French NRL activities: Results 2008- 2009 and future works

ENVL
UMAP
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Ecole Nationale Vétérinaire de Lyon

Estelle LOUKIADIS- 4th annual workshop of the NRL for *E. coli* in the EU, 30th october 2009

French NRL activities: Results 2008- 2009 and future works

- Plan

1- Results 2008- 2009

- Official controls

- Development of diagnostic methods

2- Future works

French NRL activities: Results 2008- 2009 Official controls (1/3)

Year	Matrix	Nb of samples screened	Nb of strains isolated	STEC prevalence
2007	Ground beef batch (+4°C)	3605	11 STEC (5 *eae+ E. coli*)	11/3605 0,3% CI_{95} [0,2 – 0,5%]
	Raw milk cheese	392	0	0/392 ≤ 0,9%
2008	Ground beef trimming (-20°C)	992 (4 units/samples)	10 STEC (13 *eae+ E. coli*)	10/992 1% CI_{95} [0,6 – 1,8%]
2009	Ground beef batch (+4°C)	480 out of 2000		
	Minced beef at retail	711 out of 1520	2 STEC (0 *eae+ E. coli*)	2/711
	Raw milk cheese	1334 out of 2000	14 STEC (9 *eae+ E. coli*)	14/1334

French NRL activities: Results 2008- 2009 Official controls (2/3)

Ground beef

Serotype	Nb of *E. coli* strains isolated					
	STEC			*eae+ E. coli*		
	2007	2008	2009	2007	2008	2009
O157:H7	5	2	1	ND	ND	0
O26:H11	2	**5**	0	4	**11**	0
O103:H2	3	3	1	0	2	0
O111:H8	1	0	0	0	0	0
O145:H28	0	0	0	1	0	0
Total	11	10	2	5	13	0

French NRL activities: Results 2008- 2009 Official controls (3/3)

Raw milk cheese

Serotype	Nb of *E. coli* strains isolated					
	STEC			*eae+ E. coli*		
	2007	2008	2009	2007	2008	2009
O157:H7	0	ND	1	ND	ND	0
O26:H11	0	ND	**9**	0	ND	**3**
O103:H2	0	ND	**4**	0	ND	**4**
O111:H8	0	ND	0	0	ND	0
O145:H28	0	ND	0	0	ND	2
Total	0	ND	14	0	ND	9

French NRL activities: Results 2008- 2009
Development of diagnostic method (1/11)

Optimization of raw milk and raw milk products enrichment step before O26 detection (1/6)

Nb of HUS cases in France

100 cases of HUS/year

(sporadic cases)

2005 :

122 cases of HUS

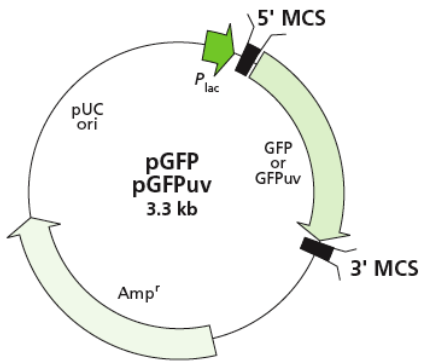
(including a major O26 outbreaks)

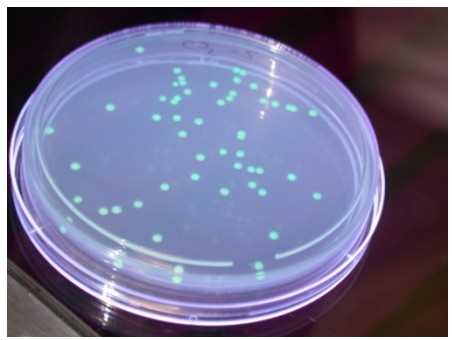
French NRL activities: Results 2008- 2009
Development of diagnostic method (2/11)

Optimization of raw milk and raw milk products enrichment step before O26 detection: material and method (2/6)

E. coli* O26
(1-8 UFC/25g)

Bacteria counts





3 of *E. coli* O26 strains (isolated from faeces) were experimentally inoculated (1-8 CFU/25g) in triplicate and in random.

In 12 different raw milk and raw milk products

French NRL activities: Results 2008- 2009
Development of diagnostic method (3/11)

Enrichment Parameters tested		
Temperature	**Broth**	**Duration**
37°C and 41.5°C	**BPW**	**4, 8, 12 and 24 hours**
	BPW+acriflavin	
	BPW+acriflavin+CT	
	BPW+acriflavin+tween	
	BPW+acriflavin+tween+CT	
	BPW+CT	
	BPW+C	
	BPW+T	
	BPW+vancomycin	
	BPW+CT transfered in Mc conkey+CT	
	mTSB+acriflavin transfered in Mc conkey+CT	

French NRL activities: Results 2008- 2009
Development of diagnostic method (4/11)

Optimization of raw milk and raw milk products enrichment step before O26 detection: Results (4/6)

BPW+acriflavin *E. coli* O26 counts (UFC/ml)

Matrices	Enrichissement duration (41.5°C)			
	4 hours	8 hours	12 hours	24 hours
Raw cow's milk (day+1)	< 10	$2\ 10^4$	$7.3\ 10^4$	$1.1\ 10^5$
Raw cow's milk (day+3)	< 10	$1.2\ 10^4$	$8.6\ 10^4$	$2.3\ 10^6$
Raw goat's milk (day+1)	< 10	$3.6\ 10^4$	$1.5\ 10^5$	$1.7\ 10^5$
Raw goat's milk (day+3)	< 10	$1.5\ 10^4$	$5.9\ 10^5$	$6.2\ 10^5$
White mould rinded soft cheese type I	< 10	$1.1\ 10^4$	$5.5\ 10^4$	$2.5\ 10^5$
Blue mould cheese	< 10	$4.7\ 10^4$	$3.3\ 10^5$	$4.2\ 10^5$
Cooked hard cheese	< 10	$1.1\ 10^4$	$7.1\ 10^5$	$1.2\ 10^6$
Washed rinded soft cheese type I	> 10	$2.6\ 10^4$	$7.6\ 10^4$	$4.3\ 10^5$
Uncooked hard cheese	89	$5.1\ 10^4$	$8.7\ 10^4$	$1.4\ 10^5$
Fresh cheese	< 10	$1\ 10^4$	$1.2\ 10^4$	$2\ 10^4$
White mould rinded soft cheese type II	10	$2\ 10^3$	$4.7\ 10^3$	$3\ 10^3$
Washed rinded soft cheese type II	69	$2.1\ 10^2$	$2.1\ 10^3$	$3.6\ 10^3$

French NRL activities: Results 2008- 2009
Development of diagnostic method (5/11)

Optimization of raw milk and raw milk products enrichment step before O26 detection: Results (5/6)

BPW+acriflavin+CT

Raw milk cheeses	Strains	Enrichment duration at 41,5°C	
		8 hours	24 hours
		O26 (UFC/ml)	O26 (UFC/ml)
White mould rinded soft cheese type II	1	$5.1\ 10^2$	$5.2\ 10^4$
	2	$3.9\ 10^2$	$6\ 10^4$
	3	$1.2\ 10^3$	$7.6\ 10^4$
Washed rinded soft cheese type II	1	$1.2\ 10^4$	$1.6\ 10^4$
	2	$3\ 10^4$	$7.4\ 10^4$
	3	$2.7\ 10^4$	$5.3\ 10^4$

French NRL activities: Results 2008- 2009
Development of diagnostic method (6/11)

Optimization of raw milk and raw milk products enrichment step before O26 detection: Results and discussion (6/6)

Optimal enrichment in dairy complex products:

24 hours at 41,5°C in BPW supplemented with acriflavin (10mg/ml) and CT (C 0,05mg/ml; T 2.5mg/ml)

Different technologies (milk composition, nature of the starters cultures, characteristics of fermentation and ripening) + different background microflora

Key step before detection and confirmation

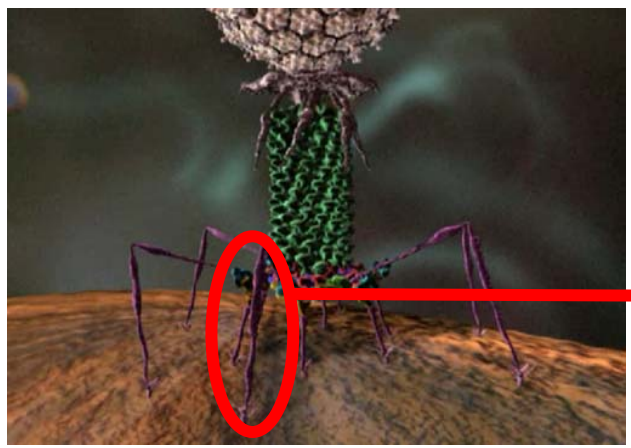
→ Optimization of raw milk and raw milk products enrichment step before *E. coli* O26 detection.
F. Savoye. C. Vernozy-Rozand, M. Bouvier, A. Gleizal, D. Thevenot.
Submitted in *Letters in Applied Microbiology*

French NRL activities: Results 2008- 2009
Development of diagnostic method (7/11)

Evaluation of different methods including a novel recombinant phage protein ligand assay for *E. coli* O157:H7 in ground beef and trimmings: (1/4)

Objectives

- improve the sampling strategy by testing up to 375g of ground beef
- decrease the incubation period



From: www.justseyet.com

Long Tail Fibers: Recombinant receptor used in the VIDAS phage method

French NRL activities: Results 2008- 2009
Development of diagnostic method (8/11)

Evaluation of different methods including a novel recombinant phage protein ligand assay for *E. coli* O157:H7 in ground beef and trimmings: (2/4)

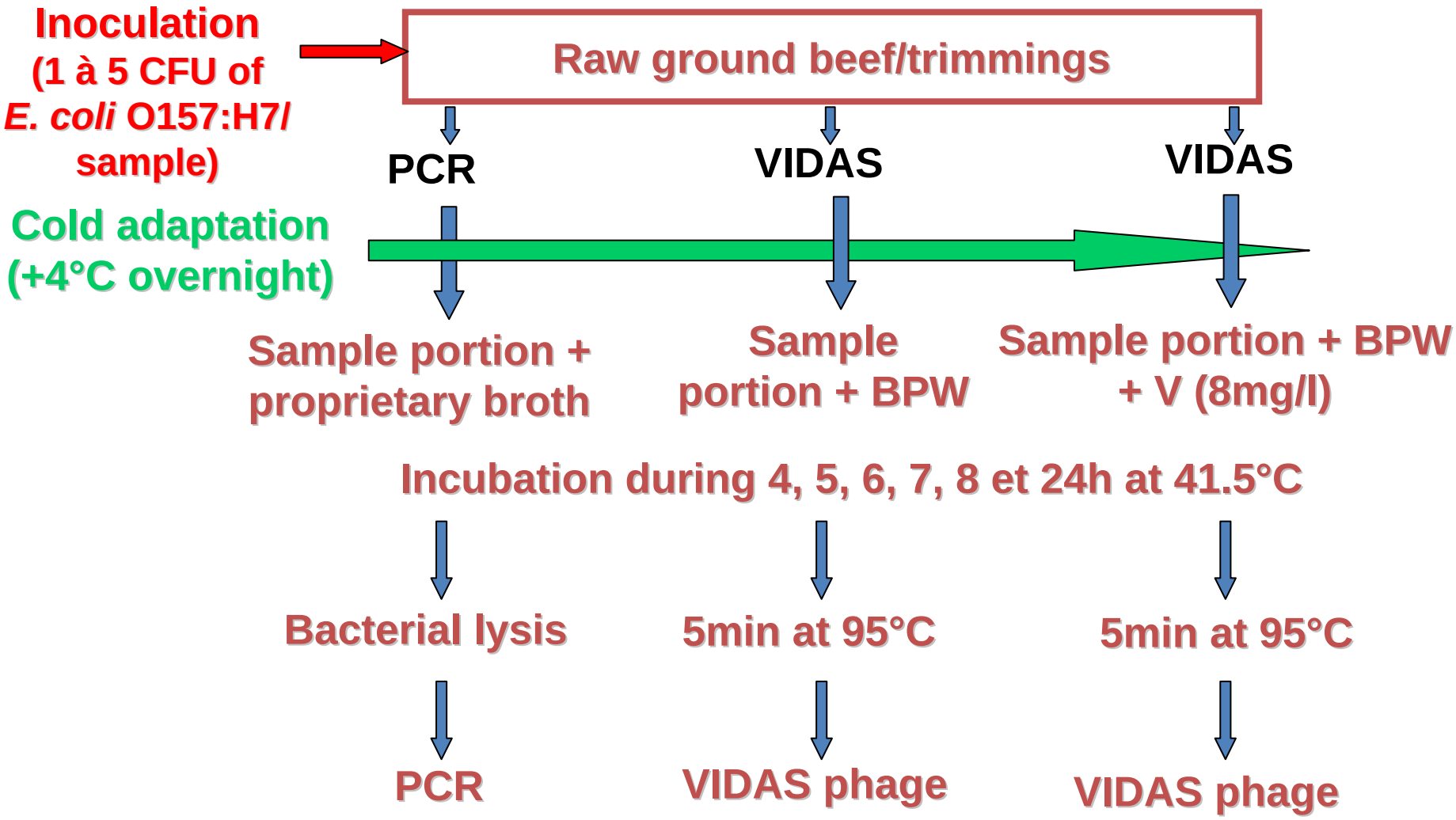
Material and methods

Sample	Inocula	Sample size	Enrichment broth volum	Sample to broth ratio
Raw ground beef	3-7 CFU/25g	25g	225ml	1/10ème
	1-8 CFU/75g	75g	225ml	1/4ème
	2-8 CFU/375g	375g	1125ml	1/4ème
Trimmings	3-6 CFU/75g	75g	225ml	1/4ème

5 *E. coli* O157:H7 strains were inoculated separately in triplicate and in a random fashion (15 tests)

French NRL activities: Results 2008- 2009
Development of diagnostic method (9/11)

Evaluation of different methods including a novel recombinant phage protein ligand assay for *E. coli* O157:H7 in ground beef and trimmings: (3/4)



French NRL activities: Results 2008- 2009
Development of diagnostic method (10/11)

Evaluation of different methods for *E. coli* O157:H7 in ground beef and trimmings: (4/4) Results

Sample portion		Detection	Broth	Enrichissement duration at 41,5°C (hours)					
				4	5	6	7	8	24
Raw ground beef	25g	PCR	PCR broth	1/15	8/15	11/15	15/15	15/15	15/15
		VIDAS PHAGE	BPW	6/15	15/15	15/15	15/15	15/15	15/15
			BPW+Vanco	3/15	14/15	15/15	15/15	15/15	15/15
	75g	PCR	PCR broth	0/15	0/15	1/15	0/15	1/15	2/15
		VIDAS PHAGE	BPW	2/15	8/15	14/15	15/15	15/15	15/15
			BPW+Vanco	1/15	12/15	15/15	15/15	15/15	15/15
	375g	PCR	PCR broth	ND	ND	0/15	2/15	0/15	6/15
		VIDAS PHAGE	BPW	ND	ND	0/15	0/15	8/15	15/15
			BPW+Vanco	ND	ND	0/15	4/15	8/15	15/15
Trimmings	75g	PCR	PCR broth	ND	ND	5/15	ND	8/15	6/15
		VIDAS PHAGE	BPW	ND	ND	5/15	ND	8/15	15/15
			BPW+Vanco	ND	ND	5/15	ND	8/15	15/15

French NRL activities: Results 2008- 2009
Development of diagnostic method (11/11)

Evaluation of different methods including a novel recombinant phage protein ligand assay for *E. coli* O157:H7 in ground beef and trimmings: Results and discussion

Shortest incubation period with the VIDAS phage assay

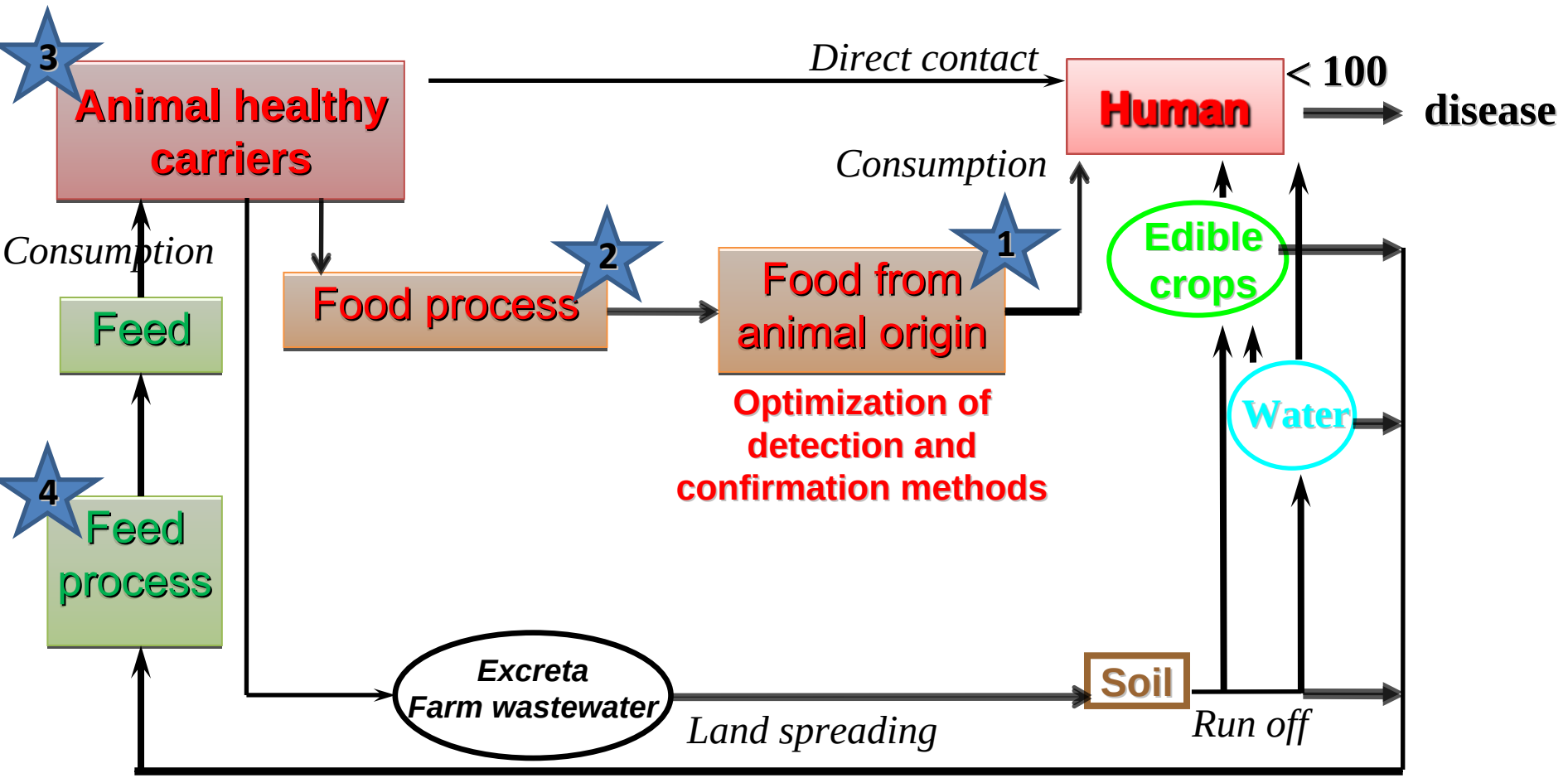
for 25 g samples, 6 h enrichment was sufficient

Recovery of *E. coli* O157:H7 in samples up to 375g with the VIDAS phage assay

but…incubation time is still of 24h for 375g! (improve the enrichment step)

➡ Novel phage ligand based enzyme linked fluorescent assay (ELFA) for same day detection of *E. coli* O157:H7 in composite samples of raw ground beef and trimmings.
F. Savoye C. Vernozy-Rozand, M. Bouvier, A. Gleizal, D. Thevenot.
Submitted in Journal of Food Microbiology

French NRL activities: Future works



French NRL activities: Future works

Project 1

Prevalence and characterization of STEC strains in food in France and optimization of the detection method

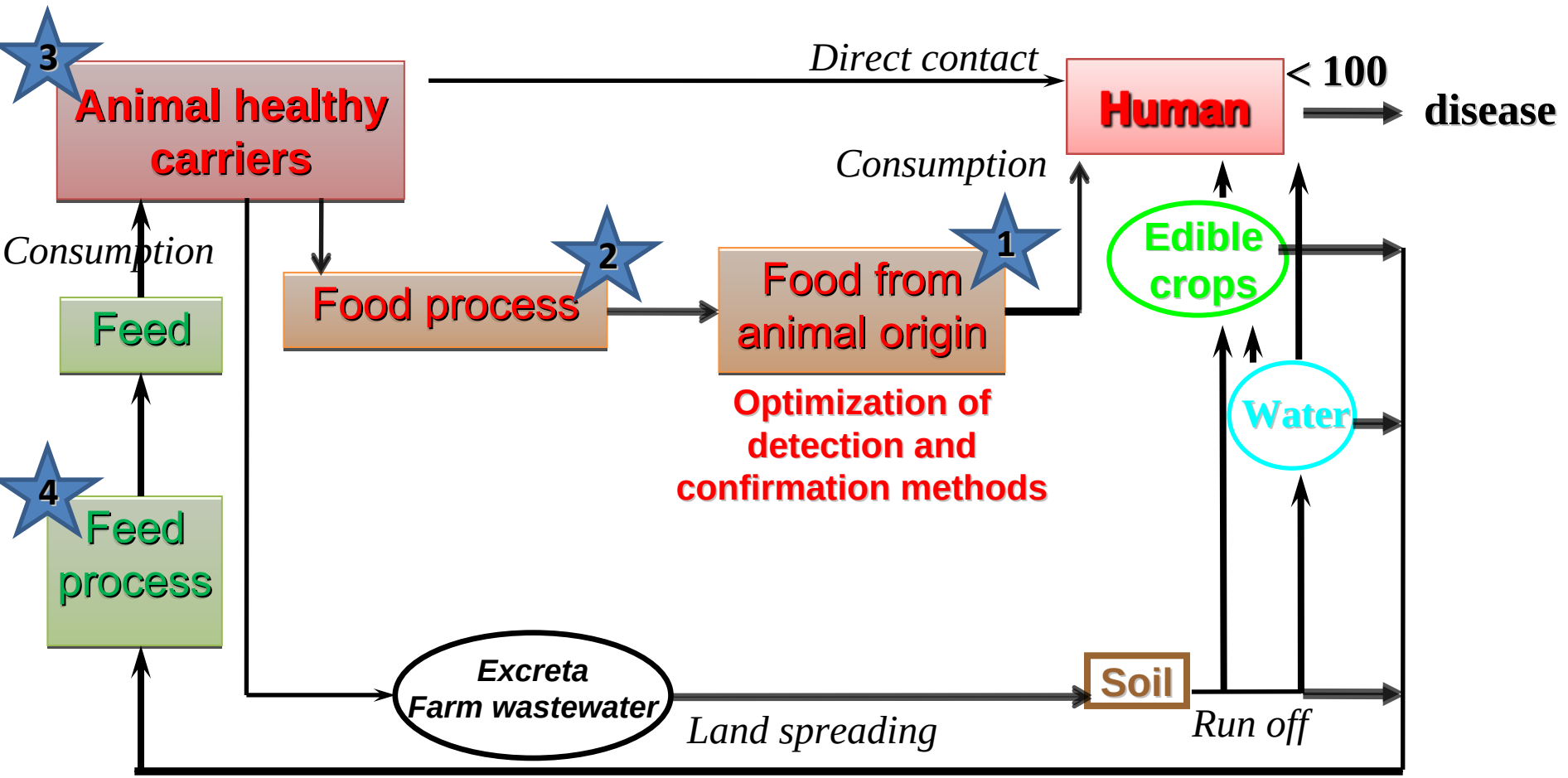
Objectives:

- Provide updated data on prevalence of STEC strains in food of animal origins in France (Gang of 5 + others)
- Optimize enrichment steps for O103, **O111**, and O145 strains
- Optimize isolation and confirmation step (automated immunoconcentration/selective media..)

Partners:

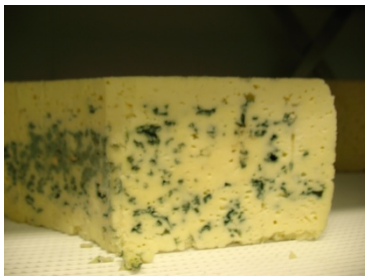
French Ministry of Agriculture
Professionnals (meat and dairy products producers)
Kits manufacturers and media providers
AFSSA LERQAP Maisons- Alfort
Others?...

French NRL activities: Future works



French NRL activities: Future works

Project 2



Growth and survival of STEC during raw milk cheese processing

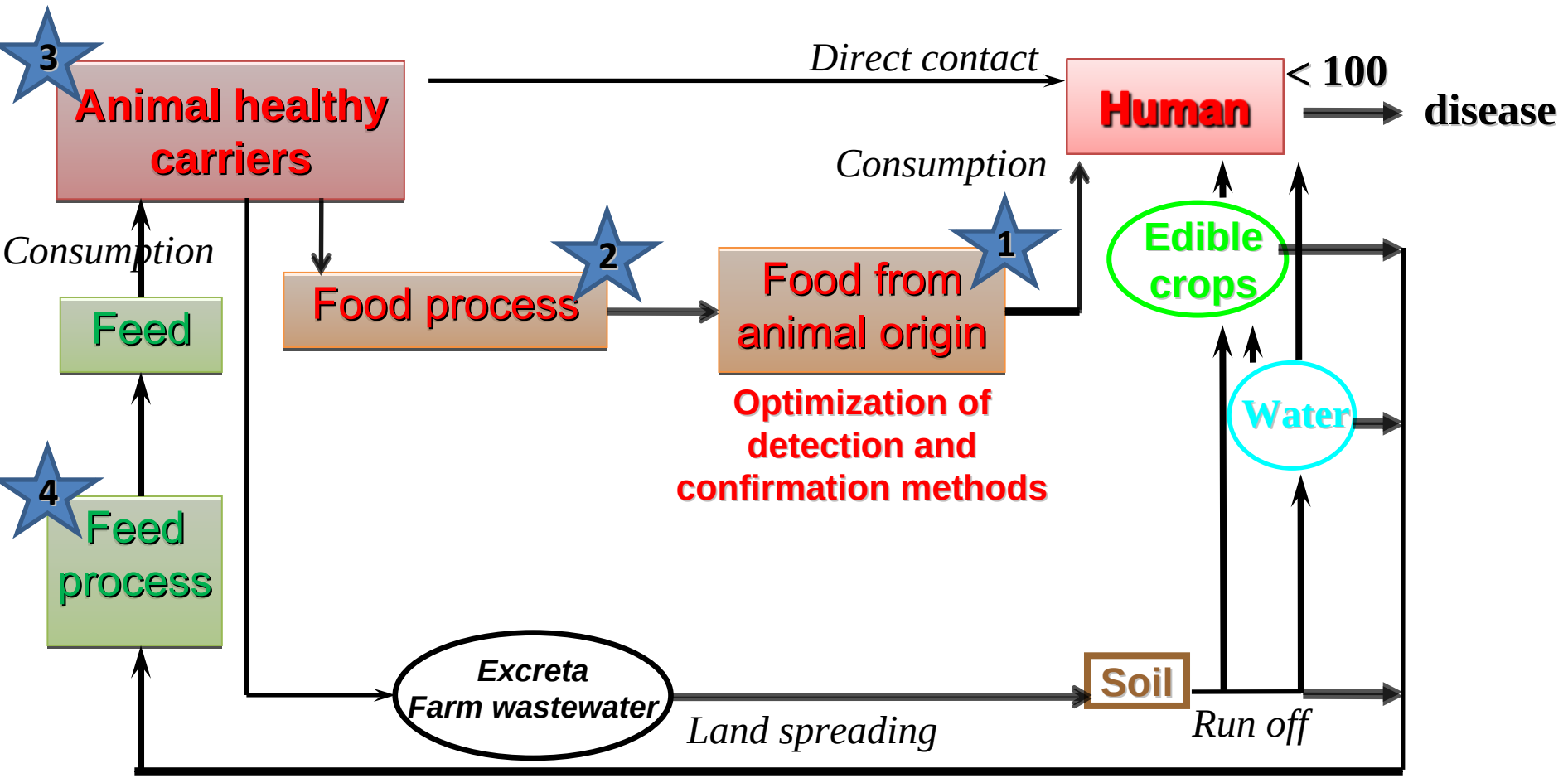
Objectives:

- Study growth and survival of different STEC strains in different types of cheese during the process (different process types, nature of stater cultures, indigenous microflora, fermentation, ripening…)
- Study the impact of biotic and abiotic parameters on STEC growth
- Define a growth model for QRA

Partners:

French Ministry of Agriculture
Professionnals and professional associations (dairy products producers)
Kits manufacturers and media providers
INRA Aurillac

French NRL activities: Future works



French NRL activities: Future works

Project 3

Carriage and excretion of EHEC strains in French livestock : Updated data for the meat producers

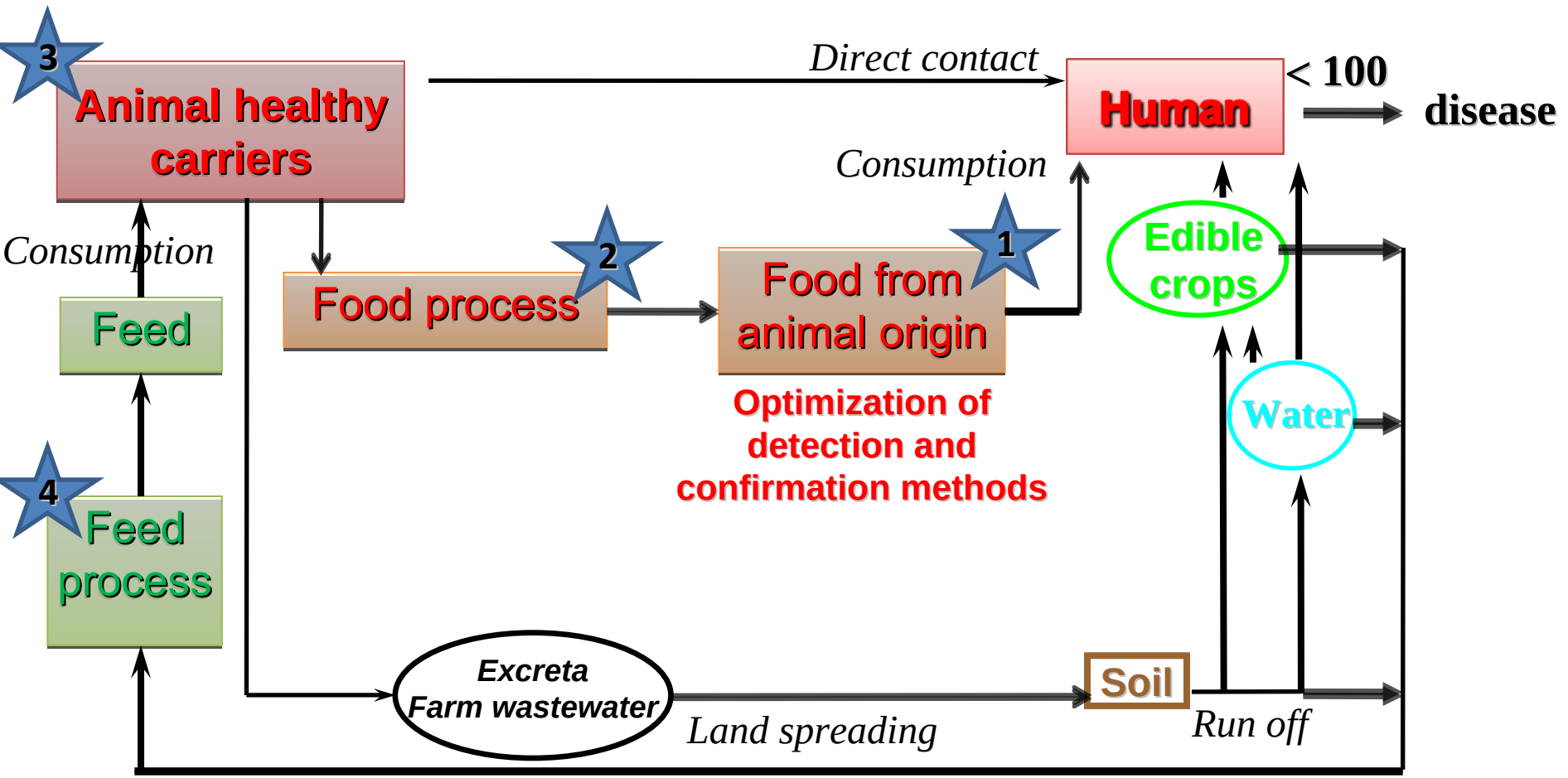
Objectives:

- Provide accurate data on bovine carriage of STEC strains belonging to the 5 major serogroups of EHEC strains (3200 animals, young cattle; suckler cows; young cattle, from different regions)
- Identify farms associated with a higher « risk »
- Optimize detection method in bovine faeces

Partners:

French association of professionals: « Institut de l'élevage »
UMR INRA ENVT Toulouse
AFSSA LERQAP Maisons- Alfort
AFSSA LERPBVH Lyon

French NRL activities: Future works



French NRL activities: Future works

Project 4

Antagonist interactions between STEC and lactic acid bacteria in silage: a way to reduce bovine carriage?

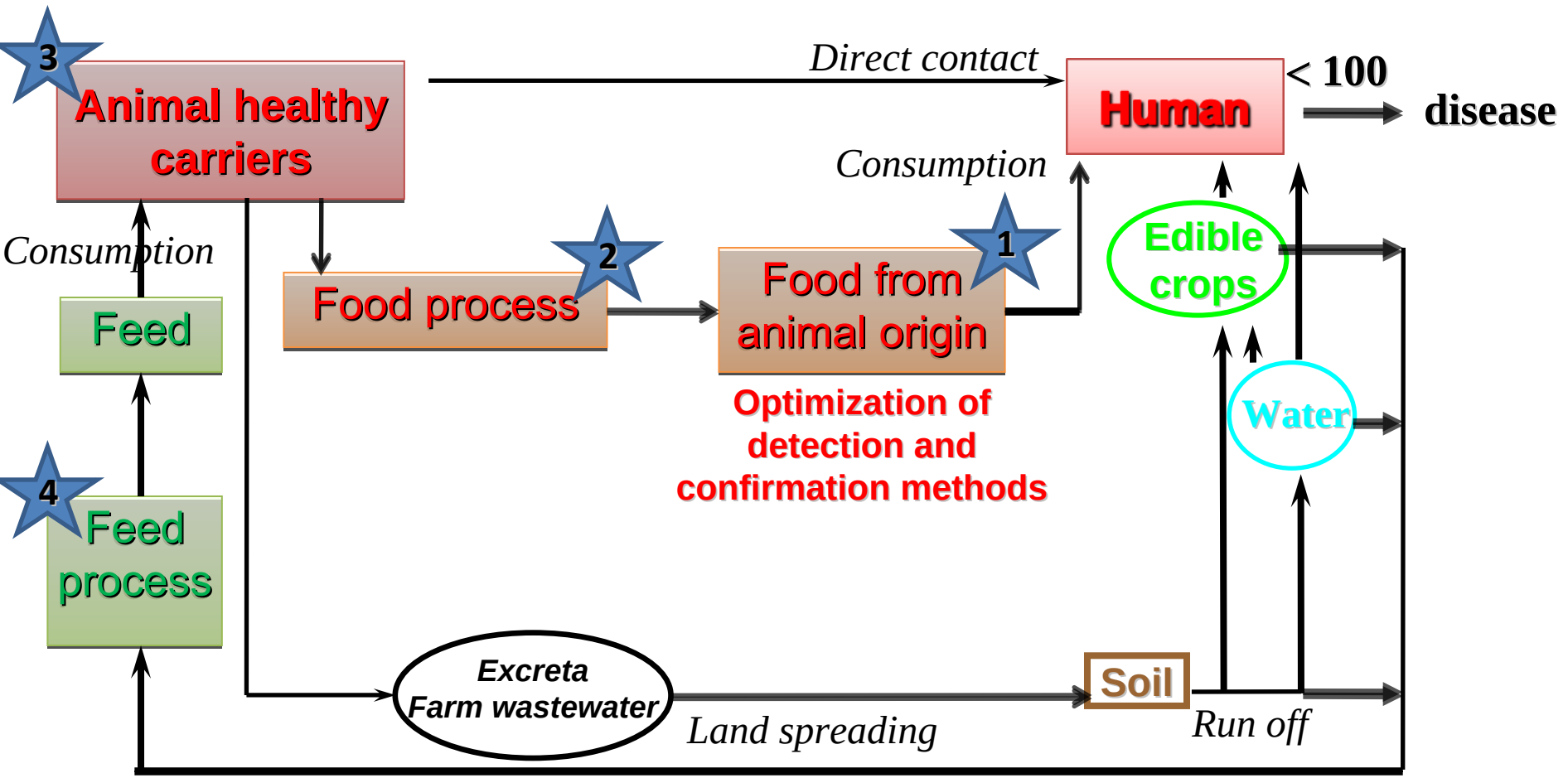
Objectives:

- Identify acid lactic bacteria that can reduce the growth or kill the STEC strains i*n vitro (*268 lactic acid bacteria/ 10 O157:H7; 10 O26 and 10 O103/O111/O145 STEC strains/ inhibition mecanisms (nutritional competition, acid inhibition, bacterocin,…)
- Study the inhibition properties of selected lactic acid bacteria in silage
- Study the inhibition properties of selected lactic acid bacteria in bovine digestive tract

Partners:

INRA Theix
ENITAC Clermont-Ferrand
Stater culture producer

French NRL activities: Future works



French NRL activities: Results 2008- 2009 and future works

Delphine Thevenot

Christine Mazuy

Christine Bavai

Lysiane Dunière

Fanny Savoye

Stéphane Miszczycha

Marie-Pierre Montet

Sarah Ganet

Marion Bouvier

Audrey Gleizal

Franck Ferre

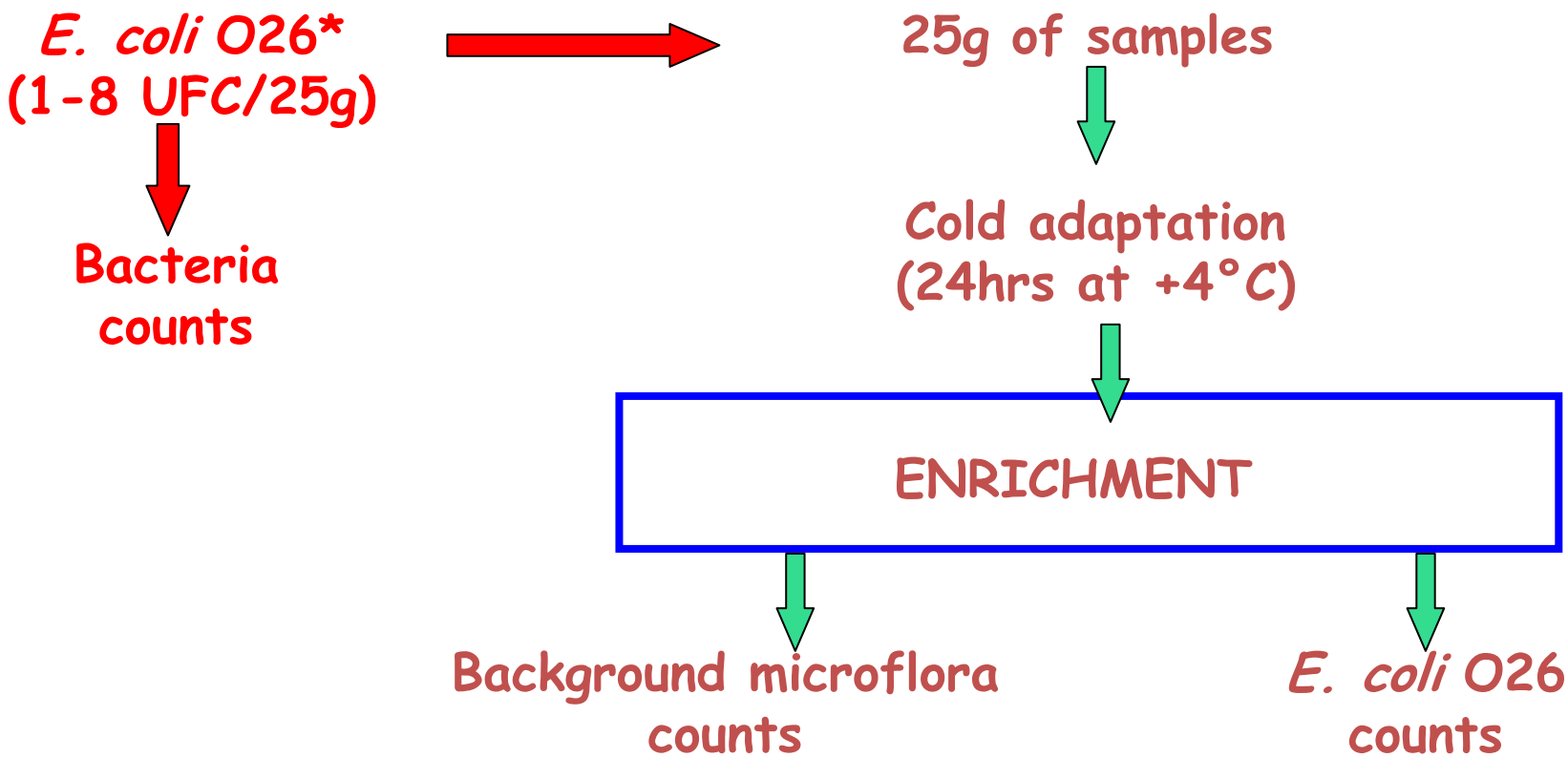
And…Cécile Dutrieux and Marie Strub !

French NRL activities: Results 2008- 2009 and future works

Thank you
for your attention!

French NRL activities: Results 2008- 2009
Development of diagnostic method

Optimization of raw milk and raw milk products enrichment step before O26 detection: material and method



French NRL activities: Results 2008- 2009
Development of diagnostic method (1/?)

Evaluation of different methods for *E. coli* O157:H7 in ground beef and trimmings: Results

	Raw groung beef						Trimmings	
Sample size	**25g**		**75g**		**375g**		**75g**	
Inoculation level	**3-7 UFC/25g**		**1-8 UFC/75g**		**2-8 UFC/375g**		**3-6 UFC/75g**	
Incubation time	**8 h**	**24 h**	**8 h**	**24 h**	**8 h**	**24 h**	**8 h**	**24 h**
VIDAS % positive results	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
USDA validated PCR method results	**100%**	**100%**	**6.8%**	**13.3%**	**0%**	**40%**	**53%**	**40%**

Calcul théorique de la probabilité de détecter *E. coli* O157:H7 en fonction de la prise d'essai effectuée

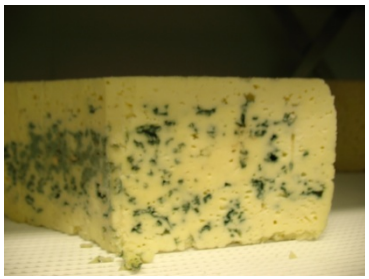
Selon la distribution de *Poisson* et en supposant une contamination homogène de la totalité de la mêlée :

$P = 1 - e^{-(C*M)}$ C, niveau de contamination et M, masse de steak haché

Niveau de contamination de la mêlée (UFC *E. coli* O157:H7.g^{-1})	Probabilité de détection de *E. coli* O157:H7 selon la prise d'essai réalisée (%)				
	25g	75g	125g	375g	750g
4.10^{-3} (0,1 UFC/25g)	10	26	39	78	95
0,04 (1 UFC/25g)	63	95	99	99,99	100
0,1	92	99,9	99,99	100	100
1	100	100	100	100	100

French NRL activities: Future works

Project 2



Growth and survival of STEC during raw milk cheese processing

Sampling strategy:

- Different types of raw milk cheese (blue mould cheese -roquefort-; white mould rinded soft cheese - camembert- etc..)
- Different STEC strains (O157:H7; O26:H11; O103:H2)
- Different step during the process

Material and method: (Challenge tests)

- Cold adapted strains
- Inocula: 100UFC/ml of raw milk
- Bacteria counts (classical counts / quantitative PCR)
- Detection after enrichment

→ Provide data on ecology of STEC in dairy complex products
Define process associated with higher risk

French NRL activities: Future works

Project 3

Carriage and excretion of EHEC strains in French livestock : Updated data for the meat producers

Sampling strategy:

- 3 different types of animals (young cattle; suckler cows; young cattle)
- 3200 animals
- Different production regions in France

Material and method:

- Detection: enrichment/DNA extraction/RT-PCR
- Confirmation: Isolation/genotypic and phenotypic characterization

→ Evaluation and comparison of carriage of STEC belonging to the 5 major serogroups

→ Identification of super excretors animals and farms associated with a higher risk

French NRL activities: Future works

Project 4

Antagonist interactions between STEC and lactic acid bacteria in silage: a way to reduce bovine carriage?

Materials and methods:

- 268 lactic acid bacteria tested
- 10 O157:H7; 10 O26 and 10 O103/O111/O145 STEC strains
- Inhibition activities *in vitro*: identification lactic acid bacteria (in solid and liquid media)
- Characterization of the inhibition mecanisms (nutritional competition, acid inhibition, bacterocin,…)
- Inhibition activities in silage: challenge tests in artificially contaminated silage
- Inhibition activities in bovine digestive tract: probiotic properties of selected lactic acid bacteria in digestive tract model

→ A way to reduce bovine carriage?