



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

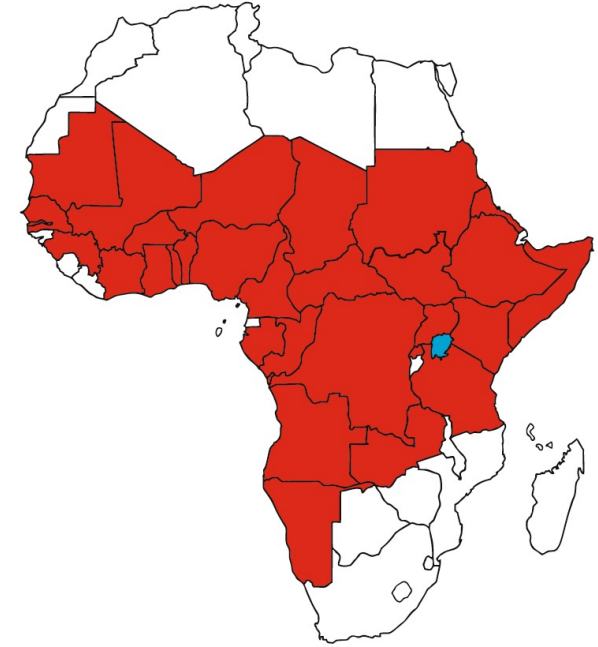
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Animal and Human Health program, ILRI*

Tuesday Scientific Seminars  
Nairobi, 7 December 2021

# 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

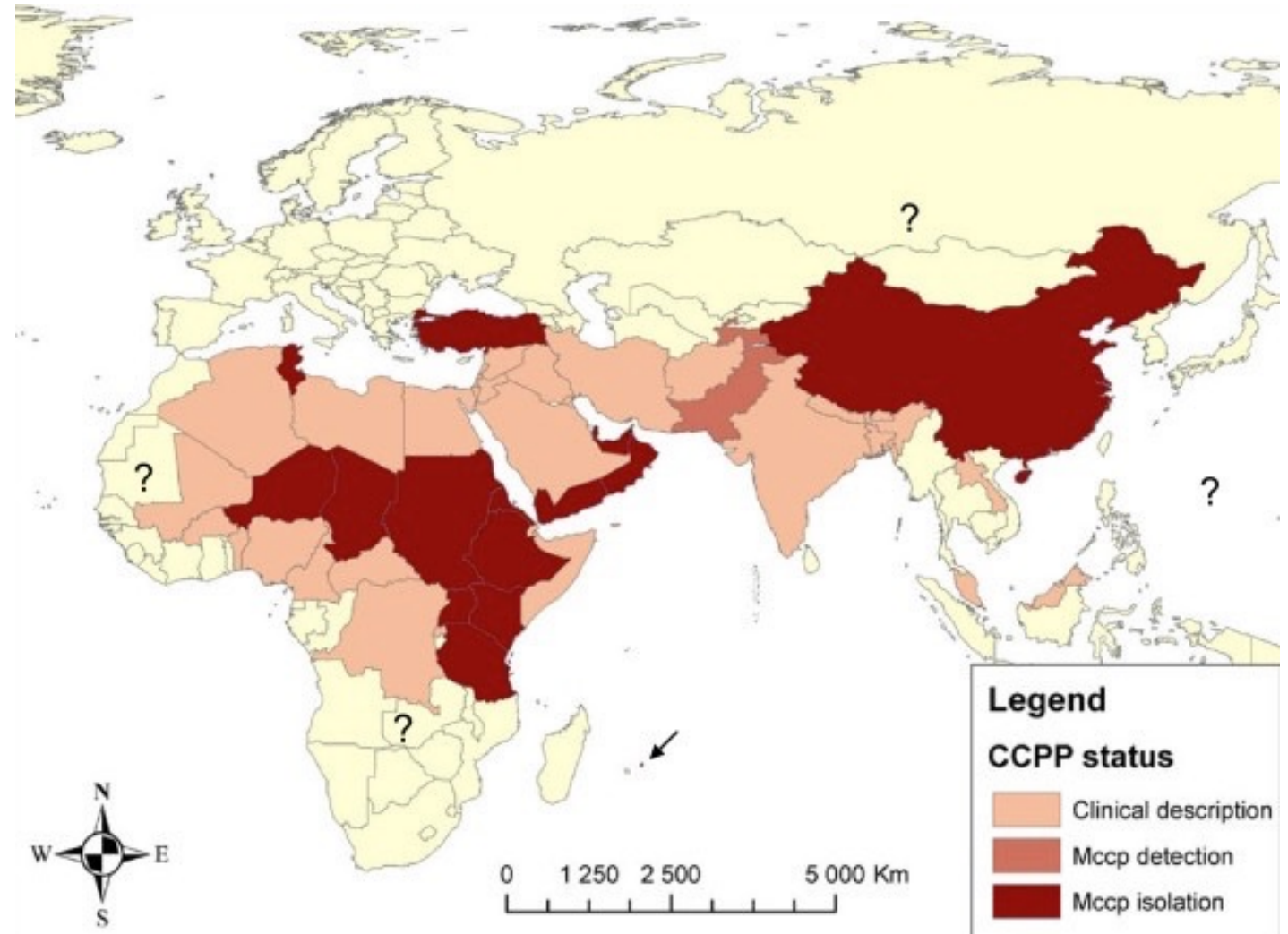
Available and OIE recommended vaccines:

- 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-Silvan et al. 2011

# Correlate of protection?

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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)

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## T-cells

- Dedieu 2005 – IFN $\gamma$  secreting CD4 T-cells correlate with protection
- Sacchini et al 2011, CD4 T-cells minor role in protection

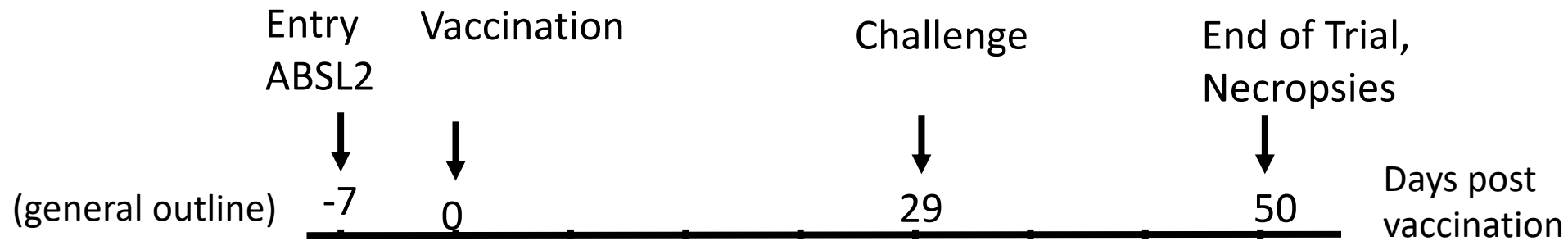
## Antigen-specific serum antibodies

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

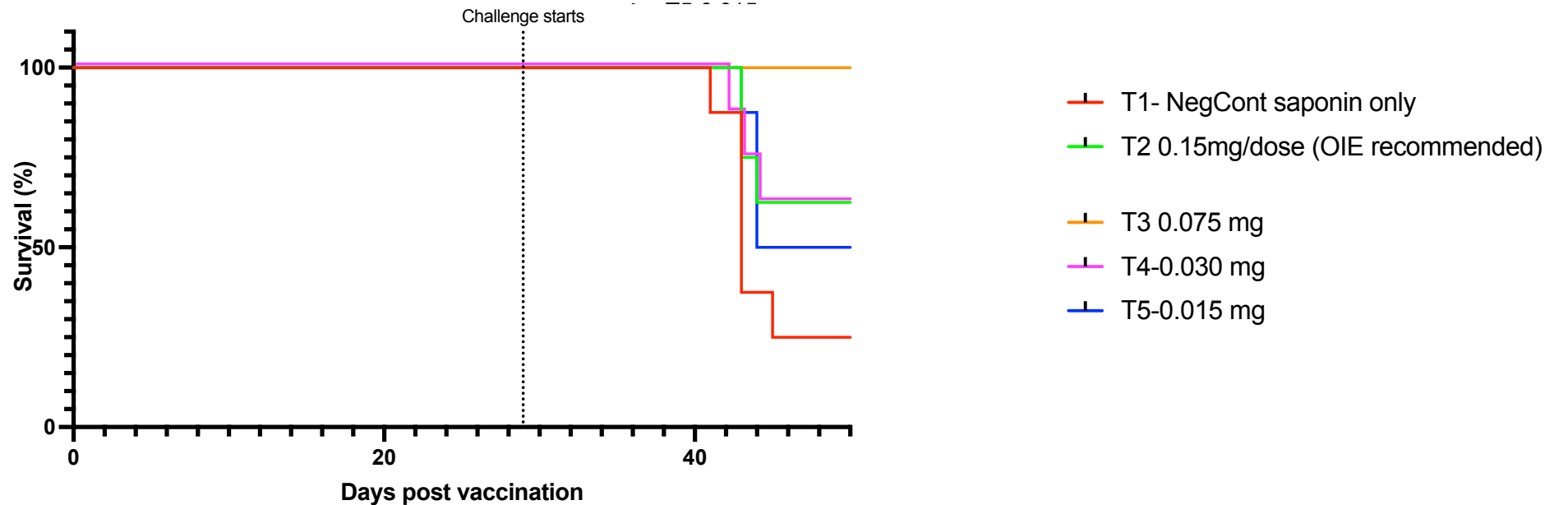
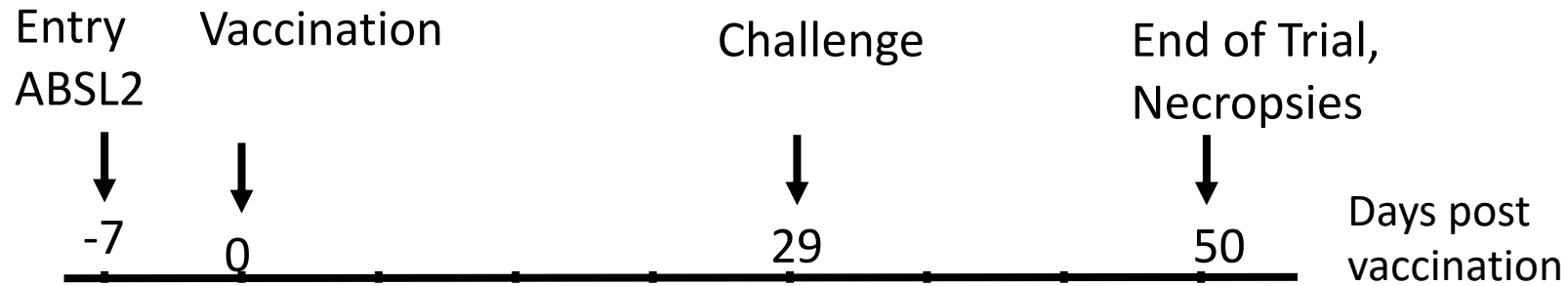
## Mucosal antibodies:

- 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

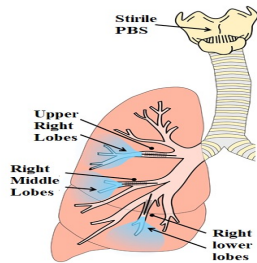
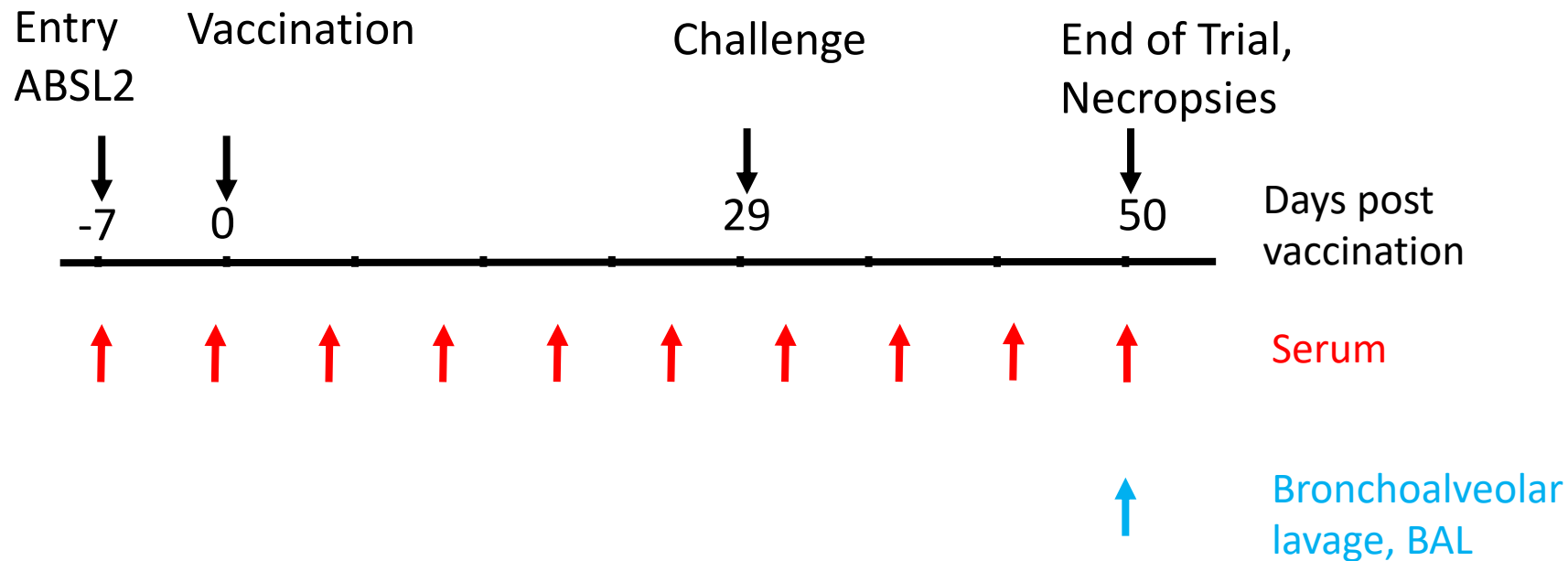


# CCPP – samples from protected and not protected goats





# CCPP – samples from protected and not protected goats

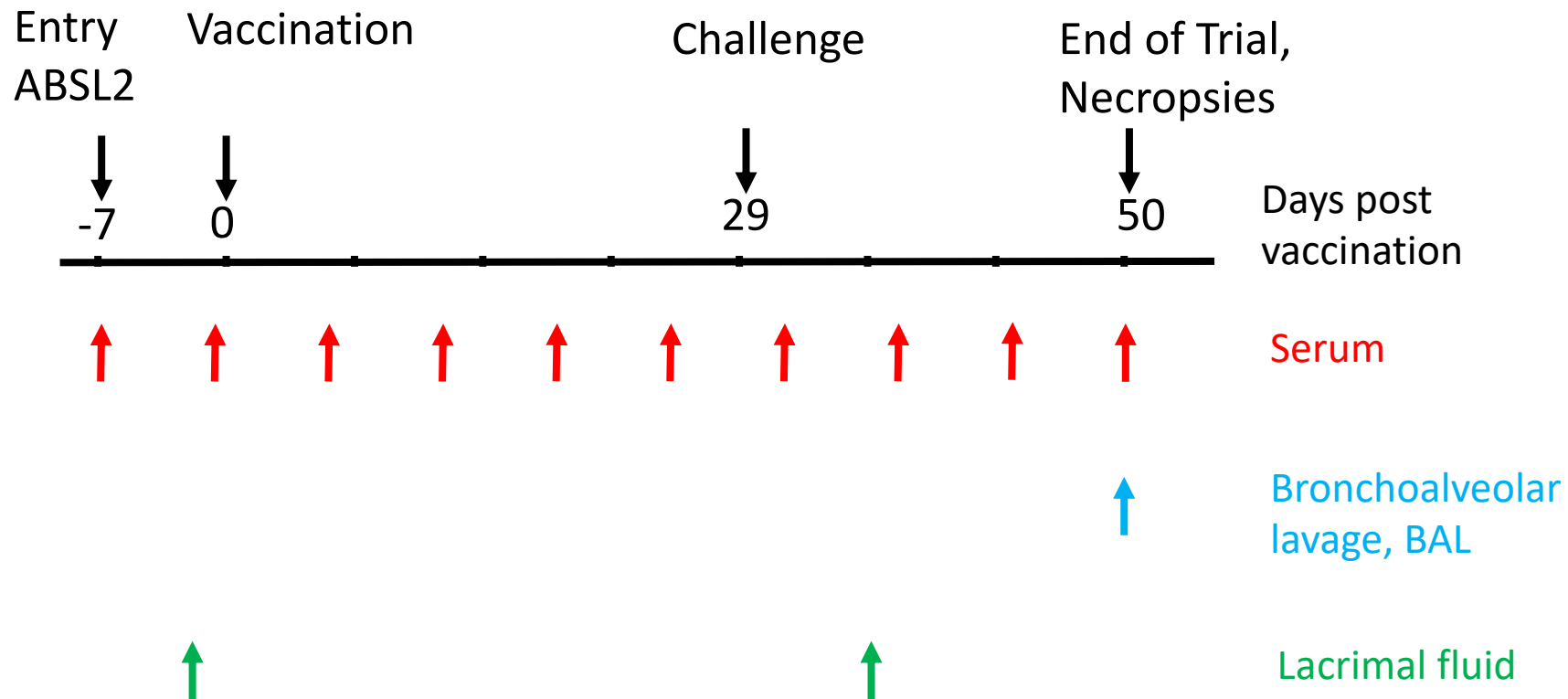


BAL (in our case):

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

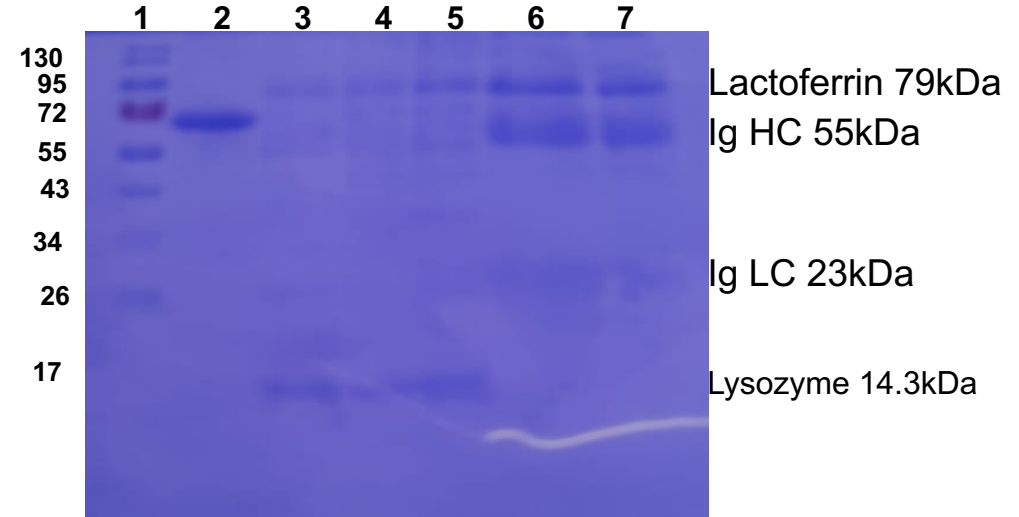


# CCPP and CBPP– Lacrimal fluid



SOP for lacrimal fluid  
collection established

## Electrophoretic profile of Goat Lacrimal fluid by SDS-PAGE



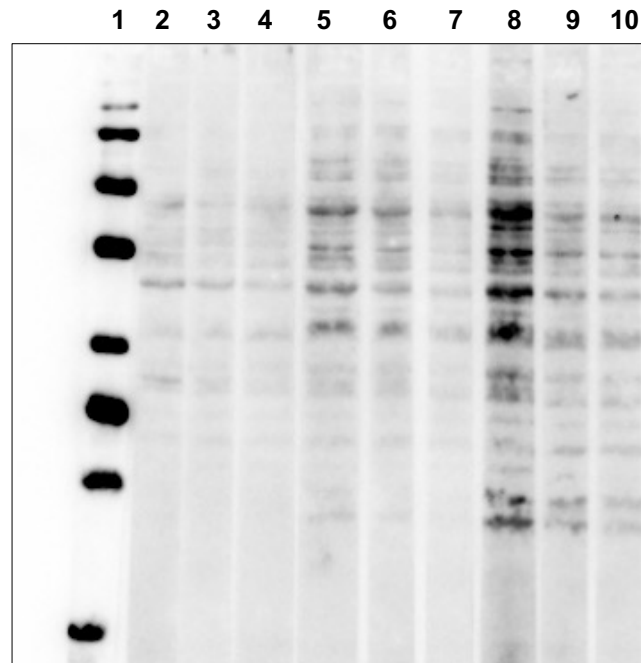
1. Protein ladder
2. BSA (66.5kDa)
3. D0 CS011 Lacrimal fluid
4. Post vaccine Lacrimal fluid
5. Post challenge lacrimal fluid
6. Bovine BS058 D1 Lacrimal fluid
7. Bovine BS058 Lacrimal fluid D15 post challenge

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



### Group T3 pooled lacrimal fluid

1.Marker

Pre-vaccination samples

2. 1:10

3. 1:20

4. 1:40

Post-vaccination samples

5. 1:10

6. 1:20

7. 1:40

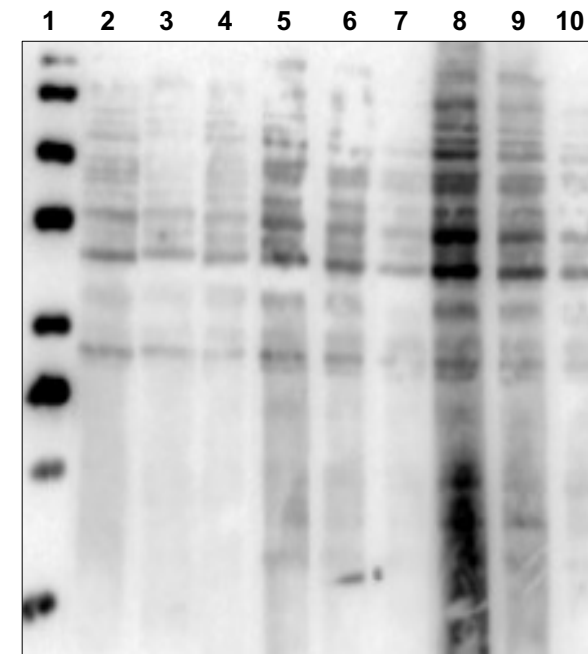
Post challenge

8. 1:10

9. 1:20

10. 1:40

## Lacrimal IgA reaction to Mccp lysate



### Group T3 pooled lacrimal fluid

1.Marker

Pre-vaccination samples

2. 1:10

3. 1:20

4. 1:40

Post-vaccination samples

5. 1:10

6. 1:20

7. 1:40

Post challenge samples

8. 1:10

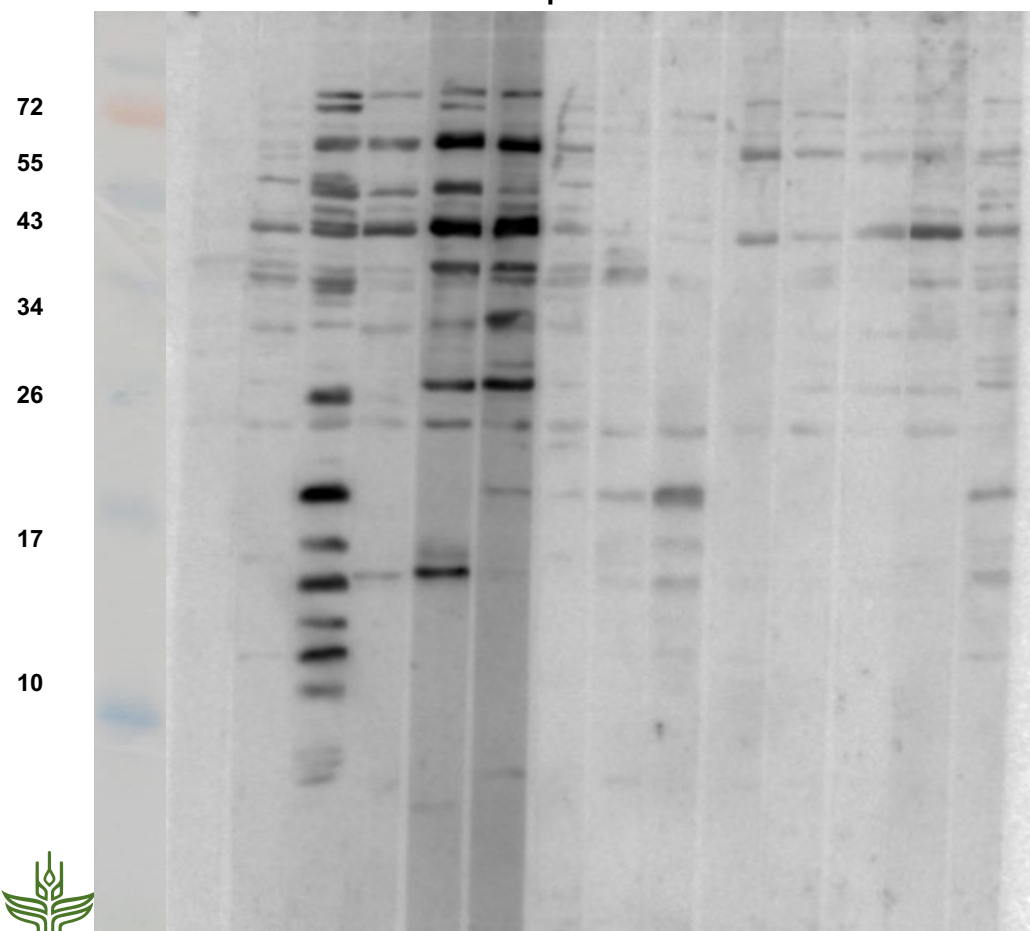
9. 1:20

10. 1:40

# CCPP – Western blot testing BAL on whole Mccp antigen

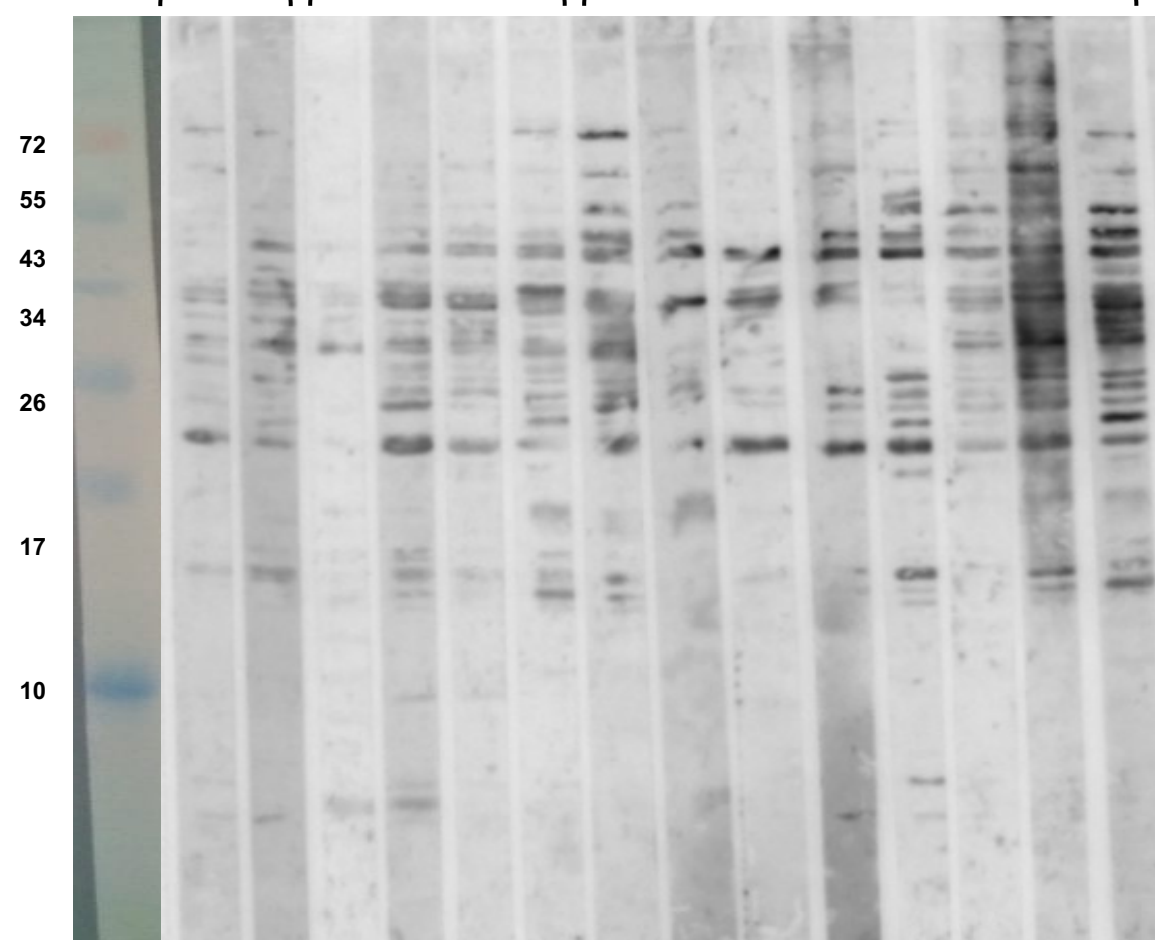
## BAL IgG

Not infected      Severely sick      Healthy (protected)



## BAL IgA

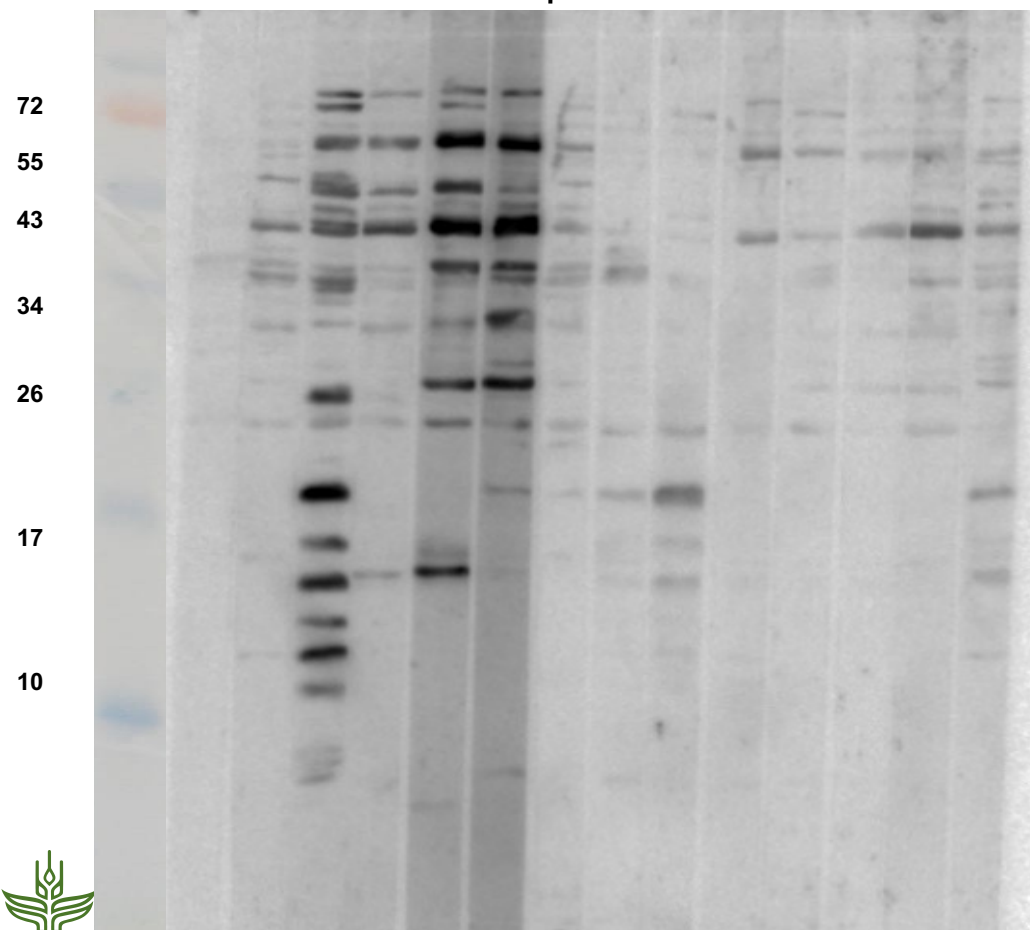
Not infected      Severely sick      Healthy (protected)



# CCPP – Western blot testing BAL on whole Mccp antigen

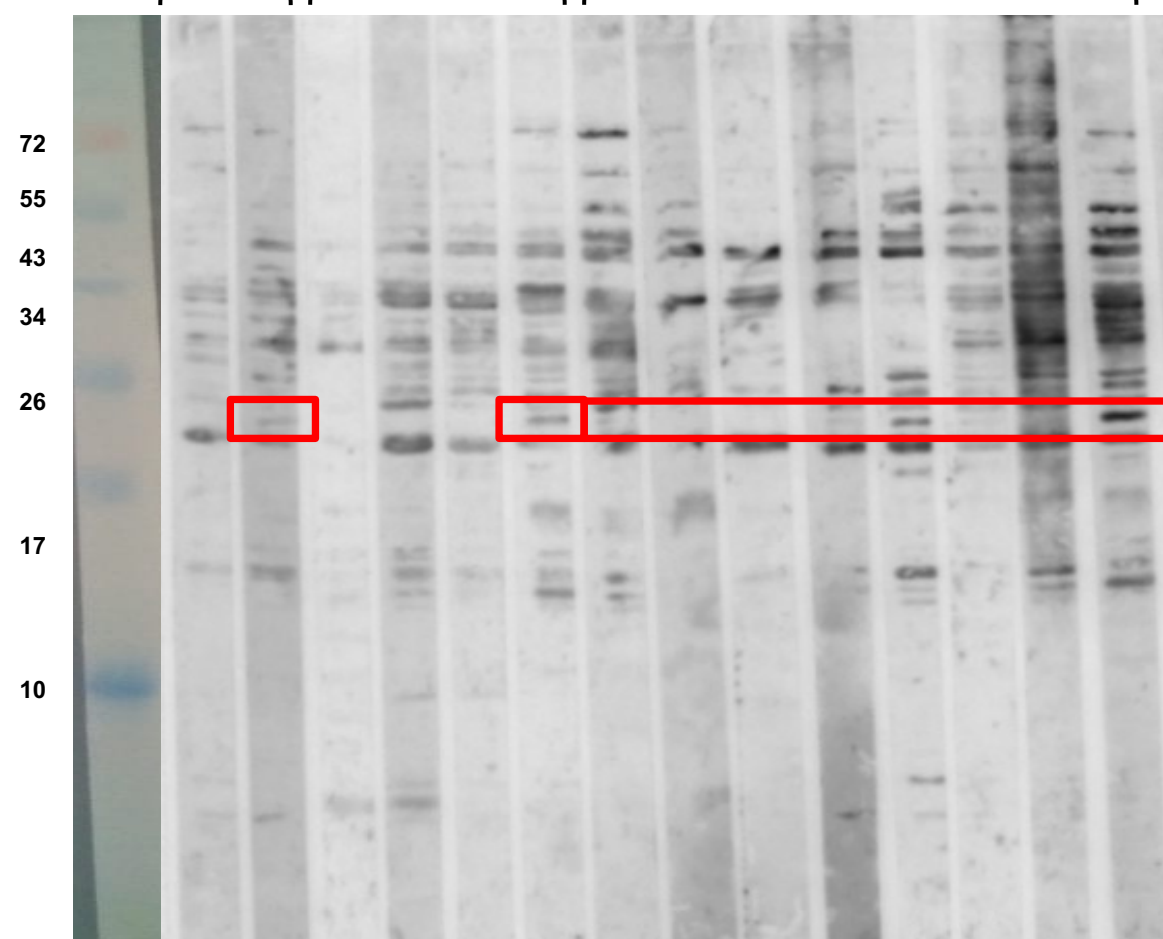
## BAL IgG

Not infected      Severely sick      Healthy (protected)



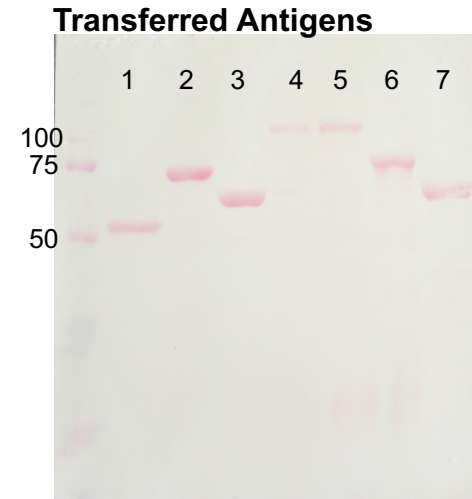
## BAL IgA

Not infected      Severely sick      Healthy (protected)



# CBPP – recombinant *Mmm* proteins

	Protein ID	Description	MW	
1	MSC -0136	Hypothetical lipoprotein	64 kDa	Subunit vaccine
2	MSC -0160	Translation elongation factor Tu	75 kDa	Nkando et al., 2016
3	MSC -0431	Prolipoprotein	68 kDa	Subunit vaccine
4	MSC -0499	Prolipoprotein	108 kDa	Subunit vaccine
5	MSC -0775	Prolipoprotein	109 kDa	Subunit vaccine
6	MSC -0816	Variable surface lipoprotein	73 kDa	Nkando et al., 2016
7	MSC-0079	Prolipoprotein, putative phosphonate ABC transport	69kDa	Hamsten et al. 2010

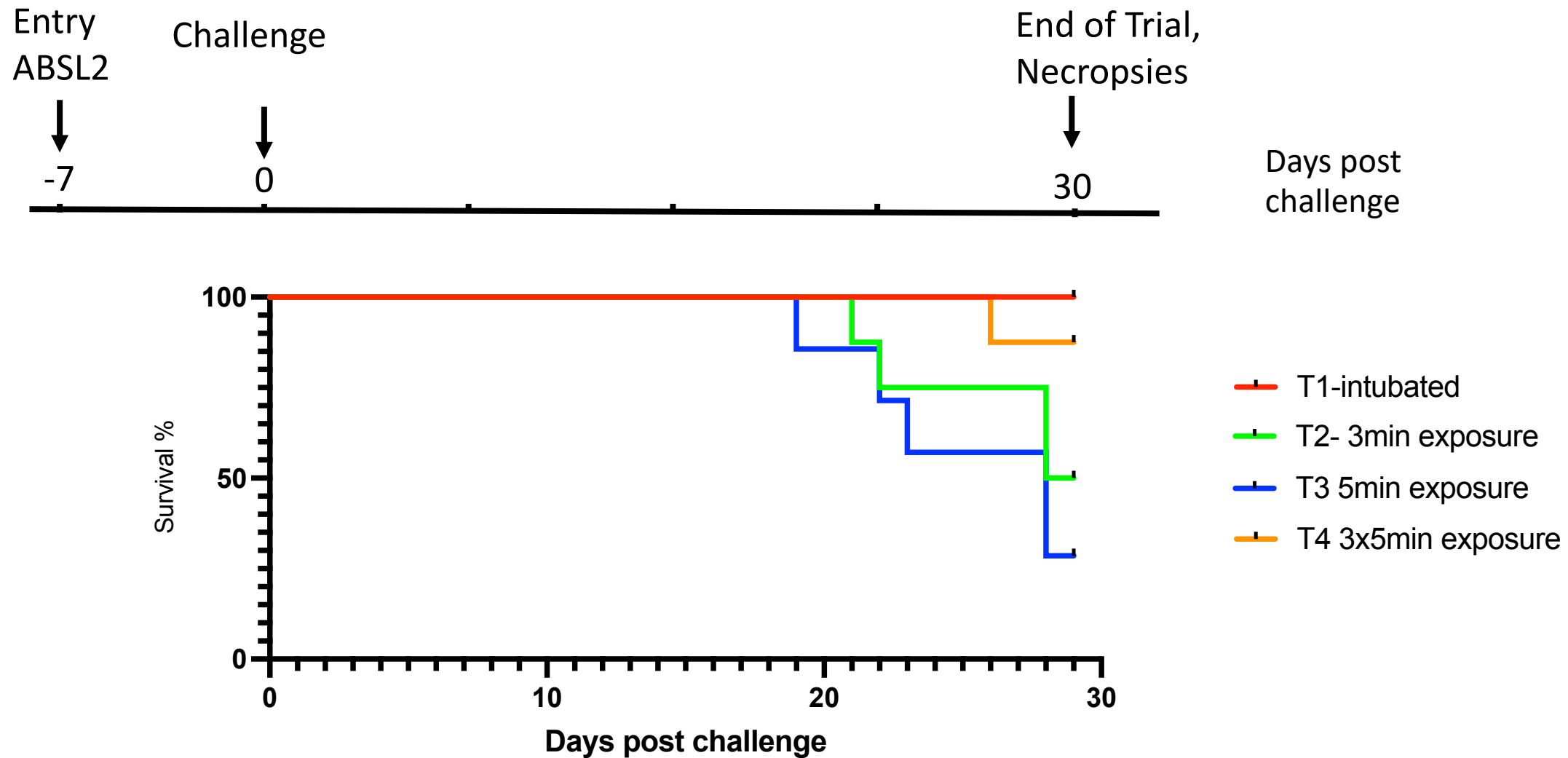


# CBPP – samples from protected and not protected cattle

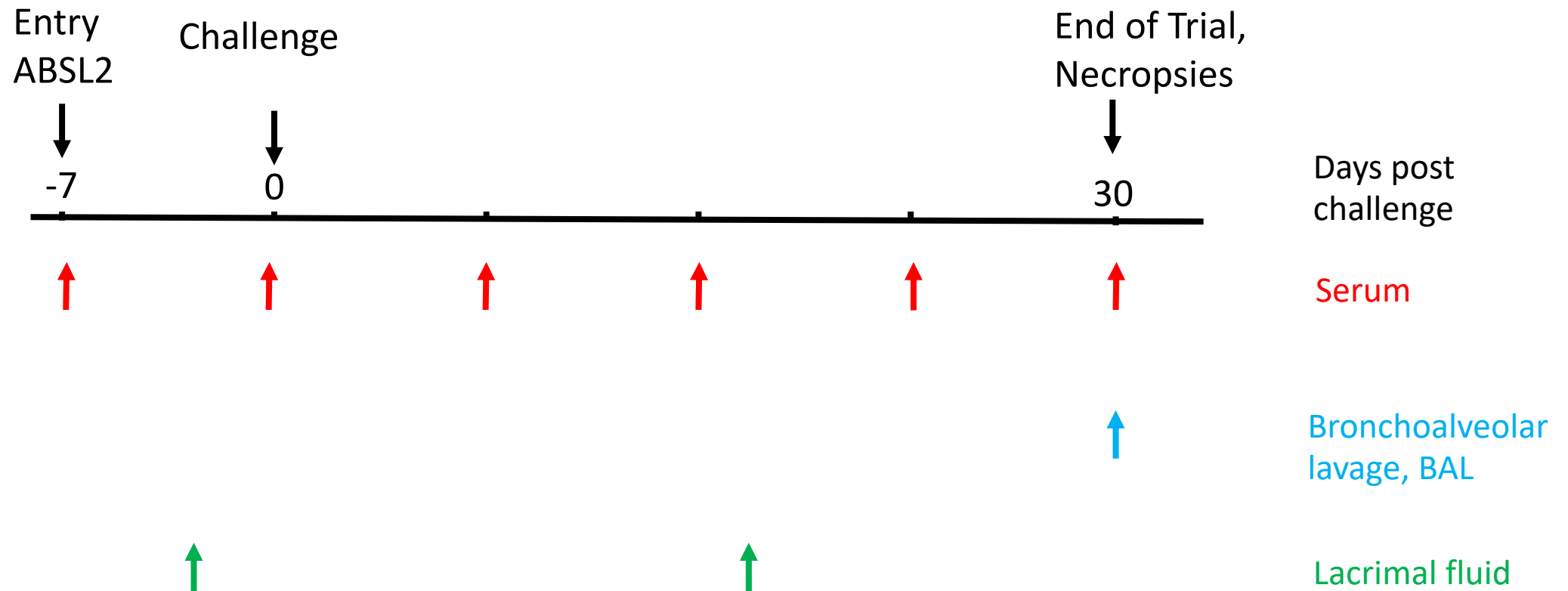




# CBPP – samples from protected and not protected cattle



# CBPP – samples from protected and not protected cattle

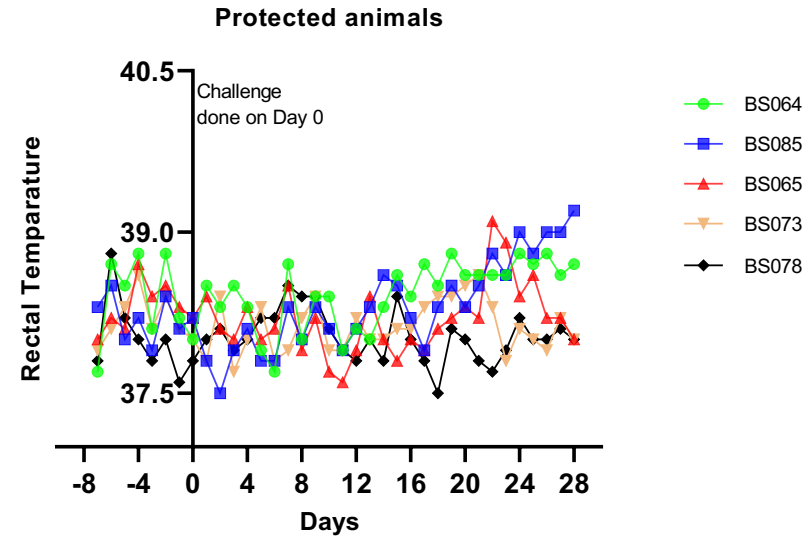


# CBPP – recombinant *Mmm* proteins - BAL

## PROTECTED ANIMALS BAL SAMPLES

Protected animals  
Pooled BAL samples

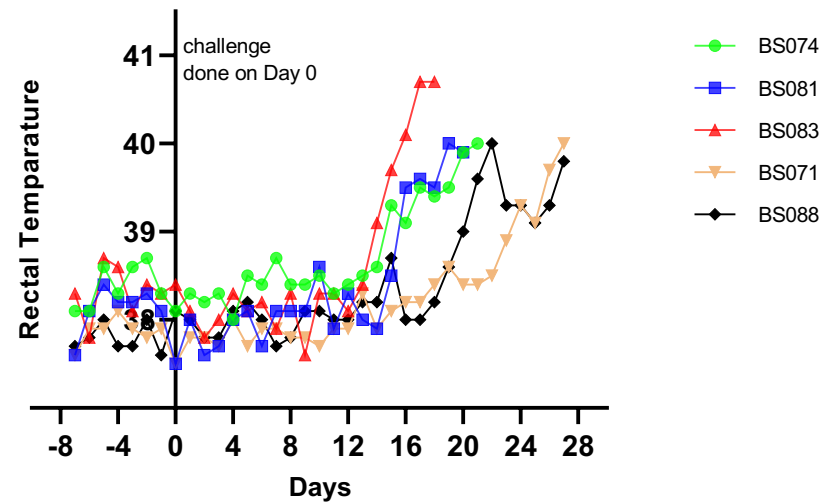
- BS064
- BS085
- BS065
- BS073
- BS078



## SEVERE DISEASED ANIMALS

Pooled BAL samples from  
severe sick animals

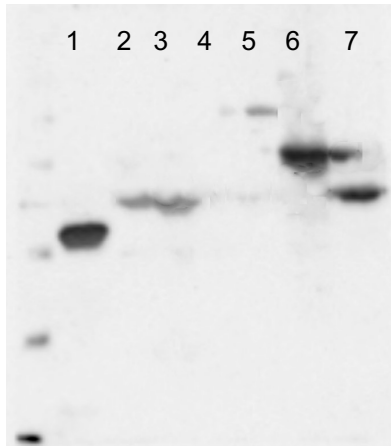
- BS074
- BS081
- BS083
- BS071
- BS088



# CBPP – recombinant *Mmm* proteins – IgA in BAL

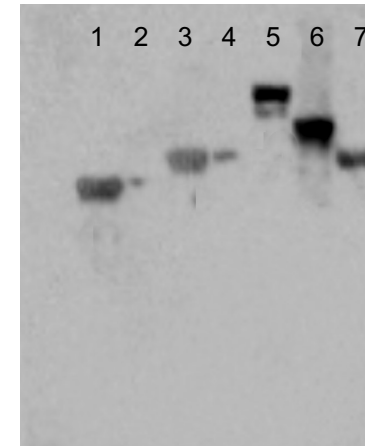
1. MSC- 0136 - 64kDa
2. MSC- 0160 - 75kDa
3. MSC- 0431 - 68kDa
4. MSC- 0775 - 108kDa
5. MSC- 0499 - 109kDa
6. MSC- 0816 - 73kDa
7. MSC- 0079 - 68kDa

## PROTECTED ANIMALS BAL SAMPLES – IgA in pooled BAL



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

## SEVERELY DISEASED ANIMALS – IgA in pooled BAL

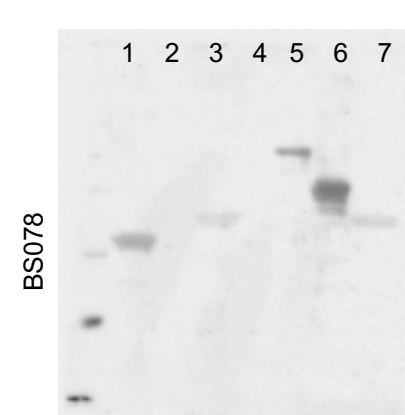
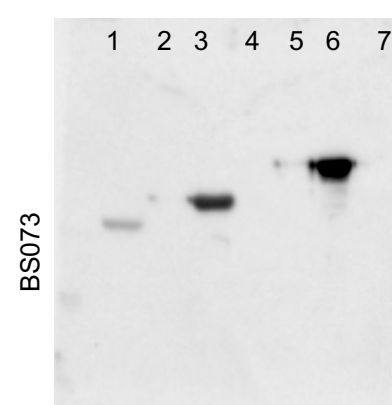
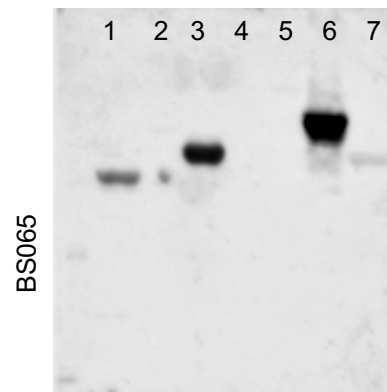
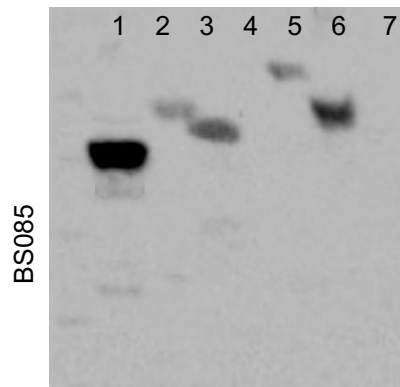
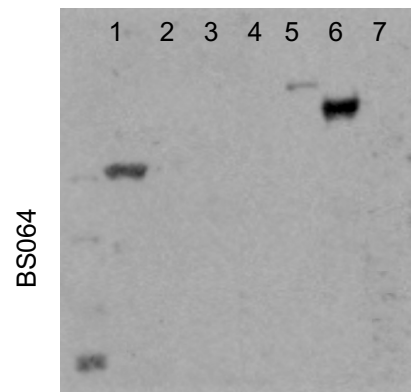


- Pooled BAL samples from  
severe sick animals
- BS074
  - BS081
  - BS083
  - BS071
  - BS088

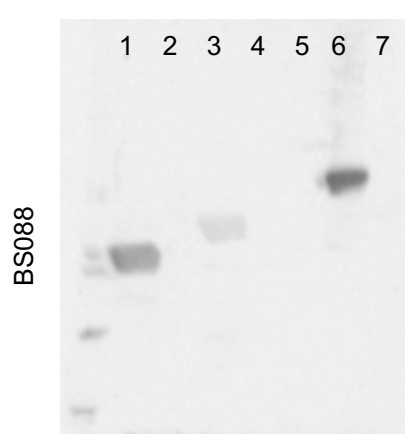
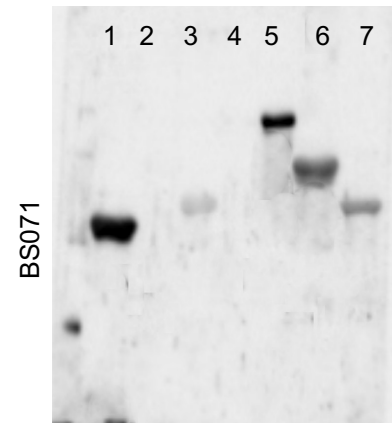
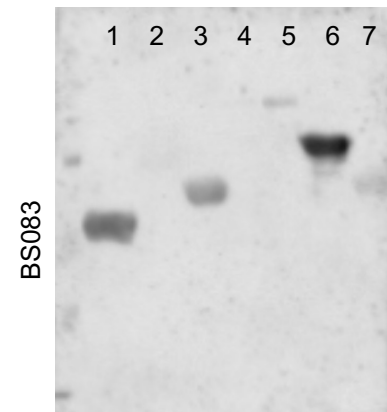
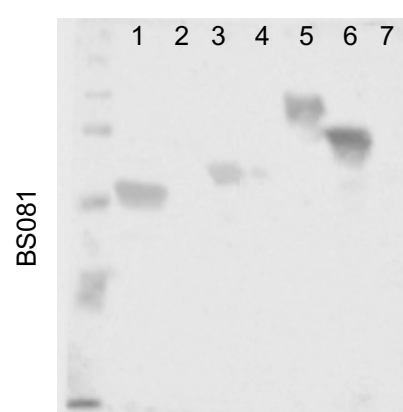
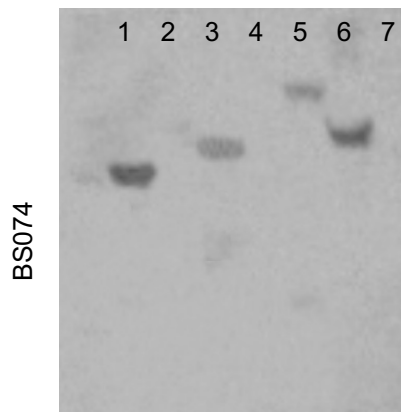
# CBPP – recombinant *Mmm* proteins – IgA in BAL

1. MSC- 0136 - 64kDa
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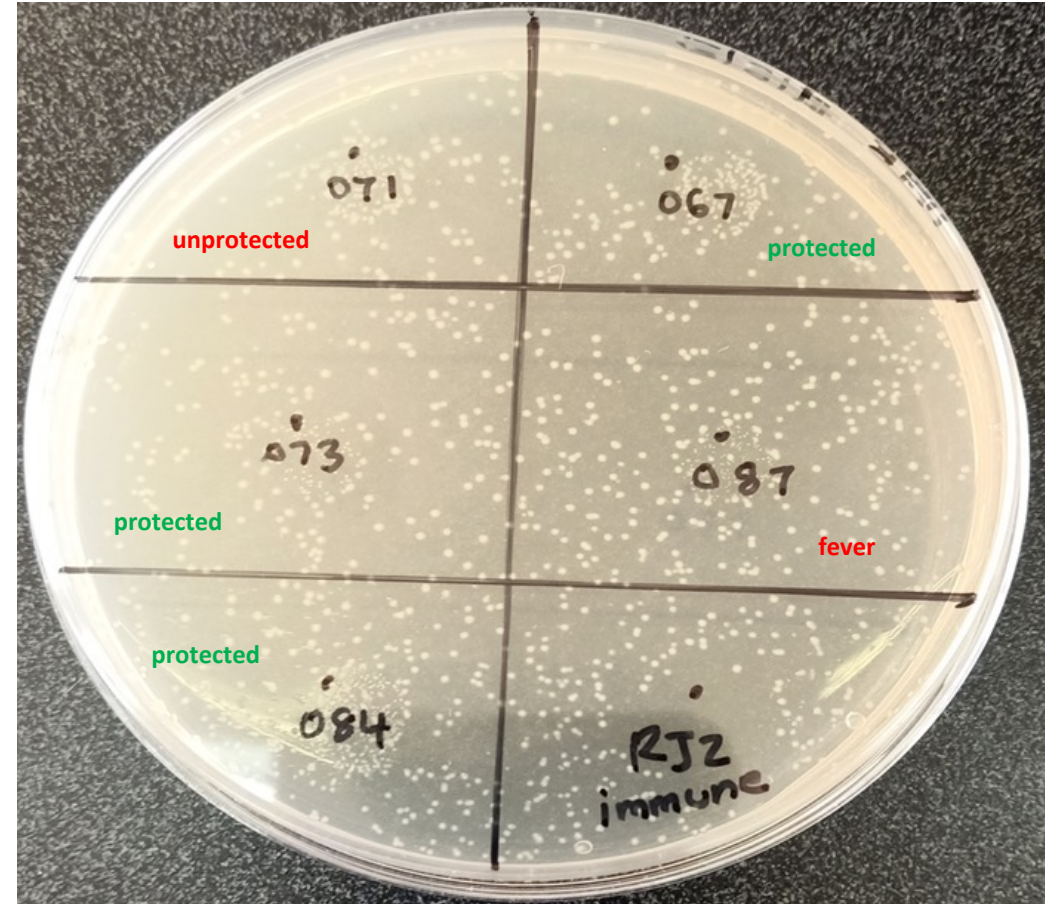
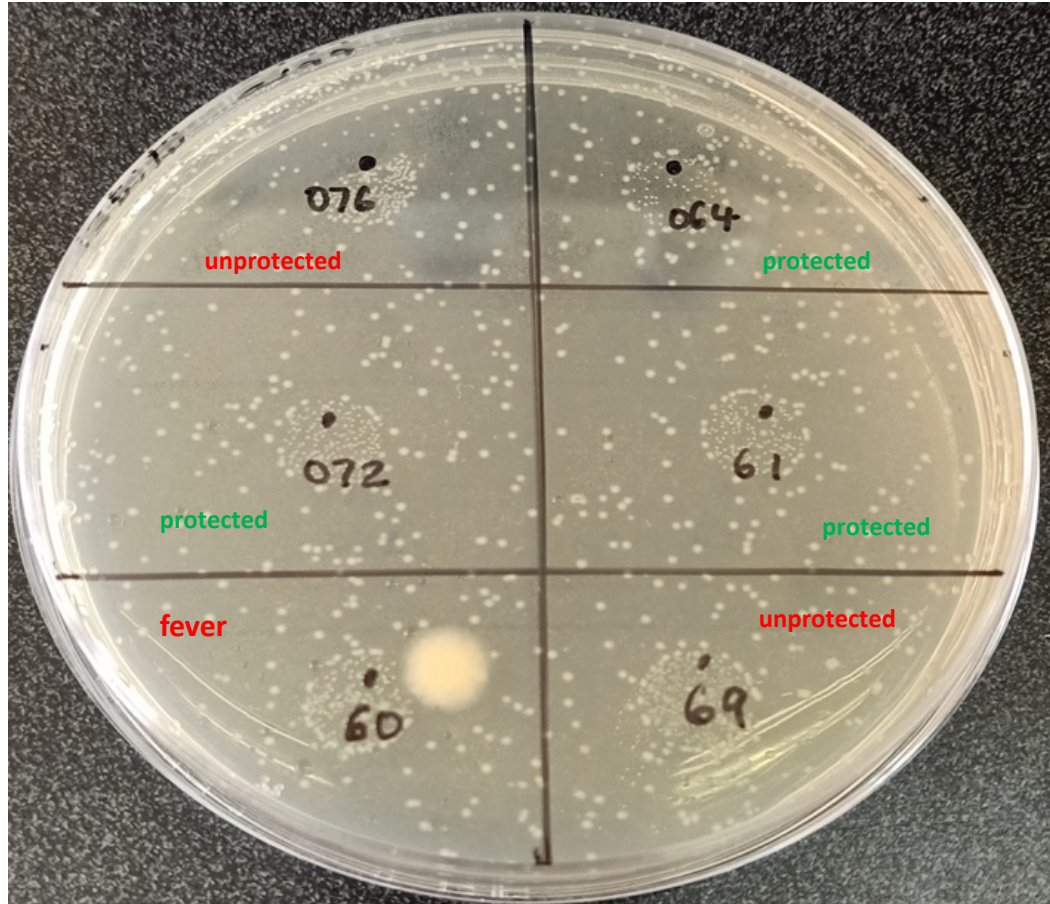
## PROTECTED ANIMALS BAL SAMPLES – IgA in BAL



## SEVERELY DISEASED ANIMALS – IgA in BAL

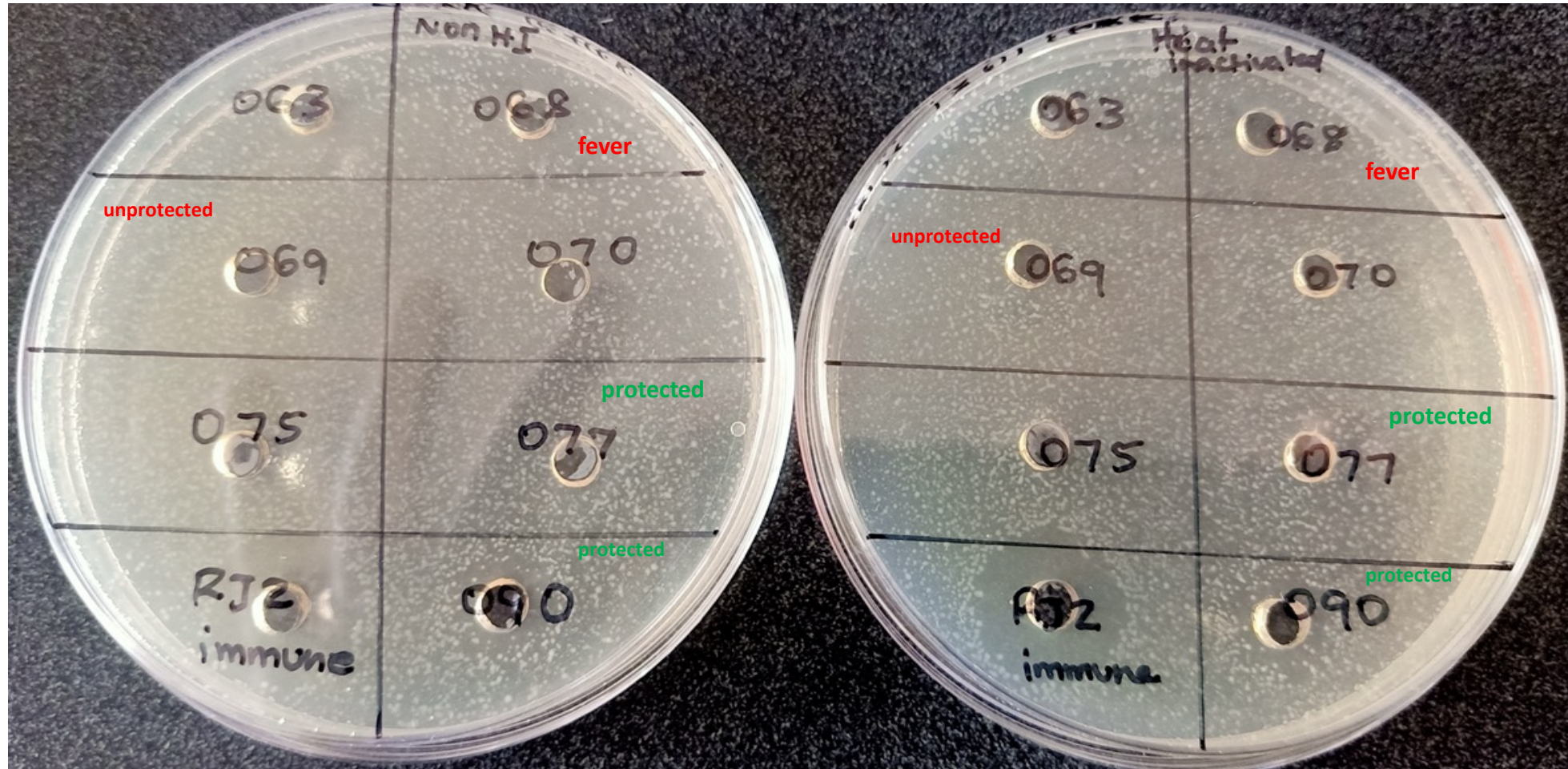


# Growth inhibition assay for *Mmm* (serum)



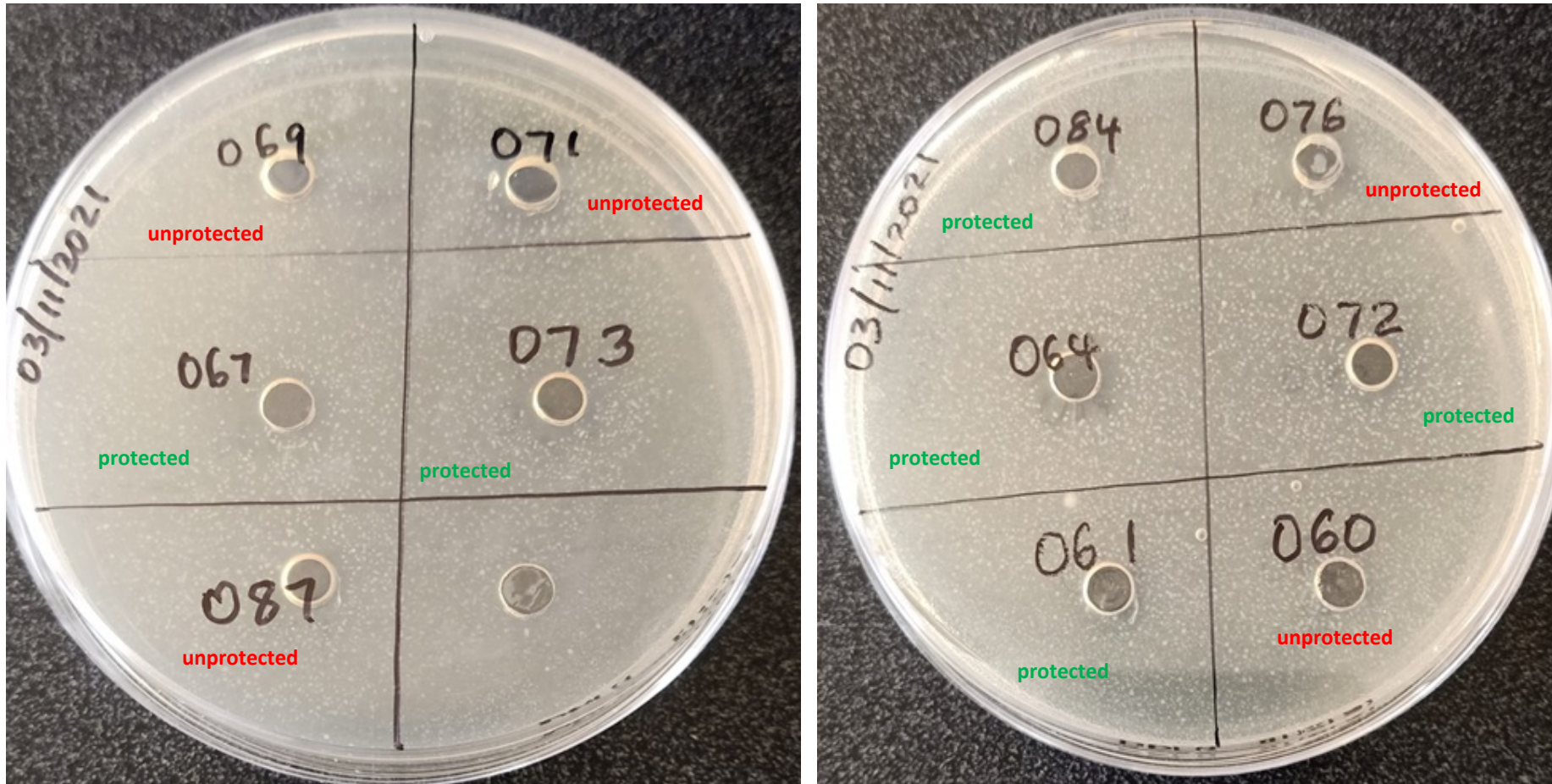
Pipette 20uL of the serum (27 dpi) on to the surface of the inoculated PPLO agar plate. Incubate at 37C.

# 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 37C for 4-7 days.

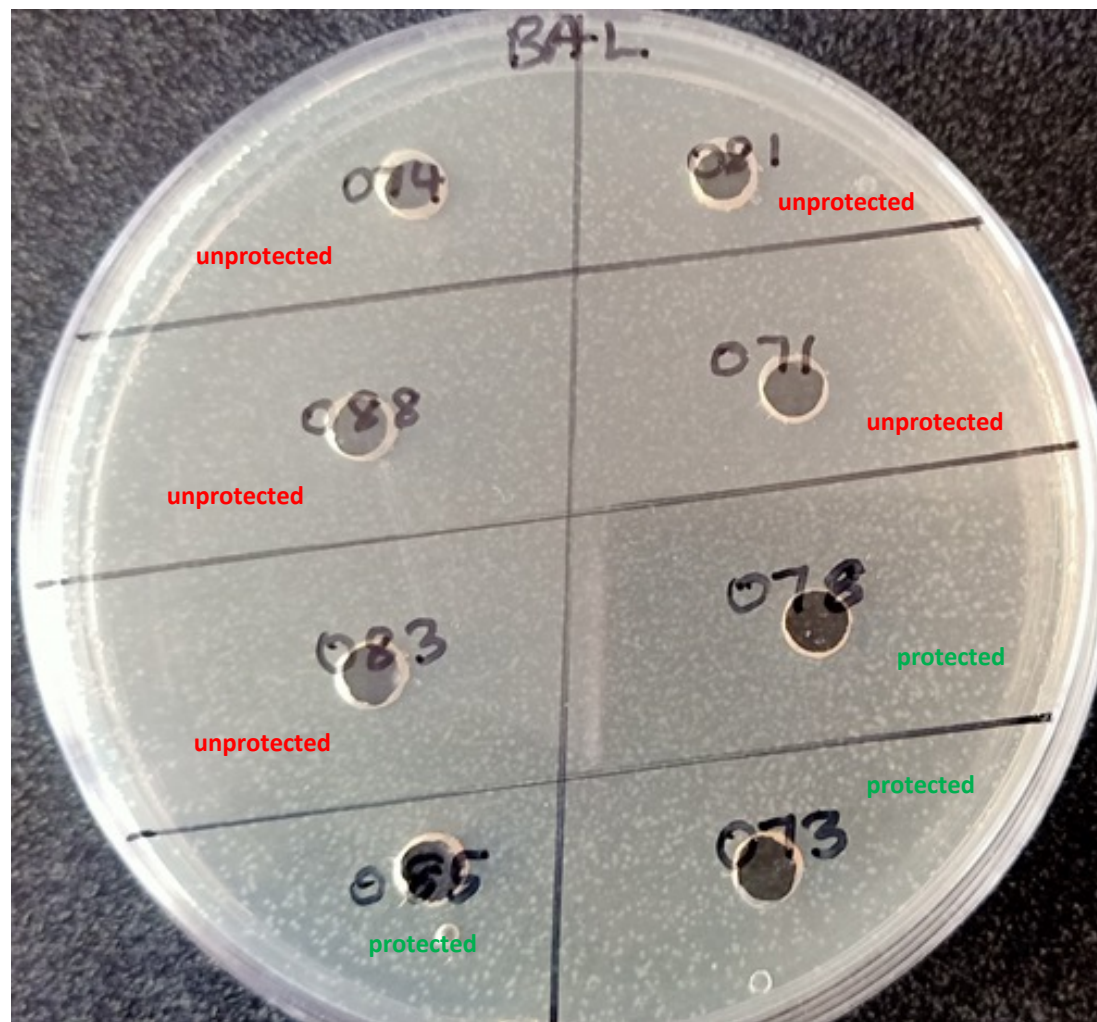
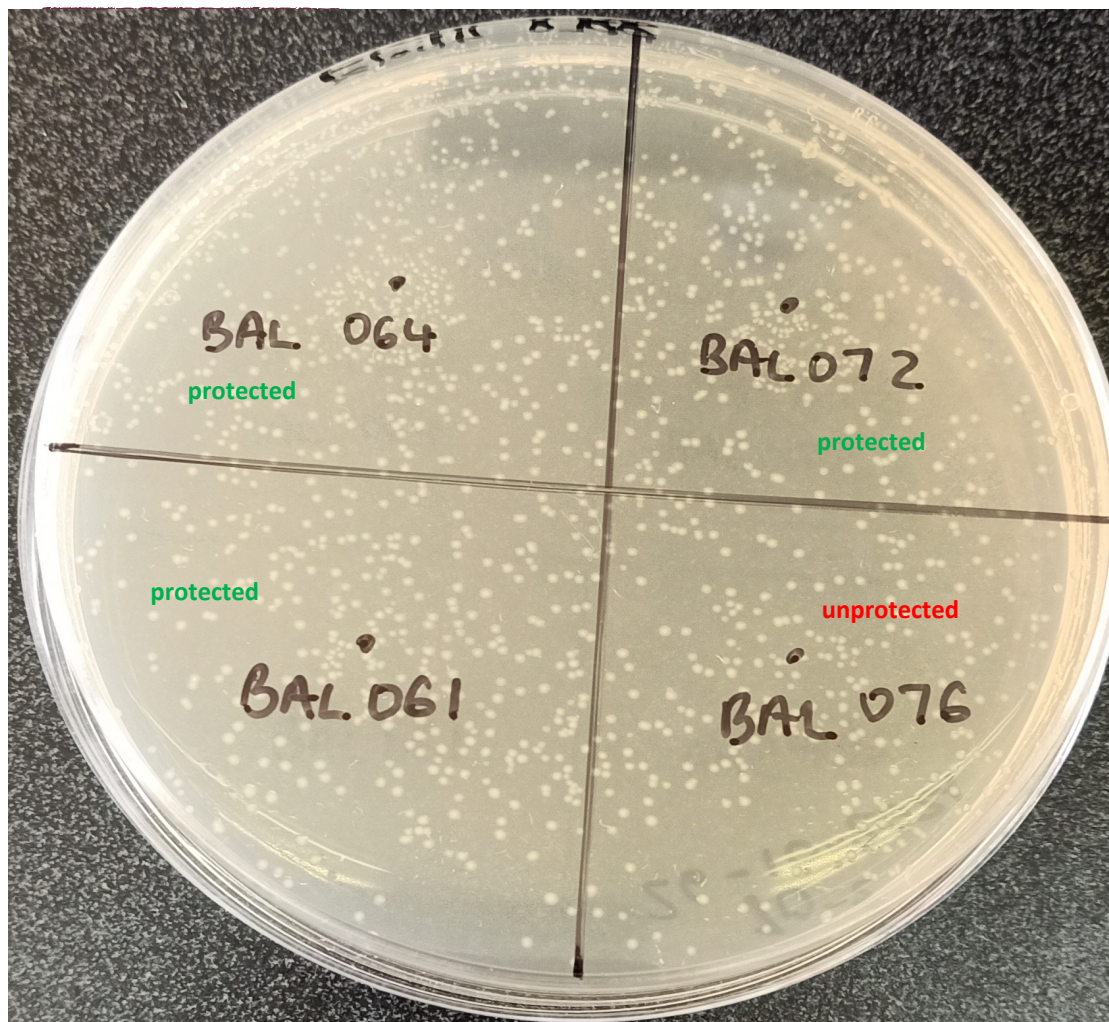
# Growth inhibition assay for *Mmm*- agar well diffusion method.



Pipette 50 $\mu$ L of the serum (27 dpi) into the well in the inoculated PPLO agar plate. Incubate at 37C for 4-7 days.



# Growth inhibition assay for *Mmm*- agar well diffusion method-BAL



# Thank you!



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Stephen Munyao, Rose Ojuok

Mycoplasma team

ILRI farm team



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