Laboratory Diagnosis of Viral Encephalitis

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Introduction

- Encephalitis is an acute inflammatory process affecting the brain
- Viral infection is the most common and important cause, with over 100 viruses implicated worldwide
- Symptoms
  - Fever
  - Headache
  - Behavioral changes
  - Altered level of consciousness
  - Focal neurologic deficits
  - Seizures
- Incidence of 3.5-7.4 per 100,000 persons per year

Viral Encephalitis

Encephalitis is an inflammatory process involving the parenchyma of the brain, although it is often associated with meningitis (meningoencephalitis).

- **Acute viral encephalitis**

Acute viral encephalitis usually begins with a fever and headache, similar to acute meningitis but with accompanying signs of cerebral involvement - altered level of consciousness (ranging from mild lethargy to coma), confusion, disorientation, mental changes.

- **Postinfectious encephalitis**

Postinfectious encephalitis is an encephalopathy which occurs relatively soon after an acute viral infection.

Causes of Viral Encephalitis

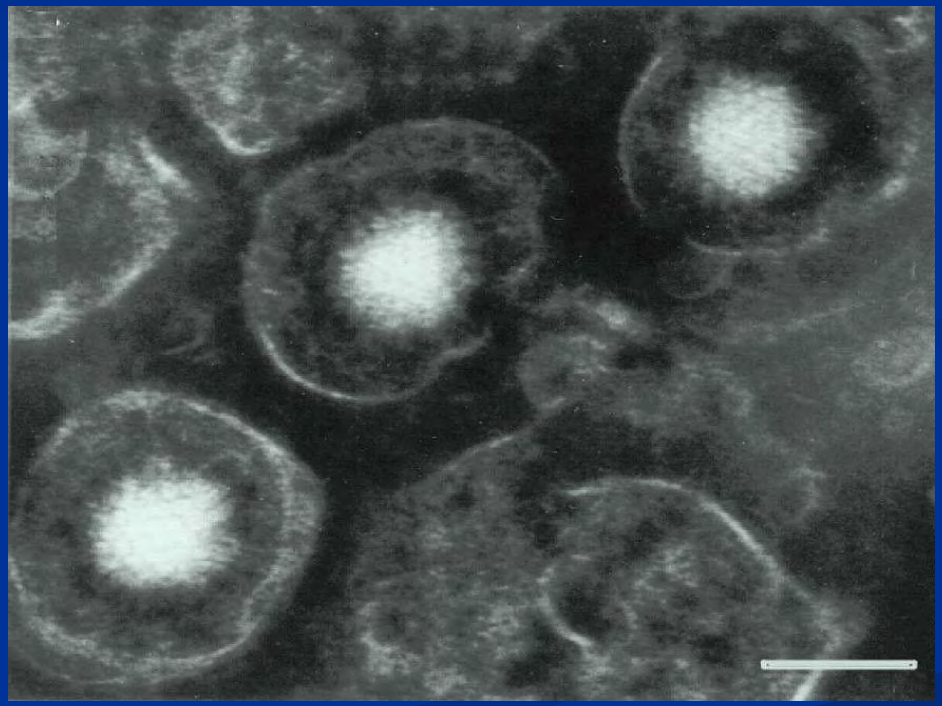
- Herpes viruses – HSV-1, HSV-2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpes virus 6
- Adenoviruses
- Influenza A
- Enteroviruses, poliovirus
- Measles, mumps, and rubella viruses
- Rabies
- Arboviruses – examples: Japanese encephalitis; St. Louis encephalitis virus; West Nile encephalitis virus; Eastern, Western and Venzuelan equine encephalitis virus; tick borne encephalitis virus
- Bunyaviruses – examples: La Crosse strain of California virus
- Reoviruses – example: Colorado tick fever virus
- Arenaviruses – example: lymphocytic choriomeningitis virus

Herpesviridae affecting CNS

- Human viruses include:
  - Herpes simplex 1
  - Herpes simplex 2
  - Varicella-zoster (covered in chickenpox talk later)
  - Cytomegalovirus
  - Epstein Barr virus
  - HHV6

Structure

- Large icosahedreal, double stranded DNA viruses
- Replicate in the nucleus of cells
- Enveloped with numerous glycoprotein spikes.
- Tendency to develop latent infections
- Most primary (initial) infections are asymptomatic (except for Varicella zoster)



Herpes Simplex Virus

- It affects CNS under several circumstances. In the rare congenital HSV infection CNS damage results from intrauterine herpes encephalitis that can occur at any gestational age.
- In the neonatal period ,HSV acquired from the mother peripartum may present as localized vesicles, encephalitis with or without skin or mucosal involvement, and as disseminated HSV infection involving the CNS and other organs.
- The mortality rate for untreated neonatal CNS disease is 50 to 80%.
- Herpes simplex encephalitis is the most common cause of sporadic encephalitis, affecting 1 to 4 per million population per year.
- Over 95% of cases are due to HSV type 1, including both primary HSV infection and reactivation of latent HSV.

Diagnosis of HSV Encephalitis

- The most reliable diagnostic strategy is: A combination of detection of intrathecal production of antibody and PCR on CSF.
- A second CSF sample for PCR is advised 14 days after treatment. If the HSV PCR is positive retreatment is recommended.

- Note: in the first few days after the onset of HSV encephalitis CSF PCR may be negative in a significant number of patients (over 25%).

- Quantitative PCR may have a role in assessing prognosis and monitoring response to therapy.

HSV-1/-2 Infection of the CNS

- Serological procedures performed on serum or CSF are not helpful early in the disease course when therapeutic decisions are needed .
- Detection of viral CSF-PCR is the diagnostic method of choice for confirmation of HSV involvement in CNS disease .
- The use of CSF-PCR instead of brain biopsy has expanded awareness of mild or atypical cases (16%-25%).
- In neonatal herpes encephalitis CSF HSV PCR has high sensitivity. Further sensitivity can be gained by testing blood as well as CSF by PCR. HSV DNA detected from neonatal dried blood spots may allow early diagnosis where symptoms are vague.
- Virus isolation or PCR from other sites such as skin vesicles, are also relevant.

Varicella- Zoster Virus

- In suspected viral CNS infection is more common than HSV or Enteroviruses .
- Varicella zoster encephalitis is a vasculopathy and often appear without a rash.
  - Large vessel VZ encephalitis
  - Small vessel VZ encephalitis

Diagnosis of VZV Encephalitis

- Both PCR on CSF and antibody testing for VZ IgG and IgM (using ELISA or similar immunoassays) on both serum and CSF. Similar tests should also be done to exclude HSV.
- Serum anti-VZV antibody is of no value since VZV antibodies persist in the serum of nearly all adults. But Testing of CSF for VZV antibodies helps to confirm the role of VZV in producing clinical syndromes of the CNS.
- Diagnosis of VZV infection of the CNS is supported by the detection of VZV antibody in the CSF, even in the absence of PCR-amplifiable VZV DNA. Therefore, Clinicians should request both PCR and antibody analysis.

- Similarly detection of virus by culture, IF, EM or PCR from skin sites or saliva can lend support to the diagnosis.

Cytomegalovirus

- Encephalitis, typically with periventricular lesions, occurs as part of the congenital CMV syndrome.
- In patients who are immunocompromised with AIDS it was until recently one of the most common causes of CNS disease (16%).
- CMV encephalitis also occurs post-transplantation but is believed to be uncommon.
- CMV DNA has been described in 50% of brains from liver transplant patients with CNS symptomatology.
- Occasional cases are described in individuals who are immunocompromised.

Diagnosis of CMV Encephalitis

- CMV PCR on CSF is the best test for diagnosis, with sensitivity around 79% or higher.
- Supporting evidence can come from serum CMV IgM reactivity blood, urine, or throat swab CMV culture and PCR.
- However, only CSF CMV PCR or intrathecal CMV antibody production are diagnostic.
- Quantitation of DNA in both CSF and brain tissue sensitively diagnoses and monitors antiviral treatment, e.g.
  - AIDS patients with HCMV-related CNS disease have high quantities of HCMV DNA in their CSF
  - Copies of HCMV DNA in CSF are higher in persons with HCMV-related polyradiculopathy than encephalitis

Epstein-Barr Virus

- Encephalitis and meningitis are recognized as uncommon complications of glandular fever.
- Acute EBV accounts for 4 to 5% of infection-related neurological disease, while reactivated EBV is reported to be an important cause of neurological complications in pediatric patients.

Diagnosis of EBV Encephalitis

- EBV is rarely cultured from CSF during CNS infection.
- Detection of EBV DNA PCR on CSF is diagnostic for CNS disease.
- When quantitative PCR is done levels are relatively high in both EBV CNS lymphoma and acute EBV encephalitis cases, and lower in post infectious EBV-related syndromes such as Guillain-Barre syndrome, acute demyelinating encephalitis and transverse myelitis.
- Antibody studies on serum may support a diagnosis of acute EBV encephalitis, for example when EBV VCA IgM is positive and anti-EBNA negative.
- Analysis by RT-PCR of specific viral mRNA

Human Herpes Virus 6 (HHV6)

- HHV6 associates with febrile convulsions in children under 2 years of age.
- It is a cause of meningitis and encephalitis in immuno-competent as well as immunocompromised patients.
- In the bone marrow transplant recipient, encephalitis presentation occurs between 10 days to 15 months (median 45 days) after transplantation.

Diagnosis of HHV6 Encephalitis

- Positive anti-HHV6 IgM serology in CSF. A fourfold rise in antibody in serum only reflects the reactivation of a virus.
- Virus culture from CSF is insensitive
- PCR from CSF is more appropriate. Interpretation may require caution, given the reported high prevalence (over 30%) of latent virus in brain detectable by PCR, it is doubtful whether HHV6 DNA is detectable in CSF except in active CNS infection.

HHV-6/-7 Infection of the CNS

- Virus Isolation and Assay
- Serological Assays
- Genomic Detection by PCR
  - Numerous PCR primer sets available for HHV-6
  - Reverse transcription–PCR (RT-PCR) assay - latent or replicating virus?
  - Quantitative PCR assay - persistence of a high HHV-6 load in the absence of apparent disease
  - Multiplex PCR method - simultaneous detection of HHV-6 and HHV-7
- CSF-PCR is the technique of choice for the diagnosis of the CNS infection
- Brain biopsy recommended to confirm diagnosis in conflicting cases

Polyomavirus

- ds DNA, Circular, Icosahedral and non enveloped, 45nm in diameter,
- Includes: SV40, BK and JC

JC Polyomavirus

- JC virus, latent in most individuals from childhood, may be associated with chronic meningoencephalitis and progressive multifocal leukoencephalopathy (PML) in immunocompromised patients.
- The insidious onset of a central nervous system illness should alert the clinician to the possibility of PML. Visual field defects and mental impairment (ranging from subtle personality changes to dementia) are typical. AIDS-associated PML progresses rapidly, with a median survival of 6 months.

Diagnosis of JC Encephalitis

- Diagnosis of PML can be made on CSF by positive JC PCR with sensitivity estimated at 75% and 98% specificity.
- Diagnosis can also be made on a serum : CSF pair demonstrating intrathecal production of JC antibody (97% sensitive,79% specific).

Measles virus

Etiology

- 1 .Pathogen is measles virus.

  It has been classed as a paramyxovirus. it is spherical in appearance ,measuring about 100~150nm in diameter. It has an outer envelope composed of M-protein, H-protein, F-protein, and internal core is RNA.

- 2 .Site of the measles virus exists

  measles can be detected from blood and nasal, pharyngeal secretions.

Measles

- **Measles causes three types of encephalitis:**

1. Postinfectious encephalitis 5 to 14 days after the rash, it is an autoimmune response characterized by perivascular inflammation and demyelination.
2. Progressive infectious encephalitis 3 to 6 months after the initial infection. It occurs in children who are immuno-deficient or immunosuppressed and is due to uncontrolled measles virus replication.
3. Subacute sclerosing pan encephalitis (SSPE) 5 to 10 years after the initial infection. Measles vaccine virus rarely causes SSPE.

Diagnosis of Measles Encephalitis

- Serology, particularly detection of IgM by ELISA is sensitive and supports the diagnosis of measles encephalitis in the appropriate context.
- Oral fluid can be used for detection of measles IgM and of virus by RT-PCR with over 90% sensitivity, while virus can be detected by RT-PCR in urine for some 5 weeks after infection.
- SSPE is diagnosed by demonstrating intrathecal production of measles IgG antibody.

Rabis

- Lyssavirus genus prototype
  - Single-stranded, negative-sense, nonsegmented RNA
- 7 rabies groups in genus
  - Classic rabies virus – common rabies
  - 6 others with less than 10 reported human cases of disease

Rabis

- Rabies is an acute progressive fatal encephalitis caused by a lyssavirus.
- It is transmitted to humans via the saliva of infected mammals, usually as a result of a bite.
- After an incubation period usually of 1 to 2 months (but which can be more than a year), an acute neurological illness develops which progresses to paralysis, coma, and death.

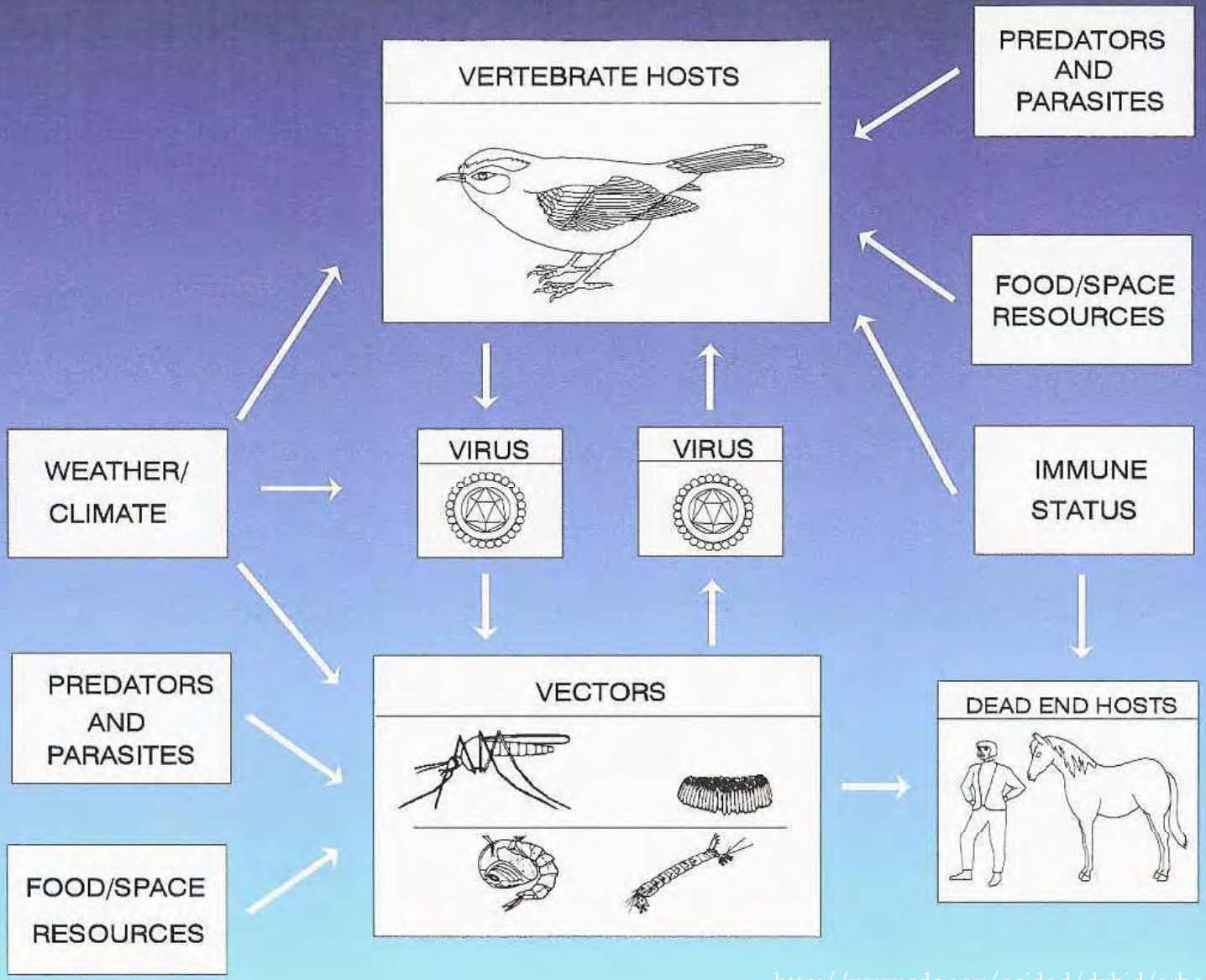
Diagnosis of Rabis Encephalitis

- Several tests should be used to attempt to diagnose rabies in a suspected case.
- Examination with fluorescent antibody to rabies antigen of the cutaneous nerves at the base of hair follicles in skin biopsies from the nape of the neck is rapid but insensitive.
- Virus isolation and RT-PCR (amplifying from the nucleoprotein gene) can be employed on saliva and on skin biopsy, with the latter being a more sensitive and rapid method.
- Rabies antibodies can be looked for in CSF and serum samples.

What Is An 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

Components in the Transmission and Maintenance of Arboviral Encephalitis

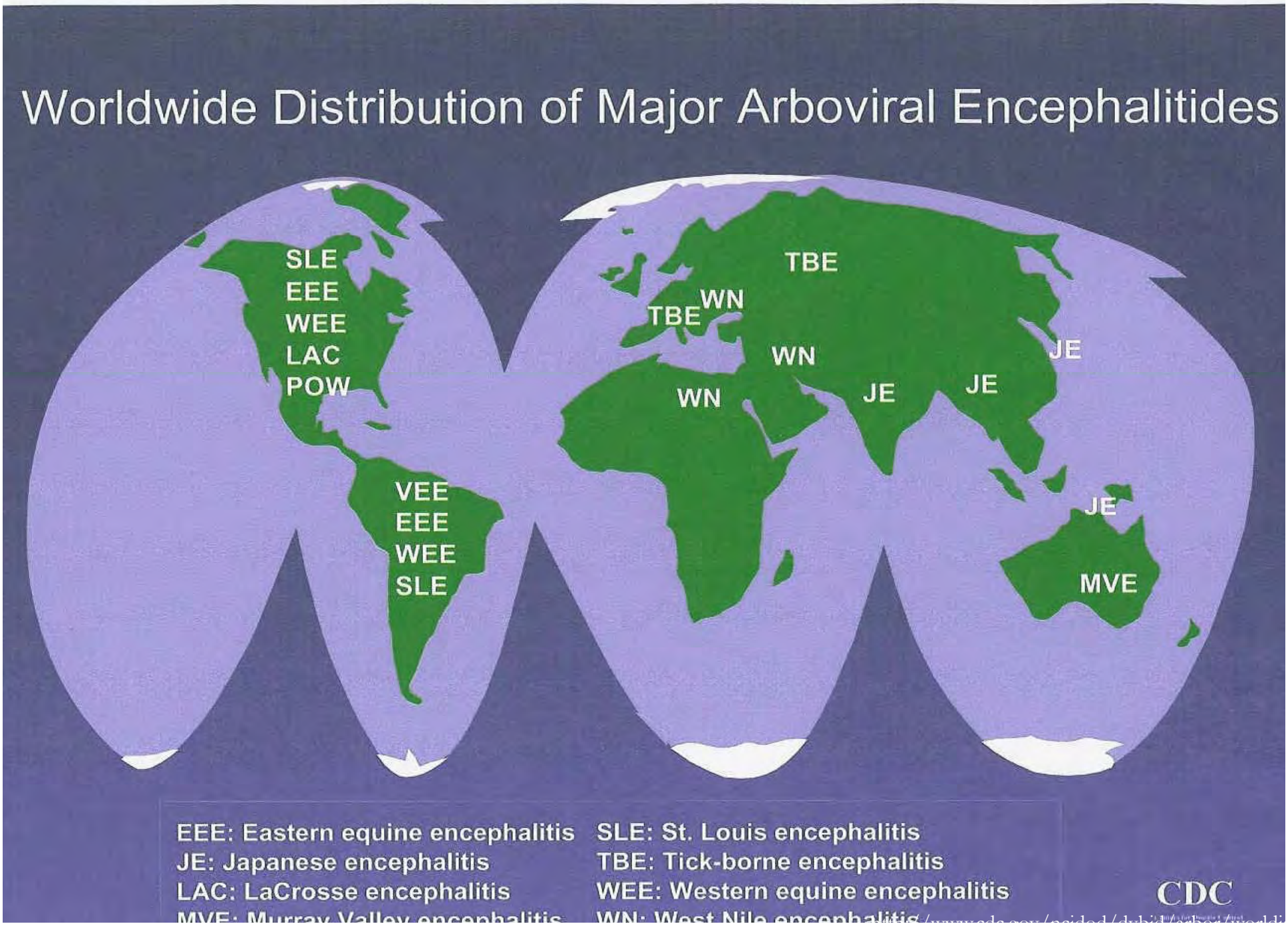


http://www.cdc.gov/ncidod/dvbid/arbor/schemat.pdf

Major Arboviruses That Cause Encephalitis

- Flaviviridae
  - Japanese encephalitis
  - St. Louis encephalitis
  - West Nile
- Togaviridae
  - Eastern equine encephalitis
  - Western equine encephalitis
- Bunyaviridae
  - La Crosse encephalitis

Worldwide Distribution of Major Arboviral Encephalitides



Flavivirus

- Japanese Encephalitis Virus
- St. Louis encephalitis virus
- West Nile Virus

Flavivirus: Virus Classification

- Family Flaviviridae
- 3 Genera
  - Flavivirus, Pestivirus, Hepacivirus
- Flavivirus - 12 Serogroups
  - Japanese encephalitis virus serogroup
    - Includes West Nile Virus (WNV), St. Louis Encephalitis, and others

Viral Genome

- Positive Strand RNA Genome, enveloped
- 1 ORF – Genome encodes single polyprotein which is subsequently cleaved
- Genome also includes 5’ and 3’ non-coding regions which have functional importance

Japanese Encephalitis

- **Flavivirus related to St. Louis encephalitis**
- **Most important cause of arboviral encephalitis worldwide, with over 45,000 cases reported annually**
- **Transmitted by culex mosquito, which breeds in rice fields**
  - Mosquitoes become infected by feeding on domestic pigs and wild birds infected with Japanese encephalitis virus. Infected mosquitoes transmit virus to humans and animals during the feeding process.

Japanese Encephalitis

- Japanese encephalitis (JE) is the most widespread of the arboviral encephalitis
- JE is caused by a mosquito-borne flavivirus carried by Culex species mosquitoes.
- Most infections are asymptomatic.

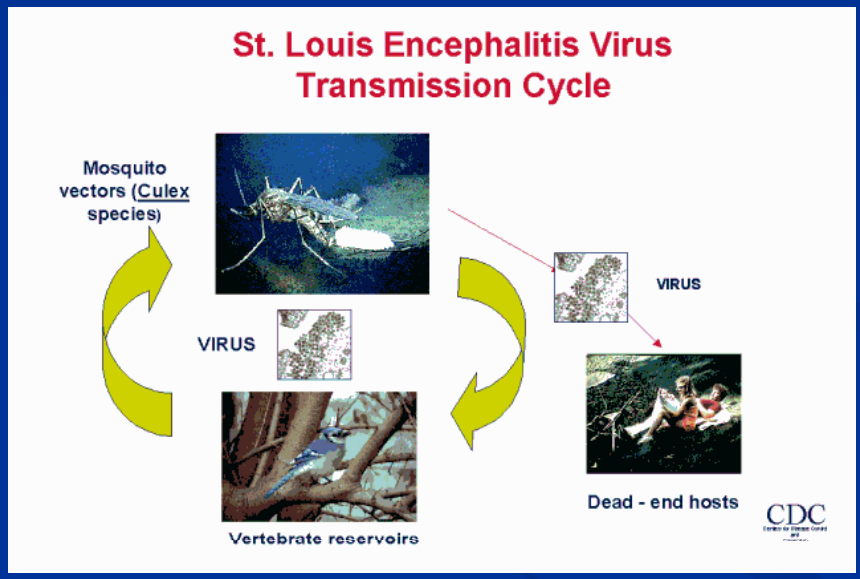
Diagnosis of JE

- Detection of IgM antibody either by capture ELISA or particle agglutination assay. Both assays have high sensitivity but specificity is less satisfactory because of cross reactivity with other flaviviruses.

- Antigen detection tests such as reverse passive haemagglutination and immunofluorescence, as well as PCR detection are also available.

St. Louis Encephalitis

- Flavivirus
- Most common mosquito-transmitted human pathogen in the US
- Leading cause of epidemic flaviviral encephalitis

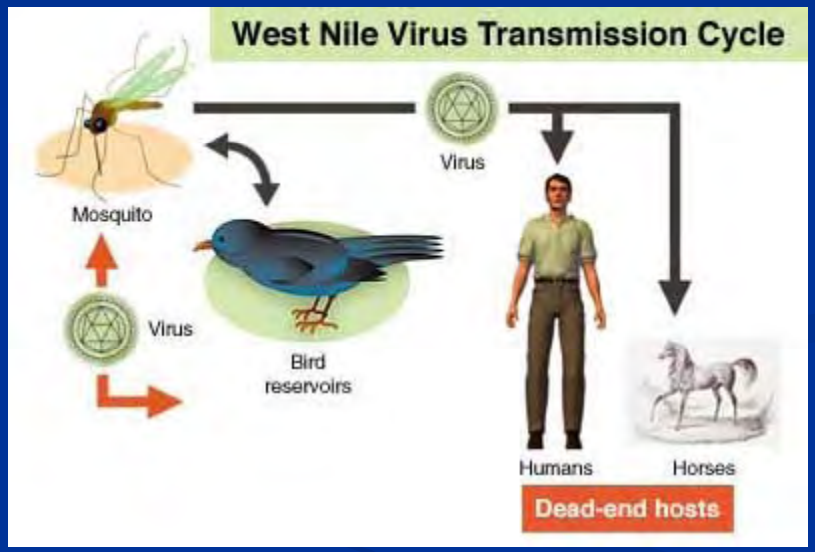


Diagnosis of SLE

- Detection of IgM antibody either by capture ELISA or particle agglutination assay. But is not reliable.
- Antigen detection tests such as reverse passive haemagglutination and immunofluorescence, as well as RTA-PCR detection are also available.

West Nile Virus

- Flavivirus
- Primary host – wild birds
- Principal arthropod vector – mosquitoes
- Geographic distribution - Africa, Middle East, Western Asia, Europe, Australia, North America, Central America



http://www.walgreens.com/images/library/healthtips/july02/westnilea.jpg

Diagnosis of WN Encephalitis

- Detection of WNV IgM antibody by capture ELISA for CSF and serum.
- IgM antibody is detectable in 90% of sera within 8 days of onset of symptoms and in 95% of CSF samples. IgM to Flaviviruses can persist for over 6 months, so in endemic areas it is important to confirm recent infection by comparing neutralizing antibody levels on paired sera. Cross reactivity occurs with other flaviviruses including St Louis encephalitis, yellow fever and Japanese Encephalitis.
- The plaque reduction neutralization assay may be needed to differentiate infection or vaccination with other flaviviruses.
- Virus culture gives low yields and PCR for West Nile virus is positive in less than 55% on CSF and 10% of serum samples.

Dengue Virus

- The incidence of CNS disease in dengue infections may be underestimated as other encephalitogenic arboviruses often coexist with dengue virus. Dengue virus should be considered in the differential diagnosis of encephalitis in areas where it is endemic.

- In a study of encephalitis in Vietnam 4% of cases seemed to be associated with the dengue virus.

Diagnosis of Dengue Virus

- Detection of serum or CSF antibody by IgM capture ELISA is recommended although similar immunoassays show satisfactory performance.
- Virus isolation is undertaken in mosquito cells, with immunofluorescence detection.
- Virus isolation from serum taken within 5 days of onset gives over 60% yield.

Louping Ill

- The only indigenous British arbovirus, louping ill disease affects mainly red grouse and sheep, and can also affect cattle and goats.
- It is thought that there may be other reservoirs such as the red deer and mountain hare in Scotland.
- It is a hazard group 3 pathogen.
- The louping ill virus is a flavivirus transmitted by the hard tick *Ixodes ricinus, the commonest tick in Northern* Europe.
- Human cases are few and seldom fatal, but should be considered in certain occupational groups in Scotland.

Diagnosis of Louping Ill

- Diagnosis is based on detection of rising haemagglutination-inhibiting antibody titres in serum.
- Early in the illness the virus may be detected in anticoagulated blood by RT-PCR.

Tick Borne Encephalitis (TBE)

- This flavivirus infection occurs across central, northern and eastern Europe and is transmitted by hard ixodid ticks.
- It is categorized as a hazard group 4 pathogen and has caused several cases in laboratory workers.
- There is usually a rash on the soft palate, as well as cervical lymphadenopathy and conjunctival injection, progressing to CNS abnormalities in 1 to 2 weeks.
- In severe cases hemorrhage occurs due to thrombocytopenia, with a death rate of 1 to 10%.

Diagnosis of TBE

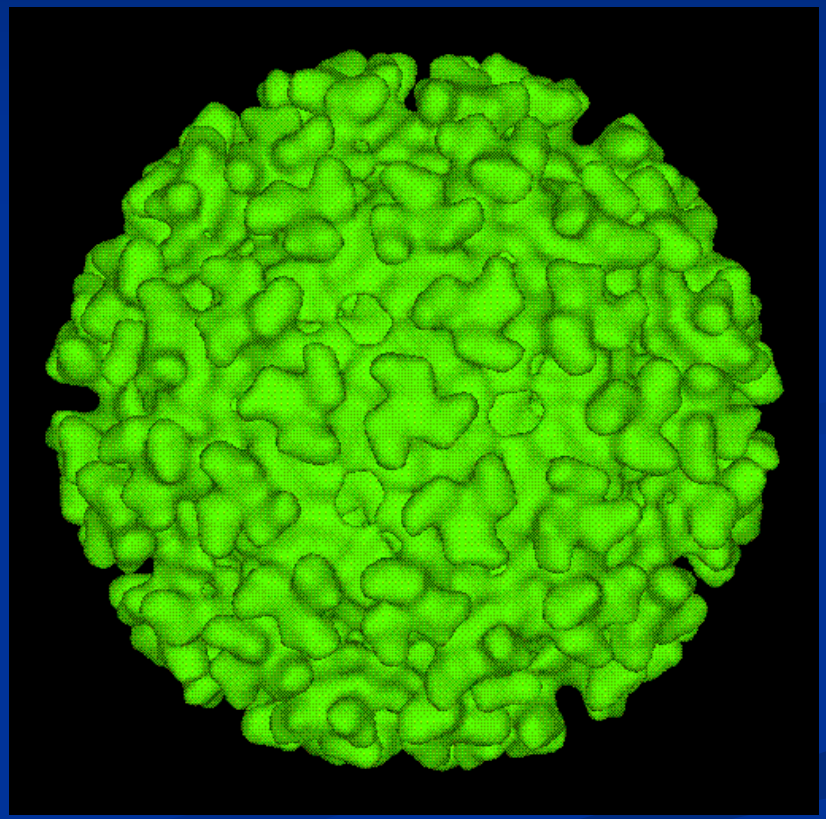
- IgG ELISA assays and haemagglutination-inhibition assays are useful screening tests but specificity can be low due to cross reactivity among flaviviruses (including vaccination against yellow fever).
- Neutralization assays required to confirm the flavivirus responsible for antibody reactivity.
- Intrathecal production of IgG against TBE is somewhat unreliable for diagnosis.
- A positive CSF IgM is reliable for diagnosis of TBE.
- PCR and virus culture in Vero cells are positive early in the infection from blood but are not usually present when CNS features develop.
- Serum IgM antibodies may persist for some 10 months.

Togavirus

- Eastern Equine Encephalitis Virus
- Western Equine Encephalitis Virus
- Venezuelan Equine Encephalitis Virus

Togavirus

- Family: Togaviridae
  - Genus: Alphavirus
- 49S Single Stranded Genome
  - ~11700 Nucleotides
- 3’ end: Five potential structural proteins
  - C, E3, E2, 6K, and E1
- 5’ end: Unknown number of non-structural proteins probably involved in replication
- Genome has an opposite orientation from the Flaviviruses



Viral Genome

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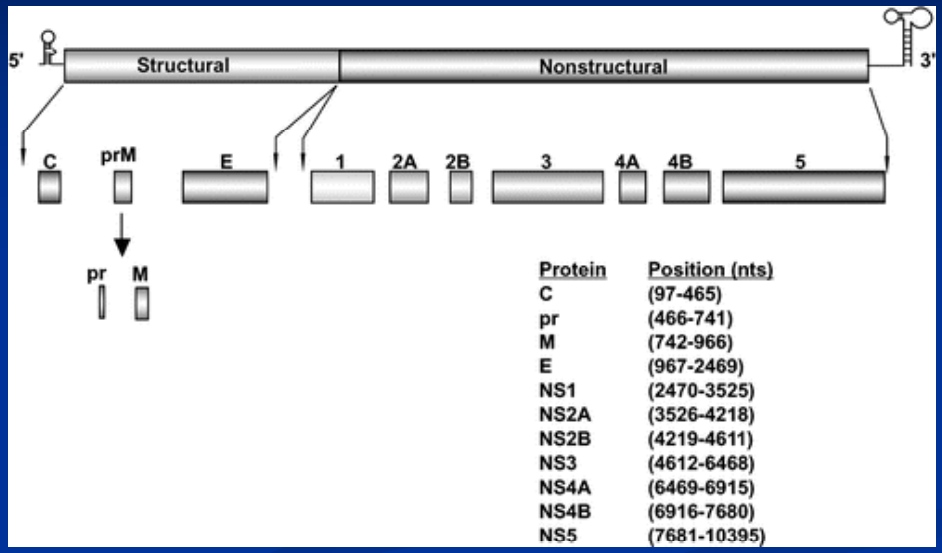
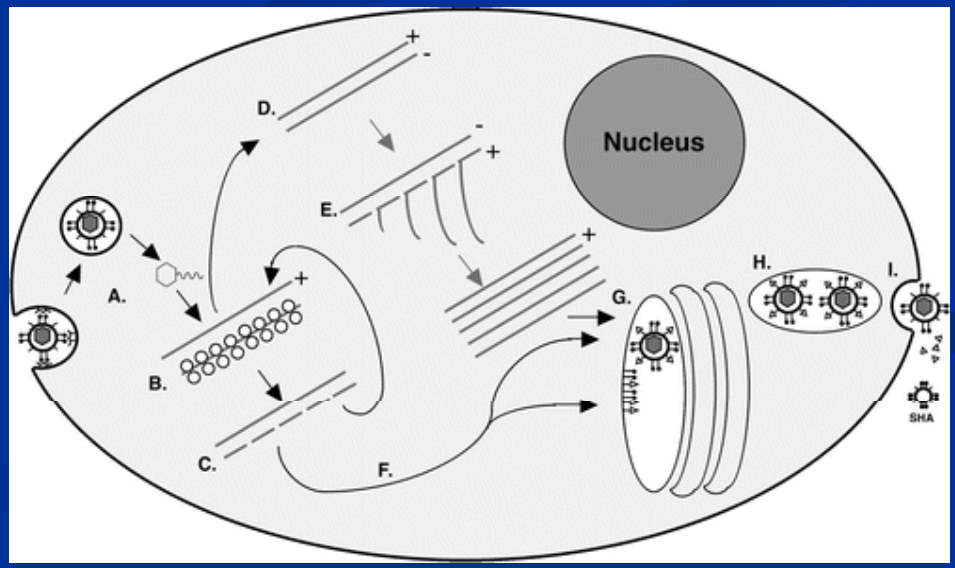


Protein	Position (nts)
C	(97-465)
pr	(466-741)
M	(742-966)
E	(967-2469)
NS1	(2470-3525)
NS2A	(3526-4218)
NS2B	(4219-4611)
NS3	(4612-6468)
NS4A	(6469-6915)
NS4B	(6916-7680)
NS5	(7681-10395)



Eastern Equine Encephalitis

- Togavirus
- Caused by a virus transmitted to humans and horses by the bite of an infected mosquito.
- 200 confirmed cases in the US 1964-present
- Average of 4 cases per year
- States with largest number of cases – Florida, Georgia, Massachusetts, and New Jersey.
- Human cases occur relatively infrequently, largely because the primary transmission cycle takes place in swamp areas where populations tend to be limited.

Western Equine Encephalitis

- Togavirus
- Mosquito-borne
- 639 confirmed cases in the US since 1964
- Important cause of encephalitis in horses and humans in North America, mainly in the Western parts of the US and Canada

Diagnosis of EEE & WEE

- Isolation of virus from blood, CSF Nasopharyngeal secretions.
- NT, CF, HI
- **virus culture**
- **PCR**

Bunyaviridae

Bunyaviruses

- Genome - single strand of negative sense RNA
- Four structural proteins
  - Two external proteins
  - Two associated with RNA to form nucleocapsid
- Matrix proteins absent from Bunyaviruses, therefore capsid proteins and envelope glycoproteins directly interact prior to budding

Rift Valley Fever

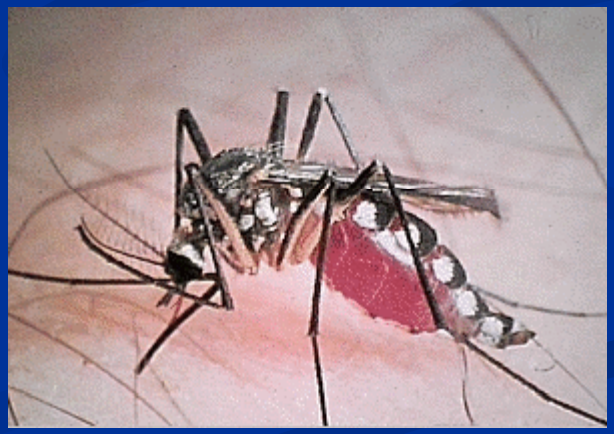
- Incubation period: 2-6 days
  - Inapparent or flu-like signs
    - Fever, headache, myalgia, nausea, vomiting
    - Recovery in 4-7 days
  - Retinopathy
  - Hemorrhagic fever
  - Encephalitis
- Overall mortality ~1%

Diagnosis of Rift Valley Fever

- ELISA, human blood
- Demonstration of viral antigen

La Crosse Encephalitis

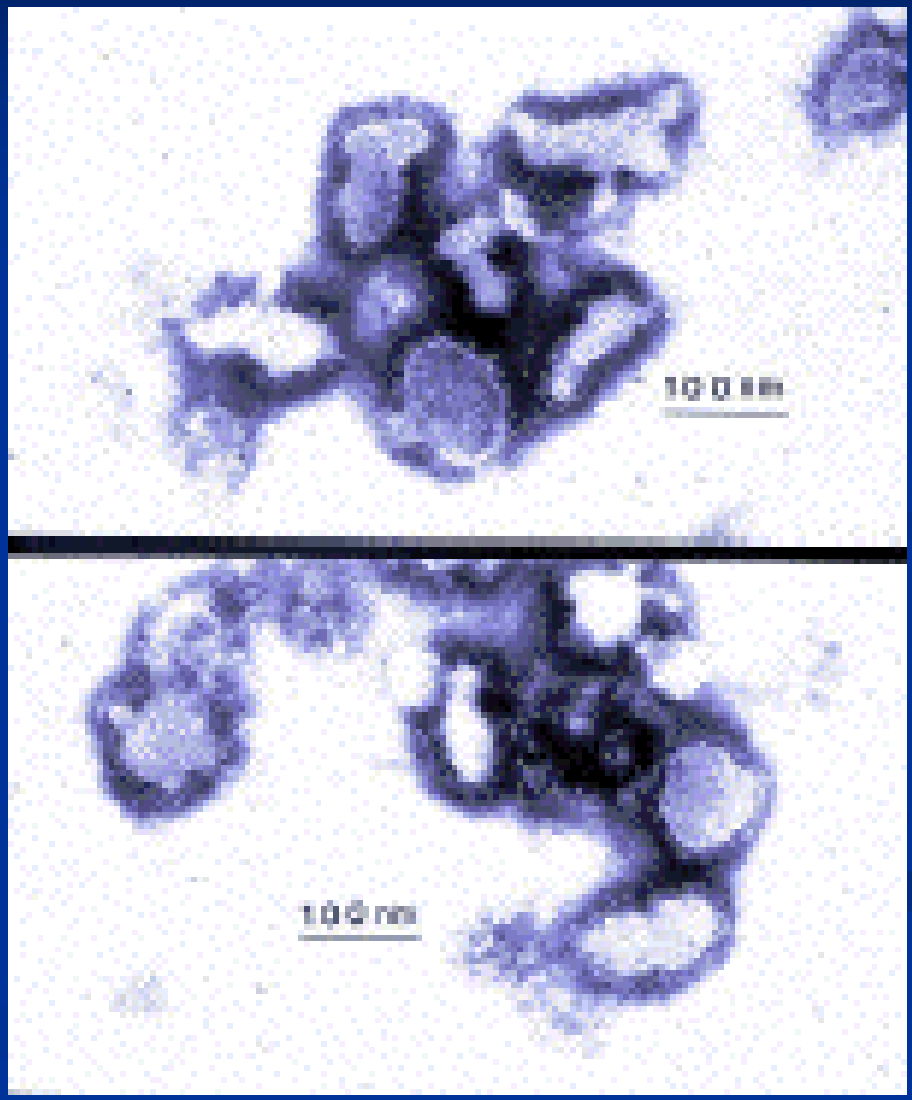
- Bunyavirus
- On average 75 cases per year reported to the CDC
- Most cases occur in children under 16 years old
- Zoonotic pathogen that cycles between the daytime biting treehole mosquito, and vertebrate amplifier hosts (chipmunk, tree squirrel) in deciduous forest habitats
- Most cases occur in the upper Midwestern state, but recently cases have been reported in the Mid-Atlantic region and the Southeast
- 1963 – isolated in La Crosse, WI from the brain of a child who died from encephalitis



La Crosse Virus

Diagnosis

- ELISA
- Antigen detection



http://www.virology.net/Big_Virology/BVRNAbunya.html

Encephalitis Study diagnostics for acute viral encephalitis[1,2]