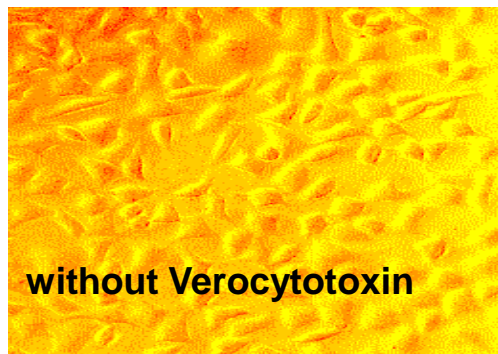


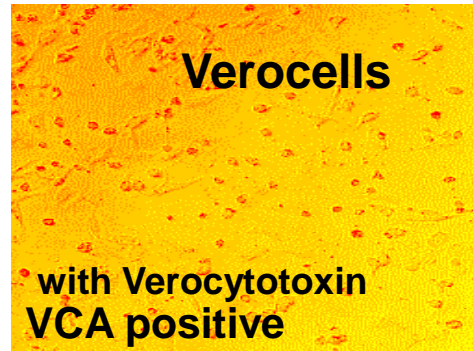
Definition of STEC

- ❖ Produce phage-encoded Shiga-toxin
 - Stx subgroups: Stx1 / Stx2
 - subtypes (Stx1a,c,d,e and Stx2a–o)
- ❖ LEE pathogenicity island that encodes a T3SS
 - intimin encoded by the *eae* gene
- ❖ Bloody diarrhoea (haemorrhagic colitis), non-bloody diarrhoea and haemolytic uremic syndrome (HUS)
 - Numerous outbreaks
 - Stx2 more important than Stx1 in the development of HUS
 - subtypes stx2a or stx2d

1977, Konowalchuk *et al.*
A cytotoxin which is lethal to Vero cells

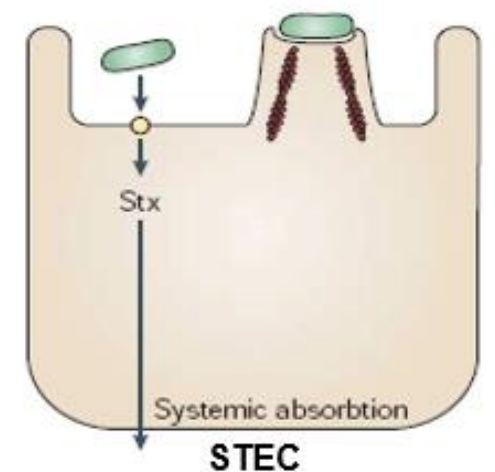


without Verocytotoxin

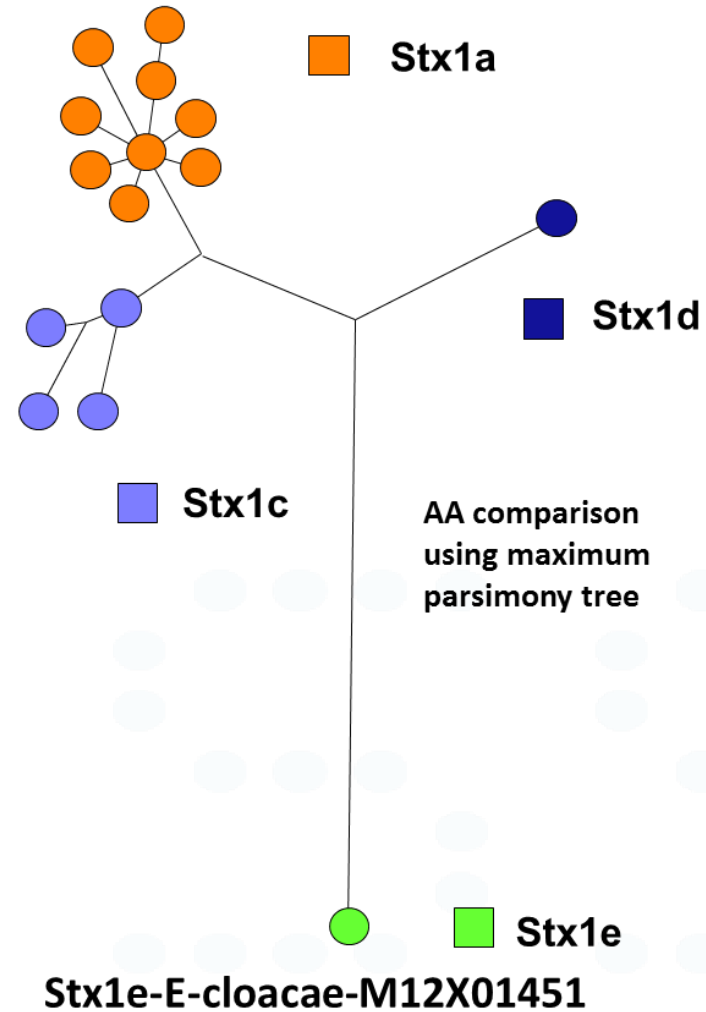
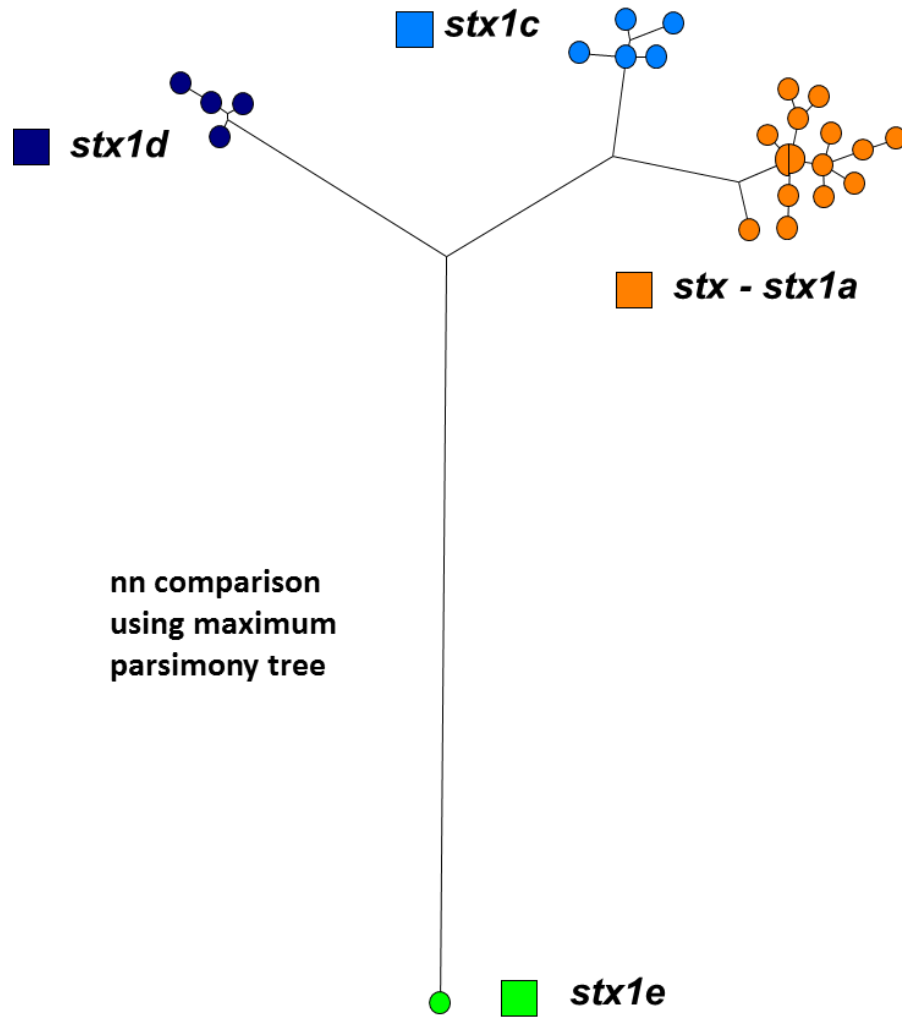


with Verocytotoxin
VCA positive

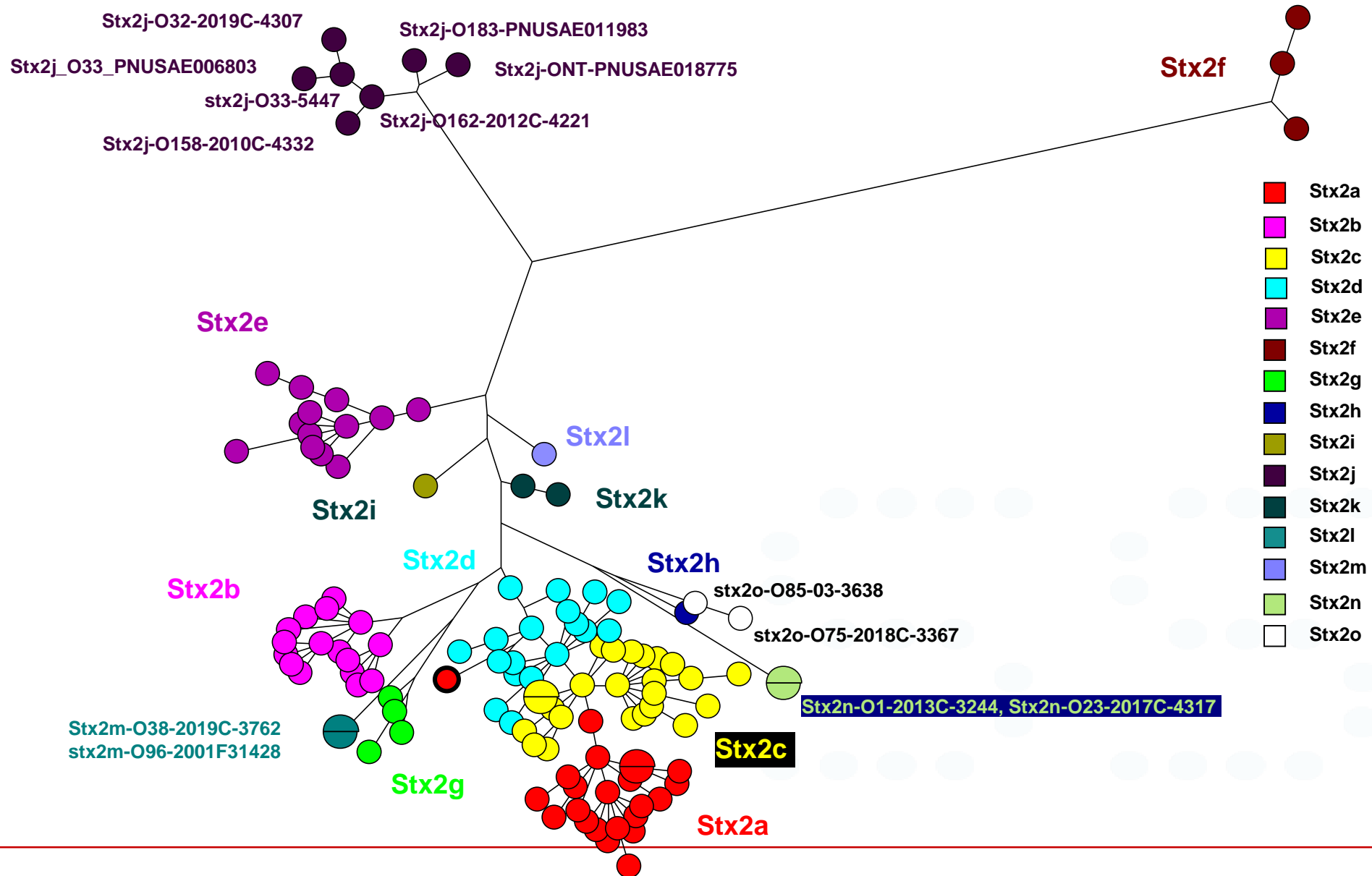
VTEC = STEC



STX1 FAMILY TREE: STX1A, STX1C-STX1E

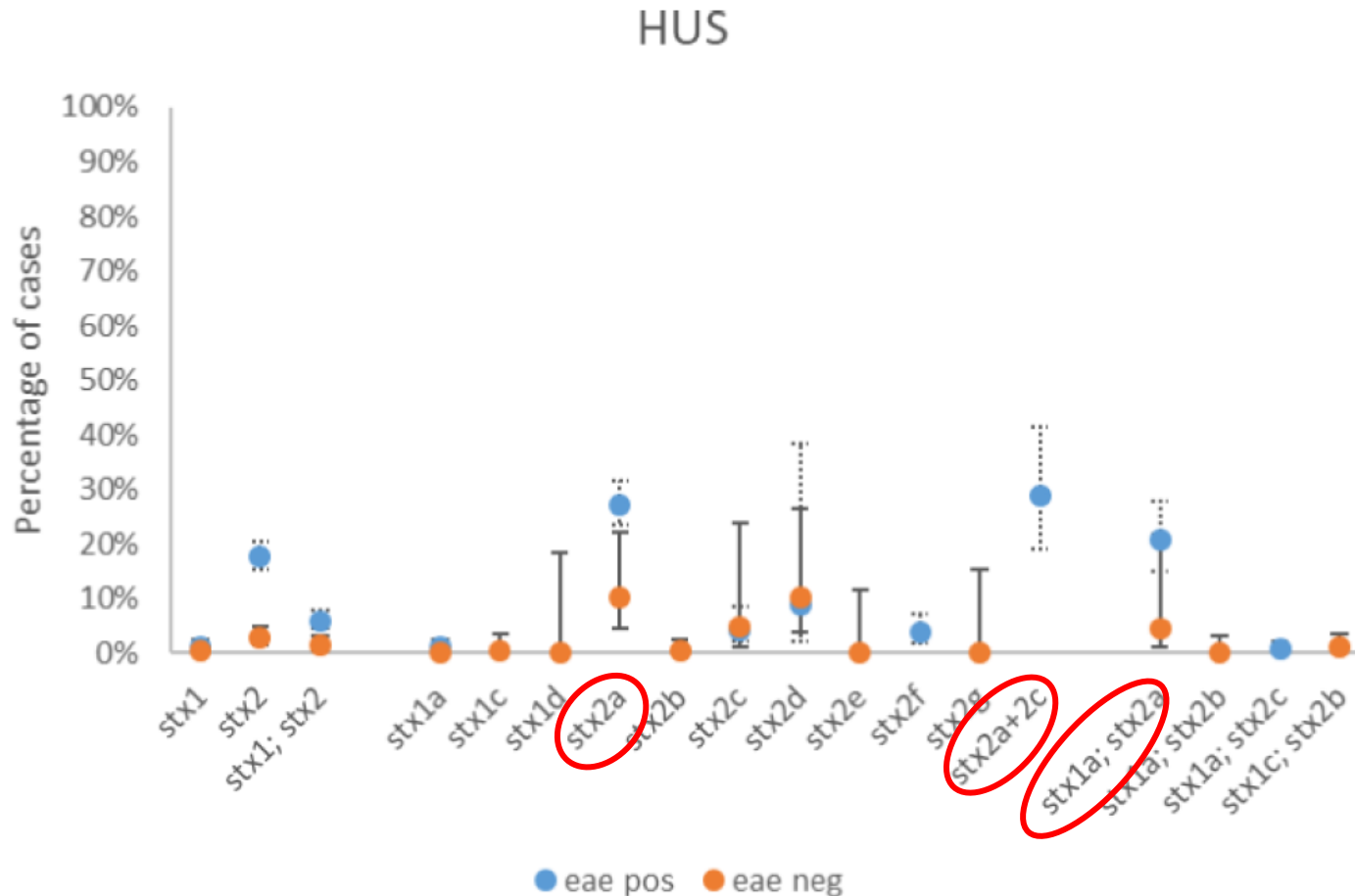


STX2 FAMILY TREE: STX2A – STX2O



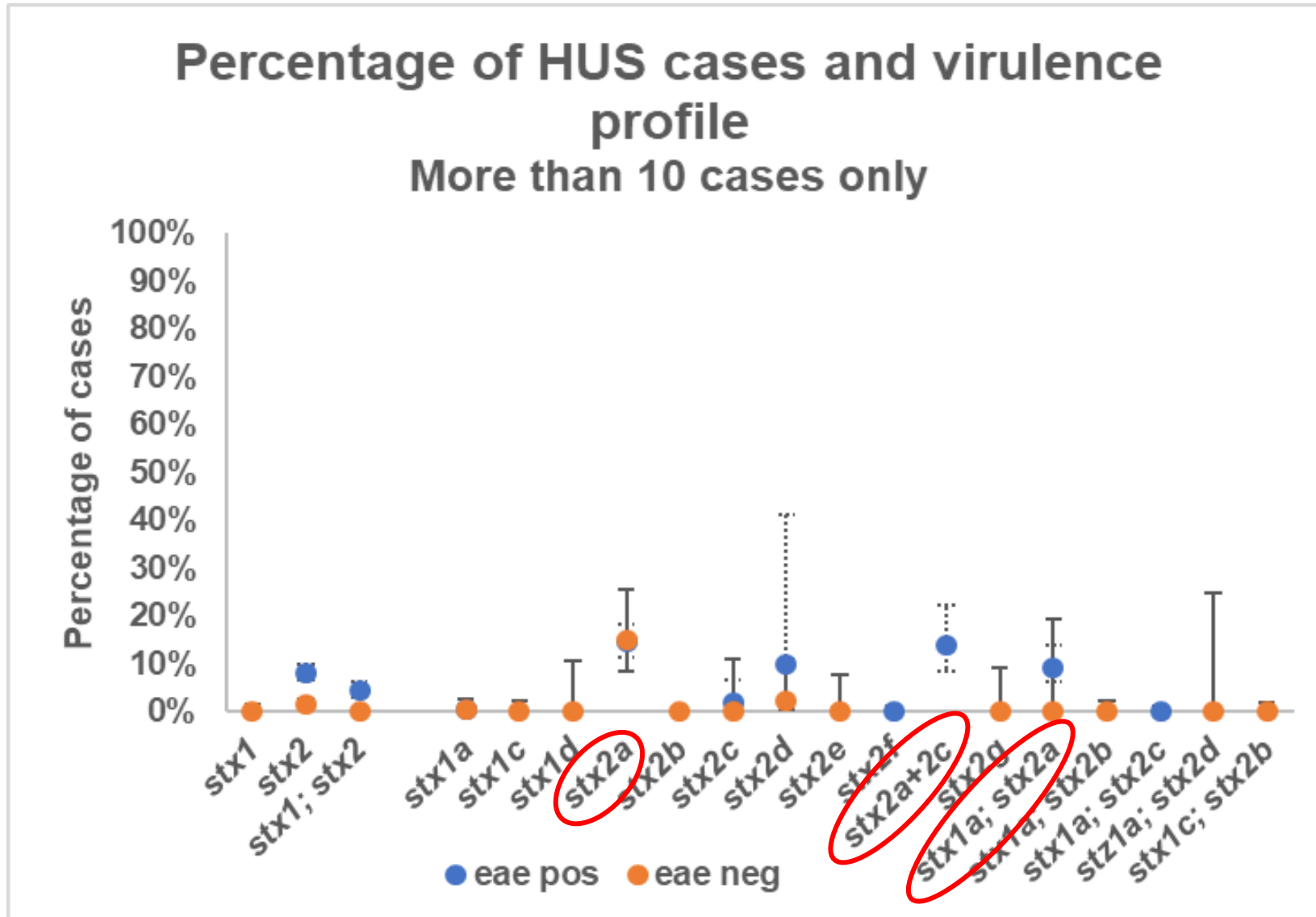
PATHOGENICITY ASSESSMENT OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) AND THE PUBLIC HEALTH RISK POSED BY CONTAMINATION OF FOOD WITH STEC

SCIENTIFIC OPINION

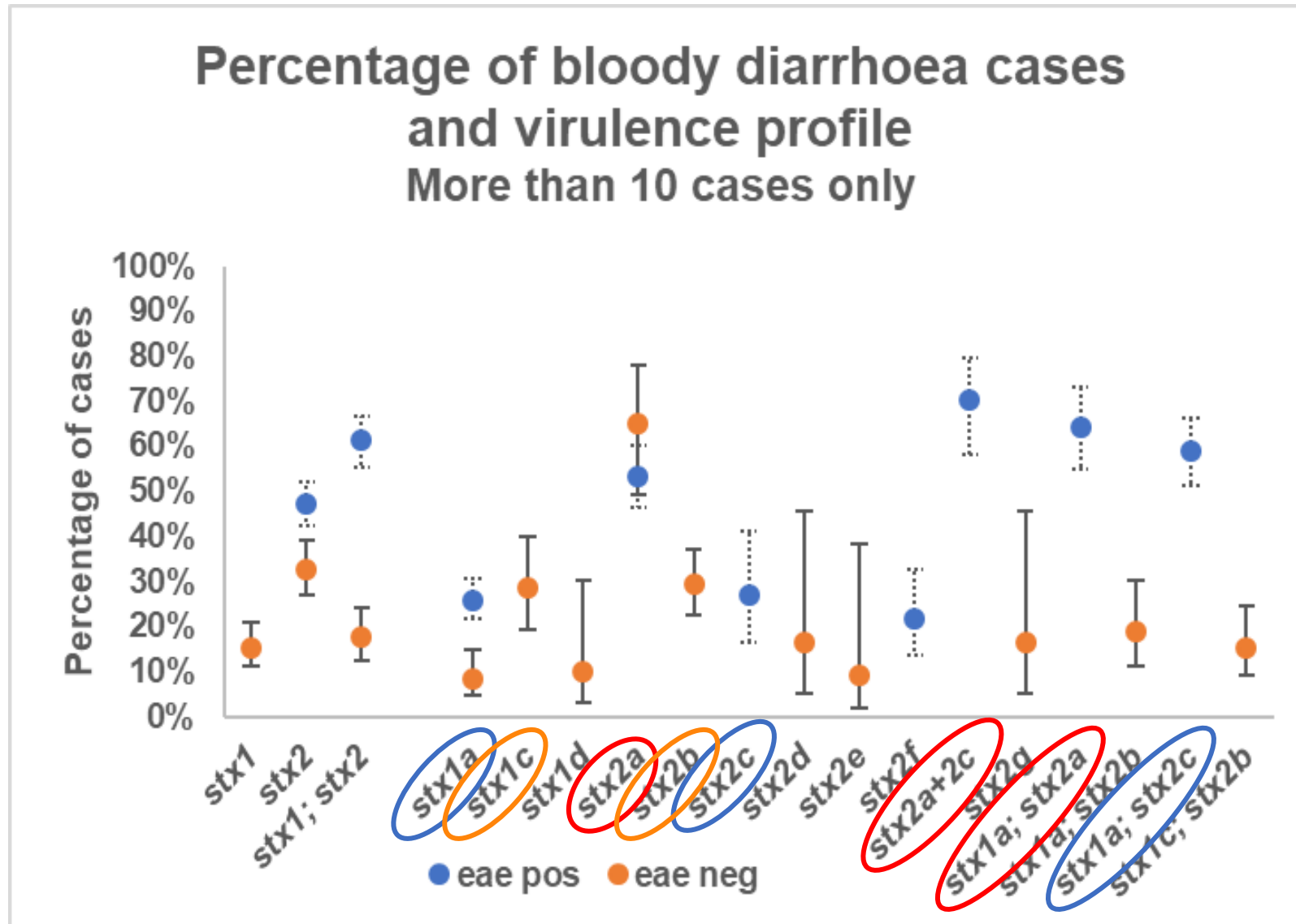


- Percentage of cases with HUS
- An estimate of the probability that a human STEC case with the given stx subtype and eae combination shows a given clinical outcome

DANISH HUS CASES: BASED ON 3.625 STEC CASES



DANISH DATA ON BLOODY DIARRHOEA: BASED ON 1.615 STEC CASES



SCIENTIFIC OPINION



ADOPTED: 11 December 2019
doi: 10.2903/j.efsa.2020.5967

Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC

EFSA BIOHAZ Panel,
Kostas Koutsoumanis, Ana Allende, Avelino Alvarez-Ordóñez, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Roland Lindqvist, Maarten Nauta, Luisa Peixe, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis, Elisabetta Suffredini, Claire Jenkins, Sara Monteiro Pires, Stefano Morabito, Maarten Nauta, Taina Niskanen, Flemming Scheutz, Maria Teresa da Silva Felício, Winy Messens and Declan Bolton

SCIENTIFIC REPORTS

OPEN Identification and pathogenomic analysis of an *Escherichia coli* strain producing a novel Shiga toxin 2 subtype

Received: 23 November 2017
Accepted: 17 April 2018
Published online: 30 April 2018

Xiangning Bai¹, Shanshan Fu¹, Ji Zhang², Ruyue Fan¹, Yanmei Xu¹, Hui Sun¹, Xiaohua He³, Jianguo Xu^{4*} & Yanwen Xiong^{1,4}

AUTHORS Meng, Q., Xiong, Y., Zhao, A. and Bai, X.
TITLE Isolation and characterization of Shiga Toxin-Producing *Escherichia coli* from slaughter pigs and swine feces
JOURNAL Unpublished



Article

Characterization of Clinical *Escherichia coli* Strains Producing a Novel Shiga Toxin 2 Subtype in Sweden and Denmark

Xiangning Bai^{1,2}, Flemming Scheutz³, Henrik Mellström Dahlgren⁴, Ingela Hedenström⁵ and Cecilia Jernberg^{5,*}



BACTERIOLOGY

Characterization of Atypical Shiga Toxin Gene Sequences and Description of Stx2j, a New Subtype

Alexander Gill,^a Forest Dussault,^b Tanis McMahon,^a Nicholas Petronella,^b Xiong Wang,^c Elizabeth Cebelinski,^c Flemming Scheutz,^d Kelly Weedmark,^a Burton Blais,^e Catherine Carrillo^e

Article

Identification and Characterization of Ten *Escherichia coli* Strains Encoding Novel Shiga Toxin 2 Subtypes, Stx2n as Well as Stx2j, Stx2m, Stx2o, in the United States

Rebecca L Lindsey^{1*}, Arjun Prasad², Michael Feldgarden², Narjol Gonzalez-Escalona³, Curtis Kapsak^{1†,4‡,5△}, William Klimke², Angela Melton-Celsa⁶, Peyton Smith¹, Alexandre Souvorov², Jenny Truong^{7‡,8†} and Flemming Scheutz⁹

SCIENTIFIC REPORTS

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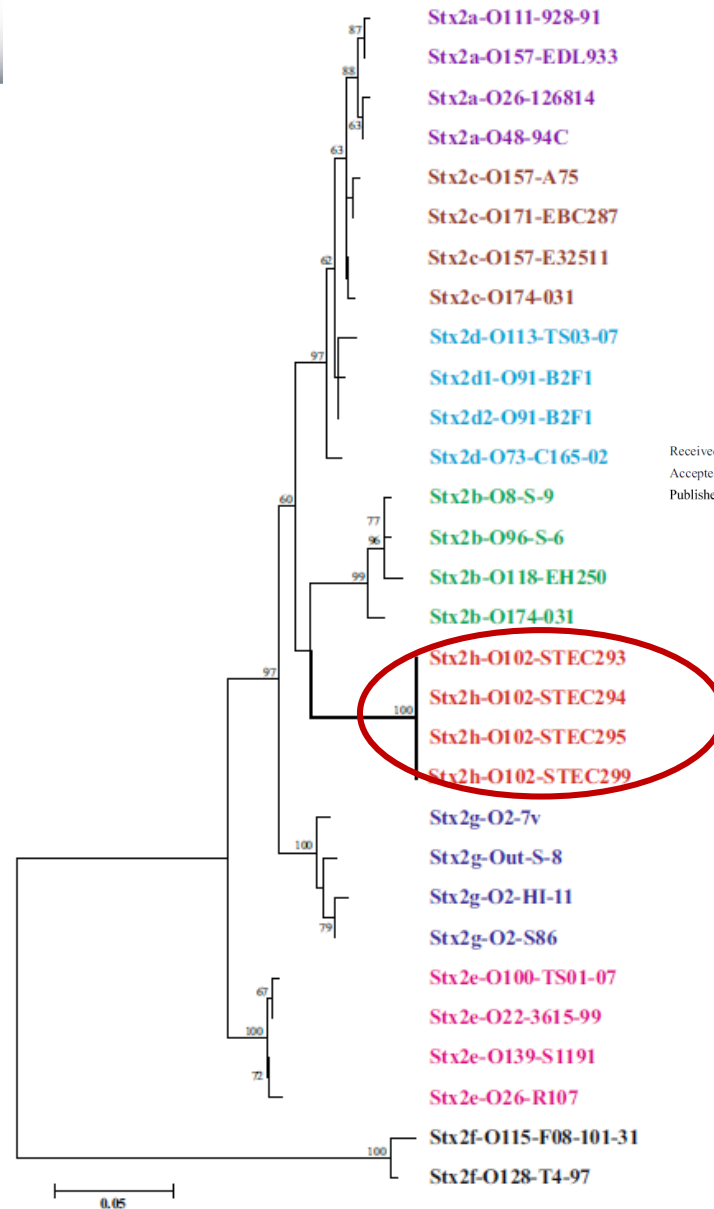
“...strains isolated from wild marmots in the Qinghai-Tibetan plateau, China.”

Isolates	Serotype	stx subtype	Virulence genes*	Sequence type	Site (above m.s. l, latitude/longitude)	Sampling time
STEC293	O102:H18	2h	<i>paa</i>	3693	Zhongdaxiang (3599 m, 33°13'/97°01')	2013-07-29
STEC294	O102:H18	2h	<i>paa</i>	3693	Dezhuotan (3025 m, 33°03'/97°11')	2013-08-02
STEC295	O102:H18	2h	<i>paa</i>	3693	Dezhuotan (3025 m, 33°03'/97°11')	2013-08-03
STEC299	O102:H18	2h	<i>paa</i>	3693	Dezhuotan (3025 m, 33°03'/97°11')	2013-08-02
STEC296	Orough:H8	2a	<i>ehxA, saa</i>	26	Dedacun (3625 m, 33°06'/97°08')	2013-08-06
STEC297	O168:H14	2g	<i>astA, saa</i>	718	Dedacun (3625 m, 33°06'/97°08')	2013-08-07

Table 1. STEC isolates recovered from intestinal contents of *Marmota himalayana*. *Virulence genes tested include *eae*, *ehxA*, *efa1*, *saa*, *paa*, *toxB*, and *astA*, among which only PCR-positive gene is listed for each isolate.

Identification of a novel Stx2 subtype in STEC strains of Marmot origin

- Six STEC isolates
 - One Stx2a, one Stx2g
 - Four novel Stx – designated Stx2h
- The Stx2h converting prophage: unique insertion site and genetic composition
- Stx2h is inducible and functional
- Hybrid spectrum of virulence genes (ExPEC)
- Human illness?



SCIENTIFIC REPORTS

OPEN

Identification and pathogenomic analysis of an *Escherichia coli* strain producing a novel Shiga toxin 2 subtype

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Figure 1. Phylogenetic tree of Stx2 subtypes by the neighbor-joining method. The neighbor-joining tree was inferred from comparison of combined (A and B) holotoxin amino acid sequences of all Stx2 subtypes. Numbers on the tree indicate bootstrap values calculated for 1000 subsets for branch points >50%. Bar, 0.05 substitutions per site. Stx2 subtypes are indicated by different colors. An extended version of this tree is available as Fig. S2.

- ❖ STECs from various foods
- ❖ STECs that had as yet undefined *stx*₂ subtypes
- ❖ ...identical to the already published subtype *stx2e*-O8-FHI-1106-1092
- ❖ *stx2i* (GenBank FN252457).”
- ❖ Isolated from shrimp, serotype ONT:H25
- ❖ Acc. No. FN252457: ONT:HNM, from raw milk
 - Isolate sent to SSI: O9:K39:H4, ST953 og *estb* German. Source unknown
 - Hybrid: ETEC-STECC
- ❖ First clinical isolate found in 2022 in Denmark: O8:H25

Journal of Food Protection, Vol. 79, No. 10, 2016, Pages 1656–1662
doi:10.4315/0362-028X.JFP-16-176
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Use of the *Escherichia coli* Identification Microarray for Characterizing the Health Risks of Shiga Toxin–Producing *Escherichia coli* Isolated from Foods

DAVID W. LACHER,¹ JAYANTHI GANGIREDLA,¹ ISHA PATEL,¹ CHRISTOPHER A. ELKINS,¹ AND PETER C. H. FENG^{2*}

¹U.S. Food and Drug Administration, Division of Molecular Biology, Laurel, Maryland 20708; and ²U.S. Food and Drug Administration, Division of Microbiology, College Park, Maryland 20740, USA

MS 16-176: Received 27 April 2016/Accepted 23 June 2016

- Stx2h (O170:H18) was identified in a Canadian sprout isolate
- Stx2j (O158:H23 and O33:H14) was found in lettuce and clinical isolates
- Stx2o (O85:H1) was identified in a clinical isolate
- Two strains possessed unreported variants of Stx2a (O8:H28) and Stx2b (O146:H21) isolated from flour

Journal of
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Published online 2022 Mar 16. doi: [10.1128/jcm.02229-21](https://doi.org/10.1128/jcm.02229-21)

PMCID: PMC8925903

PMID: [35225693](https://pubmed.ncbi.nlm.nih.gov/35225693/)

Characterization of Atypical Shiga Toxin Gene Sequences and Description of Stx2j, a New Subtype

[Alexander Gill](#),^a [Forest Dussault](#),^b [Tanis McMahon](#),^a [Nicholas Petronella](#),^b [Xiong Wang](#),^c [Elizabeth Cebelinski](#),^c [Flemming Scheutz](#),^d [Kelly Weedmark](#),^a [Burton Blais](#),^e and [Catherine Carrillo](#)^e

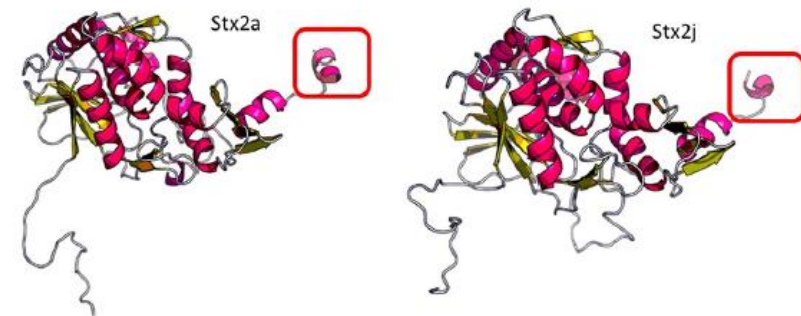


FIG 3 Predicted structure of the A subunit of Stx2a and Stx2j. Red box indicates the C terminus of the protein.

O159:H16 ST 3630,
esta, estab. Source: pig

Danish patient:
O75:H5 *eae* negative



Fig. 1. Phylogenetic tree of Stx2 subtypes by the Neighbor-Joining method. The Neighbor-Joining tree was inferred from comparison of combined A and B holotoxin amino acid sequences of all Stx2 subtypes. Numbers on the tree indicate bootstrap values calculated for 1000 subsets for branch points > 50%. Bar, 0.05 substitutions per site. Stx2 subtypes are indicated by different colors. An extended version of this tree is available as Figure S1.



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/ijmm

Escherichia coli strains producing a novel Shiga toxin 2 subtype circulate in China

Xi Yang^{a,1}, Xiangning Bai^{a,b,1}, Ji Zhang^{c,1}, Hui Sun^a, Shanshan Fu^a, Ruyue Fan^a, Xiaohua He^d, Flemming Scheutz^e, Andreas Matussek^b, Yanwen Xiong^{a,f,*}



RESEARCH ARTICLE



High Prevalence and Persistence of *Escherichia coli* Strains Producing Shiga Toxin Subtype 2k in Goat Herds

Xi Yang,^a Qian Liu,^a Xiangning Bai,^{a,b,c} Bin Hu,^d Deshui Jiang,^e Hongbo Jiao,^e Liangmei Lu,^e Ruyue Fan,^d Peibin Hou,^d Andreas Matussek,^{b,c,f} Yanwen Xiong^a

- 170 stx2k-positive STEC strains in goat herds in China
- 55% the strains were of the hybrid STEC/ETEC pathotype



Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature

Flemming Scheutz,^a Louise D. Teel,^b Lothar Beutin,^c Denis Piérard,^d Glenn Buvens,^d Helge Karch,^e Alexander Mellmann,^e Alfredo Caprioli,^f Rosangela Tozzoli,^f Stefano Morabito,^f Nancy A. Strockbine,^g Angela R. Melton-Celsa,^b Maria Sanchez,^b Soren Persson,^a and Alison D. O'Brien^b

September 2012 Volume 50 Number 9

Journal of Clinical Microbiology p. 2951–2963

jcm.asm.org 2951

TABLE 2 (Continued)

Plasmid or strain used for validation of the detection and subtyping protocol (reference) ^a	Strain serotyped as part of this study ^b (original published serotype)	Result(s) obtained using the protocol described in Table 1 and in the text
FHI-1106-1092	O8:H2 ^f	<i>stx</i> _{2a} and <i>stx</i> _{2d}
T4/97 (59)	O128ac:[H2]	<i>stx</i> _{2l}
H.I.8. (18)	O89:[H2] (O128:H2)	<i>stx</i> _{2f}
7v (33)	O2:H25	<i>stx</i> _{2g}
S86 (19)	O2:H25	<i>stx</i> _{2g}

Genomic Characterization of *Escherichia coli* O8 Strains Producing Shiga Toxin 2l Subtype

[Xi Yang](#),¹ [Qian Liu](#),¹ [Hui Sun](#),¹ [Yanwen Xiong](#),¹ [Andreas Matussek](#),^{2,3,4} and [Xiangning Bai](#)^{1,2,3,*}

Rodney A. Moxley, Academic Editor

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- ❖ Stx2l, initially designed as Stx2e
- ❖ Stx2l-STECS in diverse hosts and geographical regions
- ❖ Identified in a few clinical and sheep isolates
- ❖ Characteristics of the Stx2l-STECS strains have been poorly elucidated
- ❖ This Stx2 subtype has also been found in Norway
- ❖ Serotypes O8:H9, O8:H19 og O8:H30
- ❖ It has also been found in 6 patients with diarrhoea in Denmark
 - five (O8:H9), one O65:H16

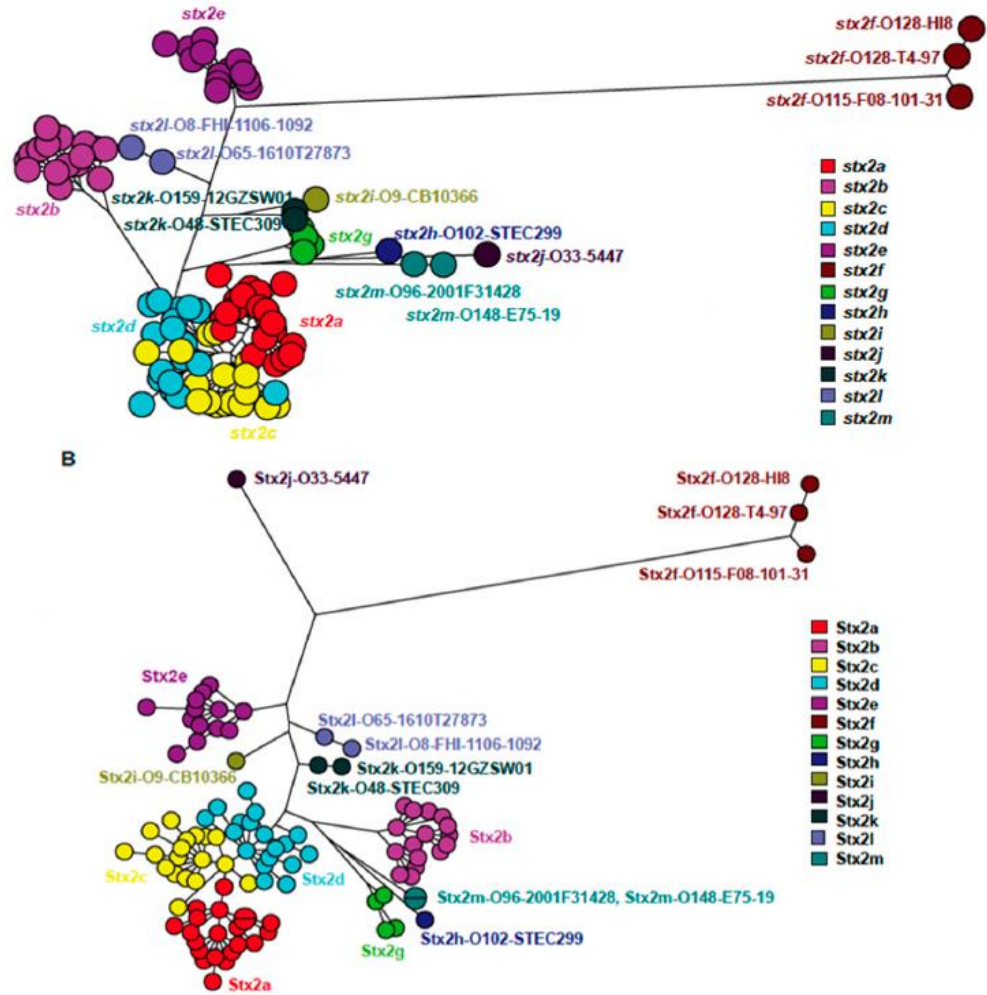


Figure 1. Comparison of nucleotide (A) and amino acid (B) sequences of different Stx2 subtypes using maximum parsimony cluster analysis. Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g adapted from Scheutz et al., 2012. Stx2h (Bai et al., 2018), Stx2i (FN252457), Stx2j (MZ571121), Stx2k (Yang et al., 2020) and Stx2l (EFSA BIOHAZ Panel, 2020).

microorganisms



Characterization of Clinical *Escherichia coli* Strains Producing a Novel Shiga Toxin 2 Subtype in Sweden and Denmark


Ying Bai ^{1,2}, Flemming Scheutz ³ , Henrik Mellström Dahlgren ⁴, Ingela Hedenström ⁵, Cecilia Jernberg ^{5,*}

Table 1. Characterization of three Stx2m-producing STEC isolates.

	E75_19	E79_19	2001F31428
Clinical information			
Source	Diarrheal patient	Healthy contact	Diarrheal patient
Infected region	Sweden	Sweden	Denmark
Isolation year	2019	2019	2020
Genetic characteristics			
Serotype	O148:H39	O148:H39	O96:H19
Stx2 subtype	Stx2m	Stx2m	Stx2m
Sequence type	5825	5825	99
Genome size (bp)	5,514,783	5,565,491	4,948,862
CG %	50.39	50.43	50.94
Accession number	SAMEA7019263	SAMEA7019264	SAMEA6873236

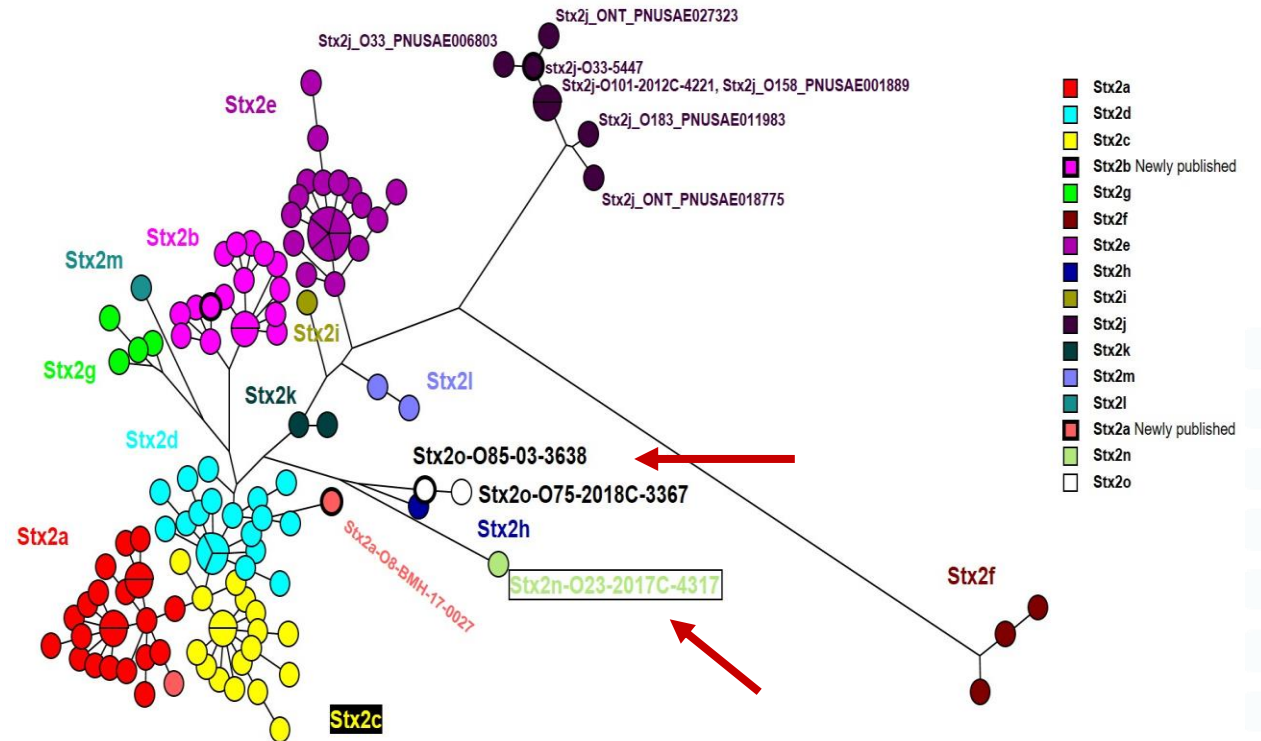
Stx2m toxin was functional and exhibited cytotoxicity in vitro

- Novel Stx-producing STEC strains were isolated from patients in clinical settings in the United States
- The virulence gene profile varied among the three isolates
- Two STECs carried the genes *fyuA*, *vat*, and *yfcV* (UPEC)
- Strains classified as multiple pathotypes can be more dangerous to human health
 - additional virulence genes related to a second pathotype, and human illness may be overlooked

Article

Identification and Characterization of Ten *Escherichia coli* Strains Encoding Novel Shiga Toxin 2 Subtypes, Stx2n as Well as Stx2j, Stx2m, Stx2o, in the United States

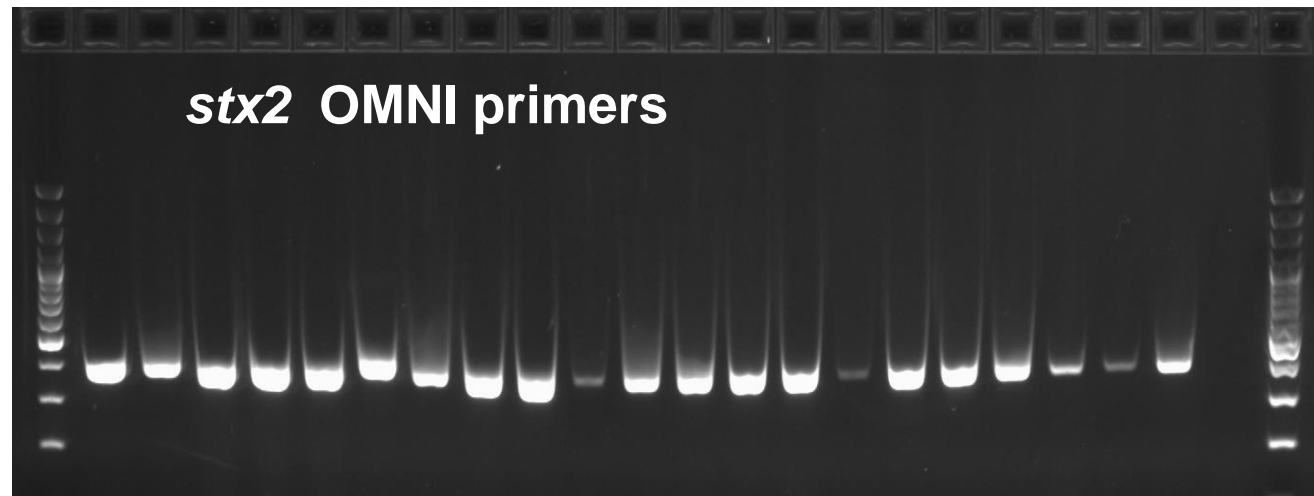
Rebecca L Lindsey ^{1,*}, Arjun Prasad ², Michael Feldgarden ², Narjol Gonzalez-Escalona ³, Curtis Kapsak ^{1,4,5*}, William Klimke ², Angela Melton-Celsa ⁶, Peyton Smith ¹, Alexandre Souvorov ², Jenny Truong ^{7,8*} and Flemming Scheutz ⁹



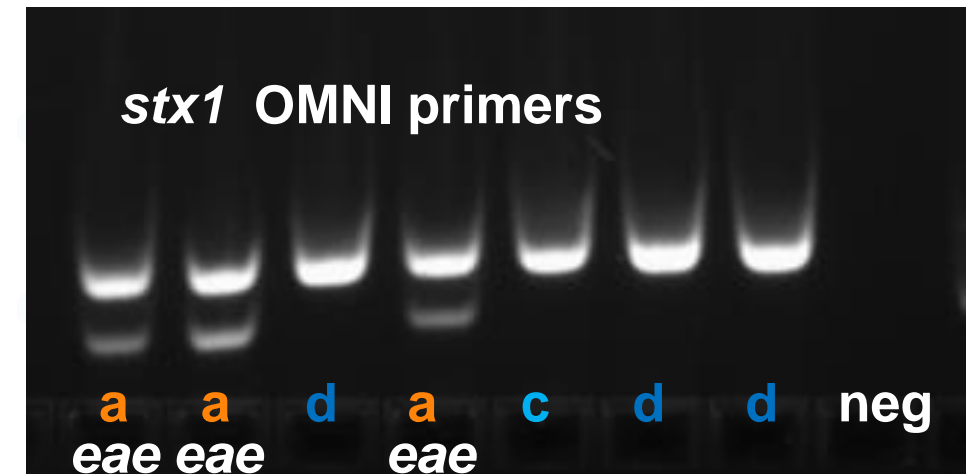
STX1 AND 2 OMNI-PRIMERS

Genes	Primer sequence	Size (bp)
PS8-F <i>stx2</i>	5'-TCACYGGTTTCATCATATCTGG	399 bp
PS7-F <i>stx2</i>	5'-GCCTGTCBCCASTTATCTGACA	399 bp
PS19 <i>vtx2f</i> F	5'-GTACAGGGATGCAGATTGGGCG	438 bp
PS20 <i>vtx2f</i> R	5'-CTTTAATGGCCGCCCTGTCTCC	438 bp

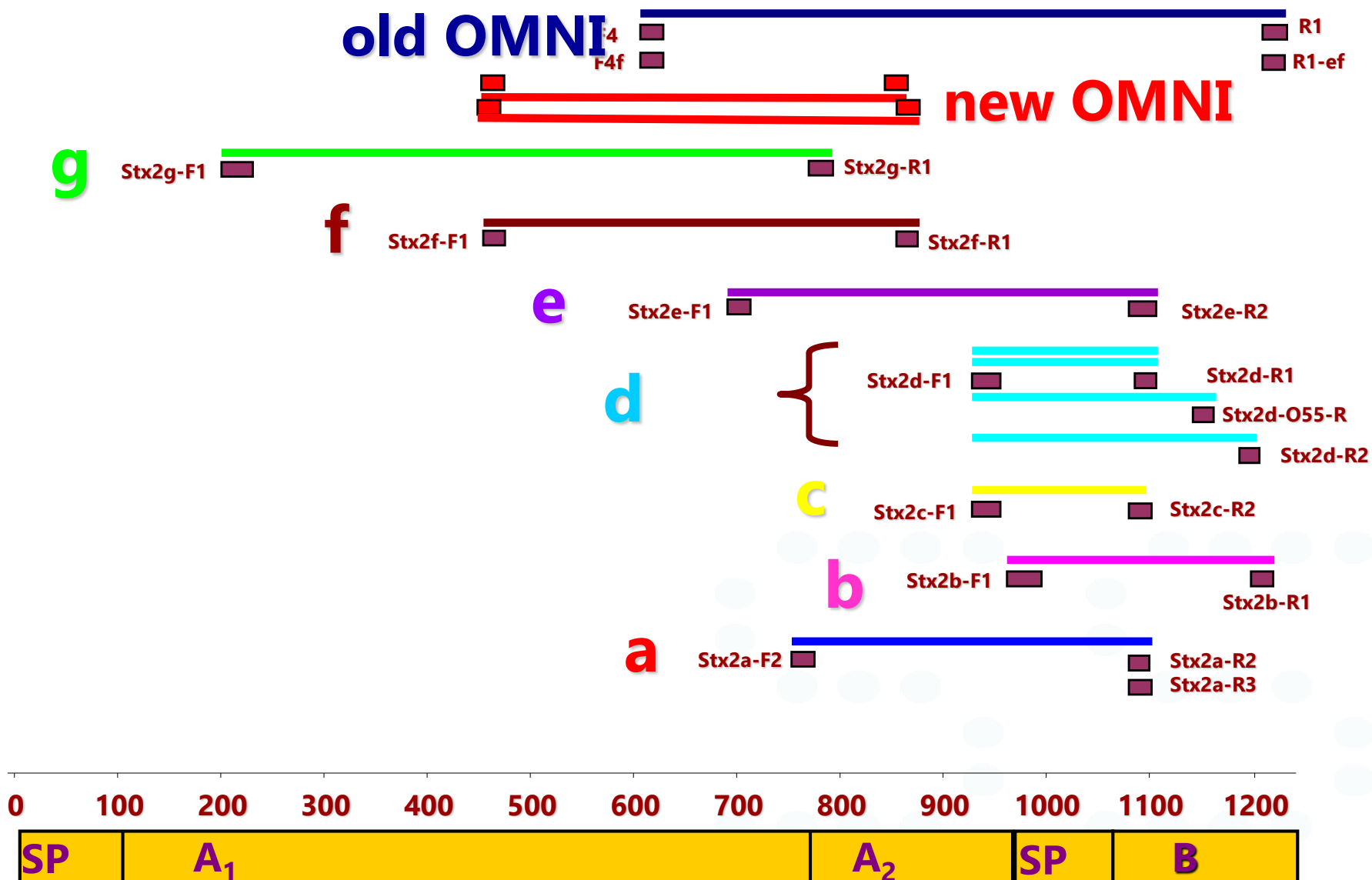
Primers 2		
stx1 F3b	5'-CTGATGATTGATAGTGGCACAGG	282bp
stx1 OMNI-R1	5'-GCGATTTATCTGCATCCCCGTAC	
Primer incl. Citrobacter		
PS17-<i>eae</i>	F 5'-CGGCTATTTCCGCATGAGCGG	222bp
PS18-<i>eae</i>-R-NEW	R 5'-AGTTDACACCAAYWGTCRCCGC	



a b c d e f g h h i j k l l n o m j n n n neg



PRIMERS FOR DETECTION AND SUBTYPING OF STX2



- ❖ Stx2a - Stx2o
- ❖ WGS: discovery of new Stx subtypes and variants
- ❖ Lacking knowledge on their prevalence and association with human illness
- ❖ Several of the new Stx subtypes have been found in combination with virulence genes from ETEC and ExPEC/UPEC
- ❖ Most of them are *eae* negative
- ❖ Nearly all reported Stx2I are O8
- ❖ The detection of all Stx is challenging (both PCR and rt-PCR)
- ❖ Stx subtyping is not in place
 - Example: stx2k variants is positive with stx2c and stx2d, or stx2a and stx2d

September 2023

- ❖ Pt: 69-year-old man admitted with diarrhoea for several weeks with thrombotic microangiopathy, haemolysis, and high creatinine levels
- ❖ Started on plasma exchange treatment and completed a total of five sessions
- ❖ Responded well to treatment: diarrhoea ceased, and the patient recovered
- ❖ Isolate: STEC - ST642, O85:H4, *stx2f*, *eae*
- ❖ Prevalence: only one other case in SSI surveillance database (*stx2a* not *stx2f*)
- ❖ Nanopore: variant of the *stx2f* gene?
- ❖ Certain serotypes of *E. coli* harbouring *stx2f* cause severe clinical outcomes, including STEC-HUS
- ❖ HUS caused by *stx2f* has been reported
 - O8:H19, O80:H2, O55:H9, O26:H11, O63:H6, O55:H9