Gap analysis workshop on Contagious Bovine Pleuropneumonia (CBPP), June 26th-28th, 2023, Wellcome-Sanger Institute, Hinxton, UK.

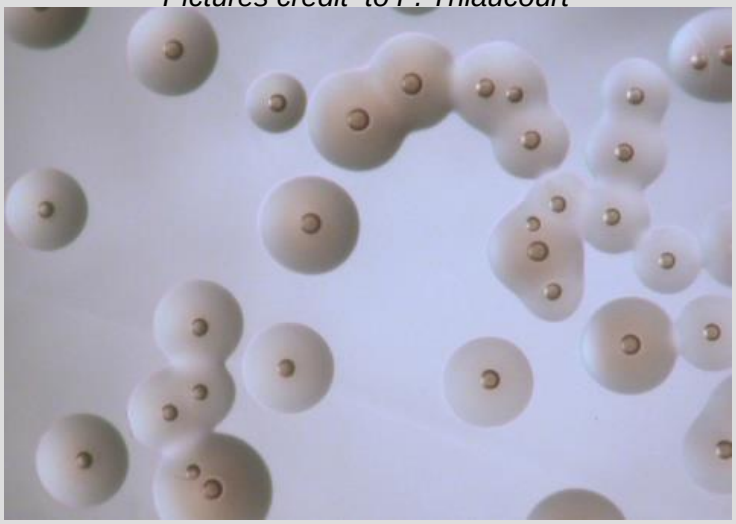
Immune responses associated with protection against CBPP and induced by immunization

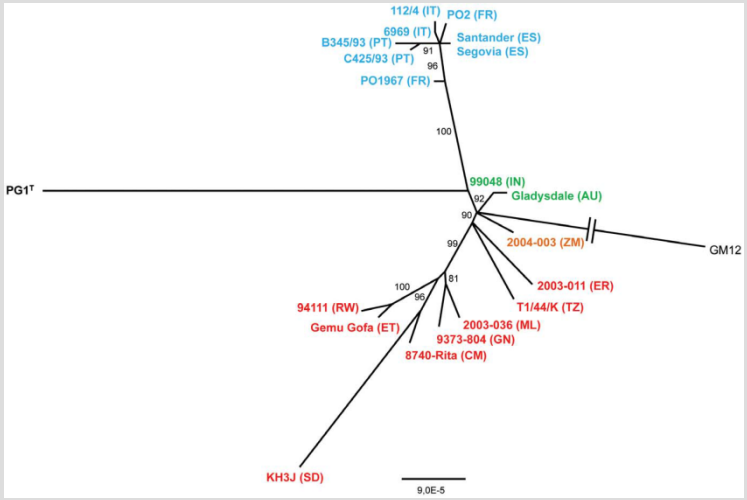
Philippe Totté
CIRAD-UMR ASTRE
« AnimalS health Territories Risks Ecosystems»

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)

Pictures credit to F. Thiaucourt



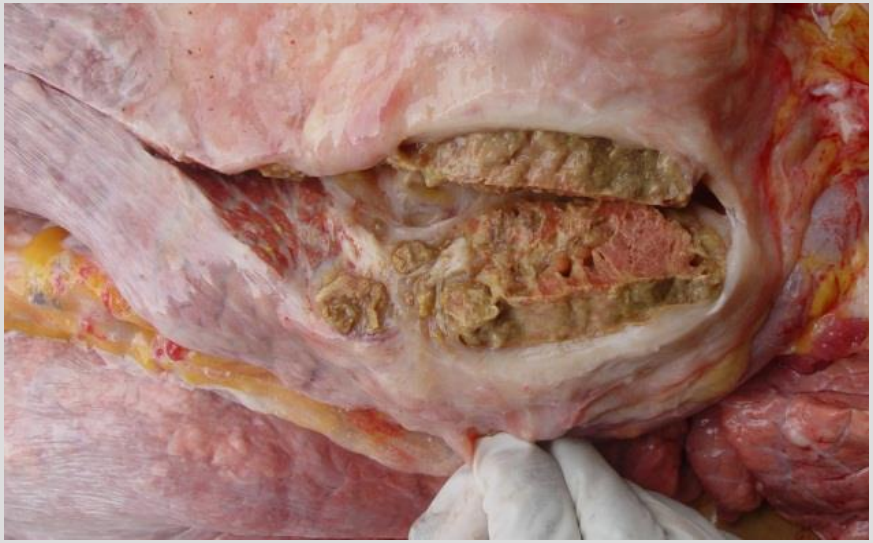


The disease

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

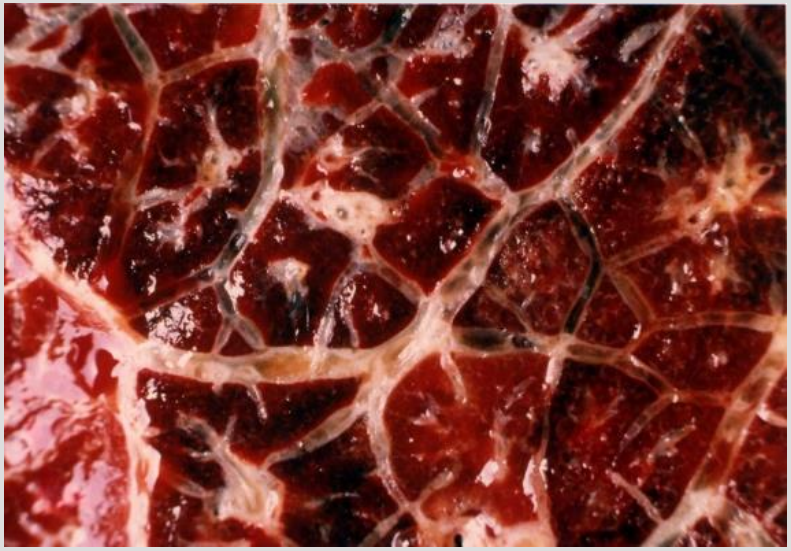
Picture credit to F. Thiaucourt

Picture credit to F. Thiaucourt



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

Picture credit to F. Thiaucourt



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

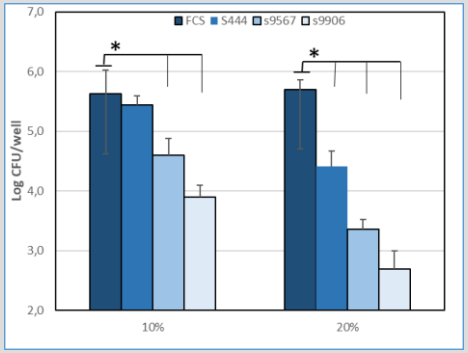
Immune responses associated with protection against CBPP and induced by immunization

Innate immunity

● Effect of complement in vitro

Totté P et al. 2023. PCI microbiol. https://microbiol.peercommunityin.org/articles/rec?id=5

→ Bactericidal capacity is highly variable between animals but conserved over time

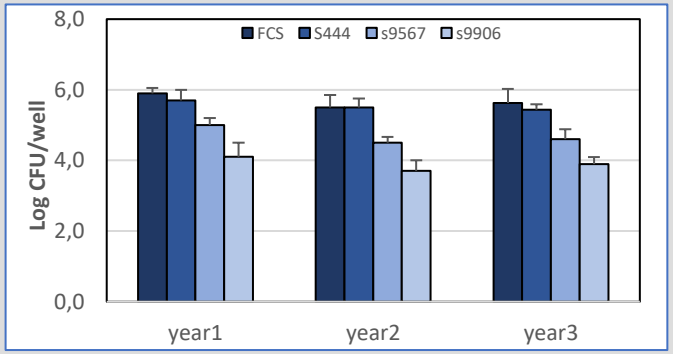


1h incubation in RPMI, 3 different cattle

-10%: ↓ 0-2 logs in CFU/ml

-20%: ↓ 1,5-3 logs in CFU/ml

No effect after decomplementation of sera



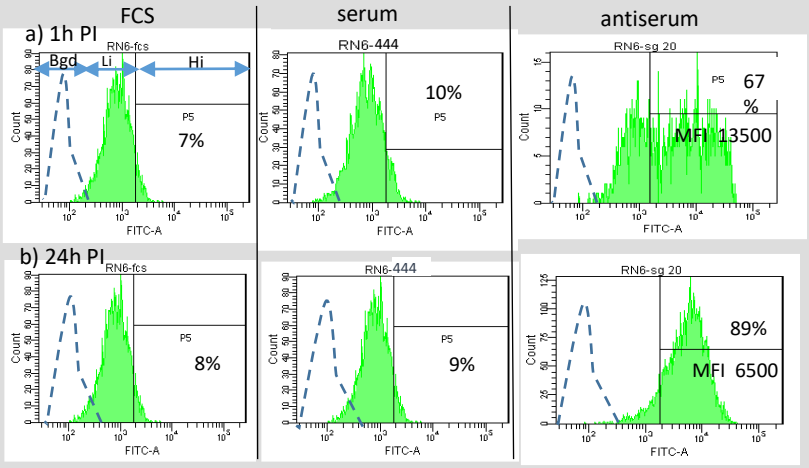
→ No opsonization effect at non bactericidal concentrations

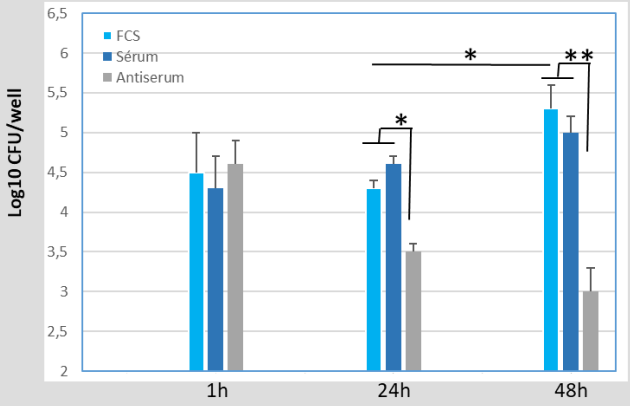
Serum = presence of complement

*Mmm*NeonGreen + macrophages (Mac) → uptake (flow cytometry)

Mmm + Mac → killing (CFU in cellular fraction)

Dashed line = *Mmm*+Mac





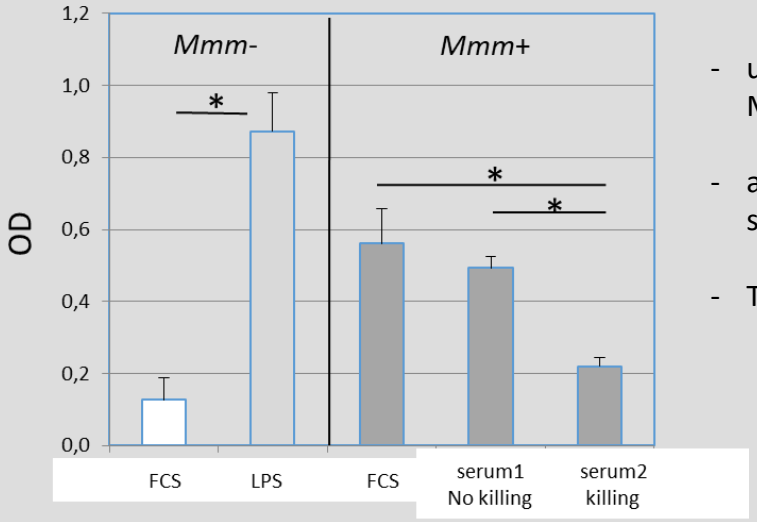
Immune responses associated with protection against CBPP and induced by immunization

Innate immunity

- **Effect of bovine complement in vitro**

→ No pro-inflammatory effect on macrophages (TNF) at non bactericidal concentrations

→ Reduces *Mmm*-induced TNF response of macrophages at bactericidal concentrations



- uninfected (Mmm-) or infected with Mmm (Mmm+) at an MOI of 500-1000
- absence (FCS) or presence of non-decomplemented bovine sera at non-bactericidal (serum1) and bactericidal (serum2)
- TNF titration by ELISA in 24h supernatants

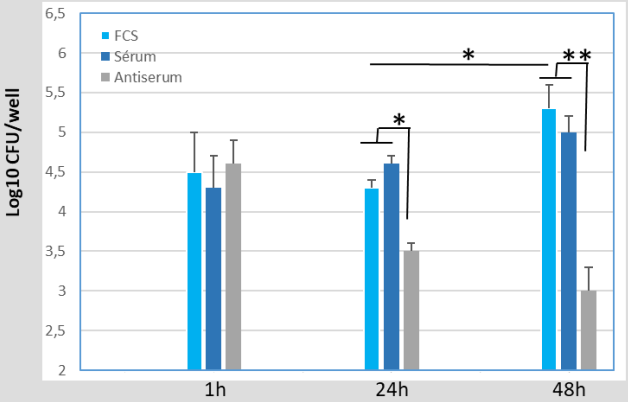
Immune responses associated with protection against CBPP and induced by immunization

Innate immunity

- **Effect of bovine complement in vitro**

Totté P et al. 2023. PCI Microbiol

- **Interactions between *Mmm* and bovine macrophages in vitro**



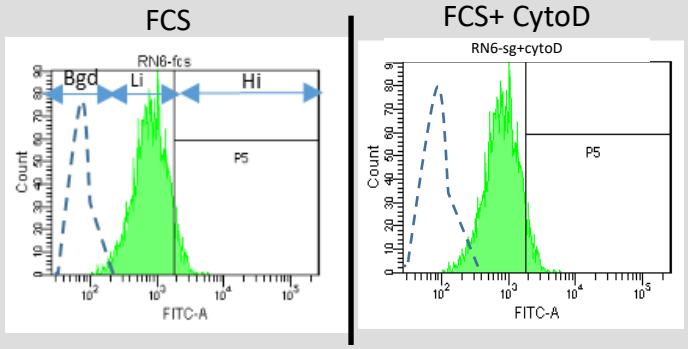
→ No killing in the absence of specific antiserum

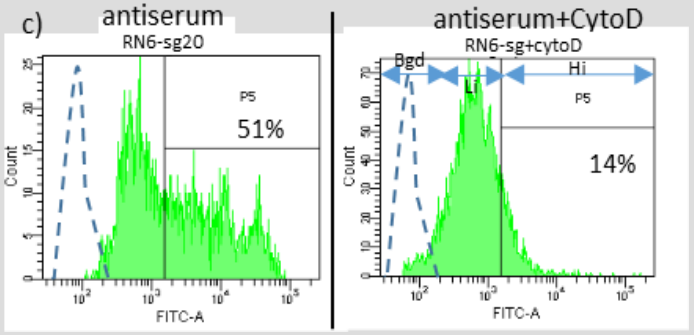
→ No intracellular survival (Gentamicin assay)

→ No opsonizing effect of complement at non bactericidal concentrations

→ Phagocytosis-independent uptake in the absence of specific antiserum

MOI = 500





→ TNF production in response to *Mmm* (MOI 500)

Immune responses associated with protection against CBPP and induced by immunization

Innate immunity (discussion & perspectives) 4/4

1- Potential role of complement

- individual bactericidal effect of complement in vitro as a marker of resistance ?
  → confirm in infection trials
- no impact on TNF response of macrophages at non bactericidal concentrations
  → confirm in alveolar macrophages, effect on neutrophils?
- reduces TNF production by macrophages at bactericidal concentrations
  → reduction of inflammation

2- Minor role of macrophages

- no killing in the absence of antiserum
  → consistent with previous work on mycoplasmas using alveolar macrophages
  → increased killing in the presence of IFN-γ ?
- at MOI>500: phagocytosis-independent uptake is associated with TNF production
  → confirm using alveolar macrophages
  → autophagy? C-type lectin receptors ?

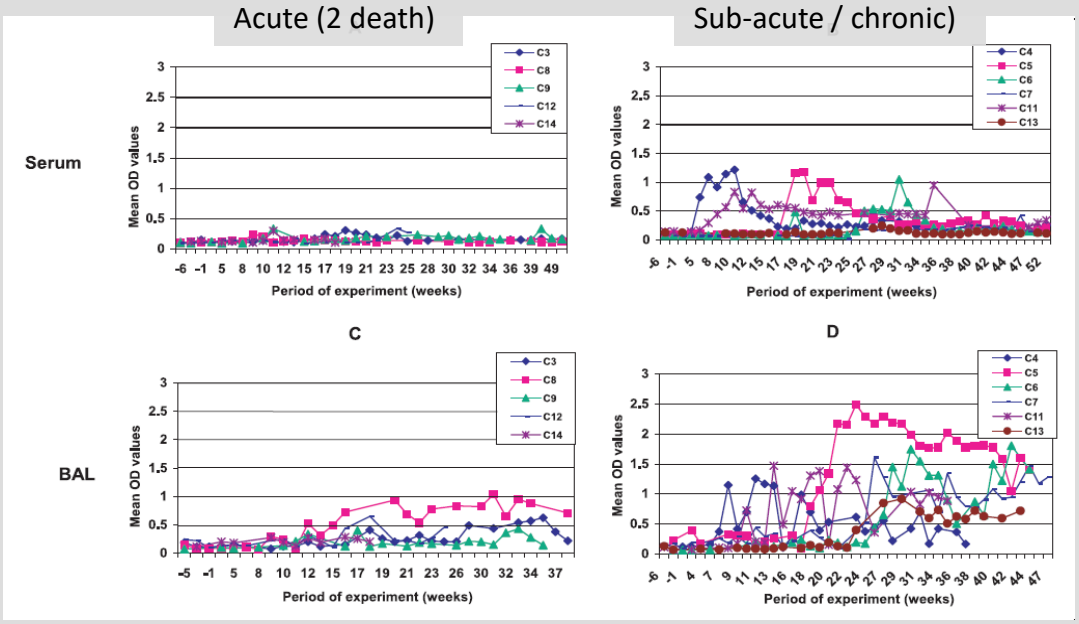
3- Role of neutrophils?

- neutrophil extracellular traps (NETs)

Immune responses associated with protection against CBPP and induced by immunization

Humoral immunity

- **during primary infection (challenge by contact and intubation)**
  - → No correlation between CFT (Bygrave *et al*., 1968) and IgM+IgG/Elisa (Le Goff and Lefevre, 1989) responses and disease severity
  - → Antibody transfer experiments using serum from convalescent animals have been inconclusive (Gourlay *et al*., 1979).
  - → Detection by ELISA of *Mmm*-specific IgA in serum and bronchoalveaolar lavage (BAL) of animals with less severe disease (Niang M *et al*. Vet Res. 2006)



- detectable for only 1-3 months in serum

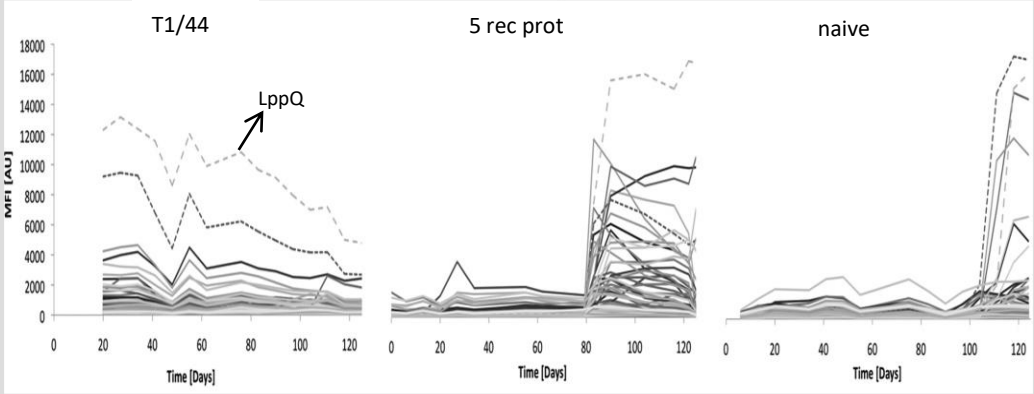
Immune responses associated with protection against CBPP and induced by immunization

Humoral immunity

- during primary infection (challenge by contact and intubation)
- during vaccination/immunization

→ Weak antibody titers (CFT, cELISA, indirect ELISA) and most animals are negative after 3 months. No correlation with reduced disease 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 bead array) in animals vaccinated with T1/44. LppQ as most prominent target of IgG (n=5)(Hamsten C *et al.* Clin Vaccine Immunol. 2010)



- IgG responses after challenge by contact for one animal representative of each group

- T1/44: only one animal with small sequestra after challenge

- recProt&Naive: 5/5 with small to large sequestra

→ Animals immunised against LppQ are not protected against challenge and show increased glomerulonephritis (Mulongo *et al*., 2015).

Immune responses associated with protection against CBPP and induced by immunization

Humoral immunity

- **during primary infection (challenge by contact and intubation)**
- **during vaccination/immunization**

in vitro studies

→ A monoclonal IgM antibody directed against a carbohydrate epitope inhibits the growth of *Mmm* in vitro in the absence of phagocytes and complement, which is the basis for the growth inhibition diagnostic assay (Kiarie *et al*., 1996).

→ 13/48 (10 IgG2, 2 IgG1, 1 IgM) anti-*Mmm* mouse Mabs induce 30 to 70% inhibition of *Mmm* adhesion to lung epithelial cells. One Mab (IgM) against galactane (capsule) is also bactericidal due to agglutination (Aye *et al*., 2018)

→ Antiserum from convalescent animals induce killing of *Mmm* by bovine macrophages (Totté *et al*., 2023)

Immune responses associated with protection against CBPP and induced by immunization

Humoral immunity (discussion&perspectives) 4/4

- No or poor correlation with milder symptoms except for IgA in broncho-alveolar lavage (BAL)
  - → confirm results for IgA in larger animal group
  - → repeat transfer experiments using improved nebulization-based challenge methods
  - → more studies in BAL

- Suspicion about antibody-antigen immunocomplexes as inducers of vasculitis and nephritis
  - → identify antigens and inactivate corresponding gene in vaccine strains

- Antibodies against capsular galactan that block adhesion and promote agglutination
  - → only IgM ? → one study shows comparable protection levels between T1 and galactan vaccines (Mwirigi et al, 2016)
  - → further explore potential of immunization against galactan

- Antibodies from convalescent animals promote phagocytosis-dependent killing of *Mmm* by macrophages
  - → confirm with alveolar macrophages
  - → identify target antigens

Immune responses associated with protection against CBPP and induced by immunization (resistance to / recovery from)

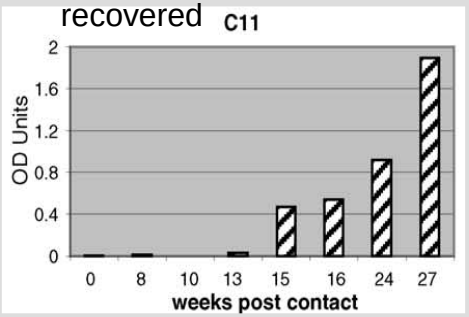
Cell mediated immunity (CMI) 1/4

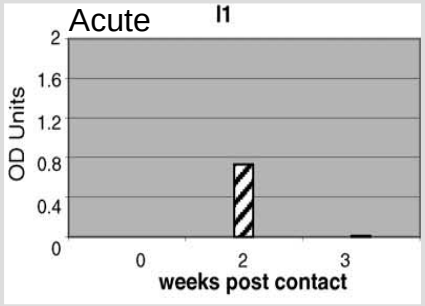
- **during primary infection (contact/intubation challenge)**

→ Positive intradermal test 3 months after infection suggests the presence of CMI (Roberts et al., 1973

→ Contradictory results with IFN-γ responses in ex vivo stimulated pbmc cultures

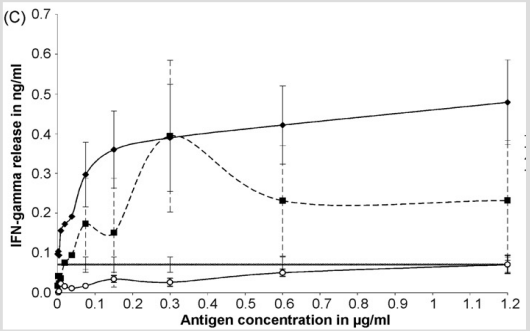
1- Dedieu et al (2005): higher IFN-γ in convalescent and fully recovered animals compared to animals with acute cbpp (hepatization, respiratory distress)

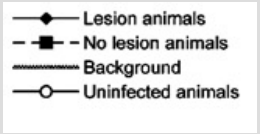




Challenge by contact: 6 animals
- 3 with sequestra (convalescent)
- 3 with scars (fully recovered)
- 3 with hepatization + respiratory distress (acute) C

2- Jores et al (2008): no difference between animals with lesions and those without any lesions





Day 45.

Challenge by intubation: 15 animals
- 5 without lesions (30%)
- 6 with sequestra
- 4 with hepatization (acute)

Immune responses associated with protection against CBPP and induced by immunization (resistance to / recovery from)

Cell mediated immunity (CMI)

recall = in vitro restimulation
With killed Mmm

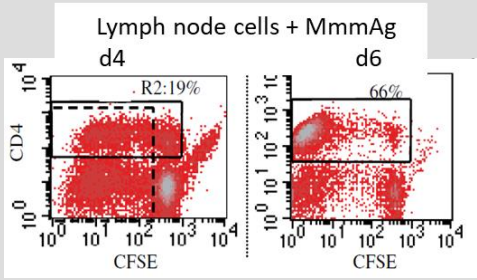
- **during primary infection (contact/intubation)**

→ Recall activation of CD4+ T cells in pbmc and draining lymph nodes of chronically infected and fully recovered animals (Dedieu *et al.*, 2003 and 2006)

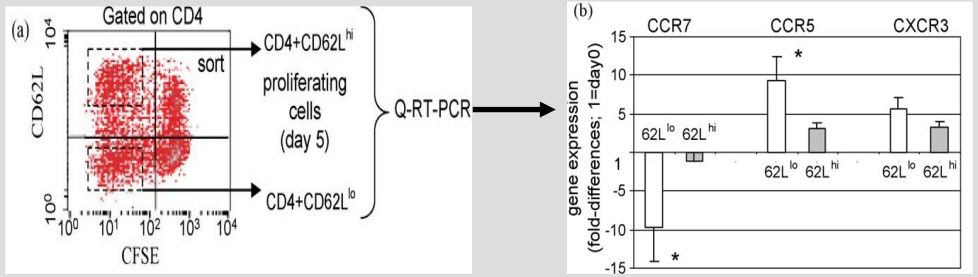
- No proliferation/enrichment of CD4 detected in day5 cultures → immune tolerance ?

→ Recall proliferation (cfse) of B and CD4 under the control of T1 type CD4+ T cells in draining lymph nodes of chronically infected and fully recovered animals (Totté *et al*, 2008)

- No proliferation and no IFN-γ after depletion of CD4
- CD4 enrichment detected in day6 cultures → active and sustained proliferation
- ↑ expression of memory marker on CD4
- IFN-γ produced by CD4 in the absence of IL-4 (elisa, intracellular staining) → T1 type



→ CD4 with effector memory (Tem: CD62Llow , CCR7-, IFN-γ+) and central memory (Tcm: CD62Lhi, CCR7+, IFN-γ+/-) are found in draining lymph nodes of chronically infected and fully recovered animals (Totté *et al*, 2010)



→ Depletion studies suggest that CD4+T lymphocytes play a minor role during primary infection (*Sacchini et al.,* 2011*)*

Immune responses associated with protection against CBPP and induced by immunization

Cell mediated immunity (CMI)

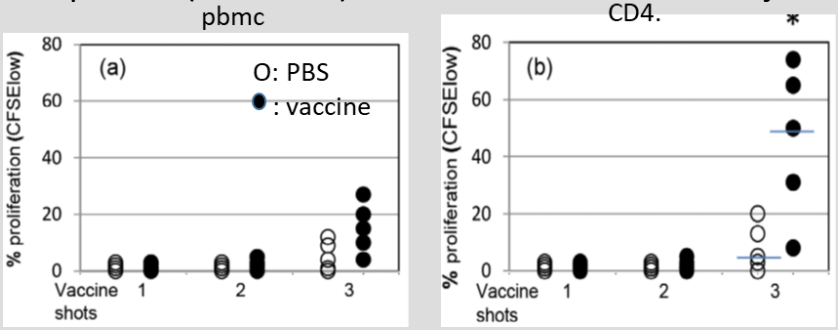
- during primary infection (contact/intubation)
- during immunization (T1 vaccines)

recall = in vitro restimulation
With killed Mmm

→ 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)

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

recall responses (cfse+Facs) one month after vaccine injections (d9 cultures)



- same for IFN-γ recall responses (Elisa,Elispot)

→ No differential regulation of bovine genes (Agilent microarray) in pbmc collected 6 months after vaccination and restimulated 24h in vitro (Totté et al., final report of the Ben-1 project funded by Galvmed, 2017)

-weak recall proliferation in pbmc detected only 4 months after vaccination (Jorg et al., personal communication)

Immune responses associated with protection against CBPP and induced by immunization

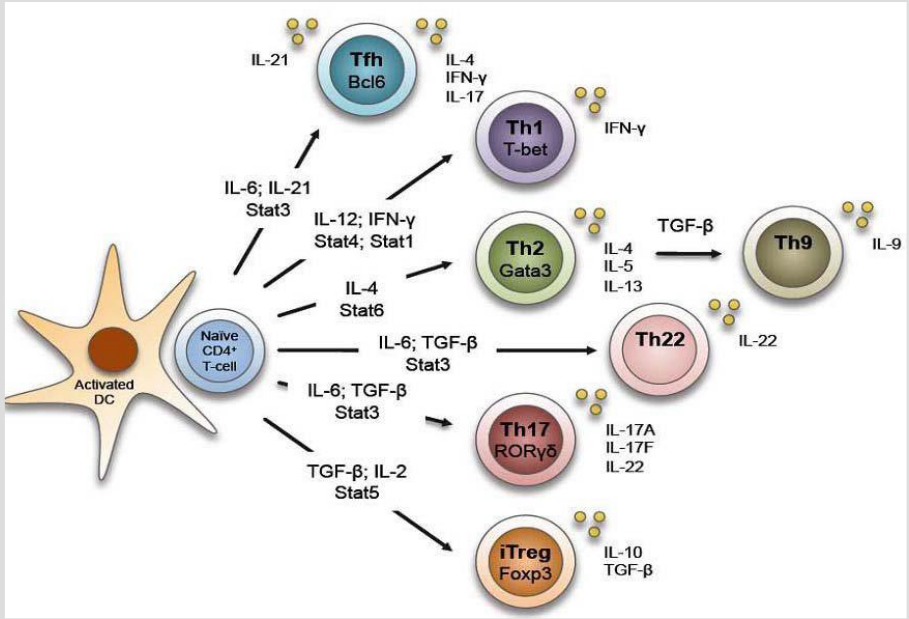
Cell mediated immunity (CMI) (Discussion & perspectives) 4/4

- Strong memory responses (short- and long-term) under the control of CD4 in fully recovered animals
  - → need to confirm protection in these animals. Is protection associated with chronic CBPP?
  - → assess these responses in animals undergoing secondary challenge
  - → further characterization of CD4 (Treg, Th17, Th22) in fully recovered animals

- Weak CD4 recall/memory responses are induced by T1/44 vaccine
  - → increase using adjuvants promoting Th1 CMI
  - → omic approach to define markers that can predict vaccine efficacy after vaccination but before challenge

lessons from the bovine tuberculosis model (Bhuju S *et al.*, 2012)

- Analysis of CMI in animals undergoing secondary challenge
  - → whole bovine genome transcriptomic approach to grasp complex acquired immunity
  - → ex vivo stimulation of blood lymphocytes to analyze long-term Tcm memory responses (IFN-γ cultured Elispot)
  - → correlates of protective immune memory to help the design/selection of better vaccines



Thanks to my colleagues at Cirad

WOAH Reference Laboratory for CBPP at CIRAD, Montpellier, France

F. Thiaucourt
L. Manso-Silvan
A. Peyraud
A. Boissière
M. Durand
L. Rousset

cirad
LA RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT

WOAH Reference Laboratory for CBPP
Reference Centre
World Organisation for Animal Health
Founded as OIE

And in Africa

LCV in Mali

ILRi and KALRO in Kenya

LANAVET in Cameroon

CVRI in Zambia

Thank you for your attention