

The role of the EU Reference Laboratory for *E.coli* in the VTEC O104:H4 outbreak



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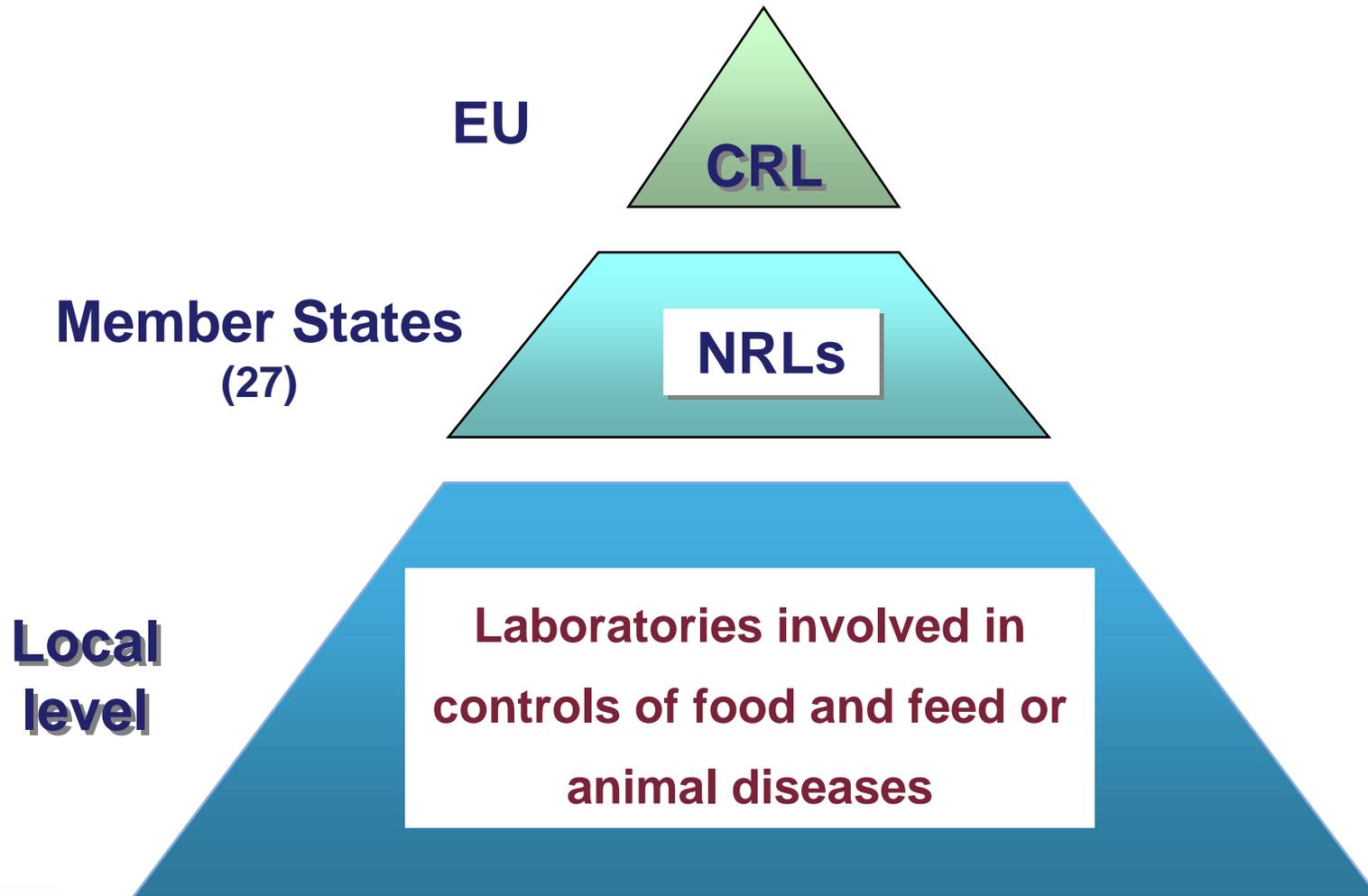
www.iss.it/vtec

www.iss.it/seu

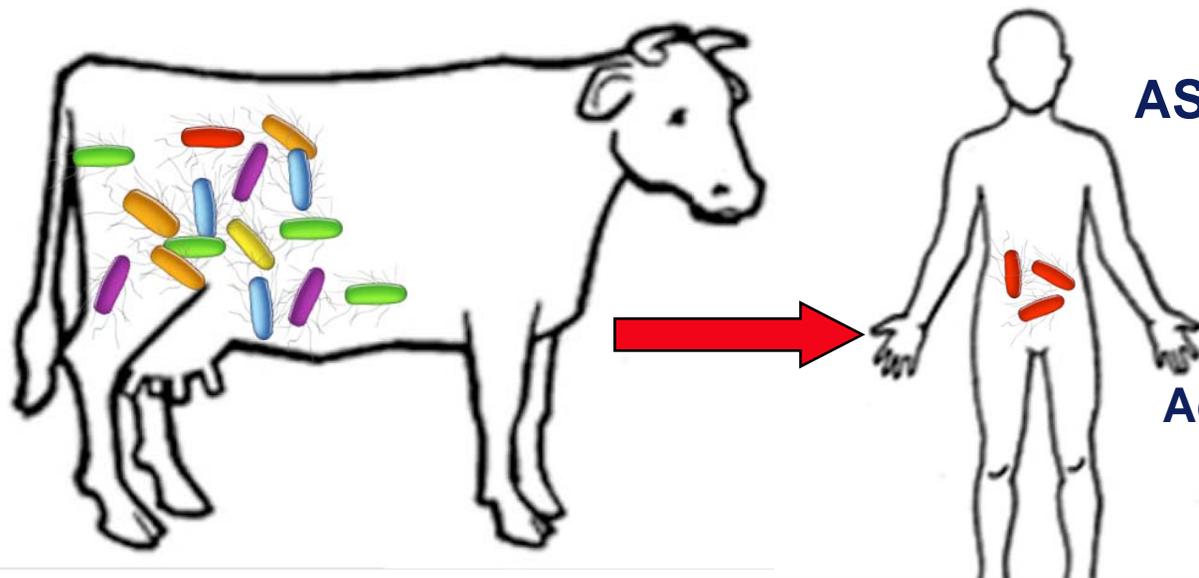
Functions and duties of EU-RLs (882/2004, Art. 32)

- ✓ To provide NRLs with analytical reference methods
- ✓ To produce and distribute reference materials
- ✓ To organise comparative testing (Proficiency Tests)
- ✓ To conduct training for NRLs, (annual workshop, courses, visits to CRL)
- ✓ To provide scientific and technical assistance to the Commission

The Network of EU Reference Laboratories (Reg. EC 882/2004)



Main VTEC serogroups pathogenic to humans



VTEC

ASSOCIATED WITH SEVERE DISEASE (BD, HUS)

Additional virulence properties
Attaching/Effacing adhesion



VTEC

>100 serogroups
in the reservoir

O157
O26, O111, O103, O145,



Detection of pathogenic non-O157 VTEC in food



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ISO/DTS 13136



Microbiology of food and animal feeding stuffs – 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) belonging to O157, O111, O26, O103 and O145 serogroups

General information

Number of Pages:

Edition: 1 (Monolingual)

ICS: [07.100.30](#)

Status:  Under development

Stage: [30.99](#) (2011-07-14)

TC/SC: [TC 34/SC 9](#)



Aimed at the “top 5” serogroups mostly associated with human disease

O157, O26, O111, O103, O145

STEP 1

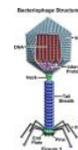
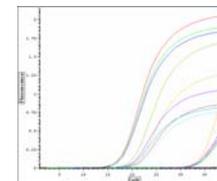


18 h



5 h

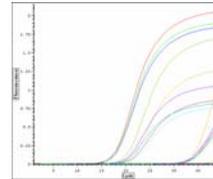
Real Time PCR for *vtx* genes



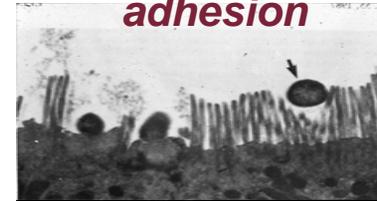
Negative samples are released in 24 h

STEP 2 (2h)

Real Time PCR for *eae* gene

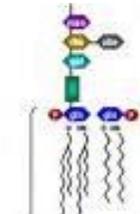
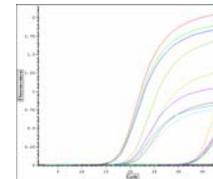


*Attaching/Effacing
adhesion*



STEP 3 (2h)

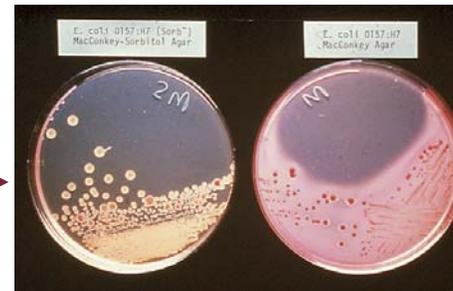
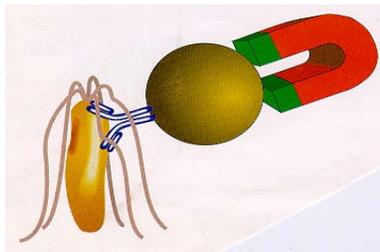
Real Time PCR for the “top 5” serogroup-associated genes



Presumptive detection of pathogenic VTEC in 30 h

STEP 4 (18 - 20 h)

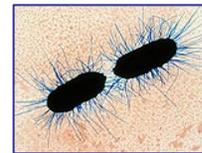
O-specific IMS



Isolation of pathogenic VTEC in 48 h

Proficiency tests organized by the EU-RL VTEC

- **2008: Bacterial strains**



- **2009: Carcass swabs**



- **2010: Milk**



- **2011: Vegetables (spinach)**



ISO/DTS 13136 for VTEC in food

Recommended by EFSA for the detection
of VTEC in food and animal samples



European Food Safety Authority

EFSA Journal 2009; 7(11):1366

SCIENTIFIC REPORT OF EFSA

**Technical specifications for the monitoring and reporting of
verotoxigenic *Escherichia coli* (VTEC) on animals and food
(VTEC surveys on animals and food)¹**

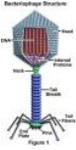
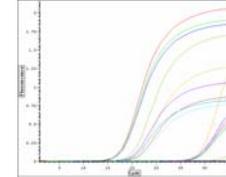
European Food Safety Authority^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

What happened with VTEC O104:H4



Real Time PCR
for *vtx* genes



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

Attaching/Effacing
adhesion



VTEC O104:H4

Real Time PCR for O104 and H4 genes

- *wzxO104* (Bugarel M. et al. 2010)
- *fliCH4* (designed on a GeneBank sequence)

Detection of VTEC O104 in food

The time line



- ✓ **Time 0** **Sample received by the lab**
- ✓ **24 h** **Sample negative for VTEC released**
- ✓ **27-29 h** **Presumptive positivity for VTEC O104**
- ✓ **47-53 h** **Colonies are available for confirmation**
- ✓ **54-56 h** **Presumptive positivity is confirmed or rejected**

Detection of VTEC O104 in food

www.iss.it/vtec



EU Reference Laboratory for *E.coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità



Detection and identification of Verocytotoxin-producing *Escherichia coli*
(VTEC) O104:H4 in food by Real Time PCR

Laboratory procedure



EU-RL involvement in the outbreak

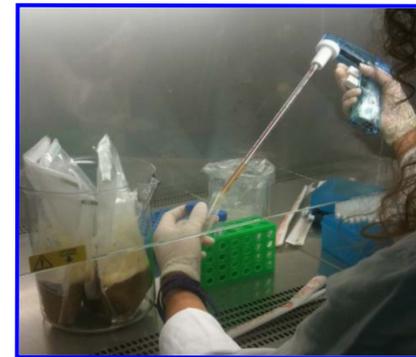


Methods



- ✓ **May 24** Report of the outbreak in ProMed
- ✓ **May 25** Alert to NRLs
- ✓ **May 27** 1st Draft method for VTEC O104 sent to NRLs
- ✓ **June 1** Outbreak strain received from Robert Koch Inst.
- ✓ **June 2** Method evaluated, revised and sent to NRLs
- ✓ **June 3-6** Reference DNA from VTEC O104 sent to 14
NRLs as positive control
- ✓ **June 9-15** Reference DNA sent to other 28 Labs

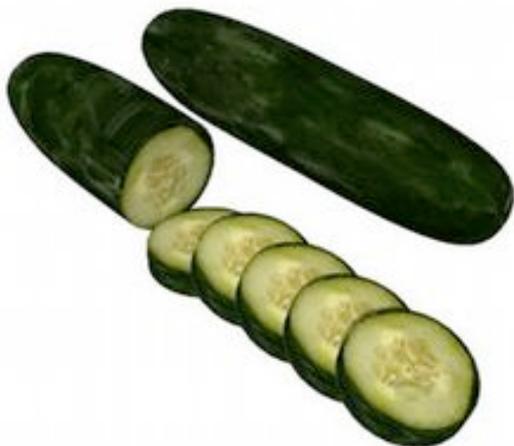
EU-RL involvement in the outbreak



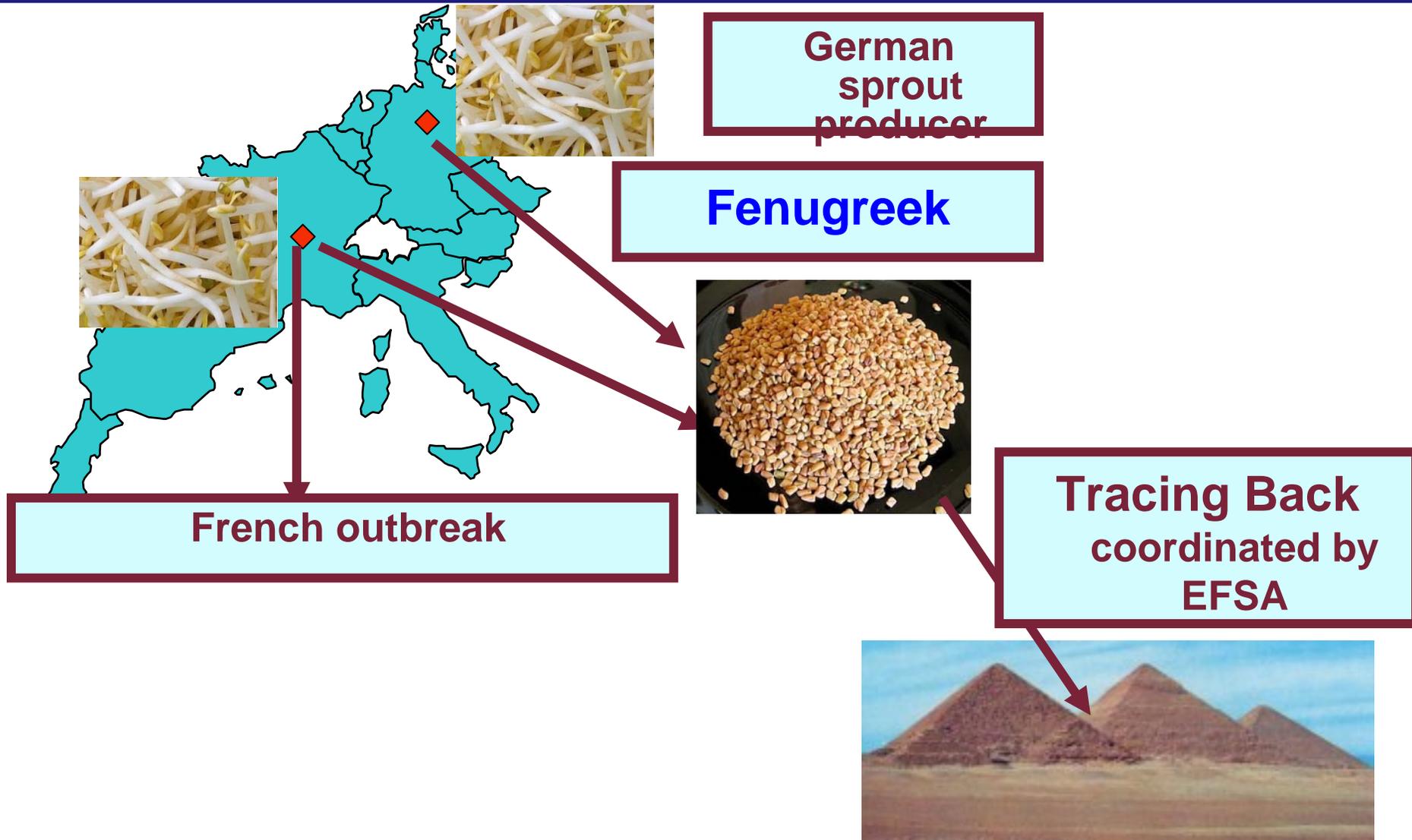
Analytical activities

✓ **May 31** Samples (cucumber) from Hamburg to EU-RL

✓ **June 2** Results sent to DG SANCO



The role of sprouts and seeds in the VTEC O104:H4 outbreak



EU-RL involvement in the outbreak



Analytical activities



- ✓ **June 14** Annex for seed analysis sent to the NRLs
- ✓ **July 8-15** Inter-laboratory study on the detection of VTEC in naturally contaminated seeds (8 NRLs)
- ✓ **September** Examination of seed samples from Egypt



Inter-laboratory study on naturally contaminated seeds

Three samples of beet seed lots sent to 8 Labs (July 2011)

Sample A *	Sample B	Sample c
Real Time PCR results expected		
<i>vtx2+</i> , <i>ehly+</i> <i>vtx1-</i> , <i>eae-</i>	Negative	Negative

* VTEC O74, *vtx2+*, *ehly+* and *vtx1-*; *eae-*
Isolated from the seed lot

Inter-laboratory study on naturally contaminated seeds

Sample (Expected results)	No. Positive / No. of Labs			
	<i>vtx1</i>	<i>vtx2</i>	<i>eae</i>	<i>e-hly</i>
A (<i>vtx2+</i> , <i>e-hly+</i>)	0/8	0/8	2/6*	0/4
B (<i>negative</i>)	0/8	1/8	0/6	0/4
C (<i>negative</i>)	0/8	0/8	0/6	0/4

* The *eae* + strain isolated by both Labs !!!!

Studies on naturally contaminated seeds

- ✓ Another lot of beet seeds
- ✓ VTEC O74, *vtx2+* isolated on 12 June 2011
- ✓ Testing of other 8 aliquots (50g) on 14 July 2011

Date of testing	Real Time PCR for vtx2 (Ct)								
	Aliquots								
	1	2	3	4	5	6	7	8	9
12 June 2011	+								
	(Ct=23)								
14 July 2011		+	-	-	+	-	-	-	-
		(Ct=36)			(Ct=37)				

Studies on naturally contaminated seeds

Conflicting results: **why** ????

- ✓ **Low level of contamination (*but strains isolated*)**
- ✓ **Death of bacteria (*but short interval*)**
- ✓ **Not homogeneous contamination of lots (*most likely*)**

EU-RL involvement in the outbreak

Support to EC structures

- ✓ Technical advice to DG SANCO and participation in the “task force” activities and conferences
- ✓ Participation in the *Food and Veterinary Office (FVO)* inspection mission to Egypt
- ✓ Participation in the ECDC/EFSA working groups:
 - ✓ *Joint fast-track assessment of the consumer exposure*
 - ✓ *Traceback exercise of seeds*
 - ✓ *Joint EFSA/ECDC assessment (“Taking stock”)*
 - ✓ *EFSA working group on MRA in seeds and sprouted seeds*

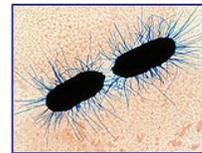
VTEC O104 outbreak: what can we learn ?

Facing food safety crisis

✓ Preparedness

Proficiency tests organized by the EU-RL VTEC

- **2008: Bacterial strains**



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- **2010: Milk**



- **2011: Vegetables (spinach)**



The EU Health and Consumer Commissioner John Dalli visits the EU-RL VTEC



ISS, 9 June 2011

VTEC O104 outbreak: what can we learn ?

Facing food safety crisis

- ✓ Preparedness
- ✓ Intersectoral collaboration

VTEC O104 outbreak: what can we learn ?



Intersectoral collaboration

- ✓ In the DG SANCO Task Force
- ✓ In the ECDC-EFSA joint working groups



VTEC O104 outbreak: what can we learn ?



Intersectoral collaboration



- ✓ The Liaison with the ECDC reference laboratory for VTEC infections (the WHO International *Escherichia* and *Klebsiella* Centre of the Statens Serum Institut, Copenhagen):
 - ✓ *Harmonization of identification and typing methods*
 - ✓ *Continuous exchange of information and strains*

