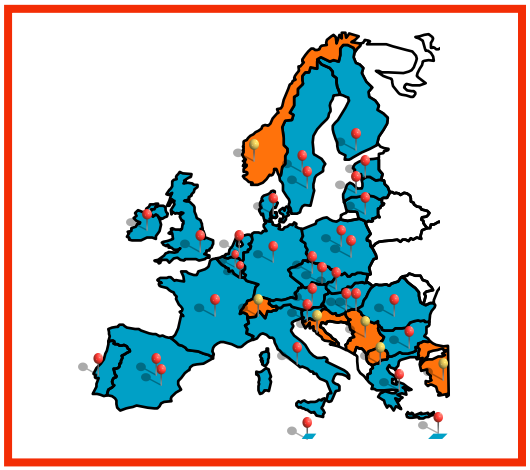
11th inter-laboratory study (PT11) on the identification and typing of VTEC and other pathogenic *E. coli*



OBJECTIVES (1)

- ✓ To further train the NRLs in the identification and typing of VTEC (virulence genes and serogroup)
- ✓ To further train the NRLs in the sub-typing of *vtx* genes
- ✓ To assess the capacity of the NRLs to identify different groups of pathogenic *E. coli* (EPEC, EAggEC, ETEC, EIEC)
- ✓ To carry out a 2nd round of EQA for PFGE, in view of the collection, of molecular typing data on VTEC by EFSA (evaluation still ongoing)

PARTICIPANTS

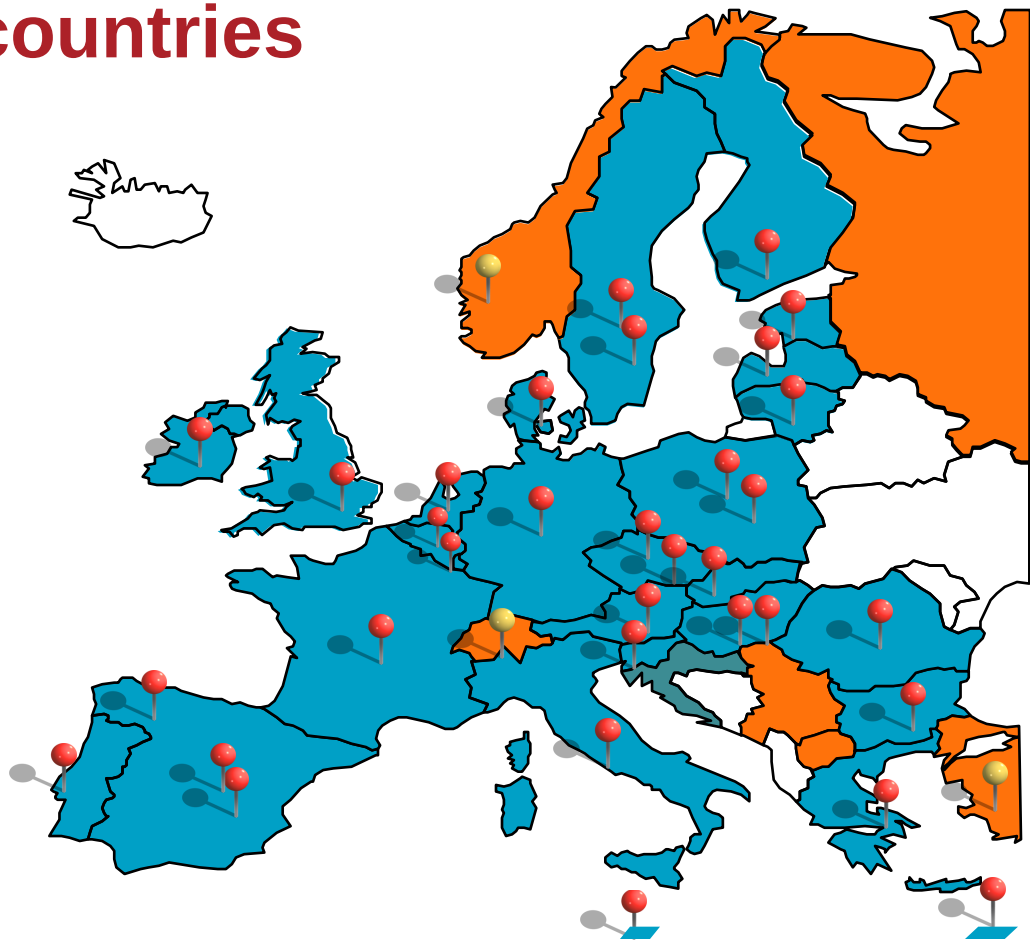
33 NRLs representing
ALL the 28 EU countries

+ the NRLs of

Norway

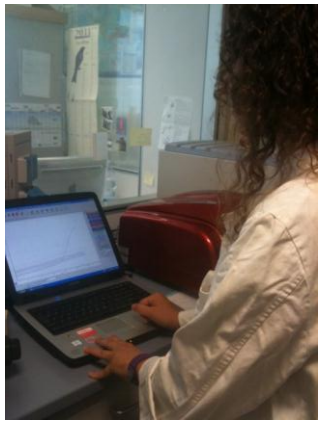
Switzerland

Turkey



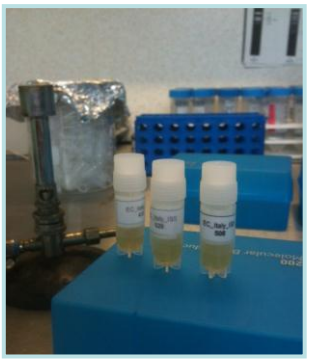
METHODS (1)

- ✓ **Real Time PCR SOPs for the detection of following virulence genes:**
  - ✓ ***vtx1* group and *vtx2* group for VTEC**
  - ✓ ***eae* for EPEC**
  - ✓ ***aaiC* and *aggR* for EAggEC**
  - ✓ ***lt*, *sth*, and *stp* for ETEC**
  - ✓ ***ipaH* for EIEC**
- ✓ **Available at the EU-RL web site and ready to use for the detection in food samples**



METHODS (2)

- ✓ 6 test strains and the needed control strains
- ✓ Samples labelled with randomly generated numerical codes
- ✓ Results submitted on-line via the web site



Characteristics of the 6 *E. coli* test strains

Strain	Patho group	Sero group	*vtx1*	*vtx2*	*eae* gene	*aggR* gene	*aaiC* gene	*ipah* gene	*sth* gene	*stp* gene	*lt* gene
1	ETEC	-	-	-	-	-	-	-	-	-	+
2	VTEC	O113	+ *vtx1c*	+ *vtx2b*	-	-	-	-	-	-	-
3	EIEC	-	-	-	-	-	-	+	-	-	-
4	VTEC	O121	-	+ *vtx2a*	+	-	-	-	-	-	-
5	EPEC	O26	-	-	+	-	-	-	-	-	-
6	EAggEC	-	-	-	-	+	+	-	-	-	-

RESULTS

At the moment, results were sent by

34 /36 NRLs

RESULTS: strain 1 (ETEC)

NRL	Detection of genes in Sample 1 (ETEC):								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	-	-	-	-	-	-	+
L105									
L151									
L162				ND	ND	ND	ND	ND	ND
L163									
L166									
L172									
L187									
L209									
L227									
L268									
L318									
L376									
L416									
L430									
L460									
L518									
L542									
L549									
L550									
L553				ND	ND	ND			
L566									
L607									
L614									
L623									
L706									
L709									
L728									
L732									
L751									
L758									
L920									
L950									
L962									
L975									

RESULTS: strain 2 (VTEC O113)

NRL	Detection of genes in Sample 2 (VTEC):									
	vtx1	*vtx2*	*eae*	Ogroup	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	+	+	-	O113	-	-	-	-	-	-
L105				-						
L151				O125ac						
L162					ND	ND	ND	ND	ND	ND
L163										
L166										
L172										
L187										
L209				-						
L227										
L268										
L318										
L376										
L416										
L430										
L460										
L518										
L542										
L549										
L550										
L553					ND	ND	ND			
L566										
L607										
L614										
L623										
L706					ND	ND	ND	ND	ND	ND
L709										
L728										
L732										
L751										
L758										
L920										
L950										
L962										
L975										

RESULTS: strain 3 (EIEC)

NRL	Detection of genes in Sample 3 (EIEC):								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	-	-	-	+	-	-	-
L105									
L151									
L162				ND	ND	ND	ND	ND	ND
L163									
L166									
L172									
L187									
L209									
L227									
L268									
L318									
L376									
L416									
L430									
L460									
L518									
L542									
L549									
L550									
L553				ND	ND	ND			
L566									
L607									
L614									
L623									
L706							ND	ND	ND
L709									
L728									
L732									
L751									
L758									
L920									
L950									
L962									
L975		+							

RESULTS: strain 4 (VTEC O121)

NRL	Detection of genes in Sample 4 (VTEC):									
	vtx1	*vtx2*	*eae*	Ogroup	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	+	+	O121	-	-	-	-	-	-
L105										
L151										
L162					ND	ND	ND	ND	ND	ND
L163										
L166										
L172										
L187										
L209				-						
L227										
L268										
L318										
L376										
L416										
L430										
L460										
L518										
L542										
L549										
L550										
L553					ND	ND	ND			
L566										
L607										
L614										
L623										
L706					ND	ND	ND	ND	ND	ND
L709										
L728	+									
L732										
L751										
L758										
L920										
L950										
L962										
L975										

RESULTS: strain 5 (EPEC O26)

NRL	Detection of genes in Sample 5 (EPEC):									
	vtx1	*vtx2*	*eae*	Ogroup	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	+	O26	-	-	-	-	-	-
L105										
L151										
L162					ND	ND	ND	ND	ND	ND
L163			-							
L166										
L172										
L187										
L209				-						
L227										
L268										
L318										
L376										
L416										
L430										
L460										
L518										
L542										
L549										
L550										
L553					ND	ND	ND			+
L566										
L607										
L614										
L623										
L706					ND	ND	ND	ND	ND	ND
L709										
L728										
L732										
L751										
L758										
L920					+					
L950										
L962										
L975										

RESULTS: strain 6 (EAggEC)

NRL	Detection of genes in Sample 6 (EAggEC):								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
True value	-	-	-	+	+	-	-	-	-
L105									
L151									
L162				ND	ND	ND	ND	ND	ND
L163									
L166									
L172									
L187									
L209					ND				
L227									
L268									
L318									
L376									
L416									
L430									
L460				-	-				
L518									
L542									
L549									
L550									
L553				ND	ND	ND			
L566									
L607									
L614									
L623									
L706						ND	ND	ND	ND
L709									
L728									
L732									
L751									
L758									
L920									
L950									
L962									
L975									

RESULTS: detection of virulence genes

NRL	Detection of virulence genes in all the 6 test strains:								
	vtx1	*vtx2*	*eae*	*aggR*	*aaiC*	*ipah*	*sth*	*stp*	*lt*
L105									
L151									
L162				ND	ND	ND	ND	ND	ND
L163			1						
L166									
L172									
L187									
L209									
L227									
L268									
L318									
L376									
L416									
L430									
L460				1	1				
L518									
L542									
L549									
L550									
L553				ND	ND	ND			1
L566									
L607									
L614									
L623									
L706									
L709									
L728	1								
L732									
L751									
L758									
L920				1					
L950									
L962									
L975		1							

RESULTS: detection of VTEC virulence genes

- ✓ **31 NRLs (91%) identified correctly the presence/absence of VTEC target genes in the test strains**

- ✓ **3 NRLs provided a total of 3 incorrect results**
  - ✓ **1 false positive for *vtx1***
  - ✓ **1 false positivefor *vtx2***
  - ✓ **1 false negative for *eae***

RESULTS: detection of virulence genes of other patho-groups

- ✓ **32 NRLs performed the detection of all the genes**
- ✓ **29 NRLs (91%) identified correctly the presence/absence of target genes in all the test strains**
- ✓ **3 NRLs provided a total of 4 incorrect results**
  - ✓ **1 false positive for *aggR***
  - ✓ **1 false positive for *lt***
  - ✓ **1 false negative for *aggR***
  - ✓ **1 false negative for *aaic***

RESULTS - O serogroup identification

Correct identification of the O serogroup:

O26: 33 /34 NRLs (97%)

O121: 33/34 NRLs (97%)

O113: 31/34 NRLs (91%)

CONCLUDING REMARKS

- ✓ 36 NRLs (28 EU MS) participated in the study, 34 sent results
- ✓ Good results in the detection of the VTEC target genes and serogroup identification
- ✓ Good results for the detection of the “new” target genes of ETEC, EIEC and EAggEC

Better preparedness toward pathogenic *E.coli* other than VTEC