A nonhuman primate model for aerosol infection with western equine encephalitis viruses

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Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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Western Equine Encephalitis Viruses (WEEV)

- Endemic in Western North America
- Naturally transmitted by mosquitoes; outbreaks are uncommon
- By mosquito bite, incapacitating illness in humans that is rarely fatal
- By aerosol, laboratory accidents suggest 40% mortality rate
- Rarely studied in nonhuman primate (NHP)
  - old reports from 1930s with rhesus
NHP Model for Aerosol Exposure to WEEV

- **Species:** *Macaca mulatta*, the rhesus macaque  
  *Macaca fascicularis*, the cynomolgus macaque

- **Virus:**  
  - WEEV (CBA-87)

- **Lethal model**  
  - Monitor physiological response by radiotelemetry  
    - Temperature, heart rate, blood pressure  
    - LD$_{50}$  
  - Disease course after aerosol exposure  
    - Viremia, CBC, clinical chemistries
Fever Response After Aerosol Exposure to WEEV in a Rhesus Macaque

Graph showing fever response with temperature on the y-axis and hour postexposure on the x-axis. Key points include:

- Tmax: 5.1°C (41.1°C)
- Duration: 72.5 hrs
- Fever-hr: 197.5

Inhaled dose: 5.4 log_{10} pfu
Fever Response to Aerosol Exposure to WEEV in a Cynomolgus Macaque

Inhaled dose: $4.5 \log_{10} \text{pfu}$

$T_{\text{max}}$: $4.03^\circ \text{C}$

$\text{Duration}$: 169 hrs

Fever-hr: 326.3
Heart Rate Increase After Aerosol Exposure to WEEV in a Rhesus Macaque

Max BPM: 265
Heart Rate Increase After Aerosol Exposure to WEEV in a Cynomolgus Macaque

Max BPM: 229
Analysis of ECG Data From Macaques

**Cyno**

**Rhesus**
Clinical Signs After Aerosol Exposure to WEEV in a Rhesus Macaque

Day Postexposure

Temperature

Heart Rate

Neurological Signs

Temperature
Heart Rate
Neurological Signs
5 = Normal
4 = Depression
3 = Occasional seizures
2 = Frequent seizures
1 = Comatose
Clinical Signs After Aerosol Exposure to WEEV in a Cynomolgus Macaque
CNS, gray matter. Comparative slide demonstrating normal on the left and WEE-infected tissue on the right. Note the increased cellularity on the infected animal, the marked perivascular cellular infiltrate, and vacuolization of the neuropil. Cynomolgus macaque, H&E, 10X.

Photo courtesy of Tom Larsen
CNS, white matter. Comparative slide demonstrating normal on the left and WEE-infected tissue on the right. Note the increased cellularity, disorganization, and vacuolization (demyelination) in the infected animal. Cynomolgus macaque, H&E, 10X.

Photo courtesy of Tom Larsen
WEEV Pathology

Rhesus brain, infiltrate, perivascular cuffs, H&E

Photo courtesy of Catherine Wilhelmsen
WEEV Pathology

Viral antigen in Purkinje cells, cerebellum, rhesus macaque

Photo courtesy of Catherine Wilhelmsen
WEEV Pathology

Viral antigen in neurons of cerebellar peduncle, cerebellum, rhesus macaque

Photo courtesy of Catherine Wilhelmsen
WEEV Pathology

Viral antigen in pontine neurons, pons, rhesus macaque

Photo courtesy of Catherine Wilhelmsen
CNS, gray matter. Note the marked immunopositive tissue (brown staining) in this focal area of inflammation, increased cellularity of the neuropil, and apoptotic bodies. Immunoperoxidase stain, 10X & 4X.

Photo courtesy of Tom Larsen
WEEV Pathology

CNS, gray matter. Note the WEE-immunopositive neurons (arrow) and the apoptotic bodies (arrowhead) in this focal area of inflammation. Immunofluorescence stain, 40X.

Photo courtesy of Tom Larsen
Summary

• WEEV can be lethal by aerosol for both cynomolgus and rhesus macaques.

• Fever onset after aerosol exposure to WEEV is delayed compared to epizootic VEEV-IA/B, similar to what was seen with VEE-IIIA.

• Clinical signs of encephalitis are not seen until fever begins to wane.

• Heart rate increases were seen with both rhesus and cynomolgus macaques. ECG analysis suggests sinus tachycardia, an increase in heart rate commonly associated with fever.

• Infection of the Purkinje cells and hypothalamus suggest direct effect by WEEV on muscle control, including heart rate.

• Pathology also shows massive cellular infiltrate into the CNS, consistent with what has been reported for fatal alphavirus infections in humans.
## Acknowledgements

<table>
<thead>
<tr>
<th>Aerobiology</th>
<th>Vet Med</th>
<th>Virology</th>
<th>DSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matt Lackemeyer</td>
<td>Mallory Tate</td>
<td>Bill Pratt</td>
<td>Larry Sullivan</td>
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<tr>
<td>David Dyer</td>
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<td>Mike Parker</td>
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<td>Keith Esham</td>
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<td>Pathology</td>
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