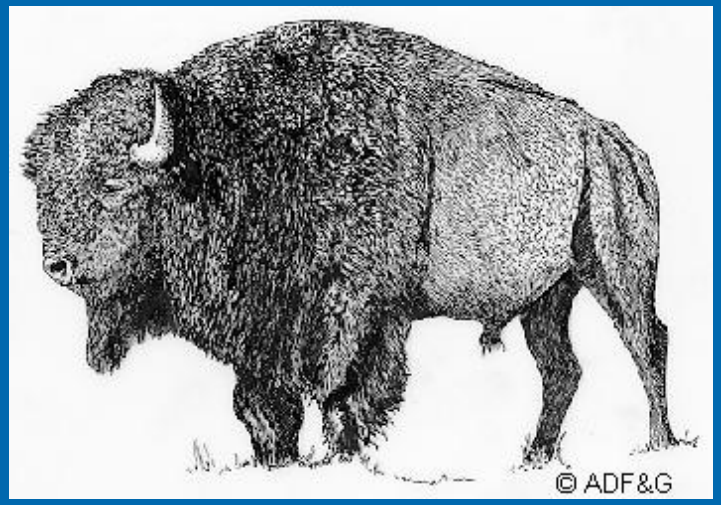
Malignant Catarrhal Fever and Vaccine Development



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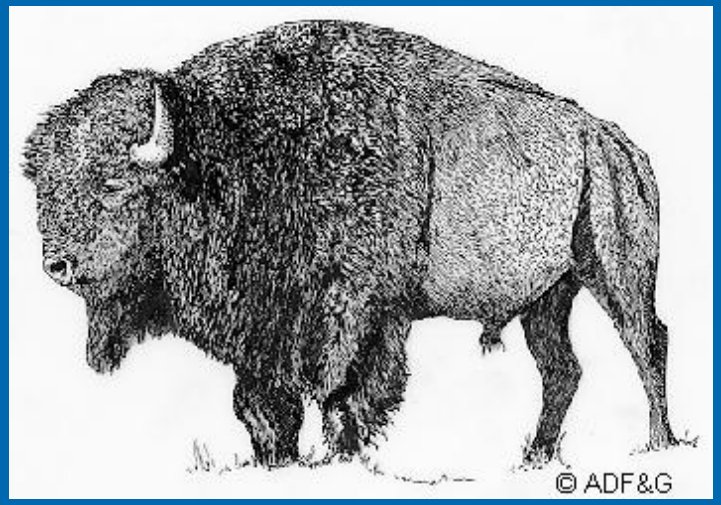
Malignant Catarrhal Fever and Vaccine Development



Outline

General background of MCF

What has been done

What we are doing specifically on vaccine development

Malignant catarrhal fever (MCF)

a frequently fatal disease primarily of ruminant species

Cattle

Deer

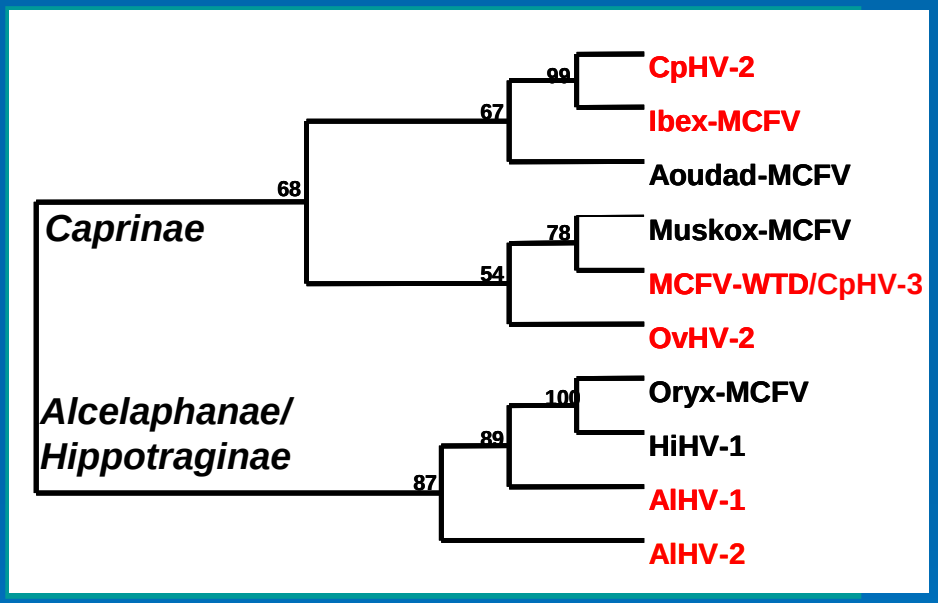
Bison

Causative agents: MCF viruses

(*Macavirus, Gammaherpesvirinae*)

10 members in MCFV group and 6 associated with disease

Goats: CpHV-2 and MCFV-WTD/CpHV-3



Oryx-MCFV

Ibex-MCFV

Roan antelope: HiHv-1

Aoudad-MCFV

Hartebeest: AlHV-2

Muskox-MCFV

Sheep: OvHV-2

Wildebeest: AlHV-1

Li, *et al.*, JGV, 2005

Transmission and outcomes

OvHV-2

Reservoir

Clinically Susceptible Hosts

Subclinical Infection

MCF → Death

→ Recovery

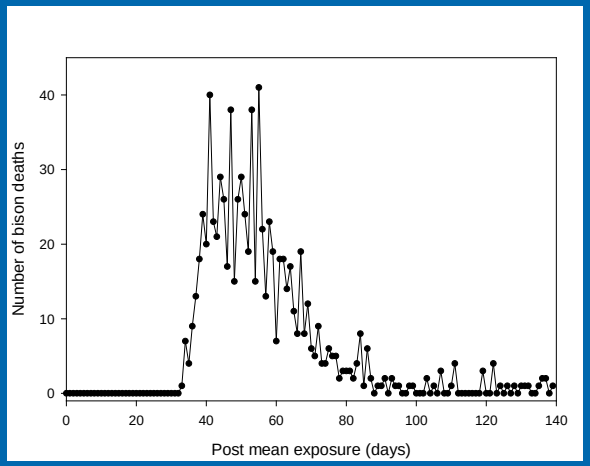
OvHV-2 X

Sheep-associated MCF by OvHV-2

One of the most important infectious diseases in North American bison

Outbreak in a bison feedlot

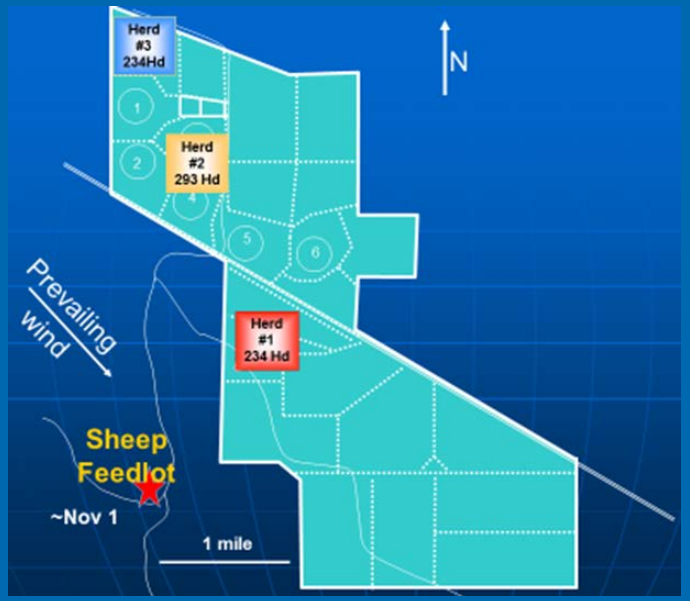
- **Total bison in lot: 1,618**
- **Total sheep: 1400 (7 mo old)**
- **Exposure days: 19 days**
- **Distance: 50 - 600 yards**
- **First case: 34 days PME (post mean exposure)**
- **Last case: 139 days PME**
- **Peak loss: 42 head/day**
- **Total dead: 835 (51.6%)**



Li, *et al.*, 2006

Outbreak in a bison ranch

- Total bison in the ranch: 761
- Total sheep: ~ 20,000 (6 -7 mo old)
- Starting exposure date: ~ Nov. 1
- Distance: 1 - 3 miles
- First case occurred in December
- Total dead: 54 (7.1%)
- Mortality rates associated with distances



Herd	No.	case	Dec	Jan	Feb	Mar	Apr	May
1	234	MCF	2	4	9	8	12	6
2	293	MCF	0	0	0	12	6	--
3	234	MCF	0	0	0	0	1	--
Total	761		2	4	9	20	19	6

Li, *et al*., 2008

SA-MCF, leading cause of death in bison

- A prospective study in a feedlot: 75% of total losses due to MCF
  - total annual loss = 8.8%
  - loss due to MCF = 6.6%

<u>additional recorded outbreaks</u>
feedlot – 9.4% (66/702)
feedlot – 8.5% (34/399)
auction – 27.6% (45/163)
ranch – 33% (300/900)
small herds - 90% (18/20)
- 94% (16/17)

Schultheiss, et al., 2000; O'Toole *et al*., 2002; Berezowski, *et al*., 2005

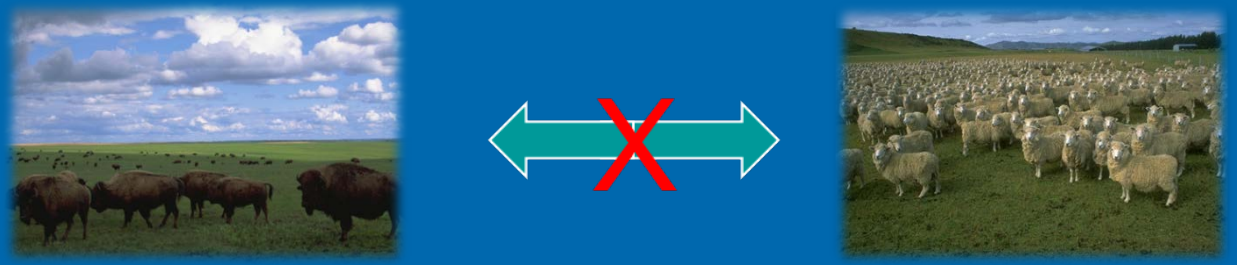
Bison are highly susceptible to MCF

Species[a]	OvHV-2 Inoculum (DNA copies)[b]							
	10^2	10^3	10^4	10^5	10^6	10^7	10^8	$>10^9$
Sheep	Neg[c]	Pos	Pos	Pos	Pos	Pos	Pos	MCF
Cattle				Neg	Pos	Pos	Pos/MCF	
Pigs			Neg	Neg	MCF	MCF	MCF	
Rabbits			Neg	Pos/MCF	MCF	MCF		
Bison		Neg	Pos/MCF	MCF	MCF	MCF	MCF	

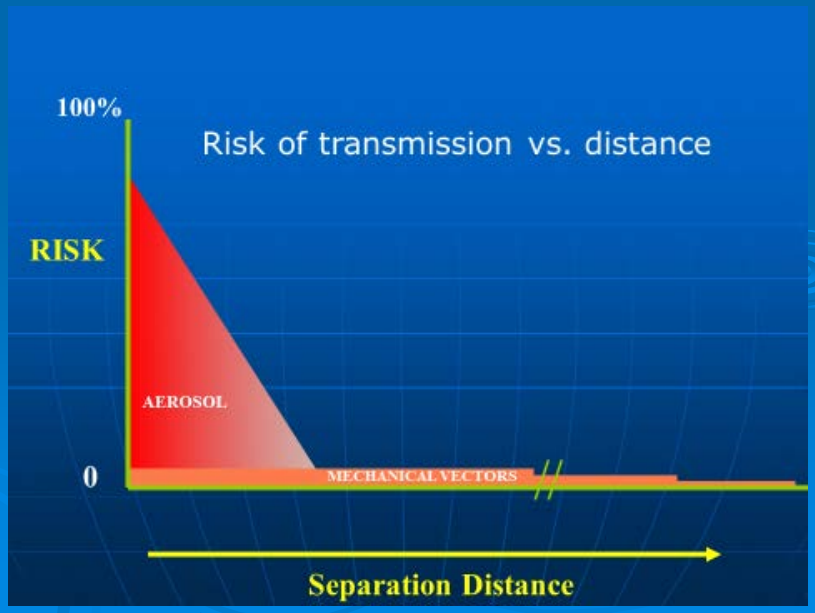
Neg = no infection; Pos = subclinical infection; MCF = clinical disease, and the gray shaded blocks indicating a minimal dose required for induction of disease.

Li, *et al*., 2014

Treatment and prevention



- No treatment available
- No vaccine available
- The only prevention measure is to separate bison from sheep



UNITED STATES ANIMAL HEALTH ASSOCIATION - 2006

RESOLUTION: 16 APPROVED

SOURCE: COMMITTEE ON INFECTIOUS DISEASES OF CATTLE, BISON AND CAMELIDS

SUBJECT MATTER: VACCINE DEVELOPMENT FOR MALIGNANT CATARRHAL FEVER IN BISON

DATES: MINNEAPOLIS, MINNESOTA, OCTOBER 12-18 2006

.

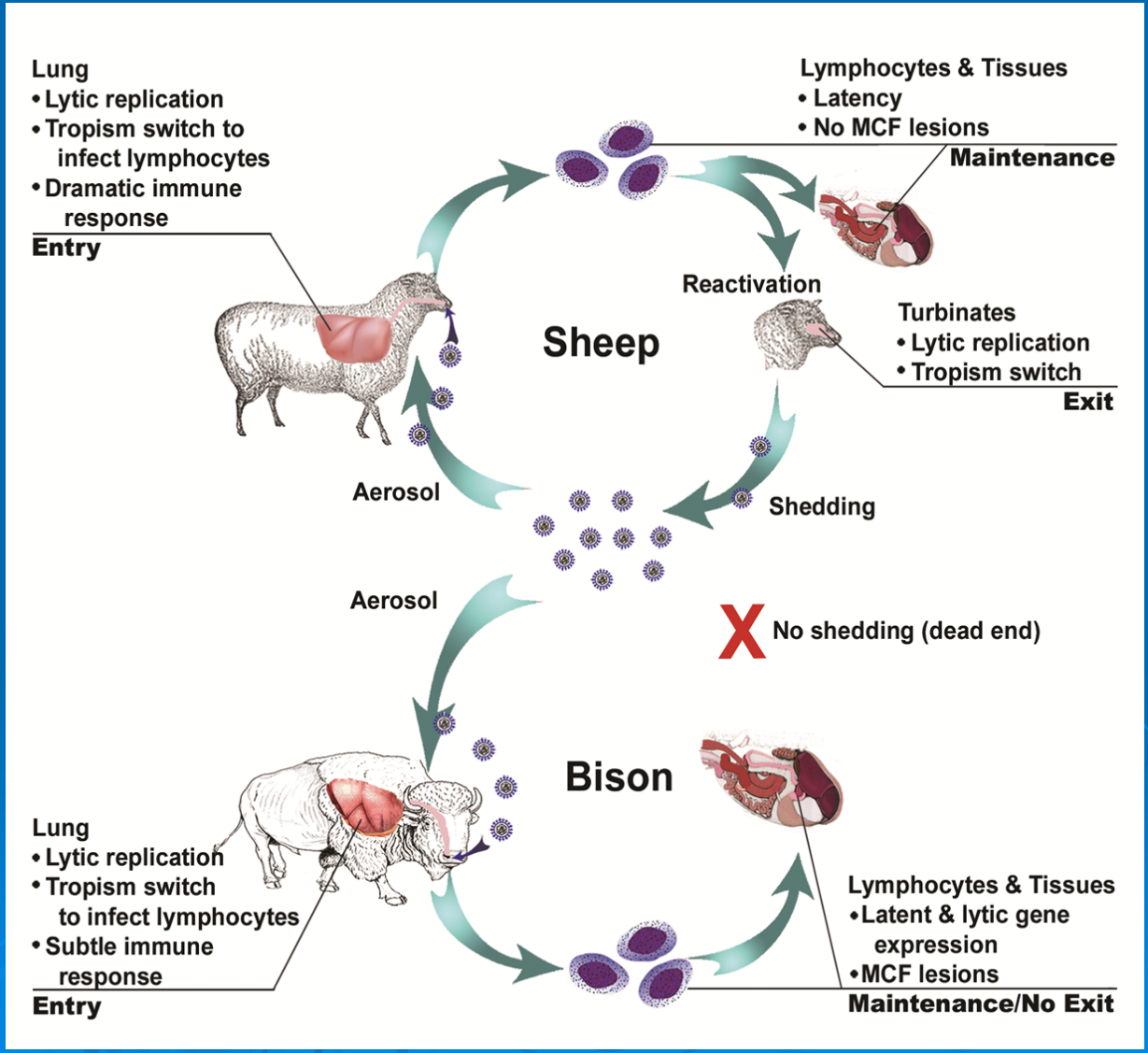
Objective

Develop an effective vaccine to protect bison from MCF

The major obstacles: no cell culture available to grow OvHV-2 and little known about OvHV-2 replication in sheep or bison

What has been done?

- Established infectious OvHV-2 stocks from sheep nasal secretions
- Sequenced OvHV-2 genome
- Developed animal models
- Evaluated OvHV-2 replications in sheep and bison
- Developed a vaccine strategy for SA-MCF

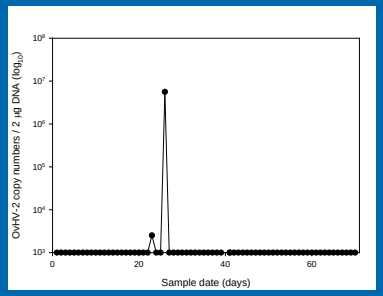


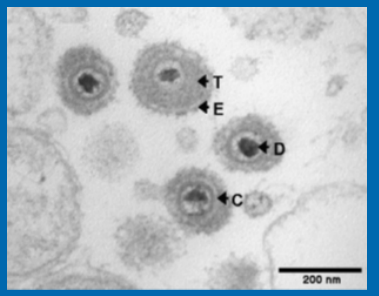
Animal models

- Defined infectious OvHV-2 inoculums
- Induced infection and disease in sheep, rabbits, bison, cattle and pigs.
- Susceptibility varies among species
- Disease is dose-dependent
- Rabbits are the suitable model for the SA-MCF research

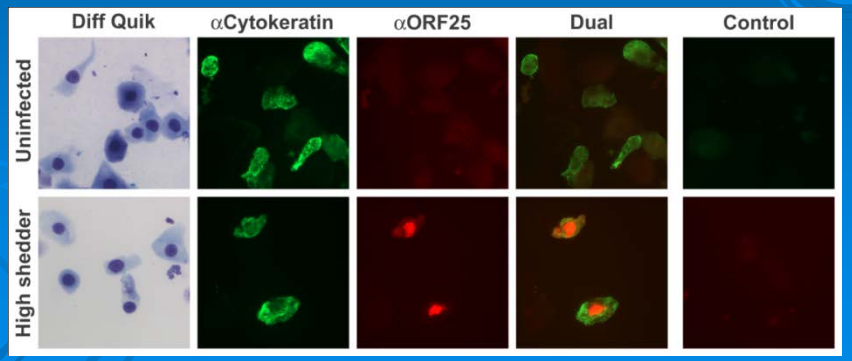
OvHV-2 replication in sheep: shedding

- OvHV-2 is shed through sheep nasal secretions
- Shedding is intense, but short-lived
- The virus replicates predominantly in turbinate of sheep
- Turbinate epithelial cells likely support OvHV-2 lytic replication





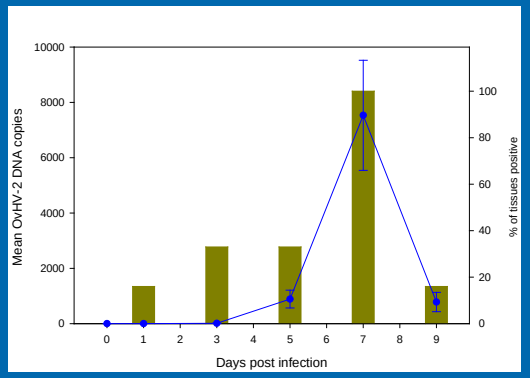
Tissues	ORF 25 transcripts *							
	High Shedder					Non-Shedder		Uninfected
	1052	1062	1059	1061	1055	1065	1066	1148
Turbinate Left Caudal	+	+	+	–	–	–	–	–
Turbinate Left Middle	+	+	+	–	–	–	–	–
Turbinate Left Rostral	–	+	+	–	–	–	+	–
Turbinate Right Caudal	+	+	–	–	–	–	–	–
Turbinate Right Middle	–	+	–	–	–	–	–	–
Turbinate Right Rostral	–	+	–	–	–	–	–	–
Trachea Caudal	–	–	–	–	–	–	–	–
Trachea Middle	–	+	–	–	–	–	–	–
Trachea Rostral	–	+	–	+	–	–	–	–
Lung Caudal	–	–	–	+	–	–	–	–
Lung Middle	–	+	–	–	–	–	–	–
Lung Cranial	–	+	–	–	–	–	–	–
Pharynx	–	–	–	–	–	–	–	–
Trigeminal ganglia	–	–	–	–	–	–	–	n/a
Tonsil	–	–	–	–	–	–	–	–
Brain	–	–	–	–	–	–	–	–
Salivary Gland	–	–	–	–	–	–	–	–
Buccal Mucosa	–	–	–	–	–	–	–	–
Lymph node Prescapular	–	–	–	–	–	–	–	–
Lymph node Retropharyngeal	–	–	–	–	–	–	–	–
Lymph node Mesenteric	–	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–	–

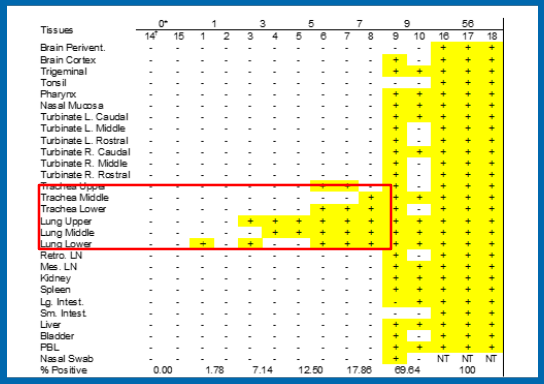


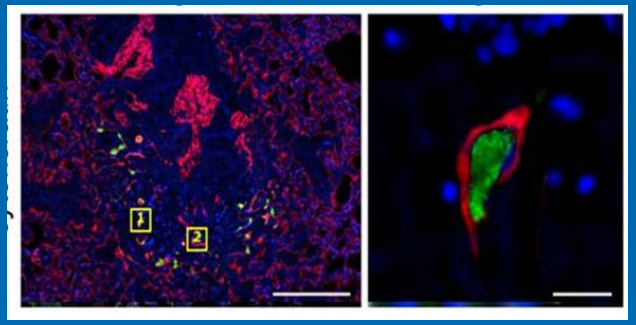
Li, *et al*., 2004; Cunha, *et al*., 2007

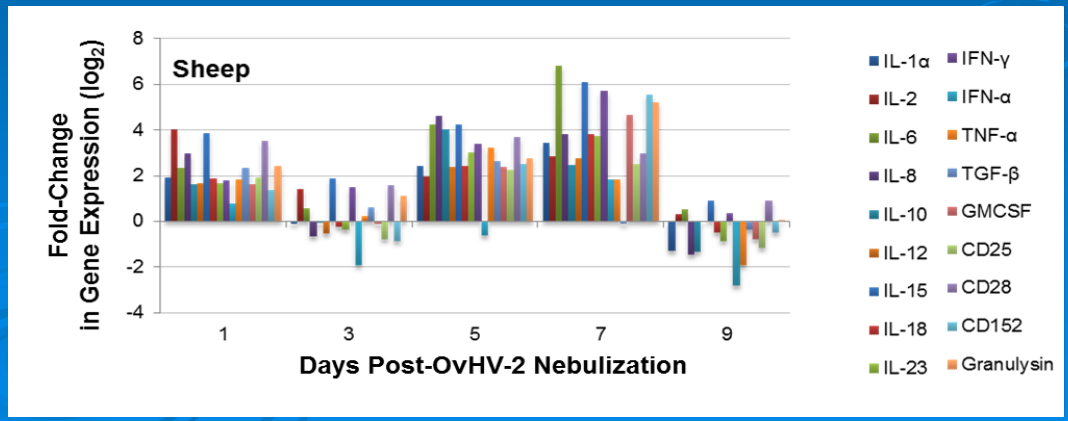
OvHV-2 replication in sheep: entry

- OvHV-2 initial replication occurs in lung and is required for infection
  - Viral DNA and ORF25 transcripts in lung
  - Viral DNA not detected in any other tissues during early infection
  - IV and IP inoculation failed to induce infection
- Lung epithelial cells support OvHV-2 lytic replication
- Strong local immune response





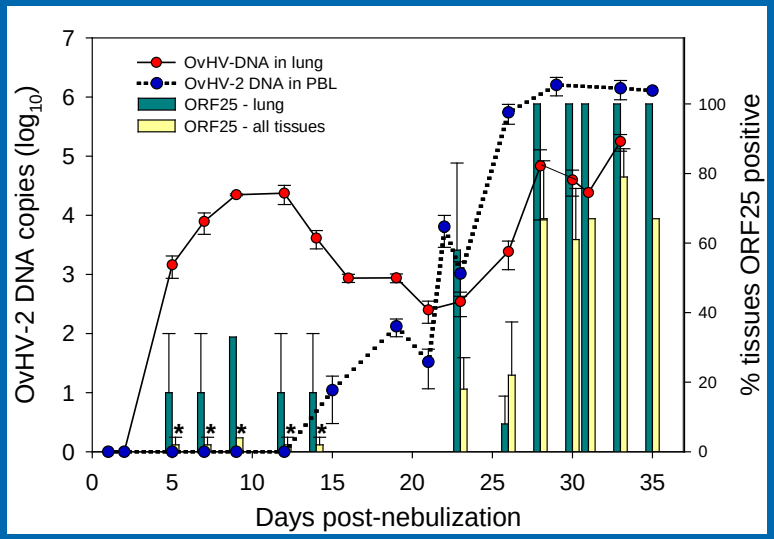


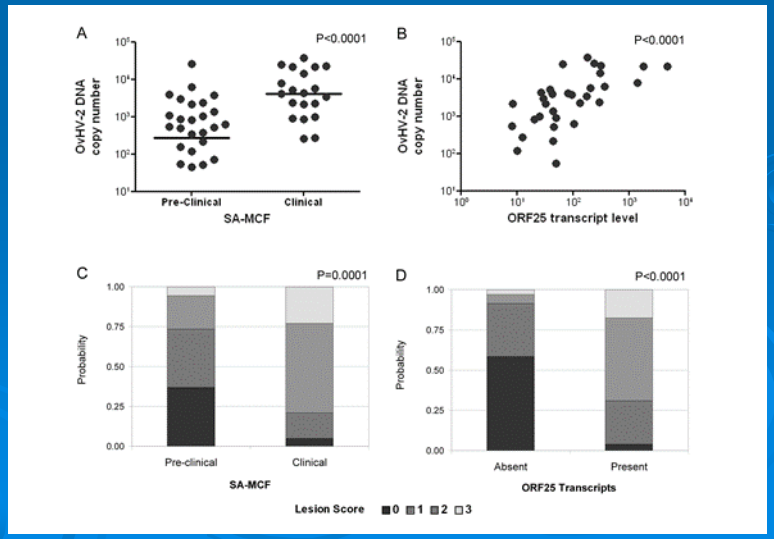


Li, *et al*., 2008; Taus, *et al*., 2010

OvHV-2 replication in bison: entry

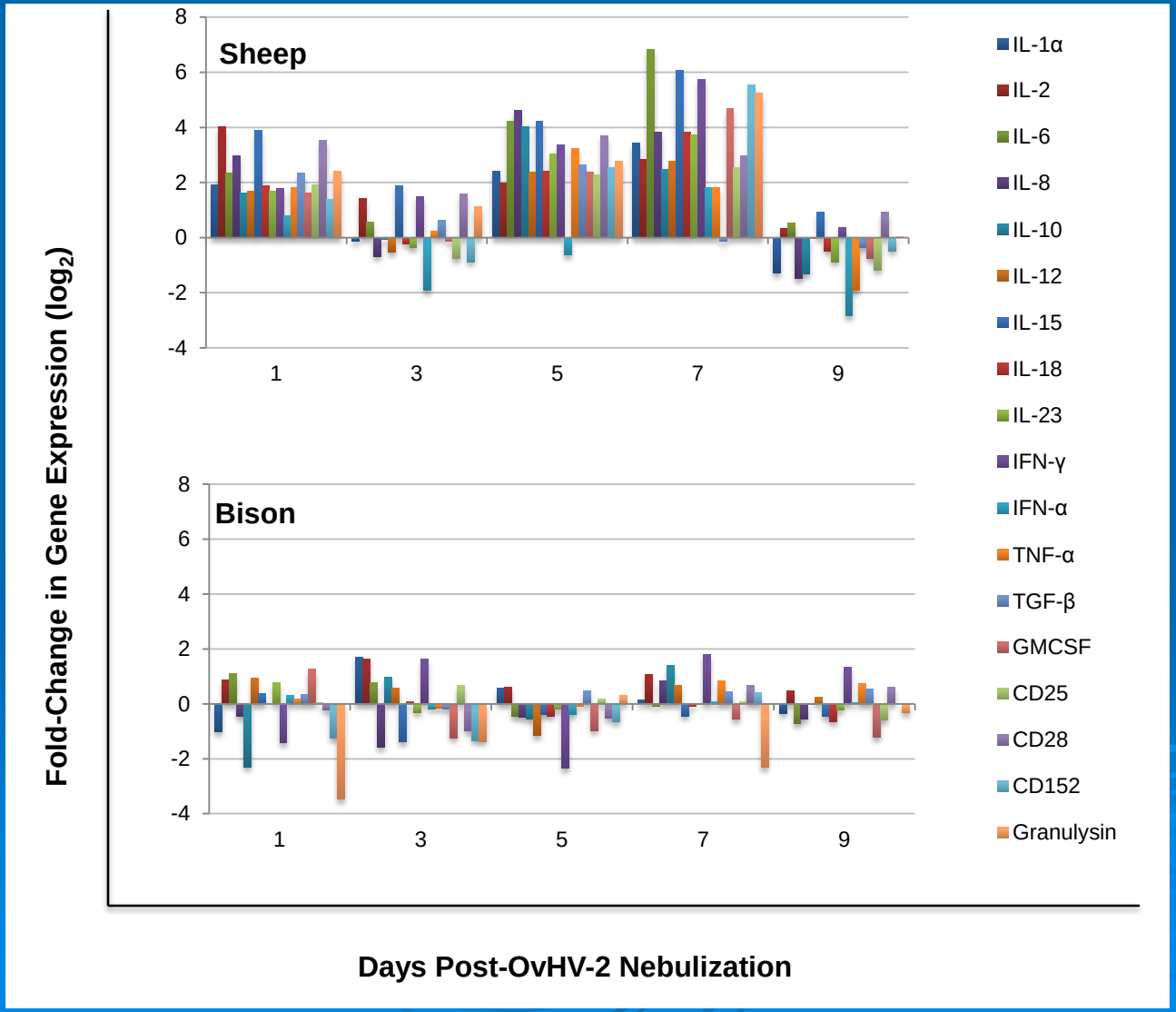
- OvHV-2 initial replication also occurs in bison lung and is required to induce disease
  - Viral DNA and ORF25 transcripts exclusively detected in lung during early infection
  - IV inoculation failed to induce MCF
- Lytic gene expression correlated with lesion development
- Weak immune response locally





Cunha, *et al*., 2012

Significant difference in local immune responses between sheep and bison



Li, *et al*., 2014

The difference in pathogenesis between sheep and bison may be due to co-evolution

Sheep	Bison
less effective immunomodulation	more effective immunomodulation
control of viral replication and maintenance of latency	unsuccessful control of viral replication
subclinical	progression to disease
host and virus survive	host and virus die
well adapted = co-evolution	poorly adapted = relatively recent exposure

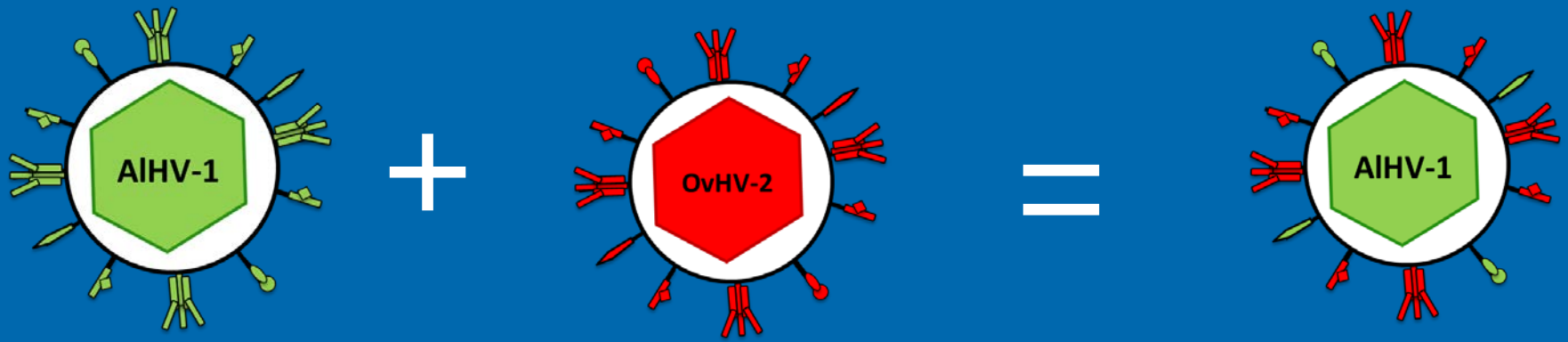
Objective

Develop an effective vaccine to protect bison from MCF

- Strategy: to generate neutralizing antibodies as a barrier to block virus at entry site
  - Respiratory transmission by cell-free virus from sheep nasal secretions
  - Initial OvHV-2 replication in lung is required for infection and clinical MCF
  - The disease is dose-dependent

Approach

Construct a recombinant virus (AlHV-1) expressing relevant OvHV-2 proteins

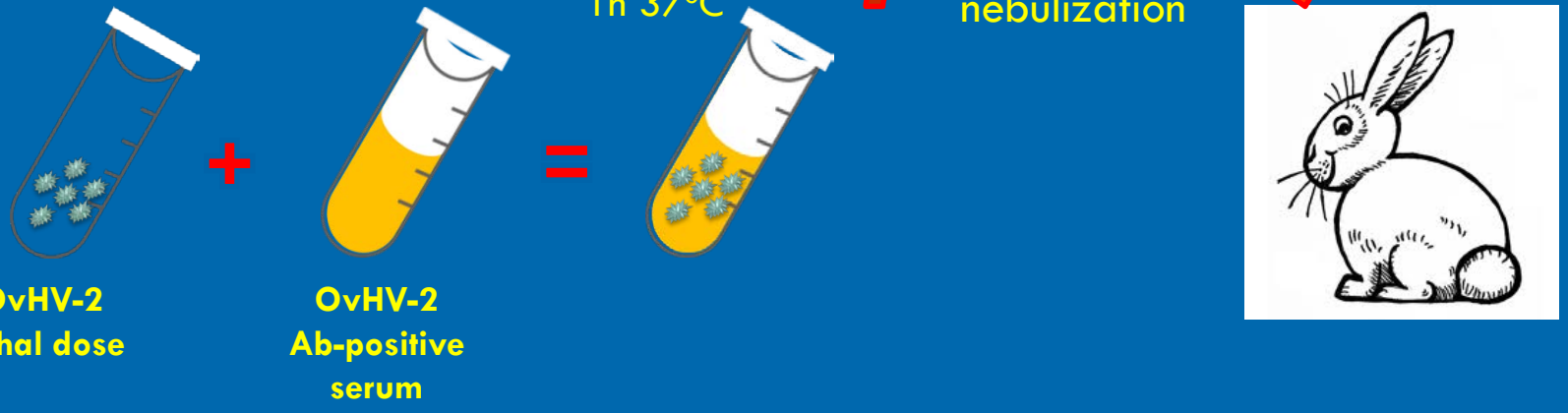


Attenuated AlHV-1

Attenuated AlHV-1 expressing OvHV-2 proteins

- Overcome the fact that OvHV-2 cannot grow in cell culture
- Obstacle is no neutralization test available for antibodies against OvHV-2 proteins

In order to identify target proteins, it was necessary to develop an *in vivo* neutralization assay



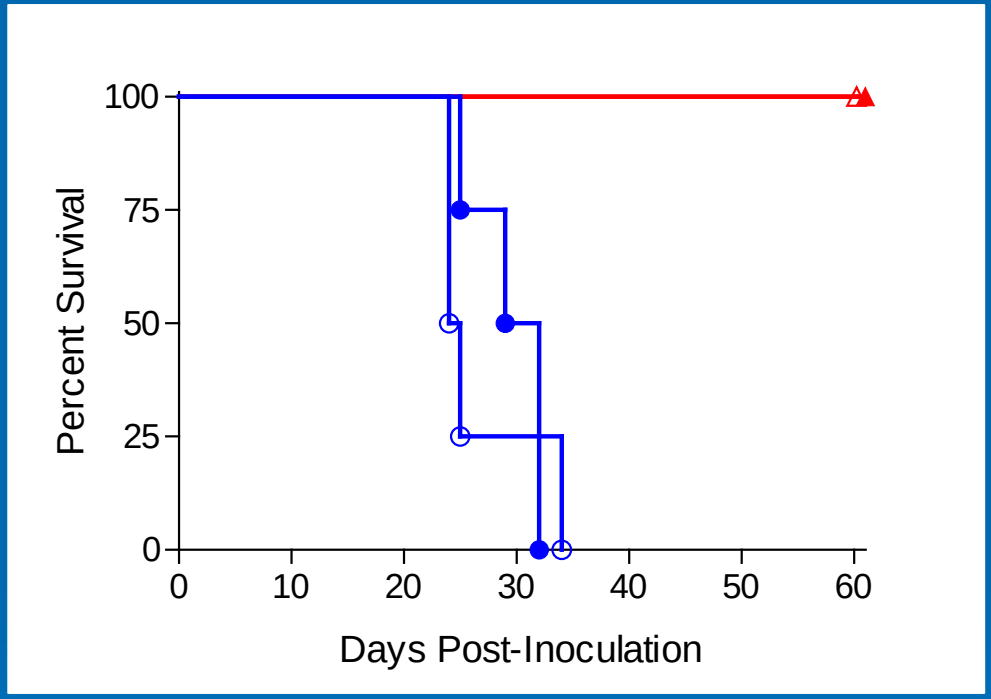
	OvHV-2 inoculum		
	10^4	10^5	10^6
Clinical outcome	neg	Pos / MCF	MCF

Virus neutralization

Clinical outcome

- **MCF**
- **Subclinical infection**
- **No infection**

Anti-OvHV-2 antibodies block virus entry



Serum positive for OvHv-2 antibodies reduces viral infectivity in a complement-independent manner

Li *et al*., 2013

First step: identify target proteins

Are antibodies against OvHV-2 glycoproteins capable of blocking virus entry?

Genes

- Sequence optimization
- Cloning into a mamalian expression vector

Sera

- Plasmid DNA immunization (rabbits)
- Production of hyper immune sera

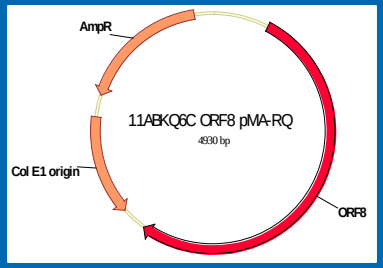
Blocking

- Treatment of OvHV-2 with hyper immune sera
- Nebulization of rabbits ➡ infection outcome

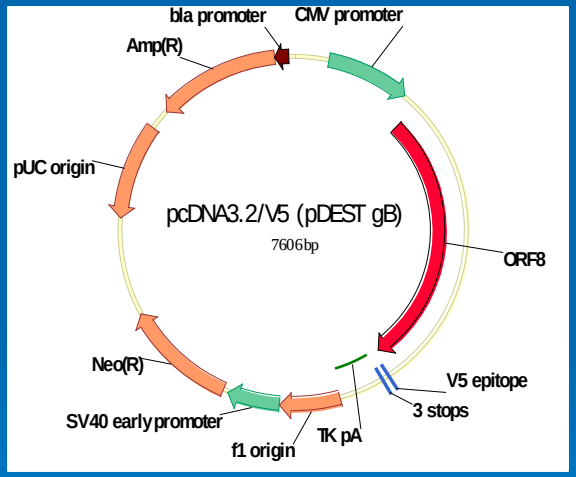
OvHV-2 target proteins

gB
gH
gL

Gene synthesis / cloning

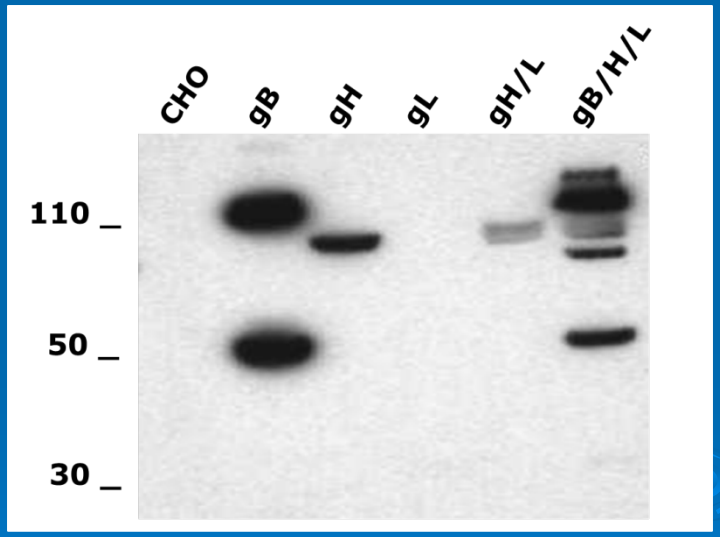


Gene sequence optimization and synthesis



Mammalian expression vector

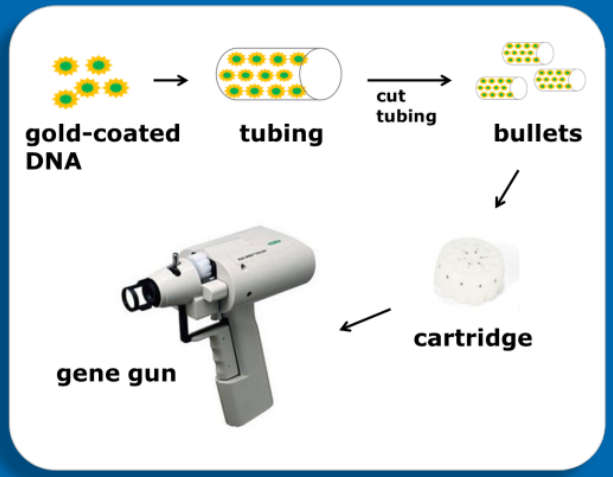
Protein expression



Western blot: transfected CHO cells lysate, V5 detection

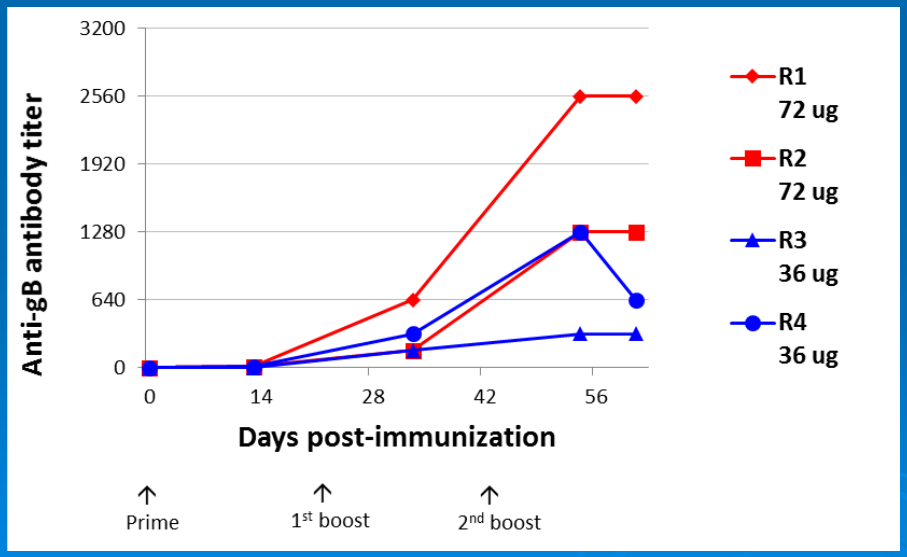
Hyper immune sera

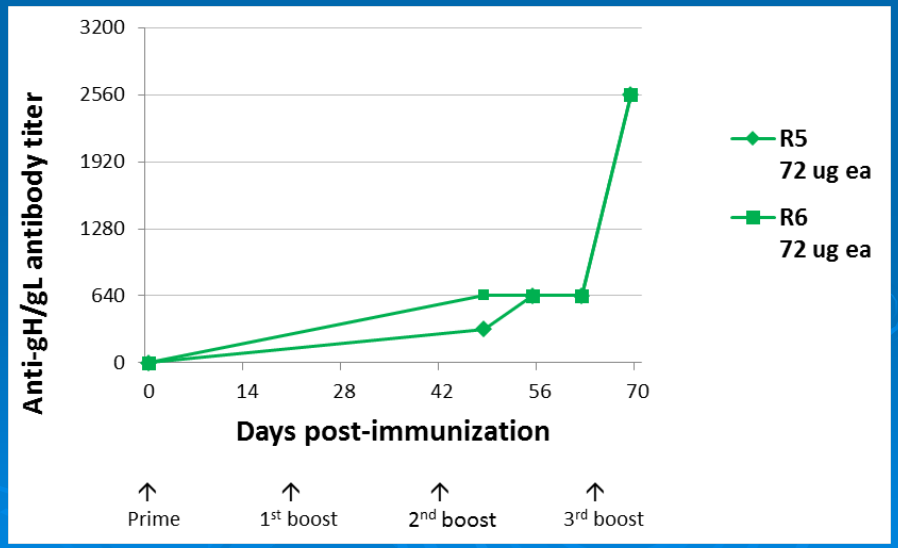
- Biolistic DNA delivery
  - gene gun system
  - 3-4 immunizations, 3-week interval
  - 24-36 µg DNA/immunization



Hyper immune sera

- Antibody response
  - gB
  - gH/gL



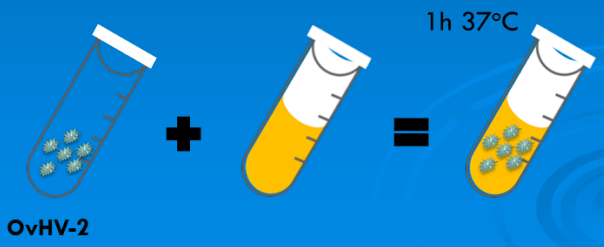


Cunha, *et al*., 2015

OvHV-2 blocking

OvHV-2 (10^6 particles) treatment

1. anti-gB sera
2. anti-gH/gL sera
3. anti-gB and anti-gH/gL sera
4. OvHV-2 infected sheep sera
5. pre-immune rabbit sera

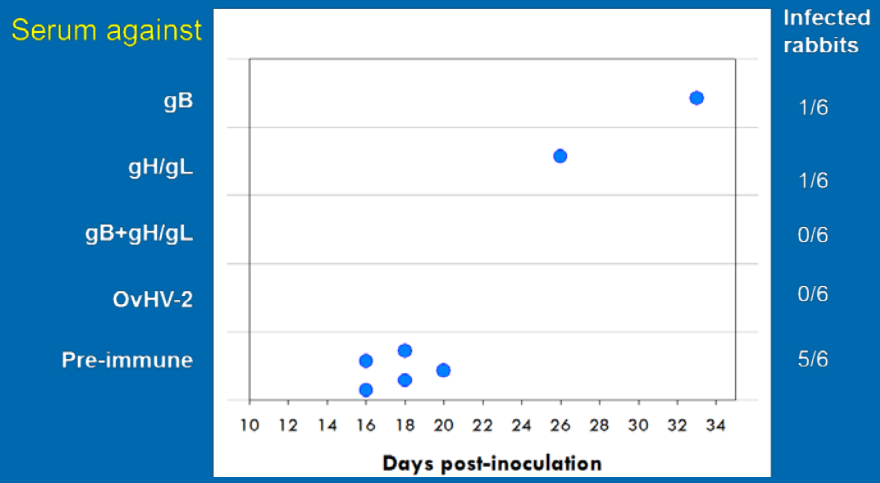


Nebulization of rabbits (n=6)

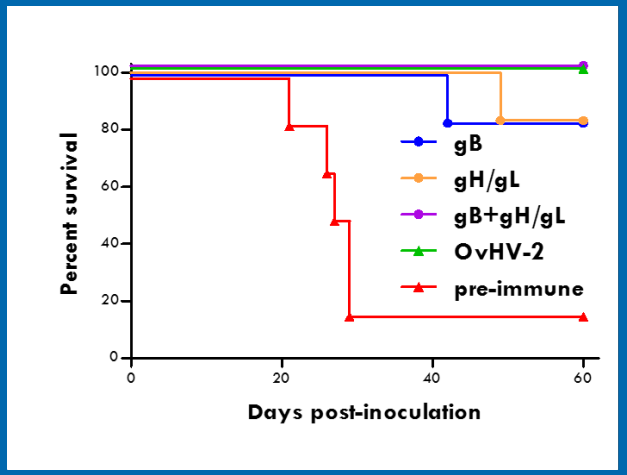
Infection outcome

1. Clinical signs - MCF
2. OvHV-2 nested PCR - PBL
3. qPCR – tissues
4. Hystopathology

Infection outcome

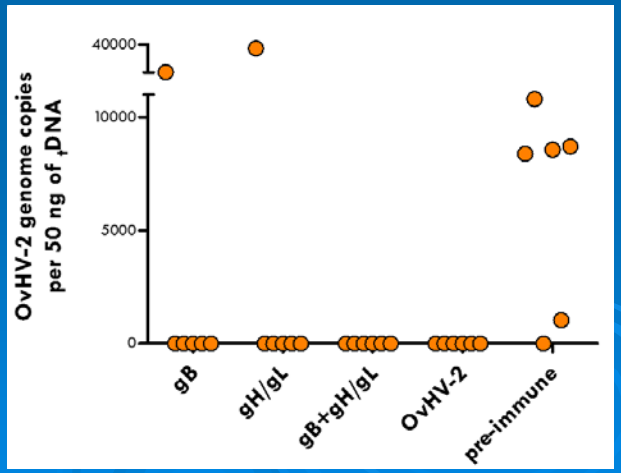


First detection of viral DNA in PBL



Rabbit death due to MCF

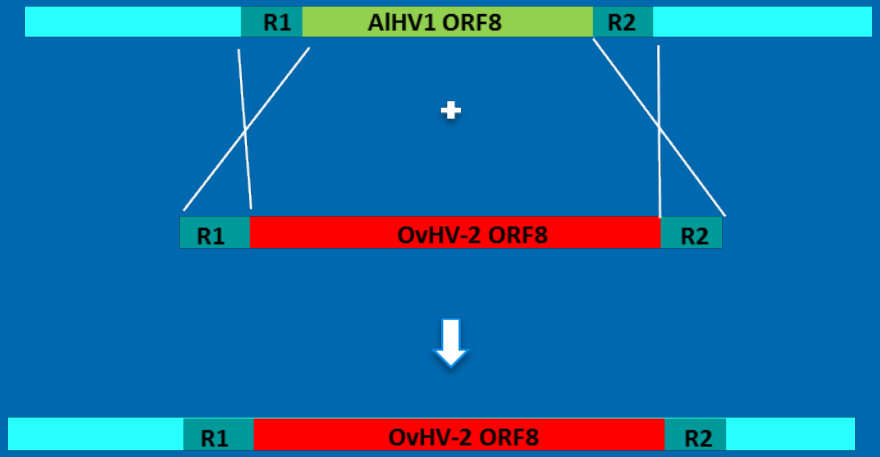
Polyclonal antibodies against OvHV-2 gB, gH/L completely block OvHV-2 infection and prevent rabbits from MCF

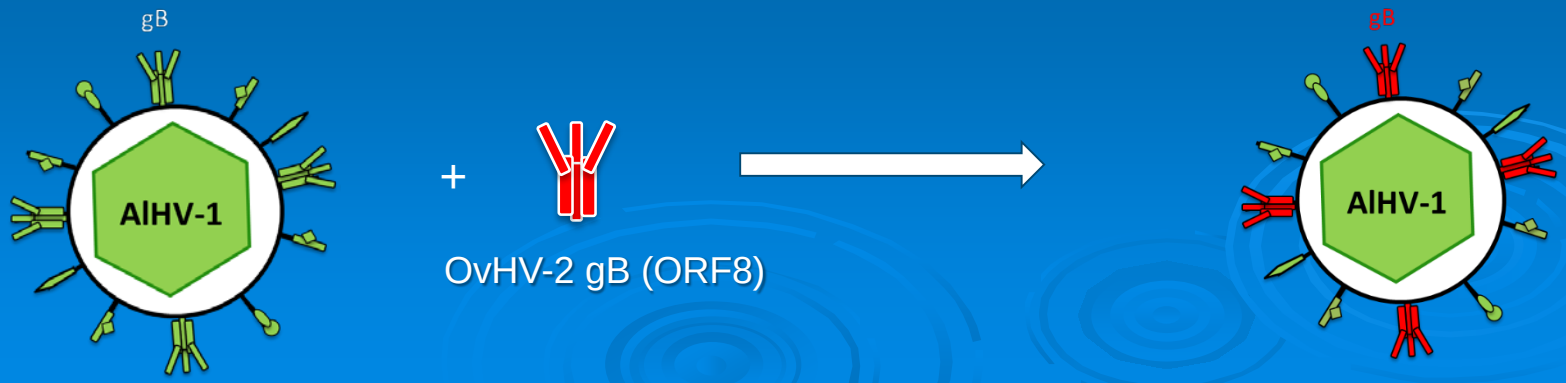


OvHV-2 DNA in liver

Cunha, *et al.*, 2015

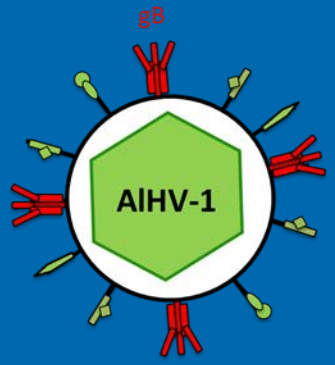
Second step: construct recombinant AlHV-1 with OvHV-2 genes

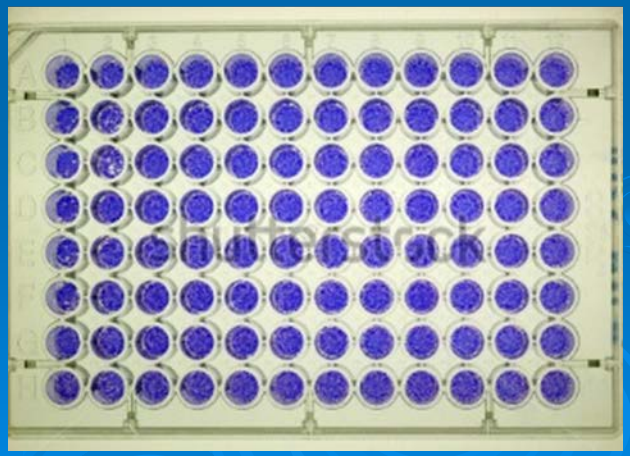




Perfect as expected!

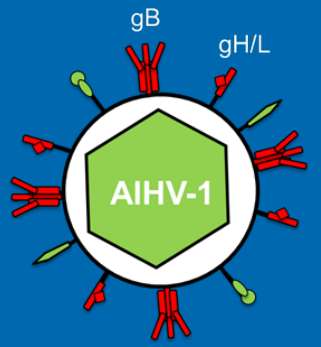
- The chimeric virus can grow in cell culture
- Antibodies against OvHV-2 gB neutralized the virus efficiently
- An in vitro assay is a critical tool for analysis of OvHV-2 neutralizing antibody response
- A potential diagnostic test specific for antibodies to MCF viruses in *Caprinae* group





Current and future experiments

- Continue to construct the chimeric viruses that express OvHV-2 genes
- Determine infectivity of the virus in rabbits
- Evaluate immune response in rabbits (eventually in bison)
- Determine MCF-protection rate in rabbits (eventually in bison) by challenge of OvHV-2
- Continue to explore a cell culture system to grow OvHV-2



Conclusions

- Sheep-associated MCF is one of the most important infectious diseases in North American bison
- The strategy for OvHV-2 vaccine development is to generate neutralizing antibodies as a barrier to block virus at entry site (lung)
- The approach is to use a recombinant virus (AlHV-1) expressing relevant OvHV-2 proteins
- Necessary steps have been achieved:
  - Animal models developed
  - A recombineering system established for generate AlHV-1/OvHV-2 chimeric viruses
  - In vitro/vivo systems to measure neutralizing antibody response
- A vaccine for sheep-associated MCF is on the horizon

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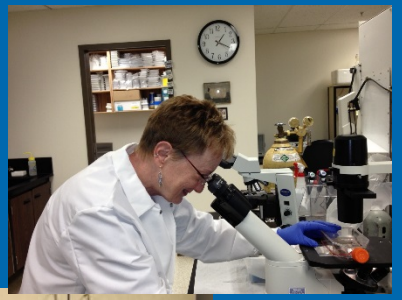
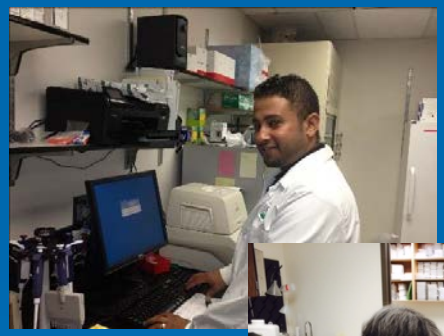


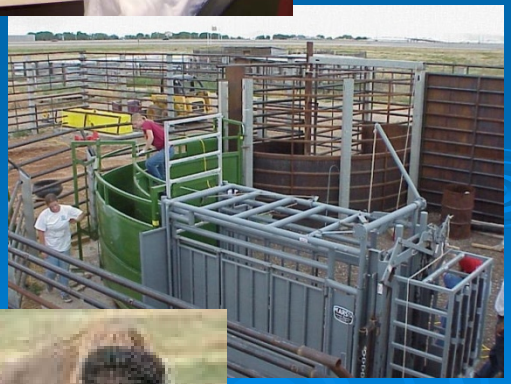

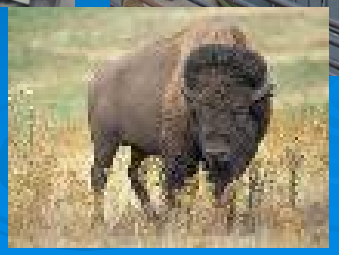

http://www.vetmed.wsu.edu/research/malignant catarrhal fever