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933.** *Infect Immun* 1995, **63**:1055-1061.

39. Bauer ME, Welch RA: **Characterization of an RTX toxin from enterohemorrhagic *Escherichia coli* O157:H7.** *Infect Immun* 1996, **64**:167-175.

40. Schmidt H, Karch H: **Enterohemolytic phenotypes and genotypes of Shiga toxin-producing *Escherichia coli* O111 strains from patients with diarrhea and hemolytic uremic syndrome.** *J Clin Microbiol* 1996, **34**:2364-2367.

41. Torres AG, Payne SM: **Haem iron-transport system in enterohaemorrhagic *Escherichia coli* O157:H7.** *Mol Microbiol* 1997, **23**:825-833.

42. •• Johnson RP, Clarke RC, Wilson JB, Read SC, Rahn K, Renwick SA, Sandhu KA, Alves D, Karmali MA, Lior H *et al.*: **Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*.** *J Food Protect* 1996, **59**:1112-1122.

This very comprehensive article reviews all aspects of non-O157:H7 enterohemorrhagic *E. coli* serotypes. The distribution of these serotypes in humans, animals, and foods is reviewed and the occurrence of potential virulence factors in these strains is discussed. Very useful tables summarizing the serotypes specifically associated with human disease and the reported outbreaks due to these serotypes are given. It is unfortunate that it was not published in a journal that is more widely available in the medical community.

43. • Tarr PI, Neill MA: **Perspective: The problem of non-O157:H7 Shiga toxin (verocytotoxin) - producing *Escherichia coli*.** *J Infect Dis* 1996, **174**:1136-1139.

This commentary briefly reviews non-O157:H7 enterohemorrhagic *E. coli* but more importantly, presents thoughtful guidance to clinicians on the diagnosis and significance of these serotypes in clinical specimens.

44. Samadpour M, Ongerth JE, Liston J, Tran N, Nguyen D, Whittam TS, Wilson RA, Tarr PI: **Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington.** *Appl Environ Microbiol* 1994, **60**:1038-1040.

45. Watanabe H, Wada A, Inagaki Y, Itoh K, Tamura K: **Outbreak of enterohaemorrhagic *Escherichia coli* O157:H7 infection in two different genotype strains in Japan, 1996.** *Lancet* 1996, **348**:831-832.

46. • Bender JB, Hedberg CW, Besser JM, Boxrud DJ, Macdonald KL, Osterholm MT: **Surveillance for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping.** *N Engl J Med* 1997, **337**:388-394.

This article describes the use of pulsed-field gel electrophoresis as a molecular-subtyping system to discriminate various strains of *E. coli* O157:H7 submitted to the Minnesota Department of Health. This method detected four outbreaks that were not detected by traditional methods and also established that sudden increases in reported cases were more commonly due to sporadic isolated cases rather than outbreaks.

47. Michino H, Araki K, Minami S, Takaya S, Sakai N: **Investigation of large-scale outbreak of *Escherichia coli* O157:H7 infection among schoolchildren in Sakai City, 1996.** In *Abstracts of the 32nd Joint US-Japan Cooperative Medical Science Program Cholera and Related Diarrheal Diseases Panel*: 1996 Nov; Nagasaki, Japan.

48. Centers for Disease Control: **Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts - Michigan and Virginia, June–July 1997.** *Morbid Mortal Weekly Rep* 1997, **46**:741-744.

49. Wallace JS, Cheasty T, Jones K: **Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds.** *J Appl Microbiol* 1997, **82**:399-404.

50. Tarr PI, Fouser LS, Stapleton AE, Wilson RA, Kim HH, Vary JC Jr, Clausen CR: **Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2.** *N Engl J Med* 1996, **335**:635-637.

51. Djafari S, Ebel F, Deibel C, Krämer S, Hudel M, Chakraborty T: **Characterization of an exported protease from Shiga toxin-producing *Escherichia coli*.** *Mol Microbiol* 1997, **25**:771-784.