FUTURE RESEARCH NEEDS FOR RVF CONTROL: VACCINES, VECTOR MONITORING AND DIAGNOSTICS

USDA, ARS
Arthropod-Borne Animal Diseases Research Laboratory
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USA

USDA United States Department of Agriculture

ARS Agricultural Research Service

ABADRL

ABADRL Scientific Staff

- Dr. Kristine Bennett- Molecular Biologist
- Dr. Barbara Drolet- Microbiologist
- Dr. James Mecham- Microbiologist
- Dr. Myrna Miller-Veterinary Medical Officer
- Dr. Will Reeves- Entomologist
- Dr. William Wilson- Microbiologist

Control of RVFV

- Diagnosis
- Vector monitoring and management
- Vaccination

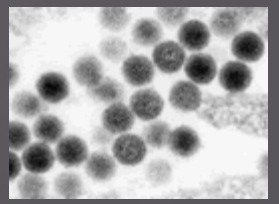
Research Objectives

- Determine vector competency of North American mosquito species for wild-type RVFV
- Determine vector competency of North American mosquito species for modified-live virus vaccines (MP12)
- Develop expression and delivery systems for RVFV vaccine candidates (replicon vaccine)
- Develop diagnostic tests for the sensitive and specific detection of RVFV

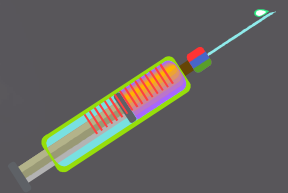
National and International Collabortions

- Vector competence
  - Susceptibility of North American mosquito species
  - Collaborator: M. Turell, USAMRIID
  - Attenuated vaccine vector competence funded by DHS

- Diagnostic development, evaluation and validation
  - Diagnostic laboratory tests as well as rapid field deployable tests
  - Collaborators:
    - J. Macharia, Kenya Central Veterinary Laboratory
    - Y. Binepal, Kenya Agriculture Research Laboratory
    - F. Weber, University of Freiburg
    - J. Paweska, National Institute for Communicable Diseases, South Africa
    - C. Schmaljohn, USAMRIID
    - H. Weingartl, Canadian Food Inspection Agency, Winnipeg



- Vaccine development, evaluation and validation
  - Planning to evaluate alphavirus replicons and attenuated vaccines
    - M. Heise, University of North Carolina
    - H. Weingartl, Canadian Food Inspection Agency, Winnipeg
    - Attenuated vaccine evaluation funded by Department of Homeland Security



Vector Competence Studies

- Susceptibility of North American mosquito species
  - Collaborator: M. Turell, USAMRIID
- Attenuated vaccine vector competence
  - Funded by DHS

Susceptibility and Vector Competence of North American Mosquito Species

- Infect mosquitoes by feeding on viremic hamsters or by needle inoculation
- Determine rate of infection and dissemination of virus in the mosquitoes
- Allow infected mosquitoes to feed on susceptible hamsters and presence of the virus in brain was determined

Susceptibility of North American Mosquito Species

- *Culex tarsalis*-highly susceptible to infection and was able to transmit wild type RFVF
- *Ae. dorsalis* and *Ae. vexans* from Colorado were susceptible to infection, but did not transmit the virus
  - Midgut and/or salivary gland barriers prevent dissemination and transmission
  - Population variation- *Ae. vexans* from Florida was shown in an earlier study to be a moderately competent vector
- *Culicoides sonorensis* not susceptible to infection, even by needle inoculation

MP12 Vector Transmission by North American Mosquitoes

- Vaccinate sheep with MP12 vaccine (USAMRIID)
- Blood feed mosquitoes on sheep on DAI 2, 3 & 4
- Blood fed females held for 10 days and then fed on hamsters
- Assay for virus or antibody to virus

Assays for Virus and Antibody

Sample	RT-PCR NSs primer (+/-)	RT-PCR G2 primer (+/-)	VI (+/-)*	Antibody
Sheep Serum	0/4	0/4	1/4	0/4 ELISA 3-4 DPV
Sheep Liver	0/4	0/4	1/4	
Sheep Spleen	0/4	0/4	1/4	
Mosquitoes	NA	0/12 pools	NA	
Hamsters, 21 DP feed			NA	0/7 (ELISA & VSN)

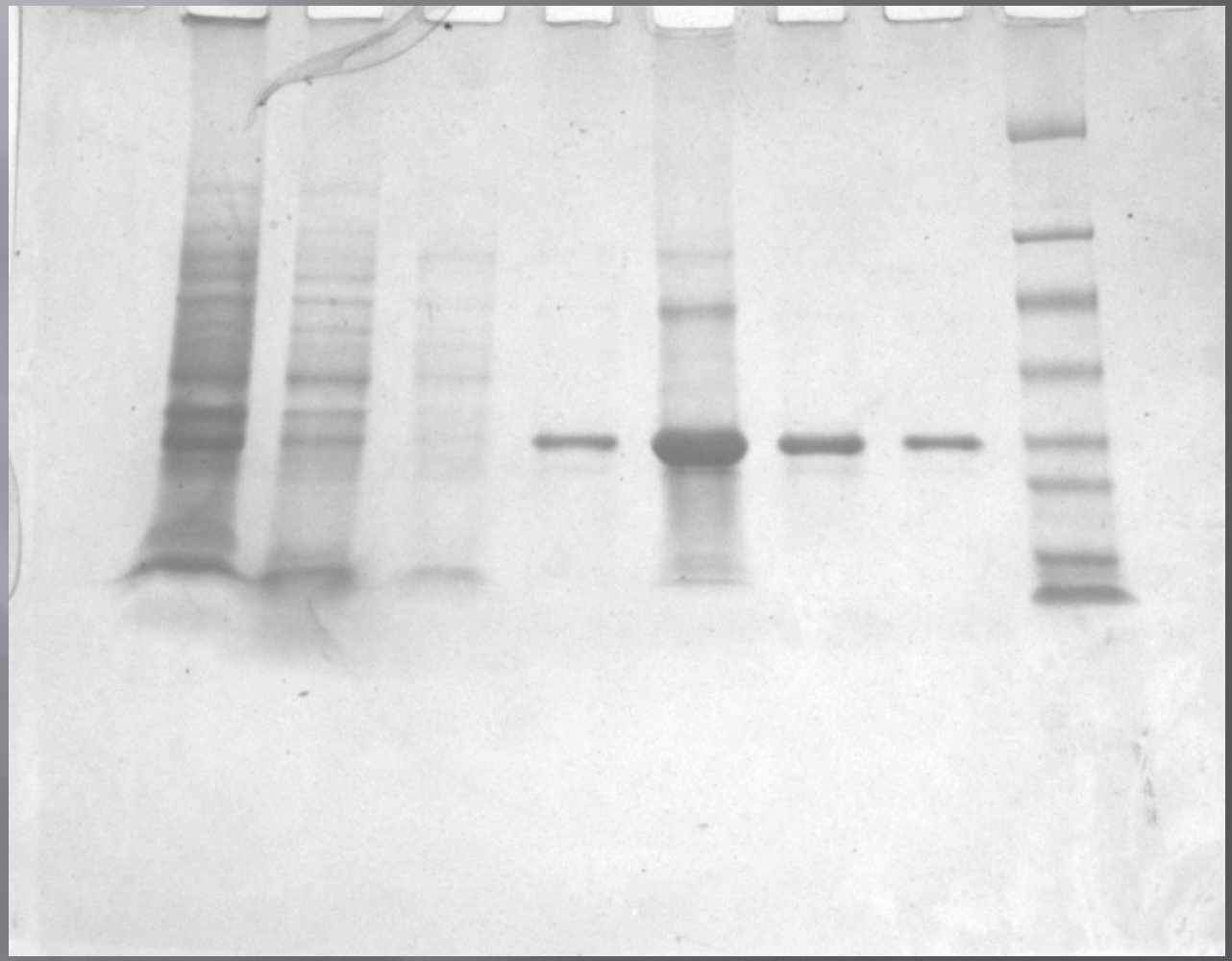
Samples from MP12 vaccinated sheep or mosquitoes fed on vaccinated sheep were tested for RVFV by RT-PCR and virus isolation on VERO cells.

- Samples positive by VI were verified by RT-PCR of cell culture supernatant.
- Hamster sera was tested for MP12 antibodies 21 days post mosquito feed

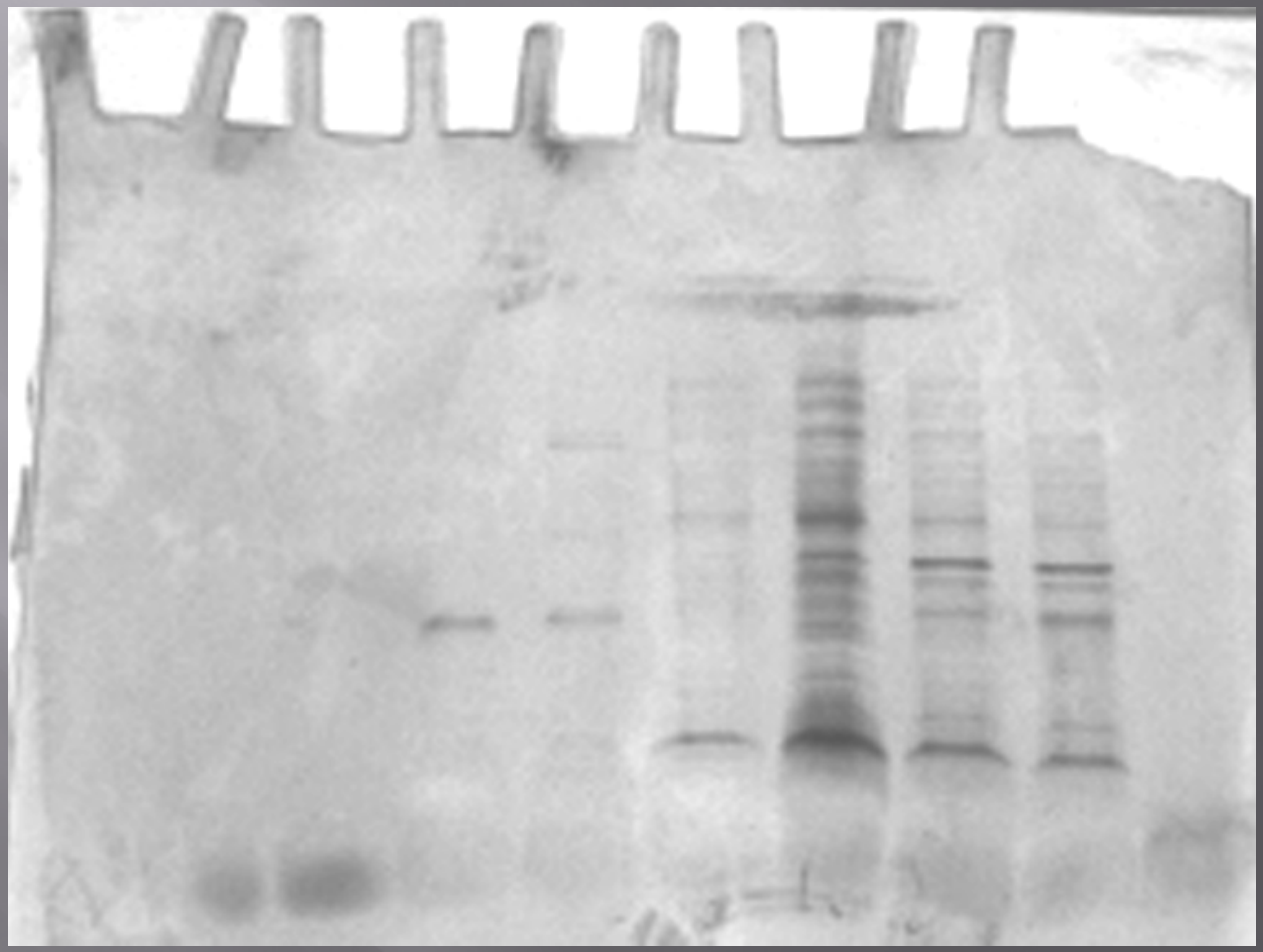
Development of Diagnostic reagents and Assays

- **G2t**. A truncated form of the gene coding for glycoprotein 2 (G2t) with immunogenic regions was inserted into the pET 30-Et/Lic vector and expressed in E. coli for use in diagnostic assays and to produce antisera.
- **NP.** The nucleocapsid gene cloned into pET30 vector was received from F. Weber, University of Freiburg and the nucleocapsid protein (NP) was was expressed in E. coli for use in diagnostic assays and to produce antisera.

Purification of Expressed NP



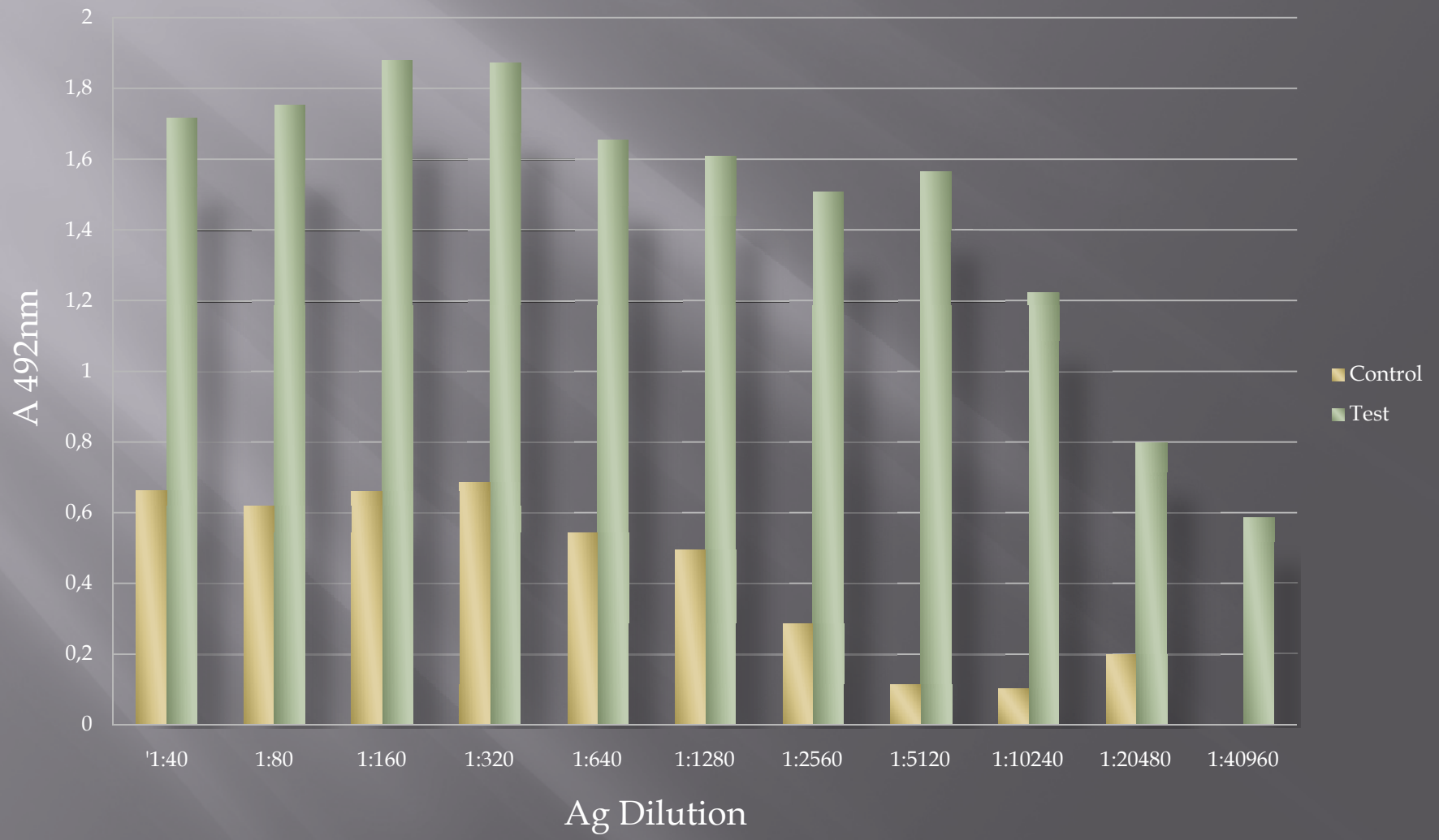
Purification of Expressed G2t



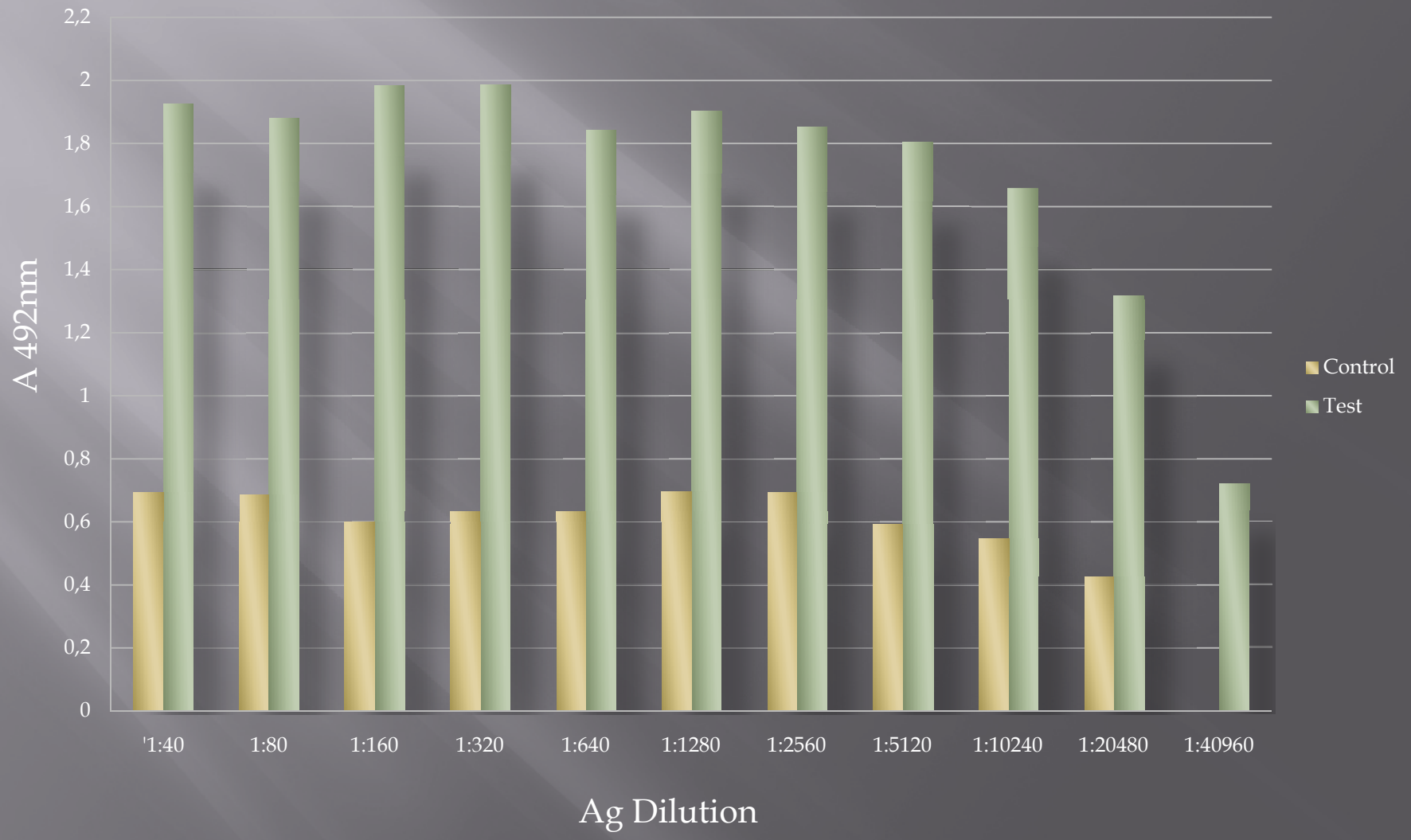
Binding ELISA using RVFV Expressed Proteins

- Dilutions of purified NP or G2t were coated onto the wells of 96 well plates
- Block un-reacted sites with 1% BSA
- Test sera from RVFV infected sheep (CFIA) and control sheep diluted 1:40 and added to duplicate wells
- React with biotinylated rabbit anti-sheep
- React with peroxidase conjugated streptavidin
- React with suitable substrate and measure adsorbance

Nucleocapsid Protein



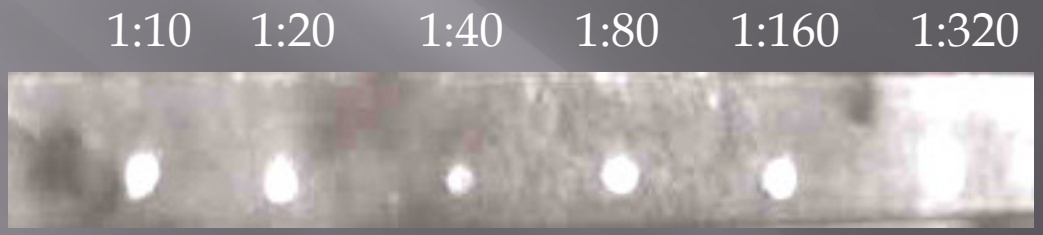
Truncated Glycoprotein



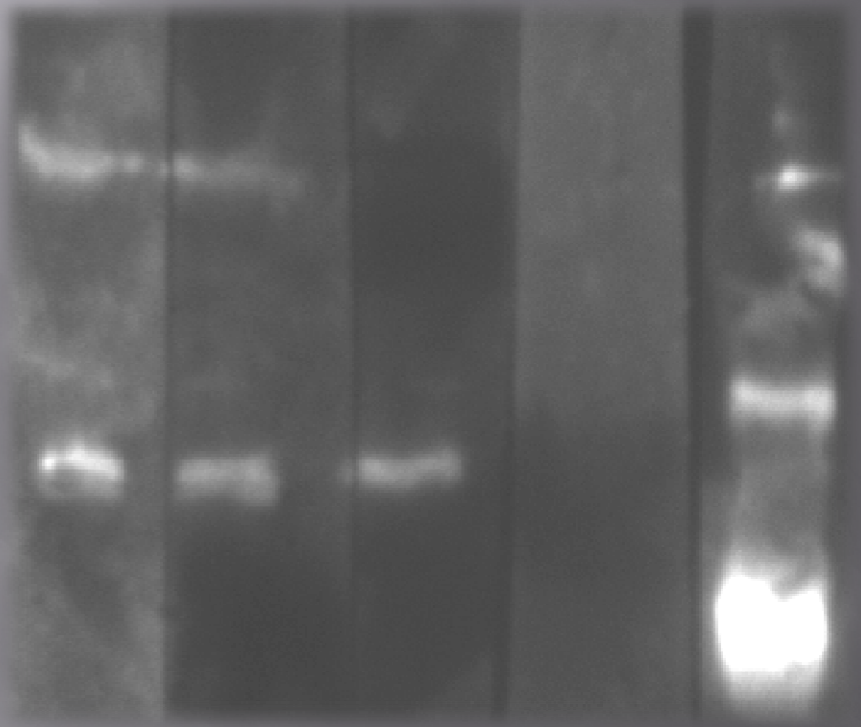
Production of Antibody Reagents

- Rabbits were immunized with the NP and G2t expressed proteins for production of mono-specific polyclonal antiserum.
  - Diagnostic development
  - Immuno-histochemical assays
- Mice have been immunized with MP12 for production of MAb.
  - Diagnostic development
  - Immuno-histochemical assays

Dot Blot of MP12 and Dilutions of Rabbit Antiserum to Expressed RVFV Nucleocapsid Protein



Western Blot of MP12 and Dilutions of Rabbit Antiserum to Expressed RVFV Nucleocapsid Protein



Nucleic Acid Detection Assays

- Evaluation of current nucleic acid based assays
- Develop multiplex real-time RT-PCR assays targeting all three RNA genome segments of RVFV

Multiplex real-time RT-PCR

- L Segment
  - (Bird et al. J. Clin. Microbiol. 45, 3506-13. 2007.)
    - Target gene cloned for positive template and assay working
- M Segment (G2)
  - Drosten et al. J. Clin. Microbiol. 40, 2323-30. 2002.
    - Target template cloned and assay working
- S Segment (NSs)
  - Garcia et al. J. Clin. Microbiol. 39, 4456-61. 2001
    - Target template cloned and assay working
- Positive RNA Control
  - Moniwa et al. J. Vet. Diag. Investig. 19, 9-20. 2007
    - Assay not working on cloned or sample templates
  - Designing new RNA control

Novel Approaches to Diagnostic Development

- **On-Probe Pyrolysis Desorption Electrospray Ionization (DESI) Mass Spectrometry**
  - Franco Basile, School of Pharmacy, University of Wyoming
- **Surface Enhanced Raman Scattering**
  - Robert Corcoran , Chemistry Department, University of Wyoming; Delta Nu Corp., Laramie, WY

DHS FUNDING ABDRL TO EVALUATE RVF VACCINES DEVELOPED BY OTHER INVESTIGATORS

Evaluation RVF MP-12

- ABADRL currently doing preliminary studies with MP-12 from USAMRIID but will switch to the Pfizer product when available
- Determining ability of mosquitoes to become infected and transmit MP-12 from vaccinates
- Potential of MP-12 to reassort with closely related phleboviruses

NSs Deletion Vaccine Candidates

- **Candidates**
  - **Onderstepoort Biological Products**
    - Clone 13 Attenuated Vaccine
    - Major deletion in NSs gene that inhibits interferon
  - **Double deletion mutants, CDC**
    - Reverse genetic deletion of NSs and NSm
- **Insect vector safety trials as with MP-12.**
- **Potential of NSs as a deletion marker**
  - Western blots
  - NSs ELISA

Alphavirus Replicon Vectors

- RVFV Gn and Gc glycoproteins expressed from Sindbis or Venezuelan equine encephalitis virus based replicon particles
  - Safe (single hit vectors)
    - Immunogenic in a wide range of species
    - Can differentiate vaccinated from naturally infected animals
      - Lack of N protein specific response
      - Unique antigenic tags can be included in the vaccine
  - Replicons produce high levels of recombinant protein for:
    - Virus free cell fusion/pseudo-typing/neutralization assays
    - Virus like particle production

- **Formal collaboration with M. Heise, U. North Carolina and R. Doms, U. Pennsylvania.**
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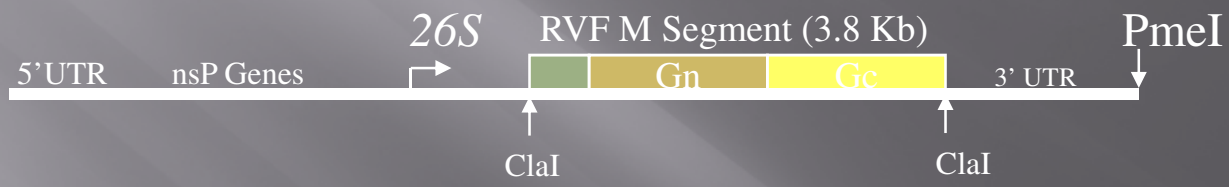
Sindbis Virus Replican with RVFV M Segment



Heise et al., submitted

Develop Challenge Model

- International Cooperation with H. Weingartl, National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Canada
- Positive samples and antibody reagents

Summary

- Initiated studies on vector competency of North American mosquito species for RVFV
- Produced operator-safe reagents and have begun development of operator-safe diagnostic tests for RVFV
- Agreements in place to evaluate RVFV DNA vaccine candidates

Questions?