Detection of Rift Valley Fever Virus Using A Plaque Reduction Neutralization Test (PRNT)

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Introduction

Rift Valley Fever Virus (RVFV) is an arbovirus endemic to Africa. Infections primarily cause abortions and neonatal death in ruminants such as sheep and cattle. However, it can also cause disease in humans, which may lead to death. Accurate and rapid diagnostic tests are needed to understand and monitor the spread of RVFV. In order to determine a new diagnostic test's sensitivity, its ability to detect small concentrations of viral antigen, and specificity, its ability to correctly identify the desired antigen, it must be compared to the current gold standard diagnostic method. The gold standard for determining RVFV infection in ruminants is an 80% plaque reduction neutralization test ($PRNT_{80}$). This test is a serological assay that detects the presence of neutralizing antibodies to RVFV in serum. Animals are typically positive by RVFV $PRNT_{80}$ once they have been infected for 3 to 4 days.

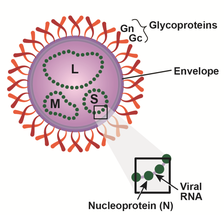

Figure 1. Structure of RVFV virion..

Currently, we are developing a fluorescence multiplexing bead-based immunoassay (FMIA) for detection of antibodies raised against multiple RVFV proteins as well as validating multiple enzyme-linked immunosorbent assays (ELISA) for RVFV. These tests are faster and easier to conduct than the $PRNT_{80}$. validation of these new assays must be conducted by comparing them to the gold standard $PRNT_{80}$. Here, we determined the $PRNT_{80}$ values for a population of serum samples for use with validation of these new diagnostic assays.

Materials & Methods

Samples:

- Positive sheep sera were obtained from an RVFV MP12 study, a wild-type RVFV strain challenge study (Kenya 2006 or Saudi Arabia 2001 strains), and an RVFV Gn/Gc subunit vaccine study with Kenya 2006 challenge
- Negative sera from sheep and cattle were obtained from locally sourced US animals

PRNT Protocol:

*conducted in a 96-well plate and 12-plate with Vero Cell (Green Monkey Kidney) monolayers

Serum
- Two fold serial dilution of serum vertically down the 8 rows
- Add MP12 virus to the diluted serum
- Shake and Incubate at 1hr at 37C 5% CO2

Virus
- Infect Vero cell monolayers (95-100% confluency)
- Incubate 5 days at 37C 5% CO2

Results
- Stain Monolayer with crystal violet fixative
- Count all plaque forming units (PFU) in the positive control plates and determine 80% cutoff
- Count PFU in wells with serum stopping if greater than 80% cutoff

Titer Calculation:

To determine the 80% cutoff for positive sera, the average number of plaques in the positive control wells is divided by 2, to account for dilution of the virus in the plates with sera. Then 80% of that number is the maximum number of plaques allowed for a well. The antibody titer is the most dilute sample that achieves 80% reduction in viral plaque formation. This value is presented as the inverse of the dilution ratio.

Results

The $PRNT_{80}$ confirmed that the samples infected with RVFV had neutralizing antibodies and those that were not exposed to RVFV had less than 1:10 neutralizing antibodies. For this project, 108 samples were used for PRNT80. Here we present a subset of the serum from the sheep population on which we conducted the $PRNT_{80}$.

Sample	Result	Titer
64d4	Positive	80
60d9	Positive	320
71d8	Negative	<10
60d10	Positive	>1280
68d8	Positive	40

Figure 2 Results of PRNT on various sheep and bovine serum. The titer is shown as the inverse of the most dilute set that shows 80% reduction of viral plaque formation.

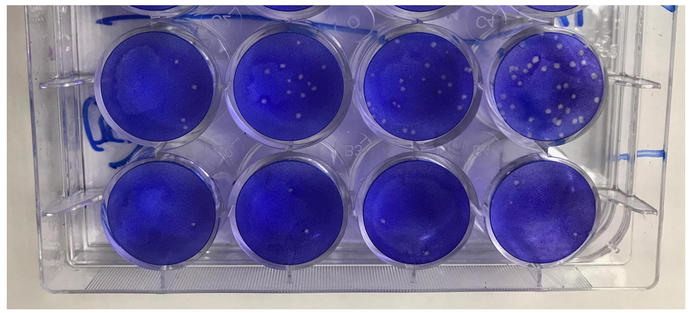

Figure 3: Results of stained vero cell monolayers for animal with positive serum. Viral plaques are white circles counted by hand. Dilution is read from right to left.

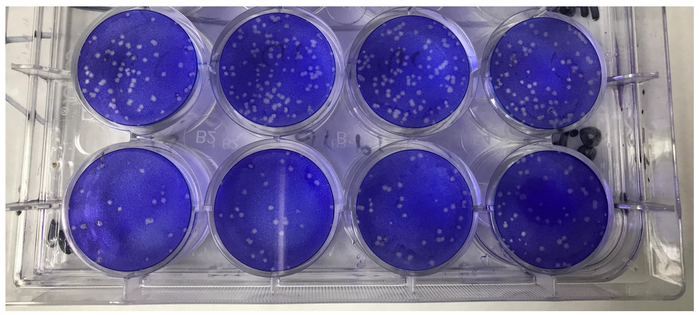

Figure 4: Results of PRNT on negative serum. The number of white plaques are far greater than the 80% reduction required for positive serum.

Controls

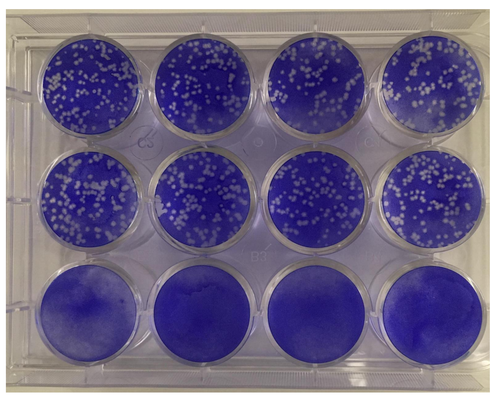

Figure 5: Control Plate. Top 2 rows are positive control (containing virus and no sera) and bottom row is negative control (containing no virus or sera).

Discussion

Our results were consistent with the data presented previously in the paper by Faburay et. al. (2). All serum samples that came from animals infected with RVFV had antibodies, with some variations in titer value; some animals having higher titer in the most recent PRNT and some lower. This variation was likely due to the thawing and freezing cycles the sera had undergone in storage.

For RVFV the accepted gold standard for antibody detection is the PRNT 80. The 80% threshold is based on the statistical logic that 100% neutralization is unreasonable to achieve but 50% neutralization could happen by chance. These results will be used to determine the sensitivity and specificity of new serological assays such as FMIA and ELISA.

References

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2. Faburary, B., et al. (2015). Development of a sheep challenge model for Rift Valley fever. Virology 489: 128-40.
3. Wilson, W.C., et al. (2014). Evaluation of lamb and calf responses to Rift Valley fever MP-12 vaccination. Vet Microbiology 6;172(1-2):44-50.

Acknowledgements

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