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Immunology of CBPP: current knowledge

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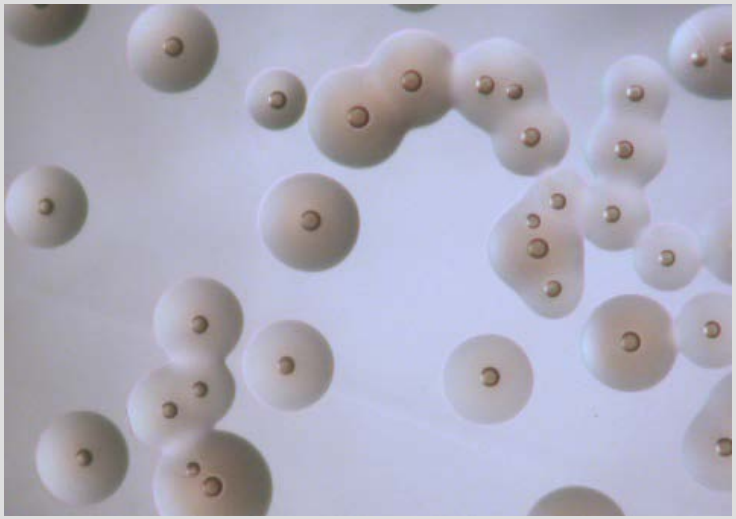
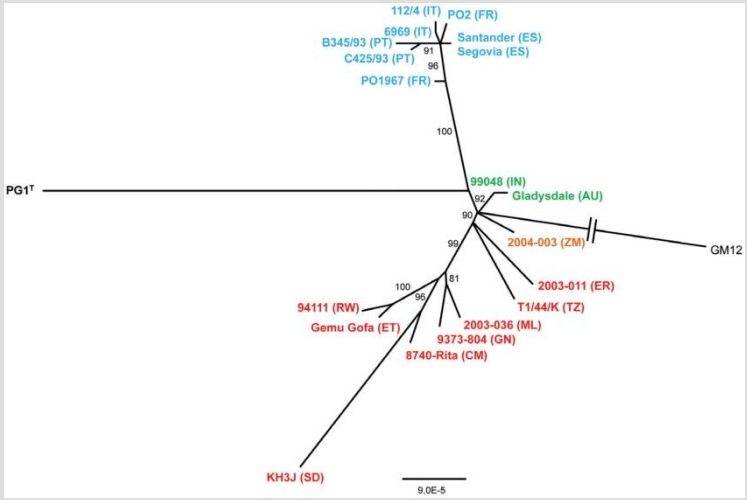
« Control of Emerging and Exotic Animal Diseases »

The pathogen

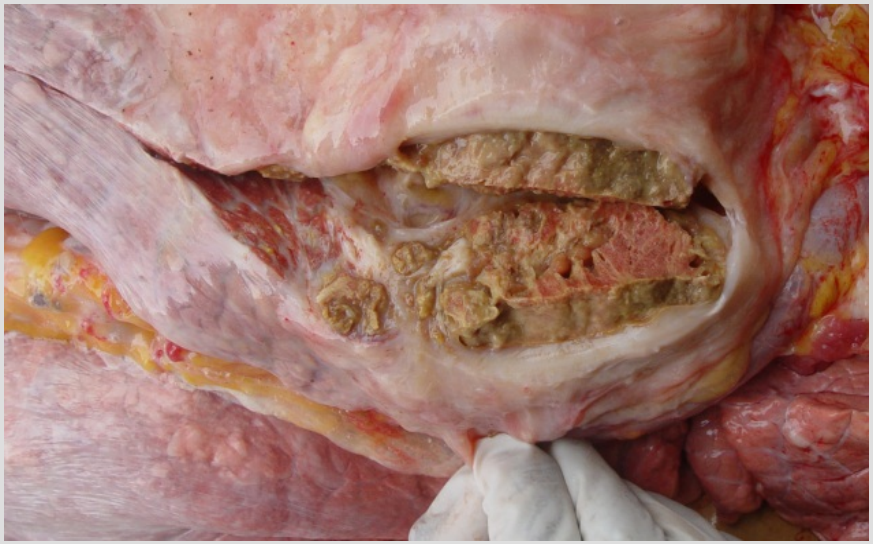
- *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) “SC”
- Class of *Mollicutes*
- ~~cell wall~~
- 2 genomes fully sequenced
- PG1 and Gladysdale
- 1,211 kb
- NGS based phylogeny
- Molecular dating (Dupuy et al 2012)



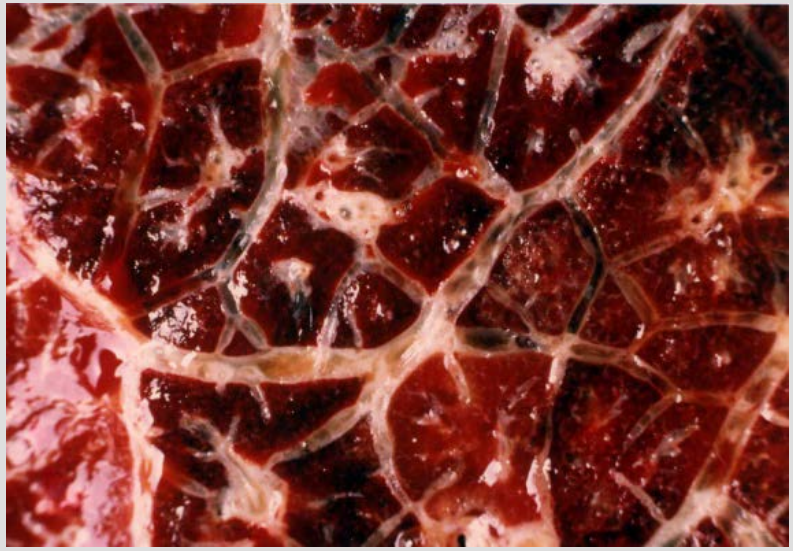


The disease

- contagious bovine pleuropneumonia (CBPP)
- Africa
- lymphatics / lungs



- chronic CBPP
- sequestrum
- long term persistance
- and excretion ?



- acute CBPP (15-30%)
- massive inflammation of lungs
- hepatisation
- pleurisy

Need for improved Vaccines

Currently: Live, empirically attenuated strains injected sub-cutaneously

Advantages

Relatively low production costs

Very long conservation at -20°C once freeze-dried

Easy administration (sub-cutaneous)

T1sr: completely safe

Transient sero-conversion (allows detection of outbreaks)

Repeated vaccinations result in good protection

Drawbacks

Thermolability (freeze-dried or reconstituted)

Freeze-drying needs industrial skill

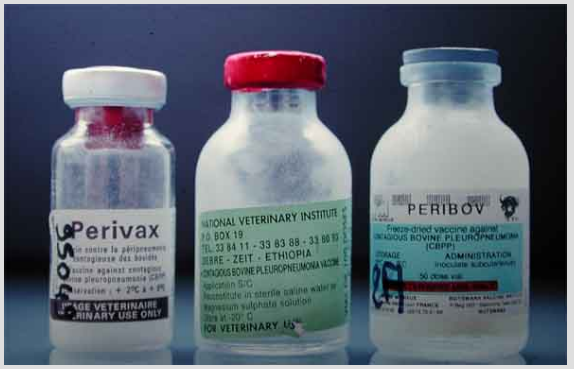
T1/44: some residual virulence (primo-vaccination)

Lack of sero-conversion does not allow sero-monitoring of vaccination campaigns

A single administration does not yield good protection

Protection is short-lived (T1sr: 6 months, T1/44: one year)

Eradication cannot be achieved with vaccination alone



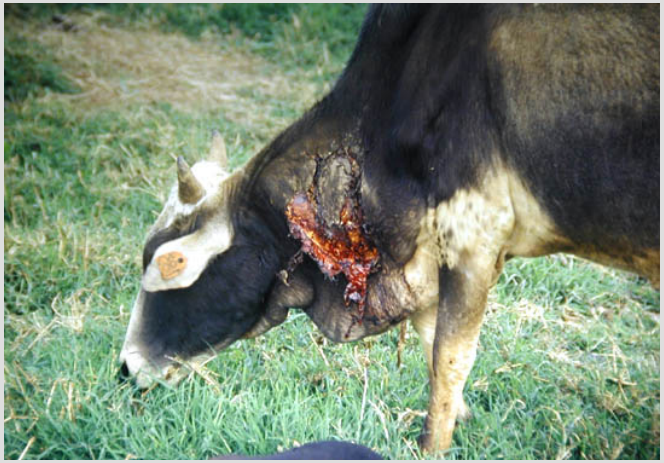
Willems reaction

With T1/44 (not T1sr)

In animals vaccinated for the first time

Appears 10-20 days post vaccination

0 to 5 % of vaccinees



Immunology of CBPP: current knowledge

1st part

	Innate immunity	Acquired immunity	
		Humoral immunity (antibodies)	Cell mediated immunity (T-cell responses)
Primary infection	+	+	+
After recovery		+	+
After vaccination		+	+
	Summary of Knowledge and gaps	Summary of Knowledge and gaps	Summary of Knowledge and gaps

2nd part

opportunities from the CBPP BEN1 vaccine project

Immunology of CBPP: current knowledge

Innate immunity

- **during primary infection (injection subcut/intubetion/contact)**

→ Lesions in lungs and locally (subcut) are typical of inflammation. Less severe when injection at the tip of the tail →importance of the lymphatic system

→ presence of myeloid cells (ilA24) in lesions.
(*Jores J et al.Vet Immunol Immunopathol 2008*)

→ TNF-α is produced in alveolar macrophages in response to both pathogenic and non pathogenic *MmmSC (in vitro)*
(*Jungi T et al. Microbial pathogenesis 1996)*

→ Archetypal inflammatory cytokines (pro and anti) are detected early in the plasma and at higher levels in acute cbpp (n=4). No effect of CD4+T cells depletion.
(*Sacchini F et al. BMC Vet Res 2012)*

Summary:

- **No endotoxins/ Mechanisms of inflammation (chimiokines)?**
- **Very little data on *MmmSC*-host cells interactions/ role of epithelial lung cells?**
- **Which cell types are producing proinflammatory cytokines ?**

Immunology of CBPP: current knowledge

Humoral immunity

- **during primary infection (contact challenge/intubation)**

→ Lesions size correlates with *Mmm*SC-specific IgM titers in serum (CFT, agglutination)

→ Detection of *MmmSC*-specific IgA in serum and bronchoalveaolar fluids (ELISA) of animals with less severe disease (n=5) / detectable for only 1-3 months in serum (*Niang M et al. Vet Res. 2006)*

→ IgG titers to 35 out of 65 recombinant surface proteins monitored (luminex) in 5 cattle (*Hamsten C et al. Clin Vaccine Immunol. 2010)*

→ higher early anti-*MmmSC* IgG1and G2 titers (ELISA) in severe cbpp after intubation (n=4) (*Sacchini F et al. BMC vet res 2012*)

Summary;

- **Possible role of immune complexes (IgM, IgG1, IgG2) in the pathology and IgA in protection**
- **Proteins targetted? → potential of luminex approach with individual surface proteins**

Immunology of CBPP: current knowledge

Humoral immunity

- **during primary infection (contact challenge/intubation)**
- **during vaccination (T144/T1sr)**

→ Weak antibody titers (CFT, cELISA) and most animals are negative after 3 months. No correlation with protection after contact challenge
(*Thiaucourt F, et al. Ann. N Y Acad Sci 2000)*

→ Strong IgG titers against 4 out of 65 recombinant surface proteins (luminex) with LppQ as potential indicator of protection after contact challenge (n=5)
(*Hamsten C et al. Clin Vaccine Immunol. 2010)*

Summary:

➢ **Potential of the luminex methodology to identify markers of vaccine success before challenge**

Immunology of CBPP: current knowledge

Cell mediated immunity (CMI)

recall = in vitro restimulation With killed MmmSC

- **during primary infection (contact/intubation challenge) / peripheral blood**

  → Sustained *MmmSC*-specific recall activation of CD4+T lymphocytes and IFN-γ production is associated with a better control of cbpp (*Dedieu L et al. Vet Immunol Immunopathol 2005*)

  → No correlation between *MmmSC*-specific recall IFN-γ production and protection after intubation. No build up of T-lymphocytes in lung lesions but presence of myeloid cells. (*Jores J et al. Vet Immunol Immunopathol 2008*)

  → Depletion studies indicate that removal of CD4+T lymphocytes has no incidence on disease progression (*Sacchini F et al. Vet Res 2011)*

Summary:

➢ **No role for CD4 in primary infection**

Immunology of CBPP: current knowledge

Cell mediated immunity (CMI)

recall = in vitro restimulation With killed MmmSC

- **during primary infection (contact/intubation challenge) / peripheral blood**

after recovery (fibrotic scars) / draining lymph nodes → memory + protection

→ *MmmSC*-specific recall activation of CD4+T lymphocytes but no proliferation (n=5) (*Dedieu L et al. Vet Res 2006*)

→ *MmmSC*-specific recall <u>proliferation</u> of B and CD4+T lymphocytes / strictly CD4-dependent / CD4 express memory markers, produce IFN-γ but no IL-4 (Th1 or Th17?) (*Totté et al. Vet Res 2008*)

→ *MmmSC*-specific memory CD4 comprise Tem (short lived) and Tcm (long lived) (*Totté et al. Dev Comp Immunol 2010)*

Summary:

- **Strong CD4 memory response after recovery → role in protection against secondary challenge ?**
- **Potential of CD4+Tcm as markers of protection as in bovine tuberculosis**

Effector memory (Tem) vs central memory (Tcm)

Tem

- blood-tissues-lymph circulation (ccr5, cxcl3)
- low proliferation
- strong IFN-γ response
- Months after infection/immunisation

local response to challenge but short-lived

Tcm

- blood-lymph circulation (cd62l, ccr7)
- high proliferation
- low IFN-γ response
- years after infection/immunisation
- differentiate into T effectors upon Ag encounter

Long-lived pool of effectors

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improved vaccines → **Tem + Tcm**

Strong challenge

long term immunity

Immunology of CBPP: current knowledge

Cell mediated immunity (CMI)

recall = in vitro restimulation With killed MmmSC

- **during primary infection (contact/intubation challenge) / peripheral blood**
- **after recovery (fibrotic scars) / draining lymph nodes → Immunological memory**

- **after vaccination (T144/T1sr) / peripheral blood**

→ no recall activation of lymphocytes in cattle vaccinated 2 months previously in the tail tip with a single dose of the T1 vaccine
(*Roberts DH, et al. Infect Immun 1973*)

→ *MmmSC*-specific recall IFN-γ and proliferation of CD4+Tem and Tcm but only after 3 shots of T1 vaccine (n=5) / lower Tcm response in comparison to animals that recovered from CBPP
(*Totté P et al. PloS One 2013*)==

- **Summary:**
- **Low T-cell immunogenicity of T1 vaccines**
- **Immunoinhibitory mechanisms?**

Immunology of CBPP: opportunities from the CBPP BEN1 vaccine project

- **confirm in larger animal groups data obtained previously**

→ IgG1, IgG2 and IgA responses against individual or groups of specific antigens as indicators of protection/pathology (ILRI)

→ Poor T-cell immunogenicity induced by live vaccines (ILRI)

→ Correlation between higher production of inflammatory cytokines early after challenge and disease severity (?)

Immunology of CBPP: opportunities from the CBPP BEN1 vaccine project

global = innate + acquired immunity

❖ **improve our current understanding of cbpp immunology**

→ characterize global immune protective mechanisms induced by attenuated CBPP vaccines (CIRAD+ILRI)

→ Involvement of various T-cells sub-types (Th orientation) in vaccinated animals undergoing challenge (ILRI+CIRAD)

→ characterize global immune responses and cell types involved in the pathology (HVRI+CIRAD)

→ Characterize the role of EBL in innate immune responses to *MmmSC* infection (HVRI)

Immunology of CBPP: opportunities from the CBPP BEN1 vaccine project

❖ **definition of markers that can predict vaccine efficacy after vaccination but before challenge (CIRAD+ILRI)**

→ Lessons from the bovine tuberculosis model (20 years of research on CMI in cattle and 10 years on correlates of protection)

➢ whole blood and T-cell based assays (recall IFN-γ response) are good markers of protection at group level but NOT at individual level

➢ IFN-γ cultured ELISPOT (Tcm) and intracellular INF-γ labeling in stimulated CD4 are good correlates of protection at individual level (R2=0.79 to 0.83) but complex and time consuming
(Hope JC et al. Clin Vaccine Immunol 2011)

➢ whole bovine genome transcriptomics has identified a 3-genes signature (*il-22, ifn-γ, mt3*) that can predict protection following vaccination (14 weeks) and before challenge → confirmed by rt-qPCR in independent experiment (n=16)
(Bhuju S et al. PLoS Pathog. 2012)

Thank you for your attention