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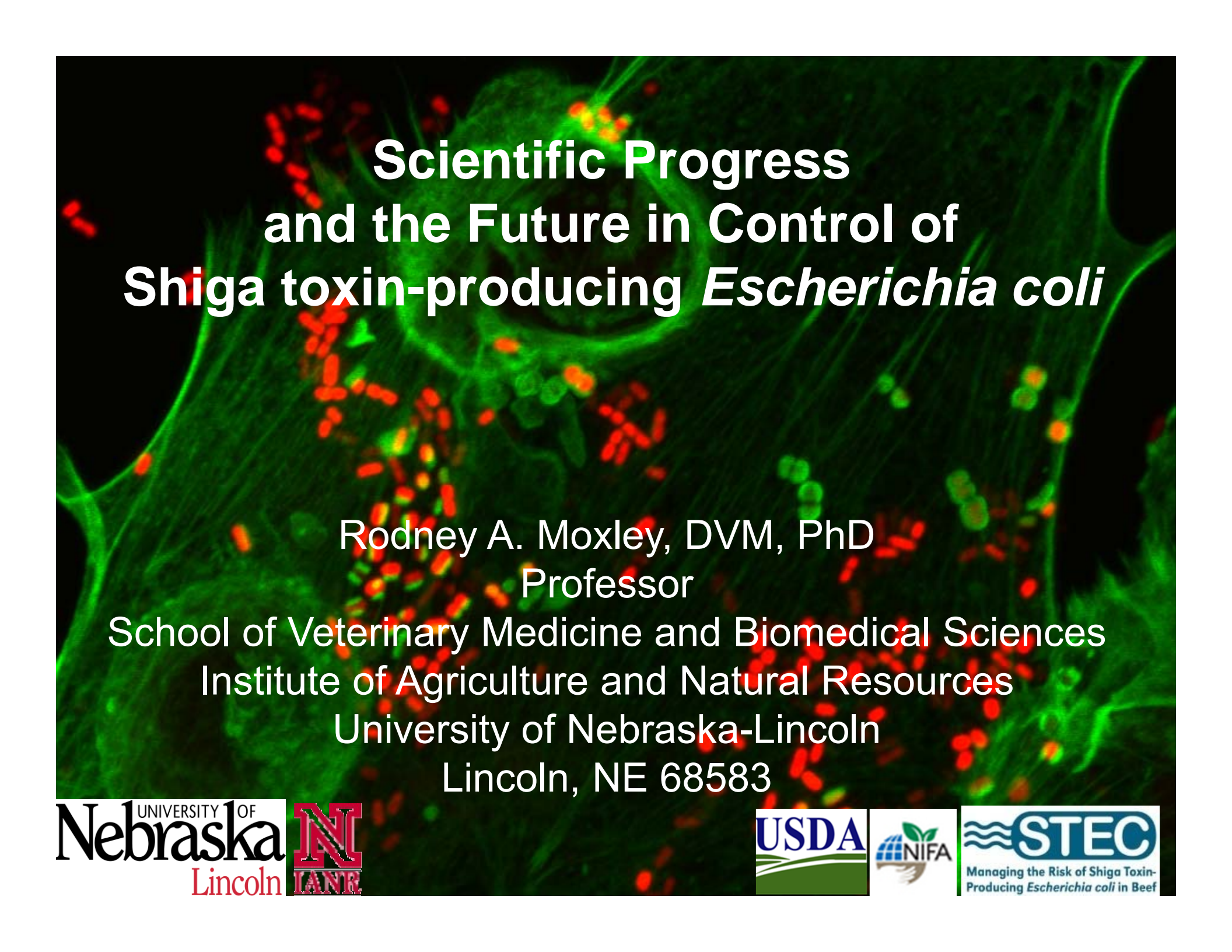
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**Scientific Progress
and the Future in Control of
Shiga toxin-producing *Escherichia coli***

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Discovery of Shiga-like toxin (SLT) in *E. coli*

O'Brien, A. D., M. R. Thompson, J. R. Canty, and S. B. Formal.
Production of a *Shigella dysenteriae*-like toxin by pathogenic *Escherichia coli*. Annual Meeting of the American Society for Microbiology, No. B103 (May 1977).

Demonstrated that some strains of *E. coli* produce activity toxic for Hela cells that can be neutralized by antibodies against *Shigella dysenteriae* type 1 (the type that produces Shiga toxin).

Discovery of Vero cytotoxin (Verotoxin) in some strains of *E. coli*

INFECTION AND IMMUNITY, Dec. 1977, p. 775-779
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Dec. 1977

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Vero Response to a Cytotoxin of *Escherichia coli*

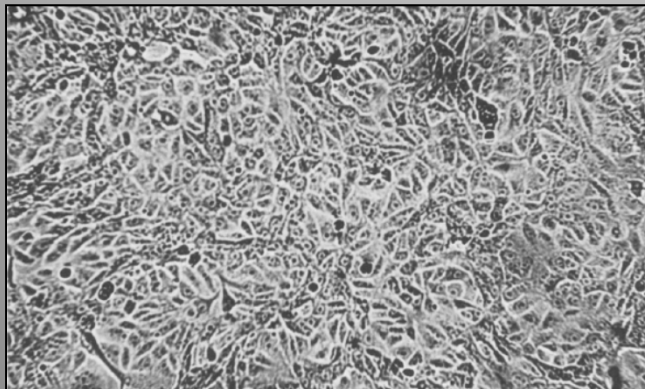
J. KONOWALCHUK,* J. I. SPEIRS, AND S. STAVRIC

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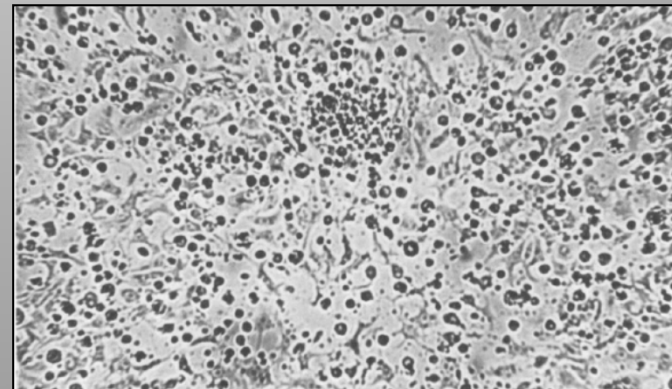
Received for publication 7 June 1977

A cytotoxin was found in culture filtrates of a number of *Escherichia coli* strains that differed from the known heat-stable and heat-labile enterotoxins of *E. coli*. It was cytotoxic for Vero but not for Y-1 or CHO cells, and its effect on Vero was distinctly different from that of heat-labile enterotoxin. It was labile to heat and antigenically different from heat-labile enterotoxin, and membrane filtration indicated a molecular weight of 10,000 to 30,000.

Control Cells



Verotoxin produced by *E. coli* O26:H11



The First *E. coli* O157:H7 Outbreak: McDonald's

February – March, 1982 (Oregon)
May – June, 1982 (Michigan)

HEMORRHAGIC COLITIS ASSOCIATED WITH A RARE *ESCHERICHIA COLI* SEROTYPE

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PAUL A. BLAKE, M.D., M.P.H., AND MITCHELL L. COHEN, M.D. (CDC)

Abstract We investigated two outbreaks of an unusual gastrointestinal illness that affected at least 47 people in Oregon and Michigan in February through March and May through June 1982. The illness was characterized by severe crampy abdominal pain, initially watery diarrhea followed by grossly bloody diarrhea, and little or no fever.

It was associated with eating at restaurants belonging to the same fast-food restaurant chain in Oregon ($P < 0.005$) and Michigan ($P = 0.0005$) and with eating any of three sandwiches containing three ingredients in common (beef patty, rehydrated onions, and pickles).

Stool cultures did not yield previously recognized pathogens. However, a rare *Escherichia coli* serotype, 0157:H7, that was not invasive or toxigenic by standard tests was isolated from 9 of 12 stools collected within four days of onset of illness in both outbreaks combined, and from a beef patty from a suspected lot of meat in Michigan. The only known previous isolation of this serotype was from a sporadic case of hemorrhagic colitis in 1975. This report describes a clinically distinctive gastrointestinal illness associated with *E. coli* 0157:H7, apparently transmitted by undercooked meat. (N Engl J Med. 1983; 308:681-5.)

Published March 24, 1983

- ≥ 47 ill, no deaths
- two outbreaks (separated in time) in different states tied to same source
- only previous knowledge of *E. coli* O157:H7 was one isolate in 1975 from a sporadic case of bloody diarrhea

Discovery that *E. coli* O157:H7 isolated from an Outbreak of Hemorrhagic Colitis in Canada in Nov. 1982 and Sporadic Cases in 1979 and 1980 makes Vero cytotoxin (Verotoxin) (Ottawa, Ontario).

Johnson, W.M., H. Lior, and G. S. Bezanson. *The Lancet*, Jan. 1/8, 1983

**CYTOTOXIC ESCHERICHIA COLI O157:H7
ASSOCIATED WITH HAEMORRHAGIC COLITIS IN
CANADA**

SIR,—*Escherichia coli* capable of producing Vero cytotoxin (VT) have been reported in classical infantile enteropathogenic (EPEC) serotypes O26 and O128,¹⁻⁴ but their role in the pathogenesis of diarrhoea has not yet been established.

Our laboratory has studied the enterotoxigenicity and cytotoxicity of over 2000 *E. coli* strains isolated from diarrhoeal patients during the five years 1978-82. 70% of the 78 cytotoxic serotypes identified were *E. coli* O 26:K60:H11; the second most common cytotoxic serotype was O157:H7, with 6 isolates from sporadic cases of gastroenteritis (9% of the total cytotoxic strains).

The U.S. Centers for Disease Control has recently reported⁵ the isolation of sorbitol-negative *E. coli* O157:H7 from cases of haemorrhagic colitis. We had the opportunity to study an outbreak of haemorrhagic colitis in November, 1982, at a Canadian institution for elderly patients. *E. coli* O157:H7 was isolated from those patients presenting with haemorrhagic colitis (bloody diarrhoea and abdominal pain) in the absence of other recognised enteric pathogens. We report for the first time Vero cytotoxin production by all *E. coli* O157:H7 isolates from the outbreak patient. Although most of the isolates were sorbitol negative some fermented sorbitol after 5 or more days' incubation. 2 of the 6 sporadic O157:H7 strains isolated in 1979 and 1980 were associated with haemorrhagic colitis. Clinical information could not be obtained for the remaining 4 cases of gastroenteritis. None of the sporadic or outbreak isolates of *E. coli* O157:H7 produced ST or LT

1. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. *FEMS Microbiol Lett* 1980; **7**: 15-17.
2. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun* 1977; **18**: 775-79.
3. Wade WG, Thom BT, Evans N. Cytotoxic enteropathogenic *Escherichia coli*. *Lancet* 1979; **ii**: 1235-36.
4. Wilson MW, Bettelheim KA. Cytotoxic *Escherichia coli* serotypes. *Lancet* 1980; **i**: 201.
5. Centers for Disease Control. Isolation of *E. coli* O157:H7 from sporadic cases of hemorrhagic colitis—United States. *Morbidity Mortal Wkly Rep* 1982; **31**: 580-85.

enterotoxins or were invasive. Neutralising anti-cytotoxin could not be detected in sera from outbreak patients taken 3 weeks after the onset of symptoms. Reactivity of the O157:H7 isolates in infant rabbit assays is now being investigated.

The plasmid DNA profiles of representative *E. coli* O157:H7 strains from the outbreak were identical (figure, lanes g-i). Three major common bands were found that corresponded to plasmids of masses 3.28, 4.25 and 65.5 megadaltons. Profiles were then compared with the *E. coli* O157:H7 VT-positive isolates from the sporadic (lanes c-f) and outbreak cases. The plasmid profiles were identical for the sporadic and outbreak strains of O157:H7 except for one sporadic isolate from 1978 (lane c) in which the two smaller plasmids were absent. No antibiotic resistance was observed in cytotoxic O157:H7 from either sporadic or outbreak sources.

This evidence for cytotoxicity of sporadic and outbreak isolates of *E. coli* O157:H7 studied to date in Canada may help to define the pathogenic potential of this serotype of *E. coli* in human diarrhoeal disease and, specifically, its association with haemorrhagic colitis.

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**AUTOLOGOUS BONE MARROW RESCUE IS
UNNECESSARY AFTER VERY-HIGH-DOSE
CYCLOPHOSPHAMIDE**

SIR,—High-dose alkylating agent chemotherapy with autologous bone marrow rescue is being investigated in the treatment of various advanced malignancies. The ability of marrow rescue to accelerate peripheral neutrophil recovery has been established for high-dose melphalan¹ and for nitrosourea-containing combinations² but its necessity for most other regimens has been presumed rather than proven.

We are investigating high-dose cyclophosphamide after four courses of conventional chemotherapy (etoposide 100 mg/m² i.v. on days 1-3, doxorubicin 40 mg/m² on day 1, and vincristine 1-4 mg/m² on day 1) in patients with small-cell carcinoma of the lung.

The Lancet, Mar. 26, 1983

Discovery that *E. coli* O157:H7 isolates from patients in United States make a Verotoxin that was chemically and biologically almost identical to Shiga toxin and neutralized by anti-Stx antiserum; named it *E. coli* Shiga-like toxin.

O'Brien, A.D., T. A. Lively, M. E. Chen, S. W. Rothman, and S. B. Formal (Uniformed Services University of the Health Sciences and Walter Reed Army Institute of Research)

702

THE LANCET, MARCH 26, 1983

ESCHERICHIA COLI O157:H7 STRAINS ASSOCIATED WITH HAEMORRHAGIC COLITIS IN THE UNITED STATES PRODUCE A SHIGELLA DYSENTERIAE 1 (SHIGA) LIKE CYTOTOXIN

SIR,—Strains of *Escherichia coli* can cause diarrhoeal disease in man either by colonising the small bowel and elaborating a heat labile or heat stable enterotoxin or, less frequently, by invading colonic epithelial cells in a manner analogous to invasion by virulent shigellae.¹ That *E. coli* must be capable of evoking gastroenteritis by other means is indicated by two observations. First, many isolates of classical enteropathogenic *E. coli* (EPEC) serotypes, although able to induce diarrhoea in children and adult volunteers, neither produce *E. coli* heat labile or heat stable enterotoxins nor are they enteroinvasive.^{2,3} Secondly, strains of the non-EPEC serotype *E. coli* O157:H7 are associated with haemorrhagic colitis^{4,5} but are not virulent by either of the two established mechanisms.⁴ The possibility that the Vero cell cytotoxin first described by Konowalchuk et al⁶ might be responsible for the bloody diarrhoea caused by some EPEC O26:K60:H11 strains or for the haemorrhagic colitis associated with some *E. coli* O157:H7 strains has lately been suggested by Wade et al⁷ and Johnson et al,⁴ respectively.

We too have been interested in the possible role of cytotoxins in the pathogenesis of certain *E. coli* diarrhoeal diseases. In 1977, we reported in a preliminary communication⁸ that extracts of certain pathogenic strains of *E. coli* were cytotoxic for hela cells and that this cytotoxic activity could be neutralised by antitoxin prepared against crude *Shigella dysenteriae* 1 (Shiga) toxin. Because we had difficulty consistently reproducing our findings and because we had no purified Shiga toxin to use as a standard, we concentrated our subsequent efforts on improving culture conditions for Shiga toxin production, purifying and characterising that toxin, and preparing antiserum to purified Shiga toxin.⁹

Recently, we used these reagents to reassess Shiga-like cytotoxin production by *E. coli*.¹⁰ We found that 11 of 13 *E. coli* strains tested produced toxin but that levels of toxin production varied from trace amounts (avirulent strains) to amounts equivalent to that of *S. dysenteriae* 1. One of the high Shiga-like toxin elaborating organisms was *E. coli* H30, an O26 strain originally described as a Vero cytotoxin producer by Konowalchuk and his colleagues.⁶ We also observed that, like bacterial extracts and culture filtrates of *S. dysenteriae* 1, such preparations of *E. coli* H30 were enterotoxic for rabbits, paralytic and lethal for mice, and able to inhibit protein synthesis in hela cells. Furthermore, when the hela cell cytotoxin of *E. coli* H30 was purified to homogeneity¹¹ it was found to have the same subunit structure, isoelectric point, and range of biological activities as the Shiga toxin.

From these data and the observation that purified Shiga toxin can kill Vero cells¹² we postulated that the Vero cell cytotoxin and the *E. coli* Shiga-like toxin were the same.

CYTOTOXICITY OF *E. COLI* O157:H7 BACTERIAL LYSATES AND CULTURE FILTRATES FOR VERO AND HELA CELLS

Strain*	Cytotoxicity for hela cells		Cytotoxicity for Vero cells		Cytotoxicity neutralised by rabbit antiserum to purified Shiga toxin
	CD ₅₀ /ml	CD ₁₀₀ /ml	CD ₅₀ /mg	CD ₁₀₀ /mg	
A	10 ⁶	2 × 10 ⁷	10 ⁶	3 × 10 ⁷	Yes
B	10 ⁶	8 × 10 ⁴	10 ⁶	2 × 10 ⁷	Yes
C	10 ⁶	8 × 10 ⁵	10 ⁶	2 × 10 ⁶	Yes

*Strain A=EDL 931 (human), strain B=EDL 932 (human), strain C=EDL 933 (Hamburger).

CD₅₀=cytotoxic dose required to kill 50% of cells in microtitre assay. Expressed as per ml culture supernatant or as per mg protein in bacterial lysates. For lysate studies organisms were cultured 48 h in 500 ml of iron-depleted modified synxase broth,¹⁴ harvested by centrifugation, washed twice in saline (0.85%), resuspended in 3 ml saline, and cells were disrupted by sonic lysis. Lysates were clarified by centrifugation and protein concentrations were estimated spectrophotometrically.*

Neutralisation of cytotoxicity defined by antiserum dilutions of ≥1/1600 completely protecting hela and Vero cells from 10 CD₅₀ of toxin in culture supernatants and cell lysates. A 1/100 dilution of prebled serum did not neutralise the toxicity of these samples. Controls included purified Shiga 60R toxin and *E. coli* H30 toxin. The titre of each toxin was 10⁶ CD₅₀/mg toxin when tested on either hela cells or on Vero cells. Both toxins were completely neutralised by antitoxin but not by prebled serum.

Earlier this year, and independently of Johnson and co-workers,⁴ we found that bacterial extracts and culture supernatants of three *E. coli* O157:H7 isolates from the recent cases of haemorrhagic colitis in the United States (kindly provided by Dr George Morris of the Centers for Disease Control, Atlanta, Georgia) were cytotoxic for hela cells and that cytotoxicity could be neutralised by rabbit antiserum to purified Shiga toxin (table). To assess the relation between the *E. coli* O157:H7 preparations and the purified toxins of *E. coli* H30 and *S. dysenteriae* 1 strain 60R, we compared the effect of the *E. coli* O157:H7 preparations on hela cells and Vero cells. The results (table) substantiate the premise that the toxins are the same and support the idea that this toxin may play an important role in the pathogenesis of some *E. coli* diarrhoeal diseases.

We propose that hereafter one name be used to describe the toxin. We prefer "*E. coli* Shiga-like toxin" to "Vero cytotoxin" because it more precisely describes the nature of the toxin.

This work was supported by the Agency for International Development grant 2212-00.

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Change in Name from SLT to Stx

Proposed SLT (VT) Nomenclature		
Current name	Proposed new nomenclature	
	Gene	Protein
Shiga toxin (Stx)	<i>stx</i>	Stx
Shiga-like toxin I (SLT-I) or verotoxin 1 (VT1)	<i>stx</i> ₁	Stx1
SLT-II or VT2	<i>stx</i> ₂	Stx2
SLT-IIc or VT2c	<i>stx</i> _{2c}	Stx2c
SLT-IIe or VT2e	<i>stx</i> _{2e}	Stx2e

Produced by:

← *Shigella dysenteriae* Type 1

← EHEC or STEC

Calderwood, et al. 1996. ASM News 62:118-9.

Conclusive Association between VTEC (STEC) with HUS

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 151, NO. 5 • MAY 1985
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May 1985

The Association Between Idiopathic Hemolytic Uremic Syndrome and Infection by Verotoxin-Producing *Escherichia coli*

Mohamed A. Karmali, Martin Petric, Corazon Lim, Peter C. Fleming, Gerald S. Arbus, and Hermy Lior

From the Departments of Bacteriology, Virology, and Pediatrics (Division of Nephrology), and the Research Institute, the Hospital for Sick Children, Toronto; the Department of Microbiology, University of Toronto; and the National Centre for Enteric Bacteriology, Laboratory Centre for Disease Control, Ottawa, Canada

Note the O groups; 5 of the 6 listed are adulterants in raw non-intact beef as declared by USDA-FSIS

Forty pediatric patients with idiopathic hemolytic uremic syndrome (HUS) were investigated for evidence of infection by Verotoxin-producing *Escherichia coli* (VTEC). Fecal VTEC (belonging to at least six different O serogroups including O26, O111, O113, O121, O145, and O157) or specifically neutralizable free-fecal Verotoxin (VT) or both were detected in 24 (60%) patients but were not detected in 40 matched controls. Ten of 15 of the former developed fourfold or greater rises in VT-neutralizing antibody titers, as did six other patients who were negative for both fecal VTEC and VT. A total of 30 (75%) patients had evidence of VTEC infection by one or more criteria. We concluded that a significant association exists between idiopathic HUS and infection by VTEC. The detection of free-fecal VT was the most important procedure for the early diagnosis of this infection because, in our study, VTEC were never isolated in the absence of fecal VT, whereas fecal VT was often present even when VTEC were undetectable.

Development of Animal Models of STEC Disease

Infect Immun. 1986 Mar;51(3):953-6.

Infection of gnotobiotic pigs with an *Escherichia coli* O157:H7 strain associated with an outbreak of hemorrhagic colitis. Francis DH, Collins JE, Duimstra JR.

J. Infect Dis. 1986 Oct;154(4):712-6.

The pathogenesis of hemorrhagic colitis caused by *Escherichia coli* O157:H7 in gnotobiotic piglets. Tzipori S, Wachsmuth IK, Chapman C, Birden R, Brittingham J, Jackson C, Hogg J.

Gnotobiotic piglets develop diarrhea as a result of colitis; also the bacteria caused attaching-effacing lesions.

Sept. 1995

The Role of the *eaeA* Gene in Diarrhea and Neurological Complications in a Gnotobiotic Piglet Model of Enterohemorrhagic *Escherichia coli* Infection

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Sept. 1995

Enterohemorrhagic *Escherichia coli* O157:H7 Requires Intimin To Colonize the Gnotobiotic Pig Intestine and To Adhere to HEp-2 Cells†

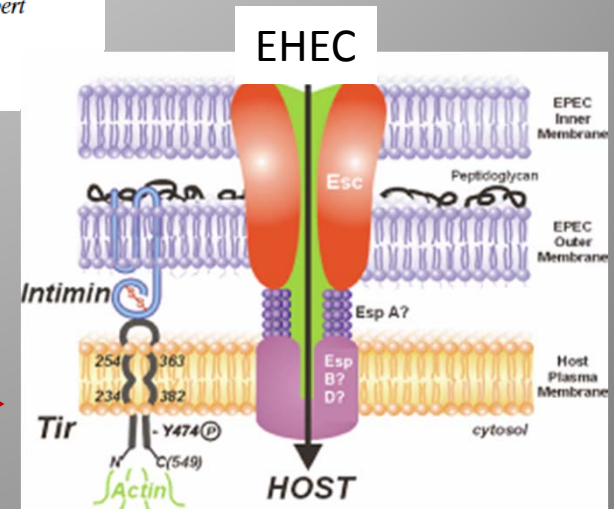
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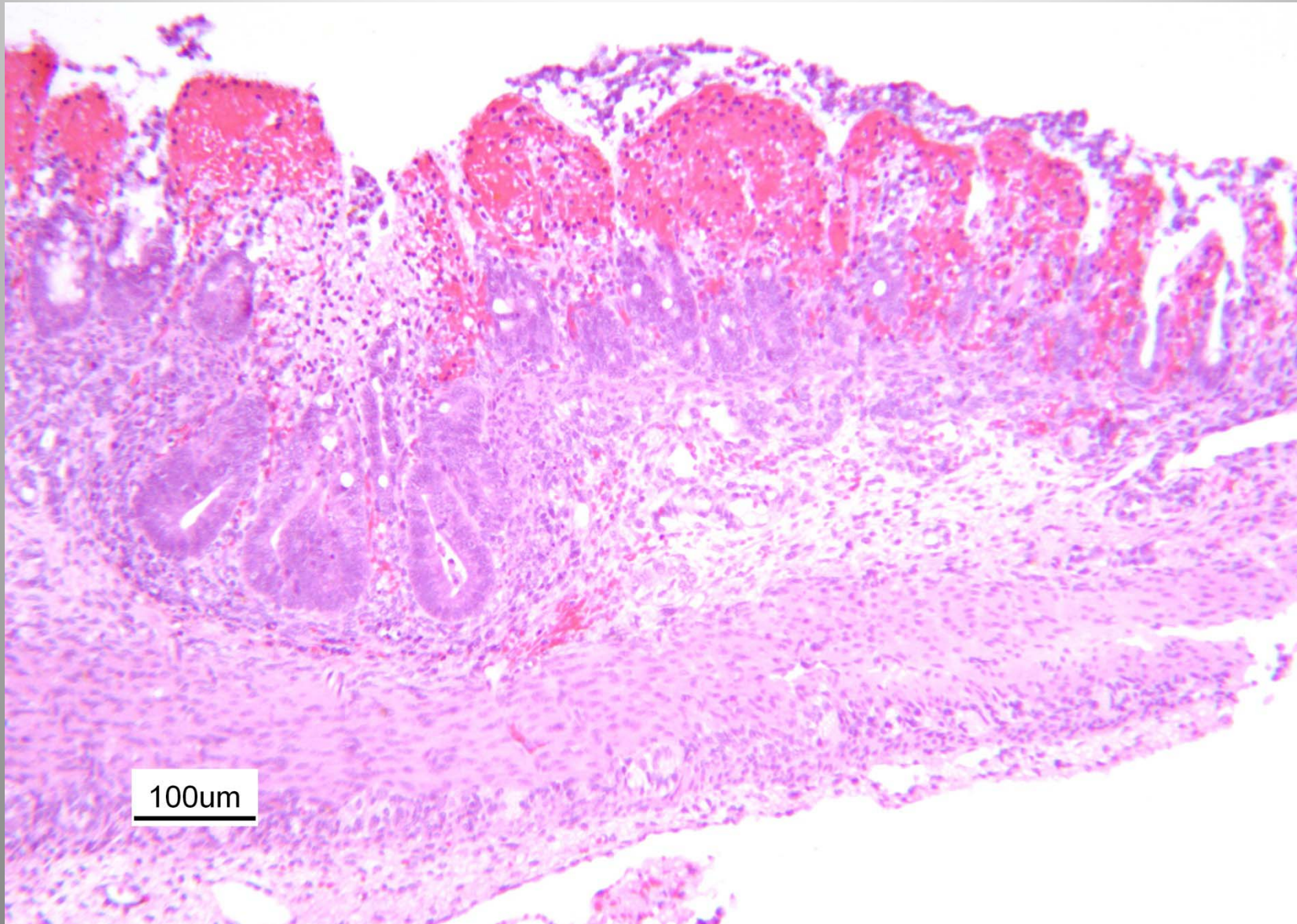
***E. coli*
O157:H7
required
intimin for
intestinal
colonization.**

The outer membrane protein intimin is required for bacterial attachment to the host cell (attaching-effacing lesions) →

Intimin attaches to Tir (type III secreted protein) →



Hemorrhagic colitis in gnotobiotic piglet caused by *E. coli* O157:H7



Aug. 1986

Natural and Experimental Infection with an Attaching and Effacing Strain of *Escherichia coli* in Calves†

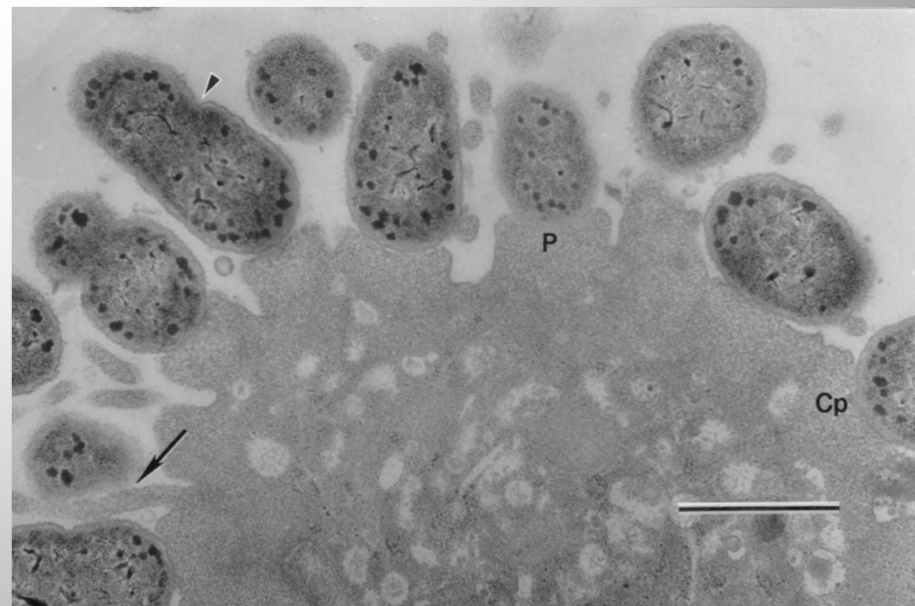
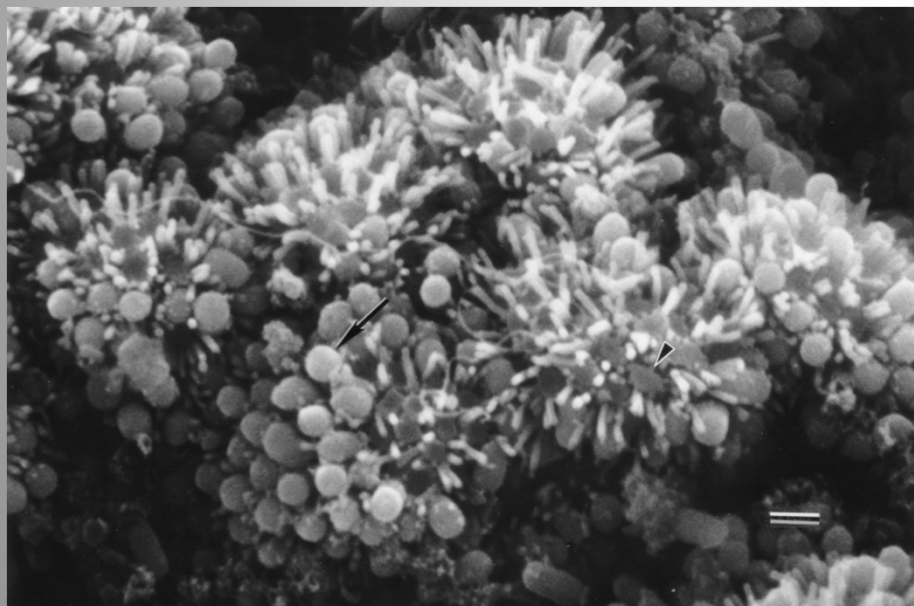
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This study involved an O5:NM, Stx1+ isolate from a calf.

Koch's postulates were satisfied, proving that non-O157 Shiga toxin-producing *E. coli* infect and cause disease in newborn calves.



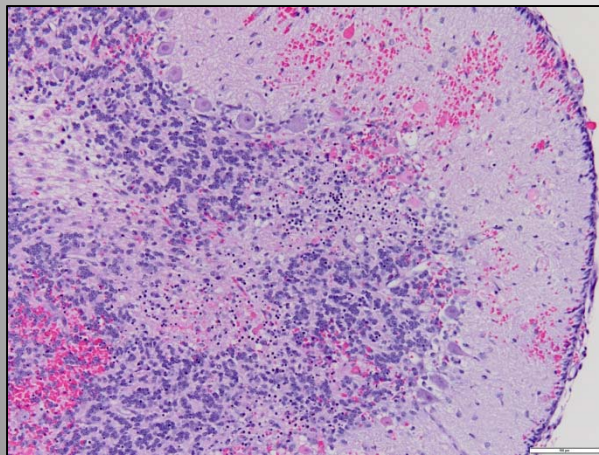
April 1989

Edema Disease-Like Brain Lesions in Gnotobiotic Piglets Infected with *Escherichia coli* Serotype O157:H7

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Received 8 September 1988/Accepted 8 January 1989



Stx2 is more likely to cause blood vessel and brain damage (stroke-like lesions) than Stx1.

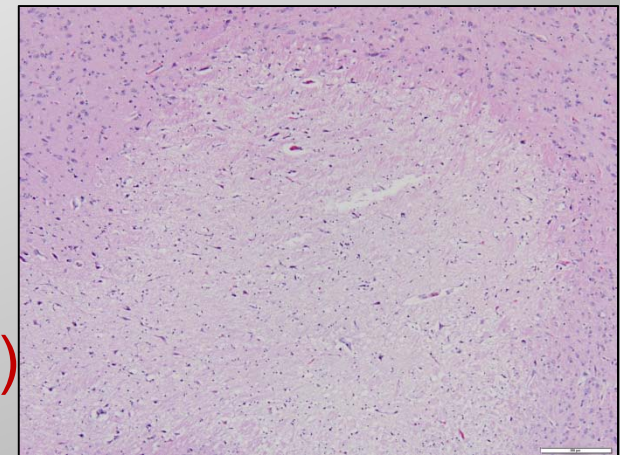


TABLE 1. Characteristics of *E. coli* strains used in piglet challenge

Strain	Source (reference)	Serotype	SLT-I ^a	SLT-II ^a
933	Hamburger isolate (18)	O157:H7	+	+
933D	Derived from 933 ^{b,c} (21)	O157:H7	+	-
B2387	Human isolate ^c	O157:H7	-	+
G58-1	Swine isolate (5)	O101:K28:NM	-	-

^a Detectable levels of toxins in culture supernatant (+, positive; -, negative).

^b Spontaneously lost ability to produce SLT-II during storage.

^c Obtained from A. D. O'Brien, Uniformed Services University of the Health Sciences, Bethesda, Md.

TABLE 2. Incidence and distribution of malacia in the brains of pigs challenged with *E. coli* strains

Brain region (reference point)	No. of pigs with lesions/no. inoculated and examined for strain:			
	933	933D	B2387	G58-1
Medulla oblongata (olivary nucleus)	5/8	0/5	2/4	0/2
Cerebellum (cerebellar peduncles)	4/9	0/5	0/6	0/3
Midbrain (corpora quadrigemina)	4/9	0/5	3/6	0/4
Cerebrum (interthalamic adhesion)	2/9	0/5	6/6	0/4
Cerebrum (genu of corpus callosum)	6/9	0/5	6/6	0/4
Total with malacia/total inoculated	8/9	0/5	6/6	0/4

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Recent Advances in Verocytotoxin-producing *Escherichia Coli* Infections
M.A. Karmali and A.G. Goglio, editors.

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241

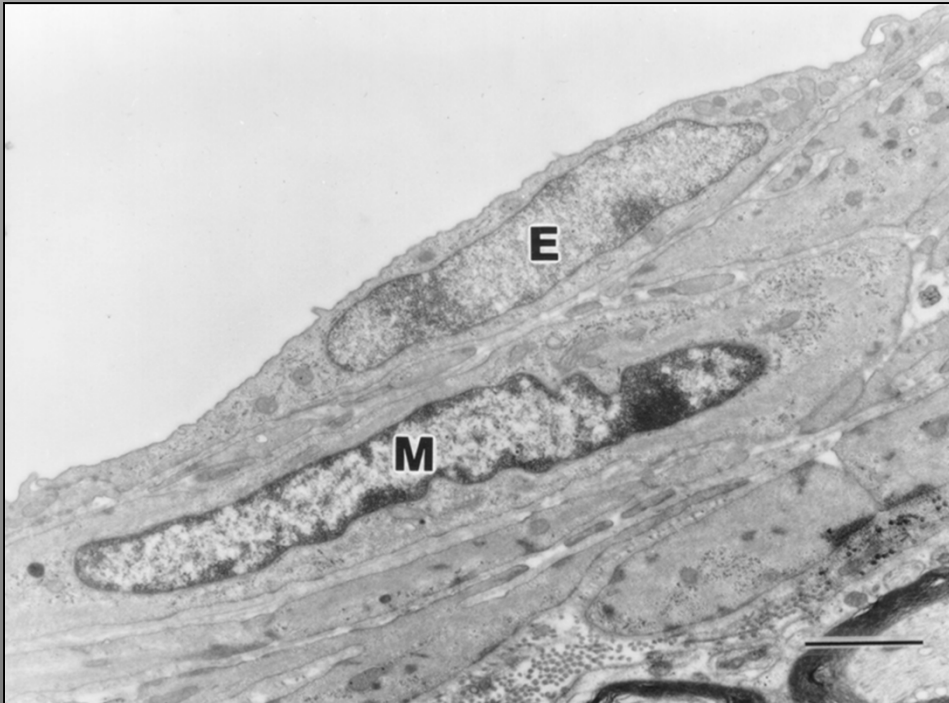
Shiga-like Toxin-II-Producing *Escherichia coli* O157:H7 Infection in Gnotobiotic Piglets: Protection against Brain Vascular Lesions with SLT-II Antiserum

C. Chae¹, R. A. Moxley¹, J. Christopher-Hennings², D. H. Francis², and M. J. Wannemuehler³

Antibodies against Stx2 protect against blood vessel and brain damage

administered anti-Stx 2 serum

administered control serum



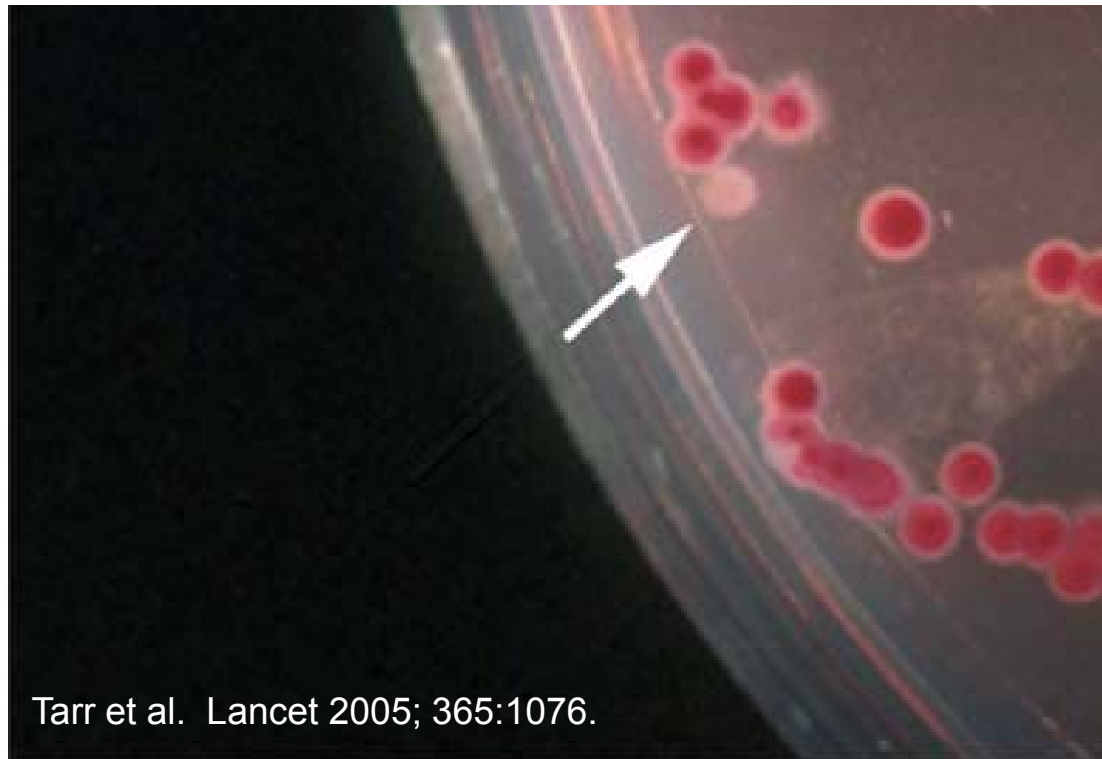
May 1986

Sorbitol-MacConkey Medium for Detection of *Escherichia coli* O157:H7 Associated with Hemorrhagic Colitis

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Newfoundland and Labrador Public Health Laboratories, St. John's, Canada A1B 3T2

Received 29 August 1985/Accepted 2 January 1986



Tarr et al. Lancet 2005; 365:1076.

Figure 2: *E coli* O157:H7 on a sorbitol-MacConkey agar plate
Arrow indicates distinctive colourless *E coli* O157:H7 colony.

Sorbitol-MacConkey became the major culture medium for detection of *E. coli* O157:H7.

First Detection of *E. coli* O157:H7 in Cattle

Borczyk AA, Karmali MA, Lior H, et al. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;1:98. (Jan. 10, 1987)

These authors reported the isolation of *E. coli* O157:H7 from a dairy cow in Canada. This was following an outbreak of *E. coli* O157:H7 in children visiting the farm who had consumed unpasteurized milk.

Orskov F, Orskov I, Villar JA. Cattle as reservoir of verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;2:276. (Aug. 1, 1987)

These authors stated that the first record of isolation of *E. coli* O157:H7 from a bovine was in Argentina in 1977 from a calf with diarrhea. This information was obtained from the records of the WHO *E. coli* Reference Laboratory at Copenhagen, Denmark.

J. Med. Microbiol. — Vol. 40 (1994), 424–427

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June 1994

A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces

P. A. CHAPMAN, D. J. WRIGHT and C. A. SIDDONS

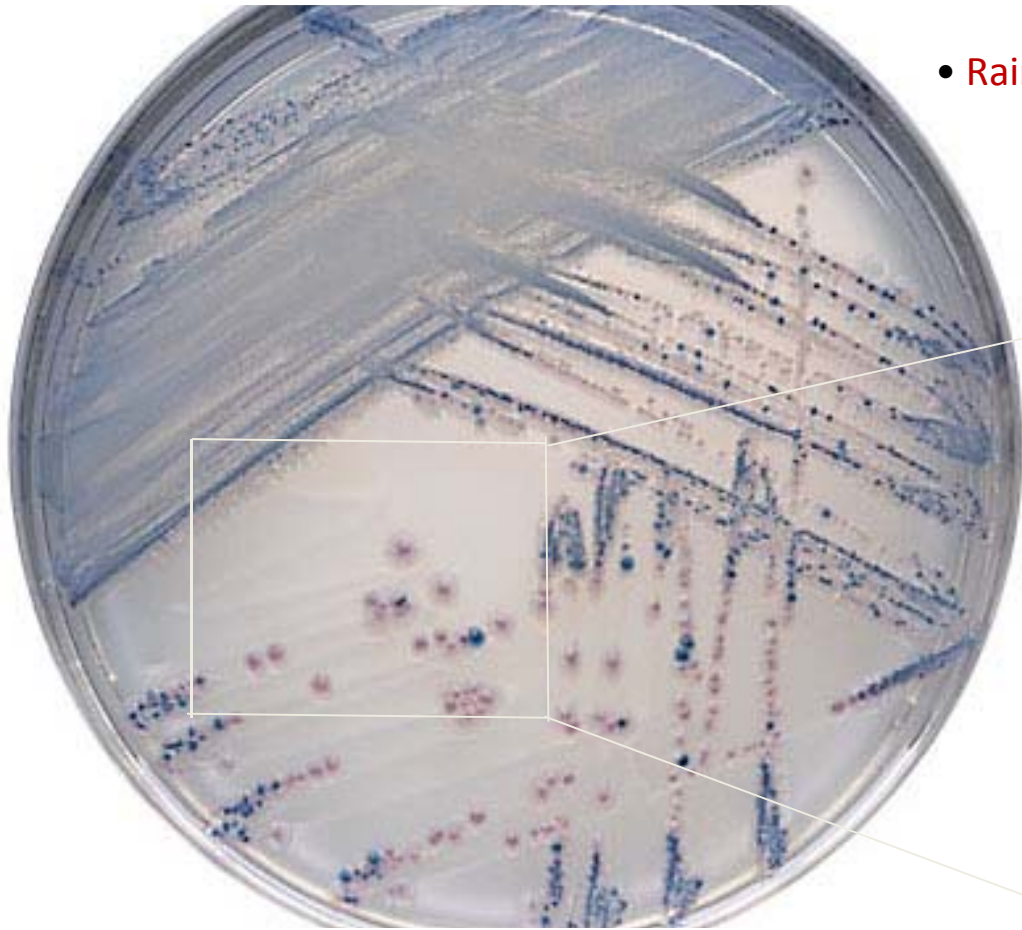
Public Health Laboratory, Herries Road, Sheffield S5 7BQ

- IMS 100-fold more sensitive than direct culture in spiked feces
- 61/84 (73%) of positive samples detected by IMS only

Chromogenic Culture Media more Specific than SMAC were Developed in mid-late 1990's

BD BBL™ CHROMagar™ O157 →

- based on enzymatic activity that differs from that of other bacteria (proprietary)
- Rainbow agar is a similar type of product and based on ability of *E. coli* O157:H7 to produce β -galactosidase but not β -glucuronidase



Optional selection: antibiotics (cefixime + cefsulodin) plus K tellurite:

- cefixime inhibits many *Enterobacteriaceae*, but especially *Proteus*
- cefsulodin inhibits *Pseudomonas aeruginosa*
- K tellurite inhibits *Providencia*, *Aeromonas* and "generic" *E. coli*

<http://www.bd.com/ds/productCenter/214984.asp>

Antibiotic selection was optimized

Jack in the Box

Nov. 1992 – Feb. 1993 (4 States)

State	Reported Sick	Meeting Case Definition	Culture-confirmed	Hospitalized	HUS	Died
WA	602	477	477	144 (30%)	30 (6%)	3* (0.6%)
ID	14	14	14	4 (29%)	1 (7%)	0
NV	58	58	1	9 (16%)	3 (5%)	0
CA	34	34	34	14 (41%)	7 (21%)	1 (3%)
Total	708	583	526	171 (29%)	41 (7%)	4 (0.7%)

~272,672 (20%) of the implicated patties were recalled. This prevented ~800 new cases.

***One patient never ate hamburger; acquired infection secondarily at day-care center.**

Morbidity Mortality Weekly Report, April 16, 1993, Vol. 42, No. 14, p. 258 - 263.



Food Safety and Inspection Service
United States Department of Agriculture
Washington, D.C. 20250-3700

Backgrounders

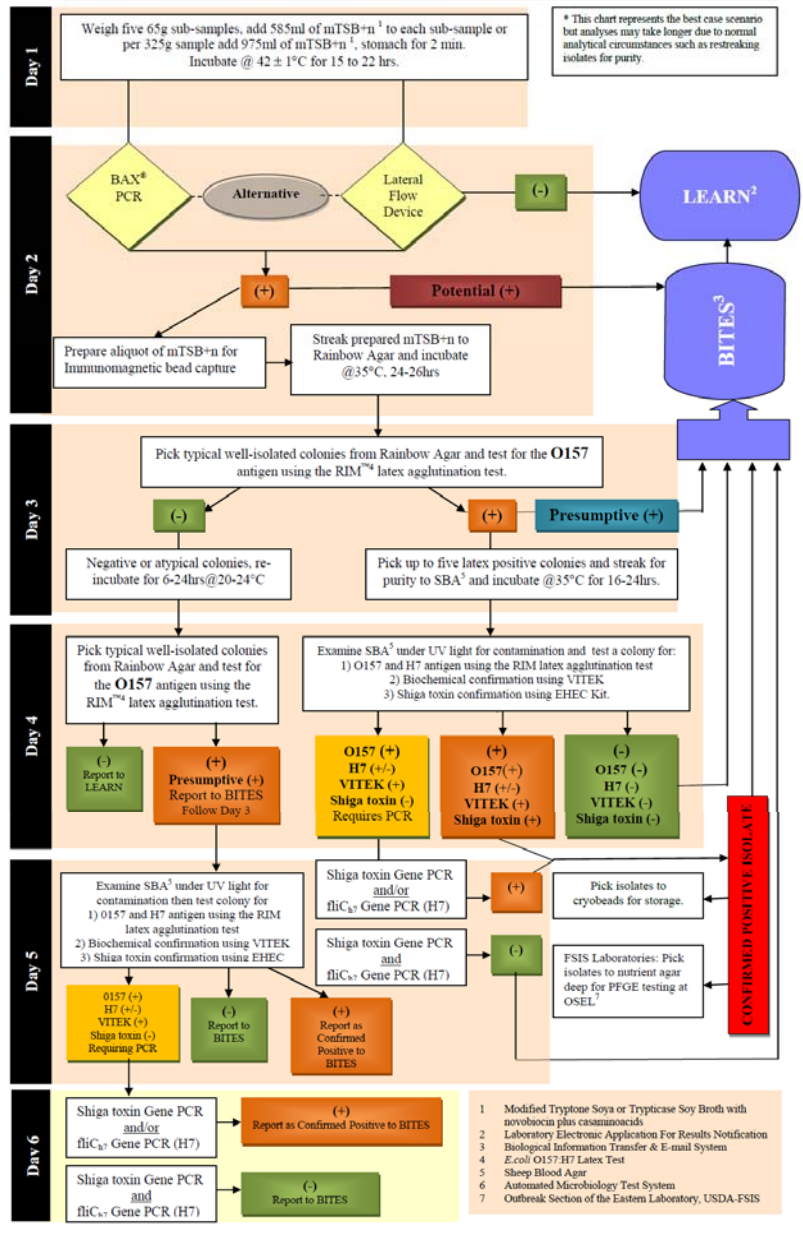
January 1999
Slightly Revised February 17, 1999

FSIS Policy on Non-intact Raw Beef Products Contaminated with *E. coli* O157:H7

In October 1994, in response to an outbreak of foodborne illness that resulted in several deaths from the consumption of undercooked ground beef contaminated with *Escherichia coli* (*E. coli*) O157:H7, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) declared *E. coli* O157:H7 an adulterant in raw ground beef and began a sampling program to test for *E. coli* O157:H7 in raw ground beef prepared in federally inspected plants and in retail stores. FSIS notified the public that raw ground beef products contaminated with *E. coli* O157:H7 are adulterated under the Federal Meat Inspection Act (FMIA) unless the ground beef is further processed to destroy this pathogen.

When the sampling program began in 1994, raw ground beef was targeted because of its strong epidemiologic link with *E. coli* O157:H7 infection. Exposure to *E. coli* O157:H7 has been linked with serious, life-threatening human illnesses (hemorrhagic colitis and hemolytic uremic syndrome). Raw ground beef products present a significant public health risk because ground beef is frequently consumed after insufficient preparation to destroy any pathogens that may have been introduced below the product's surface by chopping or grinding. Thoroughly cooking ground beef products to 160 ° F will destroy *E. coli* O157:H7 and result in a safe product. To verify that ground beef products are thoroughly cooked, a food thermometer should be used in several places to determine that the product has reached 160 ° F.

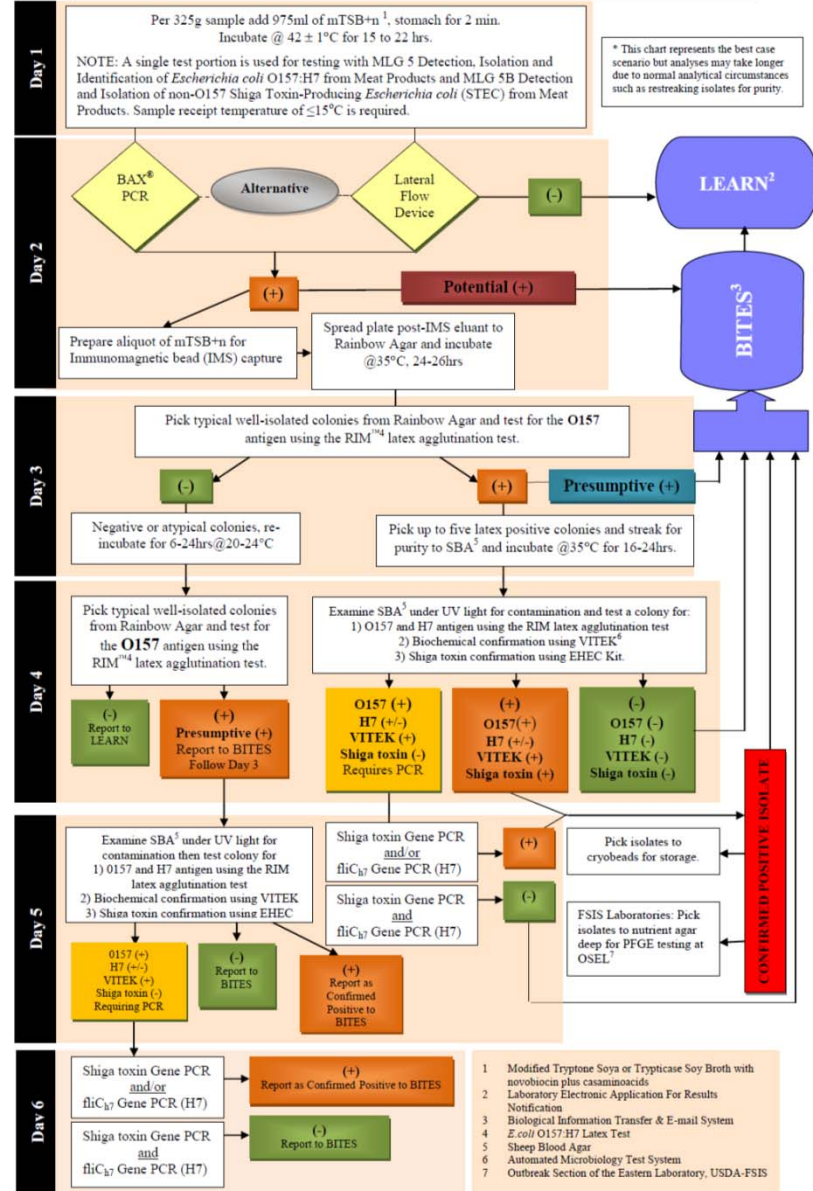
E. coli O157:H7 Ground Beef, Cooked Beef Patties, Beef Trim/components and Fermented Sausages



References: Microbiology Laboratory Guidebook MLG 5.05, MLG 5A.02

11/15/10

FSIS Laboratory Flow Chart for MLG 5.06 Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products



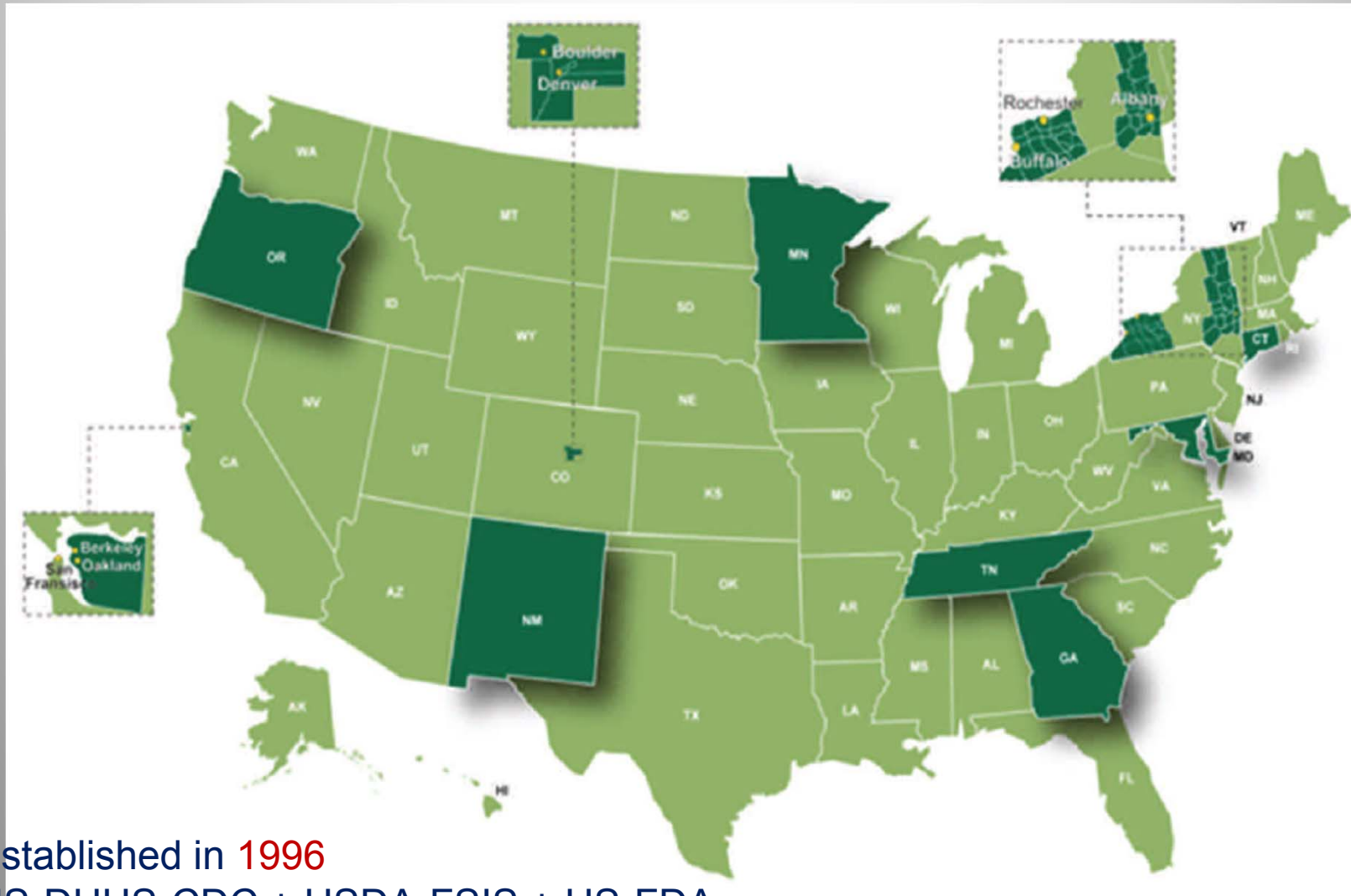
MLG 5 Appendix 1.00 *E. coli* O157:H7 Method Flow Chart

Page 1 of 1

Effective: 6/4/12

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

Foodborne Diseases Active Surveillance Network (FoodNet)

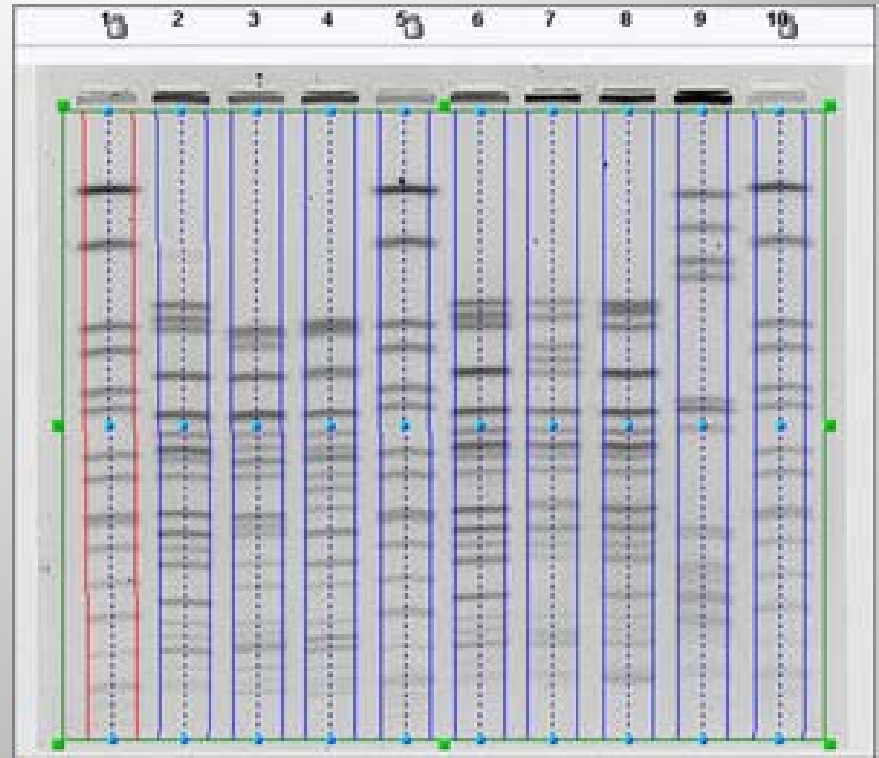


- Established in **1996**
- US-DHHS-CDC + USDA-FSIS + US-FDA
+ 10 state health departments
- 47.5 million persons (15.2% of US pop.)

PulseNet



National network of public health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC)



Network: state health departments, local health departments, and federal agencies (CDC, USDA/FSIS, FDA).

Standardized molecular subtyping (or “fingerprinting”) of foodborne disease-causing bacteria by pulsed-field gel electrophoresis (PFGE).

However, ...Hudson Foods (Nebraska) Largest Beef Recall in History at the Time then Occurred

The New York Times, page A20
Aug. 12, 1997
20,000 lbs recalled

Tons of Hamburger May Have Deadly Bacteria, U.S. Warns

By DAVID STOUT

WASHINGTON, Aug. 12 — About 20,000 pounds of frozen hamburger patties sold across the country by an Arkansas company may be contaminated with deadly E. coli bacteria, the Agriculture Department said today.

The agency said that the meat was distributed in June and early July by numerous retail and wholesale outlets, and that most of it was probably in people's freezers if it had not already been eaten. The department learned about the problem from state health officials in Colorado, where about 20 people became ill after eating some of the hamburgers in early July.

"We think we've caught this early," Jacque Knight, a spokeswoman for the Agriculture Department's Food Safety and Inspection Service, said today. She said that no deaths had been reported and that none of the people stricken in Colorado had become seriously ill.

Ms. Knight said the patties had been distributed by Hudson Foods of Rogers, Ark., which has announced a recall. She said Hudson Foods had a good record for cleanliness and had been "very cooperative" in tracing the shipments.

E. coli bacteria are associated with fecal matter and can cause death or severe illness, including bloody diarrhea, especially if the contaminated meat is undercooked. In 1993, four children died in the Northwest, and hundreds of other people became violently ill after eating contaminated hamburgers from Jack in the Box fast-food outlets.

Ms. Knight said anyone finding the patties in their freezers should take

them back to the store for refunds. If people have already eaten some of the meat and did not become ill, they probably have no reason to be alarmed, especially if they cooked the burgers thoroughly, she said.

E. coli are "very virulent in very small numbers," Ms. Knight said. Food-safety experts generally recommend cooking hamburgers so that the meat is at least 160 degrees inside. To put it another way, they

***Hope that a health
danger has been
detected in time.***

recommend against rare or medium-rare burgers.

The suspect hamburger patties are in 48-ounce packages labeled "Hudson Beef Burgers, Individually Quick Frozen," with the code 156A7; in three pound packages labeled "Hudson 100% Pure Beef Patties, Individually Frozen," with the code 156B7, and in 15-pound boxes labeled "Hudson 60 — ¼ Beef Patties Uncooked, Individually Quick Frozen," with the code 155B7. Consumers with questions can call (800) 535-4555.

In July 1996, President Clinton announced new rules for inspection and controls in the meat-processing industry. The most sweeping changes in a half-century, they were intended to do away with the old "sniff and poke method" used by meat inspectors since the Federal Meat Inspection Act was passed in 1907.

The New York Times, front page
Aug. 22, 1997
25,000,000 lbs recalled

25 Million Pounds of Beef Is Recalled

***Plant Is Closing
Over a Danger
of Bacteria***

By MICHAEL JANOFFSKY

WASHINGTON, Aug. 21 — A meat-processing company is closing its Nebraska plant indefinitely and is expanding its recall of ground beef to 25 million pounds after Federal investigators found evidence that far more meat might be contaminated by a hazardous bacteria than originally suspected. Last week, the plant recalled 1.2 million pounds of meat.

Today's actions were voluntary, but they were undertaken by the company, Hudson Foods of Rogers, Ark., under an implicit threat from the Agriculture Department that unless the processing and administrative problems at the plant were corrected, the department would force the plant to close by withdrawing food safety inspectors.

Agriculture Secretary Dan Glickman said at a news conference today that the latest recall was the largest in United States history. Mr. Glickman said Federal investigators found evidence this week that hamburger patties left over from production on June 5 — which showed evidence of the potentially deadly bacteria, E. coli 0157:H7 — were added to production the next day. As a result, the company could not guarantee that any meat produced subsequently would be free of the bacteria, leading the Agriculture Department to press for the latest recall.

Every year in the United States, bacteria in meat, poultry, seafood, eggs, fruit and vegetables kill as many as 9,000 people, mostly children and elderly people, and sicken



Associated Press

Federal investigators found weak safety standards and risky practices at this Hudson Foods Company hamburger plant in Columbus, Neb.

MEAT & POULTRY
The business journal of the meat and poultry industry

FEBRUARY 2003

**\$2.7
Billion**

**The cost of
E. coli O157:H7**

Ten-year (1993-2003) cost of *E. coli* O157:H7 to the Beef Industry

Demand for beef	\$1,584,000,000
Boneless beef prices	\$172,000,000
Packer expenditures (Capital, operating costs, recalls)	\$850,000,000
Research	\$65,000,000
Total	\$2,671,000,000

The total above does not include public health costs, which at this time were estimated at \$989,000,000/year.

Steve Kay, Meat and Poultry magazine

From 1982 – 1994: 68% of the *E. coli* O157:H7 Outbreaks were due to something other than Ground Beef

TABLE 3. Outbreaks of *Escherichia coli* O157:H7 reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1994 inclusive*

Likely vehicle or mode of spread	No. of outbreaks	No. of individuals involved
All foods†	38 (55%)	1,541 (66%)
Ground beef†	22 (32%)	1,137 (49%)
All beef products and milk†	26	1,278
Drinking water or swimming-associated	3 (4%)	276 (12%)
Person-to-person (no food identified)	9 (12%)	243 (10%)
Unknown	19 (28%)	274 (12%)
All outbreaks	69	2,334

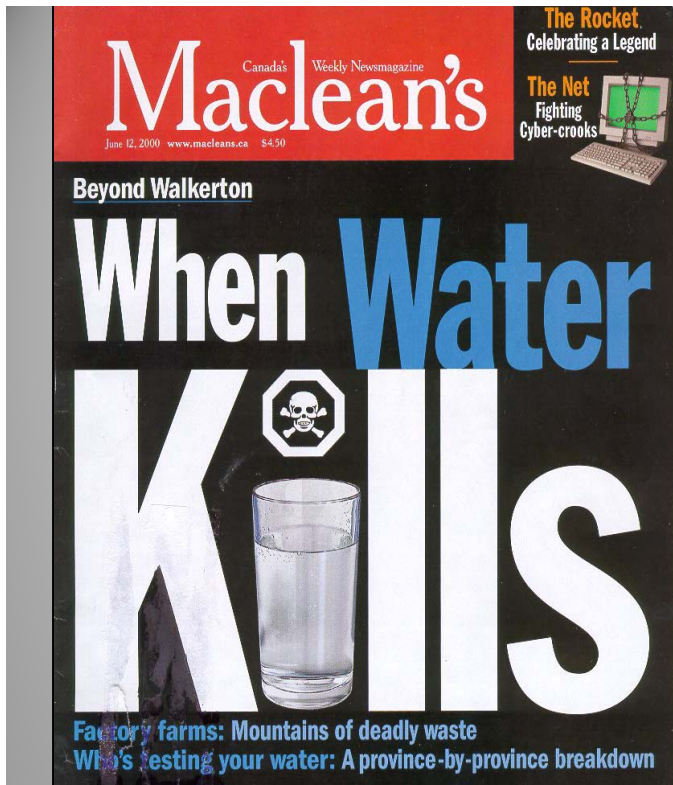
* Source: CDC surveillance data. Note: data from 1982–1992 are incomplete. Outbreaks without clear sources or sites were not tallied by the CDC during that time.

† Some of these outbreaks also involved person-to-person spread.

Armstrong, G.L. et al.

(USDA-FSIS)

Epidemiol Rev Vol. 18, No. 1, 1996



Walkerton, Ontario
May 2000

Where's the beef?

- **May 2000 Walkerton, Ontario, 5,000 pop.**
- **municipal water contamination**
- ***E. coli* O157:H7 and *Campylobacter jejuni***
- **2,300 sick; at least 7 people died**
- **excessive Spring rainfall**
- **water runoff from dairy farms**
- **cracked casings in water wells**
- **groundwater contamination of municipal water supply**
- **inadequate chlorination of municipal water**
- **laxity in regulations**
- **negligence issues by public employees**

- Contaminated water
 - Drinking water
 - Swimming pools / lakes
- Person-to-person
 - Day care centers
 - Family members
 - Nursing homes
- Direct contact with animals- farm and petting zoos
- unpasteurized milk

Spinach-associated *Escherichia coli* O157:H7 Outbreak, Utah and New Mexico, 2006

Emerging Infectious Diseases

Vol. 14, No.10, Oct. 2008

Grant, J., A. M. Wendelboe, A. Wendel, B. Jepson, P. Torres, C. Smelser,
and R. T. Rolfs

In 2006, Utah and New Mexico health departments investigated a multistate cluster of *Escherichia coli* O157:H7. A case–control study of 22 case-patients found that consuming bagged spinach was significantly associated with illness ($p < 0.01$). The outbreak strain was isolated from 3 bags of 1 brand of spinach. Nationally, 205 persons were ill with the outbreak strain.

Ecological Relationships between the Prevalence of Cattle Shedding *Escherichia coli* O157:H7 and Characteristics of the Cattle or Conditions of the Feedlot Pen[†]

DAVID SMITH,^{1*} MARK BLACKFORD,² SPRING YOUNTS,¹ RODNEY MOXLEY,¹ JEFF GRAY,^{1‡}
LAURA HUNGERFORD,¹ TODD MILTON,^{2§} AND TERRY KLOPFENSTEIN²

¹Department of Veterinary and Biomedical Sciences, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, P.O. Box 830905, Lincoln, Nebraska 68583-0905; and ²Department of Animal Science, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, P.O. Box 830908, Lincoln, Nebraska 68583-0908, USA

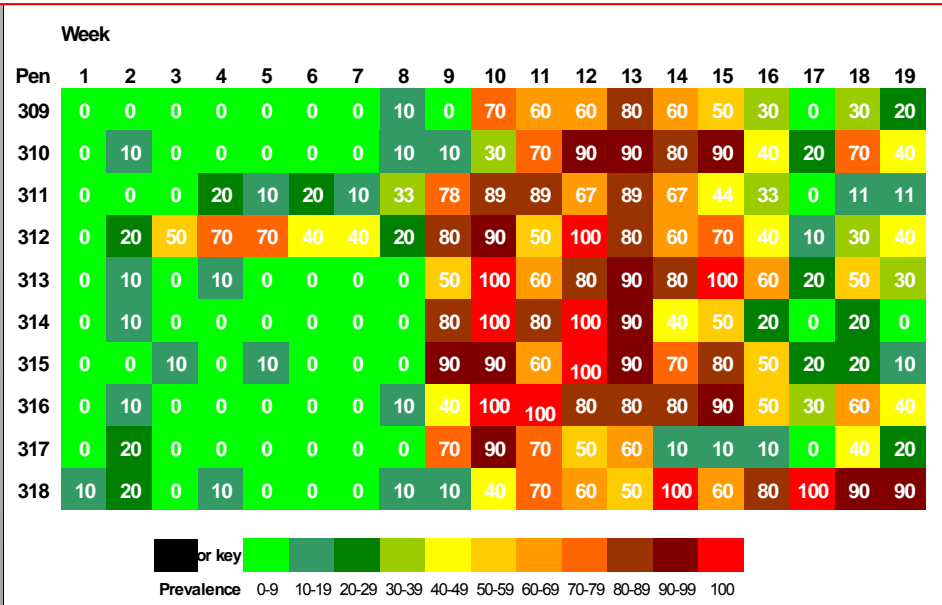
MS 01-91: Received 9 March 2001/Accepted 17 June 2001

ABSTRACT

This study was designed to describe the percentage of cattle shedding *Escherichia coli* O157:H7 in Midwestern U.S. feedlots and to discover relationships between the point prevalence of cattle shedding the organism and the characteristics of those cattle or the conditions of their pens. Cattle from 29 pens of five Midwestern feedlots were each sampled once between June and September 1999. Feces were collected from the rectum of each animal in each pen. Concurrently, samples of water

- **Cross-sectional study design**
- **one sample per animal on one day**
- **June –September 1999**
- **All feedyards positive for *E. coli* O157:H7**
- **All pens positive for *E. coli* O157:H7**
- **719 of 3,162 (23%) cattle positive with only one random sample per animal**
- **Muddy pens had higher prevalence than pens in normal condition**
- **Very dry, dusty pens had lower prevalence than muddy, but >normal**

E. coli O157:H7 causes **subclinical epidemics of infection** in feedlot cattle

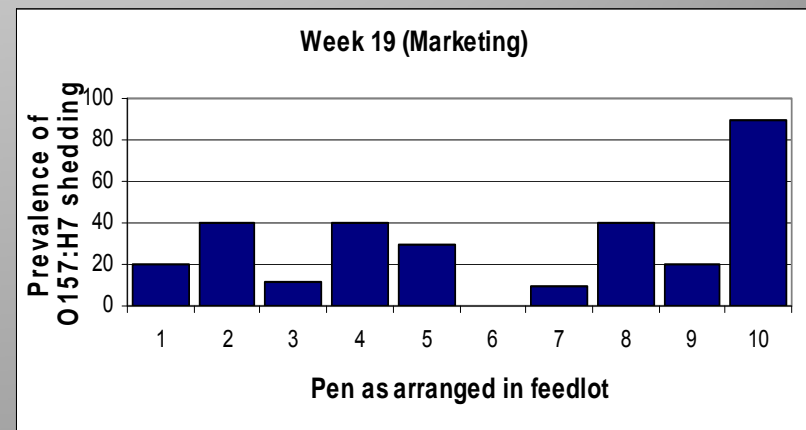
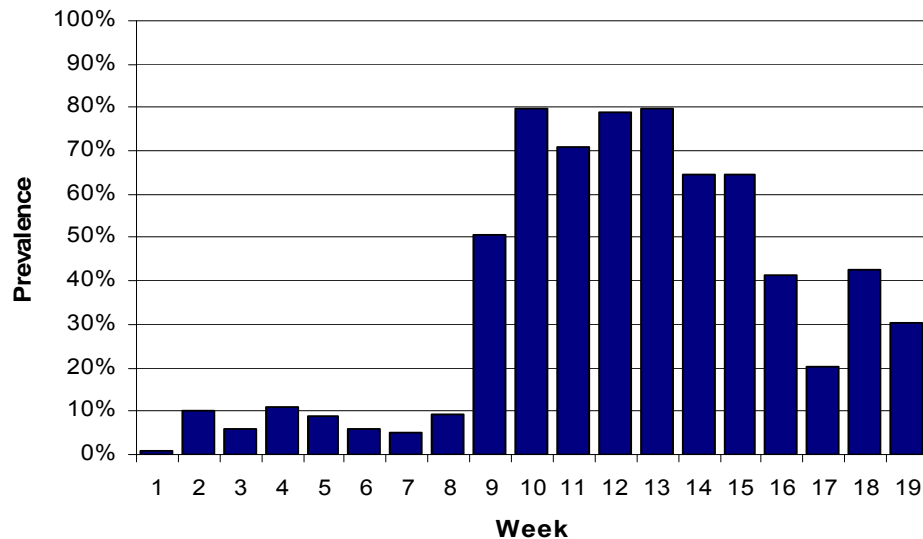


Longitudinal study Research Feedyard

Feces from 100 steers (10 pens of 10) cultured each week

E. coli O157:H7 recovered every week and at least once from every animal

Summer 2000



A diagnostic strategy to determine the Shiga toxin-producing *Escherichia coli* O157 status of pens of feedlot cattle

Studies conducted in 1999

D. R. SMITH^{1*}, J. T. GRAY^{1†}, R. A. MOXLEY¹, S. M. YOUNTS-DAHL^{1‡},
M. P. BLACKFORD², S. HINKLEY¹, L. L. HUNGERFORD^{1§}, C. T. MILTON^{2||}
AND T. J. KLOPFENSTEIN²

¹ Department of Veterinary and Biomedical Sciences, Institute of Agriculture and Forestry, University of Nebraska–Lincoln, USA

² Department of Animal Science, Institute of Agriculture and Forestry, University of Nebraska–Lincoln, USA

(Accepted 23 October 2003)

SUMMARY

Although cattle are reservoirs, no validated method for detecting *Escherichia coli* O157 (STEC O157) on farms. In 2003, we compared culturing faeces from the individual cattle or chew; and (2) culturing a composite of faecal pellets from pens classified correctly using rope devices to detect pen STEC O157 [sensitivity = 82% (57–96%); specificity = 73–98%] were classified correctly using composite samples of cattle shedding STEC O157 [sensitivity = 86% (42–96%)]. Pens were classified into three risk levels based on parallel interpretation (Spearman's $r = 0.76$, $P < 0.0001$) with the pen's prevalence of cattle posing a higher risk to food safety.

Able to detect high prevalence pens.

Culture 7 ropes instead of hundreds of steers.

Avoid running steers through chutes.



Reliable
On-
Premises
E. coli
Sampler

2005

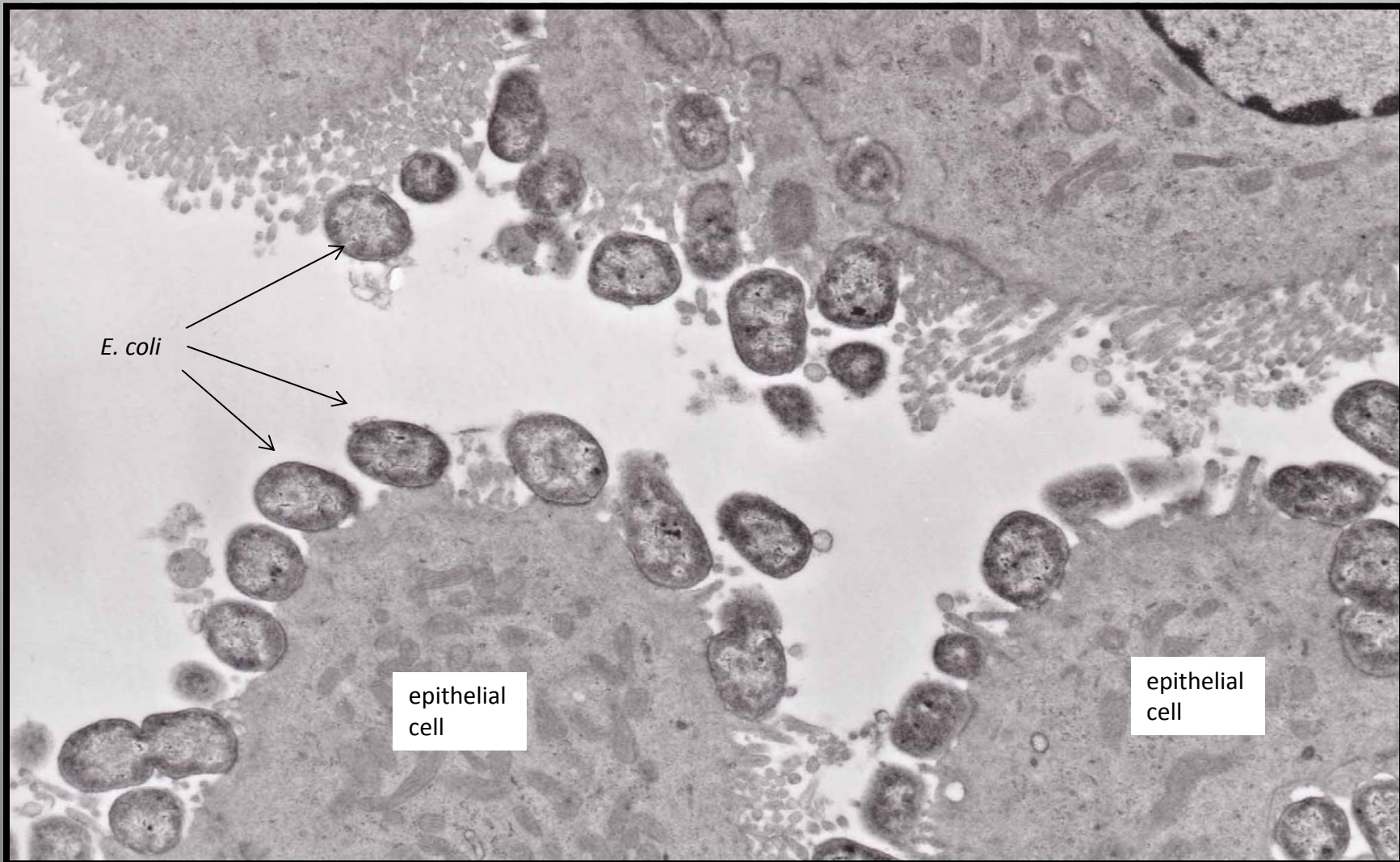
Use of Rope Devices to Describe and Explain the
Feedlot Ecology of *Escherichia coli* O157:H7
by Time and Place

D.R. SMITH,¹ R.A. MOXLEY,¹ S.L. CLOWSER,¹ J.D. FOLMER,² S. HINKLEY,¹
G.E. ERICKSON,² and T.J. KLOPFENSTEIN²

Rope-positive pens

- **classified as low and high prevalence**
- **clustered temporally**
- **summer > winter**
- **relationship with positive water tanks**
- **relationship with air temperature**
- **relationship with pen condition**

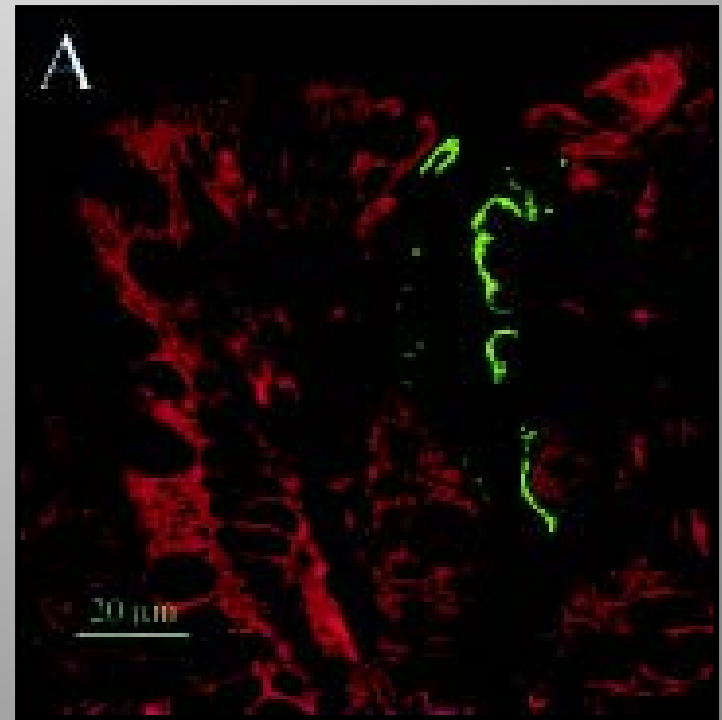
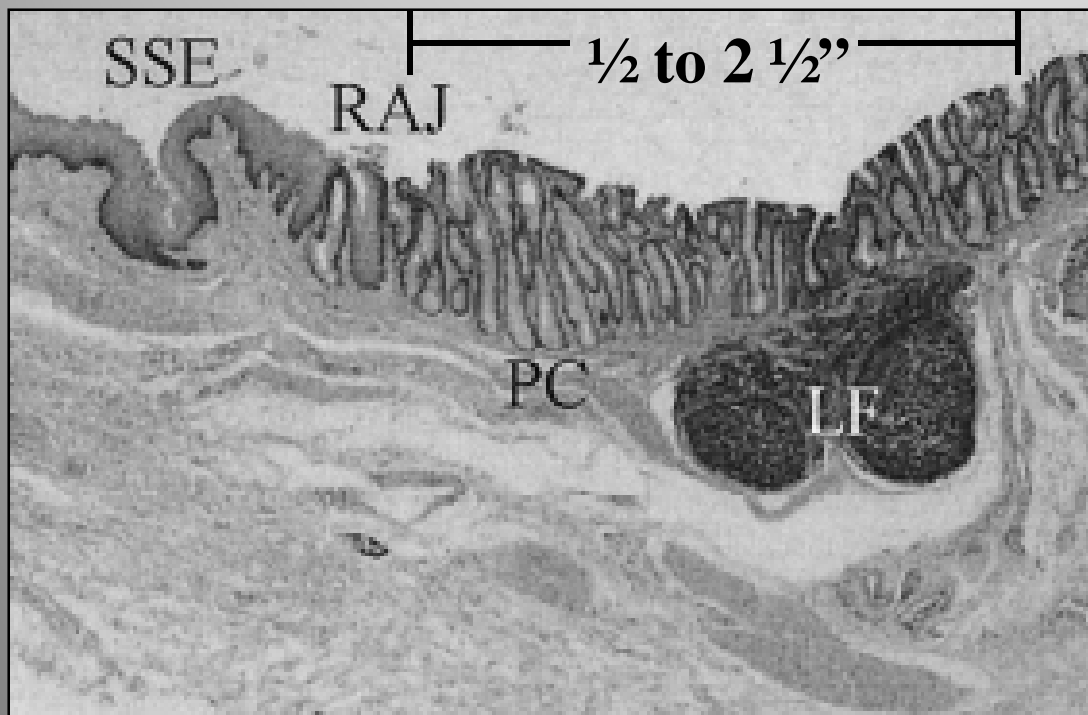
E. coli O157:H7 causes A/E lesions in rectal and colonic mucosal epithelium of adult cattle



Baehler & Moxley. 2000. FEMS Microbiol. Lett 185:239-242.

Terminal rectum is major site of *E. coli* O157:H7 colonization in cattle

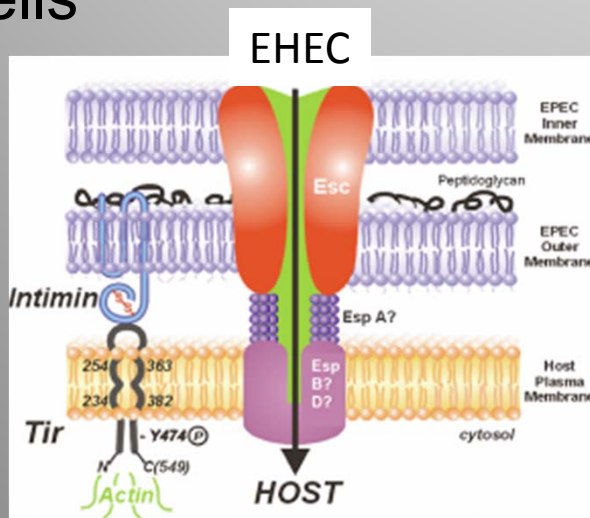
Lymphoid Follicle-Dense Mucosa at the Terminal Rectum Is the
Principal Site of Colonization of Enterohemorrhagic
Escherichia coli O157:H7 in the Bovine Host 2003



Naylor, et al. 2003. Infect. Immun. 71:1505-1512.

Vaccination as a strategy to reduce shedding of *E. coli* O157:H7 in cattle populations

Stimulate immunity against type III secreted proteins that mediate bacterial attachment to intestinal cells

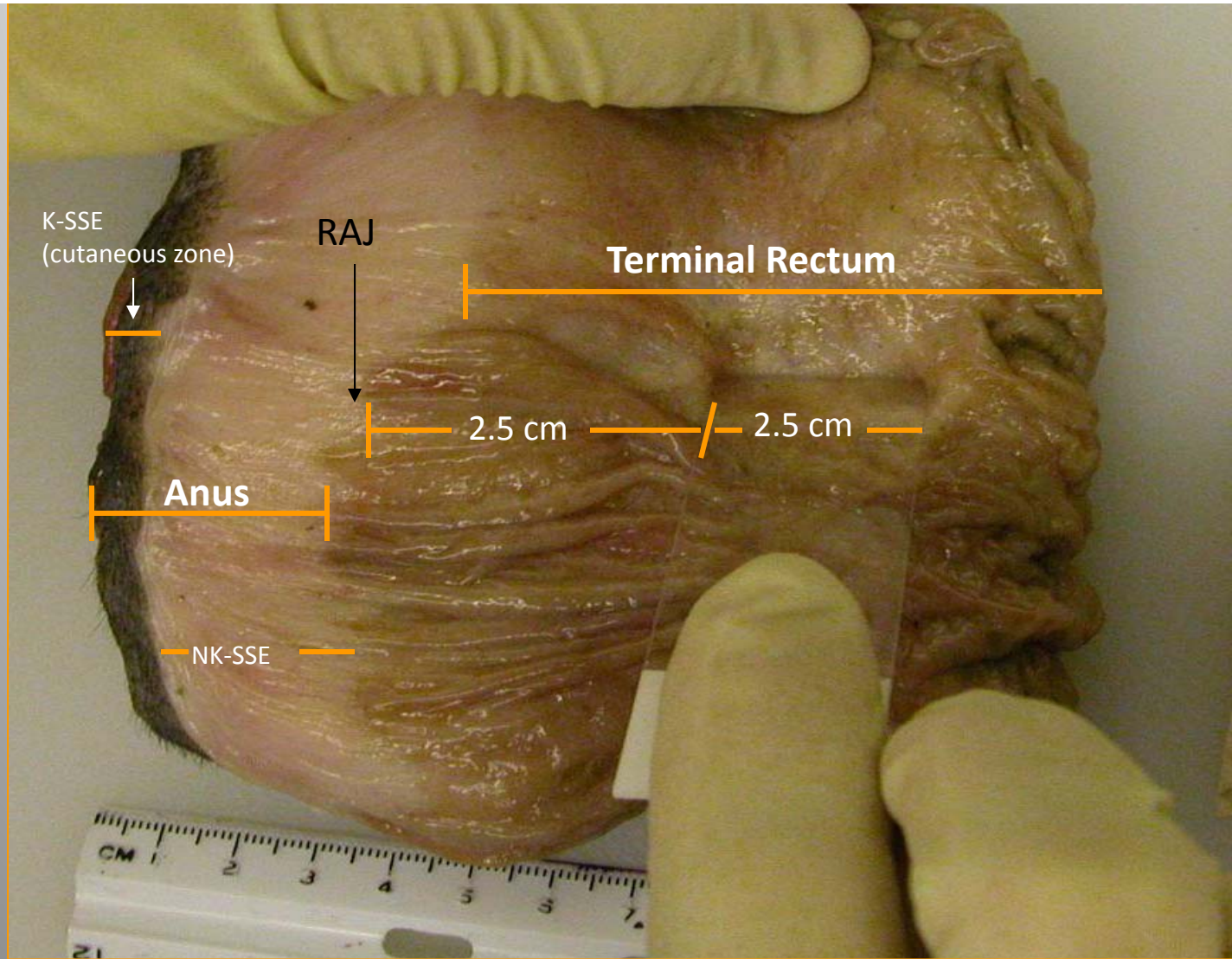


Marketed in Canada as Econiche™ by Bioniche Life Sciences



Courtesy Dr. Brett Finlay

Collection of terminal rectal cells at the abattoir for culture in a vaccine efficacy study

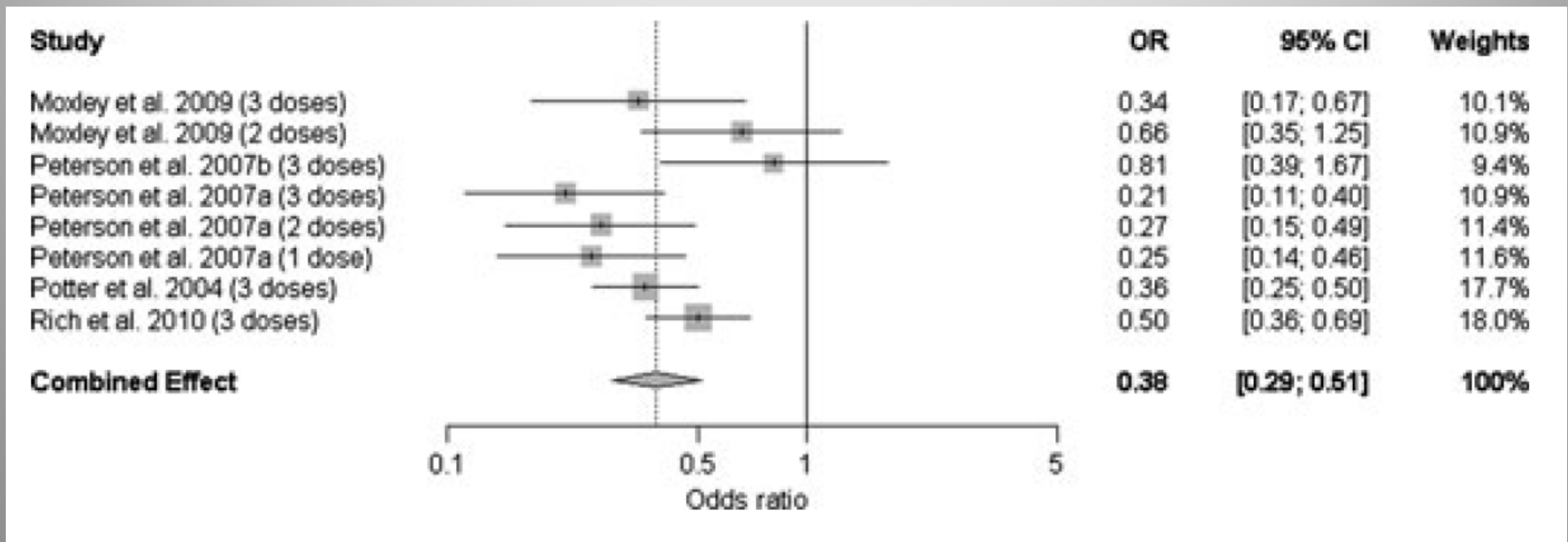


Peer-reviewed randomized controlled vaccine efficacy studies –type III secreted protein vaccine

- Potter et al. 2004. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine*. 22: 362-369.
- Peterson et al. 2007. Efficacy of dose regimen and observation of herd immunity from a vaccine against *Escherichia coli* O157:H7 for feedlot cattle. *J Food Prot*. 70(11):2561-2567.
- Peterson et al. 2007. Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. *J Food Prot*. 70(11): 2568-2577.
- Smith et al. 2008. A two-dose regimen of a vaccine against *Escherichia coli* O157:H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial beef feedlot clinical trial. *Foodborne Pathog. Dis*. 5(5):589-598.
- Smith et al. 2009. A two-dose regimen of a vaccine against type III secreted proteins reduced *Escherichia coli* O157:H7 colonization of the terminal rectum in cattle in a large-scale commercial beef feedlot clinical trial. *Foodborne Pathog. Dis*. 6(2):155-162.
- Smith et al. 2009. A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. *Foodborne Pathog. Dis*. 6(7):885-892.
- Moxley et al. 2009. *Escherichia coli* O157:H7 vaccine dose–effect in feedlot cattle. *Foodborne Pathog. Dis*. 6(7):879-884.

Forest Plot of Results of Meta-Analysis of UNL T3SP Vaccine Studies

Snedeker et al. 2011. Zoonoses and Public Health



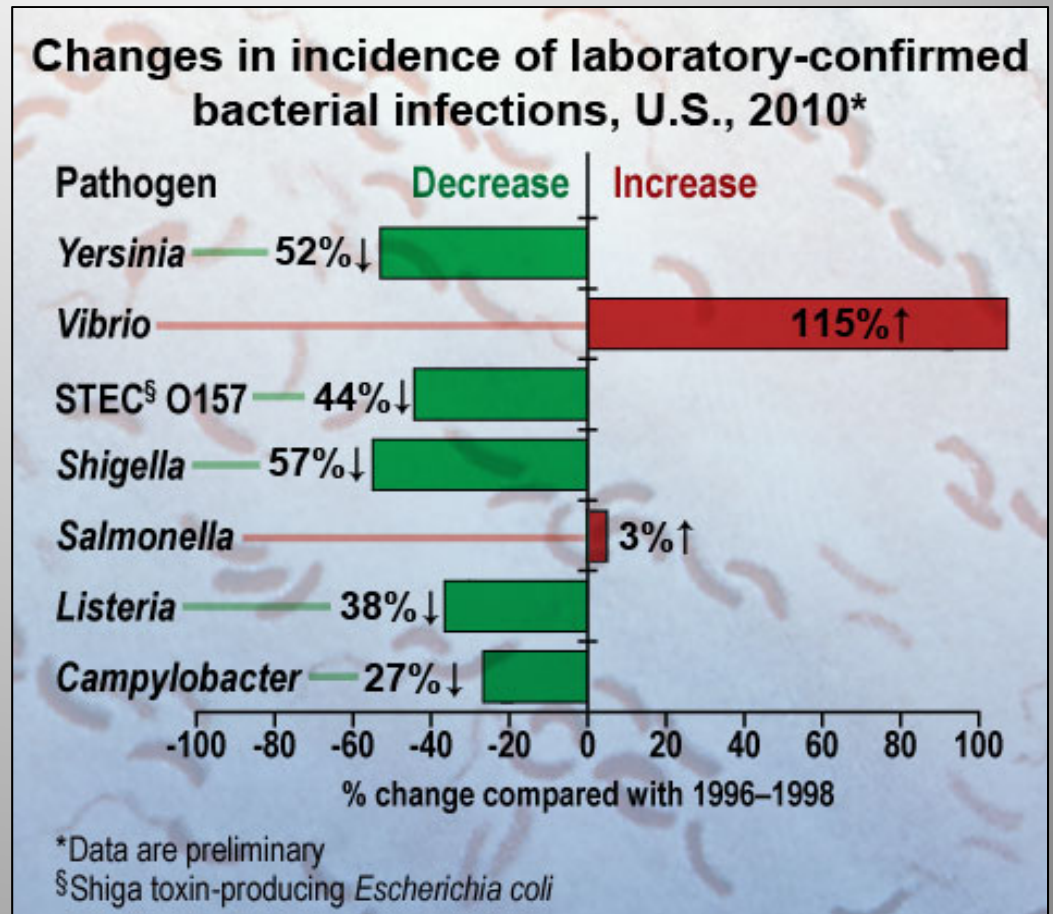
This plot includes only analyses of fecal shedding data.

Progress: 44% decrease in number of cases of STEC O157 Infections in US from 1996-98 to 2010

Incidence of STEC O157 infection has declined to reach 2010 national health objective target of ≤ 1 case per 100,000.

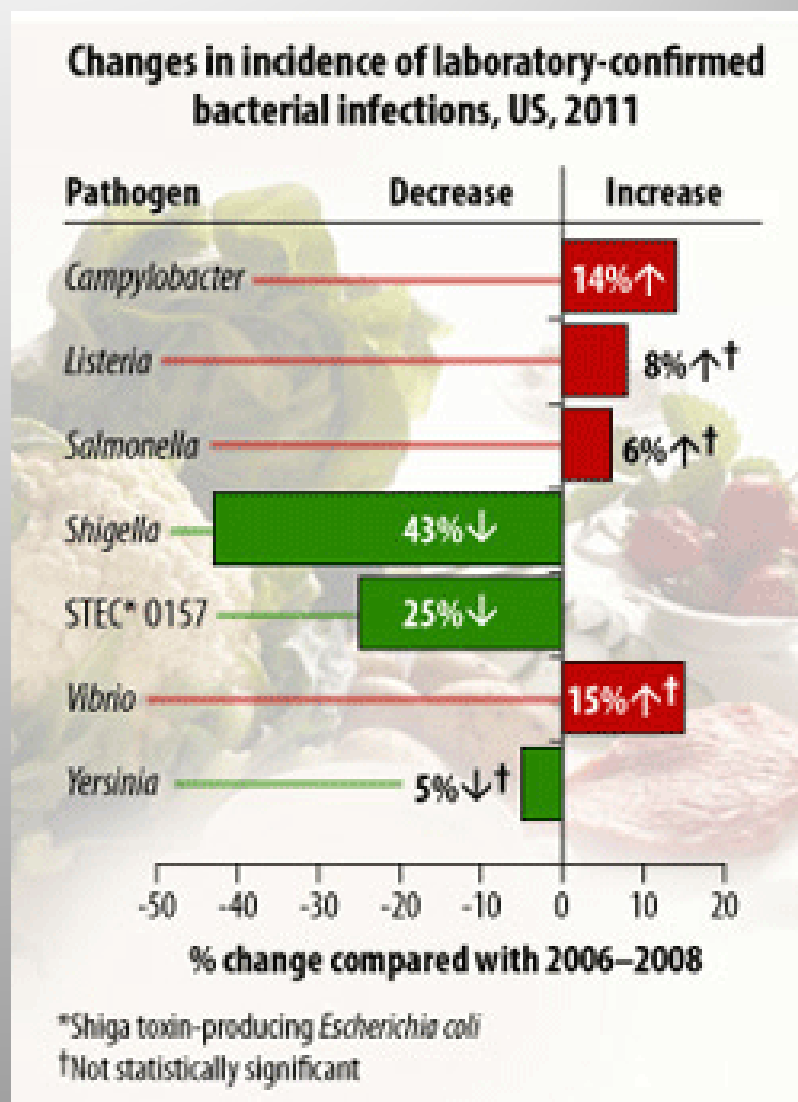
This demonstrates feasibility of preventing foodborne illnesses.

23% decrease in these overall



Progress: 25% decrease in number of cases of STEC O157 Infections in US from 1996-98 to 2011

Incidence was calculated by dividing the number of laboratory-confirmed infections in 2011 by U.S. Census estimates of the population of the FoodNet surveillance area.



What contributed to decrease in STEC O157 from 1996-98 to 2010?

- improved detection and investigation of STEC O157 outbreaks (PulseNet)
 - contaminated products removed before more persons became ill
 - enhanced knowledge about preventing contamination
- cleaner slaughter methods
- microbial testing
- better inspections in ground beef processing plants
- regulatory agency prohibition of contamination of ground beef with STEC O157 (beef recalls since STEC O157 declared an adulterant in ground beef in 1994)
- improvements in FDA model Food Code
- increased awareness in food service establishments and consumers' homes of risk of consumption of undercooked ground beef

Estimated annual number of domestically acquired STEC illnesses, hospitalizations and deaths in United States.*

	STEC O157	Non-O157 STEC	Total
All illnesses	92,872	137,502	230,374
Foodborne illnesses	63,153	112,752	175,905
Hospitalizations	2,138	271	2,409
Deaths	20	0	20

*Adapted from Scallan, E., et al. 2011. Foodborne illness acquired in the United States — Major Pathogens. *Emerg. Infect. Dis.* 17:7-15.

Human isolates of non-O157 STEC in US serotyped by CDC, 1983-2002

On September 20, 2011, the USDA declared these 6 serogroups to be adulterants in non-intact, raw beef (*Federal Register*, Vol. 76, No. 182, 58157-58165).

DEPARTMENT OF AGRICULTURE

Food Safety and Inspection Service

9 CFR Parts 416, 417, and 430

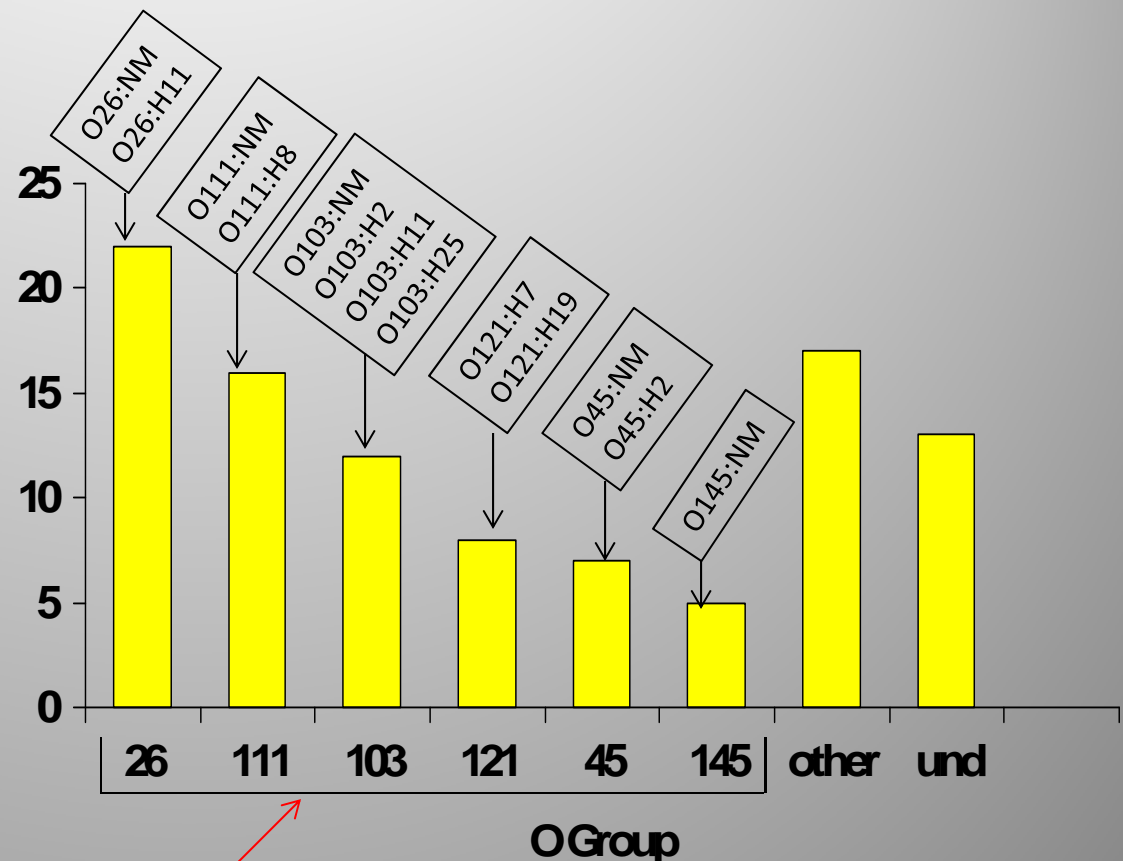
[Docket No. FSIS-2010-0023]

Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products

AGENCY: Food Safety and Inspection Service, USDA.

ACTION: Final determination and request for comments.

Progress: recognition of major serogroups by CDC; action taken by USDA-FSIS



Six O groups comprising 13 serotypes are responsible for 71% of the cases of non-O157 STEC infection in the US.

Shiga toxin-Producing Enteroaggregative *E. coli* O104:H4

- May to July 2011, outbreak in Europe
- in Germany alone: 3,816 cases, including 845 hemolytic-uremic syndrome cases and 54 deaths
- food-borne source was fenugreek seeds imported from Egypt
- Like other EAEC, O104:H4 strains lack the LEE genes, but differ, among other ways, from typical EAEC in that they have acquired genes for Shiga toxin
- culture of fecal samples from 100 cattle on 34 farms in Northern Germany, all located near the origin of the STEC O104:H4 outbreak, revealed no evidence of these organisms (Beutin, 2011)
- previous studies had not found EAEC bearing the complete virulence gene composition of isolates from human cases in any animal species

Shiga-toxigenic *Escherichia coli* (STEC) in the Beef Chain: Assessing and Mitigating the Risk by Translational Science, Education and Outreach

UNL Coordinated Agricultural Project (CAP) Grant

USDA National Institute of Food and Agriculture (NIFA)

Co-Project Directors:

Rodney A. Moxley, UNL School of Veterinary Med. & Biomedical Sciences – PD*

Harshavardhan Thippareddi, UNL Dept. of Food Science & Technology*

Randall K. Phebus, KSU Animal Science & Industry, Food Sci Institute*

John B. Luchansky, USDA ARS ERRC*

Daniel L. Gallagher, Virginia Tech, Dept. of Civil & Environmental Engineering*

Curtis Kastner, KSU Food Science Institute

Michael W. Sanderson, KSU Clinical Sciences, CVM

Daniel Thomson, KSU Beef Cattle Institute, CVM

***Executive Management Team**

50 collaborators at 12 institutions

Jill Hochstein, UNL – Project Manager

Paula Adams, UNL – Business Associate



\$25 M over 5 years






Extended team resources - more collaborators & partners



CEMMI *"Partnerships to Advance Predictive Models of Microorganisms in Food"*
USDA-ARS Eastern Regional Research Center's
Center of Excellence in Microbial Modeling & Informatics

Center of Excellence in Process Validation



CCR Center for Consumer Research
UC Davis



Veterinary Medicine Teaching & Research Center



Shiga-toxigenic *Escherichia coli* (STEC) in the Beef Chain: Assessing and Mitigating the Risk by Translational Science, Education and Outreach

Long Term STEC-CAP Goal:

Reduce occurrence and public health risks from STEC-8 (serotypes O26, O111, O103, O121, O45, O145, O157:H7/NM and O104:H4) in beef using a ***quantitative microbial risk assessment*** platform.

STEC Coordinated Agricultural Project (CAP)

Education & Training

Extension & Outreach

Pre-harvest
Research

Post-harvest
Research

Consumer
Research

Quantitative Microbial Risk Assessment

STEC-CAP Objective 1

DETECTION

LEAD: Rodney Moxley (UNL)

- i. Develop and validate reagents and assays for STEC-8;
- ii. Design valid sampling protocols for cattle and beef processing scenarios;
- iii. Optimize and implement rapid, multi-agent detection technologies for pre-harvest, post-harvest and consumer samples.

STEC-CAP Objective 2

STEC BIOLOGY

LEAD: David Renter (KSU)

- i. Characterize core microbiology and eco-epidemiology of STEC-7 versus STEC O157;
- ii. Identify modifiable biotic, abiotic and motility risk drivers of natural STEC-7 spikes (“outbreaks”) in pre-harvest, post-harvest, retail and consumer beef settings.
- iii. Determine if STEC O104:H4 is potentially present in the beef production/processing continuum.

STEC-CAP Objective 3

INTERVENTIONS

LEAD: Randall Phebus (KSU)



- i. Develop and/or validate interventions to lessen STEC-8 risk from cattle, hides, carcasses, ground and non-intact beef;
- ii. Compare energy, water and economic cost/benefit and feasibility of interventions for large, small and very small beef processors.

STEC-CAP Objective 4

RISK ANALYSIS & RISK ASSESSMENT

LEAD: Daniel Gallagher (VTU)

- i. Create beef product pathway probabilistic microbial quantitative risk assessment (QRA) models for STEC-8 from live cattle to consumption to evaluate risk mitigation strategies and their expected public health impacts.
- ii. Validate QRA and risk-based corrective actions *via* STEC-8 inoculated studies in our unique beef slaughter/processing biocontainment facility (BRI).

STEC-CAP Objective 5

RISK MANAGEMENT & COMMUNICATION

LEADs: Curtis Kastner & Daniel Thomson (KSU)

- i. Translate research into user-friendly food safety deliverables for stakeholders;
- ii. Provide and promote useful, broad risk assessment participation and understanding by non-experts;
- iii. Develop, pilot-test, execute and disseminate STEC risk assessment and risk management deliverables for food safety professionals, regulators, educators and consumers.

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