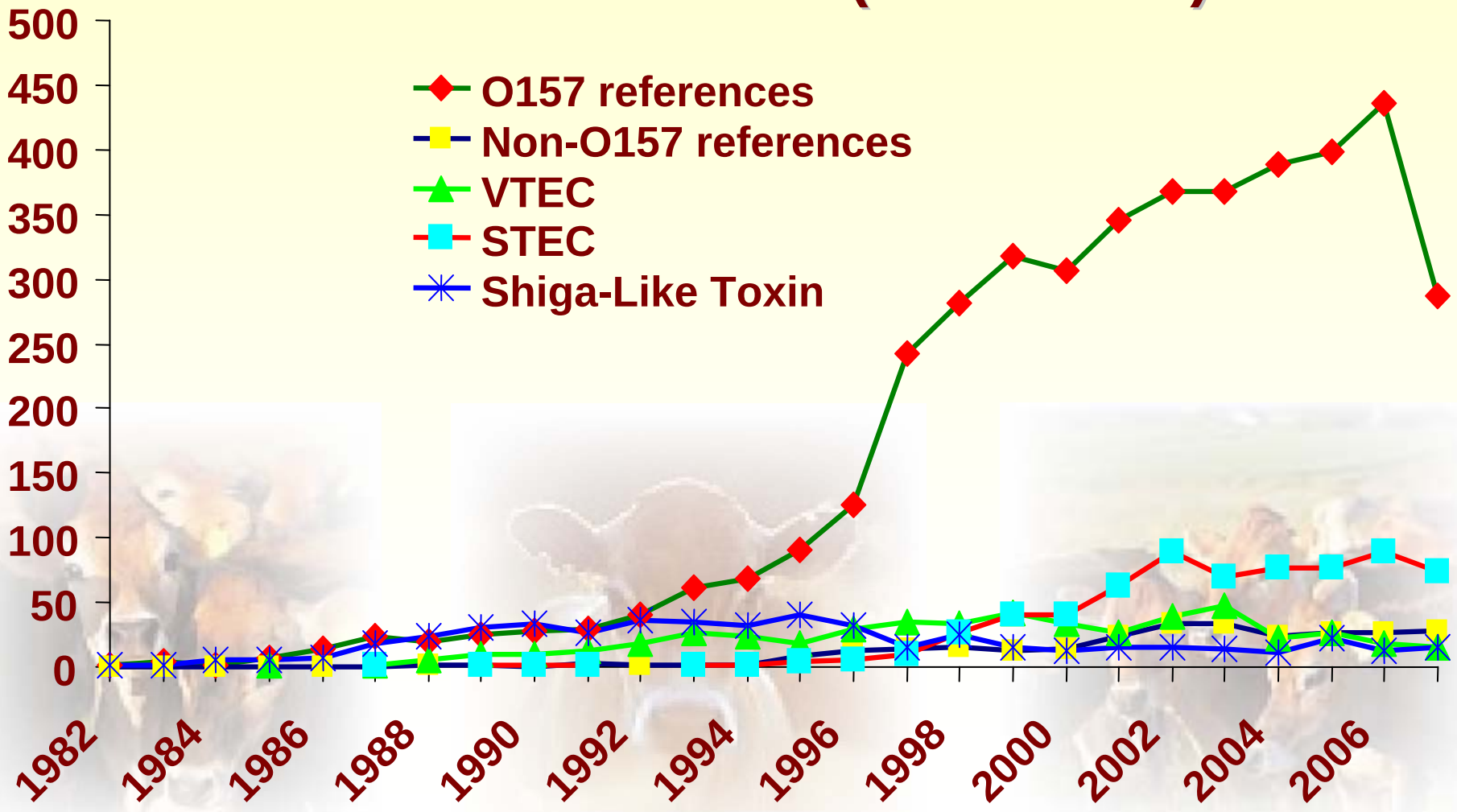
Experiences with non-O157 STEC and implications on Public Health Programs

FLEMMING SCHEUTZ

STATENS SERUM INSTITUT

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

Publications “O157”, “non-O157”, VTEC, STEC & Shiga-Like Toxin in Title/Abstract in PubMed (1982 - 2007)



Non-O157 STEC studies

K. E. Johnson et al. CID 2006;43

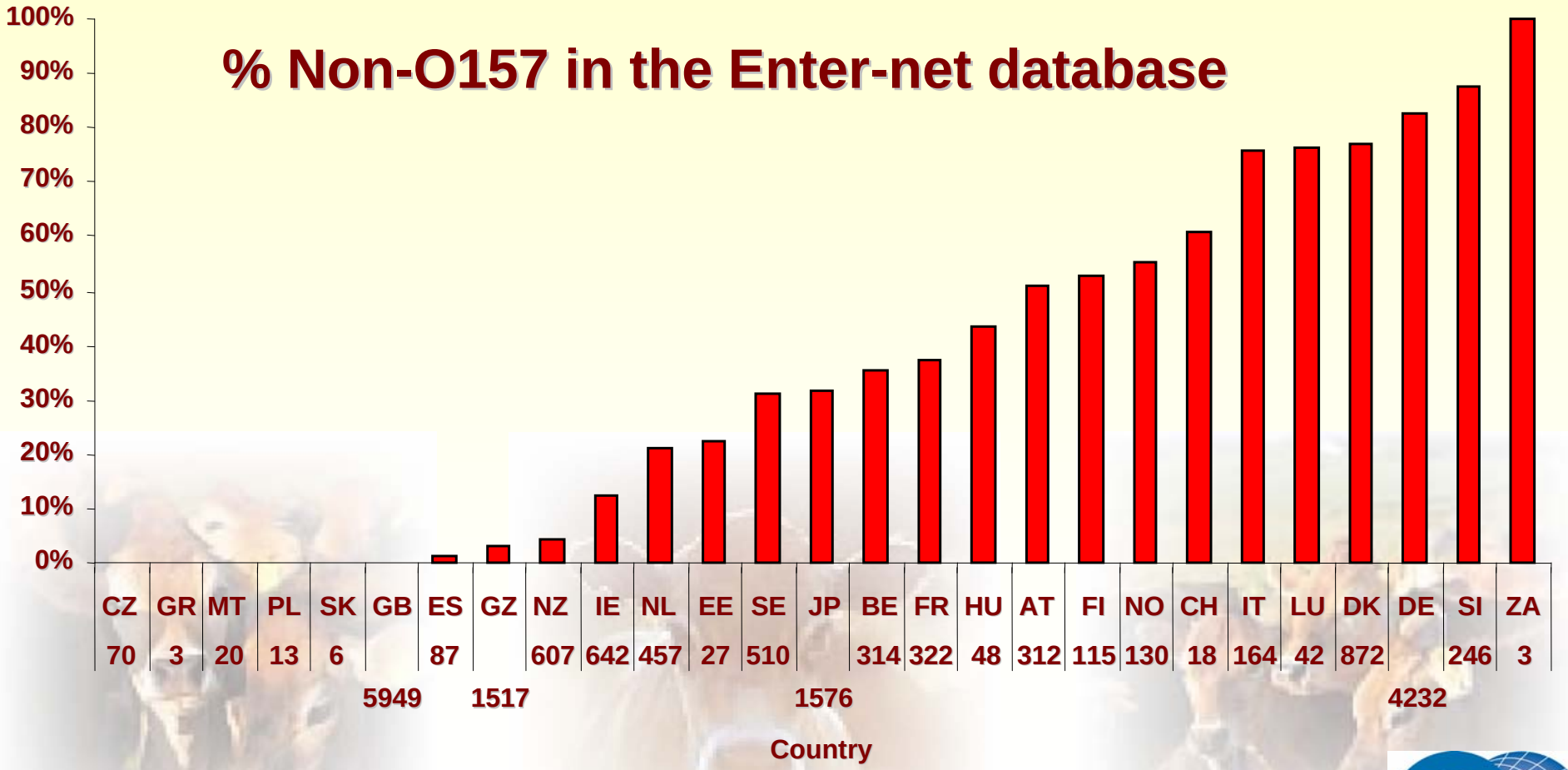
16 countries, 1988-2006 (1,402/2,892) 48%

Range 19%-100%

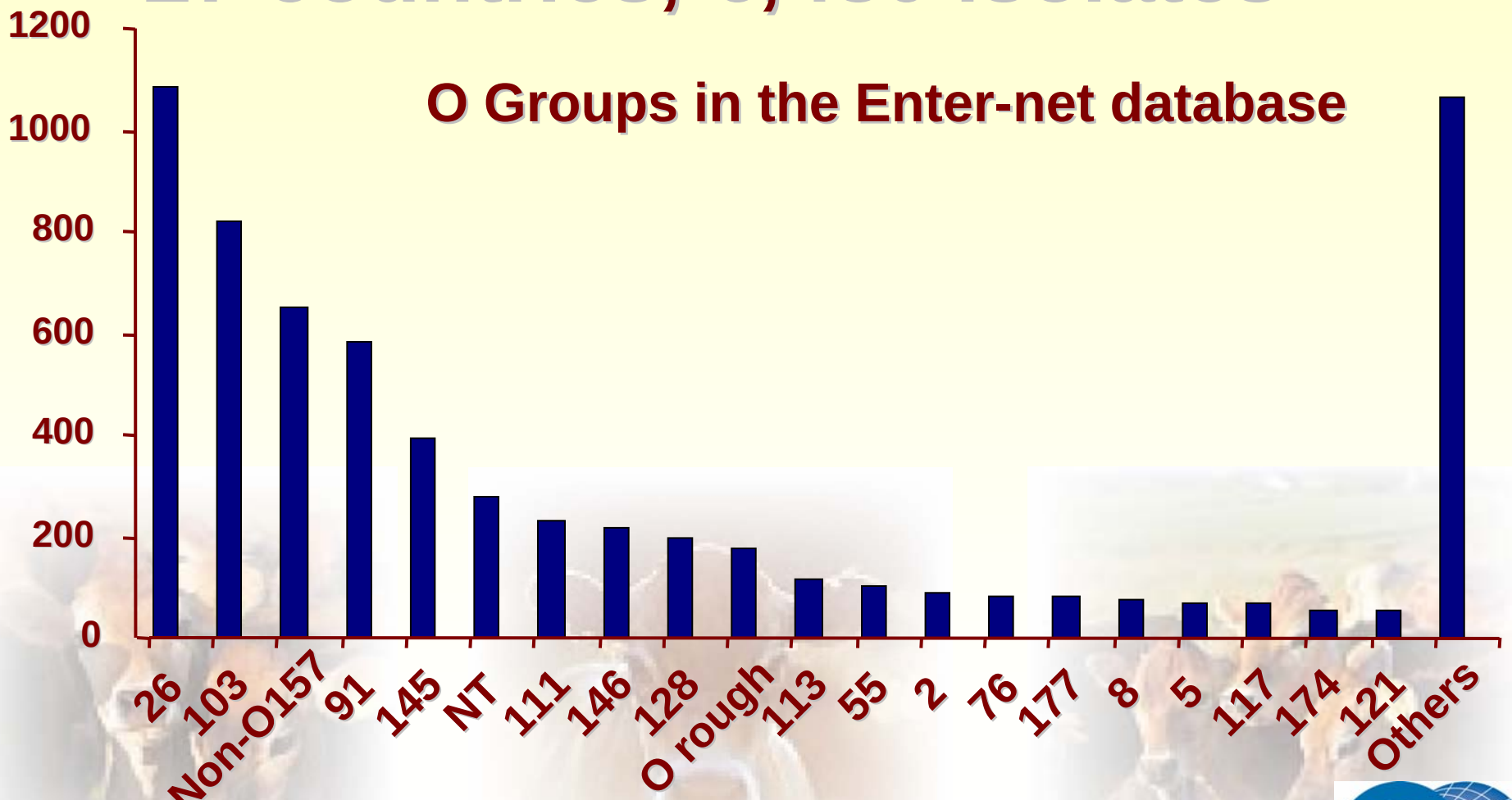
USA, Canada, UK, Germany, Spain, Italy, Czech Republic, Belgium, France, Denmark, Finland, Sweden, Australia, Chile, Argentina & Japan

Netherlands, 2006	**80%**
Australia, 2004	**64%**
Belgium, 2006	**81%**
Brazil, 2007	**100%**
Poland, 2004	**100%**
Germany, 1998	**88%**

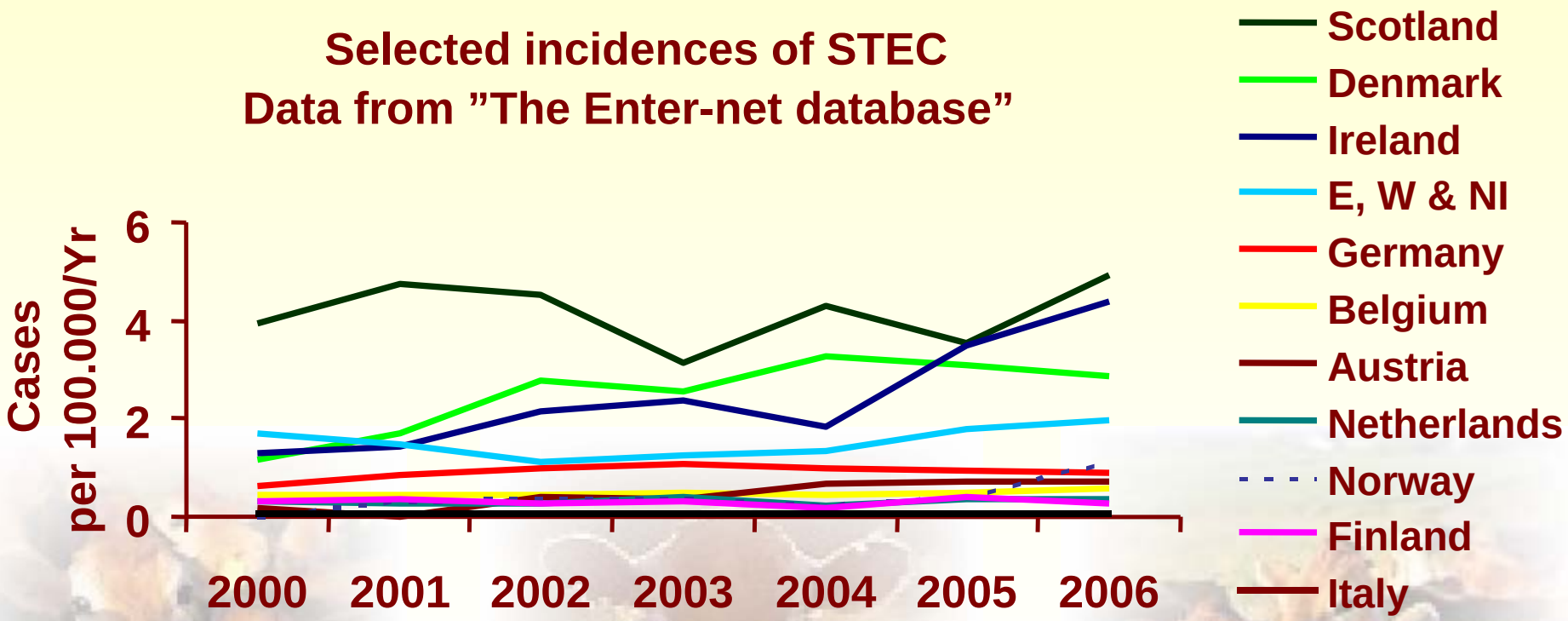
Non-O157 STEC surveillance 27 countries; 18,302 isolates



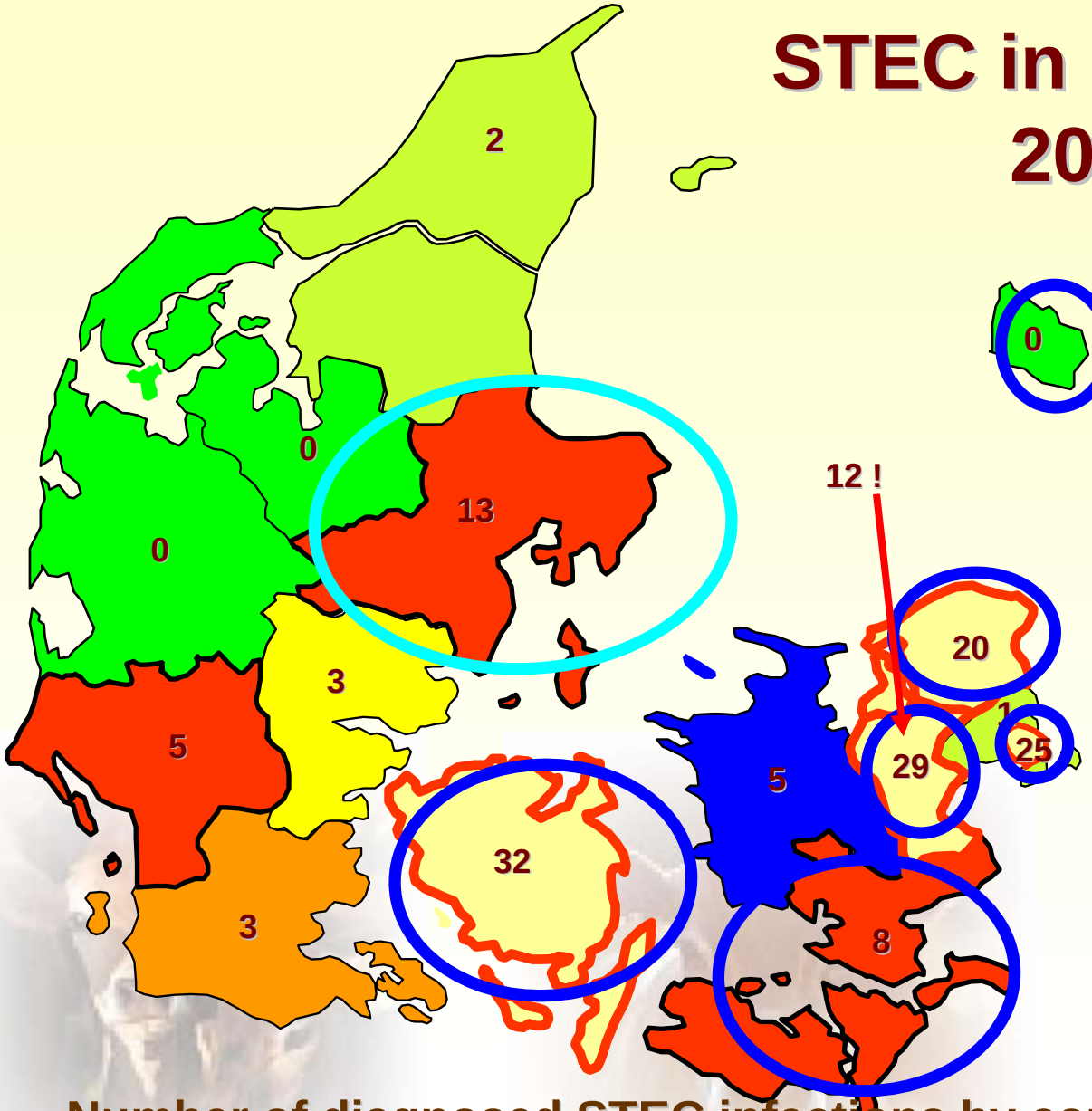
Non-O157 STEC surveillance
27 countries; 6,480 isolates



STEC incidences in Europe



STEC in Denmark 2006

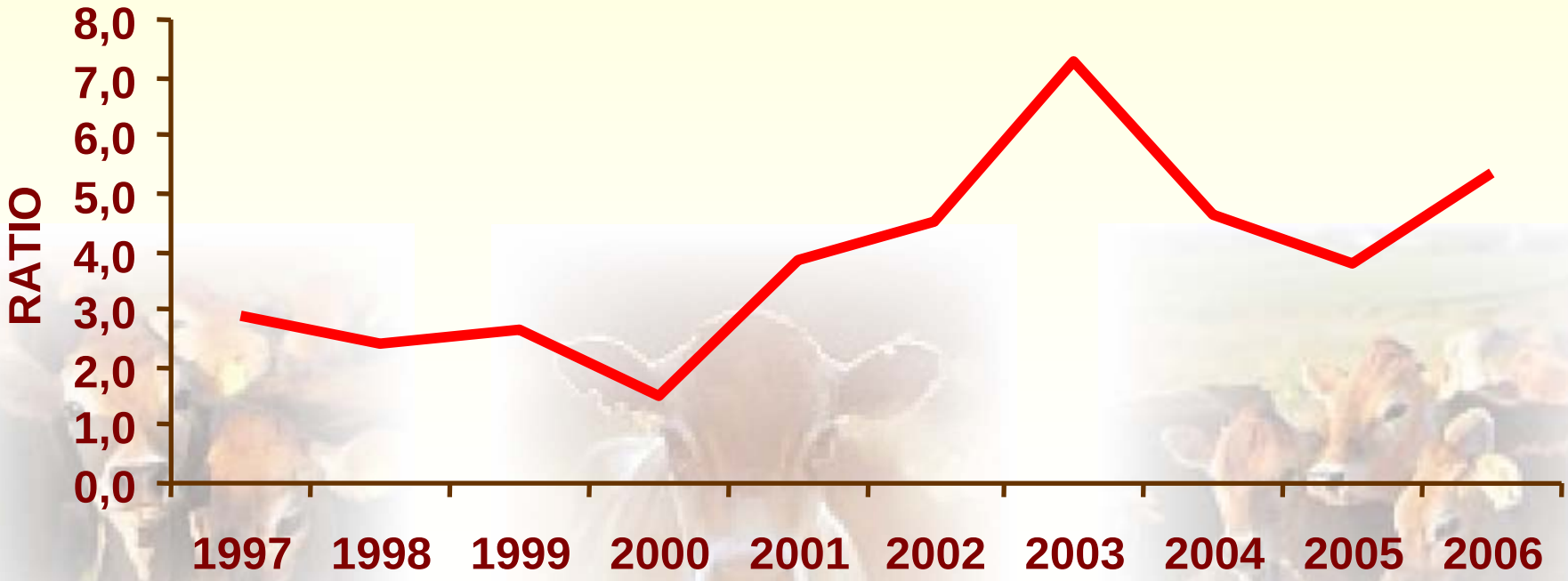


Incidence of STEC (per 100.000)

- > 4
- 2 – 3,99
- 1,5 – 1,99
- 1 - 1,49
- 0,5 - 0,99
- 0 - 0,49
- No cases

Number of diagnosed STEC infections by county, and annual incidence of all STEC infections in 2006

Detection ratio of STEC in counties using molecular methods vs "other methods" in Denmark



Non-O157 STEC 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

O103:H25 outbreak in Norway

Date of onset of disease

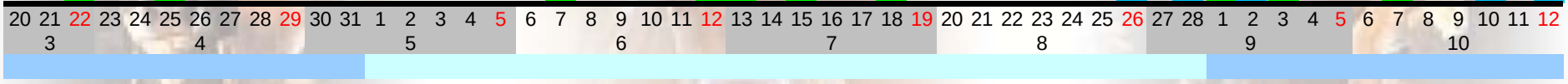
HUS cases

Diarrhea cases

HUS ”outbreak” notified

***stx2* pos**

O103 pos / *stx2* neg



January **February** **March**

Source: Sliced, dry fermented lamb's sausage

Product and environmental samples

		O103 positive
Cured meat products	2462	53
Sheep meat	171	9
Environment	296	0
Spices, additives, culture	57	0
Minced meat	1000	0

All isolates were *stx2* negative & *eae* pos. BUT clustered with patient isolates by MLVA (DNA fingerprint)

O103:H25 outbreak in Norway *stx2 & eae*

17 cases; 15 children

10 with HUS

1 child died

HUS notification

Massive media attention

O26:H11 outbreak in Denmark *stx1 & eae*

20 cases; all children

median 2 years

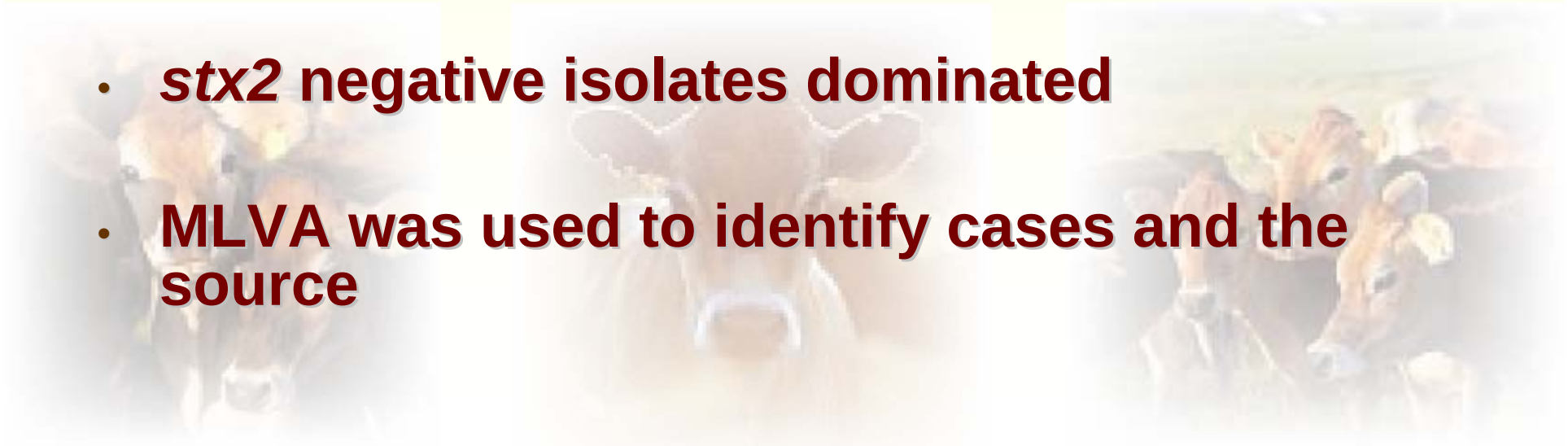
Very mild symptoms

Discovered by PFGE

Little media attention

Lessons learned in Norway

- **Outbreak discovered due to notification of cases of HUS**
- **Methods in clinical laboratories were inadeqaute for detection of non-O157 in 5 out of the six first cases of HUS**
- ***stx2* negative isolates dominated**
- **MLVA was used to identify cases and the source**



Lessons learned in Denmark

- Real-time PFGE of Danish non-O157 detected a ”mild” outbreak
- Only possible because isolates were available for typing

Source identified using

- access to purchase records
- cooperation with supermarkets searching their central computers

HUS and STEC notifications worldwide

HUS & STEC	**Australia, Cyprus, Denmark, Germany,**
7	**Hungary, Japan, Poland**
	Notification dates from 1998 – 2005
STEC only	**Austria, Canada, Estonia, Finland,**
13	**Greece,Iceland, Ireland, Luxembourg, Malta , New Zealand, Norway, Slovenia, Sweden**
	Notification dates from 1990 – 2005
Not mandatory	**England & Wales, France †, Italy ‡,**
7	**Romania, Spain, Scotland*, South Africa**
	Pediatric- nephrology network since
	† 1996 < 15 Yrs ‡ 1988 < 14 Yrs
	***Laboratory based since mid-eight'ies**

Shiga toxin 2 (*stx2*) subtype and clinical presentation

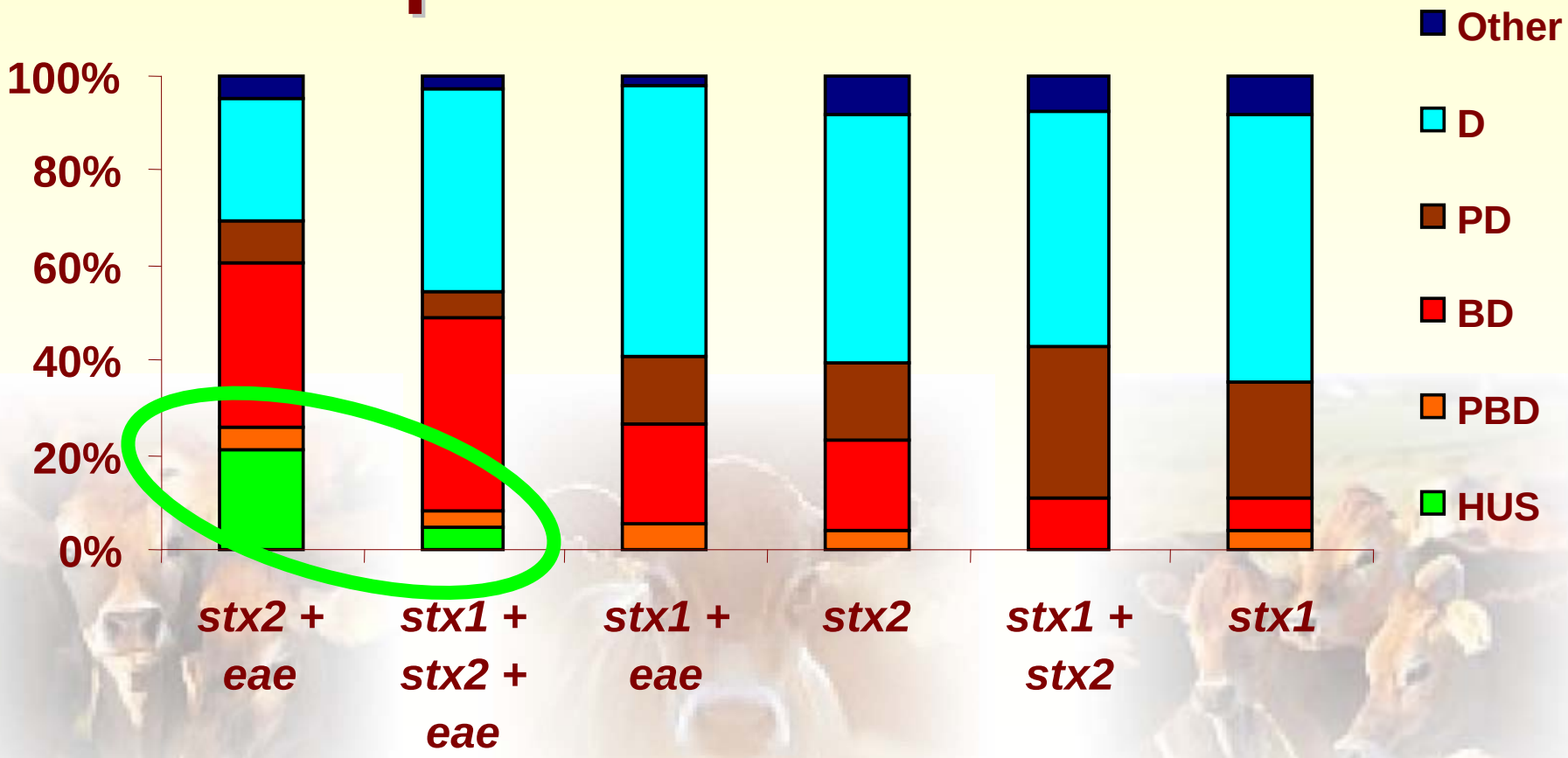
Subtype	Non-HUS *	HUS*
stx2	**60**	**11**
stx2c	**49**	**1**
***stx2d*-activatable**	**4**	
stx2d	**39**	
stx2e	**2**	
***stx2*-variant**	**3**	
stx2* + *stx2c	**23**	**7**
stx2* + *stx2d	**1**	
2x *stx2*-activatable	**4**	
***stx2c* + *stx2*-activatable**	**1**	
Total	**186**	**19**

stx2 OR* 32.5 > *stx2c* OR* 4.7 for HUS

***) OR: odds ratio; multivariant analysis adjusted for age**

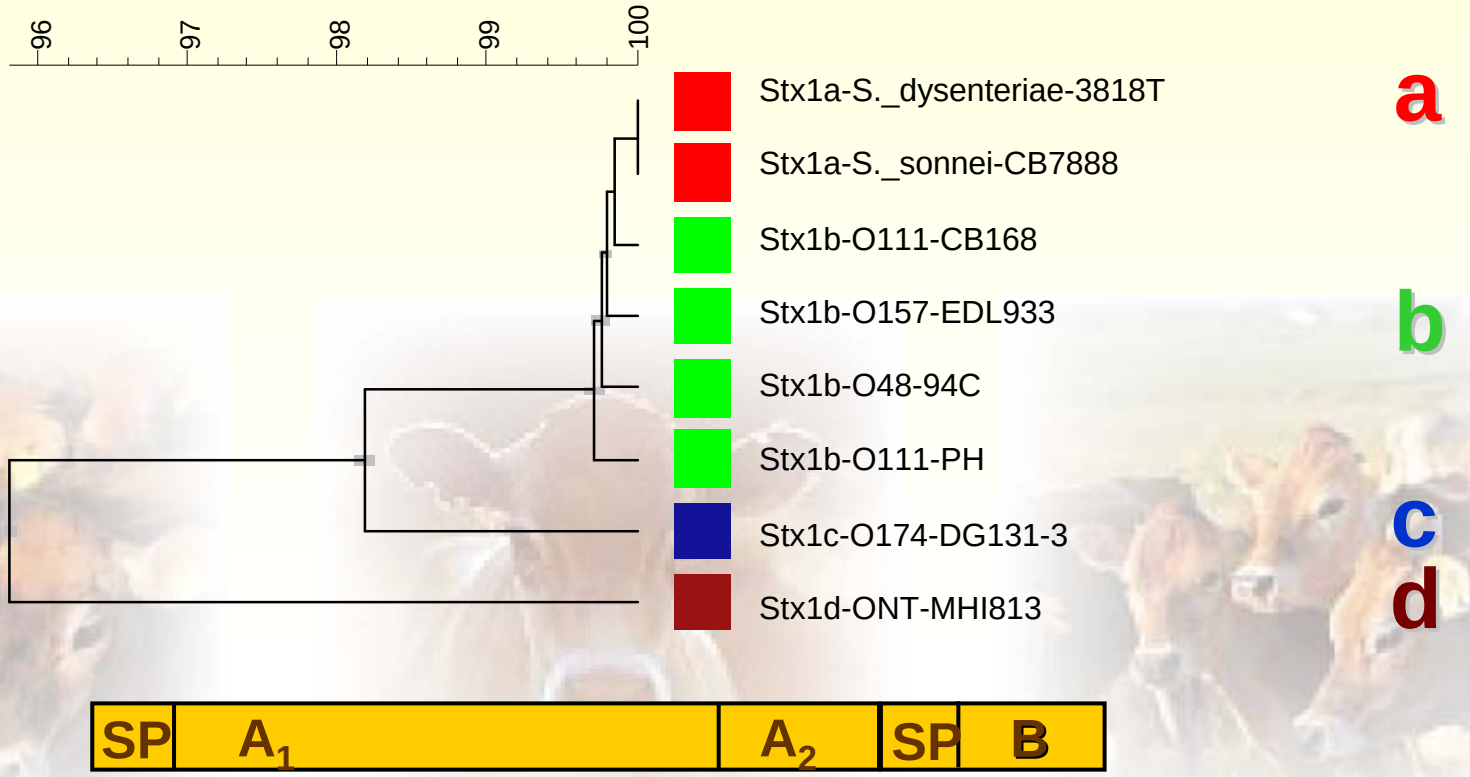
Ethelberg et al. 2004 EID; 10

Virulence profile and clinical manifestation in 559 Danish STEC patients 1994-2005



Stx1 : 4 subtypes a - d
7-8 variants

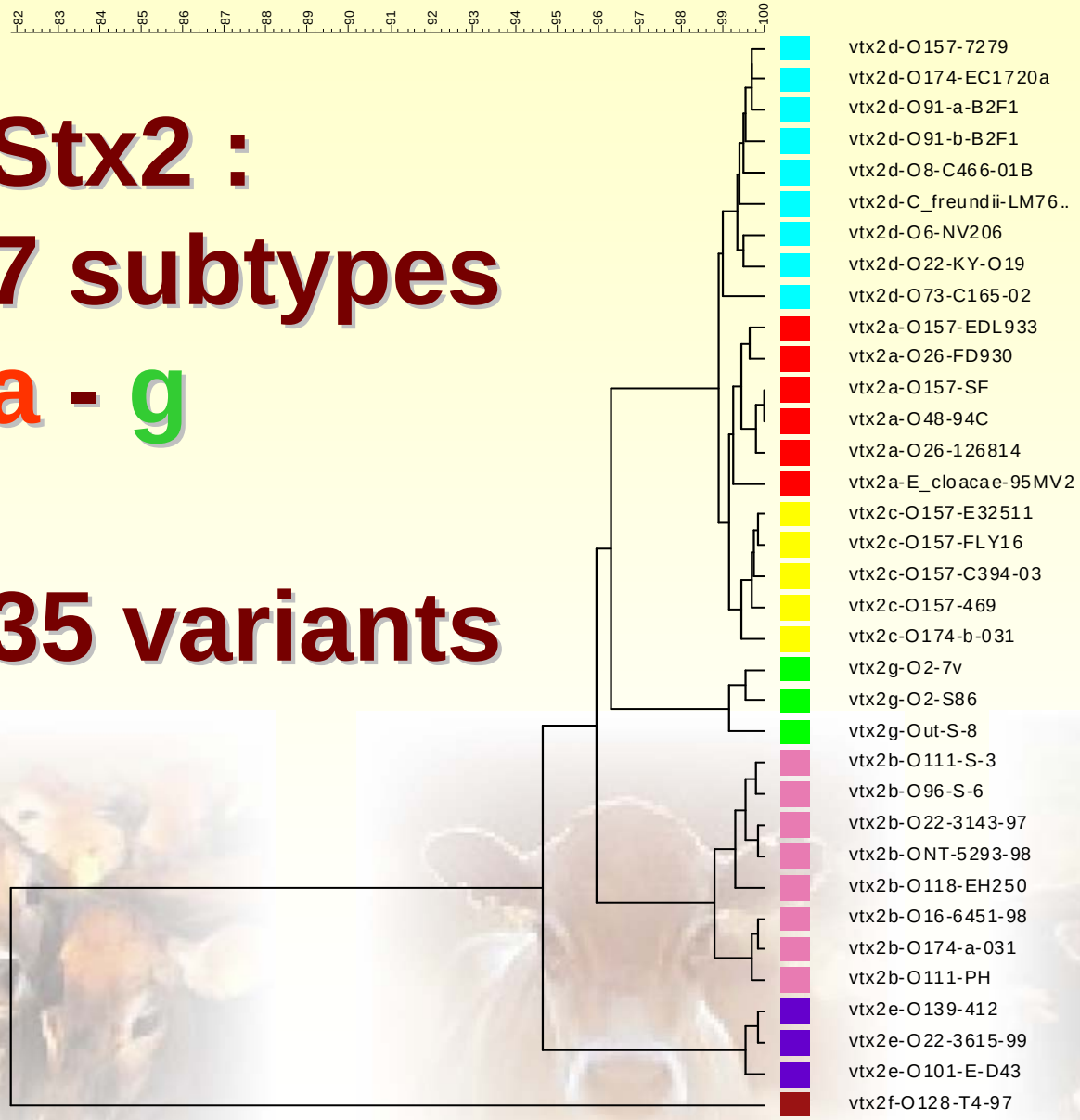
Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0%
VT1 translated sequences



Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost 0% Disc. unk.
vtx_TRANSL

Stx2 :
7 subtypes
a - g

35 variants



JOURNAL OF CLINICAL MICROBIOLOGY, June 2007, p. 2020–2024 Vol. 45, No. 6
0095-1137/07/$08.00+0 doi:10.1128/JCM.02591-06
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Subtyping Method for *Escherichia coli* Shiga Toxin (Verocytotoxin) 2 Variants and Correlations to Clinical Manifestations[▽]

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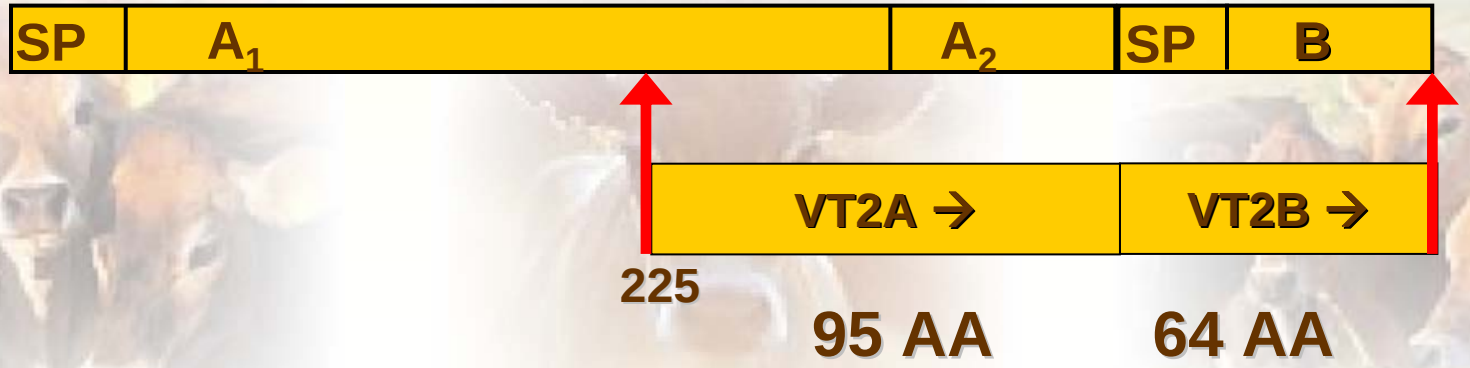
Received 27 December 2006/Returned for modification 27 January 2007/Accepted 4 April 2007

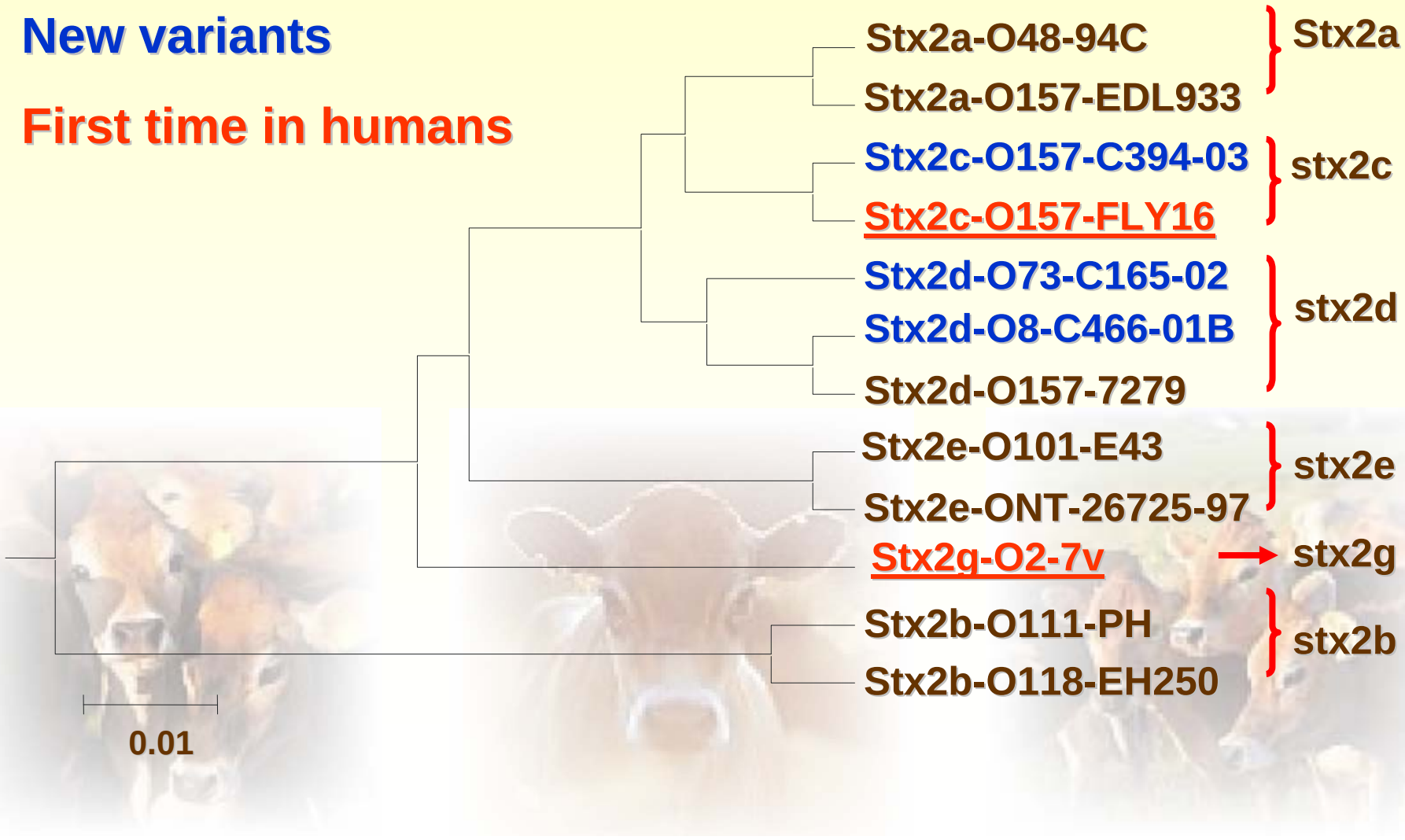
Shiga toxin 2 (Stx2) from Shiga toxin-producing *Escherichia coli* (STEC) was subtyped by a method involving partial sequencing of the *stxAB*$_2$ operon. Of 255 strains from the Danish STEC cohort, all 20 cases of hemolytic-uremic syndrome were associated with subtype Stx2 (11 cases), subtype Stx2c (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 Stx2 variants associated with HUS

O157		
Stx2a-O157-EDL933 + Stx2c-O157-FLY16	6/23	26%
Stx2a-O157-EDL933	3/17	18%
Stx2c-O157-FLY16	1/18	6%
Stx2a-O157-SF + Stx2c-O157-FLY16	1/1	-
Non-O157		
Stx2a-O48-94C	6/20	30%
Stx1b + Stx2a-O157-EDL933	1/3	-
Stx2a-O157-EDL933 + Stx2c-O157-FLY16	1/2	-
Stx1b + Stx2a-O48-94C	1/5	20%

Conclusions

Two Stx2a variants associated with HUS

Stx2a-O157-EDL933 — **in NSF O157**

(& Stx2-O157-FLY16)

Stx2a-O157-SF } **in SF O157**

Stx2a-O48-94C } **in Non-O157**

New Paradigm:

How may STEC be classified?

Are certain virulence ”cocktails” associated with severe disease rather than the serotype?

New Paradigm

Classification of STEC in 5 Sero-pathotypes

Based on the reported occurrence of serotypes in human disease, in outbreaks and/or in hemolytic-uremic syndrome (HUS)

Karmali *et al.*, 2003, J. Clin. Microbiol., 41:4930-40

Sero-pathotype	Relative incidence	Frequency of involvement in outbreaks	Association with severe disease (HUS or HC)	Serotypes
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O113: H21, O104:H21, others
D	Low	Rare	No	multiple
E	Non human only			multiple

Problems with this classification

Association with serotype and not with virulence profile

- More than 120 O:H serotypes have been associated with HUS (Bergey's Manual of Systematic Bacteriology, 2nd ed.)
- Many O:H serotypes display extensive heterogeneity

Involvement in outbreak may rapidly change

Relative incidence

- is scewed by lack of effecient detection methods
- will vary depending on the epidemiology of specific types

New Paradigm

Alternative classification

1. HUS inducing STEC and/or an epidemic outbreak potential

- *eae* and *stx2a*
- *eae* negative and *stx2d* (activatable)
- *eae* and *stx1* Less common but certain O:H serotypes have been associated with HUS

2. Diarrhea inducing in humans!

- Many different virulence profiles
- Capacity to produce Stx and association with diarrhea in humans

3. Animal-associated STEC

- High prevalence in the animal reservoir
- Seems to be their natural habitat
- No human cases
- Candidates for this group are *stx2e* positives

Questions

Should management and treatment of patients be adjusted according to virulence cocktail?

Danish Practice since 2000

ALL patients with STEC are excluded or quarantined if they are

- **Children in institutions and day care**
- **Staff of health care facilities**
- **Hospital staff or hospitalized patients**
- **Food handlers**

and until they have had two consecutive STEC negative stool samples

Prolonged shedding of STEC has resulted in huge social problems especially for parents

New Paradigm

Danish example of consequences

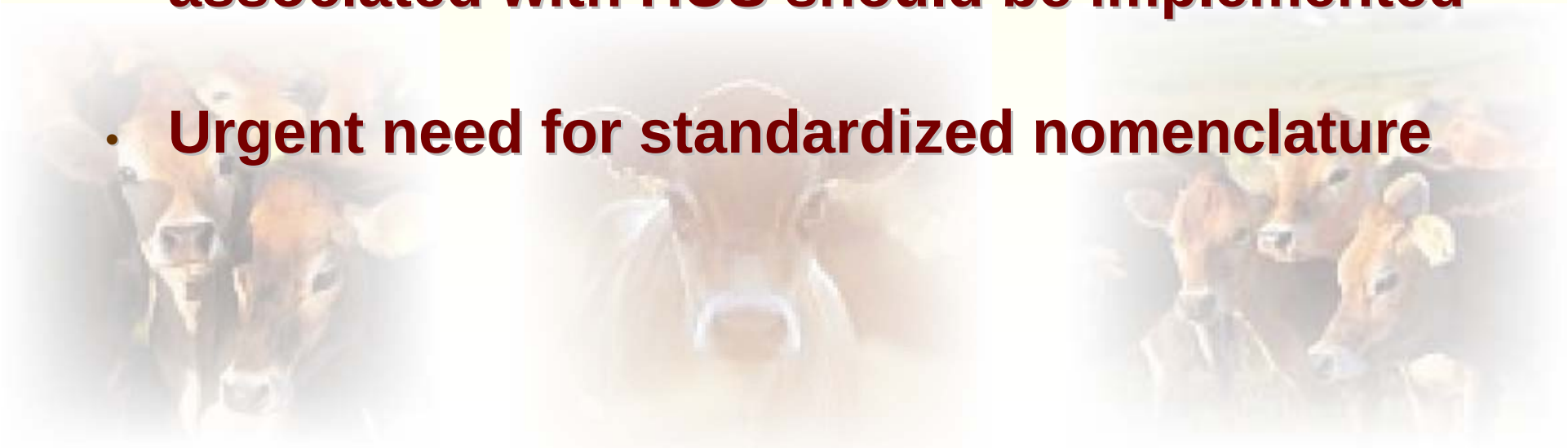
Revision of guidelines for treatment of Danish patients with STEC may include antibiotic treatment of asymptomatic patients with

- ***eae* negative STEC**
- ***eae* & *stx1*** **- except some serotypes**

Asymptomatic patients are likely to be allowed back in institutions and day care after treatment

Recommendations

- Adequate detection methods should include the isolation of bacteria
- Typing methods should be standardized
- Subtyping methods for Stx2a variants associated with HUS should be implemented
- Urgent need for standardized nomenclature



Questions

1. How much is detection and surveillance scewed?
2. Can case definitions for HUS to be notified within the Public Health system be established?
3. Will management and treatment of STEC patients depend on
   - a case-to-case based assessment?
   - an outbreak-to-outbreak approach?
   - local epidemiology?

Question

Should non-O157:H7 STECs be considered to be adulterants as *E. coli* O157:H7?

YES - some

Acknowledgements

Statens Serum Institut, Copenhagen

Joan Neverman Jensen

Søren Persson

Katharina E. P. Olsen

Co-authors on nomenclature

Lothar Beutin, Federal Institute for Risk Assessment, Berlin

Denis Piérard, Academisch Ziekenhuis Vrije Universiteit, Brussels

Nancy A. Strockbine, National Center for Zoonotic, Vector-borne and Enteric Diseases (CDC), Atlanta