

# **Swine Brucellosis: diagnostic strategies**

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<http://www.oie.int/wahis/public.php>

## OIE Official data about Swine Brucellosis

### *B. suis* biovar. 1 and 3

U.S.A	+
Argentina	+
Cuba	9
Uruguay	+
México	?
Canada	?
Ecuador	?

### *B. suis* biovar. 1 and 3

China	2
Malaysia	+
French Polynesia	2
Mongolia	+

Zoonosis

- + The disease is present but the number of outbreaks is unknown
- ? Suspected but not confirmed

# Swine brucellosis due *B. suis* to biovar 2 is an emerging disease in Europe

*B. suis* bv 2 is rarely pathogenic for humans

25-50% prevalence



Muñoz et al. 2010

Spillover from wild reservoir

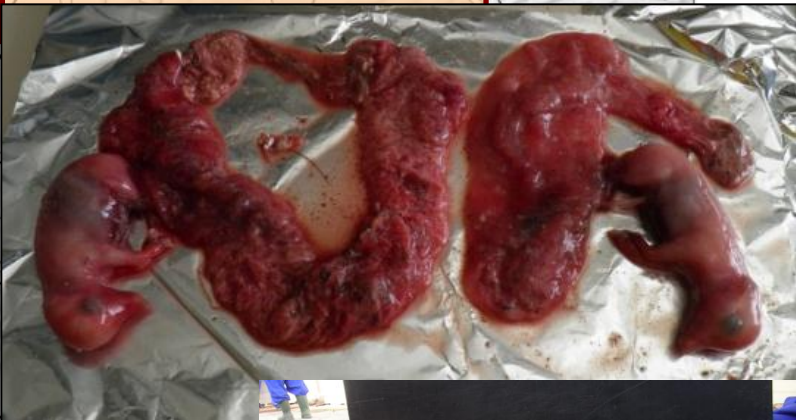


Muñoz et al. 2003

Abortions  
Infertility  
Genital lesions

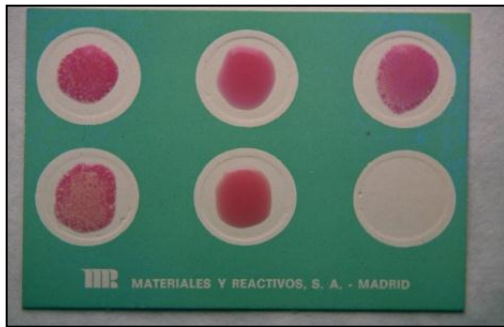


Brucellosis outbreaks reported also in intensive piggeries

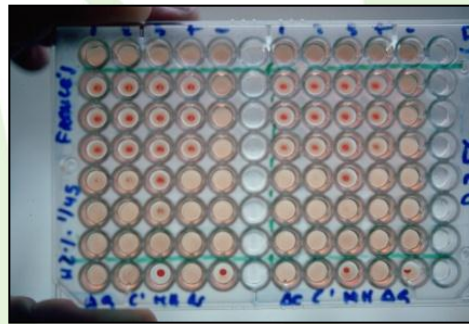


Arthritis

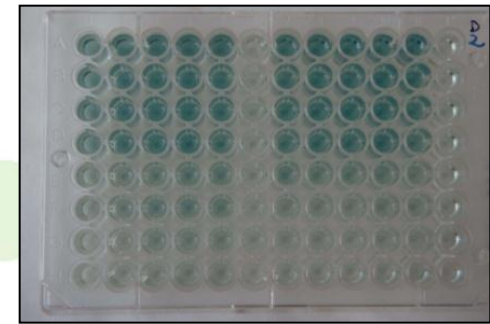
# Serological tests applied for trade (OIE) in pigs are not fully accurate for individual diagnosis



Rose Bengal Test



Complement Fixation Test



ELISA

- Moderate sensitivity
- Lack of specificity: **FPSR** mainly caused by *Yersinia enterocolitica* O:9 (common S-LPS epitopes)

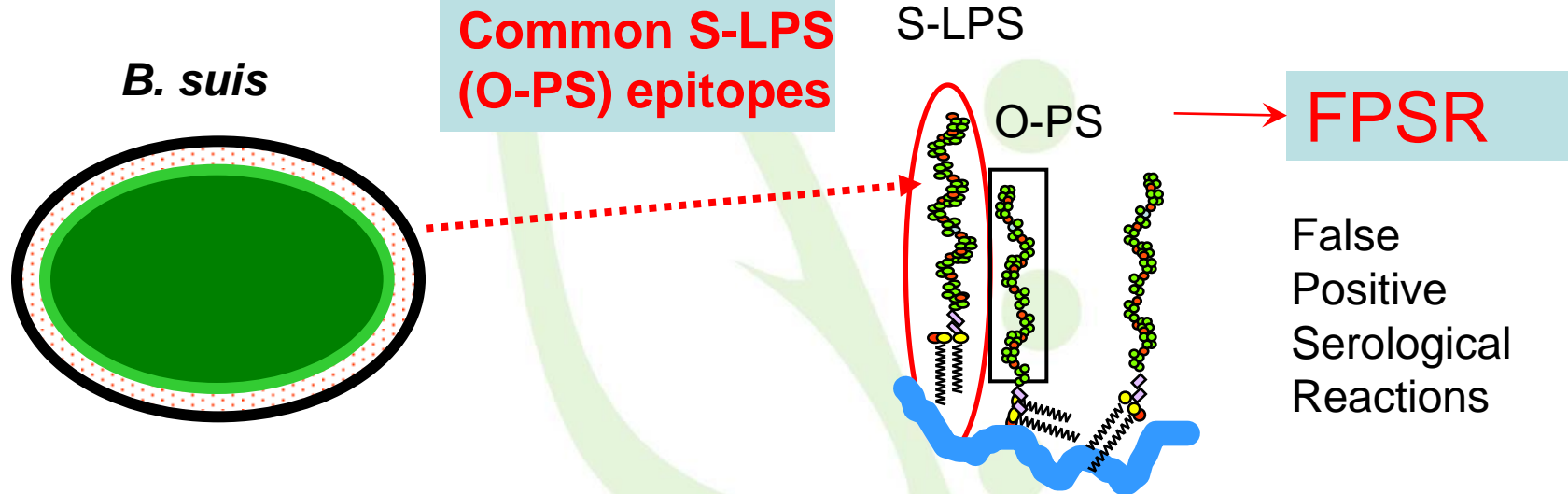


# The problem of False Positive Serological Reactions (FPSR) in swine Brucellosis

*Y. enterocolitica* O:9

S-LPS = Smooth lipopolysaccharide

O-PS = O polysaccharide



RBT; CFT; iELISA and cELISA use S/LPS or O/PS as antigens

therefore, these tests are not fully specific  
in presence of cross-reactin bacteria

# The problem of False Positive Serological Reactions (FPSR) in swine Brucellosis

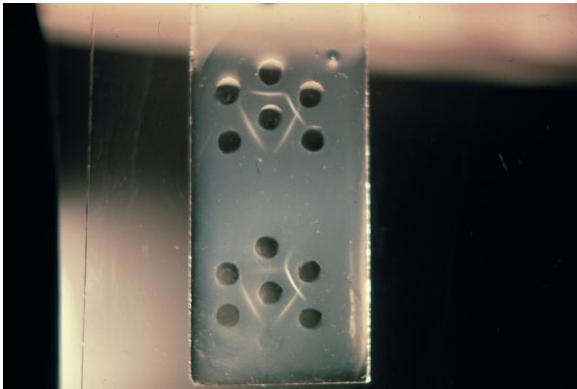
cellosis in diagnosis and the consequent effect on the export trade. Great Britain has always been free from *B. suis* infection and enjoys a thriving export trade as a result of the generally high health status of its stock. During the 7 years prior to 1988, the number of pigs tested for export certification giving a CFT reaction of greater than 20 international complement-fixation test units (icftu) never exceeded 0.004%, whereas the figures for 1988, 1989, and 1990 were 0.42%, 0.70%, and 1.5%, respectively. Since 1988, at least 4% of exporting herds have had more than 5% CFT positive reactions, with some herds reaching levels of more than 50% of animals tested failing at this level. *Y. enterocolitica* O:9 has been isolated from many herds involved, and despite extensive investigation, *B. suis* has not been recovered (Wrathall et al. 1991).

FPSR

Diseases of swine, 9th Edition, Chapter 35 p609

# Diagnostic performance of brucellosis serological tests in pigs

Serological Tests	Sensitivity <sup>1</sup>	Specificity <sup>2</sup>	Relative Specificity <sup>3</sup>
S-LPS based tests			
Rose Bengal	93.21	98.52	56.88
Complement Fixation	73.46 ↓	99.75	73.61
iELISA ("I.B Porcine", Ingenasa)	95.06	99.75	24.77
Gel Immunodiffusion	67.90 ↓	100	96.79



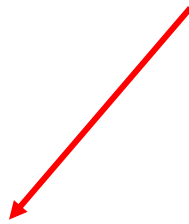
1 Calculated with 162 sera from *B. suis* biovar 2 culture positive sows

2 Calculated with 406 sera from *Brucella* free sows

3 Calculated with 218 sera of pigs from *Brucella* free herds affected by FPSR

# MAIN CONSEQUENCE of FPSR

misinterpreting and under evaluating serological results



Increased risk of  
Brucellosis  
dissemination

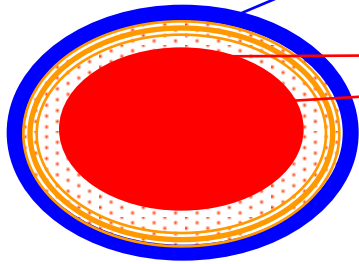


Increased prevalence  
of FPSR



**Cytosolic & periplasmic proteins**

**S-LPS /O-PS share *Y. enterocolitica* O:9 epitopes**



*B. suis*

**CYTOSOLIC-PERIPLASMIC PROTEINS  
(BRUCELLIN)**

**Are fully (mostly) genus-specific, and not shared with  
*Y. enterocolitica* O:9**

**can solve the problem  
of FPSR**

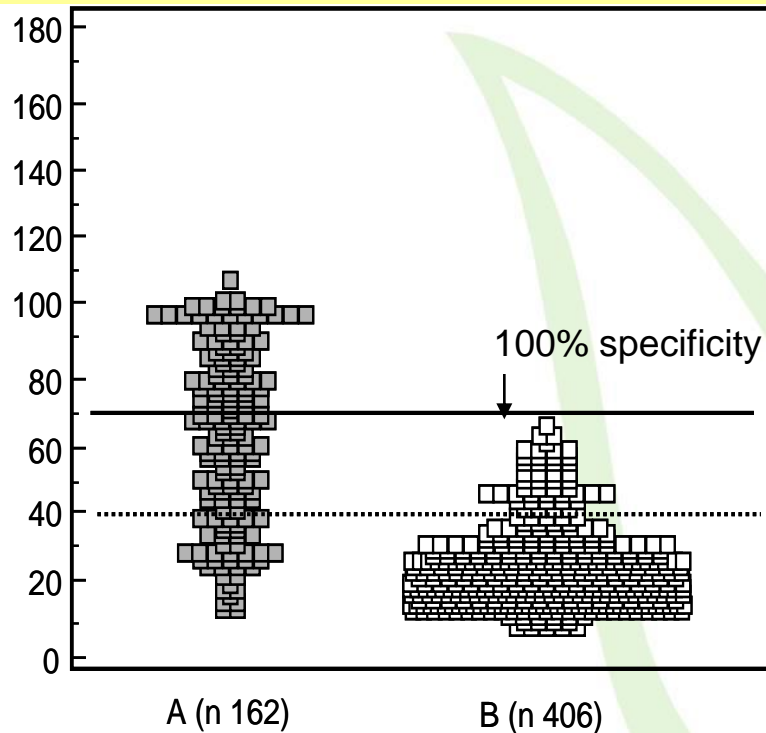
***in vitro* tests:  
ELISA**

**Gel Immunodiffusion**

***in vivo* test  
Skin test (DTH)  
(herd level)**

# I-ELISA tests using other antigens...

## ELISA with cytosolic proteins (brucellin)



Diagnostic  
sensitivity  
under 50%

- A: sera from *B. suis* biovar. 2 culture positive animals
- B: sera from Brucellosis free farms

----- cut-off resulting in the maximal Youden index ( $S_n + S_p - 1$ )  
----- cut offs allowing 100% specificity

Protein G-peroxidase conjugate and ABTS substrate

# Diagnostic performance of brucellosis serological tests in pigs

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Cytosolic Proteins based tests			
Gel Immunodiffusion	62.96	100	100
iELISA	45.06	100	100

Using gel Immunodiffusion test with S-LPS and CP in parallel we reached a combined sensitivity of 86%. Therefore this can be an useful strategy at herd level

1 Calculated with 162 sera from *B. suis* biovar 2 culture positive sows

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# The Brucellin skin-test (in vivo)



# The Brucellin skin-test (in vivo)

*Dieste-Pérez et al., 2014. Veterinary Microbiology*

	Nr. Animals	Skin Test	RBT	CFT	iELISA
Sensitivity	50	100	98	88	100
Specificity	245	100	98	99.6	99.6
Apparent prevalence in infected farm	184	41.3	33.2	25	40.8
Sp in FPSR context	31	100	41.9	74.2	19.4

High correlation with RBT and iELISA

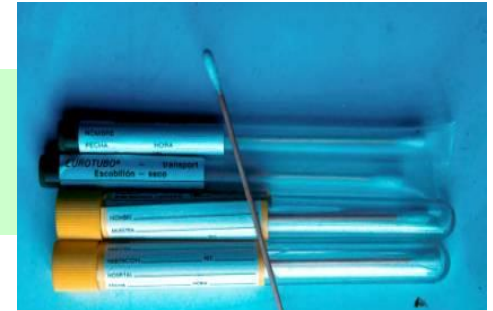
**High sensitivity in infected pigs and 100% specificity in *Brucella* free herds (including those affected by FPSR)**

No sensitization or induction of anti- O/PS antibodies were produced after repeated inoculations of this allergen

# Samples from alive animals for *B. suis* isolation

## Females

Vaginal swabs (1-2 weeks after abortion)



Swabs

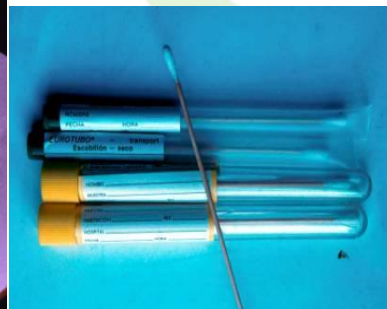
Milk (40 ml)

Change your gloves from one animal to another



Males

Semen (not diluted)

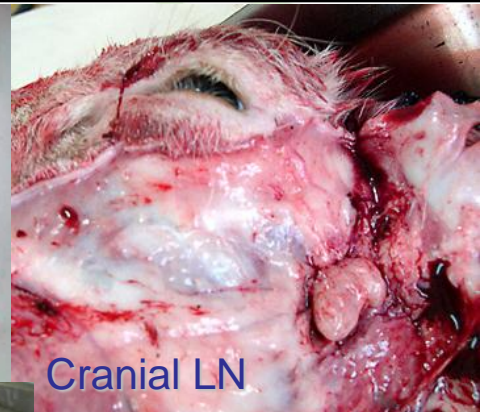
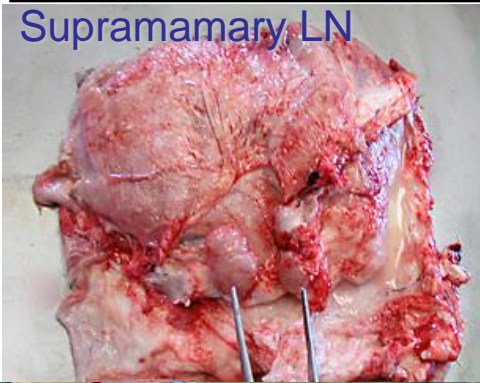


Always sera!

Blood: 5-10mL!!

# Necropsy samples

+



**Lymphnodes** (cranial, crural, mammary, testicular, Scapular...)

**Spleen**

**Mammary gland**

**Uterus**

**Epididymis**

**Sexual glands**

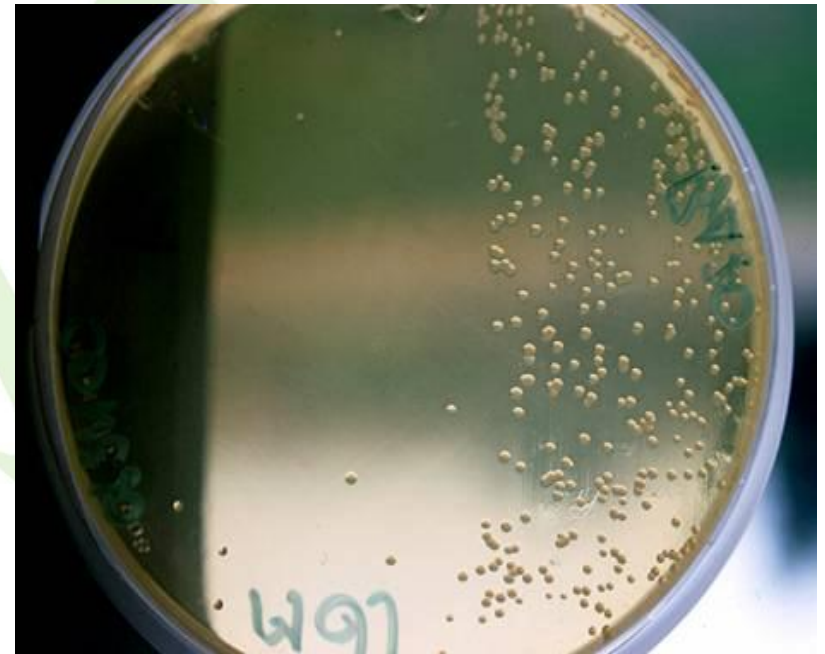


# Selective media used for *Brucella* isolation



## Farrell medium

*Farrell. Res.Vet.Sci.1974,16, 280-286*



## CITA medium

De Miguel et al, 2011 J. Clin. Microbiol . 2011, p. 1458–1463

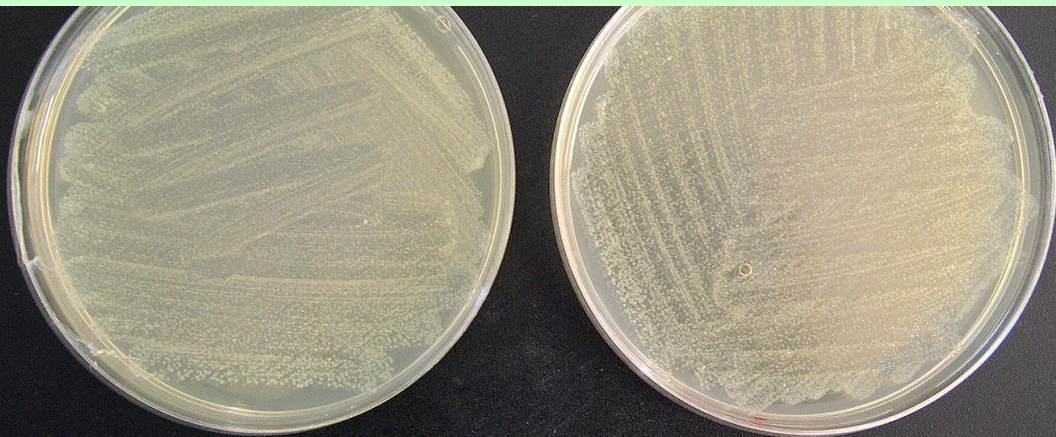
Combination giving maximum sensitivity for *B. suis* isolation

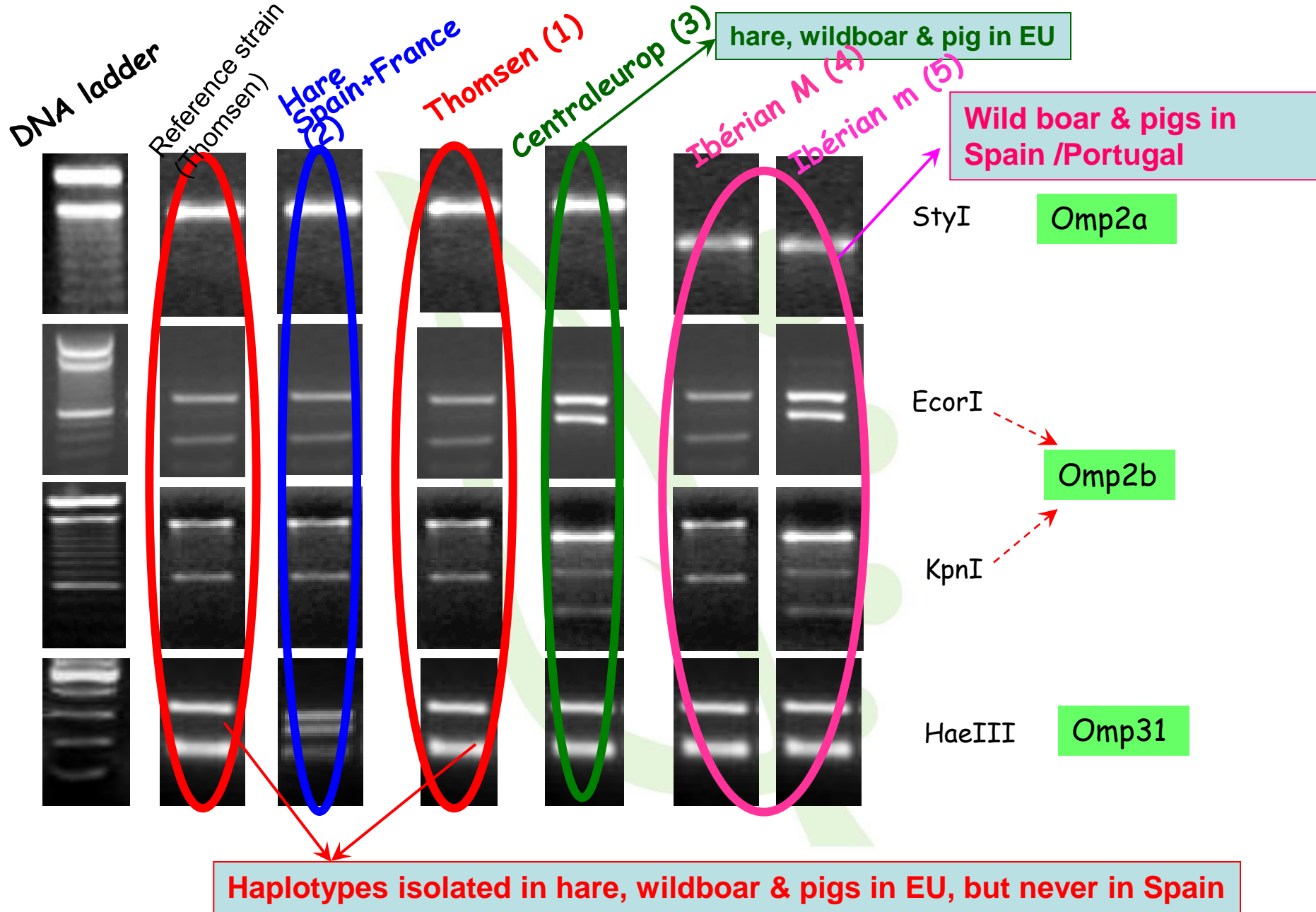




The isolation of the bacteria allows:

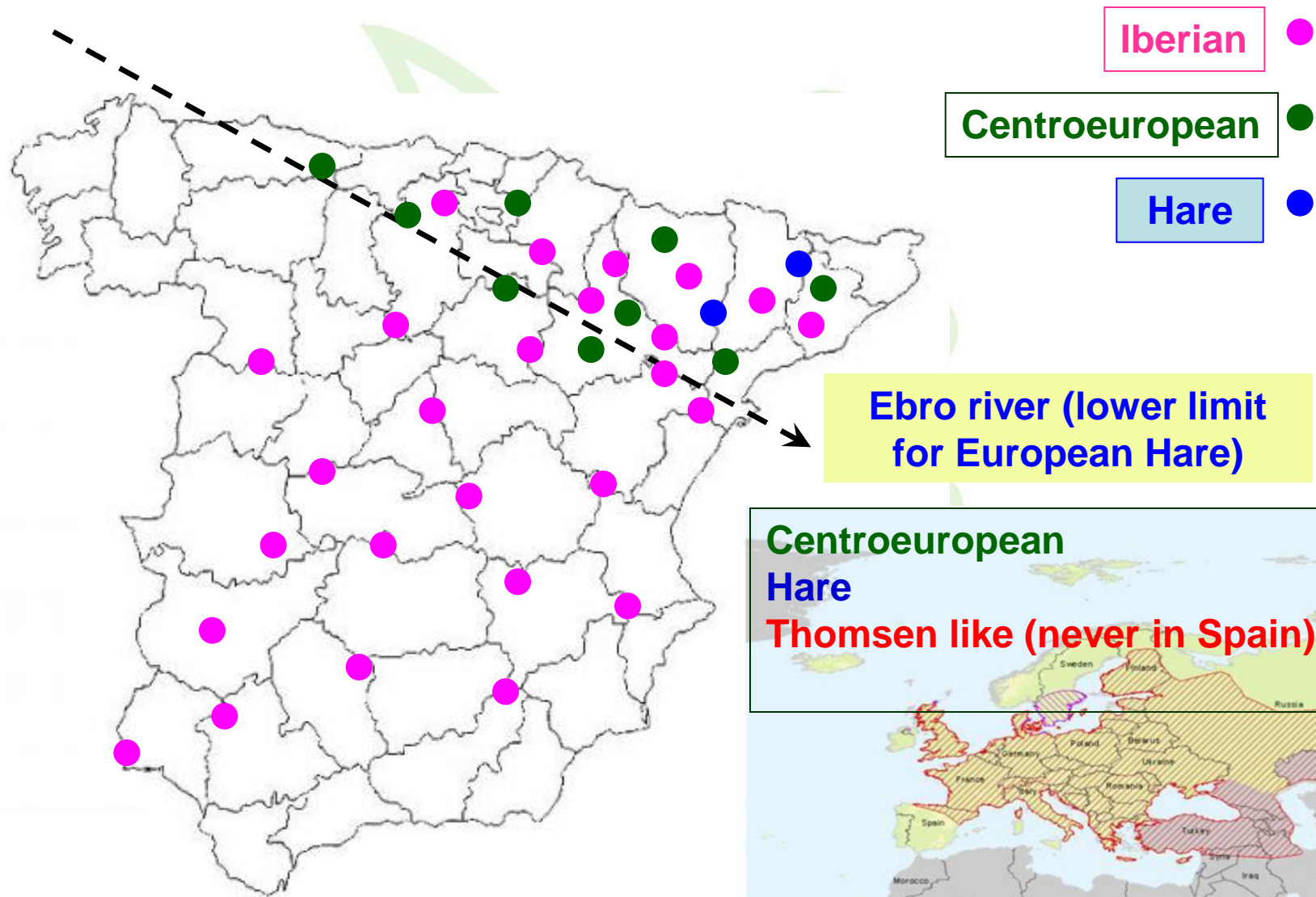
- ❖ Confirmation of the outbreak
- ❖ Epidemiological studies...





***Brucella suis* bv. 2 haplotypes (OMP PCR-RFLP) isolated in EU**

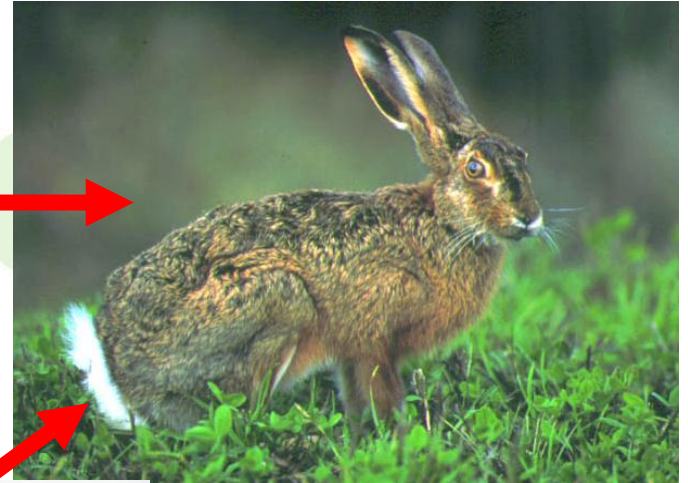
*B. suis* biovar 2 haplotypes distribution in wildboar (*Sus scrofa*), hare (*L. europaeus*) and pig in Spain



# *B. suis* biovar 2 epidemiology



Wild Boar



Sexy Pigs



**Thank you for  
your attention**



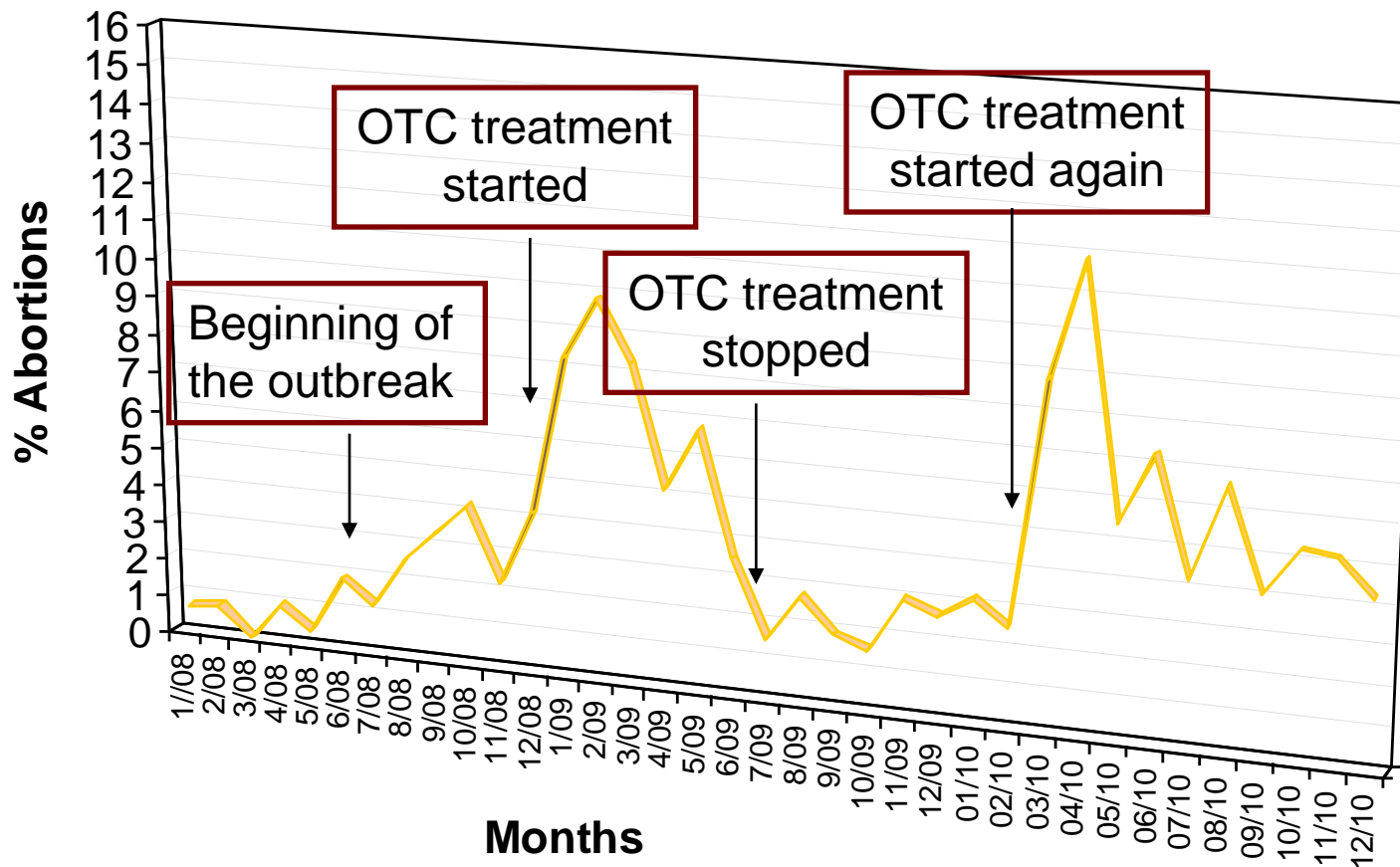
# How to deal with the problem in my farm?



- There are not effective vaccines for swine
- Depopulation? Not always possible! (think on companies with many affected farms or very large farms infected)

# Can we treat swine brucellosis?

Oxytetracyclin (OTC): 20 mg/Kg per day **during 1,5-2 years!!**  
(**cost = 19.5 €/year**)



# Antibiotic treatment of *B. suis* biovar 2 infection in pigs

Combined treatment:

OTC in pelleted feed (2000 ppm), i.e, 20 mg/Kg BW/day aprox. during 21 days + Tildipirosin (Zuprevo ®), two IM doses (4 mg/kg BW) with 10 days interval. Tildipirosin cost = 40€

Treatment	Ner. Cured sows	Infected organs	UFC per infected organ
OTC + Tildipirosin	8/8	none	0

This treatments has been used in three infected farms with satisfactory results

*Dieste-Pérez et al., 2014. Veterinary Pharmacology and Therapeutics*



# Possible Control Strategies

## WAY 1

Identifying  
infection source

Is it possible to  
prevent further  
infections?

YES

NO

Total depopulation is  
economically or practically  
unfeasible

## WAY 2

**Regular monitoring for early  
detection of infection and  
treatment with antibiotics**  
+  
**Partial depopulation, grouping  
by positive and negative  
animals (skin test + serology),  
and using only the negative  
group for replacing**

**We must learn to live with  
the disease whilst trying to  
minimize its effects**

# PROPHYLAXIS

## Go to the origin: reduce prevalence in wildboar

- No vaccines (and although having....)
- Eradication (test & slaughter) impossible (ethically and practically)

- Limiting the population density (avoid supplementary feeding and increasing hunting pressure)

- Limit the possibility of contact between wild boar and pigs (biosecurity and fences)

# Electric fence (minimum = 1.70 cm alto)



**Fine mesh in the bottom  
(hares)**

**Ideally buried (50cm) to  
avoid digging**