Overview of Shiga toxin-producing *Escherichia coli* (STEC, VTEC, EHEC)

Food Associated Pathogens, Grythyttan, August 23-26, 2010

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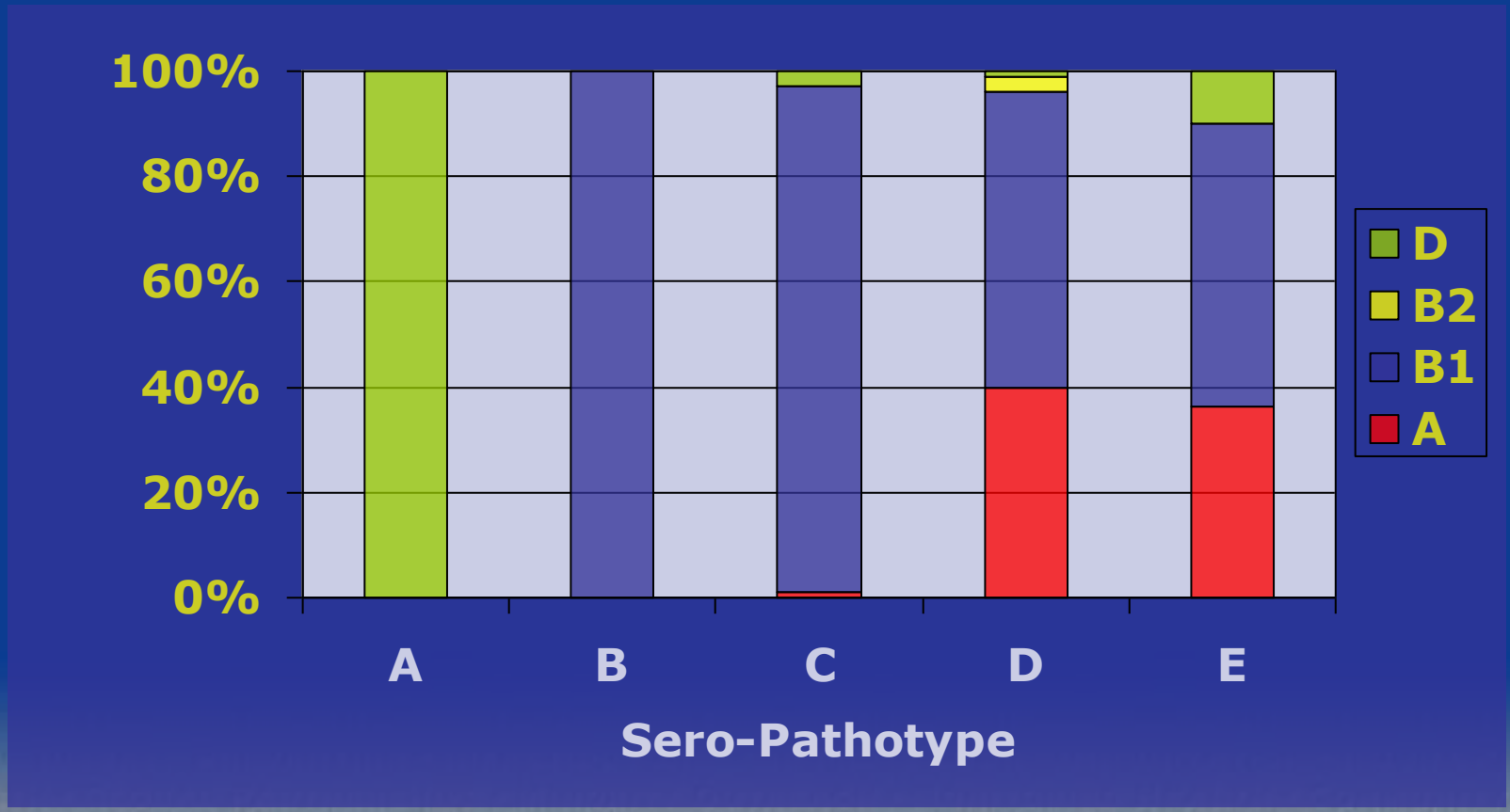
STEC facts

- **An STEC is an *E. coli* that produces Shiga toxin**
  - Irrespective of production of other virulence factors
- **Only a small subset of STEC cause disease in humans**
- **Animals are the main reservoir for STEC**
  - including the ones that do not cause infection in humans

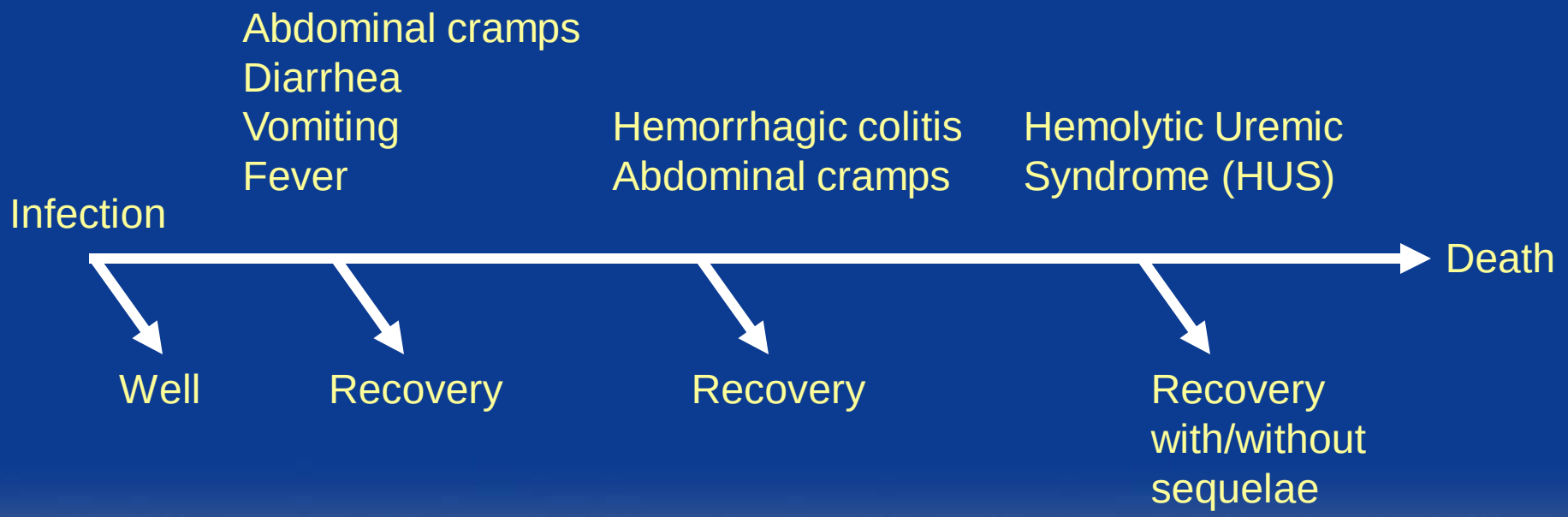
STEC are heterogenous phylogenetically

Girardeau *et al.* 2005. JCM; 43: 6098-6107



Phylogenetic groups: Selander et al. 1987, p. 1625–1648. *In* Neidhardt *et al.* (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

STEC symptoms

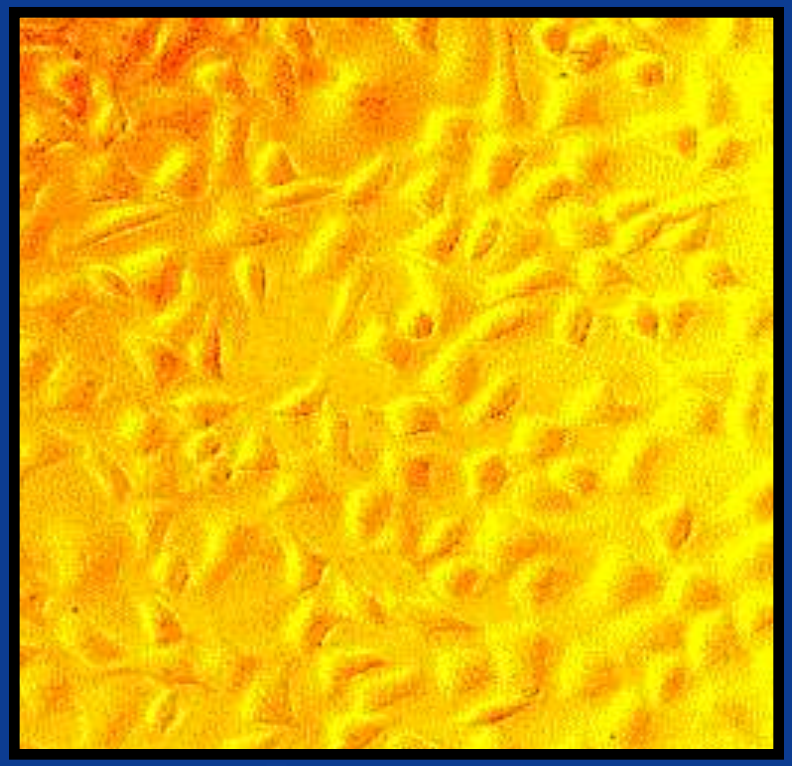


STEC diagnosis

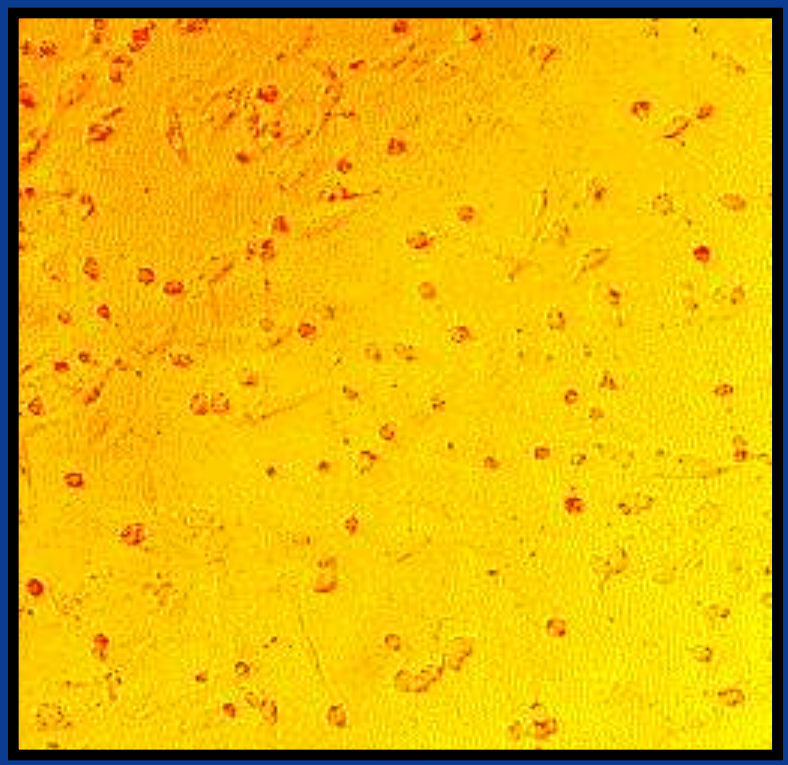
- **Humans**
  - Non-culture detection of Stx (EIA) or *stx* (PCR)
  - Culture on (CT-)SMAC (O157)
- **Animals/Food**
  - Culture on (CT-)SMAC (O157)
  - Culture of top 4-6 STEC serotypes following IMS (O157, O26, O45, O103, O111, O121, O145)

What you look for is what you will find!

Shiga Toxin



Vero cell Assay (VCA):
No Shiga toxin



VCA positive
Shiga toxin present

Konowalchuk *et al. 1977, Infect Immun;18(3):775-9*

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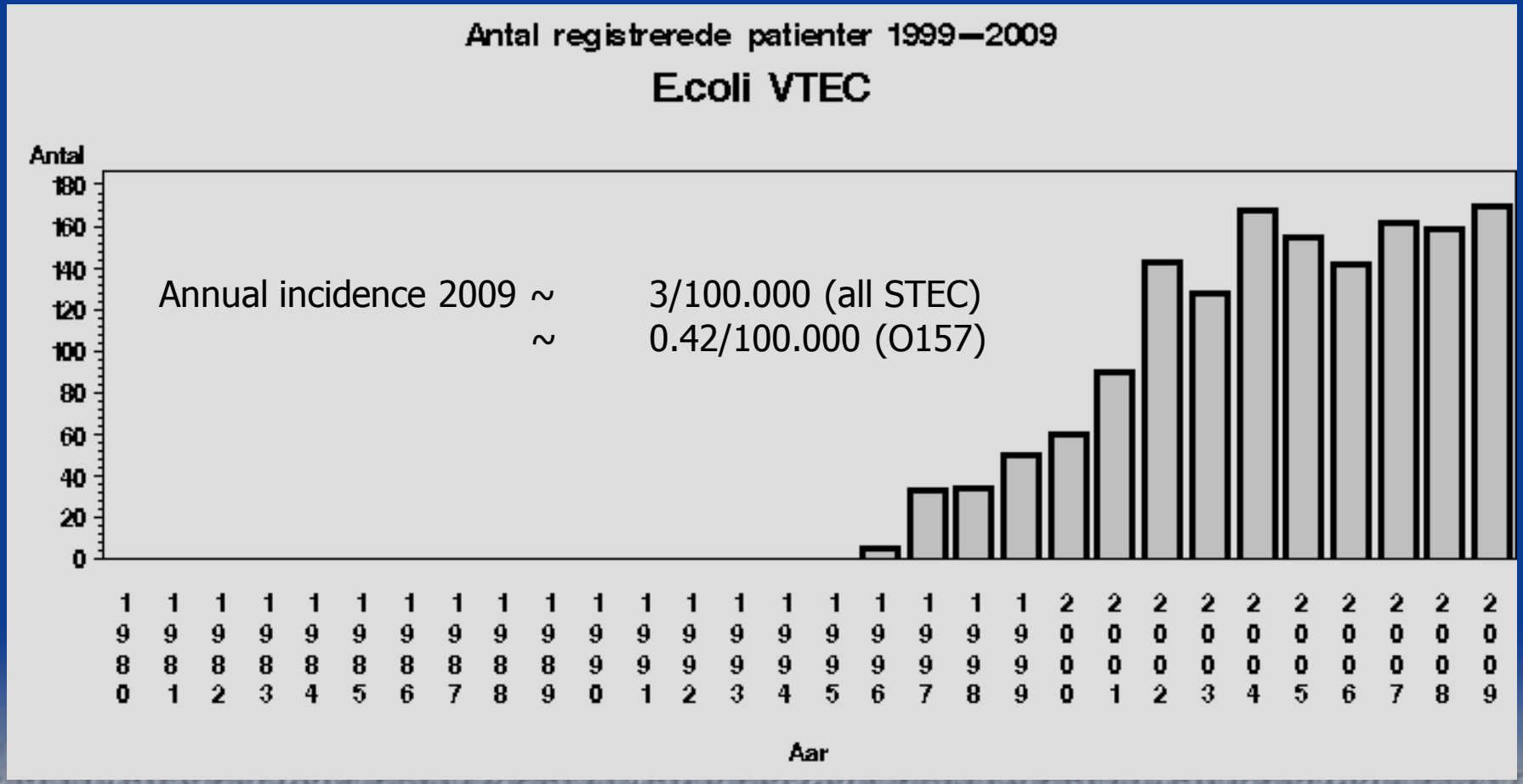
Courtesy F. Scheutz, Statens Serum Institut, Copenhagen

STEC in Denmark



Source: www.mave-tarm.dk

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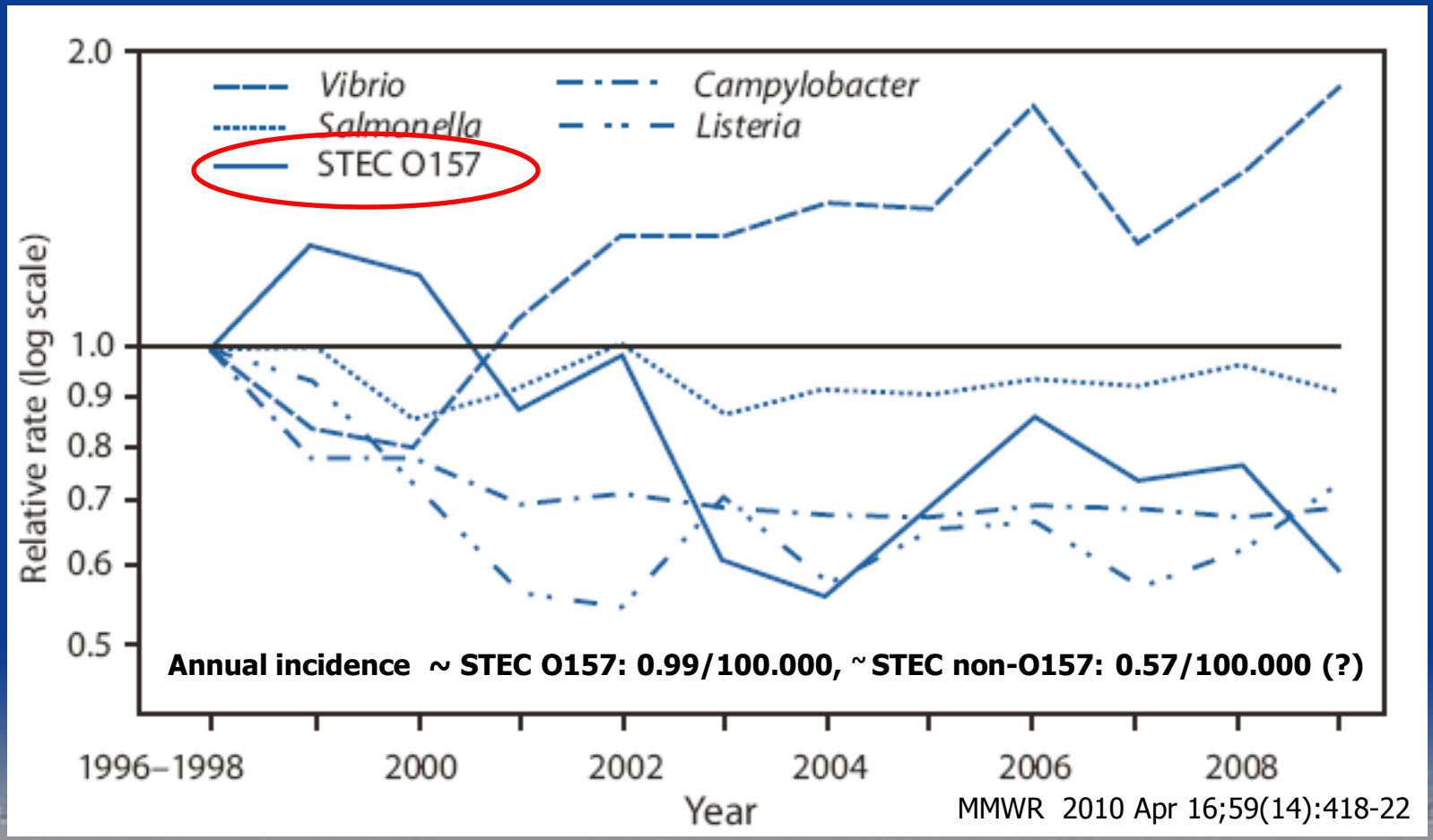
Relative rates of laboratory-confirmed infections with *Campylobacter*, STEC O157, *Listeria*, *Salmonella*, and *Vibrio* compared with 1996--1998 rates, by year -- Foodborne Diseases Active Surveillance Network (FoodNet), United States, 1996--2009



MMWR 2010 Apr 16;59(14):418-22

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Treatment: Symptomatic, Prevent HUS by rehydration

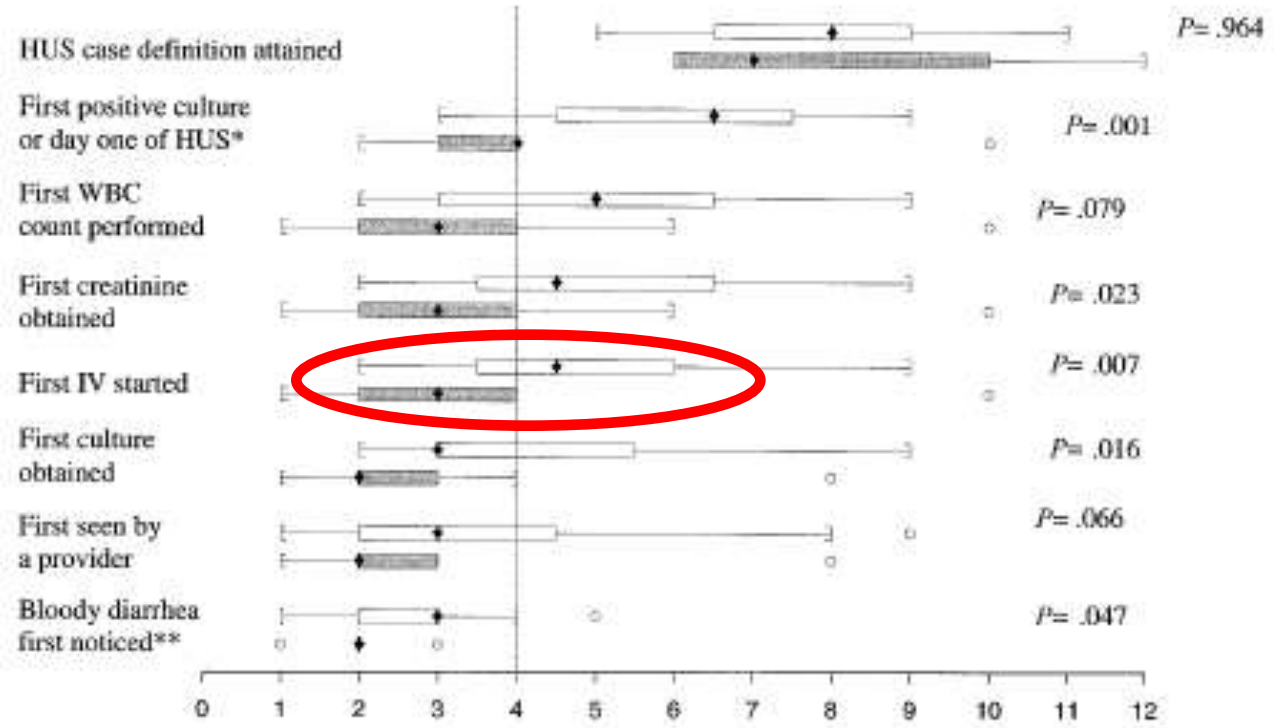

Fig 1. Timing of critical events during illness. Horizontal box-plot depictions of distribution of critical events during *E coli* O157:H7 infection, prodromal to HUS. Shown for each event is the distribution of days of occurrence of 13 children with nonoligoanuric renal failure (bottom, shaded, box plot of each pair) and the 16 children with oligoanuric renal failure (upper, open, box plot of each pair). The vertical line with a diamond within each box represents the median, and the left and right upper borders of each box represent the 25th and the 75th percentiles, respectively. The T bars represent the differences between the lower and upper borders multiplied by 1.5. Values outside these boundaries are depicted as single points. * indicates whichever event occurred first; **, among the 12 and 14 patients with nonoligoanuric and oligoanuric renal failure, respectively, whose stools were noted to contain gross blood.

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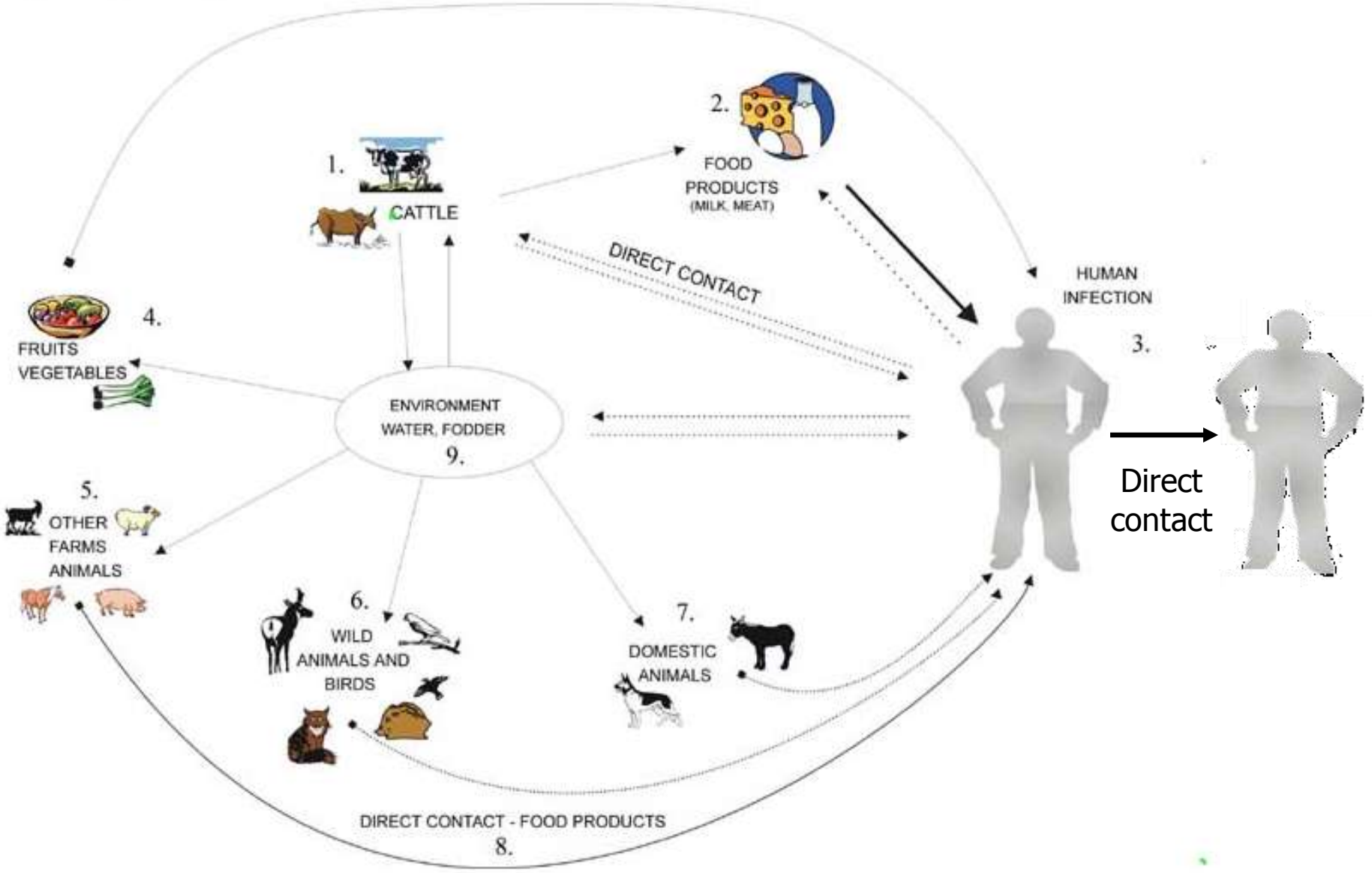
Ake *et al.*, 2005. Pediatrics; 115: e673-80

Figure 1. Epidemiology of STEC



http://www.ejpau.media.pl/volume8/issue4/art-03.html

Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002

Josefa M. Rangel,*† Phyllis H. Sparling,‡ Collen Crowe,* Patricia M. Griffin,* and David L. Swerdlow*

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 11, No. 4, April 2005

	Outbreaks		
Transmission route	n	Total %	Foodborne %
Ground beef	75	21	41
Unknown food vehicle	42	12	23
Produce	38	11	21
Other beef	11	3	6
Other food vehicle	10	3	5
Dairy product	7	2	4
Subtotal, foodborne	183	52	
Unknown transmission route	74	21	
Person-to-person	50	14	
Recreational water	21	6	
Animal contact	11	3	
Drinking water	10	3	
Laboratory-related	1	<1	
Subtotal, other routes	167	48	
Total	350		

Non-O157 in the United States

- Risk factors: As O157
  - and international travel (O26, O103)
- Outbreaks not as common as O157
  - Multistate outbreaks are rare

BUT

- Under diagnosed compared to O157

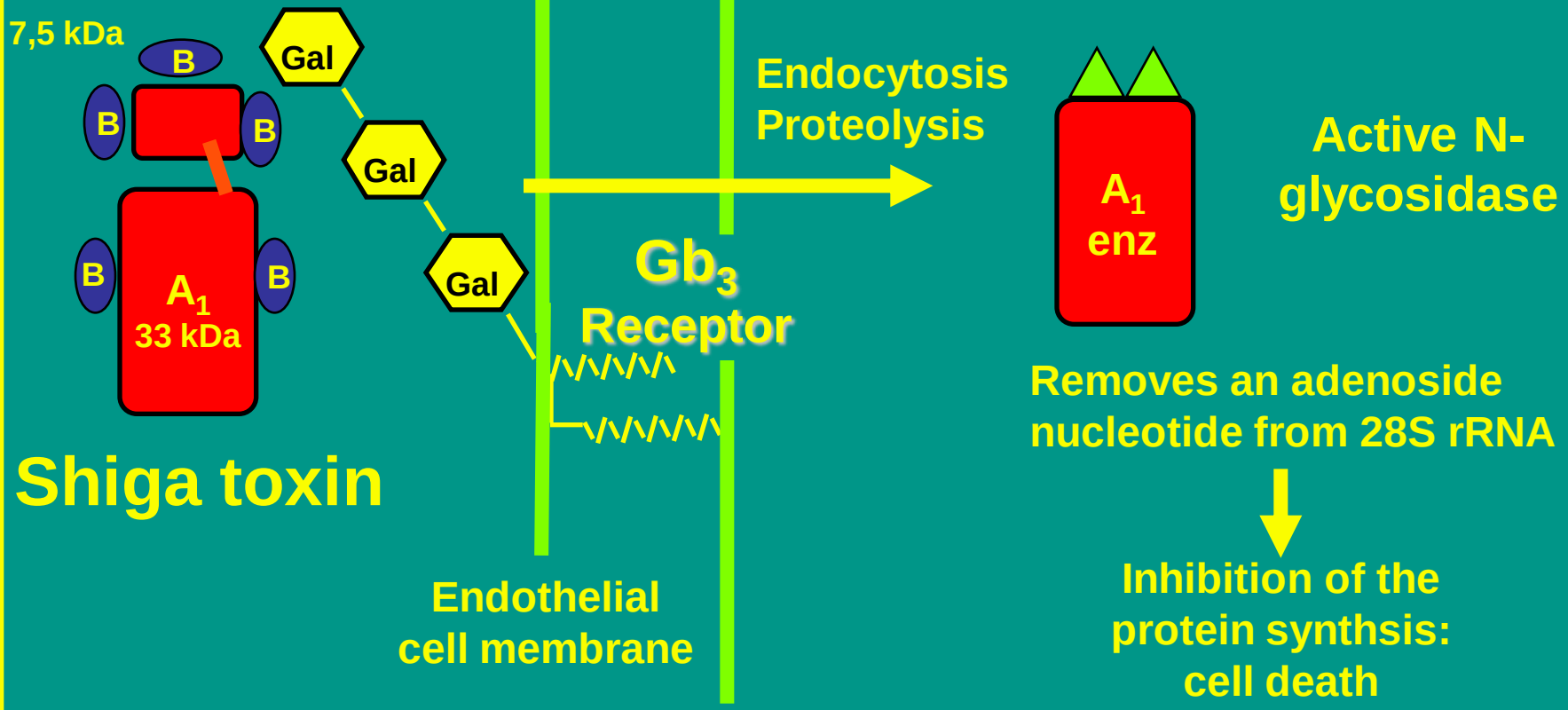
STEC O145 Multistate Outbreak 2010

- 26 confirmed cases, 3 HUS
- Young adults (students)
- First detected in MI subsequent outbreaks in OH and NY linked by PFGE (PulseNet)
- Source: shredded romaine lettuce
- Strain characteristics: O145:NM , *eae*, *stx2a,* CSSuTNa(Cip)

Preliminary data

Shiga Toxins

STRUCTURE & FUNCTION



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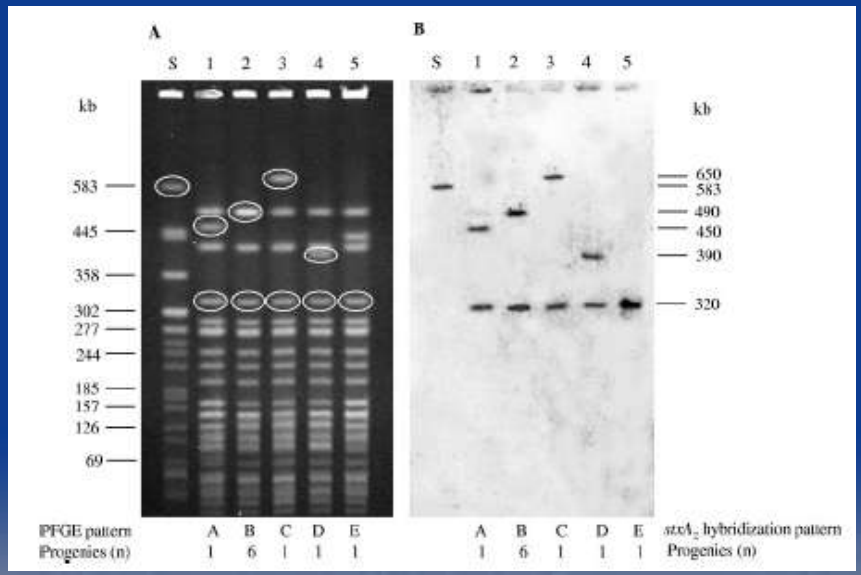
Courtesy F. Scheutz, Statens Serum Institut, Copenhagen

Shiga toxins are phage encoded

- One cell may contain more than one Stx-phage
  - The same Stx gene on more phages
  - Different Stx types
  - Different Stx subtypes/variants

The Stx phage content may cause instability of the PFGE pattern of an STEC strain



Bielaszewska *et al.* AEM 2006, 72: 1900-09

Shiga toxin nomenclature

Toxin Type	Toxin gene variant designation	Serotype of prototype organism	Previous designation or synonym for gene	Previous designation or synonym for toxin
Stx1a	*stx*$_{1a}$	*S. dysenteriae* *S. sonnei*		
Stx1b (VT1)	*stx*1b-O157-EDL933 *stx*1b-O26-H-19B *stx*1b-O26-H30 *stx*1b-O111-PH *stx*1b-O48-94C *stx*1b-O111-CB168	O157:H7 O26:H11 O26:H11 O111:H- O48:H21 O111:H-	*slt*-I	SLT-I SltI/PH SltI/O48 SltI/CB168
Stx1c (VT1c)	*stx*1c-O174-DG131/3	O174:H8	*stx1c*	
Stx1d (VT1d)	*stx*1d-ONT-MHI813	ONT:H19	*stx1d*	
Stx2a (VT2a)	*stx*2a-O157-EDL933 *stx*2a-O48-94C	O157:H7 O48:H21	*stx2*/*vtx2* *slt*-II	SLT-II SLT-II/O48
Stx2b (VT2b)	*stx*2b-O111-PH *stx*2b-O118-EH250	O111:H- O118:H12	*stx*$_{2vO111}$	SLT-II/O111 / VT2d-O111 Stx2d/VT2d-Ount
Stx2c (VT2c)	*stx*2c-O157-E32511 *stx*2c-O157-FLY16 *stx*2c-O157-C394-03 *stx*2c-O157-TK-51 *stx*2c-O174/a-031 *stx*2c-O174/b-031 *stx*2c-O22-KY-O19	O157:H- O157:H- O157:H- O157:H7 O174:H21 O174:H21 O22:H-	*stx2c*/*vtx2c* *slt*-IIc *stx2* *stx*$_{2vOX392}$ *stx*$_{2vOX393}$	Stx2v/VT2v SLTIIc VT2v(pKTN1050) =Stx2vhd ? SLT-II/OX3 / VT2d-OX3 SLT-II/OX3/2 / VT2d-OX3/2 VT2v(pKTN1054) = Stx2vhc ?

Shiga toxin nomenclature

Toxin Type	Toxin gene variant designation	Serotype of prototype organism	Previous designation or synonym for toxin gene	Previous designation or synonym for toxin
Stx2d (VT2d)	*stx*2d *stx*2d-O91/a-B2F1 *stx*2d-O91/b-B2F1 *stx*2d-O157-7279 *stx*2d-O73-C165-03 *stx*2d-O8- C466-01B	O91:H21 O91:H21 O91:H21 O157:H7 O73:H18 O8:H19	*stx*2vha/*vtx*2vha stx_{2d1}/*vtx*2d1 *stx*2ha *stx*2vhb/*vtx*2vhb stx_{2d2}/*vtx*2d2 *stx*2hb *stx*2vhc	SLT-IIvh Stx2vha/VT2vha Stx2d1/VT2d1 Stx2vh-a/VT2vh-a VT2v-a SLT-IIvha Stx2vhb/VT2vhb Stx2vh-b/VT2vh-b Stx2d1/VT2d1 VT2v-b SLT-IIvhb Stx2vhc
Stx2e (VT2e)	*stx*2e-O139-412 *stx*2e-O139-S1191 *stx*2e-ONT-26725-97 *stx*2e-O101-E-D43	O139:K12:H1 O139:H1 ONT:H- O101:H14	*slt*-IIv *slt*-IIva *slt*-IIe	SLT-IIv SLT-IIva SLTIIe/VTe VT2vp VT2vp1
Stx2f (VT2f)	*stx*2f-O128-H.I.8 *stx*2f-O128-T4/97	O128:H2 O128:H2	stx_{2ev}/*vtx*2ev *slt*-IIvhc *slt*-IId [d]	Stx2ev/VT2ev Stx2vp2/VT2vp2 VTev SLTIIvhc SLT-IId/VT2d SLTIIva
Stx2g (VT2g)	*stx*2g-O2-7v	O2:H25		
Stx2h (VT2h)	*stx*2h-Out-S-8	Out:H25		

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0095-1137/07/$08.00+0 doi:10.1128/JCM.02591-06

Subtyping Method for *Escherichia coli* Shiga Toxin (Verocytotoxin) 2 Variants and Correlations to Clinical Manifestations[▽]

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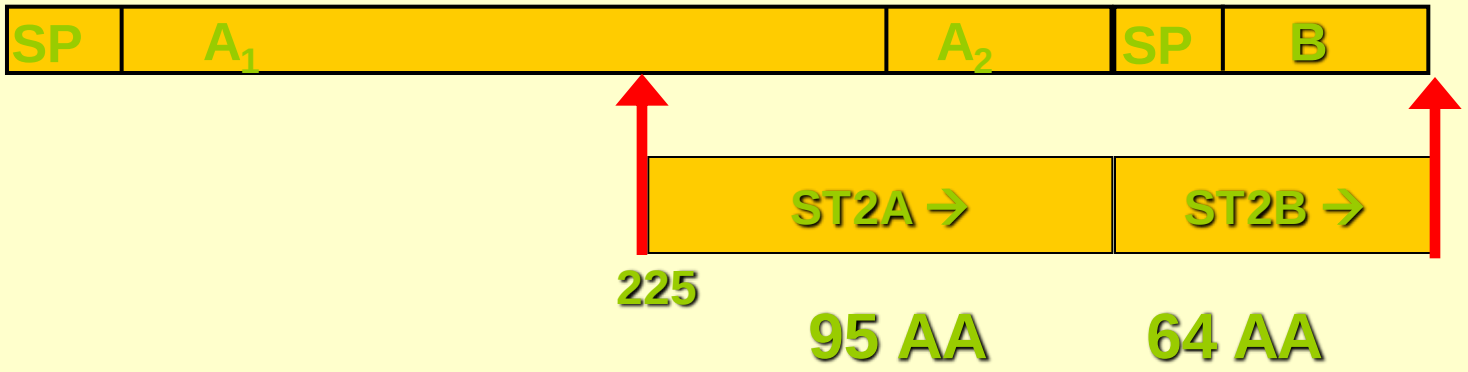
Department of Bacteriology, Mycology and Parasitology, Unit of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark,[1] and The International Escherichia and Klebsiella Centre (WHO), Unit of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark[2]

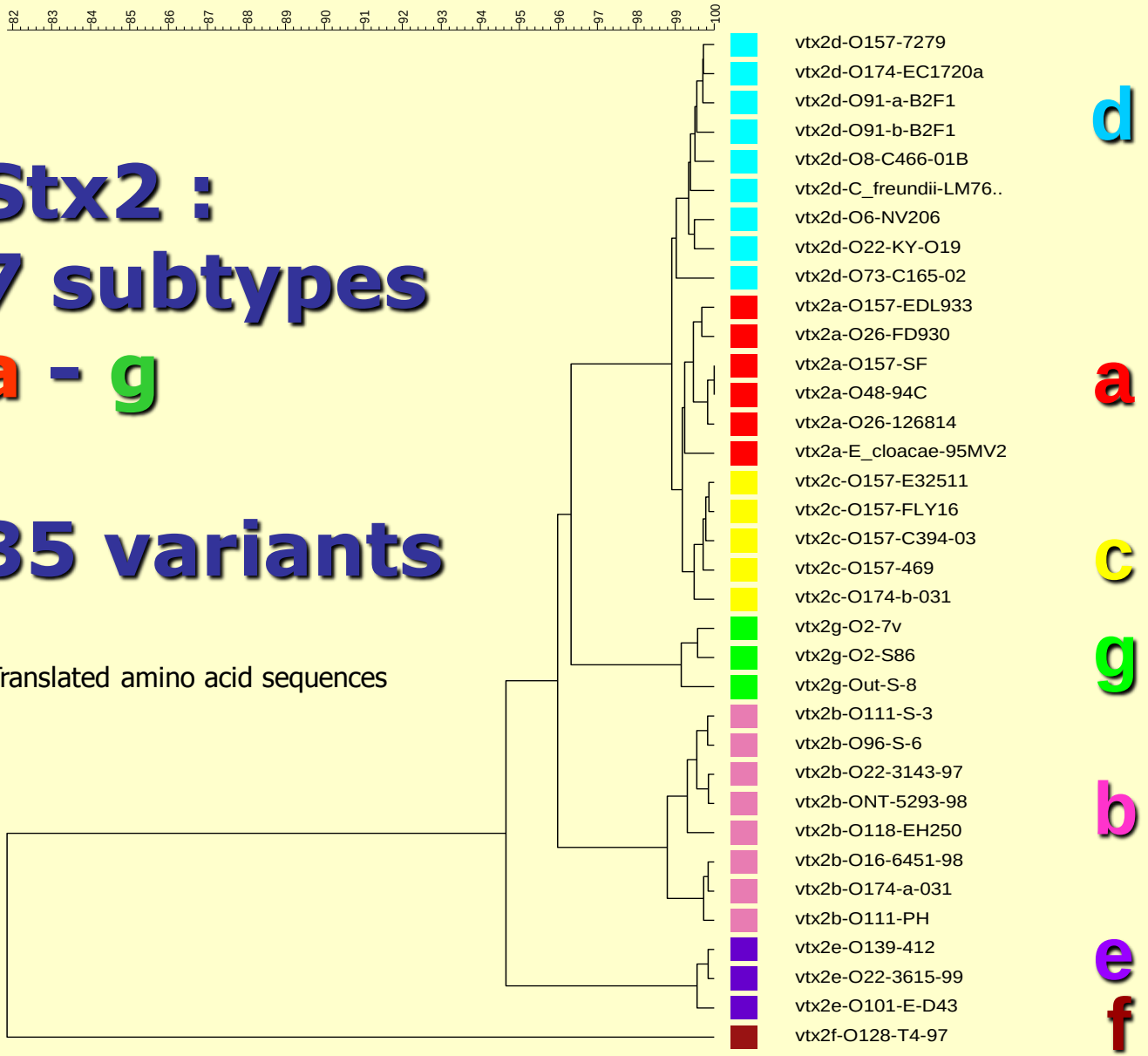
Received 27 December 2006/Returned for modification 27 January 2007/Accepted 4 April 2007

Shiga toxin 2 (Stx2) from Shiga toxin-producing *Escherichia coli* (STEC) was subtyped by a method involving partial sequencing of the *stxAB*$_2$ operon. Of 255 strains from the Danish STEC cohort, all 20 cases of hemolytic-uremic syndrome were associated with subtype Stx2 (11 cases), subtype Stx2c (1 case), or the two combined (8 cases).



F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0% Disc. unk.
vtx_TRANSL



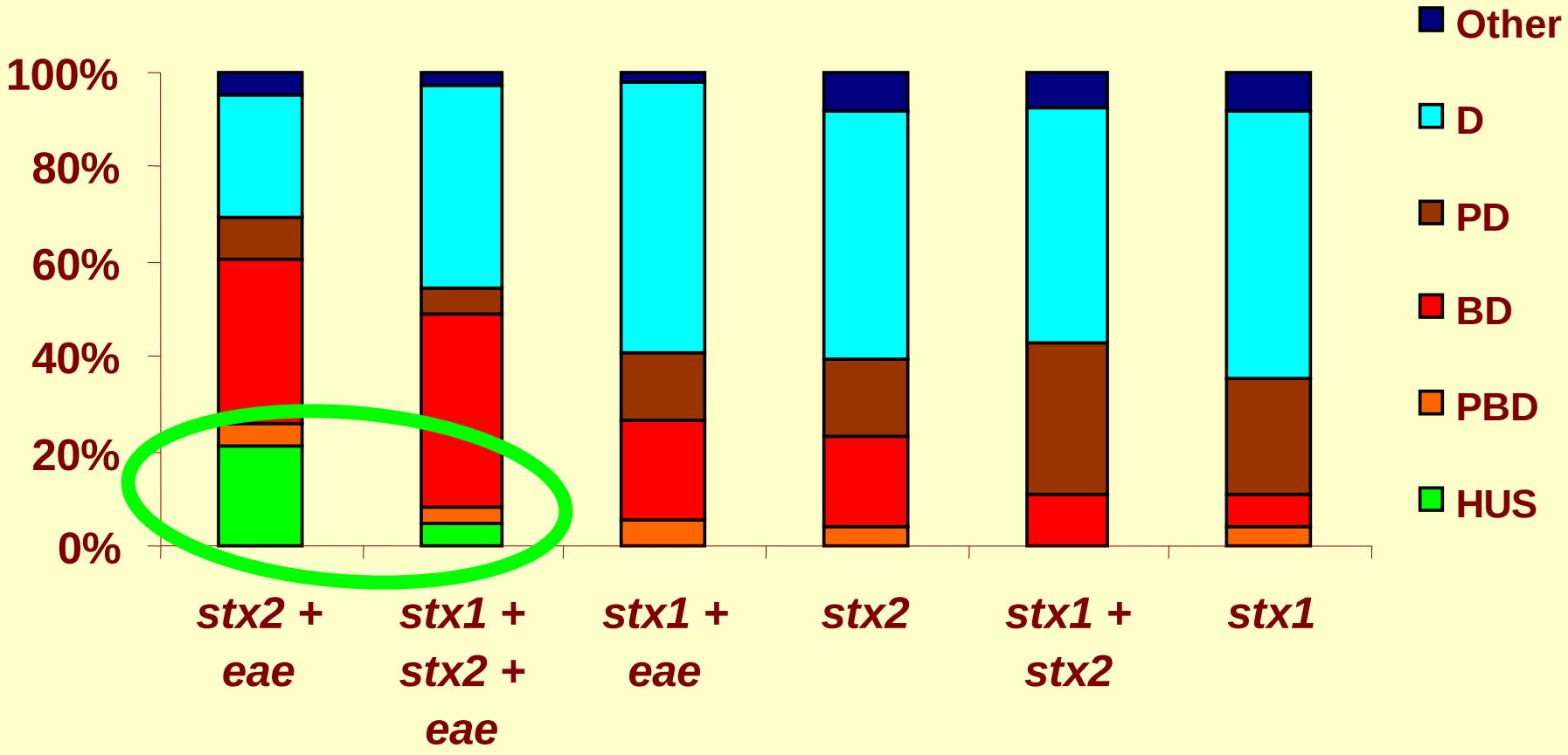
Stx2 :
7 subtypes
a - g

35 variants

Translated amino acid sequences

F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

Virulence profile and clinical manifestation in 559 Danish STEC patients 1994-2005



F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

Attack rate of Stx2 subtypes associated with HUS (DK)

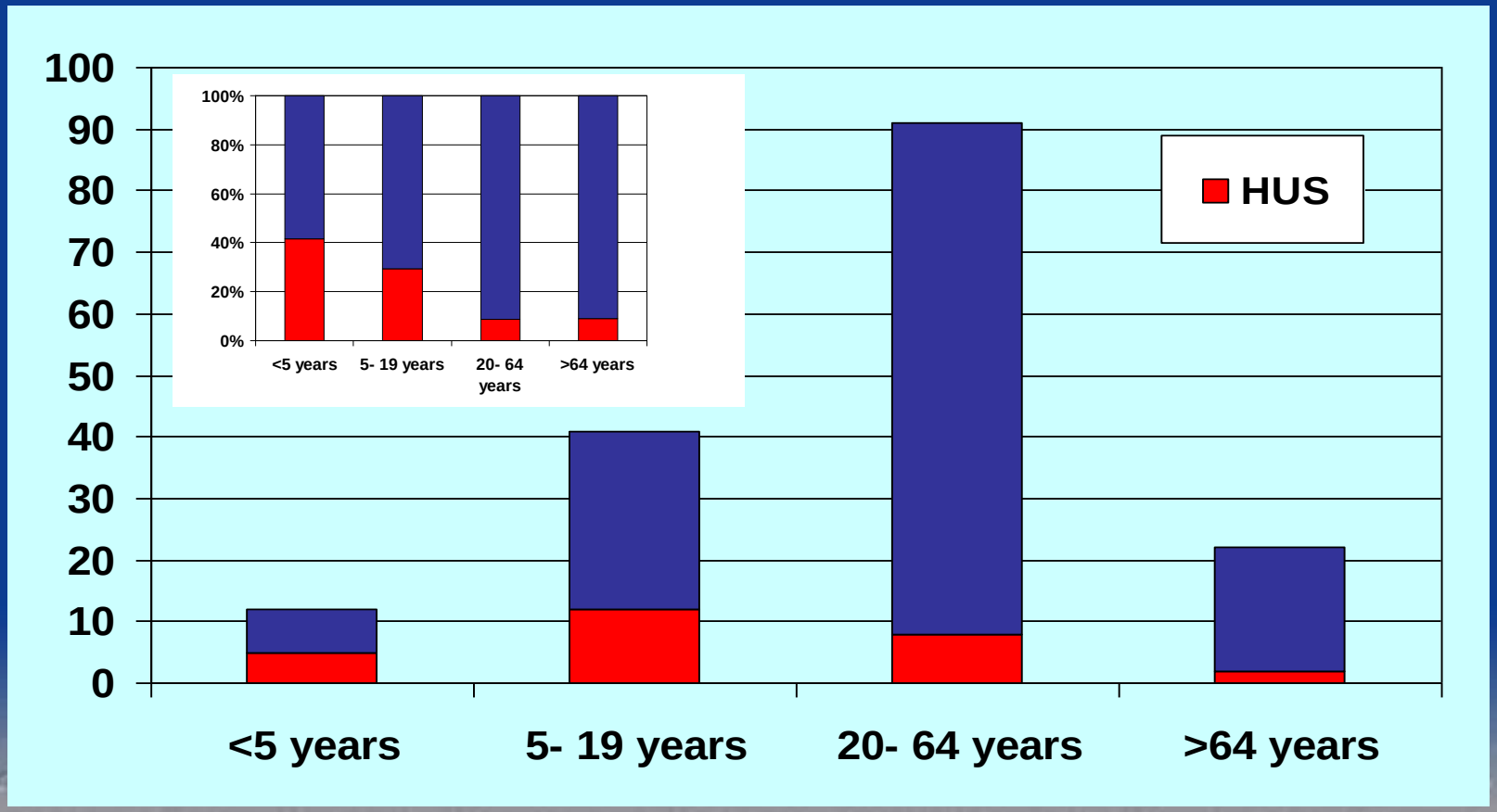
O157		
Stx2a + Stx2c	7/24	29%
Stx2a	3/17	18%
Stx2c	1/18	6%

Non-O157		
Stx2a	6/20	30%
Stx1b + Stx2a	2/8	25%
Stx2a + Stx2c	1/2	50%

Adapted from F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

HUS in the "spinach outbreak" USA 2006

(O157:H7, *Stx2a+Stx2c*)



Preliminary data

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Stx2d$_{activatable}$, *eae*-negative STEC cause severe disease

Clinical outcome	Total isolates	No. (%) of *eae* negative	No. (%) of *eae*-negative STEC producing	
			Stx2d$_{activatable}$*	non-activatable Stxs
HUS	337	11 (3)	7 (64)	4 (36)
BD	50	6 (12)	6 (100)	0 (0)
D	397	113 (29)	17 (15)	96 (85)
A	108	79 (73)	0 (0)	79 (100)

* Most common serotypes O91:H21, O113:H21
Important Stx2d$_{activatable}$ synonyms: Stx2-vha, Stx2-vhb

Bielaszewska et al., Clin. Infect. Dis. 2006 43:1160-7

Virulence Factors in STEC

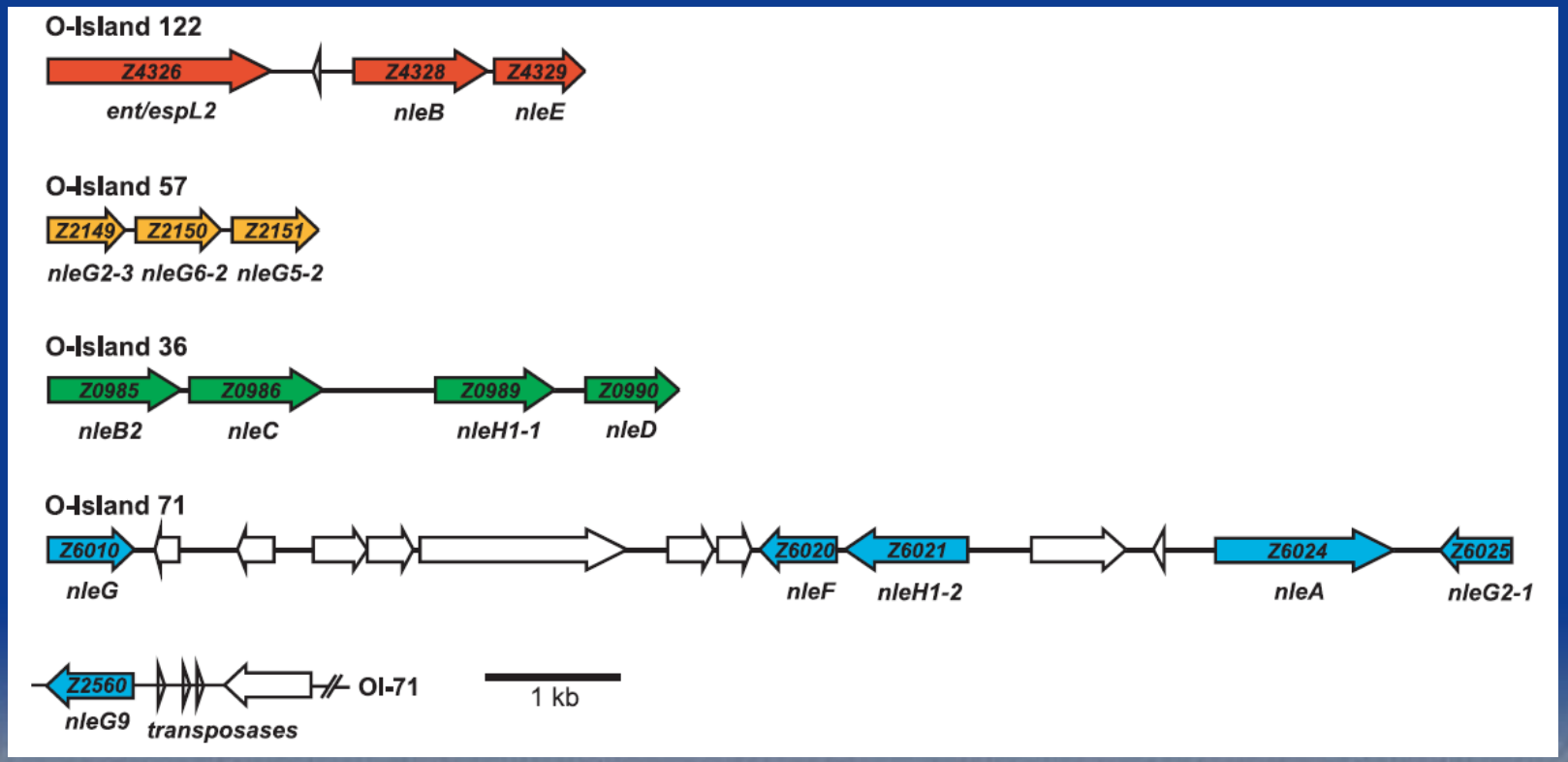
Virulence factor	Gene Location
Shiga toxin (*stx*)	Phage
Intimin (*eae*)	PAI (LEE)
Enterohemolysin (EhxA, HlyA)	Plasmid (pO157)
Non-LEE effectors (*nle*)	PAI's
Saa adhesin (STEC autoagglutinating adhesin)	Plasmid
Subtilase	Plasmid

Locus for Enterocyte Effacement (LEE)

- Pathogenicity island (PAI) in EPEC and STEC
- Contains genes encoding intimin *(eae),* intiminreceptor (*tir*), type III secretion system, regulators
- Adherence to the enteric epithelium and attaching and effacing enteric lesions

Non-LEE effectors (*nle*)

- At least 16 *nle* genes in at least four PAI's



Karmali *et al.* JCM 2003, 41: 4930- 40; Coombes *et al.* AEM 2008, 74: 2153-60

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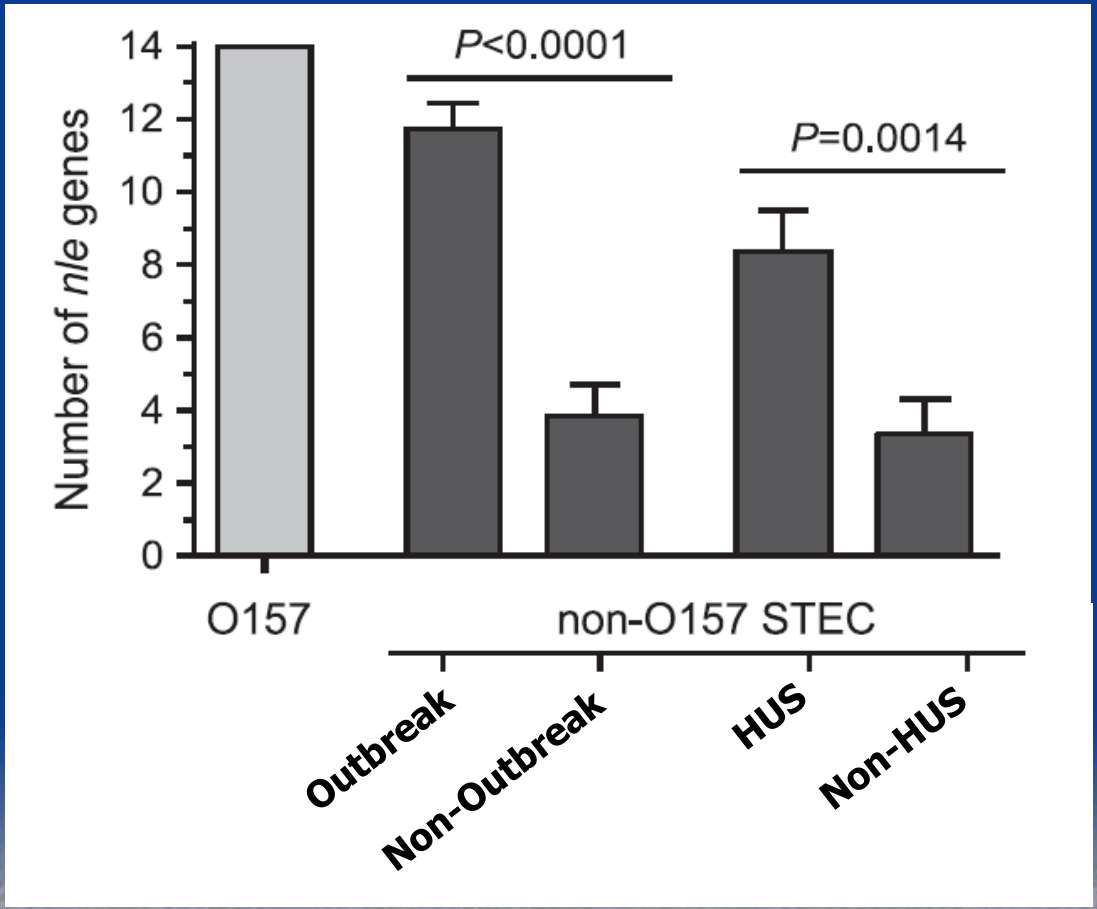
Non-LEE effectors (*nle*) and Disease



Wickham *et al.* 2006; 194:819–27

New Paradigm

Classification of STEC in 5 Sero-pathotypes

Karmali et al. , 2003, J. Clin. Microbiol., 41:4930-40

Based on the reported occurrence of serotypes in human disease, in outbreaks and/or in hemolytic-uremic syndrome (HUS)

Sero-pathotype	Relative incidence	Frequency of involvement in outbreaks	Association with severe disease (HUS or HC)	Serotypes
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O113: H21, O104:H21, others
D	Low	Rare	No	multiple
E	Non human only			multiple

New Paradigm

Problems with the sero-pathotype classification

- **Association with serotype and not with virulence profile**
  - O157 is virulent because it almost invariably contains Stx2a and or Stx2c
  - More than 120 O:H serotypes have been associated with HUS (Bergey's Manual of Systematic Bacteriology, 2nd ed.)
  - Many O:H serotypes display extensive heterogeneity
- **Involvement in outbreak may rapidly change**
- **Relative incidence**
  - is scewed by lack of efficient detection methods
  - will vary depending on the epidemiology of specific types

F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

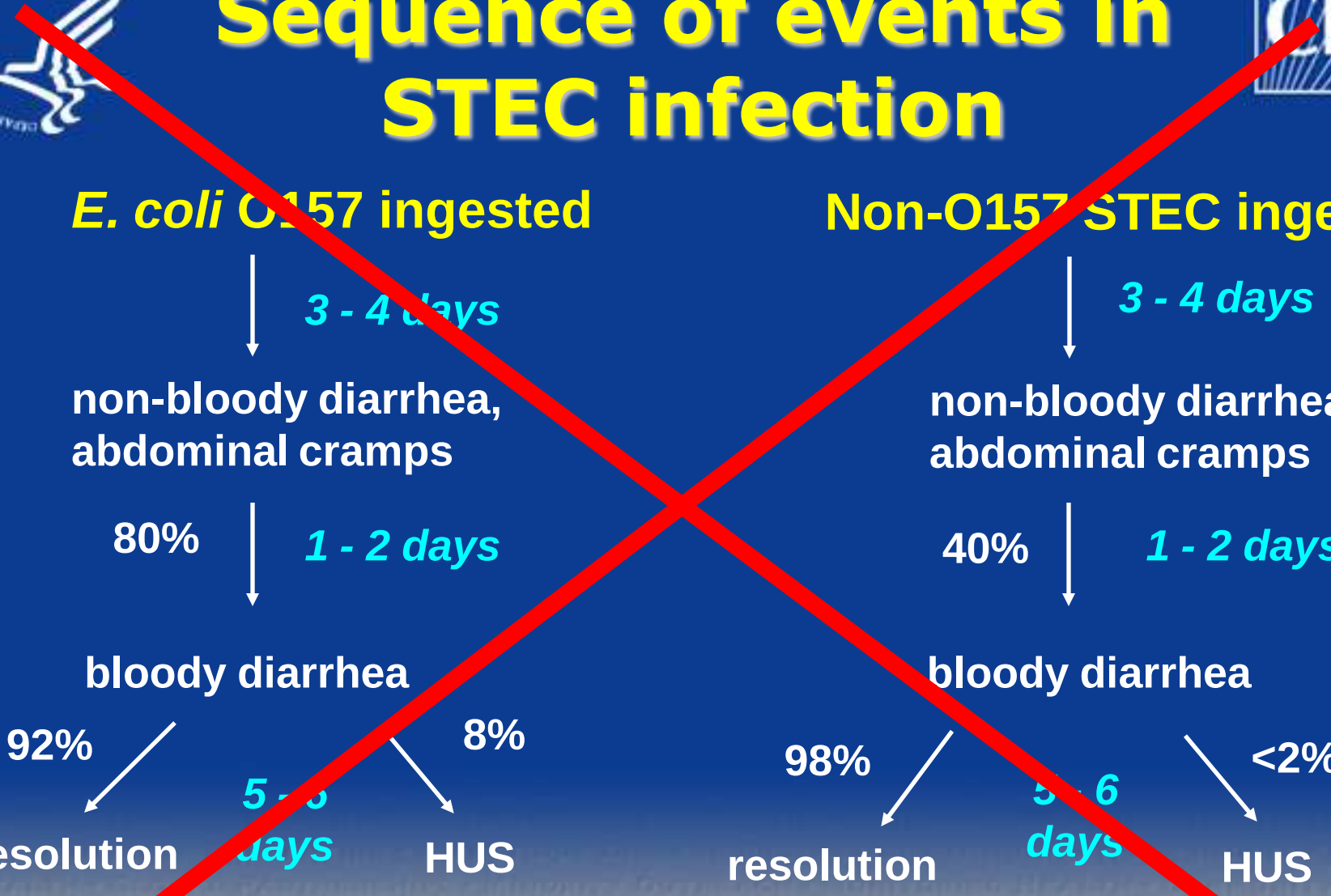
Alternative Classification

To be developed

- **Pathogenecity index (PI)**
  - Stx profile
  - *eae*
  - Other virulence genes/factors (e.g. *nle, saa,* subtilase)

1. Severe disease – PI high
2. Diarrhea in humans – PI intermediate
3. Animal STEC's – PI low

Sequence of events in STEC infection



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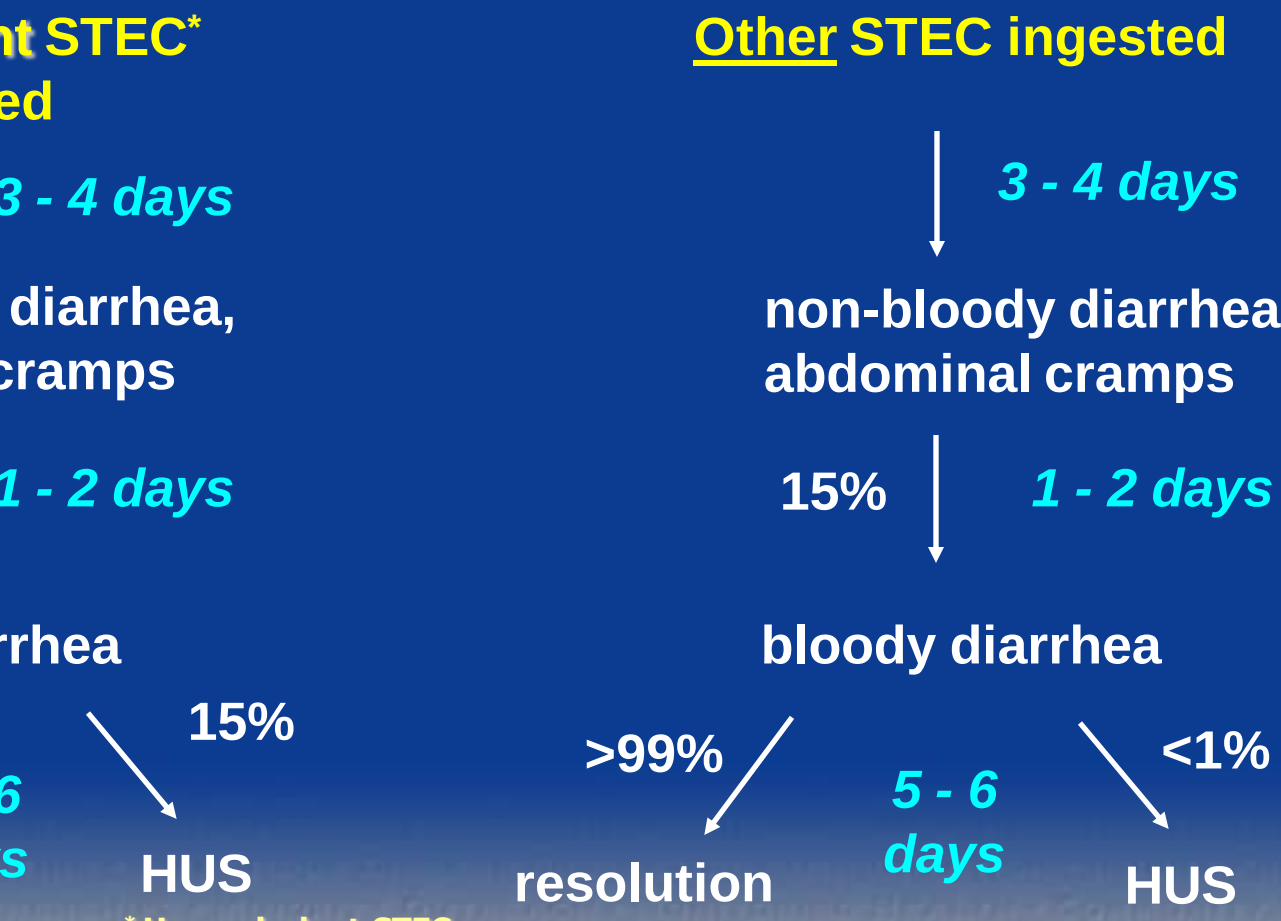
Sequence of events in STEC infection



* Hypervirulent STEC:
- Stx2a and/or Stx2c, eae, many nle
- Stx2d and NO eae, many nle

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STEC Characterization in the 21st Century

DNA array:

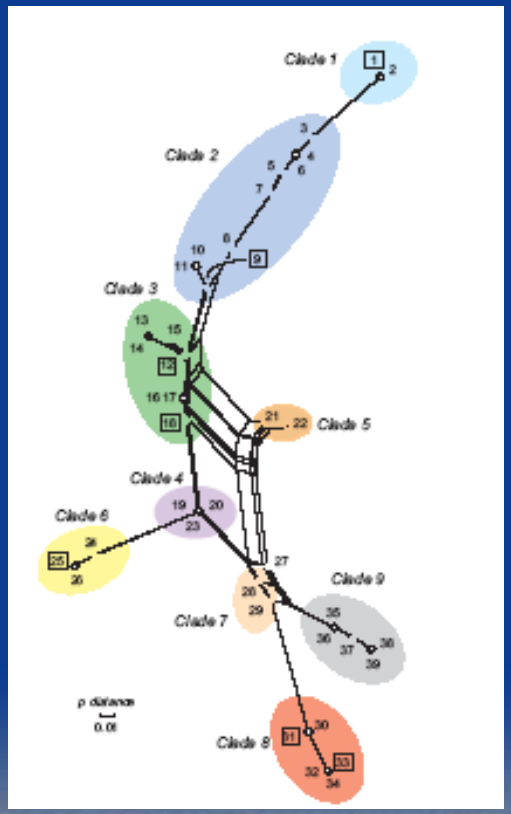
1. Virulence Profile
2. Molecular Serotype
3. SNP-Type

e.g. *stx1*, *eae*, *nleA*, *nleB*, O26, H2, A-G-C-A-A-T-G-C-C-C

- Virulence Information - Clinician
- Cluster Detection – Public Health
- Follow Trends – Public Health

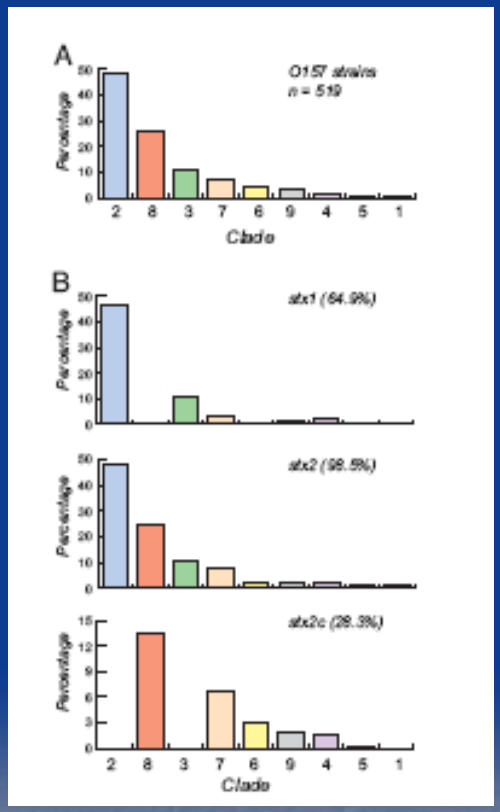
O157 Lineages, Stx subtypes and Pathogenecity

Lineages based on 96 SNP's



HUS
Clade 2: 3/154 (2%)
Clade 8: 7/63 (11%)
Other clades: 0/116

Stx subtypes in clades



Manning *et al.* PNAS 2008;105(12):4868-73

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