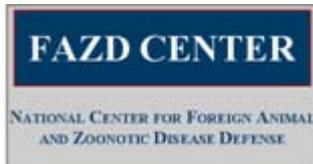


Development of modern detection & diagnostic capabilities for Rift Valley Fever & Foot & Mouth Disease



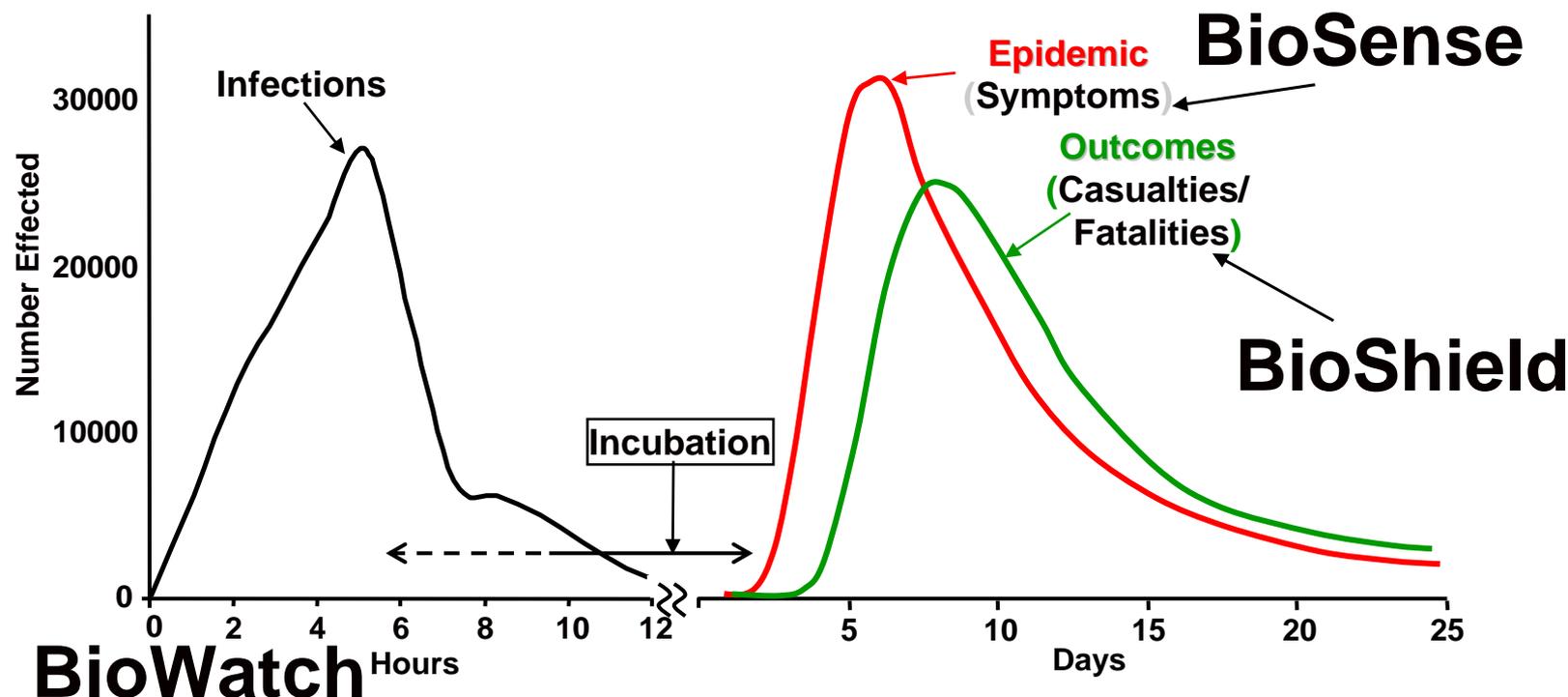
**Peter Mason, C. J. Peters, Tilahun
Yilma & L. Garry Adams***

**University of Texas-Medical Branch, University of
California, Davis, & Texas A&M University**

Washington, DC

March 16, 2007

Public Health National Detection Architectures



Prevent	Warn	Protect	Contain / Decon	Treat
Intel	Facilities Transport	Wide area monitoring	Restoration Portable detection	Early diagnostics
Intel/Law PHO	Bldg. owner	Mayor	First responders Hazmat lead Forensics	Public Health Org.

FMD is the #1 foreign animal disease threat to the US

- FMDV spreads rapidly, exists in multiple serotypes and subtypes, complicating detection and control.
- Outbreaks destroy consumer confidence, and produce billion-dollar losses in productivity.

Our project and its deliverables will help DHS and USDA to minimize the losses resulting from a deliberate (or natural) introduction of FMDV.



A VERY SILENT SPRING

FOOT-AND-MOUTH DISEASE RETURNS TO EUROPE



FMD Diagnostics

- **Development of a Rapid and Inexpensive Diagnostic Kit for FMD**
- **ELISA Tests for FMD using Sub-Genomic Replicons**
- **Mass scale ELISA with new diagnostic antigens**

Development of a Rapid and Inexpensive Antigen-Detection “Chute-Side” Diagnostic Kit for FMD

FAZD CENTER

NATIONAL CENTER FOR FOREIGN ANIMAL
AND ZOONOTIC DISEASE DEFENSE

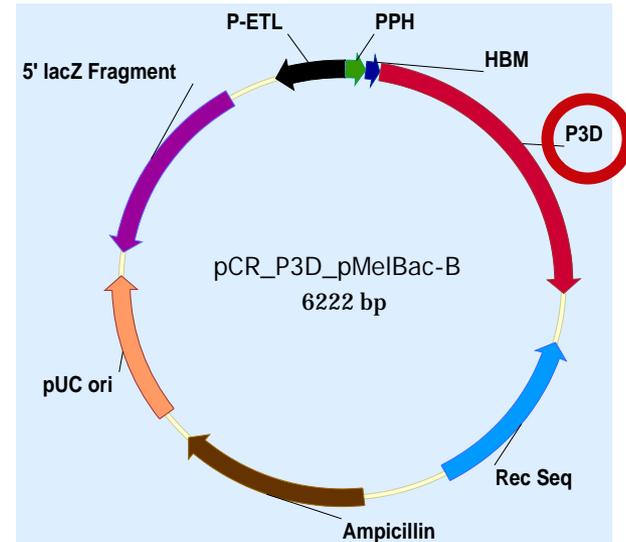
- **Characteristics of the kit:**

- Antigen detection chromatographic strip test based on anti-FMD-P3D monoclonal antibodies
- The P3D protein is highly conserved in all 7 types of FMDV

- **Progress toward “Chute-side” test:**

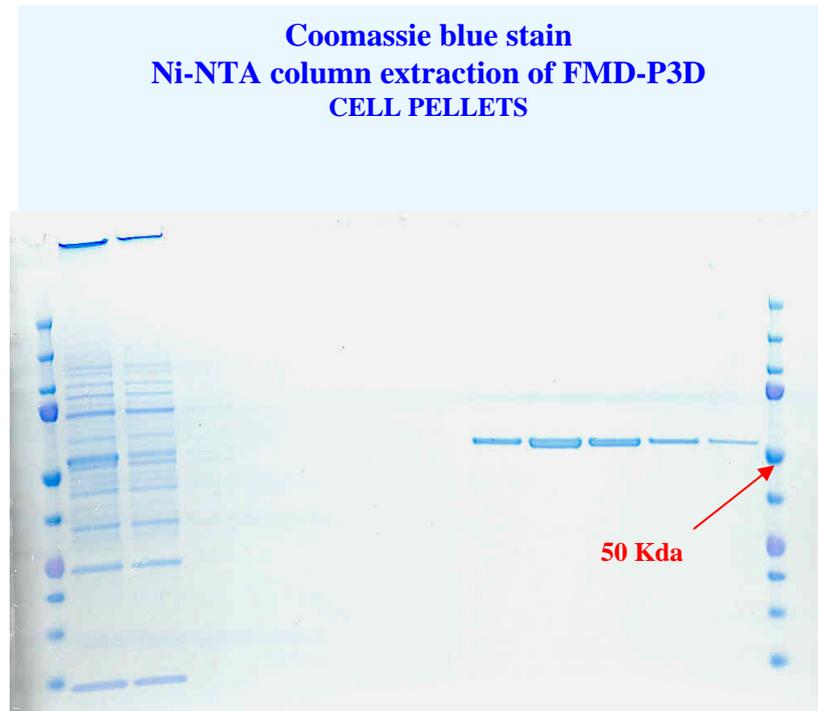
- FMDV P3D was subcloned into the baculovirus transfer vector to produce pCRP3DpMelBacB & co-infection of Bac-N-Blue baculovirus expression producing cell lysates containing high levels of expressed P3D

pCRP3DMelBac-B
transfer vector (6.3 kb)



"Chute-side" antigen detection kit

- **Monoclonal antibody production is underway to develop the chromatographic strip test to be evaluated in US negative cohorts & positive cohorts in Brazil**



15 μ l sample load; 45 min run at 200 V

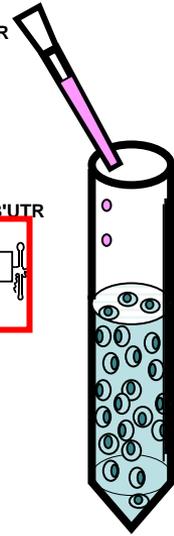
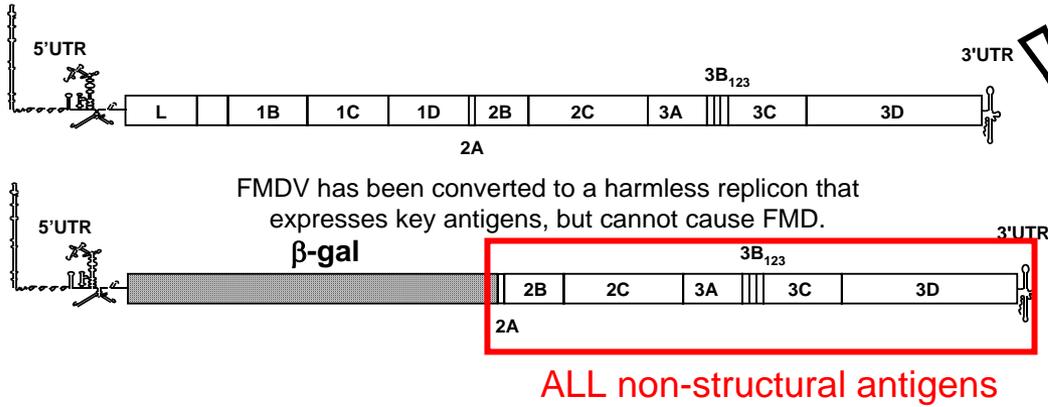
- **Mixtures of Mab's will be used to capture FMDV-P3D from test samples in a rapid strip test which will become available in the spring 2008**

Novel antigens for FMD ELISA-based diagnosis

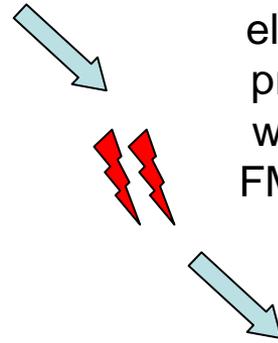
RATIONALE: Cells expressing FMDV non-structural protein encoding replicons (repRNAs) can be produced outside of biocontainment. These should produce all of the viral antigens found in infected cells, including antigens that are broadly cross-reactive among serotypes. Cells that persistently express these replicons, should be a cheap, safe, and abundant source of antigen.

IMPACT: These assays will provide for screening of potentially infected herds or premises, to demonstrate freedom of infection, allowing for return of animals, and farms to production.

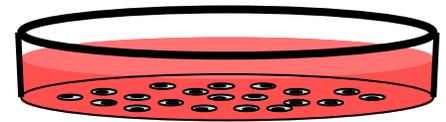
Replicons will be used to make FMD diagnostic antigens



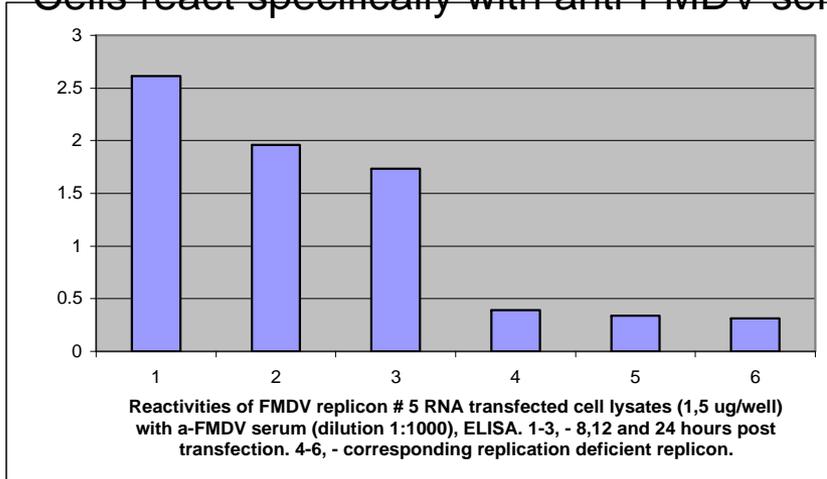
This replicon RNA is added to cells, and they are subjected to electroporation, producing cells with replicating FMDV genomes



Cells are harvested and used in a standard ELISA assay



Cells react specifically with anti-FMDV sera



Antigen also show strong reactivity with MAbs to 3D and 3B

However, there is a time dependent loss of antigen from these cells, so antigen production is limited

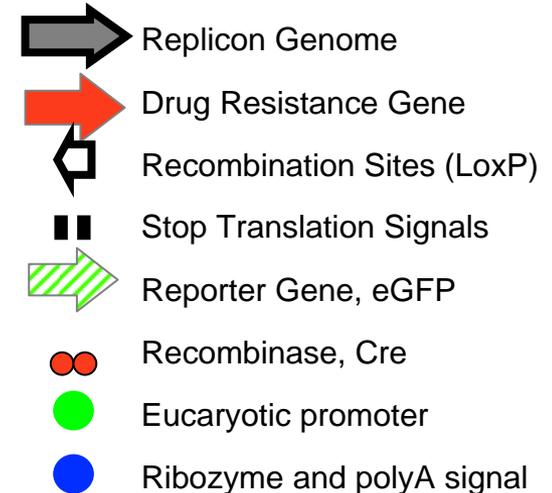
As an alternative strategy, cell lines are being generated that encode a "dormant" DNA that can "launch" a replicon



- **Dormant bi-partite replicon genome has an antibiotic resistance gene inserted, permitting selection of cells with the genome.**



- **Addition of Cre protein brings about recombination between two LoxP sites, which removes the drug resistance gene and restores integrity of replicon genome.**
- **Restored replicon is now replication competent and produces antigen in cells.**
- **This will produce a limitless source of antigen!**



Evaluation of Mass-Scale Robotic FMD ELISA Tests

- **State Diagnostic Lab/Veterinary School Infrastructure for "Scaled-up" FMD Detection**
 - **As replicon-based FMDV antigens become available, the ELISA technology will be transferred to Texas & California Veterinary Medical Diagnostic Laboratory Regional HUB National Animal Health Laboratory Networks**
 - **FMDV antigens will be implemented & tested on Mass-Scale Robotic ELISA units**

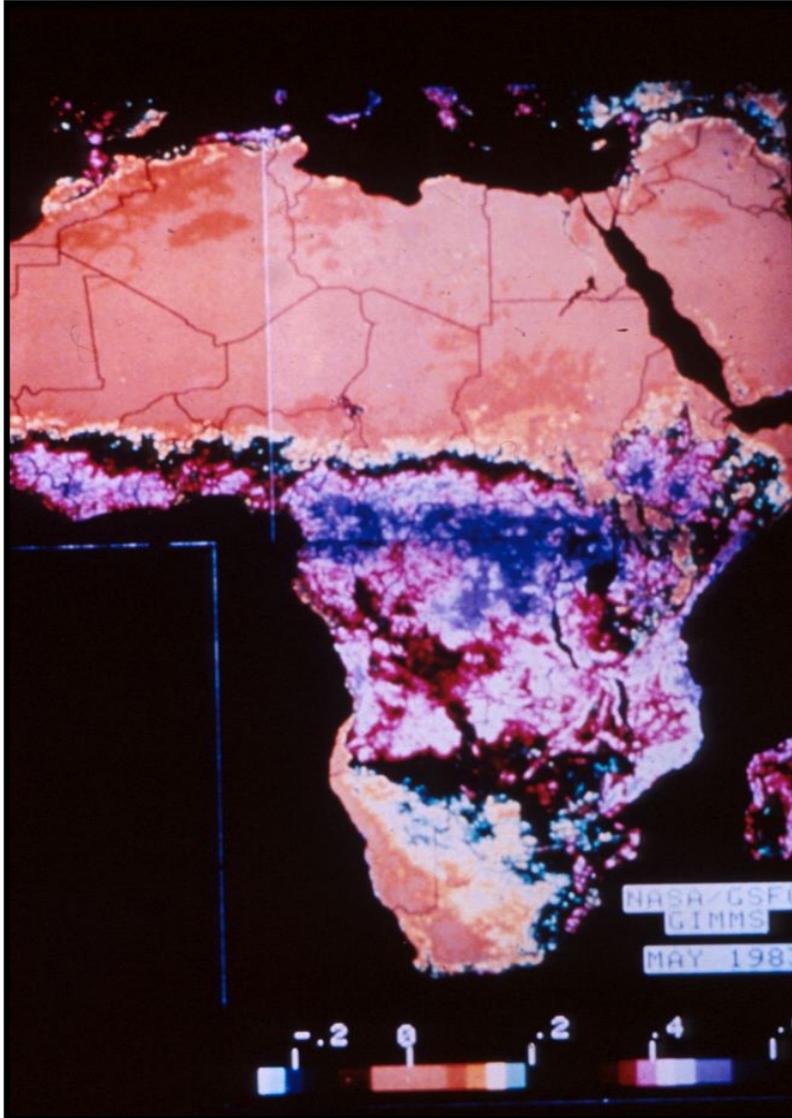


Brazil 2001

Texas 1920s



Rift Valley Fever



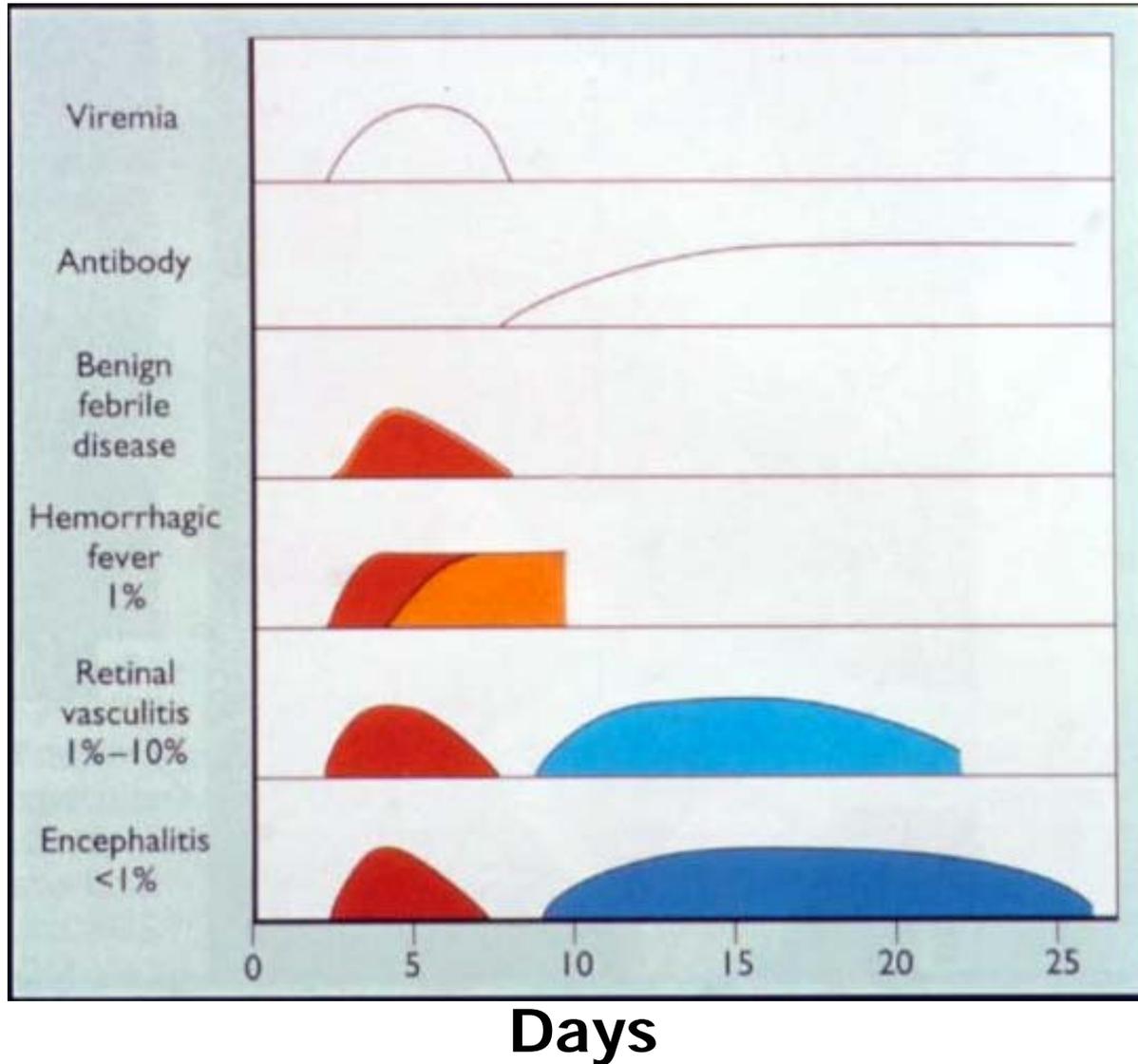
RVF virus is endemic and intermittently epidemic throughout sub-Saharan Africa with distant spread to Egypt and Arabia



Why is RVF a Concern?

- Epizootic disease in sheep and cattle has direct and indirect (trade) effects
- Disease occurs in humans with about 0.5% of infected humans dying further complicating control
- Introduction through natural or bio-argoterrorism is considered feasible
 - Many mosquitoes can be vectors in the lab and in the field at blood meal virus concentrations found in sheep, cattle, humans
 - Virus can cause epidemics whenever vertebrates with high viremias are present (sheep, cattle)
 - Humans visit endemic/epidemic areas and return to their homes within an incubation period
 - Both arthropod and direct transmission from blood efficient
 - RVFV epidemics in new territory: Egypt, Saudi, Yemen
 - Current sever epidemics n Kenya

RVF Human Clinical Schema



RVFV in Domestic Animals

- SHEEP: ~20-30% mortality, abortion
- CATTLE: ~10-15% mortality, abortion
- GOATS: ~5-10% mortality, abortion
- CAMELS: survive, low viremia, ?abortion
- WATER BUFFALO: survive, low viremia
- AFRICAN BUFFALO: survive, ?abortion
- OTHER AFRICAN UNGULATES: Antibody only

- **Mortality depends on breeds, other health and stress factors.**
- **Infections of adult animals end in death if viremia high.**
- **Immature animals have higher viremias and mortality.**
- **Abortion seems to be a complication of most viremic infections.**

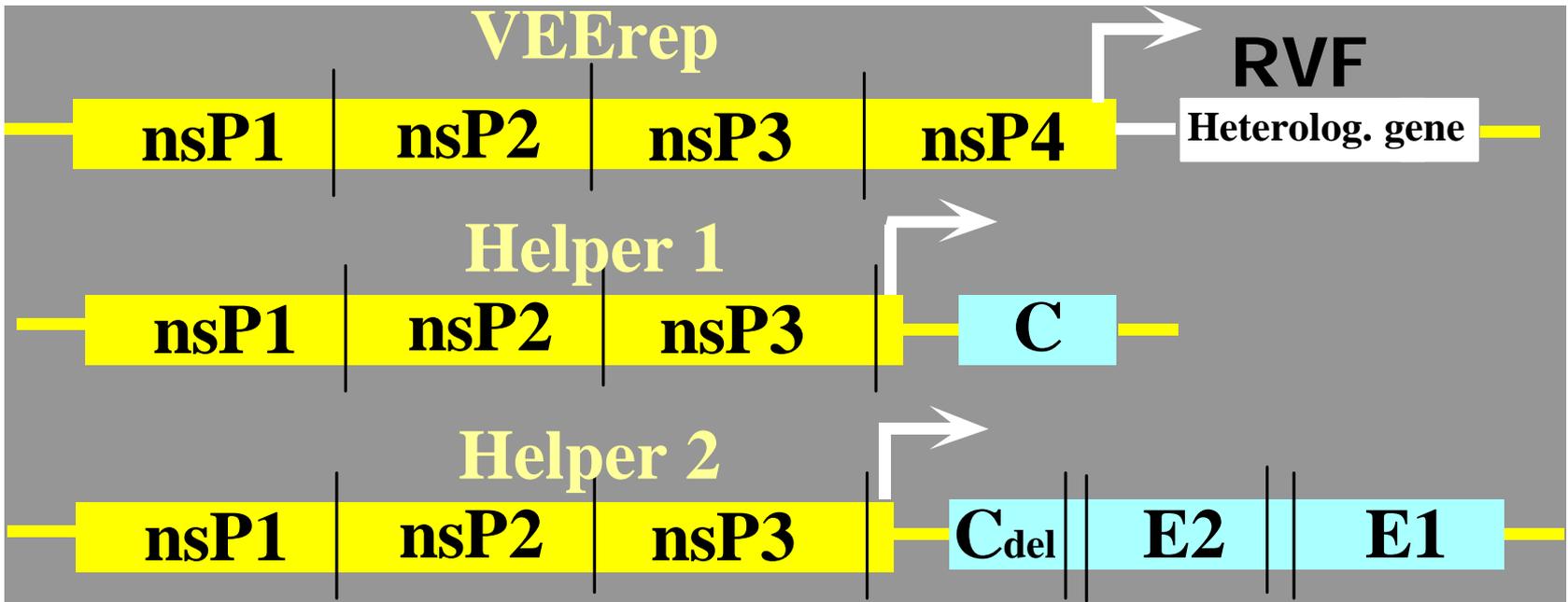
Rift Valley Fever Diagnostics

- **Rift Valley fever virus (RVFV) is an emerging zoonotic disease with high morbidity and mortality transmitted by several mosquito species native in America.**
- **In the event of an outbreak of RVF in the USA, rapid diagnostic techniques would be required to control the disease.**
- **The nucleoprotein (N) of RVFV will be used for the detection of both antibodies and antigen to the virus.**

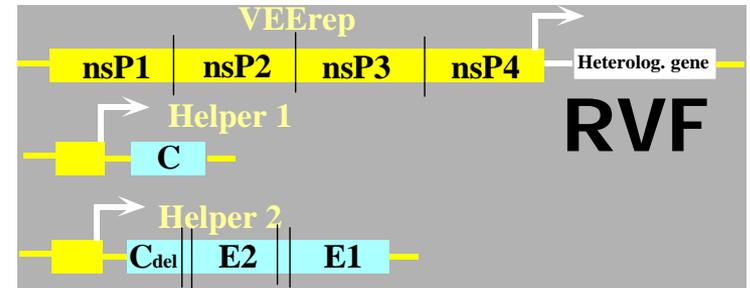
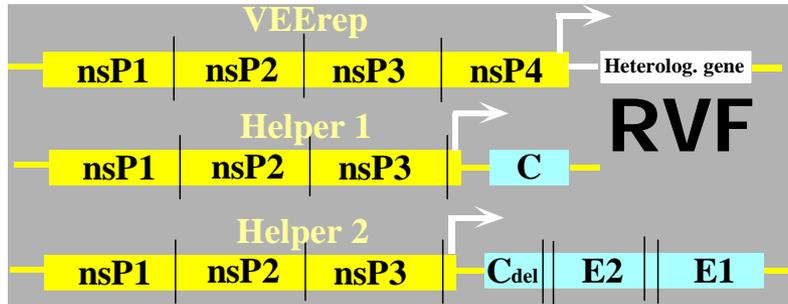
Rift Valley Fever Diagnostics

- **Development of Rapid and Inexpensive Diagnostic Kit for RVF**
- **Provide ELISA kit for rapid diagnosis of RVF**
- **Development of an ELISA kit for detection of antibody to the N protein of RVFV**

Tri-component genome replicon VEE RVF N protein



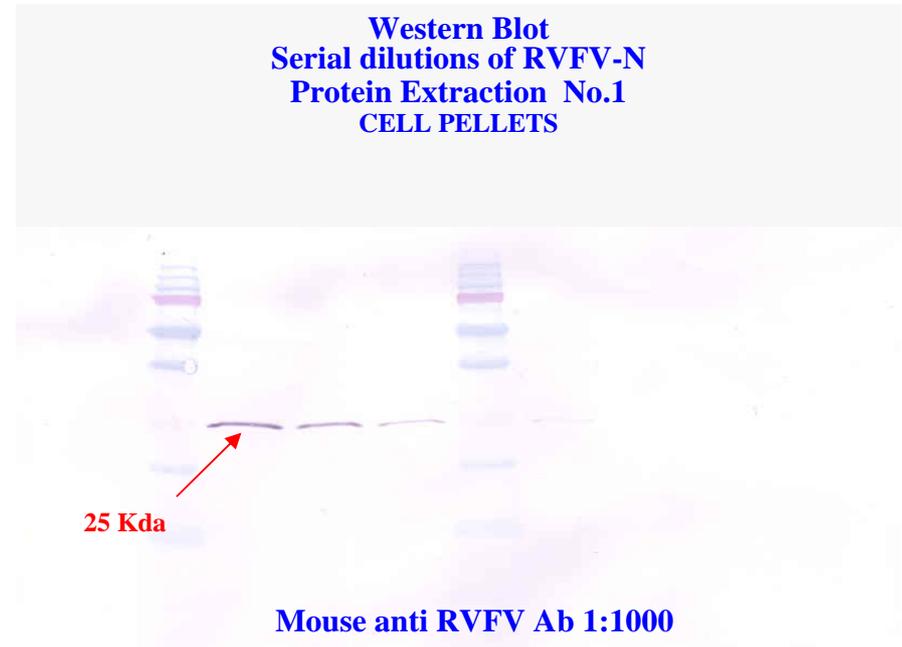
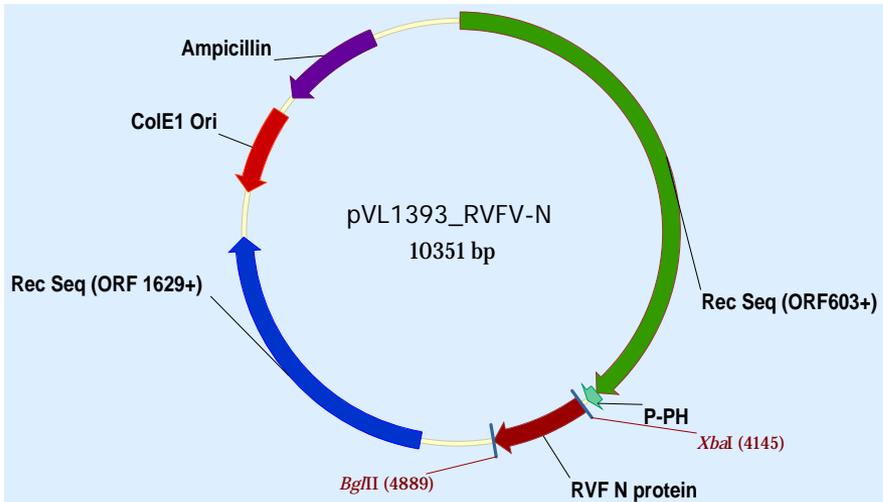
Tri-component genome replicon VEE RVF N protein



Passage	Titers of packaged replicons(inf.u/ml) self-packaging helpers	Titers of packaged replicons(inf.u/ml) traditional helpers
Passage 1 (elporation)	$1-2 \times 10^8$	$1-2 \times 10^9$
Passage 2 (1:100)	$1-8 \times 10^8$	$1-2 \times 10^6$
Passage 3 (1:100)	$1-2 \times 10^8$	Not detectable

Baculovirus expressed RVF N protein antigen

The gene for the RVFV-N protein was subcloned into the baculovirus transfer vector pVL1393 to produce the plasmid pVL1393RVFVN



bRVFV-N protein is ~ 28 KDa

10 µl sample load; 45 min run at 200 V

Cloning was confirmed by sequence analysis and transfer vectors co-transfected with Bac-N-Blue baculovirus expression system

Summary

- Baculovirus & replicon-based FMD viral antigens produced under BSL2 conditions
- Chromatographic strip antigen 'chute-side' & antibody laboratory-based mass scale robotic FMD ELISA in spring '08
- Baculovirus & VEE replicon-based RVF viral antigens produced under BSL2 conditions
- Laboratory-based antibody RVF ELISA in spring '08
- RVF & FMD 'chute-side' & laboratory ELISAs tested in US, South American or African populations of ruminants in '08 & '09



Homeland Security