



Typing of *vtx* genes

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Confusion illustrated

vtx2d-O91/a-B2F1

vtx2vha

vtx2d1

stx2ha

SLT-IIvh

SLT-IIvha

VT2vha

VT2d1

VT2vh-a

VT2v-a





Confusion illustrated

vtx2f-O128-H.I.8.

vtx2ev

s/t-IIvhc

s/t-IIId

VT2ev

VT2vp2

VTev

SLTIIvhc

SLT-IIId/VT2d

SLTIIva





Main groups

Stx1/VT1 and VT2

Subtypes

Suffixed with small Arabic letters

Stx1: Shiga toxin from *Shigella stx1a*

VT1: *E. coli* Verocytotoxins *vtx1b* to *vtx1d*

VT2: *vtx2a* to *vtx2g*

Variant designations

Subtype - first published O group - strain No.

Phylogenetic sequence based nomenclature for the combined A- and B-subunits

SP	A ₁	A ₂	SP	B
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Stx

slt-1 or VT1

slt-2 or VT2

slt-llv or e

stx2c or VT2v

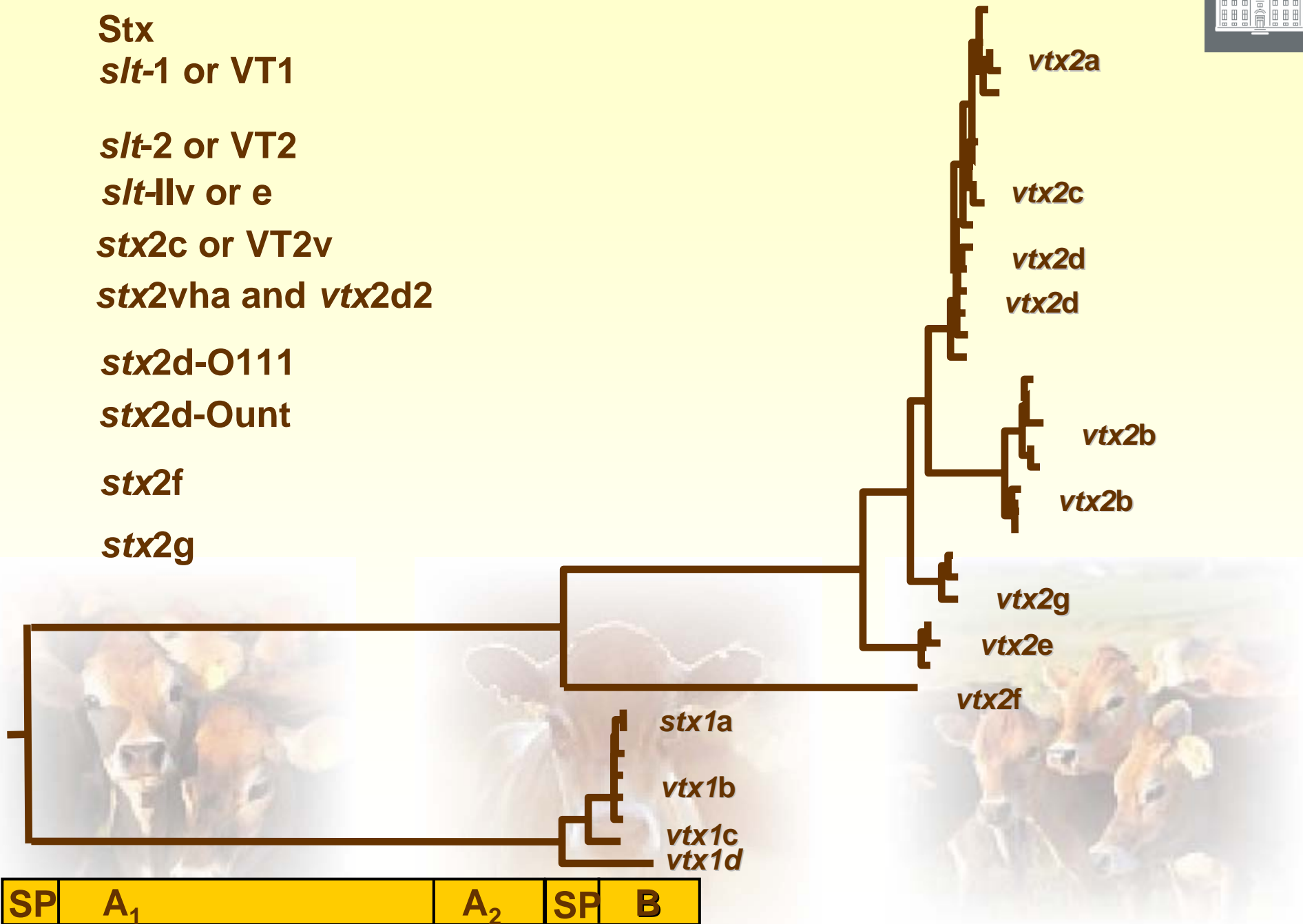
stx2vha and *vtx2d2*

stx2d-O111

stx2d-Ount

stx2f

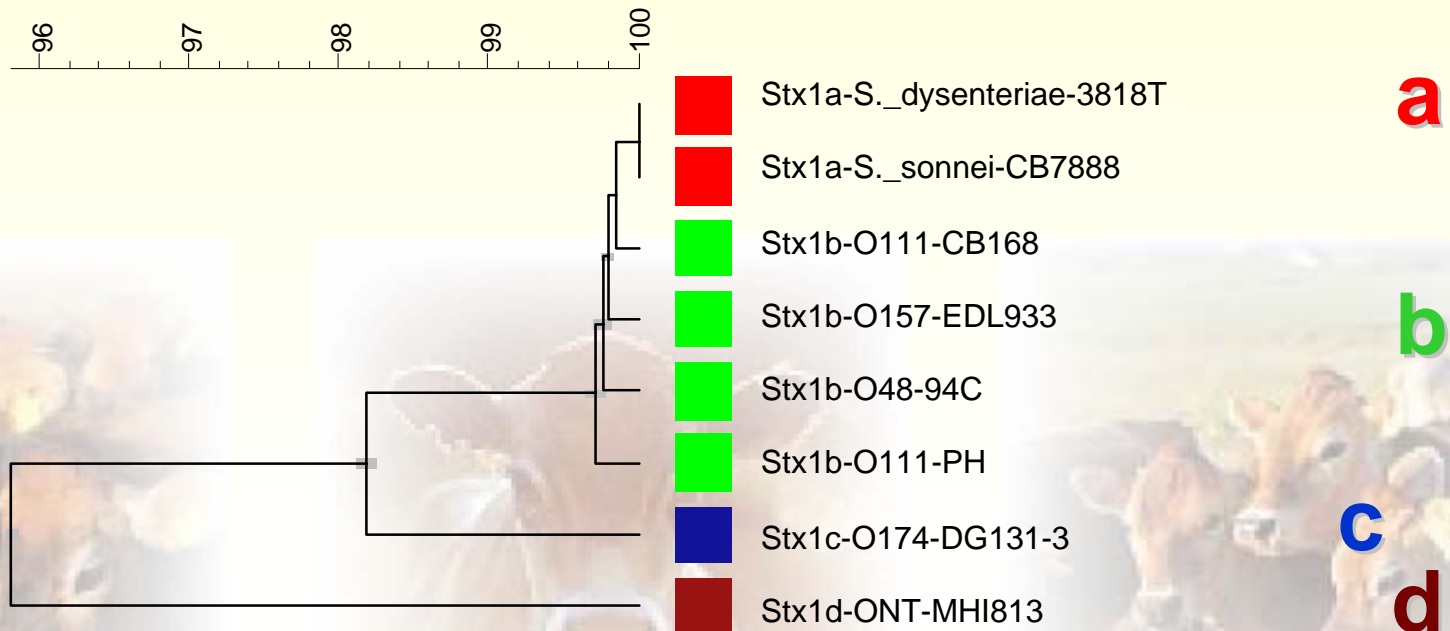
stx2g





Stx1 : 4 subtypes a - d 7-8 variants

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0%
VT1 translated sequences

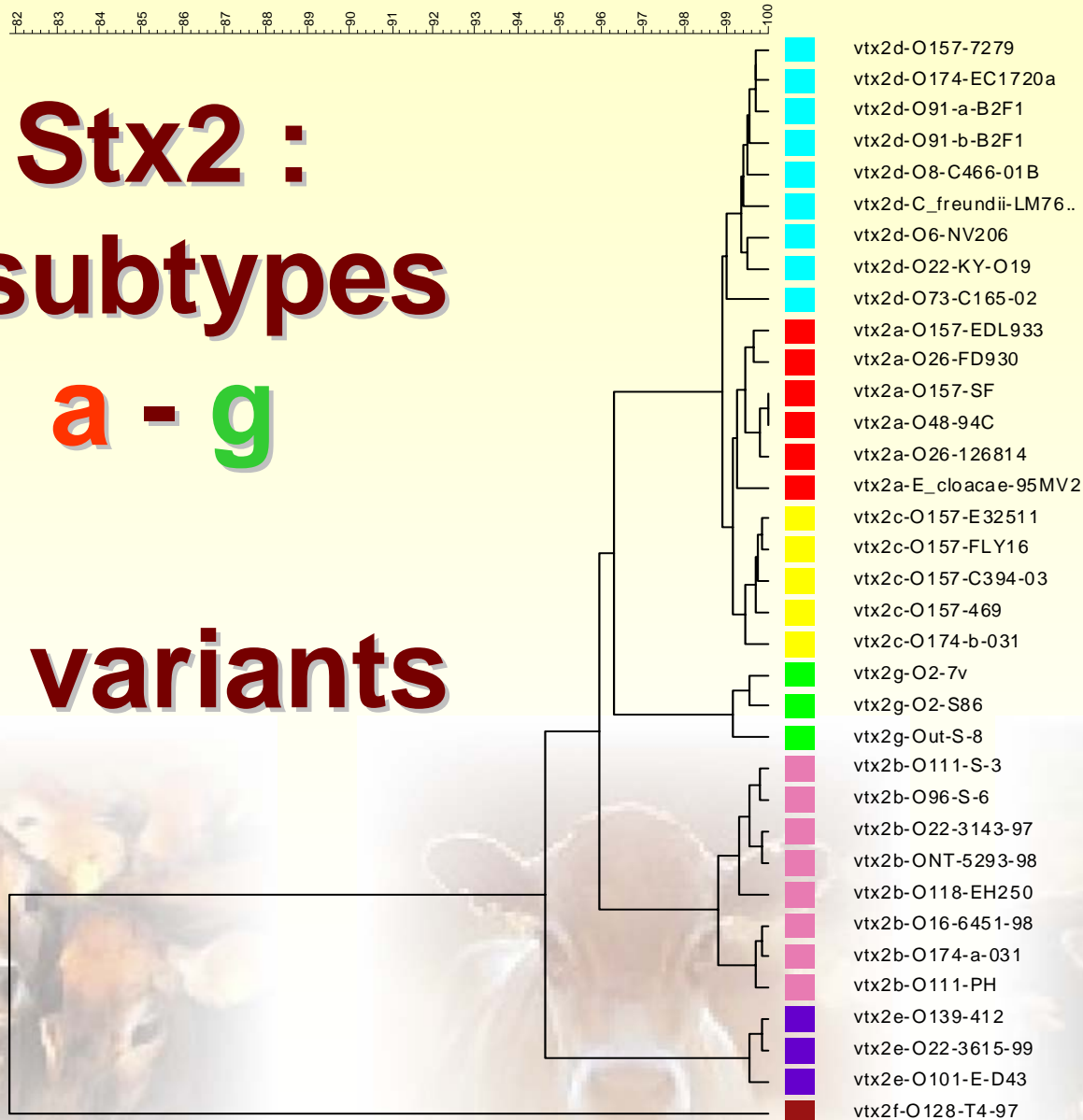




Stx2 : 7 subtypes

a - g

36 variants



2006 Enter-net ringtrial results

Serotype	Subtype	32 laboratories
O38:H26	Stx1c	2 negative
O128ab:H2	Stx1c	1 negative
O154:H31	Stx1d	9 negative
O103:H25	Stx2a	5 negative
O38:H26	Stx2b	5 negative
O128ab:H2	Stx2b (twice)	3 negative
O157:H7	Stx2a+c	4 negative
O145:H4	Stx2a	4 negative
O113:H4	Stx2d	7 negative
O51:H49	Stx2e	7 negative

2007 Enter-net ringtrial results

Serotype Subtype 35 laboratories

O145:H34 Stx2f 23 negative

= 66% !





Prevalence of *vtx2f*

Netherlands, 2006 Nationwide survey

1.4% of 211 stools positive for *vtx2f*

O63:H6 eae +

Denmark

2004	2	O128ab:H- & O119:H-
2005	2	O145:H- & O?:HR
2006	4	O145:H34 (3) & O128ab:H-
2007	2	O145:H34 & O 26:H34



Typing by RFLP

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Paris 1998

Res. Microbiol.
1998, 149, 457-472

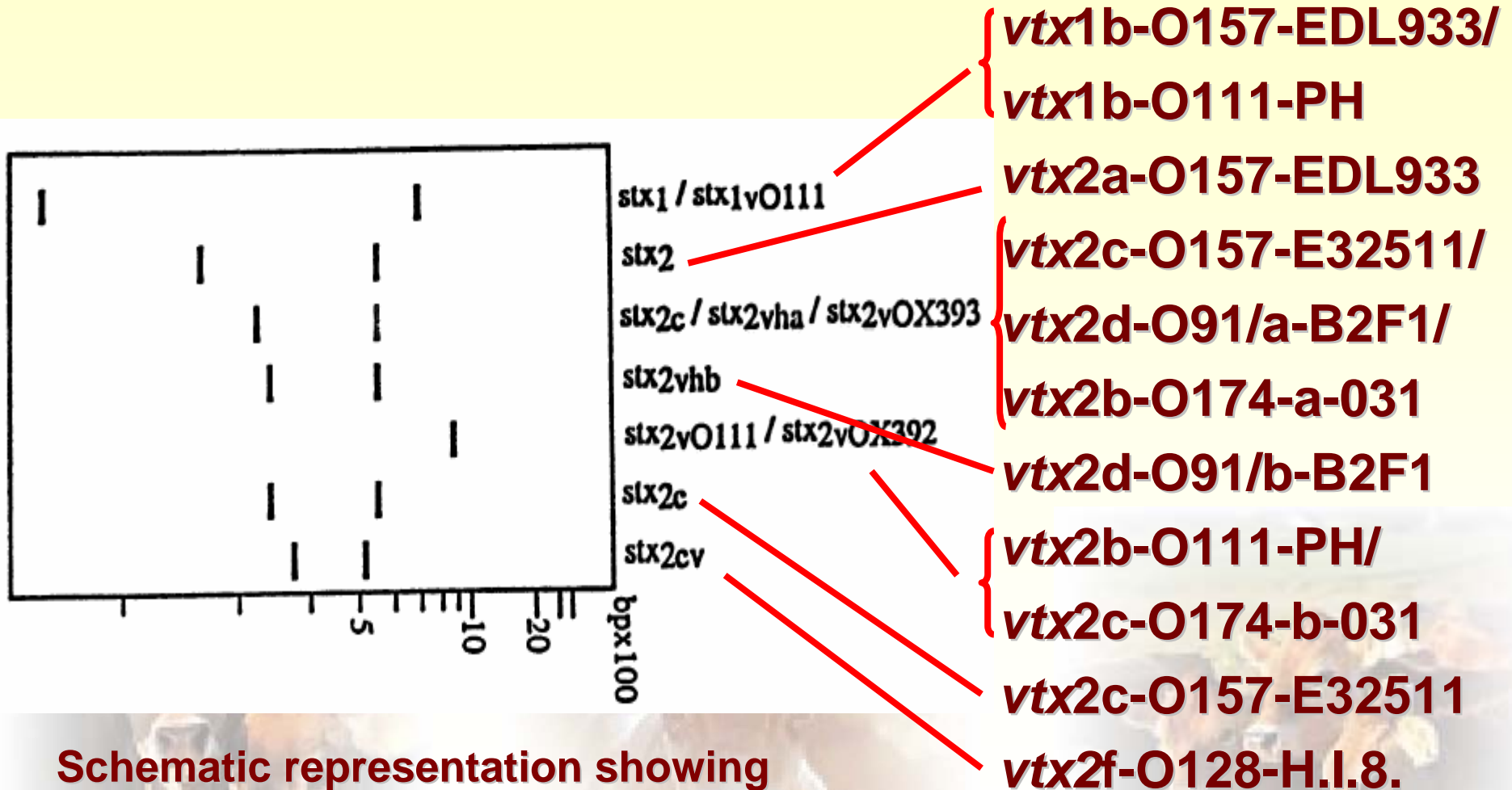
Comparison of 14 PCR systems for the detection and subtyping of *stx* genes in Shiga-toxin-producing *Escherichia coli*

S.N. Bastian^(1, 2), I. Carle⁽¹⁾ and F. Grimont^{(1) (*)}





Typing by PCR-RFLP



Schematic representation showing the patterns of Lin-all PCR products restricted by *HincII*



Typing by PCR-RFLP

Method	Primers	Restriction enzyme
Bastian <i>et al.</i>	Lin	<i>HincII</i> & <i>AccI</i>
Tyler <i>et al.</i>	VT2-c/VT2-d	<i>HaeIII</i> , <i>RsaI</i> & <i>NciI</i>
Piérard <i>et al.</i>	VT2-e/VT2-f	<i>HaeIII</i> & <i>PvuII</i>

Test by Ziebell *et al.*:

20 % (49/249 strains) contradictory results



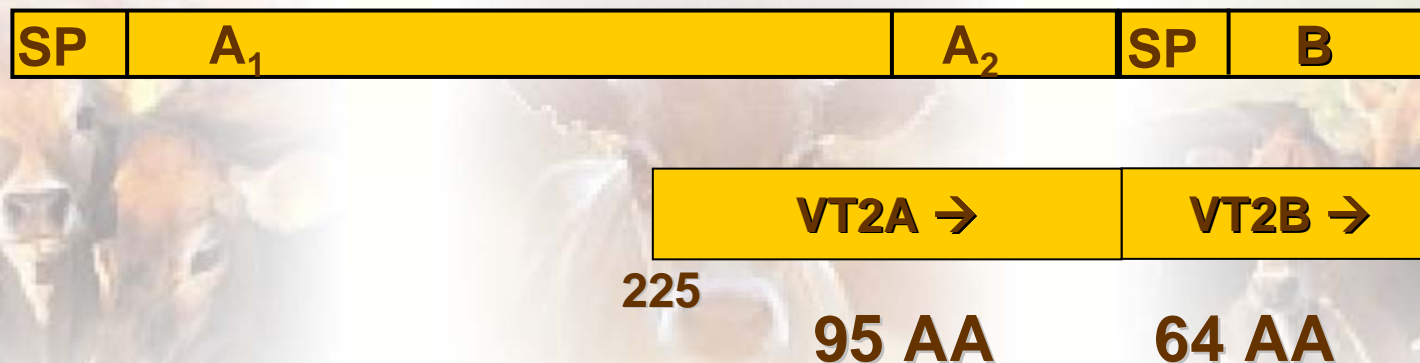
Subtyping Method for *Escherichia coli* Shiga Toxin (Verocytotoxin) 2 Variants and Correlations to Clinical Manifestations[∇]

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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).

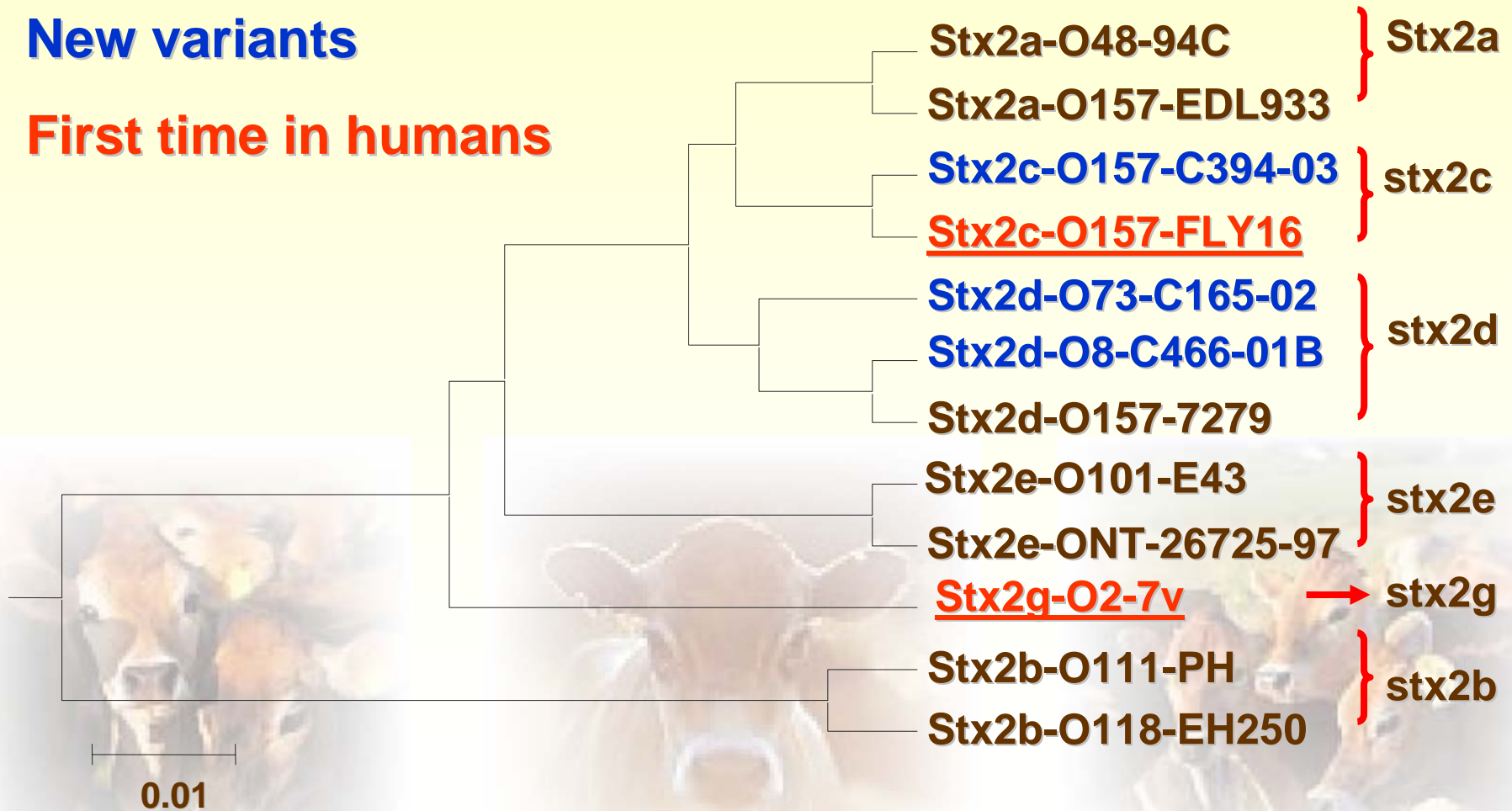




Only 12 Stx2 variants found in Danish patients

New variants

First time in humans





Attack rate of vtx2 variants associated with HUS

O157

vtx2a-O157-EDL933 +/- vtx2c-O157-FLY16	9/40	23%
vtx2c-O157-FLY16	1/18	6%
vtx2a-O157-SF + vtx2c-O157-FLY16	1/1	-

Non-O157

vtx2a-O48-94C +/- vtx1b	7/25	28%
vtx2a-O157-EDL933 +/- vtx1b	1/3	-
vtx2a-O157-EDL933 +/- vtx2c-O157-FLY16	1/2	-



stx genes detection and typing by PCR

DNA template preparation.

PCR for:

stx1

(*stx1com-F/stx1com-R*)

If +:*stx1* subtyping

***stx1c*:**

Lion-up/10X3

***stx1d*:**

VT1AvarF/VT1AvarR

Both PCRs negative:

stx1

stx2

(F4/R1/F4f/R1e-f)

If +:*stx2* subtyping

1

***stx2*, *stx2c*:**

GK3/GK4

2

***stx2*:** Stx2-a/Stx2-b

***Stx2c*:** Stx2c-a/Stx2c-b

3

***stx2d*:** VT2cm/VT2f

4

***stx2e*:**

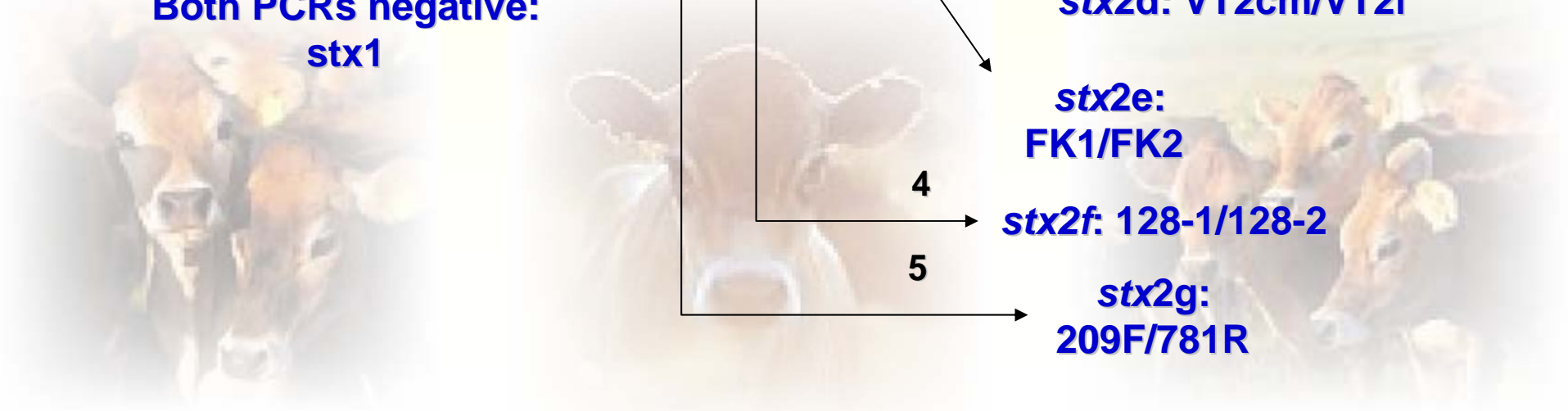
FK1/FK2

5

***stx2f*:** 128-1/128-2

***stx2g*:**

209F/781R

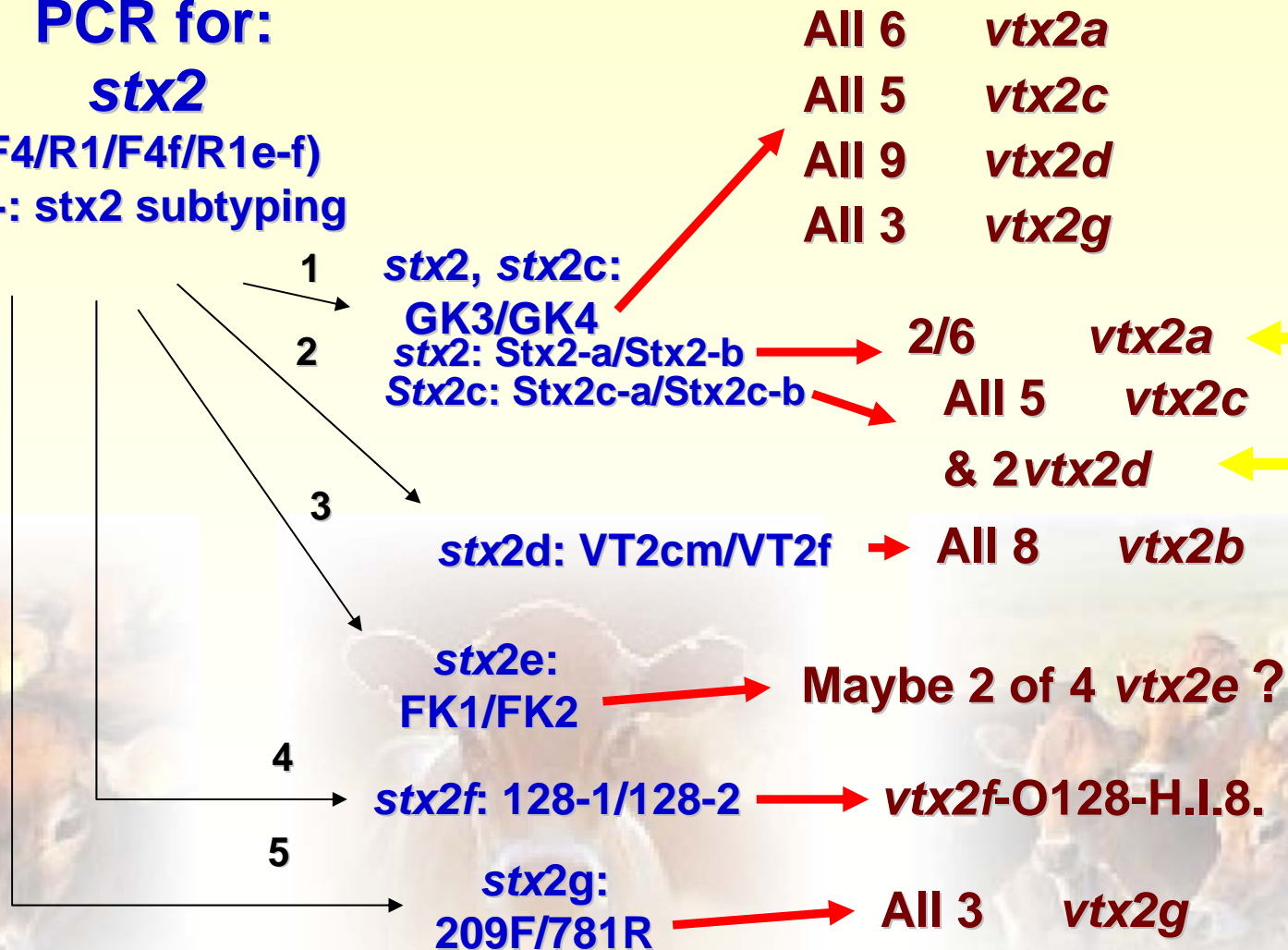




stx genes detection and typing by PCR

DNA template preparation.

PCR for:
stx2
(F4/R1/F4f/R1e-f)
If +: stx2 subtyping





"Semi" Draft Proposal

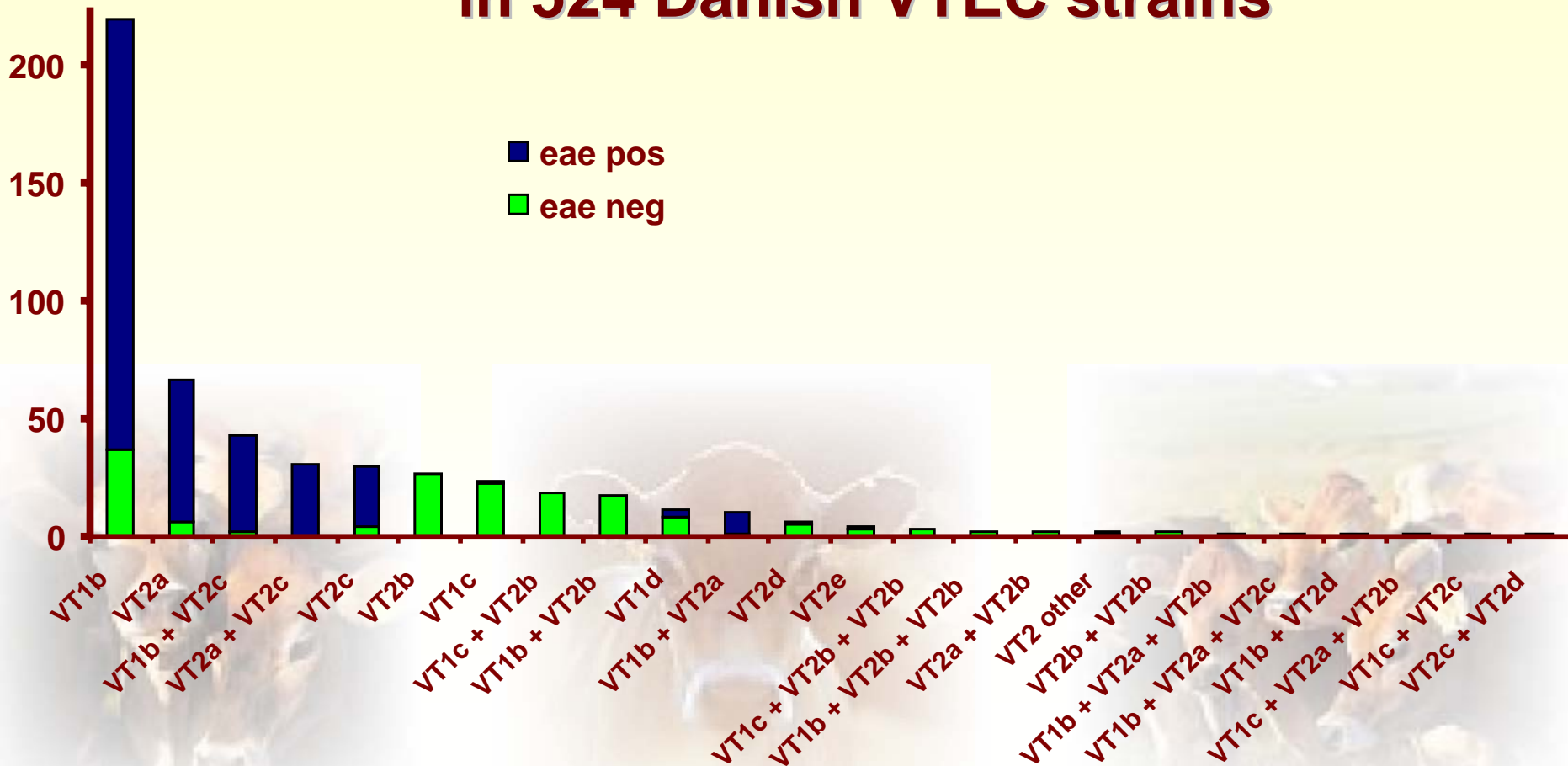
Primer pairs	Subtype	<i>vtx2a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
F4 / R1		+	+	+	+			+
F4f / R1e-f						+	+	
GK3/GK4		+		+	+			+
Stx2-a/Stx2-b		2/6!!						
Stx2c-a/Stx2c-b					+	2/9		
VT2cm/VT2f			+					
128-1/128-2							+	
209F /781R								+





What would be the use?

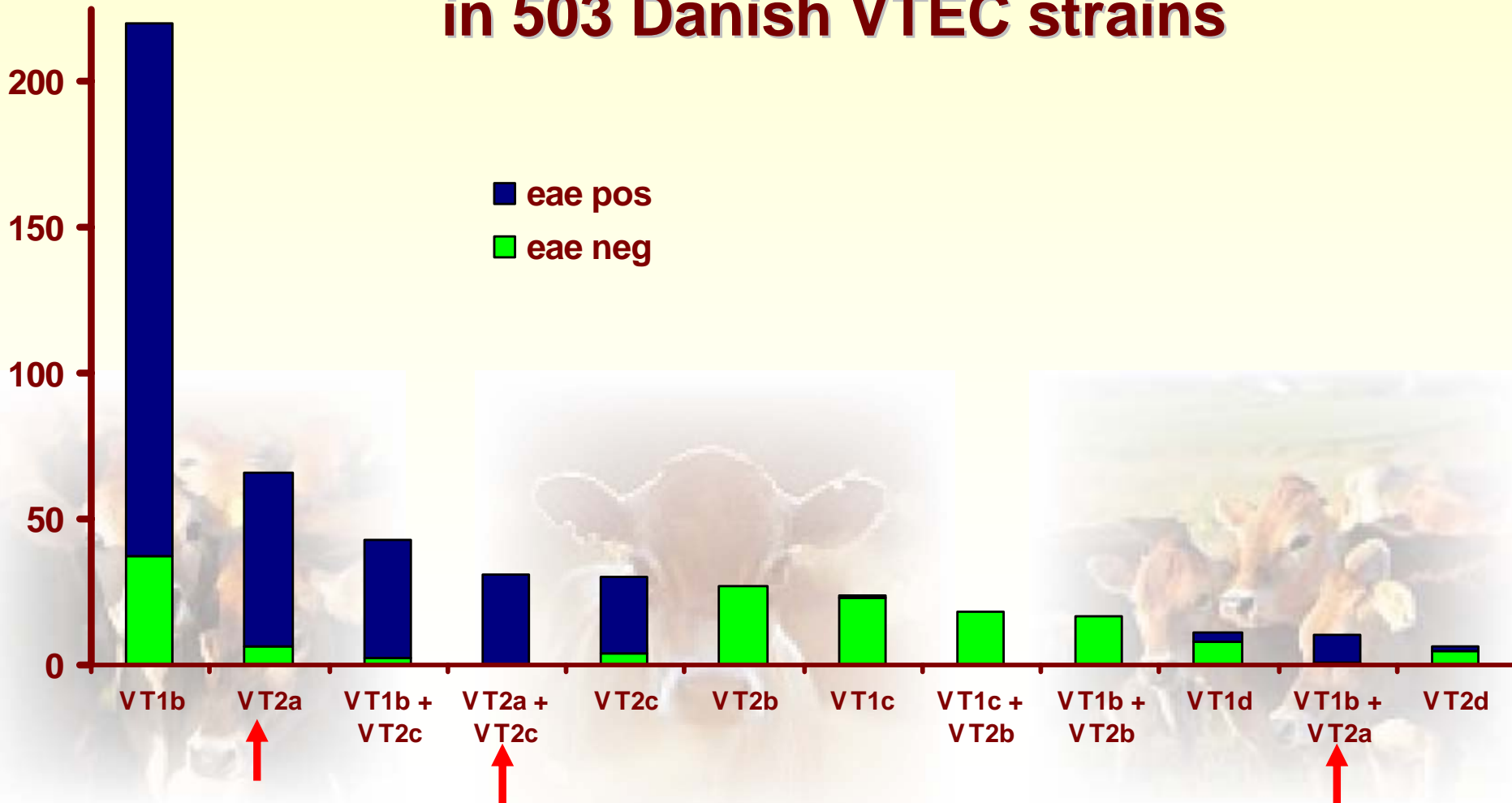
Association between eae and VT subtype in 524 Danish VTEC strains





What would be the use?

Association between *eae* and VT subtype in 503 Danish VTEC strains

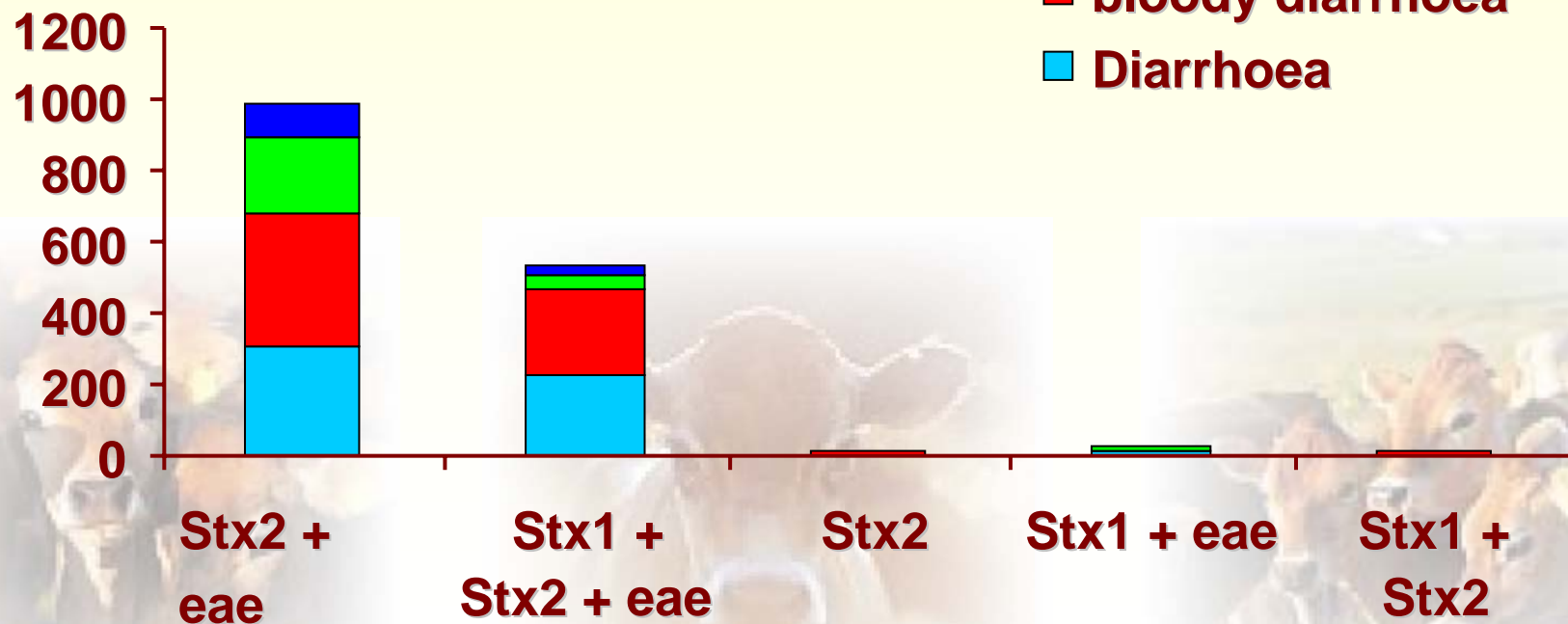


What would be the use?

ae and VT types in 1,596 VTEC O157 strains

O157

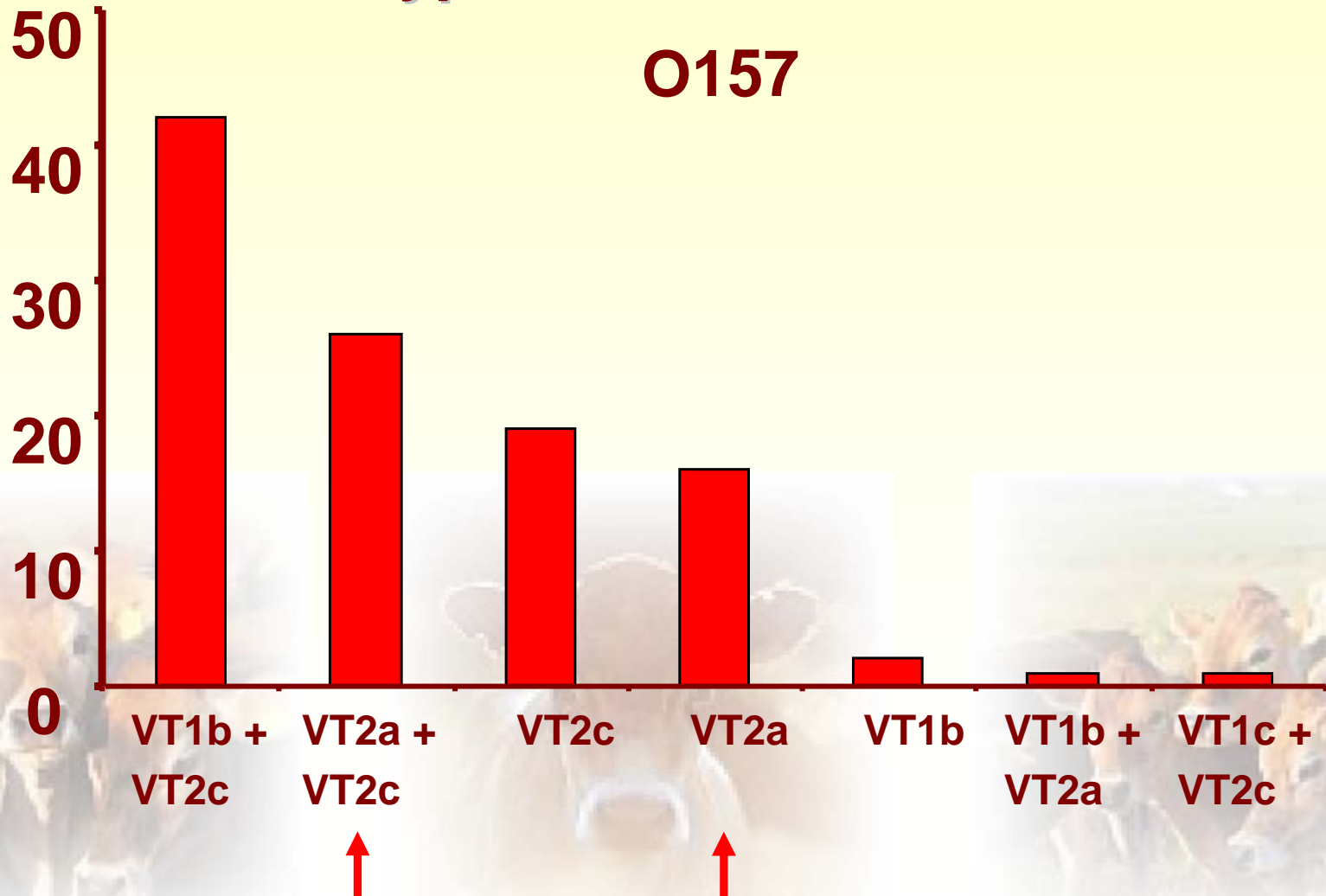
- asymptomatic
- HUS
- bloody diarrhoea
- Diarrhoea





What would be the use?

VT subtype in 107 Danish VTEC strains

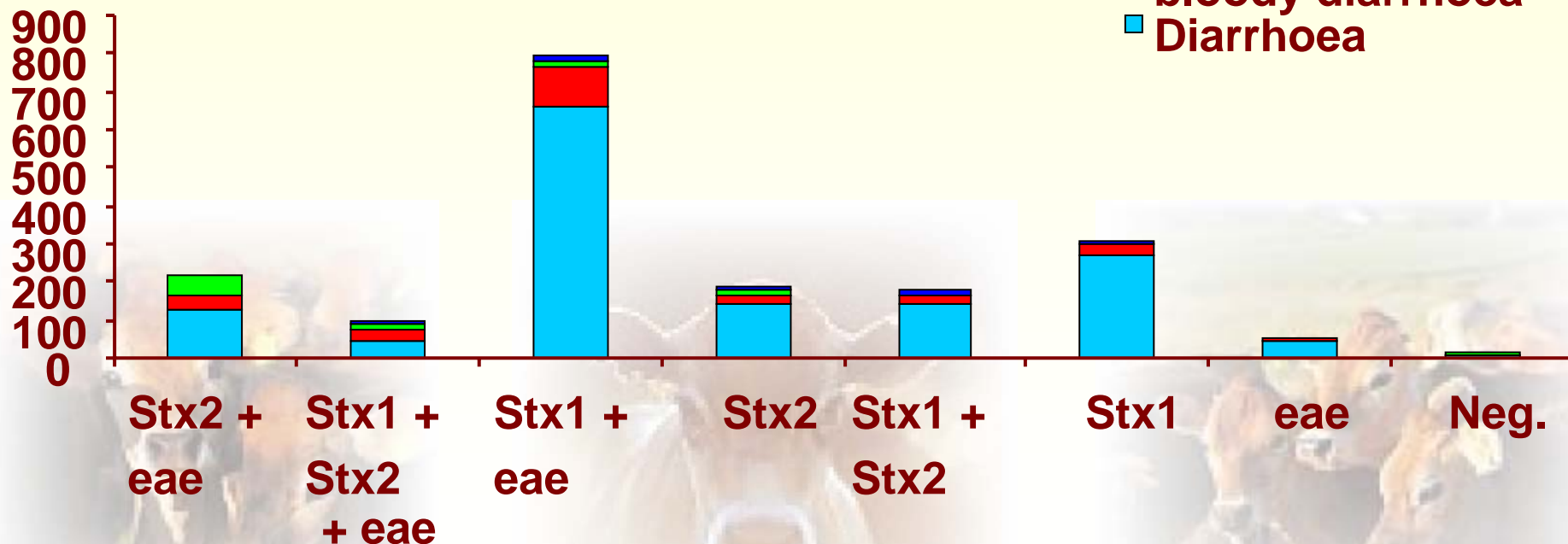


What would be the use?

eae and VT types in 1,859 VTEC non-O157 strains

Non-O157

- asymptomatic
- HUS
- bloody diarrhoea
- Diarrhoea





What would be the use?

VT subtype in 375 Danish non-O157 VTEC

