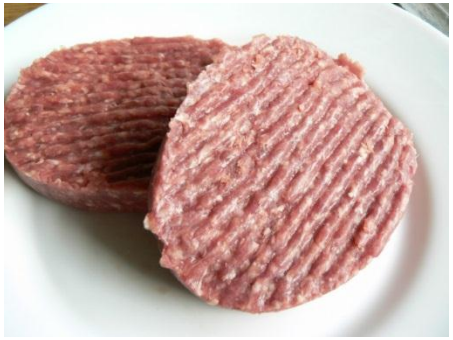

Prevalence of the 7 major serogroups of enterohemorrhagic *Escherichia coli* (EHEC) in fresh minced beef in France: A novel real-time PCR strategy for their early detection in food

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7th annual workshop of the NRL for *E. coli* in the EU
Rome, 8th -9th November 2012

1- Lyon University, VetAgro Sup, LMAP, French National Reference Laboratory for E. coli, FRANCE

2- French Food Safety Agency (ANSES)-Laboratoire de Sécurité des aliments, FRANCE

Introduction

Precise virulent profiles of EHEC strains are still unknown.

2 approaches to defining EHEC strains outside a clinical context in humans:

Epidemiological criteria

Identify serotypes more frequent and more associated with outbreaks and severe disease (Karmali *et al*., 2003)

O157:H7, O26:H11, O103:H2, O111:H8, O145:H28, O121:H19, O45:H2
and non motile derivates

Top 5

Top 7

Molecular criteria

On the basis of molecular markers associated with pathogenicity

Association of *eae* variants with specific EHEC serotypes
Association of genomic O islands with major EHEC strains (notably OI122)

Bugarel *et al.,* 2010, Appl. Environ. Microbiol. (76) 1:203-11; Coombes *et al*., 2008. Appl. Env. Microbiol. (74) 2153-60; Karmali *et al.,* 2003, J. Clin. Microbiol. (41) 4930-40; Immamovic *et al.,* 2010, Infect. Immun. (78) 11: 4697-704.

Introduction

Identification of the major typical EHEC strains

	Major EHEC	Serogroups maker	*stx* genes	LEE marker	*eae* variants	OI 122 marker
Top 5	EHEC O157:H7/H-	*rfbE*-O157	*stx1* or *stx2*	*eae*	γ	*nleB*
	EHEC O26:H11/H-	*wzx*-O26	*stx1* or *stx2*	*eae*	β	*nleB*
	EHEC O103:H2/H-	*wzx*-O103	*stx1* or *stx2*	*eae*	ε	*nleB*
	EHEC O111:H8/H-	*wbdI*-O111	*stx1* or *stx2*	*eae*	θ	*nleB*
	EHEC O145:H28/H-	*ihp1*-O145	*stx1* or *stx2*	eae	γ	*nleB*
Top 7	EHEC O121:H19/H-	*wzx*-O121	*stx1* or *stx2*	*eae*	ε	*nleB*
	EHEC O45:H2/H-	*wzx*-O45	*stx1* or *stx2*	*eae*	ε or β	*nleB*

ISO TS 13136 and MLG 5B

? ?

New tools for EHEC screening in meat?

Bugarel *et al.,* 2010, Int. J. Food. Microbiol. (142) 318- 29 ; Madic *et al.,* 2011, Appl. Environ. Microbiol. (77) 6:2035-41; Nielsen *et al.,* 2003, J. Clin. Microbiol. (41) 7 :2884-93;Perelle *et al.,* 2004, Mol. Cell Probes (18) 185-92

Objectives

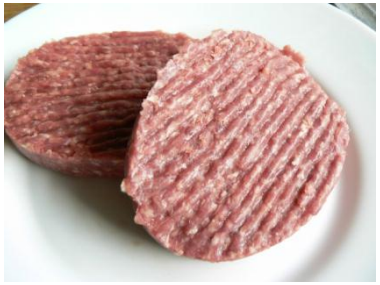
with permission of Caprioli A.

- **Evaluate the prevalence of the top 7 EHEC in fresh minced beef samples collected in France**
- **Propose a novel detection strategy for their early detection in minced meat.**

Materials and methods

Food samples

2476 fresh minced beef samples



collected in supermarkets of 92 French departments

from March to December 2010

ORDRE DE SERVICE D'INSPECTION

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RÉPUBLIQUE FRANÇAISE

MINISTERE DE L'AGRICULTURE, DE L'ALIMENTATION, DE LA PÊCHE
DE LA RURALITE ET DE L'AMENAGEMENT DU TERRITOIRE

Materials and methods

Food PCR detection

Test portion 25g

Enrichment
in 225 ml BPW 37C 18/24h

Test portion (1ml)
DNA extraction purification

ISO TS 13136
MLG 5B

RT-PCR detection
of *stx* and *eae* genes

GD1

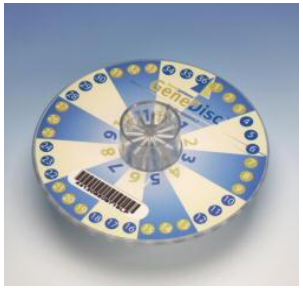
stx/eae +

RT-PCR detection
of top 7 associated genes

GD2

stx/eae/top7 +

Isolation



New approach

RT-PCR detection
of *stx* and *eae* genes

New GD1

stx/eae +

RT-PCR detection
of *nleB* genes

stx/eae/nleB +

RT-PCR detection
of top 7 associated genes

New GD2

stx/eae/nleB/top7 +

RT-PCR detection
of top 7 associated *eae* variants

stx/eae/nleB/top7
/eae variants +

Isolation

Materials and methods

Isolation and characterization procedure

ISO TS 13136

Serogroup specific enrichment or direct plating (O121; O45)

↓

Enrichment broth streaked on to suitable solid media (3 per serogroup)

↓

Incubation for 18h to 24h at 37C

↓

Selection of 50 colonies with *E. coli* morphology

↓

PCR detection of EHEC virulence markers and serotypes-associated genes

Results and discussion

Top 5 prevalence: 6/2476 (0,2 % CI_{95} [0,09%- 0,5%])

Results and discussion

Top 5 prevalence: 6/2476 (0,2 % CI_{95} [0,09%- 0,5%])

- **Low prevalence**
- **In accordance with previous results observed in France**

Year	Type of minced beef	Top 5 prevalence
2007	Frozen (production)	**0,3%** (11/3605) CI_{95} [0,2 – 0,5%]
2008	Frozen trims (production)	**0,4%** (15/4000) CI_{95} [0,2 – 0,6%]
2009	Fresh (at retail)	**0,1%** (2/1527) CI_{95} [0,04 – 0,5%]

Top 7 prevalence: 7/2476 (0,3 % CI_{95} [0,1%- 0,6%])

- **First data in French minced beef**
- **Low prevalence (comparison with other areas?** USA: 0,2% CI_{95} [0,05 – 0,3%] (6/4133) (Bosilevac and Koohmaraie, 2011))

Results and discussion

Top 5 screening

N = 2476 samples (25g)

Markers/strains detected	Nb of positive samples
stx+ *eae*+	109
stx+ *eae*+ top 5+	79
EHEC strains isolated (top 5)	6

ISO TS 13136

4,4%

3,2%

0,2%

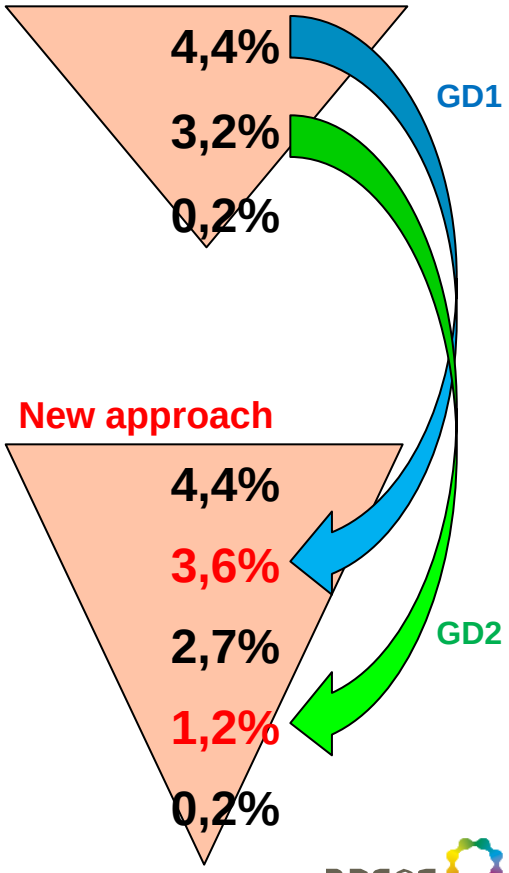
Results and discussion

Top 5 screening

N = 2476 samples (25g)

Markers/strains detected	Nb of positive samples
stx+ eae+	109
stx+ eae+ top 5+	79
EHEC strains isolated (top 5)	6

Markers/strains detected	Nb of positive samples
stx+ eae+	109
stx+ eae+ nleB+	**88**
stx+ eae+ nleB+ top 5+	67
***stx+ eae+ nleB+* top 5*leae* assoc.+**	**29**
EHEC strains isolated (top 5)	6



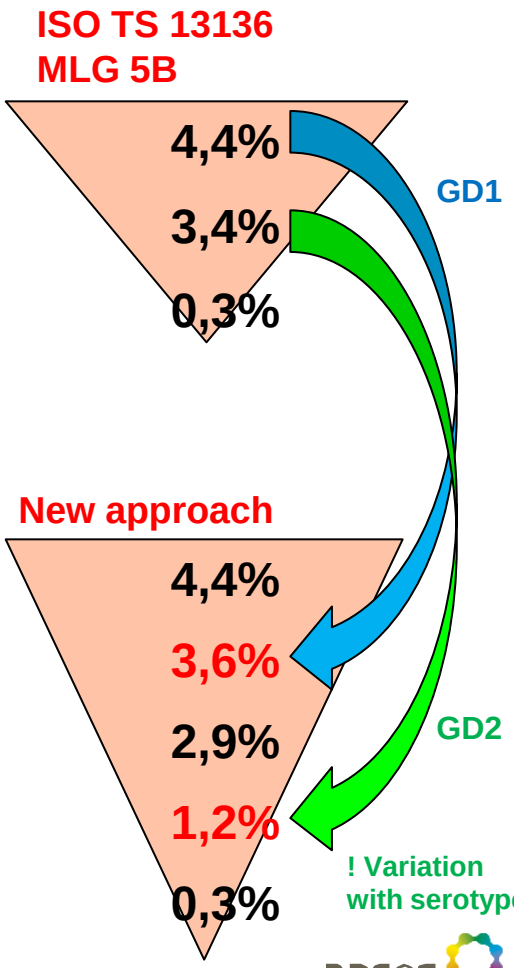
Results and discussion

Top 7 screening

N = 2476 samples (25g)

Markers/strains detected	Nb of positive samples
stx+ eae+	109
stx+ eae+ top 7+	84
EHEC strains isolated (top 7)	7

Markers/strains detected	Nb of positive samples
stx+ eae+	109
stx+ eae+ nleB+	**88**
stx+ eae+ nleB+ top 7+	71
***stx+ eae+ nleB+* top 7/*eae* assoc.+**	**29**
EHEC strains isolated (top 7)	7



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Results and discussion

Characteristics of the 7 EHEC strains isolated

	Serotype	*stx* genes	*eae* variants	OI 122 marker	Nb of strains isolated
Top 5	EHEC O157:[H7]	*stx2*	*eae γ*	*nleB*	1
	EHEC O26:[H11]	*stx1*	*eae β*	*nleB*	4
	EHEC O103:H2/H⁻	*stx1* or *stx2*	*eae ε*	*nleB*	0
	EHEC O111:H8/H⁻	*stx1* or *stx2*	*eae θ*	*nleB*	0
	EHEC O145:[H28]	*stx1*	eae γ	*nleB*	1
Top 7	EHEC O121:H19/H⁻	*stx1* or *stx2*	*eae ε*	*nleB*	0
	EHEC O45:[H2]	*stx1*	*eae ε*	*nleB*	1

No EHEC O103, O111 nor O121

Only 1 EHEC O157:H7 and a majority of EHEC O26:H11 (4/7)

Results and discussion

9 particular AEEC strains isolated

Isolated in samples in which *stx* genes were detected by PCR
Resemble the major EHEC strains (top 5 / top 7)

Serotype	*eae* variants	OI 122 marker	Nb of strains isolated
AEEC O157:**non**H7	***no*** *eae γ*	***no*** *nleB*	2
AEEC O26:[H11]	***eae β***	***nleB***	**7**
AEEC O103:[H2]	***eae ε***	***nleB***	**2**
AEEC O103:**non**H2	***no*** *eae ε*	***no*** *nleB*	2
AEEC O111:H8/H^{-}	*eae θ*	*nleB*	0
AEEC O145:H28/H^{-}	*eae γ*	*nleB*	0
AEEC O121:H19/H^{-}	*eae ε*	*nleB*	0
AEEC O45:H2/H^{-}	*eae ε*	*nleB*	0

Public health significance of these particular strains?

Conclusion

Prevalence of major EHEC in fresh minced meat in France

The 5 and the 7 major EHEC serogroups (top 5 and top 7) were detected at a low prevalence (0,2% and 0,3% respectively)

O26 was the main serogroup isolated in meat
None O111 nor O121 strains were isolated

A novel real-time PCR strategy for EHEC detection in food

using OI122 marker and association of serotype with specific eae variants

A reliable strategy (specific and sensitive)
Based upon complementary tools to those described in the ISO TS 13136
A cost effective screening (reduces the number of PCR suspicions without increasing time analysis)

Thanks !

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