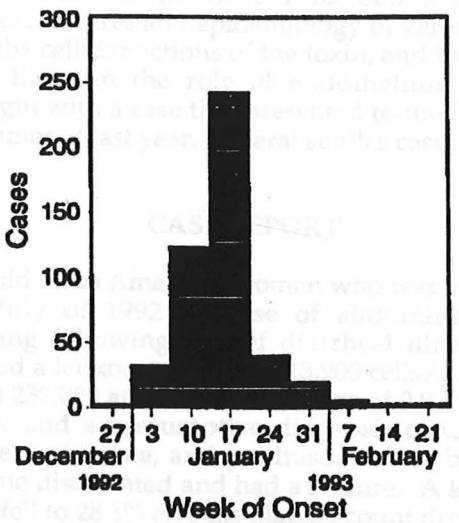
SHIGA- AND SHIGA-LIKE TOXINS IN HEMOLYTIC-UREMIC SYNDROME AND THROMBOTIC THROMBOCYTOPENIC PURPURA



MEDICAL GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER
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INTRODUCTION

Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are overlapping syndromes that have puzzled hematologists for decades. Recent evidence has shown that the overwhelming majority of cases of HUS (and a smaller but growing proportion of cases of TTP) are caused by a toxin produced by enteric bacteria. The toxin, referred to as verotoxin or Shiga-like toxin, causes direct damage to endothelial cells and produces the clinical syndrome. Recent epidemics of hemorrhagic colitis associated with a verotoxin-producing E. coli strain O157:H7 have occurred in several states in the Far West. Between November 15, 1992 and February 28, 1993, more than 500 confirmed infections with E. coli O157:H7 occurred in Washington, Idaho, California, and Nevada. Forty patients developed HUS and four died, all of them children. Nearly all of the cases were associated with the ingestion of ground meat eaten at a particular fast food restaurant chain. The purpose of this medical grand rounds is to illustrate the clinical features and epidemiology of verotoxin-mediated HUS and TTP, to delineate the cellular actions of the toxin, and to investigate whether the toxin sheds any light on the role of endothelium in anti-thrombotic mechanisms. We'll begin with a case that presented to the Hematology Consult Service during the summer of last year. Several similar cases were seen in the fall and winter of '92-'93.

CASE REPORT

F. G. is a 66 year-old Latin American woman who was admitted to the PMH Surgical Service in July of 1992 because of abdominal pain and lower gastrointestinal bleeding following a brief diarrheal illness. Her admitting laboratory data included a leukocyte count of 13,900 cells/cu mm, hematocrit of 47.9%, platelet count of 232,000 and serum creatinine of 0.8 mg/dl. Colonoscopy revealed severe colitis, and a presumptive diagnosis of Crohn's disease was made. Azulfadine, metronidazole, and prednisone were begun. On the fifth hospital day, she became disoriented and had a seizure. A low-grade fever was noted. The hematocrit fell to 28.1% and the platelet count dropped to 18,000 over the course of less than 48 h. A peripheral blood smear showed fragmented erythrocytes. A rise in serum creatinine to 1.8 was noted, and she became anuric. The prothrombin time was 13.6 seconds and the partial thromboplastin time remained unchanged at 25.0 seconds. The patient was treated emergently with dialysis, fresh frozen plasma, daily plasma exchange, and intravenous gamma-globulin. Despite multiple complications including bacterial sepsis, prolonged intubation, and candidemia, necessitating a two month hospital stay, she recovered fully and has remained well since discharge.

HUS AND TTP: CLINICAL FEATURES

TTP was described as a distinct clinicopathologic entity by Moschcowitz nearly 70 years ago (1). In this classic paper, entitled "An Acute Febrile Pleiochromic Anemia With Hyaline Thrombosis Of Terminal Arterioles," the pathological hallmark of TTP (arterial hyaline thrombi) was first noted. The

classic "pentad" of findings in TTP were brought to the fore in 1966 in an important review article in Medicine by Amorosi and Ultmann (2). These pentad features are microangiopathic hemolytic anemia, thrombocytopenia, neurological manifestations, fever and renal disease (Fig. 1).

TTP "PENTAD"

1. **MICROANGIOPATHIC HEMOLYTIC ANEMIA**
2. **THROMBOCYTOPENIA**
3. **RENAL: HEMATURIA, PROTEINURIA, ACUTE RENAL FAILURE**
4. **NEUROLOGIC: HEADACHE, PARESIS, COMA, SEIZURES**
5. **FEVER**

ONLY 40% OF PATIENTS WILL HAVE ALL FIVE!

Fig. 1

Of course, there is no "gold standard" for the diagnosis of TTP; it is a constellation of signs and symptoms occurring in an appropriate clinical setting. One would be hard-pressed to make the diagnosis in the absence of either microangiopathic hemolytic anemia and some degree of thrombocytopenia; the other features (fever, neurological signs/symptoms, and renal dysfunction) may be fluctuating or mild. Actually, a minority of patients will have all five of the features during the clinical course.

The mean hematologic values for the patients described in the Amorosi review are presented in Fig. 2. The anemia and thrombocytopenia are usually quite severe. The findings of depressed fibrinogen level and prolongation of the PT and PTT should make one consider the diagnosis of disseminated intravascular coagulation (DIC) instead. However, some patients with TTP will exhibit an elevated titer of fibrin split products, presumably as a result of local thrombin production and fibrinogenolysis.

Mean Laboratory Values For 25 Patients With TTP

Hct	**21.3**	**fibrinogen**	**225**
platelet count	**20,500**	**PT**	**(normal in 22/25)**
wbc	**14,300**	**PTT**	**(normal in 24/25)**
retic count	**14%**	**FDPs**	**(25% > 1:32)**

Fig. 2 (adapted from (3))

The renal dysfuction in TTP is mild, in contrast to the picture seen in HUS, in which renal failure is an invariant feature, with about 70% of patients requiring dialysis (4). Only 12% of patients with classic TTP develop frank renal failure (Fig. 3). Of note, about 13% of 250 patients in this review complained of abdominal pain, and melena was a feature in 8%.

RENAL MANIFESTATIONS OF TTP

Microscopic hematuria	**61%**
Gross hematuria	**15%**
Proteinuria-any	**59%**
> 2 g/day	**1.5%**
> 5g/day	**1.5%**
Cr > 2.0	**45%**
Cr > 5.0	**12%**
Acute renal failure	**12%**

Fig. 3. (adapted from (3))

HUS was first described by Gasser, et al. in 1955 in a paper entitled "Hemolytic-Uremic Syndrome: Bilateral Renal Cortical Necrosis And Acute Acquired Hemolytic Anemia" (translated from the German). In this paper the cardinal triad of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failute are described. Interestingly, a fourth feature (neurologic

dysfunction similar to that seen in TTP) was also included in this original description. HUS is commonly thought of as a disease of children. A prodrome consisting of a diarrheal illness (occurring in 90% of cases (5)), often with bloody stools, has also been noted, and it is interesting that this has not been emphasized as a cardinal feature of the disease. (In retrospect, it is an important finding.) HUS is the leading cause of acute renal failure in children and carries with it a 10% mortality and a 25% chance of residual renal or neurologic deficits. The relapse rate is low (4).

Fig. 4 provides a comparison of some distinguishing features of HUS and TTP.

Feature	HUS	TTP
Age:	Children	Adults
Diarrhea:	Yes	Occasional
Renal:	ARF	Mild
Neuro:	Rare	Common
Relapses	V. Rare	Common (8-37%)

Fig. 4

The relapse rate in TTP is increasing because more patients are surviving the initial episode. In a more recent study, the rate of relapse approached 37% (6). The relapse rate in HUS is very low. Interestingly, a hereditary form has been reported, with HUS or TTP occurring in multiple family members (7).

"VEROTOXIN" AS THE ETIOLOGIC AGENT IN HUS

In 1977, Konowalchuk, et. al. (8) took filtrates of E. coli cultures and placed them on monolayers of Vero cells (an African green monkey kidney cell line). Certain E. coli strains appeared to produce a potent cytotoxin that slowly killed the cells over the course of about four days (see Fig 5). The toxin was antigenically distinct from other E. coli enterotoxins, which did not share this type of cytotoxicity. They called the active agent "Verotoxin" (VT). Around the same time, O'Brien and coworkers (9) described a similar cytotoxin found in enteropathogenic strains of E. coli that was lethal for HeLa cells. They called the toxin Shiga-like toxin because, like Shiga toxin, it inhibited protein synthesis, was enterotoxic in the rabbit intestine, and killed mice. Later, it was appreciated that Shiga-like toxin and verotoxin were the same; subsequently, an antigenically distinct verotoxin, VT2 was characterized. We now know that Shiga toxin and Shiga-like toxin (VT1) differ only by one amino acid in their primary sequence (10, 11), whereas VT1 and VT2 share a 57% identity at the nucleotide level (12).

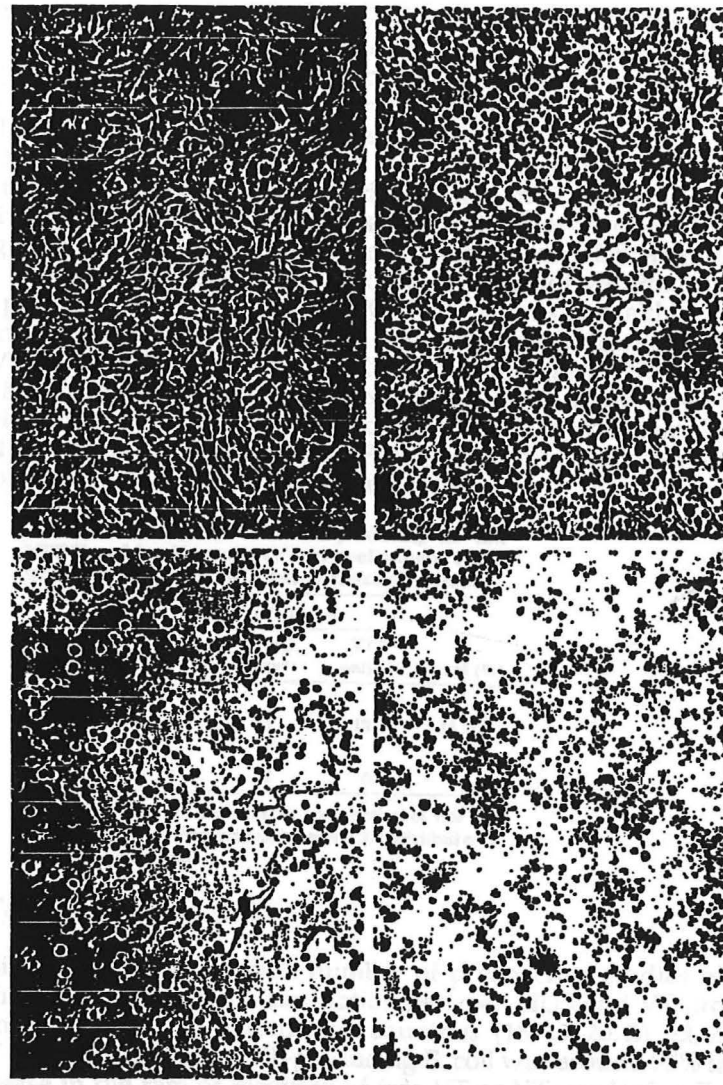

Fig. 5. Verotoxin cytopathic effect observed over the course of four days. From ref (8).

The pathological significance of verotoxins was uncertain, and would have remained so, if it weren't for two outbreaks of hemorrhagic colitis in 1982 that were associated with a rare E. coli serotype, O157:H7 (13). The two outbreaks, which occurred in early 1982 in Oregon and Michigan, affected 47 people and was characterized by severe cramping abdominal pain and initially watery diarrhea followed by grossly bloody diarrhea. All but two of the cases interviewed had eaten a hamburger at one local fast food restaurant chain, at a median of four days prior to the attack. Screening for routine enteric pathogens

was negative, but a rare E. coli O157:H7 serotype was isolated from 12 cases and from a hamburger from the restaurant chain. Only one infection due to this serotype had been reported previously, in a woman with bloody diarrhea in 1975. Shortly thereafter, O'Brien and colleagues documented the production of high levels of shiga-like toxin by this serotype (9).

The key observation linking verotoxin production to HUS came from investigators at the Hospital for Sick Children in Toronto (14, 15). A verotoxin-positive strain of E.coli was isolated from the bowel of an 18-month old boy dying of HUS. Given the previous association of HUS with bloody diarrhea, these investigators decided to look for evidence of verotoxin prospectively in their patients with HUS. Fully 60% of 40 patients had either free verotoxin or verotoxin-producing E. coli isolated from the stool, and an additional 16 patients had VT-neutralizing antibodies in their sera; therefore, 75% of the patients with HUS had evidence of verotoxin-producing E. coli. None of 40 control patients had either free verotoxin or VT-producing E. coli in stool samples; sera was not tested for neutralizing antibodies. Free verotoxin was a more sensitive test than the ability to culture the offending organism.

Evidence of Verotoxin-producing *E. coli* (VTEC) and free Verotoxin (VT) in stool samples of patients with idiopathic HUS and in matched controls.

	No. with following:			
Subjects	VTEC and VT	VTEC only*	VT only†	Total (%)
Patients (n = 40)	9	3	12	24 (60)
Controls (n = 40)	0	0	0	0

* Stool samples not available for investigation of free VT.
† VTEC were not detected despite testing 20 individual colonies from primary agar.

Table 1. From ref. (15).

Since this initial study, a number of prospective studies of HUS patients have firmly established the association between HUS and VT-producing E. coli, both in the U.S. (16, 17), and in other countries (18, 19, 20, 21, 22, 23). The overall rate of finding evidence of VT-producing E. coli was around 75% in these studies; a decrease in the rate of recovery of free VT or VT-producing E. coli with time has been observed (Fig. 6) and one explanation for the low rate of recovery in some studies may be the age of the samples. A more recent study (18) that relied heavily on serologic data found evidence of infection in 22 out of 22 consecutive cases (Table 2). The predominant organism responsible for producing the toxin was E. coli O157:H7, although other serotypes have been isolated (24). Interestingly, O157:H7 has been responsible for the many outbreaks of bloody diarrhea in the Pacific Northwest, upper Midwest, and Canada (see below); however, the highest reported frequency of HUS in the world in is Argentina, which has an extemely high frequency of summer diarrheal illness among children--E. coli O157:H7 is distinctly uncommon in HUS in Argentina (25) and

Chile (20), although evidence of verotoxin in invariably present. Other bacterial strains must account for these outbreaks.

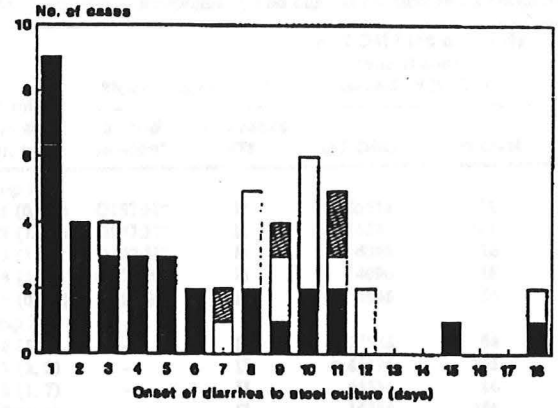

Fig. 6. Recovery of E. coli O157:H7 in 52 HUS patients. Black bars=culture positive. White bars=culture negative. Hatched bars=culture negative in group setting with culture positive patients. From ref. (17).

What about children with HUS who do not present with the classic prodrome of bloody diarrhea? Is VT-producing E. coli a factor in these illnesses as well? In a prospective study of 49 children in Italy (19), the answer appears to be affirmative. Twenty patients had bloody diarrhea, 15 had non-bloody diarrhea, and 14 had no diarrhea. Almost all (95%) of the first group had VT-producing E. coli infection, whereas fully 60% and 65% of the other patients also had evidence for infection. The etiology for HUS in the minority remaining is unknown.

Is VT-producing E. coli responsible for TTP in adults as well? Several case reports have appeared recently (26, 27) in which elderly patients with bloody diarrhea and TTP (similar to F. G. above) have had documented E. coli O157:H7 infection. In addition, an outbreak of bloody diarrhea in a nursing home was associated with a rate of HUS/TTP of over 20%. About half of these patients had a syndrome indistiguishable from classic TTP (28). It is probably safe to say that most adults presenting with TTP and a "classical" prodrome of watery followed by bloody diarrhea will be shown to have infection with VT-E. coli. Whether this organism is responsible for TTP in patients lacking this prodrome awaits further prospective study. Interestingly, free VT has never been found in the blood of any HUS or TTP patient. Rapid clearance by tight binding to target tissues appears to be the explanation for this observation.

The frequent finding of VT-producing E. coli in patients striken with the disease, and the absence of the organism in normal controls fulfill the two of the three Koch-Henle criteria (29) for microbial causation of disease. I would like to turn our attention now to the third criteria: experimental demonstration of the pathogenicity of VT-producing E. coli, concentrating on the mechanism of action of the verotoxin. Later we will come back to the clinical aspects and epidemiology of the disease.

Table. Serologic, microbiologic, and clinical features in 22 children with classic HUS

Patient No. and age (yr, mo)	Stool examination		Anti-O157 LPS antibody (initial serum) detected by IHA‡		Time after onset of diarrhea/HUS (days)	Clinical features		
	E. coli isolate*	Free fecal VT†	LPS O157	LPS O111		Diarrhea	Leukocytes§	Outcome
Group 1								
1 (0, 11)	O157:H⁻	II	≥65536	16	5/2	Watery	15.3	Good
2 (7, 11)	O157:H⁻	II	16384	NT	8/5	Bloody	26.0	Poor‖
3 (1, 5)	O157:H⁻	II	4096	16	5/1	Bloody	9.7	Hematuria
4 (1, 0)	O157:H⁻	II	4096	16	11/3	Bloody	13.7	Good
5 (0, 11)	O157:H⁻	II	4096	64	4/0	Watery	15.8	Good
Group 2								
6 (5, 5)	—	II	≥65536	64	7/1	Bloody	16.6	Good
7 (2, 2)	—	II	≥65536	256	6/1	Watery	15.4	Good
8 (1, 7)	—	II	65536	16	7/1	Bloody	17.3	Good
9 (4, 4)	—	II	16384	256	9/1	Bloody	16.6	Good
10 (6, 9)	—	II	4096	64	6/4	Watery	17.5	Good
11 (2, 2)	—	II	4096	64	7/2	Watery	11.0	Good
12 (6, 2)	—	II	4096	64	5/1	Bloody	15.8	Hypertension
Group 3								
13 (1, 8)	—	—	≥65536	64	7/1	Bloody	11.1	Good
14 (1, 4)	—	—	≥65536	64	8/1	Bloody	14.8	Good
15 (8, 9)	—	—	65536	256	6/1	Bloody	12.2	Good
16 (3, 9)	—	—	16384	NT	6/2	Bloody	43.5	Good
17 (3, 7)	—	—	16384	256	8/2	Bloody	13.7	Good
18 (6, 0)	—	—	16384	NT	5/2	Bloody	8.8	Good
19 (3, 7)	NT	NT	16384	64	5/2	Bloody	?	Fatal
Group 4								
20 (1, 6)	O26:H11	I	64	NT	5/1	Bloody	43.9	Good
21 (4, 8)	O55:H6	II	64	64	3/2	Watery	9.5	Good
22 (0, 10)	O111:H8	II	1024	256	8/3	Watery	20.0	Good

IHA, Indirect hemagglutination assay; *NT*, not tested.
*Production of VT was confirmed by HeLa and Vero cell tests of the bacterial lysates.
†Cytotoxicity was completely neutralized by SLT-I– or SLT-II–specific antisera.
‡Reciprocal titers of indirect hemagglutination assay with sheep erythrocytes sensitized with purified LPS from *E. coli* O157:H7 strain 933 and *E. coli* O111.
§Peripheral blood leukocytes ($\times 10^9$/L) at the time of diagnosis of HUS.
‖Laparotomy, chronic renal failure.

Table 2. From ref. (18).

"VERO" TOXIN AS THE ETIOLOGIC AGENT IN HUS:

- **HUS as a complication of Shigellosis**
  - **1974 Castro, Rosal, and Sanchez (30)**
  - **1974 Guillen-Alvarez and Bolanos (31)**
  - **1978 Koster, et. al. (32)**

- **Discovery of "Vero" toxin from E. coli. Assays developed.**
  - **1977 Konowalchuk, Speirs and Stavric (8)**

- **Verotoxin-producing E. coli (serotype O157:H7) causes human disease**
  - **1982 Riley, et. al. (CDC--Atlanta) (13)**

- **Verotoxin-producing E. coli infection found in 75% of cases of HUS**
  - **1985 Karmali, et. al. (15)**

- **Verotoxin-producing E. coli infection in sporadic TTP.**
  - **1986 Morrison, Tyrrell, and Jewell (27)**
  - **1990 Kovacs, et. al. (26)**

RELATED DEVELOPMENTS:

- **Discovery of Shiga toxin**
  - **1903 Conradi (33)**

- **Enterotoxicity of Shiga toxin**
  - **1972 Keusch, et al. (34)**

- **Purification of Shiga toxin**
  - **1980 O'Brien, et al. (35)**
  - **1980 Olsnes and Eiklid (36)**

- **Purification of Shiga-like toxin; similarity to Vero toxin**
  - **1983 O'Brien and LaVeck (37)**
  - **1983 O'Brien, et. al. (9)**

- **Cloning of structural genes encoding Shiga-like toxins**
  - **1986 Huang, et. al. (38)**
  - **1985 Newland, et. al. (39)**
  - **1985 Willshaw, et. al. (40)**

- **Mechanism of action of Shiga and Shiga-like toxins**
  - **1988 Endo, et. al. (41)**

STRUCTURE OF SHIGA AND SHIGA-LIKE TOXINS

The discovery of Shiga toxin is credited to Conradi in 1903 (33). The active principle was purified in 1980 by O'Brien and coworkers (35) and by Olsnes and Eiklid (36). In 1983, O'Brien and coworkers purified the verotoxin from a verotoxin-producing strain of E. coli, and discovered that it shared immunological cross-reactivity to Shiga-toxin. They called this verotoxin "Shiga-like toxin I", or SLT-I, and this nomenclature had predominated in the U. S. literature. Outside the U. S., the term verotoxin 1, or VT 1 is used. In this paper, I will use them interchangeably. A second form of verotoxin, immunologically distinct from VT1, was soon discovered, and named SLT-II or VT2. With the cloning of the structural genes for SLT-I and SLT-II (38, 39, 40), the relationships between Shiga toxin and SLT-I and SLT-II became apparent: Shiga toxin and SLT-I differ by only one amino acid in the B subunit (10), while SLT-II shares an overall 57% and 60% identity in the A and B subunits, respectively (12). Studies on the structure-function relationships between Shiga and Shiga-like toxins, including a variant, SLT-IIv, which causes edema disease in pigs, have been reviewed recently (42).

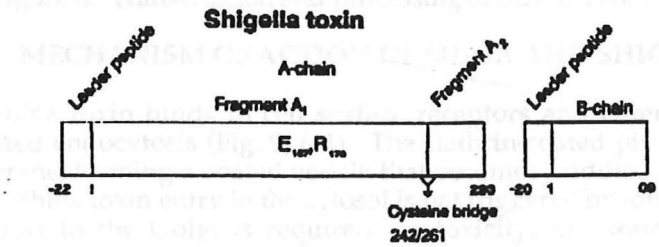

Fig. 7. Structure of Shiga toxin.

The structure of Shiga toxin is shown in Fig. 7. As discussed above, SLT-I differs by only one amino acid from this structure and SLT-II is highly homologous. The toxin is composed of a 33 kDa A subunit, which possesses catalytic activity, and a 7.7 kDa B subunit, which mediates binding to target cells.

The genes encoding SLT-I and SLT-II reside on a bacteriophage that infects E. coli. (In contrast, the Shiga toxin gene of Shigella resides on a bacterial chromosome). The genes are transcribed as part of a polycistronic message, and translated separately (Fig. 8). The precursor polypeptides are secreted into the periplasm of the bacterium, and the leader sequences are removed. Next, four or five B subunits (the stoichiometry is still uncertain) associate with one A subunit to form the inactive holotoxin. It is presumed that the toxin is released following rupture and death of the bacterium.

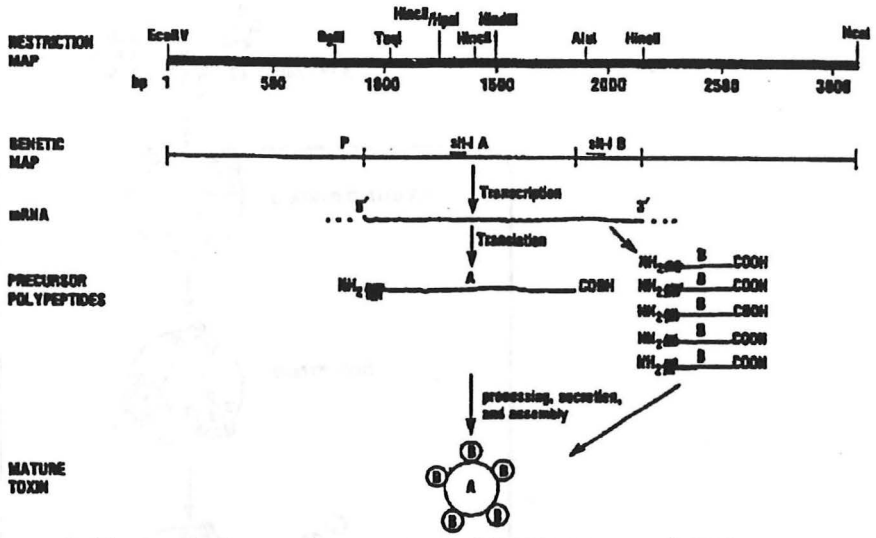

Figure 8. Transcription and processing of SLT-I. From ref. (43).

MECHANISM OF ACTION OF SHIGA AND SHIGA-LIKE TOXINS

Shiga toxin binds to cell surface receptors and enters cells by receptor-mediated endocytosis (Fig. 9) (44). The clathrin-coated pit pinches off from the membrane, forming a coated vesicle that becomes acidified. In contrast to other toxins, Shiga toxin entry in the cytosol is not triggered by low pH. It appears that transport to the Golgi is required for toxicity; for example, brefeldin A, a compound that dissolves the Golgi apparatus, blocks intoxication by Shiga toxin (45). The mechanism for the translocation of the A chain into the cytosol is still mysterious; calcium ions may be involved. One model, developed for a toxin of similar subunit structure (E. coli heat labile toxin) suggests that the multiple B subunits form a pore within the membrane that opens like the iris of a camera, and that the sides of the pore have binding pockets for extended peptide chains, like the peptide binding cleft of HLA molecules (46). Once the A chain enters the cytoplasm of the cell, it is cleaved by cytosolic proteases into two fragments (A1 and A2), the disulfide bond between the two fragments is reduced, and the catalytically active Fragment A1 is released into the cytoplasm.

Once inside the cell, the active Fragment A1 disrupts protein synthesis by inactivating ribosomes through an amazing mechanism. The toxin cleaves an adenine residue from a specific nucleotide of the 28 S rRNA component of the large ribosomal subunit (41). The loss of the single adenine residue blocks peptide elongation by blocking elongation factor 1 (EF-1)-dependent aminoacyl tRNA binding (see Fig. 10 and 11). Amazingly, this is exactly the mechanism of action of the potent plant toxin ricin. Even the same adenine residue (A-4324) is cleaved by both toxins! The 28 S RNA N-glycosidase activity of Shiga toxin, SLT-IIv and ricin have been verified by microinjection experiments in Xenopus oocytes (47). Interestingly, the A chain of ricin and the A chains of the Shiga and Shiga-like toxins share only small, but presumably important, regions of homology.

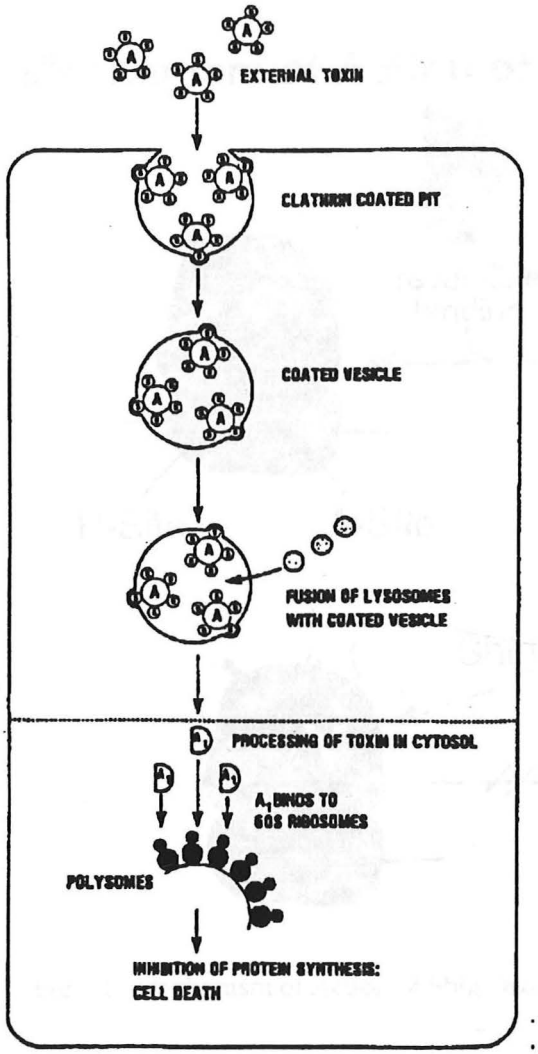

Fig. 9. Intracellular processing of Shiga toxins. From ref. (43).

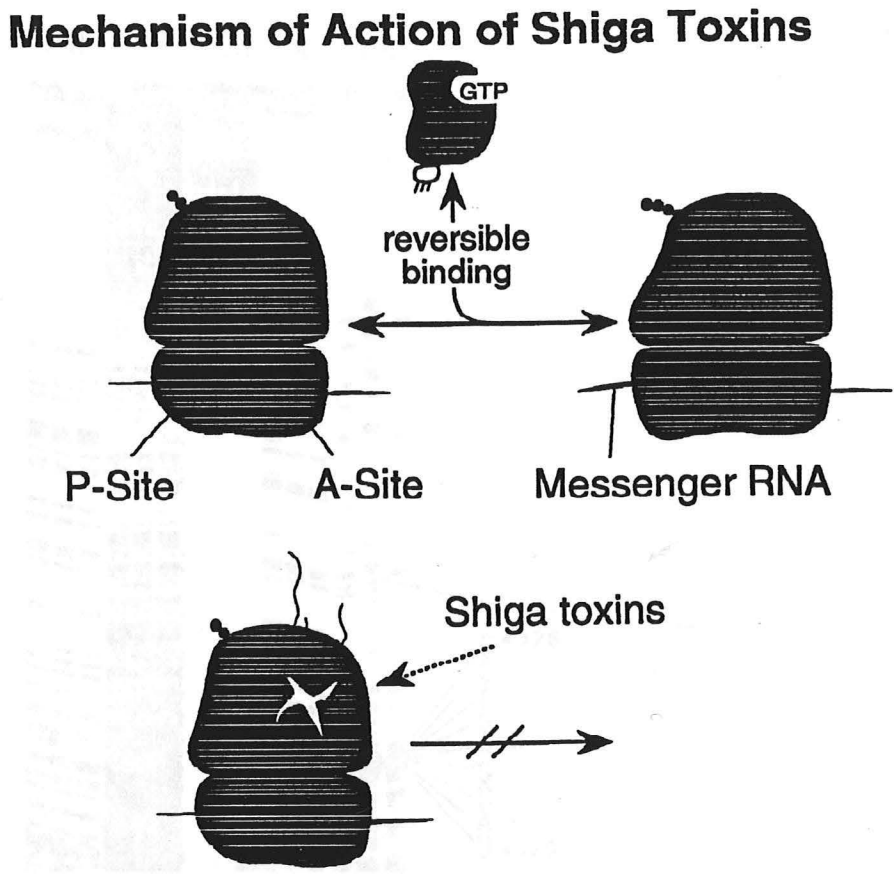

Fig. 11. Mechanism of Action of Shiga toxins.

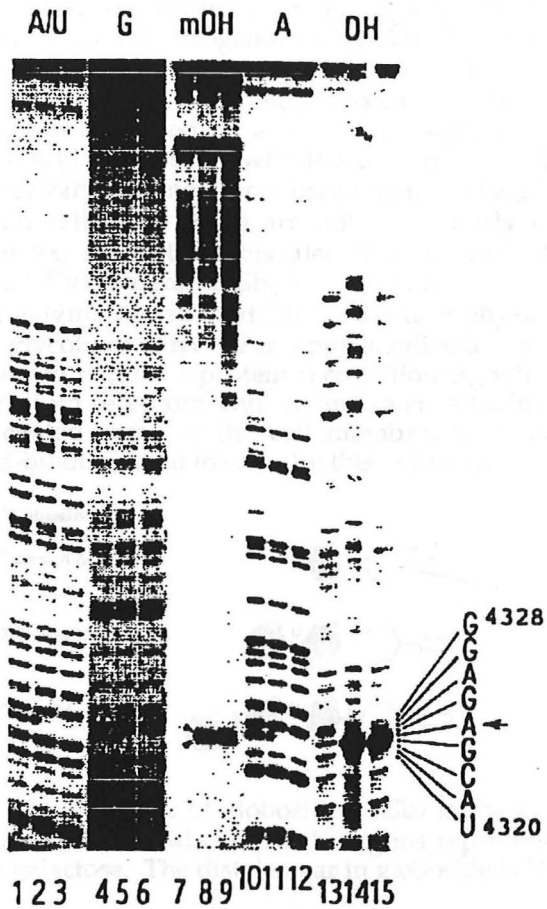

Fig. 11. Site of adenine removal on 28 S rRNA by ricin and Shiga toxin. From ref. (41).

THE CELLULAR RECEPTOR FOR SHIGA AND SHIGA-LIKE TOXINS

The cellular receptor for Shiga and Shiga-like toxins appears to be a neutral glycolipid, globotriaosyl ceramide, or Gb_3. Gb_3 consists of a ceramide base (analogous to diglyceride) linked to three sugar moieties (Fig. 12). Early reports (48, 49) questioned whether this binding site was the natural "functional receptor" capable of mediating not only binding but internalization and cellular toxicity, largely because there is an unexplained poor correlation between the number of binding sites and sensitivity to toxin among cell lines. However, further evidence supports Gb_3 as the physiologically relevant receptor: Cells

lines completely lacking the receptor are never sensitive to the toxin (50); cell mutants selected for resistance to verotoxin have lost Gb_3 from the cell surface (51); and these insensitive mutants regain close to normal sensivitity upon supplementation with exogenous Gb_3 (52). In addition, a precedent exists for glycolipids as bacterial cytotoxin receptors, as the ganglioside G_{M1} is believed to be the functional receptor for cholera toxin. Interestingly, binding of verotoxin to HeLa cells was found to vary about 10-fold within one cell cycle (53); cell surface Gb_3 was found to fluctuate with the cell cycle, but the total amount of cellular Gb_3 did not vary. Human renal tissue is rich in Gb_3 (54). Human umbilical vein endothelial cells (HUVECs) are not particularly rich in Gb_3 under normal circumstances; more about this later. The SLT variant that causes edema disease in pigs (SLT-IIv) recognizes Gb_4 as its receptor.

One enigma remains: if Gb_3 is the true physiological receptor, then how does this glycolipid enter the receptor-mediated endocytic pathway, which thus far appears to require a protein recognition signal? The two other toxins that have glycolipid receptors, cholera and tetanus toxin, are internalized from non-clathrin coated areas of the cell membrane; thus, Shiga toxin is the only glycolipid-binding toxin to enter by this pathway.

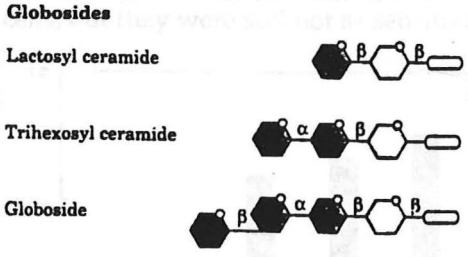

Fig. 12. Structure of globosides. Gb_3 is the middle structure. The small ovals represent ceramide. Open hexagons represent glucose moieties; closed represent galactose. The distal sugar in globoside is N-acetylgalactosamine.

THE PATHOPHYSIOLOGY OF HUS: VEROTOXIN AND THE ENDOTHELIAL CELL

Research into HUS has been hampered by the lack of a suitable animal model (reviewed in (55)). Following exposure to either SLT or to verotoxin-producing E. coli, rabbits develop diarrhea and hemorrhagic gut lesions, but do not develop renal or other vascular lesions. (The explanation for this failure may be the lack of Gb_3 in the renal tissue of all other mammals except man.) Therefore, what we know about HUS has been learned from renal biopsies, autopsies on patients dying of HUS, or cell culture experiments. If patients with active HUS are subjected to renal biopsy, a characteristic histopathologic picture is observed. The glomerular endothelial cells are swollen, and fibrin deposition and inflammatory cells are seen in the lumen of the glomerular vessels (56). These observations suggest that direct endothelial cell damage is the initial insult. However, initial studies on the sensitivity of human endothelial cells in

culture to the toxic effect of verotoxins have been disappointing: the cells are some million-fold less sensitive as compared to Vero cells (57). This is attributable to the very low level of toxin binding to the cells, as illustrated in Table 3.

Cell type	nmol Gb_3/mg
HUVEC	0.06
HSVEC	0.03
Vero	80
HeLa	25

Table 3.Verotoxin receptors on cultured cells.

Other investigators later went on to show that the cytokines TNFα and IL-1 sensitize HUVECs to the action of verotoxin by at least 100-fold (58). After treatment, the number of receptors for verotoxin increases dramatically, and there is a corresponding increase in the Gb_3 content of these cells, so the effect appears to be mediated by an increase in the number of functional receptors on the cell surface (Fig 13). Concentrations as low as 17 pM inhibit protein synthesis in these cells, but they were still not as sensitive as Vero cells.

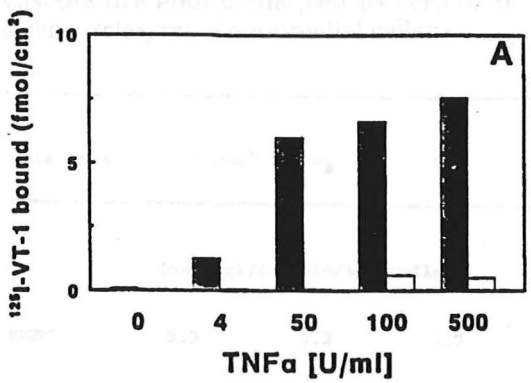

Fig. 13. TNF induces ligand for Shiga-like toxin on human umbilical vein endothelial cells. From ref. (58).

Dr. Tom Daniel (Vanderbilt University Medical Center) and colleagues have recently provided a major insight into the pathophysiology of the renal lesion in verotoxin-produced HUS (Obrig,T.G., Louise, C. B., Lingwood, C. A., Boxyd, B., Barley-Maloney, and Daniel, T. O., J. Biol. Chem., in press). These investigators have been able to grow human microvacular endothelial cells in culture and show that they are exquisitely sensitive to Shiga toxin. Renal endothelial cells are killed by < 1 pM of Shiga toxin, while umbilical vein cells are not killed by > 1 nM of toxin (Fig 14). Basal levels of Gb_3 were about 50 times higher in renal cells than in HUVECs (Table 4). Cytokines did not alter the sensitivity of the renal microvascular cells.

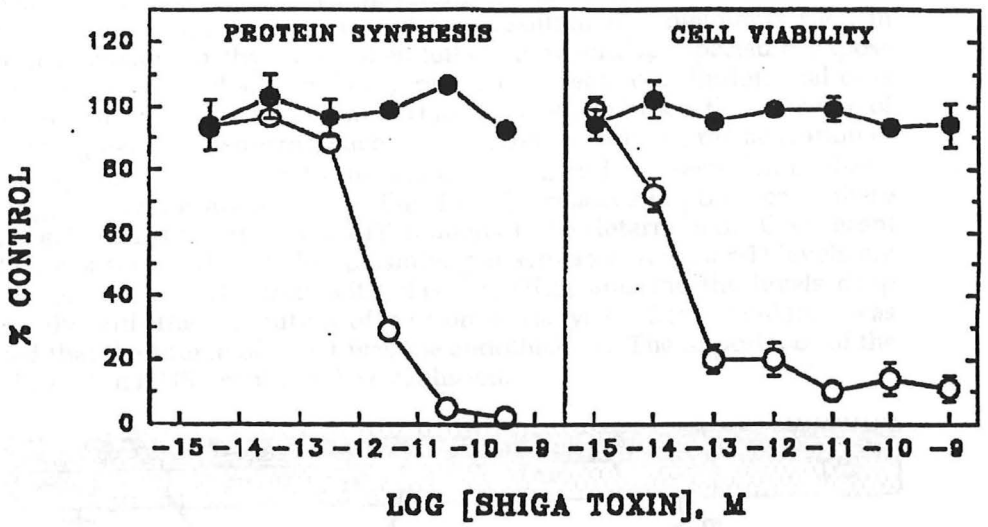

Fig. 14. Differential sensitivity of human umbilical vein and human renal microvasculature endothelial cell to verotoxin. Closed circles, umbilical vein cells; open circles, renal endothelial cells.

Cell type	Lac cer[a]	Gb_3	Gb_4
	(nmol glycolipid/10^6 cells)		
RMEC	2.3	3.2	7.7
UVEC	0.24	0.067	0.48
Ratio (RMEC/UVEC)	9.6	48	16

[a]Lac cer, lactosyl ceramide

Table 4. Neutral glycolipid levels in renal microvascular (RMEC) and umbilical vein (UVEC) endothelial cells.

There should be no doubt that the killing of endothelial cells could result in local platelet thrombus formation and the resultant derangements seen in thrombotic microangiopathy. Loss of endothelium would be expected to expose collagen to circulating blood, initiating platelet aggregation. Endothelial cells also provide an active anticoagulant surface over which blood flows; some of these anticoagulant mechanisms, such as the secretion of PGI_2, the activation of Protein C by thrombin bound to thrombomodulin, and the secretion of tissue plasminogen activator are shown in Fig. 15. The relative importance of these several mechanisms in HUS and TTP remains to be determined. One recent study (59) has shown that PAI-1 (plasminogen activator inhibitor-1) levels are markedly elevated in children with classical HUS, and the the levels drop dramatically with the institution of peritoneal dialysis. Some evidence was presented that the source of PAI-1 was the endothelium. The importance of the elevated PAI-1 in HUS remains to be established.

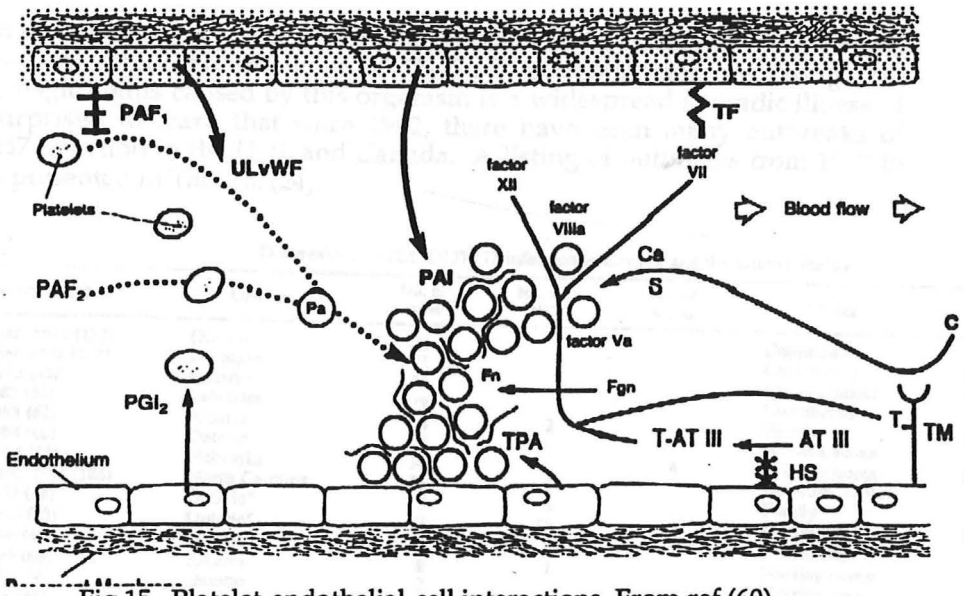

Fig.15. Platelet-endothelial-cell interactions From ref (60).

CLINICAL FEATURES AND EPIDEMIOLOGY OF HUS CAUSED BY E. COLI O157:H7 INFECTION

Let's return now to the clinical features and epidemiology of verotoxin-induced disease. E. coli O157:H7 infection is a new and virulent pathogen; before the two outbreaks of dysentery due to this organism in 1982, it was isolated in only a single case of diarrhea in 1975. Beginning in 1982, the CDC established a surveillance system for this infection, asking for reports of illnesses consisting of crampy abdominal pain, bloody diarrhea, and negative stool

cultures for routine pathogens. Some of the methods used to diagnose these infections are listed in Table 5.

DIAGNOSIS OF ENTEROHEMORRHAGIC *E. COLI*

Methods Useful for All Enterohemorrhagic *E. coli* (EHEC)
Free fecal toxin, toxin production by isolated colonies, or polymyxin extract of mixed colonies
ELISAs for toxins in culture or fecal extract
Colony hybridization using large fragment of genome or synthetic nucleotides
Polymerase chain reaction amplification
Serologic response to SLT-I/II
Methods Specific for *E. coli* 0157:H7
Sorbitol media
Latex agglutination for 0157 LPS
H determination
Serologic response to 0157 LPS

Table 5. Diagnosis of enterohemorrhagic E. coli. From ref. (61).

In the subsequent 2 year period, 28 people from 11 states met these criteria and had microbiologic evidence of O157:H7 infection, demonstrating that hemorrhagic colitis caused by this organism is a widespread sporadic illness. I was surprised to learn that since 1982, there have been many outbreaks of O157:H7 infection in the U. S. and Canada. A listing of outbreaks from 1982 to 1987 is presented in Table 6. (24).

Outbreaks of VTEC O157:H7 infection in Canada and the United States[a]

Date (reference)	Location	No. of cases	No. with HUS	No. of deaths	Setting	Source
Feb.–Mar. 1982 (158)	Oregon[c]	26			Community	Hamburg
May–June 1982 (158)	Michigan[c]	21			Community	Hamburg
Nov. 1982 (68)	Ontario	31			Nursing home	Ground l
May 1983 (68)	Labrador	19			Community	
Aug. 1983 (68)	Alberta	4	2		Family	Hamburg
Mar. 1984 (68)	Ontario	7			Nursing home	
Sept. 1984 (166)	Nebraska	34		4	Nursing home	Hamburg
Sept.–Oct. 1984 (186)	North Carolina	36	3		Day-care center	
Aug. 1985 (68)	Ontario[d]	5	5		Family	
Sept. 1985 (22)	Ontario[e]	73	12	17	Nursing home	Sandwic
Apr. 1986 (43)	Ontario	30	3		School trip	Raw mil
June 1986 (68)	Alberta	8	2		Nursing home	
June 1986 (68)	Ontario	2			Nursing home	
July 1986 (68)	British Columbia	20			Community	
Oct.–Nov. 1986 (196)[f]	Washington	37	3	2	Restaurant	Ground l
Dec. 1986 (68)	Ontario	4			? Restaurant	
June 1987 (68)	Alberta	15		2	Nursing home	Ground l
July 1987 (68)	Ontario	9			Nursing home	
July 1987 (68)	Ontario	6			Girls' camp	
Aug. 1987 (68)	Ontario	9		2	Nursing home	

[a] This table is based in design and content on an excellent summary of VTEC 0157:H7-associated outbreaks in Canada, 1982 to 1987, by Hockin and Lior (reprinted by permission). The table has been suitably expanded to include U.S. data.
[b] Sources highly suspected or confirmed.
[c] Case definition included only hemorrhagic colitis.
[d] Ish-Shalom et al., Pediatr. Res. 20:228A, 1986.
[e] The 73 affected cases included 55 (32.5%) of 169 elderly residents and 18 (13%) of 137 staff members. All cases of HUS and all fatalities occurred in residents.
[f] Ostroff et al., Abstr. Int. Symp. Workshop Verocytotoxin-Producing *Escherichia coli* Infections 1987, CEP-8.

Table 6. From ref. (24).

The hallmark of O157:H7 infection is bloody diarrhea, with the finding of hemorrhagic colitis on colonoscopy. However, nonbloody diarrhea, asymptomatic infection, as well as HUS and TTP lie within its broad clinical spectrum. Associated symptoms include severe abdominal cramps, and nausea and vomiting, with relatively little fever. Fig. 16 shows the cumulative incidence of symptoms during the outbreak in Washington state in 1987. Typically, the bloody diarrhea continues for 2 to 4 days, and the entire illness resolves in about a week. Interestingly, in one study, over 80% of patients who later developed HUS or TTP sought advice from a physician several days before the onset of these syndromes. From studying contacts of patients with hemorragic colitis, it is clear that nonbloody diarrhea and even asymptomatic infection are frequent. Risk factors for diarrhea caused by O157:H7 infection appear to be very young and very old age (Fig. 17), previous gastrectomy, and possibly, recent antibiotic use (62).

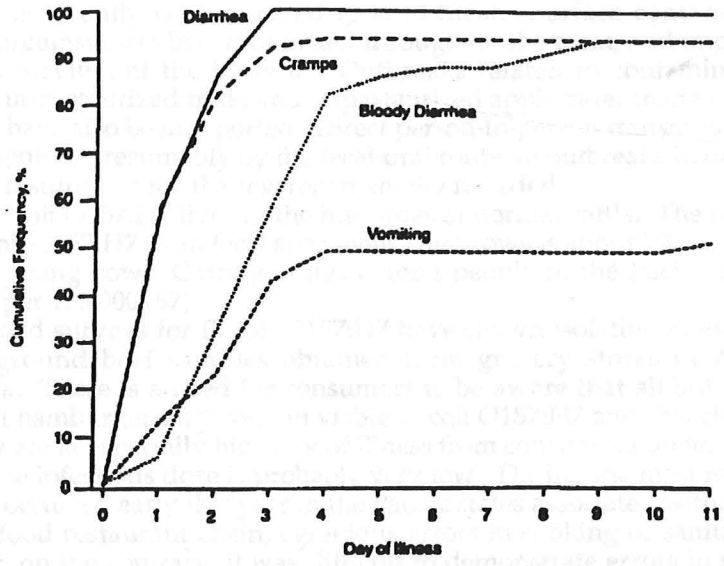

Fig. 16. Cumulative incidence of symptoms during E. coli O157:H7 infection. From ref. (63)

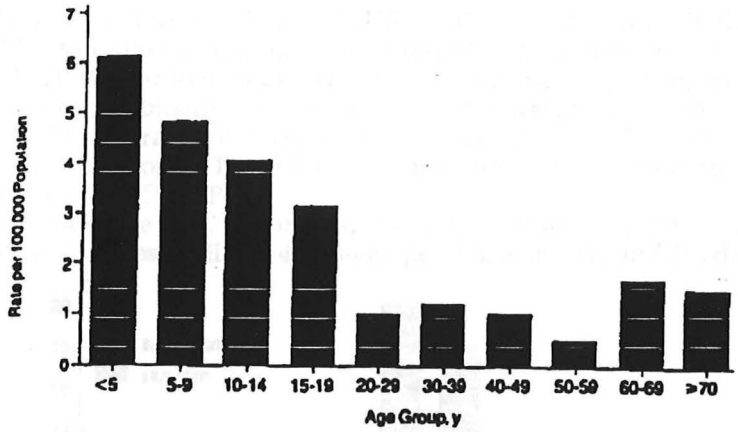

Fig. 17. Age-specific rates of infection with E. coli O157:H7.

Most outbreaks have been associated with the ingestion of bovine food products, usually contaminated ground meat. Surface contamination under these circumstances becomes mixed throughout the meat, and improper cooking allows survival of the bacteria. Outbreaks related to contaminated drinking water, unpasteurized milk, and unpasteurized apple cider made from unwashed apples have also been reported. Direct person-to-person transmission is also well documented, presumably by the fecal-oral route, in outbreaks in day-care centers and in institutions for the severely mentally retarded.

E. coli O157:H7 lives in the intestines of normal cattle. The rate of isolation of E. coli O157:H7 from fecal specimens from cows is about 1% in adult cows and 5% in young cows. Carriage rates among people in the Pacific Northwest are only 8 per 100,000 (62).

Food surveys for E. coli O157:H7 have shown isolation rates as high as 4% from ground beef samples obtained from grocery stores in Wisconsin and Canada. There is a need for consumers to be aware that all but the most well-cooked hamburger may contain viable E. coli O157:H7 and that children and the elderly are at especially high risk of illness from consuming undercooked beef.

The infectious dose is probably very low. During the most recent outbreak, which occurred early this year in the Pacific states associated with hamburgers at a fast food restaurant chain, egregious errors in cooking or sanitation were not found; on the contrary, it was difficult to demonstrate errors in food handling, except that certain areas of the grills during peak hours may have not reached killing temperatures. Subsequently, restaurants have been asked to raise temperatures to 155°F to insure that all bacteria are killed.

How frequently is diarrhea caused by E. coli O157:H7 in North America? In each of five major studies, it was the second or third most frequent known pathogen. In one major study, it was easily the most frequent pathogen in cases of bloody diarrhea, much more frequent than Shigella (62).

What proportion of patients develop HUS/TTP as a sequela of O157:H7 infection? If only patients with bloody diarrhea are considered, the rate is approximately 10% (reviewed in (62)). Fig. 18 shows the number of cases of

O157:H7 and the number of HUS/TTP cases per month during the 1987 Washington state outbreak as an example. Risk factors for development of HUS/TTP appear to include very young or very old age, P antigen expression on red blood cells, bloody diarrhea, fever, and elevated leukocyte count early in the illness. Some strains of E. coli O157:H7 produce both VT1 and VT2 while others produce VT2 alone. These latter strains appear to be associated with a higher incidence of HUS/TTP.

Among the U.S. outbreaks, the mean number of cases was 56; 18% of pateints were hospitalized, 4% developed HUS or TTP, and 2% died (62).

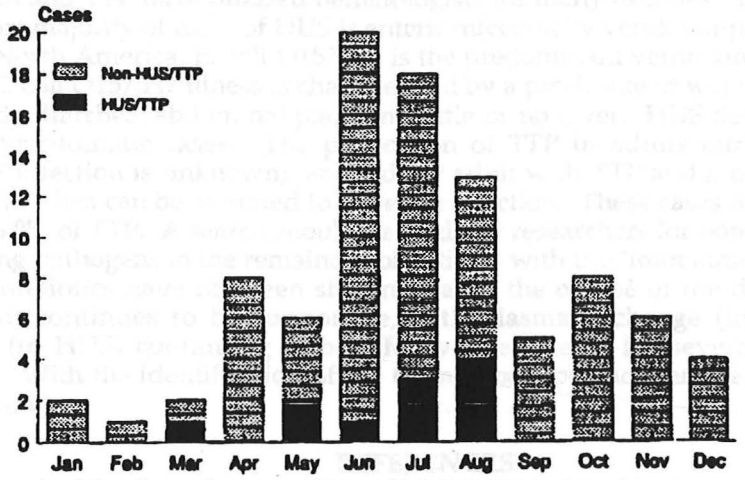

Fig. 18. Number of cases of E. coli O157:H7 in Washington state in 1987. From ref. (63).

PROSPECTS FOR TREATMENT, PREVENTION AND CONTROL

How does knowing the etiology of HUS and diarrhea-associated TTP affect our management of these patients? In the short run, it probably doesn't. A prospective, randomized, controlled trial of trimethoprim-sulfamethoxazole (to which verotoxin-associated E. coli are uniformly sensitive) in 47 children presenting with diarrhea and documented E. coli O157:H7 infection was performed from June 1, 1989 to June 1, 1990 in Montreal (64). No effect on symptoms, duration of fecal pathogen excretion, or incidence of HUS were seen. In fact, there is evidence that exposure to subinhibitory concentrations of antibiotics may actually increase toxin production by the bacteria (65). As is the case in other toxin-mediated syndromes (for example, toxic shock syndrome), it may prove that antibiotic treatment is not very useful in the acute phase of the illness.

On the other hand, identification of the toxin involved opens up the possiblity for the development of specific antitoxin or specific immune globulin. Some commercial immunoglobulin preparations contain some neutralizing activity against Shiga toxin (and SLT-I), perhaps explaining occasional responses to IV gammaglobulin. It is possible that a more specific, high-titer antitoxin

could be developed. Immunization against the toxin is also a possibility. These avenues are currently under investigation.

In the meantime, public education programs and programs to control VT-producing E. coli contamination are underway to prevent further outbreaks of illness caused by this new pathogen.

SUMMARY AND CONCLUSION

HUS and TTP have puzzled hematologists for many decades. The etiology of the vast majority of cases of HUS is enteric infection by verotoxin-producing E. coli. In North America, E. coli O157:H7 is the predominant verotoxin-producing strain. E. coli O157:H7 illness is characterized by a prodrome of watery followed by bloody diarrhea, abdominal pain, and little or no fever. HUS develops in 2-7% of symptomatic cases. The proportion of TTP in adults attributable to O157:H7 infection is unknown; any elderly adult with TTP and a prodrome of bloody diarrhea can be assumed to have the infection. These cases may account for up to 8% of TTP. A search should be made by researchers for potential toxin-producing pathogens in the remainder of patients with the "idiopathic" variety of TTP. Antibiotics have not been shown to alter the course of the disease, and treatment continues to be supportive, with plasma exchange (in TTP) and dialysis (in HUS) continuing to be effective treatments for severely affected patients. With the identification of the toxin, more specific therapies should be forthcoming.

REFERENCES

1. Moschcowitz, E. 1925. An acute febrile pleiochromic anemia with hyaline thrombosis of terminal arterioles and capillaries; an undescribed disease. *A.M.A. Arch. Intern. Med.* 36: 89.

2. Amorosi, E. L. and J. E. Ultmann. 1966. Thrombotic thrombocytopenic purpura: Report of 16 cases and review of the literature. *Medicine.* 45: 139-159.

3. Ridolfi, R. L. and W. R. Bell. 1981. TTP, report of 25 casis and review of the literature. *Medicine.* 60: 413-428.

4. Loirat, C., E. Sonsino, A. V. Moreno, G. Pillion, J. C. Mercier, F. Beaufils and H. Mathieu. 1984. Hemolytic-uremic syndrome: An analysis of the natural history and prognostic features. *Acta Pediatr. Scand.* 73: 505-514.

5. Neild, G. 1987. The haemolytic uraemic syndrome. *Q. J. Med.* 63: 367-376.

6. Rose, M. and A. Eldor. 1987. High incidence of relapses in TTP. *Amer. J. Med.* 83: 437-444.

7. Berns, J. S., B. S. Kaplan, R. C. Mackow and L. G. Hefter. 1992. Inherited hemolytic uremic syndrome in adults. *Am. J. Kid. Dis.* 19: 331-334.

8. Konowalchuk, J., J. I. Speirs and S. Stavric. 1977. Vero response to a cytotoxin of Escherichia coli. *Infect. Immun.* 18: 775-779.

9. O'Brien, A. D., G. D. LaVeck, M. R. Thompson and S. B. Formal. 1982. Production of a Shigella dysenteriae type 1-like cytotoxin by Escherichia coli. *Journal of Infectious Disease.* 146: 763-769.

10. Calderwood, S. B., F. Auclair and A. E. A. Donohue-Rolfe. 1987. Nucleotide sequence of the Shiga-like toxin genes of Escherichia coli. *Proc. Natl. Acad. Sci. U.S.A.* 84: 4364-4368.

11. Strockbine, N. A., M. P. Jackson and L. M. Sung et al. 1988. Cloning and sequencing of the genes for Shiga toxin from Shigella dysenteriae type 1. *J. Bacteriol.* 170: 1116-1122.

12. Jackson, M. P., R. J. Neill and A. D. e. a. O'Brien. 1987. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from Escherichia coli 933. *FEMS Microbiol.* 44: 109-114.

13. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. H. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. *New Engl. J. Med.* 308: 681-685.

14. Karmali, M. A., B. T. Steele, M. Petric and C. Lim. 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing Escherichia coli in stools. *Lancet.* 1: 619-620.

15. Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus and H. Lior. 1985. The association between idiopathic hemolytic uremic syndrome and infection by Verotoxin-producing Escherichia coli. *J. Infect. Dis.* 151: 775-782.

16. Neill, M. A., P. I. Tarr, C. R. Clausen, D. L. Christie and R. O. Hickman. 1987. Escherichia coli O157:H7 as the predominant pathogen associated with the hemolytic uremic syndrome: A prospective study in the Pacific Northwest. *Pediatrics.* 80: 37-40.

17. Tarr, P. I., M. G. Neill, C. R. Clausen, S. L. Watkins, D. L. Christie and R. O. Hickman. 1990. Escherichia coli O157:H7 and the hemolytic uremic syndrome: Importance of early cultures in establishing the etiology. *J. Infect. Dis.* 162: 553-556.

18. Bitzan, M., E. Moebius, K. Ludwig, D. E. Muller-Wiefel, J. Heesemann and H. Karch. 1991. High incidence of seum antibodies to Escherichia coli O157 lipopolysaccaride in children with hemolytic-uremic syndrome. *J. Pediatr.* 119: 380-385.

19. Caprioli, A., I. Luzzi, F. Rosmini, P. Pasquini, R. Cirrincione, A. Gianviti, M. C. Matteucci and G. Rizzoni. 1992. Hemolytic-uremic syndrome and Vero cytotoxin-producing Escherichia coli infection in Italy. The HUS Italian Study Group. *J. Infect. Dis.* 166: 154-8.

20. Cordovez, A., V. Prado, L. Maggi, J. Cordero, J. Martinez, A. Misraji, R. Rios, G. Soza, A. Ojeda and M. M. Levine. 1992. Enterohemorrhagic Escherichia coli associated with hemolytic-uremic syndrome in Chilean children. *J. Clin. Microbiol.* 30: 2153-2157.

21. Kleanthous, H., H. R. Smith and S. M. Scotland. 1990. Haemolytic uraemic syndrome the the British Isles, 1985-1988: Association with verocytotoxin-rpoducing Escherichia coli. Part 2: microbiological aspects. *Arch. Dis. Child.* 65: 722-727.

22. Lopez, E. L., M. Diaz, S. Devoto, S. Grinstein, M. Woloj, B. E. Murray, E. Rubeglio, F. Mendilaharzu, M. Turco, M. Vasquez, L. K. Pickering and T. G. Cleary. 1991. Evidence of infection with organisms producing Shiga-like toxins in household contacts of children wiht the hemolytic uremic syndrome. *Pediatr. Infect. Dis. J.* 10: 20-24.

23. Scotland, S. M., B. Rowe, H. R. Smith, G. A. Willshaw and R. J. Gross. 1988. Vero cytotoxin-producing strains of Escherichia coli from children with haemolytic uraemic syndrome and their detection by specific DNA probes. *J. Med. Microbiol.* 25: 237-243.

24. Karmali, M. A. 1989. Infection by verocytotoxin-producing Escherichia coli. *Clin. Micro. Rev.* 2: 15-38.

25. Lopez, E. L., M. Diaz, S. Grinstein, S. Devoto, F. Mendilaharzu, M. B. E., S. Ashkenazi, E. Rubeglio, M. Woloj, M. Vasquez, M. Turco, L. K. Pickering and T. G. Cleary. 1989. Hemolytic uremic syndrome and diarrhea in Argentine children: The role of Shiga-like toxins. *J. Infect. Dis.* 160: 469-475.

26. Kovacs, M. J., J. Roddy, S. Gregoire, W. Cameron, L. Eidus and J. Drouin. 1990. Thrombotic thrombocytopenic purpura following hemorrhagic colitis due to Escherichia coli 0157:H7. *Amer. J. Med.* 88: 177-179.

27. Morrison, D. M., D. L. J. Tyrrell and L. D. Jewell. 1986. Colonic biopsy in verotoxin-induced hemorrhagic colitis and thrombotic thrombocytopenic purpura (TTP). *Am. J. Clin. Pathol.* 86: 108-112.

28. Carter, A. O., A. A. Borczyk, J. A. K. Carson., B. Harvey, J. C. Hockin, M. A. Karmali, C. Krishnan, D. A. Korn and H. Lior. 1987. A severe outbreak of Escerichia coli O157:H7-associated hemorrhagic colitis in a nursing home. *New Engl. J. Med.* 317: 1496-1500.

29. Evans, A. S. 1991. Epidemiologic concepts. *In* Bacterial infections of humans: epidemiology and control. Evans and Brachman. Plemun Medical Book Company, New York. 3-57.

30. Castro, F., J. E. Rosal and E. Sanchez. 1974. Hallazgos anatomopatologios en 173 casos de disenteria bacilar. *In* Simposio sobre disenteria Shiga en Centroamerica. Mata. Pan American Health Organization, Washington, D.C. p17-26.

31. Guillen-Alvarez, G. and E. V. Bolanos. 1974. Sindrome hemolotico uremico: reporte de 60 cases asociados a una epidemic de enterocolitis hemorrhagica. *Rev. Columb. Pediatr.* 28: 414-417.

32. Koster, F., J. Levin, L. Walker, K. S. K. Tung, R. H. Gilman, M. M. Rahaman, A. Majid, S. Islam and R. C. Williams. 1978. Hemolytic-uremic syndrome after shigellosis: Relation to endotoxemia and circulating immune complexes. *New Engl. J. Med.* 298: 927-933.

33. Conradi, H. 1903. Uber loslishe, durch aseptische autolyse, erhaltene giftstoffe von ruhr- and typus bazillen. *Deutsch. Med. Wochenschr.* 29: 26-28.

34. Keusch, G. T., G. F. Grady, M. L. J. and J. McIver. 1972. The pathogenesis of Shigella diarrhea: l. Enterotoxin production by Shigella dysenteriae 1. *J. Clin. Invest.* 51: 1212-1218.

35. O'Brien, A. D., G. D. LaVeck, D. E. Griffin and M. R. Thompson. 1980. Characterization of Shigella dysenteriae 1 (Shiga) toxin purified by anti-Shiga toxin affinity chromatography. *Infect. Immun.* 30: 170-179.

36. Olsnes, S. and K. Eiklid. 1980. Isolation and characterization of Shigella shigae cytotoxin. *J. Biol. Chem.* 255: 284-289.

37. O'Brien, A. D. and G. D. LaVeck. 1983. Purification and characterization of a Shigella dysenteriae 1-like toxin produced by Escherichia coli. *Infect. Immun.* 40: 675-683.

38. Huang, A., S. de Grandis, J. Friesen, M. Karmali, R. Petric, R. Congi and J. L. Brunton. 1986. Cloning and expression of the genes specifying Shiga-like toxin production in Escherichia coli H19. *J. Bacteriol.* 1666: 375-379.

39. Newland, J. W., N. A. Strockbine, S. F. MIller, A. D. O'Brien and R. K. Holmes. 1985. Cloning of Shiga-like toxin structural genes from a toxin converting phage of Escherichia coli. *Science.* 230: 179-181.

40. Wilshaw, G. A., H. R. Smith, S. M. Scotland and B. Rowe. 1985. Cloning of genes determining the production of Vero cytotoxin by Escherichia coli. *J. Gen. Microbiol.* 131: 3047-3053.

41. Endo, Y., K. Tsurugi, T. Yutsudo, Y. Takeda, T. Ogasawara and K. Igarashi. 1988. Site of action of a Vero toxin (VT2) from Escherichia coli O157:H7 and of Shiga toxin on eukaryotic ribosomes: RNA N-glycosidase activity of the toxins. *Eur. J. Biochem.* 171: 45-50.

42. Jackson, M. P. 1990. Structure-function analysis of Shiga toxin and the Shiga-like toxins. *Microb. Pathogen.* 8: 235-242.

43. O'Brien, A. D. and R. K. Holmes. 1987. Shiga and Shiga-like toxins. *Micro. Rev.* 51: 206-220.

44. Sandvig, D., K. Prydz, M. Ryd and B. van Deurs. 1991. Endocytosis and intracellular transport of the glycolipid-binding ligand Shiga toxin in polarized MDCK cells. *J. Cell Biol.* 113: 553-562.

45. Sandvig, K., K. Prydz and B. van Deurs. 1991. Endocytic uptake of ricin and Shiga toxin. Springer, New York.

46. Olsnes, S., J. V. Kozlov, B. van Deurs and K. Sandvig. 1991. Bacterial protein toxins acting on intracellular targets. *Semin. Cell Biol.* 2: 7-14.

47. Saxena, S. K., A. D. O'Brien and E. J. Ackerman. 1989. Shiga toxin, shiga-like toxin II variant, and ricin are all single-site RNA N-glycosidases of 28 S RNA when microinjected into Xenopus oocytes. *J. Biol. Chem.* 264: 596-601.

48. Jacewicz, M., H. Clausen, E. Nudelman, A. Donohue-Rolfe and G. T. Keusch. 1986. Pathogenesis of Shigella diarrhea XI. Isolation of a Shigella toxin-binding glycolipid from rabbit jejunum and HeLa cells and its identification as globotriaosylceramide. *J. Exp. Med.* 163: 1391-1404.

49. Lindberg, A. A., J. E. Brown, N. Stromberg, M. Westling-Ryd, J. E. Schultz and K. A. Karlsson. 1987. Identification of the carbohydrate receptor for Shiga toxin produced by Shigella dysenteriae type 1. *J. Biol. Chem.* 262: 1779-1785.

50. Cohen, A., V. Madrid-Marina, Z. Estrov, M. Freedman, C. Lingwood and H.-M. Dosch. 1990. *Int. J. Immunol.* 2: 1-8.

51. Cohen, A., M. V. Madrid, Z. Estrov, M. H. Freedman, C. A. Lingwood and H. M. Dosch. 1990. Expression of glycolipid receptors to Shiga-like toxin on human B lymphocytes: a mechanism for the failure of long-lived antibody response to dysenteric disease. *Int. Immunol.* 2: 1-8.

52. Waddell, T., A. Cohen and C. A. Lingwood. 1990. Induction of verotoxin sensitivity in receptor-deficient cell lines using the receptor glycolipid globotriosylceramide. *Proc. Natl. Acad. Sci. (USA)* 87: 7898-901.

53. Pudymaitis, A., G. Armstrong and C. A. Lingwood. 1991. Verotoxin-resistant cell clones are deficient in the glycolipid globotriosylceramide: differential basis of phenotype. *Arch. Biochem. Biophys.* 286: 448-52.

54. Boyd, B. and C. A. Lingwood. 1989. Verotoxin receptor glycoprotein in human renal tissue. *Nephron.* 51: 207-210.

55. O'Brien, A. D., V. L. Tesh, R. A. Donohue, M. P. Jackson, S. Olsnes, K. Sandvig, A. A. Lindberg and G. T. Keusch. 1992. Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. *Curr. Top. Microbiol. Immunol.* 180: 65-94.

56. Richardson, S. E., M. A. Karmali, L. E. Becker and C. R. Smith. 1988. The histopathology of the hemolytic uremic syndrome associated verocytotoxin produced Escherichia coli infections. *Hum. Pathol.* 19: 1102-1108.

57. Tesh, V. L. and A. D. O'Brien. 1991. The pathogenic mechanisms of Shiga toxin and the Shiga-like toxins. *Mol.Microbiol.* 5: 1817-22.

58. van de Kar, N. C. A. J., L. A. H. Monnens, M. A. Karmali and V. W. M. van Hinsbergh. 1992. Tumor necrosis factor and interleukin-1 induce expression of the verocytotoxin receptor globotriaosylceramide on human endothelial cells: implications for the pathogenesis of the hemolytic uremic syndrome. *Blood.* 80: 2755-64.

59. Bergstein, J. M., M. Riley and N. U. Bang. 1992. Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *New Engl. J. Med.* 327: 755-9.

60. Anonymous. 1991. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 30-1991. An 85-year-old woman with renal failure, neurologic deterioration, and seizures. *New Engl. J. Med.* 325: 265-73.

61. Cleary, T. G. 1992. Escherichia coli that cause hemolytic uremic syndrome. *Infect. Dis. Clin. N. A.* 6: 163-76

62. Griffin, P. M. and R. V. Tauxe. 1991. The epidemiology of infections caused by Escherichia coli O157:H7, other enterohemorrhagic E. coli, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13: 60-98.

63. Ostroff, S. M., J. M. Kobayashi and J. H. Lewis. 1989. Infections wtih Escherichia coli O157:H7 in Washington state: the first year of statewide disease surveillance. *JAMA.* 262: 355-359.

64. Proulx, F., J. P. Turgeon, G. Delage, L. Lafleur and L. Chicoine. 1992. Randomized, controlled trial of antibiotic therapy for Escherichia coli O157:H7 enteritis. *J. Pediatr.* 121: 299-303.

65. Walterspiel, J. N., S. Ashkenazi, A. L. Morrow and T. G. Cleary. 1992. Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. *Infection.* 20: 25-9.