EMERGING VIRUSES

ARTHROPOD-BORNE VIRUS DISEASES

"Arboviruses" are the old terminology for a group of RNA viruses that were classified together based on their mode of transmission by insects and arthropod vectors. These viruses are now classified on the basis of physical, chemical and morphologic properties into the following families

- Flaviridae
- Togaviridae-Alphaviruses
- Bunyaviridae
- Arenaviridae
- Reoviridae (Orbivirus)
- Rhabdoviridae

Note: The Arenaviridae and Rhabdoviridae (rabies) are generally transmitted via mammalian vectors, not arthropods.

EPIDEMIOLOGY OF ARBOVIRUSES

Arboviruses multiply in both vertebrates and arthropods. The arthropods (mosquitos, ticks, sandfly, etc.) act as vectors for the virus. The virus is transmitted via the saliva of the arthropod when it bites the vertebrate host. The vertebrate host may then act as a reservoir for the virus. This means that there is usually a prolonged viremia important to the cycle. Usually the infected reservoir recovers and develops lasting immunity to the virus.
DISEASES OF ARBOVIRUSES - THREE CLINICAL SYNDROMES

Undifferentiated Fever - This is characterized by fevers (usually 102-105 F), headaches, myalgias (muscle ache), arthralgias (joint pain) and malaise. The symptoms usually last 3-10 days and they resolve without sequelae.

Encephalitis - This is characterized by fever, headache, stiff neck and alteration of the level of consciousness. Encephalitis may be complicated by seizures, strokes, and may result in permanent neurologic sequelae. Certain types of encephalitis have a high mortality rate.

Hemorrhagic Fever - This is characterized by all the features of an undifferentiated fever plus profuse bleeding into the skin and GI tract secondary to DIC, hypotension, shock and leukopenia (low WBC).

LABORATORY DIAGNOSIS

The arboviruses may be isolated in various culture systems; for most agents, however, isolation is by intracerebral inoculation of newborn mice, which often results in
encephalitis and death. The viruses may be found in the blood (viremia) from a few days before onset of symptoms through the first 1 to 2 days of illness; attempts at isolation from the blood are generally useful only when viremia is prolonged, as in dengue, Colorado tick fever, and some of the hemorrhagic fevers. Virus is not present in the stool and is rarely found in the throat; virus may be detected in cerebrospinal fluid using RT-PCR. Specific diagnosis is usually accomplished by serologic techniques using acute and convalescent sera. PCR-based methods are now becoming available for more rapid and specific diagnosis (2017 film array PCR of CSF is not yet available for arboviruses).

CHARACTERISTICS OF SELECTED ARBOVIRUS ENCEPHALITIDES

<table>
<thead>
<tr>
<th></th>
<th>Eastern Equine</th>
<th>St. Louis</th>
<th>LaCrosse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geographical</strong></td>
<td>East, Gulf Coast, South</td>
<td>Central, West, South</td>
<td>Central, East</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age Group Affected</strong></td>
<td>Children</td>
<td>Adults &gt; 50 years old</td>
<td>Children</td>
</tr>
<tr>
<td><strong>Mortality (%)</strong></td>
<td>50-75</td>
<td>2-20</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Sequelae</strong></td>
<td>80% of survivors</td>
<td>20% of survivors</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>Headache, altered consciousness, seizures (fulminant)</td>
<td>Headache, nausea, vomiting, disorientation, stupor irritability</td>
<td>Seizures, paralysis, focal weakness</td>
</tr>
</tbody>
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PREVENTION AND CONTROL OF ARBOVIRUS INFECTION

Breakage of transmission cycle: eradicating vector with insecticides

Avoidance of endemic areas by nonimmune population

Immunization

- Especially important for travelers to endemic areas.
- Yellow fever vaccine - live, attenuated vaccine - very effective
- Killed virus vaccines are available for WEE, EEE, and VEE for horses and have been given to laboratory personnel who work on the viruses.
DR. BAKER'S LIST OF EMERGING OR RE-EMERGING VIRUSES
Best interactive resource (with cool pictures) is www.cdc.gov; find the search box and put in name of virus in the search box

YELLOW FEVER VIRUS
Yellow fever virus is a Flavivirus (+RNA, env).
Epidemics had huge impacts in history, including on the building of the Panama Canal.
Yellow fever virus is spread by Aedes aegypti mosquitoes.
Fever and Jaundice are primary symptoms. Asymptomatic infection is possible, more likely in younger individuals. Additional clinical symptoms are degenerative changes in the liver, kidney and heart. Appearance of Councilman Bodies in the liver is characteristic of Yellow fever.
Detection is by RT-PCR for viral RNA from the serum and/or by detection of NS1 protein in the serum (using an ELISA to detect the protein). No specific therapies. Supportive care only.

Reservoir - monkey
Vector - mosquito (Aedes aegypti in most urban settings)
Cycle 2 - man can serve as reservoir for urban cycle.

Live Attenuated vaccine is available. All travelers going to endemic areas should be vaccinated!! (range is mostly around the equator, especially in Africa and SE Asia.

DENGUE VIRUS

There are 4 distinct serotypes of Dengue virus. Primary infection generally results in Dengue Fever. High fever, aches, no specific therapy, and recovery is generally complete. However, reinfection with a different serotype can result in more severe disease termed "breakbone fever" - headache, backache, fever, pains in joints, muscles, eyeballs, maculopapular eruptions persisting 3-4 days. Sometimes also causes Dengue hemorrhagic fever - most frequently seen in children (severe hemorrhage or shock).

Reservoir - monkey
Vector - mosquito
Cycle 2 - man can serve as reservoir for urban cycle

Vaccine studies indicate that vaccination with one serotype may predispose that individual to a more severe disease upon reinfection with another serotype. Vaccine trials were halted pending development of attenuated or killed virus vaccines to all serotypes. Current studies are in progress to develop a live-attenuated tetravalent vaccine that is designed to produce a "balanced" neutralizing response to all four serotypes.

WEST NILE VIRUS
In August of 1999, an outbreak of arboviral encephalitis associated with mosquitoes was recognized to be occurring in New York City. The disease was clinically similar to St. Louis Encephalitis (SLE), but initial laboratory tests for SLE were negative. The CDC
was called in and serology and sequence analysis revealed that the causative agent was a strain of West Nile Virus which had never been seen in the USA. Analysis of mosquitoes and birds in the surrounding area showed that the virus infected at least 2 strains of mosquitoes and several species of birds. With the onset of colder weather in September and October, there was a drop in the number of cases of West Nile Virus encephalitis. However, since 1999, the virus has become endemic in the U.S.

For every five humans infected with West Nile virus, one has a mild, febrile illness usually lasting three to six days; meningitis or encephalitis develops in approximately 1 in 150 infected persons. The incubation period typically ranges from 2 to 14 days. Symptoms of the mild illness include malaise, headache, eye pain, gastrointestinal problems, and rash. Meningoencephalitis is rare in young persons, but its incidence is markedly higher among persons older than 50 years of age. Anecdotal data suggest that immunosuppression may increase the risk of severe disease. Severe muscle weakness is a common symptom and may provide a diagnostic clue.

Demonstration of the presence of WNV RNA in the serum, CSF or other tissue confirms the diagnosis of a current infection (RT-PCR analysis of patient sample). In addition, serology can also be used to confirm the diagnosis. Identification of WNV-specific IgM in cerebrospinal fluid confirms the presence of a current WNV infection. However, identification of WNV-specific IgM in serum indicates only a probable infection and necessitates further testing, including the use of serum specimens from the acute and convalescent phases of illness to identify a change by a factor of four or more in the antibody titer. Because of serologic cross-reactions with other closely related flaviviruses (e.g. St. Louis encephalitis virus), virus-neutralization tests are used to confirm a diagnosis of West Nile virus infection only when multiple serum specimens are available.

Currently, there are no effective anti-viral agents or vaccines for West Nile Virus. Vaccines in clinical trials are based on chimeric viruses with the YF17D backbone expressing West Nile envelope proteins.

**Reservoir** – marsh birds  
**Vector** – mosquito (Culex pipiens in the Chicago area)  
**Cycle 2** - man can serve as reservoir for urban cycle, but viremia is low

**ZIKA VIRUS**
Zika virus was first described in the 1940s when it was identified in Africa, and thought to cause only mild fever/rash disease. Zika is a flavivirus, transmitted by mosquitoes (cycle 2). Zika virus recently emerged in Brazil and is associated with microcephaly in children born to mothers who experienced infection with Zika during pregnancy. Another unusual characteristic of Zika virus is that it persists in the semen of infected men and can be transmitted by sexual contact. The exact time of persistence is unknown, but current recommendations indicate that the virus may persist for 6 months.

See the cdc.gov and NEJM web sites for updates. Antiviral drugs and vaccines (chimeric with yellow fever 17D vaccine) are under investigation.
ST. LOUIS ENCEPHALITIS
St. Louis encephalitis was the most common cause of epidemic encephalitis in the United States prior to the coming of West Nile virus in 1999. There are still outbreaks of SLE in the US in late summer/early Fall. It is a member of the Flaviviridae of positive strand RNA viruses with an envelope. Detection of infection is generally by seroconversion (usually after patient has recovered).

Reservoir - bird  
Vector – mosquito (culex pipiens)  
Cycle 2 - man can serve as reservoir for urban cycle

CHIKUNGUNYA VIRUS (+ RNA virus, togavirus)
Chikungunya virus is transmitted to people by mosquitoes. The most common symptoms of chikungunya virus infection are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Outbreaks have occurred in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans. In late 2013, chikungunya virus was found for the first time in the Americas on islands in the Caribbean. There is a risk that the virus will be imported to new areas by infected travelers. There is no vaccine to prevent or medicine to treat chikungunya virus infection. Travelers can protect themselves by preventing mosquito bites. When traveling to countries with chikungunya virus, use insect repellent, wear long sleeves and pants, and stay in places with air conditioning or that use window and door screens.

Reservoir - bird  
Vector – mosquito (Aedes aegypti and albopictus)  
Cycle 2 - man can serve as reservoir for urban cycle

EQUINE ENCEPHALITIS VIRUSES
Venezuela Equine Encephalitis virus (VEE), Western EE (WEE), Eastern EE (EEE)  
These viruses are togaviruses. The virion is an icosahedral nucleocapsid surrounded by a lipid bilayer. Genome is single stranded RNA, positive sense. An additional subgenomic mRNA is made during the infectious cycle. Genome is translated into a polyprotein which contains a protease that cleaves the polyprotein into subunits. The viral RNA-dependent-RNA-polymerase synthesizes the full length viral genomic RNA and a subgenomic mRNA. The subgenomic mRNA encodes the viral structural proteins.

Reservoir - bird (VEE, EEE), rodent (VEE)  
Vector - mosquito  
Cycle 1 - man and horse dead end host
Key feature of a dead end host - high, quick viremia that can be lethal. There is no prolonged viremia so humans are not good hosts for urban cycles of transmission.

CALIFORNIA ENCEPHALITIS VIRUS

California Encephalitis Viruses, such as LaCrosse Viruses, are members of the bunyavirus family of segmented RNA viruses. Most patients with infections have mild illness. However, onset of encephalitis may occur suddenly after 1 week incubation. Seizures occur in 50% of patients with encephalitis, usually early in illness. On average, illness lasts 7 days (fatalities < 1%, but 20% may have seizure disorders as sequelae). Detection is by serology (increased IgG).

Reservoir – squirrel and chipmunk
Vector - mosquito
Cycle 4 – man is dead end host, in insect, there is transovarial transmission.

Japanese Encephalitis Virus
Japanese encephalitis (JE) virus is the leading cause of vaccine-preventable encephalitis in Asia and the western Pacific. For most travelers to Asia, the risk for JE is very low but varies based on destination, duration of travel, season, and activities. JE virus is maintained in a cycle involving mosquitoes and vertebrate hosts, mainly pigs and wading birds. Humans can be infected when bitten by an infected mosquito. Most human infections are asymptomatic or result in only mild symptoms. However, a small percentage of infected persons develop inflammation of the brain (encephalitis), with
symptoms including sudden onset of headache, high fever, disorientation, coma, tremors and convulsions. About 1 in 4 symptomatic cases are fatal. There is no specific treatment for JE. Patient management focuses on supportive care and management of complications. Steps to prevent JE include using personal protective measures to prevent mosquito bites and vaccination (killed virus vaccine approved in 2009 for those 17 and older and approved in 2013 for use in children).

**COLORADO TICK FEVER**

The virion is icosahedral with no envelope. The genome is segmented, double-stranded RNA (Reoviridae).

**This is the most common arboviral infection in the U.S.** Incubation period 3-6 days followed by sudden onset of fever, chills, headache and prostration. Ten percent also show rash. Fever may be biphasic. Recovery is usually complete

- **Reservoir** - squirrels
- **Vector** - ticks
- **Cycle 4** - virus is transmitted transovarial

**TICK-BORNE FLAVIVIRUSES**

- **Russian Spring-Summer encephalitis virus, Omsk hemorrhagic fever.**
- **Reservoir** - small mammals or goats
- **Vector** - ticks
- **Cycle 3** - virus may be transmitted through goat milk

A live-attenuated vaccine is available and is widely used in Austria. (It needs to be used more widely in Eastern Europe and Russia).

**EBOLA VIRUS HEMORRHAGIC FEVER**

Members of the family Filoviridae, which currently consists of Ebola and Marburg viruses, cause severe and often fatal hemorrhagic fevers in humans and non human primates. Filoviruses (negative strand RNA viruses which look like a shepherd’s hook under the electron microscope) are among the most mysterious groups of viruses known because their natural history and reservoirs remain undefined and their pathogenesis is poorly understood. Fruit bats have been implicated as the reservoir. Transmission is by contact with body fluids (no evidence of respiratory transmission).

The most up-to-date information is available at an interactive site at [www.nejm.org](http://www.nejm.org) then look for the Ebola Outbreak link (this link in not live in the notes, sorry!). During my lecture, I will go to the interactive site for the latest news.

**Ebola vaccines in development:** live-attenuated VSV-ebola glycoprotein (rVSV-ZEBOV); Chimpanzee adenovirus-ebola glycoprotein (ChAd3-ZEBOV); Ebola-deltaVP30, and modified vaccinia-ebola glycoprotein (MVA-EOBV). All vaccines elicit protective immunity in animal models. Fast track studies are evaluating the safety and level of antibody response elicited by the vaccines.
Therapeutics in development: neutralizing antibody (ZMapp); plasma from Ebola virus survivors; siRNAs and others…but none are likely to be inexpensive enough to use in Africa…Up to date info available on WHO website
http://www.who.int/medicines/empEbola_q_as/en/
Information about the latest outbreak is at www.who.int/ebola/en/

SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

A new disease, termed Severe Acute Respiratory Syndrome (SARS), emerged into the human population during the winter of 2002-2003. The virus emerged in the Guangdong province of southern China, and then spread to cause major outbreaks in Hong Kong, Vietnam, Singapore, and Toronto. More than 8000 people were infected and over 770 died of the disease. Rapid work by teams of scientist assembled by the World Health Organization led to the identification of a novel coronavirus, termed SARS-CoV, as the causative agent of SARS. Epidemiologic data indicated that the earliest infections occurred in people working at exotic animal markets. Samples taken from animals in the markets suggested that Masked Palm Civets (and other animals such as the raccoon dog) can be infected with SARS-CoV, and may serve as an animal reservoir for the virus. Infection control measures were ultimately successful in controlling the 2002-2003 epidemics, but the existence of an animal reservoir (such as Chinese Horseshoe Bats) may allow re-introduction of the virus into humans.

Transmission: Transmission occurs through respiratory droplets and contact with virus on surfaces. Virus particles may be stable for as long as 24 hours on surfaces. In addition, tests indicate that people shed virus in their feces for up to 30 days, so transmission through fecal contamination is also possible. The incubation period for SARS is approximately 10-14 days.

Pathogenesis: The virus initially replicates in the respiratory tract. Viremia (virus in the blood stream) spreads the virus to multiple organs. Dramatic immune response, particularly in the lungs, leads to inflammation and pneumonia. Analysis of patient data indicates that fatality is more frequent in the older age groups (~1% fatality in people less than 24 years old, ~over 10% fatality in those over 65) and in individuals with pre-existing health conditions (diabetes, lung, heart or kidney disease, etc).

Detection: RT-PCR assays of respiratory specimens and/or serum can be used to detect infection. In addition, patient serum can be tested for seroconversion to SARS-CoV antigens. The best data is from comparison of sera from early (day 1-4 of fever) and late (day 12-16 after onset of fever) and detection of rising titer of IgM and IgG antibodies to viral antigens. For diagnosis of acute infection, RT-PCR for detection of viral RNA is the most rapid test available. However, the virus may not be present in the upper respiratory tract, and “false negatives” are possible. In addition, collection of the nasal/respiratory swab was one of the most common ways that SARS-CoV was transmitted to health care workers.
Protection: Currently there are no FDA approved vaccines or anti-viral agents for SARS-CoV. Watch for rapid developments in this field!!

MERS-CoV: Middle East Respiratory Syndrome coronavirus emerged into humans in 2012. Most cases detected to date are derived from the Middle East, predominately Saudi Arabia. Camels are implicated as the reservoir for the virus, and drinking camel milk is implicated as one possible way to acquire the infection. Person to person spread led to an outbreak in South Korea. Current confirmed cases result in ~30% mortality; mostly in older individuals with co-morbidities. Are there asymptomatic infections? Efforts are underway with vaccine and antiviral development.

For current information:

NIPAH VIRUS and HENDRA VIRUS ENCEPHALITIS
A new disease characterized by acute encephalitis emerged in Nipah, Malaysia. Mortality was over 50% in those affected. Epidemiological studies showed that the initial infections occurred in people associated with raising pigs in the countryside. Further studies showed that some of the pigs had respiratory infections with a "mile long cough." A novel paramyxovirus (negative strand RNA with envelope) could be isolated from pigs and humans. The reservoir for the virus was found to be flying foxes that fed on mangos in trees that shaded the pig sty. The virus went from bat saliva/urine to infect pigs to infect humans. There was no strong evidence for human to human transmission. Culling of infected pigs (and the uninfected pigs on the same farm) eliminated the transmission to humans.

Note: Hendra virus, which is distantly related to Nipah virus, emerged in Australia where infected horses transmitted the virus to veterinarians (50% mortality). It turns out the horses were bitten by BATS that harbored the virus.....

SO...how come bats don't die of all these intense viral diseases??! WE DON'T KNOW!!! Studies are being done to determine if bats generate antioxidants that limit disease. Watch for future developments to determine if some of these strategies can be translated to the clinic. (If you are interested, google Ian Mendenhall bats reveal clues to viral immunity to see a cool video).

HANTAVIRUS PULMONARY SYNDROME

In early 1993, an outbreak of fulminant respiratory disease with high mortality (67%) occurred in the southwestern United States. The CDC quickly determined that the disease was caused by a Hantavirus of the family Bunyaviridae. (Bunyaviruses are ambisense RNA viruses which are enveloped). The virus is endemic in deer mice and is transmitted to humans by inhalation of virus particles present in mouse feces and urine. Human to human spread has not been encountered. Hantavirus infection is detected by the presence of Hantavirus specific IgM in patient serum or cultivation of virus in mouse cell lines, but these tests are only available at the CDC. Treatment for Hantavirus
Pulmonary syndrome involves aggressive respiratory support (Intravenous ribavirin is also being tested). Public health measures to control Hantavirus include informing inhabitants of routes of spread and to reduce the rodent population in areas where there is outbreaks of the disease.

STUDY QUESTIONS
1. What are the main disease types caused by the arboviruses?

2. Why is it important in the U.S. for the CDC to monitor the number of horses which suffer from viral encephalitis during the summer months?

3. What infection control measures should be taken in the event of an outbreak of Hantavirus?

EXAMPLE OF TEST QUESTION
Factors important in the prevention and control of arbovirus infection include all of the following except:

A. Vaccination with live, attenuated or killed virus arbovirus vaccines.

B. Wearing appropriate clothing (long pants, boots, etc.) when hiking in endemic areas.

C. Use of insecticides to control virus vectors.

D. Immunization of horses to control spread of both Western and Eastern equine encephalitis virus.

E. Avoidance of endemic areas.

CORRECT ANSWER TO ABOVE QUESTION: D