Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency




Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Diagnostic Developments for High Consequence Zoonotic Animal Pathogens

Hana Weingartl
Special Pathogens Unit
National Centre for Foreign Animal Disease
Canadian Food Inspection Agency

Canada

Special Pathogens Unit

BSL4
Henipaviruses,
Ebola virus,
“unknown”

Rift Valley fever virus;
HPAI (H5 and H7)
Zoonotic AgBSL3

Capripox
Dr. Babiuk
AgBSL3

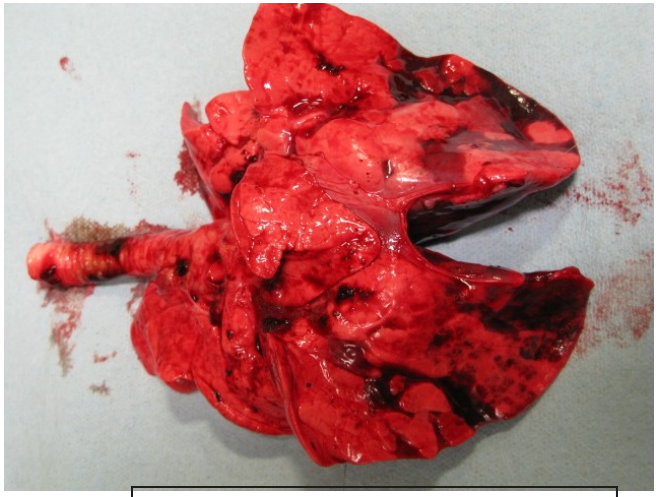
AgBSL3 agents, recombinant work, inactivated material

Emerging or neglected diseases: understanding the infection in the target host species requires *in vivo* studies

- Pathogenesis studies
  - sampling strategy
  - testing strategy

- Immune response
  - antibody development

- Development of control samples



Inoculation of pigs with NiV and HeV

Challenges:
laboratory set up, design of animal experiments

Availability of experimental samples – need for animal inoculations in high containment (Zoonotic BSL3, BSL4)(age /size/number/duration restrictions)

Lack of field samples to evaluate/validate the test performance (e.g. duration of antibody response)

Work with live virus – assay, reagent production in high containment

development of test suited for
low containment, including reagent production

Etiological agents of henipavirus diseases

Family: *Paramyxoviridae*

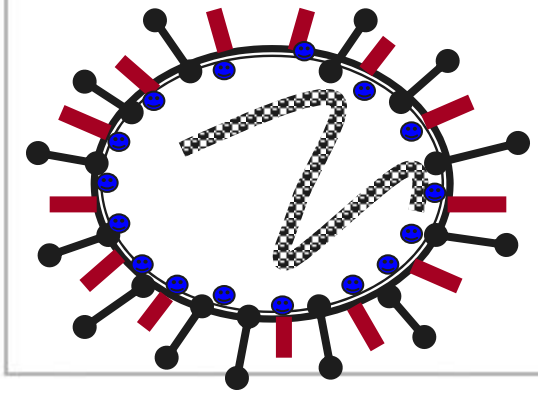
Genus: *Henipavirus*

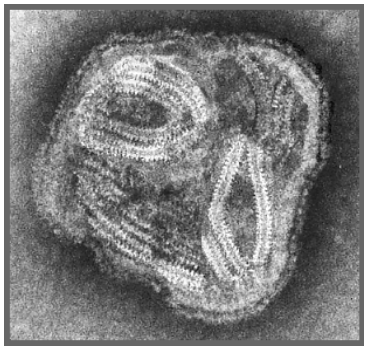
Species:

Hendra virus

Nipah virus

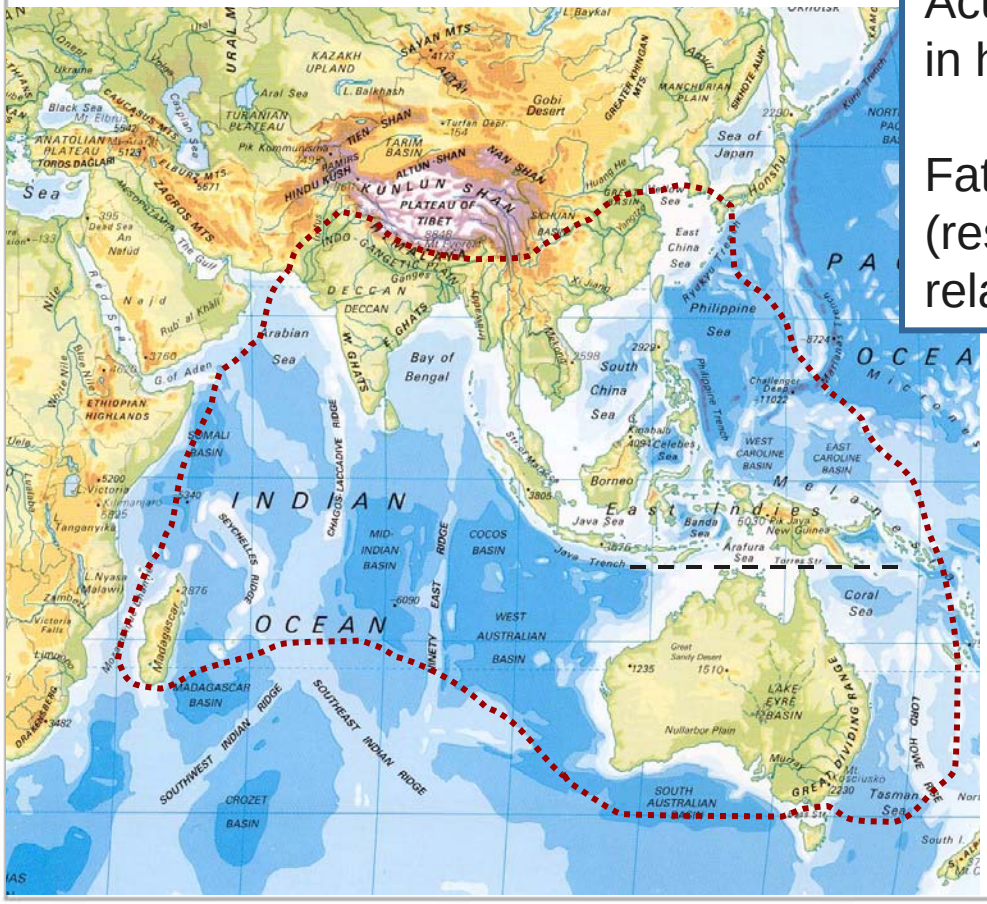
Negative single strand RNA genome
nonsegmented (18kb)
Virions are enveloped





Natural reservoir: bats from the genus ***Pteropus***
(fruit bats/flying foxes)

Seropositive/ virus isolation; no clinical signs; no pathology



Hendra virus

Acute fatal respiratory disease in horses; may resemble AHS

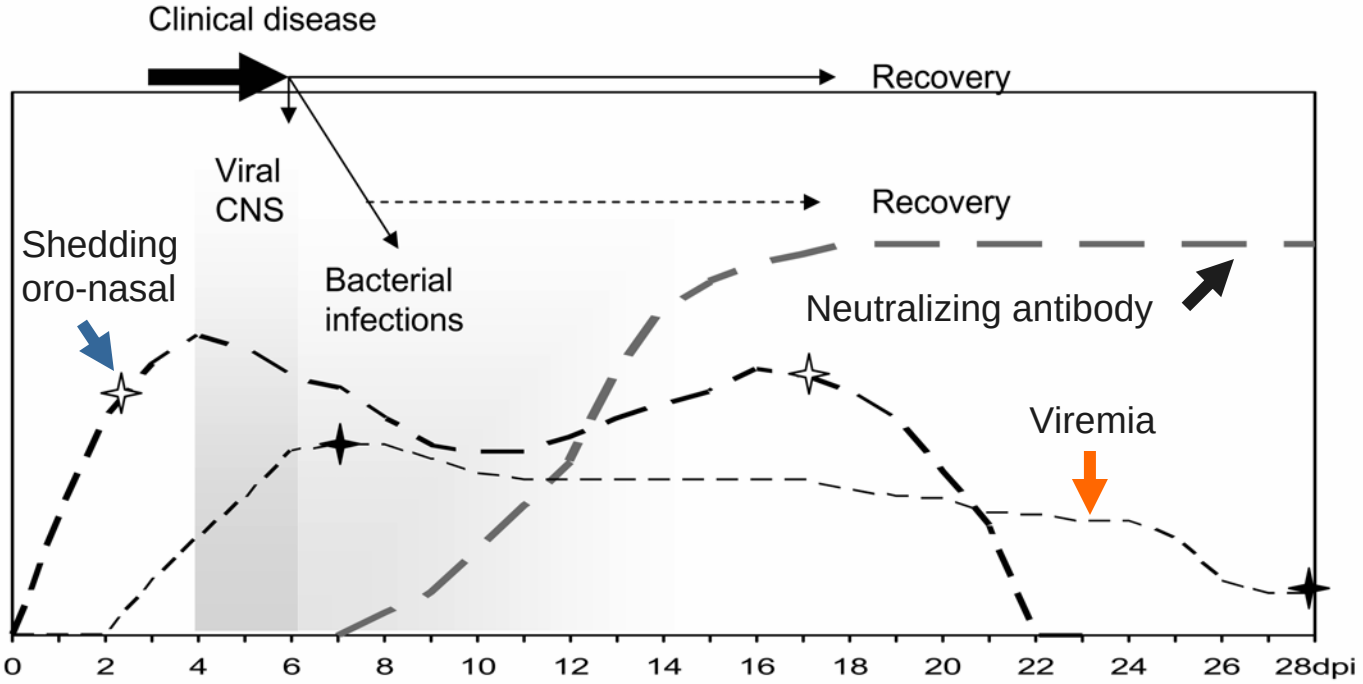
Fatal disease in humans (respiratory and renal failure, relapsing encephalitic disease)

Nipah virus

Respiratory (CNS signs) in pigs highly contagious; low mortality

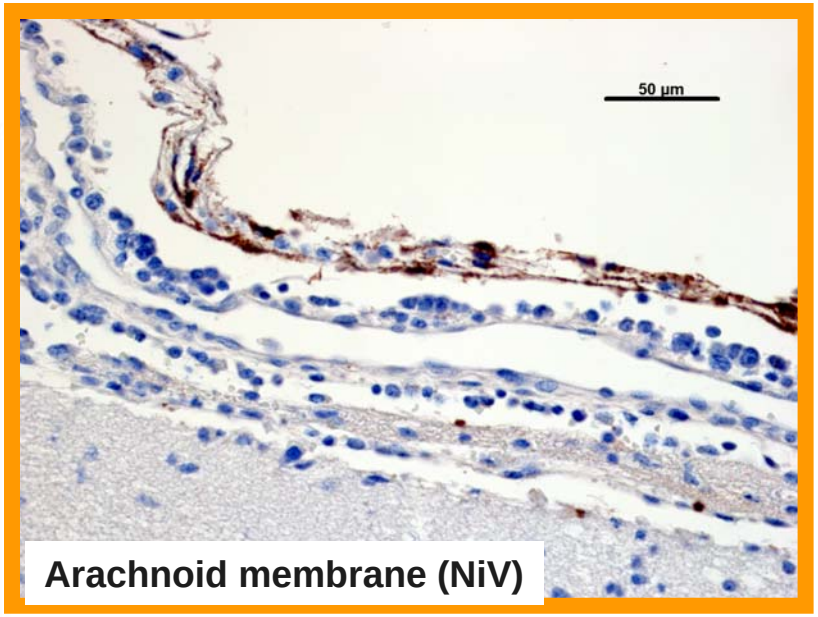
Fatal encephalitis and Pulmonary edema in humans

NiV infection in pigs

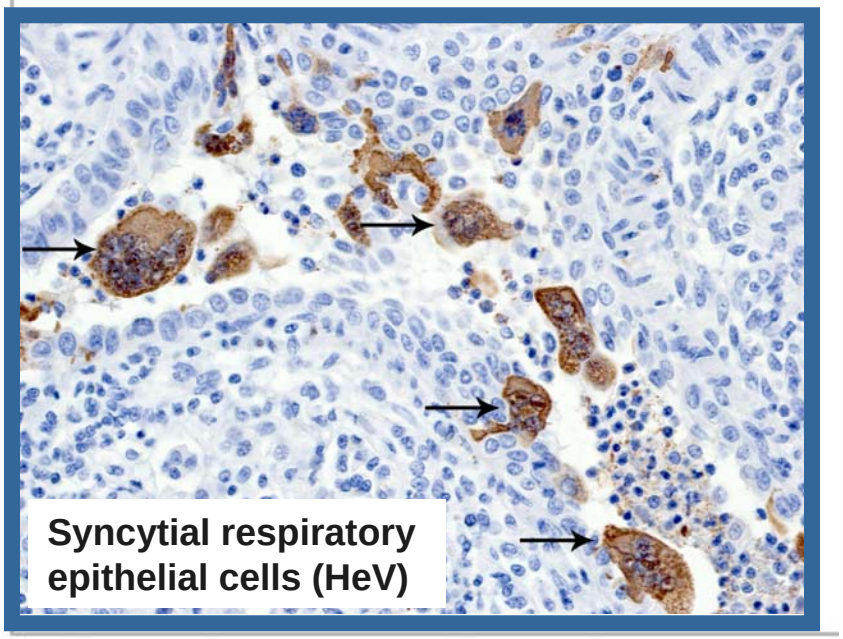


Lateral flow (NiV)
Real-time RT-PCR (NiV; HeV)
Virus isolation

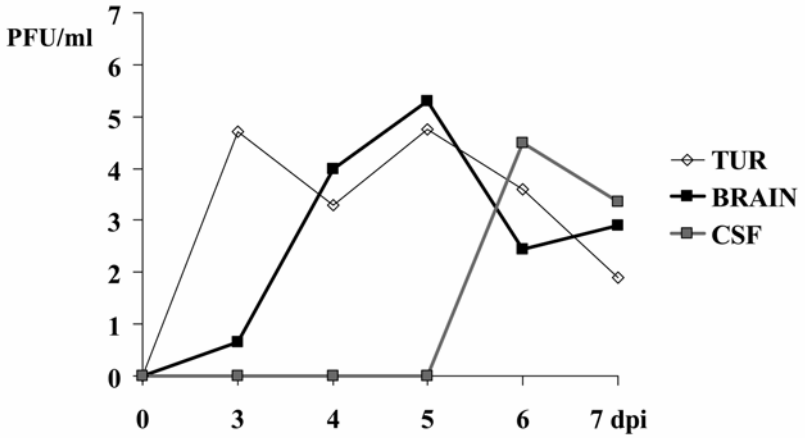
Virus neutralization assay – microPRNT (**NiV**; HeV)
ELISA – whole virion (BEI-inactivated, semipurified, concentrated)
ELISA – recombinant (N, P proteins) (NiV)

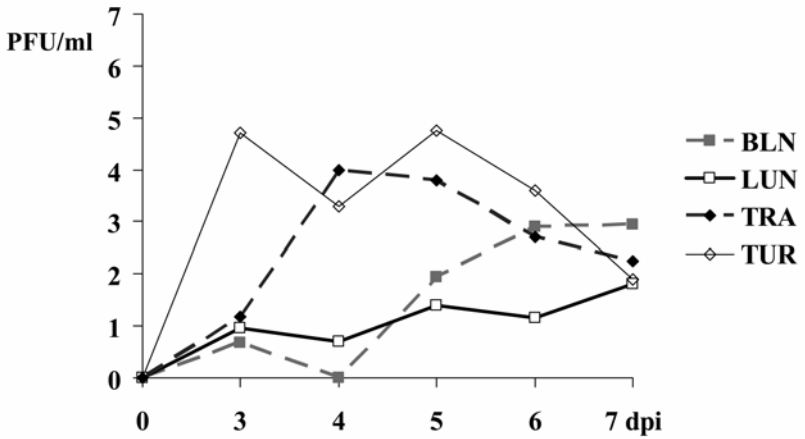


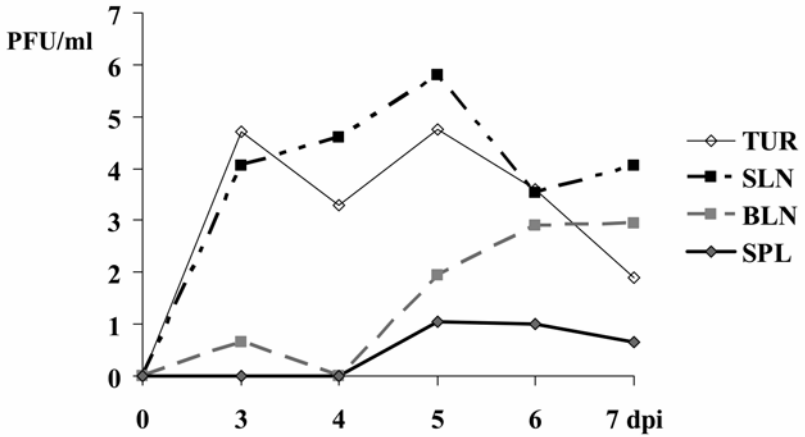
Arachnoid membrane (NiV)



Syncytial respiratory epithelial cells (HeV)







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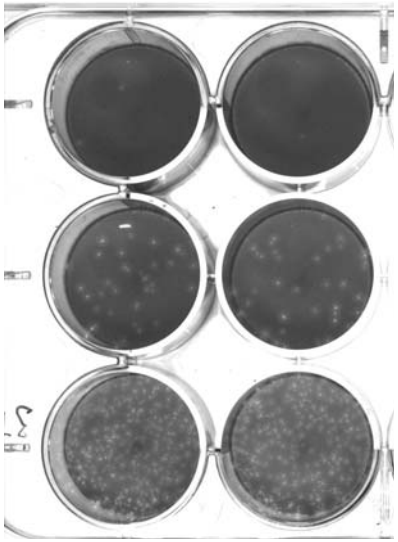
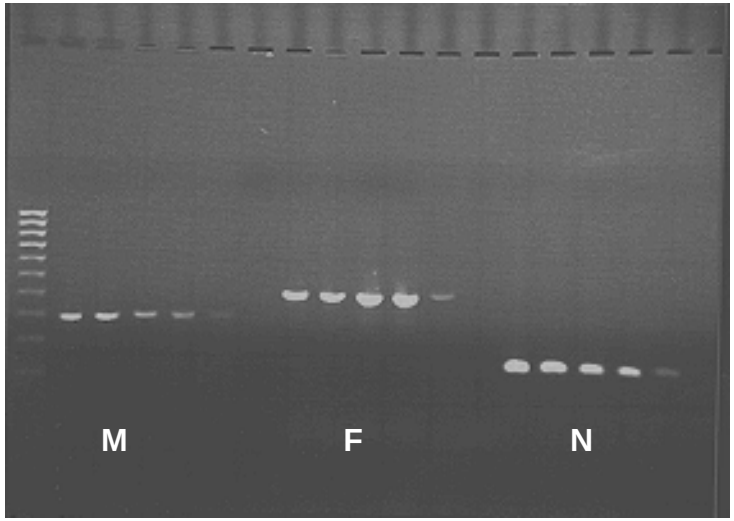
Virus Detection

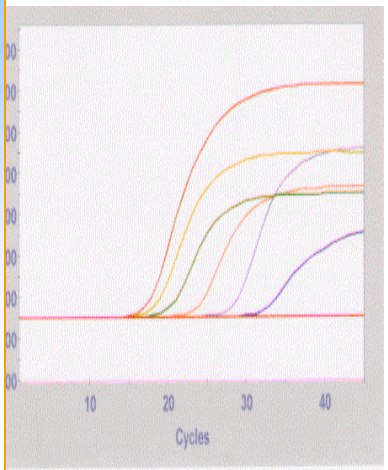


Virus isolation:
Vero cells, 37°C, 5% CO_2
plaque assay (CMC overlay) or CPE

RT-PCR (confirmatory)
Tri-Pure (Roche Dx)
Qiagen OneStep RT-PCR kit
primers: N gene (395 Nt amplicon)
F gene (289 Nt amplicon)
M gene (360 Nt amplicon)

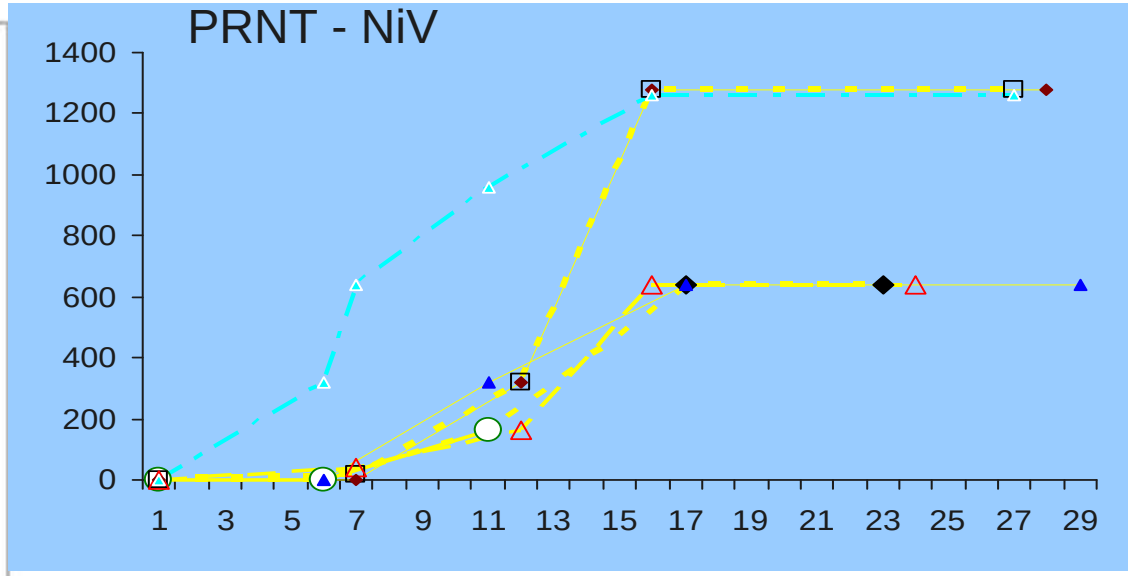


Real-time RT-PCR:
RNA isolation – TriPure
primers and probe
located in the **N** gene region(**NiV**)(105bp product)
(Guillaume et al., 2004)
or the **M** gene region (**HeV**) (69bp product)
(Smith et al., 2001)
probe labelled with FAM (TAMRA quencher)
Standard curve using plasmids with cloned corresponding sequences to semi-quantify the RNA
Taqman and RotorGene-3000 platforms
Exogeneous internal control:
armoured enterovirus RNA (probe labelled with TET or JOE; NFQ quencher)

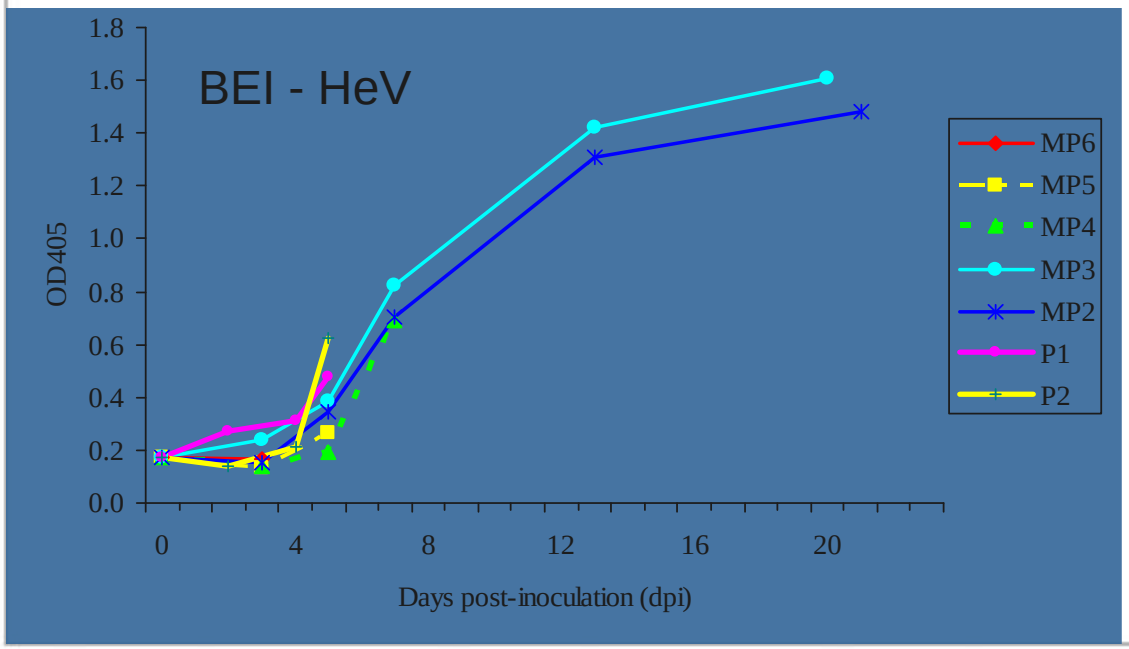


ID	Sample ID	FAM Ct	Protocol
	1/10	32.14	Nipah-FAM
	1	27.27	Nipah-FAM
	10	23.05	Nipah-FAM
	100	19.42	Nipah-FAM
	1000	17.43	Nipah-FAM
	10,000	16.24	Nipah-FAM
	-ve control	0.00	Nipah-FAM

Antibody response



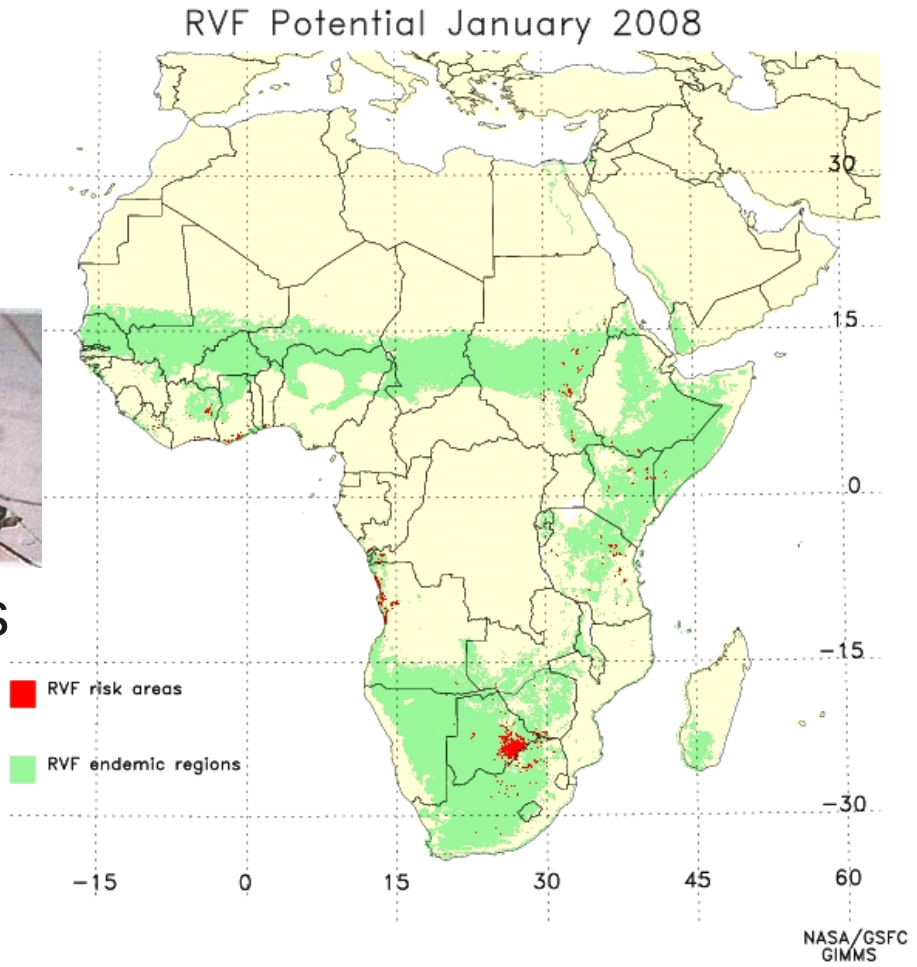
Microtiter PRNT (CPE reduction)



BEI-inactivated whole virus antigen indirect ELISA

Recombinant NiV N, P protein indirect ELISA

Rift Valley fever



Reservoir: mosquitoes

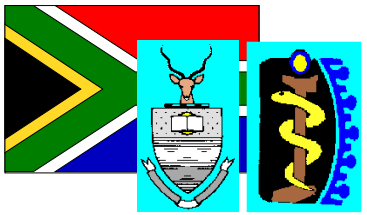
ruminants

Zoonotic disease

up to 36% case-fatality rate
(South Africa, 2010)

Transmission to humans by handling infected tissues and blood, mosquito bites, ingesting raw milk? (3%)

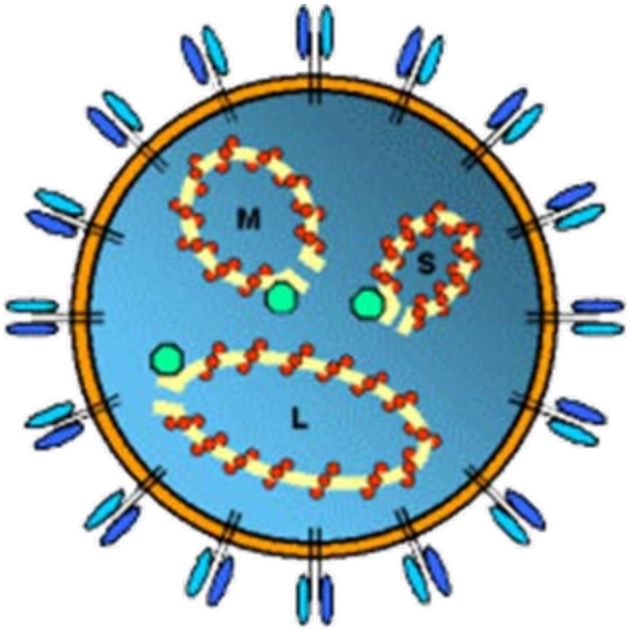
R. Swanepoel



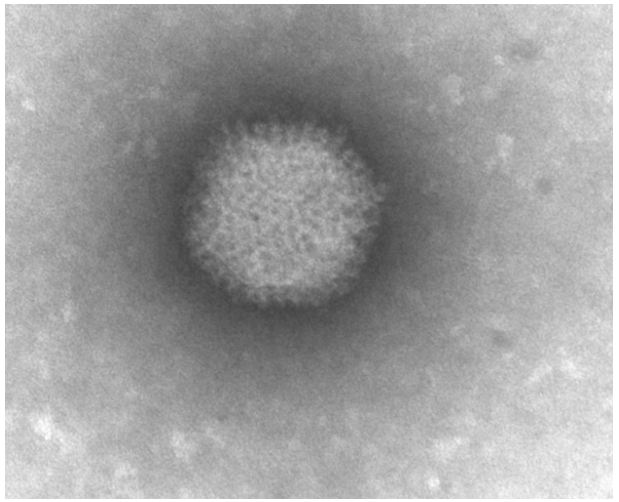
Rift Valley fever virus

Etiological agent:

Family *Bunyaviridae*,
Genus *Phlebovirus*



Segmented ssRNA virus, Enveloped



CRTI: development of operator safe assays

Concern:
if introduced to North America – will not be timely recognized

Clinical signs in older ruminants are **not specific**, or not present (non-pregnant animals, older then 2 – 3 week)

If the introduction of RVFV occurs outside the lambing/calving/kidding season
it will be not be recognized despite **high viremia.**

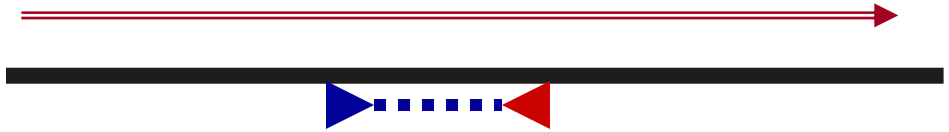
Samples from abortion cases or newborn deaths in ruminants (**high virus load** in the tissues and blood)
will be submitted to provincial laboratories (BSL2)

Workers at slaughter houses, **veterinarians,** etc.

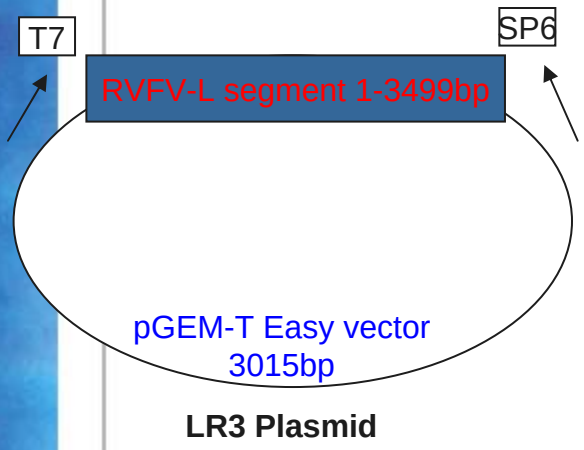
Recognition of RVF outbreak – through a human case?
(Egypt – majority human cases by mosquito bite)

Real-time RT-PCR

L genome segment
RNA-dependent-RNA polymerase **L**



The primer and the probe set (Bird *et al.*, 2007 – CDC)
- developed based on over 70 RVFV isolates)



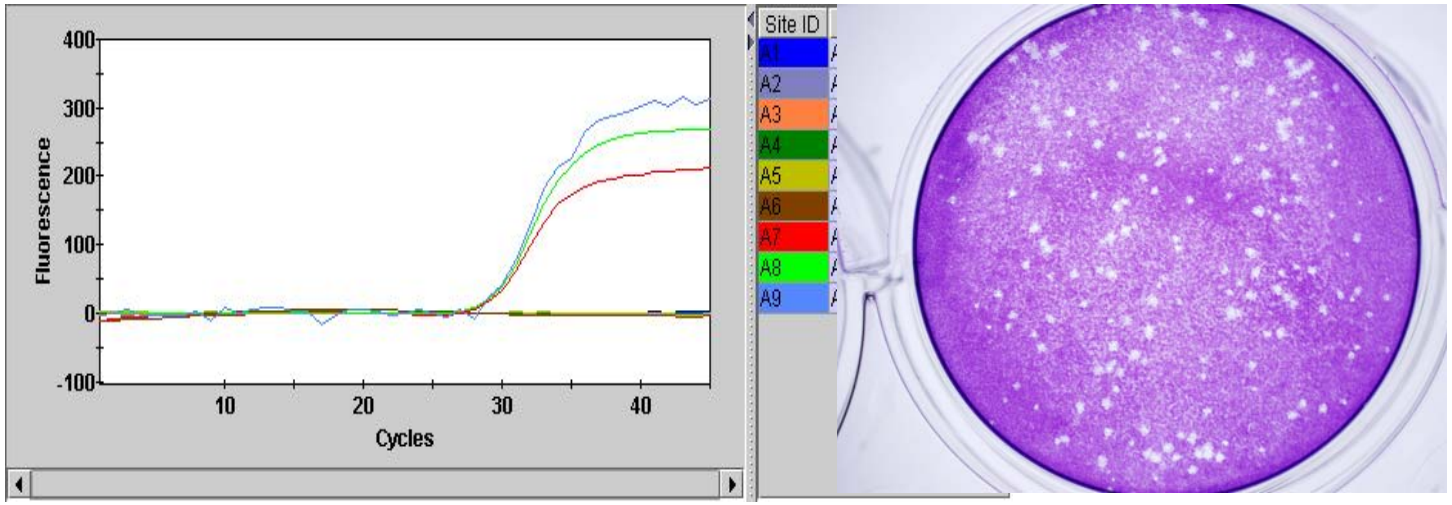
LR3 Plasmid

Standard curve to estimate copy numbers was based on plasmid containing cDNA of partial nucleotide sequence of the L gene .
Armoured enterovirus RNA as an exogenous **internal control**

analytical and diagnostic evaluation of one-step RVFV real-time RT-PCR

Virus isolation:
confluent Vero E6 cells, 2% CMC in EMEM/2% FCS to determine PFU/ml.

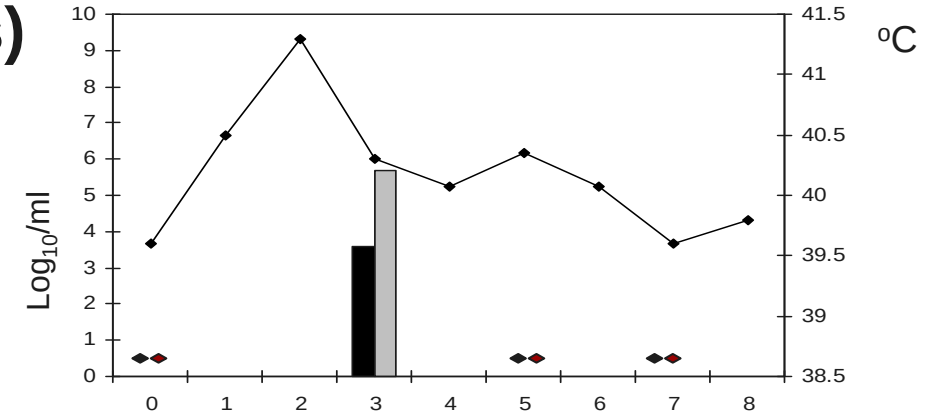
One-step protocol
Rotor-Gene 3000 thermocycler, probe labelled at the 5' end with the reporter dye 6-FAM and at the 3' end with the quencher TAMRA
Other platforms tested (ABI 7900, Smart Cycler) could be used as well



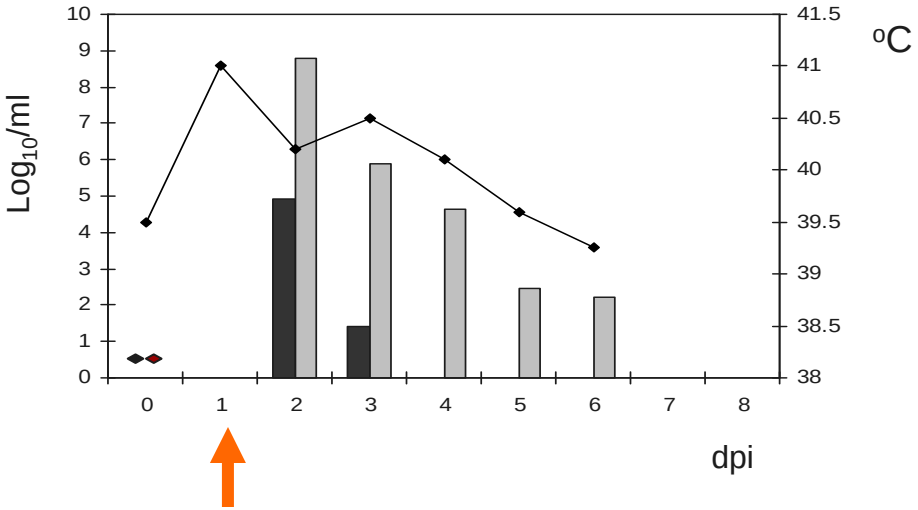
Evaluation of the one-step RVFV real-time RT-PCR in serum samples

Sheep (calves, goats)

Group A: age – 4 month, **SC** inoculation with $5x10^5$ PFU/animal



Group B: age- 14 days, **SC** with 10^6 PFU/animal



Black line:
Rectal temperatures
Black columns:
Virus isolation from serum (log10 PFU/ml)
Gray columns:
Virus RNA detection in serum (log10 copy number/ml)

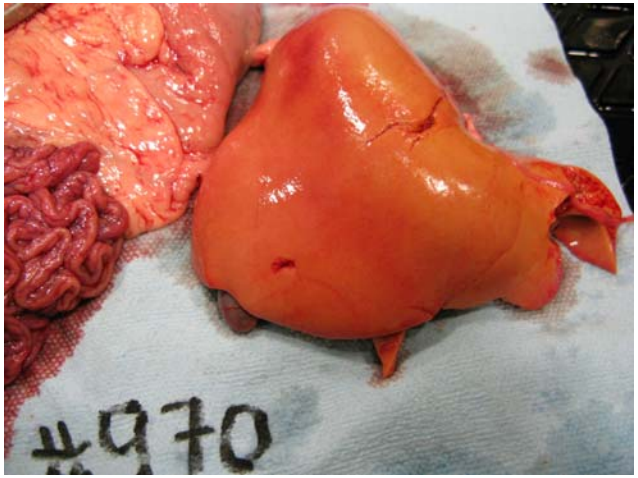
Sample collection:

For virus detection
(Virus isolation and real time RT-PCR):

Serum
Blood (in an anticoagulant: EDTA, sodium citrate, heparin.)
Liver, spleen, brain, lymph nodes,
any organs of **aborted fetuses, and placenta**

"double – isolation" needed for severely affected organs

Combination of steps using TriPure and RNeasy RNA isolation kits



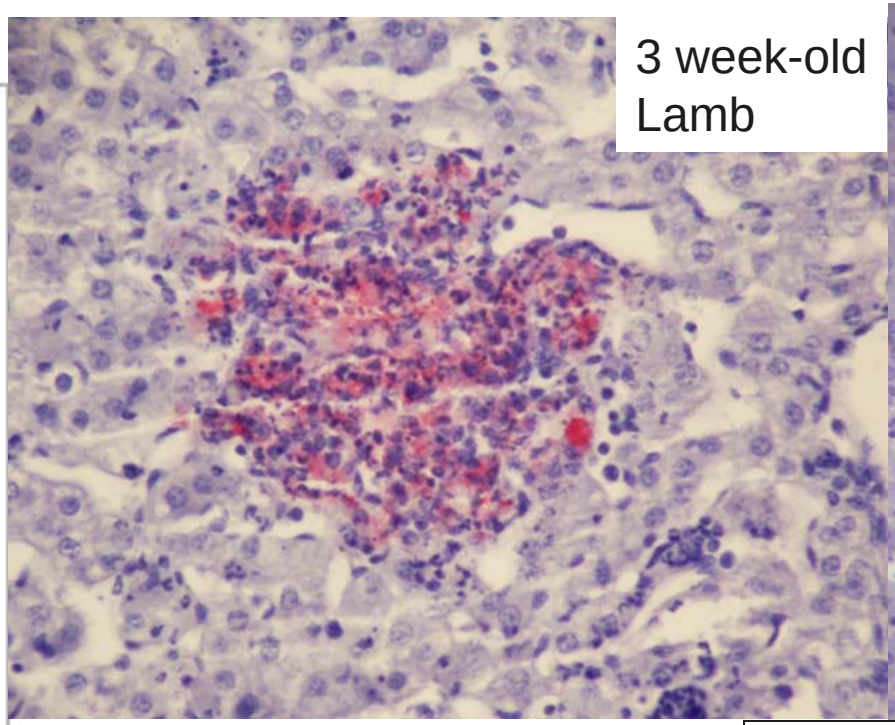
RVFV immunohistochemistry (IHC) developed at ABADRU

Dr. Miller, Dr. Drolet, RVFV-N gene construct from F. Weber

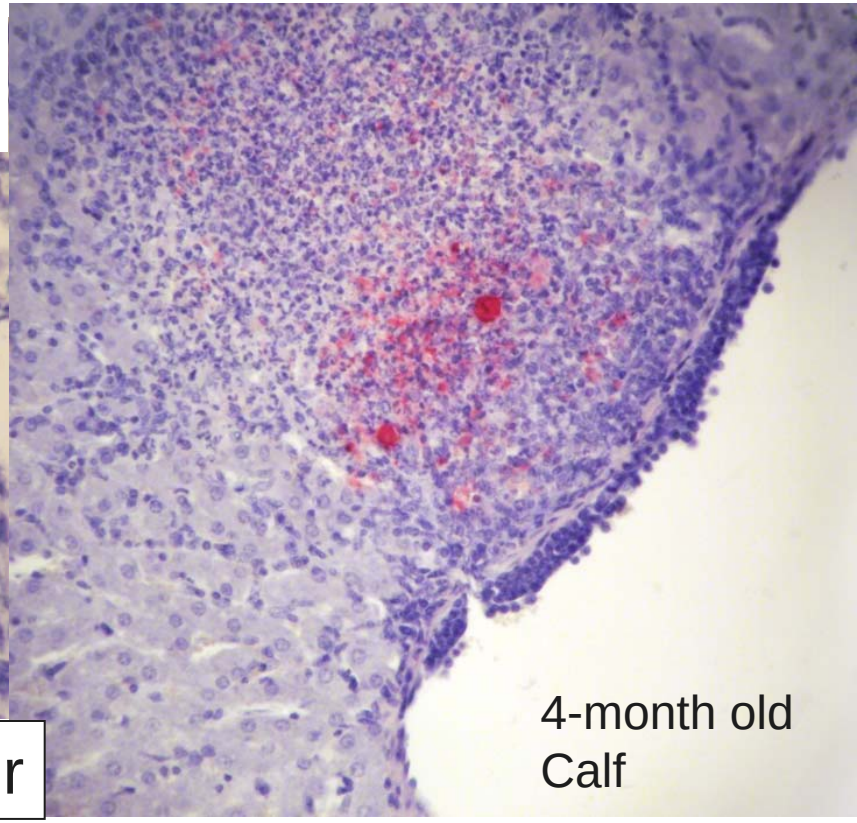
Expressed in E. coli, purified, used to make antibody in rabbits

Rabbit anti-RVFV N used to develop immunohistochemical assay for detection of vaccine strain and wild type strains of RVFV in cell cultures, mammalian tissues and insect tissues

Protocol and polyclonal antibodies transferred to NCFAD

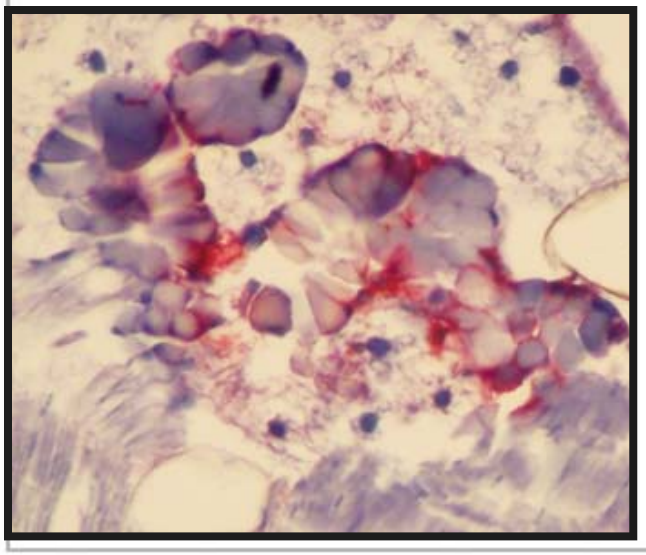


3 week-old Lamb



4-month old Calf

Liver



RVFV in mosquito salivary glands

ELISA using recombinant antigens to detect antibodies against RVFV

Serum samples may due to high viremia contain live RVFV

virus inactivation

Serum mixed with 0.5% Tween 20 in PBS, and heat inactivated at 56^{o}C for 30 min

5x10^{7} PFU/ml to 0 PFU/ml

RVFV is genetically stable (for an RNA virus): **one serotype**

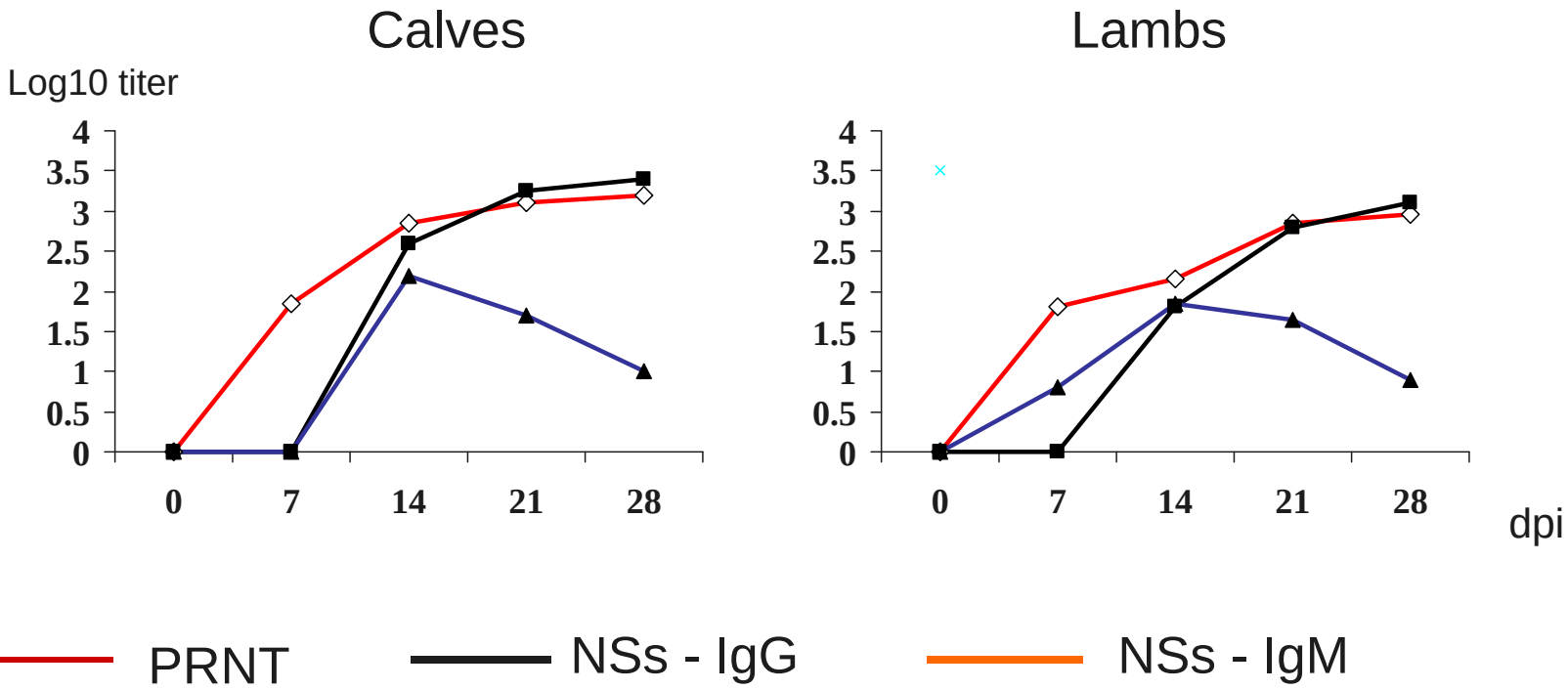
L genome segment
RNA-dependent-RNA polymerase **L**

M genome segment
Surface glycoproteins: **Gn, Gc**
nonstructural proteins: **NSm1, NSm2**

S genome segment
Nucleopcapsid protein **N**; nonstructural protein **NSs**

Rift Valley fever virus – protein coding

Anti-RVFV antibody detection in the serum



Recombinant NSs ELISA suitable as DIVA tests,
especially in endemic countries or in recovery

Ebola – Reston in pigs

Ebola-Reston present in the non-human primate population in Philippines (fatal to NHP)

REBOV does not cause disease in humans

Family: *Filoviridae*
Genus: *Filovirus*

Ebola species isolated in **Africa**:
Zaire, Sudan, Ivory Coast, Uganda
cause **fatal hemorrhagic fever in humans**
Case fatality rate up to 90%

High morbidity and
mortality in pigs in Philippines in 2008

Clinical disease:
severe respiratory syndrome

Suspected outbreak of highly pathogenic PRRS

Lung
Liver
Spleen
Lymph nodes
Tonsil

USDA, FADDL Plum Island

Array of molecular diagnostic assays
- confirmed PRRSV, (and porcine circovirus)
- **virus isolation**

↓

CPE in Vero cells (nonpermissive to PRRSV)

↓

Panviral microarray
(**NGS alternative**) → **REBOV** in lung and lymph nodes with PRRSV

Confirmed by CDC Atlanta

Serological surveys in the area of submissions in Philippines

↓

presence of REBOV antibodies in serum of farm workers

NCFAD veterinary diagnostic capability for EBOV in swine

Virus isolation

Virus neutralization

Real time RT-PCR (transferred from SP, NML, PHAC)

Whole virion ELISA (transferred from SP, NML, PHAC)

Infected cell lysate ELISA (transferred from CDC, Atlanta Dr. Pierre Rollin)

↓

Evaluation/modifications for swine samples

Pathogenesis of ZEBOV in swine
(collaboration with Dr. Kobinger, SP, PHAC)

Field lab deployment cross training, February 26 – March 12, 2012

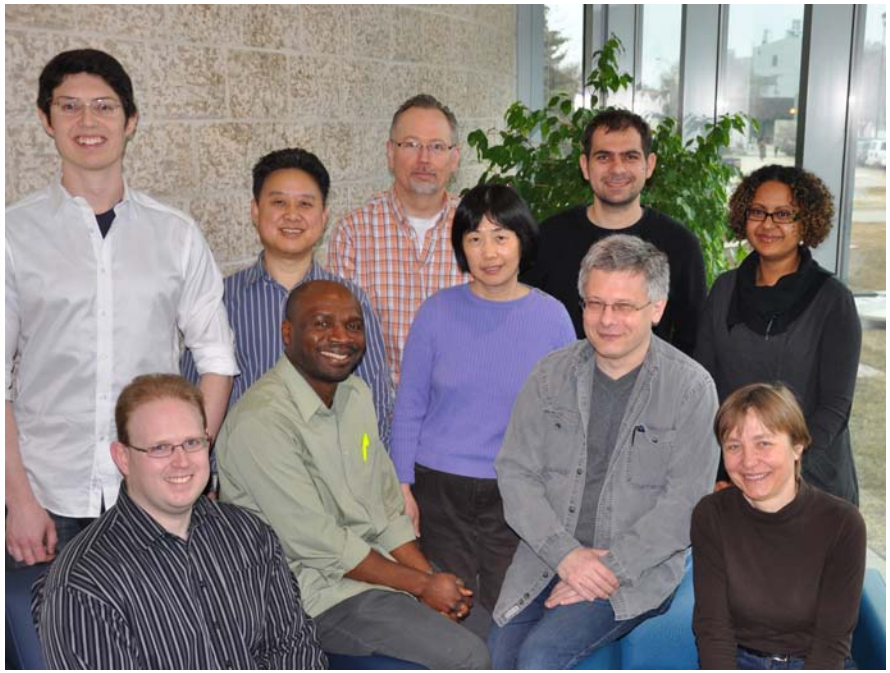
Lead – PHAC (Corbett, Kobinger)

Performance of the assays in the field

Swine and goat samples (tissues and blood from markets, “live” bleeding of pigs)

Acknowledgements

Main collaborators in areas related to diagnostics:
USDA (William Wilson, Barbara Drolet,Myrna Miller)
PHAC (Gary Kobinger, Markus Czub)



Animal Care Unit

Special Pathogens Unit

Pathology Unit

Funding: CFIA, CRTI, USDA