

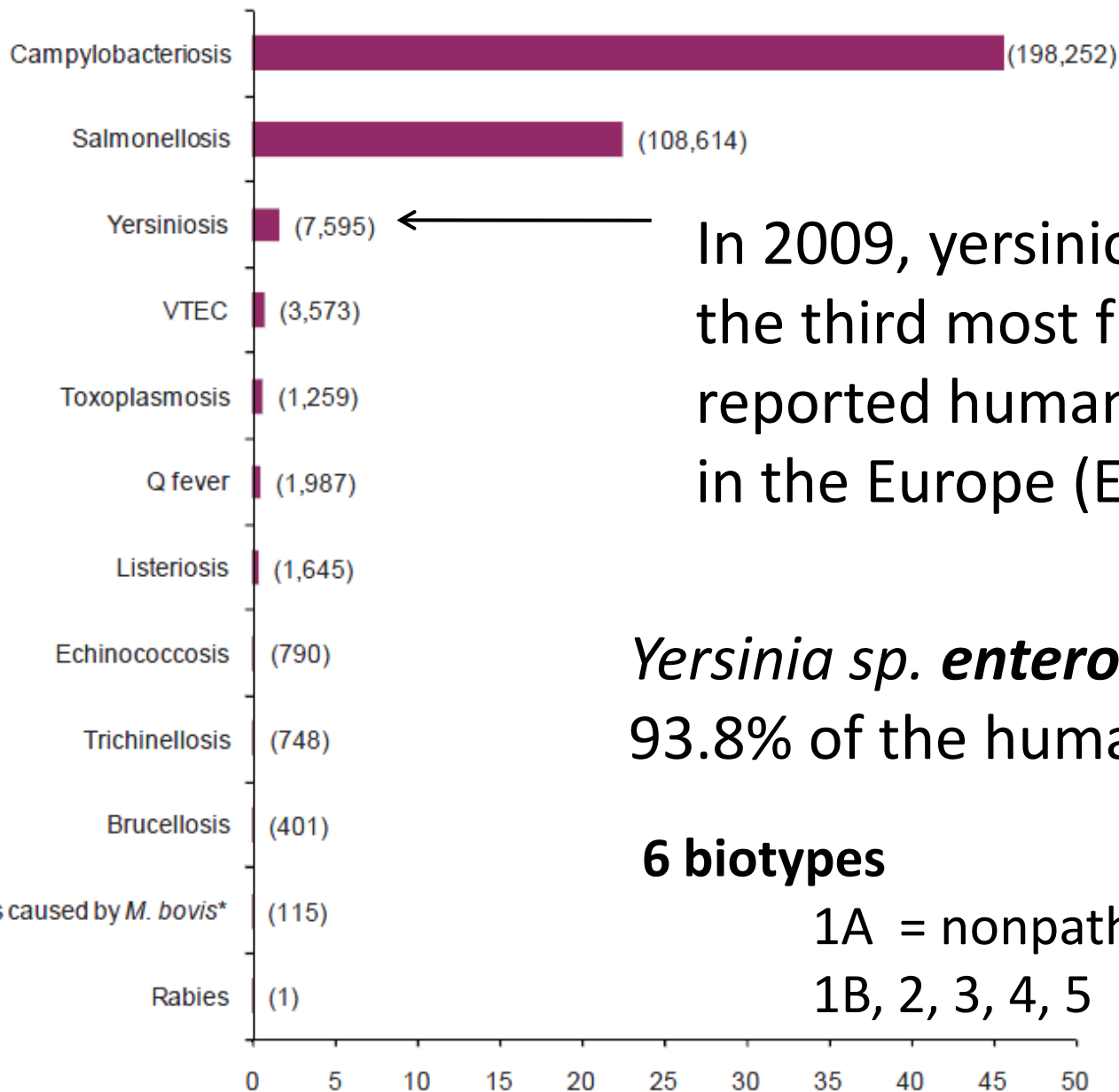


A selective chromogenic plate, YECA, for the detection of pathogenic *Yersinia enterocolitica*

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INTRODUCTION



In 2009, yersiniosis was the third most frequently reported human zoonosis in the Europe (EFSA, 2011)

Yersinia sp. enterocolitica in 93.8% of the human infections.

6 biotypes

1A = nonpathogenic

1B, 2, 3, 4, 5 = pathogenic

Pig : principal reservoir of pathogenic *Y. enterocolitica*

Pigs do not develop clinical signs

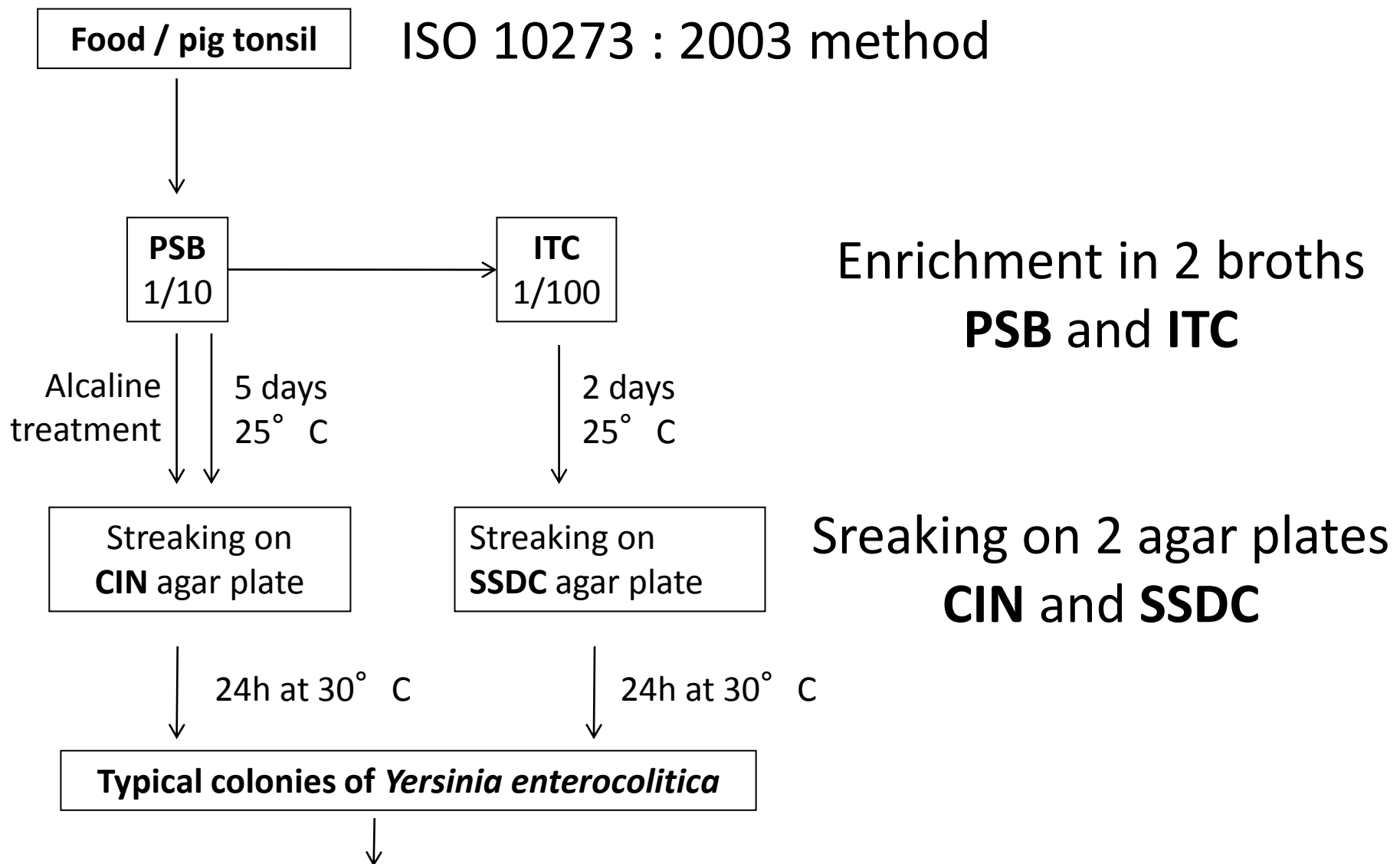
carry *Y. enterocolitica*
in their oral cavity,
on tongues and tonsils,
and in lymph nodes,



and excrete this
bacterium in their
feces.

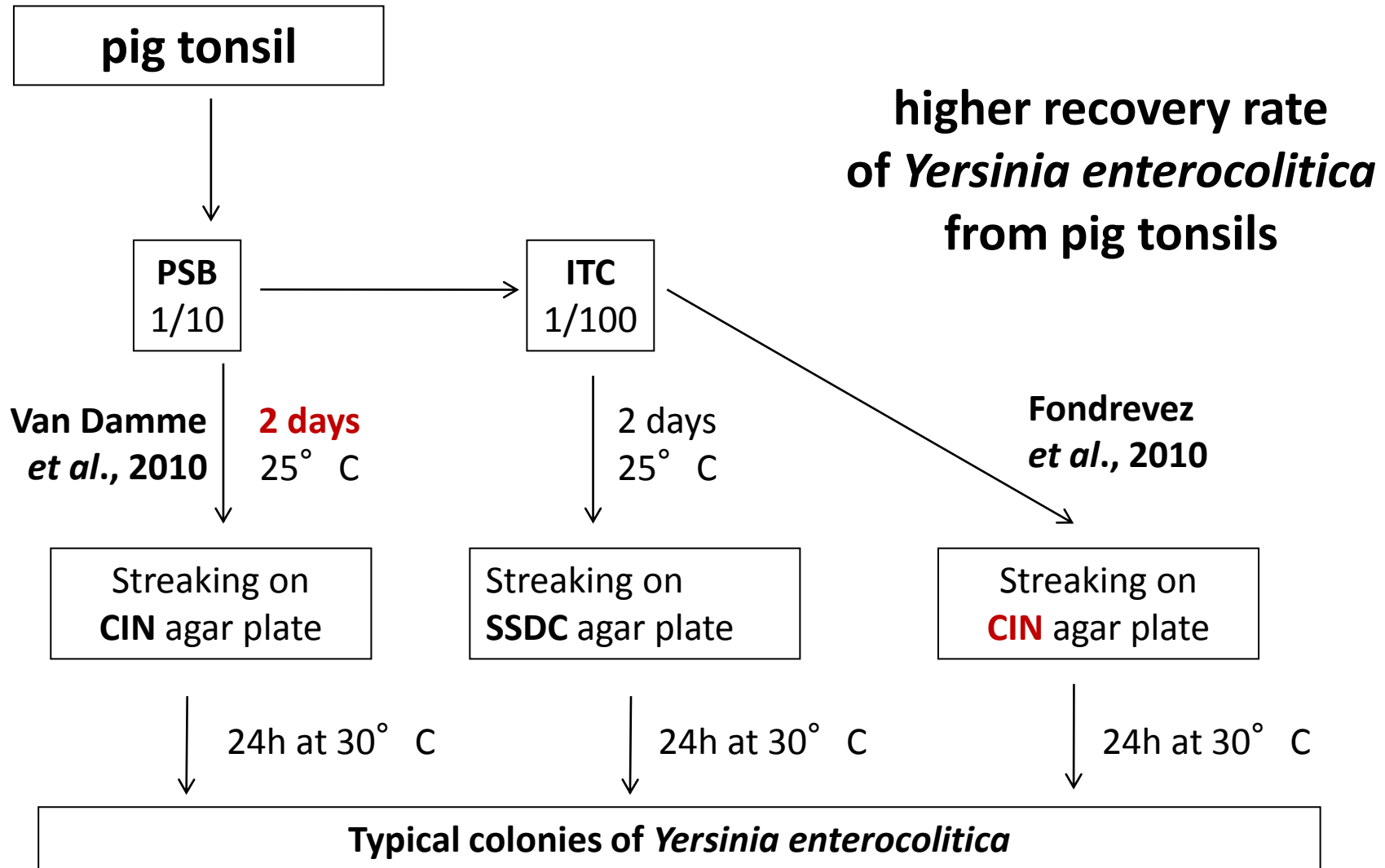
Bioserotype 4/0:3 :
the most prevalent pathogenic bioserotype
isolated from pigs.

Detection of *Yersinia enterocolitica*



Confirmation of *Yersinia enterocolitica* and biotyping

Detection of *Yersinia enterocolitica*

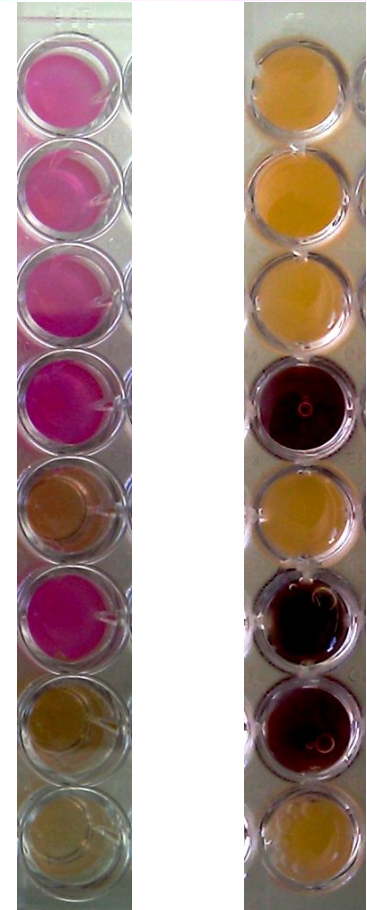


**SSDC and CIN do not differentiate
pathogenic biotypes from non-pathogenic biotype**

Confirmation of *Yersinia enterocolitica*

Table C.1 — Caractéristiques biochimiques, à 30 °C, des *Yersinia*

Essai	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia enterocolitica</i>	Espèces apparentées
Glucose	+ ^a	+	+
Gaz à partir du glucose	-	- (ou quelques bulles)	- (ou quelques bulles)
Lactose	-	-	-
ONPG	+	+/-	+/-
Adonitol	-	-	-
Cellobiose	-	+	D
Dulcitol	-	-	-
Mannitol	+	+	+
Mélibiose	+/-	-	D
Rhamnose	+	-	D
Saccharose	-	+	D
Sorbitol	-	+/-	D
Tréhalose	+	+/-	+
Xylose	+	D	+
Esculine	+	D	D
Salicine	+	D	D
Urée	+	+	+
Indole	-	D	D
Voges Proskauer	-	+*/-	D
Sulfure d'hydrogène	-	-	-
Désaminase (APP)	-	-	-
Lysine	-	-	-
Ornithine	-	+/-	+
Citrate (Simmons)	-	-	D
Lipase (Tween 80™)	-	D	D
Mucate	-	-	D



Urea TDA

**degradation of urea
absence of tryptophan
desaminase**

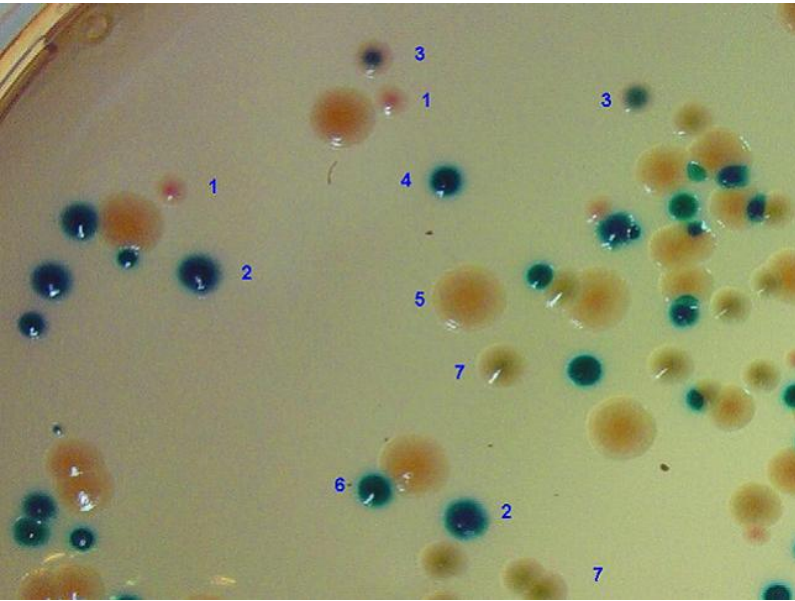
Identification of biotype

Tableau D.1 — Biotypes de *Yersinia enterocolitica*

Biotypes	Tween™-estérase	Esculine	Pyrazinamidase	Indole	Xylose	Tréhalose
1A ^a	+	+	+	+	+	+
1B	+	-	-	+	+	+
2	-	-	-	(+) ^b	+	+
3	-	-	-	-	+	+
4	-	-	-	-	-	+
5	-	-	-	-	D ^b	-

differentiation of
pathogenic biotypes from non-pathogenic biotype

Chromogenic medium YeCM (Weagent, 2008)



1, pathogenic *Y. enterocolitica*
2, *Y. enterocolitica* biotype 1A

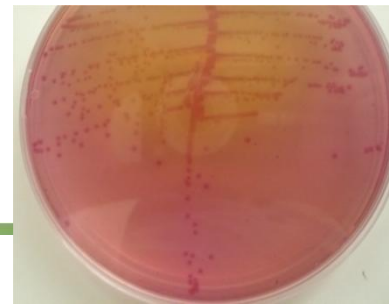
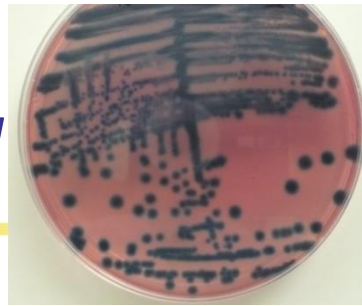
**Difficulties to isolate YE
from non-YE colonies
after PSB and ITC**

ITC

Fondrevez *et al.*, 2010


↓
Streaking on
CIN agar plate

↓
Typical colonies on YeCM



**Biotype 1A
Yersinia enterocolitica
« bleu purple
colonies »**

**pathogenic
Yersinia enterocolitica
« red-bull's-eyes-like
colonies »**



YECA : *Yersinia enterocolitica* chromogenic agar,
developed by AES-Chemunex (Combourg, France)
isolate specifically **pathogenic *Yersinia enterocolitica***
colonies small and red fuchsia after 24H at 30° C

Sensitivity of YECA

Numeration from a 10-fold-serial dilution of culture
of *Y. enterocolitica* strains from biotype 1A, 2, 3, and 4.
on 3 plates : PCA, CIN and YeCM

Specificity of YECA

Streaking from 26 strains at 10^8 cells/ml on **CIN, YeCM and YECA**:

4 ***Yersinia enterocolitica*** : biotypes 1A, 2, 3 and 4

8 ***Yersinia*-like** : *Y. aldovae*, *Y. bercovieri*, *Y. frederiksenii*, *Y. kristensenii*,
Y. massiliensis, *Y. mollaretii*, *Y. rohdei*, *Y. ruckeri*.

14 **non-*Yersinia*** strains

→ Characteristics of the colonies

→ Importance of growth in a scale from 0 (no growth) to 5 (colonies on all the plate).

Capacity of YECA

for detecting pathogenic *Yersinia enterocolitica* from pig tonsils

50 pig tonsils collected from a slaughterhouse

10 g for Enrichment in PSB and ITC

Streaking on **CIN** and **YECA** at different times :

- direct streaking
- after 24h and 48H of incubation of ITC
- after 48h of incubation of PSB

typical colonies on CIN and YECA confirmed and biotyped (ISO method)

Sensitivity of YECA from 10-fold serial dilution of 10^8 cells/ml

Dilution of the culture	Biotype 1A				Biotype 2 / Biotype 3 / Biotype 4			
	PCA	CIN	YeCM	YECA	PCA	CIN	YeCM	YECA
-1				150 VC				NN
-2				32 VC				NN
-3				2 VC				NN
-4				0				NN
-5	> 200	>200	>150	0	>300	>300	>300	>300 RF
-6	53	39	46	0	34	53	50	67 RF
-7	3	3	2	0	3	5	6	9 RF
-8	1	1	1	0	0	1	1	1 RF
-9	0	0	0	0	0	0	0	0

Pathogenic *Y. enterocolitica* strains :

Numeration until dilution -8 on YECA / PCA / CIN / YeCM
small and red fuchsia colonies on YECA

Biotype 1A : numeration until dilution -3 on YECA \neq PCA / CIN / YeCM

YECA high inhibitor effect on the growth of B1A , violet colonies

RESULTS : Specificity of YECA from 10⁸ cells/ml ≈ CIN and YeCM

Name of the strains	CIN Growth / color of colonies	YeCM Growth / color of colonies	YECA Growth / color of colonies
<i>Y. enterocolitica</i> biotype 2	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
<i>Y. enterocolitica</i> biotype 3	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
<i>Y. enterocolitica</i> biotype 4	+++++ red with a translucent rim	+++++ red bull's-eye-like	+++++ small red fuchsia
<i>Y. enterocolitica</i> biotype 1A	+++++ red with a translucent rim	+++++ blue-purple	+ violet colonies (5)
<i>Yersinia aldovae</i>	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ small red fuchsia (1)
<i>Yersinia bercovieri</i>	+++++ red with translucent rim	+++++ yellow/red with translucent rim	++ yellow/small red fuchsia
<i>Yersinia frederiksenii</i>	+++++ red with translucent rim	+++++ blue to green	++ green/small red fuchsia
<i>Yersinia kristensenii</i>	+++++ red with translucent rim	++++ red with translucent rim	++ pink/small red fuchsia
<i>Yersinia massiliensis</i>	+++++ red with translucent rim	+++++ green	++ green/small red fuchsia
<i>Yersinia mollaretii</i>	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ small red fuchsia (1)
<i>Yersinia rohdei</i>	+++++ red with translucent rim	+++++ yellow/red with translucent rim	+ pink (1)
<i>Yersinia ruckeri</i>	No growth	No growth	No growth
<i>Salmonella</i> Typhimurium	No growth	No growth	No growth
<i>Campylobacter jejuni</i>	No growth	No growth	No growth
<i>Enterococcus faecalis</i>	No growth	No growth	No growth
<i>Lactobacillus plantarum</i>	No growth	No growth	No growth
<i>Pseudomonas fluorescens</i>	+++++ yellow	+++++ yellow	+ pink
<i>Brochothrix thermosphacta</i>	No growth	No growth	No growth
<i>Listeria monocytogenes</i>	No growth	No growth	No growth
<i>Escherichia coli</i>	No growth	No growth	No growth
<i>Staphylococcus aureus</i>	No growth	No growth	No growth
<i>Klebsiella</i> sp.	No growth	No growth	No growth
<i>Proteus mirabilis</i>	No growth	No growth	No growth
<i>Morganella morganii</i>	+++++ yellow	+++++ yellow	++ yellow/pink
<i>Pseudomonas</i> sp.	+++++ yellow	+++++ yellow	+ pink
<i>Serratia liquefaciens</i>	+++++ pink with translucent rim	+++++ green	++++ green/blue/pink

Detection of pathogenic *Yersinia enterocolitica* from pig tonsils

Streaking	Number of tonsils / 50 with pathogenic YE using CIN	Number of tonsils / 50 with pathogenic YE using YECA
Direct	17	15
ITC-24H	21	22
ITC-48H	28	28
PSB-48H	7	5

enrichment in **ITC-48 hours**

- ➔ higher recovery rate of samples positive in pathogenic *Y. enterocolitica*
- ➔ same number of positive tonsils recovered on CIN and YECA

141 pathogenic strains on YECA :

- 135 strains biotype 4 (12 after PSB; 123 after ITC)
- 2 strains biotype 3
- 4 strains biotype 2.

CONCLUSION

We described a simplified method (ITC-48H / YECA-24H) that efficiently detects pathogenic *Y. enterocolitica* in pig tonsils and that it is less time-consuming than the ISO 10273: 2003 method.
➔ **positive test for pathogenic *Yersinia enterocolitica* in 72 hours.**

In this study, we used this method on pig tonsils but studies has to be carried out on pig feces, carcasses and pork meat.

Anses - HQPAP



Thank you
for your attention !

