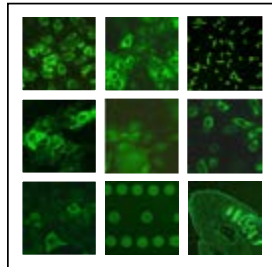




# Investigation of serological cross-reactivity within the alphavirus genus using IFA Biochip Mosaics



**Dr. Erik Lattwein**

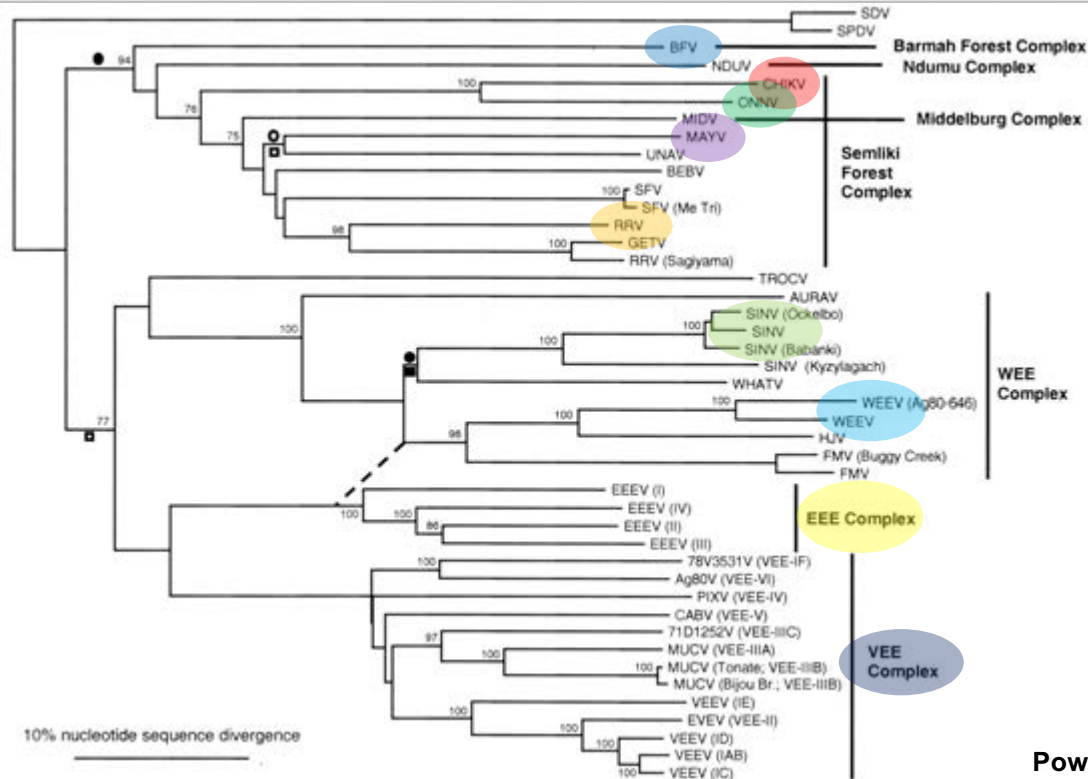
11 November 2018

# Alphavirus Pathogens



| Virus  | Disease                  | Distribution                    |
|--|--------------------------|---------------------------------|
| Chikungunya virus <b>CHIKV</b>                   | Arthralgia/<br>Arthritis | Africa, America, Asia(, Europe) |
| O'nyong-nyong virus <b>ONNV</b>                  |                          | Africa                          |
| Sindbis virus <b>SINV</b>                        |                          | Africa, Europe, Asia            |
| Ross River virus <b>RRV</b>                      |                          | Australia, Oceania              |
| Barmah Forest virus <b>BFV</b>                   |                          | Australia                       |
| Mayaro virus <b>MAYV</b>                         |                          | Central and South America       |
| Western equine encephalitis virus <b>WEEV</b>    | Encephalitis             | North and South America         |
| Eastern equine encephalitis virus <b>EEEV</b>    |                          | North and South America         |
| Venezuelan equine encephalitis virus <b>VEEV</b> |                          | Central and South America       |

# Alphavirus Phylogeny



Phylogenetic tree  
generated from partial  
E1 envelope  
glycoprotein gene  
sequences

# Alphavirus Serology

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ELISA or IFA for serological alphavirus diagnostics are **cross-reactive!**

Using a **monospecific assay** alone can lead to a **false** result:

Patient A: **RRV** IgG 1:1000 / IgM neg.

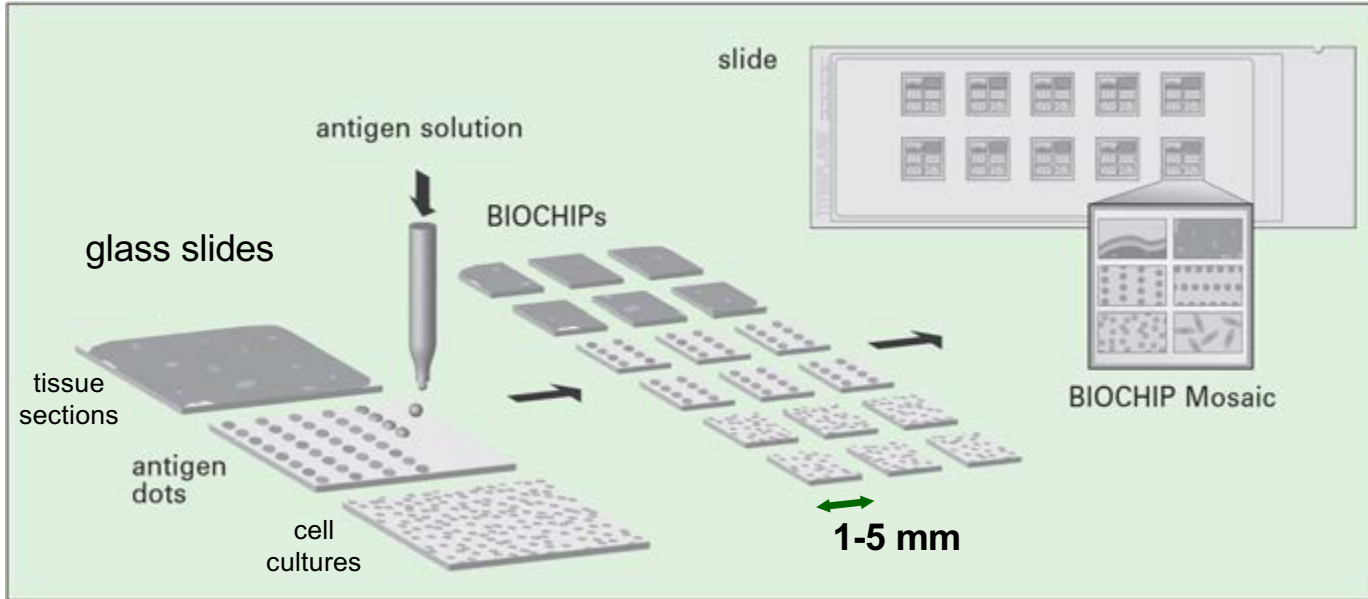
**CHIKV** IgG 1:32,000 / IgM 1:320

→ **Parallel investigation for different alphavirus antibodies to be aware of cross-reactivities**



**IFA BIOCHIP Mosaics for  
serological differential diagnostics  
of  
alphavirus infections**

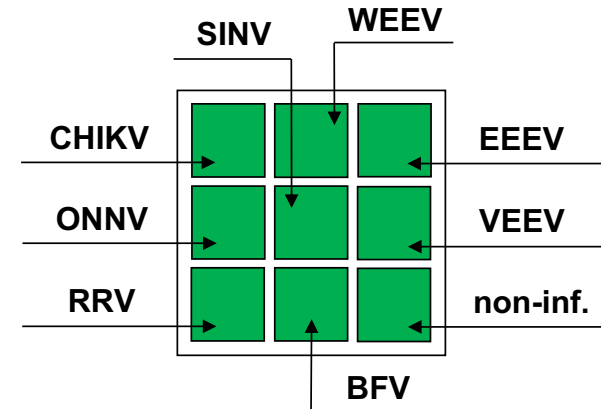
# BIOCHIP Mosaics



# Alphavirus Mosaic (IgG, IgM)



virus-infected cells



→ Investigation of different alphavirus sample collectives

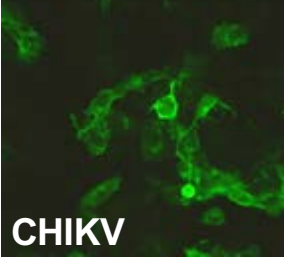
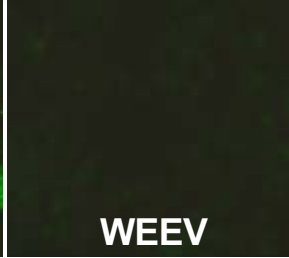

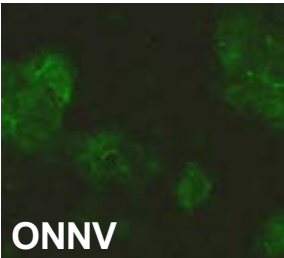


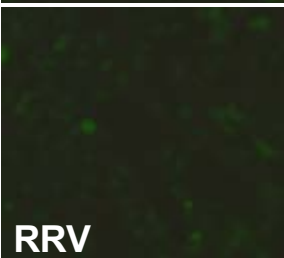
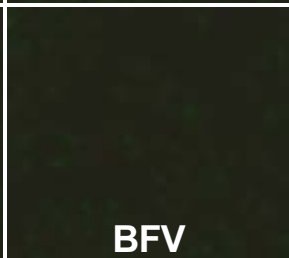



Samples from  
**Chikungunya virus (CHIKV)**  
infected patients



# CHIKV Case Example – IgG 1:100

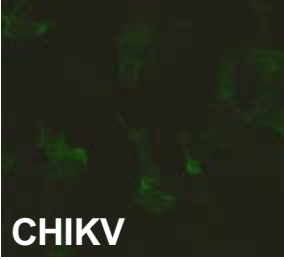
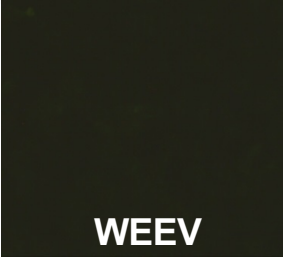

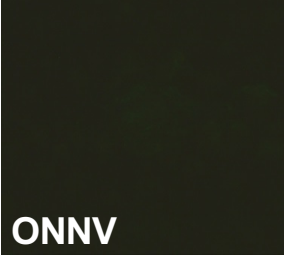
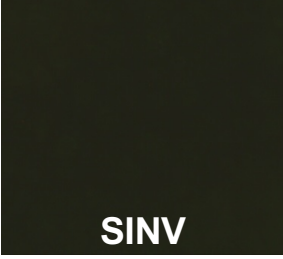

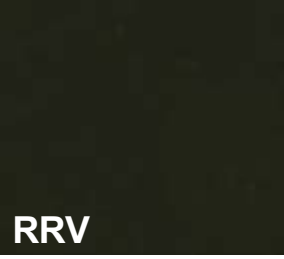




|  |   |   |
|--|---|---|
| <br>CHIKV | <br>WEEV | <br>EEEV      |
| <br>ONNV  | <br>SINV | <br>VEEV      |
| <br>RRV  | <br>BFV | <br>non-inf. |

**POSITIVE**  
**CHIKV**  
**+ ONNV**  
**IgG**

# CHIKV Case Example – IgG 1:1000



|  |   |   |
|--|---|---|
| <br>CHIKV | <br>WEEV | <br>EEEV      |
| <br>ONNV  | <br>SINV | <br>VEEV      |
| <br>RRV  | <br>BFV | <br>non-inf. |

**POSITIVE**  
**CHIKV**  
**IgG**

## CHIKV samples IgG (n=18)



| Antigen | Positive in 1:100 dilution |            | Positive after endpoint titration (less than 10x diff.) |            |
|---------|----------------------------|------------|---|------------|
| ONNV    | 17/18                      | <b>94%</b> | 5/18  | <b>28%</b> |
| RRV     | 5/18                       | <b>28%</b> | 0/18  | 0%         |
| BFV     | 2/18                       | <b>11%</b> | 0/18  | 0%         |
| SINV    | 6/18                       | <b>33%</b> | 0/18  | 0%         |
| WEEV    | 8/18                       | <b>44%</b> | 0/18  | 0%         |
| EEEV    | 1/18                       | <b>6%</b>  | 0/18  | 0%         |
| VEEV    | 6/18                       | <b>33%</b> | 0/18  | 0%         |

## CHIKV samples **IgM** (n=18)



| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |            |
|---------|---------------------------|------------|---|------------|
| ONNV    | 17/18                     | <b>94%</b> | 9/18  | <b>50%</b> |
| RRV     | 3/18                      | <b>17%</b> | 1/18  | <b>6%</b>  |
| BFV     | 0/18                      | 0%         | 0/18  | 0%         |
| SINV    | 0/18                      | 0%         | 0/18  | 0%         |
| WEEV    | 1/18                      | <b>6%</b>  | 0/18  | 0%         |
| EEEV    | 0/18                      | 0%         | 0/18  | 0%         |
| VEEV    | 0/18                      | 0%         | 0/18  | 0%         |

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# Australian Alphaviruses and CHIKV

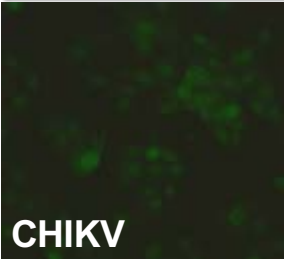
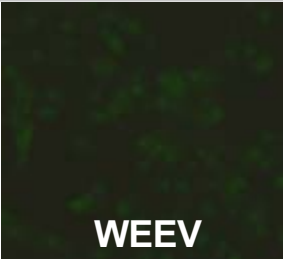
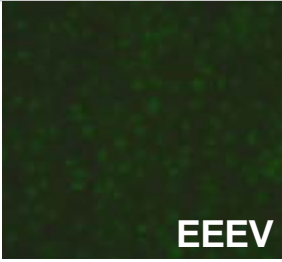

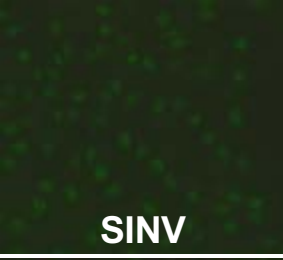

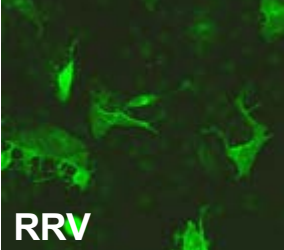


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Samples from Australian patients  
precharacterized by in-house ELISA for  
Ross River virus (RRV) and Barmah Forest virus (BFV)  
antibodies

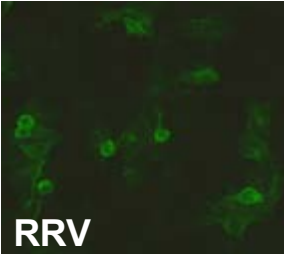
# RRV Case Example – IgG 1:100



|  |   |   |
|--|---|---|
|  <p>CHIKV</p> |  <p>WEEV</p> |  <p>EEEV</p>      |
|  <p>ONNV</p>  |  <p>SINV</p> |  <p>VEEV</p>      |
|  <p>RRV</p>  |  <p>BFV</p> |  <p>non-inf.</p> |

# RRV Case Example – IgG 1:1000



|   |      |          |
|---|------|----------|
| CHIKV   | WEEV | EEEV     |
| ONNV  | SINV | VEEV     |
| <br>RRV | BFV  | non-inf. |

**POSITIVE**  
**RRV**  
**IgG**

## RRV samples



### IgG (n=85)

| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |            |
|---------|---------------------------|------------|---|------------|
| CHIKV   | 79/85                     | <b>93%</b> | 10/85   | <b>12%</b> |
| BFV     | 19/85                     | <b>22%</b> | 0/85  | 0%         |

### IgM (n=72)

| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |    |
|---------|---------------------------|------------|---|----|
| CHIKV   | 8/72                      | <b>11%</b> | 0/72  | 0% |
| BFV     | 0/72                      | 0%         | 0/72  | 0% |



# BFV Case Example – IgG 1:10



|       |      |          |
|-------|------|----------|
| CHIKV | WEEV | EEEV     |
| ONNV  | SINV | VEEV     |
| RRV   | BFV  | non-inf. |

# BFV Case Example – IgG 1:100



|       |      |          |
|-------|------|----------|
| CHIKV | WEEV | EEEV     |
| ONNV  | SINV | VEEV     |
| RRV   | BFV  | non-inf. |

**POSITIVE**  
**BFV**  
**IgG**

## BFV samples



### IgG (n=46)

| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |    |
|---------|---------------------------|------------|---|----|
| CHIKV   | 14/46                     | <b>30%</b> | 0/46  | 0% |
| RRV     | 13/46                     | <b>28%</b> | 0/46  | 0% |

### IgM (n=40)

| Antigen | Positive in 1:10 dilution |             | Positive after endpoint titration (less than 10x diff.) |             |
|---------|---------------------------|-------------|---|-------------|
| CHIKV   | 1/40                      | <b>2.5%</b> | 0/40  | 0%          |
| RRV     | 1/40                      | <b>2.5%</b> | 1/40  | <b>2.5%</b> |

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## Summary and outlook

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- **Cross-reactivity** of IgG and IgM antibodies plays an important role in serological alphavirus diagnostics.
- Parallel investigation using a **biochip mosaic** can identify the causative virus in many cases (depending on the antigenic relatedness).
- Studies need to be extended with **more samples**.
- Samples from patients with MAYV, ONNV, WEEV, EEEV or VEEV infection have still **to be tested**.



Robert Koch Institute, Berlin, Germany

Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

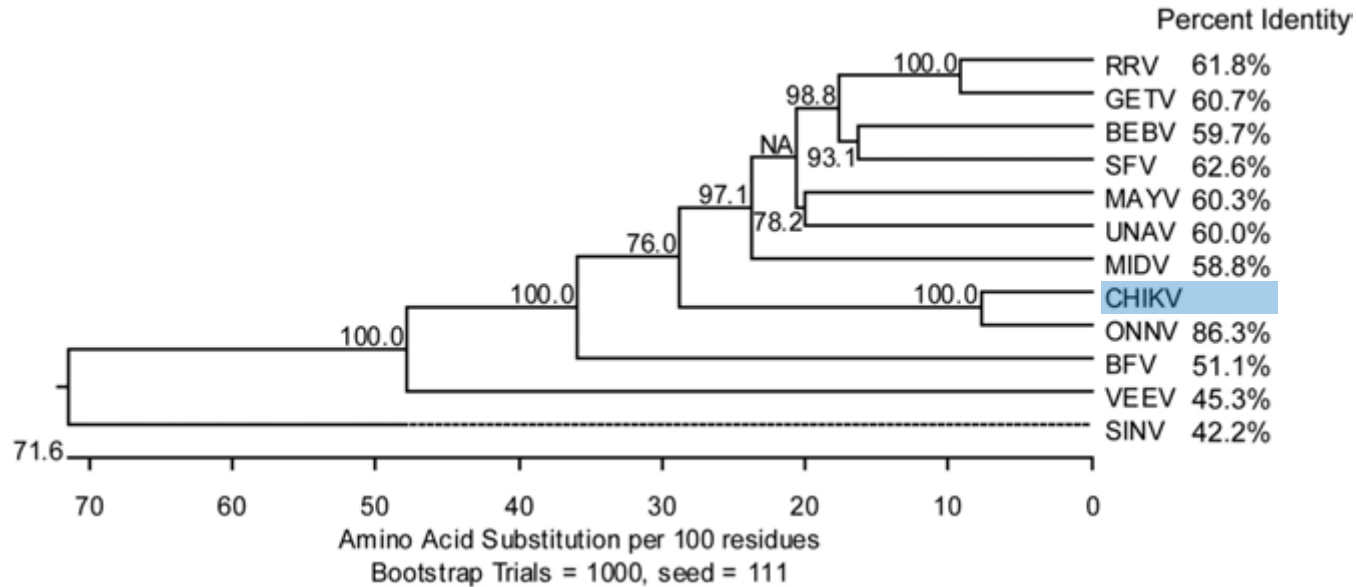
Institute for Virology, Charité, Berlin, Germany



**Thank you for your attention!**



## Alignment of the C-E3-E2-6K-E1 amino acid sequences



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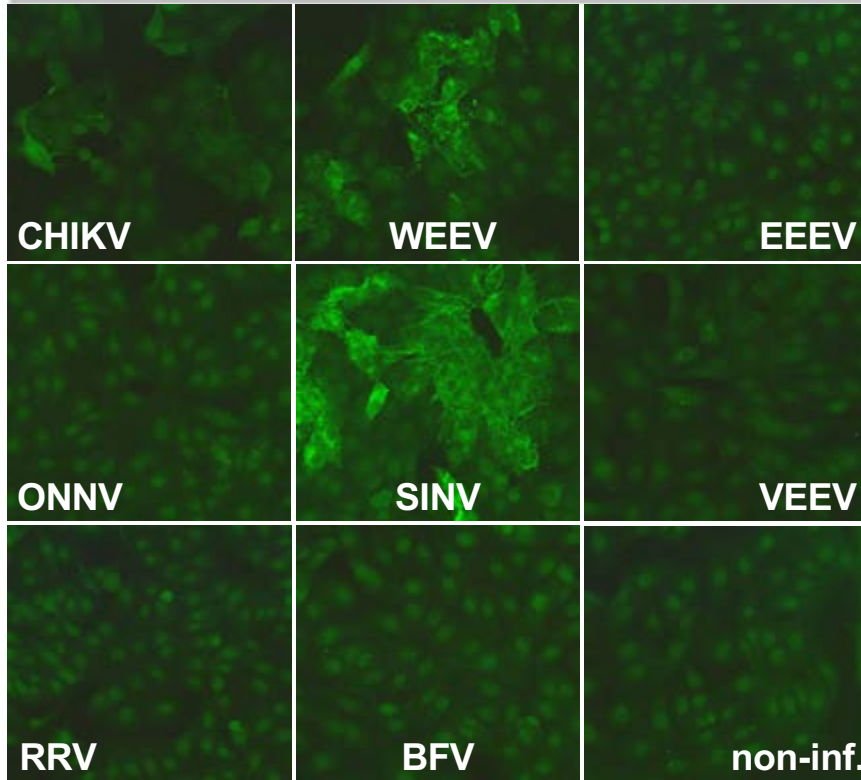
## Sindbis virus and CHIKV

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Study with samples from Finnish patients  
precharacterized by in-house IFA for  
**Sindbis virus** (SINV) antibodies.

# SINV Case Example – IgG 1:10



**POSITIVE**  
**SINV**  
**+ CHIKV**  
**+ WEEV**  
**+ VEEV**  
**IgG**



# SINV Case Example – IgG 1:100



|       |      |          |
|-------|------|----------|
| CHIKV | WEEV | EEEV     |
| ONNV  | SINV | VEEV     |
| RRV   | BFV  | non-inf. |

**POSITIVE**  
**SINV**  
**IgG**

# SINV Case Example – IgM 1:10



|       |      |          |
|-------|------|----------|
| CHIKV | WEEV | EEEV     |
| ONNV  | SINV | VEEV     |
| RRV   | BFV  | non-inf. |

**POSITIVE**  
**SINV**  
**IgM**

## SINV samples IgG (n=15)



| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |            |
|---------|---------------------------|------------|---|------------|
| CHIKV   | 6/15                      | <b>40%</b> | 0/15  | 0%         |
| ONNV    | 2/15                      | <b>13%</b> | 0/15  | 0%         |
| RRV     | 0/15                      | 0%         | 0/15  | 0%         |
| BFV     | 0/15                      | 0%         | 0/15  | 0%         |
| WEEV    | 14/15                     | <b>93%</b> | 4/15  | <b>27%</b> |
| EEEV    | 0/15                      | 0%         | 0/15  | 0%         |
| VEEV    | 4/15                      | <b>27%</b> | 0/15  | 0%         |

## SINV samples **IgM** (n=7)



| Antigen | Positive in 1:10 dilution |            | Positive after endpoint titration (less than 10x diff.) |    |
|---------|---------------------------|------------|---|----|
| CHIKV   | 0/7                       | 0%         | 0/7   | 0% |
| ONNV    | 0/7                       | 0%         | 0/7   | 0% |
| RRV     | 0/7                       | 0%         | 0/7   | 0% |
| BFV     | 0/7                       | 0%         | 0/7   | 0% |
| WEEV    | 4/7                       | <b>57%</b> | 0/7   | 0% |
| EEEV    | 0/7                       | 0%         | 0/7   | 0% |
| VEEV    | 0/7                       | 0%         | 0/7   | 0% |