Infections with Shiga toxin producing *E.coli* (STEC): emerging issues and reflections on the global food trade

Alfredo Caprioli

EU Reference Laboratory for Escherichia coli

Dip. Sanità Pubblica Veterinaria e Sicurezza Alimentare

Istituto Superiore di Sanità, Roma

www.iss.it/vtec

www.iss.it/seu

Content

- ✓ **Pathogenesis and epidemiology of STEC infections.**
- ✓ **The role of STEC serogroups other than O157 (non-O157 STEC)**
- ✓ **The *E. coli* O104:H4 outbreak in Germany:**
  - ✓ **What happened ?**
  - ✓ **Who is the “killer bacterium” and where did it come from ?**
- ✓ **Surveillance and control of STEC in the EU:**
  - ✓ **Organization**
  - ✓ **Detection methods**
  - ✓ **Legislation**

VTEC are nasty bugs !!

One of the most dangerous foodborne pathogens !

Life-threatening clinical manifestation

Very low infectious dose (10-100 CFU !)

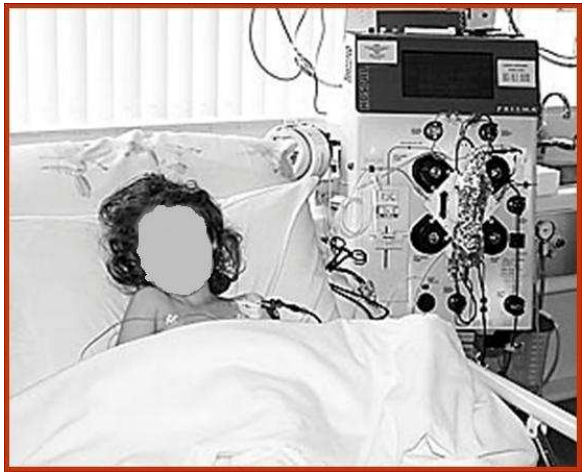
Very large community outbreaks

VTEC: Clinical manifestation

- **Intestinal**
  - **Asymptomatic**
  - **Watery Diarrhoea**
  - **Hemorrhagic Colitis**

- **Systemic** • **Hemolitic Uremic Syndrome (HUS)**

Hemolytic Uremic Syndrome (HUS)



- Hemolytic Anemia
- Thrombocytopenia
- Acute Renal failure
- Main cause of renal failure in childhood and usually requires dialysis
- Possible neurological involvement
- Antibiotic treatment is not effective and is not recommended !!

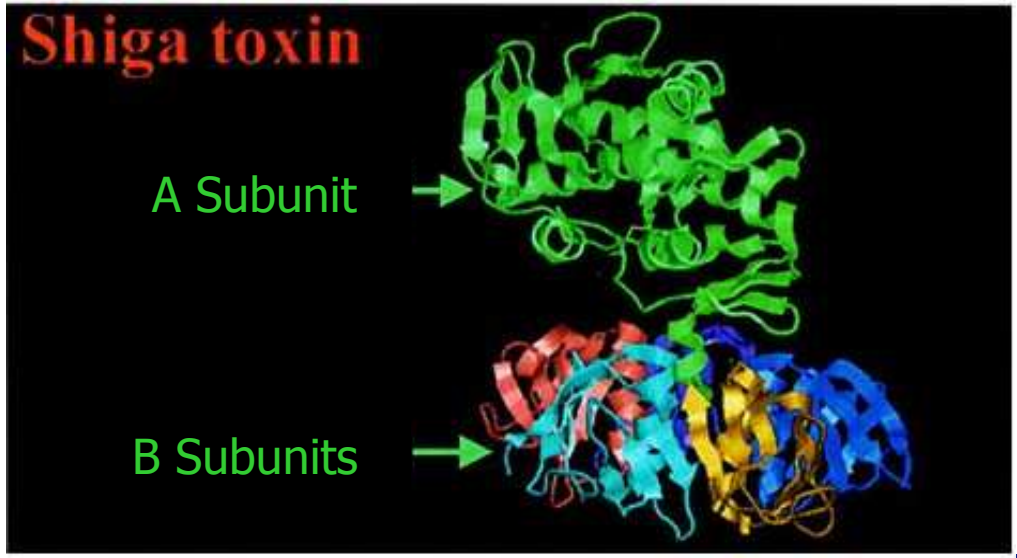
Shiga-toxins

Lethal for laboratory animals

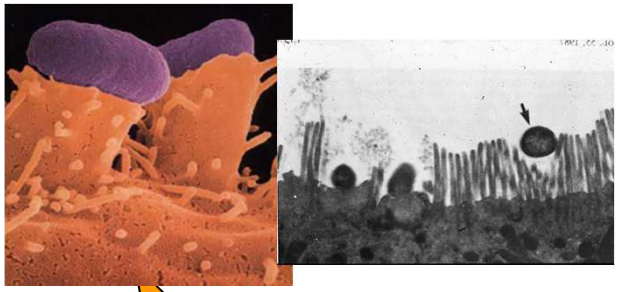
Inhibition of protein synthesis

Cythopatic effect on many cell lines

Particularly active on endothelial cells



E.coli O157 virulence factors



Adhesion

Hemolysin

LEE

E-hly

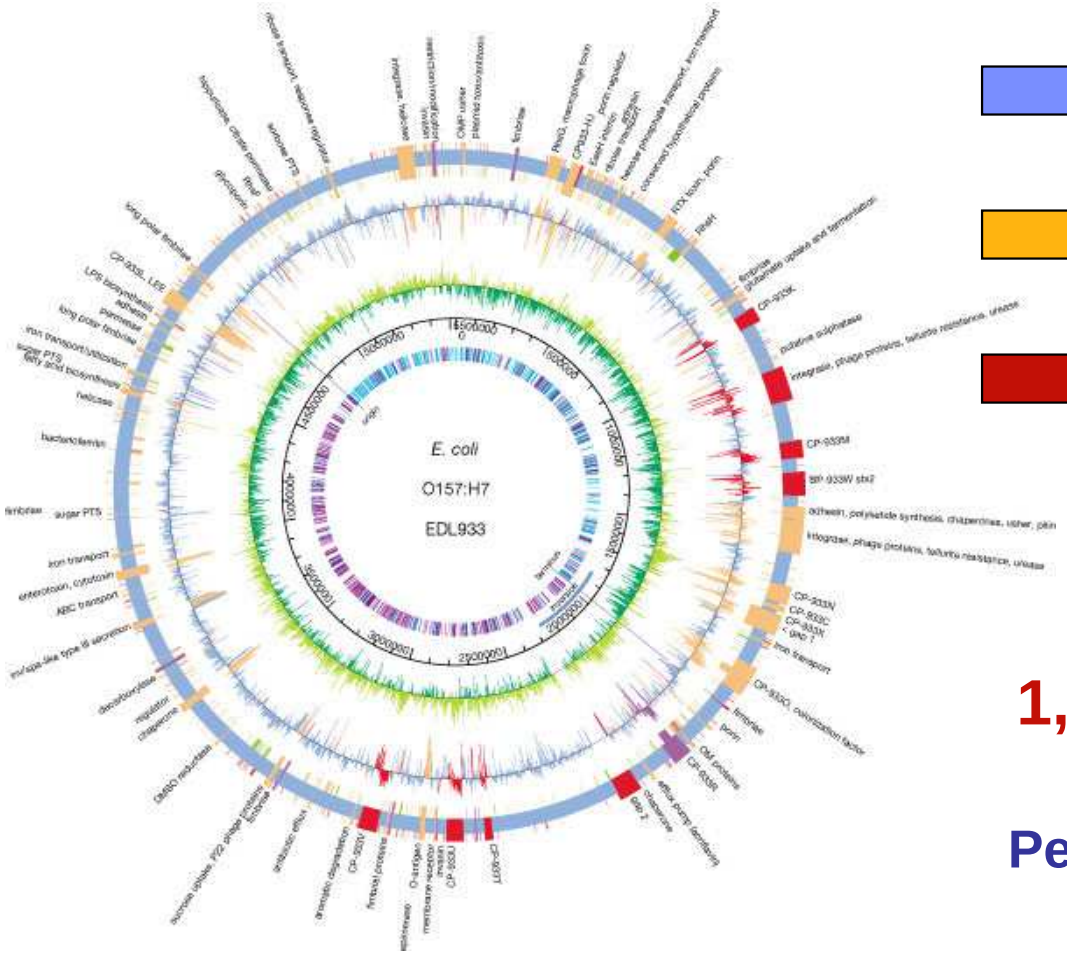
toxB

Mobile genetic elements:

- **Phages**
- **Genomic islands**
- **Plasmid**

Shiga toxin-Production

E.coli O157 chromosome



E.coli K12 sequences

Genomic islands

Integrated prophages

1,387 genes absent in E.coli K12

Perna et al. Nature, 409: 529-33, 2001

VTEC are nasty bugs !!

One of the most dangerous foodborne pathogens !

Very large community outbreaks

1996 - Japan and Scotland


Scozia, 6 morti intossicati da colibatteri nelle carni

LONDRA - E' salito ancora e rischia di aumentare, secondo le autorità sanitarie, il numero delle persone morte nella contea del

Le vittime sono tutte persone anziane: altri 100 in ospedale

Carni avariate, cinque morti

Scozia, strage causata da un batterio


LA PIÙ GRAVE EPIDEMIA REGISTRATA TRA I MINORI

Giappone, settemila bambini intossicati

Chiuse scuole e piscine, ma il contagio non si ferma


L'epidemia ha già contagiato 5 mila persone e 3 bambini sono morti

Un batterio-killer uccide i giapponesi

2005 - Italy

Mediatic impact !

Socio-economic consequences on:

- ✓ **Agri-Food industry**
- ✓ **Tourism**
- ✓ **……**

Outbreak of Shiga toxin-producing *E. coli* in Germany

REUTERS EDITION: U.S.

Killer bacteria claims victims in Germany, Sweden

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Spain mulls action over blame for E.coli outbreak
Tue, May 31 2011

Europe trades blame over deadly E.coli in Germany
Tue, May 31 2011

By Eric Kelsey

BERLIN | Tue May 31, 2011 5:31pm EDT

(Reuters) - A killer bacteria linked to contaminated

Regulation EC No 882/2004 on official controls

The EU Reference Laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC)

Since 2006

Unit for Foodborne Zoonoses and Veterinary Epidemiology

Department of Veterinary Public Health and Food Safety

Istituto Superiore di Sanità, Rome, Italy

www.iss.it/vtec

Regulation (EC) 882/2004 on official controls

TITOLO III

REFERENCE LABORATORIES

- **Community Reference Laboratories (EU-RL) for specific food and feed hazards and for specific animal diseases (Art. 32).**

- **National Reference Laboratories (NRL), which are designated by Member States for each CRL (Art. 33)**

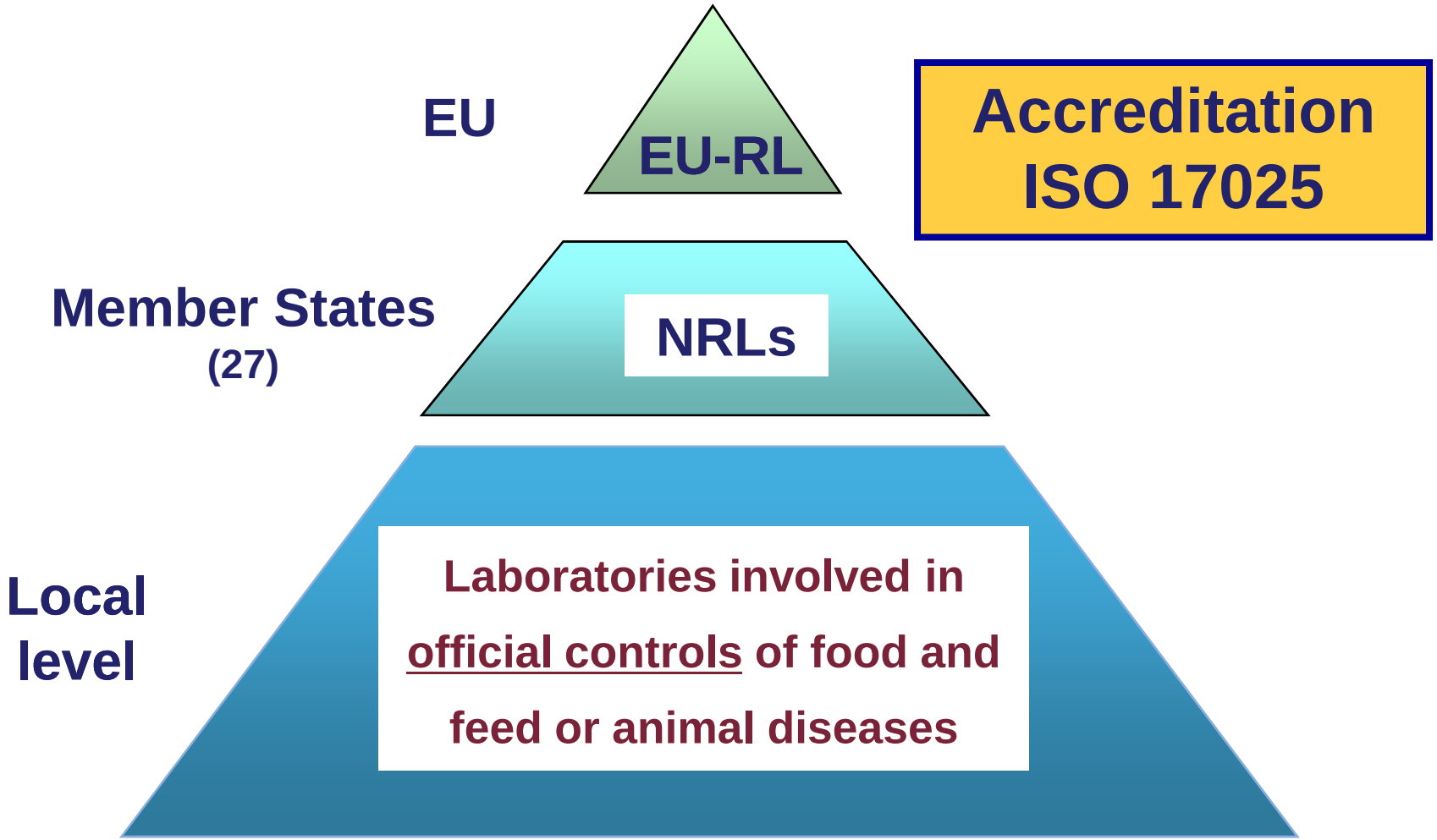
Functions and duties of EU-RLs (882/2004, Art. 32)

- ✓ To provide NRLs with analytical reference methods
- ✓ To provide NRLs with reference materials
- ✓ To organise comparative testing (ring tests)
- ✓ To conduct training for NRLs, (annual workshop, courses, visits to EU-RL)
- ✓ To provide scientific and technical assistance to the Commission

Functions and duties of NRLs (882/2004, Art. 33)

- ✓To collaborate with their EU-RL
- ✓To coordinate the activities of laboratories involved in official food controls in their own country
- ✓To organise comparative tests for those labs
- ✓To disseminate the information received from EU-RLs
- ✓To provide scientific support to their Authorities

The Network of EU Reference Laboratories



Regulation EC No 776/2006

- **41 EU-RLs, selected and designated for 5-years periods upon public calls**

- **14 EU-RLs for animal health**

- **14 EU-RLs for chemical risks in food and feed**

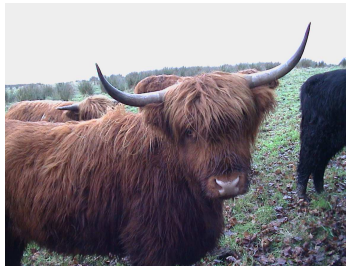
- **13 EU-RLs for biological risks in food and feed**

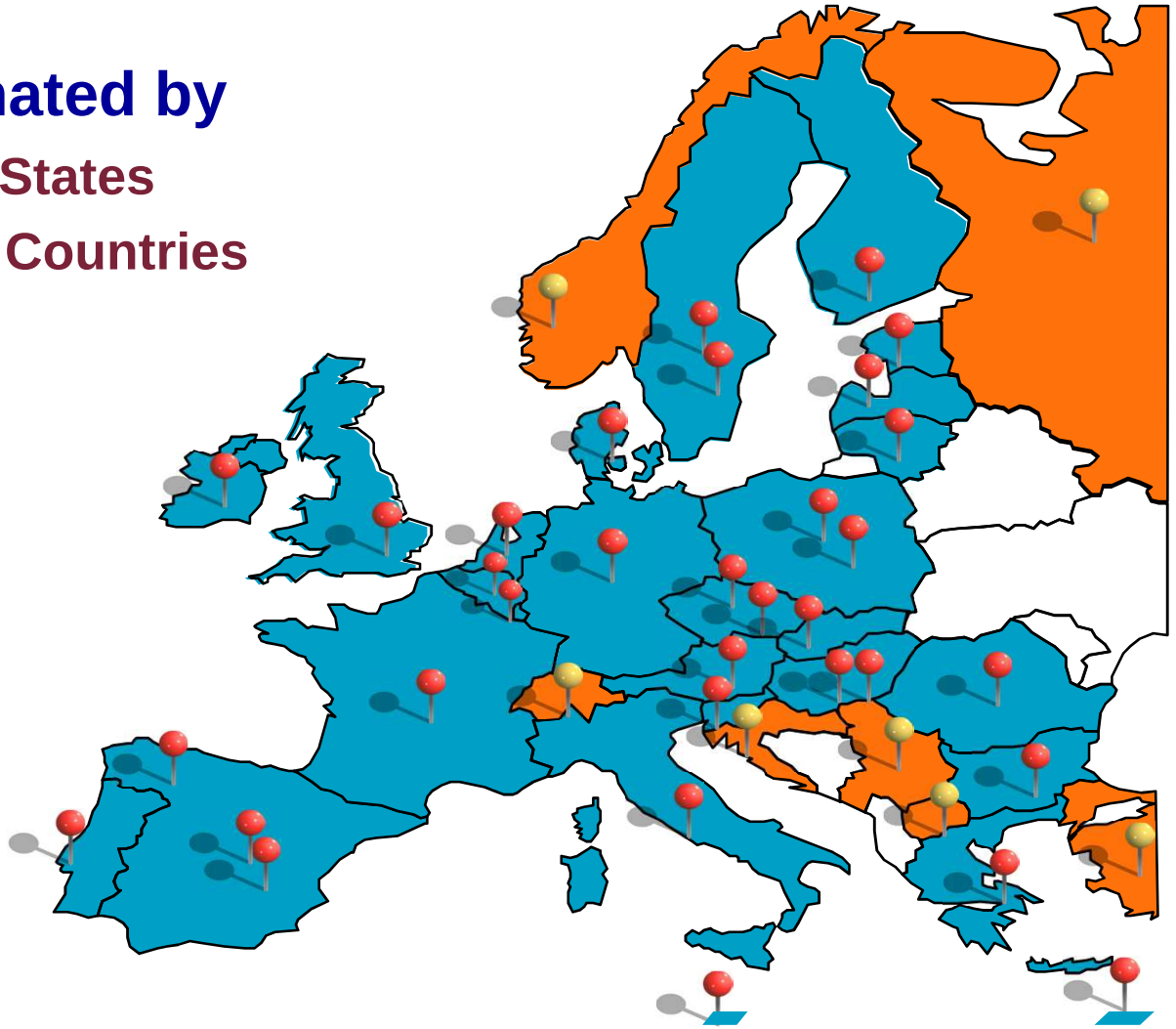
The network of RLs for *Escherichia coli* (VTEC)

41 NRLs designated by

27 EU Member States

+ 7 other European Countries





VTEC: Zoonotic origin

Asymptomatic carriage/colonization

VTEC: Zoonotic origin

Animal reservoir : Cattle

Sheep

ter buffalo

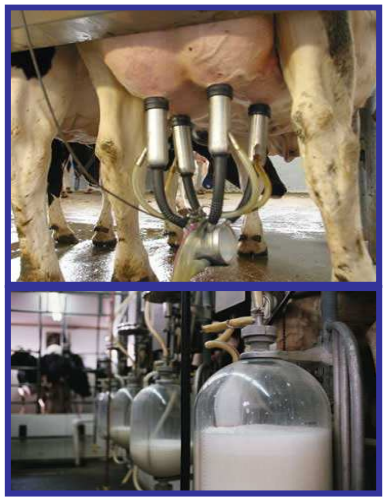
Wild ruminants

✓ VTEC in > 50% of the animals

✓ > 100 *E.coli* serogroups reported

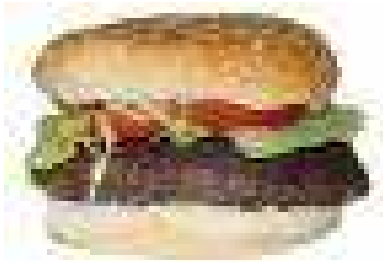
VTEC: routes of transmission

Foodborne:Primary contamination of meat and milk

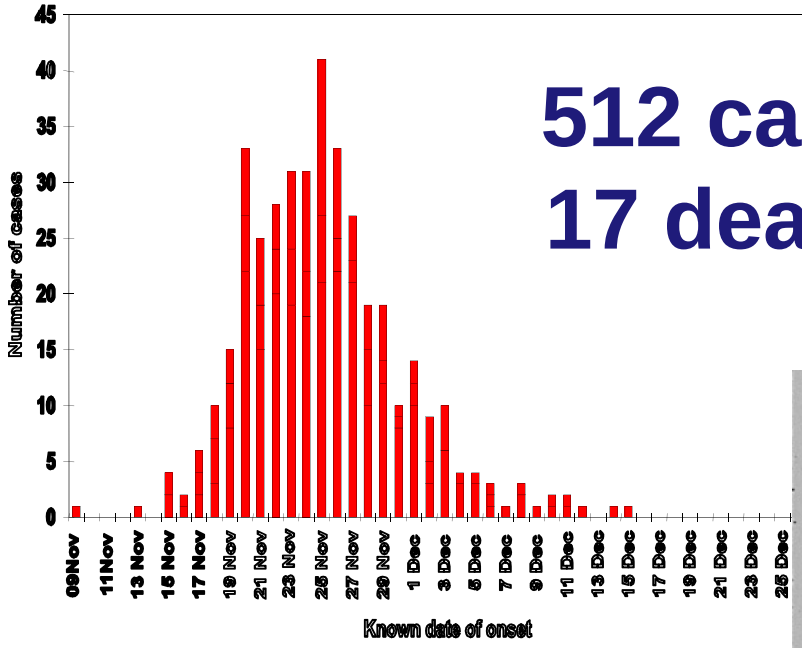


USA, *E.coli* O157 multi-state outbreak, 1993

- **Fast food chain, several States in the west coast**
- **Burgers (not properly cooked)**
- **4 deaths**
- **700 ill**

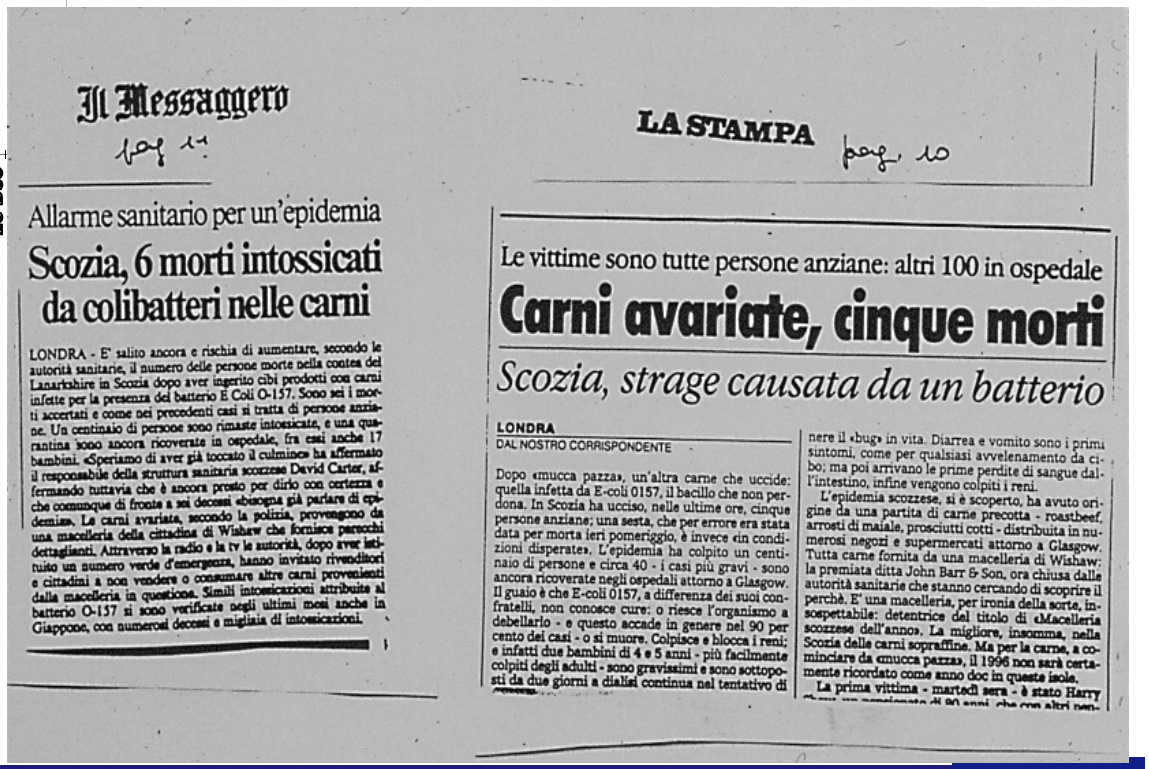


Central Scotland Outbreak of *E.coli* O157, 1996



512 cases
17 deaths

- **Contaminated "steak pie"**
- **Cross contamination with raw meat**



Il Messaggero

Allarme sanitario per un'epidemia

Scozia, 6 morti intossicati da colibatteri nelle carni

LONDRA - E' salito ancora e rischia di aumentare, secondo le autorità sanitarie, il numero delle persone morte nella contea del Lanarkshire in Scozia dopo aver ingerito cibi prodotti con carni infette per la presenza del batterio E Coli O-157. Sono sei i morti accertati e come nei precedenti casi si tratta di persone anziane. Un centinaio di persone sono rimaste intossicate, e una quarantina sono ancora ricoverate in ospedale, fra essi anche 17 bambini. «Speriamo di aver già toccato il culmine» ha affermato il responsabile della struttura sanitaria scozzese David Carter, affermando tuttavia che è ancora presto per dirlo con certezza e che comunque di fronte a sei decessi «bisogna già parlare di epidemia». Le carni avariate, secondo la polizia, provengono da una macelleria della cittadina di Wishaw che fornisce parecchi dettaglianti. Attraverso la radio e la tv le autorità, dopo aver istituito un numero verde d'emergenza, hanno invitato rivenditori e cittadini a non vendere o consumare altre carni provenienti dalla macelleria in questione. Simili intossicazioni attribuite al batterio O-157 si sono verificate negli ultimi mesi anche in Giappone, con numerosi decessi e migliaia di intossicazioni.

LA STAMPA

Le vittime sono tutte persone anziane: altri 100 in ospedale

Carni avariate, cinque morti

Scozia, strage causata da un batterio

LONDRA
DAL NOSTRO CORRISPONDENTE

Dopo «mucca pazza», un'altra carne che uccide: quella infetta da E-coli 0157, il bacillo che non perdona. In Scozia ha ucciso, nelle ultime ore, cinque persone anziane; una sesta, che per errore era stata data per morta ieri pomeriggio, è invece «in condizioni disperate». L'epidemia ha colpito un centinaio di persone e circa 40 - i casi più gravi - sono ancora ricoverate negli ospedali attorno a Glasgow. Il guaio è che E-coli 0157, a differenza dei suoi confratelli, non conosce cure: o riesce l'organismo a debellarlo - e questo accade in genere nel 90 per cento dei casi - o si muore. Colpisce e blocca i reni; e infatti due bambini di 4 e 5 anni - più facilmente colpiti degli adulti - sono gravissimi e sono sottoposti da due giorni a dialisi continua nel tentativo di

nere il «bug» in vita. Diarrea e vomito sono i primi sintomi, come per qualsiasi avvelenamento da cibo; ma poi arrivano le prime perdite di sangue dall'intestino, infine vengono colpiti i reni.

L'epidemia scozzese, si è scoperto, ha avuto origine da una partita di carne precotta - roastbeef, arrosti di maiale, prosciutti cotti - distribuita in numerosi negozi e supermercati attorno a Glasgow. Tutta carne fornita da una macelleria di Wishaw: la premiata ditta John Barr & Son, ora chiusa dalle autorità sanitarie che stanno cercando di scoprire il perchè. E' una macelleria, per ironia della sorte, insospettabile: detentrice del titolo di «Macelleria scozzese dell'anno». La migliore, insomma, nella Scozia delle carni sopraffine. Ma per la carne, a cominciare da «mucca pazza», il 1996 non sarà certamente ricordato come anno doc in queste isole.

La prima vittima - martedì sera - è stato Harry

E.coli - unpasteurized milk

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Reports Matching Search

02-MAR-07	PRO/AH/EDR> E. coli O157, unpasteurized milk, 2005 - USA (WA)	20070302.0741
29-SEP-06	PRO/AH/EDR> E. coli O157, unpasteurized milk - USA (CA)(03)	20060929.2791
	PRO/AH/EDR> E. coli O157, unpasteurized milk - USA (WA): recall	20060929.2790
27-SEP-06	PRO/AH> E. coli O157, unpasteurized milk - USA (CA)(02): background	20060927.2761
22-SEP-06	PRO/AH/EDR> E. coli O157, unpasteurized milk - USA (CA)	20060922.2706
21-JAN-06	PRO/AH/EDR> E. coli O157, unpasteurized milk - USA (OR,WA)(04)	20060121.0199
20-DEC-05	PRO/AH/EDR> E. coli O157, unpasteurized milk - USA (OR,WA)(03)	20051220.3647
19-DEC-05	PRO/AH/EDR> E . coli O157, unpasteurized milk - USA (OR, WA)(02)	20051219.3633
16-DEC-05	PRO/EDR> E. coli O157, unpasteurized milk - USA (OR,WA)	20051216.3622
11-APR-05	PRO/AH/EDR> E. coli O157: H7, unpasteurized milk - Canada (ONT)	20050411.1047
13-MAY-04	PRO/EDR> E. coli O157, pasteurized milk - Denmark (Copenhagen)	20040513.1297
31-OCT-98	PRO/AH> E. coli, unpasteurized milk - UK (England) (02)	19981031.2126
22-OCT-98	PRO/EDR> E. coli, unpasterized milk - UK (England)	19981022.2080

VTEC: routes of transmission

Environmental contamination

Soil

Surface water

VTEC: routes of transmission

Waterborne: Drinking and recreational water

Contamination of spring and well water

Waterborne outbreak *of E.coli* O157, Walkerton Canada, 2000

Walkerton **E. coli TRAGEDY**

Contamination of municipal drinking water

- population: 5,000
- ill persons: 2,300
- deaths: 7

THE GLOBE AND MAIL

CANADA'S NATIONAL NEWSPAPER • FOUNDED 1844 • FRIDAY, MAY 26, 2000

'This could have been prevented'

Accusations fly that Ontario town's tainted water supply was covered up for five days; death toll continues to climb

Desperately ill children

VTEC: routes of transmission

Contamination of vegetables

Outbreak of *E.coli* O157, Japan 1996

CNN interactive CNN.com

WORLD NEWS STORY PAGE

Health officials search for the source of the outbreak (CNN)

Radish sprouts in school meal

- 10,000 school children ill
- 8 deaths

Giappone: ancora una vittima del colibatterio «O-157»

L'epidemia ha già contagiato 5 mila persone e 3 bambini sono morti

Un batterio-killer uccide i giapponesi

In coma due bimbe colpite dal virus-killer "O-157"

Giappone si aggrava l'epidemia

TOKYO — Si allarga in Giappone l'epidemia da vi-

l'Unità pagina 15

Il batterio piega il Giappone

8mila contagiati tra bimbi e anziani. 4 morti

LA PIÙ GRAVE EPIDEMIA REGISTRATA TRA I MINORI

Giappone, settemila bambini intossicati

Chiuse scuole e piscine, ma il contagio non si ferma

bilancio diffuso dalle autorità parla di 6.784 ne che ha un'incubazione di circa due set

Contamination of vegetables

Department of Health and Human Services
Centers for Disease Control and Prevention

CDC en Español

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E. coli O157:H7 Outbreak from Fresh Spinach

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Update on Multi-State Outbreak of *E. coli* O157:H7 Infections From Fresh Spinach, September 28, 2006

VTEC: routes of transmission

Direct contact with ruminants
farm visits - petting zoos

Direct contact with ruminants

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Page last updated at 16:23 GMT, Sunday, 20 September 2009 17:23 UK

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Farm E. coli cases increase to 64

The number of cases of E. coli in an outbreak linked to a children's petting farm in Surrey has risen to 64.

The Health Protection Agency said seven more people had been affected by the O157 strain of the bacteria connected to Godstone Farm.

There are now nine children in hospital, all of whom are described as stable and improving.

Godstone Farm in Surrey has now been linked to 64 cases of E.coli

Direct contact with ruminants

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News › UK news › E coli

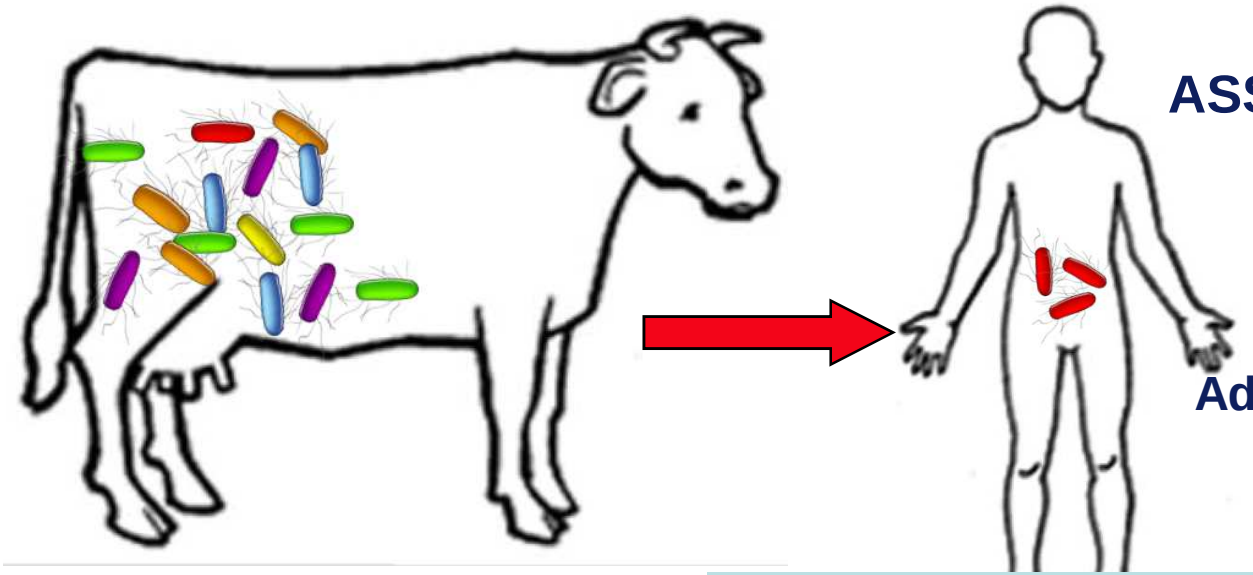
E coli outbreak: call for petting farms to stop children stroking the animals

Young children are risking the spread of E coli by touching the animals at zoos and farms, say medical experts

Children feed goat at petting zoo, Blair Drummond Safari Park, Stirling, Scotland, May 2008. Photograph: Rex Features

A sign near an animal shed at Godstone Farm in Godstone, Surrey, which has been hi by an outbreak of E Coli. Photograph: Gareth Fuller/PA

Are all VTEC pathogenic to humans?



VTEC

ASSOCIATED WITH SEVERE DISEASE (BD, HUS)

Additional virulence properties

Attaching/Effacing adhesion

VTEC
>100 serogroups
in the reservoir

O157
which others ?? ….

efsa
European Food Safety Authority

The EFSA Journal (2007) 579, 1-61

Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types [1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-036)

Adopted on 18 October 2007

Recommendations

Monitoring of animal populations and foodstuffs

…. examining food and animal reservoirs for a defined range of VTEC serotypes, which have been selected using human surveillance data.

The European Centre of Disease Prevention and Control (ECDC)

European Centre for Disease Prevention and Control

ECDC is the EU agency to fight against infectious diseases

Surveillance of VTEC infections in the EU

THE COMMUNITY SUMMARY REPORT ON TRENDS AND SOURCES OF ZOONOSES AND ZOONOTIC AGENTS AND FOOD-BORNE OUTBREAKS IN THE EUROPEAN UNION IN 2008

Issued on 23 December 2009
Published on 28 January 2010

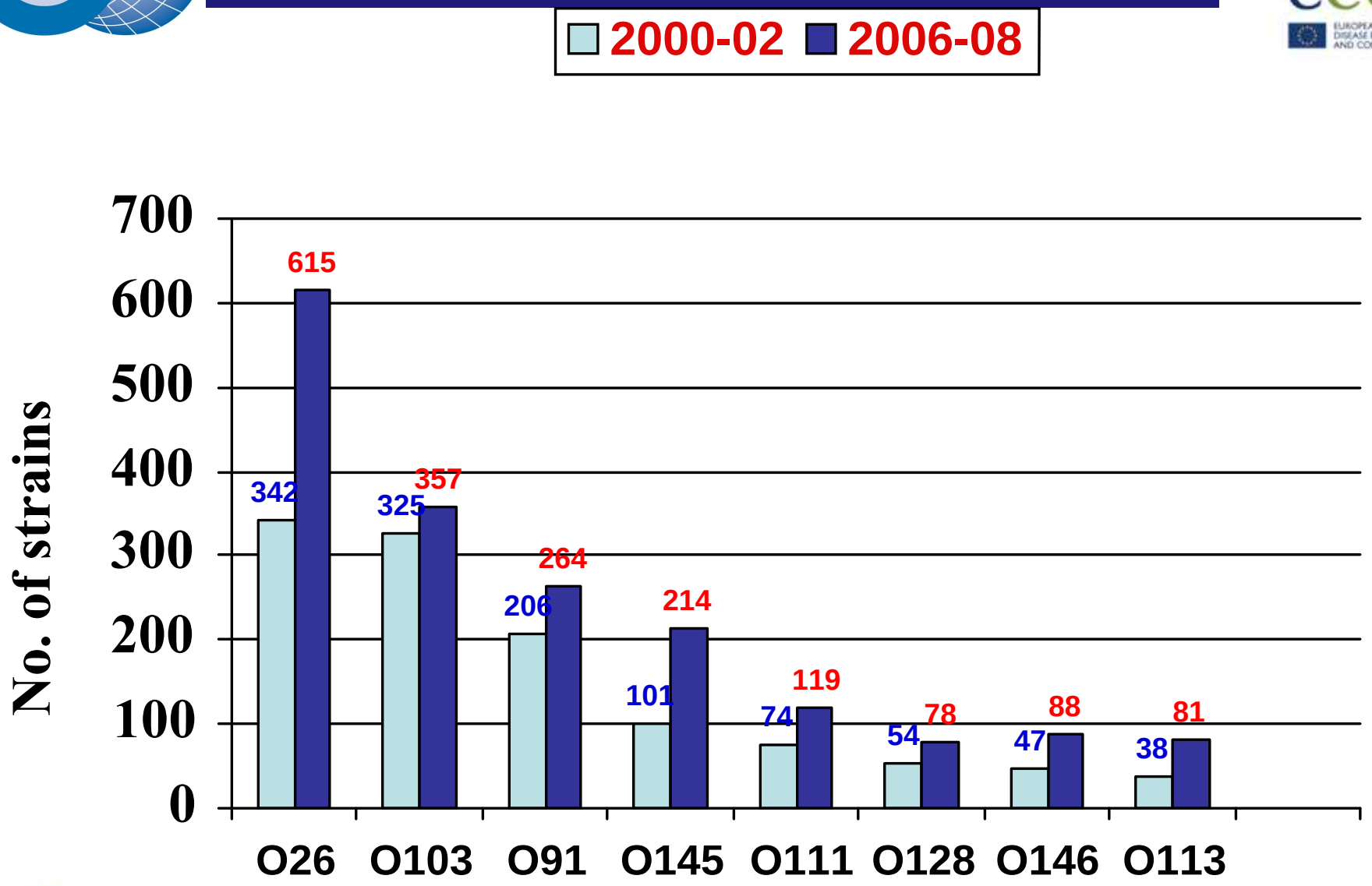
O157	52.7 %
O26	6.5 %
O103	3.8 %
O91	2.8 %
O145	2.3 %
O111	1.3 %
O146	0.9 %

VTEC infections by serogroup

2006 - 2008

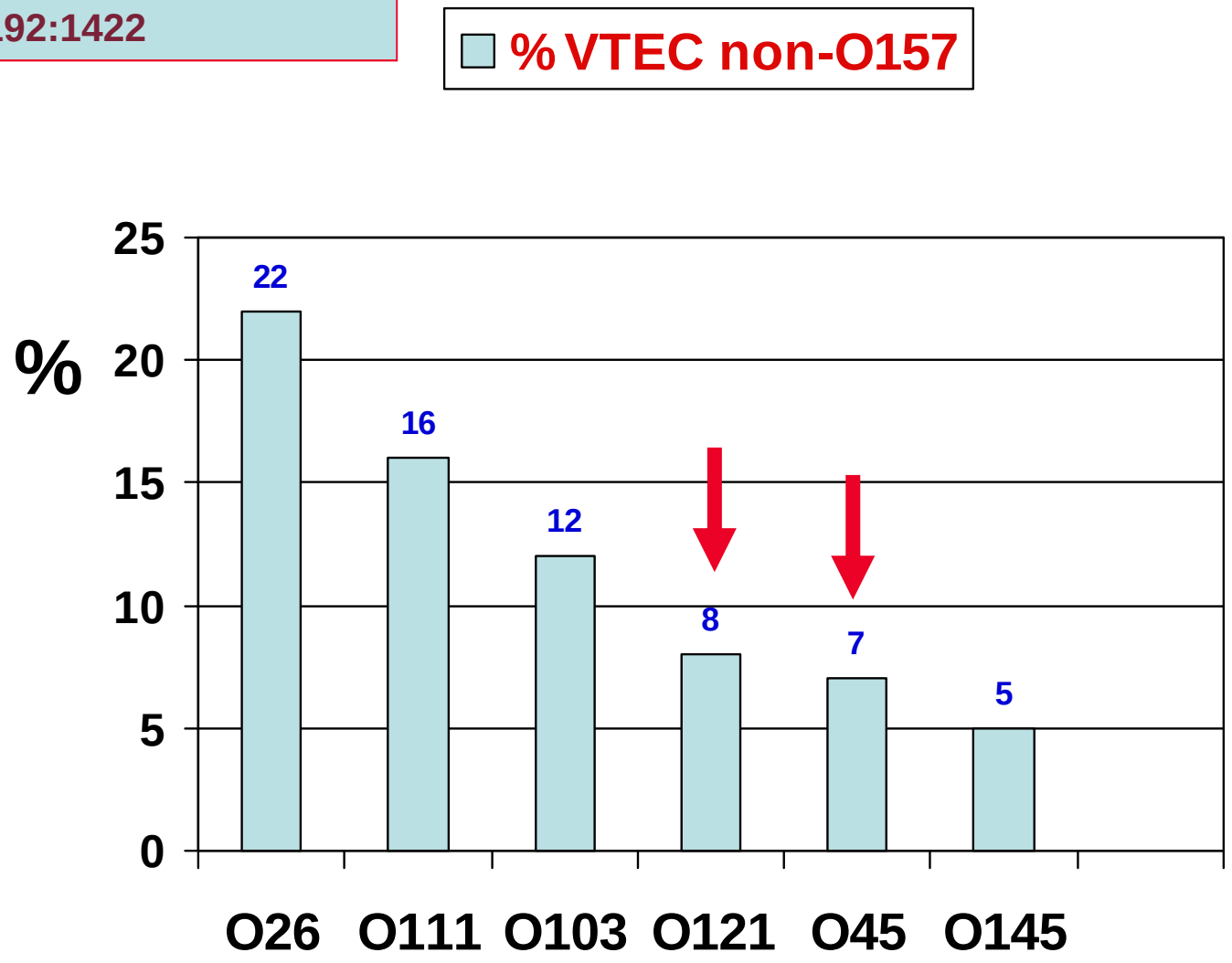
N = 9,421

VTEC non-O157 in Europe



VTEC non-O157 in the US, 1993 - 2002

Brooks et al. J Infect Dis. 2005;192:1422



VTEC infections in the European Union 2002-2006

	O157	O26	O103	O91	O145	O111	O146
Total Cases	7,227	732	603	425	312	180	153
HUS Cases	310	52	20	1	27	18	0

VTEC non-O157 outbreaks: O26 – Denmark, 2007

Eurosurveillance
Europe's journal on infectious disease epidemiology, prevention and control

Eurosurveillance, Volume 12, Issue 22, 31 May 2007

Articles

Citation style for this article: Ethelberg S, Smith B, Torpdahl M, Lisby M, Boel J, Jensen T, Mølbak K. An outbreak of Verocytotoxin-producing Escherichia coli O26:H11 caused by beef sausage, Denmark 2007. Euro Surveill. 2007;12(22):pii=3208. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3208

An outbreak of Verocytotoxin-producing *Escherichia coli* O26:H11 caused by beef sausage, Denmark 2007

S Ethelberg (SET@ssi.dk)[1,5], B Smith[1], M Torpdahl[1], M Lisby[2], J Boel[3], T Jensen[4] and K Mølbak[5]

VTEC non-O157 Outbreaks: O103 – Norway,

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Journal List > BMC Infect Dis > v.8; 2008

BMC Infect Dis. 2008; 8: 41.
Published online 2008 April 3. doi: 10.1186/1471-2334-8-41.
PMCID: PMC2335110

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Outbreak of haemolytic uraemic syndrome in Norway caused by *stx*$_2$-positive *Escherichia coli* O103:H25 traced to cured mutton sausages

Barbara Schimmer,[1,2] Karin Nygard,[1] Hanne-Merete Eriksen,[1] Jørgen Lassen,[3] Bjørn-Arne Lindstedt,[3] Lin T Brandal,[3] Georg Kapperud,[3,4] and Preben Aavitsland[1]

VTEC non-O157 outbreaks: O111 – USA, 1999

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Weekly

April 21, 2000 / 49(15);321-4

Escherichia coli O111:H8 Outbreak Among Teenage Campers --- Texas, 1999

In June 1999, the Tarrant County Health Department reported to the Texas Department of Health (TDH) that a g
attending a cheerleading camp during June 9--11 became ill with nausea, vomiting, severe abdominal cramps, a
of which e hospitalized with hemolytic uremic syndrome (HUS), and two others
appende om eight ill persons failed to yield a pathogen. Stools subsequently we
laborato lealth and CDC, where *Escherichia coli* O111:H8 was isolated from tw
report su s outbreak.

VTEC non-O157 outbreaks: O111 – USA, 2008

VTEC non-O157 outbreaks: O145 – USA, 2010

MAY 6, 2010

FOOD SAFETY INFOSHEET

Romaine lettuce linked to *E. coli* O145 outbreak

CLUSTERS OF ILLNESSES IN THE NORTH EAST AND MIDWEST

60 ILL IN MICHIGAN, NEW YORK AND OHIO

LEAFY GREENS HAVE A HISTORY

LETTUCE, SPINACH, GREENS LINKED TO AT LEAST 30 OUTBREAKS OF PATHOGENIC *E. COLI* SINCE 1993

Freshway FOODS

www.foodsafetyinfosheets.com

VTEC non-O157 outbreaks: O121 – USA, 1999

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PEDIATRICS Vol. 108 No. 4 October 2001, p. e59

ELECTRONIC ARTICLE:

Hemolytic-Uremic Syndrome and *Escherichia coli* O121 at a Lake in Connecticut, 1999

Abstract FI

VTEC non-O157 outbreaks: O121 – USA, 2006

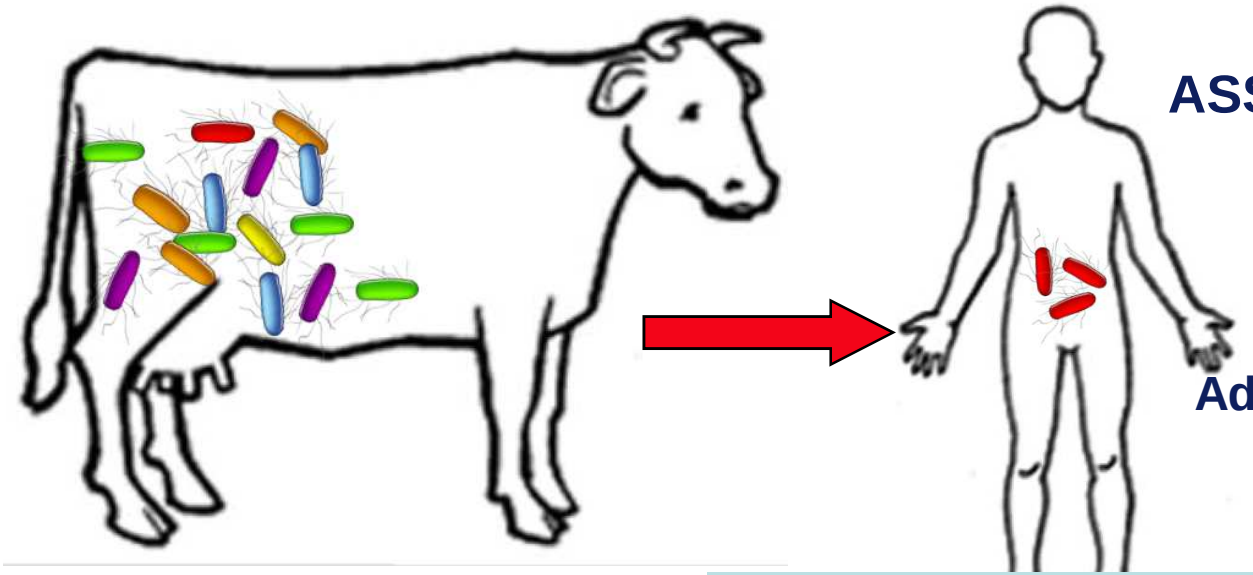
About E. Coli

Wendy's E. Coli Outbreak (Utah)

OUTBREAK NEWS

Lettuce served in dishes prepared by a Wendy's restaurant in Ogden, Utah, was the source of an E. coli outbreak in June of 2006. Following an investigation into an E. coli outbreak among attendees of a CORE Academy luncheon held at Orion Junior High School in Harrisville, Utah, on June 30, the Weber-Morgan Health Department (WMHD) announced that four people had become ill [redacted] infections after eating iceberg lettuce prepared at the Wendy's restaurant located at 2500 N 400 E in North Ogden, Utah. WMHD announced that three o the four people who were confirmed ill with E. coli infections after eating the food prepared by Wendy's had developed hemolytic uremic syndrome (HUS).

Are all VTEC pathogenic to humans?



VTEC

ASSOCIATED WITH SEVERE DISEASE (BD, HUS)

Additional virulence properties

Attaching/Effacing adhesion

VTEC
>100 serogroups
in the reservoir

O157
which others ?? ….

Recommendations

Monitoring of animal populations and foodstuffs

2009

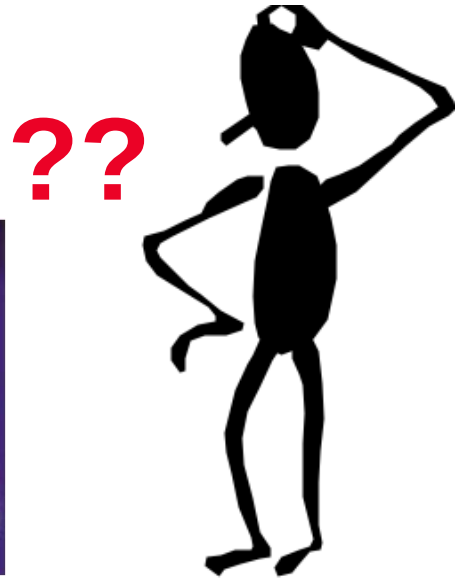
Initially mon s serotype
is most fre ncluding
HUS) in th
seropatho O111)
that are id eriodical
analysis of human disease and epidemiological data.

- Attaching / effacing
- Cause HUS
- Cause outbreaks

Detection of pathogenic non-O157 VTEC in food

- **No peculiar features to discriminate non-O157 VTEC from other *E. coli***

- **No standard methods available yet**



International Organization for Standardization

Technical specification approved by CEN and by 22 ISO Member Bodies

afnor NORMALISATION

« Food products – Microbiology »	CEN/TC 275 WG 6
Date: **2009-03-03**	Doc. Number: **N 426 (replaces doc N 425)**
Assistant: *Nathalie BRUNET* *Direct line: + 33 (0)1 41 62 85 09* *nathalie.brunet@afnor.org*	***Your contact:*** *Sandrine ESPEILLAC* *Direct line : + 33 (0)1 41 62 86 02* *sandrine.espeillac@afnor.org*

Registration of the STEC project

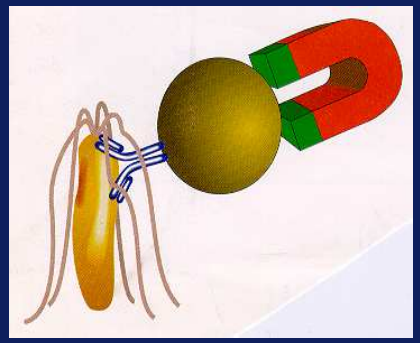
Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157, O111, O26, O103 and O145 serogroups - Qualitative Real-time polymerase chain reaction (PCR)-based Method

CEN ISO PRF 13136

A MULTI-STEP SEQUENTIAL PROCEDURE

ISOLATION OF THE STRAIN

Serogroup- specific enrichment by immuno-capture with O-specific antibodies



Detection of “pathogenic” VTEC in food

United States Department of | Food Safety and Inspection | Office of Public Health | Laboratory QA/QC Division 950 College Station Road

Aimed at the so called “Top 7”

O157, O26, O45, O111, O121, O103, O145, ….

MLG 5B.00 of the FSIS Microbiology Laboratory Guidebook

http://www.fsis.usda.gov/PDF/Mlg_5B_00.pdf

Chapt

Title:
Strain

Effect

li

Detection of VTEC in food by ISO/TS 13136

Proficiency tests organized by the EU-RL VTEC

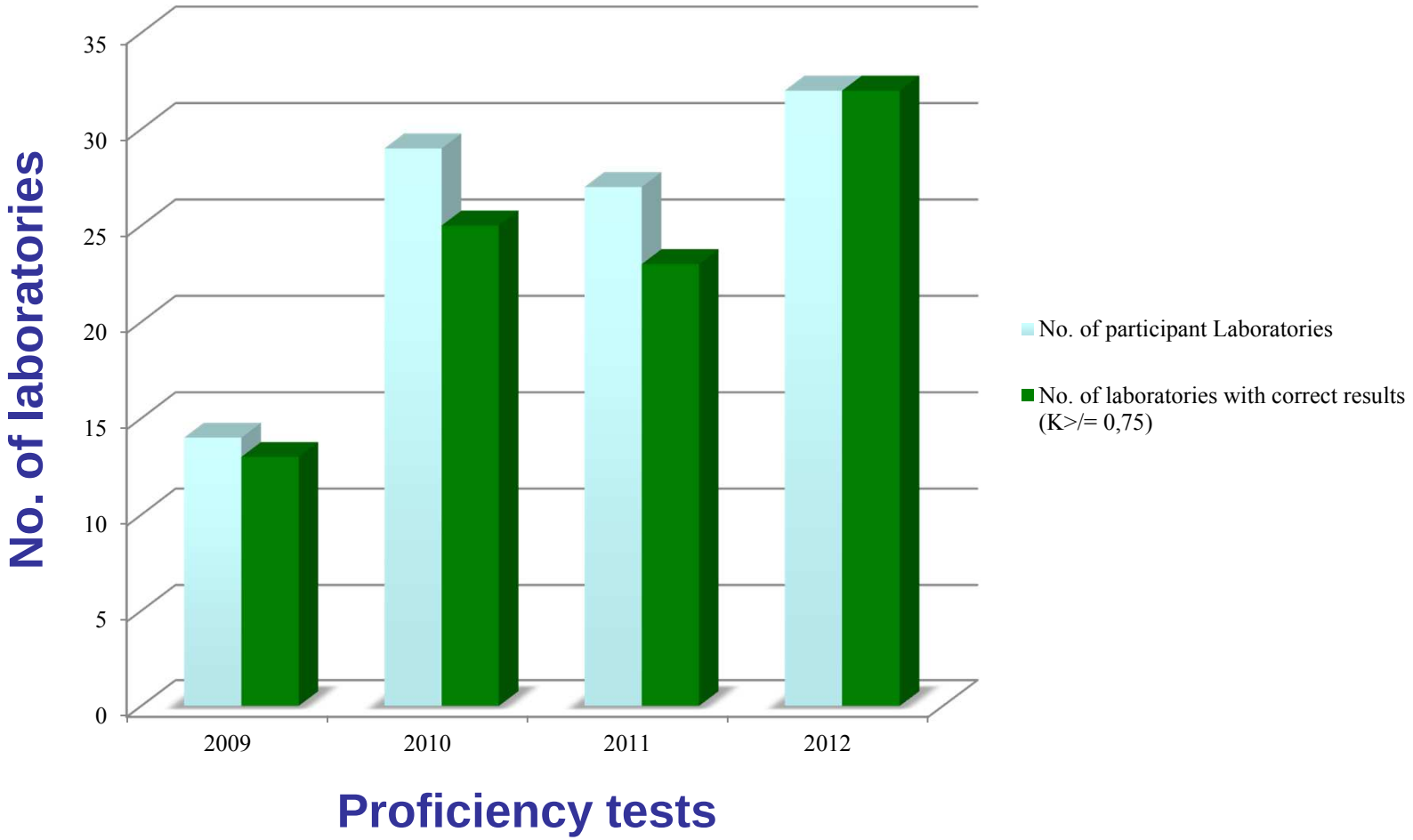
- **2008: Bacterial strains**

- **2009: Carcass swabs**

- **2010: Milk**

- **2011: Vegetables (spinach)**
- **2012: Water samples**

Proficiency testing: Detection of VTEC by ISO/TS 13136



No. of laboratories includes EU, EFTA and other third countries NRLs who participated in the PT programs

Outbreak of Shiga toxin-producing *E. coli* in Germany

REUTERS EDITION: U.S.

Killer bacteria claims victims in Germany, Sweden

Consiglia 379 consigli. Registrazione per vedere cosa consigliano i tuoi amici.

Tweet 46

Share 22

Share this

+1 0

Email

Print

Related News

Spain mulls action over blame for E.coli outbreak
Tue, May 31 2011

Europe trades blame over deadly E.coli in Germany
Tue, May 31 2011

By Eric Kelsey

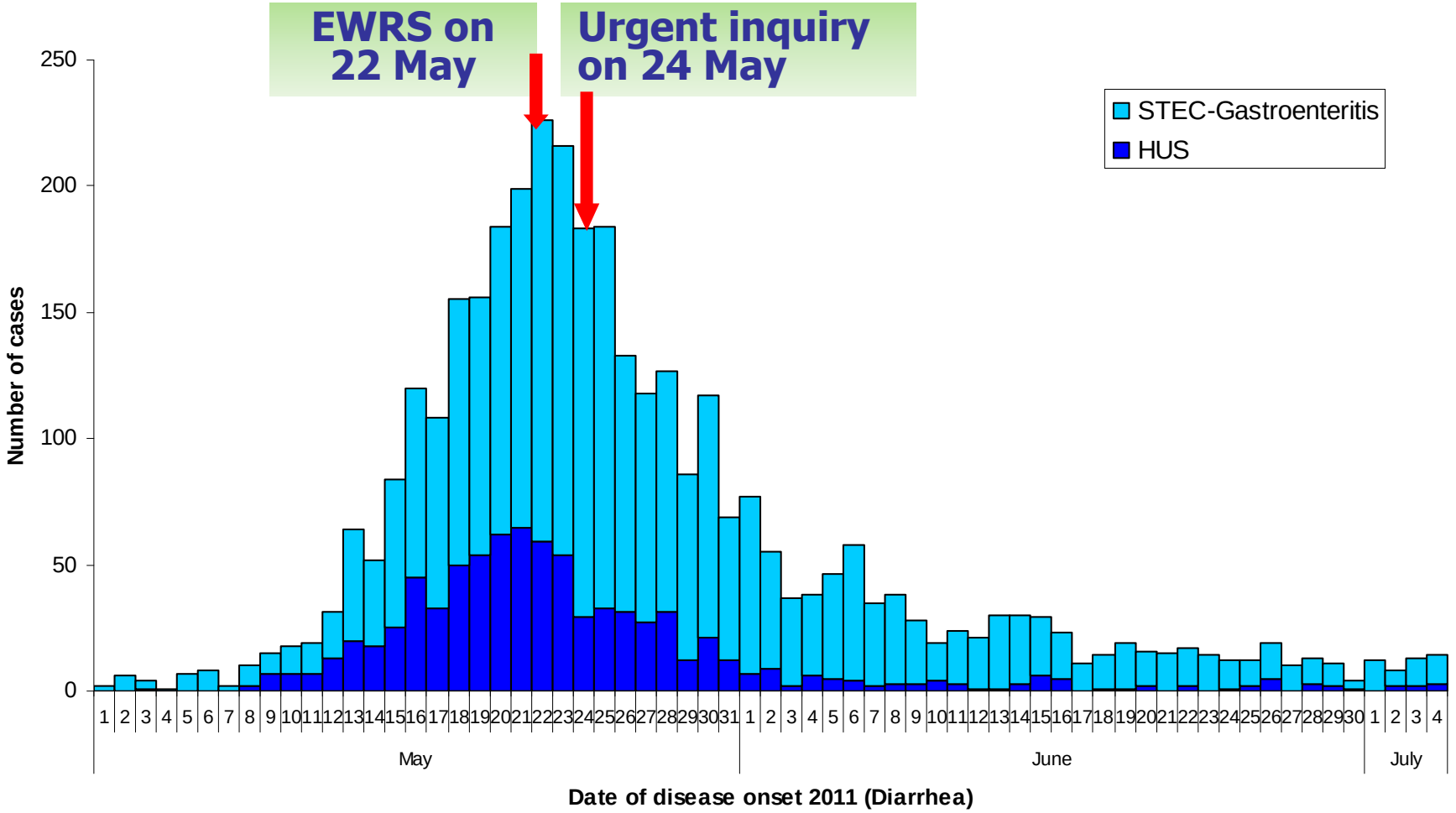
BERLIN | Tue May 31, 2011 5:31pm EDT

(Reuters) - A killer bacteria linked to contaminated

ROBERT KOCH INSTITUT

STEC O104:H4 outbreak, Germany 2011

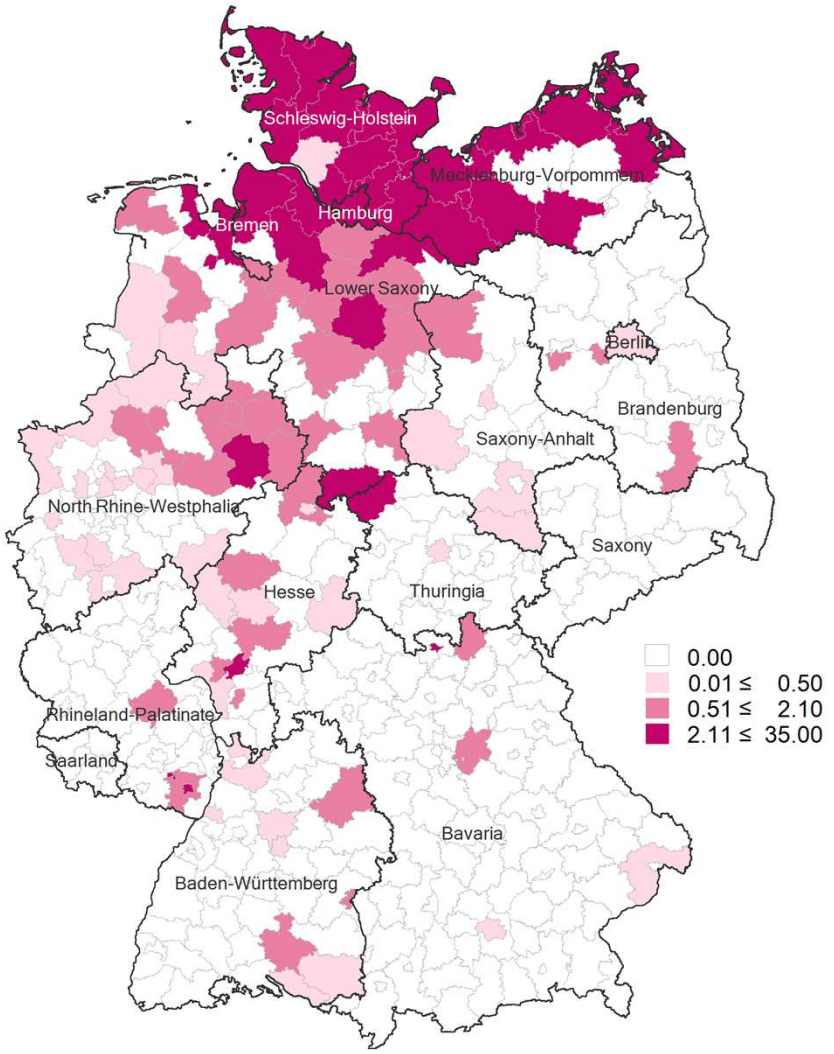
Source: Robert Koch Institute. Report: Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 outbreak, Germany 2011. Berlin 2011.



ROBERT KOCH INSTITUT

Incidence of HUS during the STEC O104 outbreak

German cases /100,000 population



Source: Robert Koch Institute. Report: Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 outbreak, Germany 2011. Berlin 2011.

STEC O104:H4 outbreak, Germany 2011

3,842 cases

2987 non-HUS STEC	855 HUS
18 deaths (0.6%)	35 deaths (4.1%)

Of HUS cases,

- 68% women

- Bloody diarrhoea in 79%

Eurosurveillance
Europe's journal on infectious disease epidemiology, prevention and control
NOW LISTED FOR IMPACT FACTOR!

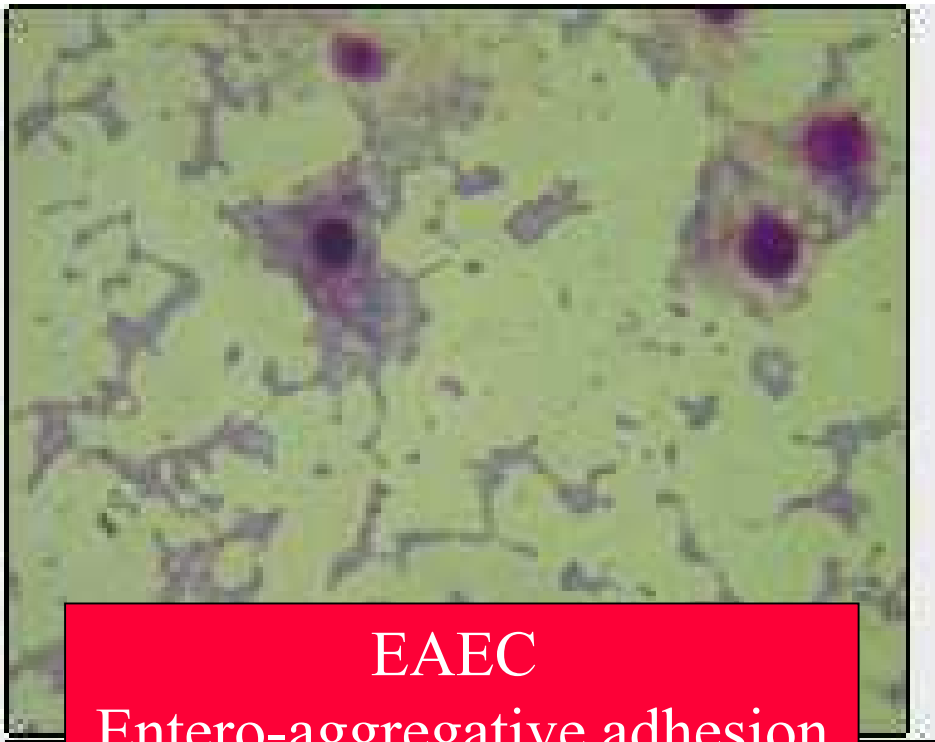
16 June

Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011

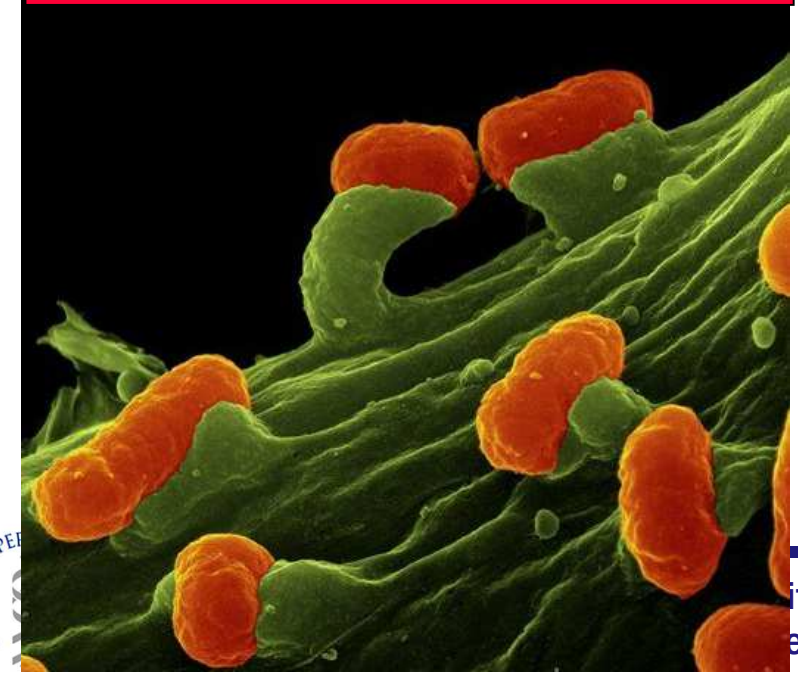
F Scheutz (fsc@ssi.dk)[1,2], E Møller Nielsen[2], J Frimodt-Møller[1,3], N Boisen[1,2], S Morabito[4], R Tozzoli[4], J P Nataro[5], A Caprioli[4]

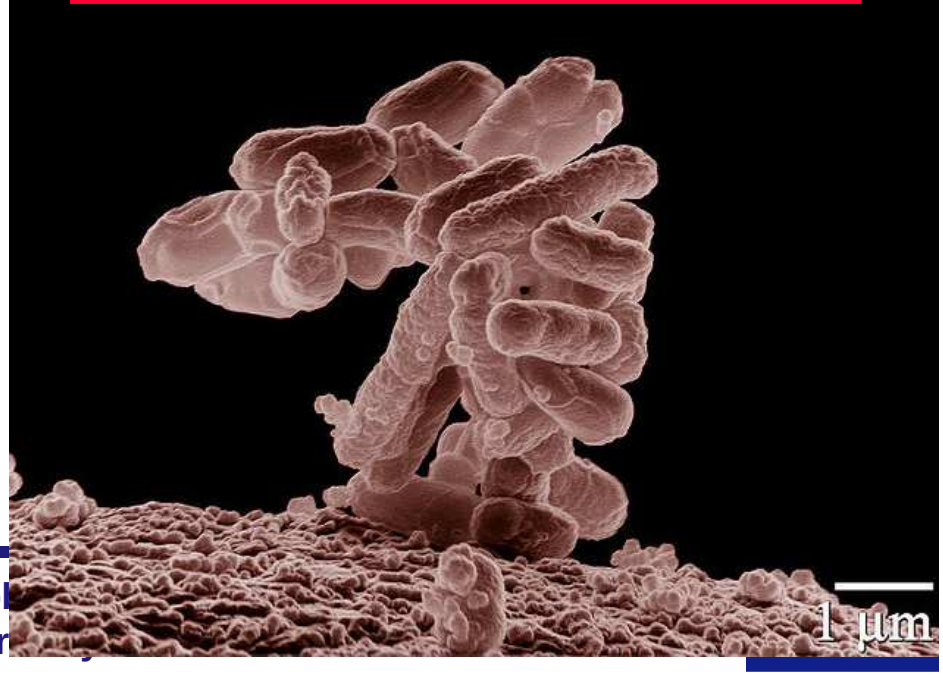
1. World Health Organization Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Department of Microbiological Surveillance and Research, Copenhagen, Denmark
2. Food-borne pathogens and typing, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark
3. Department of Clinical Microbiology, Hillerød Sygehus, Hillerød, Denmark
4. European Union Reference Laboratory for Escherichia coli, Department of veterinary public health and food safety, Istituto Superiore di Sanità, Rome, Italy
5. University of Virginia School of Medicine, Charlottesville, United States

Pathogenic VTEC
attaching/effacing adhesion

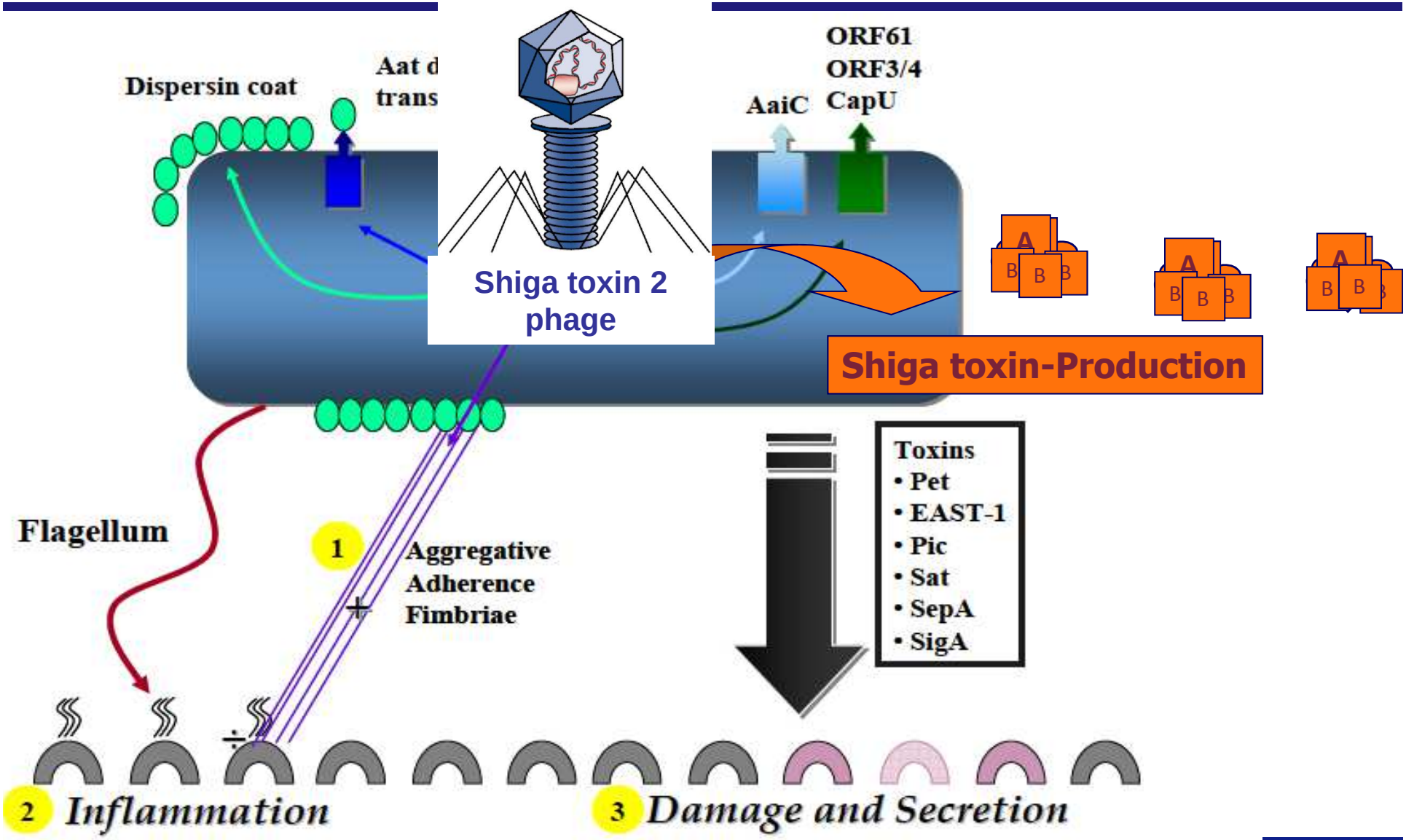


EAEC
Entero-aggregative adhesion

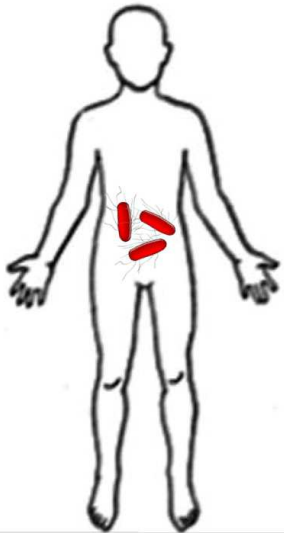


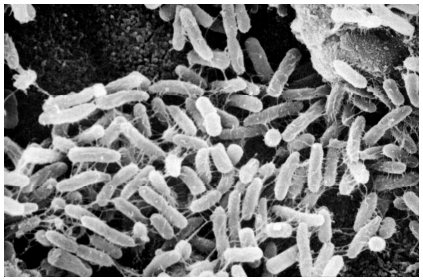


Enteroaggregative - Shiga toxin-producing *E. coli* O104:H4

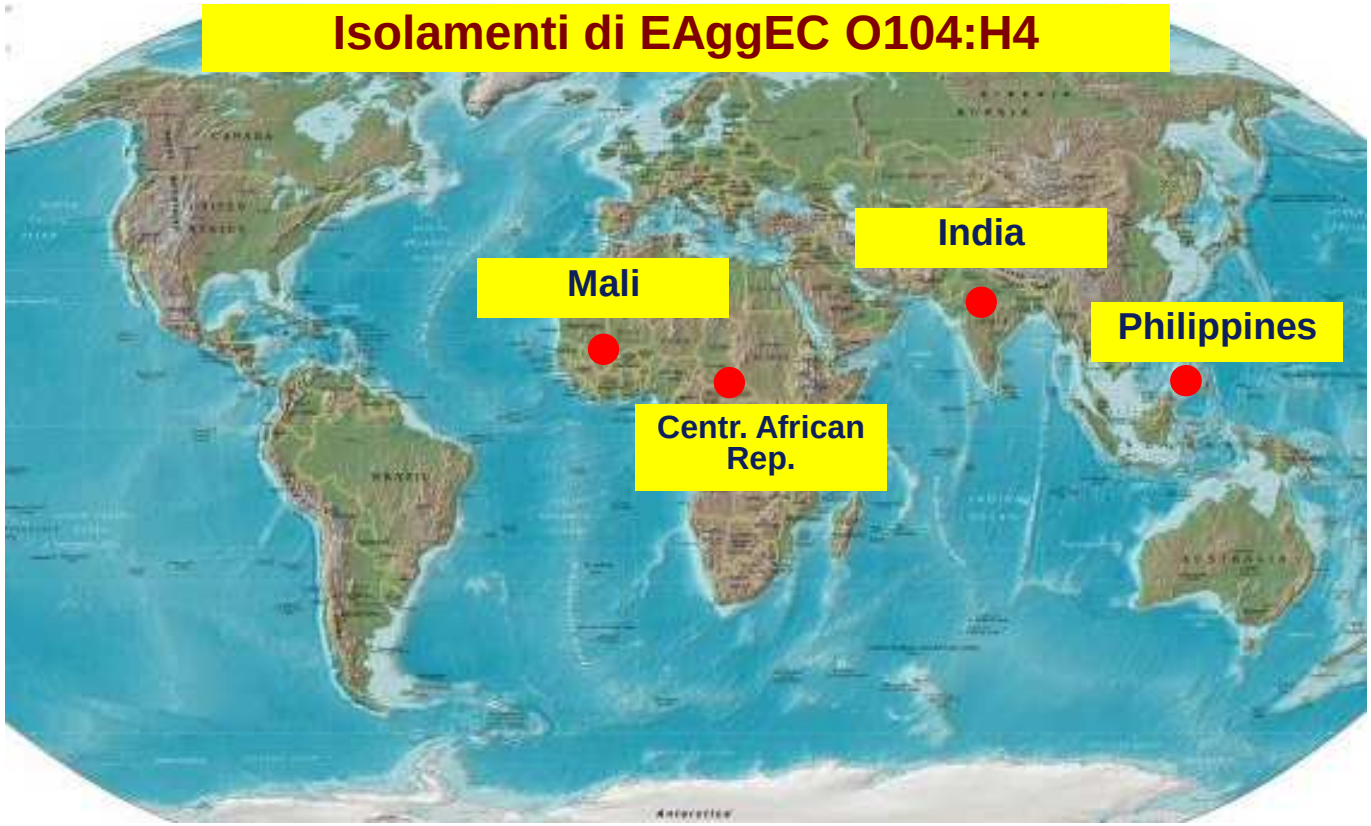


Enteroaggregative *E. coli* O104:H4

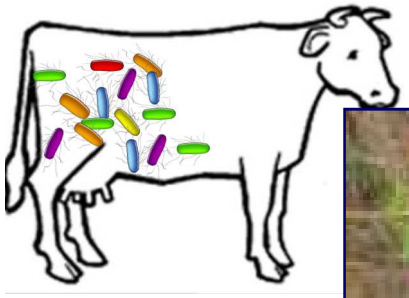




- ✓ Human origin
- ✓ Developing countries



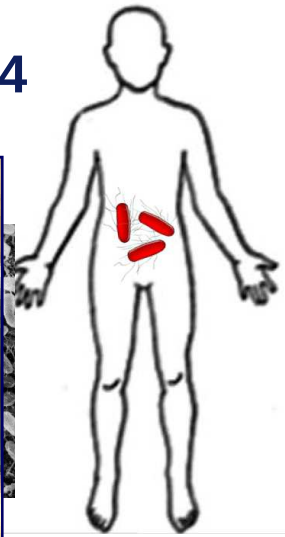
Origin of the Enteroaggregative - Shiga toxin-producing *E. coli* O104:H4



Stx2-converting Phage

EAggEC O104:H4

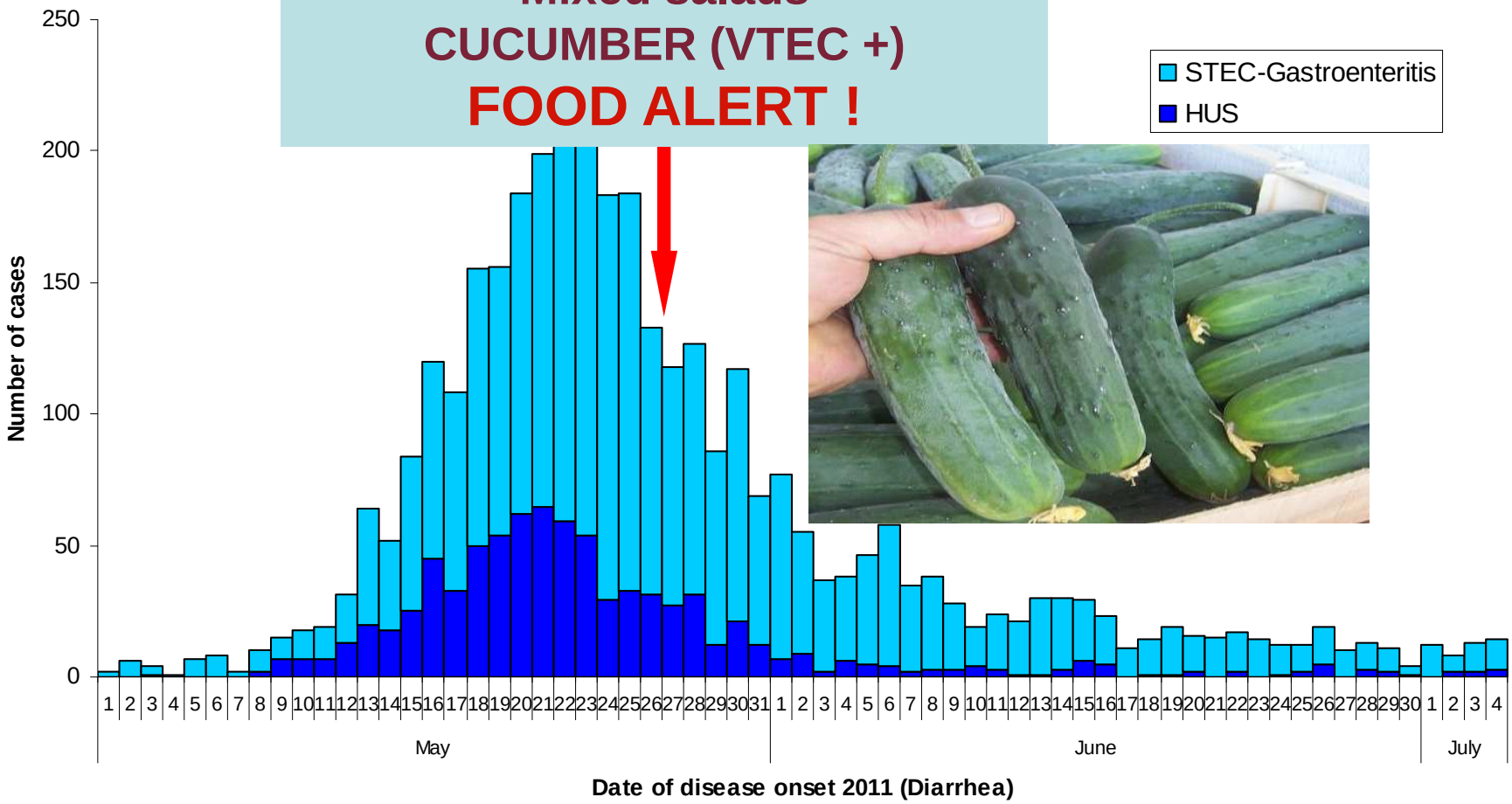
Human origin



ROBERT KOCH INSTITUT

STEC O104:H4 outbreak

26 May
Case control studies:
Mixed salads
CUCUMBER (VTEC +)
FOOD ALERT !



The cucumber lead - 26 May

Do, 22.09.11 | 15°C Wetter | English | Stadtplan | HVV Fahrplan | Schiffe | Mobil | Branchenbuch | Registrieren | Login

MOIN, BEIM STADTPORTAL FÜR HAMBURG

Suchen

hamburg.de

HOTELS & TOURISMUS | KULTUR & TICKETS | JOBS & WOHNEN | ERLEBNIS HAMBURG | SHOPPING & GASTRO | VERKEHR & WIRTSCHAFT | BÜRGER-SERVICE | STADT & STAAT

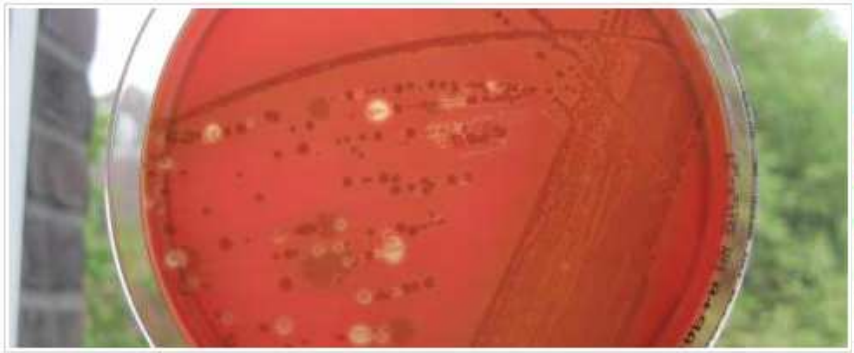
30 MaY

Cucumbers negative for VTEC O104

Ham
iden

66 HUS-Erkrankungen in Hamburg - Verdacht auf O104 im EHEC-Stamm durch HU bestätigt

26.05.2011



Agarmedium mit EHEC-Kolonien (Bild: HU)

DETTE billede er taget den 26. maj på et marked i Hamborg. Agurkerne er spanske. Disse og alle andre agurker fra Spanien er nu fjernet fra markeder og butikshylder. - Foto: MARIUS ROEER/AP

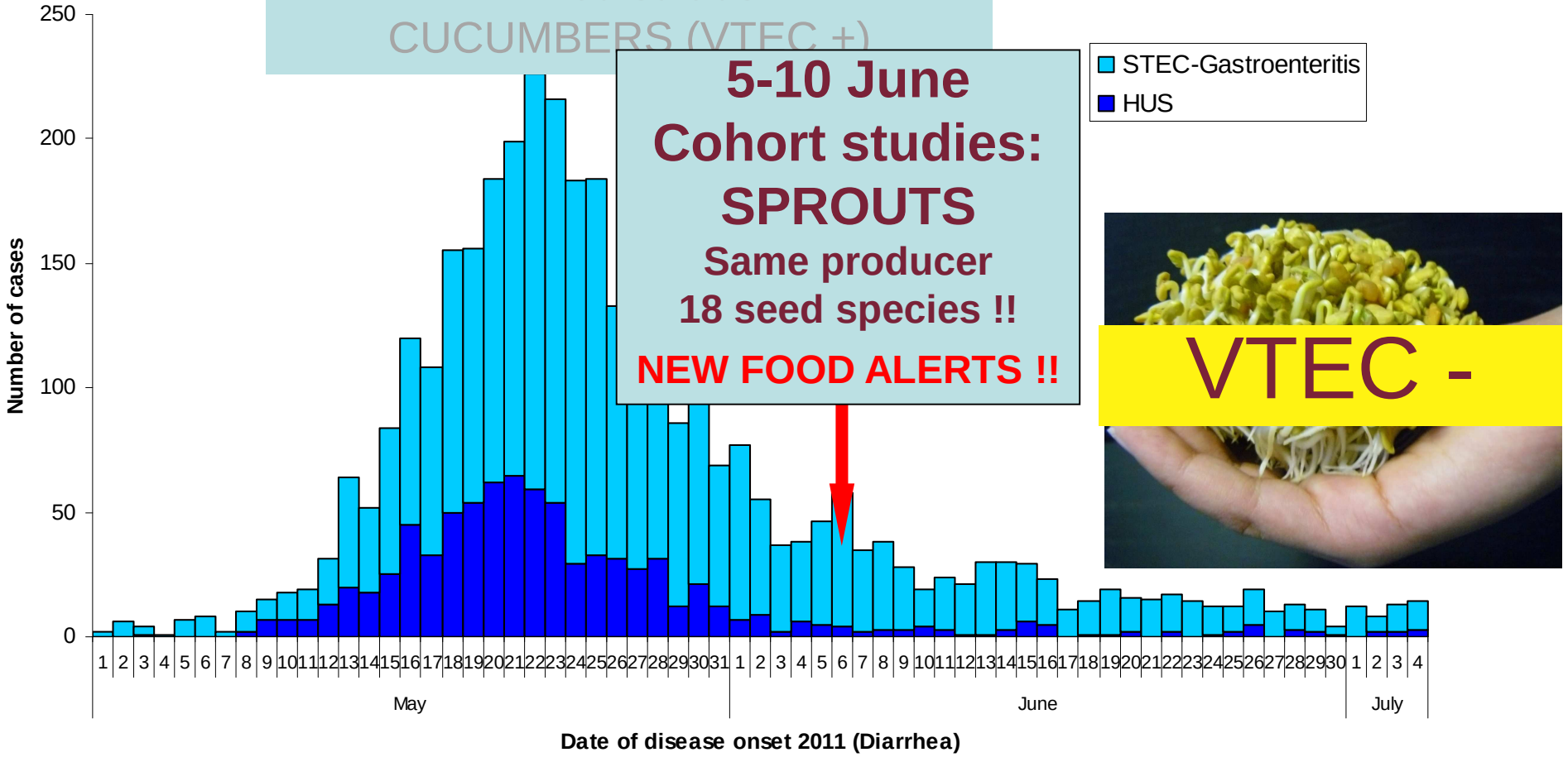

Strasbourg
7 June 2011
clear that
have rush
se
SVENSKA
There was no coordination, no leadership.

ROBERT KOCH INSTITUT

STEC O104:H4 outbreak

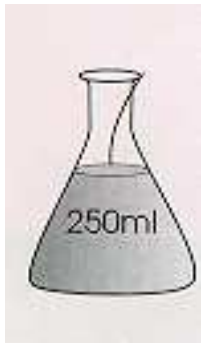
26 May
Case-control studies:
Mixed salads
CUCUMBERS (VTEC +)

5-10 June
Cohort studies:
SPROUTS
Same producer
18 seed species !!
NEW FOOD ALERTS !!



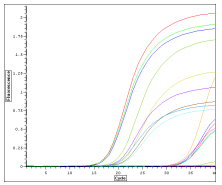
VTEC -

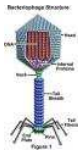
Detection of VTEC O104:H4 in food by ISO/TS 13136



STEP 1

Real Time PCR for *vtx* genes

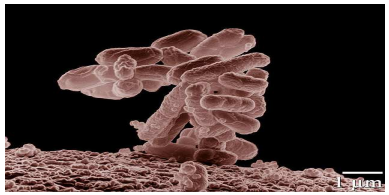




STEP 2

~~Real Time PCR for *eae* gene~~

Attaching/Effacing adhesion



VTEC O104:H4

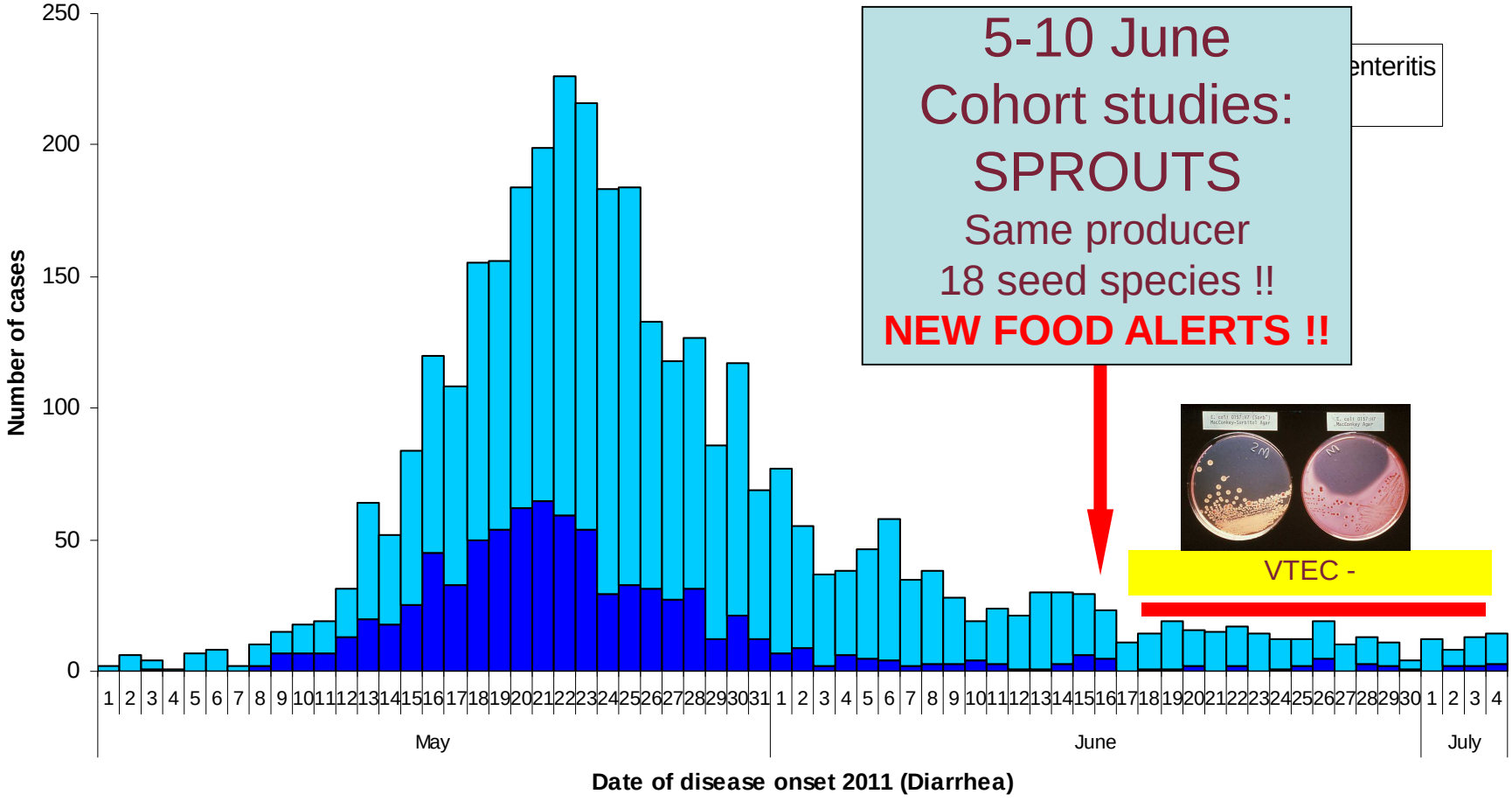
Real Time PCR for O104 and H4 genes

- *wzx*O104 (Bugarel M. et al. 2010)
- *fliCH4 (designed on a GeneBank sequence)*

ROBERT KOCH INSTITUT

STEC O104:H4 outbreak

Epi curve by exposure (8-10 days incubation time)



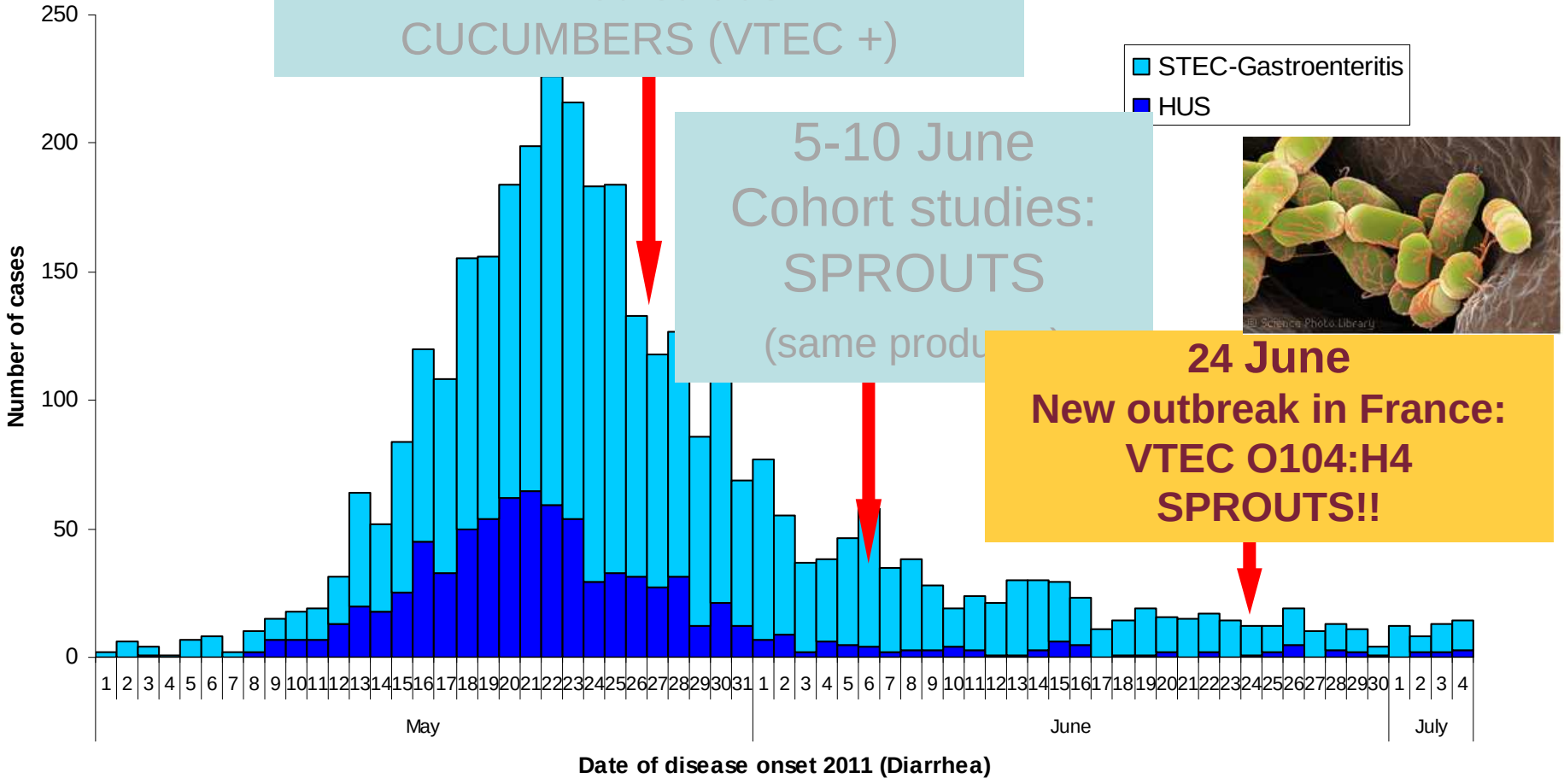
ROBERT KOCH INSTITUT

STEC O104:H4 outbreak

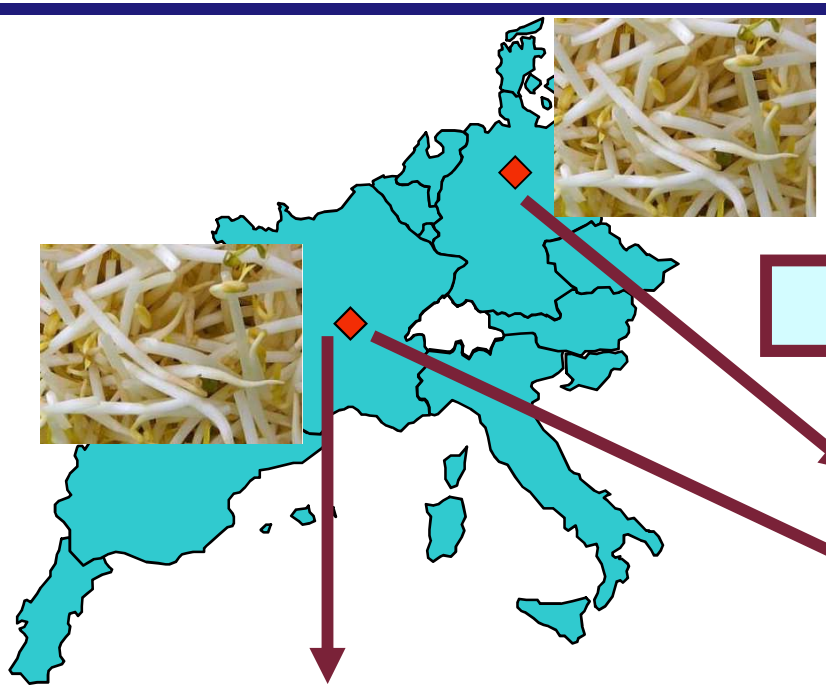
26 May
Case-control studies:
Mixed salads
CUCUMBERS (VTEC +)

5-10 June
Cohort studies:
SPROUTS
(same produ

24 June
New outbreak in France:
VTEC O104:H4
SPROUTS!!



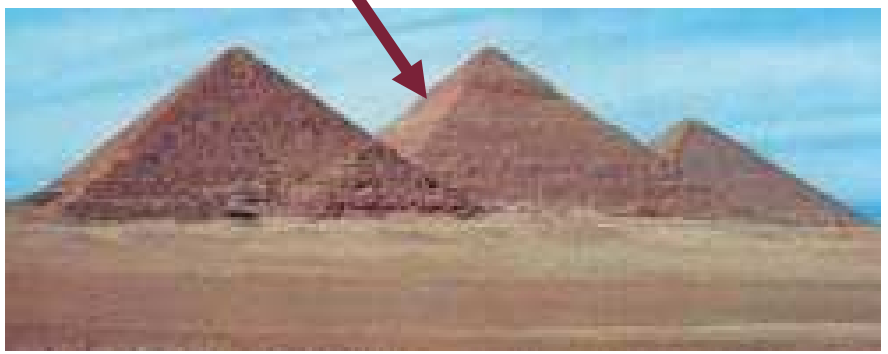
E.Coli O104 outbreak: the role of seeds



German producer associated with the outbreak (18 different species of seeds !)

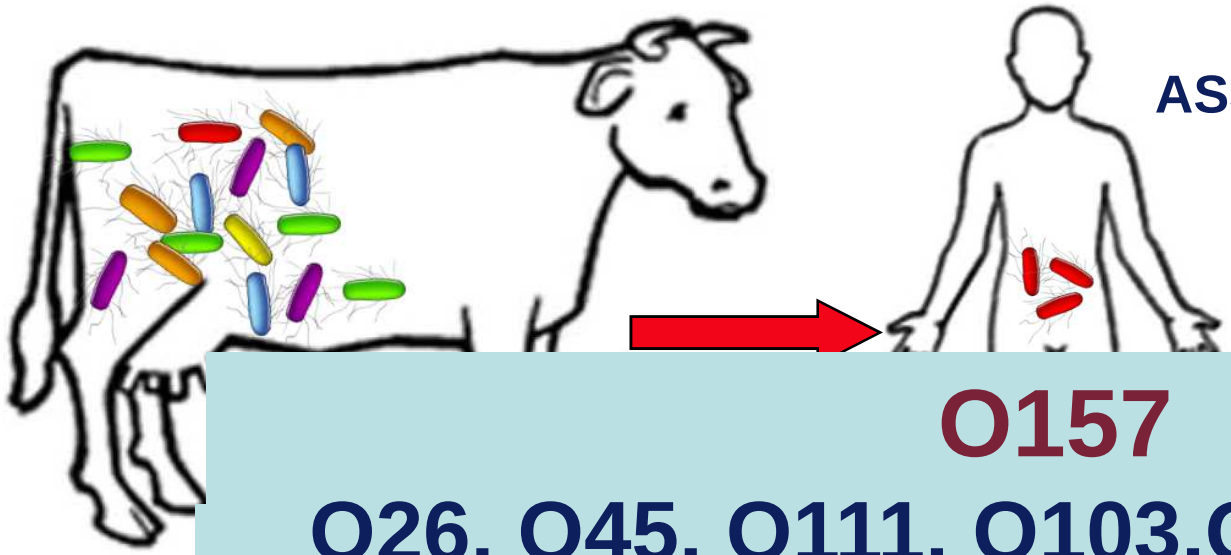
Fenugreek

Tracing Back coordinated by EFSA: **Same lot from Egypt**



HUS outbreak in the French school (3 different species of seeds)

Are all VTEC pathogenic to humans?



VTEC

ASSOCIATED WITH SEVERE DISEASE (BD, HUS)

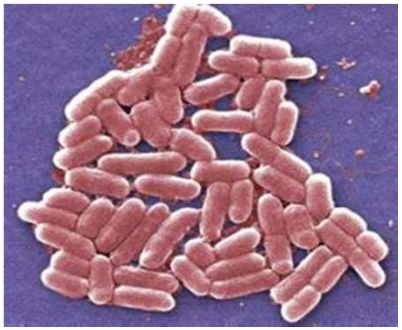
O157

O26, O45, O111, O103,O145, O121, ….

O104 ……and which others ??

>100
in th

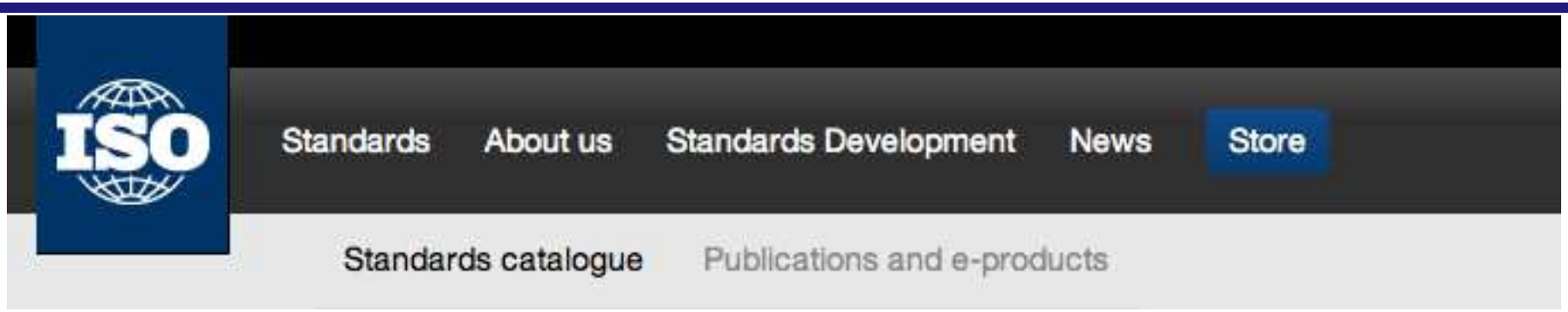
A broader view when testing food for VTEC



VT-producing EAggEC O104:H4!!!!

- ✓ **Some VTEC serogroups may fall outside the top 5-7 and still cause SEVERE disease in humans**
- ✓ **It may be worth identifying *eae*-negative VTEC, at least in particular matrices**
- ✓ **VTEC causing diarrhoea are pathogens!!!**

Detection of VTEC in food



ISO/TS 13136:2012

Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

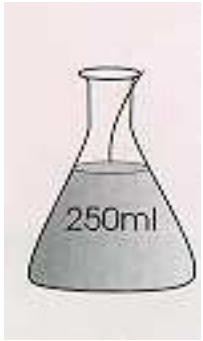
First draft in 2008
Published on 7 November 2012

ISO/TS 13136

Detection of VTEC in food

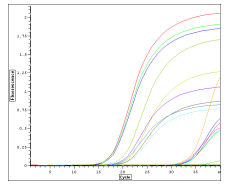
STEP 1 (24h)

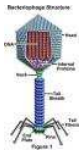
18 h



5 h

Real Time PCR
for *vtx* genes





Negative samples: released

Positive samples: presumptive detection of VTEC go to isolation

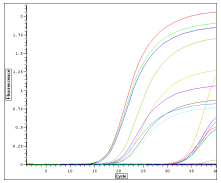
ISO/TS 13136

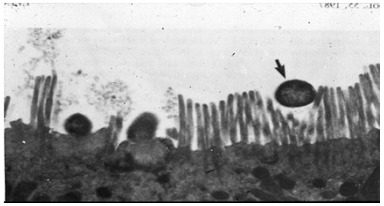
Detection of VTEC in food and identification of the top five serogroups

STEP 2 (2h)

Attaching/Effacing adhesion

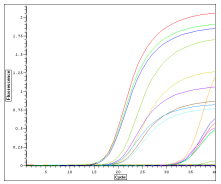
Real Time PCR for *eae* gene

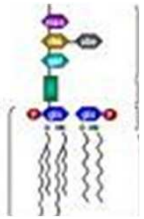




STEP 3 (2h)

Real Time PCR for serogroup-associated genes





Step 2 and 3 are carried out to drive the selection of reagents to ease the isolation of the strain

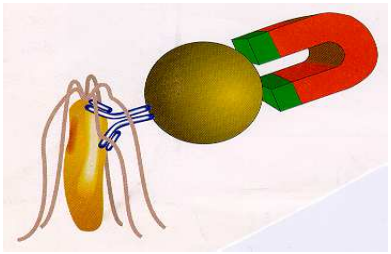
Informative to the risk manager

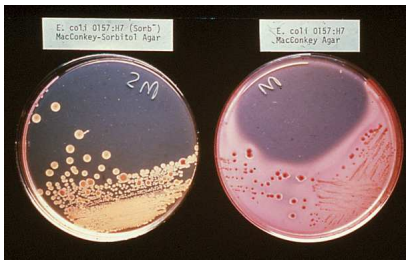
ISO/TS 13136

Isolation of VTEC from food

STEP 4 (18 - 20 h)

O-specific IMS





<u>Isolation</u> of VTEC belonging to one of the serogroups identified in the PCR screening is accomplished by IMS

ISO/TS 13136

Isolation of VTEC from food

Up to 50 colonies screened by PCR for the presence of stx genes

<u>Isolation</u> of VTEC that do not belong to the "top 5" serogroups accomplished by screening colonies from an *E. coli* agar medium (e.g. TBX)

Concluding remarks on methods

- ✓ ISO/TS 13136 published in November 2012
- ✓ ISO/TS 13136 scope has been enlarged (all VTEC)
- ✓ Top 5 serogroups determination is still in the method but all VTEC+ samples must be subjected to isolation
- ✓ A new definition of pathogenic VTEC is under discussion at EFSA (upon request of Austria, supported by Germany)
- ✓ USDA MLG 5B:00 in use since June 2012 but is focused on top 7 serogroups (Beef)

From Methodology to Regulations

- ✓ **USA enforced rules on beef since June 2012**

- ✓ **EU approved microbiologic criteria for VTEC in sprouts in October 2012 (enforced from March 2013)**

Microbiological criteria in EU legistation:

Regulation (EC) 2073/2005

- ✓ **Two types of criteria:**
  - ✓ Food safety criteria (pathogenic microrganisms)
  - ✓ Pprocess hygiene criteria (indicator microrganisms)
- ✓ **Addressed to food business operators**
- ✓ **Official controls: must be consistent with food safety criteria**
- ✓ **Purposes: harmonization !**
  - ✓ same food safety standards in all Member States
  - ✓ same acceptance of risks in all Member States
  - ✓ avoid discussions at intra-EU trade and import.

Regulation (EC) No 2073/2005

VTEC and many other pathogens not included in 2073/2005

(No microbiologic criteria)

WHY ??

- ✓ **If high prevalence in certain food: clearly a risk management decision not to include (too high economic impact, no cost efficient control options available, other priorities, …): e.g. Campylobacter in poultry meat**
- ✓ **If low prevalence (too high sampling costs to detect the pathogen): other approaches considered more appropriate e.g. (certain) VTEC in beef: Process hygiene criterion for Enterobacteriaceae to control fecal contamination**

EU Legislation

Are Member States allowed to withdraw food from the market if there is no food safety criterion in Regulation (EC) No 2073/2005?

- ✓ **YES if in line with Art. 14 of Reg. 178/2002 (food considered as unsafe):**
  - ✓ **Taking into account further processing and labelling (risk profile of the food)**
  - ✓ **Based on a case by case risk assessment by MS**

REGULATION (EC) No 178/2002

Laying down the general principles and requirements of food law, establishing the EFSA, and laying down procedures in matters of food safety

SECTION 4 - GENERAL REQUIREMENTS OF FOOD LAW

Article 14 – Food safety requirements

1. **Food shall not be placed on the market if it is unsafe.**
2. **Food shall be deemed to be unsafe if it is considered to be:**
   - a) **injurious to health;**
   - b) **unfit for human consumption.**

Specific new rules on sprouts:

VTEC food safety criterion

- ✓ *October 2011: EFSA opinion*
- ✓ *November 2011-January 2012: internal reflections*
- ✓ *February-June 2012: Consultations/discussions/ WG meetings with MS and private sector.*
- ✓ *July 2012: Technical agreement at Standing Committee*
- ✓ *August-September 2012: WTO consultation*
- ✓ *15 October 2012: final opinion at standing committee*
- ✓ *By end of November 2012: translations*
- ✓ *Until end of January 2013: right of scrutiny of Council and European Parliament*
- ✓ *Beginning of February 2013: publication in Official Journal*
- ✓ *Applicable from 1 March 2013.*

Specific new rules on sprouts

- ***Traceability of sprouts and seeds intended for sprouting***
- ***Import certification***
- ***Approval of sprouts establishments***
- ***Specific guidance on hygiene requirements***
- ***VTEC food safety criterion***

WHICH FUTURE ??

Will we have a food safety criterion for (certain pathogenic) VTEC in beef?

- ✓ First step: EFSA opinion. Pathogenic VTEC clearly defined?
- ✓ Second step: demonstration if such criteria would result in a cost efficient further reduction of human infections?
- ✓ Third step: support of MS by qualified majority needed

Thank you for attention !

The EU-RL VTEC folks