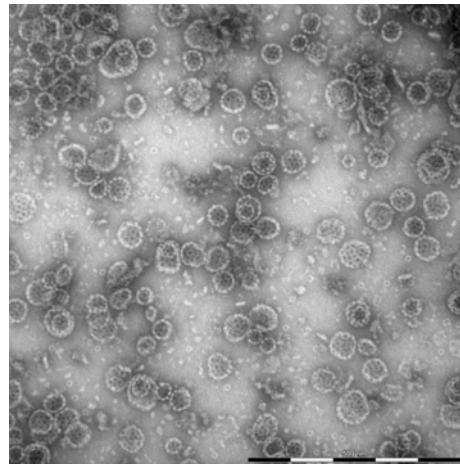


BRSV-ISCOMs induce clinical and virological protection in young calves with maternal antibodies

S Hägglund, K-F Hu, K Vargmar, L Poré, A-S Olofson, K Blodörn, J Anderson, P Ahooghalandari, K Ellencrona, K Lövgren Bengtsson, G Taylor, S Riffault, J-F Eléouët, J Pringle and J-F Valarcher

*Swedish University of Agricultural Sciences
Swedish National Veterinary Institute
Host Pathogen Interaction Group*



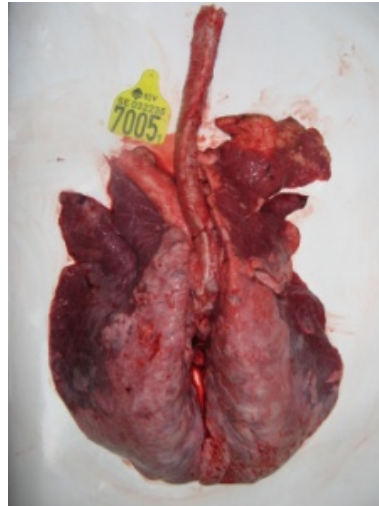
Bovine respiratory syncytial virus (BRSV)

Enveloped single stranded RNA virus



Clinical impact

Major cause of acute respiratory disease in calves and young stock



✓ *Need to protect animals at a young age*

Commercial BRSV vaccines



Inactivated adjuvanted, Live attenuated

When tested experimentally

- tested in calves without BRSV-specific maternal antibodies that inhibit immune responses to vaccination
- *or* against mild disease

Variable effect in the field

Vaccination can aggravate RSV disease

Formalin-inactivated HRSV vaccine

(Kim et al *Am J Epidemiol* 1969)

β -propiolactone inactivated BRSV vaccine

(Schreiber et al *J Vet Med* 2000)

increased morbidity and mortality compared to controls
after natural RSV infection



↓ Mucosal immunity
↓ Neutralizing antibodies

– *allowing infection*

↓ Th1 response (cytotoxic T lymphocytes)

– *poor virus clearance*

↑ Th2 response

– *cell influx (eosinophils), inflammation and bronchoconstriction*

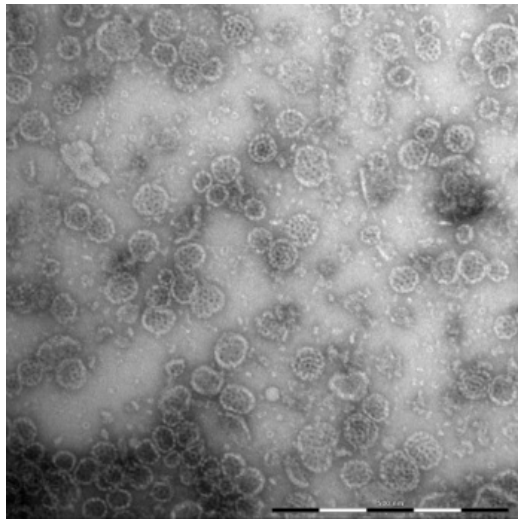
A new vaccine :

- Efficient in calves with maternal antibodies
- Virological protection, stop virus circulation
- Long duration of protection
- No spread of vaccine strain
- Good safety – no exaggerated immunological reactions against infection after vaccination

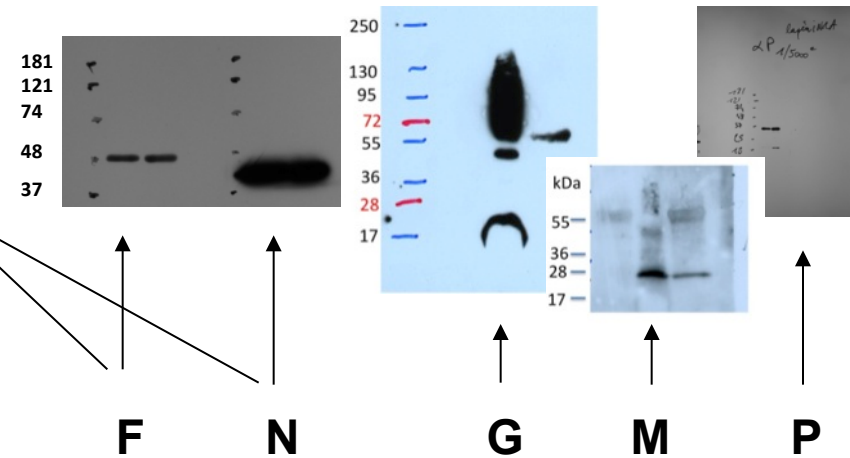


Experimental BRSV-ISCOM vaccine

Quillaja saponin + cholesterol + phospholipids + antigen
= Immunostimulating complexes (ISCOMs)



56,4 nm (SD 11.1nm)



Mass spectrometry: BRSV F and N and cellular proteins

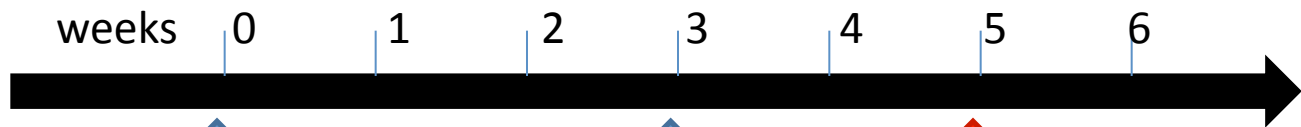
188 ug total protein per dose, ~50% BRSV proteins

Previous comparison with commercial vaccine in
7–15 weeks old calves with maternal antibodies
→ **highly significant improvement of protection**

(Hägglund et al 2004)

Aim of the present study:

Characterize immune responses and protection in
younger animals



1st s.c. vaccination

2nd s.c. vaccination

BRSV challenge by aerosol

Pathology



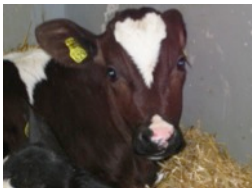
BRSV-ISCOM n=5



BRSV protein n=5



Adjuvant n=5

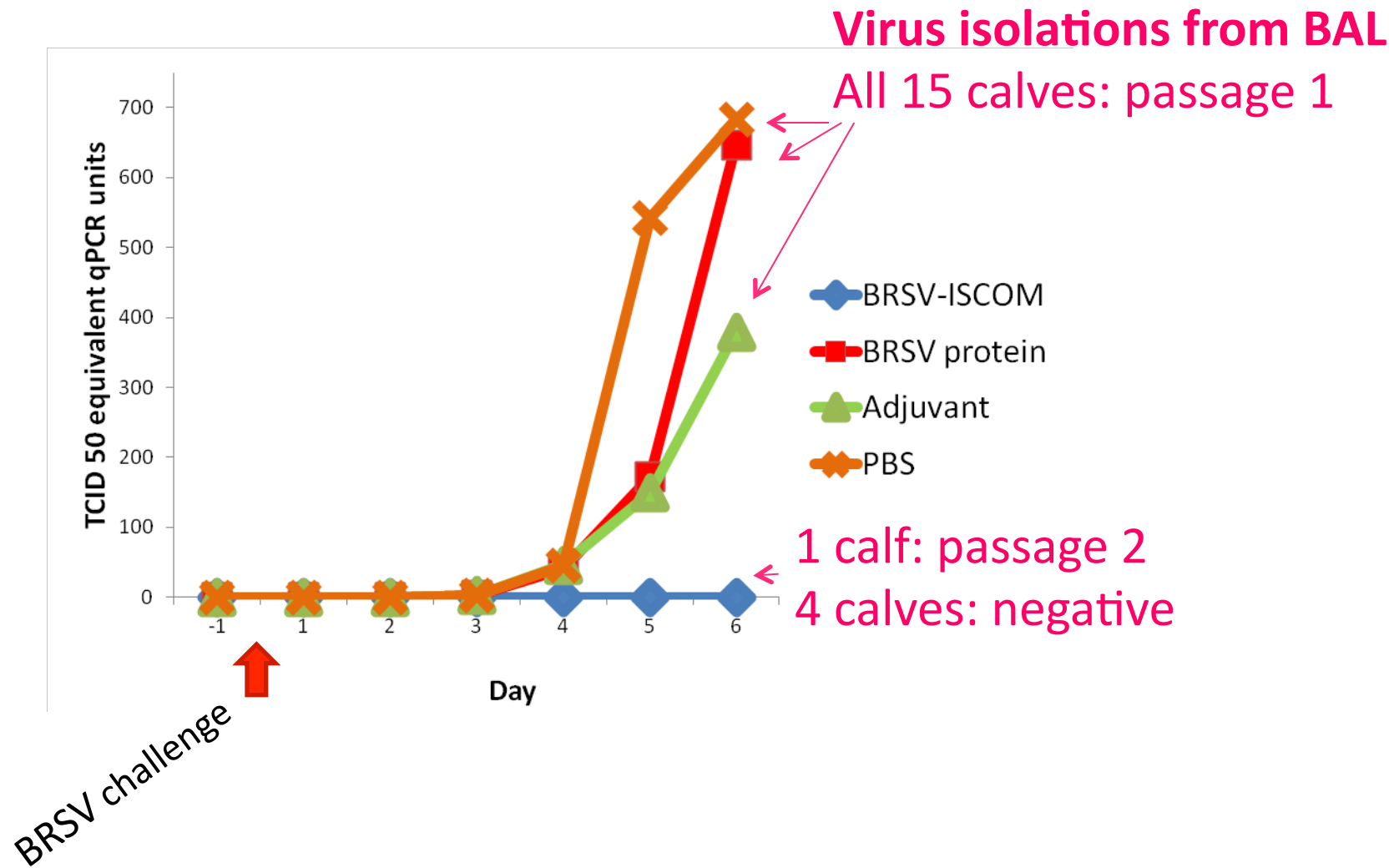


PBS n=5

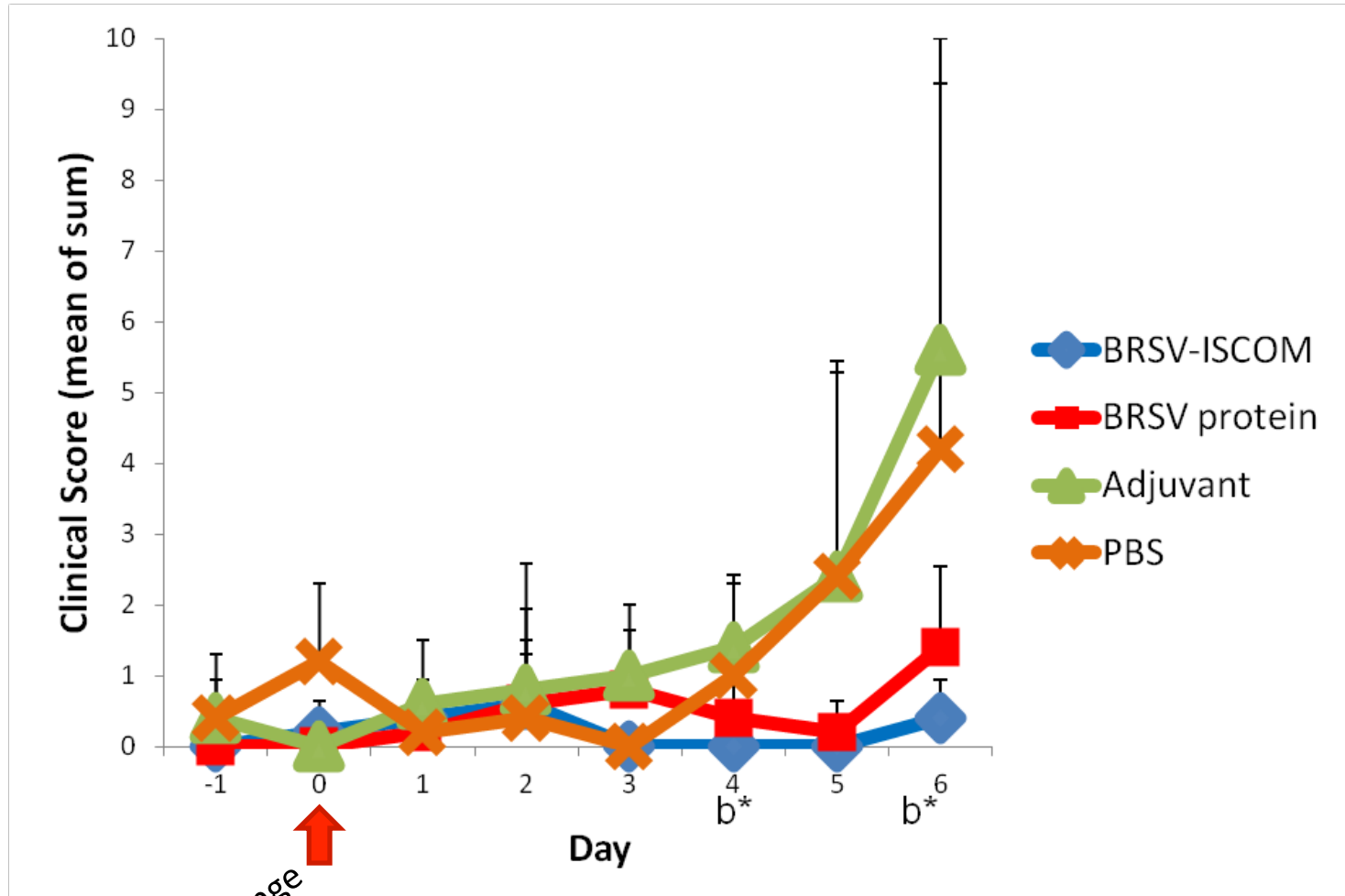


✓ 3–8 week old calves with variable levels of BRSV-specific maternal antibodies

Detection of BRSV by PCR in nasal secretions



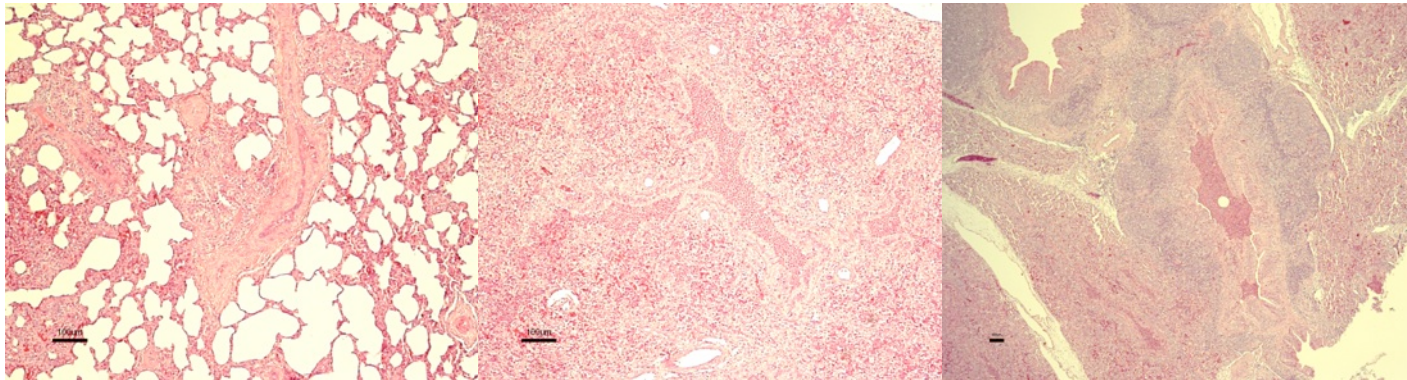
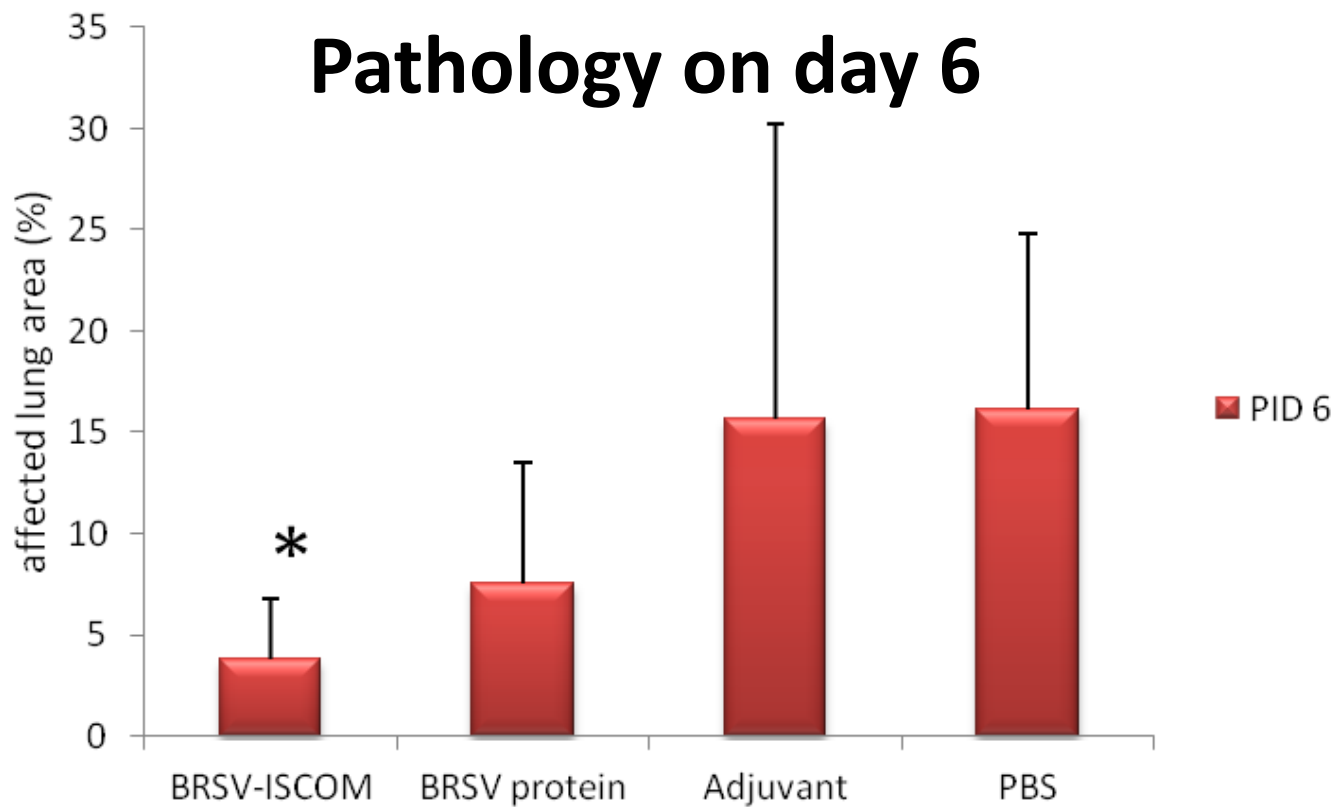
Clinical signs of disease after BRSV challenge



BRSV challenge

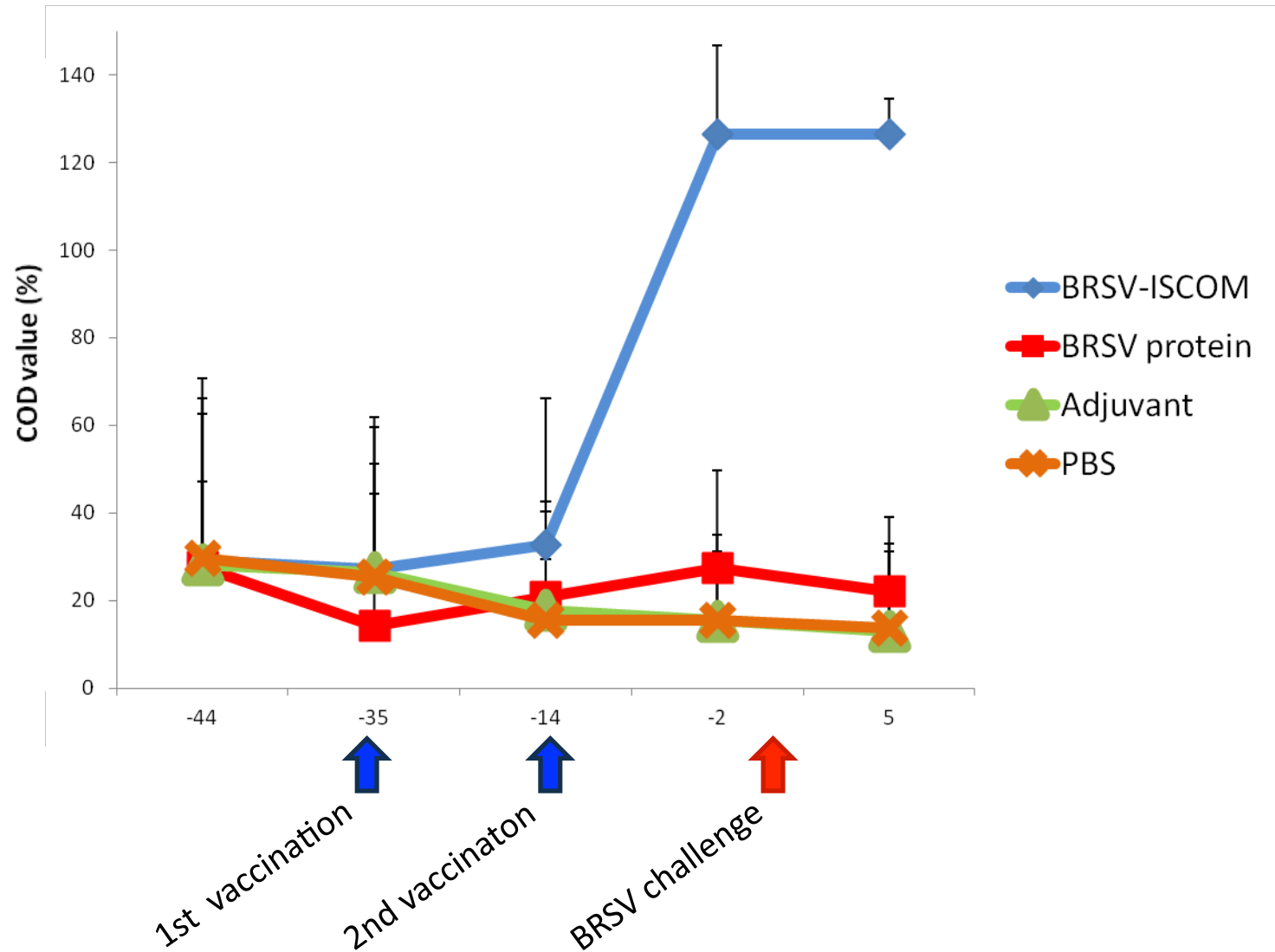
- ✓ Rectal temperatures up to 41°C in controls
- ✓ Respiratory rates up to 76/min in controls
- ✓ Significant clinical protection

Pathology on day 6



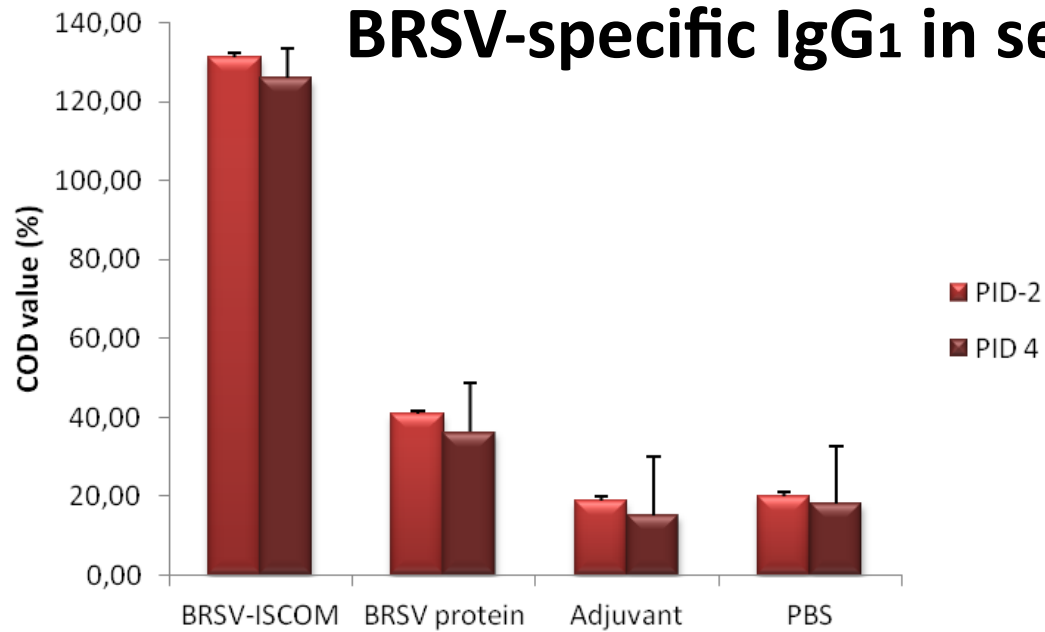
- ✓ Reduction of pathology (no aggravation)
- ✓ No increase in cellular infiltrate or eosinophils

BRSV-specific IgG in sera

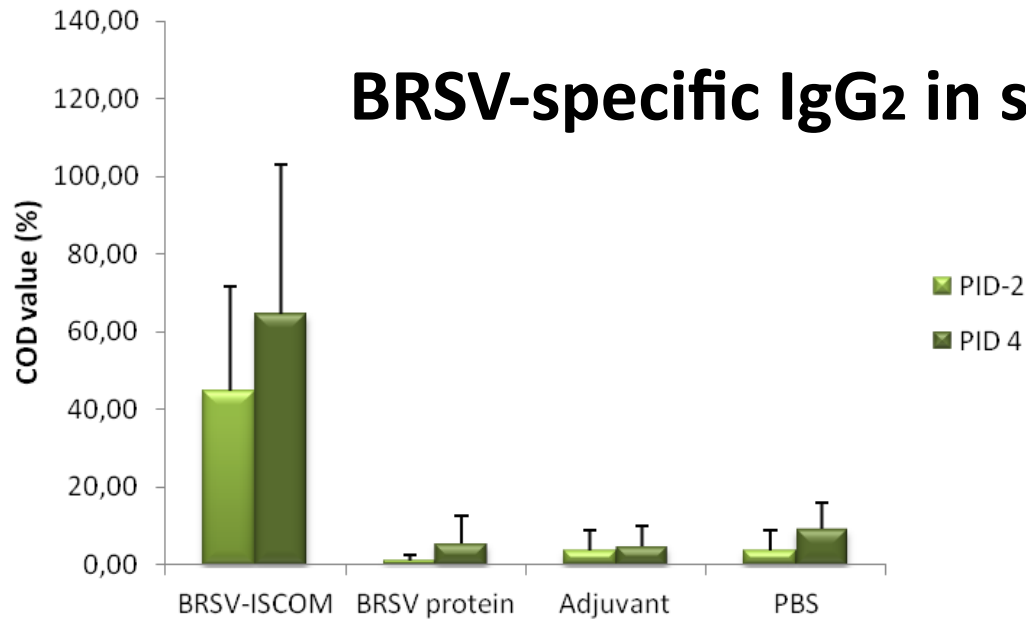


✓ **Rapid and strong systemic IgG responses**

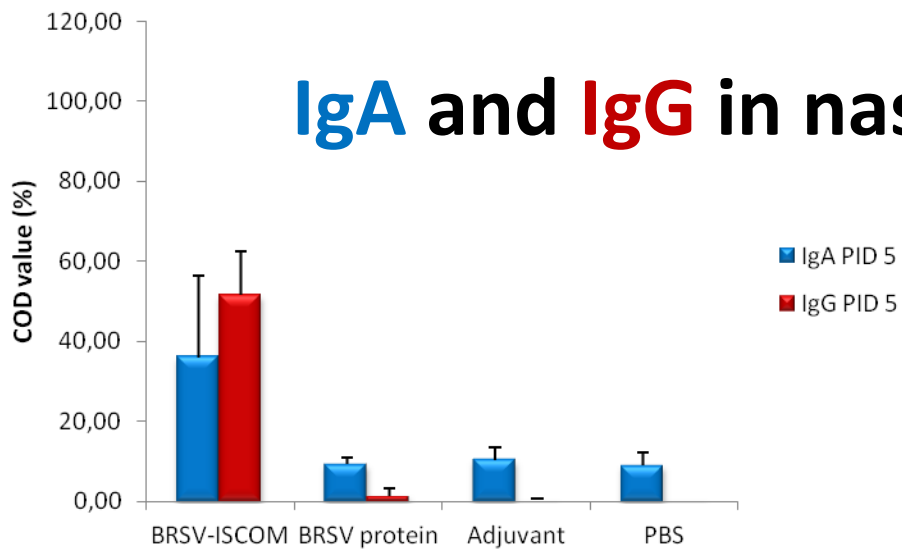
BRSV-specific IgG1 in sera



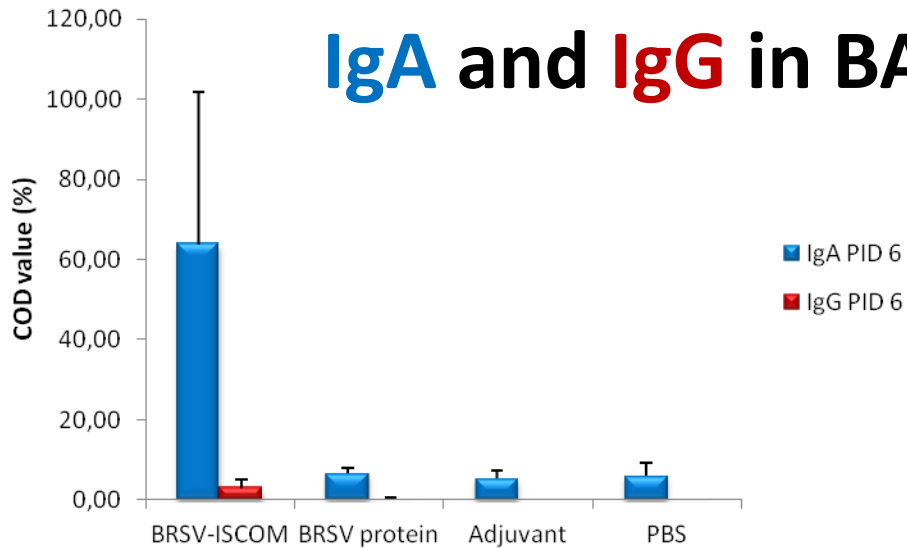
BRSV-specific IgG2 in sera



IgA and IgG in nasal fluids

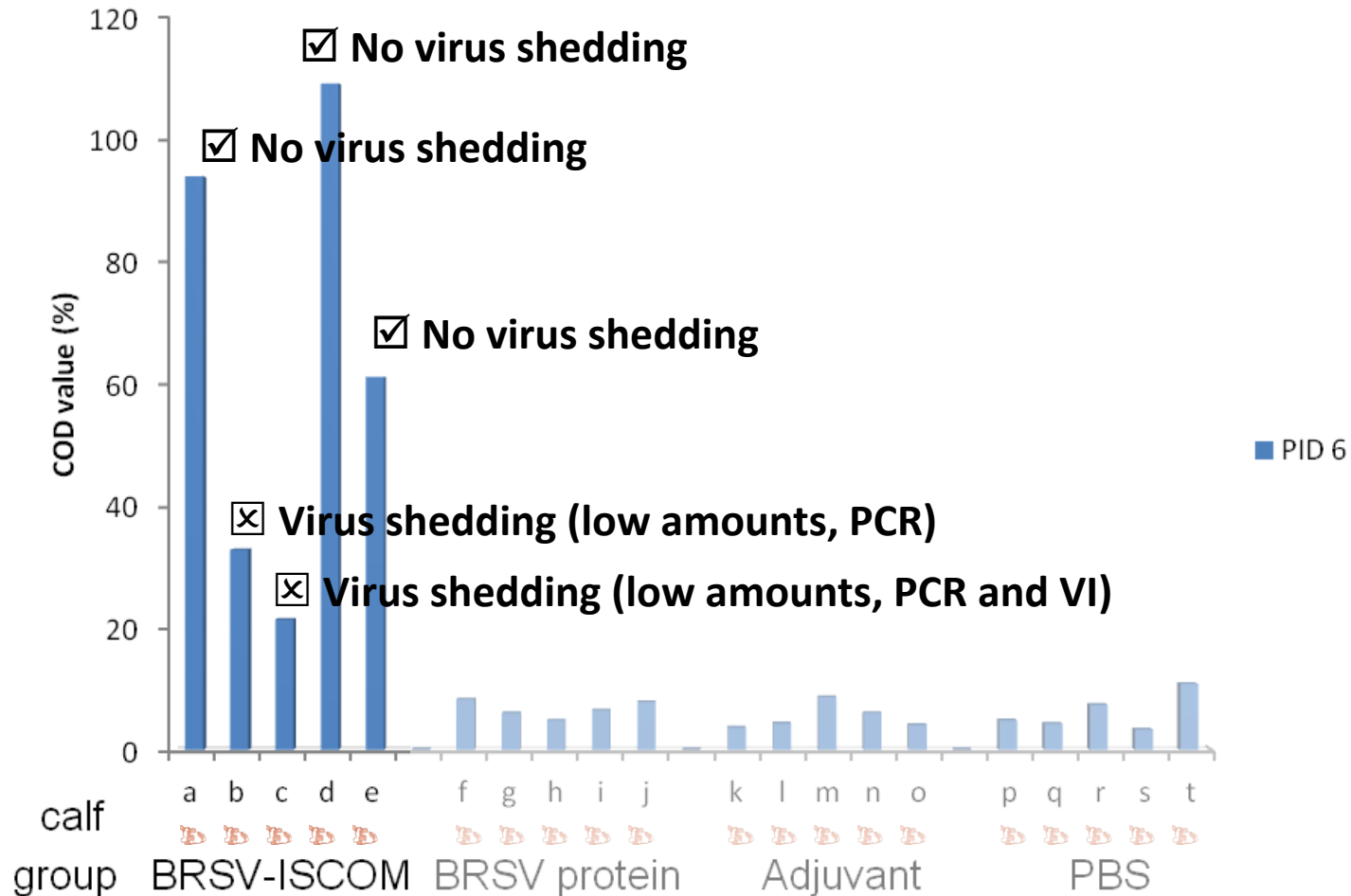


IgA and IgG in BAL



✓ *Very high amounts of IgA in lung*

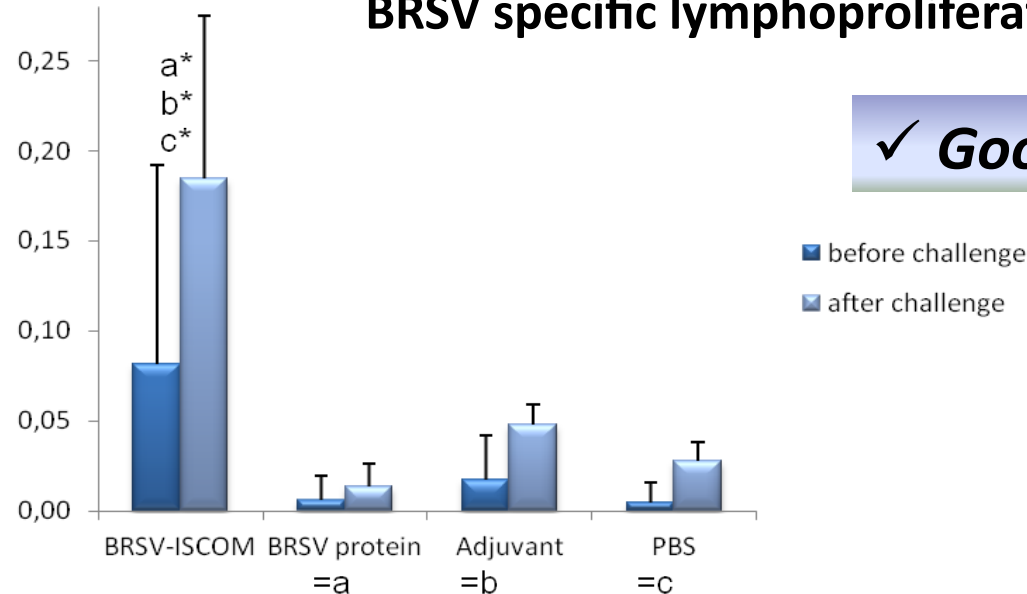
individual values of IgA in BAL day 6



✓ Lung IgA levels correlated with viral protection

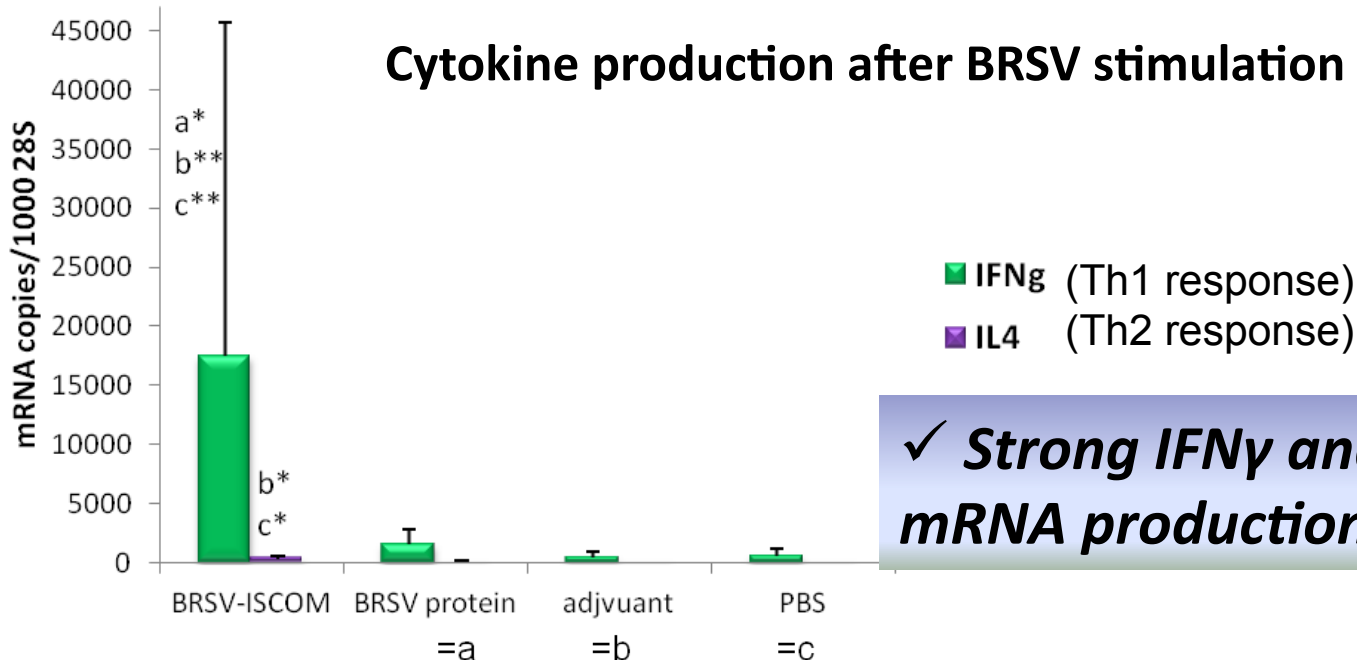
BRSV specific lymphoproliferation

✓ *Good lymphocyte expansion*



Cytokine production after BRSV stimulation in vitro

✓ *Strong IFN γ and moderate IL-4 mRNA production per cell*



Conclusions

Efficient in calves with
maternal antibodies



BRSV-ISCOMs

induce

virological and clinical protection

due to

rapid humoral responses

strong mucosal immunity (IgA)

balanced T helper cell responses (Th1/Th2)

**-A tool to reduce
virus circulation?**

- No exaggerated inflammatory response upon RSV infection

Acknowledgement



Funded by Swedish Farmers' Foundation fo Agricultural Research

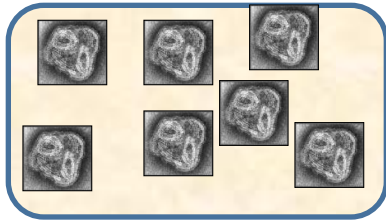
Thanks also to:

B. Norén and staff for rearing and maintenance of animals

L. E. Larsen DTU, Denmark for sharing the BRSV DK9402022 strain

B. Morein and ISCONOVA AB, Sweden for material and advice on vaccine preparation

BRSV infected cell culture



Uninfected cell culture



Purification by ultracentrifugation



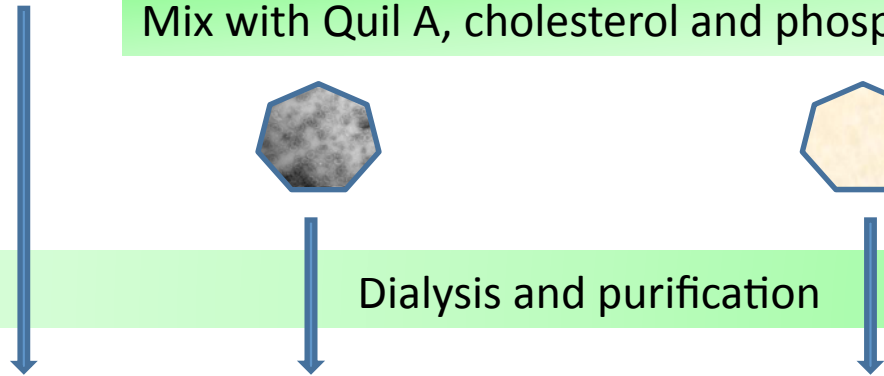
Solubilization , ultracentrifugation and recovery of one fraction



Mix with Quil A, cholesterol and phospholipids



Dialysis and purification



BRSV proteins = P **BRSV-ISCOMs = I** **control ISCOMs = C**

Detection of BRSV by PCR in nasal secretions, individual data

Different scale

Virus isolation from BAL on day 6

