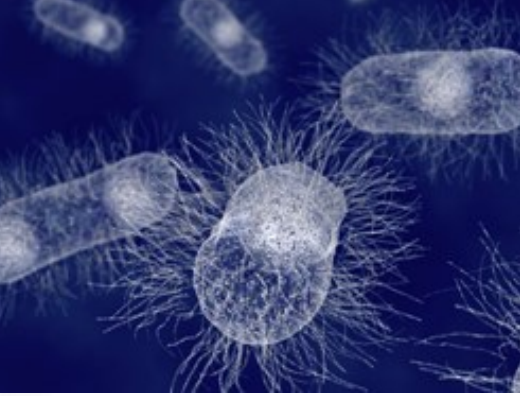




**The contribution of typing  
methods to risk assessment  
- the case of  
Vero cytotoxin- (Shiga toxin-)  
producing  
*E. coli* (VTEC/STEC)**



**Flemming Scheutz**

**WHO Collaborating Centre for  
Reference and Research on  
*Escherichia* and *Klebsiella***

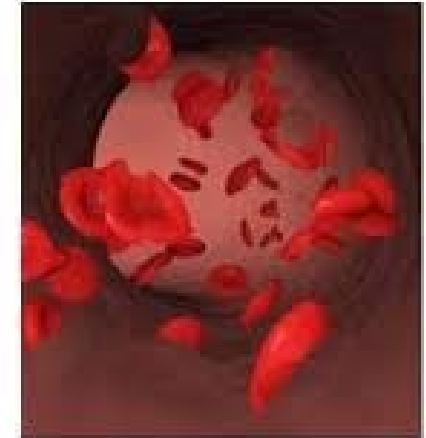
**Foodborne bacterial infections  
Microbiology & Infection control  
STATENS SERUM INSTITUT**

# Haemolytic uraemic syndrome

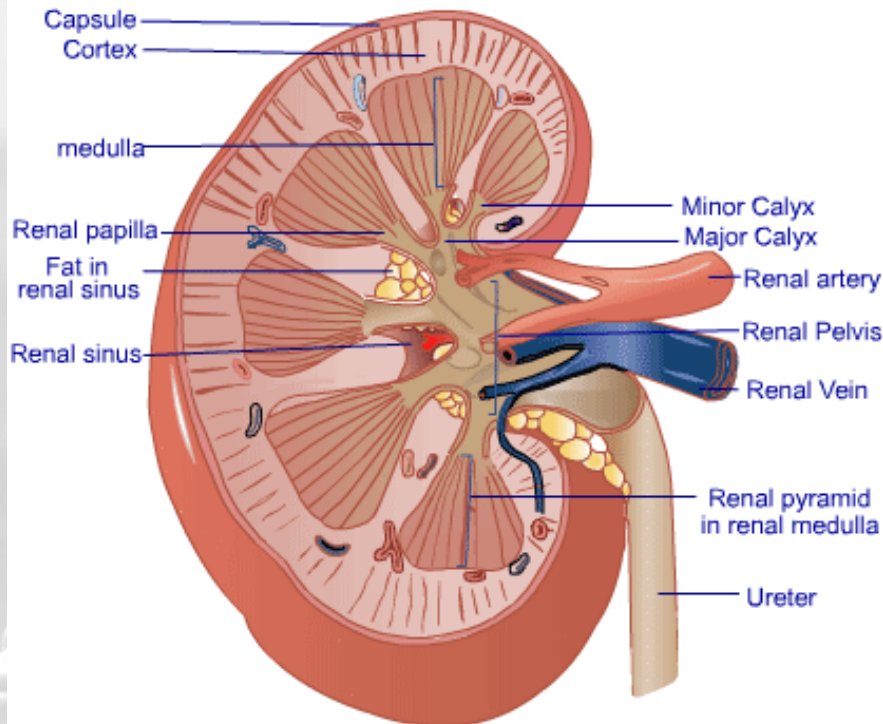
## HUS



- Acute renal failure
- Microangiopathic haemolytic anaemia (Intravascular haemolysis and destruction of the red blood cells)
- Thrombocytopenia (Drop in the blood platelet count)



Cut Section of Kidney





Once upon a time....



The NEW ENGLAND  
JOURNAL of MEDICINE

Hemorrhagic Colitis Associated with a Rare *Escherichia coli*  
Serotype

Lee W. Riley, M.D., Robert S. Remis, M.D., M.P.H., Steven D. Helgerson, M.D., M.P.H., Harry B. McGee, M.P.H., Joy G. Wells, M.S., Betty R. Davis, M.S., Richard J. Hebert, M.D., Ellen S. Olcott, R.N., Linda M. Johnson, R.N., M.S., Nancy T. Hargrett, Ph.D., Paul A. Blake, M.D., M.P.H., and Mitchell L. Cohen, M.D.

N Engl J Med 1983; 308:681-685 | [March 24, 1983](#) | DOI: [10.1056/NEJM198303243081203](#)

## **Non-O157s now share the scene with O157**

**from cases of HUS**

**O26:H11 in Italy (*stx2a* highly virulent)**

**O80:H2 in France (*stx2a*, *stx2a2d*, *stx2c2d*, *stx2d*, *eae*  $\xi$ )**

**O104:H4 in the Republic of Georgia**

**O73:H18 in Spain**

**O91:H21 in Spain**

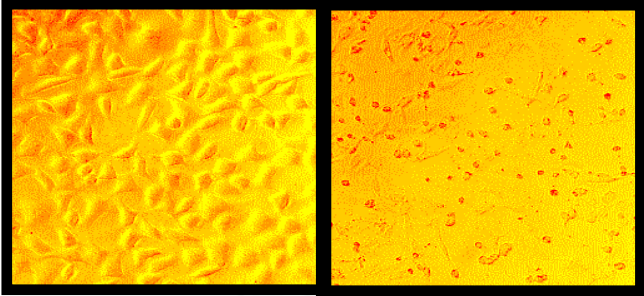
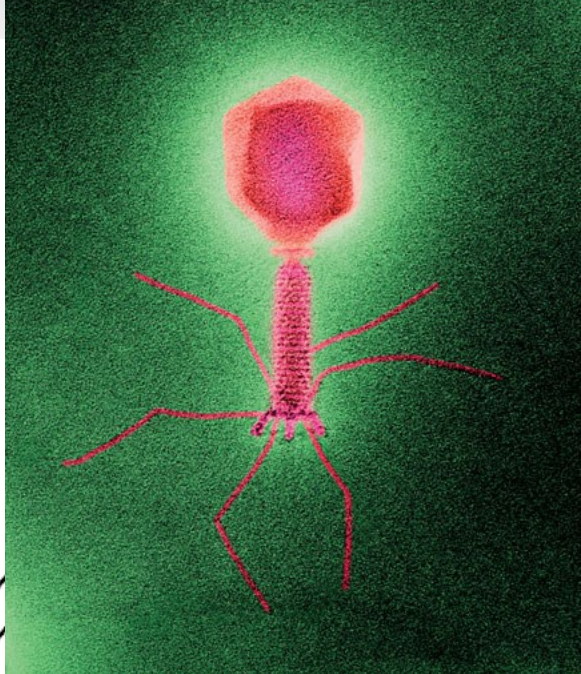
**O59:H19 EAEC in Argentina**

**O174:H8, H21 and H28 in Argentina**





# VTEC



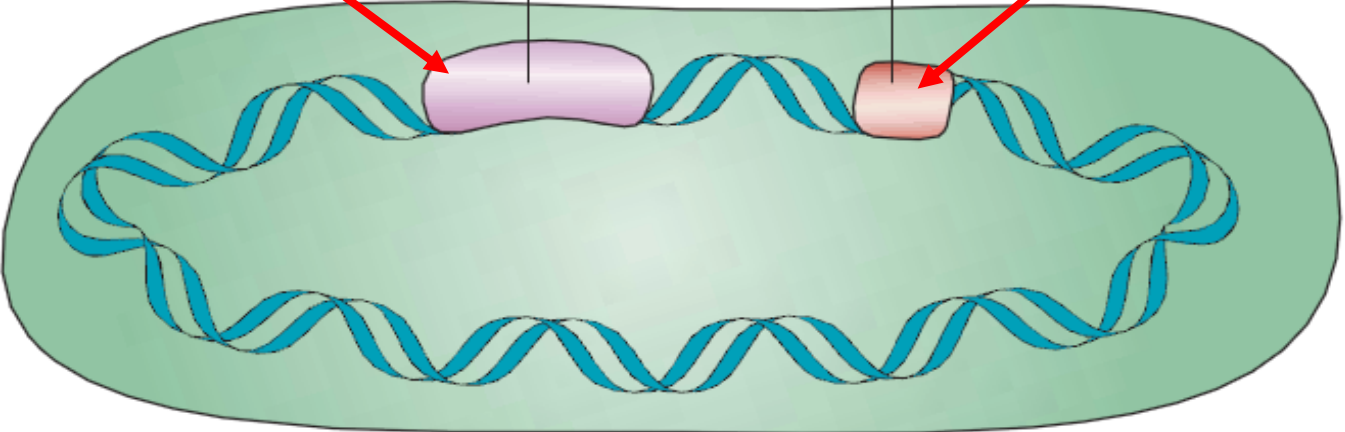
***vtx1***  
**and/or**  
***vtx2***

Phage

***eae***

LEE PAI

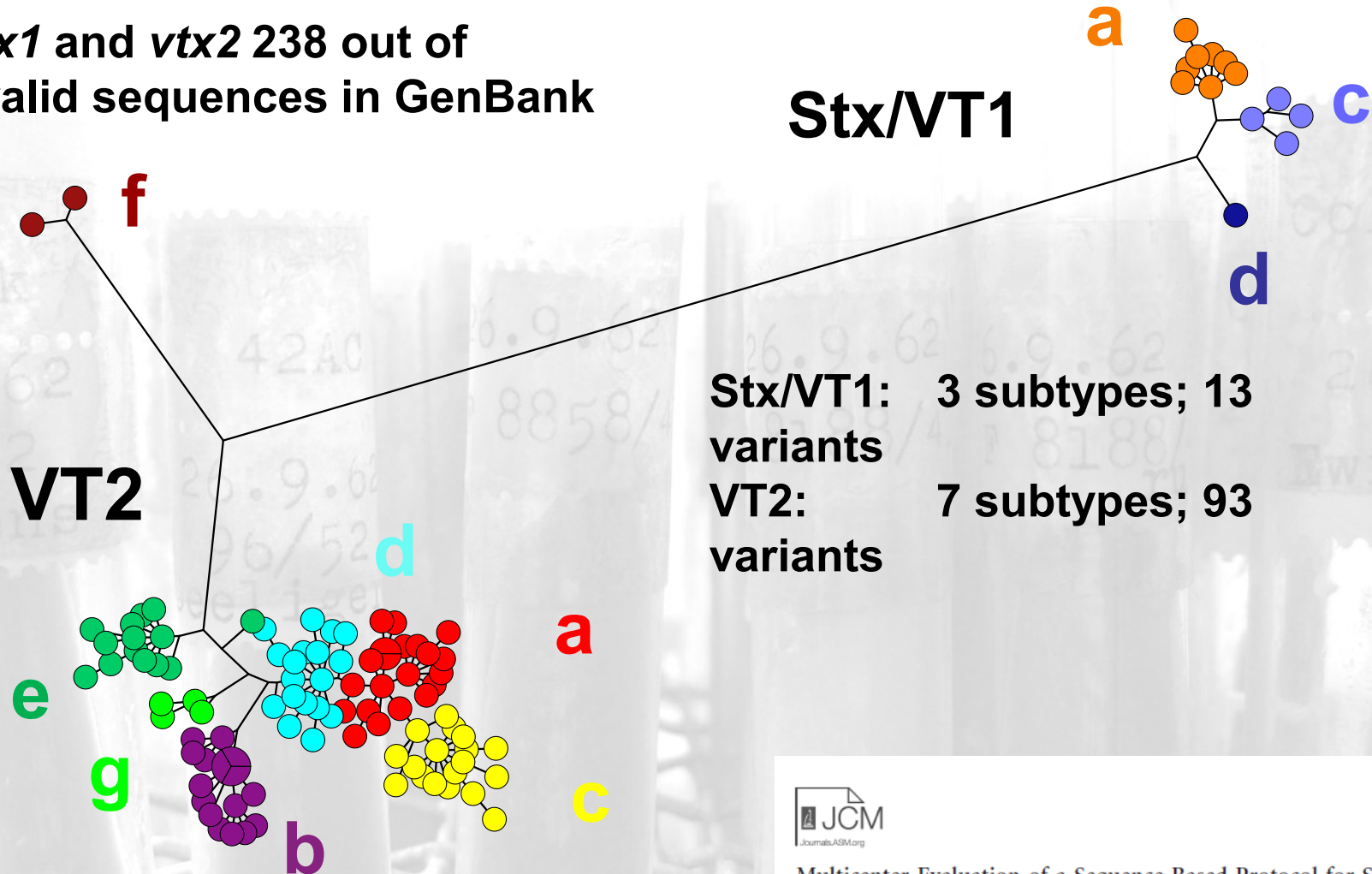
Shiga toxin





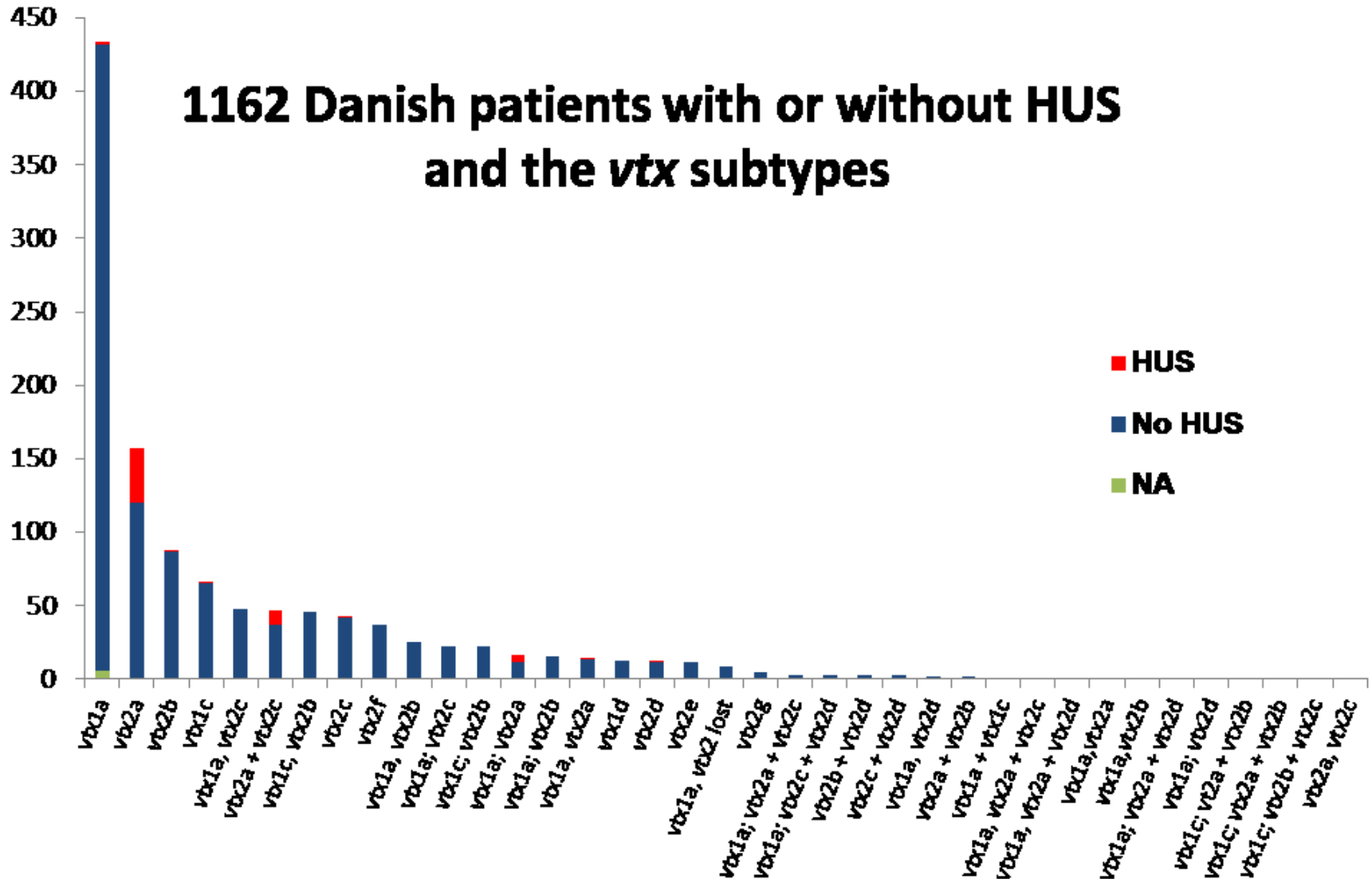
# Stx subtypes and variants

47 *vtx1* and *vtx2* 238 out of  
285 valid sequences in GenBank



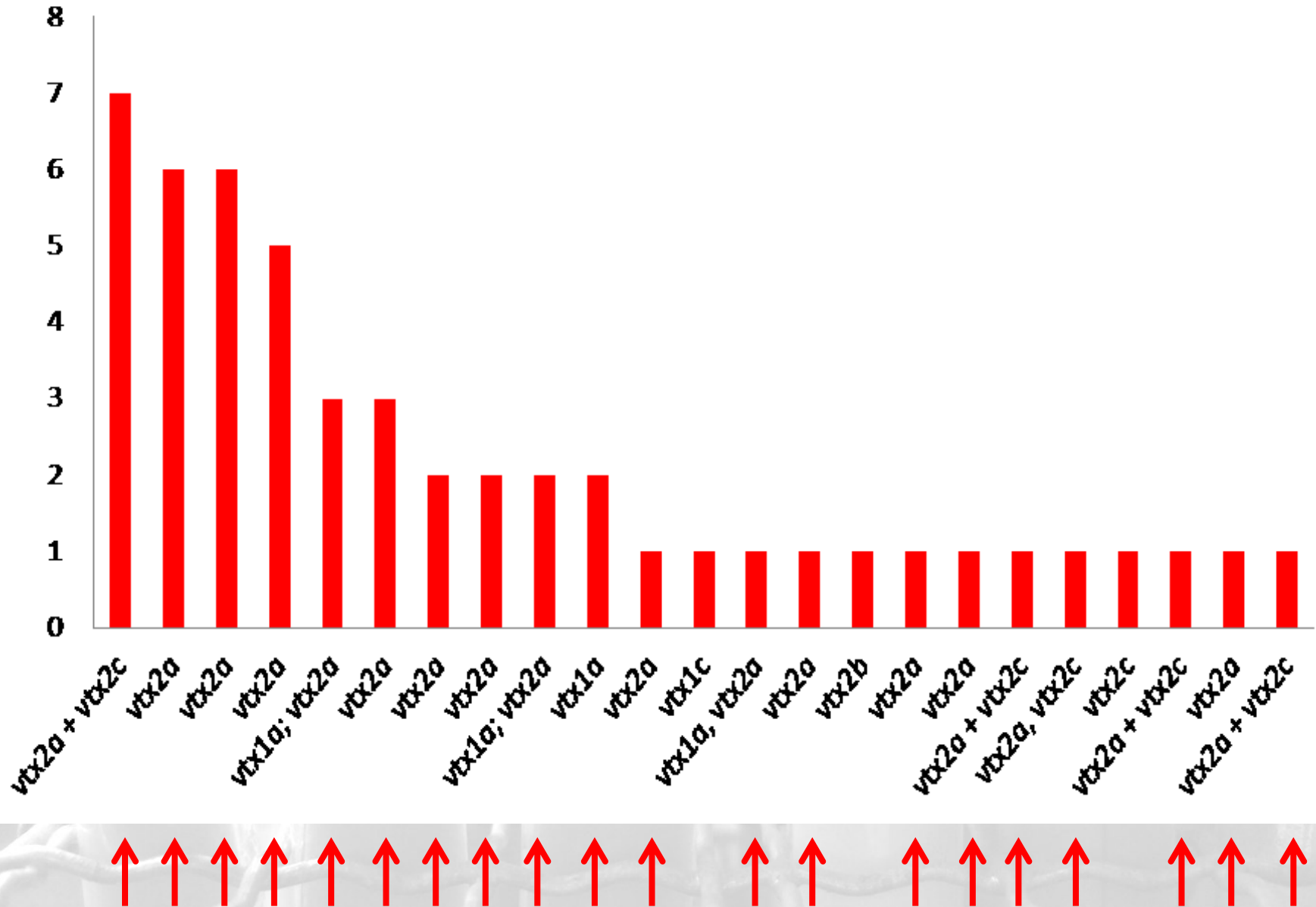


# HUS & vtx subtypes





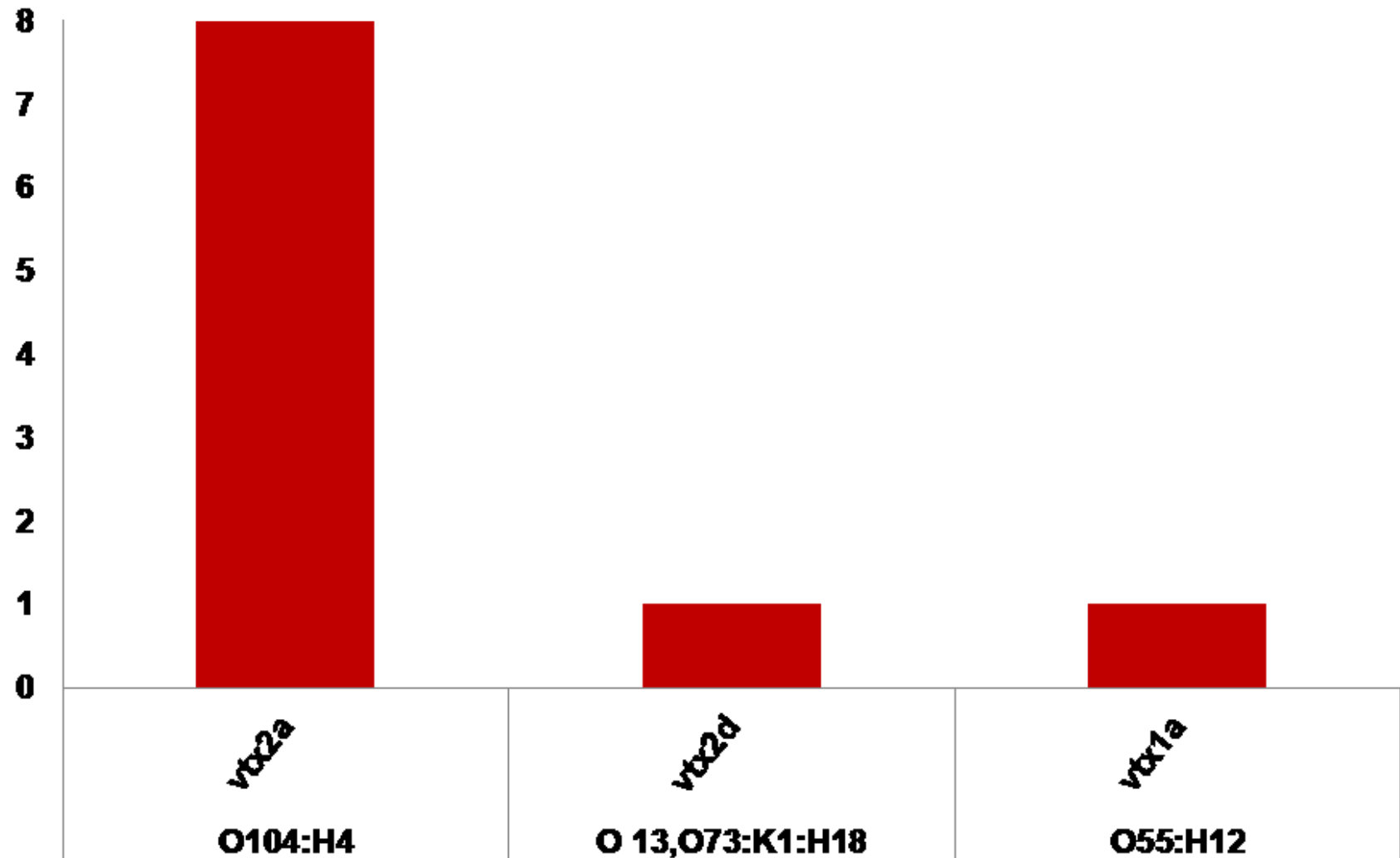
# HUS & *vtx* subtypes; all *eae* positives







# HUS; Sero- & vtx subtypes; all eae negatives



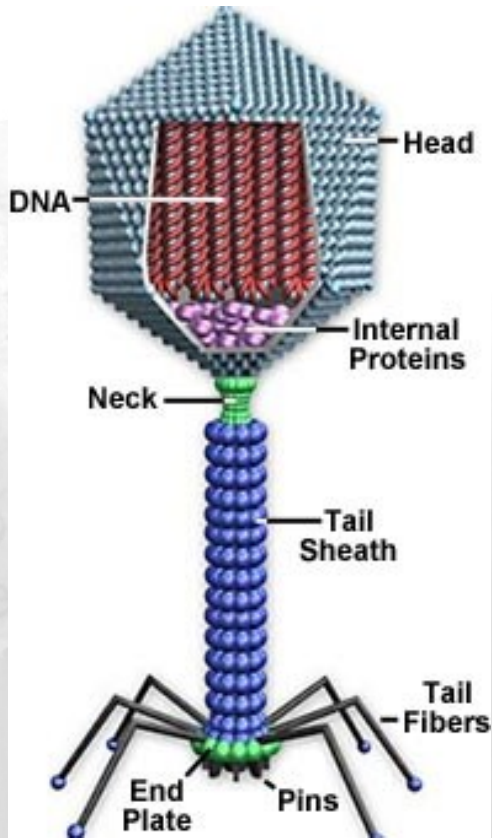


**HUS associated VTEC  
= HUSEC**

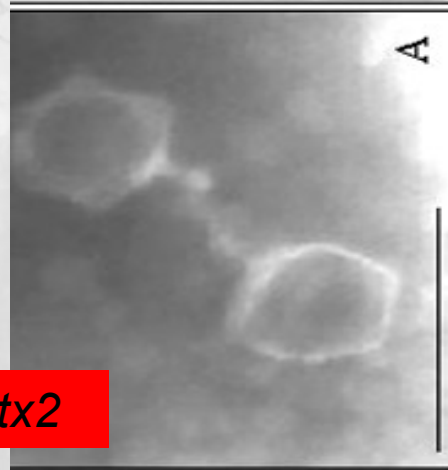
***vtx2a***

**and to a lesser degree  
*vtx2d* in eae negative**

# *vtx1* and *vtx2* bacteriophages

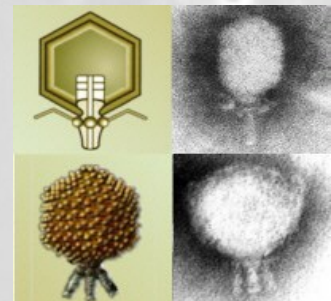
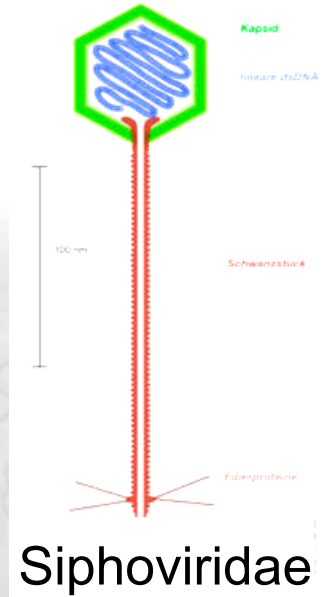


*vtx1*



*vtx2*

Schematischer Querschnitt des Lambda-Phagen



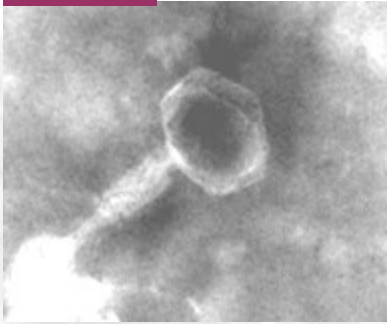
Podoviridae

Courtesy of Maite Muniesa



# Diversity: Morphology

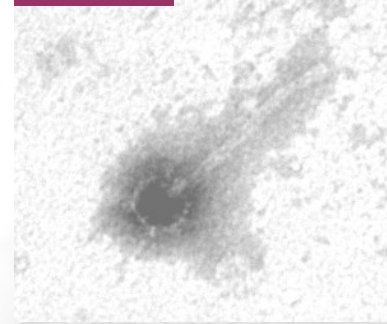
$\phi 1a$



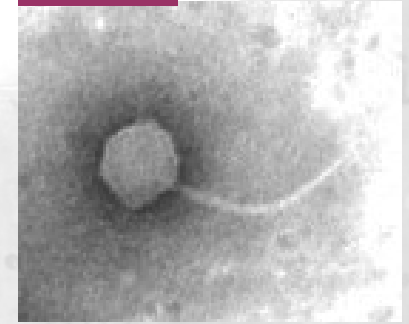
$\phi 4$



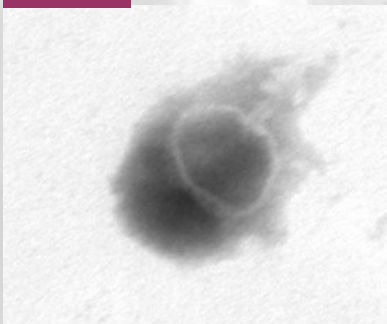
$\phi 13a$



$\phi 16a$



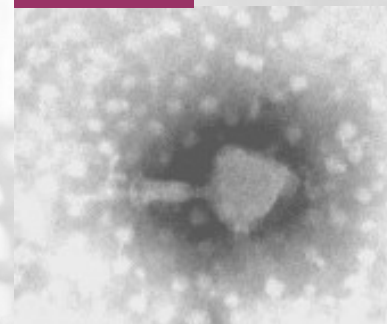
$\phi 1b$



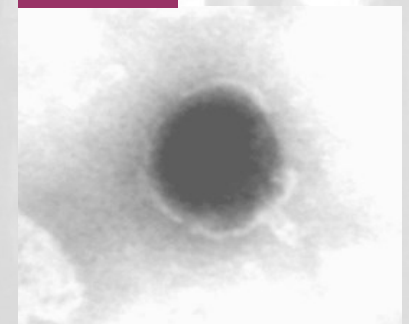
$\phi 12$



$\phi 13b$



$\phi 16b$





# Bacteriophages in healthy humans



62% of 100 fecal samples from 100 healthy individuals showing no enteric symptoms carried Stx phages, with an average value of  $2.6 \times 10^4$  Stx phages/g

Martinez-

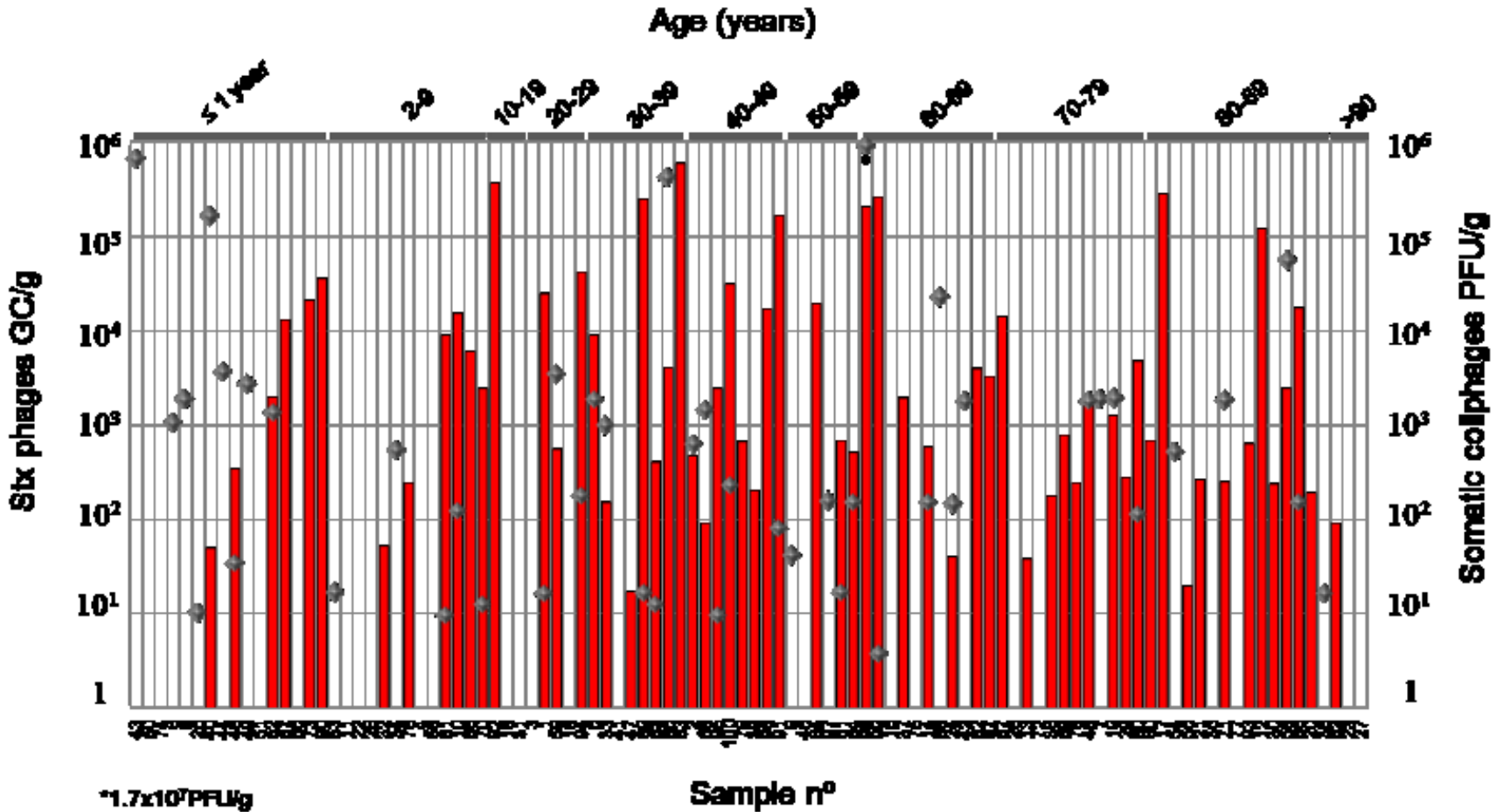


FIG 1 Quantification of Stx2 bacteriophages (GC/g of feces) (black bars) and infectious somatic coliphages (PFU/g) (gray diamonds) in 100 human fecal samples.

**Prevalence of VTEC in healthy humans  
including food handlers or workers in daycare  
centers during 2010-2012 in Japan**

**Healthy carriers**

**84.2  
/100,000**

**Diarrhoeal cases**

**2.1  
/100,000**



# Stx phages in food

## Food



Microbiological criteria EU regulation



$10^2$ - $10^5$  Stx phages / 25 g minced beef. 100% of the samples  
 $10^2$ - $10^3$  Stx phages / 25 g fresh salad. 69 % of the samples

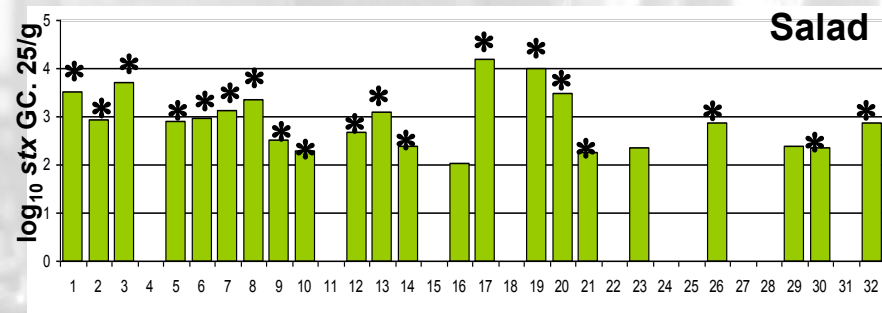
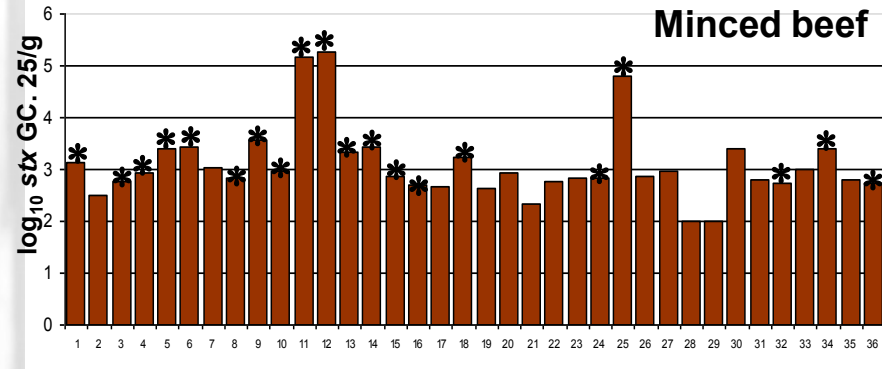
Bar: qPCR  
\*: + end point PCR



At least 50 % of the Stx phages in these samples were infectious

Able to propagate in host strains

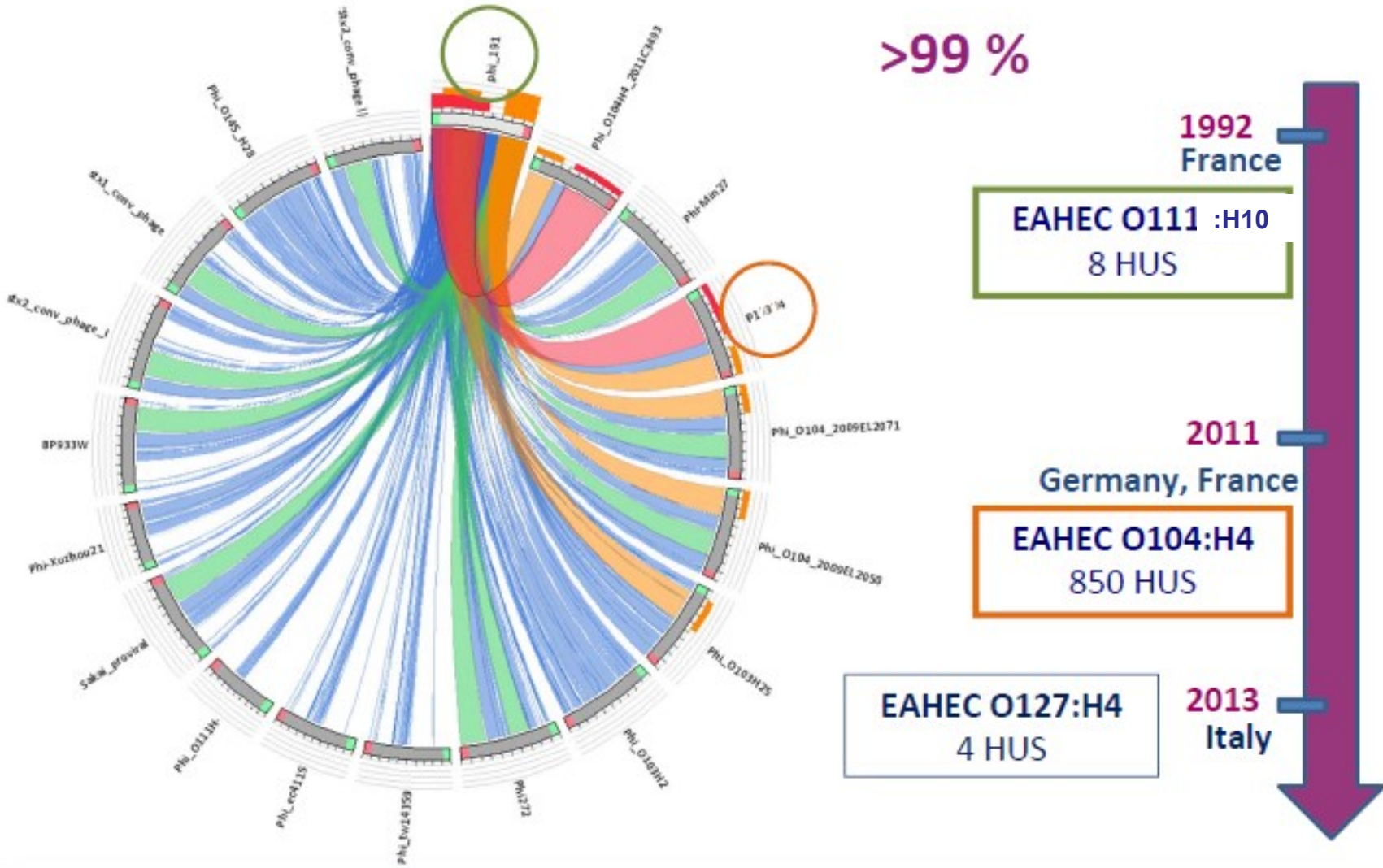
Barcelona area has a low incidence of VTEC infections



Imamovic & Muniesa, AEM. 2011 Sample n°

Courtesy of Maite Muniesa

# Whole genome comparison of Vtx-phages from EAHEC



20 years time-span: two events of Vtx-phage acquisition





**Next generation sequencing**

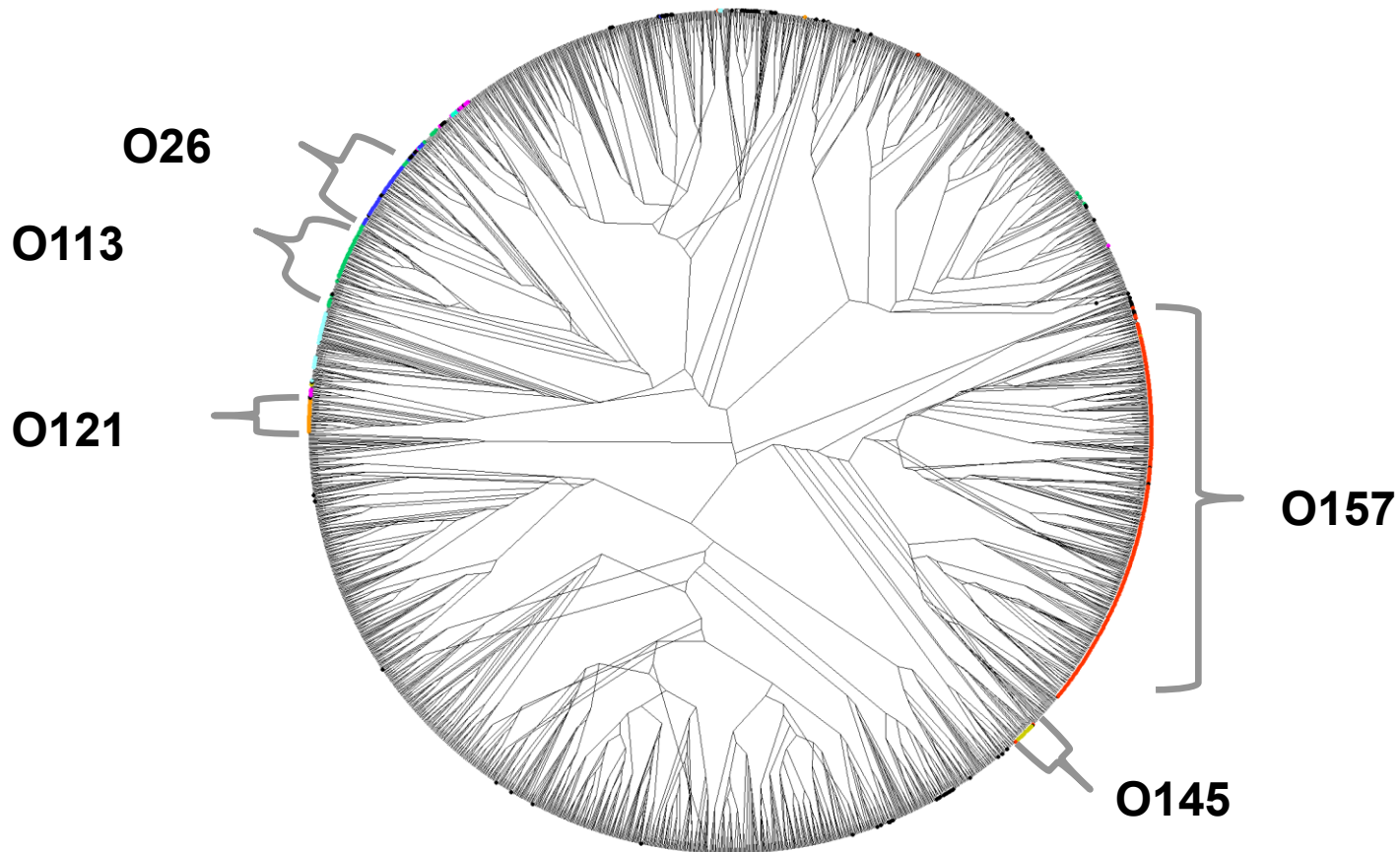
**NGS**

**or**

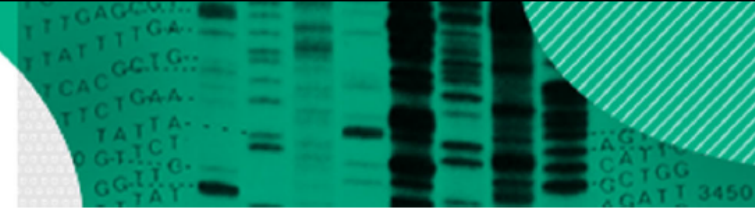
**Whole genome sequencing**

**WGS**

# 2324 *E. coli* genomes – Panseq SNP phylogeny (ML)



Courtesy of Chad Laing



## Applying phylogenomics to understand the emergence of Shiga-toxin-producing *Escherichia coli* O157:H7 strains causing severe human disease in the UK

Timothy J. Dallman,<sup>1</sup> Philip M. Ashton,<sup>1</sup> Lisa Byrne,<sup>1</sup> Neil T. Perry,<sup>1</sup> Liljana Petrovska,<sup>2</sup> Richard Ellis,<sup>2</sup> Lesley Allison,<sup>3</sup> Mary Hanson,<sup>3</sup> Anne Holmes,<sup>3</sup> George J. Gunn,<sup>4</sup> Margo E. Chase-Topping,<sup>5</sup> Mark E. J. Woolhouse,<sup>5</sup> Kathie A. Grant,<sup>1</sup> David L. Gally,<sup>6</sup> John Wain<sup>7</sup> and Claire Jenkins<sup>1</sup>

<sup>1</sup>Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK

<sup>2</sup>Animal Laboratories and Plant Health Agency, Woodham Lane, Surrey KT15 3NB, UK

<sup>3</sup>Scottish *E. coli* O157/VTEC Reference Laboratory, Department of Laboratory Medicine, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, UK

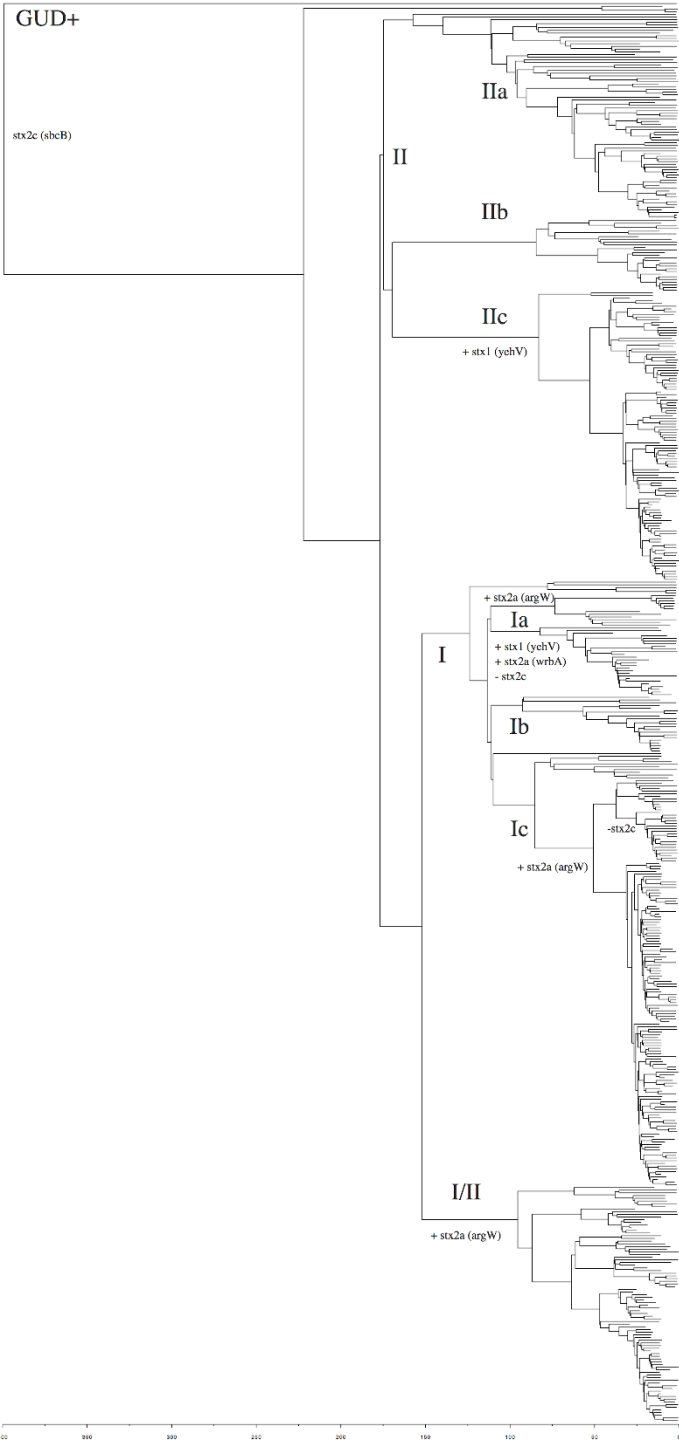
<sup>4</sup>Future Farming Systems, R&D Division, SRUC, Drummondhill, Stratherrick Rd., Inverness IV2 4JZ, Scotland, UK

<sup>5</sup>Centre for Immunity, Infection and Evolution, Kings Buildings, University of Edinburgh, Edinburgh EH9 3FL, UK

<sup>6</sup>Division of Infection and Immunity, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin EH25 9RG, UK

<sup>7</sup>University of East Anglia, Norwich NR4 7TJ, UK

**1,075 strains of STEC O157 from clinical and animal isolates from England, Northern Ireland, Wales and Scotland collected from 1985 to 2014**

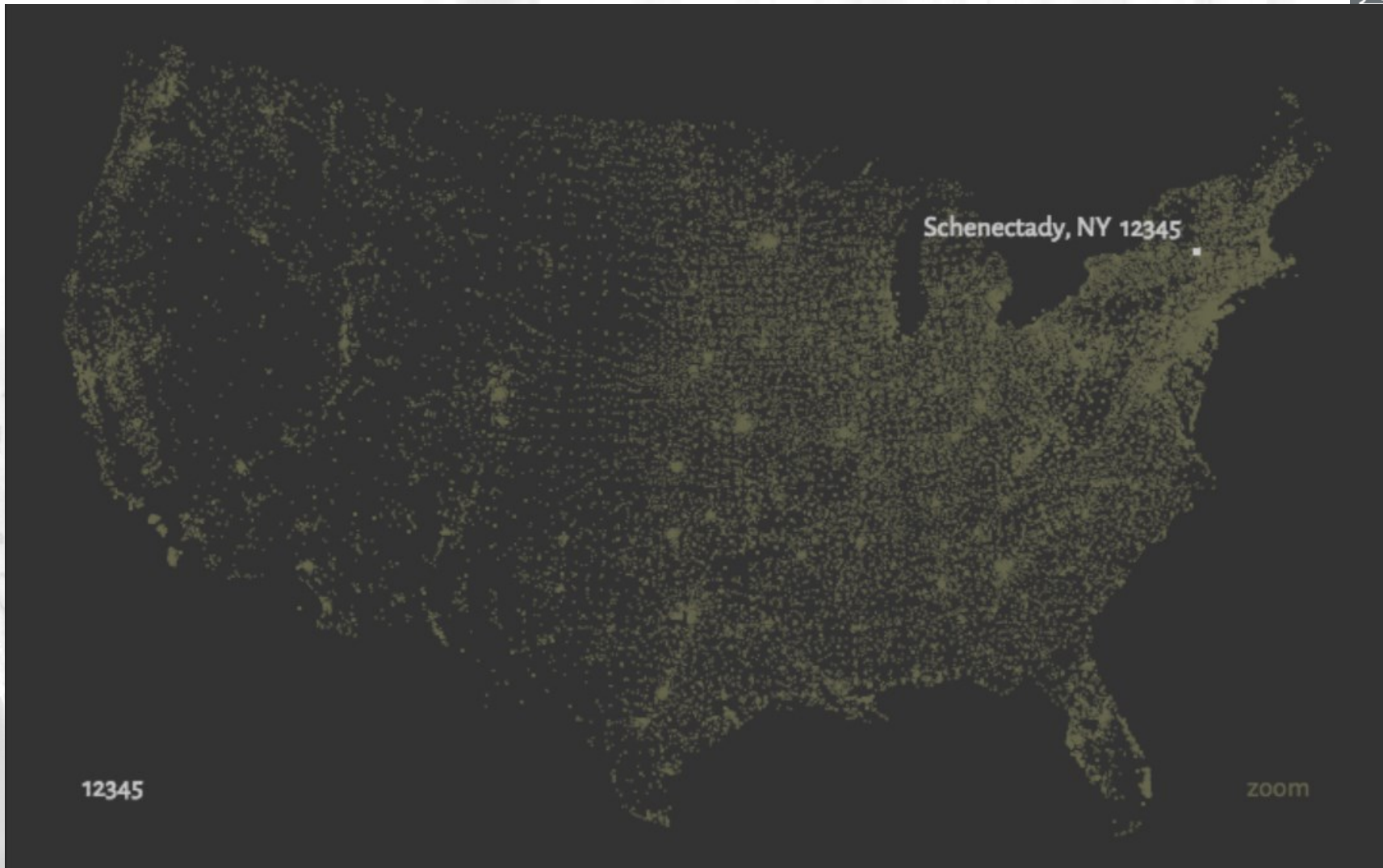


Maximum clade credibility  
tree of 530  $\Delta 25$  SNP  
representatives of O157:H7

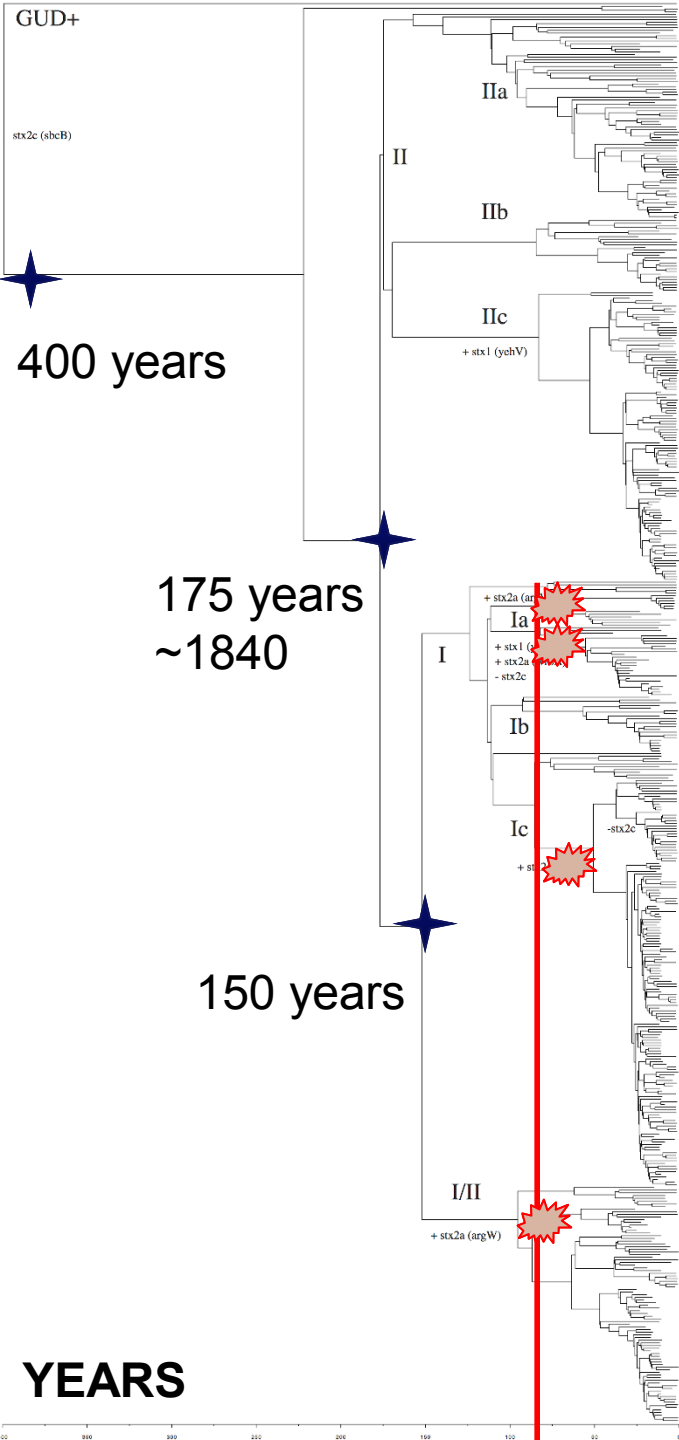
= Family Tree of O157:H7  
using  $\Delta 25$  SNP addresses

**SNP = Single Nucleotide  
Polymorphism**





<http://benfry.com/zipdecode/>



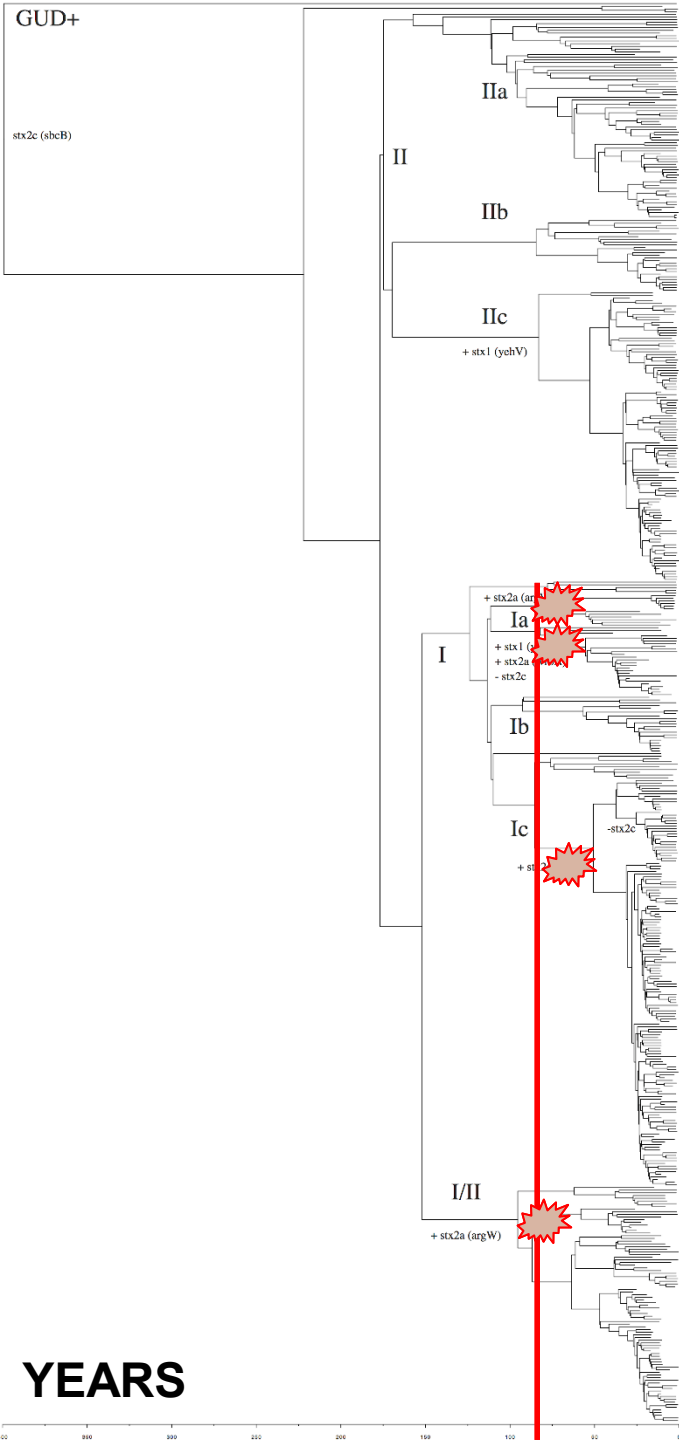
The ancestral O157:H7 state is stx2c (lineage II)

Early rapid diversification

Shiga toxin complement and insertion sites are relatively stable

Stx2a is relatively recent and has been acquired ~60 years ago on multiple occasions

Courtesy of Tim Dallman



Sublineage	Mild		Severe*		Total		HUS†	
	n	(%)	n	(%)	n	(%)	n	(%)
II a	42	56.8	32	43.2	74	100	1	1.4
II b	18	81.8	4	18.2	22	100	0	0.0
II c	31	23.7	100	76.3	131	100	1	0.8
I a	3	17.7	14	82.3	17	100	0	0.0
I b	7	77.8	2	22.2	9	100	0	0.0
Ic ( <i>stx2a</i> )	9	20.9	34	79.1	43	100	8	18.6
Ic ( <i>stx2a/2c</i> )	35	30.2	81	69.8	116	100	10	8.6
Ic ( <i>stx2c</i> )	1	25.0	3	75.0	4	100	0	0.0
I/II ( <i>stx2a</i> )	7	18.4	31	81.6	38	100	2	5.3
I/II ( <i>stx2a/2c</i> )	12	30.8	27	69.2	39	100	4	10.3
All strains	165	33.5	328	66.5	493	100	26	5.3

\*Includes cases with bloody diarrhoea or cases who were hospitalized.

† The lineage IIa strain isolated from a patient with HUS possessed *stx2a/2c*; the lineage IIc strain possessed *stx1a/2a/2c*.

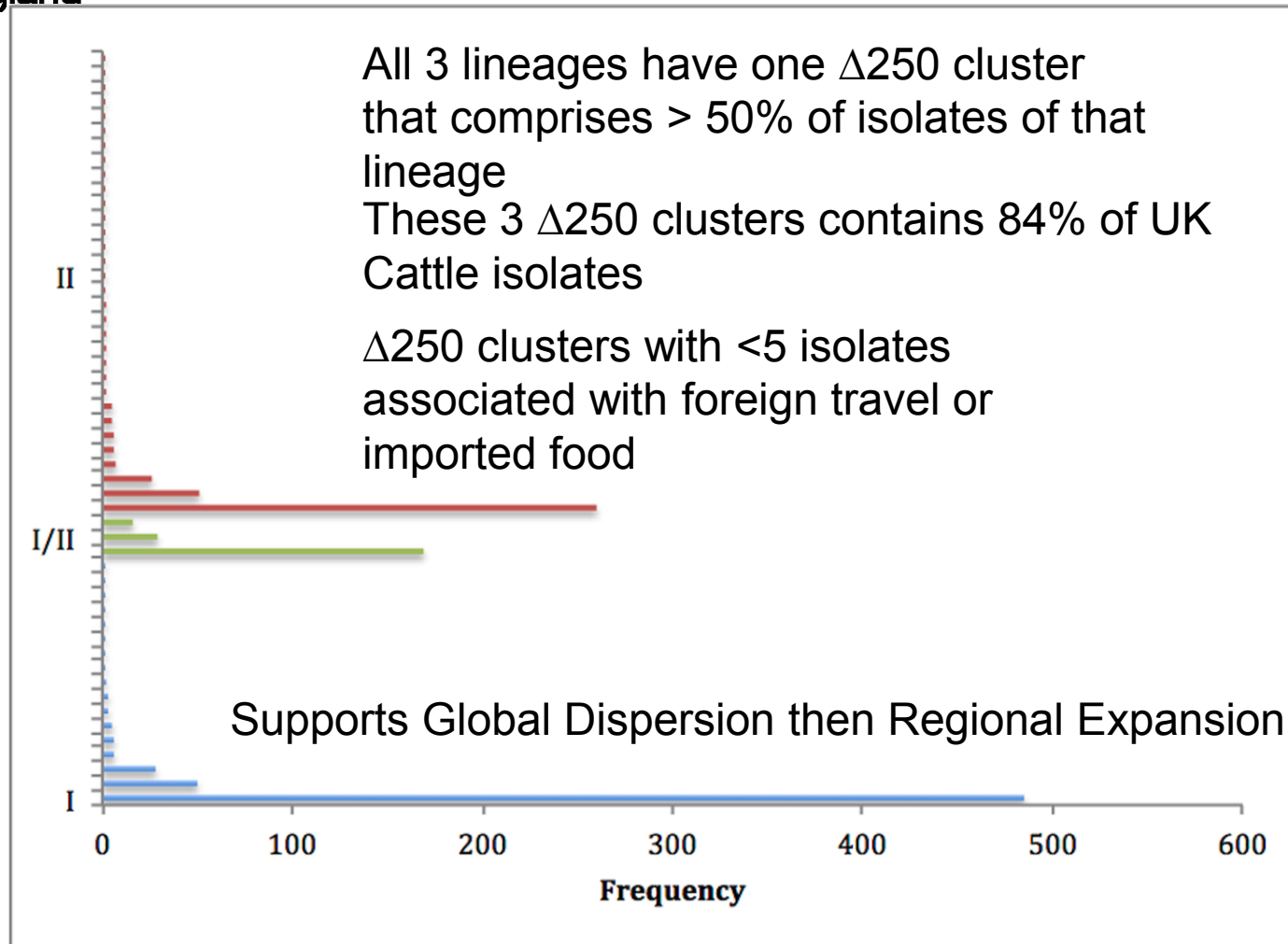
O157:H7 has become more pathogenic to humans over its evolution

Strong association with *stx2a* and severe disease

Courtesy of Tim Dallman



## Uneven Diversity of O157



54  $\Delta 250$  Clusters

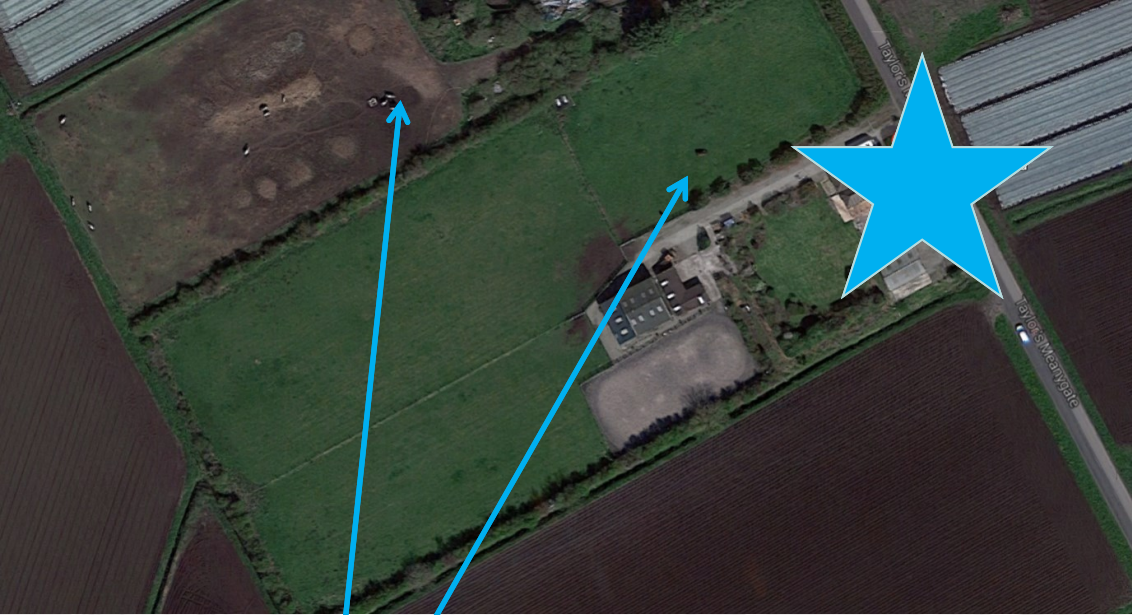


Food item	Proportion exposed (%)	95% Confidence Intervals
Prepacked salad	87.1	[74.6 - 99.6]
Pasteurised milk	80.6	[65.9 - 95.4]
Other salad	74.2	[57.9 - 90.5]
Raw fruit	74.2	[57.9 - 90.5]
Fish	67.7	[50.3 - 85.2]
Hard cheese	67.7	[50.3 - 85.2]
Raw vegetables	64.5	[46.7 - 82.4]
Cooked poultry	58.1	[39.7 - 76.5]
Yoghurt	54.8	[36.3 - 73.4]
Juice	51.6	[33 - 70.2]
Cooked beef	41.9	[23.5 - 60.3]
Cured products	41.9	[23.5 - 60.3]
Raw poultry	38.7	[20.5 - 56.9]
Other foods	38.7	[20.5 - 56.9]
Cooked pork	32.3	[14.8 - 49.7]
Herbs	29	[12.1 - 46]
Soft cheese	25.8	[9.5 - 42.1]
Processed food	22.6	[7 - 38.2]
Raw beef	19.4	[4.6 - 34.1]
Raw pork	16.1	[2.4 - 29.8]
Cooked lamb	16.1	[2.4 - 29.8]
Sandwiches	16.1	[2.4 - 29.8]
Cream	12.9	[0.4 - 25.4]
Raw lamb	9.7	[-1.3 - 20.7]
Cooked meats other	6.5	[-2.7 - 15.6]
Raw meats other	3.2	[-3.4 - 9.8]
Unpasteurised milk	3.2	[-3.4 - 9.8]
Fromage frais	0	[0 - 0]





**The implicated O157 strain with the SNP address 18.35.397.765 had a strong NW geographical signal including some possible environmental exposures**





# Once upon a time....



**Hamburgers =  
ground beef**

## **These days:**

### **Outbreaks:**

- **Watercress**

### **Risk factors:**

- **Swimming**
- **Contact with ruminants**
- **Contact with another person with diarrhoea**
- **Visiting farms**
- **Steaks and ground beef**
- **Travel to Africa (Turkey and Marocco)**

### **Host factors:**

- **Antacids**
- **Cardiovascular disease**
- **Gastrointestinal infection**
- **Hispanic ethnicity (in the US)**





# Microbiological risk assessment (MRA)

(i) hazard identification:

- ***vtx/stx* subtyping by PCR or WGS**

(ii) hazard characterization:

- **WGS of bacteriophages and their hosts;**
- **WGS assignment of host SNP addresses**

(iii) exposure assessment &

(iv) risk characterisation

- **WGS & EPIDEMIOLOGY**



# ACKNOWLEDGEMENTS

- **Tim Dallman, Public Health England**
- **Chad Laing; Public Health Agency of Canada**
- **Stefano Morabito; Istituto Superiore di Sanità, Rome**
- **Maite Muniesa; University of Barcelona**

# THANK you for your attention

