Detection of La Crosse Virus in Cerebrospinal Fluid and Autopsied Tissues by Reverse Transcription- Polymerase Chain Reaction

Brett Slater, Cinnia Huang, Karen Bloch, Tim Jones, Gina Woodlief and Todd McPherson
Arboviruses

(Arthropod-borne Viruses)

Viruses Transmitted to humans or other mammals by mosquitoes, ticks, or sandflies

There are more than 100 arboviruses known to be human pathogens
Distribution of Arboviruses

Worldwide

Their occurrence in a given area depends on the presence of the particular mosquito or tick species that can serve as an effective arthropod vector, and

The presence of an animal reservoir, often birds or small mammals.
Clinical Signs and Symptoms of Arbovirus Infections

Most infected people show no signs of illness

When symptoms occur, they usually consist of:
  - Sudden fever, chills, headache, muscle aches, and tiredness

Symptoms of hemorrhagic fever involve:
  - Signs of internal bleeding, which can lead to shock and sometimes death

Some infections can lead to severe and even fatal encephalitis
  - Symptoms include drowsiness, stiff neck, confusion, convulsions, tremors, and coma
La Crosse Encephalitis

CLINICAL FEATURES
  Majority of Infections are subclinical or result in mild illness

ETIOLOGIC AGENT
  La Crosse virus - California serogroup virus in the family Bunyaviridae

INCIDENCE
  Approximately 70 cases reported per year

SEQUELAE
  Case-fatality ratio <1%
  Hospitalization for CNS infection
  Neurological sequelae that resolve within several years

TRANSMISSION
  Vector: tree hole mosquito (Aedes triseriatu)

RISK GROUPS
  Children <16 years
  Residence in woodland
  Outdoor activities
Aedes triseriatus, primary vector for La Crosse Virus

Close up: Characteristic white spots
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Serogroup</th>
<th>Complex</th>
<th>Virus</th>
<th>Subtype</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlebovirus</td>
<td>(8 groups: ≥ 23 viruses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nairovirus</td>
<td>(7 groups: ≥ 33 viruses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bunyavirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bunyaviridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hantavirus</td>
<td>(1 Group: ≥ 6 Viruses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tospovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- California encephalitis
  - California encephalitis
    - La Crosse → [La Crosse Snowshoe Hare, Snowshoe Hare]
    - San Angelo
    - Tahyna → [Tahyna Lumbo, Lumbo]

- California
  - Melao
    - Jamestown Canyon
      - Jamestown Canyon South River
    - Keystone
    - Serra do Navio
    - Inkoo
    - Jerry Slough

- Guaroa
  - Guaroa

- Trivittatus
  - Trivittatus
Human Cases of La Crosse Encephalitis
1964-2001

Darker color indicates greater frequency in recent years
Diagnosis of La Crosse Viral Infections

- Virus isolation
  - Virus rarely recovered from clinical samples
  - only 3 isolates from post-mortem brain tissues
  - only 1 isolate from CSF
  - only 1 isolate from brain biopsy sample

- Traditionally based on serology (detection of specific antibodies)
  - Complement Fixation
  - Hemagglutination-inhibition
  - Neutralization

- New technology based on genome amplification: mainly PCR
Objectives

- To evaluate a rapid and sensitive diagnostic tool for detecting La Crosse virus in CNS infections
- Use PCR results to supplement serological results
Flow Chart for Detection of La Crosse Virus

- CSF or Brain Tissues
- RNA
- cDNA, made with random primers
- PCR, 5 ul cDNA per rxn.
  - CAL serogroup primers
  - La Crosse specific primers
Genome of La Crosse Virus

<table>
<thead>
<tr>
<th>Segment</th>
<th>Length (nt)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>L segment</td>
<td>6875</td>
<td>(Polymerase)</td>
</tr>
<tr>
<td>M segment</td>
<td>4458</td>
<td>G2 NSm G1</td>
</tr>
<tr>
<td>S segment</td>
<td>961</td>
<td>(Nucleocapsid)</td>
</tr>
</tbody>
</table>

PCR, CAL Serogroup and LAC Specific
Detection of California Serogroup Viruses
(Using Group-Specific Primers)
Clinical features of patients with La Crosse encephalitis

<table>
<thead>
<tr>
<th>Pt#</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Specimen</th>
<th>Onset</th>
<th>Coll. Date</th>
<th>Group-specific</th>
<th>LAC-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>F</td>
<td>CSF</td>
<td>9/3/96</td>
<td>9/6/96</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>M</td>
<td>CSF</td>
<td>8/31/96</td>
<td>9/10/96</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>M</td>
<td>CSF</td>
<td>9/3/97</td>
<td>9/11/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>M</td>
<td>CSF</td>
<td>8/4/97</td>
<td>8/4/97</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>M</td>
<td>CSF</td>
<td>8/5/97</td>
<td>8/5/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>F</td>
<td>CSF</td>
<td>8/22/97</td>
<td>8/25/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>F</td>
<td>CSF</td>
<td>9/11/97</td>
<td>9/14/97</td>
<td>Neg</td>
<td>not done*</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>F</td>
<td>CSF</td>
<td>7/9/00</td>
<td>7/17/00</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>F</td>
<td>frontal lobe</td>
<td>6/22/00</td>
<td>6/30/00</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>spinal cord</td>
<td></td>
<td></td>
<td>Neg</td>
<td>Pos</td>
</tr>
</tbody>
</table>

*: not done (insufficient sample)
Case 1
- lanes 2, 3, 4

Case 2
- lane 5

Figure 1A

Figure 1B

Primers: CAL-group

Primers: LAC-specific
Case 9: fatal
2, 6: Frontal lobe
3, 7: Spinal cord

Figure 2

CAL-Group  LAC-specific
Clinical features of patients with La Crosse encephalitis

<table>
<thead>
<tr>
<th>Pt#</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Specimen</th>
<th>Onset</th>
<th>Coll. Date</th>
<th>Group-specific</th>
<th>LAC-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>F</td>
<td>CSF</td>
<td>9/3/96</td>
<td>9/6/96</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>M</td>
<td>CSF</td>
<td>8/31/96</td>
<td>9/10/96</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>M</td>
<td>CSF</td>
<td>9/3/97</td>
<td>9/11/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>M</td>
<td>CSF</td>
<td>8/4/97</td>
<td>8/4/97</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>M</td>
<td>CSF</td>
<td>8/5/97</td>
<td>8/5/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>F</td>
<td>CSF</td>
<td>8/22/97</td>
<td>8/25/97</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>F</td>
<td>CSF</td>
<td>9/11/97</td>
<td>9/14/97</td>
<td>Neg</td>
<td>not done*</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>F</td>
<td>CSF</td>
<td>7/9/00</td>
<td>7/17/00</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>F</td>
<td>Frontal lobe</td>
<td>6/22/00</td>
<td>6/30/00</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spinal cord</td>
<td></td>
<td></td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: not done (insufficient sample)
Sequences of ORF of La Crosse virus M segment
Summary

• **La Crosse Specific PCR primers** can be used to detect La Crosse virus from human CSF and brain samples.

• **La Crosse specific primers** are more sensitive than **Cal serogroup primers** in detecting La Crosse virus.

• PCR can be an effective and timely diagnostic tool for detection of La Crosse virus in human specimens.

• More extensive sequencing studies of La Crosse virus from different geographic regions are required to provide the basis for the development of improved primers.
Collaborators

Illinois Dept. of Public Health
Tennessee Dept. Of Health & Human Services
North Carolina Dept. Of Health & Human Services
Florida Dept. of Health
Jim McJunkin, M.D., Charleston, WV
M. Marcon, Ohio State Univ. College of Medicine
CDC - Ft. Collins

Acknowledgment

CDC - Emerging Infectious Program grant, Rene Hull, Rocco Ferrera, Michelle Dupuis, Blair Rosen, Leo Grady, Wayne Campbell and Charles Trimarchi