



EXTERNAL QUALITY ASSESSMENT SCHEME RESULTATER

- VTEC & andre diaréfremkaldende *E. coli* (DEC)

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Fødevarebårne Infektioner Statens Serum Institut

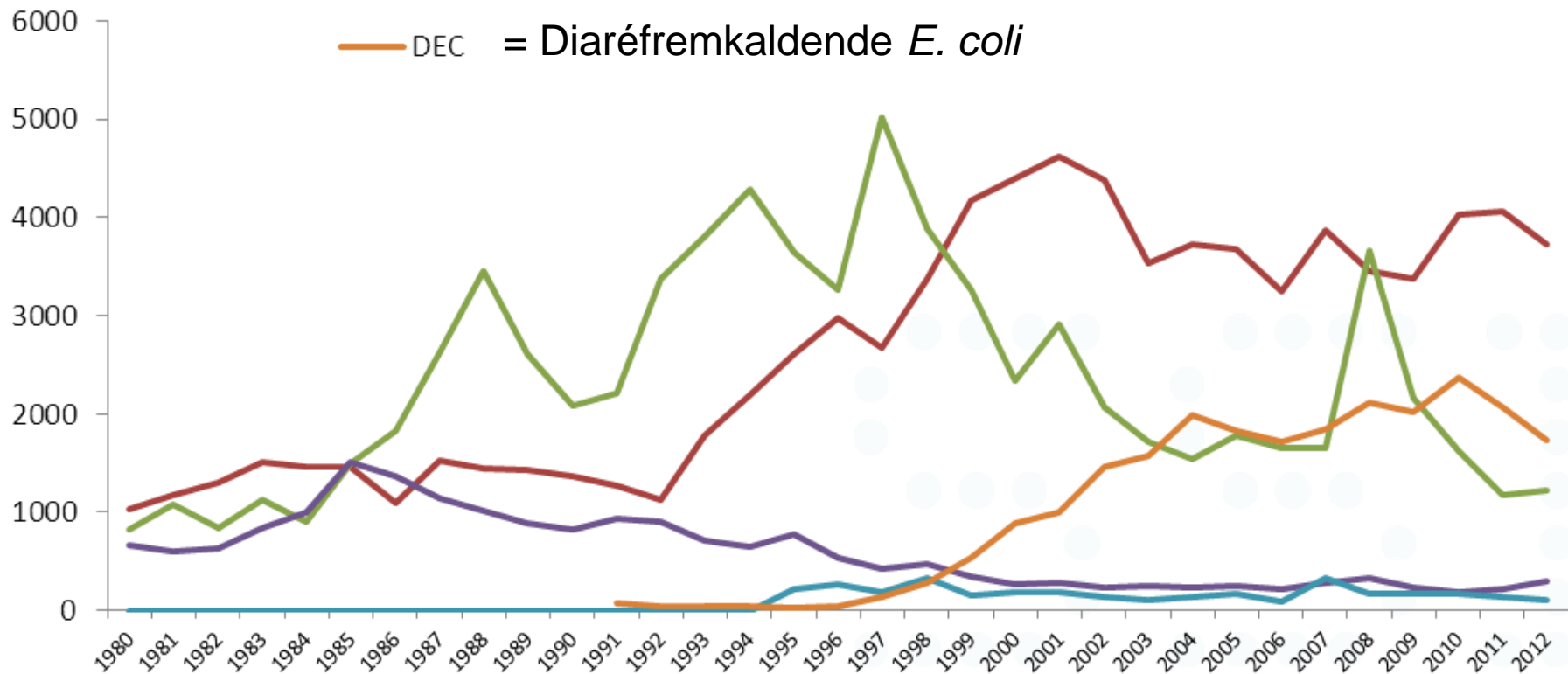


Maj 2013

Enteropatogene bakterier: Tendenser i Danmark 1980- 2012

DEC er fra 2003 den 2. mest hyppige årsag til diaré i Danmark

— Campylobacter
— Salmonella
— Yersinia enterocolitica
— Shigella
— DEC = Diaréfremkaldende *E. coli*





EPI-NYT

Overvågning og forebyggelse af smitsomme sygdomme

Uge 1/2 - 2013

Smitsomme sygdomme 2012

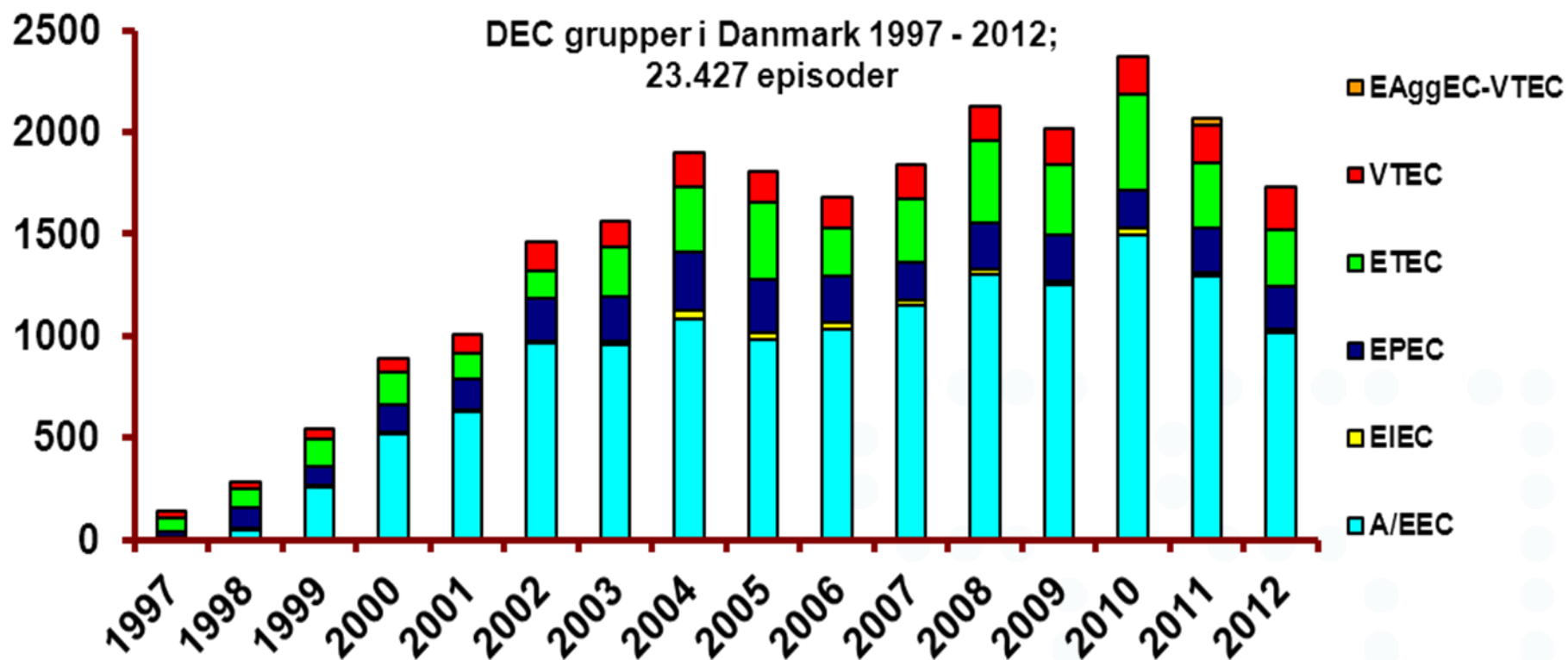
Diaréfremkaldende *E. coli*

Danmark har hidtil været forskånet for generelle fødevarebårne sygdomsudbrud forårsaget af ”burgerbakterien” *E. coli* O157 af den særligt aggressive variant, der medfører høj risiko for komplikationer i form af hæmolytisk uræmisk syndrom (HUS). Udbruddet i september-oktober 2012, [EPI-NYT 45/12](#), var det første danske fødevarebårne udbrud af en sådan HUS-associeret type og havde et meget højt antal tilfælde af HUS i forhold til antallet af patienter med diaré. Udbruddet fik relativt begrænset omfang, idet smitekilden sandsynligvis var hakket oksekød, som har kort holdbarhed, og som oftest anvendes i retter, hvor det gennemsteges, inden det spises. Havde smitekilden været en vare med længere holdbarhed, eller havde der været en kontinuerlig smitekilde, var omfanget og konsekvenserne af udbruddet formentlig blevet større.

Udbruddet understreger, at det er vigtigt, at HUS-tilfælde anmeldes uden unødigt forsinkelse, ligesom en hurtig og rettidig diagnostik af verocytotoksinproducerende *E. coli* (VTEC) er essentiel for både patientbehandling og smitteopsporing. I forbindelse med udbruddet var der dog en del debat om indikationer for undersøgelse af fæces for VTEC eller andre typer af diaréfremkaldende *E. coli*.

Afgræsning af ”tarmpatogene bakterier” til salmonella, shigella, *Yersinia enterocolitica* og campylobacter er en forældet konvention og er ikke udtryk for evidens. De diaréfremkaldende *E. coli* er samlet set hyppigere årsag til diarré end de nævnte bakterier, fraset *Campylobacter jejuni* som er den mest almindelige årsag til bakteriel gastroenteritis i Danmark. Ved at fravæge diagnostik af diaréfremkaldende *E. coli* er der et markant mindre diagnostisk udbytte af fæcesdyrkning. Såfremt der er klinisk eller epidemiologisk indikation for at undersøge for tarmpatogene bakterier, vil det i de fleste tilfælde være relevant at undersøge for diaréfremkaldende *E. coli*. **På denne baggrund anbefaler Dansk Selskab for Klinisk Mikrobiologi, at alle børn under 7 år med diaré, samt alle med blodig diaré – uanset alder – undersøges for diaréfremkaldende *E. coli* ved hjælp af molekylærbiologisk diagnostik.**

DEC GRUPPER I DANMARK



- Pulsed Field Gel Electrophoresis (PFGE of 11 strains)
- serotyping O:H
- virulence determination:
 - genotyping of VTEC virulence genes: *vtx1*, *vtx2*, *eae*, *aaiC* and *aggR*
 - subtyping of *vtx1* (*vtx1a*, *vtx1c* and *vtx1d*) and *vtx2* (*vtx2a* to *vtx2g*)
- phenotypic testing
 - production of Verocytotoxin/Shiga toxin
 - production of Extended Spectrum Beta Lactamases (ESBL)
 - production of β -glucuronidase
 - production of enterohaemolysin
 - Fermentation of sorbitol
- Reference subtypes strains (9)
- PFGE reference S. Braenderup



Hillerød Sygehus	Hillerød
Afsnit for bakteriel specialdiagnostik	SSI, København
Hvidovre Hospital	Hvidovre
Odense Universitetshospital	Odense
Sygehus Vestsjælland	Slagelse
Vejle sygehus	Vejle
Aalborg Sygehus Syd	Aalborg
Århus Universitetshospital, Skejby	Århus N

VTEC & DEC – 15 STAMMER

RESULTAT TABEL

	O group	H type	Vero Cell assay	ESBL prod.	Haemolysin prod.	Beta-glucuronidase prod.	Sorbitol ferm.	eae gene	ehxA gene	vtx1 gene	vtx2 gene	vtx Subtypes			Additional virulence genes	Pathogenic group
AA1	O113	H4	Pos.	Neg.	Pos.	Pos.	Pos.	Neg.	Pos.	Pos.	Pos.	<i>vtx1c</i>		<i>vtx2b</i>		VTEC
BB2	O177	H25	Neg.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.					A/EEC
CC3	O121	H19	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	Pos.		<i>vtx2a</i>			VTEC
DD4	O128	H2/H-	Pos.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Pos.	Neg.	<i>vtx1c</i>				VTEC
EE5	O41	H26	Pos.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Pos.	Neg.	<i>vtx1d</i>				VTEC
FF6	O26	H11	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	Pos.		<i>vtx2a</i>			VTEC
GG7	O111	H8/H-	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	<i>vtx1a</i>				VTEC
HH8	O104	H4	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.				<i>aggR, aaiC, aatA</i>	EAggEC
II9	O157	H7	Pos.	Neg.	Pos.	Neg.	Neg.	Pos.	Pos.	Neg.	Pos.		<i>vtx2a</i>	<i>vtx2c</i>		VTEC
JJ10	O146	H21	Pos.	Neg.	alfa	Pos.	Pos.	Neg.	Neg.	Neg.	Pos.			<i>vtx2d</i>		VTEC
KK11	O103	H2	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	<i>vtx1a</i>				VTEC
LL12	O157	H7	Pos.	Pos.b)	Pos.	Neg.	Neg.	Pos.	Pos.	Neg.	Pos.		<i>vtx2a</i>			VTEC
MM13	O166	H15	Pos.	Pos.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Pos.			<i>vtx2d</i>	<i>elt</i>	ETEC/VTEC
NN14	O78	H11	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.				<i>estAp, elt</i>	ETEC
OO15 a)	O124	H30	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.				<i>ipaH</i>	EIEC

a) Laktose negative

b) Denne stamme kan have tabt ESBL plasmidet. Begge resultater er accepteret.

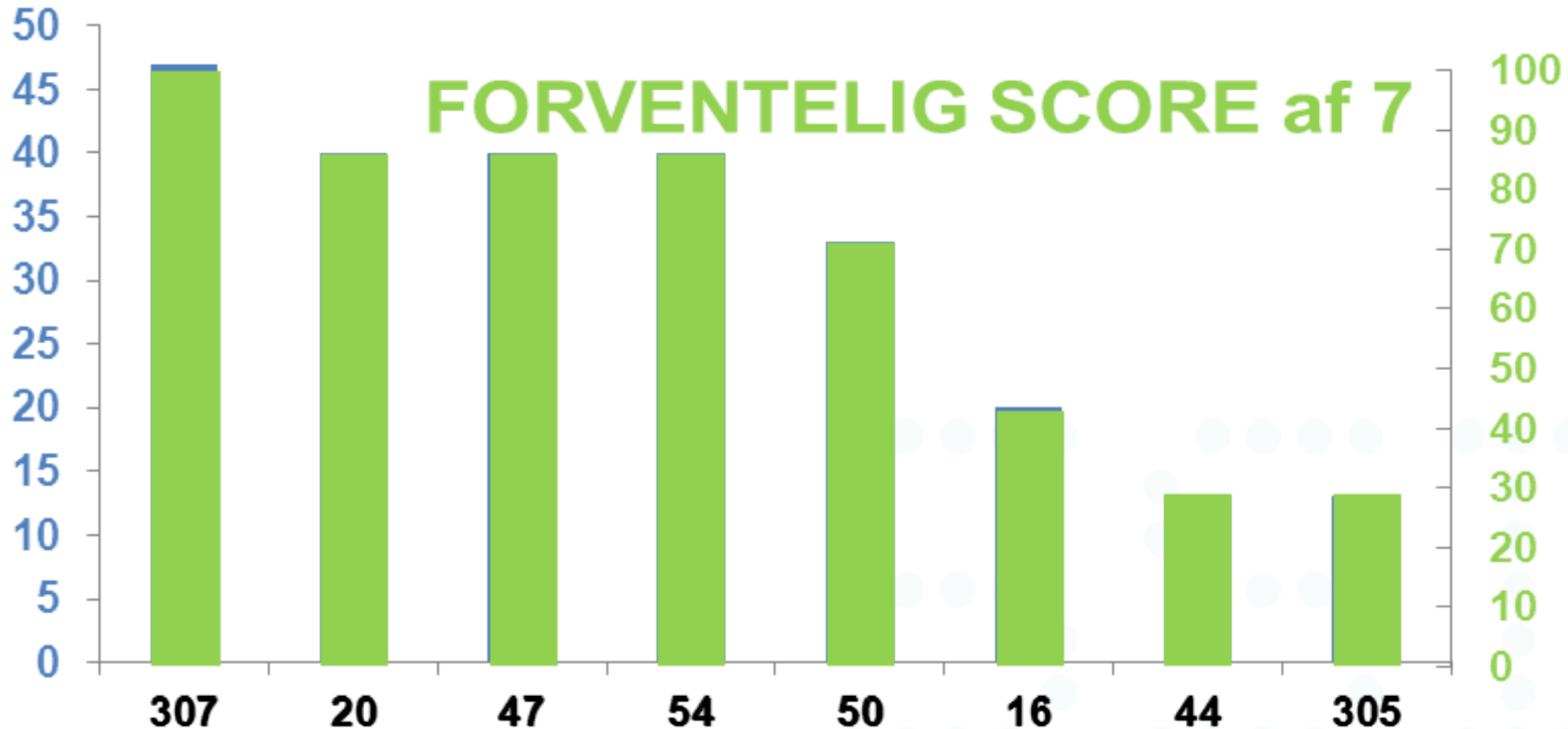
Forventelig (O gruppe: 7 stammer)

Burde også kunne detekteres?

O GRUPPERING – 8 DELTAGERE



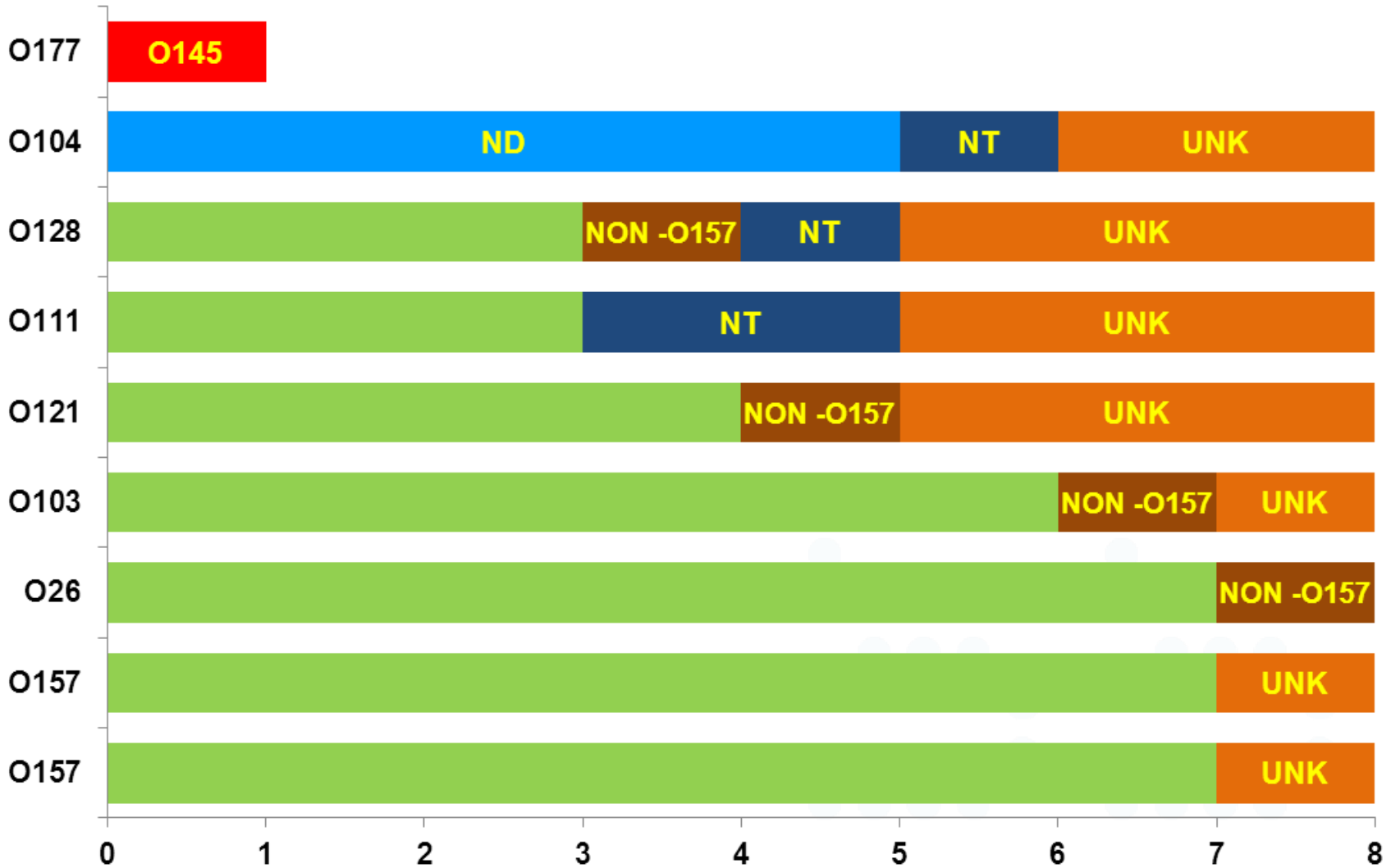
SCORE af 15



O GRUPPERING – PER STAMME



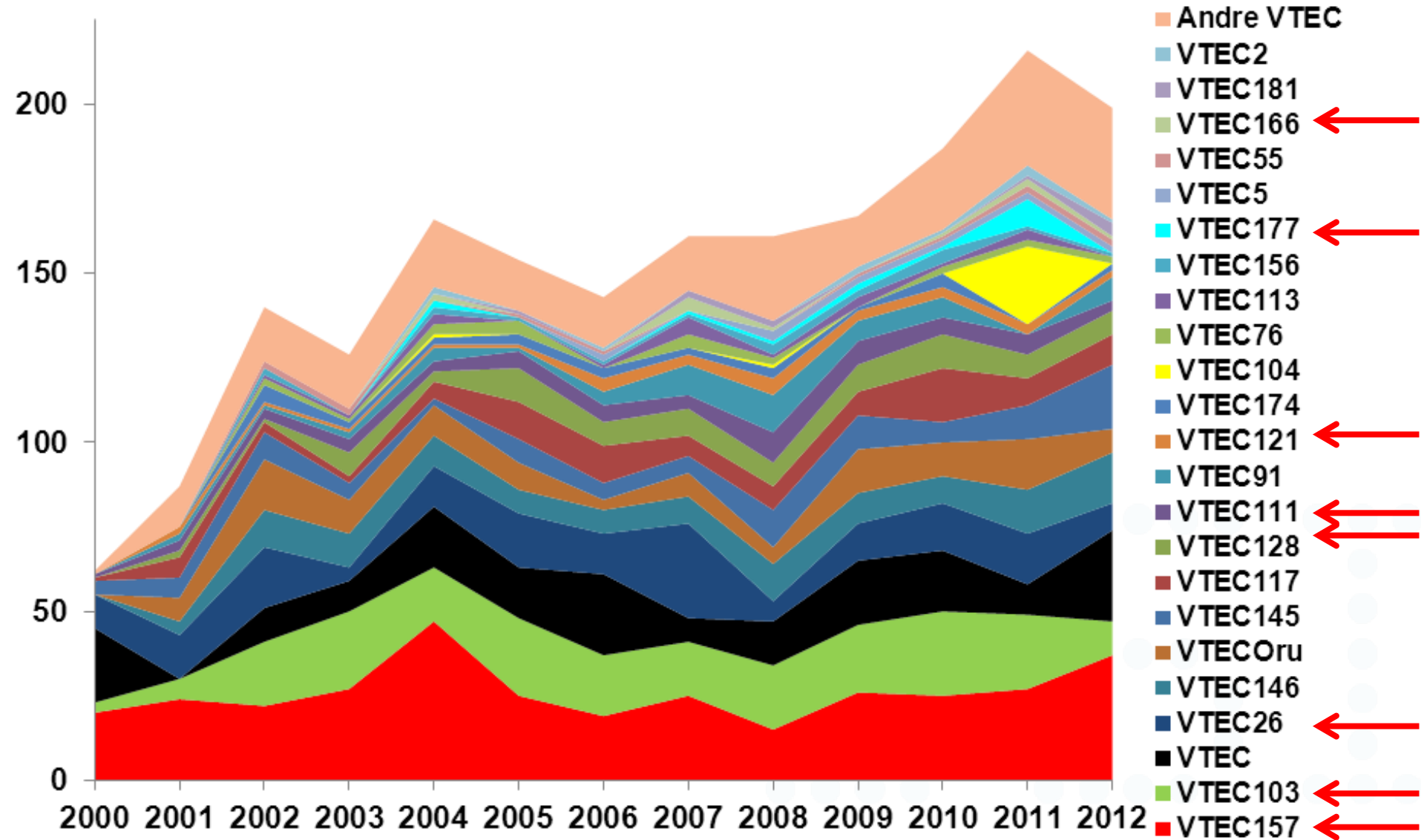
Grøn: KORREKT Not done Non-typeable Unknown



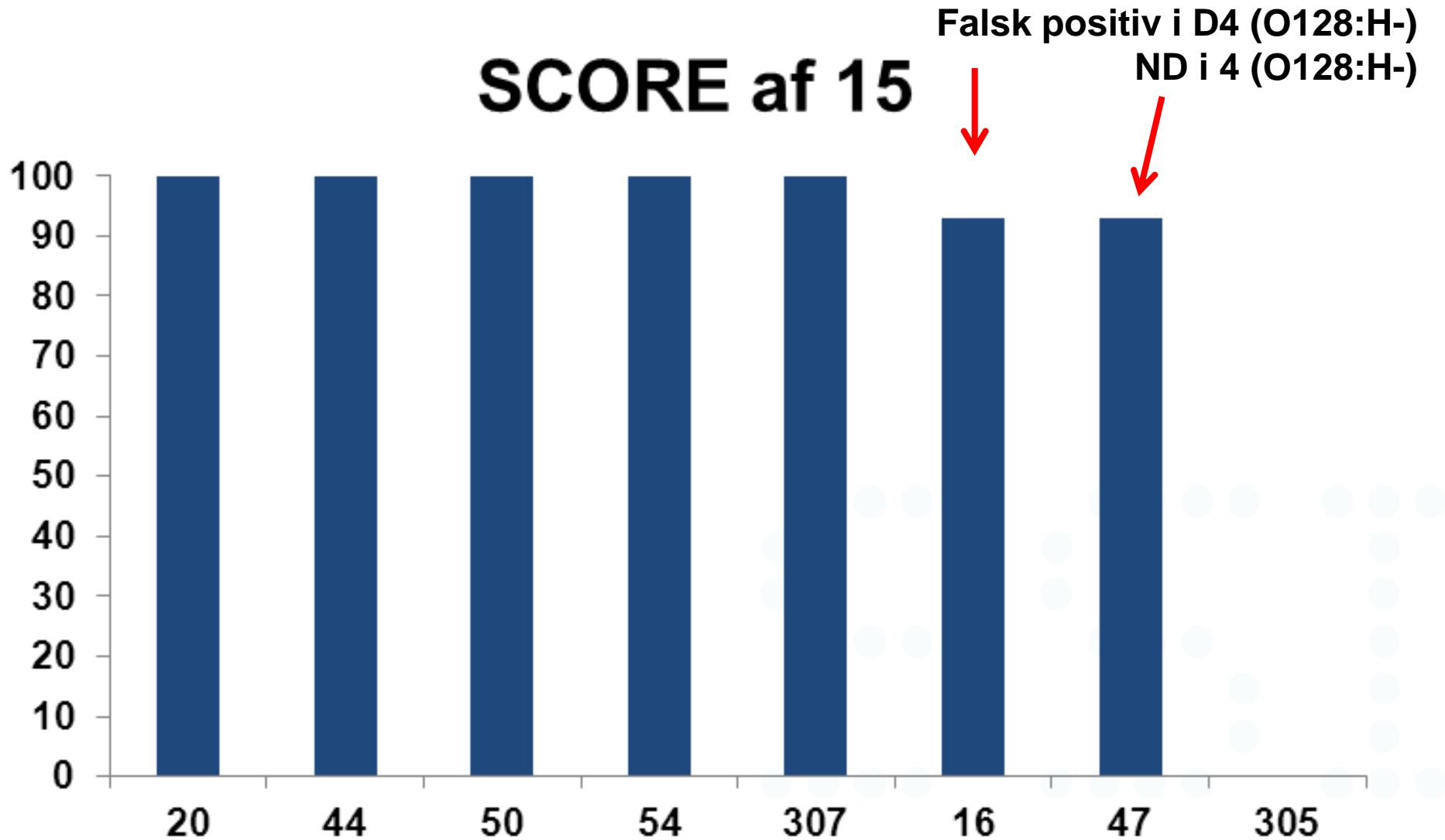
Tarmbakteriologisk register

O gruppe	VTEC	EPEC
O177	1% (18)	
O104	1% (14)*	
O128	4% (9)	10% (5)
O111	3% (10)	5% (7)
O121	1% (12)	1% (17)
O103	11% (2)	7% (6)
O26	8% (4)	14% (2)
O157	17% (1)	12% (4)
SUM	43%	49%

VTEC O GRUPPER 2000 - 2012

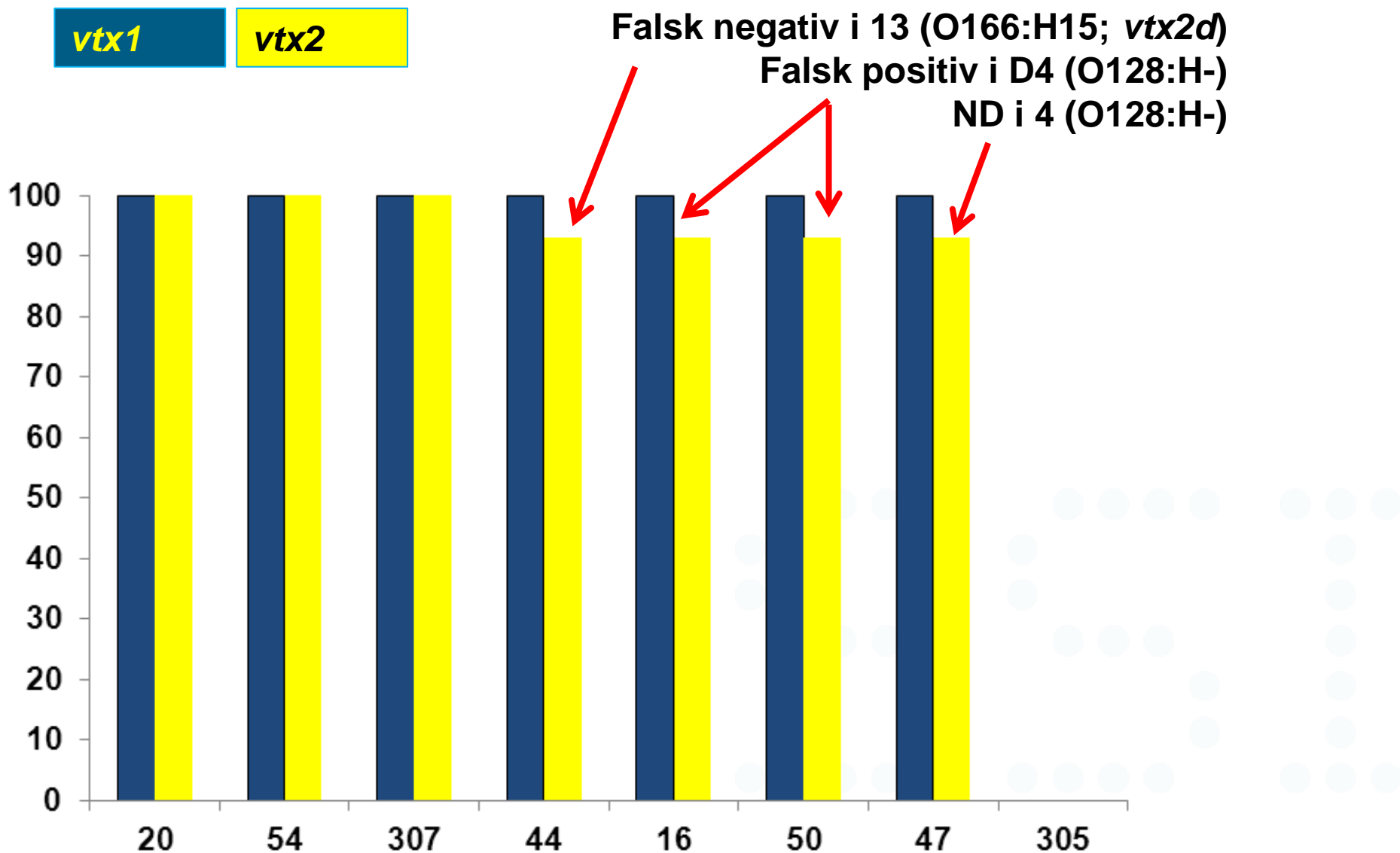


eee – 8 DELTAGERE – 15 STAMMER



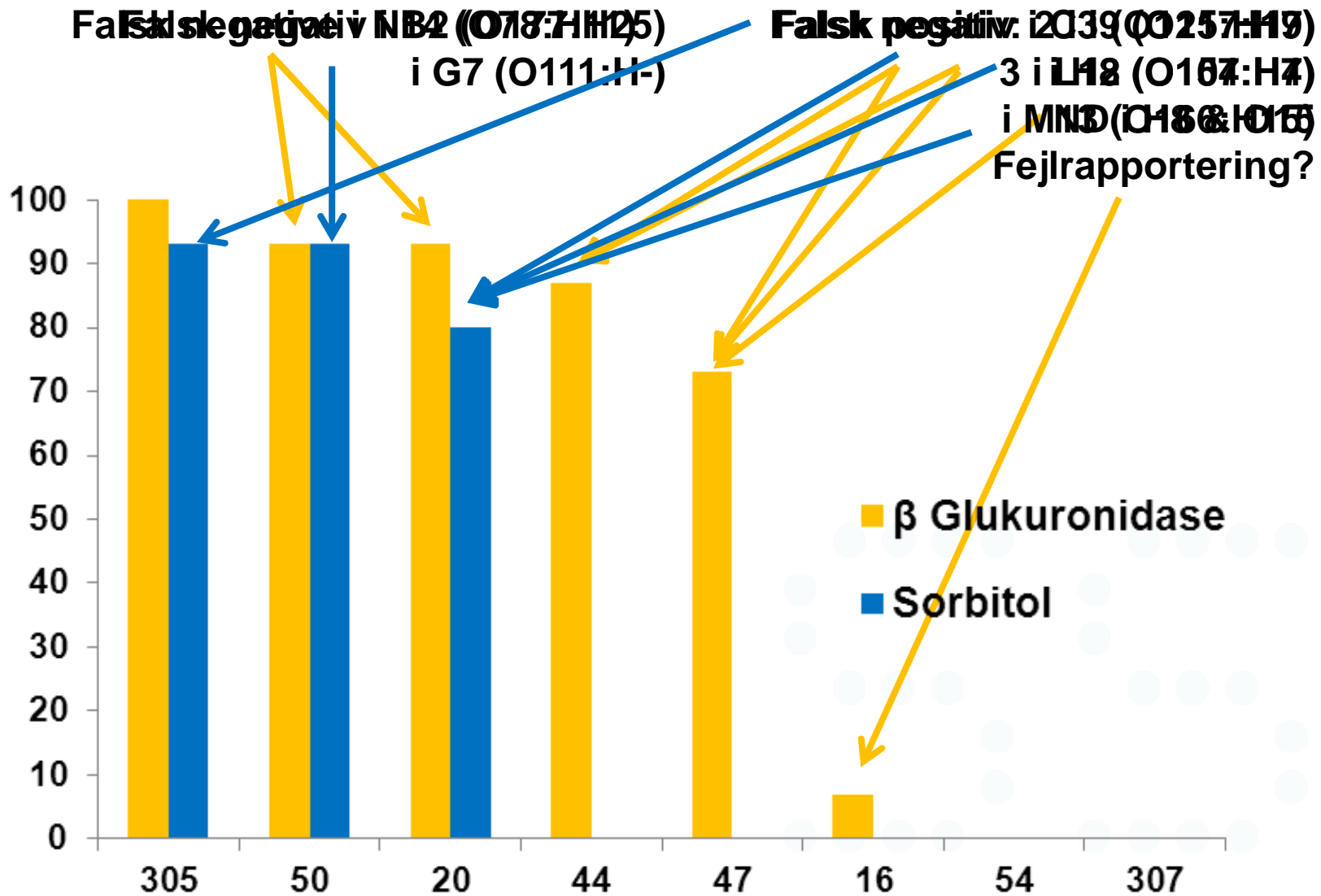
vtx1 - *vtx2* DETEKTION

8 DELTAGERE – 15 STAMMER



FÆNOTYPISK DETEKTION

8 DELTAGERE – 15 STAMMER

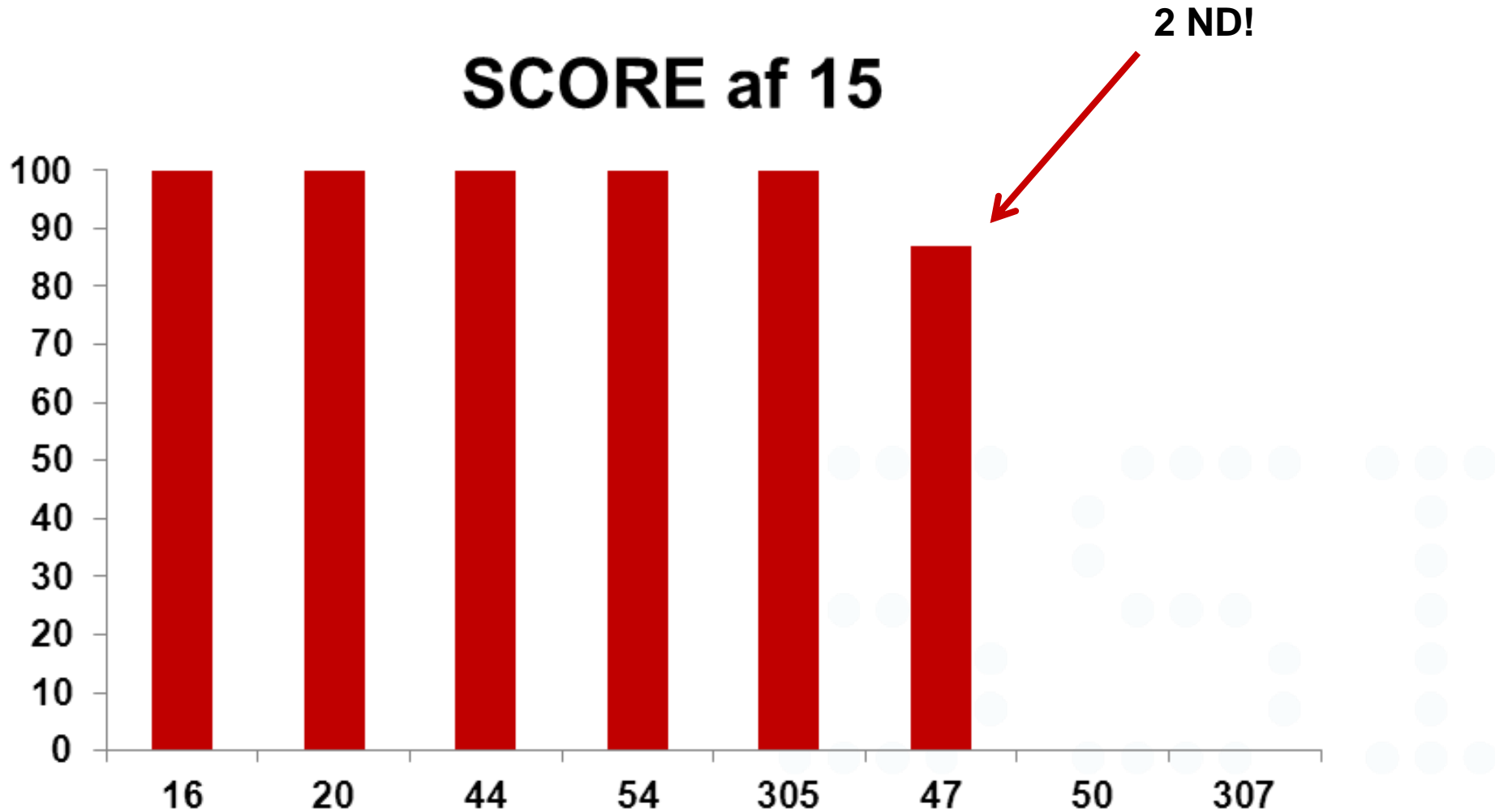


ESBL DETEKTION

8 DELTAGERE – 15 STAMMER



SCORE af 15





Fænotypning

	2008-9	2010	2013
	8	12	8
O gruppering			
O157	92%	100%	88%
O26	75%	100%/86%	88%
O103	83%	100%	75%
O111	-	100%	38%
O121	-	-	50%
O128	67%	50%	38%
β-glucuronidase	98%	96%	89% n=5
Sorbitol	96%	92%	89% n=3



Genotypning

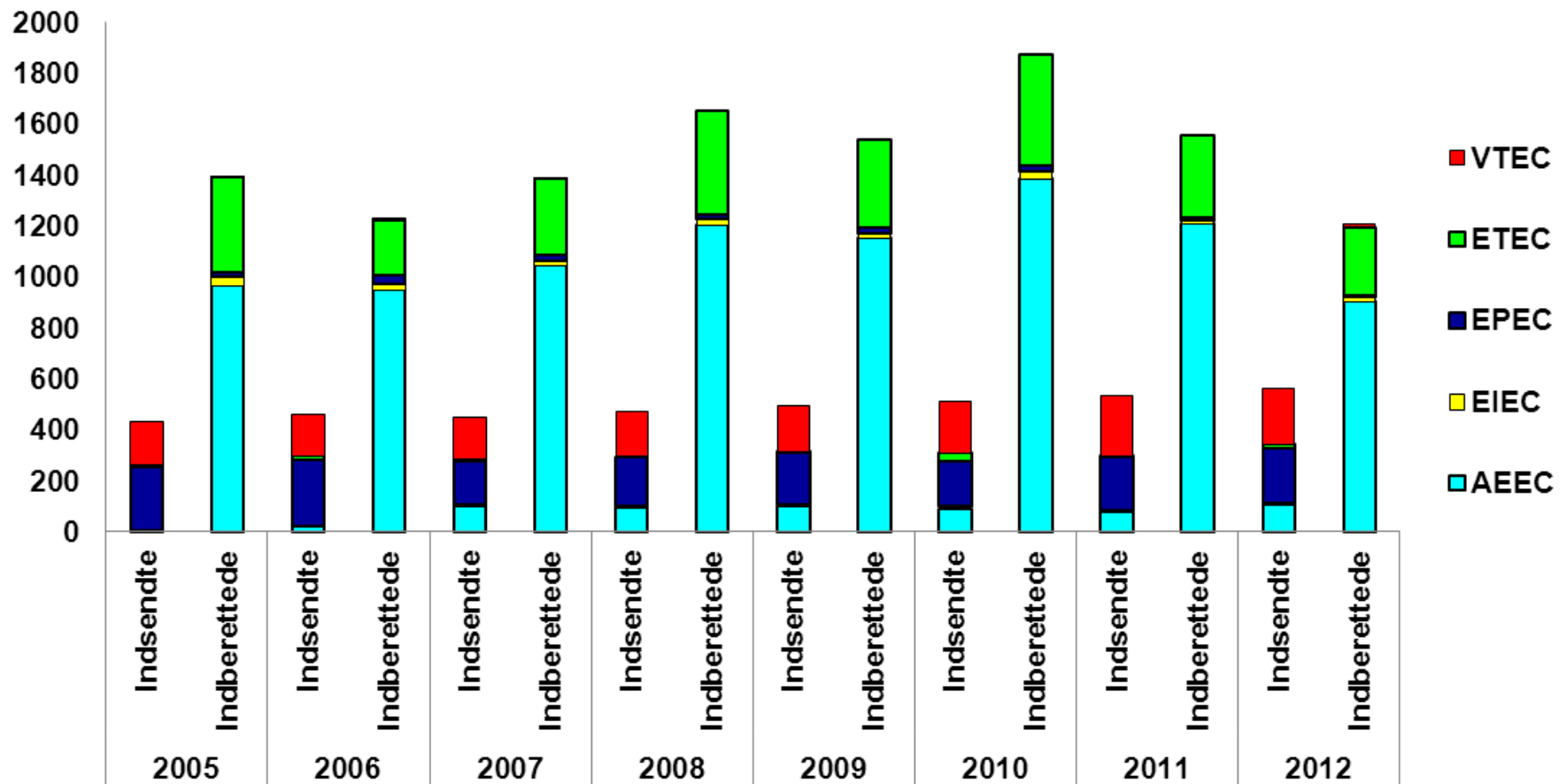
	2008-9 8	2010 12	2013 8
<i>eae</i>	100%	100%	98%
<i>vtx1</i>	96%	98%	100%
<i>vtx2</i>	95%	92%	96%
ESBL	ND	ND	98%
EAggEC	ND	ND	71%
ETEC	ND	67%	100% (57%)
EIEC	ND	67%	100%



• Er antallet af stammer (15) passende?

- Mangler der nogen?
- Er der nogen, der skal udelades?
- Hvordan fungerede indtastning af resultater?
- Kommentarer og forslag er meget velkomne

Indsendte versus indberettede DEC grupper 2005 - 2012





INCIDENS PER 100.000 INDBYGGERE PÅ BAGGRUND AF RAPPORTERINGER TIL TBR

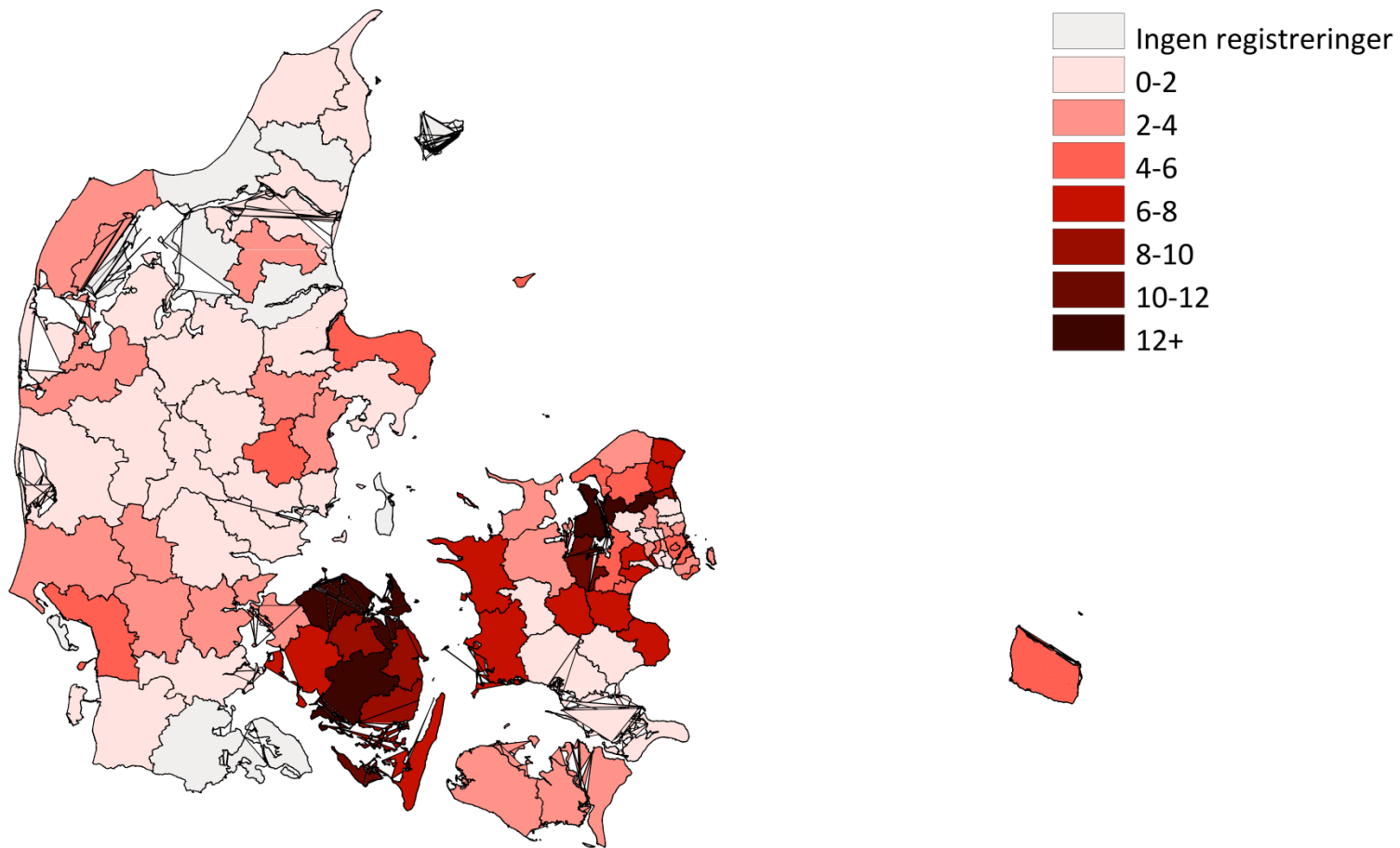
Gennemsnit



VTEC

Rapporteret incidens per 100.000

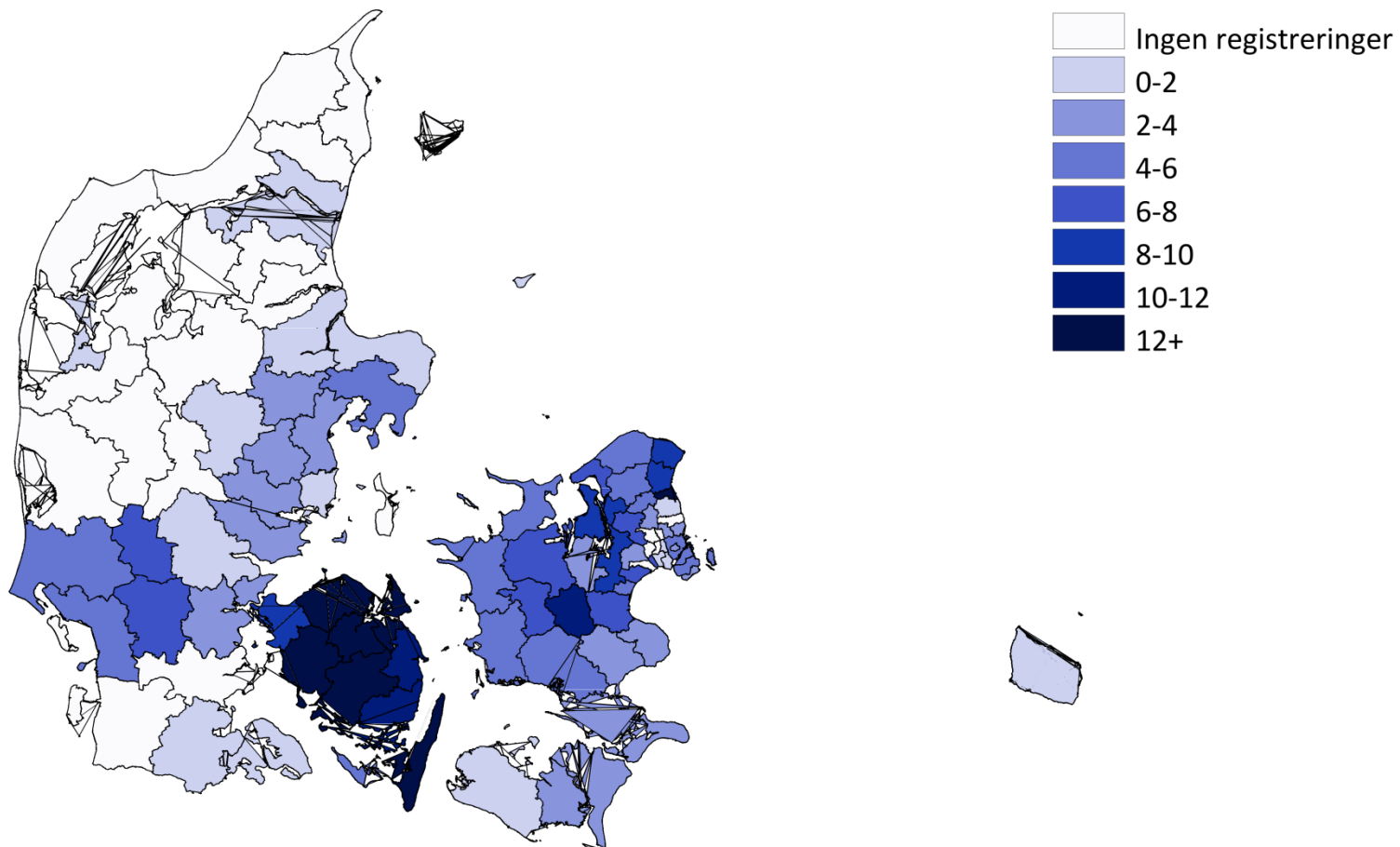
gennemsnit 2010-2012
Tarm bakteriologisk register



EPEC

Rapporteret incidens per 100.000

gennemsnit 2010-2012
Tarm bakteriologisk register

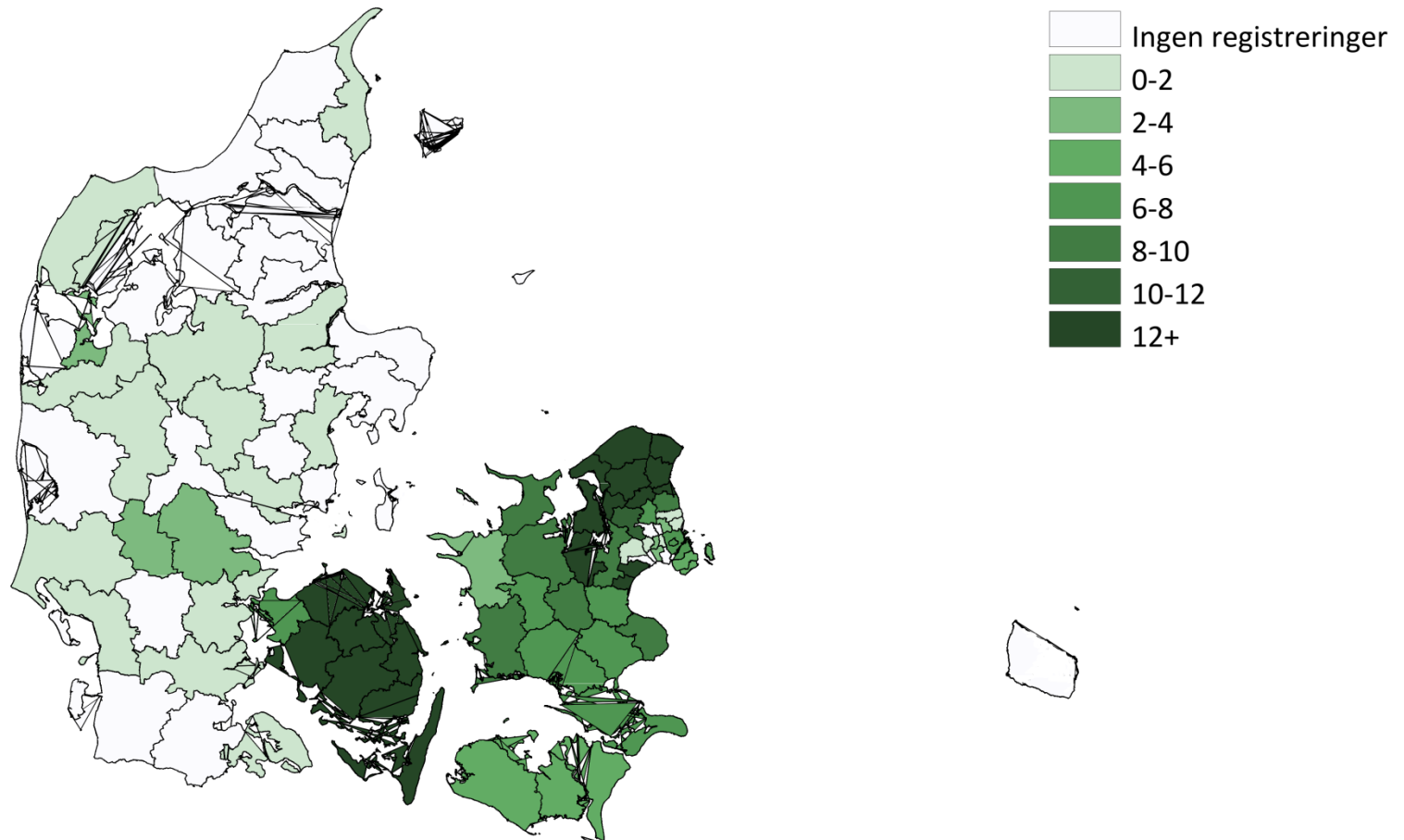


ETEC

Rapporteret incidens per 100.000

gennemsnit 2010-2012

Tarm bakteriologisk register



INCIDENS BLANDT BØRN UNDER 5 ÅR PER 100.000 - PÅ BAGGRUND AF RAPPORTERINGER TIL TBR

Gennemsnit 2010 -2012

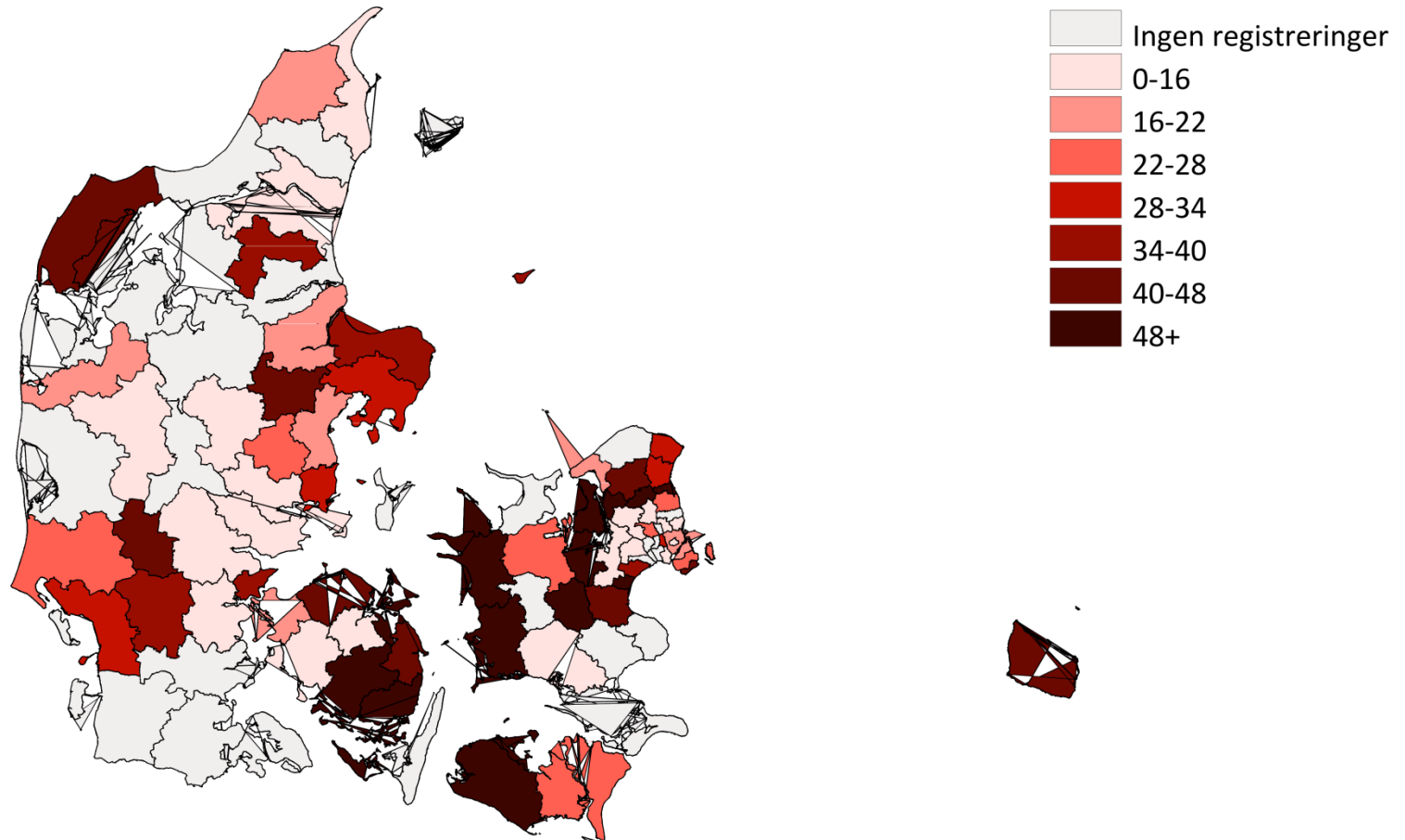


VTEC

Rapporteret incidens blandt børn under 5 år

per 100.000 - gennemsnit 2010-2012

Tarm bakteriologisk register

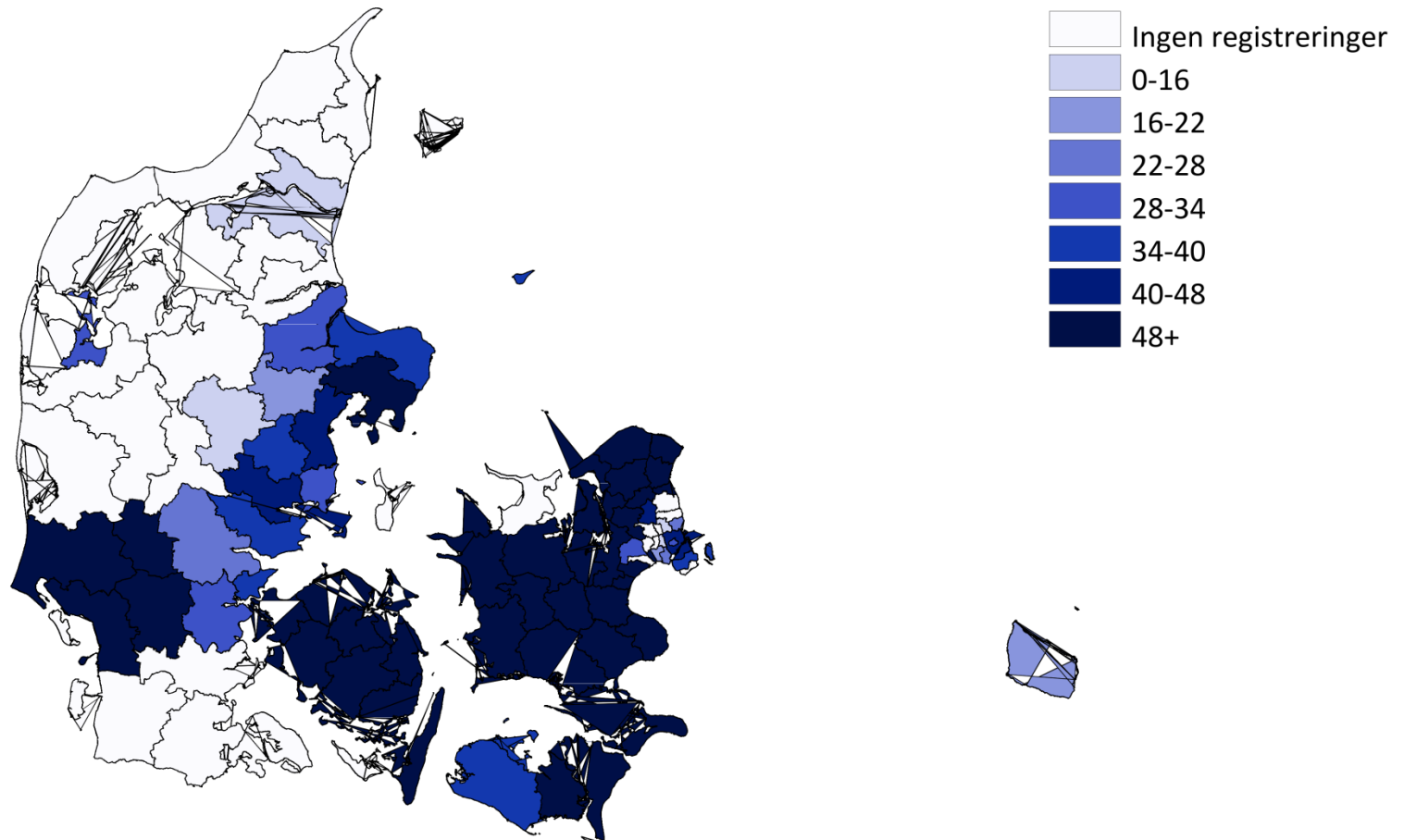


EPEC

Rapporteret incidens blandt børn under 5 år

per 100.000 - gennemsnit 2010-2012

Tarm bakteriologisk register



ETEC

Rapporteret incidens blandt børn under 5 år

gennemsnit 2010-2012

Tarm bakteriologisk register



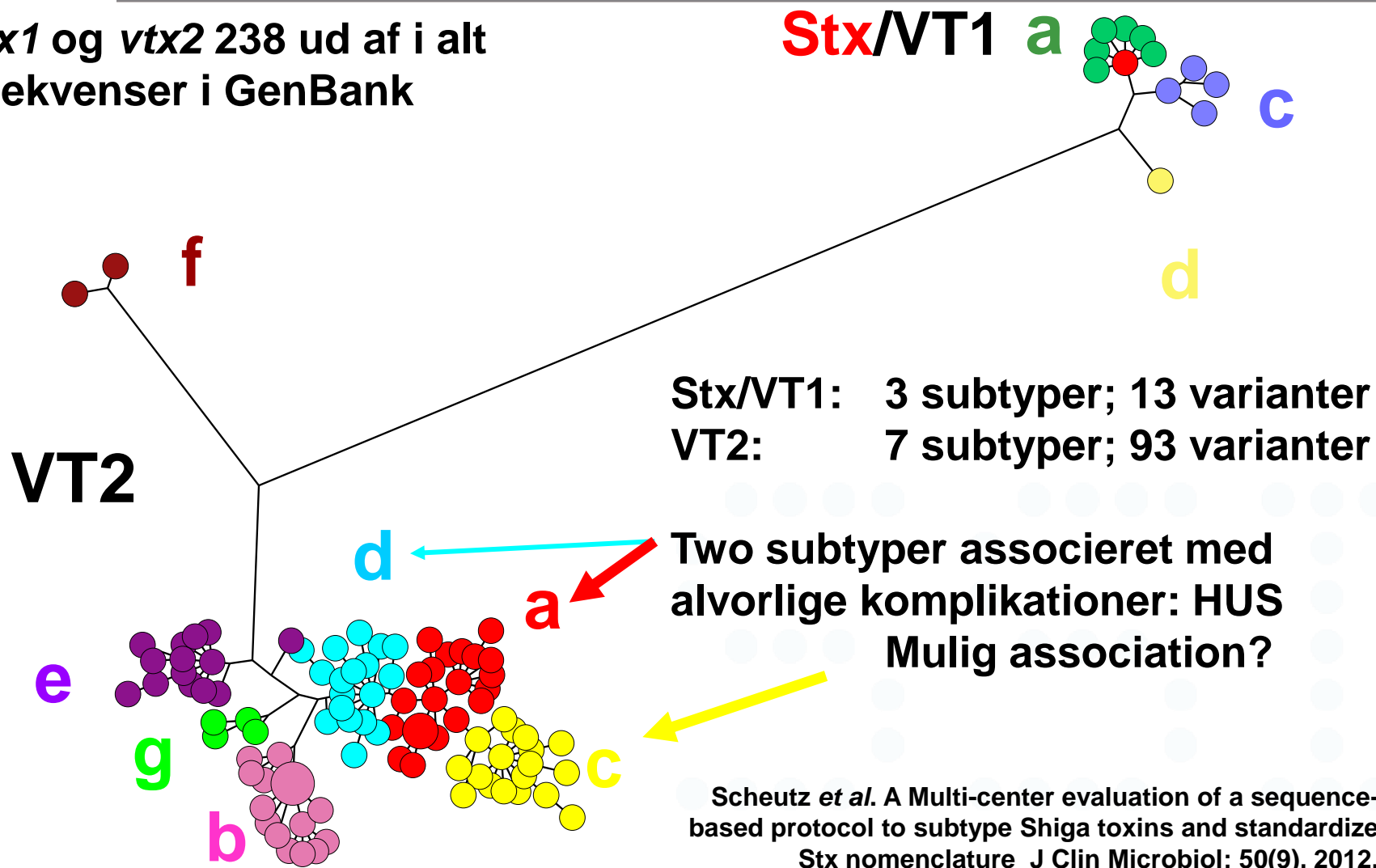
NYE VIRULENSGENER





stx SUBTYPER OG VARIANTER

47 *vtx1* og *vtx2* 238 ud af i alt
398 sekvenser i GenBank





Journal of
Clinical Microbiology

Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature

Flemming Scheutz, Louise D. Teel, Lothar Beutin, Denis Piérard, Glenn Buvens, Helge Karch, Alexander Mellmann, Alfredo Caprioli, Rosangela Tozzoli, Stefano Morabito, Nancy A. Strockbine, Angela R. Melton-Celsa, Maria Sanchez, Søren Persson and Alison D. O'Brien
J. Clin. Microbiol. 2012, 50(9):2951. DOI:
10.1128/JCM.00860-12.
Published Ahead of Print 3 July 2012.



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Reference- og speciallaboratorier

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> Parasitter

> Svampe

> Virus



WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella

Laboratoriet er det nationale referencelaboratorium for typning af *E. coli* og *Klebsiella*. Centeret indsamler overvågningsdata til nationale og internationale myndigheder. Centeret koordinerer typning og deltager i forskningsprojekter i ind- og udland, samt rådgiver vedrørende detektion og typning. Det fungerer som ECDC's mikrobiologiske støttelaboratorium og organiserer eksterne kvalitetsprogrammer (EQA) i samarbejde med European Centre for Disease Prevention and Control (ECDC) i Stockholm, European Union Reference Laboratory (EU-RL) for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC), Istituto Superiore di Sanità (ISS) i Rom, Global Foodborne Disease Network (GFN) og WHO's Department of Food Safety and Zoonoses (FOS) i Genève

Undersøgelser

Kontakt

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WHO Collaborating Centre for Reference and Research on Escherichia Klebsiella
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Relaterede links

[SOP for O & H serotyping and reference strains \(pdf\)](#)

[Vtx detection & subtyping protocol \(pdf\)](#)

[Nordic VTEC Report \(pdf\)](#)

[ECDC](#)

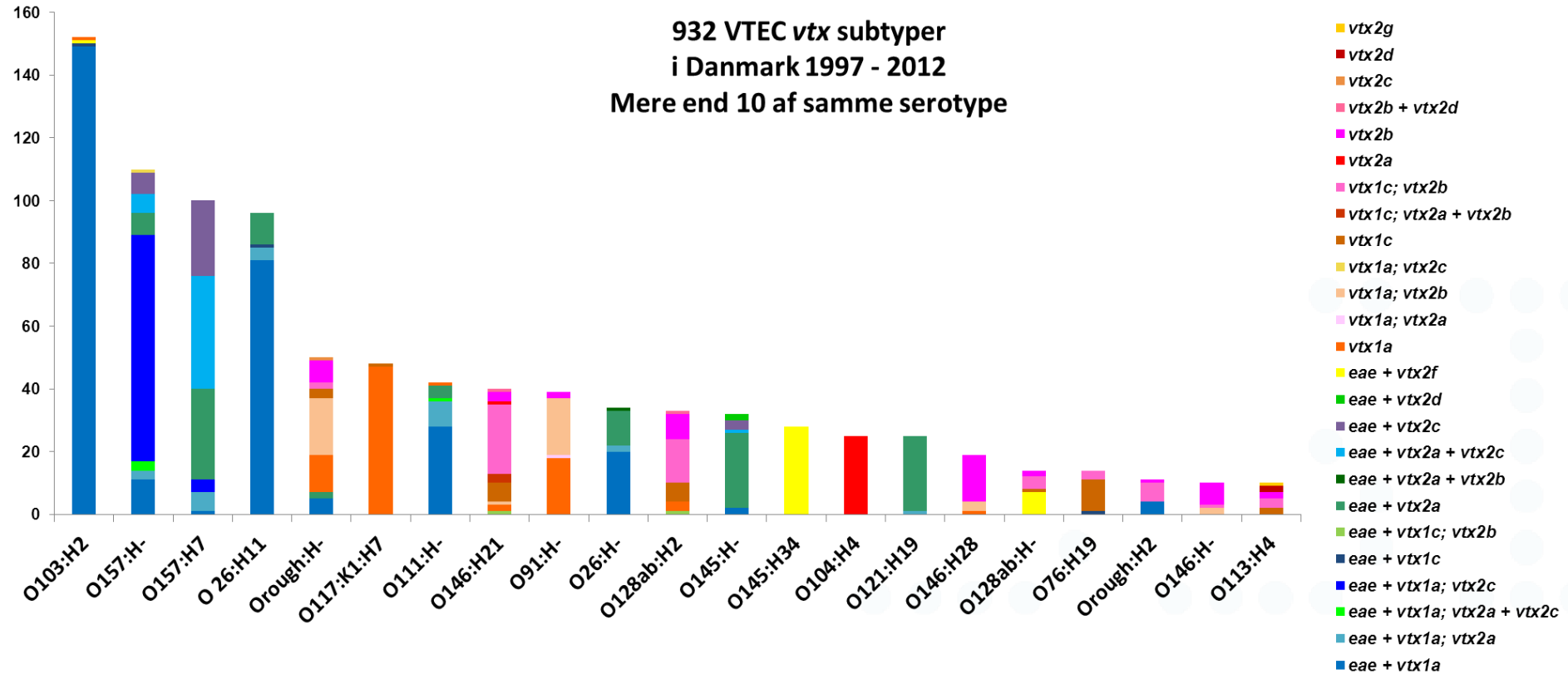
[EU-RL](#)

[GFN](#)

[WHO FOS](#)

SEROTYPER, *vtx* SUBTYPER & *eae*

932 VTEC *vtx* subtyper
i Danmark 1997 - 2012
Mere end 10 af samme serotype



"Attack rate" af vtx subtyper associeret med HUS

O157	20/195	10%
Non-O157	36/928	4%
<i>eae</i> positive	27/472	6%
<i>vtx2a</i>	18/85	21%
<i>vtx2a</i> + <i>vtx2c</i>	1/5	20%
<i>vtx1a</i> + <i>vtx2a</i>	4/23	17%
<i>vtx1c</i>	1/6	17%
<i>vtx1a</i>	3/305	1%
<i>eae</i> negative	9/465	2%
<i>vtx2a</i>	8/34	24%
<i>vtx2d</i>	1/13	8%
<i>vtx2a</i> in EAggEC-VTEC O104:H4	827/3793	22%



HUS associerede VTEC

...

Følgende virulensprofiler har en klinisk relevant association til HUS;

1. *vtx1* og *eae* og O103:H2;
2. *vtx1* og *vtx2* og *eae*;
3. *vtx2* og *eae*;
4. *vtx2* i en enteroaggregativ *E. coli* (EAaggEC) fx. O104 og O111.
5. *vtx2d* i *eae* negative VTEC.

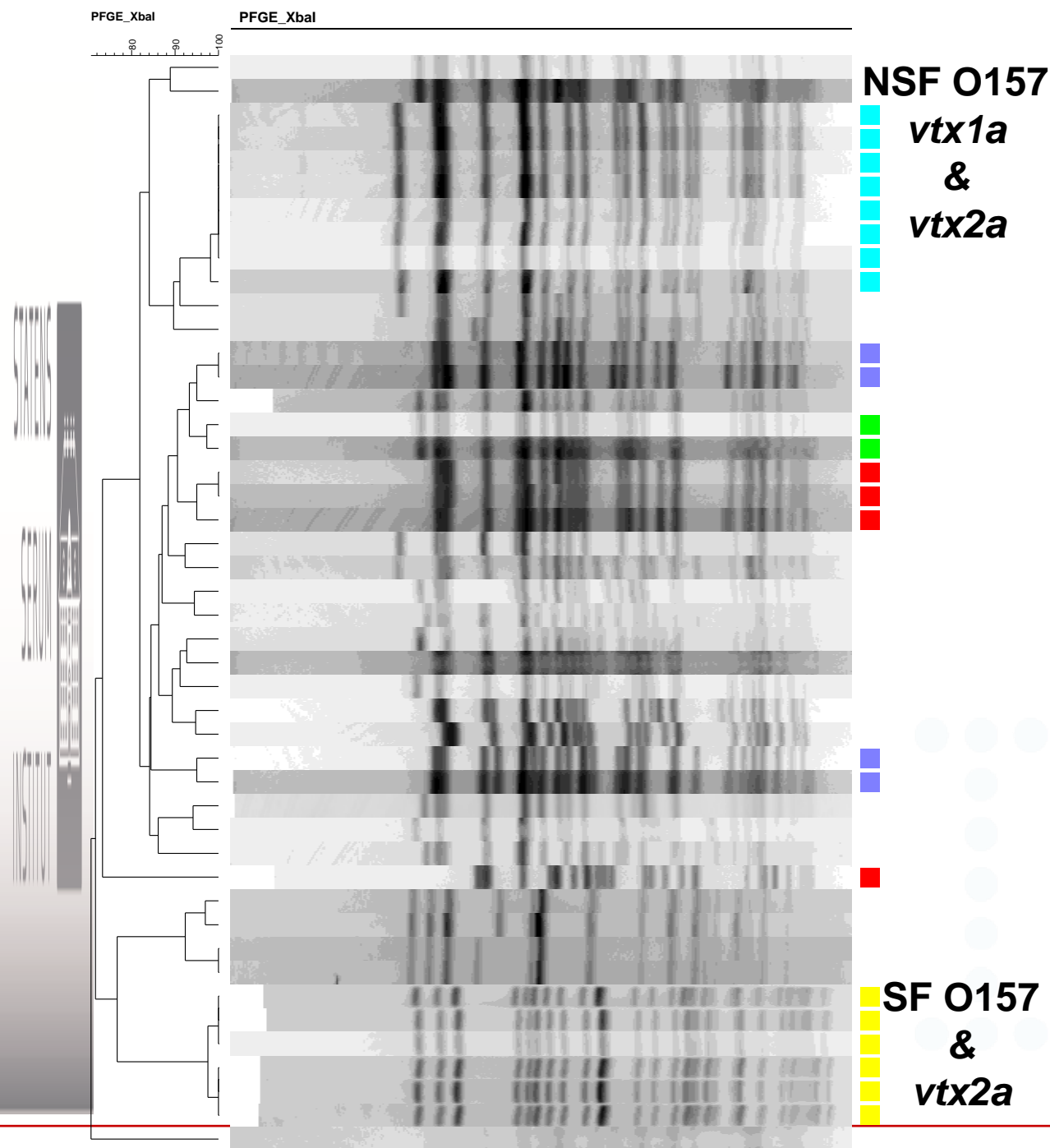
Med disse kriterier ville 61% (1111/1827) have fået besked om infektion med "lav risiko VTEC"

39% (716/1827) ville have en mulig HUSEC infektion

Efter *vtx* subtypning ville dette kunne reduceres til ~21% (234/1134)

PFGE PROFILER AF VTEC O157 I EFTERÅRET 2012

I
FORÅRET
2012



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Eurosurveillance, Volume 18, Issue 2, 10 January 2013

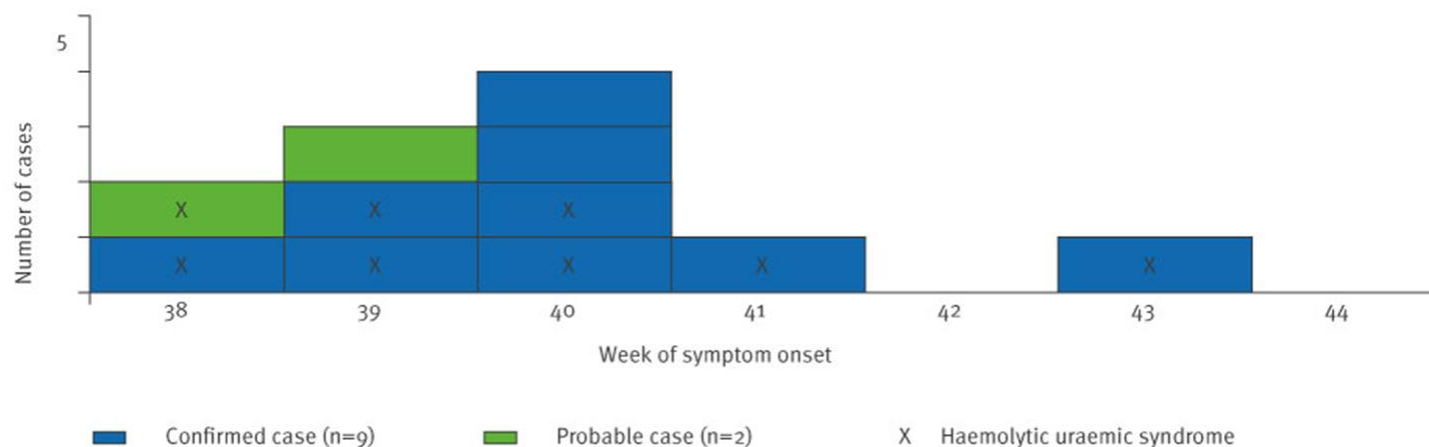
Rapid communications

A VEROCYTOTOXIN-PRODUCING *E. COLI* OUTBREAK WITH A SURPRISINGLY HIGH RISK OF HAEMOLYTIC URAEMIC SYNDROME, DENMARK, SEPTEMBER-OCTOBER 2012

B Soborg (bot@ssi.dk)^{1,2}, S G Lassen¹, L Müller¹, T Jensen³, S Ethelberg^{1,4}, K Mølbak¹, F Scheutz⁴

FIGURE

Probable and confirmed cases of symptomatic verocytotoxin-producing *E. coli* O157 by date of onset, Denmark, September –October 2012 (n=11)



The two asymptomatic cases found by screening are not depicted in the figure.

TABLE

Verocytotoxin-producing *E. coli* O157 cases and proportion of haemolytic uraemic syndrome by toxin profile, Denmark, 1 January 1997–31 July 2012 (n=212)

Toxin profile	Total number of cases	Number of HUS cases	Proportion of HUS
<i>eae + vtx1a</i>	8	0	0
<i>eae + vtx1a + vtx2c</i>	85	0	0
<i>eae + vtx2c</i>	31	1	3%
<i>eae + vtx2a + vtx2c</i>	45	11	24%
<i>eae + vtx2a</i>	37	11	30%
<i>eae + vtx1a + vtx2a</i>	6	2	33%

HUS: haemolytic uraemic syndrome.

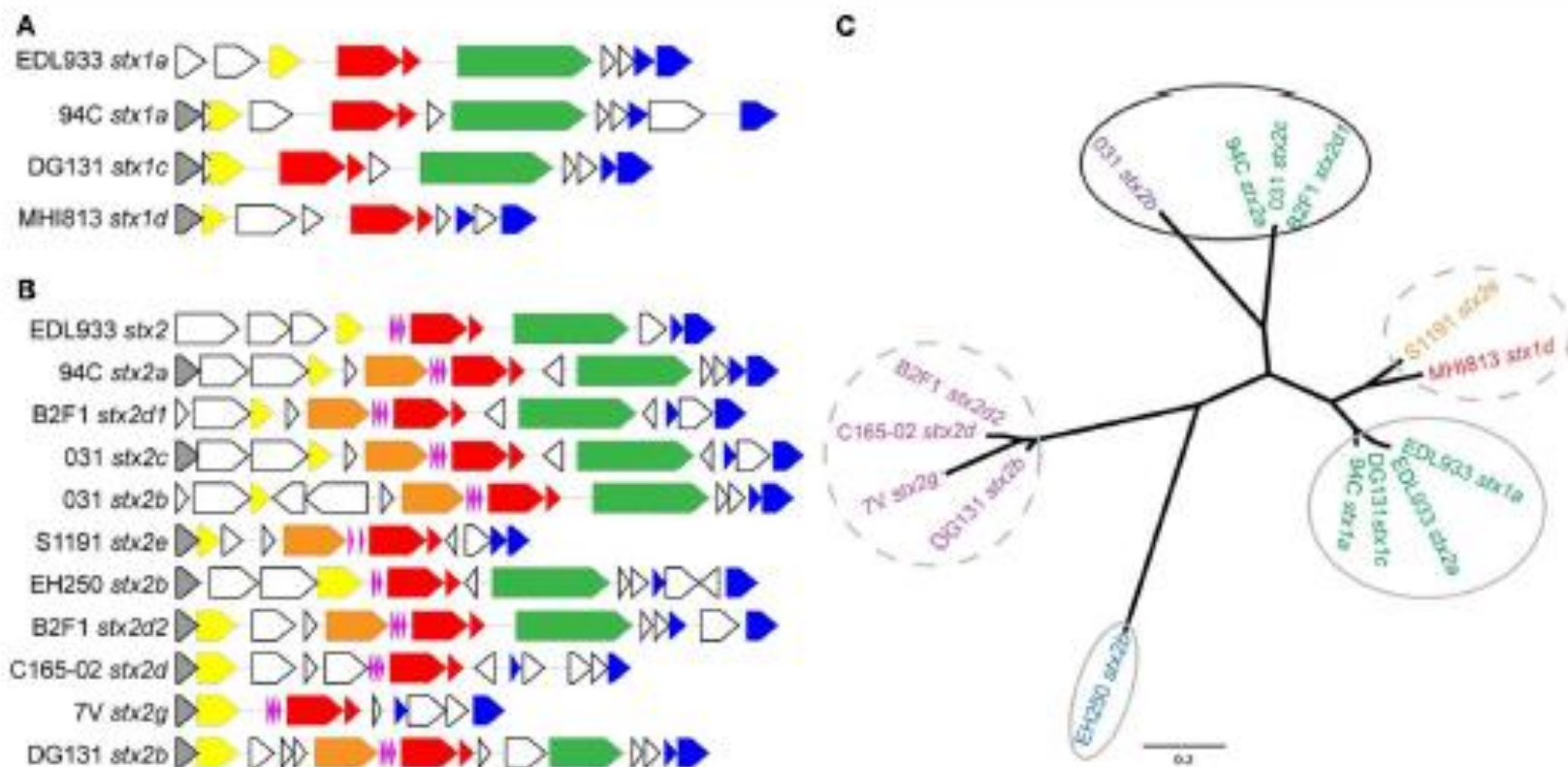


FIGURE 5 | Gene organization flanking the *stx* genes and *Q* gene phylogeny for the *stx* phages in the LEE-negative STEC isolates and LEE-positive O157:H7 EDL933. Gene organization comparisons are shown for **(A)** *stx*_i-encoding phages and **(B)** *stx*_j-encoding phages. The colors correspond to the following gene designations: gray, *rusA*; yellow, *Q*; orange, DNA methylase; pink, tRNA genes; red, *stxAB*; green, *yjH5*; blue, lysis *S*, and endolysin genes; white, all other genes, predominantly

encoding hypothetical proteins. A cluster diagram based on the *Q* gene sequences was determined **(C)** and primers **(Table 2)** were designed to be specific for each cluster according to the colors: O1 green, O2 purple, O3 turquoise, O4 blue, O5 magenta, O6a orange, and O6b red. Clusters circled by a solid black line denote a high level of *stx* induction, gray circles denote intermediate level induction, and broken lines denote lack of induction.

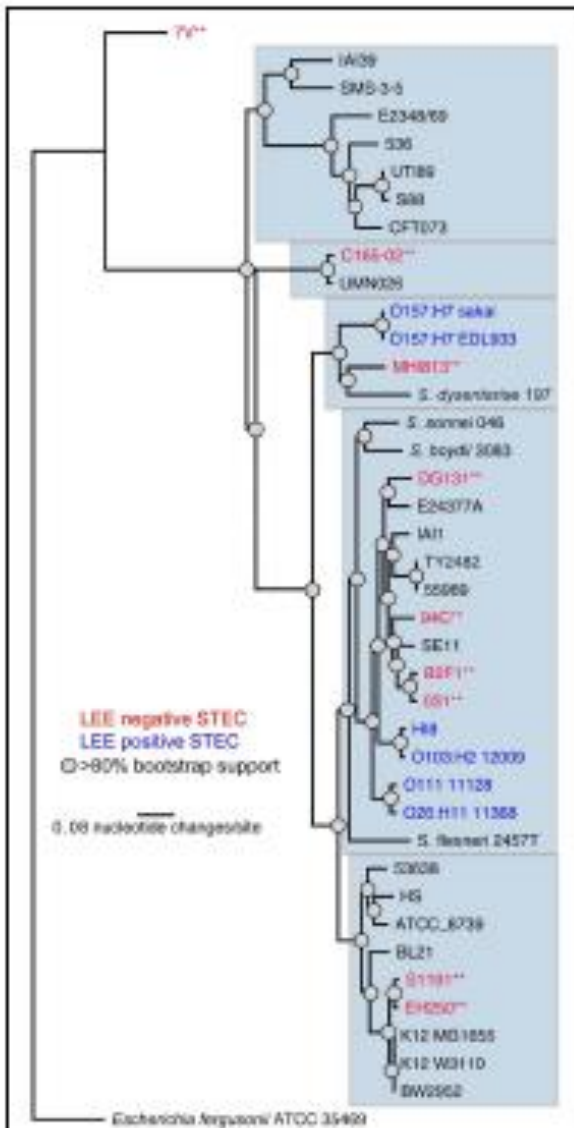
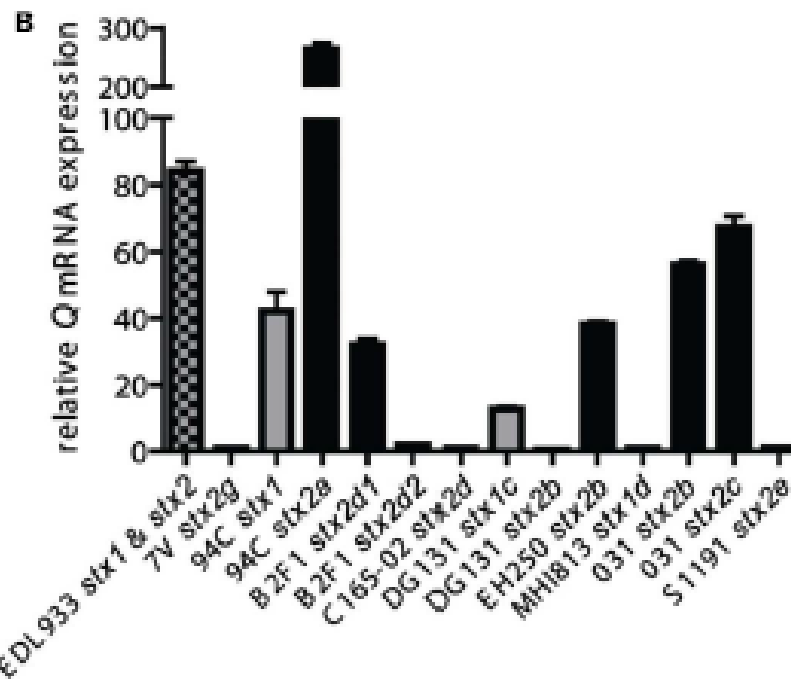
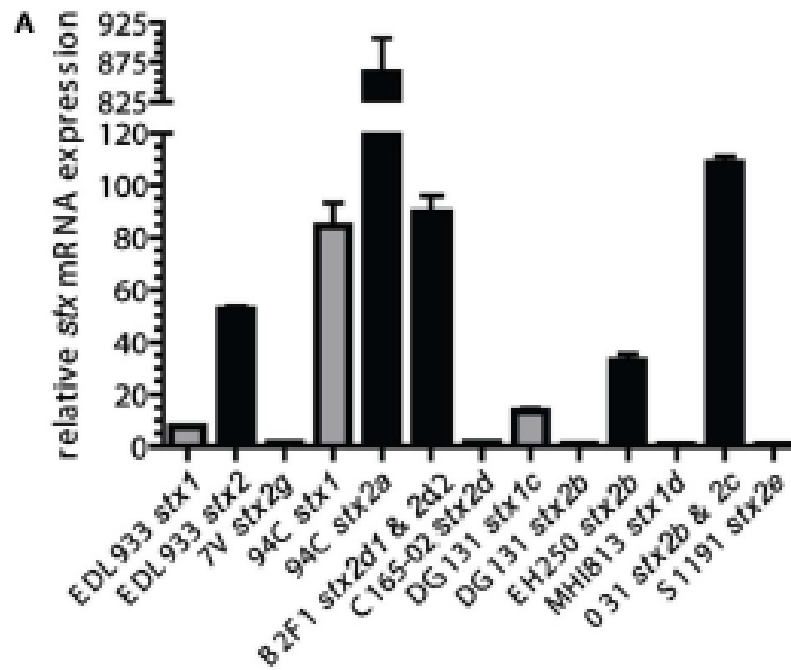


FIGURE 1 | A whole genome phylogeny of nine LEE-negative (red) and four LEE-positive (blue) STEC compared in this study. Whole genome sequences for the LEE-negative STEC sequenced in this study (indicated by asterisks) was combined with sequence data obtained from GenBank for *E. coli*/*Shiga* genomes representing the major pathotypes (Table A1 in Appendix), and aligned based on concatenated regions of shared sequence as determined from analysis using Mugsy (Anguich and Selberg, 2017). The phylogenetic tree was inferred with *E. fergusonii* isolate 35469 as the outgroup.



A comparison
of induced
stx

and

Q gene
expression



∴ TTSS is necessary for expression of *nleB* & *nleE*

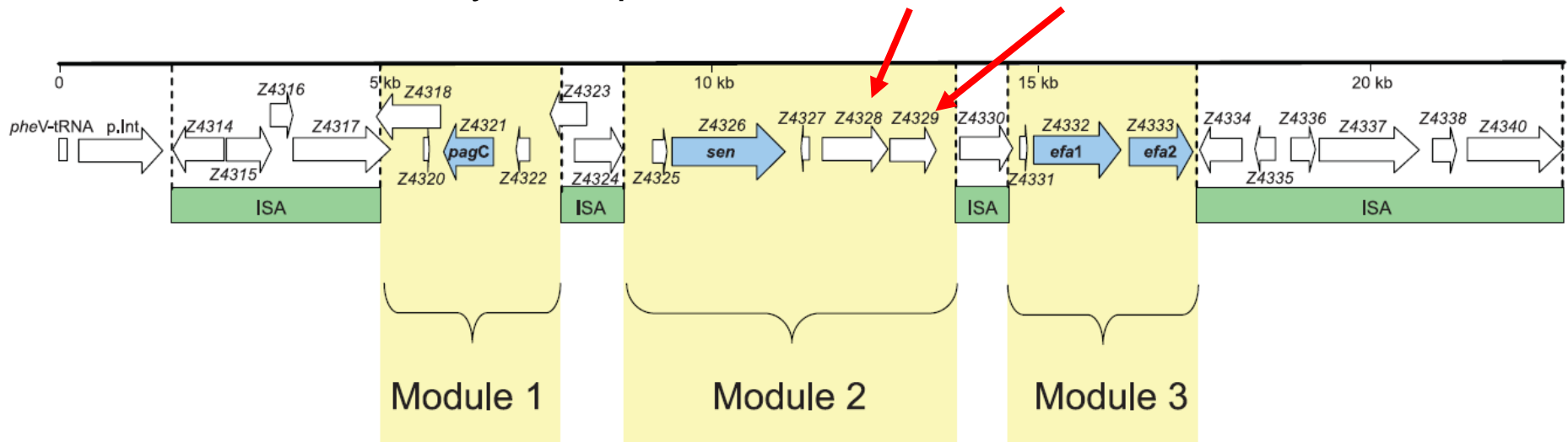
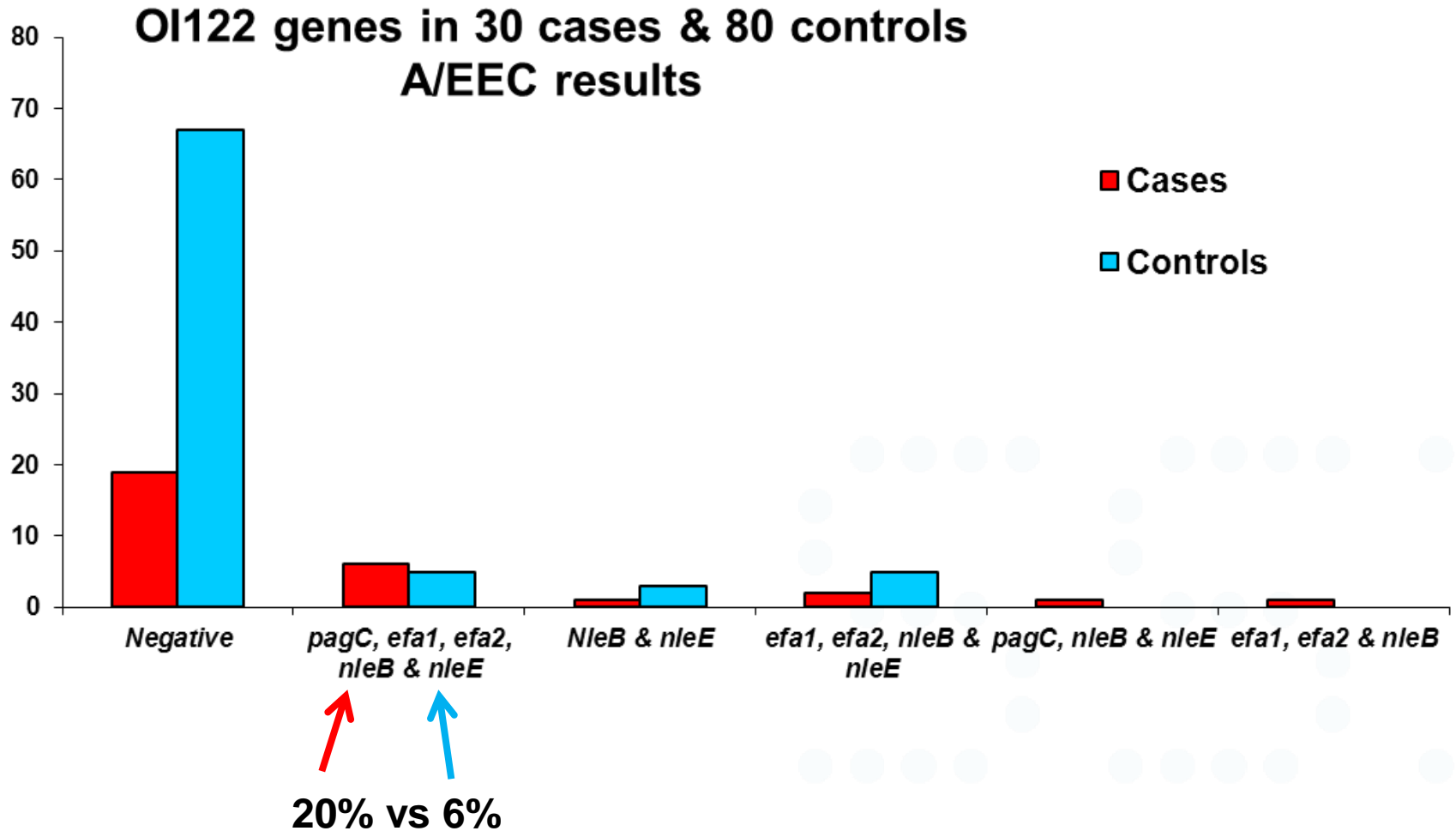
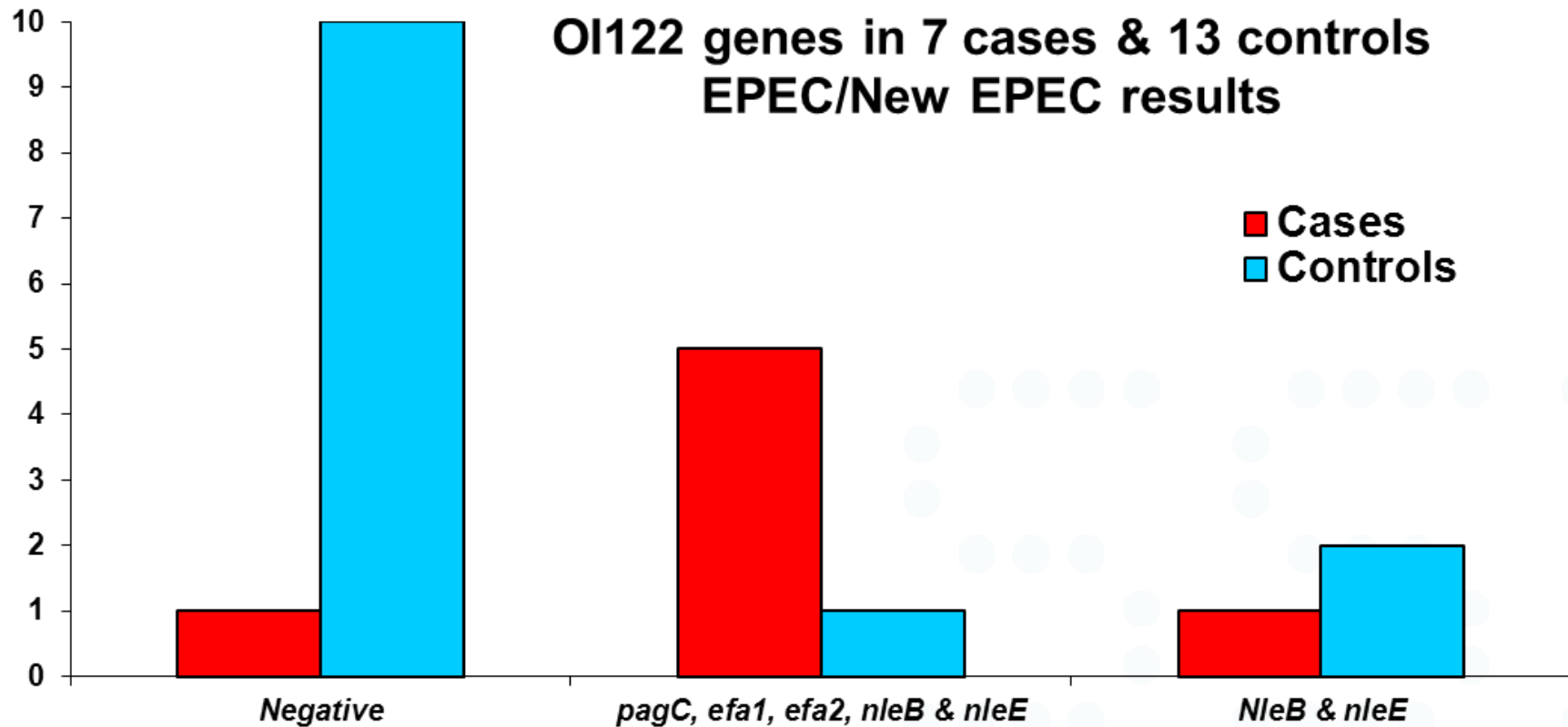


FIG. 1. Modular components of OI-122. ISA, insertion sequence-associated elements (or putative transposases) between the three modules. The PCR gene markers used to detect the presence of modules are indicated by bold type and blue.

FLUIDIGM HIGH THROUGHPUT RT-PCR USING 96 REACTIONS; A/EEC



FLUIDIGM HIGH THROUGHPUT RT-PCR USING 96 REACTIONS; EPEC/NEW EPEC



Cases

O55:[H7]	4	<i>pagC, efa1, efa2, nleB & nleE</i>
O114:H-	1	<i>pagC, efa1, efa2, nleB & nleE</i>
O127:H-	1	<i>nleB & nleE</i>

Controls

O26:H-	1	<i>pagC, efa1, efa2, nleB & nleE</i>
O103:H-	1	<i>nleB & nleE</i>
O127:H40	1	<i>nleB & nleE</i>

**O86:H8, O88:H- (3), O114:H-, O125ab:H6, O128:H2,
O142:H34 and O157:H16 ALL negative!**



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DEMONSTRATION OF ANTIBODIES AGAINST ENTERO- PATHOGENIC ESCHERICHIA COLI IN SERA OF CHILDREN OF VARIOUS AGES

Sk

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EXTENSIVE investigations into the etiology of epidemic diarrhea of the newborn and young infant carried out in various parts of the world during the past decade have revealed that a few (0111, 055, 0127) of the numerous serogroups of *Escherichia coli* are responsible for many outbreaks. Serogroup 026 is associated with sporadic cases. The conclusion of the etiologic role of these microorganisms is based on extensive epidemiological data and the results of feeding studies in adults and infants. In addition, the efficacy of anti-

solved. Attempts to isolate in human volunteers the virus responsible for mild gastroenteritis (Gordon) from material obtained during 1947 outbreaks of epidemic diarrhea of the newborn yielded negative results.⁵ One of the enteropathogenic serogroups of *E. coli*, namely serogroup 0111, was found in 1952 to be present in all available fecal specimens from the same outbreaks and was, in fact, the predominant coliform organism in both fatal and non-fatal cases.⁶ An intensive search for a virus by means of animal inoculations and the

804 NETER — ANTIBODIES AGAINST ENTEROPATHOGENIC E. COLI

ESCHERICHIA COLI HEMAGGLUTINININS IN RANDOM HUMAN SERA

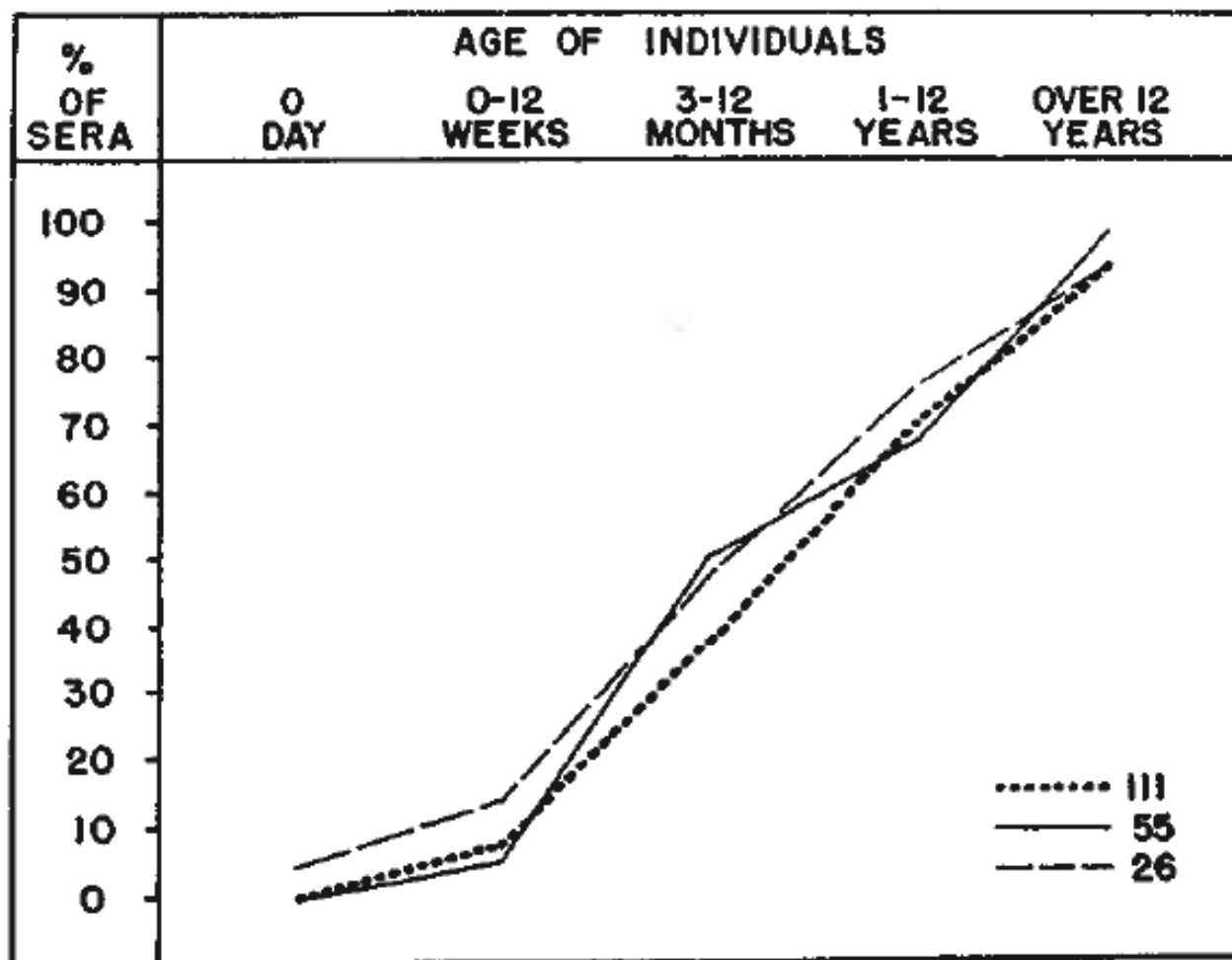


FIG. 1.

- ❖ ***Hafnia alvei***, a probable cause of diarrhea in humans - 1992
- ❖ Først beskrevet som eae positive *Hafnia alvei* hos børn i Bangladesh med diaré og hos rejsende fra Marokko – 1994
- ❖ eae positive skilles fra andre *H. alvei* på:
 - negative 2-ketogluconate & histidine assimilation tests
 - positive i 3- hydroxybenzoate assimilation test
- ❖ Tilhører genus *Escherichia* - 1999
 - laktose, sorbitol ? og β -glukuronidase negative
- ❖ *E. albertii* - 2003
 - indole positive
 - D-mannitol positiv
 - D-xylose og dulcitol negativ
 - Vil ofte identificeres som *Hafnia*, *Salmonella*, *Escherichia coli* eller *Yersinia ruckeri*

- Ooka, T., K. Seto, K. Kawano, H. Kobayashi, Y. Etoh, S. Ichihara, A. Kaneko, J. Isobe, K. Yamaguchi, K. Horikawa, T. A. Gomes, A. Linden, M. Bardiau, J. G. Mainil, L. Beutin, Y. Ogura, and T. Hayashi. 2012. Clinical Significance of *Escherichia albertii*. *Emerg. Infect. Dis.* 18:488-492.
- **Abstract: Discriminating *Escherichia albertii* from other *Enterobacteriaceae* is difficult. Systematic analyses showed that *E. albertii* represents a substantial portion of strains currently identified as *eae*-positive *Escherichia coli* and includes Shiga toxin 2f-producing strains. Because *E. albertii* possesses the *eae* gene, many strains might have been misidentified as enterohemorrhagic or enteropathogenic *E. coli***
- β -glukuronidase, D-xylose og dulcitol negativ
- *cdtB* og *vtx2f* fundet i nogle af stammerne

SHIGELLA INTERNATIONAL MEETING

May, 28-29, 2012 – Buenos Aires – Argentina

Funding for this meeting was made possible by the Bill & Melinda Gates Foundation, PATH and PAHO/WHO

“as a long term goal, it was proposed to revise the classification of *Shigella* spp. including it in the same scheme as *Escherichia coli*, given the evidence of close genetic relation between these organisms...”

2nd version: . . . “As a long term goal, **some participants** proposed that the classification of *Shigella* spp. should be revised and **perhaps** to fold it into the same scheme as *Escherichia coli* . . . “!!

Classical & **New** EIEC O:H serotypes

O group

H antigen

O28ac

H-

= *S. boydii* 13

O29

H-

= *S. dysenteriae* 11

O96

H19

O112ac

H-

= *S. dysenteriae* 2

O112ab ~ *S. boydii* 15

O115

H-

O121

H-

= *S. dysenteriae* 7

O124

H-; H7; H30; H32

= *S. dysenteriae* 3

O135

H-

O136

H-

O143

H-

= *S. boydii* 8

O144

H-; H25

= *S. dysenteriae* 10

O152

H-

= *S. dysenteriae* 12

O159

H2

O164

H-

O167

H-; H4; H5

= *S. boydii* 3

O173

H-

Shigella IDs & **New** EIEC O groups

O9	H-		
O13	H-		
O15	H-		
O36		H14	
O40			~ <i>S. dysenteriae</i> 9 ?
O44	H-		
O58			<i>S. dysenteriae</i> 5
O89	H19		
O112ab			<i>S. boydii</i> 15
O130	H-		~ <i>S. dysenteriae</i> 6 (SR form)
O132		H21	
O147			<i>S. flexneri</i> 6 & 1-5)
O148			~ <i>S. dysenteriae</i> 1 ?
O149			<i>S. boydii</i> 1
O150			<i>S. dysenteriae</i> 13
O168			<i>S. dysenteriae</i> 4

TAK FOR JERES OPMÆRKSOMHED



Eva Møller Nielsen Mia Torpdahl Susanne Schjørring Flemming Scheutz