Togaviridae

VMC 321: Systematic Veterinary Virology
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Baltimore Classification

- Group IV: (+) sense single-stranded RNA viruses
Family: Togaviridae

- Togaviridae
  - Genus:
    - Alphavirus
    - Rubivirus
  - Species
    - Eastern equine encephalitis virus (EEE virus)
    - Western equine encephalitis virus (WEE virus)
    - Venezuela equine encephalitis virus (VEE virus)
ICTV classification

- Family: *Togaviridae*
- Genus: *Alphavirus*
- Complex: New World
Arbovirus

• Arboviruses = arthropod-borne viruses
• Arboviruses are maintained in nature through biological transmission between susceptible vertebrate hosts by blood-feeding arthropods
• Vertebrate infection occurs when the infected arthropod takes a blood meal
# Important species in the genus Alphavirus

**Prototype virus: Sindbis virus (SINV),**

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Disease</th>
<th>Vector</th>
<th>Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern equine encephalitis virus</td>
<td>Mammals, Birds, Reptiles, amphibians</td>
<td>Encephalitis</td>
<td>Aedes, Culex</td>
<td>Birds</td>
</tr>
<tr>
<td>Western equine encephalitis virus</td>
<td>Humans, Horses, Birds</td>
<td>Encephalitis</td>
<td>Culex tarsalis, Aedes</td>
<td>Birds</td>
</tr>
<tr>
<td>Venezuelan equine encephalitis virus</td>
<td>Humans, Horses, Birds</td>
<td>Encephalitis</td>
<td>Aedes, Culex</td>
<td>Horses</td>
</tr>
<tr>
<td>Chikungunya virus</td>
<td>Humans</td>
<td>Fever &amp; arthritis</td>
<td>Aedes aegypti</td>
<td>Monkeys</td>
</tr>
<tr>
<td>Semliki Forest virus</td>
<td>Humans, mosquitoes, and animals, including wild birds, rodents, domestic animals and non-human primates</td>
<td>Fever &amp; arthralgia</td>
<td>Aedes</td>
<td>Birds, rodents</td>
</tr>
<tr>
<td>Species</td>
<td>Host</td>
<td>Disease</td>
<td>Vector</td>
<td>Reservoir</td>
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<td>---------------------------------</td>
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</tr>
<tr>
<td><em>Sindbis virus</em></td>
<td>Humans, mosquito and birds</td>
<td>Fever, Arthralgia, and rash,</td>
<td>Culex</td>
<td>Birds, mammals</td>
</tr>
<tr>
<td><em>Middelburg virus</em></td>
<td>Horses, Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Zikavirus</em></td>
<td>Human</td>
<td>Fever and arthritis</td>
<td><em>Aedes aegypti</em></td>
<td></td>
</tr>
<tr>
<td><em>Japanese B encephalitis virus</em></td>
<td>Human</td>
<td>Encephalitis</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>Pigs, Birds</td>
</tr>
<tr>
<td><em>Dengue virus</em></td>
<td></td>
<td><em>Hemorrhagic fever</em></td>
<td><em>Aedes aegypti</em></td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Morphology

- Virion appear round 65-70 nm in diameter
- Capsid: 40 nm in diameter
- Capsid contains 42 capsomeres arranged in T4 symmetry
- Enveloped – consist of 240 copies of two virally encoded glycoproteins, E2 and E1
- Four nonstructural proteins nsP1, nsP2, nsP3, and nsP4 (viral RNA-dependent -RNA polymerase)
Alphaviruses: Protein Function

- E1 and E2 glycoprotein heterodimers form trimers that appear as knobs on the surface of the virion
  - E1 - transmembrane glycoprotein with 2 to 3 N-linked glycosylation sites
  - E2 - glycoprotein with 1 to 2 N-linked glycosylation sites, contains short intracytoplasmic tail and hydrophobic stretch of amino acids that serves as the fusion peptide for viral entry

- Capsid protein has a conserved N-terminal region which binds RNA and a C-terminal region which interacts with the cytoplasmic tail of E2 as well as capsid proteins

- E3 and 6K proteins are signal sequences for E2 and E1, respectively, and are largely cleaved off from the mature virion
Genome organisation

Figure: Genome organization of representative members of the Togaviridae
Viral Genome

- Genomic RNA (+ sense)
- Non-segmented - poly (A) tail
- Size ranges from
  - 9.7 to 11.8 kb for the Alphavirus
  - 9.8-10.0 kb for Rubivirus.
- RNA contains a 5′-terminal m7G cap and a 3′-terminal poly-A tail
- genome encodes non-structural proteins in a single ORF immediately after a 5′ non-coding region
  - Non structural proteins include nsP1, nsP2 and nsP3
  - Structural proteins include the capsid (C/CP), E3, E2, 6K, and E1 proteins
Biological properties

• Alphaviruses are:
  – arthropod-borne viruses
  – replicate in invertebrate vectors and their vertebrate hosts.
  – cytopathic to vertebrate cells

• RUBV
  – only infect mammals
  – develop persistent infections.
Replication Cycle

- Proposed Model: E1 glycoprotein interacts with proteins on the cell surface. E2 binds to cellular proteins and receptor-mediated endocytosis takes place.
- In acidified endosomal compartment, glycoproteins fuse with membrane and the nucleocapsid is released.
- Virion RNA serves as mRNA, translation of non-structural proteins begins
- Structural proteins are transcribed as polyprotein
- E2 and E1 travel from ER to the Golgi
- At cellular membrane regions containing E1 and E2 heterodimers interact with nucleocapsids and viral particles bud from the cell surface
Disinfectants

- Chemical disinfectant:
  - effective disinfectants - 1% sodium hypochlorite and 70% ethanol

- Physical disinfectant:
  - Inactivated by UV light and at pH below 6.0
The Viruses

- EEE, WEE, and VEE viruses
  - Family Togaviridae
  - Genus Alphavirus
- Mosquito-borne
- Disease
  - Encephalitis in humans and horses
  - Other mammals and birds are occasionally affected
### Equine Encephalitides: Classification and Distribution

<table>
<thead>
<tr>
<th></th>
<th>Family</th>
<th>Genus</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Togaviridae</td>
<td>Alphavirus</td>
<td>EEE</td>
</tr>
<tr>
<td>2</td>
<td>Togaviridae</td>
<td>Alphavirus</td>
<td>WEE</td>
</tr>
<tr>
<td>3</td>
<td>Togaviridae</td>
<td>Alphavirus</td>
<td>VEE</td>
</tr>
</tbody>
</table>
Togaviridae: Replication

- RNA is capped and polyadenylated
- serves as mRNA -> nonstructural proteins
- sense RNA -> antisense RNA
- Antisense RNA template for progeny RNA
- antisense RNA--> mRNA -> structural proteins
- mature at intracytoplasmic membranes
Transmission
Transmission

[Diagram showing the transmission cycle with labels for Enzootic Host, Enzootic Vector, Epizootic Host, and Epizootic Cycle.]
Transmission

Vertebrate Hosts → Virus Particles → Dead End Hosts → Mosquito Vector → Vertebrate Hosts
## Vectors of the Equine Encephalitides

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mosquito Vector</th>
</tr>
</thead>
</table>
| EEE     | *Culiseta melanura*  
|         | *Aedes* spp.  
|         | *Culex (Cx.) nigrapalpus, Coquilletidia* spp. |
| WEE     | *Culex tarsalis*  
|         | *Aedes melanimon*  
|         | *Aedes dorsalis*  
|         | *Aedes campestris* |
| VEE     | *Culex (Melanoconion)* spp. |
Summary of Equine Encephalitis

Distribution, Magnitude, and Outcomes
Eastern Equine Encephalitis
EEE Transmission

- Aedes spp. Coquillettidia perturbans
- Dead end hosts: Horses, humans, other mammals
- Pecking transmission
- Culiseta melanura

Summer Swampy areas

Over wintering?

Spring Reintroduction

Bird migration
Wild-bird reservoir hosts and incidental hosts

- Wild-bird reservoir hosts and exposure of wild-bird reservoir hosts causes EEEV
• Incubation period: 5 to 14 days
• Clinical signs in horses
  • Fever, anorexia, depression
  • CNS signs
    • Hypersensitivity, aimless wandering, head pressing, circling, ataxia, paresis, paralysis
• Death may occur within days
• Asymptomatic or mild infections also occur
• Equine vaccine available
Clinical signs of EEE in a horse

Sleepy facial expression, the lax muzzle, and the abrasions between the eye and the base of the ear.
EEE in Birds

- Asymptomatic in most bird species
- Clinical signs
  - Depression, tremors, leg paralysis, somnolence
  - Emus, ostriches
    - Hemorrhagic enteritis, emesis
  - Death 24 hours after onset
- Vaccination
  - Some birds are vaccinated for EEE
Diagnosis

• **Ante mortem**: serology
  • Virus neutralization
  • Hemagglutination inhibition test
  • Enzyme-linked immunosorbent assay (ELISA)
  • Complement fixation
  • Virus isolation
  • Immunofluorescence
  • Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay

• **Post mortem**
  • Virus identification in tissues (brain)
  • Immunohistochemistry, ELISA, RT-PCR
Diagnosis

- **Clinical diagnosis**
  - Fever

- **Laboratory confirmation**
  1. Probable case: Detection of virus specific IgM antibodies in serum sample AND confirmation by neutralisation;
  2. Isolation of chikungunya virus from a clinical specimen;
  3. Detection of chikungunya viral nucleic acid from a clinical specimen;
  4. Detection of chikungunya specific IgM antibodies in a single serum sample
Western Equine Encephalitis
WEE Transmission

- **Secondary Amplifiers**: Blacktail Jackrabbit
- **Primary Vertebrate Hosts**: House Sparrow, House Finch
- **Primary Vector**: *Culex tarsalis*
- **Dead-end hosts**: Horses, humans
WEE in Animals

• Asymptomatic
  • Blacktail jackrabbit, kangaroo rat, Western gray squirrel, prairie dog, birds

• Horses with clinical signs
  • Fever, depression, altered mentation, head pressing, ataxia, dysphagia
  • Progress to paralysis, convulsions, death
  • Mortality rate <30%
WEE in Animals

• Diagnosis
  • Serology
    • Can differentiate EEE and WEE using the virus neutralization or ELISA tests
  • Post mortem
    • Immunohistochemistry, ELISA, RT-PCR

• Treatment is supportive care
• Vaccine available
Venezuelan Equine Encephalitis
VEE Viral Strains

• Epizootic/Epidemic
  • I-A, I-B, and I-C
  • Disease in humans and horses
  • Transmission by many mosquito species
  • Natural reservoir unknown
  • Horses and donkeys act as amplifiers

• Enzootic/Endemic
  • Disease in humans
  • Transmission mainly by Culex (Melanoconion) species
  • Natural reservoir is rodents living in swamps and forests
VEE Epizootic Transmission

Primary Vector
- multiple mosquito species

Other species
- naturally infected but not amplifiers

Dead-end hosts
- Humans

Vertebrate Host
- Horses
VEE Enzootic Transmission

Primary Vector
*Culex*
*(Melanoconion)*
species

Vertebrate Host
Rodents

Dead end hosts
Humans
VEE in Horses

- Incubation period: 1 to 5 days
- Horses most susceptible
  - Fever, anorexia, depression, flaccid lips, droopy eyelids and ears, incoordination, and blindness
  - Death 5 to 14 days after clinical onset
- Case-fatality rate: 50 to 90%
- In utero transmission results in abortion, stillbirth
VEE in Animals

- Most domestic animals do not show clinical signs or amplify the virus
- Experimentally
  - Infected rabbits and dogs die after inoculation
  - Laboratory animals susceptible
    - Act as sentinels
    - Guinea pigs, mice, hamsters
- Enzootic strains do not cause disease in animals
VEE in Animals

- Diagnosis
  - Virus isolation
  - Serology
    - Paired sera with rising titer
    - ELISA IgG or IgM

- Vaccine available for horses
Prevention and Control
Management of Mosquito-Borne Diseases

• Source reduction
• Surveillance
• Biological control
• Chemical control
  • Larvicide
  • Adulticide
• Educating the public
  • How to protect themselves
Biological Control

• Predators, natural and introduced, to eat larvae and pupae
  • Mosquito fish
    • *Gambusia affinis*,
    *G. holbrooki*
  • *Fundulus* spp.,
    *Rivulus* spp., killifish

• Other agents have been used but are not readily available

• Copepods
Chemical Control

• Essential when:
  • Source reduction not effective
  • Surveillance shows increased population of virus-carrying mosquitoes
• Requires properly trained personnel
• Larvicides, adulticides
• Toxic to many birds, fish, wildlife, aquatic invertebrates, honeybees
• Human exposure is uncommon
Chemical Control

- Federal Food Drug and Cosmetic Act limits the quantity of adulticide used
  - Due to wind drift onto agricultural crops
- Method used varies
  - Type of target mosquito
  - Type of targeted habitat
  - Aerial spraying covers wide area
- Funding provided by state or local government
  - Rarely federal
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Thanks