Summary

- Arboviruses: Arthropod-borne viruses are viruses that can be transmitted to man by arthropod vectors (mosquitoes, ticks, flies)
- Over 500 virus species can be transmitted by arthropods, approximately 150 of those cause human disease
- Arboviruses belong to 5 different Families (Togaviridae, Flaviviridae, Bunyaviridae, Rhabdoviridae and Reoviridae)
- The main clinical syndromes are skin rash, arthralgia, neurological and/ or hemorrhagic manifestations.
- Clinical distinction between arboviral infections is difficult, because symptoms are often non-specific. Clinical presentation is also similar to many non-arboviral infections.
- Geographical distribution of arboviral infections varies and is often related to outbreaks; knowledge of possible exposure is important for recognition of clinical cases and for choosing diagnostic tests.
- Laboratory diagnosis is required for confirmation of arboviral infections; the timeline of infection (date of exposure, date of symptom onset) is required to choose the appropriate diagnostic assays.
- Treatment is mainly supportive
- Preventive measures include personal protective measures like the use of protective clothing and insect repellents. Vaccination for selected arboviral infections is available.

General

Transmission

The WHO definition of arthropod-borne viruses (arboviruses) is as follows: “Viruses that are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagous arthropods or through trans-ovarian and possibly venereal transmission in arthropods.” Over 500 virus species can be transmitted by arthropods, and approximately 150 of those cause human disease. The viruses multiply in the vector, migrate towards the salivary glands and are transmitted via the saliva to the vertebrate host during a blood meal. There is therefore no simple mechanical transmission (e.g. the mosquito as a flying injection needle). Different arthropod

species often have different vector competence for a particular (strain of) arbovirus. Transovarial transmission in the vector has epidemiological significance, because it allows the arthropod to act both as a vector and as a reservoir.

Aetiologic agents of arboviral diseases are primarily zoonotic pathogens. Spillover from the enzootic cycle to humans occurs when humans enter areas of zoonotic transmission or when enzootic transmission is increased near humans. Examples include Eastern (EEEV) and Western equine encephalitis viruses (WEEV), as well as West Nile (WNV), St. Louis encephalitis (SLEV) and Yellow fever viruses. Spillover may involve direct transmission to humans by primary enzootic vectors (e.g. WNV, SLEV and WEEV) or by bridge vectors, i.e. vectors that take bloodmeals across species, including humans (e.g. EEEV). Some viruses, such as Rift Valley fever, Japanese encephalitis and Venezuelan equine encephalitis viruses (VEEV) infect livestock animals, resulting in increased risk of infection in persons living in rural communities. Two of the most important human arboviral pathogens, Yellow fever and dengue viruses (DENV) have adapted to replication in humans only, allowing for urban transmission.

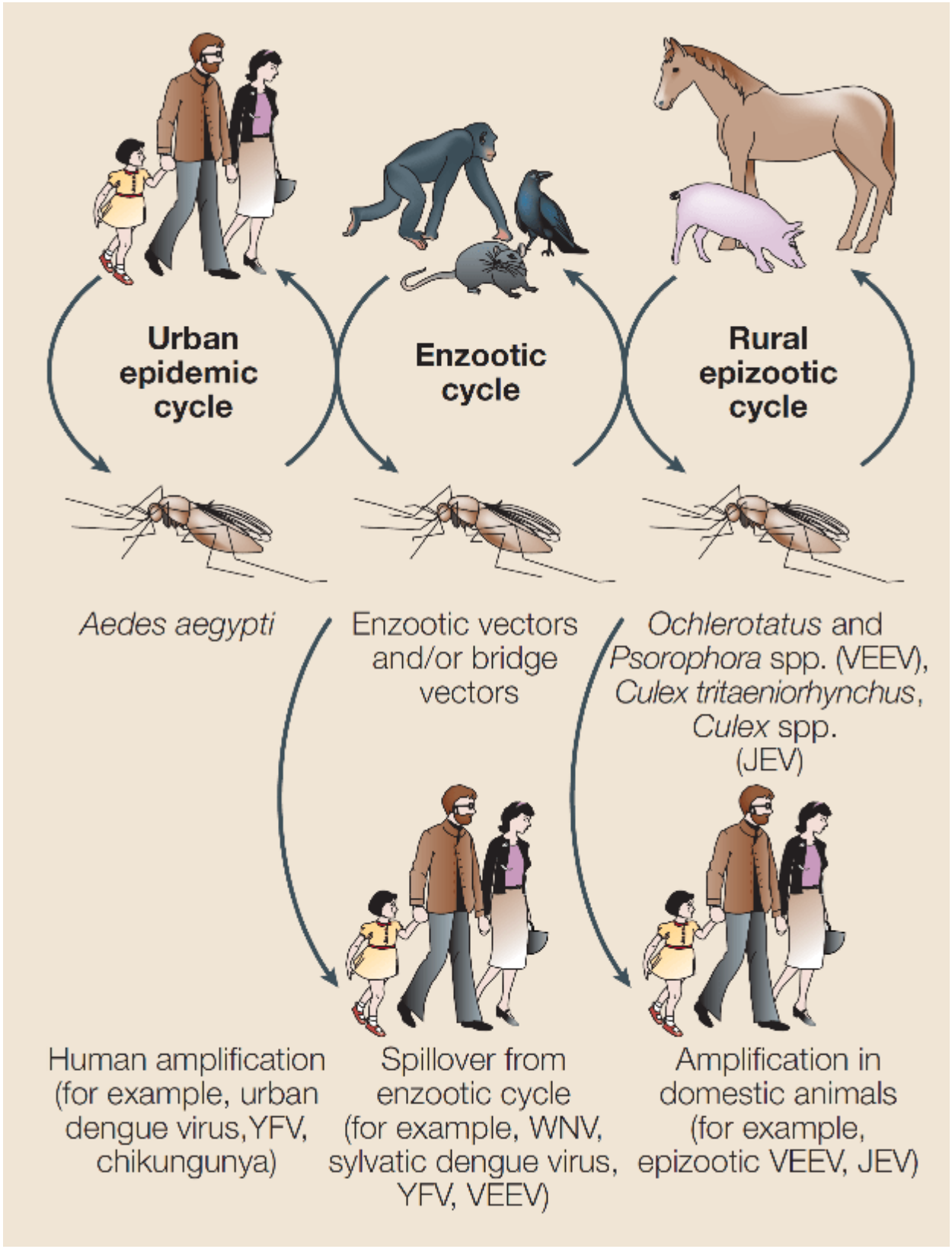
Urban epidemic cycle
Enzootic cycle
Rural epizootic cycle
Aedes aegypti
Enzootic vectors and/or bridge vectors
Ochlerotatus and Psorophora spp. (VEEV), Culex tritaeniorhynchus, Culex spp. (JEV)
Human amplification (for example, urban dengue virus, YFV, chikungunya)
Spillover from enzootic cycle (for example, WNV, sylvatic dengue virus, YFV, VEEV)
Amplification in domestic animals (for example, epizootic VEEV, JEV)

Figure: Typical mechanisms of arboviral emergence (Weaver et al, Nat Rev Microbiol. 2004)

Virology

Thus, the acronym ‘arbovirus’ does not refer to a virological classification, but rather to the main mode of transmission. Taxonomy divides Arboviruses 4 different classes, that all have a single stranded RNA genome:

1. Togaviridae (genus Alphavirus, not Rubivirus); examples Chikungunya virus (CHIKV), Eastern/ Western/ Venezuelan equine encephalitis viruses (E,W,VEEV), Ross river virus (RRV), Mayaro virus
2. Flaviviridae (genera Flavivirus and Pestivirus and not Hepacivirus); examples Dengue virus (DENV), Japanese encephalitis (JEV), West Nile virus (WNV), Yellow fever virus (YFV), Zika virus (ZIKV), Tick-borne encephalitis (TBEV), Kyasanur forest disease virus
3. Bunyaviridae (genera Bunyavirus, Phlebovirus, and Nairovirus but not Hantavirus); examples Crimean-Congo hemorrhagic fever (CCHKV), Toscana virus (TOSV), Sandfly fever virus (SFV), Rift Valley fever virus (RVFV), Oropuche (OROV)
4. Rhabdoviridae; example Indiana vesiculovirus
5. Reoviridae; example Colorado tick fever virus

This Chapter will focus on the arboviral families most important in human medicine: Togaviridae, Flaviviridae and Bunyaviridae.

Vectors

Aedes mosquitoes are the most important vector species of arbovirus infections in Africa, America and Asia.

Aedes aegypti prefers peridomestic settings, where water containers are a typical example of preferred breeding sites. It also enters houses to feed. Aedes mosquitoes bite during the day, mainly in the late afternoon (unlike Anopheles). The adult mosquitoes buzz a little but do not keep people awake in their siesta (unlike night biting Culex mosquitoes).

Mosquito biology

Traditionally it was thought that Aedes aegypti had limited flying ability (100 m). This was called into question by more recent data. Studies with labelled mosquitoes revealed an area of ± 840 meters in diameter in which eggs were laid. In order to study the density of vectors in an area, entomological surveys are used. A frequently used index is the number of positive water containers per 100 houses ("Breteau index"). Dumps of old car tyres are favourite breeding sites.

Aedes albopictus is another dengue vector. The mosquito is recognisable because unlike A. aegypti, it has one longitudinal white stripe down its back. This vector breeds in all kinds of water reservoirs, from lucky bamboo stems to septic tanks, which is important for control purposes. This mosquito also called Asian Tiger mosquito, has been recognized among the world's most invasive species. Its territorial expansion has already been associated with dengue and other arboviral outbreaks in non-tropical countries, like France and Croatia in 2010 and on the Madeira islands of Portugal 2012 (over 2000 cases).

Culex species are the vector of Japanese Encephalitis and West Nile virus.

Vector control

If only the adult mosquitoes are to be controlled, for example with so-called "adulticides", very rapid reduction in the number of adult mosquitoes can be achieved. This reduction will however only be for a short time. The insecticides soon lose their effect, after which mosquitoes that have hatched occupy the ecological niche that has been vacated. It is therefore strongly advised that the breeding sites are controlled also using larvicides. Slow-release formulations of methoprene (Altocid®) can be used here for this purpose.

Aedes aegypti is a peridomestic mosquito and this means that the population can be controlled. The elimination of small water reservoirs (=breeding sites) near housing (cans, car tyres, vases, bottles, buckets, snail shells, coconut shells, bamboo stubble, hollows in plants, waste gullies, etc.) by clearing away rubbish and by having a "dry" day systematically once a week is important in controlling Aedes aegypti. On "dry days", all small water containers (buckets, vases) are emptied to interrupt the cycle of the mosquitoes. The larvae

and pupae of the insects are destroyed before adult mosquitoes can emerge. Large reservoirs – drinking water for example – cannot of course be emptied quite so simply. Because large water containers have such a great epidemiological importance in some areas (Thailand for example) covering these with a fine-mesh net is effective in considerably reducing the population of Aedes mosquitoes (much better than a normal cover). Temephos pellets (Abate®, a larvicide) can be placed in water containers and is non-toxic for humans.

If Aedes albopictus plays an important role, appropriate measures are necessary for this (for example by expandable polystyrene beads that float on the water of septic tanks).

In epidemics the vector can also be controlled by using insecticides such as Bacillus thuringiensis H-14 or organophosphate larvicides (eg. Temephos pellets= Abate®).

Vector control for Culex mosquitoes consists of reducing contact with the vector by use of personal protective measures, such as protective clothing, mosquito repellents and impregnated mosquito nets.

Insecticide can also be sprayed indoors. In the case of large epidemics, outdoor vector control is also important (larvicides and adulticides). Today, several biological control methods can be used to diminish mosquito populations: the sterile insect technique (SIT) is a form of insect birth control where male mosquitoes are sterilized through irradiation. They are then released to mate with wild females that will lay non-viable eggs. RIDL (Release of Insects carrying Dominant Lethals) is a new tool to control *Aedes aegypti.* Genetically engineered mosquitos carry a lethal gene that is inherited by all offspring of RIDL mosquitoes. The lethal gene, which has an on and of switch, is switched on when the insects are released in the environment. The RIDL genes will then kill the larvae and pupae. Incompatible Insect Technique (IIT) makes us of the *Wolbachia* gram-negative bacteria that competes with viruses like dengue, zika, chikungunya and yellow fever in *Aedes aegypti. Wolbachia*-carrying mosquitoes are bread and then released into areas affected by mosquito-borne diseases.

Ixodes ticks are the vector of Tick-borne Encephalitis viruses. The main prevention is vaccination. Vector control measures are not very effective. They include the use of tick repellents in combination with the wearing of appropriate clothing (for example, long

trousers) and avoidance of the tick habitat if possible, although a recent study has shown that tick repellents are only moderately effective.

Hyalomma ticks are involved in the transmission of Crimean-Congo Haemorrhagic Fever (CCHF) virus, although sometimes other up to 31 tick species are involved (e.g. Rhipicephalus, Haemaphysalis, Amblyomma and Dermacentor sp). The virus can survive in a tick population because it is transmitted both by the transovarial and the transstadial route.

Geographical Distribution

Geographical distribution of arboviral infections varies and is often related to outbreaks; knowledge of possible exposure is important for recognition of clinical cases and for choosing diagnostic tests. When the distribution of arthropod vectors for pathogens overlaps, the distribution of the arboviruses can be similar (see also Figure). Co-infections (eg. two different serotypes of DENV, two different arboviruses and co-infections of malaria with arboviral infections) do occur. Online resources should be used to obtain up-to-date information concerning ongoing epidemics (eg. www.cdc.gov, www.who.int, http://ecdc.europa.eu/, http://www.promedmail.org/).

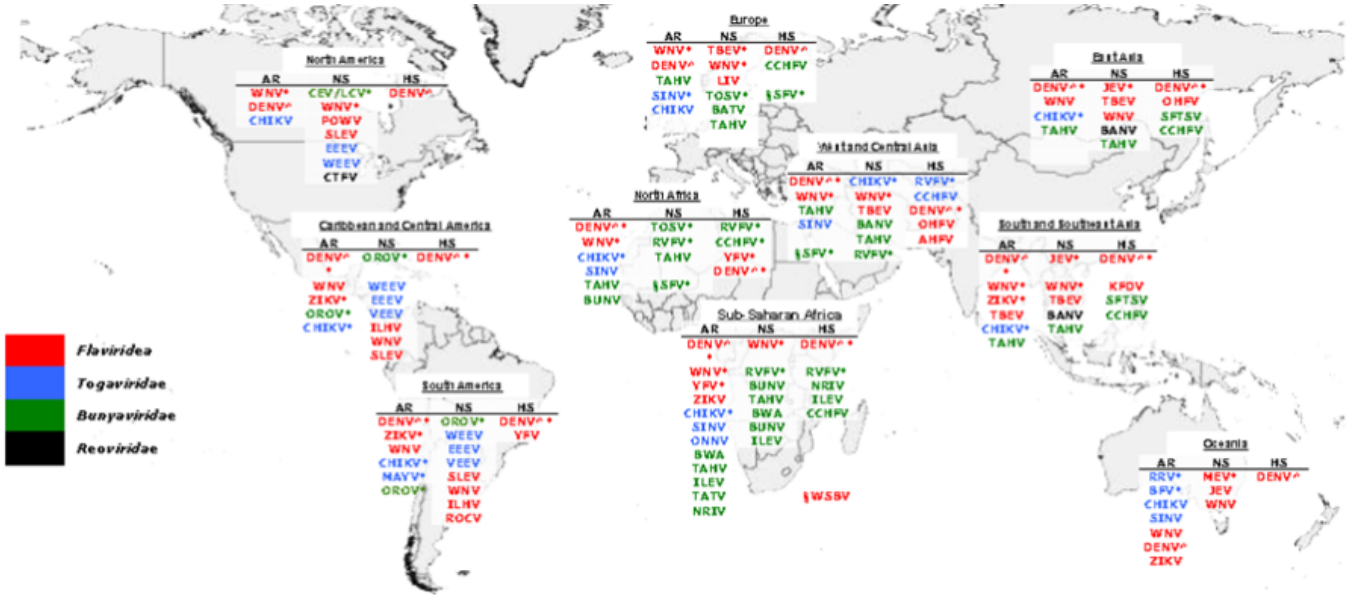

Figure: Overlapping distribution of selected arboviruses (Cleton *et al*, PLoS Negl Trop Dis)

Arbovirus include different families of viruses, as presented in this figure with colours. The Flaviviridea family, which is coloured in red, includes DENV, ZIKV and other species. The blue coloured viruses belong to the Togaviridea family, which include CHIKV among other species. The viruses belonging to the Bunyaviridea family are coloured in green and the viruses belonging to the Reoviridea family are coloured in black.

Clinical aspects

The clinical presentation of arbovirus infection varies from asymptomatic to critical illness with organ failure and death. It is not possible to distinguish between arboviral infections clinically, because symptoms are often non-specific. The clinical presentation is also similar to that of many non-arboviral infections.

However, a number of clinical syndromes may be distinguished. These are:

- Fever
- Skin rash
- Arthralgia
- Neurological manifestations
- Haemorrhagic manifestations

Skin rash

A non-pruritic skin rash tends to occur frequently. It can be maculopapular or morbilliform. Skin desquamation is uncommon. Skin vesicles can form in Sindbis virus infection.

Arboviral-induced arthritis

Arthralgia is a frequent finding in mosquito-borne arboviral disease, but some of them play a more prominent role than others. The six main mosquito-borne viruses associated with arthritis in humans belong to the Family of Togoviridae, genus Alphavirus. They are: Chikungunya, Sindbis, O'nyong-nyong, Mayaro, Ross River and Barmah Forest virus.

All these viruses are transmitted via culicine mosquitoes, such as Aedes or Culex spp, except O'nyong-nyong virus, which is transmitted via anopheline spp. Incubation is usually 2 to 10 days. The illness begins suddenly. The most common symptoms are fever, arthralgia and rash. Fever is usually low grade in O'nyong-nyong, Sindbis and Ross River virus infections, but high in Mayaro and Chikungunya infections.

Headache, photophobia, retro-orbital pain, myalgia and backache occur frequently. Anorexia, nausea and vomiting are also part of the clinical spectrum. Weakness can persist for several weeks, sometimes even months.

The severity of arthralgia can vary from vague stiffness to excruciating pain. Patients with Alphavirus infections (Chikungunya, Ross River virus) often have swollen tender joints; this does not occur in dengue or West Nile fever. Fingers, wrists, elbows, toes, ankles and knees are the most common affected. In most cases, the symptoms persist for several days and complete recovery follows. However, arthralgias may persist for several months and even for years. This results in prolonged disability. Intermittent attacks of joint pain and swelling can occur.

Incidence of arthralgia after Chikungunya virus infection varies greatly with factors such as genetic susceptibility of populations, cultural perceptions, and quality of study. In some cohorts, over 50% of patients develop chronic arthralgias and clinically detectable joint swelling at 3 years after their acute infection, so called post- Chikungunya rheumatic disorder. A 6-year retrospective study in La Réunion looked at patients referred to a rheumatologist due to rheumatic symptoms lasting more than 4 months following CHIKV infection. Out of 159 cases, they found that 59% met the criteria for de novo chronic inflammatory rheumatism (CIR) like rheumatoid arthritis, spondylarthropathy, and undifferentiated polyarthritis, and 31% had pre-existing rheumatic musculoskeletal disorders. Amongst those with de novo rheumatoid arthritis, 80% developed joint damage within 3–4 years. They found that some patients remained symptomatic for 6–8 years.

In those with persistent symptoms, there is little evidence on effective therapies. Several disease modifying drugs (DMARDs) have been studied with varying success. Chloroquine has some antiviral effect but has not been found to be more effective than other anti-inflammatories like meloxicam in acute and chronic CHIKV arthralgia. Methotrexate has been

widely used, particularly in patients who present with a systemic polyarthritis. Up to 75% of patients may have a positive clinical response to this. Sulfasalazine has been shown to have good clinical efficacy, particularly when combined with methotrexate.

There are no vaccines against Togaviridae. Vector control and personal protection are the only effective preventive measures.

O'nyong-nyong virus

Poorly understood epidemiology. It was first isolated in East Africa in 1961. In this period, there was a massive epidemic involving millions of people. The virus is transmitted via anopheline mosquitoes, which is very unusual for an arbovirus.

Mayaro virus

This virus has been reported from Brazil, Colombia, Bolivia, Trinidad and Surinam. Most infections seem to occur in the forest. Forest-dwelling mosquitoes of the genus Haemagogus are believed to be the principal vector. Rodents or monkeys probably serve as reservoir.

Ross River virus

Human infection has been documented from Australia, New Guinea, the Solomon Islands, Fiji, Samoa and a number of South Pacific Islands. New Zealand seems to be spared. In Australia, infection with this virus is known as epidemic polyarthritis. The first recorded outbreak was described in 1928. A major epidemic occurred in 1979-80 on a number of South Pacific Islands. The disease occurs in both an endemic and epidemic form. In Australia, the virus seems to be maintained in a wild vertebrate-mosquito cycle, with Culex annulirostris and Aedes vigilax serving as the principal vectors. In the Pacific the virus can be transmitted via Aedes polynesiensis.

Barmah Forest virus

This virus is so far only found in Australia. Barmah Forest virus was first isolated in 1974 from Culex annulirostris mosquitoes collected in the Barmah Forest of northern Victoria. It has also been isolated from numerous other mosquitoes including the coastal species Ochlerotatus vigilax and O. camptorhynchus, which have a salt marsh habitat, and from the midge Culicoides marksi in the Northern Territory. The virus was found to be pathogenic for man since 1988. Infections with this virus are less common than infections with Ross River virus. Wallabies and kangaroos are thought to form the reservoir.

Sindbis virus

Sindbis is the most widely geographically distributed of the six alphaviruses causing arthritis. It has been recovered from Europe, Africa, Asia, Australia and the Philippines. It has a broad host range. The basic life cycle involves Culex mosquitoes and wild birds. Because the vectors are mainly ornithophilic (“bird-loving”), human infection is uncommon.

Neurological symptoms

Although neurological symptoms may occur with many arboviral infections, the most important causes of neurological symptoms belong to the genus Flavivirus, of the family Flaviviridae. Important Flavivirus species which frequently cause neurological symptoms belong to the Japanese Encephalitis serogroup (Japanese encephalitis virus (JEV), West Nile virus (WNV), St Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV)) and Tick-borne encephalitis virus (TBEV). Zika virus also has marked neurotropism.

Because of the clinical importance and vast distribution, these viruses are discussed in separate sections.

Laboratory diagnosis of arboviral infections

Laboratory diagnosis is required for confirmation of arboviral infections. As explained below, information regarding the timeline of infection (date of exposure, date of symptom onset) is

required to choose the appropriate diagnostic assays. This is illustrated for dengue virus in Figure 3.

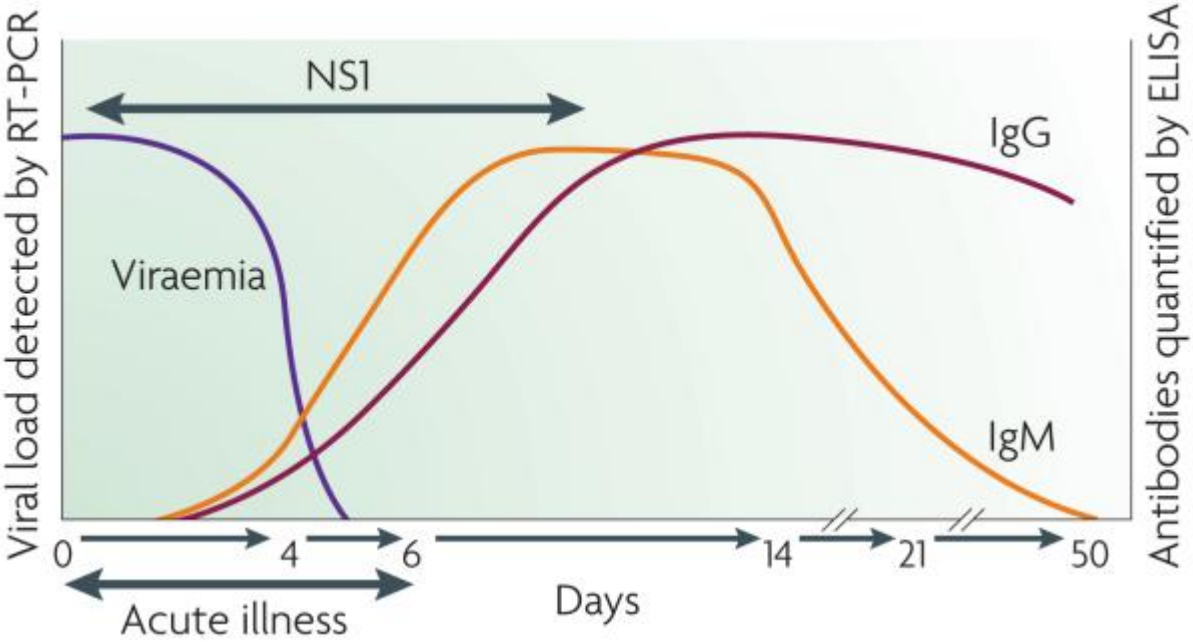

Figure 1 Typical timeline of arboviral infection (Dengue) (Guzman et al, Nat Rev Microbiol)

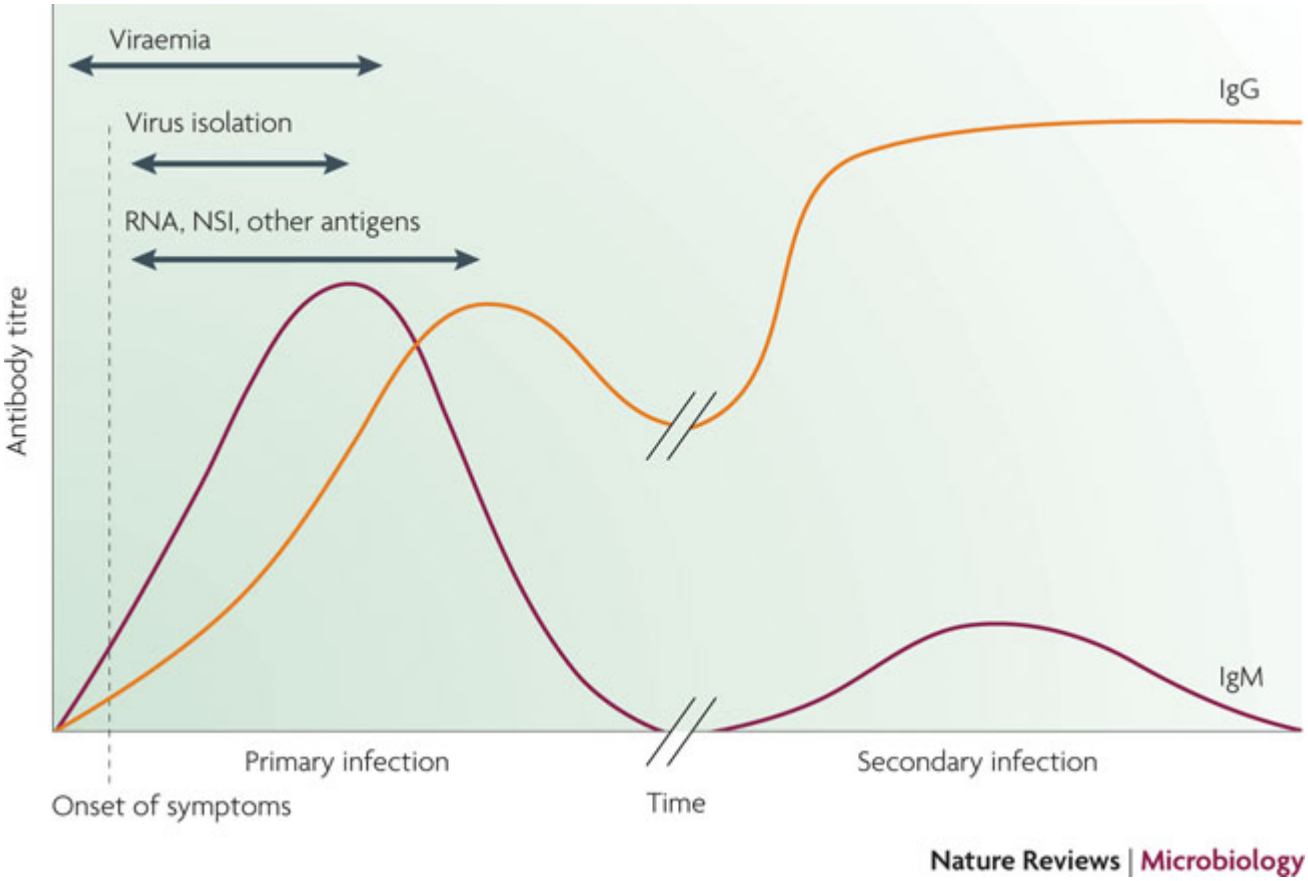

Figure: kinetics of anti-arbovirus antibodies (Dengue) (Peeling et al, Nat Rev Microbiol)

Direct tests

After the incubation period, the arbovirus is viraemic (ie. it circulates in human blood). In the acute phase of infection, the virus can be detected in serum or whole blood by molecular detection assays that target virus-specific sequences, such as real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The viraemic phase is usually short-lasted (up to 7 days after symptom onset, depending on the arbovirus). RT-PCR may be used for detection of the arbovirus in other body fluids, thus possibly extending the diagnostic window. In addition, antigen detection tests have been developed. In the case of dengue virus infection, a rapid test targeting NS1, a glycoprotein that is essential for viral replication and viability has been introduced; this test can be used within 7 days after onset of dengue virus infection. Virus isolation from body fluids or tissues in cell lines is another means of confirming infection, but due to high costs and sophisticated technical requirements, its use is restricted to research settings.

Indirect tests

Antibody detection assays such as Enzyme Linked Immuno Sorbent Assay (ELISA) or Immune Fluorescence Assays (IFA) are available for detection of arbovirus-specific antibodies. Only after developing a humoral immune response to an arbovirus, these tests can be used for detection of that virus. This generally limits their use in the acute phase of arboviral illness. Apart from limited sensitivity in the early course of the disease, serological assays that detect immunoglobulins present challenges to interpretation; specificity is frequently affected by cross-reactivity (particularly with other flavivirus infections or previous flavivirus vaccinations). It may also be difficult to discriminate subsequent infections because of persistence of IgG-class antibodies (see Figure 4).

A single indirect test can rarely confirm the diagnosis. To confirm a case by antibody detection assays, demonstration of seroconversion is required. Both seroconversion from negative to positive IgM antibody detection as well as a demonstration of a fourfold or greater increase in IgG antibody titers in paired sample analysis can be used to this end. Consecutive samples should ideally be taken at least 14 days apart.

To confirm the specificity of an antibody reaction to an arbovirus, Virus Neutralization Tests (VNT) can be used. Neutralization of a virus is defined as the loss of infectivity by binding to virus-specific antibody. Virus and serum are mixed and then inoculated into cell culture. Sera that contain antibodies that neutralise the virus will then prevent infection of the cells in culture. When little or no neutralizing antibody to the virus is present, the virus remains infectious. This can be observed microscopically by demonstrating a CytoPathogenic Effect (CPE) in the cell line, or by detecting higher viral loads using RT-PCR.

Table 1 Advantages and limitations of arboviral diagnostics tests (Peeling et al, Nat Rev Microbiol)

* virus neutralisation test is not included in this comparison

Diagnostic tests	Advantages	Limitations

Virus isolation and identification	Confirmed infection Specific Identifies serotypes	Requires acute sample Requires expertise and appropriate facilities Does not differentiate between primary and secondary infection Expensive
Viral RNA detection	Confirmed infection Sensitive and specific Identifies serotype and genotype Results within hours	Potential false-positives owing contamination Requires acute sample Doesn’t differentiate between primary and secondary infection Expensive
Antigen detection		
	Confirmed infection Easy to perform Less expensive	Not as sensitive as virus isolation or RNA detection
Serological tests		
IgM or IgG seroconversion (paired samples)	Confirmed infection Least expensive Easy to perform Can differentiate between primary and secondary infection	IgM levels can be low in secondary infections Confirmation requires two or more serum samples
IgM detection (single sample)	Identifies probable cases Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions	IgM levels can be low in secondary infections

Medically Important Arboviruses

Table2 Medically Important Arboviruses

Family Genus	Virus	Vector	Host	Transmission cycle	Incubation period	Clinical syndrome
Bunyaviridea						
Nariovirus	Crimean-Congo hemorrhagic fever	Tick	Birds, small mammals	R; H2H	1–3 (1–9)	FD, HS, (NS)
Orthobunya virus	Bwamba virus	Mosquito	Unknown	R	1–14	FD, AR, (NS)
	Bunyamwera virus	Mosquito	? rodents	R	Unknown	FD, AR, (NS)
	Guaroa virus	Mosquito	Unknown	R	Unknown	FD, AR
	Ilesha virus	Mosquito	Unknown	R (U)	Unknown	FD, AR, (NS, HS)
	Ngari virus	Mosquito	Unknown	R	Unknown	FD, AR, HS
	La Cross virus	Mosquito	Small mammals	R	5–15	FD, NS
	Tahyna virus	Mosquito	Rodents, small mammals	U	3–7	FD, AR, (NS), conjunctivitis, pneumonia
	Oropouche virus	Midge	Humans, Sloths, ? primates/ birds	R, U	4–8	FD, AR, NS
	Tataguine virus	Mosquito	Unknown	R	Unknown	FD, AR
Phlebovirus	Toscana virus	Sandfly	Humans, bats	R	2–14	FD, NS, (AR)
	Sandfly fever Naples/Sicilian	Sandfly	Humans, rodents	R	2–14	FD
	Rift valley fever virus	Mosquito	Rodents, bats, cattle	R; H2H	1–7	FD, HS, NS, hepatitis
Flavivirus	Dengue virus	Mosquito	Primates, humans	R, U; H2H	4–7 (3–14)	FD, HS, AR

Family Genus	Virus	Vector	Host	Transmission cycle	Incubation period	Clinical syndrome
	Japanese encephalitis virus	Mosquito	Ardeid birds, pigs	R, U	5–14	FD, NS
	West Nile virus	Mosquito	Birds	R, U; H2H	3–5 (2–14)	FD, NS, (AR)
	St. Louis encephalitis virus	Mosquito	Birds	R, U	2–21	FD, NS
	Murray Valley virus	Mosquito	Ardeid birds	R	1–28	FD, NS
	Kyasanur Forest disease virus	Tick	Small mammals, humans	R	3–8	FD, HS, conjunctivitis, pneumonia
	Alkhurma hemorrhagic fever virus	Tick	Small mammals	R	3–12	FD, HS
	Tick-borne encephalitis virus	Tick	Small mammals, birds	R; H2H	7–14	FD, NS, (HS)
	Ilheus virus	Mosquito	Birds	R	Unknown	FD, NS
	Yellow fever virus	Mosquito	Primates, humans	R, U; H2H	3–6	FD, HS, hepatitis
	Zika virus	Mosquito	Primates, humans	R, U; H2H	3-12	FD, AR, NR, conjunctivitis, congenital syndrome
Reoviridea						
Coltivirus	Colorado tick fever virus	Tick	Small mammals	R; H2H	3–5 (0–20)	FD, NS, AR, HS
Seadronvirus	Banna virus	Mosquito	Unknown	R	Unknown	FD, AR, NS
Togaviridea						
Alphaviruses	Barmah Forest virus	Mosquito	Birds, marsupials	R, U	7–9 (5–2)	FD, AR

Family Genus	Virus	Vector	Host	Transmission cycle	Incubation period	Clinical syndrome
	Eastern equine encephalitis virus	Mosquito	Birds, small mammals, marsupials	R	3–10	FD, NS
	Chikungunya virus	Mosquito	Primates, humans	R, U	3–7 (1–12)	FD, AR, HS, NS, Conjunctivitis
	Mayaro virus	Mosquito	Primates	R, U	6–12 (3–12)	FD, AR, HS
	O'Nyong Nyong virus	Mosquito	Primates, humans	R, U	>8	FD, AR
	Ross river virus	Mosquito	Marsupials, mammals	R, U	7–9 (3–21)	FD, AR, HS
	Sindbis virus	Mosquito	Birds	R	1–7	FD, AR
	Western equine encephalitis virus	Mosquito	Birds, small mammals	R	2–10	FD, NS
	Venezuelan equine encephalitis virus	Mosquito	Small mammals	R	<1–5	FD, NS

A. Clinical syndromes: FD = febrile disease; AR = atralgia/ rash; HS = haemorrhagic syndrome; NS = neurological syndrome; () = less frequent.

B. Transmission cycle: R = rural, U = urban; H2H = human transmission reported

(adapted from Cleton[10])