



# ***E. coli* other than VTEC: which role in food safety ?**

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**& Infection Control**

**STATENS SERUM INSTITUT**



# **DETECTION OF FOOD-BORNE PATHOGENS**

**Immediate recognition and notification of  
the (suspected) outbreak**

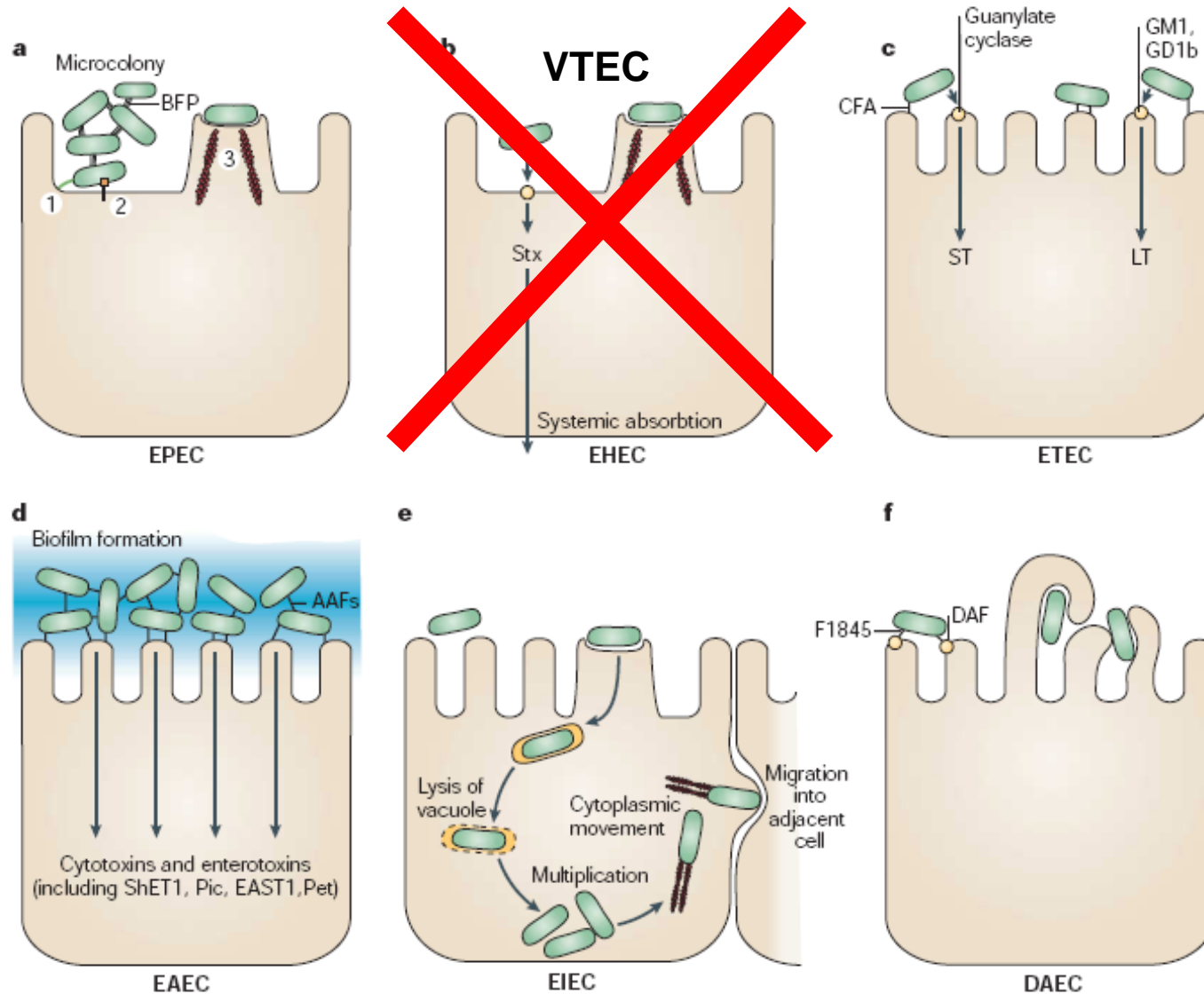
**Adequate detection methods must be  
available in all the concerned institutions  
and agencies**

**Epidemiological follow-up must be available**

**ASSOCIATION WITH DISEASE MUST BE  
RECOGNISED**



# Diarrhoeagenic *E. coli* DEC



Kaper *et al*,  
Nature  
Reviews;  
Microbiology  
2004



# EPEC definition:

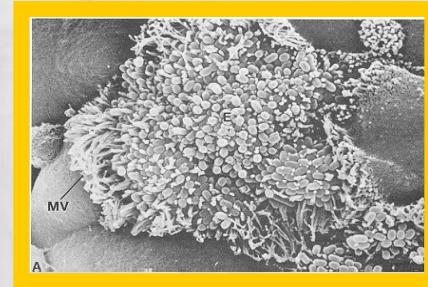
The Second International EPEC Symposium , 1996

EPEC defined as *E. coli* that :

- are diarrhoeagenic
- produce the A/E lesion on intestinal cells
- do not produce Verocytotoxin



Typical EPEC are bfp/EAF plasmid positive  
Atypical EPEC are bfp/EAF plasmid negative



Add to this definition that:

”The majority of typical EPEC strains fall into certain well-recognised O:H serotypes”

# Classical & **New** EPEC O:H serotypes



O26	H-;	H11;		H34
<b>O39</b>	<b>H-</b>			
O55	H-;	H6;	H7	
O86	H-;		<b>H8</b>	H34
<b>O88</b>	<b>H-?;</b>			<b>H25</b>
O111	H-; H2;		<b>H8; H9</b>	H25
O114	H-; H2			
O119	H-; H2; H6			
O125ac	H-;	H6;		H21
O126	H-; H2;		<b>H12</b>	H21; H27
O127	H-; <b>H4</b>	H6;		H21 <b>H40</b>
O128ab	H-; H2;		H7;	H12
O142	H-;	H6		
<b>O145</b>	<b>H-;</b>			<b>H45</b>
<b>O157</b>	<b>H-;</b>		<b>H8</b>	<b>H16</b> <b>H45</b>
O158	H-;			H23

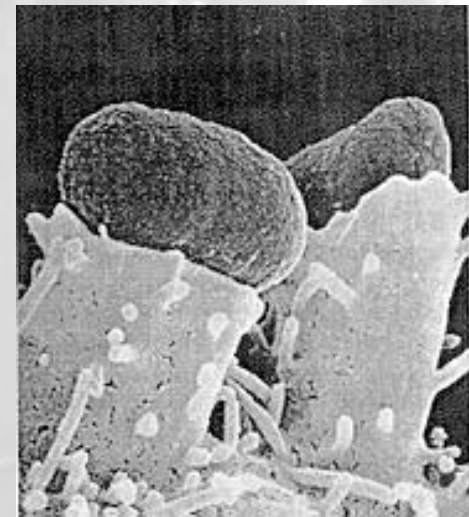


# Definition of A/EEC

***eae* positive (non-VTEC)**

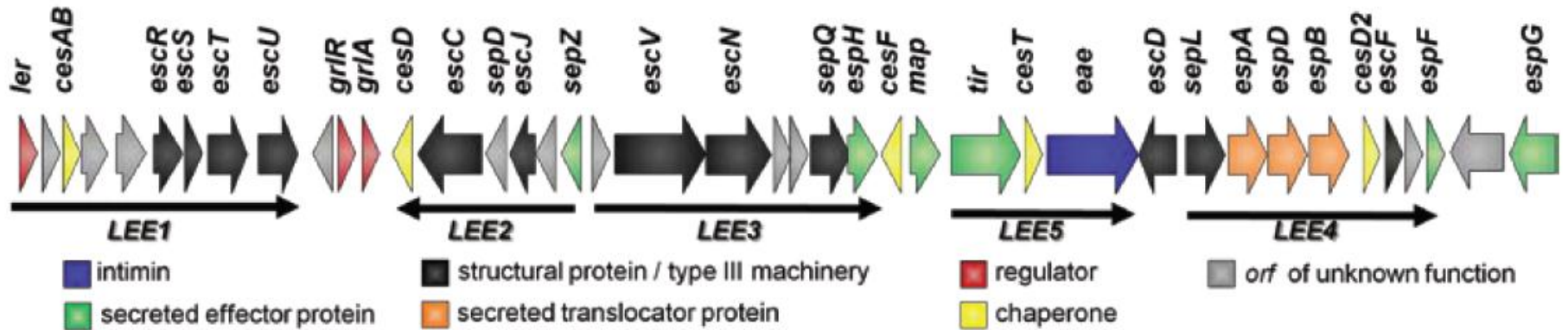
***E. coli* strains not belonging to classical or new EPEC serotypes**

***i.e.* for which there is no obvious association with disease!!!**





# LEE & OI122



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

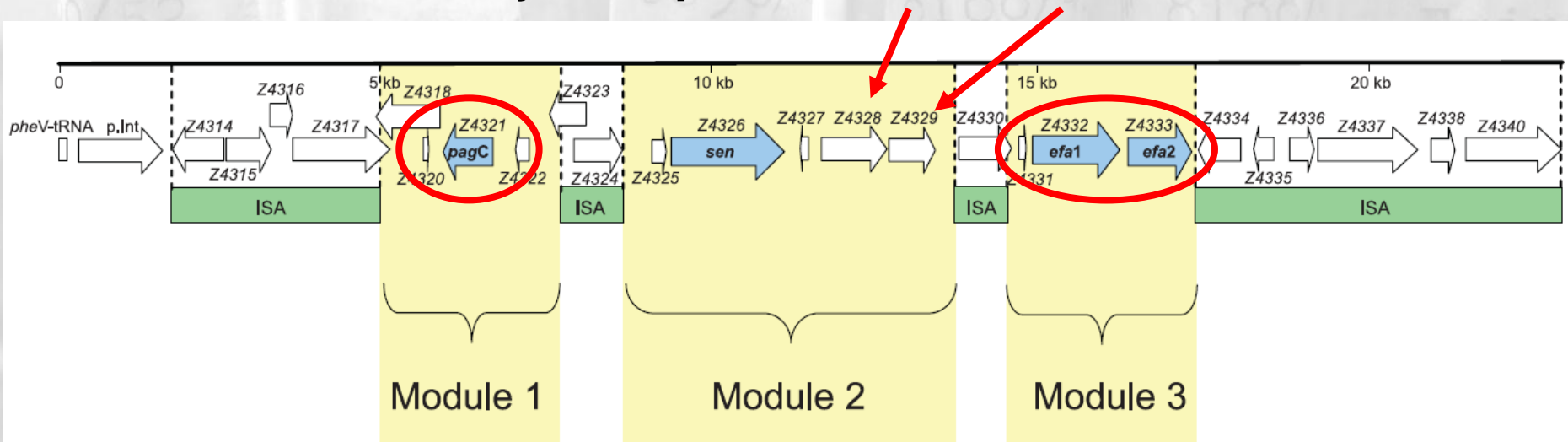
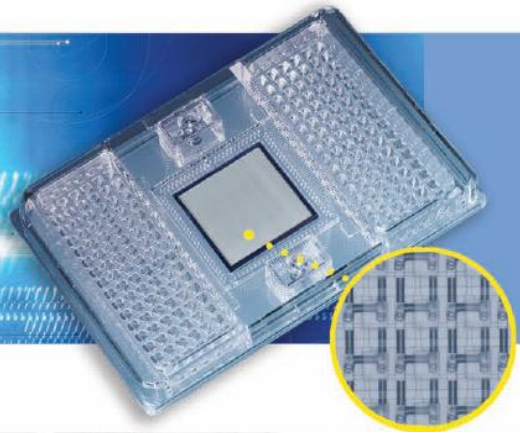
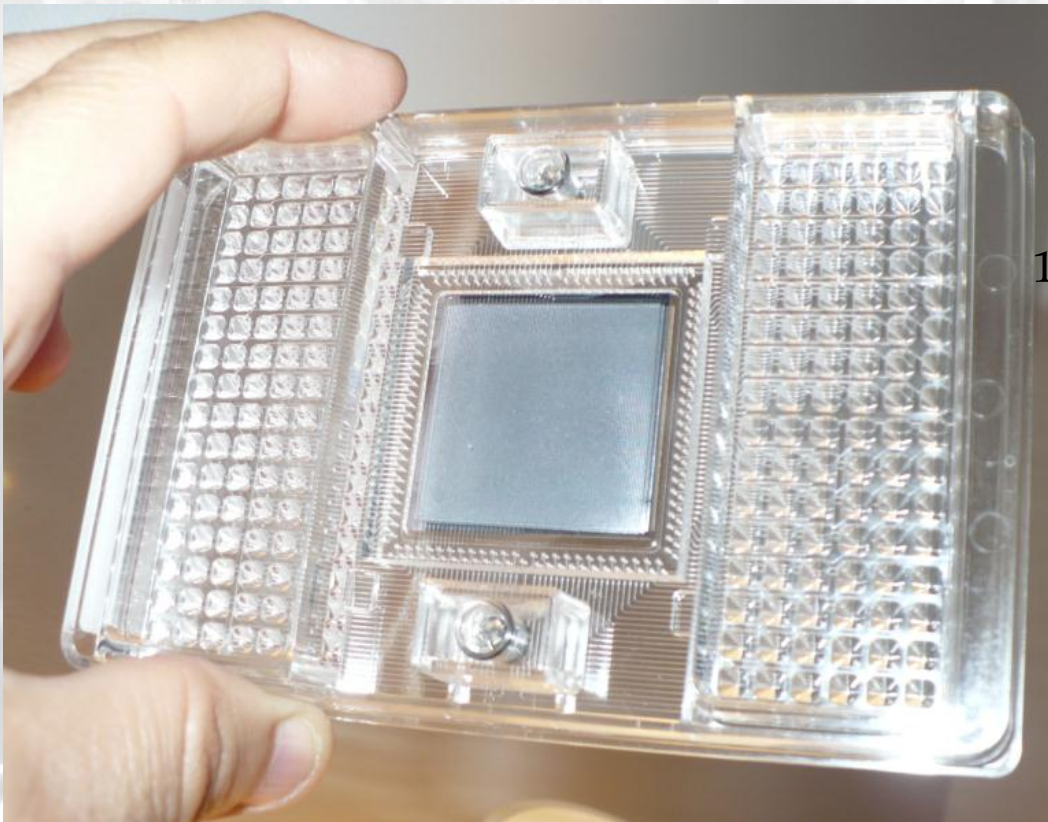


FIG. 1. Modular components of OI-122. ISA, insertion sequence-associated elements (or putative transposases) between the three modules. The PCR gene markers used to detect the presence of modules are indicated by bold type and blue.



96.96 Dynamic Array  
- Gene Expression 15

# VTEC



12

8

Gene	Gene Description
stx1	Shiga toxin 1
stx2	Shiga toxin 2
eae	intimin
eae-gamma	intimin-gamma
eae-beta	intimin-beta
eae-epsilon	intimin-epsilon
eae-theta	intimin-theta
tir-gamma	translocated intimin-gamma receptor protein
tir-beta	translocated intimin-beta receptor protein
tir-epsilon	translocated intimin-epsilon receptor protein
tir-theta	translocated intimin-theta receptor protein
tox B	toxin B
espA	type III secretion system
ehxA	entero-haemolysin A, plasmid encoded
katP	catalase peroxidase, plasmid encoded
espP	putative exoprotein-precursor, plasmid encoded
etpD	type II secretion protein, plasmid encoded
Saa	STEC auto-agglutinating adhesin
subA	subtilase cytotoxin, subunit A
ent	non LEE encoded effector ent, OI#122
nleB	non LEE encoded effector B, OI#122
nleE	non LEE encoded effector E, OI#122
nleF	non LEE encoded effector F, OI#71
nleH	non LEE encoded effector H, OI#71
nleA	non LEE encoded effector A, OI#71
rfbeO157	O157-antigen, seropathotype A
wzxO26	O26-antigen, seropathotype B
wzxO103	O103-antigen, seropathotype B
wbd1O111	O111-antigen, seropathotype B
ihp1O145	O145-antigen, seropathotype B
wzxO121	O121-antigen, seropathotype B
wzy O113	O113-antigen, seropathotype C
wzy O91	O91-antigen, seropathotype C
wzx O104	O104-antigen, seropathotype C
wzy O118	O118-antigen
wzx O45	O45-antigen
wbgN O55	O55-antigen
fliC H7	H7-antigen
fliC H2	H2-antigen
fliC H8	H8-antigen
fliC H11	H11-antigen
fliC H28	H28-antigen
fliC H21	H21-antigen
fliC H19	H19-antigen
fliC H16	H16-antigen





# Preliminary results with Fluidigm high throughput RT-PCR using 96 reactions

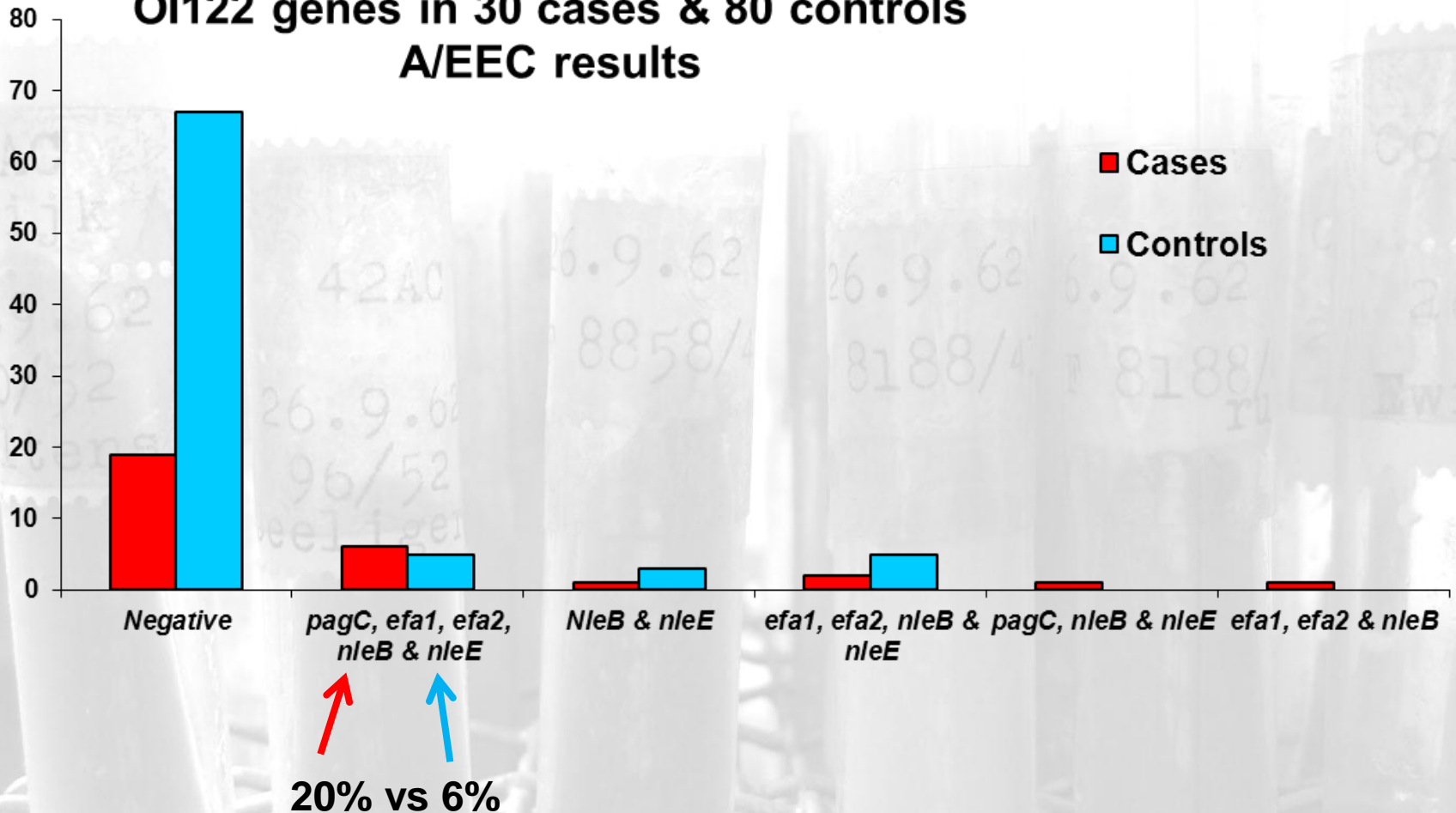
## Virulence factor scores

	<b>Cases</b>	<b>Controls</b>
<b>A/EEC</b>	<b>30</b>	<b>80</b>
	<b>11,3</b>	<b>9,3</b>
<b>EPEC and New EPEC</b>	<b>7</b>	<b>13</b>
	<b>21,0</b>	<b>9,8</b>



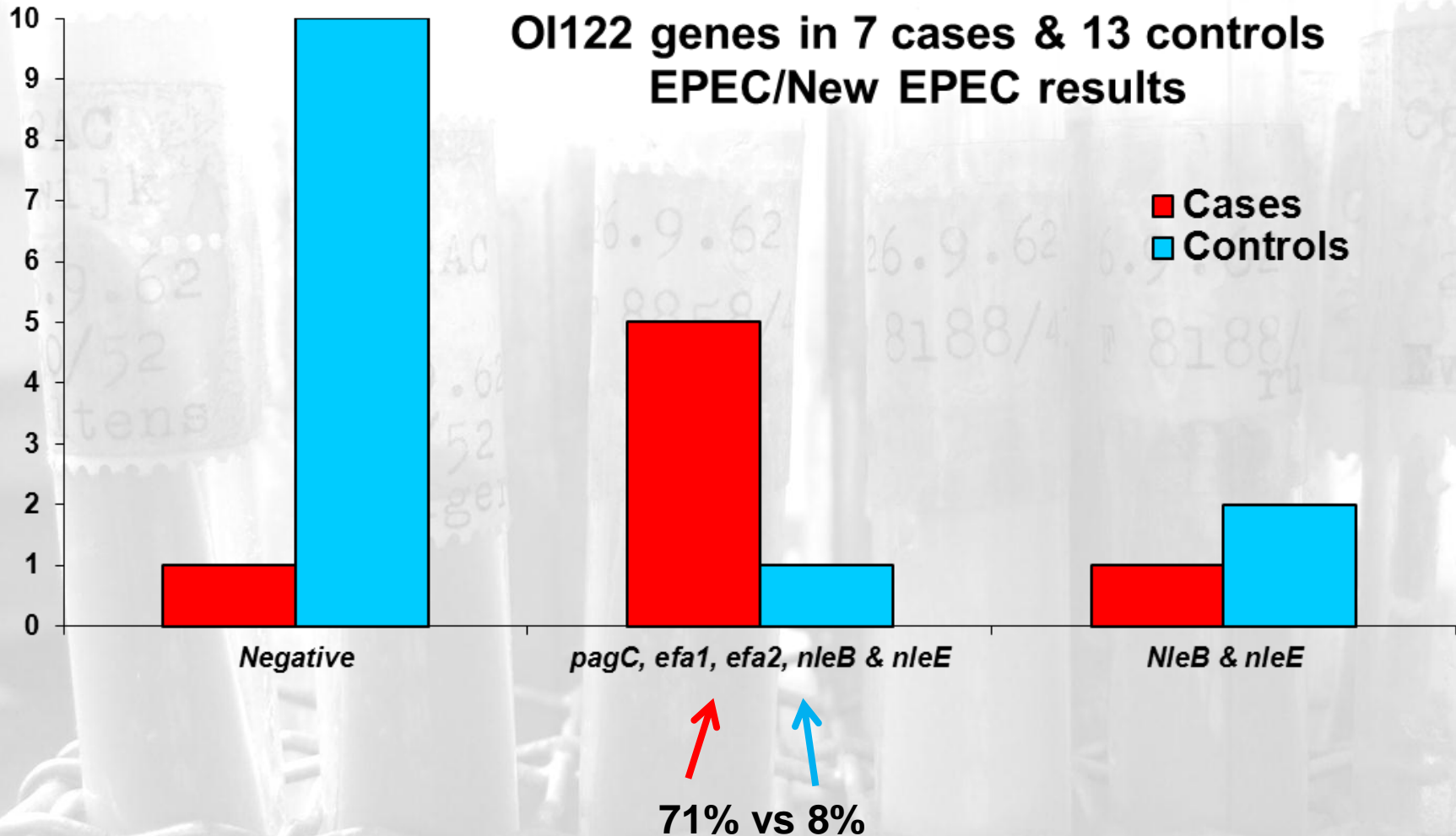
# Fluidigm high throughput RT-PCR using 96 reactions; A/EEC

O122 genes in 30 cases & 80 controls  
A/EEC results





# Fluidigm high throughput RT-PCR using 96 reactions; EPEC/New EPEC





# EPEC serotypes

## Cases

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

## Controls

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

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



# **ETEC: Enterotoxigenic *E. coli***

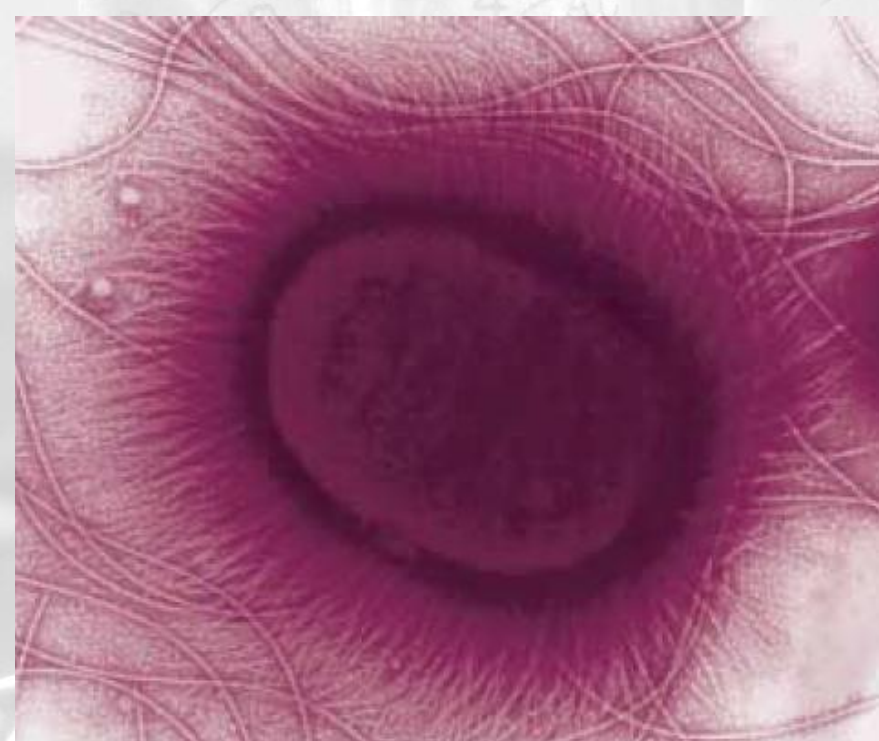
**Heat-labile enterotoxin (LT) ~ Cholera toxin**

**Heat-stable enterotoxin (ST)**

**Colonization Factors :**

**CFA/I-IV or CS1-CS21 (in humans)**

**F4, F5, F6, F41 & F18 in pigs and calves**

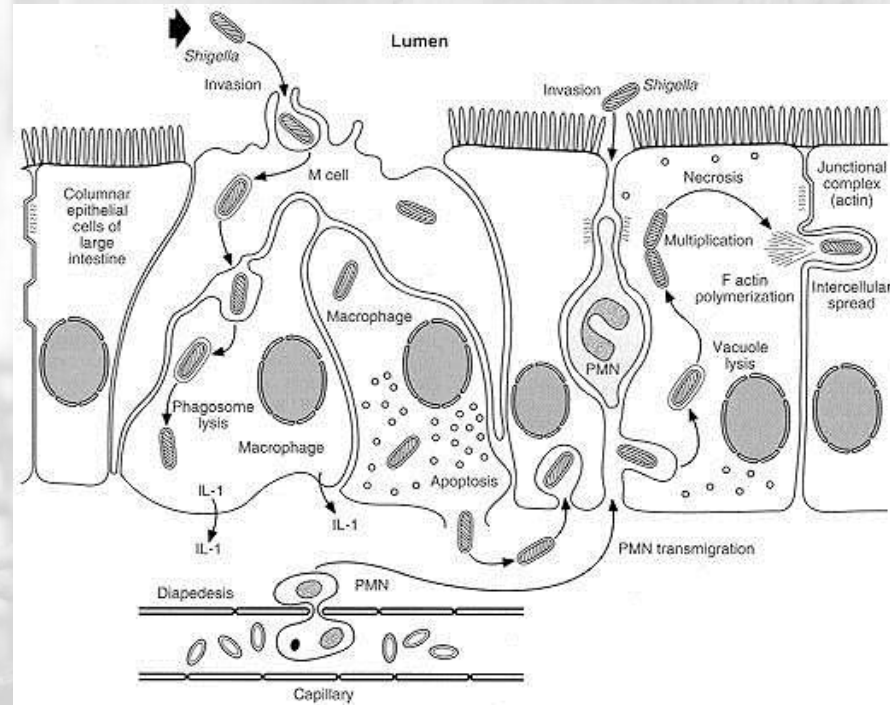




# EIEC: Enteroinvasive *E. coli*

## Clinical features = Shigellosis

~ *Shigella* (lactose negative)  
"Invasive" plasmids  
EIEC probe is from a large invasive plasmid  
*ipaH* is an invasion associated plasmid- and chromosomal gene  
The *Shigella* life cycle begins with penetration of colonic mucosa. This results in degradation of the epithelium and acute inflammatory colitis in the lamina propria. This causes leakage of blood, inflammation in the colon, and mucus into the intestinal lumen





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

				Shigella IDs	
O28ac	H-				
O29	H-			D11	
<b>O96</b>		<b>H19</b>			
O112ac	H-			D2	
O115	H-				
O121	H-			D7	
O124	H-;	H7	H30	H32	D3
O135	H-				
O136	H-				
O143	H-			B8	
O144	H-;	H25			
O152	H-			D12	
O159		H2			
O164	H-				
O167	H-;	H4	H5	B3	
O172	H-				

# Shigella IDs & **New** EIEC O groups



<b>O9</b>	<b>H-</b>		
<b>O13</b>	<b>H-</b>		
<b>O15</b>	<b>H-</b>		
<b>O36</b>		<b>H14</b>	
<b>O40</b>			<b>~D9 ?</b>
<b>O44</b>	<b>H-</b>		
<b>O58</b>			<b>D5</b>
<b>O89</b>	<b>H19</b>		
<b>O112ab</b>			<b>B15</b>
<b>O130</b>	<b>H-</b>		<b>~D6 (SR form)</b>
<b>O132</b>		<b>H21</b>	
<b>O147</b>			<b>F6 &amp; F1-5)</b>
<b>O148</b>			<b>~D1 ?</b>
<b>O149</b>			<b>B1</b>
<b>O150</b>			<b>D13</b>
<b>O168</b>			<b>D4</b>





## SHIGELLA INTERNATIONAL MEETING

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

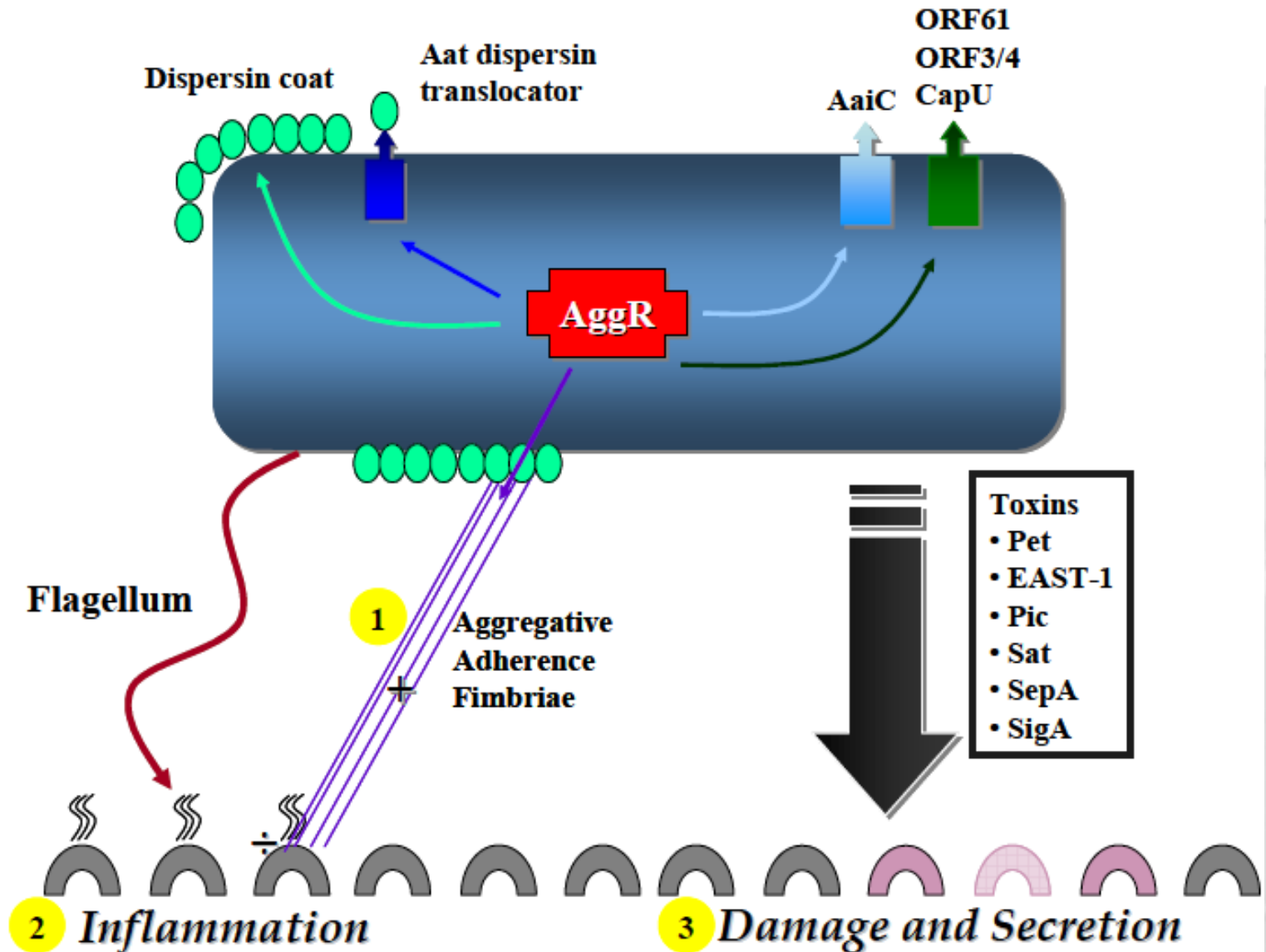
Funding for this meeting was made possible by the Bill & Melinda Gates Foundation, PATH and PAHO/WHO

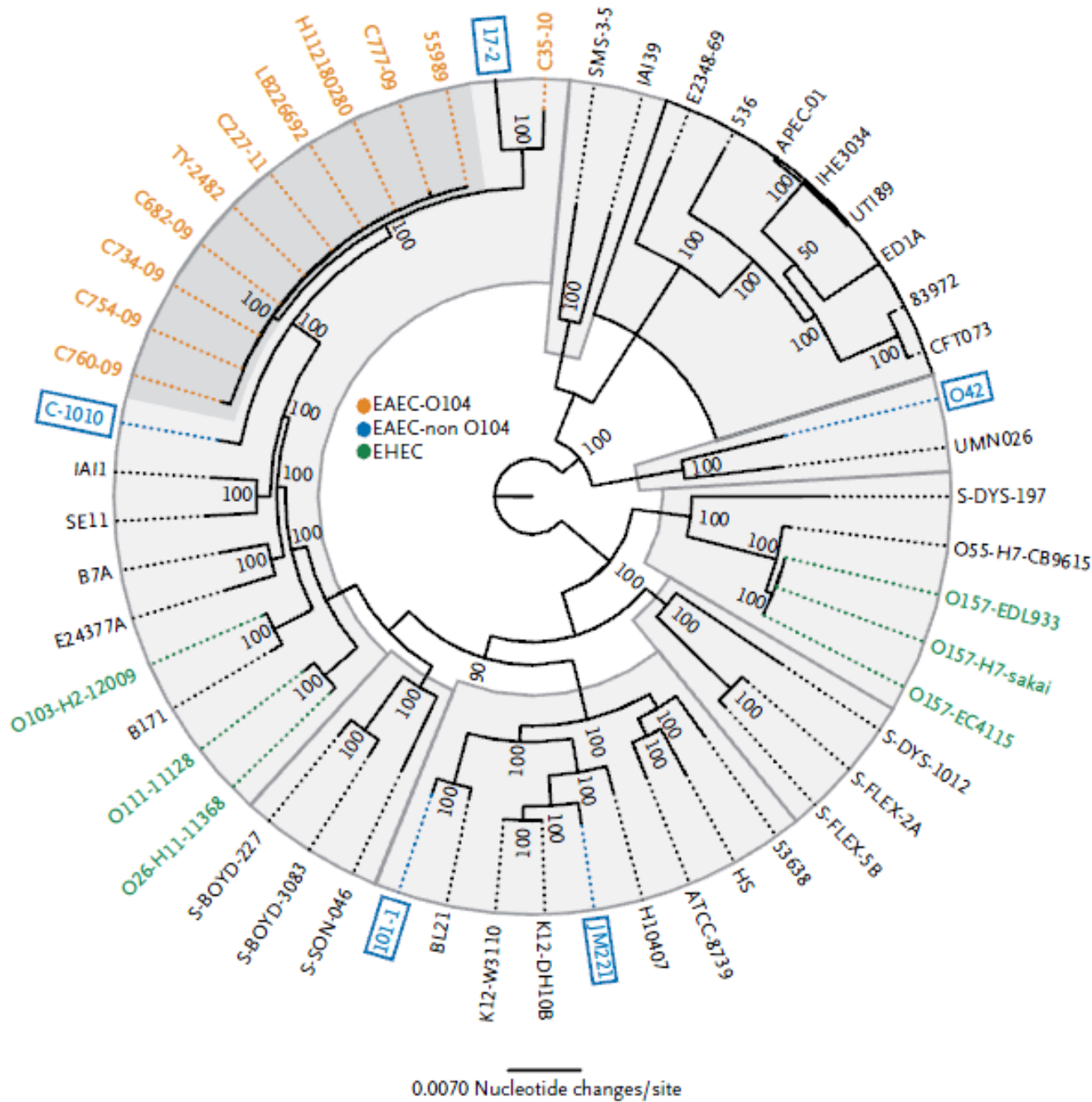
“as a long term goal, it was proposed to revise the classification of *Shigella* spp. including it in the same scheme as *Escherichia coli*, given the evidence of close genetic relation between these organisms...”

2<sup>nd</sup> 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* . . . “!!



# Enteroaggregative *E. coli* (EAaggEC)





# The Five test strains for EaggEC

**Figure 2. Phylogenetic Comparisons of 53 *Escherichia coli* and *Shigella* Isolates.**

Genomic sequences were compared with the use of 100 bootstrap calculations, as described by Gohl et al.<sup>35</sup> The

# DIARRHOEAGENIC *E. coli* (DEC)



**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*





**Infectious  
dose**

**Incubation  
time  
(in hours)**

**Duration  
(in days)  
Average**

---

<b>EPEC</b>	<b><math>10^6 - 10^{10}</math></b>	<b>9-19</b>	<b>5 (3-14)</b>
<b>A/EEC</b>	<b>?</b>	<b>?</b>	<b>14 (3-chronic?)</b>
<b>ETEC</b>	<b><math>10^8 - 10^{10}</math></b>	<b>3-166</b>	<b>4-7 (1-53)</b>
<b>EIEC</b>	<b><math>10^8</math></b>	<b>&lt;24</b>	<b>2-4 (1-12)</b>
<b>VTEC</b>	<b>50 - 700</b>	<b>24-336</b>	<b>4-5 (6-9)</b>
<b>EAggEC/VTEC O104 ? but low</b>		<b>7- 9 days</b>	<b>2 – 3 weeks</b>
<b>EAggEC</b>	<b><math>\sim 10^{10}</math></b>	<b>14-46</b>	<b>5 (3-&gt;14)</b>
<b>DAEC</b>	<b>?</b>	<b>?</b>	<b>?</b>



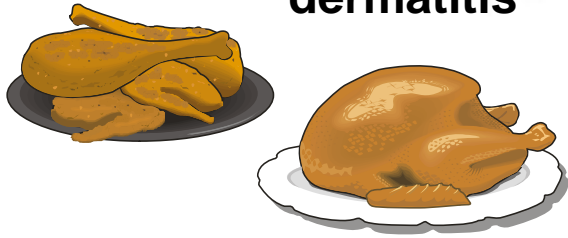
# ExPEC as a zoonotic agent?

- 24% (396/1648) of foods samples *E. coli* positive:
- 9 % in miscellaneous foods (= "non-meat")
- 69% in beef / pork
- 92% in poultry

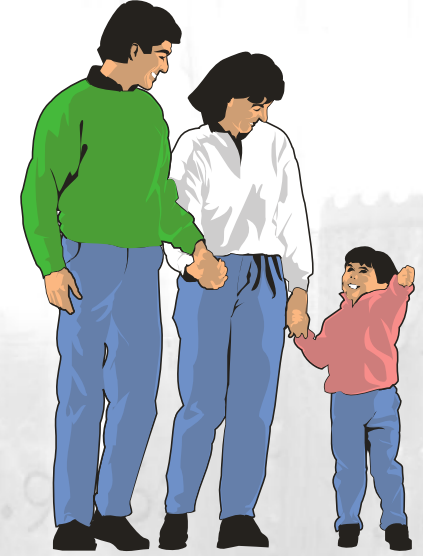
	<b>ExPEC</b>	<b>AMR</b>
■ Miscellaneous foods :	4%	27%
■ Beef / pork :	19%	85%
■ Poultry:	46%	94%



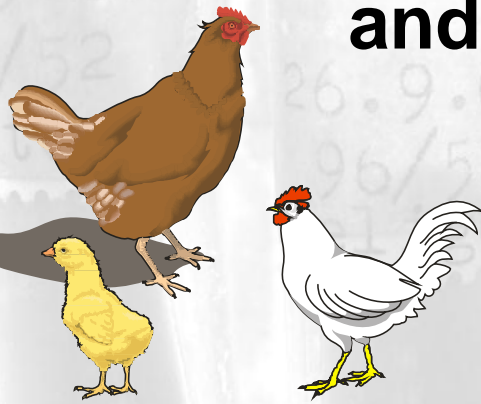
**dermatitis**



**colonisation**



***E. coli* O45:K1:H7  
causing an outbreak  
of neonatal meningitis  
and sepsis in Germany**



**septicaemia**

**colonisation  
septicaemia  
meningitis**



**Non-published data by Rita Prager, Helmut Tschäpe *et al.***



# Food-Borne Origins of *Escherichia coli* Causing Extraintestinal Infections

Amee R. Manges and James R. Johnson

Clinical Infectious Diseases Advance Access published June 7, 2012

**Table 1. Antimicrobial Resistance and Food Animal Reservoir Summary for Major Human Extraintestinal Pathogenic *Escherichia coli* Groups**

Human ExPEC Groups	Associated Characteristics and Food Animal Sources					No Known Food Animal Reservoir
	Antimicrobial Resistance	ESBL Production	Poultry	Pork/Pigs	Beef/Cattle	
O25:H4-B2-ST131	✓	✓	✓			
O11/O17/O77:K52:H18-D-ST69	✓		✓	✓	✓	
O15:K52:H1-D-ST393	✓					
Serotype (Various)-A-ST10 <sup>a</sup>	✓	✓	✓	✓		
Serotype (Various)-D-ST117	✓	✓	✓			
O1/O2/O18:K1:H7-B2-ST95	✓	✓	✓			
O6:H1-B2-ST73	✓	✓				✓
Serotype (Various)-D-ST405	✓	✓				✓
O75:K + :H5-B2-ST14	✓					✓

This table summarizes general information available on these groups from the literature. It is not exhaustive and does not distinguish the quantity or quality of the data.

Abbreviations: ESBL; extended-spectrum  $\beta$ -lactamase; ExPEC, extraintestinal pathogenic *Escherichia coli*.

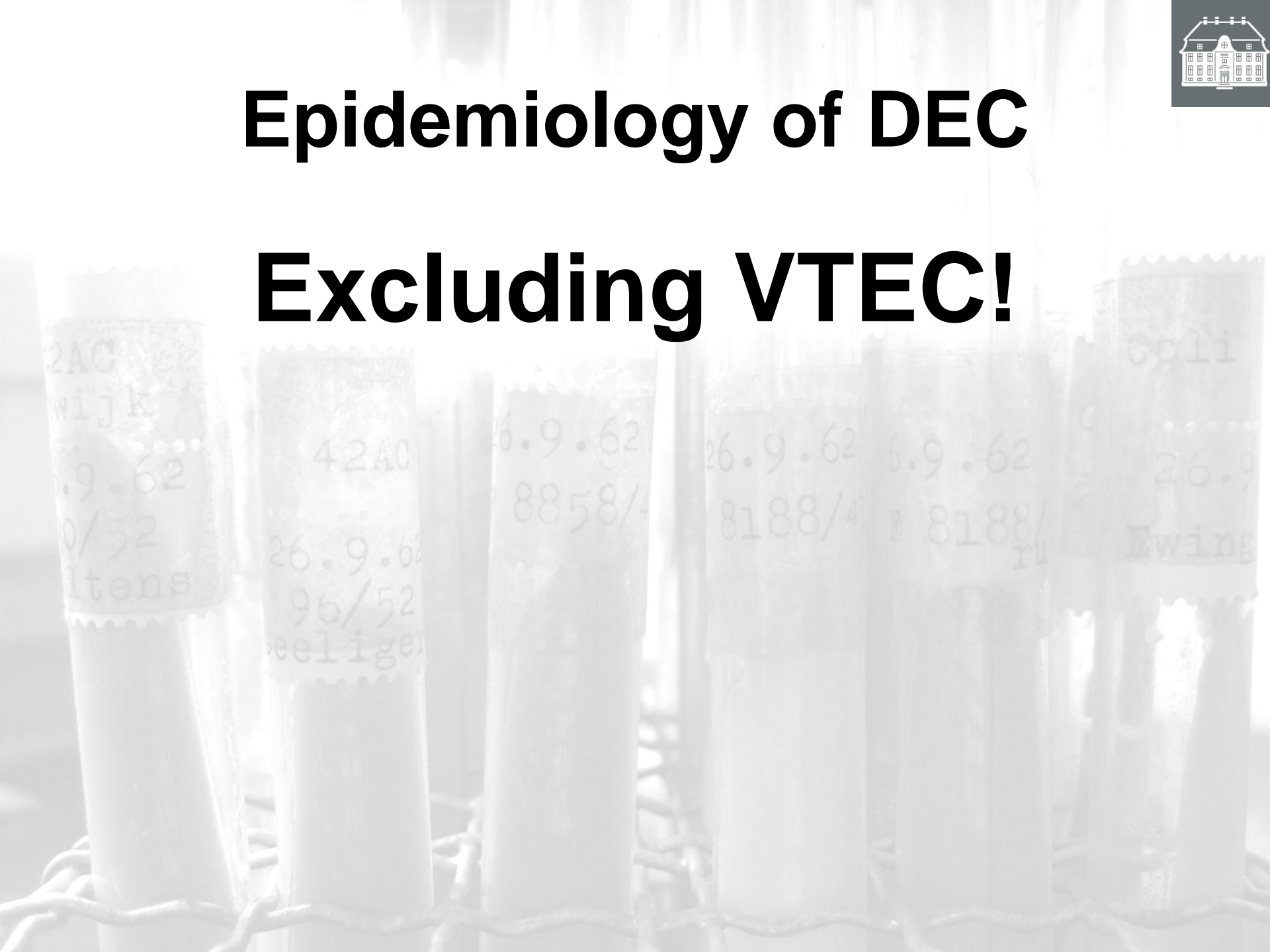
<sup>a</sup> Many *E. coli* ST10 strains are not ExPEC; those that are ExPEC tend to be extensively antimicrobial resistant.





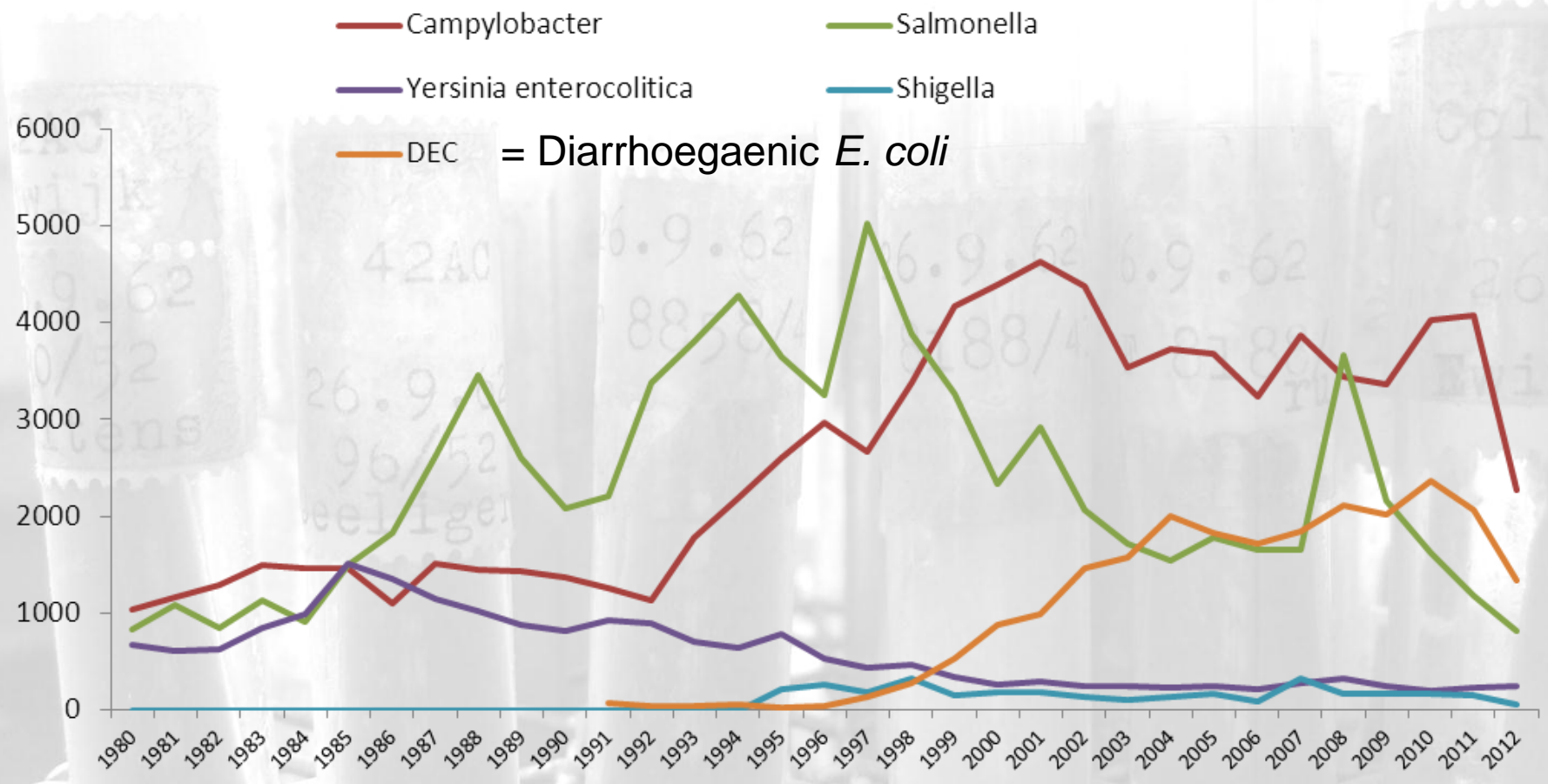
# Epidemiology of DEC

## Excluding VTEC!



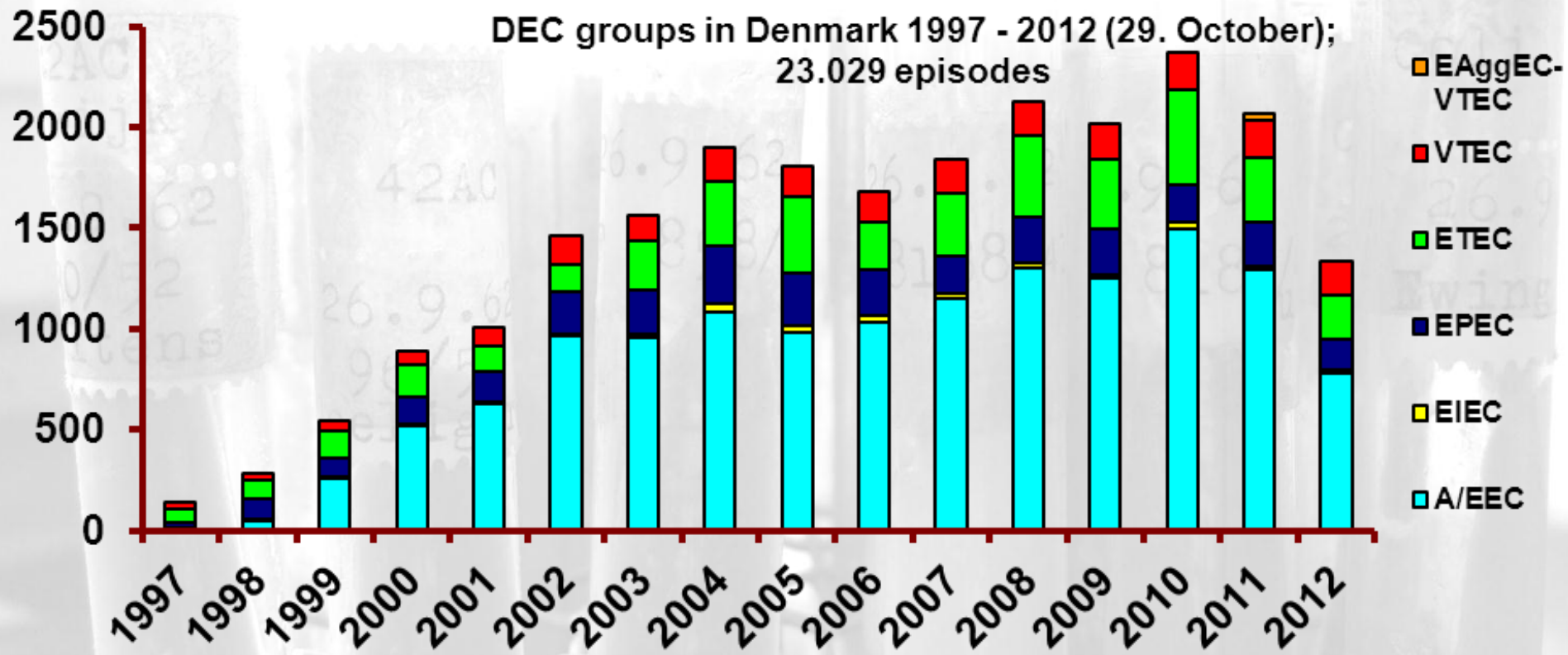


# Enteropathogenic bacteria: Trends in Denmark 1980- 2012





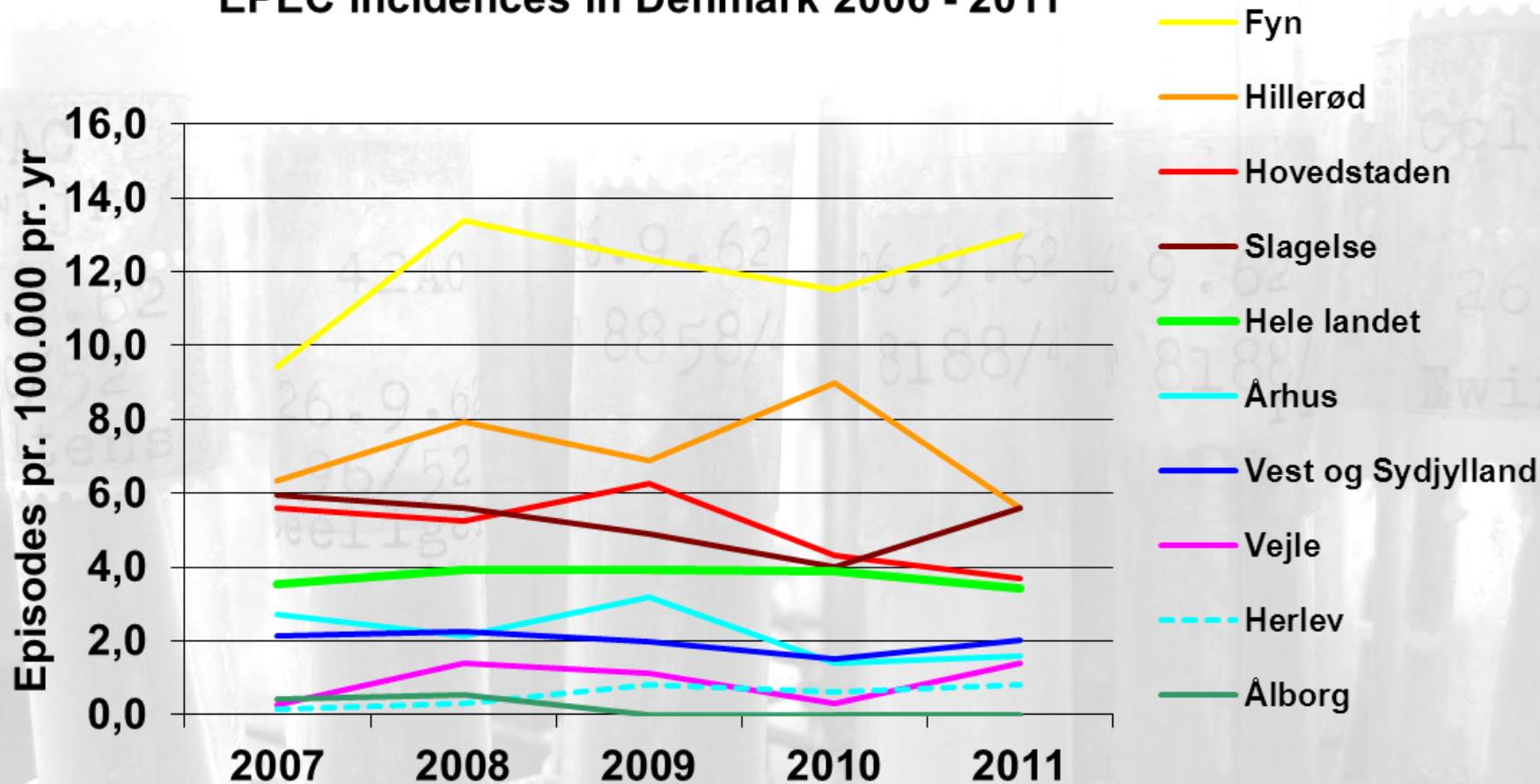
# Cases with diarrhoeagenic *E. coli* (DEC) groups



# EPEC incidences in Denmark 2007-2011



EPEC incidences in Denmark 2006 - 2011

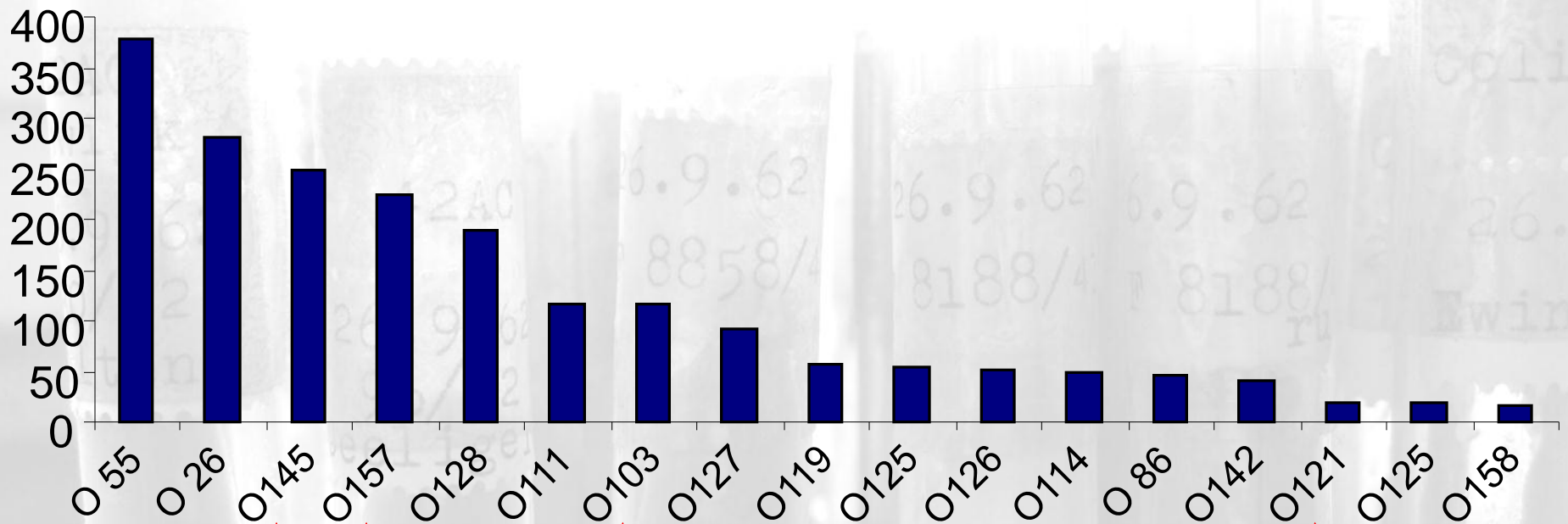






# EPEC O groups

## EPEC O groups in the Danish Germ register

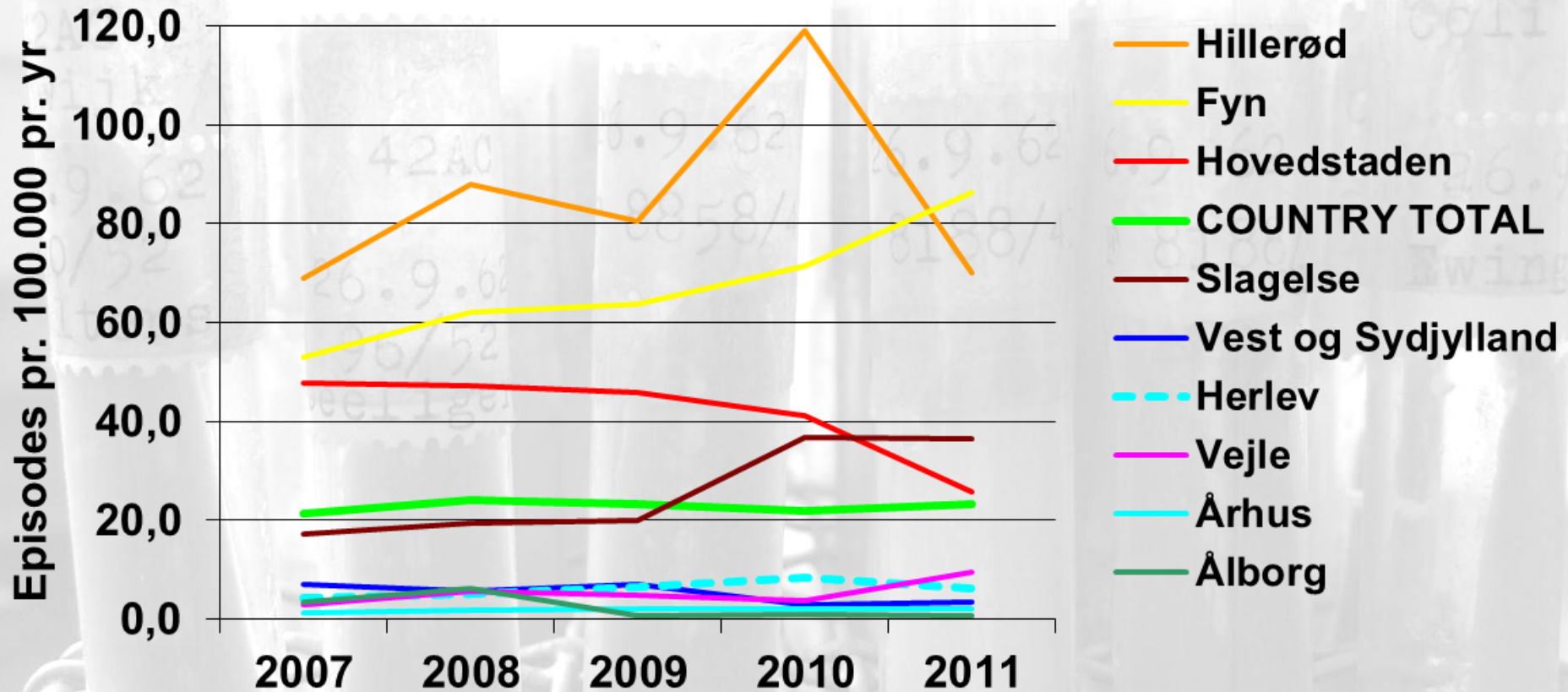


May also be VTEC



# A/EEC incidence in Denmark 2007 - 2011

A/EEC incidences in Denmark 2007- 2011

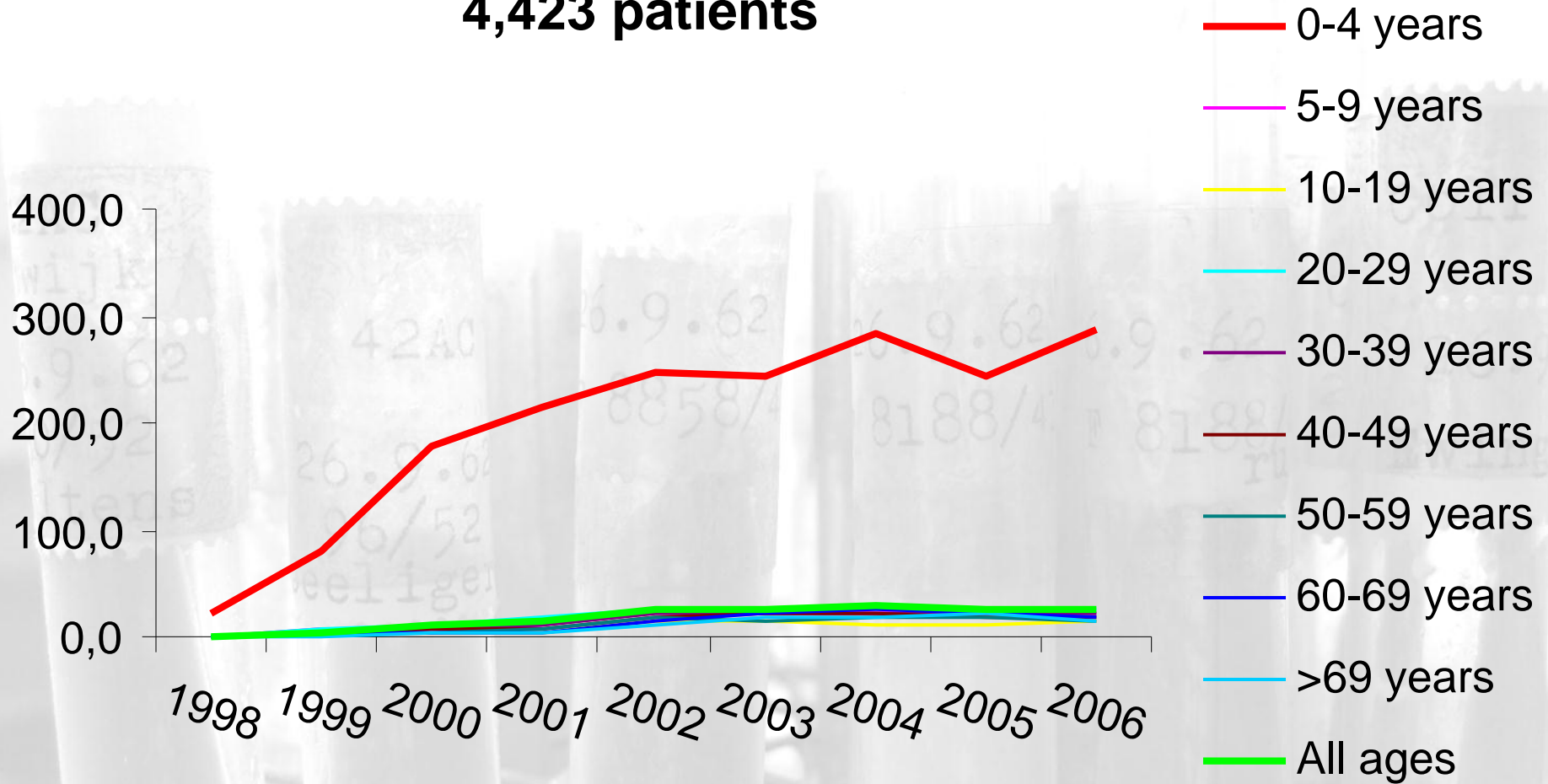




# A/EEC:

## Age-specific incidences in counties using molecular detection methods

4,423 patients



Cases registered 2004-2006

2,453

Estimate

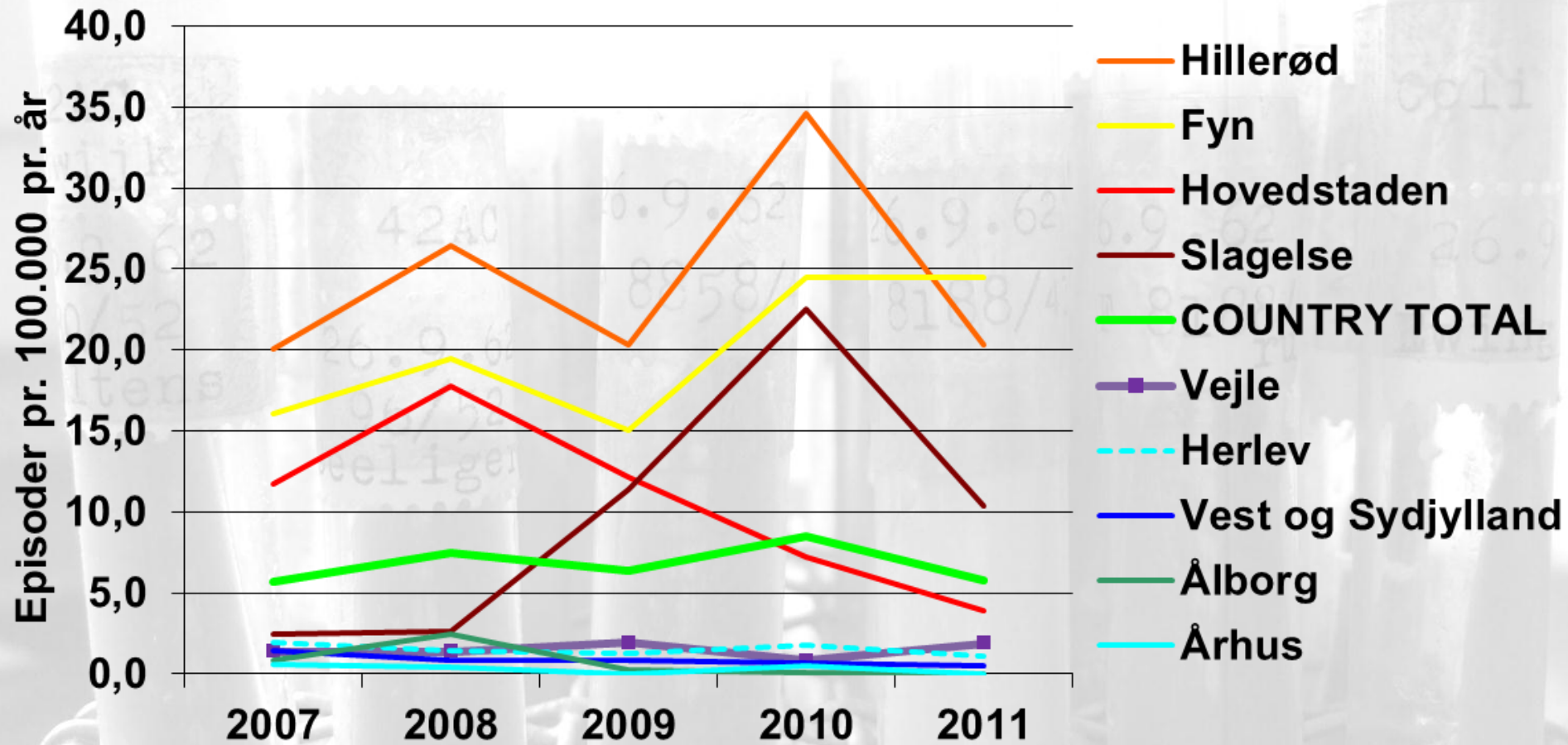
4,410





# ETEC incidences in Denmark 2007 - 2011

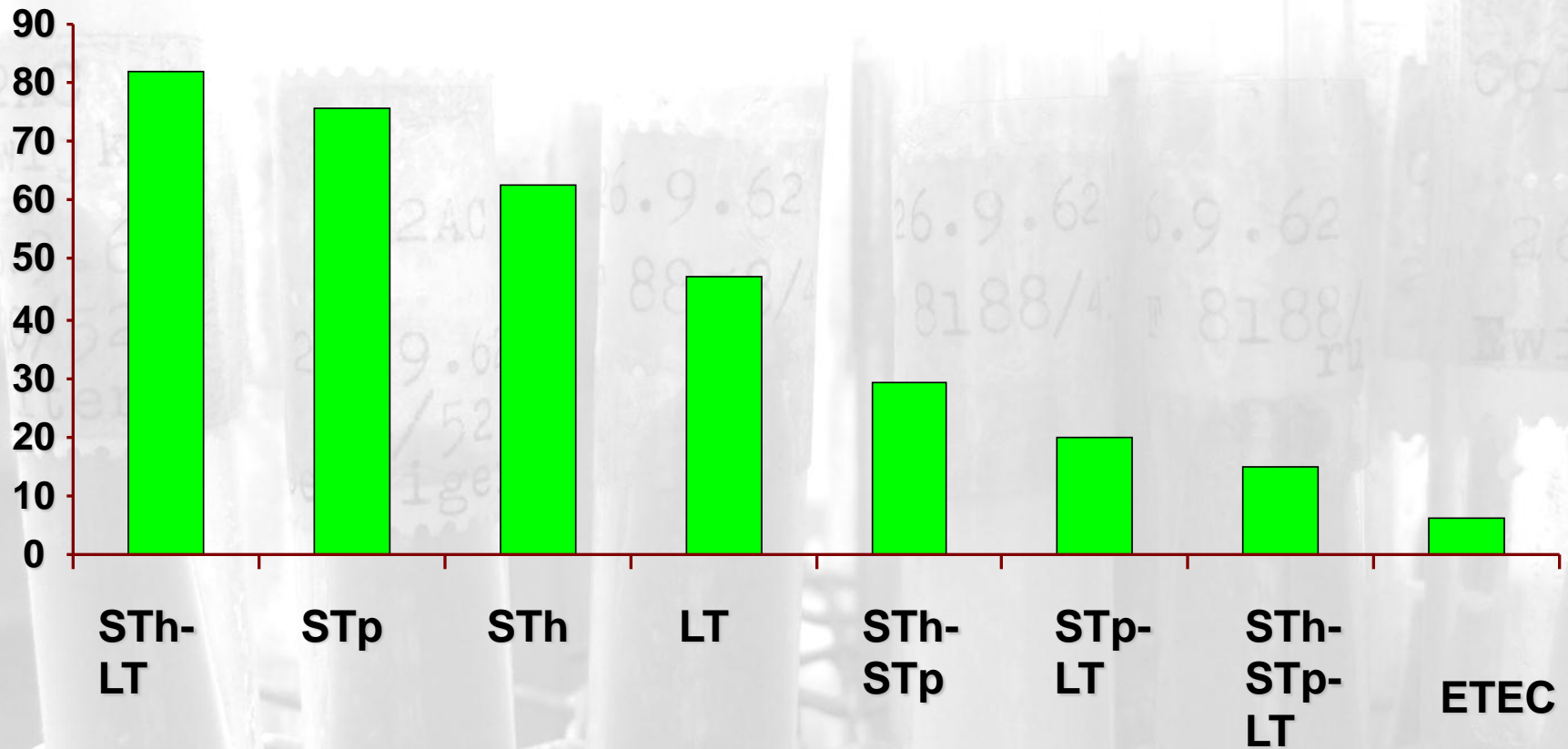
## ETEC incidences in Denmark 2007 - 2011





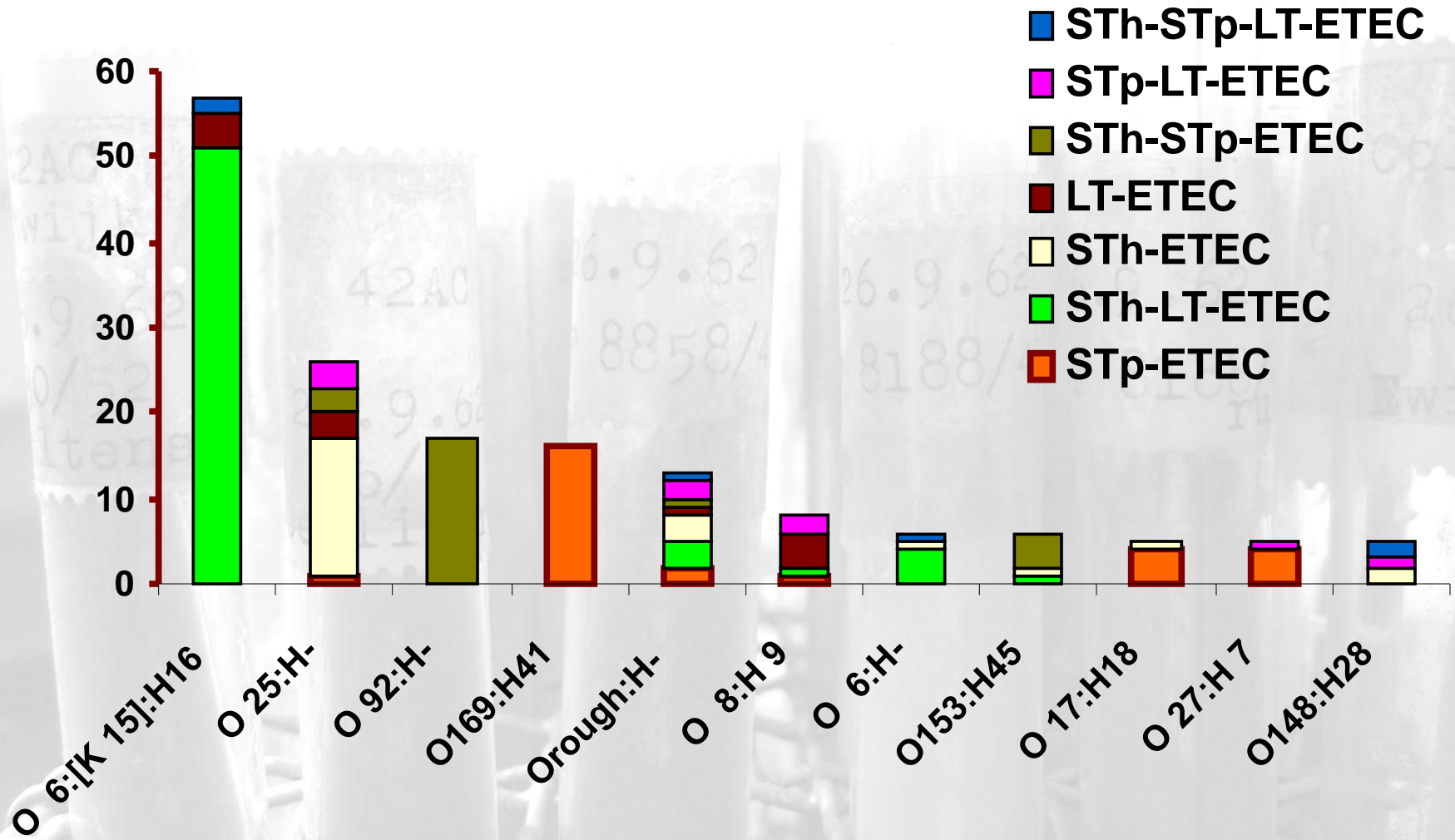


# Toxin profile of ETEC from 338 Danish patients





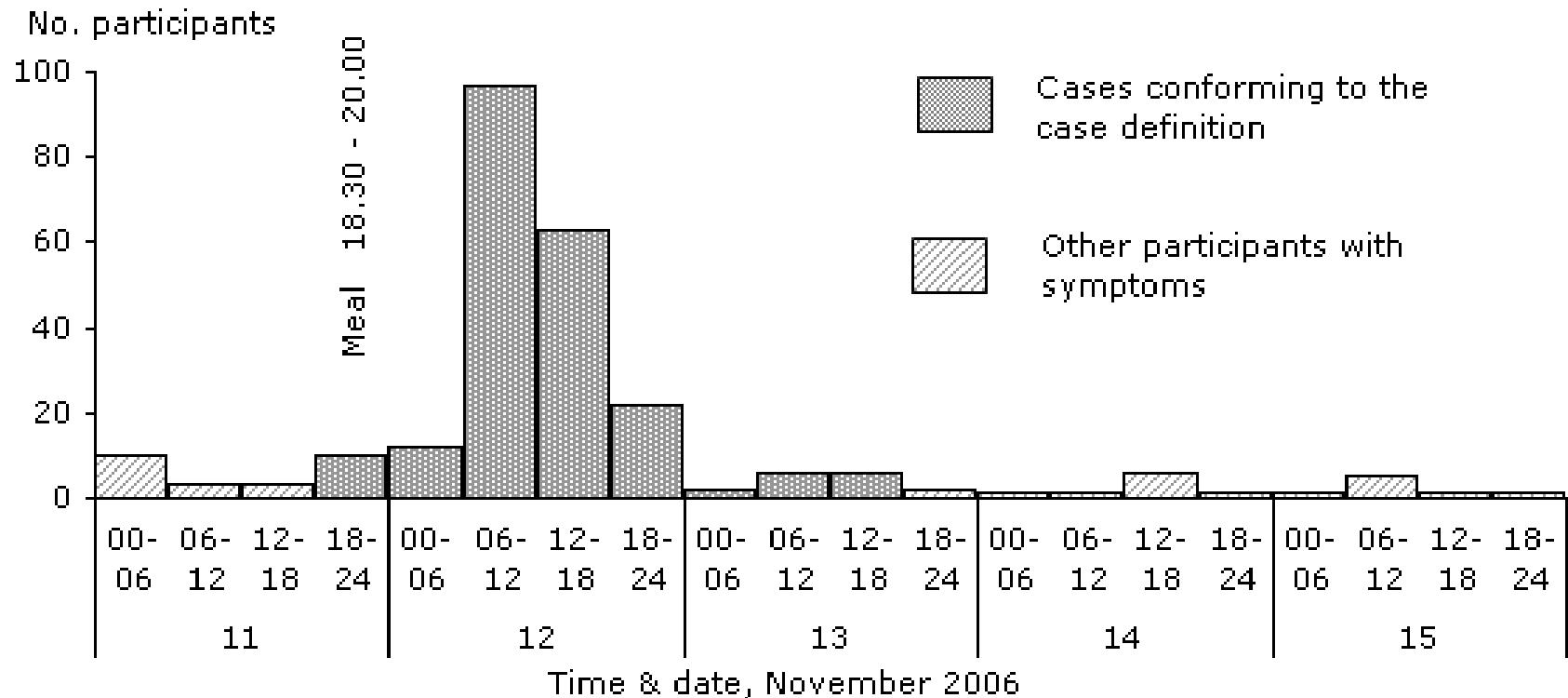
# ETEC serotypes & toxin profile in 156 Danish patients





# Food-borne ETEC and salmonella outbreak Denmark 2006

Figure 1. Dinner participants with diarrhoea and/or vomiting by time of onset of symptoms (n=253)



(Source: EPI-NEWS 51/06)



# Microbiological analyses

***E. coli* (>10<sup>5</sup> bacteria/gram)**

**Salmonella serotype Anatum**

**were found in left-overs of the pesto used to prepare the pasta salad**

**Samples from 48 persons were examined:**

**18 samples with enterotoxigenic *E. coli* (ETEC) of two different serotypes O92:H- (17 pts); O153:H2 (1 pt)**

**4 samples with Salmonella Anatum**

**PFGE typing demonstrated that these strains were identical to the food isolate**

# **Eurosurveillance, Volume 15, Issue 6, 11 February 2010**

## **Rapid communications**

# **OUTBREAKS OF GASTROENTERITIS LINKED TO LETTUCE, DENMARK, JANUARY 2010**

**S Ethelberg ( )<sup>1,2</sup>, M Lisby<sup>3</sup>, B Böttiger<sup>4</sup>, A C Schultz<sup>5</sup>, A Villif<sup>3</sup>, T  
Jensen<sup>6</sup>, K E Olsen<sup>2,7</sup>, F Scheutz<sup>2</sup>, C Kjelsø<sup>1</sup>, L Müller<sup>1</sup>**

**Eleven outbreaks; Five with ETEC O6:K15:H16 LT & ST<sub>h</sub>**



# Recent Danish outbreaks of ETEC

## Suspected sources:

- Basil from Israel (O92:H- & O153:H2)
- Sugar snaps from Kenya
- Green beans (O27:H7)
- Sprouts in sandwiches (O169:H41)

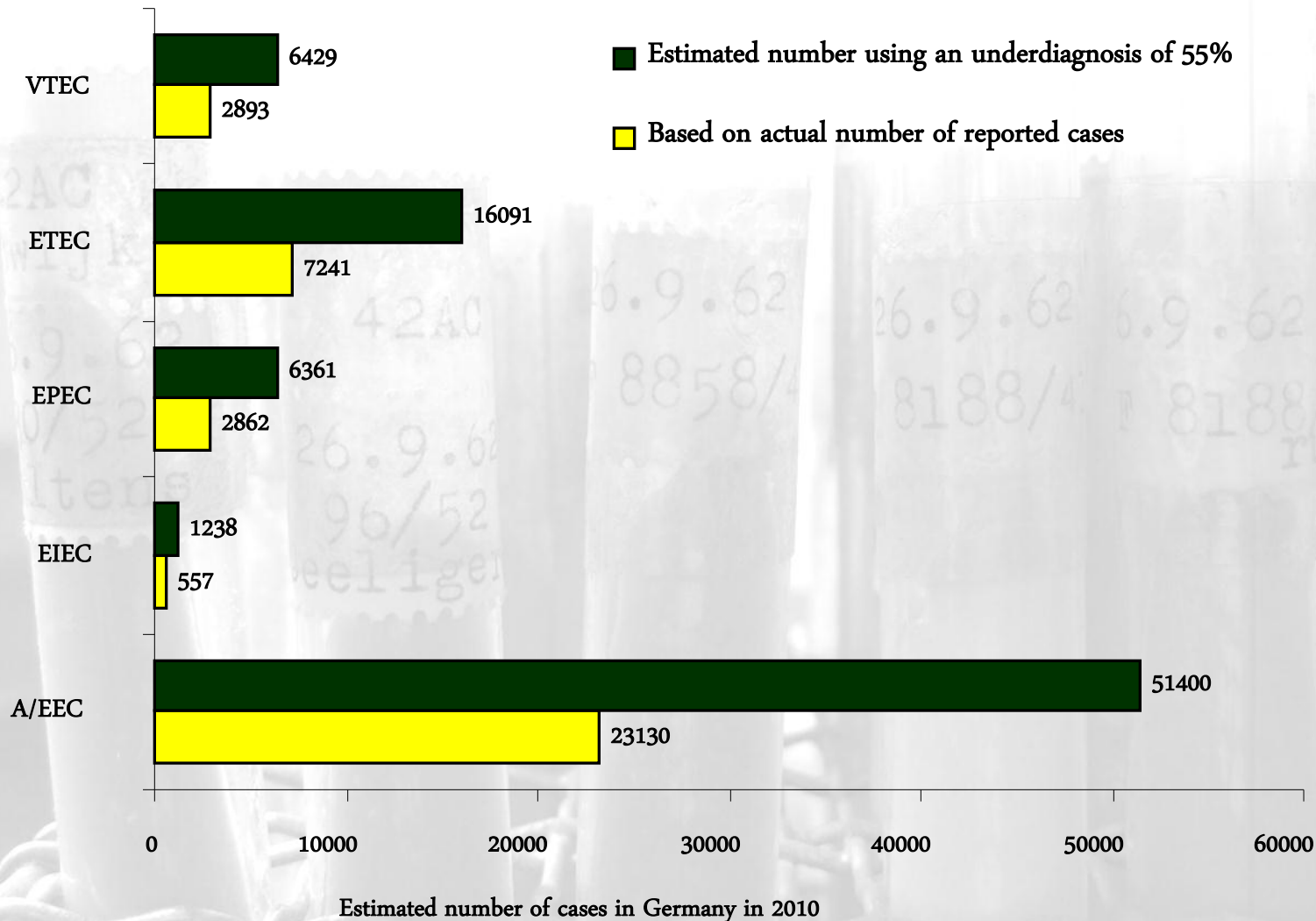




# Recent Danish outbreaks mixed

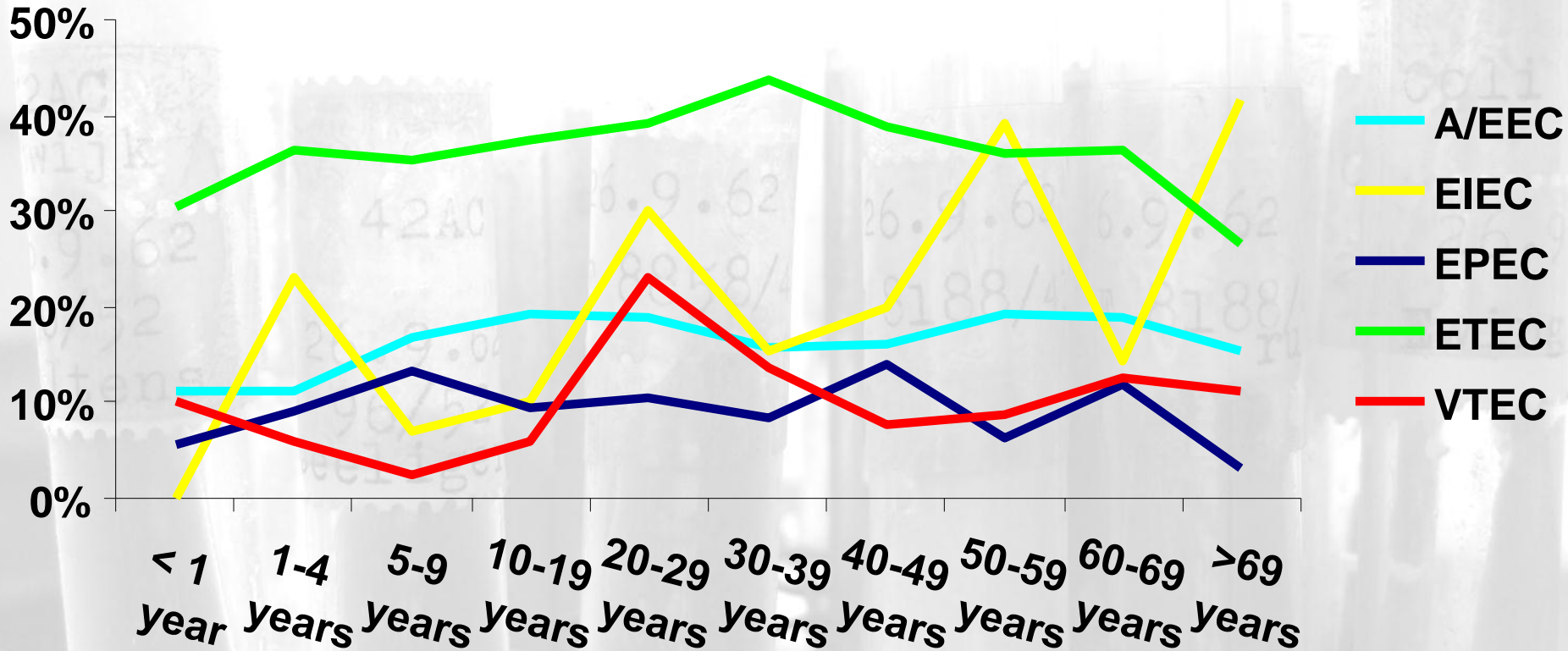
- **Lollo Bionda salad from France:**
  - ETEC; vira; bacteria**
- **Water (Køge):**
  - ETEC O159:H21**
  - EPEC O119:H- & O55:H7**
  - New EPEC O88:H25**
  - A/EEC Eight O:H types**
  - EAggEC Six O:H types**
- **Water (Klarup)**
  - Campylobacter**
  - EAggEC O21:H10 & O130:H27/H18**

# Hypothetical extrapolation of Danish DEC incidence data to Germany - based on 2010

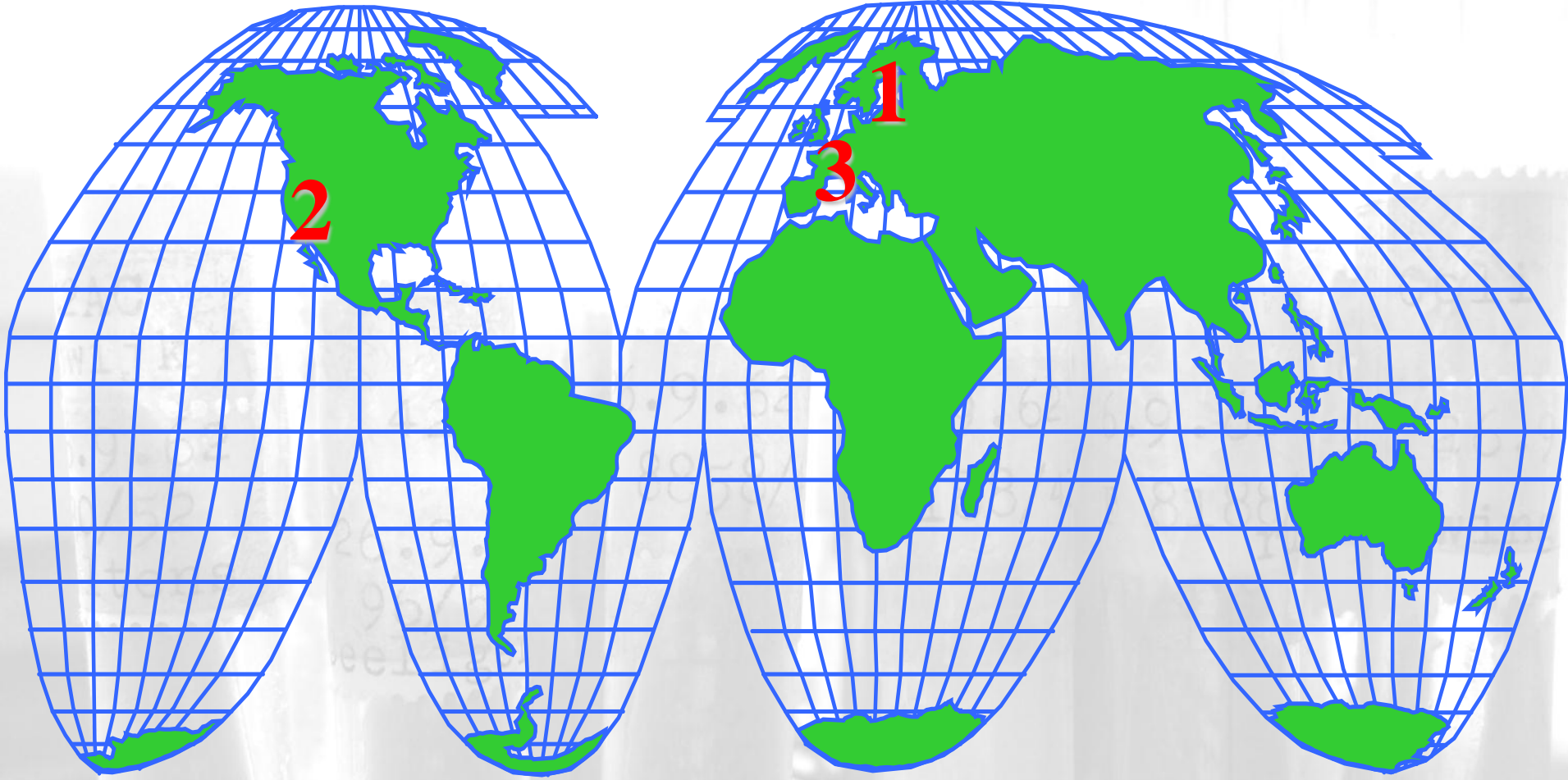




# Travel associated DEC group and age



# 1) THE FINNISH SCHOOL 1987



- 2) THE CRATER LAKE OUTBREAK 1975
- 3) THE FRENCH COACH TRIPS 1995



# 1) THE FINNISH EXPERIENCE

**Six days in November 1987**

**Halikko, Finland 8,200 inhabitants**

## **School complex**

**611 (72%) pupils 7 - 19 years**

**39 (45%) adults 23 - 57 years**

**137 household members**

**No source was identified**

**99% had eaten food served at the school**

***E. coli* O111:NM (later typed as H9) isolated**

**from 30 patients**

**EAS positive**



## **2) THE CRATER LAKE OUTBREAK**

**June and July 1975**

**Crater Lake, American national park**

**> 200 staff members**

**2,000 visitors**

**Source: Park water ( $P < 0.001$ )**

**Contaminated by raw sewage**

**06:K15:H16 ETEC isolated from 20/49 ill park residents and from the park's water supply**

**First waterborne epidemic due to ETEC**

**Documented one mode of transmission**



# 1) THE FRENCH COACH TRIPS

November 1995

Weekend coach trips to Northern France

**37/48** (77%) 9 -72 years

**Diarrhoea within 4 days of the trip**

**Source: Prawn mayonnaise vol au vents (P < 0,05)**

December 1995

**22/37** (59%) 45 - 82 years

**Second party also became ill**

**Source: Lettuce (P < 0,024)**

**Gherkins (P < 0,006)**



## 2) THE FRENCH COACH TRIPS

**EPEC O111 *eae***

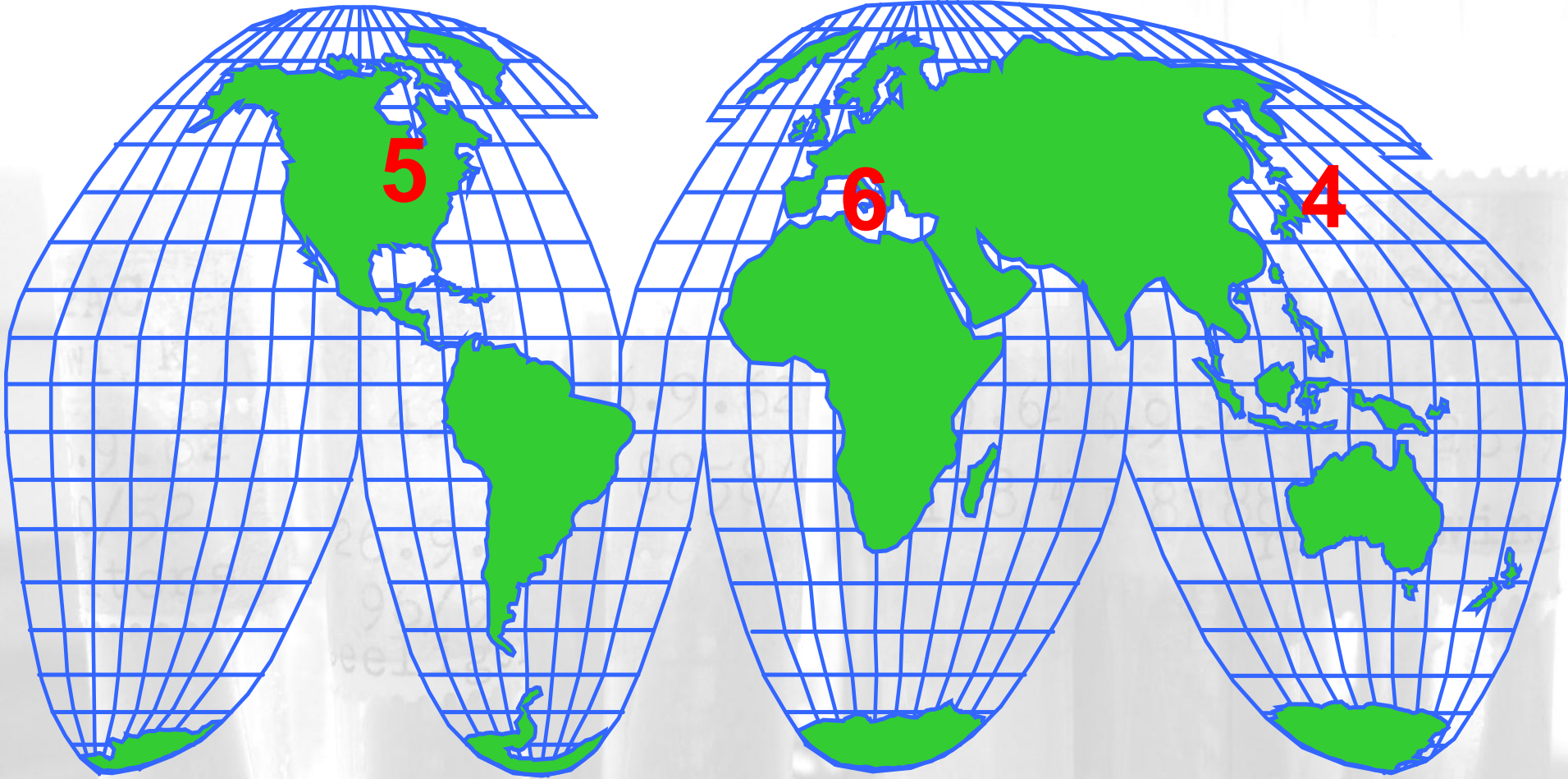
**Isolated from 30 cases (first trip)  
and 1 case (second trip)**

**The French restaurant was closed  
in January 1996**



# 4) THE JAPANESE EXPERIENCES

1993 & 1996



5) THE AMERICAN FRUSTRATION

1991

6) THE ITALIAN SURPRISE



## **4a) THE JAPANESE EXPERIENCES**

**21 - 24 June 1993**

**Tajimi city, Gifu prefecture schools**

**2,697 / 6,636 (40.6% attack rate)**

**14 / 20 schools affected**

**6 schools not affected were closed on the  
21st June**

**Official report:**

**No source or pathogen was identified!**



## 4a) THE JAPANESE EXPERIENCES

### Laboratory investigation

**EAggEC      O Untypeable:H10    *astA***

**Isolated from 12 / 30 children with severe, protracted diarrhoea**

**O Untypeable??**

**The laboratory only examined for 43 / 173 O groups**



## 4b) THE JAPANESE EXPERIENCES

23 July 1996

Osaka, Meeting in office building

54 / 91 (59 %)

Only common food: Lunch at the office

O166:H? EAST1 was isolated from 29 / 33

First report of an outbreak caused by an  
EAST1-only-producing *E. coli*  
New subgroup of ETEC



## 5) THE AMERICAN FRUSTRATION

29 April 1991

Local restaurant, hotel and conference centre

9 / 11 (82 %) of patrons had diarrhoea

O39:NM *eae*

FAS positive

EAST1

Isolated from 10 patrons who all ate a particular meal at the restaurant



## 5) THE AMERICAN FRUSTRATION

**O39:NM *eae* FAS positive EAST1**

**First report of an outbreak caused by an *E. coli*:**

**“Not fitting into the Existing Scheme for Classifying Diarrheogenic *E. coli*”**

**New subgroup = New EPEC**



# 6) THE ITALIAN SURPRISE = next presentation

## Episodio epidemico tra vigili del fuoco a Milano

la Repubblica **MILANO.it**

Martedì 17 Aprile 2012 – Aggiornato Alle 11.35

### Milano, decine di pompieri in ospedale i Nas chiudono la mensa della centrale

Sessanta vigili del fuoco (il numero è provvisorio) sono stati ricoverati per una intossicazione alimentare: tutti avevano pranzato in via Messina. La più grave è un aiuto cuoco di 34 anni

di FRANCO VANNI



Dissenteria, vomito e febbre fino a 40. Sono almeno 60 i vigili del fuoco che nelle ultime 24 ore sono stati visitati e ricoverati negli ospedali milanesi per quella che sembra essere un'intossicazione alimentare. La Asl e i Nas dei carabinieri hanno chiuso - "a titolo precauzionale", spiegano - la cucina e la mensa della centrale dei pompieri in via Messina. Il sospetto è che a causare i

What are we looking for?







# CONCLUSIONS

- **Some serotypes are clearly associated with diarrhoea**
- **Some pathotypes are also clearly associated with diarrhoea**
- **A clear definition of "diarrhoeagenic" is warranted especially for **A/EEC & EAaggEC****



# DETECTION OF FOOD-BORNE PATHOGENS

**Immediate recognition and notification of  
the (suspected) outbreak**

**Are adequate detection methods available  
in all the concerned institutions and  
agencies?**

**Are action plans available?**



# The evidence is there...!



**Thank yous**

**To the Regional hospitals  
– primary diagnostic laboratories**



**Thank yous**

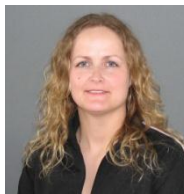
# To my SSI colleagues



**Susanne  
Jespersen**



**Pia Møller  
Hansen**



**Pernille  
Gymoese**



**Steen Ethelberg**



**Luise Müller**



**Kåre Mølbak**



**Charlotte Kjelsø**



**Eva Møller Nielsen**



**Flemming Scheutz**