# PRODUCTION and CONTROL OF VACCINE AGAINST ABORTION CAUSED BY *COXIELLA BURNETII* IN RUMINANTS

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# Background



- Coxiella – zoonosis
- Disease prevalence is underestimated *(Rousset et al., 2007)*
- More and more cases in animals (*Arricau-Bouvery and Rodolakis, 2005*)
  - Refined diagnostic methods such as ELISA and PCR
- The risk of human infection is increasing *(Arricau-Bouvery and Rodolakis, 2005, Schimmer et al., 2009)*

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## Seroprevalence of Q-fever in animals

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*(Arricau-Bouvery and Rodolakis, 2005)*

| COUNTRY | YEAR | NO. OF ANIMALS TESTED | SEROPREVALENCE (%) |
|---|---|---|---|
| **CATTLE** | | | |
| Cyprus | 2006 | 974 | 24 % |
| Chad | 1999-2000 | 195 | 4 % |
| Turkey | 1998 | 416 | 6 % |
| Germany | 1998 | 21191 | 8 % |
| Italy | 1998 | 1185 | 11.6 % |
| **SHEEP** | | | |
| Cyprus | 2006 | 974 | 18.9 % |
| Italy | 1999-2002 | 7194 | 9 % |
| Chad | 1999-2003 | 142 | 11 % |
| Germany | 1999 | 1346 | 1.3 % |
| Germany | 1999 | 100 | 57 % |
| Turkey | 1998 | 411 | 10.5 % |

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# Seroprevalence of Q-fever in animals (continued)



*(Arricau-Bouvery and Rodolakis, 2005)*

| COUNTRY | YEAR | NO. OF ANIMALS TESTED | SEROPREVALENCE (%) |
|---|---|---|---|
| GOAT | | | |
| Cyprus | 2006 | 974 | 48.2 % |
| Italy | 1999-2002 | 2155 | 13 % |
| Chad | 1999-2000 | 134 | 13 % |
| Germany | 1998 | 278 | 2.5 % |
| OTHER | | | |
| Japan | 2003 | 310 pet cats | 14 % |
| Japan | 2003 | 36 stray cats | 42 % |
| Korea | 2003 | 116 pet cats | 9 % |
| Canada | 1993 | Hare | 49 % |
| Canada | 1993 | Moose | 16.5 % |
| Canada | 1993 | Racoon | 7.1 % |
| Canada | 1993 | Deer | 1.5 % |

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# Seroprevalence of Q-fever in humans

*(Arricau-Bouvery and Rodolakis, 2005, Schimmer et al., 2009)*

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| COUNTRY | YEAR | NO. OF EXPOSED PERSONS | SEROPREVALENCE (%) |
|---|---|---|---|
| Poland | 2003 | 90 (farming)<br>30 (urban) | 18 %<br>0 % |
| Canary Island | 2003 | 662 | 36 % |
| Japan | 2001 | 267 (veterinarian)<br>352 (Medical workers)<br>2003 (blood donors) | 13.5 %<br>5 %<br>4 % |
| Japan | 2000 | 200 (pregnant) | 4 % |
| Taiwan | 2000 | 616 | 4 % |
| Chad | 1999-2000 | 368 | 1 % |
| Turkey | 1998 | 102 | 8 % |
| Canada | 1997-1998 | 7658 (pregnant) | 4 % |
| Spain | 1996-1997 | 1654 | 5 % |
| France | 1996 | 12716 | 0.15 % |
| France | 1995 | 790 (blood donors) | 0.4 % |
| France | 1996 | 620 (blood donors)<br>785 (general population) | 3 %<br>3 % |
| Netherlands | 2007<br>2008<br>2009 | 167<br>1000 (associated with dairy goat farms)<br>333 | |

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# Transmission of the pathogen



In the moment of **abortion** or during **parturition** the ***placenta, foetal fluids, vaginal discharge, faeces*** and ***milk*** **are highly infective**

**Transmission**

***C. burnetii*** can survive long time in the environment (aerosol, dust)

– The main route is the aerogen dissemination; even to long distances (wind)

– Rarely, by bite of ticks

**Transmission to humans (zoonosis)**

## Prevention is needed

# Prevention



## Non-specific measures

- **Isolation of affected animals and their progeny**
- **Elimination of contagious material (e.g. placenta)**
- **Disinfection of the infected area**

## Specific measures

- **Antibiotherapy**
  - **Oxytetracycline and its derivatives can limit the abortions but it does not suppress the bacterial excretion** *(Berri et al., 2002; Astobiza et al., 2009)*
- **Vaccination**
  - **The most effective solution to control the infection**

# Existing vaccines



- **Q-Vax**

  Inactivated, purified whole cells of Henzerling strain of *C. burnetii* Phase I for human use

- **Chlamyvax FQ**

  A combined vaccine containing *C. burnetii* Phase II and *Chlamydophyla abortus* for animal use

- **Coxevac**

  Inactivated, purified whole cells of Nine Mile strain of *C. burnettii* Phase I for animal use (Under TMA)

# Production of vaccines



## Essential points

**Zoonotic agents** → Health risks for humans

***C. burnetii*: *Class III***

- Production of live material in confined Class III area
- Well-trained staff (vaccinated against Coxiellosis, pregnant women excluded)

- Detailed SOPs for each phase of production
- Control
  - In process
  - On finished product
- GMP Certificate
- Seed lot system

# Difference between phase I and II LPS

- The **only protective antigen** is the non-truncated **phase I LPS**
- Purified particles in **phase I** with the LPS structure of highly infectious form protects **100 - 200 times** more against challenge with virulent **phase I** *C. burnetii* than **phase II** vaccines

(*Ormsbee et al., 1964; OIE 2009*)

# Phase variation



**Related to mutational variation of LPS ( ~ smooth / rough variation)**

| | PHASE I | PHASE II |
|---|---|---|
| OCCURENCE | The natural phase in infected animals, arthropods, humans | Obtained in vitro only upon serial passages in cell culture or embryonated eggs |
| INFECTIVITY | High | Less |
| GENETIC DIFFERENCES | | Chromosomal deletions |
| DIFFERENCES IN PHENOTYPE | | • Truncated LPS<br>• Lack of some surface proteins<br>• Lack of some sugars (L-virenose, dihydroxystreptose, galactosamine uronyl-α-(1,6)-glucosamine) |

# Vaccine strains



***Coxiella burnetii* strains used so far in the existing phase I vaccines:**

- **Nine Mile phase I**
- **Henzerling phase I**

## Simplified flow chart

**The vaccine is produced in SPF eggs (specific pathogen-free)**

**Inoculation**
**6 - 7 day-old embryonated eggs**

↓

**Incubation during 8 - 9 days**

↓

**Harvest (egg yolk sac membrane)**

↓

**Homogenisation**

↓

**Control of titre**

**Class III area**

# Production of vaccine



## Simplified flow chart (continued):

***C. burnetii***

**Inactivation**

**Purification**
**(organic solvent and ultracfg)**

**Control & quantification of active ingredient**

**Preparation of vaccine suspension (Dilution)**

**Filling**

**Secondary packaging**

# Control of vaccines by Quality Standards

## Seed lot

- **Extraneous agents**
- **Purity:** direct inoculation in thioglycolate medium (TY) and tripcasein soy broth (TSB) medium
- **Infective titre:** $10^4$ $EID_{50}$/mL

## Antigen

- **Infective titre**
- **Purity**
- **Inactivation**
- **Concentration**
- **Phase**

## Finished product

- **Sterility**
- **Physico-chemical characteristics**
- **Potency**
- **Safety**
- **Inactivation**
- **Concentration**

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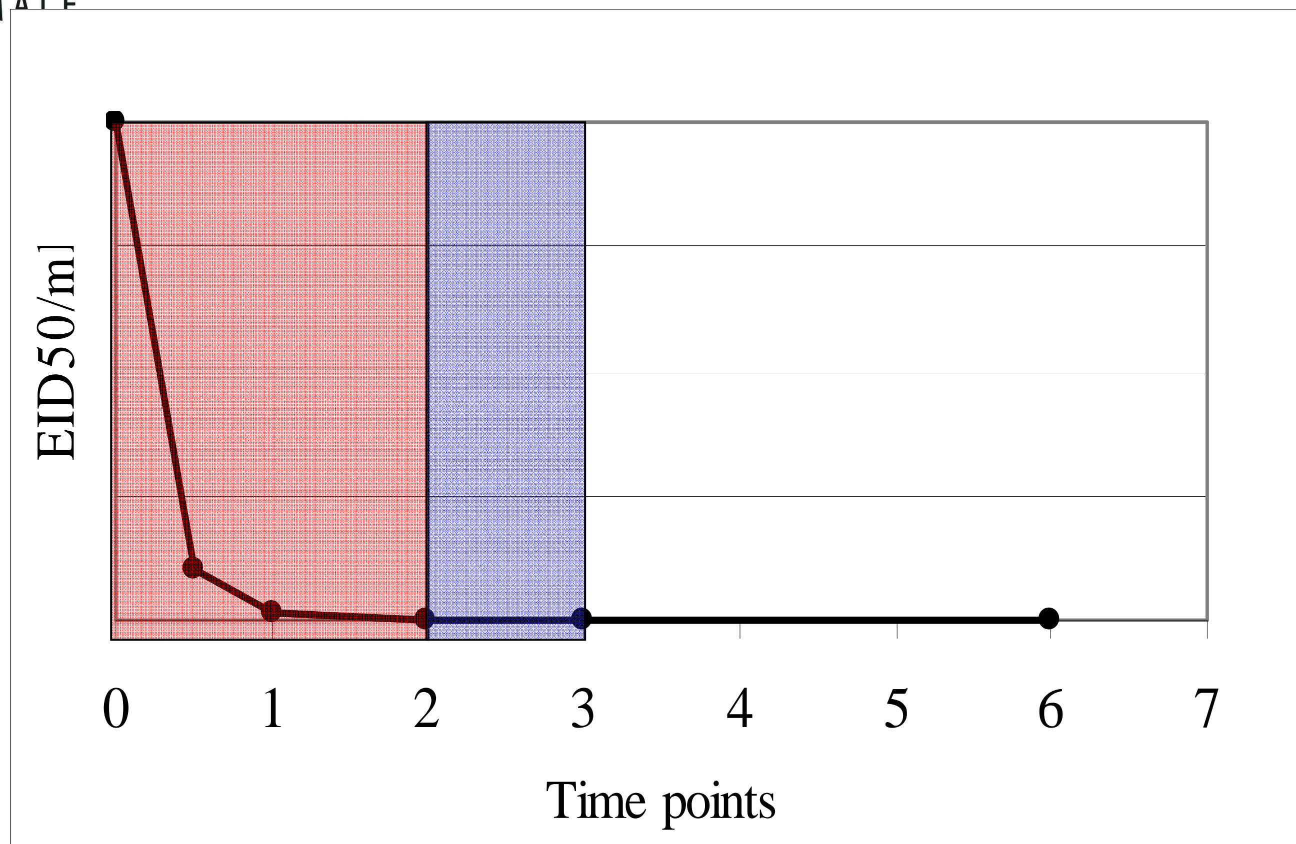
# Kinetics of inactivation





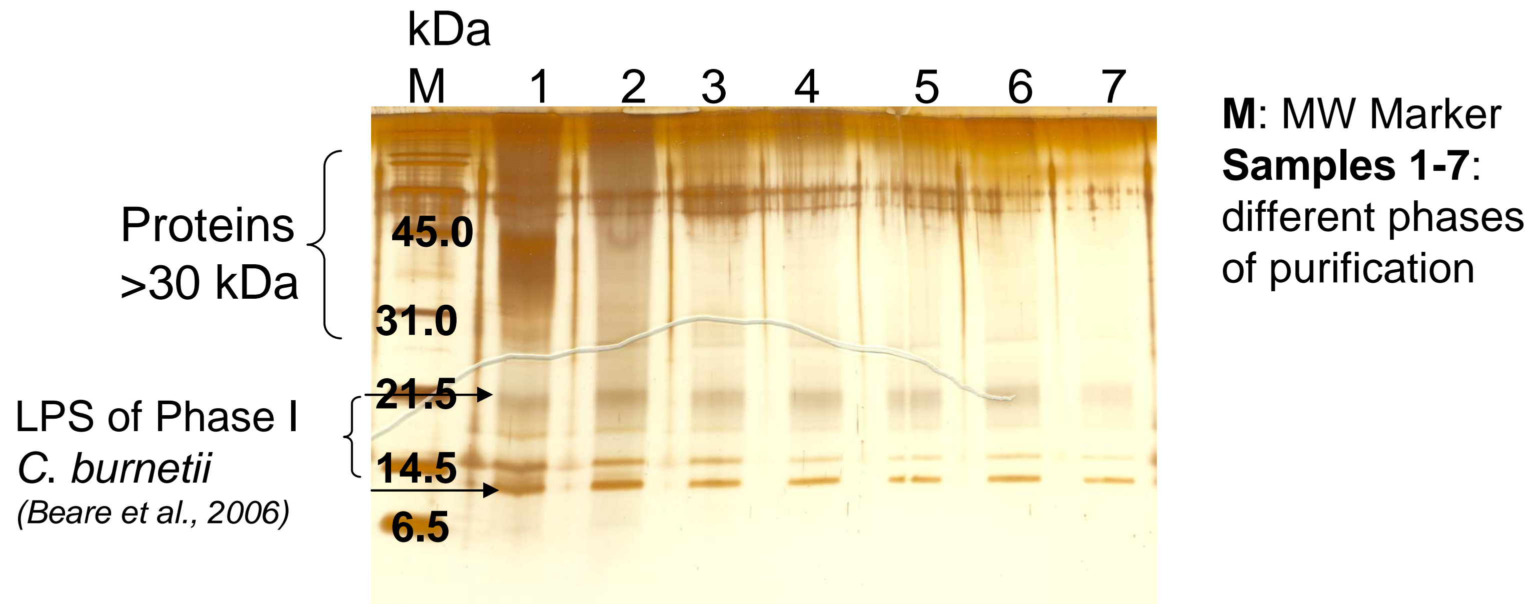

**Time of complete inactivation: 48 hours.**

**Time of inactivation with safety margin (+ 50%): 72 hours.**

# Control of egg-derived residuals



## *Test 1:* 15% SDS-PAGE, silver staining




**M**: MW Marker
**Samples 1-7**: different phases of purification

**During the purification process proteins bigger than 30 kDa are removed**

## *Test 2:* ELISA - Quantitative measurement of egg-derived material

# Summary of vaccine technology



- Protective antigen – full-length Phase I LPS
- Grown in eggs (better yield than cell culture) – harvest
- Inactivation
- Chemical process – purification steps with organic solvent
- Separation by ultracentrifuge
- Control for in-process and release

--------Control of inactivation

  - Quantitative antigen ELISA Phase I
  - Quantitative ELISA for the egg-derived mat measurement
- No adjuvant is used
- Lead time for production and control of one batch: min. 5 months (1 batch ~ 27,000 vials)

# Control efficacy / potency



## Efficacy control:

### 1. Challenge in pregnant animals:

- Goat (*Souriau et al., 2003*)
- Ewes (in collaboration with INRA - Nouzilly): data to be published

### 2. Follow-up of shedding parameter in cattle:

- non-pregnant vaccinated cattle had 5 times lower probability to become shedder than non-vaccinated (*Guatteo et al., 2008*)

## Potency:

- *C. burnetii* - specific serum ELISA – in lab animals
- Antigen ELISA (Phase I - specific)

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# Efficacy in the field



- Ultimately, the vaccine efficacy is proven under **field conditions**
  - Decreasing the prevalence & incidence
  - Pharmacovigilance

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# Perspectives



- Investigation of **cell mediated immunity** (CMI)

| CMI METHODS | MOUSE | RUMINANTS |
|---|---|---|
| IFN-γ ELISA | + | + |
| IFN-γ ELISPOT | + | + |
| Serum IgG1 vs. IgG2a | + | + |
| DTH (skin test) | - | + |
| T cell proliferation | + | + |

- Long term: investigation of **DIVA system**

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# Collaboration



- **Between human and veterinary medicine public institutions**

- **Between public institutions and industry**

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# Coxevac

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## THANK YOU FOR YOUR ATTENTION!

**Bacteriological Development Directorate**

**CEVA-Phylaxia, Budapest**