Analysis of the role of mucosal antibodies in protection against contagious caprine pleuropneumonia and contagious bovine pleuropneumonia: Update

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Introducing CBPP

• Contagious bovine pleuropneumonia, CBPP, caused by *Mycoplasma mycoides* subsp. *mycoides (Mmm)* is a highly contagious disease that affect cattle in many countries of sub-Saharan Africa.

• CBPP are among the most serious livestock diseases in Africa.

• Imposes an estimated minimal cost of >50,000,000 €/year in Africa and restricts trade

• Clinical signs include fever, coughing, respiratory distress and anorexia with unilateral lung lesions and pleural fluid - acute, subacute or chronic disease
Introducing CBPP – available vaccines ghtd

Available and OIE recommended vaccines:

• Live attenuated vaccine (mostly T1/44)
• Low efficacy
• Short duration of protection
• Remaining virulence causing occasional post-vaccination reactions (Willem’s reactions) at site of injection
• Continued attenuation: better safety profile, lower protection
• Inactivated vaccines not working so far
Introducing CCPP

• Contagious Caprine Pleuropneumoniae
• Caused by *Mycoplasma capricolum* subsp. *capripneumoniae*
• Widespread in Central and East Africa, Middle East and Asia
• Affects domestic goats and wild ruminants
• Aerosol infection
• Mortality can reach 80%
• Clinical signs similar to CBPP
• Vaccine: inactivated whole bacterin

Manso-Silván et al. 2011
Correlate of protection?

Correlate of protection still unknown

- Vaccine candidates
- Only way to test vaccine efficacy today: challenge studies
  - costly, cumbersome and animal welfare issues
Correlate of protection? (CBPP – none for CCPP)

T-cells
• Dedieu 2005 – IFNg secreting CD4 T-cells correlate with protection
• Sacchini et al 2011, CD4 T-cells minor role in protection

Antigen-specific serum antibodies
• Hamsten et al., 2010, 5 candidate antigens
• Schieck et al., 2014 - none
• VIDO, KALRO, ILRI subunit vaccine (need to be confirmed)

Mucosal antibodies:
• Niang et al. 2006: Humoral and mucosal (BAL) levels of IgM, IgG1 and IgG2 do not correlate with severity of disease. All animals with high BAL levels of IgA were characterized by reduced disease severity. (Did not identify specific IgA targets).
• Karst et al. 1972: Intranasal vaccination using live attenuated strain showed protection
CCPP – samples from protected and not protected goats

(general outline)

Entry
ABSLO2

Vaccination

Challenge

End of Trial, Necropsies

Days post vaccination

-7
-0
29
50

70
29
CCPP – samples from protected and not protected goats

Entry
ABSL2
-7
Vaccination
0
Challenge
29
End of Trial,
Necropsies
50

Days post vaccination

Survival (%)

T1- NegCont saponin only
T2 0.15mg/dose (OIE recommended)
T3 0.075 mg
T4-0.030 mg
T5-0.015 mg

Challenge starts
CCPP – samples from protected and not protected goats

**Entry**
-7

**Vaccination**
0

**Challenge**
29

**End of Trial, Necropsies**
50

**Days post vaccination**

**Serum**

**Bronchoalveolar lavage, BAL**

**BAL (in our case):**
Briefly, following slaughter, the trachea together with the lungs is cut out and lavaged by introducing sterile PBS, into the lungs. This is followed by gentle massage of the lungs before the fluid is re-collected into a beaker and frozen in 50ml falcon tubes.

The BAL are diluted 1:10 with TBST/5% low fat skimmed milk and used as primary antibodies in w/b protocol.
CCPP – Lacrimal fluid

Entry ABSL2 Vaccination Challenge End of Trial, Necropsies
-7 0 29 50

Days post vaccination

↑ ↑ ↑ ↑ ↑ ↑ ↑
Serum

↑ ↑
Bronchoalveolar lavage, BAL

↑ ↑
Lacrimal fluid
CCPP and CBPP—Lacrimal fluid

SOP for lacrimal fluid collection established

Electrophoretic profile of Goat Lacrimal fluid by SDS-PAGE

1. Protein ladder
2. BSA (66.5kDa)
3. D0 CS011 Lacrimal fluid
4. Post vaccine Lacrimal fluid
5. Post challenge lacrimal fluid
6. Bovine BS058 D1 Lacrimal fluid
7. Bovine BS058 Lacrimal fluid D15 post challenge

Gel load:
1. 10ul all Lacrimal fluids samples
2. 2ul Loading dye
3. 5ug BSA
CCPP–Lacrimal fluid western blot

Lacrimal IgG reaction to Mccp lysate

1. Marker
2. Pre-vaccination samples
3. 1:10
4. 1:20
5. 1:40

Post-vaccination samples
6. 1:10
7. 1:20
8. 1:40

Post challenge
9. 1:10
10. 1:20

Lacrimal IgA reaction to Mccp lysate

Group T3 pooled lacrimal fluid
1. Marker
2. Pre-vaccination samples
3. 1:10
4. 1:20
5. 1:40

Post-vaccination samples
6. 1:10
7. 1:20
8. 1:40

Post challenge
9. 1:10
10. 1:20

CCPP – Western blot testing BAL on whole Mccp antigen

BAL IgG

Not infected  Severely sick  Healthy (protected)

72  55  43  34  26  17  10

BAL IgA

Not infected  Severely sick  Healthy (protected)

72  55  43  34  26  17  10
CCPP – Western blot testing BAL on whole Mccp antigen

BAL IgG
Not infected  Severely sick  Healthy (protected)

BAL IgA
Not infected  Severely sick  Healthy (protected)
CBPP – recombinant *Mmm* proteins

<table>
<thead>
<tr>
<th>Protein ID</th>
<th>Description</th>
<th>MW</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSC -0136</td>
<td>Hypothetical lipoprotein</td>
<td>64 kDa</td>
<td>Subunit vaccine</td>
</tr>
<tr>
<td>MSC -0160</td>
<td>Translation elongation factor Tu</td>
<td>75 kDa</td>
<td>Nkando et al., 2016</td>
</tr>
<tr>
<td>MSC -0431</td>
<td>Prolipoprotein</td>
<td>68 kDa</td>
<td>Subunit vaccine</td>
</tr>
<tr>
<td>MSC -0499</td>
<td>Prolipoprotein</td>
<td>108 kDa</td>
<td>Subunit vaccine</td>
</tr>
<tr>
<td>MSC -0775</td>
<td>Prolipoprotein</td>
<td>109 kDa</td>
<td>Subunit vaccine</td>
</tr>
<tr>
<td>MSC -0816</td>
<td>Variable surface lipoprotein</td>
<td>73 kDa</td>
<td>Nkando et al., 2016</td>
</tr>
<tr>
<td>MSC-0079</td>
<td>Prolipoprotein, putative phosphonate ABC transport</td>
<td>69 kDa</td>
<td>Hamsten et al. 2010</td>
</tr>
</tbody>
</table>

![Transferred Antigens](image.png)
CBPP – samples from protected and not protected cattle

Entry ABSL2 | Challenge | End of Trial, Necropsies
---|---|---
-7 | 0 | 30
Days post challenge
CBPP – samples from protected and not protected cattle

Entry ABSL2 | Challenge | End of Trial, Necropsies
---|---|---
-7 | 0 | 30

Days post challenge

Survival %

Days post challenge

- T1-intubated
- T2- 3min exposure
- T3 5min exposure
- T4 3x5min exposure
CBPP – samples from protected and not protected cattle

- Entry ABSL2
- Challenge
- End of Trial, Necropsies

Days post challenge:
- Serum
- Bronchoalveolar lavage, BAL
- Lacrimal fluid
CBPP – recombinant *Mmm* proteins - BAL

**PROTECTED ANIMALS BAL SAMPLES**

Protected animals
Pooled BAL samples
- BS064
- BS085
- BS065
- BS073
- BS078

**SEVERE DISEASED ANIMALS**

Pooled BAL samples from severe sick animals
- BS074
- BS081
- BS083
- BS071
- BS088
CBPP – recombinant *Mmm* proteins – IgA in BAL

**PROTECTED ANIMALS BAL SAMPLES**
- IgA in pooled BAL

1. MSC-0136 - 64kDa
2. MSC-0160 - 75kDa
3. MSC-0431 - 68kDa
4. MSC-0775 - 108kDa
5. MSC-0499 - 109kDa
6. MSC-0816 - 73kDa
7. MSC-0079 - 68kDa

**SEVERELY DISEASED ANIMALS**
- IgA in pooled BAL

1. MSC-0136 - 64kDa
2. MSC-0160 - 75kDa
3. MSC-0431 - 68kDa
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Protected animals
- BS064
- BS085
- BS065
- BS073
- BS078

Pooled BAL samples from severe sick animals
- BS074
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CBPP – recombinant *Mmm* proteins – IgA in BAL

**PROTECTED ANIMALS BAL SAMPLES – IgA in BAL**

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**SEVERELY DISEASED ANIMALS – IgA in BAL**
Growth inhibition assay for *Mmm* (serum)

Pipette 20μL of the serum (27 dpi) on to the surface of the inoculated PPLO agar plate. Incubate at 37°C.
Growth inhibition assay for *Mmm*- agar well diffusion method.

Pipette 50µL of the serum (27 dpi) into the well in the inoculated PPLO agar plate. Incubate at 37°C for 4-7 days.
Growth inhibition assay for *Mmm*- agar well diffusion method.

Pipette 50µL of the serum (27 dpi) into the well in the inoculated PPLO agar plate. Incubate at 37°C for 4-7 days.
Growth inhibition assay for *Mmm*- agar well diffusion method-BAL
Thank you!

Stephen Munyao, Rose Ojuok

Mycoplasma team

ILRI farm team
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