

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

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# 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 triseriatus*)

## RISK GROUPS

Children <16 years

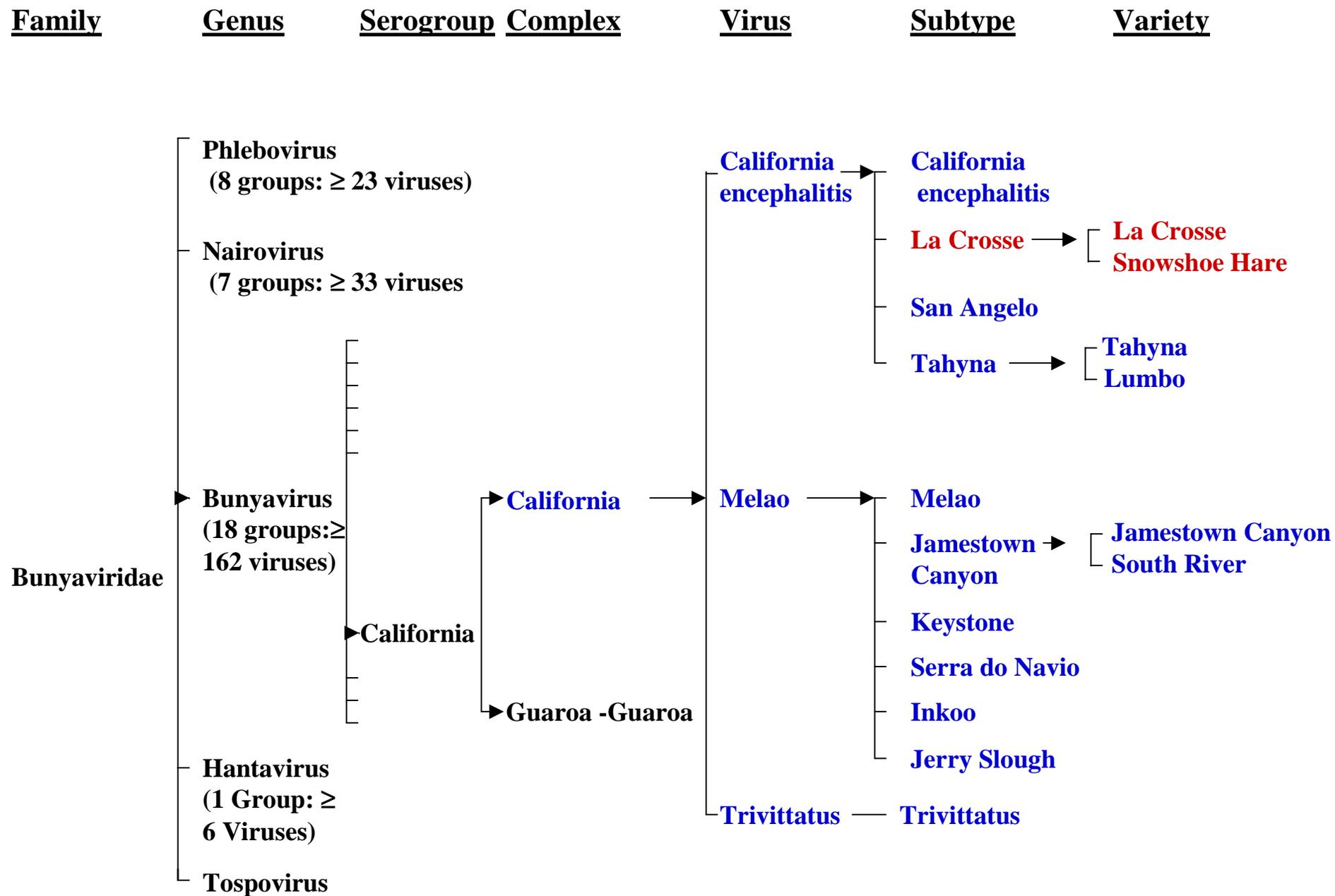
Residence in woodland

Outdoor activities

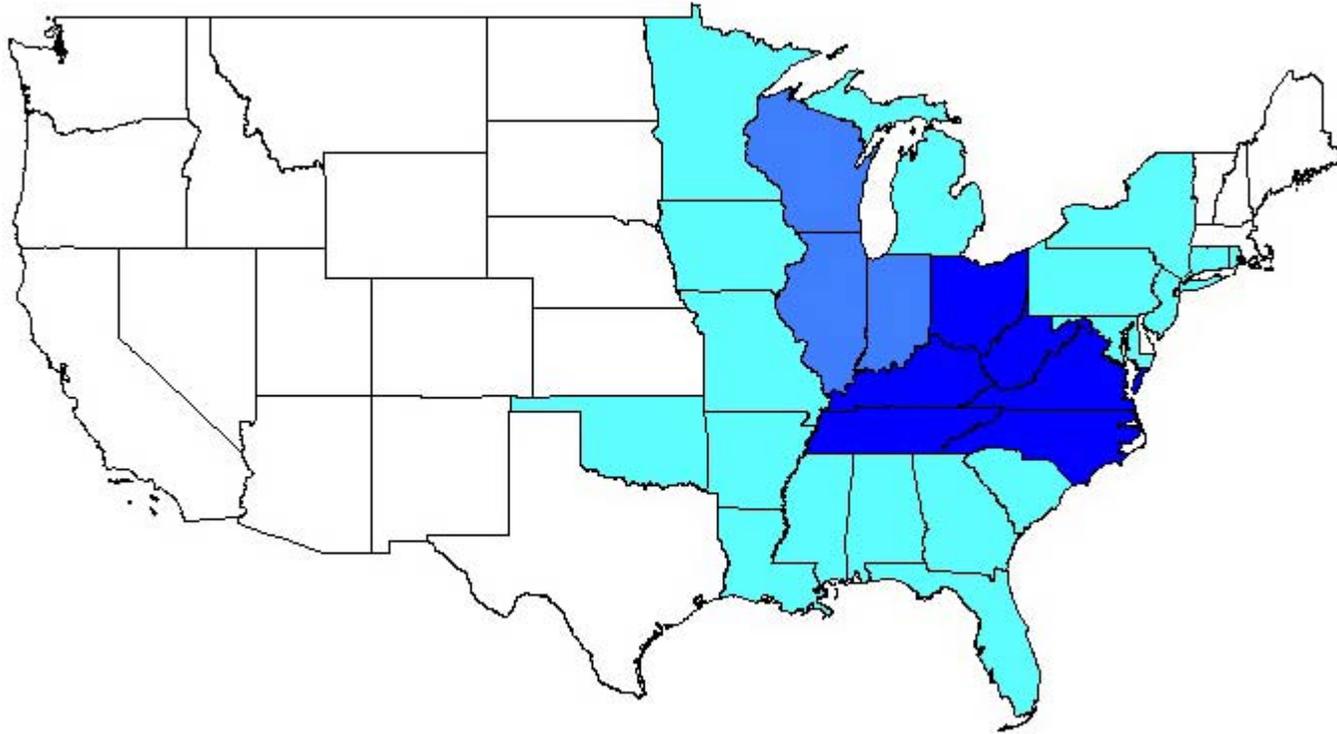
# **Aedes triseriatus, primary vector for La Crosse Virus**



**Close up: Characteristic white spots**



# Human Cases of La Crosse Encephalitis 1964- 2001



Darker color indicates greater  
frequency in recent years

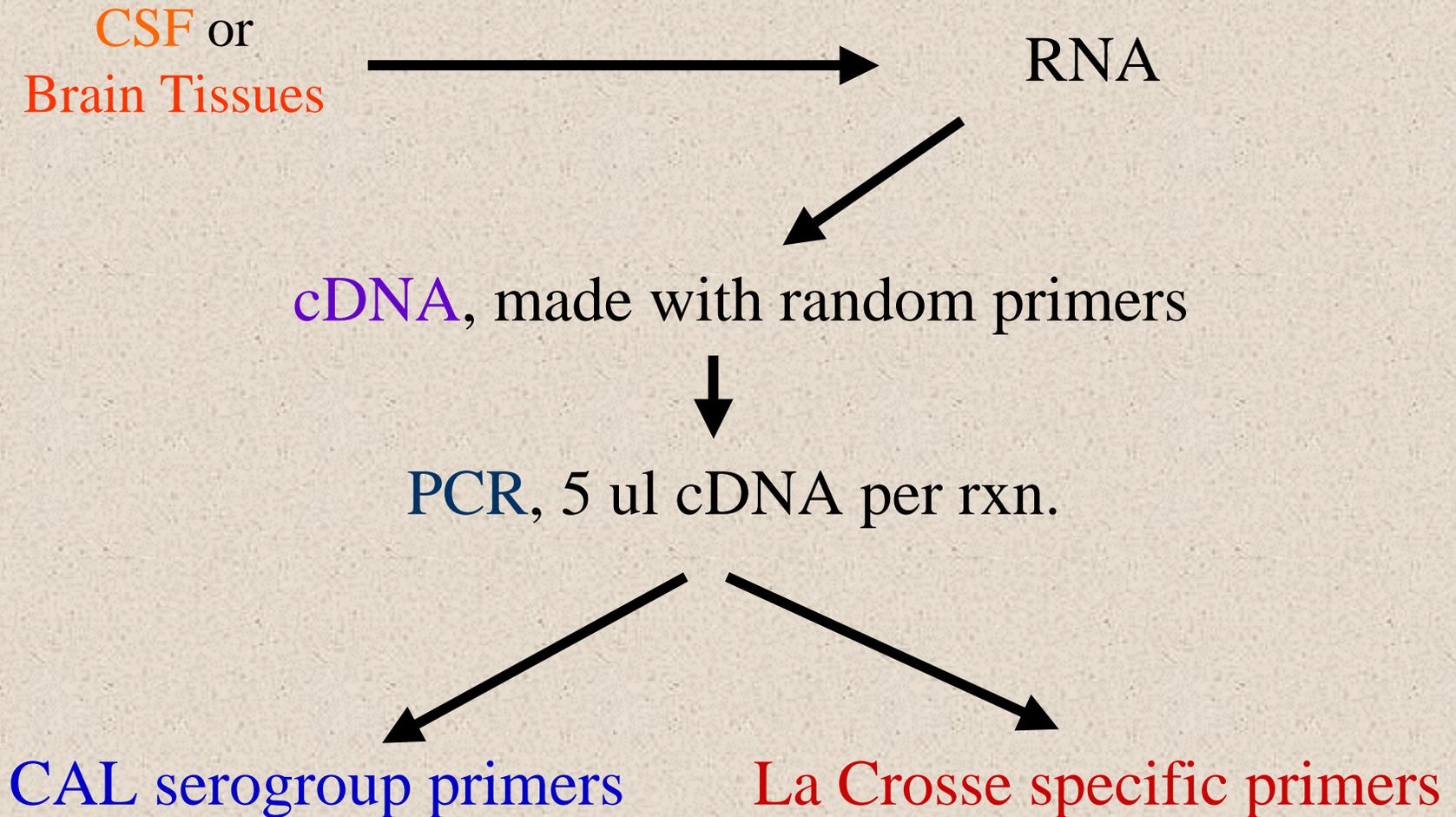
# Diagnosis of La Crosse Viral Infections

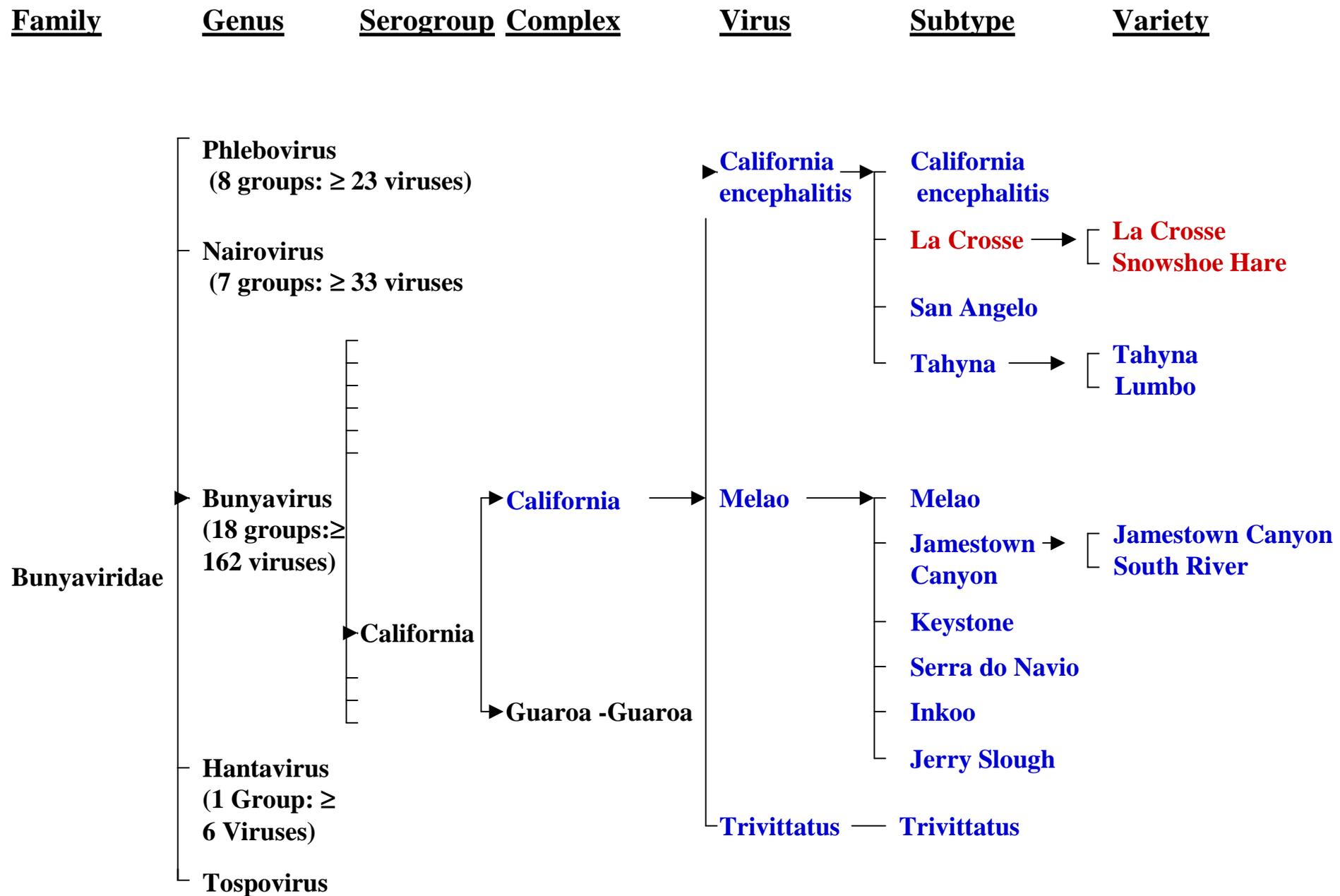
- Virus isolation
  - Virus rarely recovered from clinical samples
  - only 3 isolates from post-mortem brain tissues
  - only 1 isolate from CSF
  - only 1 isolate from brain biopsy sample
- Traditionally based on serology (detection of specific antibodies)
  - Complement Fixation
  - Hemagglutination-inhibition
  - 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





# Genome of La Crosse Virus

**6875 nt**

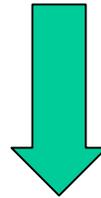
**L segment  
(Polymerase)**

**4458 nt**

**M segment**

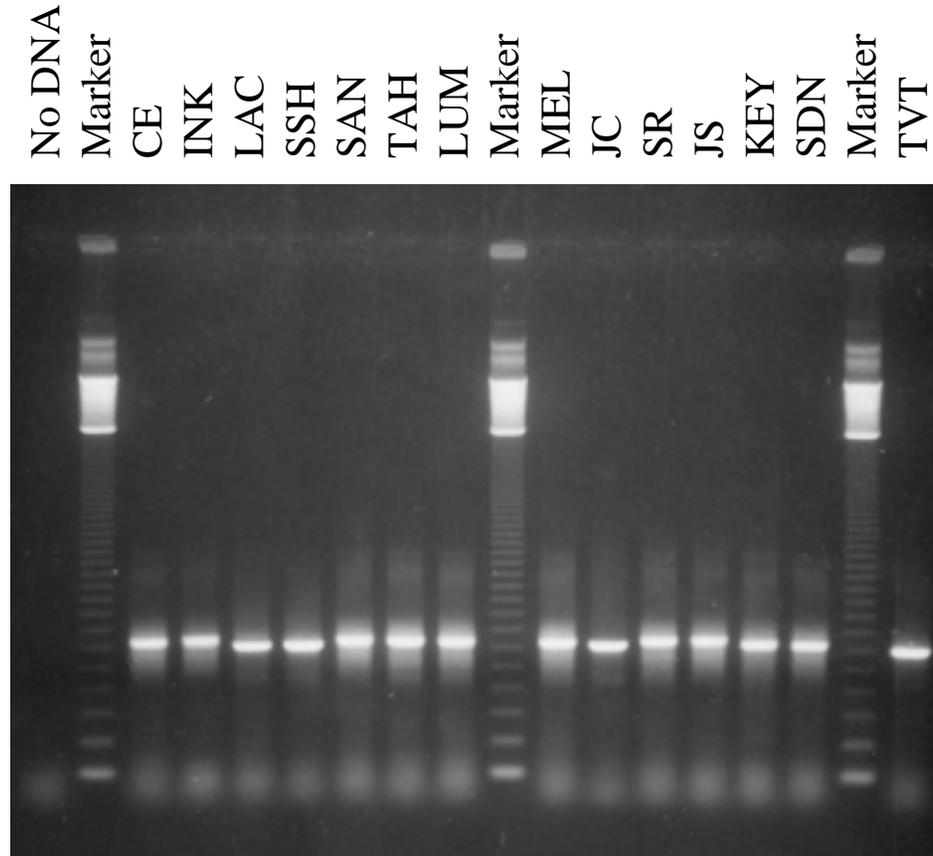
**961 nt**

**S segment  
(Nucleocapsid)**



**PCR, CAL Serogroup and LAC Specific**

# Detection of California Serogroup Viruses (Using **Group-Specific Primers**)



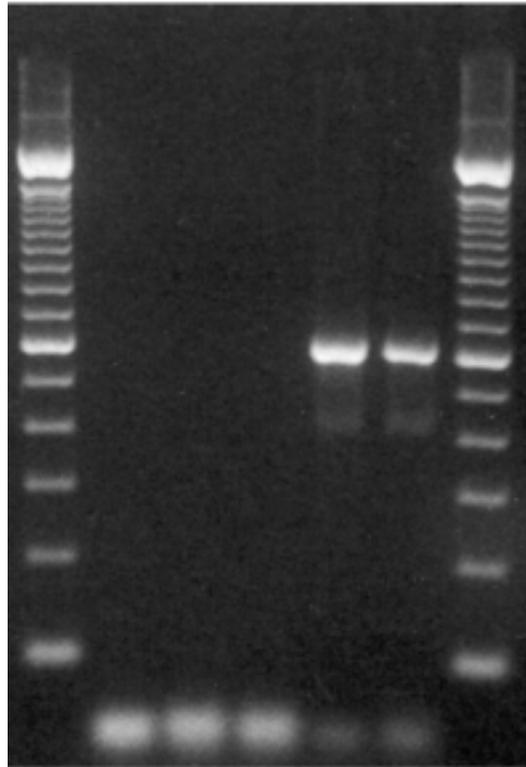
# Clinical features of patients with La Crosse encephalitis

Pt#	Age (yr)	Sex	Specimen	Onset	Coll. Date	Group-specific	LAC-specific
<b>1</b>	<b>12</b>	<b>F</b>	<b>CSF</b>	<b>9/3/96</b>	<b>9/6/96</b>	Neg	Pos
			<b>CSF</b>		<b>9/10/96</b>	Neg	Pos
			<b>CSF</b>		<b>9/16/96</b>	Pos	Pos
<b>2</b>	<b>6</b>	<b>M</b>	<b>CSF</b>	<b>8/31/96</b>	<b>9/10/96</b>	Pos	Pos
3	4	M	CSF	9/3/97	9/11/97	Neg	Pos
4	8	M	CSF	8/4/97	8/4/97	Pos	Pos
5	4	M	CSF	8/5/97	8/5/97	Neg	Pos
6	5	F	CSF	8/22/97	8/25/97	Neg	Pos
7	6	F	CSF	9/11/97	9/14/97	Neg	not done*
8	7	F	CSF	7/9/00	7/17/00	Neg	Pos
<b>9</b>	<b>9</b>	<b>F</b>	<b>frontal lobe</b>	<b>6/22/00</b>	<b>6/30/00</b>	Pos	Pos
			<b>spinal cord</b>		<b>6/30/00</b>	Neg	Pos

\*: not done (insufficient sample)

**Figure 1A**

M 1 2 3 4 5 M

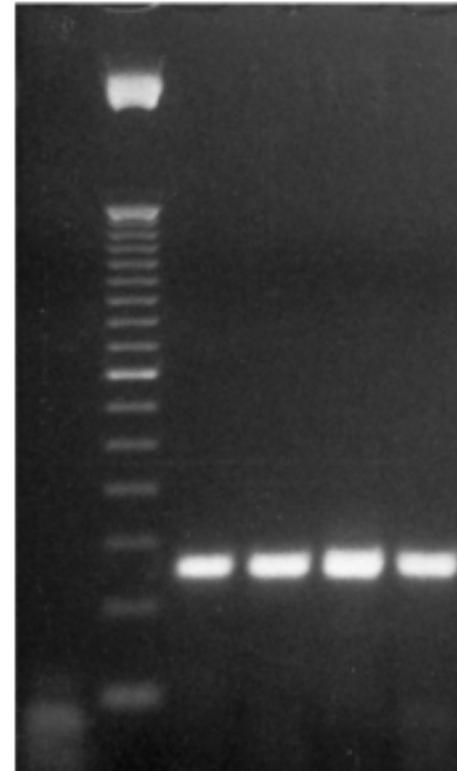


Case 1  
- lanes 2, 3, 4

Case 2  
- lane 5

**Figure 1B**

1 2 3 4 5 6



Case 1  
- lanes 3, 4, 5

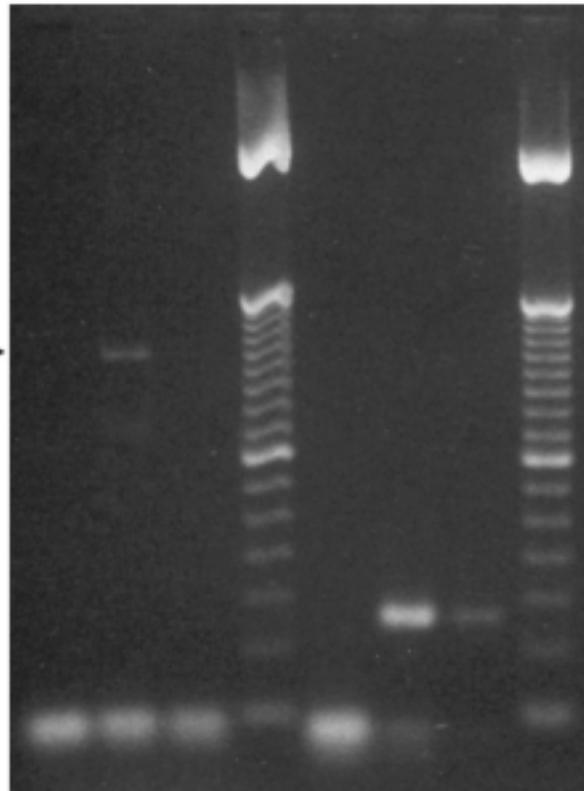
Case 2  
- lane 6

Primers: CAL-group

LAC-specific

**Figure 2**

1 2 3 4 5 6 7 8



**Case 9: fatal**  
**2, 6: Frontal lobe**  
**3, 7: Spinal cord**

**CAL-Group**

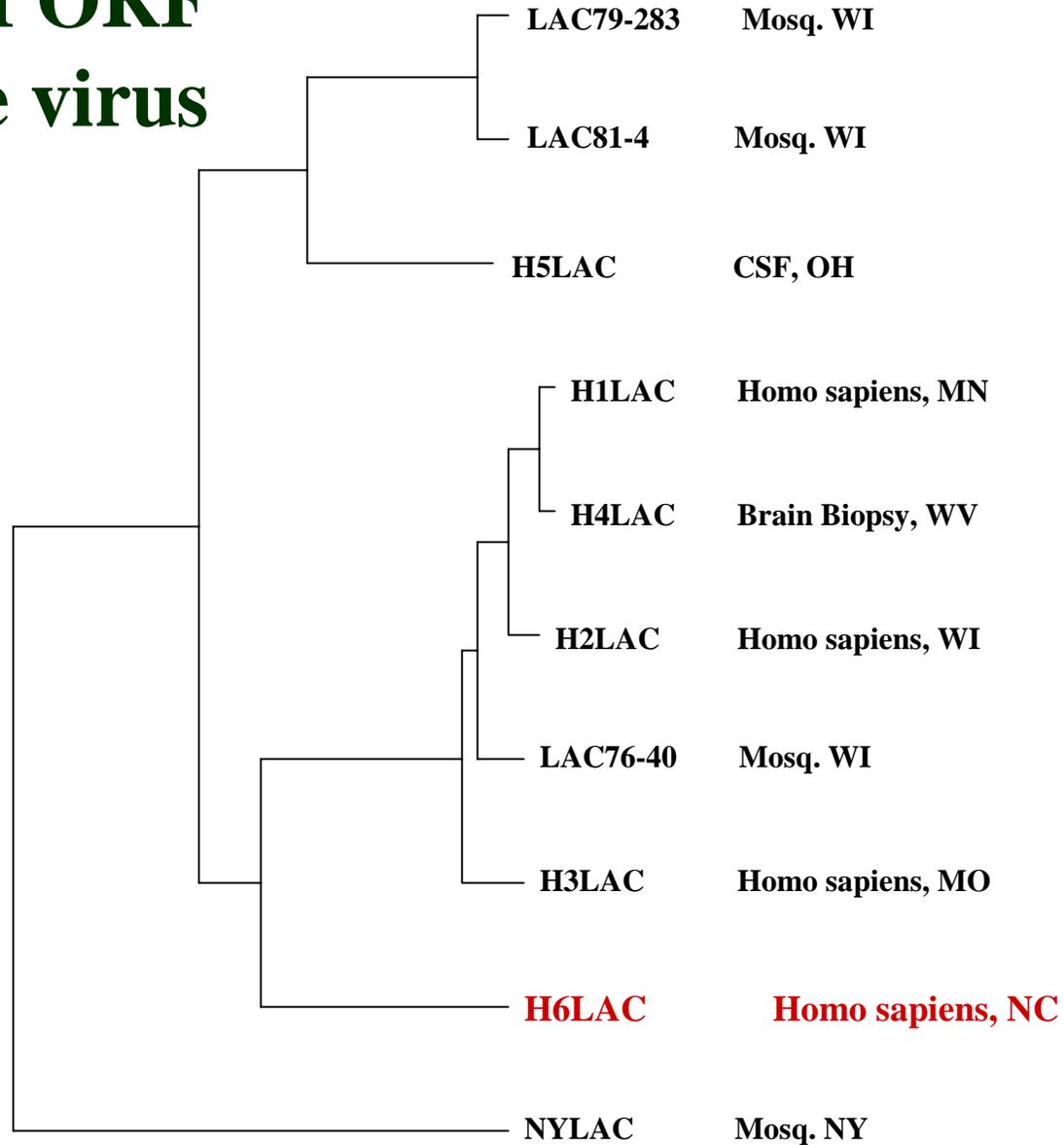
**LAC-specific**

# Clinical features of patients with La Crosse encephalitis

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<b>1</b>	<b>12</b>	<b>F</b>	<b>CSF</b>	<b>9/3/96</b>	<b>9/6/96</b>	Neg	<b>Pos</b>
			<b>CSF</b>		<b>9/10/96</b>	Neg	<b>Pos</b>
			<b>CSF</b>		<b>9/16/96</b>	Pos	<b>Pos</b>
<b>2</b>	<b>6</b>	<b>M</b>	<b>CSF</b>	<b>8/31/96</b>	<b>9/10/96</b>	Pos	<b>Pos</b>
3	4	M	CSF	9/3/97	9/11/97	Neg	<b>Pos</b>
4	8	M	CSF	8/4/97	8/4/97	Pos	<b>Pos</b>
5	4	M	CSF	8/5/97	8/5/97	Neg	<b>Pos</b>
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7	6	F	CSF	9/11/97	9/14/97	Neg	not done*
8	7	F	CSF	7/9/00	7/17/00	Neg	<b>Pos</b>
<b>9</b>	<b>9</b>	<b>F</b>	<b>frontal lobe</b>	<b>6/22/00</b>	<b>6/30/00</b>	Pos	<b>Pos</b>
			<b>spinal cord</b>		<b>6/30/00</b>	Neg	<b>Pos</b>

\*: 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

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M. Marcon, Ohio State Univ. College of Medicine

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