Brucellosis: Implementation of control strategies to prevent animal and human infections

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Factors accounting for brucellosis increase in fast growing economies and developing countries

- **Exponential growth** of livestock numbers, directly related to the economical growth, because of the preference for food of animal origin.
- **Extensive & nomadic breeding systems** dominant, with many domestic & wildlife species potentially involved, and in which brucellosis epidemiology is mostly **unknown**.
- **Lack of experience** of vet. Services in disease control and eradication, aggravated by the **practical absence** of true skilled international experts.
- Under poverty, brucellosis is **only one disease more in the list**, then unreported, misdiagnosed and neglected.
1. *B. melitensis* (bv 1 and 3) is GLOBALLY the most important zoonotic agent and causes also huge economical losses in animals

2. *B. suis* (bv 1 and 3) is the second, but can be the first in many countries because the high zoonotic and economical importance, and because this infection is mostly (fully) neglected

   (*B. suis* biovar 2 is also of high economical importance in domestic swine in the EU but not relevant as zoonotic agent)

3. *B. abortus* is the third in the ranking, having moderate zoonotic effect but high economical impact, particularly in fast growing countries with a lack of biosafety experience in livestock intensification
- No available vaccines

- Awareness campaigns are suitable to reduce the impact, but insufficient to prevent the problem

- Most cases transmitted by eating animal products (FOOD SAFETY important) and/or direct contact with animals

Controlling/eradicating the disease in animals is the most suitable way to prevent/eliminate the disease in humans
BRUCELLOSIS IS NOT A SOURCE FOR GOOD BUSINESS ACTIVITY OF PRIVATE SECTOR

↓

AN OFFICIAL INTERVENTION IS COMPULSORY
Part 1. Swine Brucellosis

**Brucella suis**

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

- **Non (few) zoonotic**
  - B. suis biovar 2
    - 200 mill.
  - B. suis biovars 1 and 3
    - 550 mill.

- **Highly zoonotic**
  - B. suis biovars 1 and 3
    - China
      - Malaysia
    - French Polynesia (25%)
    - Indonesia
    - Vietnam
    - 550 mill.
  - U.S.A
    - Argentina
      - Cuba
    - Uruguay
      - México
    - Canada
      - Ecuador....
    - 150 mill.

Prevalence unknown or underestimated
SITUATION IN ASIA AND AMERICA

- Biovars 1 and 3 prevalent (**highly zoonotic**)
- Lack of data in most countries (except USA): **FULLY neglected**

### MAIN PROBLEMS
- Full ignorance of epidemiology and the real impact
- Lack of adequate formation (veterinarians\&physicians)
- Practical absence of true swine brucellosis experts (**worldwide problem**)  
- Extensive or smallholder breeding systems in most countries
- Inability to control feral pig populations (USA)

<table>
<thead>
<tr>
<th>Country</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>470,960,950</td>
</tr>
<tr>
<td>Korea</td>
<td>10,525,000</td>
</tr>
<tr>
<td>India</td>
<td>9,500,000</td>
</tr>
<tr>
<td>Indonesia</td>
<td>7,758,000</td>
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<tr>
<td>Japan</td>
<td>9,768,000</td>
</tr>
<tr>
<td>Malaysia</td>
<td>1,695,000</td>
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<tr>
<td>Myanmar</td>
<td>9,416,360</td>
</tr>
<tr>
<td>Nepal</td>
<td>1,108,470</td>
</tr>
<tr>
<td>Papua Guinea</td>
<td>1,785,000</td>
</tr>
<tr>
<td>Philippines</td>
<td>12,303,100</td>
</tr>
<tr>
<td>Thailand</td>
<td>7,660,000</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>27,056,000</td>
</tr>
<tr>
<td>Cambodia</td>
<td>2,000,000</td>
</tr>
<tr>
<td></td>
<td><strong>Ca 550 mill</strong></td>
</tr>
<tr>
<td>Argentina</td>
<td>2,350,000</td>
</tr>
<tr>
<td>Bolivia</td>
<td>2,712,800</td>
</tr>
<tr>
<td>Brazil</td>
<td>39,306,700</td>
</tr>
<tr>
<td>Venezuela</td>
<td>3,450,000</td>
</tr>
<tr>
<td>USA</td>
<td>66,361,000</td>
</tr>
<tr>
<td>Paraguay</td>
<td>1,241,030</td>
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<tr>
<td>Peru</td>
<td>3,263,250</td>
</tr>
<tr>
<td>Mexico</td>
<td>15,547,300</td>
</tr>
<tr>
<td>Haiti</td>
<td>1,001,000</td>
</tr>
<tr>
<td>Guatemala</td>
<td>2,798,590</td>
</tr>
<tr>
<td>Ecuador</td>
<td>1,831,070</td>
</tr>
<tr>
<td>Cuba</td>
<td>1,518,000</td>
</tr>
<tr>
<td>Colombia</td>
<td>1,900,000</td>
</tr>
<tr>
<td>Chile</td>
<td>2,824,460</td>
</tr>
<tr>
<td></td>
<td><strong>Ca. 150 mill</strong></td>
</tr>
</tbody>
</table>
Epidemiology of swine brucellosis due *B. suis* biovars 1/3

Wild reservoirs?

Mostly UNKNOWN

established endemically in smallholder-backyard farms (representing ca. 70% of pig production in Asia)

Abortions, genital lesions and Infertility

can be introduced in intensive farms due to biosafety failures and the poor performance of diagnostic tests

*B. suis* bv 1 and 3 are highly pathogenic for humans
Swine brucellosis due *B. suis* to biovar 2 is emerging in Europe

Mostly in outdoor breeding systems or backyard farms as consequence of spillover from wild reservoir

25-50% prevalence

- Abortions
- Infertility
- Genital lesions

*B. suis* bv 2 is not (or few) pathogenic for humans

Brucellosis can be introduced in intensive farms due to the poor performance of diagnostic tests
B. suis biovar 2 EPIDEMIOLOGY

Wild Boar

??

?
Abortion (5–30%; 70–95 days) and increased perinatal mortality

Infertility

Arthritis
Endometritis

Orchitis

Reduced fertility

Not pathognomonic
DIRECT (Bacteriological) ⇒ essential for confirmation

INDIRECT

- Antibody detection (SEROLOGICAL)
  - Detection of cellular immunoreponses
    - *in vivo* (SKIN TEST) ⇒ BRUCELLIN TEST
    - *in vitro*:
      - Blastogenesis
      - detecting IL / IFNg

LABORATORY DIAGNOSIS REQUIRED
INDIRECT DIAGNOSTIC TESTS

EXTREMELY SIMPLISTIC ("ADMINISTRATIVE") CLASSIFICATION (INTERPRETATION) OF BRUCELLOSIS TESTS:

- SCREENING
- CONFIRMATORY

HOWEVER, SCIENTIFICALLY SPEAKING, NONE OF SEROLOGICAL TESTS CAN CONFIRM THE DISEASE

CONFIRMATION DEPENDS EXCLUSIVELY ON BACTERIOLOGY
The immunological test labyrinth in swine brucellosis

RBT, SAT, CFT ⇒ official in UE, and are OIE prescribed tests for trade (OIE Manual)

i-cELISA, FPA ⇒ are also OIE prescribed tests

Brucellin ⇒ used sometimes in the EU and appears in “other tests” in the OIE Manual but not prescribed for trade
Tests prescribed for trade are not accurate for individual diagnosis

- **Moderate** sensitivity (**CFT** in particular)

- **Lack of specificity**: **False Positive** **Serological Responses**
The problem of FPSR

Cellulosis 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).
Specificity of RBT in OBF regions in France

• 1.017 herds
• 7.814 sows
  - sows+ : 2.77%
  - herds+ : 13.3%

A similar situation in Spain
The origin of FPSR problem

B. suis

common O-PS epitopes

Y. enterocolitica O:9

Escherichia coli O157
Salmonella N (O:30)
Vibrio cholerae O:1
Stenotrophomonas maltophilia strains
Escherichia hermani strains
Brucellosis tests detect mostly antibodies against O-PS.

Then, none is able to differentiate brucellosis from FPSR.
MAIN CONSEQUENCES

Veterinary Services under-estimate serological results

Increased risk of Brucellosis dissemination

Increased prevalence of FPSR
Veterinary Services over-estimate serological results

False declaration of disease and unnecessary restrictions for trade
Each arrow represents a trade link
Line thickness proportional to log10 (#consignments)
The only solution: Tests using cytosoluble Proteins

S-LPS common O/PS epitopes with \textit{Y. enterocolitica} O:9

**CYTOSOLIC-PERIPLASMIC PROTEINS (BRUCELLIN) ARE SPECIFIC**

- close phylogenetic relatives (\textit{Rhizobiaceae} and \textit{\alpha-2 Proteobacteria})
- no crossreactions?

**can solve FPSR**

- serological tests
- moderate results

- CMI tests \textit{in vitro}
- IL/IFNg
- poor results

- CMI \textit{in vivo}
- Skin Test (DTH)
- (the best option)
**BRUCELLIN SKIN TEST**

• GOOD Sensit./Spec. ratios in ruminants and pigs
• Differentiate brucellosis from *Y. enterocolitica* O:9 infections in pigs

BUT.....not registered specifically for swine, manufacturing frequently discontinued, very expensive (2 euros/dose)....and a potential problem (strain Bm 115B contains O/PS biosynthetic precursors)
Performance of skin tests with allergens from *B. melitensis* B115 and rough *B. abortus* mutants for diagnosing swine brucellosis

L. Dieste-Pérez\(^a\), J.M. Blasco\(^a\), M.J. De Miguel\(^a\), C.M. Marín\(^a\), M. Barberán\(^b\), R. Conde-Álvarez\(^c\), I. Moriyón\(^c\), P.M. Muñoz\(^a,\ast\)
Inoc. ID (50-100μg/0.1 ml)
(cleaning inoculating area with soap)

48 h. after inoculation
(no skin measurements required)
Palpation advisable
Several inflammatory reactions from mild to moderate with hyperaemia
severe inflammation with haemorrhage

Easy to interpret (ca. 100% agreement among 6 vets in 8 different assays)
How dealing with the problem in infected farms?

- *No vaccines available for swine (or are useless - S2 in China)*

- **Depopulation**: Frequently impossible
  - companies with many affected farms or very large premises infected
  - endangered breeds
  - smallholders
Can swine brucellosis be treated?

Oxytetracycline (OTC): 20 mg/Kg/day given orally during 1 - 2 years!! (cost = 19.5 €/year)

OTC treatment started
OTC treatment started again
Beginning of the outbreak
OTC treatment stopped
Withdrawal time: 21 days

% Abortions
Months
Blasco et al, unpublished
Treatment of infection by *B. suis* biovar 2

Bacteriological results after treatment of *B. suis* bv 2 naturally infected sows with oxytetracycline (OXT) given alone (20 mg/kg BW/day, orally for 21 days), or combined with tildipirosin - Zuprevo- (two intramuscular administrations of 4 mg/kg BW) at the first and tenth day of treatment (OXT / TIL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Infected sows / total</th>
<th>Infected samples / total</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXT</td>
<td>4 / 8 (50)</td>
<td>13 / 56 (23.2)</td>
</tr>
<tr>
<td>OXT + TIL</td>
<td>0 / 8 (0)</td>
<td>0 / 56 (0)</td>
</tr>
</tbody>
</table>

Cost = c.a. 20 €/animal  
Withdrawal time: 47 days

*(Dieste et al., in press)*
Eradication/Control Strategies

ERADICATION

Identifying infection source

Is it possible to prevent further infections?

YES

Total depopulation

FEASIBLE?

depopulation and replace with OBF pigs

NO

CONTROL

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

learn to live with the disease BUT minimizing its effects
**PROPHYLAXIS**

Biovars 1 and 3: ??

**URGENT need of studies to clarify epidemiology and true prevalence**

- No suitable vaccines available
- Eradication (test & slaughter) unfeasible

- Intensifying swine production **BUT** under strict biosafety conditions

- Few (if any) alternatives in small familiar back-yard farms
- Epidemiology well known = wild reservoirs
- No eradication possible in wild reservoirs
- No vaccines available neither for domestic pigs nor wild reservoirs

- Identify the existence of the problem
- Limit the possibility of contact between wild boar and pigs (biosafety and fences)
Electric fence (minimum = 1.70 cm high)

- Fine mesh in the bottom (hares)
- Ideally buried (50cm) to avoid digging
1. Swine brucellosis due to \textit{B. suis} biovars 1 and 3 is a fully-or almost-neglected disease needing many epidemiological research to clarify its true prevalence and how it is transmitted and maintained.

2. Current serological tests are not specific, and only bacteriology and brucellin skin test can differentiate brucellosis from FPSR.

3. No vaccines are available (and if developed should be of DIVA nature).

4. Intensification and suitable biosafey (and fencing -outdoor farms-) are essential for adequate prophylaxis.

5. Full depopulation is the only available strategy for eradication.

6. Antibiotic treatment could minimise the economical impact of disease in affected farms.