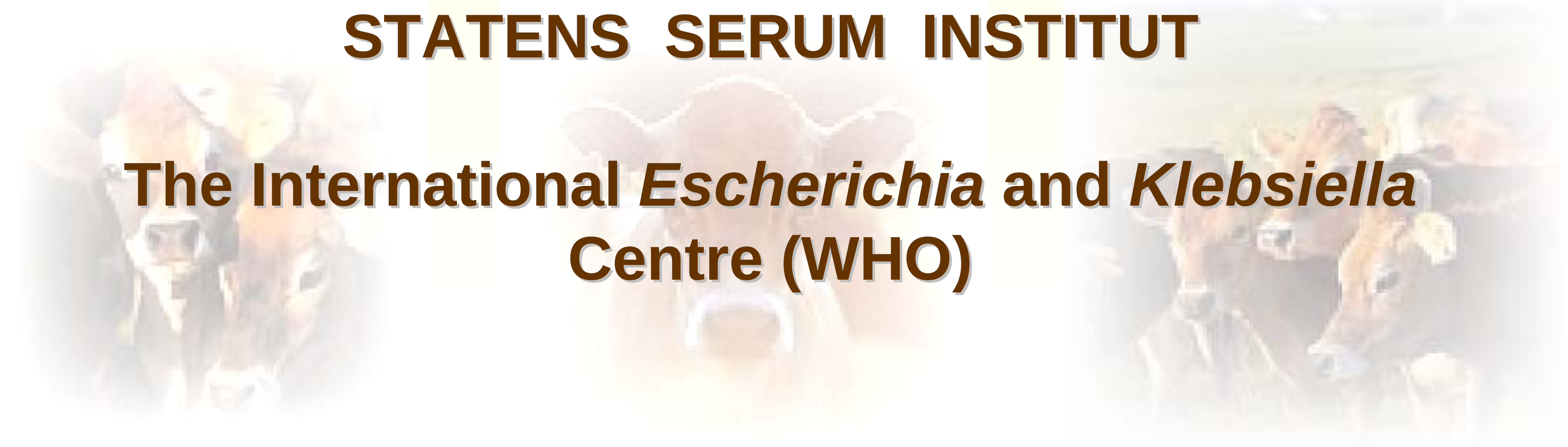
E. coli
Typing, characterization and nomenclature of VTEC

FLEMMING SCHEUTZ

STATENS SERUM INSTITUT

The International *Escherichia* and *Klebsiella* Centre (WHO)

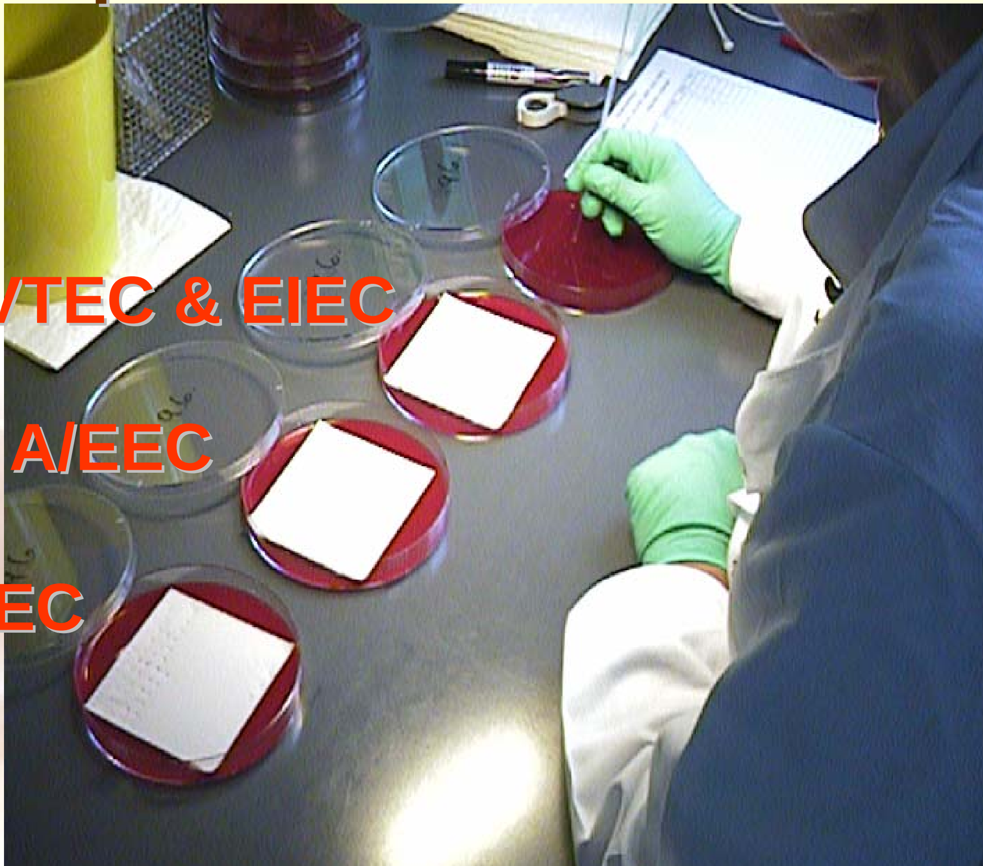


DIARRHOEAGENIC *E. coli* (DEC) & EXTRAINTESTINAL PATHOGENIC *E. coli* (ExPEC)

A/EEC:	**Attaching and effacing (A/E) *E. coli***
EPEC:	**Enteropathogenic *E. coli***
ETEC:	**Enterotoxigenic *E. coli***
EIEC:	**Enteroinvasive *E. coli***
VTEC:	**Verotoxin producing *E. coli***
STEC:	**Shigatoxin producing *E. coli***
SLTEC:	**Shiga-like toxin producing *E. coli***
EHEC:	**Enterohaemorrhagic *E. coli* - subgroup**
EAggEC:	**EnteroAggregative *E. coli***
DAEC:	**Diffusely Adherent *E. coli***
ExPEC:	**Extraintestinal pathogenic *E. coli***

Clinical detection of *E. coli* in Denmark - Methods

Colony dot blot hybridisation with DNA polynucleotide probes in pools:



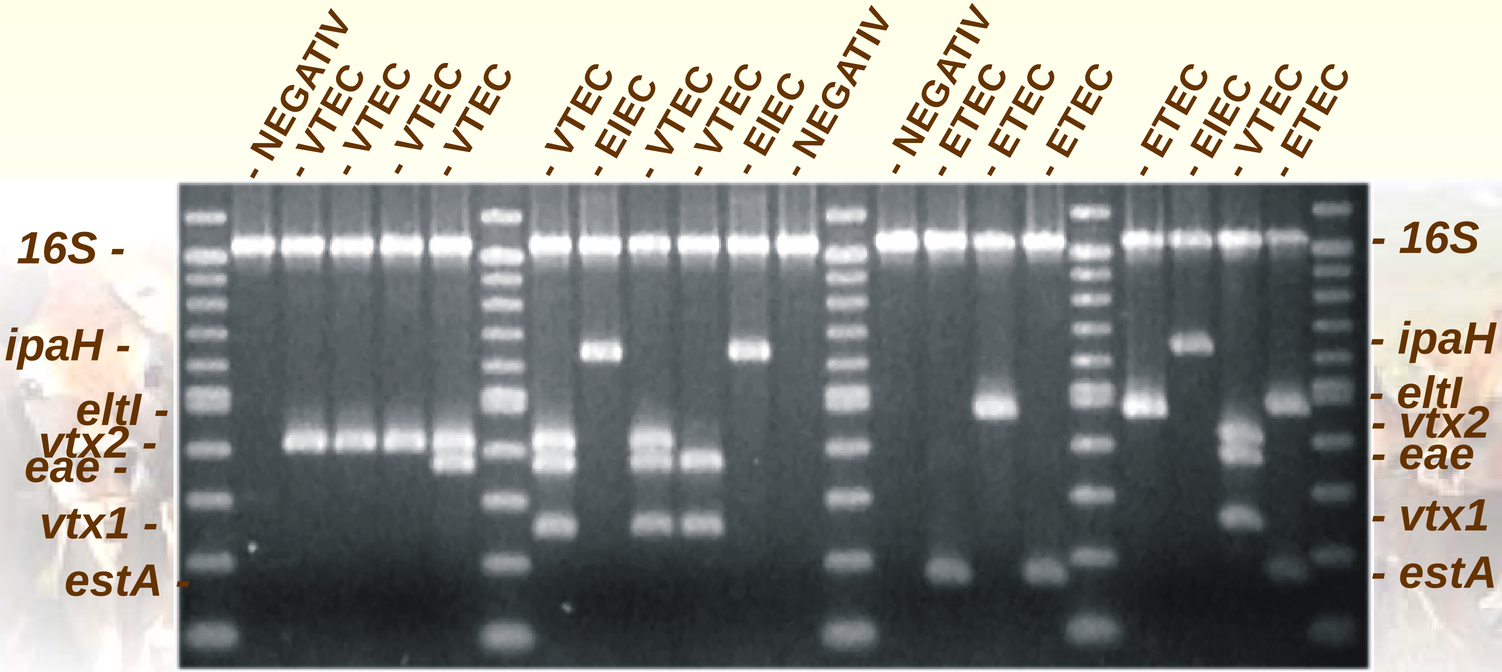
POOL 1: *vtx*1, *vtx*2, *ipa*H VTEC & EIEC

eae EPEC & A/EEC

POOL 2: LT, ST_h, ST_p ETEC

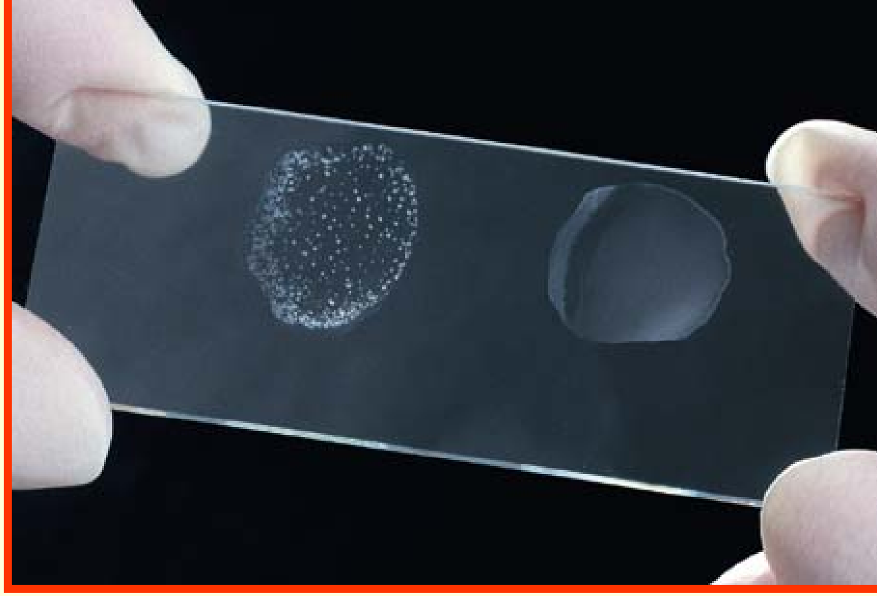
E. coli multiplex-PCR: specificity

E. coli **group**		**Virulence gene(s)**	
ETEC	**enterotoxigenic *E. coli***	***eltI* and/or** ***estAp / estAh***	**heat-labile enterotoxin** **heat-stable enterotoxin**
A/EEC **EPEC**	**attaching and effacing *E. coli*** **enteropathogenic *E. coli***	***eae***	**intimin**
VTEC	**verocytotoxin producing *E. coli***	***vtx1* and/or *vtx2***	**verotcytooxin 1** **verocytotoxin 2**
EIEC	**enteroinvasive *E. coli***	***ipaH***	**invasive plasmid antigen**



PHENOTYPIC TYPING of *E. coli*

- O:H serotyping
- Complete serotyping : O:K:H; F
- Adherence patterns
- Haemolysin production
- Haemagglutination patterns
- Toxin production
- Phage typing
- Biochemical reaction patterns



*vtx*1, *vtx*2 or *eae* positive colonies are live slide agglutinated with OK antisera against common VTEC and EPEC O groups:

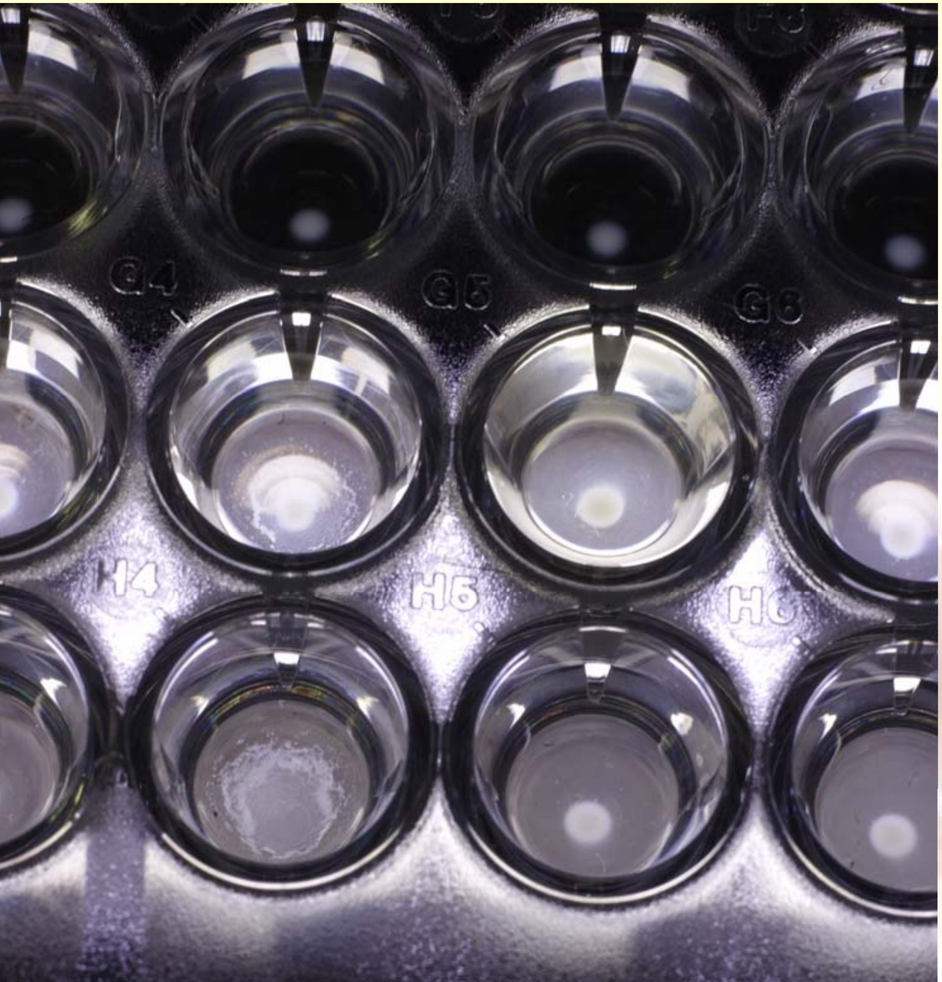
VTEC/EPEC
Pool 1: O26, O103, O111, O145 & O157
Supplemeted by: single antiserum O121 & O45
EPEC
Pool 2: O55, O119, O125ac, O127, O128ab
Pool 3: O86, O114, O126, O142, O158

All strains should be serotyped and characterised

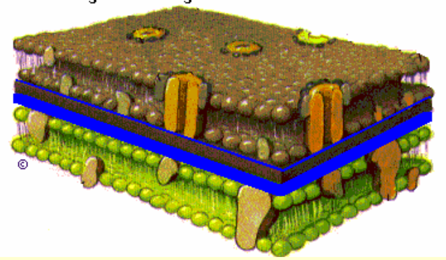
O grouping

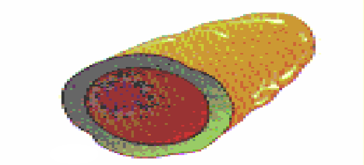
Boiled culture mixed with O antiserum

in microtiterplate **or** **in tubes**

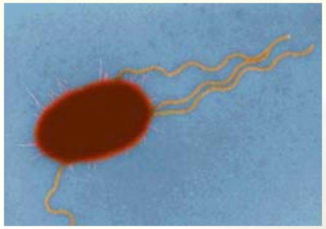


Serotyping of *E. coli*

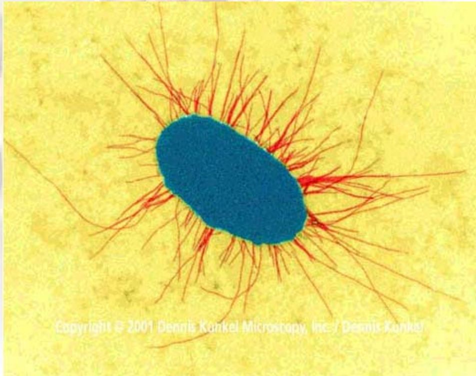




O antigen	**Lipopolysaccharide; LPS**	**181**
K antigen	**Acidic polysaccharide capsules**	**~ 60**
H antigen	**Protein; Flagella**	**53**



Complete serotype: O:K:H; F



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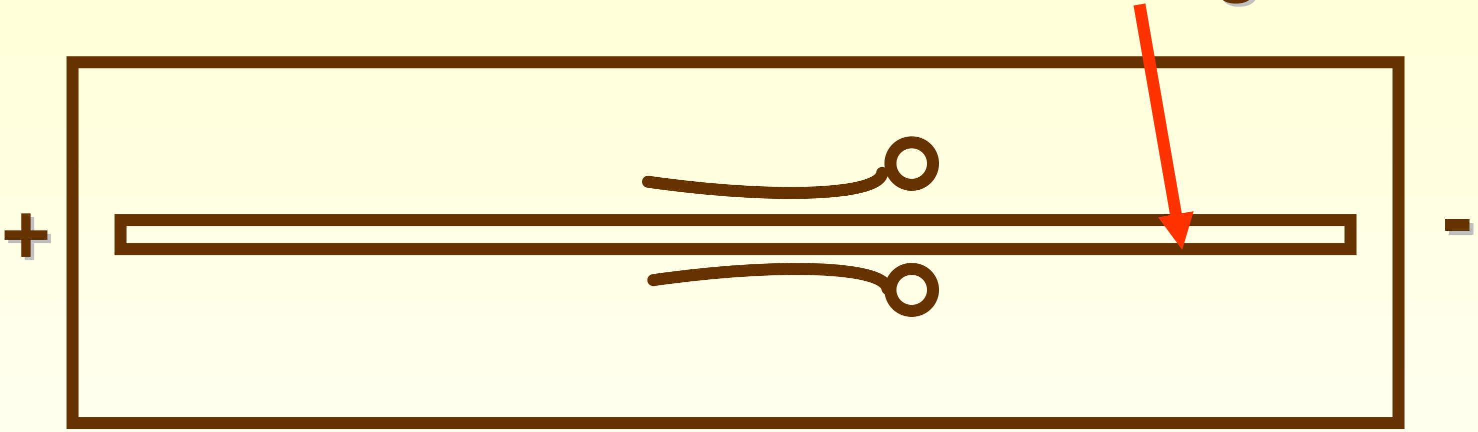
Serotyping of *E. coli* today . . 2

O antigen Lipopolysaccharide; LPS

Divided into

Antiserum in trough

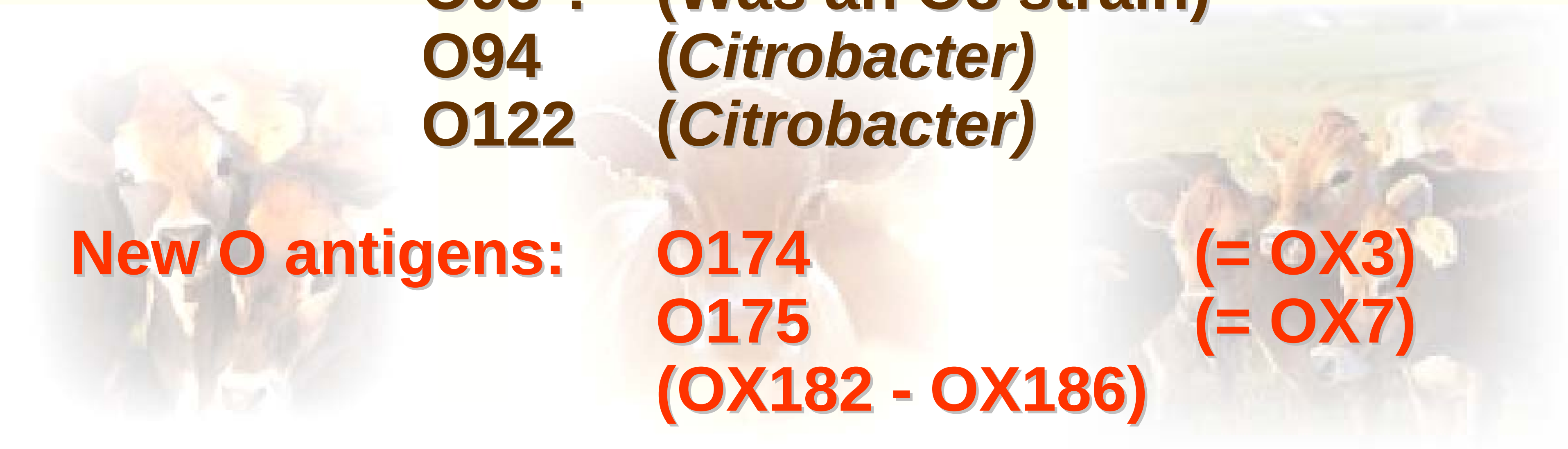
Acidic



and

Neutral

Serotyping of *E. coli* today . . 3



O antigen Lipopolysaccharide; LPS

Official number is 181 Actual: 175

Removed:

O31	
O47	
O67	(*Citrobacter*)
O72	(*Citrobacter*)
O93 ?	(Was an O8 strain)
O94	(*Citrobacter*)
O122	(*Citrobacter*)

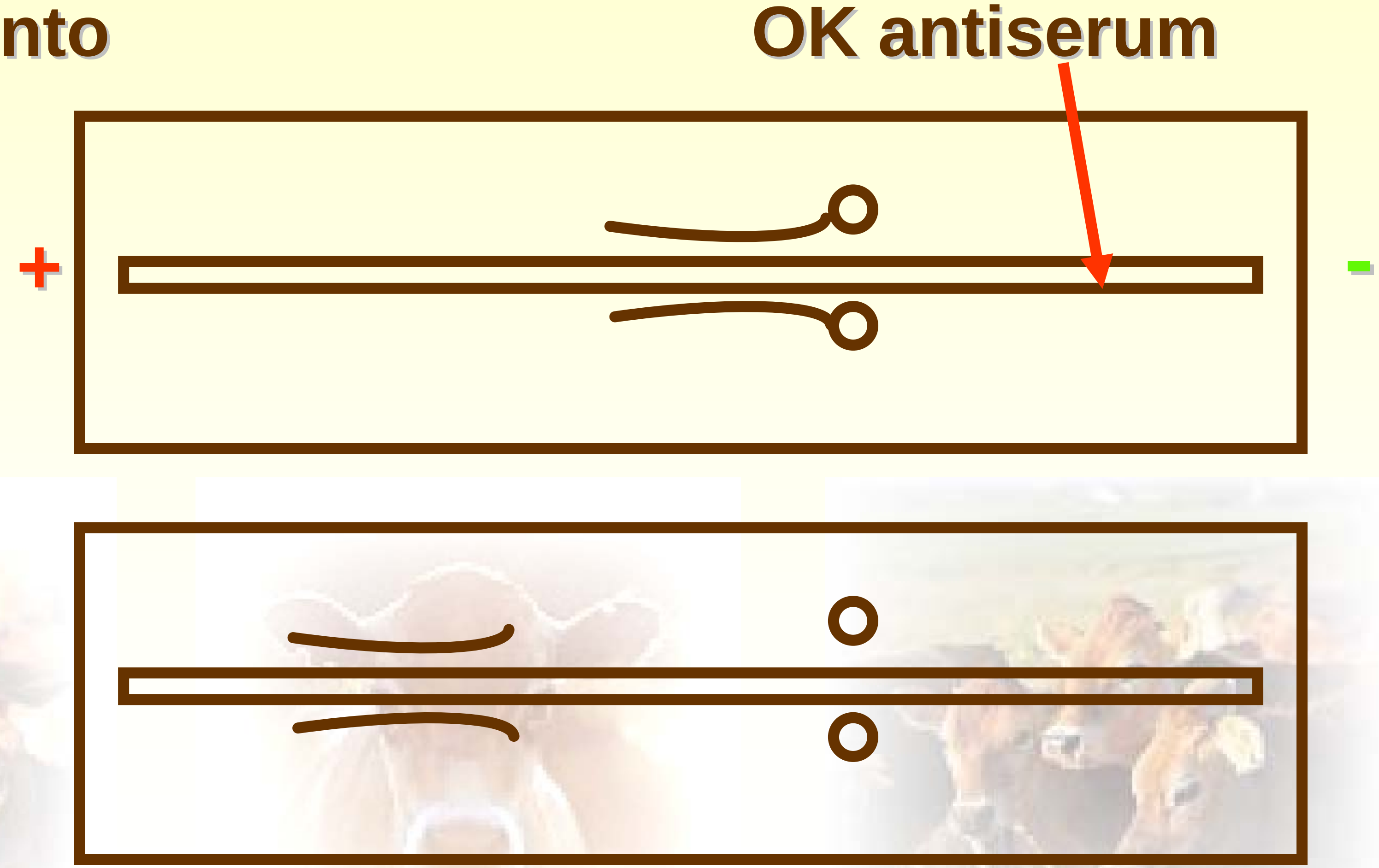
New O antigens:

O174	(= OX3)
O175	(= OX7)
(OX182 - OX186)	

Serotyping of *E. coli* today . . 4

K antigen Capsular polysaccharide; CPS

Divided into



Group I & IV

and

Group II & III

Serotyping of *E. coli* today . . 5

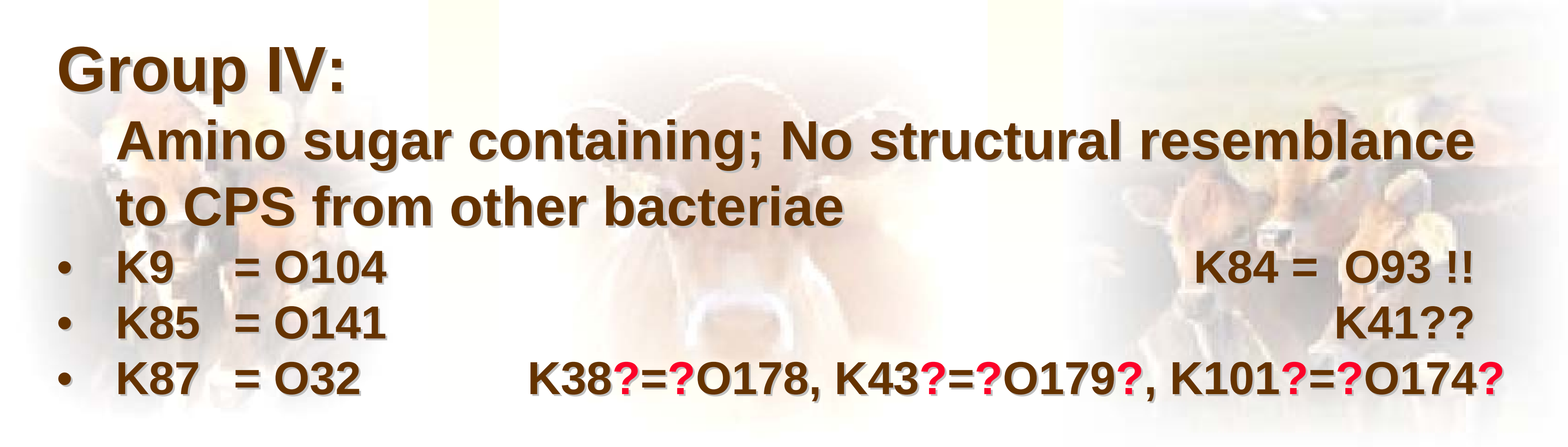
K antigen Capsular polysaccharide; CPS

Group I:

Only found in O groups O8, O9, O20 og O101

Amino sugar free; Structural resemblance to CPS from *Klebsiella* spp.

- **K28 = *Klebsiella* K54**
- **K30 = *Klebsiella* K20**
- **K55 = *Klebsiella* K5**



Group IV:

Amino sugar containing; No structural resemblance to CPS from other bacteriae

- **K9 = O104** **K84 = O93 !!**
- **K85 = O141** **K41??**
- **K87 = O32** **K38?=?O178, K43?=?O179?, K101?=?O174?**

Serotyping of *E. coli* today . . 6

K antigen Capsular polysaccharide; CPS

Many group IV K antigens are bound to lipid A

Because they are heat-stable the ”cover” the O antigen, thus making the O determination appear negative.

Either you need to autoclave the culture or to isolate K minus forms:

Serotyping of *E. coli* today . . 8

K antigen Capsular polysaccharide; CPS

K minus form

Serotyping of *E. coli* today . . 9

K antigen Capsular polysaccharide; CPS

Group II:

Found in many O groups

Structural resemblance to CPS from Gram-positives
20-50% of the sidechains are lipidbound

Subgroup: I/II or III

K2a, ac, (K62); K3; K10; K11; K19; K54; K96 ?; K98 (?)

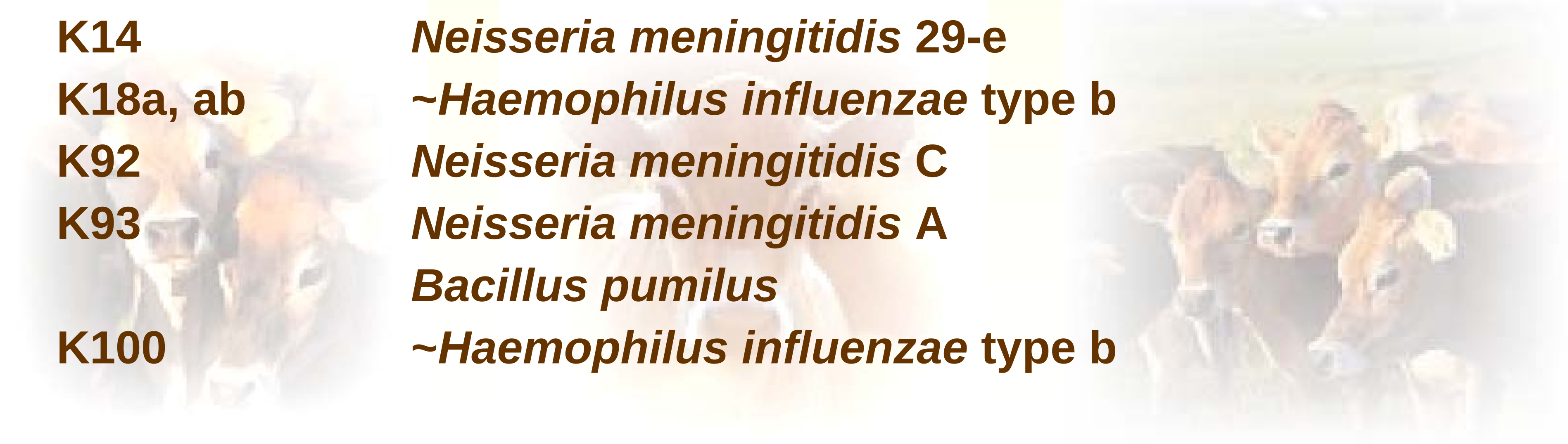
Serotyping of *E. coli* today . . 10

K antigen Capsular polysaccharide; CPS

Group II:

Structural resemblance to CPS from Gram-posistives

K2a, ac, (K62)	***Neisseria meningitidis* H**
K1 (~K92)	***Neisseria meningitidis* C** ***Pasteurella haemolytica* A2** ***Moraxella nonliquefacius***
K7, (56)	***Streptococcus pneumonia* type 3**
K14	***Neisseria meningitidis* 29-e**
K18a, ab	**~*Haemophilus influenzae* type b**
K92	***Neisseria meningitidis* C**
K93	***Neisseria meningitidis* A** ***Bacillus pumilus***
K100	**~*Haemophilus influenzae* type b**



Serotyping of *E. coli* today . . 11

K antigen Capsular polysaccharide; CPS

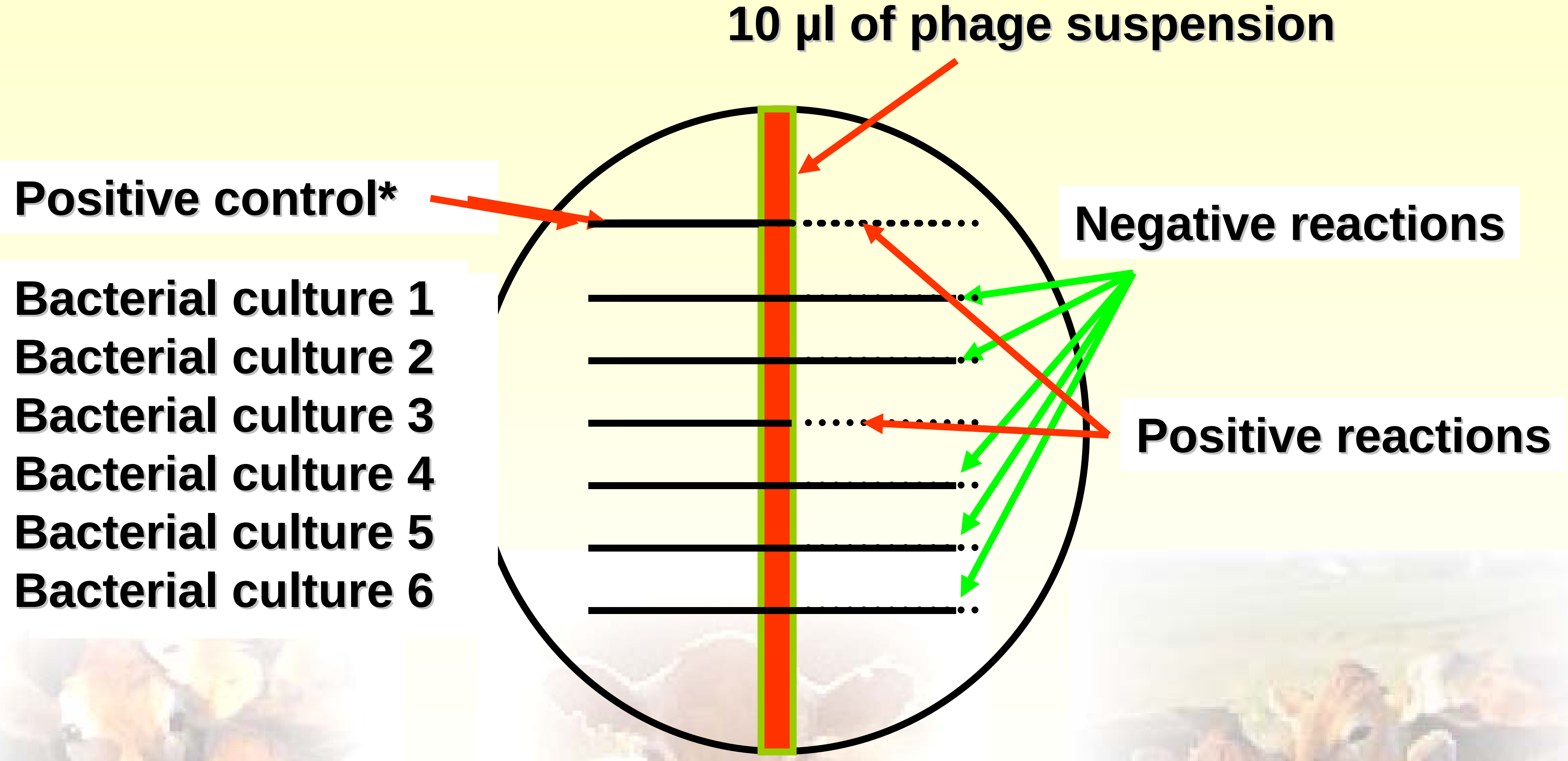
Official number is 101

Actual number is 60

***i. e.* IF grouped by backbone polysaccharide structure**

NOTE: The B antigens are obsolete!

Escherichia coli K-determination with phage cross-brush



10 µl of phage suspension

Positive control*

Bacterial culture 1
Bacterial culture 2
Bacterial culture 3
Bacterial culture 4
Bacterial culture 5
Bacterial culture 6

Negative reactions

Positive reactions

Incubation for 4 hours

*) Positive control strains:

	Origination	Serotype
K1	U 9-41	O2:K1:H4
K5	Bi8337-41	O10:K5:H4

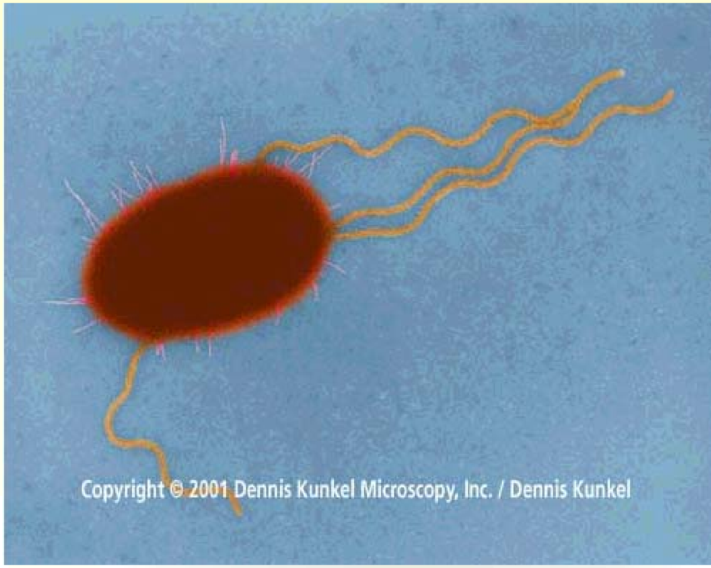
Serotyping of *E. coli* today

H antigen Flagella, protein structure

Official number is 56

Actual number is 53

Removed: H13
H22
H50 (= H10)



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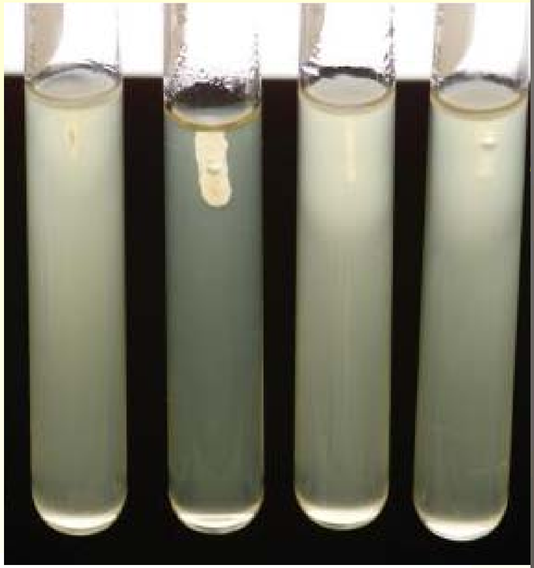
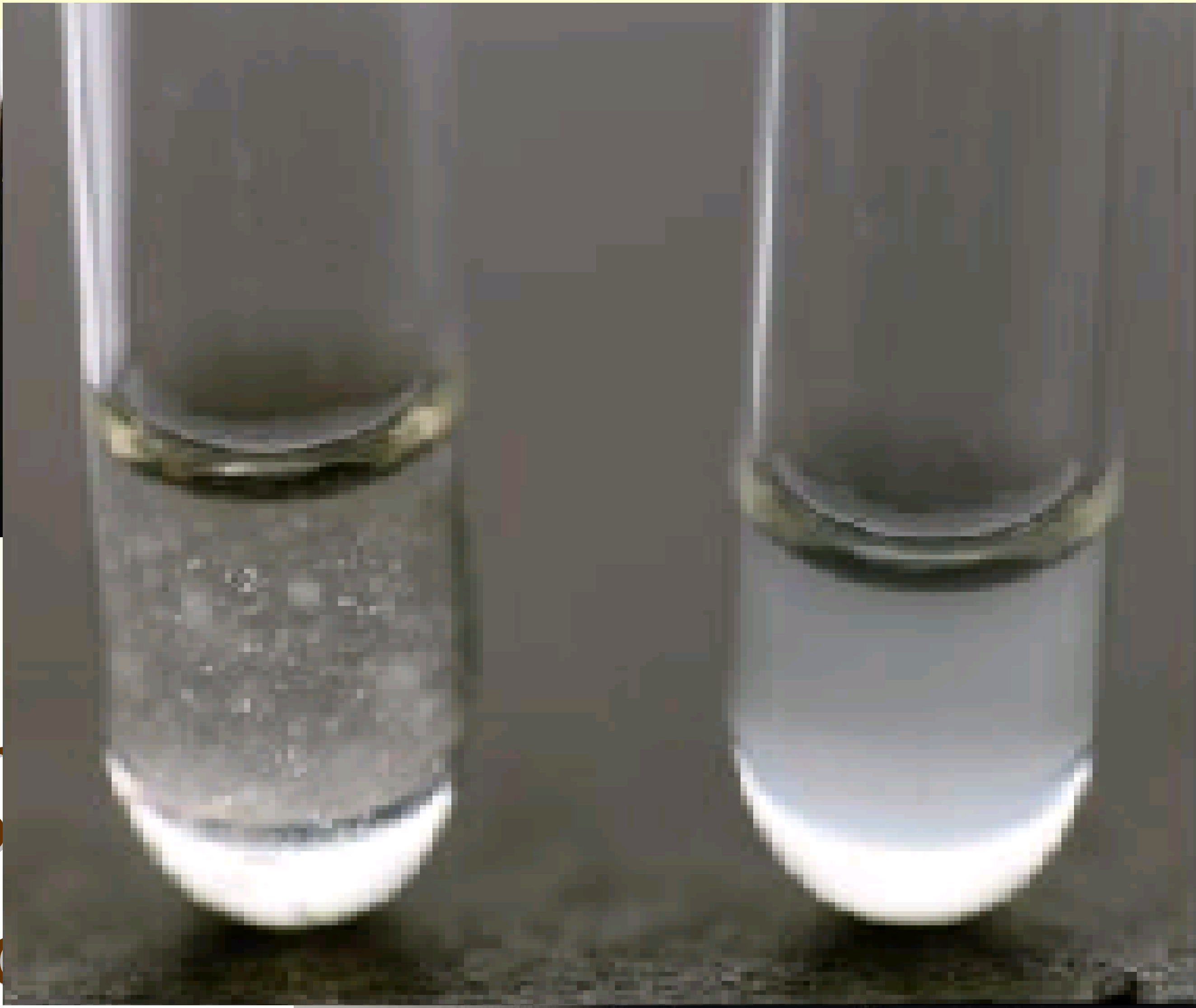
Serotyping of *E. coli* today . . 14

H antigen Flagella, protein structure

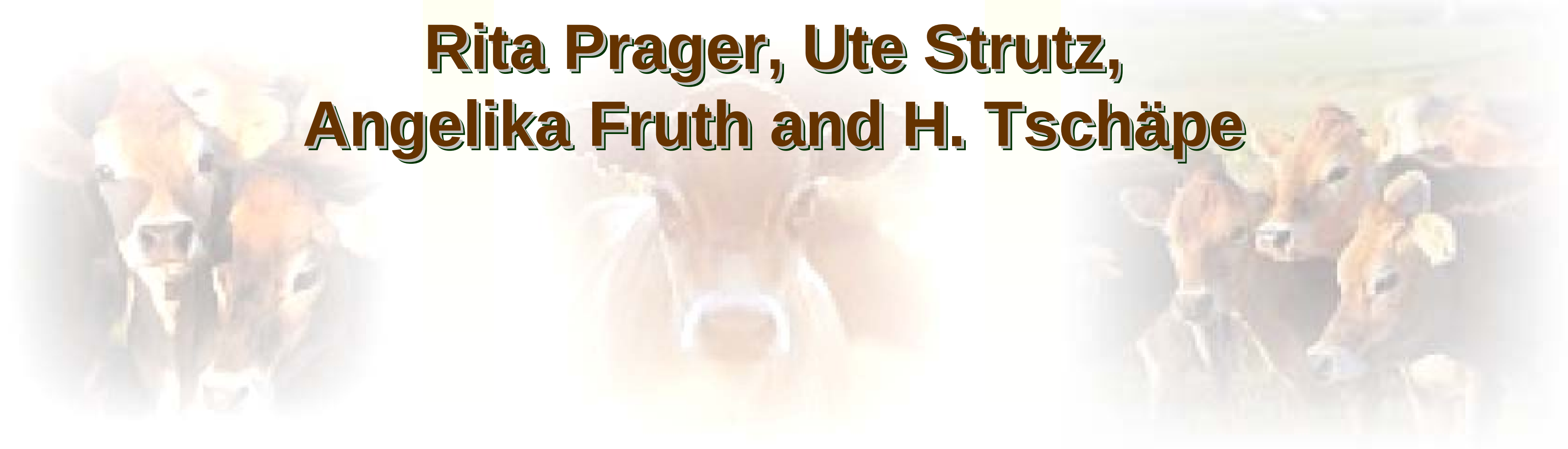




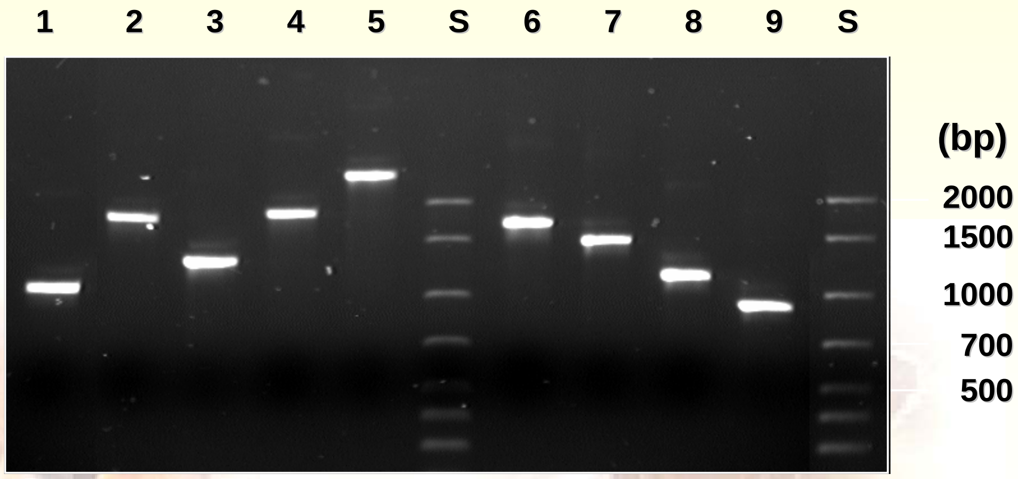
After passage
solid agar it is
important to m
motility in a ro
broth!
6 hours at 37ºC

Typing of pathogenic *E. coli* strains using flagellar (H)-antigens: serotyping vs fliC-polymorphisms

Rita Prager, Ute Strutz,
Angelika Fruth and H. Tschäpe



PCR-products derived from the *fliC*-gene PCR using different primers

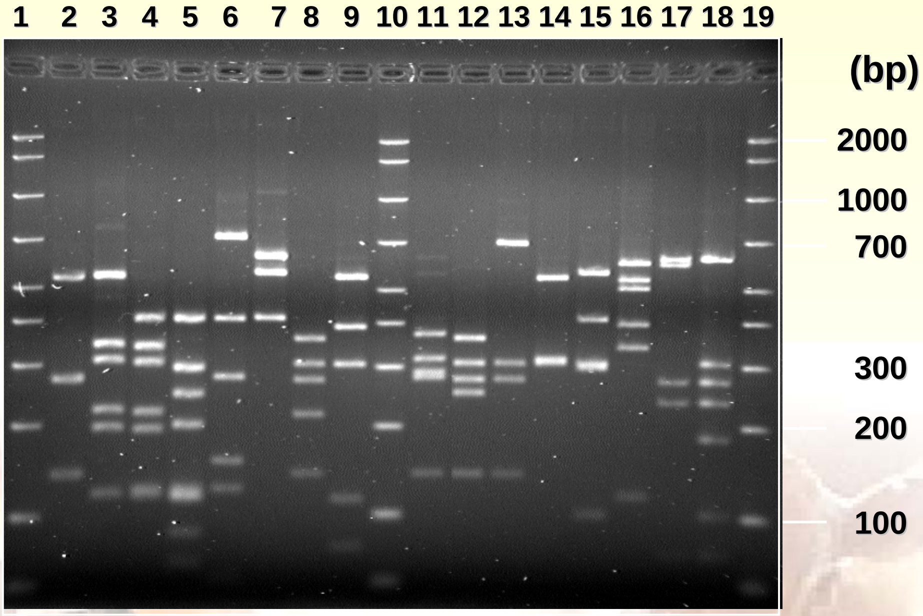


lanes:

1	O7:H4	fliC-F
2	O15:H9	fliC-F
3	O92:H33	fliC-F
4	O8:H19	fliC-F
5	O164:H$^-$	fliC-F
6	O157:H12	fliC-M
7	O118:H16	fliC-M
8	O111:H2	fliC-M
9	O7:H4	fliC-M
S	molecular standard	

Some *Rsa*I restriction profiles of amplified *fliC*-genes

according to Fields *et al*



lanes:		fliC-RFLP type
2	O26:H11	1
3	O157:H7	2
4	O156:H9	2-1
5	O157:H45	3
6	O157:H12	4
7	O91:H14	5
8	O46:H21	6
9	O128:H2	7
11	O118:H16	8
12	Ont:H^-	8-1
13	O22:H8	9
14	O115:H16	10
15	O126:H^-	11
16	O164:H^-	12
17	O76:H19	13
18	O121:H19	13-1

1, 10, 19 = molecular standard

Summary of *fli*C*(F)* restriction pattern and their genetic distance

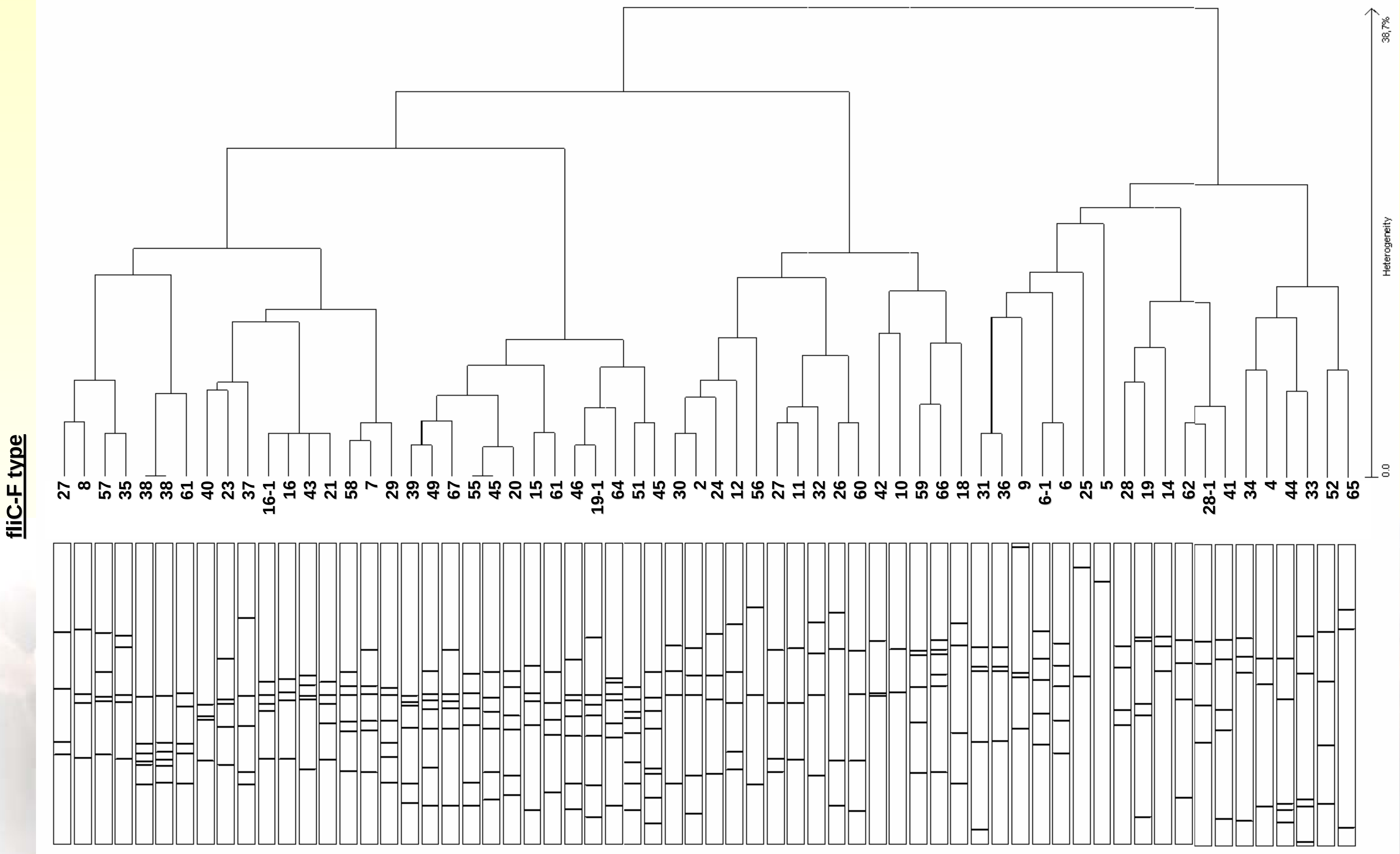
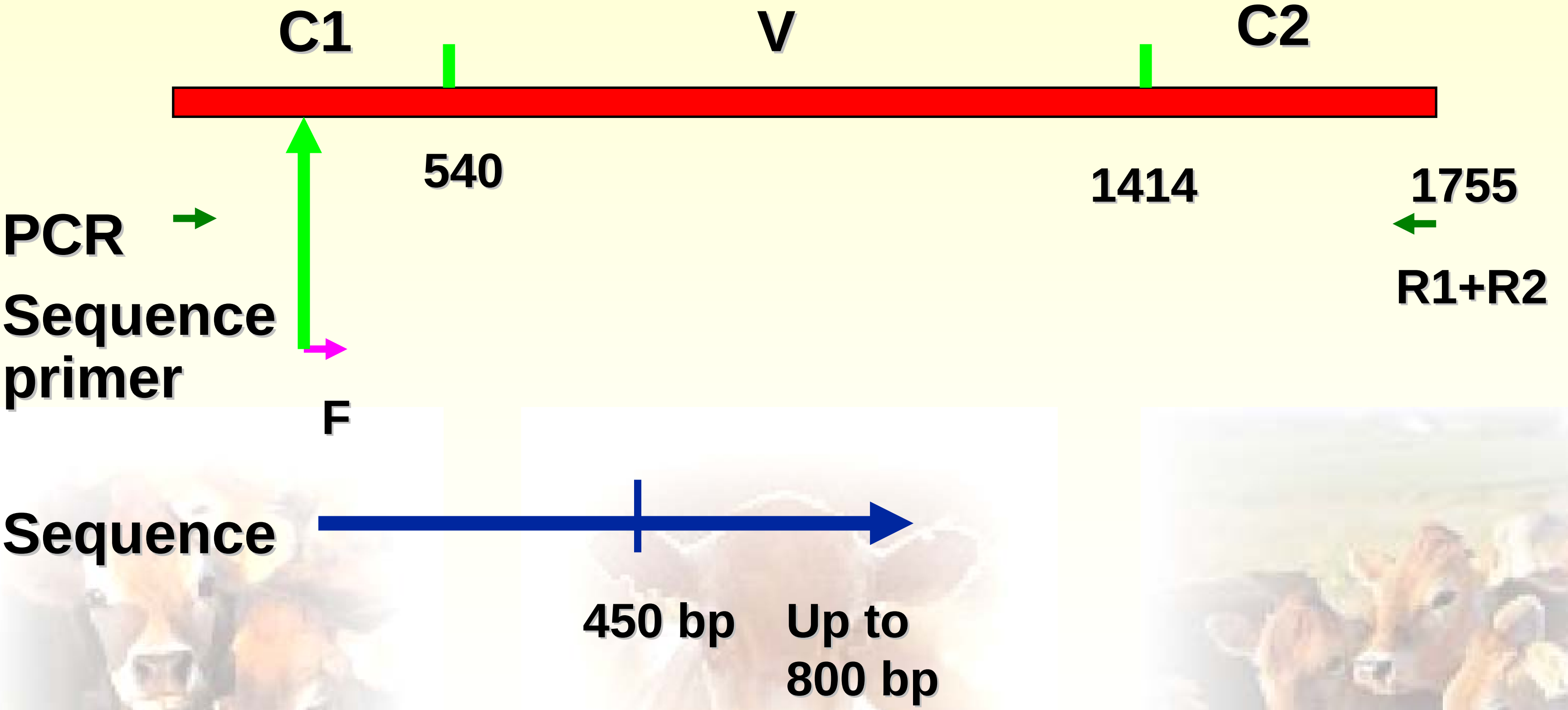


Diagram *fliC*



C1

V

C2

540

1414

1755

PCR

Sequence primer

F

R1+R2

Sequence

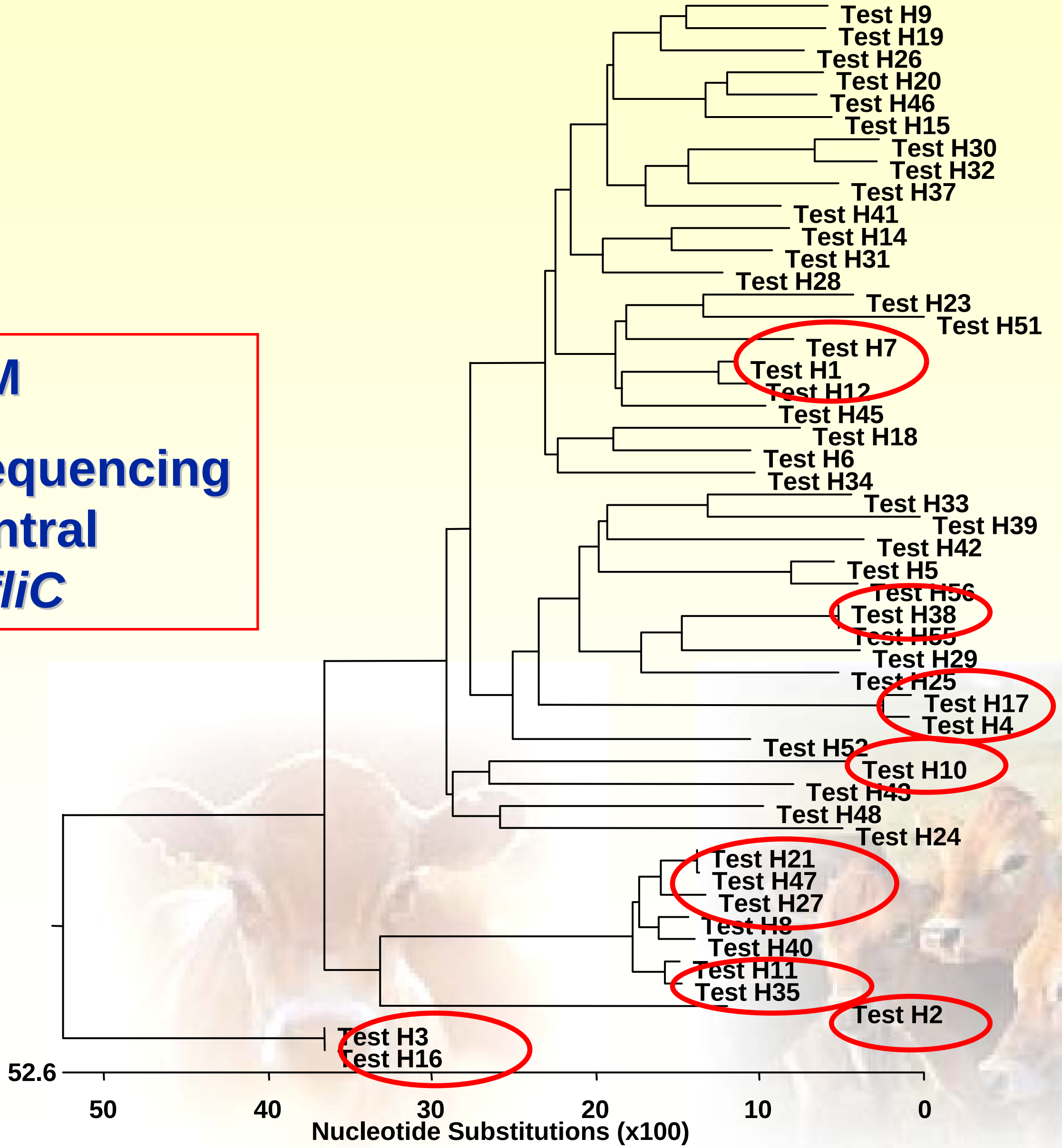
450 bp

Up to 800 bp

E. coli test strains

DENDROGRAM

based on partial sequencing of 450 bp in the central variable region of *fliC*

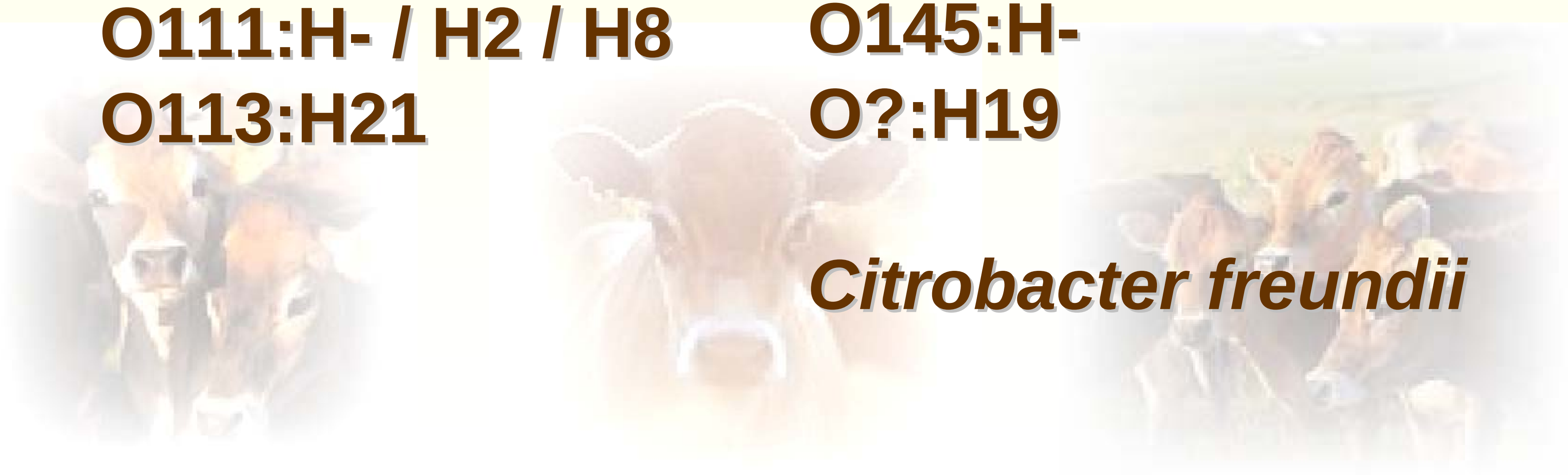


Comparison of partial *fliC* molecular typing with previous studies

phenotype	Genotypes			
	RFLP [a]		Sequencing	
Antigen	*fliC* (F)	*fliC* (M)	Entire *fliC* [b]	Partial *fliC* [c]
H1	12	12-1	1	1/12/16
H3	16	16	16	16
H17	n.a.	17	4	4
H35	35	35	11	11
H36	37	71	n.a.	n.a.[d]
H40	40	8-2	8	40
H44	44	17	4	2 Bands [e]/38
H47	16-1	47a	21	21/11/12/2
H53	n.a.	53	n.a.	n.a.[f]
H54	n.a.	54	21	n.a./10/11
H55	55	55	38	38

n.a. no PCR amplification; [a] Typing of *fliC* by Restriction Fragment Length Polymorphism by Prager *et al*.; [b] Sequences obtained by Wang *et al.;* [c] This study; [d] Four strains tested; [e] Two strains tested; [f] Three strains tested

Non-O157 VTEC causing outbreaks



O22:H8

O26:H11

O103:H2

O103:H25

O104:H21

O111:H- / H2 / H8

O113:H21

O117:H4

O118:H2

O119

O121:H19 / H21

O128:H2

O145:H-

O?:H19

Citrobacter freundii

VIRULENCE FACTORS IN *E. coli*

- on chromosome (Pathogenicity Islands)
- plasmids, transposons & phages

LT	heat-labile toxin
ST_p	heat-stable toxin (porcine)
ST_h	heat-stable toxin (human)
CFA	colonization factors
inv	enteroinvasive *E. coli*
ipaH	invasion plasmid antigen
saa	STEC autoagglutinating adhesin
EAgg	aggregative adherence
DA	diffuse adherence
lpf	Long Polar Fimbriae
cnf	Cytotoxin necrotising factor

EAF factor	EPEC adherence
*bfp*A	Bundle forming pilus
LEE	locus for enterocyte effacement genes
eae	attaching and effacing
*ehx*A	enterohaemolysin
*hly*A	haemolysin
*ast*A	EAST1
*chu*A	OMP iron uptake
*vtx*1	verocytotoxin 1
*vtx*2	verocytotoxin 2

GENE SUB-TYPING

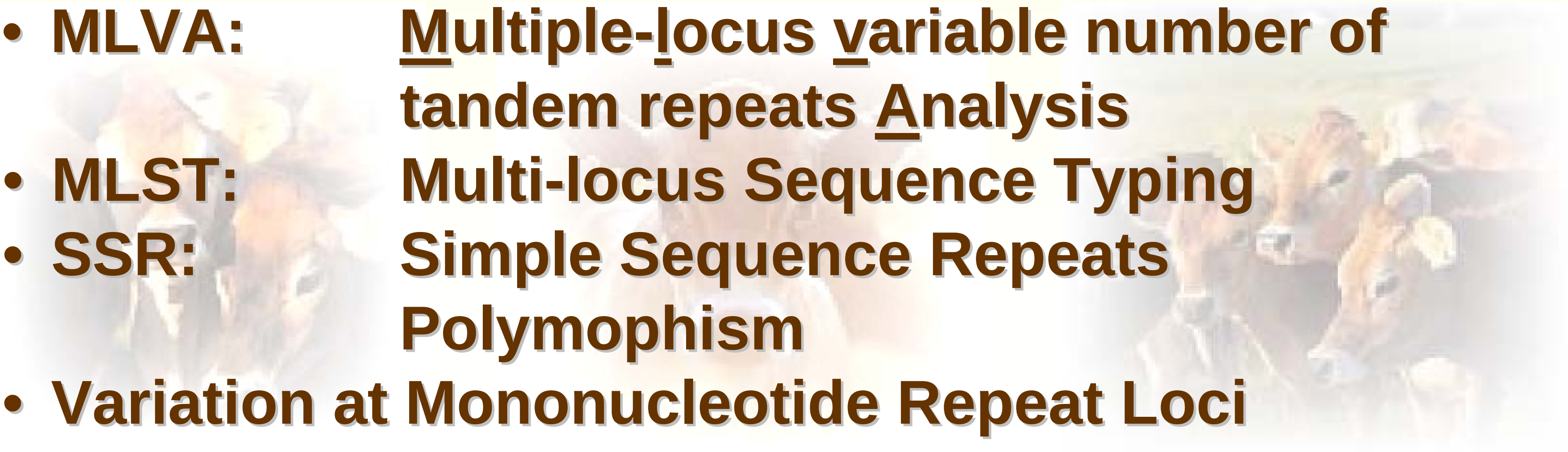
- **Specific PCR**
- **RFLP: restriction fragment length polymorphism**
- **Sequencing**

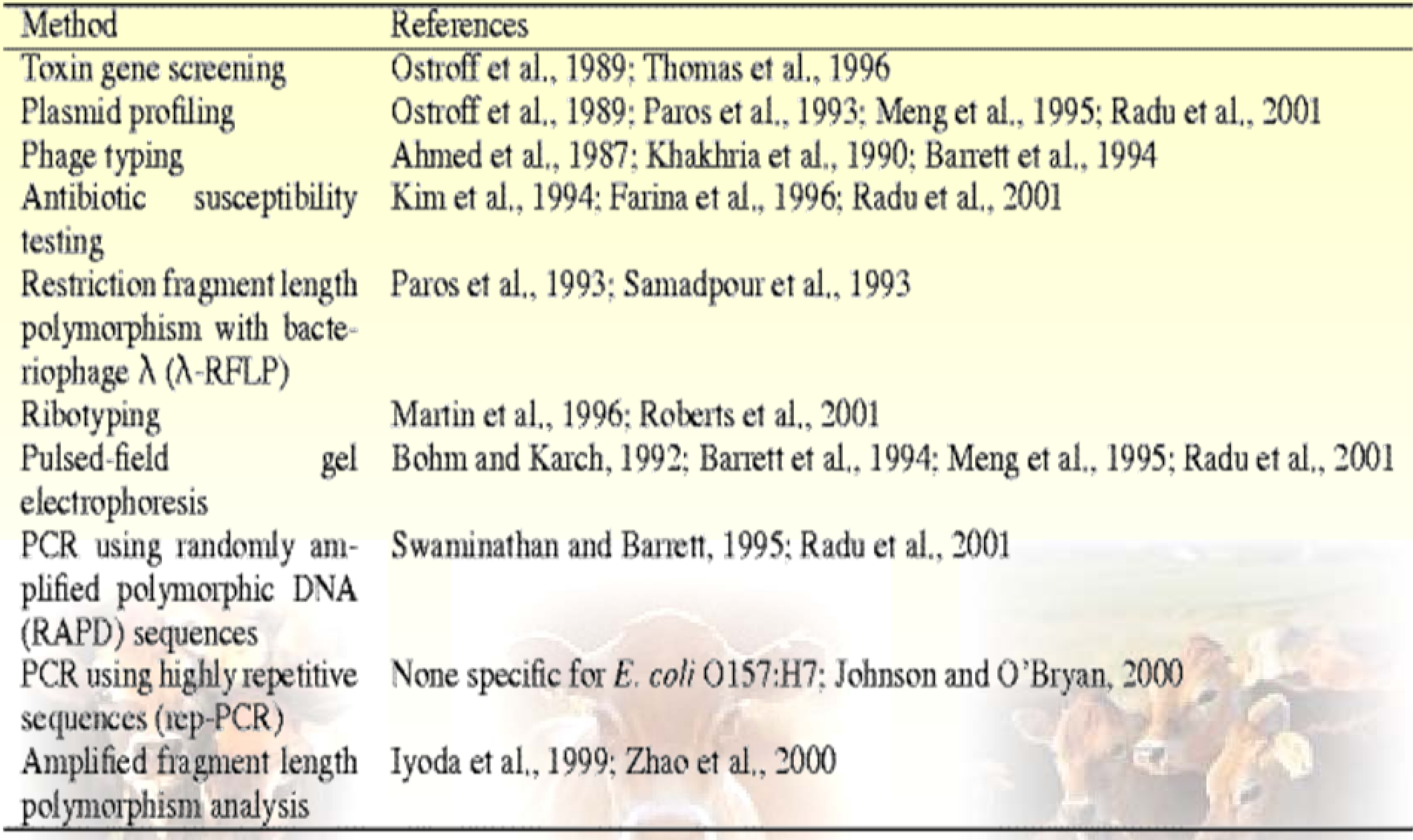
eg *fliC*

vtx

DNA TYPING or FINGERPRINTING

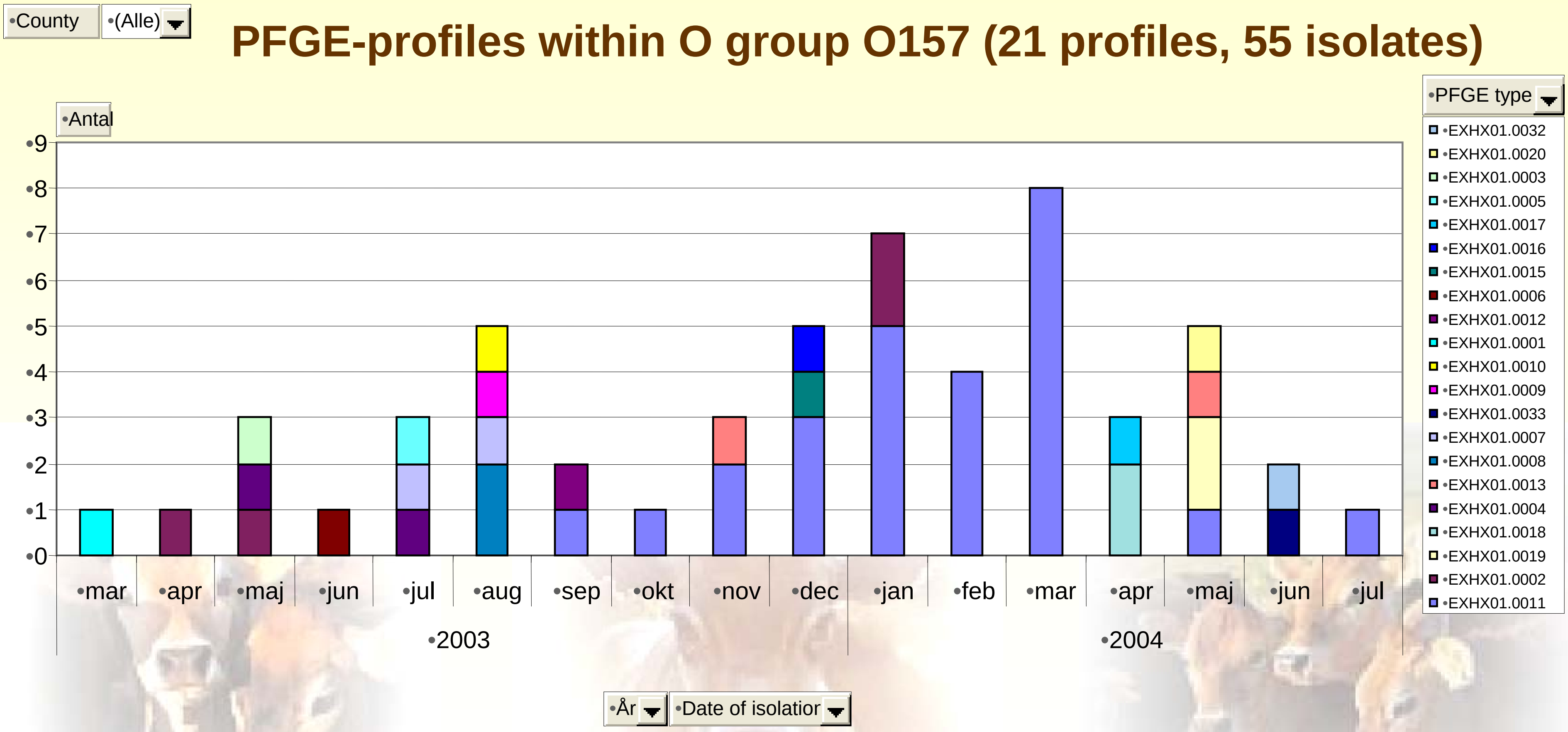
- **PFGE: Pulsed Field Gel Electrophoresis**
- **RiboPrinting: Automated rRNA typing**
- **RAPD: Random Amplified Polymorphic DNA**
- **Box-ERIC: Enterobacterial Repetitive Intergenic Consensus sequence**
- **Rep-PCR: Repetitive Extragenic Palindromic sequence PCR**
- **VNTR: Variable Number of Tandem Repeats**
- **MLVA: Multiple-locus variable number of tandem repeats Analysis**
- **MLST: Multi-locus Sequence Typing**
- **SSR: Simple Sequence Repeats Polymophism**
- **Variation at Mononucleotide Repeat Loci**



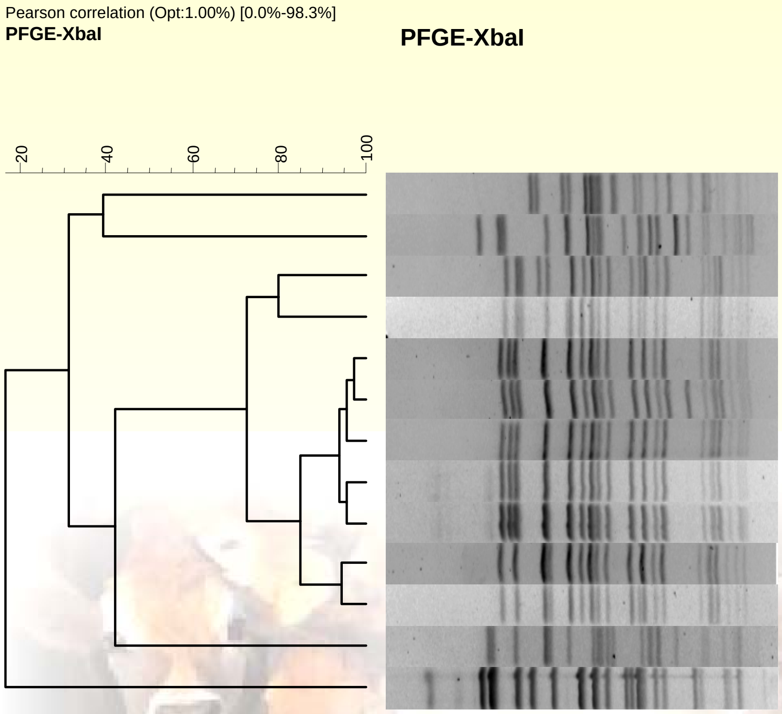


Method	References
Toxin gene screening	Ostroff et al., 1989; Thomas et al., 1996
Plasmid profiling	Ostroff et al., 1989; Paros et al., 1993; Meng et al., 1995; Radu et al., 2001
Phage typing	Ahmed et al., 1987; Khakhria et al., 1990; Barrett et al., 1994
Antibiotic susceptibility testing	Kim et al., 1994; Farina et al., 1996; Radu et al., 2001
Restriction fragment length polymorphism with bacteriophage λ (λ-RFLP)	Paros et al., 1993; Samadpour et al., 1993
Ribotyping	Martin et al., 1996; Roberts et al., 2001
Pulsed-field gel electrophoresis	Bohm and Karch, 1992; Barrett et al., 1994; Meng et al., 1995; Radu et al., 2001
PCR using randomly amplified polymorphic DNA (RAPD) sequences	Swaminathan and Barrett, 1995; Radu et al., 2001
PCR using highly repetitive sequences (rep-PCR)	None specific for *E. coli* O157:H7; Johnson and O'Bryan, 2000
Amplified fragment length polymorphism analysis	Iyoda et al., 1999; Zhao et al., 2000

PFGE types of Danish O157


PFGE-profiles within O group O157 (21 profiles, 55 isolates)

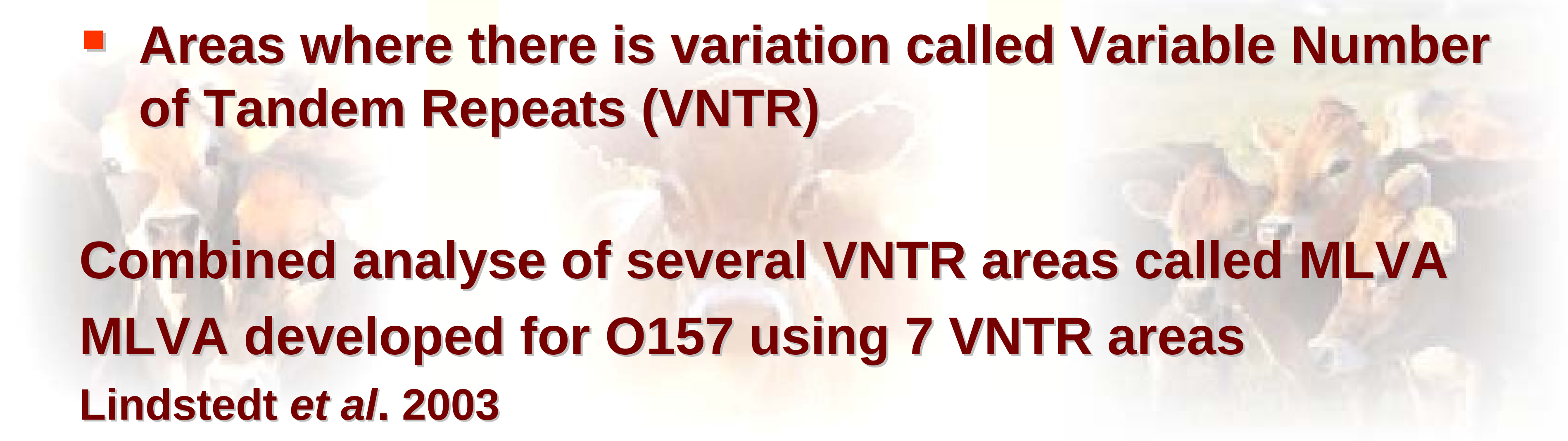
PFGE profiles of farm-related VTEC isolates



C 526-04	O 2	H29		-	+	-	2004-05-19.	64	M
C 532-04	O 15	H 8		-	+	+	2004-05-24.	1	K
C 527-04	O157	H-	EXHX01.0011.DK	+	+	+	2004-05-19.	2	M
C 547-04	O157	H-	EXHX01.0032.DK	+	+	+	2004-06-01.	1	K
C 511-04	O157	H 7	EXHX01.0019.DK	-	+	+	2004-05-11.	5	K
C 512-04	O157	H 7	EXHX01.0020.DK	-	+	+	2004-05-17.	2	K
C 481-04	O157	H 7	EXHX01.0019.DK	-	+	+	2004-05-06.	5	•K
goats_1A2a	O157		EXHX01.0019.DK						
goats_8.1	O157		EXHX01.0019.DK						
C 488-04	O157	H 7	EXHX01.0013.DK	-	+	+	2004-05-11.	5	•K
C 546-04	O157	H 7	EXHX01.0035.DK	-	+	+	2004-06-01.	1	•K
C 540-04	Oru	H 8		-	+	+	2004-05-27.	43	•M
goats_7									

Multiple Locus Variable number repeats Analysis (MLVA)

Repetetive DNA sequences



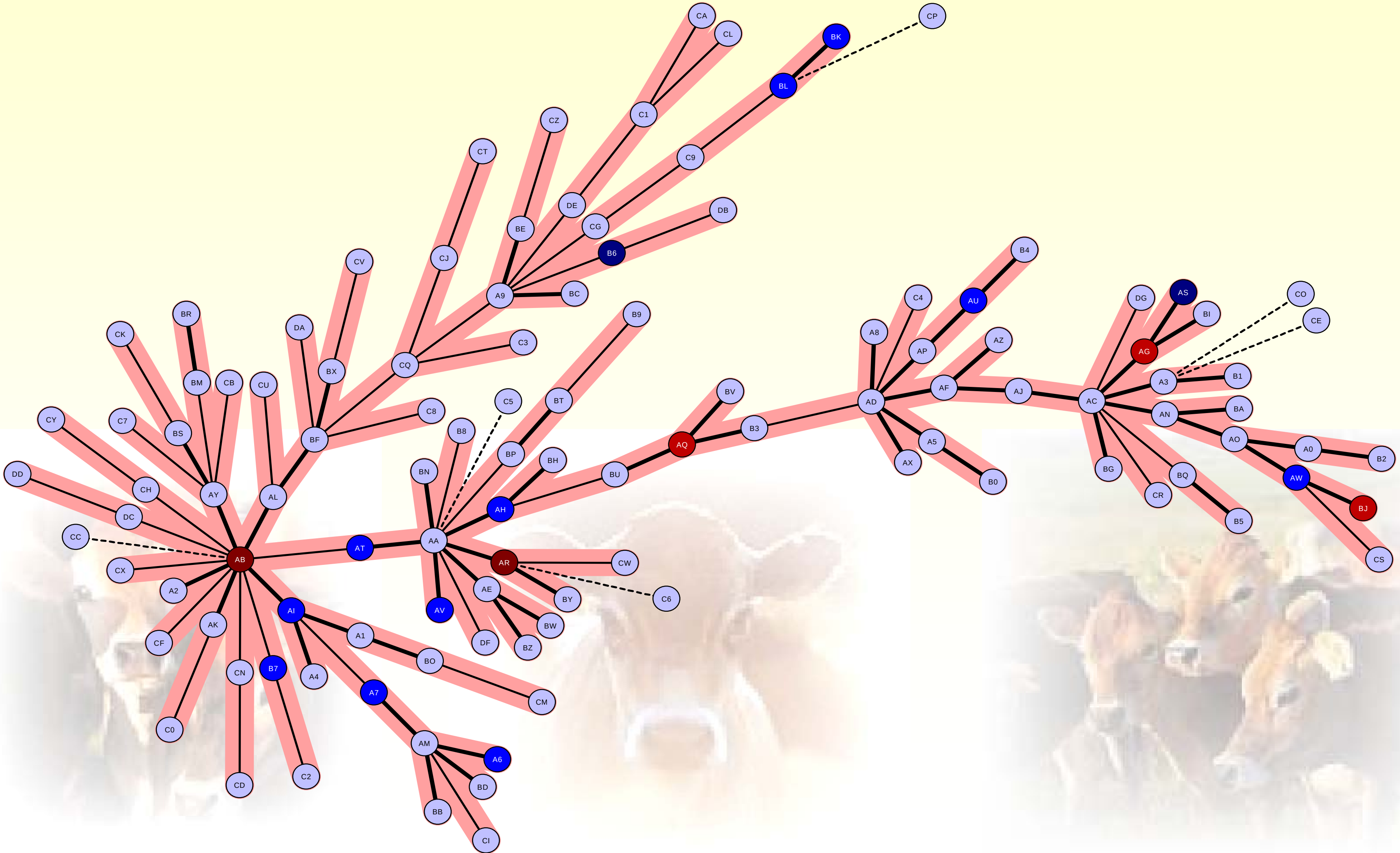
- **Linked to modulation of gene expression in bacteria**
- **From 1bp repeats**
- **Number of repeat units varies from strain to strain because of mispairing during replication that results in introduction or deletion of repeat units**
- **Areas where there is variation called Variable Number of Tandem Repeats (VNTR)**

Combined analyse of several VNTR areas called MLVA

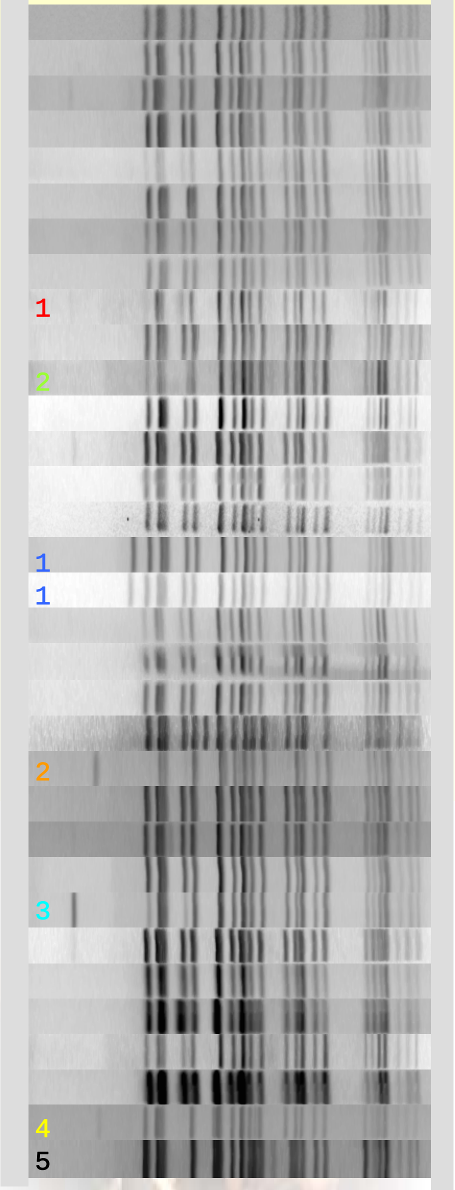
MLVA developed for O157 using 7 VNTR areas

Lindstedt *et al*. 2003

Minimum spanning tree



PFGE XbaI



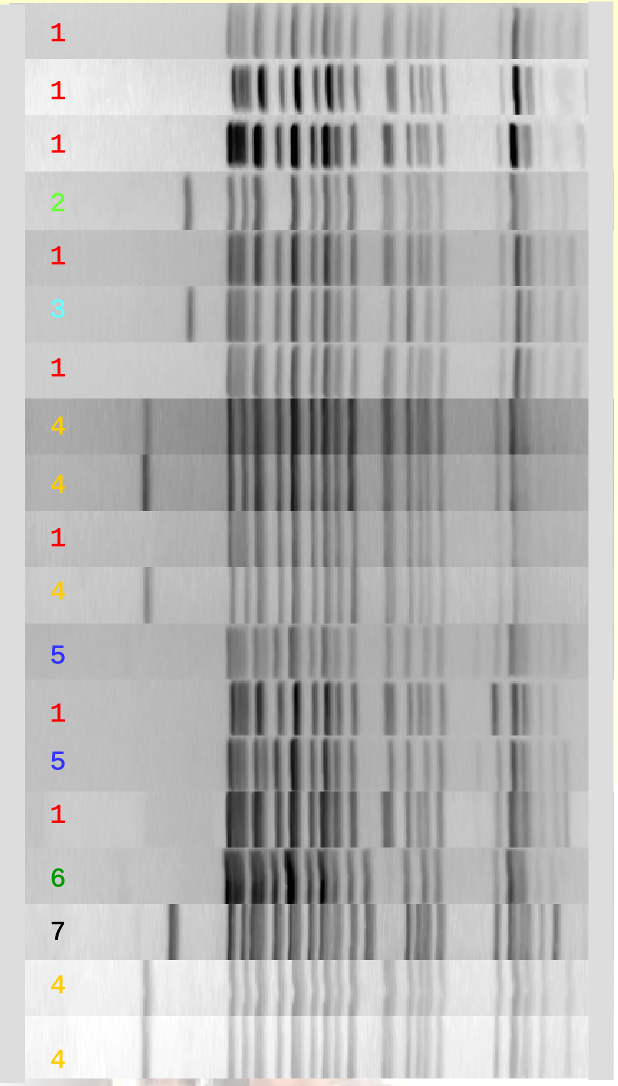
MLVA

Vhec1	Vhec2	Vhec3	Vhec4	Vhec5	Vhec6	Vhec7	Isolate#	Purification
522	434	0	537	470	330	269	1	A
521	433	0	537	470	329	269	1	B
523	434	0	538	469	331	270	2	A
521	434	0	536	470	329	269	2	B
523	434	0	538	470	331	270	3	A
521	433	0	537	470	329	269	3	B
523	435	0	538	470	331	270	4	A
520	433	0	537	470	329	269	4	B
523	434	0	538	470	331	270	5	A
521	433	0	537	470	329	269	5	B
510	434	0	538	470	331	270	6	A
508	433	0	537	469	329	269	6	B
522	435	0	538	469	331	270	1	A
521	433	0	537	470	330	269	1	B
516	434	0	537	470	330	270	2	A
515	434	0	537	470	330	269	2	B
515	434	0	537	470	330	269	2	C
516	435	0	538	470	331	270	3	A
517	434	0	537	470	330	269	3	B
517	434	0	537	470	330	269	3	C
516	434	0	538	469	331	270	4	A
515	433	0	537	470	329	269	4	B
515	433	0	537	470	330	269	4	C
516	434	0	538	469	331	270	5	A
516	437	0	537	469	330	269	5	B
515	434	0	537	470	330	269	5	C
517	435	0	538	469	331	270	6	A
515	433	0	537	470	330	269	6	B
515	434	0	537	470	330	269	6	C
516	435	0	538	469	331	270	7	A
515	434	0	537	470	330	269	7	B
516	435	0	538	469	331	270	8	A
515	433	0	537	470	330	269	8	B

Patient 1

Patient 2

PFGE and MLVA results from siblings involved in the milk associated O157:H- outbreak
MLVA types that differ from the epi-type are indicated with colours.
PFGE types that differ are shown with coloured numbers.



PFGE XbaI	Vhec1	Vhec2	Vhec3	Vhec4	Vhec5	Vhec6	Vhec7	Isolate#	Purification
1	577	417	530	520	0	331	325	1	A
1	576	416	529	520	0	330	324	1	B
1	576	416	529	519	0	330	324	1	C
2	571	417	530	520	0	331	325	2	A
1	570	417	529	520	0	330	324	2	B
3	570	417	529	520	0	330	324	2	C
1	564	416	529	519	0	330	324	2	D
4	571	417	530	520	0	331	325	3	A
4	570	417	530	520	0	331	325	3	B
1	570	417	529	520	0	330	324	3	C
4	570	417	529	519	0	330	324	3	D
5	565	417	529	520	0	331	325	4	A
1	570	417	529	519	0	331	324	4	B
5	563	417	529	520	0	330	324	4	C
1	570	417	529	519	0	330	324	4	D
6	571	417	529	520	0	331	325	5	A
7	569	416	529	519	0	330	324	5	B
4	570	416	529	520	0	330	324	5	C
4	570	416	529	519	0	330	324	5	D

MLVA

PFGE and MLVA results for five isolates from one patient with an O157:H7 infection.
Different MLVA alleles are shown in colours
PFGE types are shown in coloured numbers

Nomenclature of Verocytotoxins

vtx’s

&

VTEC

Main groups

Stx1/VT1 and VT2

Subtypes

Suffixed with small Arabic letters

Stx1: Shiga toxin from *Shigella* *stx1*a

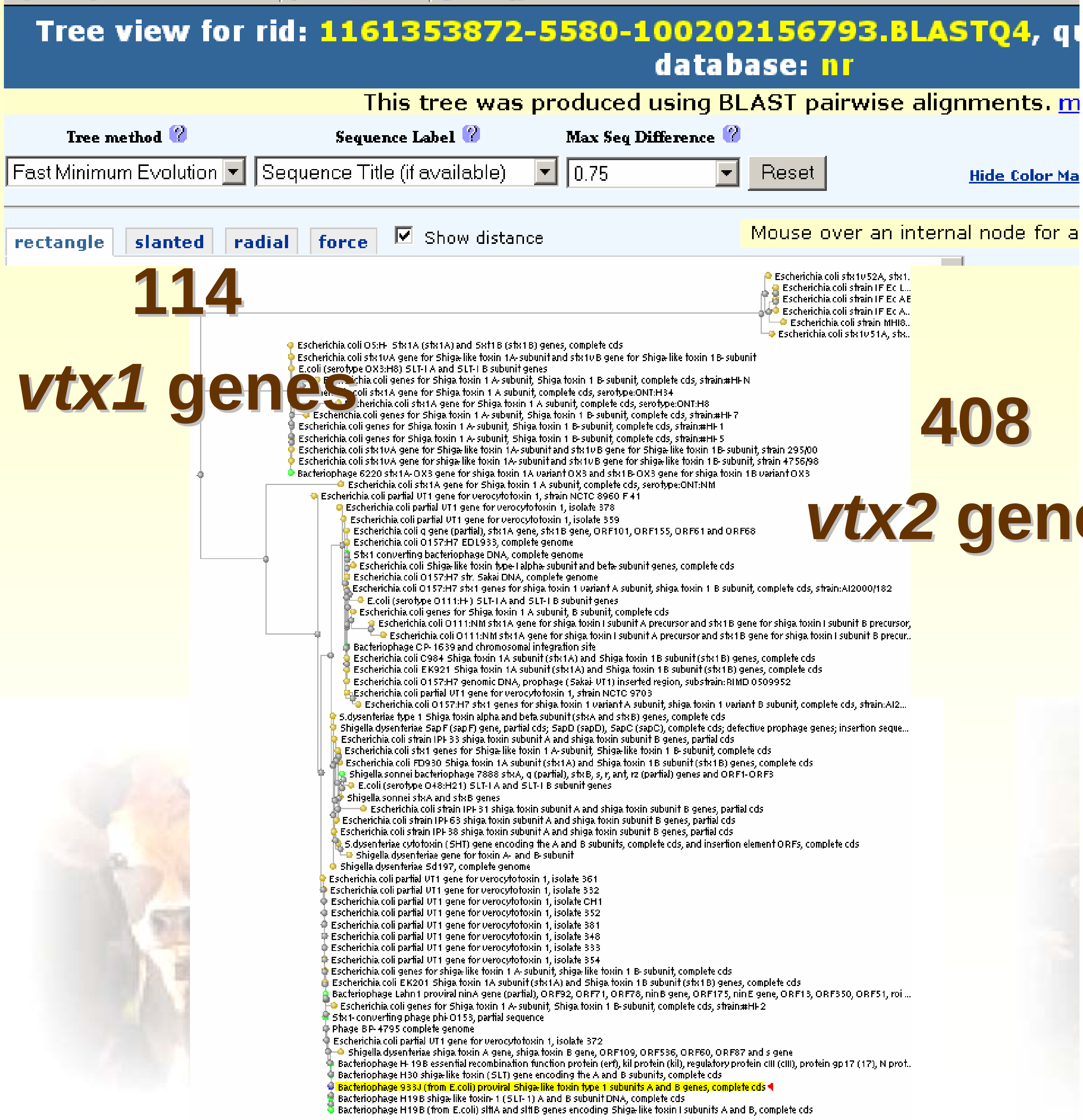
VT1: *E. coli* Verocytotoxins *vtx1*b to *vtx1*d

VT2: *vtx2*a to *vtx2*g

Variant designations

Subtype - first published O group - strain No.

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



Tree view for rid: 1161353872-5580-100202156793.BLASTQ4, q

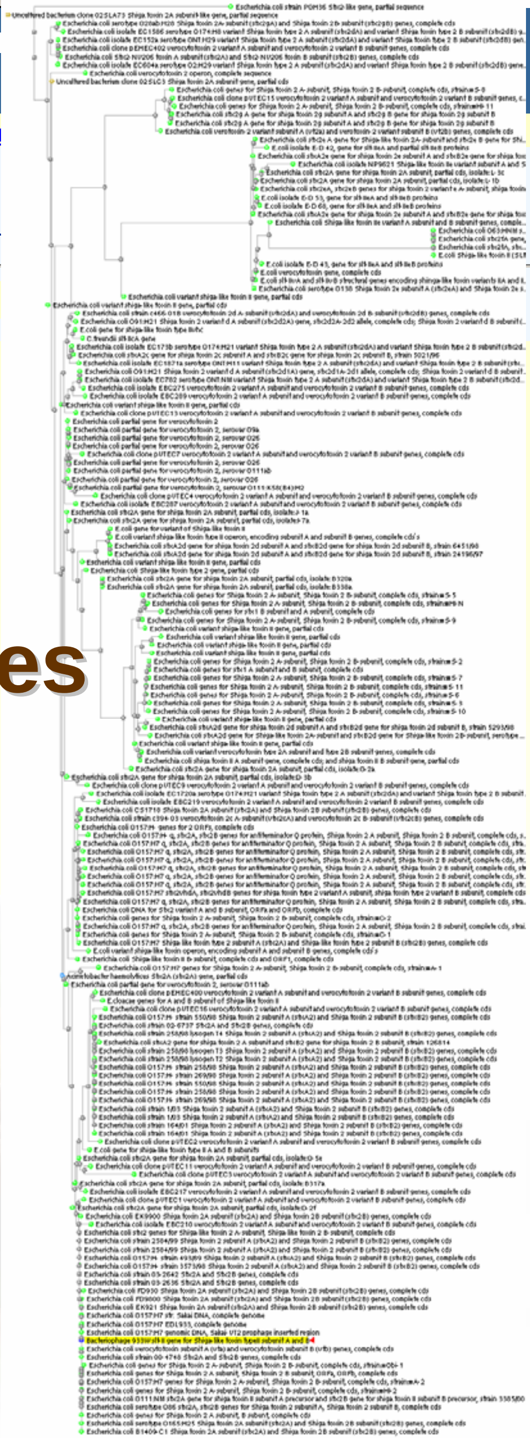
database: nr

This tree was produced using BLAST pairwise alignments. m

☑ Show distance

Mouse over an internal node for a

- Escherichia coli stx1v52A, stx1.
- Escherichia coli strain IF Ec L...
- Escherichia coli strain IF Ec AE
- Escherichia coli strain IF Ec A...
- Escherichia coli strain MHI8..
- Escherichia coli stx1v51A, stx..
- Escherichia coli O5:H- Stx1A (stx1A) and Sxt1B (stx1B) genes, complete cds
- Escherichia coli stx1vA gene for Shiga-like toxin 1A-subunit and stx1vB gene for Shiga-like toxin 1B-subunit
- E.coli (serotype OX3:H8) SLT-I A and SLT-I B subunit genes
- E[illegible]chia coli genes for Shiga toxin 1 A-subunit, Shiga toxin 1 B-subunit, complete cds, strain:#H-N
- [illegible] coli stx1A gene for Shiga toxin 1 A subunit, complete cds, serotype:ONT:H34
- [illegible]cherichia coli stx1A gene for Shiga toxin 1 A subunit, complete cds, serotype:ONT:H8
- Escherichia coli genes for Shiga toxin 1 A-subunit, Shiga toxin 1 B-subunit, complete cds, strain:#H-7
- Escherichia coli genes for Shiga toxin 1 A-subunit, Shiga toxin 1 B-subunit, complete cds, strain:#H-1
- Escherichia coli genes for Shiga toxin 1 A-subunit, Shiga toxin 1 B-subunit, complete cds, strain:#H-5
- Escherichia coli stx1vA gene for Shiga-like toxin 1A-subunit and stx1vB gene for Shiga-like toxin 1B-subunit, strain 295/00
- Escherichia coli stx1vA gene for shiga-like toxin 1A-subunit and stx1vB gene for shiga-like toxin 1B-subunit, strain 4756/98
- Bacteriophage 6220 stx1A-OX3 gene for shiga toxin 1A variant OX3 and stx1B-OX3 gene for shiga toxin 1B variant OX3
- Escherichia coli stx1A gene for Shiga toxin 1 A subunit, complete cds, serotype:ONT:NM
- Escherichia coli partial VT1 gene for verocytotoxin 1, strain NCTC 8960 F41
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 378
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 359
- Escherichia coli q gene (partial), stx1A gene, stx1B gene, ORF101, ORF155, ORF61 and ORF68
- Escherichia coli O157:H7 EDL933, complete genome
- Stx1 converting bacteriophage DNA, complete genome
- Escherichia coli Shiga-like toxin type-I alpha-subunit and beta-subunit genes, complete cds
- Escherichia coli O157:H7 str. Sakai DNA, complete genome
- Escherichia coli O157:H7 stx1 genes for shiga toxin 1 variant A subunit, shiga toxin 1 B subunit, complete cds, strain:AI2000/182
- E.coli (serotype O111:H-) SLT-I A and SLT-I B subunit genes
- Escherichia coli genes for Shiga toxin 1 A subunit, B subunit, complete cds
- Escherichia coli O111:NM stx1A gene for shiga toxin I subunit A precursor and stx1B gene for shiga toxin I subunit B precursor,
- Escherichia coli O111:NM stx1A gene for shiga toxin I subunit A precursor and stx1B gene for shiga toxin I subunit B precur..
- Bacteriophage CP-1639 and chromosomal integration site
- Escherichia coli C984 Shiga toxin 1A subunit (stx1A) and Shiga toxin 1B subunit (stx1B) genes, complete cds
- Escherichia coli EK921 Shiga toxin 1A subunit (stx1A) and Shiga toxin 1B subunit (stx1B) genes, complete cds
- Escherichia coli O157:H7 genomic DNA, prophage (Sakai-VT1) inserted region, substrain:RIMD 0509952
- Escherichia coli partial VT1 gene for verocytotoxin 1, strain NCTC 9703
- Escherichia coli O157:H7 stx1 genes for shiga toxin 1 variant A subunit, shiga toxin 1 variant B subunit, complete cds, strain:AI2...
- S.dysenteriae type 1 Shiga toxin alpha and beta subunit (stxA and stxB) genes, complete cds
- Shigella dysenteriae SapF (sapF) gene, partial cds; SapD (sapD), SapC (sapC), complete cds; defective prophage genes; insertion seque...
- Escherichia coli strain IPH33 shiga toxin subunit A and shiga toxin subunit B genes, partial cds
- Escherichia coli stx1 genes for Shiga-like toxin 1 A-subunit, Shiga-like toxin 1 B-subunit, complete cds
- Escherichia coli FD930 Shiga toxin 1A subunit (stx1A) and Shiga toxin 1B subunit (stx1B) genes, complete cds
- Shigella sonnei bacteriophage 7888 stxA, q (partial), stxB, s, r, ant, rz (partial) genes and ORF1-ORF3
- E.coli (serotype O48:H21) SLT-I A and SLT-I B subunit genes
- Shigella sonnei stxA and stxB genes
- Escherichia coli strain IPH31 shiga toxin subunit A and shiga toxin subunit B genes, partial cds
- Escherichia coli strain IPH63 shiga toxin subunit A and shiga toxin subunit B genes, partial cds
- Escherichia coli strain IPH38 shiga toxin subunit A and shiga toxin subunit B genes, partial cds
- S.dysenteriae cytotoxin (SHT) gene encoding the A and B subunits, complete cds, and insertion element ORFs, complete cds
- Shigella dysenteriae gene for toxin A- and B-subunit
- Shigella dysenteriae Sd197, complete genome
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 361
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 332
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate CH1
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 352
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 381
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 348
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 333
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 354
- Escherichia coli genes for shiga-like toxin 1 A-subunit, shiga-like toxin 1 B-subunit, complete cds
- Escherichia coli EK201 Shiga toxin 1A subunit (stx1A) and Shiga toxin 1B subunit (stx1B) genes, complete cds
- Bacteriophage Lahn1 proviral ninA gene (partial), ORF92, ORF71, ORF78, ninB gene, ORF175, ninE gene, ORF13, ORF350, ORF51, roi ...
- Escherichia coli genes for Shiga toxin 1 A-subunit, Shiga toxin 1 B-subunit, complete cds, strain:#H-2
- Stx1-converting phage phi-O153, partial sequence
- Phage BP-4795 complete genome
- Escherichia coli partial VT1 gene for verocytotoxin 1, isolate 372
- Shigella dysenteriae shiga toxin A gene, shiga toxin B gene, ORF109, ORF536, ORF60, ORF87 and s gene
- Bacteriophage H-19B essential recombination function protein (erf), kil protein (kil), regulatory protein cIII (cIII), protein gp17 (17), N prot..
- Bacteriophage H30 shiga-like toxin (SLT) gene encoding the A and B subunits, complete cds
- Bacteriophage 933J (from E.coli) proviral Shiga-like toxin type 1 subunits A and B genes, complete cds
- Bacteriophage H19B shiga-like toxin-1 (SLT-1) A and B subunit DNA, complete cds
- Bacteriophage H19B (from E.coli) sltIA and sltIB genes encoding Shiga-like toxin I subunits A and B, complete cds

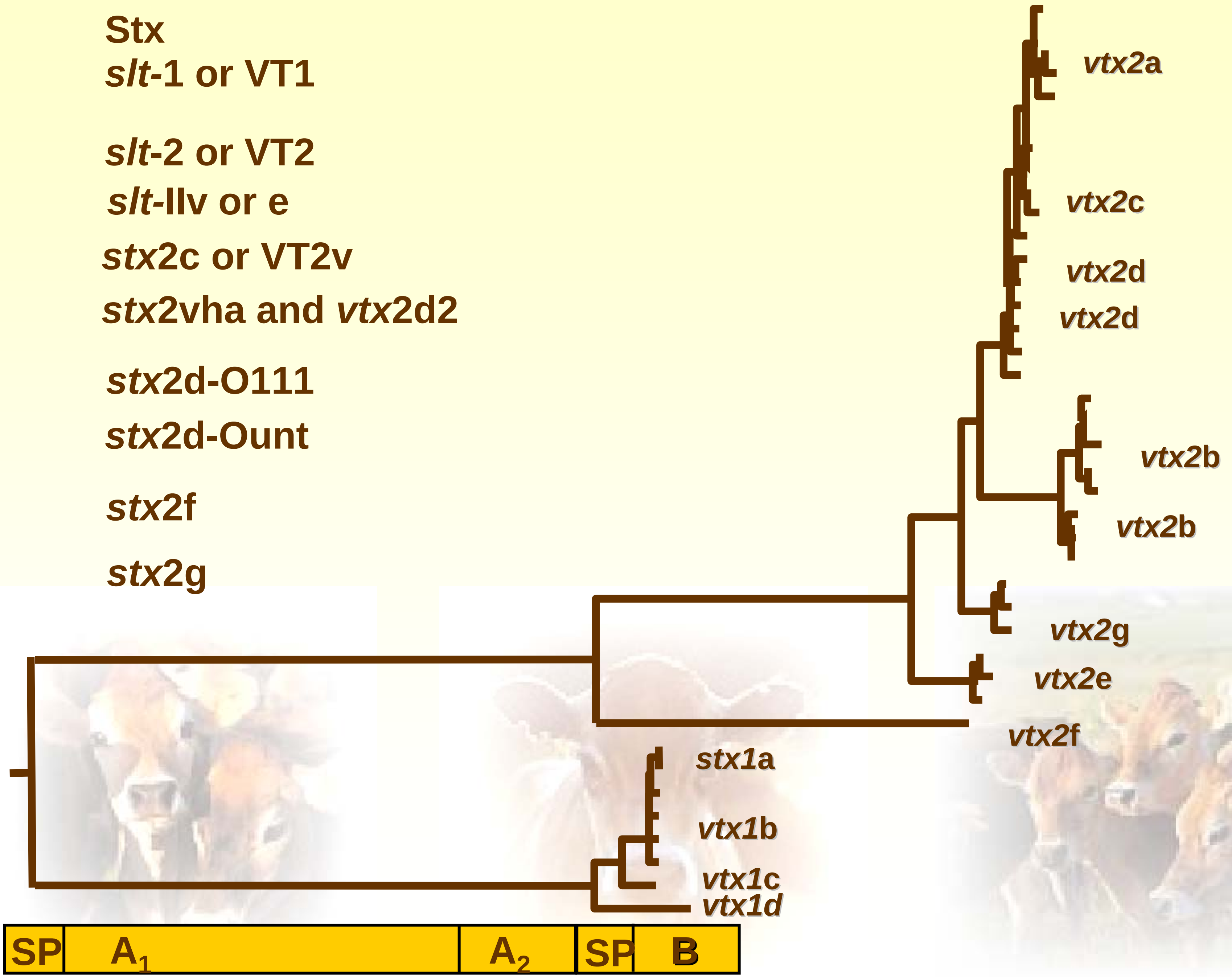


114

***vtx1* genes**

408

***vtx2* genes**



Stx

slt-1 or VT1

slt-2 or VT2

slt-IIv or e

*stx*2c or VT2v

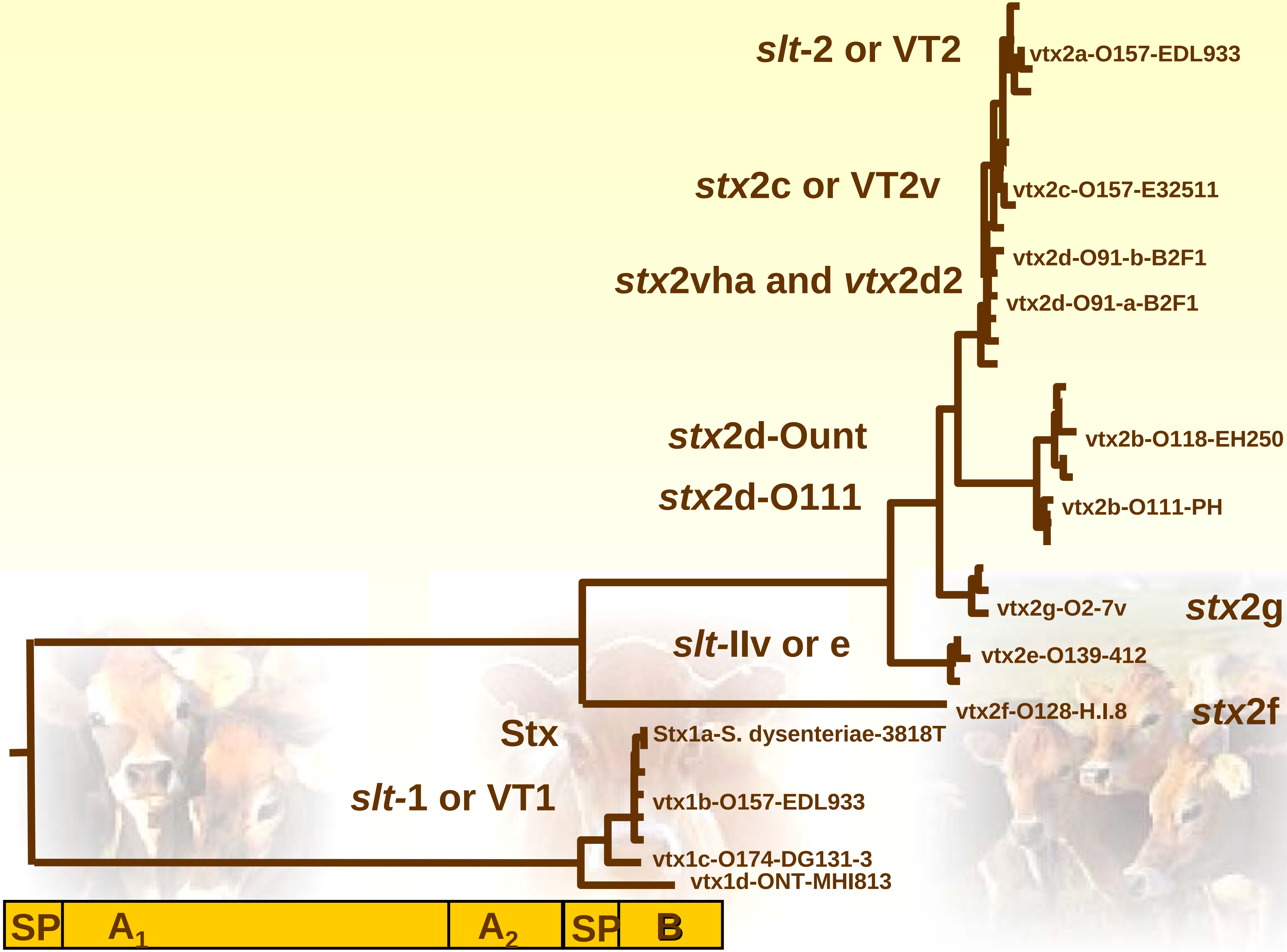
*stx*2vha and *vtx*2d2

*stx*2d-O111

*stx*2d-Ount

*stx*2f

*stx*2g



***slt*-2 or VT2**

vtx2a-O157-EDL933

***stx*2c or VT2v**

vtx2c-O157-E32511

vtx2d-O91-b-B2F1

***stx*2vha and *vtx*2d2**

vtx2d-O91-a-B2F1

***stx*2d-Ount**

vtx2b-O118-EH250

***stx*2d-O111**

vtx2b-O111-PH

vtx2g-O2-7v

***stx*2g**

***slt*-IIv or e**

vtx2e-O139-412

vtx2f-O128-H.I.8

***stx*2f**

Stx

Stx1a-S. dysenteriae-3818T

***slt*-1 or VT1**

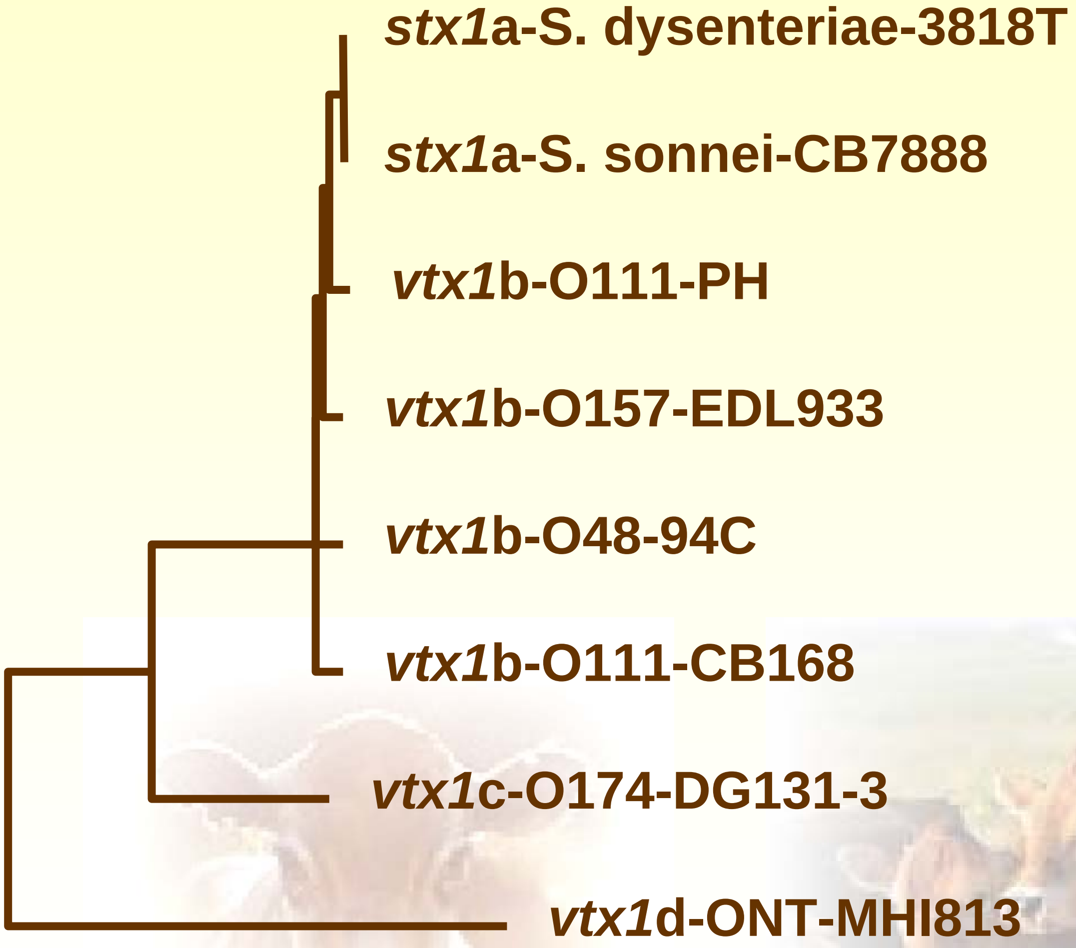
vtx1b-O157-EDL933

vtx1c-O174-DG131-3

vtx1d-ONT-MHI813

SP **A_1** **A_2** **SP** **B**

8 *vtx1* variant designations



*stx1*a-S. dysenteriae-3818T

*stx1*a-S. sonnei-CB7888

*vtx1*b-O111-PH

*vtx1*b-O157-EDL933

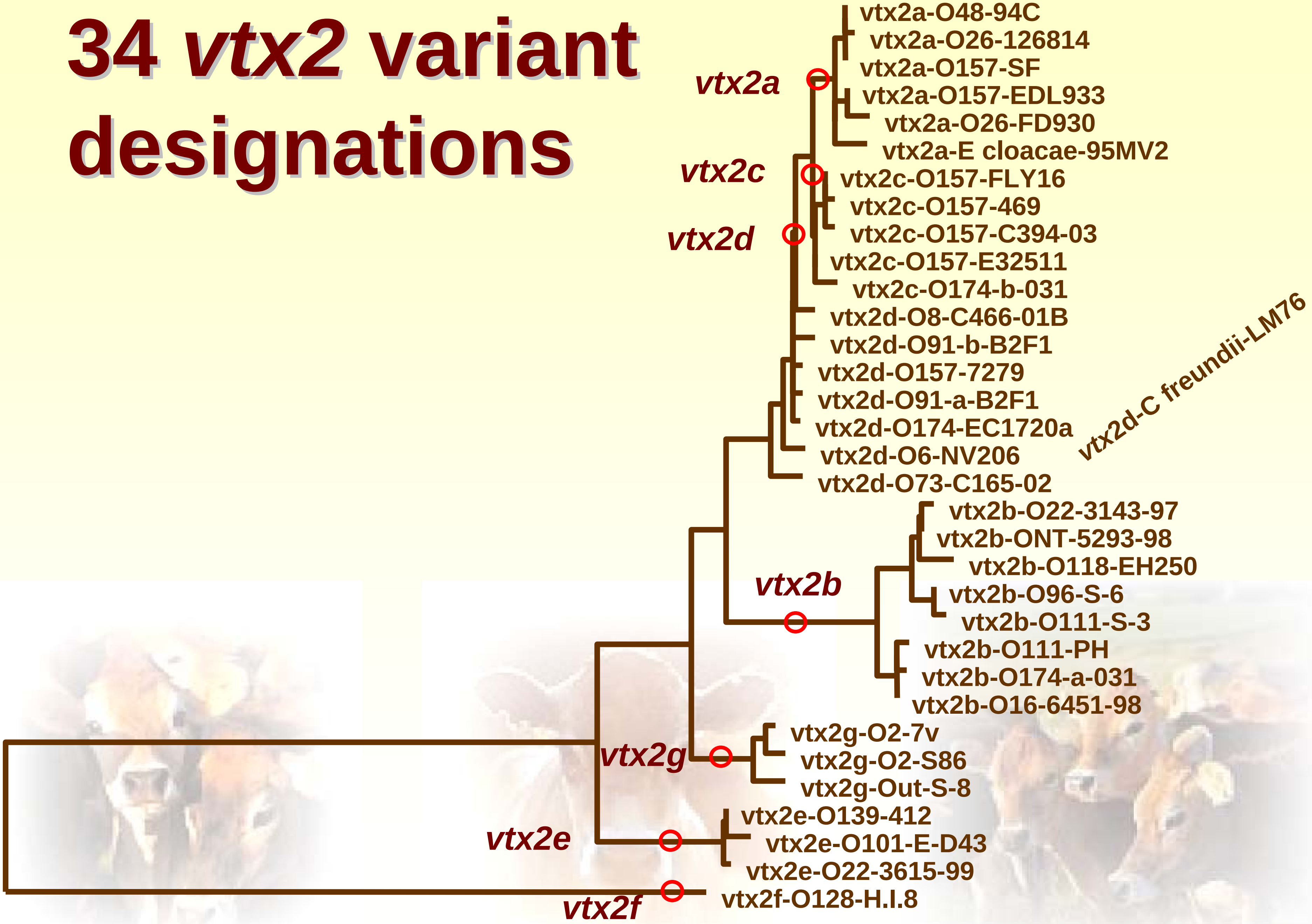
*vtx1*b-O48-94C

*vtx1*b-O111-CB168

*vtx1*c-O174-DG131-3

*vtx1*d-ONT-MHI813

34 *vtx2* variant designations



vtx2a
vtx2a-O48-94C
vtx2a-O26-126814
vtx2a-O157-SF
vtx2a-O157-EDL933
vtx2a-O26-FD930
vtx2a-E cloacae-95MV2

vtx2c
vtx2c-O157-FLY16
vtx2c-O157-469
vtx2c-O157-C394-03
vtx2c-O157-E32511
vtx2c-O174-b-031

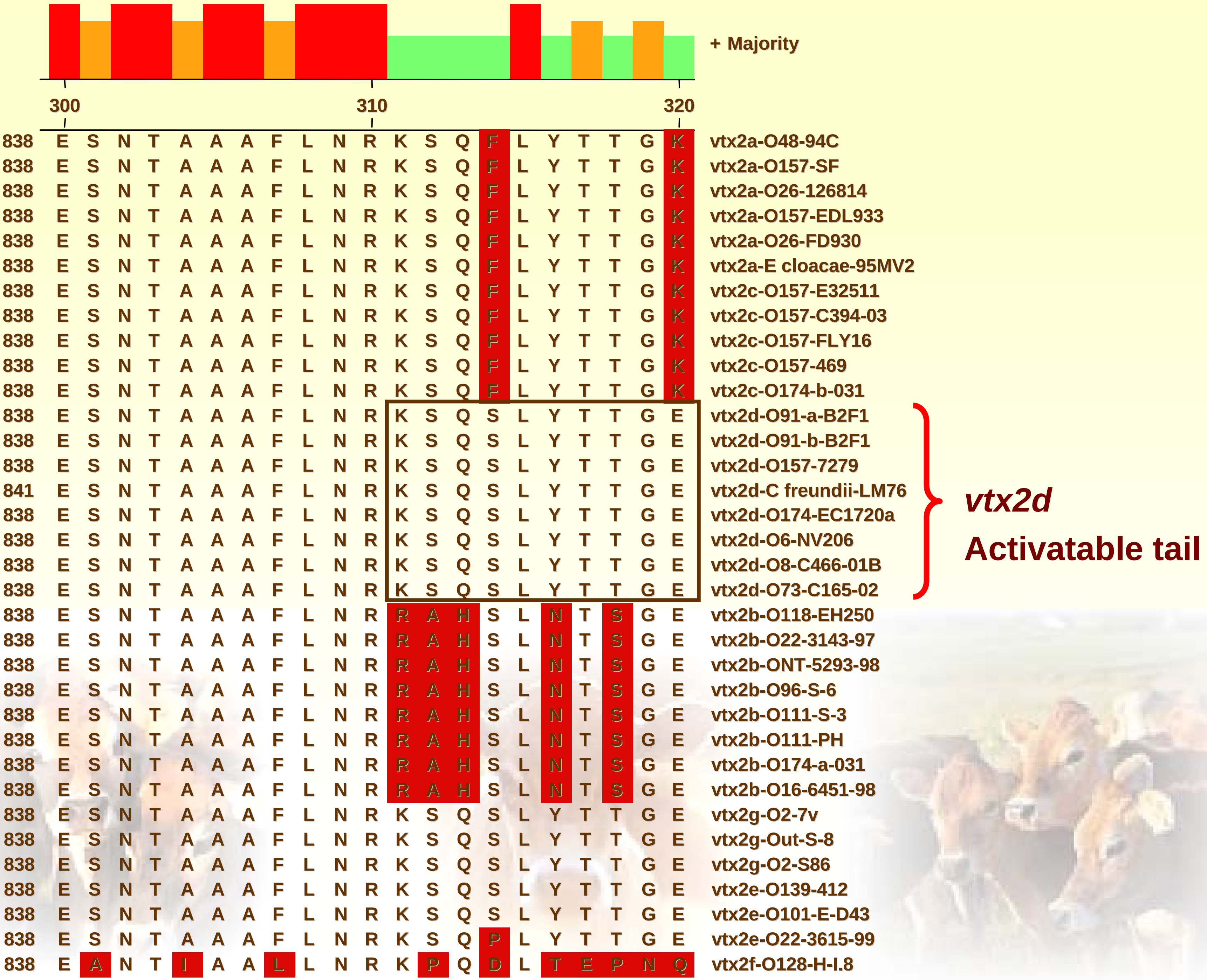
vtx2d
vtx2d-O8-C466-01B
vtx2d-O91-b-B2F1
vtx2d-O157-7279
vtx2d-O91-a-B2F1
vtx2d-O174-EC1720a
vtx2d-O6-NV206
vtx2d-O73-C165-02
vtx2d-C freundii-LM76

vtx2b
vtx2b-O22-3143-97
vtx2b-ONT-5293-98
vtx2b-O118-EH250
vtx2b-O96-S-6
vtx2b-O111-S-3
vtx2b-O111-PH
vtx2b-O174-a-031
vtx2b-O16-6451-98

vtx2g
vtx2g-O2-7v
vtx2g-O2-S86
vtx2g-Out-S-8

vtx2e
vtx2e-O139-412
vtx2e-O101-E-D43
vtx2e-O22-3615-99

vtx2f
vtx2f-O128-H.I.8



838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-O48-94C
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-O157-SF
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-O26-126814
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-O157-EDL933
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-O26-FD930
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2a-E cloacae-95MV2
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2c-O157-E32511
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2c-O157-C394-03
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2c-O157-FLY16
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2c-O157-469
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	F	L	Y	T	T	G	K	vtx2c-O174-b-031
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O91-a-B2F1
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O91-b-B2F1
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O157-7279
841	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-C freundii-LM76
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O174-EC1720a
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O6-NV206
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O8-C466-01B
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2d-O73-C165-02
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O118-EH250
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O22-3143-97
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-ONT-5293-98
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O96-S-6
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O111-S-3
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O111-PH
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O174-a-031
838	E	S	N	T	A	A	A	F	L	N	R	R	A	H	S	L	N	T	S	G	E	vtx2b-O16-6451-98
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2g-O2-7v
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2g-Out-S-8
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2g-O2-S86
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2e-O139-412
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	S	L	Y	T	T	G	E	vtx2e-O101-E-D43
838	E	S	N	T	A	A	A	F	L	N	R	K	S	Q	P	L	Y	T	T	G	E	vtx2e-O22-3615-99
838	E	A	N	T	I	A	A	L	L	N	R	K	P	Q	D	L	T	E	P	N	Q	vtx2f-O128-H-I.8

vtx2d

Activatable tail

*vtx2*a variant designations

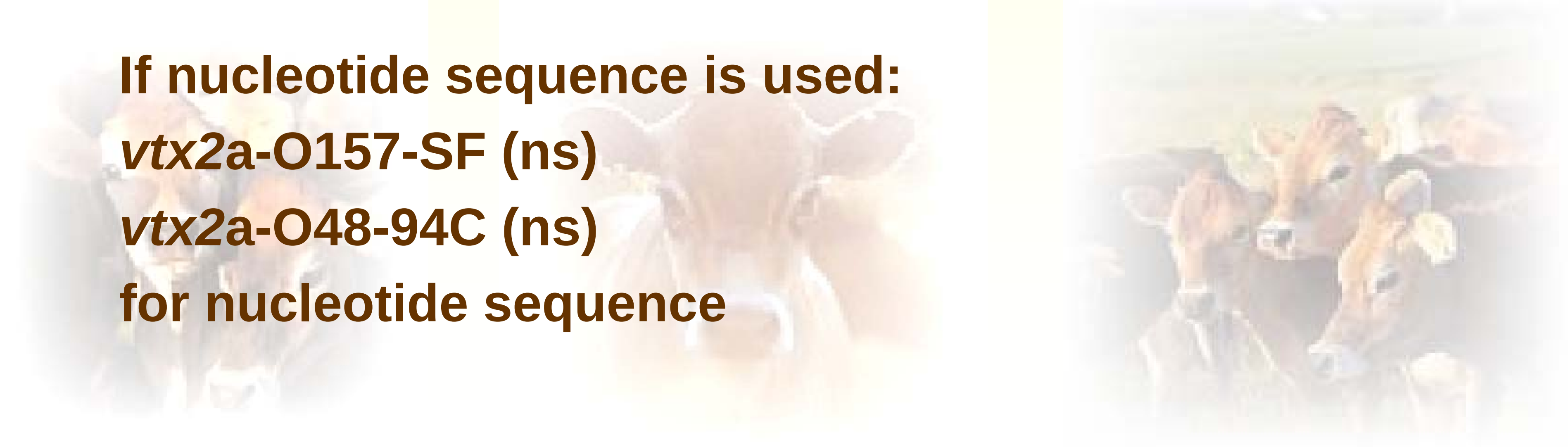
Non-O157 VTEC

*vtx2*a-O48-94C

*vtx2*a-O26-126814

O157- Sorbitol fermenting

*vtx2*a-O157-SF



If nucleotide sequence is used:

*vtx2*a-O157-SF (ns)

*vtx2*a-O48-94C (ns)

for nucleotide sequence

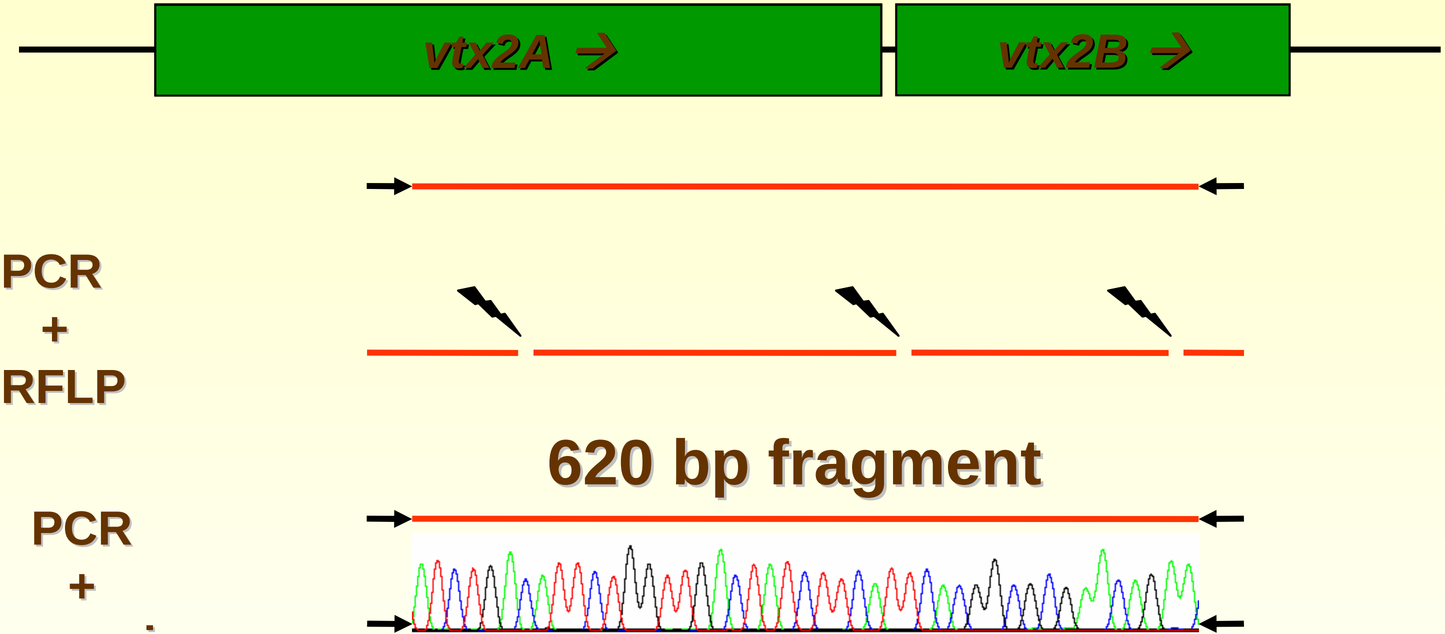
2006 Enter-net ringtrial results

Serotype	*vtx1*	32 laboratories
O38:H26	*vtx1*c	2 negative
O128ab:H2	*vtx1*c	1 neagtive
O154:H31	*vtx1*d	9 negative
O157:H7	*vtx1* negative	1 positive
O145:H4	*vtx1* negative	1 positive
O113:H4	*vtx1* negative	2 positive

2006 Enter-net ringtrial results

Serotype	*vtx2*	32 laboratories
O103:H25	*vtx2*a-O157-EDL933	5 negative
O38:H26	*vtx2*b-O111-PH	5 negative
O128ab:H2	*vtx2*b-O111-PH	3 negative
	*vtx2*b-O118-EH250	3 negative
O154:H31	*vtx2* negative	4 positive
O157:H7	*vtx2*a-O157-EDL933	4 negative
	*vtx2*c-O157-FLY16	
O145:H4	*vtx2*a-O48-94C	4 negative
O113:H4	*vtx2*d-O157-7279	7 negative
O51:H49	*vtx2*e-ONT-26725-97	7 negative

vtx2 subtyping



PCR
+
RFLP

620 bp fragment

PCR
+
sequencing

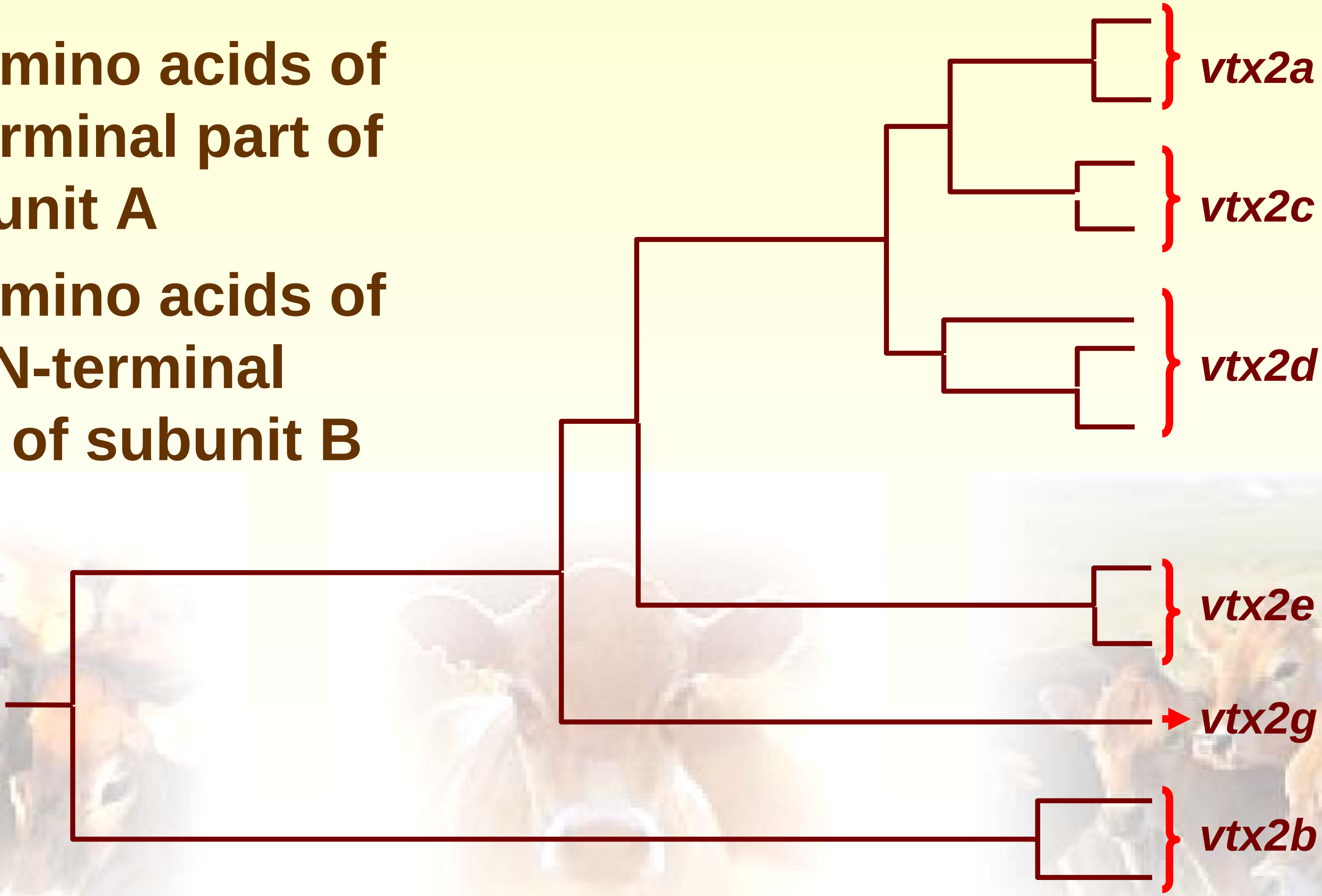
491 nucleotide sequences translated into aminoacids were used for comparison and phylogenetic trees

Søren Persson’s Ph.D.

Phylogenetic tree of *vtx*2 subgroups found in Danish patients by alignment of the 159 aa (491 bp) fragment

95 amino acids of C-terminal part of subunit A

64 amino acids of the N-terminal part of subunit B

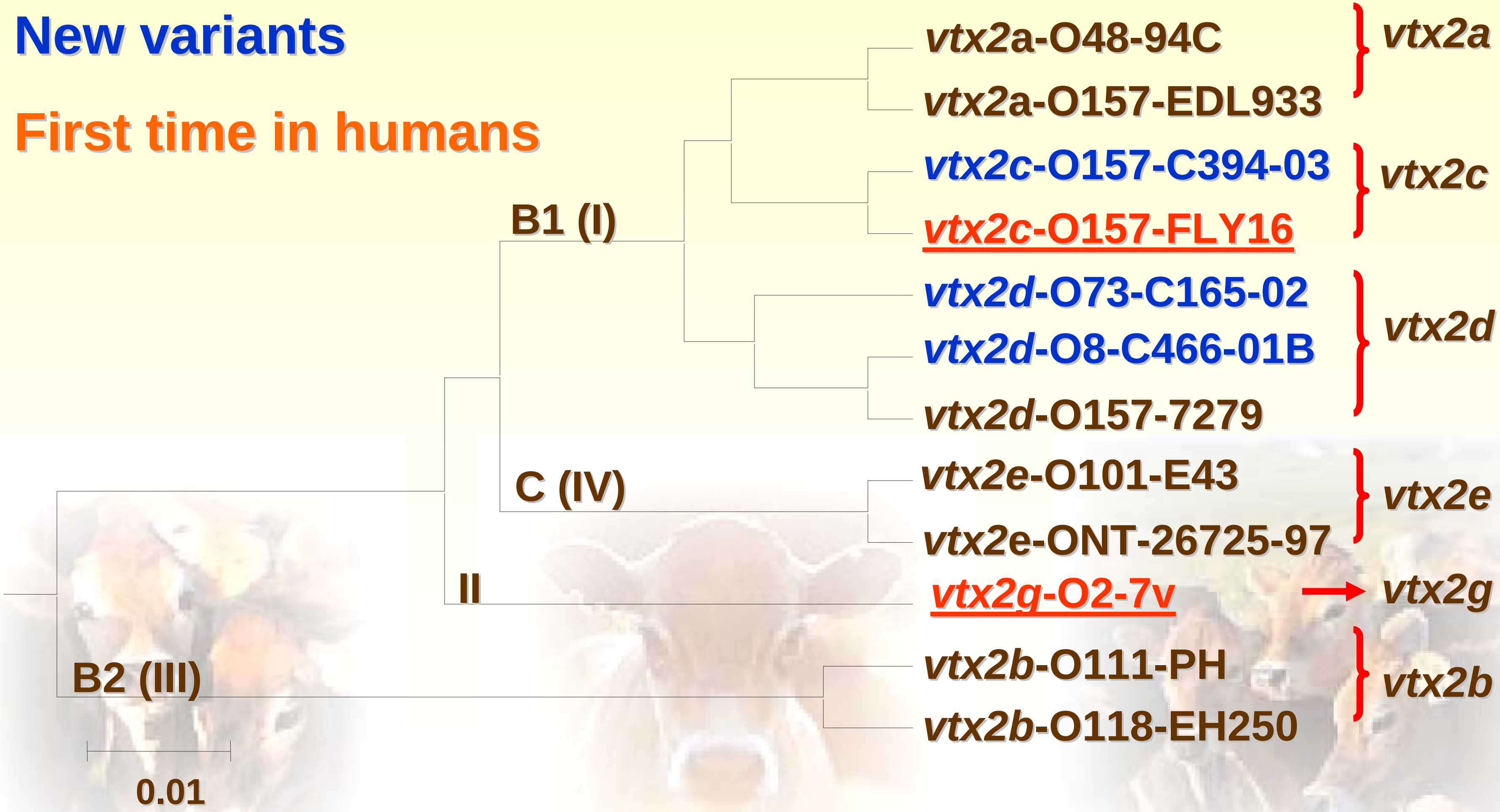


Søren Persson's Ph.D.

The 12 *vtx2* genes found in Danish patients

New variants

First time in humans



B1 (I)

C (IV)

II

B2 (III)

0.01

*vtx2*a-O48-94C

*vtx2*a-O157-EDL933

vtx2c-O157-C394-03

vtx2c-O157-FLY16

vtx2d-O73-C165-02

vtx2d-O8-C466-01B

vtx2d-O157-7279

*vtx2*e-O101-E43

*vtx2*e-ONT-26725-97

vtx2g-O2-7v

vtx2b-O111-PH

vtx2b-O118-EH250

vtx2a

vtx2c

vtx2d

vtx2e

vtx2g

vtx2b

Søren Persson’s Ph.D.

Prevalence of *vtx1* subtypes & *vtx2* variants in 102 O157

*vtx1*b + *vtx2*c-O157-FLY16	27
*vtx1*b + *vtx2*c-O157-C394-03	14
*vtx1*b + *vtx2*a-O157-EDL933	1
*vtx1*c + *vtx2*c-O157-FLY16	1

*vtx2*a-O157-EDL933 + *vtx2*c-O157-FLY16	23
*vtx2*a-O157-EDL933	17
*vtx2*c-O157-FLY16	18
*vtx2*a-O157-SF + *vtx2*c-O157-FLY16	1

Prevalence of *vtx1* subtypes & *vtx2* variants in 183 *eae* positive non-O157

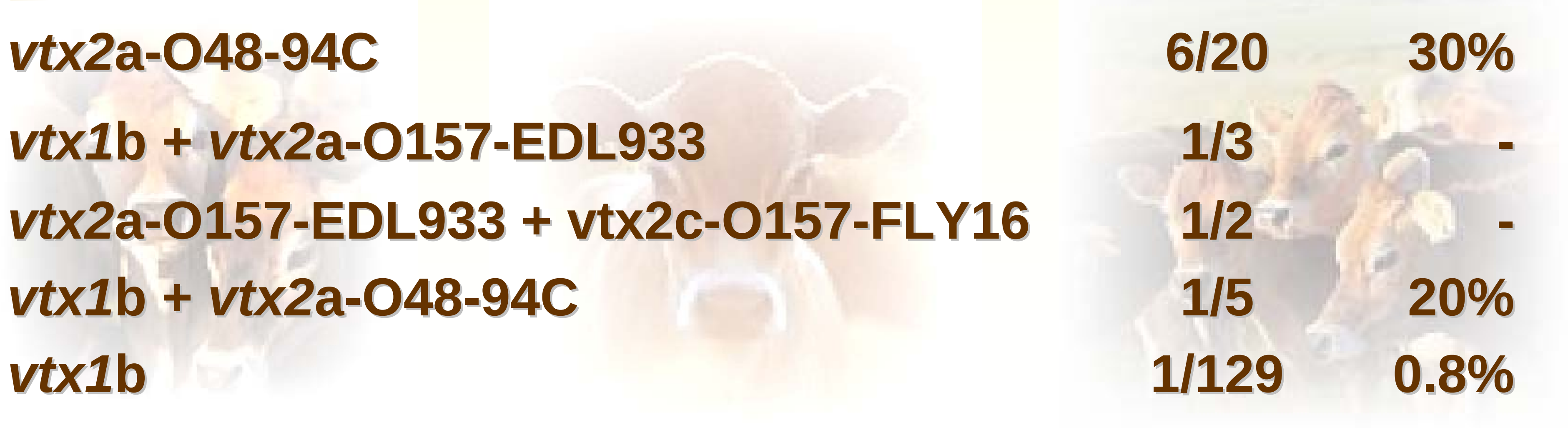
*vtx1*b	129
*vtx2*a-O48-94C	20
*vtx2*a-O157-EDL933	16
*vtx1*b + *vtx2*a-O48-94C	5
*vtx1*b + *vtx2*a-O157-EDL933	3
*vtx2*c-O157-FLY16	3
*vtx2*a-O157-EDL933 + vtx2c-O157-FLY16	2
*vtx1*c	1
*vtx1*d	1
*vtx2*d-O8-C466-01B	1
*vtx2*e-ONT-26725-97	1
vtx2g-O2-7v	1

Prevalence of *vtx1* subtypes & *vtx2* variants in 144 *eae* negative non-O157

***vtx1*b**	**32**
***vtx2*b-O111-PH**	**18**
***vtx1*c**	**17**
***vtx1*c + *vtx2*b-O118-EH250**	**15**
***vtx1*b + *vtx2*b-O118-EH250**	**10**
***vtx1*c + *vtx2*b-O111-PH**	**8**
***vtx1*d**	**6**
***vtx1*b + *vtx2*b-O111-PH**	**5**
***vtx2*a-O48-94C**	**4**
***vtx1*c + *vtx2*b-O111-PH + *vtx2*b-O118-EH250**	**4**
***vtx2*c-O157-FLY16**	**4**
***vtx2*d-O157-7279**	**3**
***vtx1*b + *vtx2*b-O118-EH250 + *vtx2*b-O111-PH**	**2**
***vtx2*e-ONT-26725-97**	**2**

***vtx1*b + *vtx2*d-O157-7279**	**1**
***vtx1*b + *vtx2*c-O157-FLY16**	**1**
***vtx1*b + *vtx2*a-O48-94C**	**1**
***vtx1*b + *vtx2*a-O48 + *vtx2*b-O111-PH**	**1**
***vtx1*c + *vtx2*a-O157-EDL933 + *vtx2*-O118-EH350**	**1**
***vtx2*a-O48-94C + *vtx2*d-O157-7279**	**1**
***vtx2*e-O101-E-D43**	**1**
***vtx2*b-O118-EH250**	**1**
***vtx2*b-O111-PH + *vtx2*b-O118-EH250**	**1**
***vtx2*c-O157-FLY16 + *vtx2*d-O8-C46601B**	**1**
***vtx2*d-O73-C165-02**	**1**
***vtx2*a-O48-94C** + ***vtx2*d-O8-C466-01B**	**1**
***vtx2*d-O8-C466-01B**	**1**
***vtx2*g-O2-7v**	**1**

Attack rate of *vtx2* variants associated with HUS



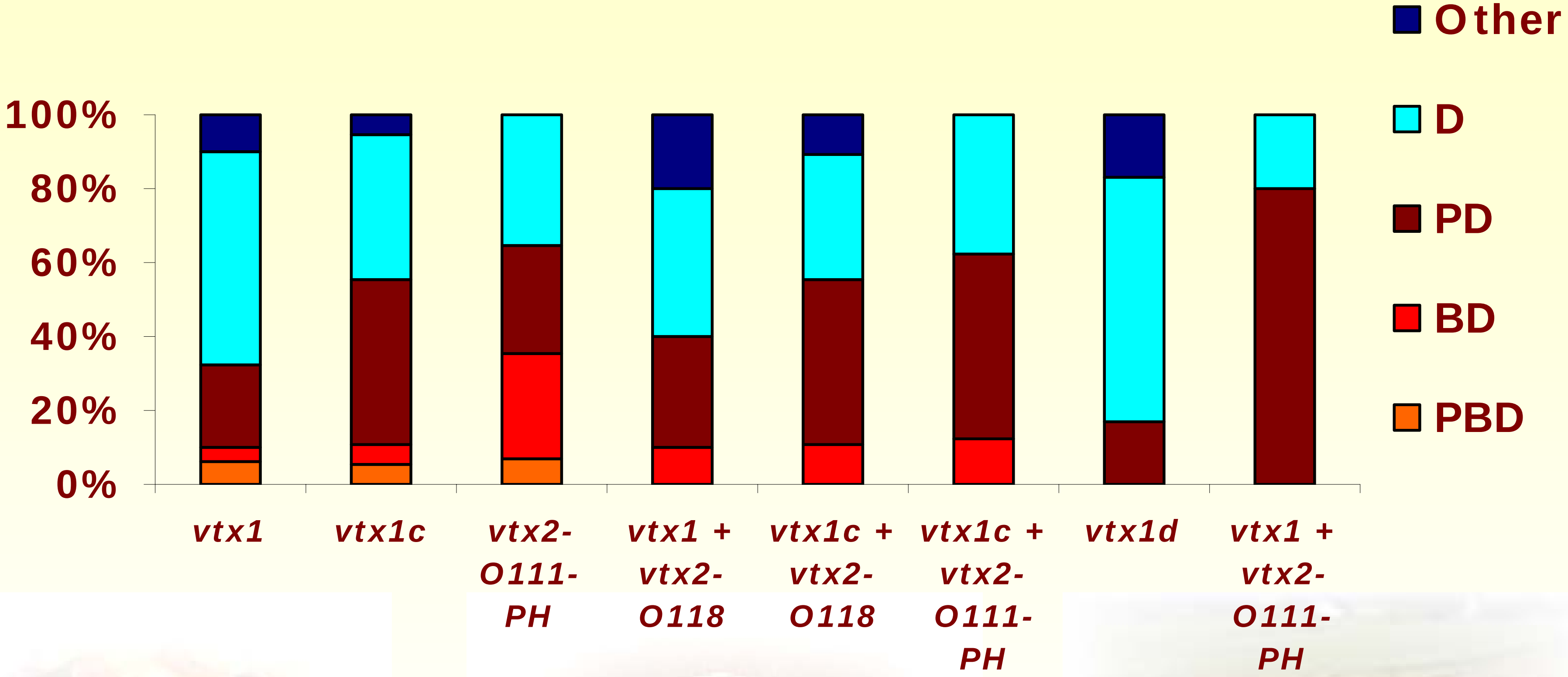
O157

*vtx2*a-O157-EDL933 + *vtx2*c-O157-FLY16	6/23	26%
*vtx2*a-O157-EDL933	3/17	18%
*vtx2*c-O157-FLY16	1/18	6%
*vtx2*a-O157-SF + *vtx2*c-O157-FLY16	1/1	-

Non-O157

*vtx2*a-O48-94C	6/20	30%
*vtx1*b + *vtx2*a-O157-EDL933	1/3	-
*vtx2*a-O157-EDL933 + vtx2c-O157-FLY16	1/2	-
*vtx1*b + *vtx2*a-O48-94C	1/5	20%
*vtx1*b	1/129	0.8%

Virulenceprofile of 101 Danish *eae* negative non-O157 VTEC and clinical manifestation



- ■ **Other: Asymptomatic carriers and non-diarrhoeal symptoms**
- ■ **D: Diarrhoea**
- ■ **PD: Persistent diarrhoea (more than 2 weeks)**
- ■ **BD: Bloody diarrhoea**
- ■ **PBD: Persistent bloody diarrhoea (more than 2 weeks)**

Conclusions

There is no definitive typing method for VTEC

Standard methods:

- O grouping
- O:H serotyping
- Virulo-typing
- Sero-pathovar typing
- PFGE
- MLVA

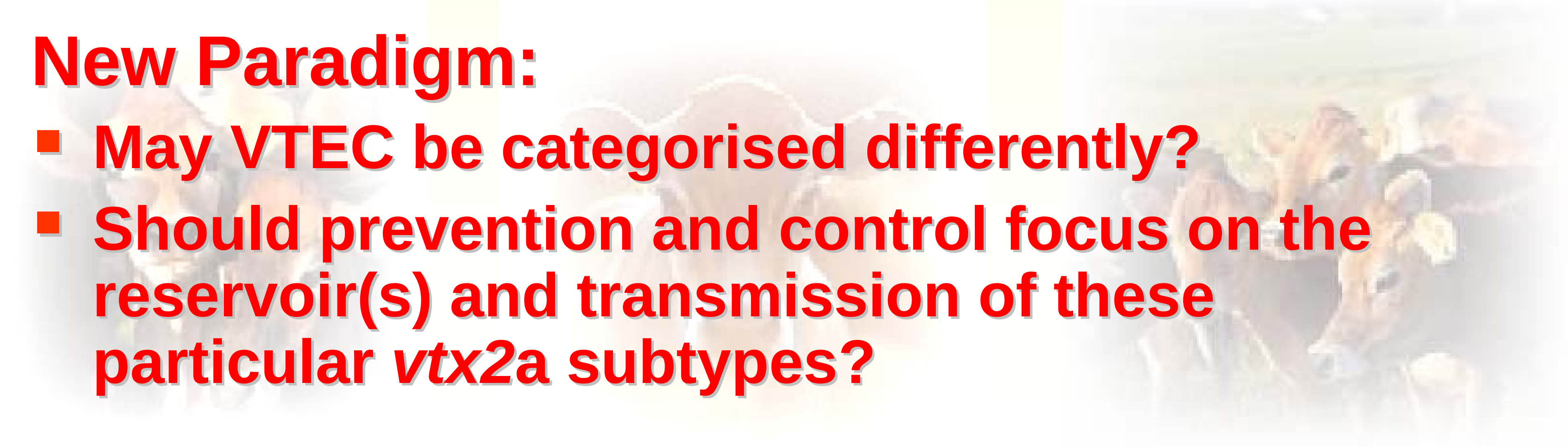
- Are these adequate?

Conclusions

Subtyping of *vtx2* genes has shown that:

Two *vtx2*a variants associated with HUS:

*vtx2*a-O157-EDL933 (& *vtx2*-O157-FLY16)	in NSF O157
*vtx2*a-O157-SF	in SF O157
*vtx2*a-O48-94C	in Non-O157



New Paradigm:

- May VTEC be categorised differently?
- Should prevention and control focus on the reservoir(s) and transmission of these particular *vtx2*a subtypes?

Conclusions

Certain virulence ”Cocktails” including *eae* positive or negative, and *vtx* subtypes/variants seem to be associated with specific clinical manifestations

Should management and treatment be adjusted according to virulence cocktail?

How is detection and surveillance scewed by the lack of specific detection and typing of variants?

Is EHEC obsolete?

- Wide clinical spectrum regardless of serotype
  Evident in person-to-person transmission
- There is increasing evidence that even within the same serotype, O157:H7/NM included, there are differences in the clinical outcome. This seems to be much closer related to the virulence profile than to the serotype.
- Animals will often carry types that have been referred to as EHEC without any clinical symptoms. The clinical course of disease is therefore host associated and the term EHEC misleading.
- Furthermore, there is no internationally accepted definition of EHEC and it is therefore used very differently in many countries

New nomenclature

1. **HUS inducing and outbreak associated VTEC (HI/OA VTEC/STEC)**

- **- *eae* and *vtx2a***
- **- *eae* negative and *vtx2*d (activatable)**
- **- *eae* and *vtx1* in this group are less common but O103:H2, O111:[H8], O115:H10, O118:H16, O145:[H28], O26:H11, O2:H44, O5:NM, O18:NM, O55:H? and O91:NM have been associated with HUS. We have published Significant HUS-inducing capacity plus significant epidemic outbreak potential or capacity**

2. **HUS inducing, NOT outbreak associated (HI VTEC/STEC)**

   VTEC with significant HUS-inducing, but low epidemic inducing capacity! No outbreaks detected with these many heterogeneous types.

3. **Diarrhoea inducing (in humans!) VTEC (DI VTEC/STEC)**
   Many different virulence profiles and by far dominated by *vtx1* strains. There is no one common denominator for this group except the capacity to produce VT and observations of clinical cases of diarrhoea in humans.

4. **Animal-associated VTEC (AA VTEC/STEC)**

   VTEC that are widely spread out and exclusively found in the animal reservoir; yet they don't show up in human cases. Seems to be their natural habitat.

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