

Nomenclature of Verocytotoxins: a review, a proposal, and a a protocol for typing *vtx* genes

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WHY?

- **Detection and diagnostics**
- **Clinical manifestation**
- **Antibiotic treatment**
- **Patient handling and quarantine**
- **Epidemiology**
- **Risk assessment**
- **Surveillance**
- **External quality assurance (EQA) programmes**
- **Daily routine practice**

BENEFITS

- **Results will be comparable**
- **Pitfalls of misinterpretation can be described and avoided**
- **Wrong conclusions will be minimized**
- **Significant associations will be revealed**
- **Prediction of clinical outcome**
- **Public health actions can be adjusted**
- **Monitor trends and emergence of new types**
- **EQA programmes will be strengthened**

BACKGROUND

Already existing subtype designations should be respected as much as possible because:

- specific subtypes in have been shown to exhibit significant differences in biological activities
- specific subtypes have been shown to exhibit differences in the association to HUS (and HC)
- existing subtyping methodology will identify many of these subtypes
- of respect for the historical designations thereby avoiding the introduction of additional confusion to the nomenclature of these toxins

1) Types

Stx from Shigella

Stx1 = VT1 (synonymous)

Stx2 = VT2 (synonymous)

2) Subtypes

Stx, Stx1a, Stx1c & Stx1d

Stx2a to Stx2g

3) Variants

Amino acid differences within a subtype

A variant is defined by one AA difference in the analysed sequence compared to the other sequences.

Methods

Neighbour Joining cluster analysis

Evolutionary, unrooted trees from maximum parsimony cluster analysis using 100 bootstrap simulations on

- the combined Amino acid A- and B subunits = **Stx**



- the full nucleotide sequences including the spacer between the A- and B subunit genes = **stx**

The first published, valid sequence represents each specific subtype and variant.

Accession numbers analysed analysed

333 sequences

83 Stx1

250 Stx2



Stx1

Stx2

invalid

1

9

partial

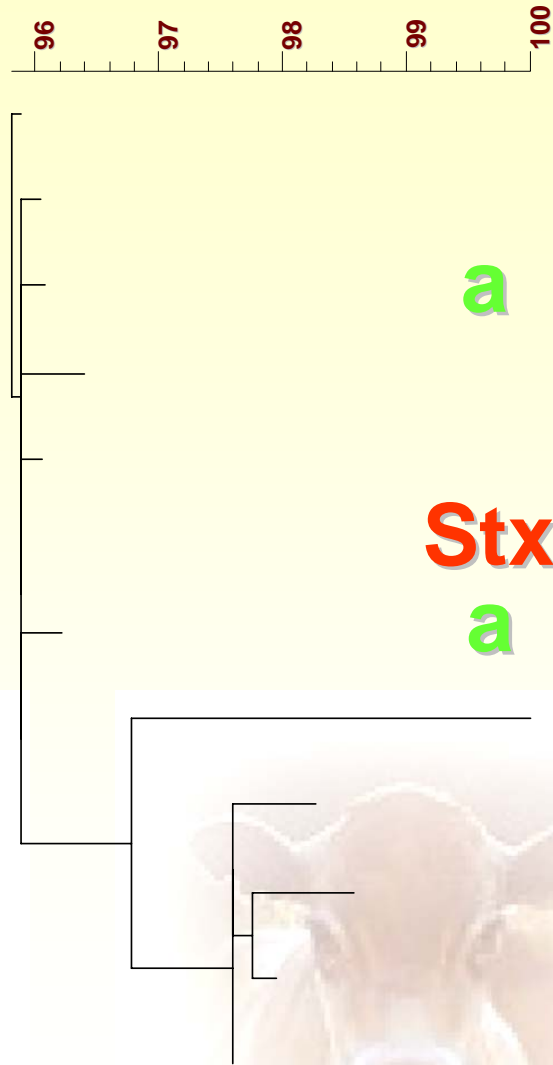
6

40

Stx & Stx1 : Three clusters

12 AA variants

Holotoxin vtx_TRANSLATED (minus spacer)



Key

- vtx1a-O157-EDL933
- vtx1a-O111-CB168
- vtx1a-O111-PH
- vtx1a-O111-04-06263
- vtx1a-O48-94C
- Stx-S_dysenteriae-3818T
- vtx1a-O157-AI2001-52
- vtx1d-ONT-MHI813
- vtx1c-Out-HI-N
- Stx1c-ONT-HI-A
- Stx1c-ONT-HI-C
- vtx1c-O174-DG131-3

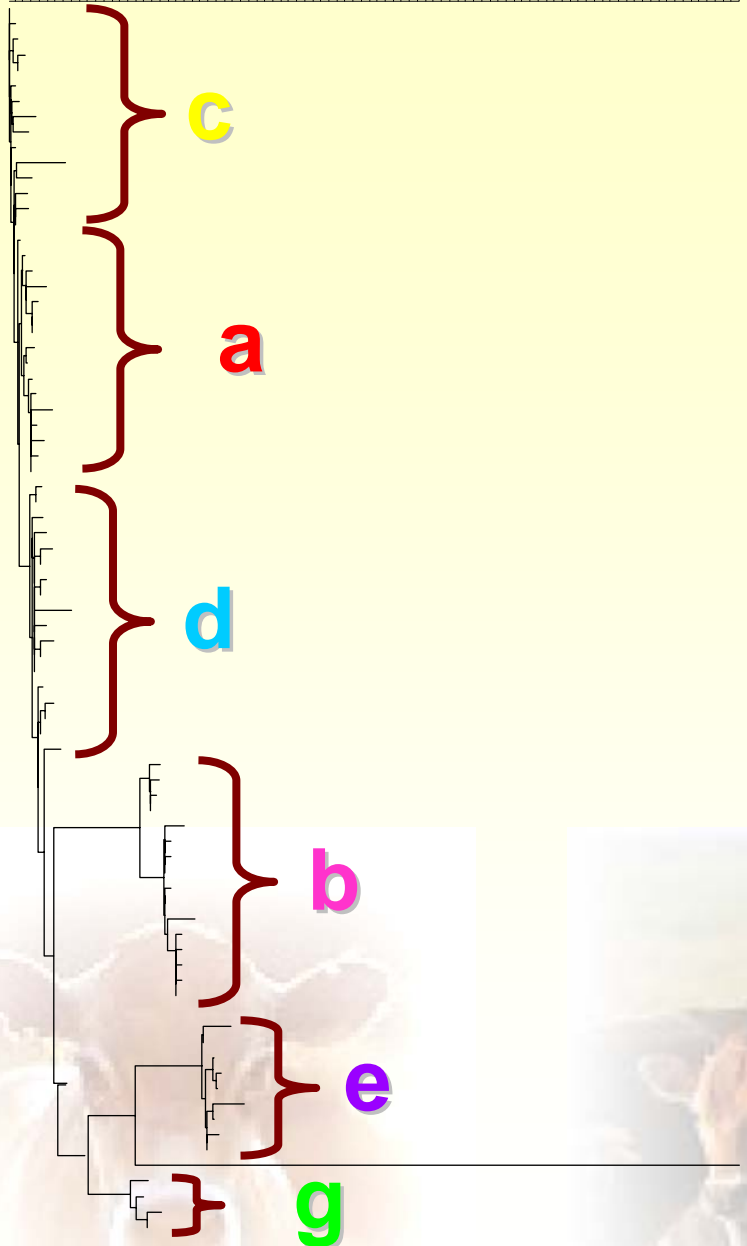


Stx2 : Seven clusters

80 AA variants

Fairyrise (QG:100% UG:0%) (FAST:2,10) Gapcost:0%

Stx2_TRANSLATED (minus spacer)



Stx2c-O157-E32511

Stx2a-O157-EDL933

Stx2d1-O91-B2F1
Stx2d2-O91-B2F1

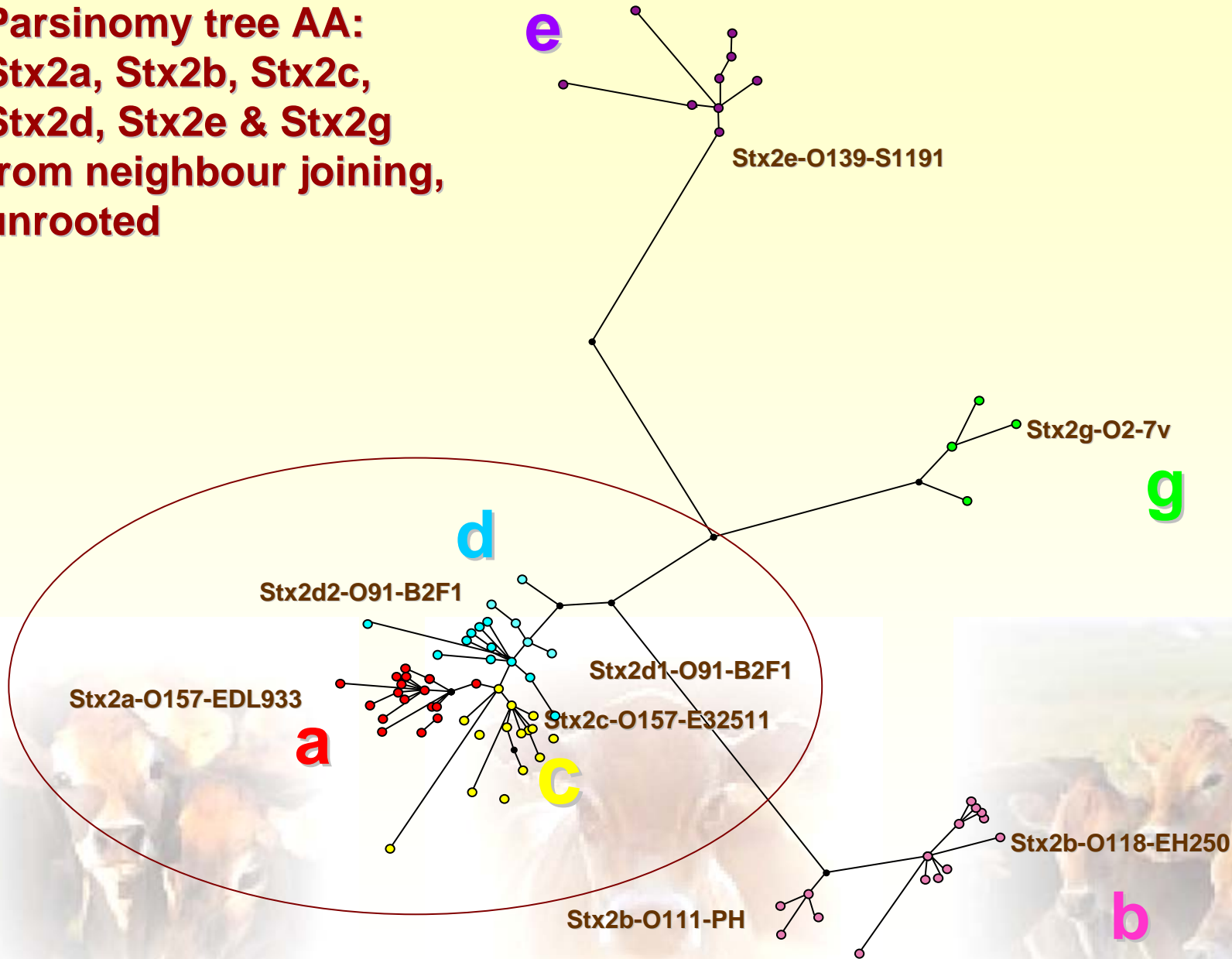
Stx2b-O111-PH
Stx2b-O118-EH250

Stx2e-O139-S1191

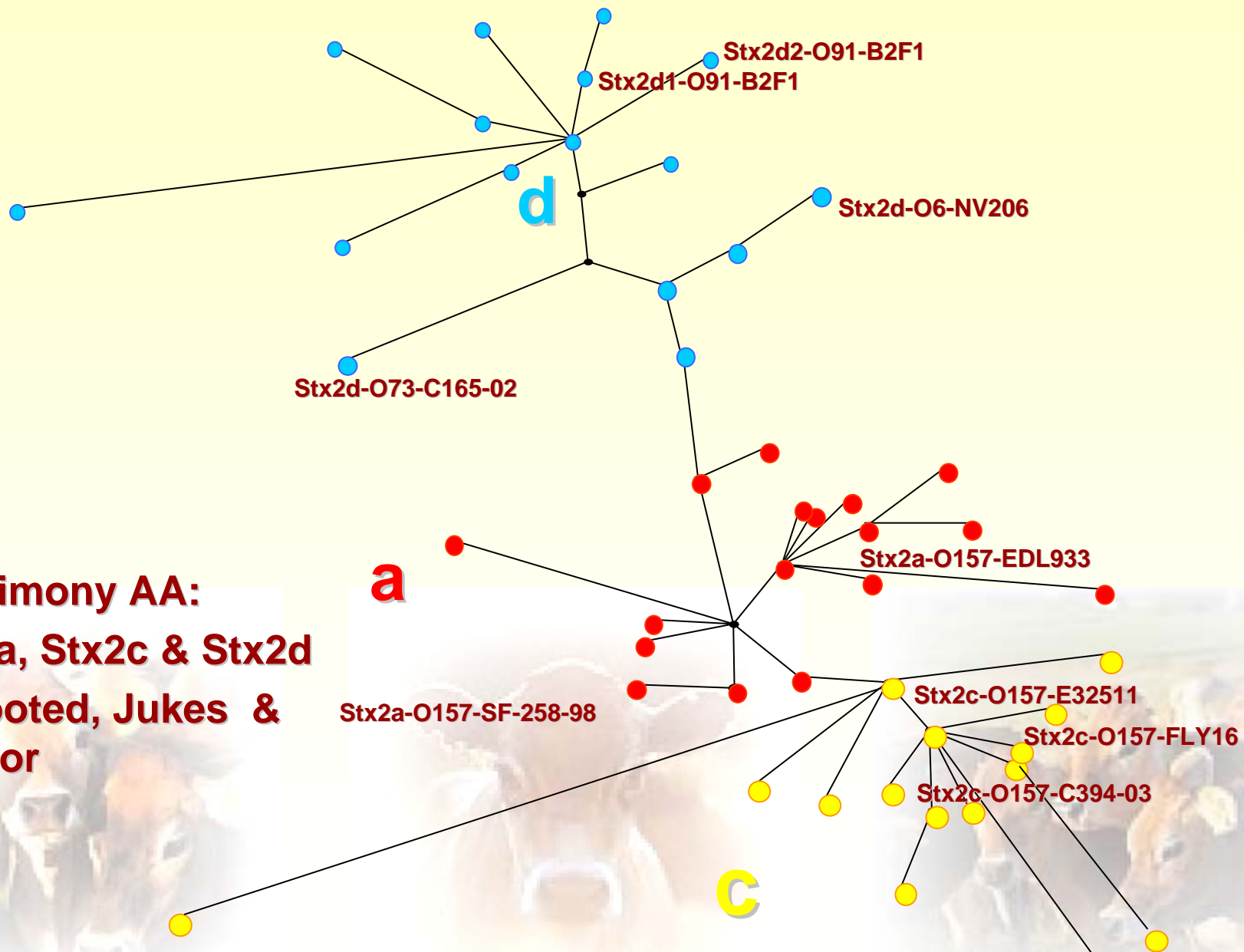
Stx2f-O128-H.I.8.
Stx2g-O2-7v



**Parsimony tree AA:
Stx2a, Stx2b, Stx2c,
Stx2d, Stx2e & Stx2g
from neighbour joining,
unrooted**



**Parsimony AA:
Stx2a, Stx2c & Stx2d
Unrooted, Jukes &
Cantor**



C-terminus of A₂ & B Subunits of Stx2

290	397			.10	- .30	-.50	-.62
K S Q F L Y T T G K	Stx2a	15 variants	}	SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q F L Y T M G K	Stx2a	1 variant		SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q F L Y T T G K	Stx2c	15 variants		SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
R A H S L N T S G E	Stx2b	16 variants [⊠]		SKYNE DD T-TNR-I <u>K</u> SNTCAS-AEVQFN(ND)			
K S Q S L Y T T G E	Stx2a	2 variants	}	SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q S L Y T T G E	Stx2d1-O91-B2F1 *			SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q S L Y T T G E	Stx2d2-O91-B2F1 *			SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q S L Y T T G E	Stx2d	14 variants ‡		SKYNE DD T-TSR-I <u>K</u> SSTCES-AEVQFNND			
K S Q S L Y T T G E	Stx2g	4 variants		SKYNE DD T-TNR-I <u>K</u> SNTCES-AEVQFNND			
K S Q S L Y T T G E	Stx2e	3 variants	}	SKYNE DD T-TNR-I <u>I</u> SNTCSS-AQVKFN			
K S Q P L Y T T G E	Stx2e	6 variants		SKYNE DD T-TNR-I <u>I</u> SNTCSS-AQVKFN			
K P Q D L T E P N Q	Stx2f	1 variant		SKYNE DD T-TNR-I <u>I</u> SNTCSS-AQVKFN			

Stx2d
Activatable tail

Stx2d
"Activatable" associated
B subunit??

- ⊠ At least Stx2b-O118-EH250 is not activatable
- * Variants have been shown to be activatable
- ‡ Includes Stx2_{c2} which has been shown to be activatable

(Louise Teel, pers. comm.)

(Angela Melton-Celsa, pers. comm.)

Jelacic *et al.* 2003

4th Annual CRLs October 2009

Activatable tail

.280 .290 Signal peptide
 012345678901234567.1234567890123456789

SNTAAAF LN RKSQSL YTTGE . MKKMFMAVLFALVSVNAMA

Stx2c, 15 variantsF.....K.....
 Stx2a, 1 variantF...M.K.....
 Stx2a, 15 variantsF.....K.....A.....
 Stx2a, 2 variantsS.....E.....
 Stx2d, 11 variantsS.....E.....
 Stx2d, 5 variantsS.....E.....
 Stx2b, 16 variantsRAHS.N.S.E...I.V.A...F.....
 Stx2g, 4 variantsS.....E.....
 Stx2f, 1 variant A..I..L...P.D.TEPNQ...II...G.F.A.S..
 Stx2e, 3 variantsS.....E.....I.....
 Stx2e, 6 variantsP.....E.....I.....

B-subunit

.0 .10 .20 .30 .40 .50 .60 .70
 012345678901234567890123456789012345678901234567890123456789012345678

ADCAK GKIEF SKYNENDTFTVKVAGKEYWTSRWNLQPLLQSAQLTGMTVTIKSSTCESGSGFAEVQFNNDXX

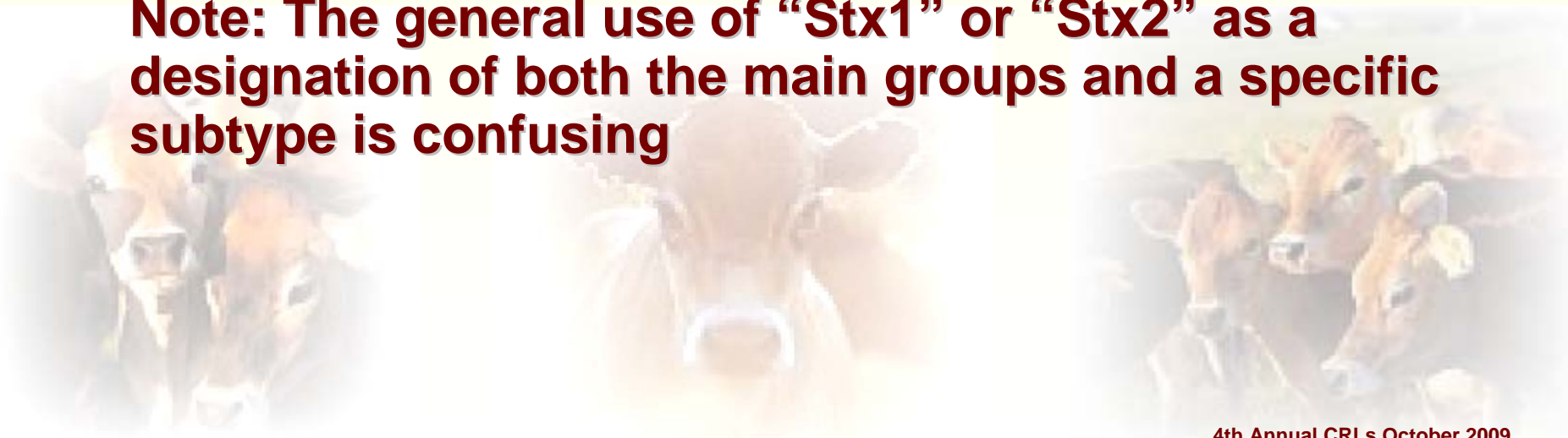
cEND.....A.....*...N..
 aEDD.....D.....N..
 aEDD.....* *.....* *.....*.....N..
 aEDD.....D.....*...N..
 dEND.....D.....N..
 dEND.....*.....N.....A.....N..
 bEND.....A.....N.....N.A.....
 gGDN.....D.....N.....N.....N..
 fV.....EDD.....S.R.....N.....ISN..S.....Q.K..
 eEDN.....S.R.....N.....ISN..S.....Q.K..
 eEDN.....S.R.....N.....ISN..S.....Q.K..

1) Types of Shiga toxin (Stx) (Stx)

Stx1 and Stx2

- No cross-neutralization
- No DNA-DNA cross-hybridization

Note: The general use of “Stx1” or “Stx2” as a designation of both the main groups and a specific subtype is confusing



2) Subtypes

Suffixed with small Arabic letters

- Share cross-hybridization

Show significant differences in:

- Biologic activity, including the capacity to be activated
- Serologic reactivity
- Receptor binding

Stx: Shiga toxin from *Shigella*

Stx1: *E. coli* Shiga toxins

Stx2:

Stx
Stx1a, Stx2c & Stx1d
Stx2a to Stx2g

3) Variants – Toxins - proposal

Subtype - first published O group - strain No.

examples

Subtype	Variant designation
Stx1a	Stx1a-O157-EDL933
	Stx1a-O111-PH
Stx2a	Stx2a-O157-EDL933
	Stx2a-O48-94C
Stx2c	Stx2c-O157-E32511
Stx2dact	Stx2d ¹ -O91-B2F1
	Stx2d ² -O91-B2F1
	Stx2d ³ -O28- MT71
Stx2e	Stx2e-O139-S1191
Stx2f	Stx2f-O128-H.I.8

Whittam, T. S. 1998. Evolution of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains, p. 195-209. In J. B. Kaper and A. D. O'Brien (eds.), *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* Strains. ASM Press, Washington

3) Variants - *genes* - proposal proposal

Subtype - first published O group - strain No.

e.g.

Subtype Variant *gene* designation

Stx2a *stx2a*-O157-EDL933

stx2a-O121- I5518

Toxin identity to Stx2a-O157-EDL933

gene difference at position 867 = C (instead of T)

Found in O121:H19

Accession No. EF441611

3) Stx2d variants

Variant designation +/- biological activity

Activatable

Stx2d**1**-O91-B2F1-act

Stx2d**2**-O91-B2F1-act

Stx2d**3**-O28-MT71-act

Non-activatable

Stx2d-"**O?-n**"-nonact ?

...

Not Tested:

Stx2d-O73-C165-02-NT

(Stx2d**16**)



Subunits A & B

StxA

StxB

StxA2a

StxB2a

StxA1a

StxB1a

StxA2b

StxB2b

StxA1c

StxB1c

StxA2c

StxB2c

StxA1d

StxB1d

StxA2d

StxB2d

StxA2e

StxB2e

StxA2f

StxB2f

StxA2g

StxB2g



Please use:

- _ = Underscore
- = hyphen

Avoid the use of:

#

/

;

”””

:

,

.

()

[]

?

SPACE



1st generation Stx & Stx1 specific PCR

PCR

Primer pairs	Subtype	Stx & Stx1	a	c	d
--------------	---------	------------	---	---	---

Stx1-seq-F1 / Stx1-seq-R1 Stx1-det-F1 / Stx1-det-R1		OMNI	+	+	+
			+	+	+

Stx1a-F1 / Stx1a-R1

+

Stx1a-F1 / Stx1a-R2

+

Stx1c-F1 / Stx1c-R1

+

Stx1c-F1 / Stx1c-R2

+

Stx1d-F1 / Stx1d-R1

+



Primers for sequencing, detection and subtyping of Stx & Stx1

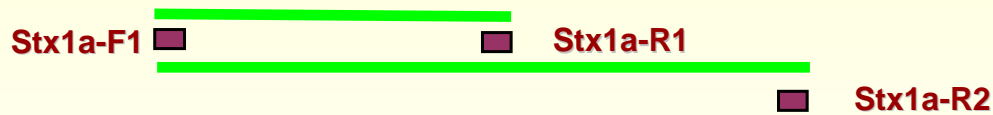
Stx1 OMNI-seq



Stx1 OMNI-det



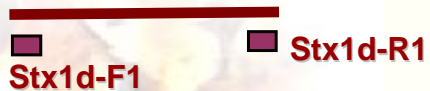
Stx & 1a



1c



1d



0 100 200 300 400 500 600 700 800 900 1000 1100 1200



Stx1 Triplex PCR

Stx1a-F1
Stx1a-R2

Stx1c-F1
Stx1c-R1

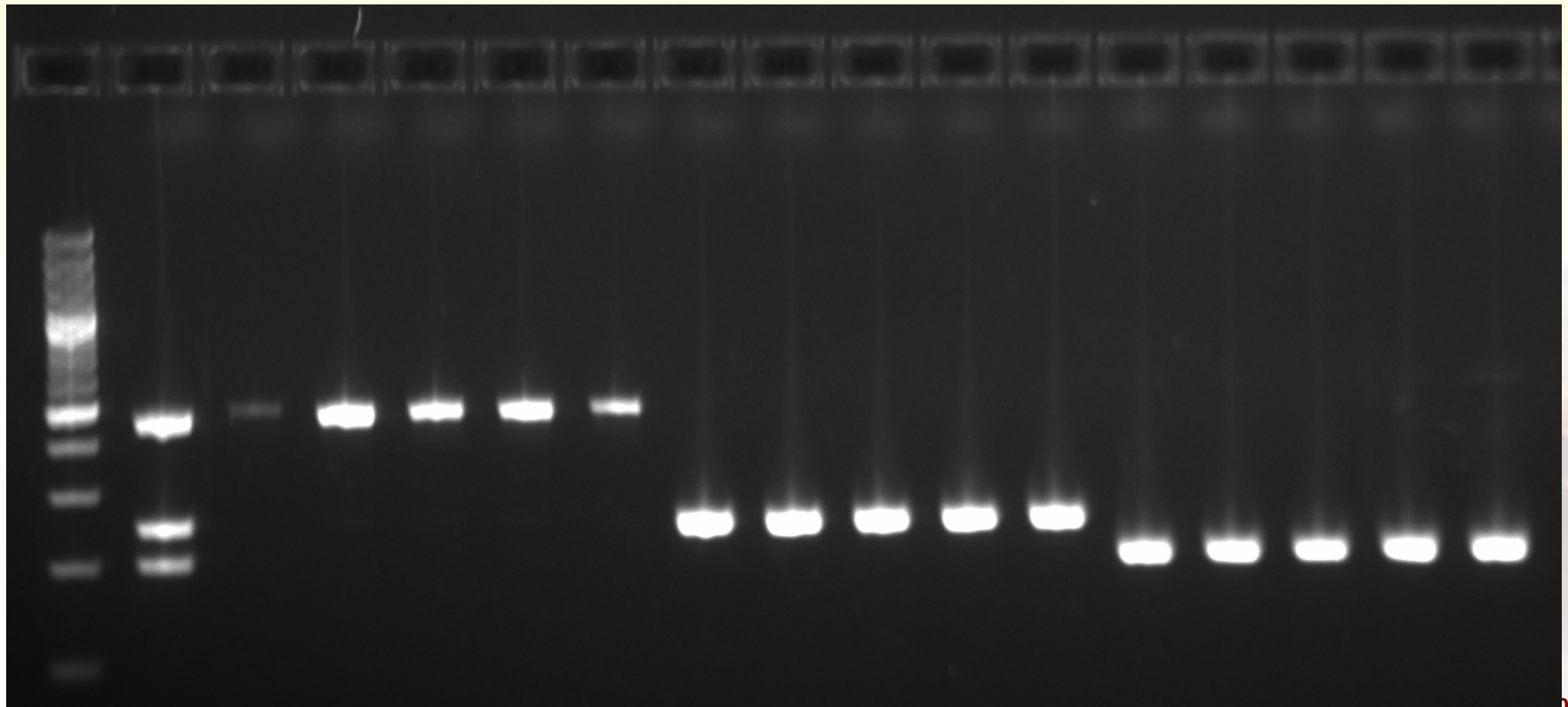
Stx1d-F1
Stx1d-R1

M Control

stx1a

stx1c

stx1d



3rd generation Stx2 specific PCR

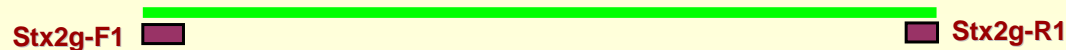
Primer pairs	Subtype	Stx2	a	b	c	d	e	f	g
F4 / R1	} Stx2 OMNI		+	+	+	+			+
F4f / R1e-f								+	
F4 / R1e-f							+		
Stx2a-F2/R2/R3			+						
Stx2b-F1/R1				+					
Stx2c-F/R					+				
Stx2dF1/R1/Stx2d-O55-R/Stx2d-R2						+			
Stx2e-F1/R1							+		
Stx2f-F1/R1								+	
Stx2g-F1/R1									+

Primers for detection and subtyping of Stx2

OMNI



g



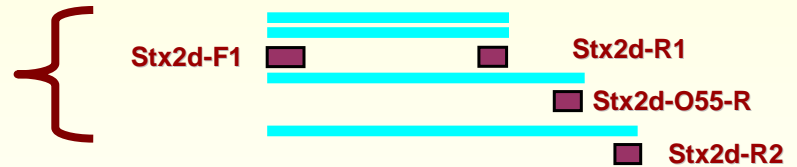
f



e



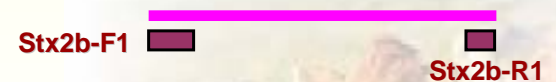
d



c



b

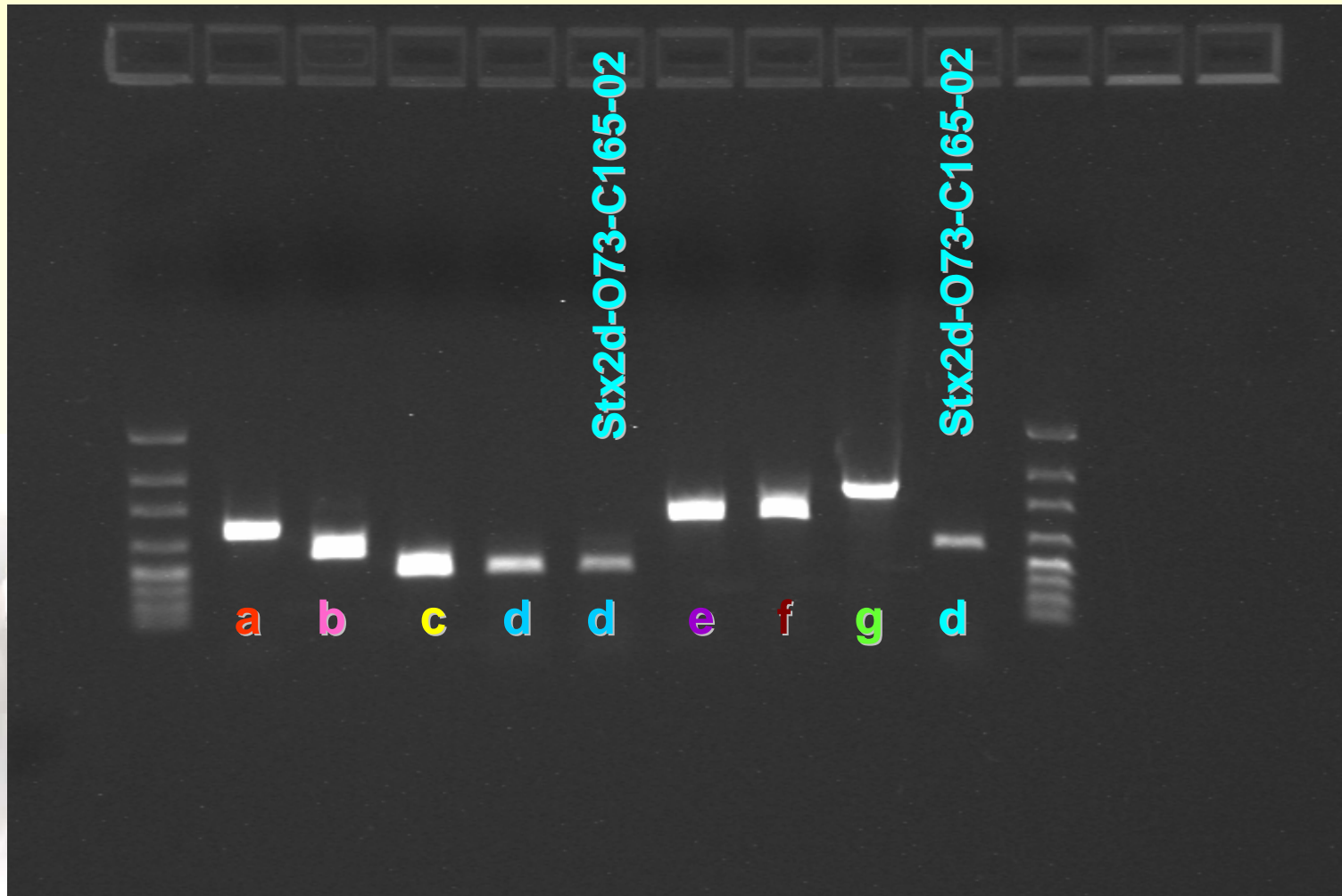


a

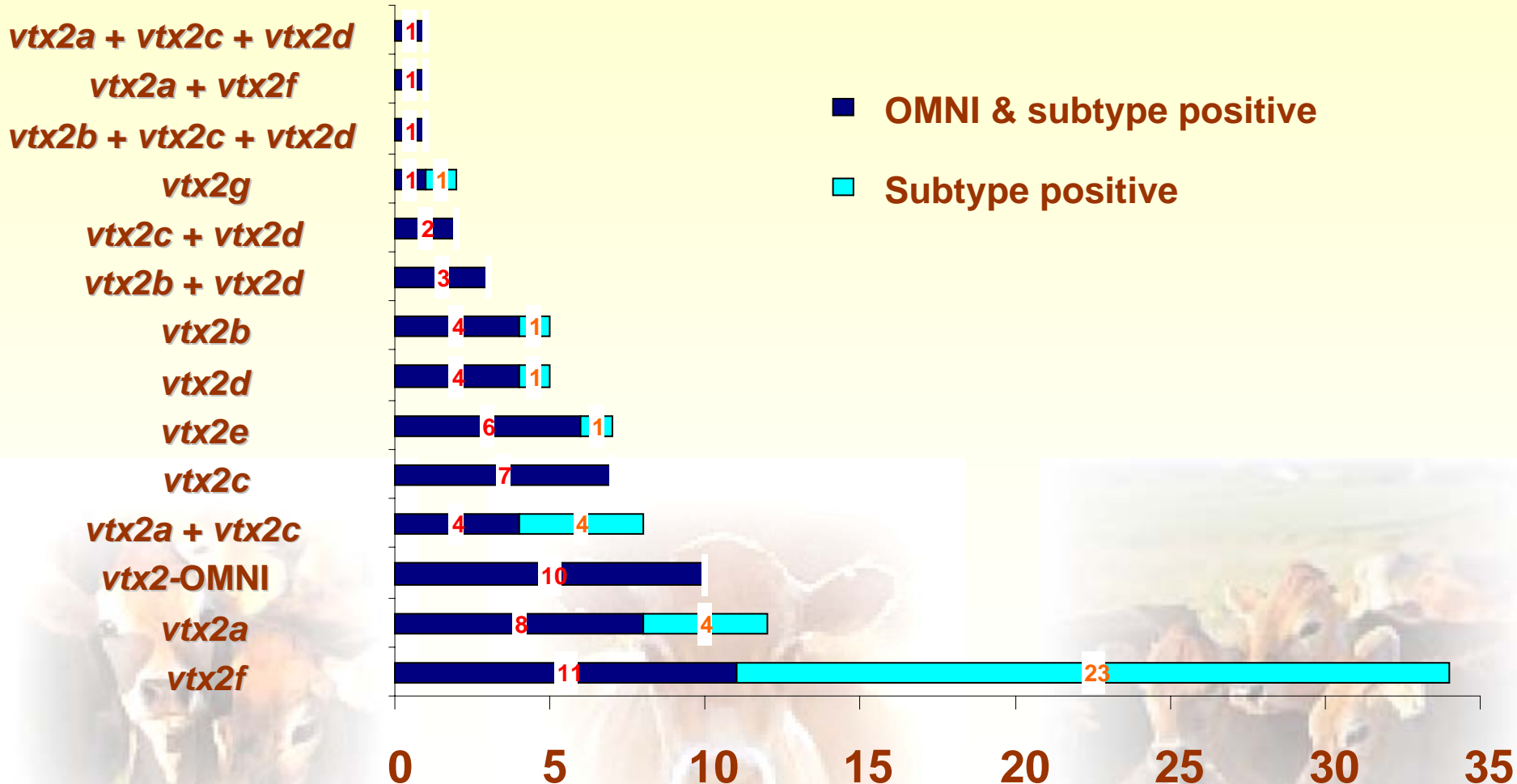


Preliminary test subtyping Stx2

Stx2



vtx2 PCR: OMNI and subtyping 98 VTEC strains



Prevalence of *vtx2f*

Denmark

2004	3	O2:H29, O128ab:H- & <u>O119:H-</u>
2006	5	O145:H34 (4) & O128ab:H-
2007	4	O145:H34 (3) & <u>O26:H34</u>
2008	7	O145:H34 (5), O103:H- & O125ac:H6
2009	5	O145:H34 (3) & O128:H- (2)

New variants of *vtx2f* may be found in these two strains





WORD

Table X

Establishment of reference collection



Please do not Annoy, Torment, Pester, Molest, Worry,
Badger Harry, Harass, Hackle, Persecute, Irk, Rag,
Vex, Bother, Tease, Nettle, Tantalise or Ruffle the Animal.

भंडावू , अस्वस्थ, झुलवू , निराश, हाल, व्याकूळ करू नका दुःख, त्रास, इजा,
डिवचू , छळू , मारू, हेळसांड, चिडवू नका. क्षुब्ध करू नका.
गोंगाट, आवाज टाळा. हुसकू नका. हल्ला करू नका. मारू नका. उपद्रव देऊ नका.
सताऊ नका यातना वेदना देऊ नका. पिडा, छेडू , खोड्या काढू नका.

PLEASE!

**ELVIS
IS DEAD
SINATRA
IS DEAD
AND ME I FEEL
ALSO NOT SO GOOD**