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Arboviruses

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.

Aetiological 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.
Viruses | 8

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)
Virology

Thus, the acronym ‘arbovirus’ does not refer to a virological classification, but rather to the main mode of transmission. Taxonomy divides Arboviruses into 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)
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
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

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)
Direct tests

After the incubation period, the arbovirus is viraemic (i.e. 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-lasting (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 titres 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)**

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<td>Virus isolation and identification</td>
<td>Confirmed infection</td>
<td>Requires acute sample</td>
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<td></td>
<td>Specific</td>
<td>Requires expertise and appropriate facilities</td>
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<tr>
<td></td>
<td>Identifies serotypes</td>
<td>Does not differentiate between primary and secondary infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expensive</td>
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</table>
### Viral RNA detection
- Confirmed infection
- Sensitive and specific
- Identifies serotype and genotype
- Results within hours
- Potential false-positives owing to 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

**Table 2: Medically Important Arboviruses**

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<tr>
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<th>Virus</th>
<th>Vector</th>
<th>Host</th>
<th>Transmission cycle</th>
<th>Incubation period</th>
<th>Clinical syndrome</th>
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<td>Tick</td>
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<td>R; H2H</td>
<td>1–3 (1–9)</td>
<td>FD, HS, (NS)</td>
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<td>Small mammals</td>
<td>R</td>
<td>5-15</td>
<td>FD, NS</td>
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<tr>
<td>Tahyna virus</td>
<td>Mosquito</td>
<td>Rodents, small mammals</td>
<td>U</td>
<td>3-7</td>
<td>FD, AR, (NS), conjunctivitis, pneumonia</td>
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<tr>
<td>Oropouche virus</td>
<td>Midge</td>
<td>Humans, Sloths, ? primates/birds</td>
<td>R, U</td>
<td>4-8</td>
<td>FD, AR, NS</td>
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<td>Toscana virus</td>
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<td>Humans, bats</td>
<td>R</td>
<td>2-14</td>
<td>FD, NS, (AR)</td>
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<td>Sandfly</td>
<td>Humans, rodents</td>
<td>R</td>
<td>2-14</td>
<td>FD</td>
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<td>Mosquito</td>
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<td>R; H2H</td>
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<td>FD, HS, NS, hepatitis</td>
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<td>Mosquito</td>
<td>Primates, humans</td>
<td>R, U; H2H</td>
<td>4-7 (3-14)</td>
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<td>Mosquito</td>
<td>Ardeid birds, pigs</td>
<td>R, U</td>
<td>5-14</td>
<td>FD, NS</td>
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<td>Mosquito</td>
<td>Birds</td>
<td>R, U; H2H</td>
<td>3-5 (2-14)</td>
<td>FD, NS, (AR)</td>
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<td>2-21</td>
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<td>Virus</td>
<td>Vector</td>
<td>Host</td>
<td>Transmission cycle</td>
<td>Incubation period</td>
<td>Clinical syndrome</td>
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<td>Mosquito</td>
<td>Ardeid birds</td>
<td>R</td>
<td>1-28</td>
<td>FD, NS</td>
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<td>Tick</td>
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<td>R</td>
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<td>FD, HS, conjunctivitis, pneumonia</td>
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<td>R</td>
<td>3-12</td>
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<td>7-14</td>
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<td>R, U; H2H</td>
<td>3-6</td>
<td>FD, HS, hepatitis</td>
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<td>Mosquito</td>
<td>Primates, humans</td>
<td>R, U; H2H</td>
<td>3-12</td>
<td>FD, AR, NR, conjunctivitis, congenital syndrome</td>
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<td>R; H2H</td>
<td>3-5 (0-20)</td>
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<td>Banna virus</td>
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<td>Barmah Forest virus</td>
<td>Mosquito</td>
<td>Birds, marsupials</td>
<td>R, U</td>
<td>7-9 (5-2)</td>
<td>FD, AR</td>
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<td>Eastern equine encephalitis virus</td>
<td>Mosquito</td>
<td>Birds, small mammals, marsupials</td>
<td>R</td>
<td>3-10</td>
<td>FD, NS</td>
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<td>Mosquito</td>
<td>Primates, humans</td>
<td>R, U</td>
<td>3-7 (1-12)</td>
<td>FD, AR, HS, NS, Conjunctivitis</td>
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<td>Family Genus</td>
<td>Virus</td>
<td>Vector</td>
<td>Host</td>
<td>Transmission cycle</td>
<td>Incubation period</td>
<td>Clinical syndrome</td>
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<td>Mosquito</td>
<td>Primates</td>
<td>R, U</td>
<td>6–12 (3–12)</td>
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<td>O’Nyong Nyong virus</td>
<td>Mosquito</td>
<td>Primates, humans</td>
<td>R, U</td>
<td>&gt;8</td>
<td>FD, AR</td>
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<td>Ross river virus</td>
<td>Mosquito</td>
<td>Marsupials, mammals</td>
<td>R, U</td>
<td>7–9 (3–21)</td>
<td>FD, AR, HS</td>
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<td>Sindbis virus</td>
<td>Mosquito</td>
<td>Birds</td>
<td>R</td>
<td>1–7</td>
<td>FD, AR</td>
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<td>Mosquito</td>
<td>Birds, small mammals</td>
<td>R</td>
<td>2–10</td>
<td>FD, NS</td>
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<td>Venezuelan equine encephalitis virus</td>
<td>Mosquito</td>
<td>Small mammals</td>
<td>R</td>
<td>&lt;1–5</td>
<td>FD, NS</td>
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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\textsuperscript{10})

LAST UPDATED BY ADMIN ON AUGUST 16TH, 2023

## Dengue

### Summary

- Up to 390 million infections annually, 500 thousand cases of severe dengue, with an estimated 36,000 deaths
- Vector: mosquitoes, Aedes species
• Flavivirus, 4 main serotypes (DEN 1-4)
• Infection with one serotype produces lifelong immunity against this serotype, but only short-lasting cross-protection against other serotypes.
• Main clinical presentations: fever, arthralgia-rash, hemorrhagic syndrome (FD, AR, HS)
• Plasma leakage is the hallmark of severe dengue
• WHO 2009 classification: Dengue and Severe Dengue (D/SD); Warning signs (WS) help clinicians identify cases in need of closer surveillance (dengue with warning signs [D +/- WS])
• No antiviral treatment is available at present, but mortality is greatly reduced by appropriate supportive treatment
• 2 Dengue vaccines are licensed: CYD-TDV (Dengvaxia®) should only be administrated to seronegative people, TAK-003 (Qdenga®) may be used in seropositive and seronegative individuals. Future efficacy and safety monitoring is warranted

**Virus**

Dengue viruses belong to the family of Flaviridae (yellow viruses), genus *Flavivirus*. The virus has a positive sense single-stranded RNA genome. It is translated into a large precursor protein, which is then cleaved by host-cell and viral proteases into three structural and seven non-structural proteins (See Figure 5).
Dengue virus has 4 main serotypes. Infection with one serotype results in lifelong immunity to subsequent infection with that particular serotype (homologous immunity). There is no lasting cross-protection between the serotypes (heterologous immunity).

In 2013, a 5th serotype (DEN-5) was described, of which the clinical significance is not yet understood. Contrary to DEN 1-4, it has a sylvatic transmission cycle, which may hamper current dengue control efforts.

**Epidemiology and transmission**

Dengue prevalence increased over 15-fold over the last two decades, attributable to three principal drivers: urbanization, globalization and lack of effective mosquito control. Dengue viruses have fully adapted to a human-*Aedes aegypti*-human transmission cycle in large, crowded urban centers in the tropics. In rapidly developing suburbs, running tap water is often lacking, and people depend on fetching water in small reservoirs. Sewage systems are often open and are ideal breeding sites for mosquitoes. Increased mobilization with more car tires, together with a surge in the use of plastics, contributes to mosquito propagation since water containing mosquito larvae is co-transported.
International travel can transport the virus to new regions with little mosquito control. Transported rubber car tires and lucky bamboo plants have been shown to carry *Aedes* spp. larvae.

Dengue virus infects an estimated 300-530 million cases annually, of which almost 100 million manifest clinically. The estimated annual death rate of 36,000 deaths due to dengue virus is relatively low, but high numbers of less sick dengue patients can overburden health structures. Dengue occurs in 129 countries and 70 percent of the burden is in Asia. As with other arboviruses, the geographic distribution of dengue is determined by the distribution of its vectors (See Figure 6). The main reservoir of the dengue serotypes 1-4 is probably man.

![Figure 6: Probability of dengue occurrence](image)

The bite of infected female *Aedes* mosquitoes transmits dengue. The virus develops a life-long non-cytocidal infection in the mosquito. It may infect the mosquito ovaries and offspring (transovarial transmission). *Aedes* eggs can withstand dehydration for several months, and eggs of some *Aedes* species survive for several years. This cycle can be repeated for multiple generations and drive new outbreaks. It takes at least one week from the egg’s hatching to the mosquito’s adult stage. This is essential information for understanding the “dry day” principle (see below). Infection of humans occurs when dengue virus is introduced into the skin via the insect’s saliva during a bite of female mosquitoes. *Aedes albopictus* is a less competent vector for dengue virus but survives in a more temperate climate. Global warming might therefore increase the population at risk for dengue.

Dengue transmission follows two patterns that are not mutually exclusive. “Epidemic” dengue occurs
when a single virus strain is introduced into a new region. Adults and children are affected, but dengue hemorrhagic fever is rare. In “hyperendemic” dengue, there is continuous circulation of multiple dengue serotypes. Seasonal variation is common and urban areas are particularly affected. Children are more at risk than adults, with a higher risk of dengue hemorrhagic fever.

**Clinical aspects**

Three-quarters of the estimated 390 million dengue virus (DENV) infections annually are clinically unapparent. These asymptomatic cases have the potential to contribute significantly more to virus transmission to mosquitoes than previously recognized, as high levels of viremia have been detected in infected people who do not have an interruption of their daily routine and who continue to have exposure to the virus’ vectors.

Symptomatic dengue infection begins with a sudden onset of a flu-like syndrome. The febrile phase lasts 2-7 days, and the fever is biphasic (saddleback fever) in 5 percent of cases. Skin rash, headache, myalgia and arthralgia are frequent symptoms. The rash may have a dengue-specific appearance of “white isles in a red sea” (Figure), but this finding has low sensitivity (up to 20%).
There may be marked muscle pain (breakbone fever), especially in the back and in the extraocular eye muscles (pain around and behind the eyes when looking sideways).

According to the 2009 WHO guidelines for diagnosis, treatment, prevention and control of dengue, a positive tourniquet test (aka. Rumpel-Leede or capillary fragility test) increases the probability of dengue in acute febrile illness. The sphygmomanometer is inflated around the upper arm to mid-systolic blood pressure. After the cuff is left in place for 5 minutes, more than 20 petechiae in a 3 cm diameter circle in the crease of the elbow indicate a positive test. Recent literature suggests that the tourniquet test is more effective in detecting true negative than true positive cases, and the test should not be used for diagnosing dengue.
Severe dengue

Severe dengue may be rapidly fatal and usually results from a second dengue infection more than 18 months after a resolved first infection. An estimated 500,000 people with severe dengue require hospitalization each year, a large proportion of whom are children.

Complications may develop after 3 to 5 days when the first fever subsides (defervescence), and endothelial dysfunction may lead to hemoconcentration. Patients may develop hemorrhage, ranging from petechiae, ecchymosis and purpura to overt bleeding from mucosal surfaces (epistaxis, melena), injection sites and cerebrovascular accidents. They may develop shock with plasma leakage; pleural or pericardial effusion or ascites can be observed by ultrasonography. Detection of an oedematous gallbladder wall by serial ultrasonography identifies patients at risk for developing severe dengue.

Prediction of severe dengue remains challenging, mainly because the determinants of a complicated course of dengue virus infection are poorly understood. Severe dengue was observed to occur more frequently in secondary dengue infections. In 1977, this led to the development of the concept of ‘Antibody-Dependent Enhancement (ADE). Secondary dengue infections were found to be correlated with higher levels of viremia. A molecular model to support the ADE hypothesis was described by Dejnirattisai et al. Briefly: Dengue infection leads to the development of homologous neutralizing and protective antibodies. Upon subsequent infection with a different serotype, these antibodies may enhance the replication of even immature virus particles. This results in higher levels of viremia (replication of both mature and immature virions), thereby increasing the release of pro-inflammatory cytokines and, thus, the severity of the disease.

The prevailing dengue serotype may be a determinant of severe dengue. This should probably also be evaluated against existing population immunity to previous dengue serotypes. In a recent meta-analysis, Soo et al. compared the percentage of severe cases of both primary and secondary infections with different serotypes of dengue virus. They found that the presence of certain serotypes, including primary infection with DENV-3 from the SEA region and secondary infection with DENV-2, DENV-3, and DENV-4 also from the SEA region, as well as DENV-2 and DENV-3 from non-SEA regions, increased the risk of severe dengue infections.

Apart from the fever, rash, arthralgia, hemorrhage and symptoms related to the plasma leakage syndrome, additional manifestations of dengue infection are described:

- Liver failure, which is caused by hypoperfusion or hypoxia rather than direct viral liver damage
- Neurological symptoms such as encephalopathy and seizures
**WHO dengue classification**

Recognizing severe dengue remains a challenge for the clinician. In 2009, WHO adopted a new classification of symptomatic dengue infections i.e., dengue with or without warning signs (WS +/-). While the performance of the triage based on the presence of warning signs (WS) need further validation across different clinical settings, this practical classification helps clinicians identify those patients in need of closer surveillance and/or hospitalization. Dengue warning signs include spontaneous or provoked bleeding, severe abdominal pain, persistent vomiting, painful hepatomegaly, dyspnoea, lethargy and effusions (see Figure 8). Severe dengue is defined by the occurrence of plasma leakage and/or fluid accumulation leading to shock or respiratory distress, and/or severe bleeding, and/or severe organ impairment.

![The WHO Classification (2009)](image_url)

Figure: WHO dengue Classification 2009

The former WHO classification (1975, revised in 1997) was derived from a pediatric population. It

- Cardiac manifestations, including myocarditis, arrhythmias and heart failure
- Secondary hemophagocytic lymphohistiocytosis

There is no specific treatment for dengue or severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. To facilitate the clinical management of patients with dengue virus infections, a new classification system was introduced by the WHO in 2009.
identified Dengue fever, dengue hemorrhagic fever and dengue shock syndrome (DF/ DHF/ DSS). It was used to classify disease severity for surveillance purposes. The main criticisms are summarized below:

1. poorly related to disease severity
2. misdirecting clinicians in identifying severe disease
3. difficult to use (tests required are often not available/difficult to apply)
4. does not help for triage in outbreaks
5. leads to different reporting globally due to the difficulties in using the classification for reporting clinicians.

Further comparison of the usefulness of the 1997 and 2009 WHO Dengue Case Classifications can be found in recent publications.

**Diagnosis**

(see also the section: Laboratory diagnosis of arboviral infections).

Common hematological abnormalities include leukopenia and thrombocytopenia. Both are poor predictors of disease severity. Increased hematocrit (≥20% increase) indicates severe disease since it can point towards plasma leakage syndrome and evolution to shock syndrome.

Biochemical abnormalities correlate with disease severity and organ failure. Increased transaminase levels and hypoproteinemia are observed in severe dengue. Proteinuria, where proteins as large as albumin are lost, occurs and is consistent with disruption in the function of the endothelial glycocalyx layer. Hyperferritinemia in dengue-infected patients is associated with immune activation and coagulation disturbances and may reflect macrophage activation.

Patients with dengue or other febrile illness usually seek medical attention within several days of fever onset. Documenting the day of symptom onset (day 0) is essential to choose a single specimen diagnostic approach. DENV viremia occurs 3–5 days before fever onset and continues for approximately 5 days into the febrile illness. Viremia can be detected by molecular assays targeting DENV RNA (such as RT-PCR) or by immunoassays targeting DENV nonstructural protein 1 (NS1) antigen. An anti-DENV IgM response becomes detectable by IgM-capture immunoassays (Enzyme-Linked Immuno Sorbent Assay (ELISA) or Immune Fluorescence Assays (IFA)) 3–5 days after onset of fever. IgM levels peak 6–10 days after fever onset and may persist for up to 90 days. IgG antibodies can be detected from day 7 onwards and may persist for life. Anti-dengue IgG-antibodies may
increase sooner in the event of secondary dengue infection. In view of these kinetics, laboratory diagnostic tests in a patient with suspected dengue infection should consider the day of symptom onset (Figure 9).

Flaviviruses share antigenic epitopes, which elicit cross-reacting antibodies. This cross-reactivity may result in false positive test results. To identify false positive test results or confirm true positives, virus neutralization tests can be performed. Because of the costs and technical expertise required, the use of these tests is mainly restricted to reference laboratories.
**Treatment**

No antiviral compounds are available for the treatment of dengue virus infections. Corticosteroids are not effective.

Most cases can be treated on an outpatient basis. Symptomatic treatment should avoid aspirin and NSAIDs (risk of bleeding), but paracetamol can be used. The patient or the parents of the sick child should be counseled on dengue complications. In-patient care is required if warning signs appear as these may predict severe dengue to occur on days 4-7 after symptom onset.

In the case of warning signs, isotonic crystalloid fluids such as Ringer’s lactate should be used to restore circulating blood volume. Fluid resuscitation requires observation in intensive care units. When the endothelial function recovers, fluid overload may cause iatrogenic complications. In patients with severe dengue infection, adjuvant therapy, including vasopressor and inotropic therapies, renal-replacement therapy and further treatment of organ impairment may be necessary.

Blood transfusion and fresh frozen plasma are sometimes required to treat severe bleeding. In case of massive bleeding, platelet transfusion may be needed in addition to packet cell transfusion. Platelet transfusion is warranted in patients with a platelet count <10,000/µl and active bleeding, but there is no benefit in prophylactic platelet transfusion without active bleeding.

**Prevention**

**Personal protection**

Contact with *Aedes* mosquitoes can be reduced using insect repellents. Sleeping at night under a bed net does not give any protection against *Aedes* sp. that bite during the day but can be useful for e. g., children sleeping during the day.

**Vaccination**

Immunity to dengue virus infections is complex, as is the development of dengue vaccines. As discussed under the section ‘Severe dengue’, dengue infection with one serotype leads to the development of lasting homologous neutralizing and protective antibodies, but it induces only short-term immunity against other (heterologous) serotypes. Because of antibody-dependent enhancement (ADE), infection with a second serotype may lead to more severe illness. Hence there is concern over increasing the risk of severe dengue by vaccination. After infection with 2 different serotypes, broad
immunity is observed.

Chimeric Yellow Fever-Dengue-Tetravalent Dengue Vaccine or CYD-TDV (Denvaxia®) is a tetravalent, live attenuated, chimeric vaccine and combines four chimeric yellow fever 17D-dengue vaccine viruses, where the premembrane and envelope proteins from each of the four DENV types replace the same proteins in a yellow fever 17D backbone virus. Three doses at months 0, 6 and 12 are administered. CYD-TDV is now used in about 20 countries in Latin America and Southeast Asia as part of their dengue control program after a study had shown an 80.3% efficacy against hospitalization and a 56.5 – 60.8% efficacy in contracting dengue disease in children. An additional analysis with retrospective determination of serostatus at the time of vaccination showed that children that were seronegative at the time of the first vaccination had a higher risk of developing severe dengue. Vaccination is therefore limited to people living in endemic areas ranging from 9-45 years of age who have had at least 1 documented dengue virus infection previously. This pre-vaccination screening for past dengue disease complicates the rollout of this vaccine in many low-resource settings.

TAK-003 (Qdenga®) is a tetravalent live attenuated DENV-2 virus with chimeras replacing the premembrane- and envelop genes of the DENV-2 with those from wild-type DENV-1, DENV-3 and DENV-4 strains. Two doses at months 0 and 3 are administered. The overall vaccine efficacy in children and adolescents 4 to 16 years of age was 80.9 % and 73.3 % at 12 and 18 months of follow-up, respectively. There was a 90.4 % efficacy against hospitalization for dengue. The vaccine efficacy was slightly higher among the baseline seropositive than baseline seronegative, without increased risk of severe dengue. Since DENV-2 was the backbone of TAK-003, efficacy was highest against DENV-2. There was no efficacy against DENV-3, and data were insufficient to evaluate efficacy against DENV-4.

Vector control

See general section.

LAST UPDATED BY ADMIN ON OCTOBER 6TH, 2023

Chikungunya
Summary

- Togavirus family, genus alphavirus
- Vector: mosquito, Aedes species
- Main clinical presentation: arthralgia/rash, febrile disease (AR, FD), frequently post-Chikungunya rheumatic syndrome

Virus

Chikungunya virus (CHIKV) is a single-strand RNA virus of positive polarity; its genome encodes 4 non-structural (nsP1-4) and three structural proteins (C, E1, E2). Phylogenetically, there are 3 distinct genotypes: the West African, the Asian and the Eastern-Central-South African genotype.

Figure: Structure of Chikungunya virus (Weaver et al, NEJM)
Transmission

Chikungunya virus was isolated during an epidemic in Tanzania in 1952 from both patients and mosquitoes. It has since been isolated frequently from humans and mosquitoes in tropical Africa, India and Southeast Asia, where large epidemics occur from time to time. Non-human reservoir species have not been identified unequivocally. Both Aedes aegypti and A. albopictus are vectors.

In 2004, there was an outbreak of Chikungunya fever in Kenya. The next year it reached the Comores. In 2005-6, outbreaks followed in Reunion (with 265,000 clinical cases out of a population of 770,000), Mauritius, Madagascar and other islands in the Indian Ocean. In Reunion, mortality was 237 deaths, about 1 per 1000 clinical cases. A single mutation (A236V) was identified in chikungunya virus strains in the 2005-2006 Reunion Island outbreak, that facilitated transmission by the Asian tiger mosquito (A. albopictus). CHIKV was capable of spreading via travellers, as was witnessed in July 2007, when about 160 people in Ravenna province, Italy fell ill. This was the first example of Chikungunya transmission via exotic mosquitoes (Aedes albopictus) outside the tropics.

Figure: Distribution of Chikungunya (Weaver et al, NEJM)
Contrary to expectations and reports of introduction of so-called Indian Ocean Lineage of the ECSA genotype by travellers into the Americas, it was an Asian-lineage Chikungunya virus strain that caused a major epidemic in the Americas. The strain was introduced into the island of St. Martin in October 2013.

Clinical aspects
The clinical picture resembles that of classic dengue fever with which chikungunya fever is often confused. After a brief incubation period of 2 to 5 days, there is sudden onset of fever followed by crippling joint pains that may temporarily incapacitate the patient. In the Makonde language, “chikungunya” means “doubled up; that which bends up”, referring to this important arthralgia. Conjunctivitis and skin rash are common. Arthralgias occur in around 70 percent of cases and can persist for weeks to months. If no complications ensue, recovery takes 5 to 7 days. New severe clinical forms were reported in Reunion, including cases caused by peripartum mother-to-infant transmission. Rare complications include meningoencephalitis (also in newborns) and probably hepatic failure (possible role of high doses of acetaminophen). Common hematologic abnormalities in the acute phase include lymphopenia and thrombocytopenia that may lead to bleeding. Hepatic enzymes are commonly increased.

Chronic joint pains can be persistent or relapsing. These arthralgias are located mostly in the distal joints and may be associated with arthritis and may mimic rheumatoid arthritis (chronic inflammatory, erosive, and rarely deforming polyarthritis) in up to 50% of patients.

Diagnosis
Diagnosis in endemic areas is clinical, although it is very difficult to discriminate from co-circulating arboviral infections. A definitive diagnosis relies on virus detection through reverse-transcriptase–polymerase-chain-reaction (RT-PCR) testing during the viraemic phase (the first week). RT-PCR can be designed in a multiplex format to simultaneously detect several other arboviruses, such as dengue virus, which can be very useful for triage of patients. Chikungunya virus culture in a variety of cells permits further virologic characterization but has no added value over RT-PCR in clinical practice and is not performed routinely.

Sero-diagnosis is facilitated by the limited antigenic diversity of chikungunya virus and extensive cross-reactivity of the antibodies induced by different strains. Serum IgM is detectable from day 5 (and even earlier) to several months after the onset of illness and is also considered diagnostic. Seroconversion can also be detected as a fourfold increase in IgG between acute-phase and
convalescent-phase serum samples.

Figure: Chikungunya diagnostics in relation to kinetics of viremia and antibody response (Johnson et al, J Infect Dis)

**Treatment**

Treatment is symptomatic. Post-chikungunya rheumatism may require long-term treatment with nonsteroidal anti-inflammatory drugs or Disease Modifying Anti Rheumatic Drugs (DMARDs) such as methotrexate, although their safety and efficacy also have yet to be demonstrated in clinical trials.

LAST UPDATED BY ADMIN ON JULY 14TH, 2022

**Zika virus**
Summary

- Flavivirus, belongs to Spondweni serogroup
- Vector: mosquito, Aedes species; human to human transmission occurs (sexually)
- Main clinical presentation: Artralgia/ rash, Febrile disease, neurological syndrome (AR, FD, NS), conjunctivitis, congenital syndrome
- WHO declared the Zika virus epidemic in the Americas a Public Health Emergency of International Concern (PHEIC), because of its association with microcephaly and other neurodevelopmental disorders

Virus

Zika virus (ZIKV) is a member of the virus family Flaviviridae, genus Flavivirus. It is a 40-nm virus and has icosahedral symmetry. ZIKV has a non-segmented, single-stranded, positive sense RNA genome.

Transmission

Prior to the 2007 outbreak in the Yap islands (Micronesia), no outbreaks and only 14 cases of human ZIKV disease had been recorded, although sero-surveillance studies in Africa had already indicated anti-ZIKV antibody presence of ca. 6% in some populations. The Yap outbreak indicated that the virus could now spread in human communities and establish a so-called urban transmission cycle. The biggest epidemic occurred in 2015-2017 in the Americas with spread to several countries in Asia. In 2016 the incidence peaked in the Americas and declined substantially throughout 2017 and 2018 probably due to herd immunity. In 2020, a total of 87 countries have had evidence of autochthonous transmission of Zika virus.

The reservoir of ZIKV are primates, both human and non-human. The virus is primarily transmitted by mosquitoes from the genus Aedes, most commonly Aedes aegypti. However sexual transmission of the virus (male to female, male to male, female to male) from symptomatic or asymptomatic persons is now well established. Uncertainty remains over the duration of infectivity of one person.

Table 4 A brief history of Zika virus infections

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1947</td>
<td>ZIKV was first detected from rhesus monkey in Uganda.</td>
</tr>
<tr>
<td>1952</td>
<td>First human case has been identified in Uganda.</td>
</tr>
<tr>
<td>1968</td>
<td>ZIKV has been reported from Nigeria.</td>
</tr>
</tbody>
</table>
1951-1981 | Incidences of this virus have been reported from various countries of Asia and Africa.

2007 | The first outbreak was reported in Yap Islands, part of the Federated States of Micronesia. Prior to this event, no outbreaks and only 14 cases of human Zika virus disease had been documented worldwide. Zika virus infection is estimated to be asymptomatic in 80% of cases.

2012-2014 | Cases have been reported from Thailand.

2013 | The virus spread to French Polynesia with an estimated 28 000 cases. ZIKV rapidly spreads to the Cook Islands and Easter Island. An association of Zika virus with Guillain Barré Syndrome is observed.

2015 | Zika virus infection was first diagnosed in Brazil. It was found to be associated with microcephaly in the infants of mothers with suspected ZIKV infection.

February 2016 | WHO declares the Zika virus epidemic in the Americas a Public Health Emergency of International Concern (PHEIC) because of its association with microcephaly and other neurodevelopmental disorders.

2015-2017 | Epidemic in the Americas with 500,000 symptomatic cases reported at the peak of the pandemic in 2016.
Alternative transmission routes: Perinatal, sexual, breastfeeding, or blood transfusion

Sylvatic cycle

Urban cycle

Figure: Important transmission routes of Zika virus (Blázquez A-B et al, World J Virol)
Clinical aspects

Symptomatic ZIKV infections

After a mosquito bite, the incubation period is 3-12 days, with a mean of 5.9 days (95% credible interval, CrI: 4.4–7.6), and 95% of people who developed symptoms doing so within 11.2 days (95% CrI: 7.6–18.0) after infection. Approximately 20% of patients are symptomatic. They can present with acute onset of low-grade fever with maculopapular rash, arthralgia or non-purulent conjunctivitis. These symptoms feature in the (E)CDC case definition. Other commonly reported clinical manifestations are lymphadenopathy and ulcers on the mucous membrane are less common. Thrombocytopenia, palatal petechiae, and uveitis have been reported. In adults, ZIKV infection generally produces very mild disease. Infants and young children may present with irritability, walking with a limp, difficulty moving an extremity. There may be pain on palpation, or pain with active or passive movement of the affected joint.
**Guillain Barré syndrome**

Guillain-Barré Syndrome (GBS) is a post-infectious peripheral autoimmune neuropathy, characterized by progressive weakness of the limbs and absent or depressed deep tendon reflexes and cytoalbuminologic dissociation in cerebrospinal fluid (CSF) examination. Several electro-myographic (EMG) types exist. Up to 25% of those affected may require mechanical ventilation. Mortality is estimated at 3-5%. Global incidence of GBS varies from 0.8-1.9/100,000.

The incidence of ZIKV-associated GBS is estimated to be 2 to 3 cases per 10,000 ZIKV infections. The median time before onset of neurological symptoms was 6 days.

**Neurodevelopmental disorders**

The most disconcerting finding is the association of ZIKV infection with neurodevelopmental disorders. Health care personnel and authorities in Brazil observed a sharp increase in the number of neonates born with congenital microcephaly and found an epidemiological association with the ZIKV epidemic which hit Brazil early in 2015.

![Source: Data published by the Pernambuco State Secretary of Health, Brazil.](image-url)
Microcephaly is defined as Head Circumference (HC) at birth less than the 3rd percentile for gestational age and sex.

Maternal-fetal ZIKV transmission can occur in all trimesters of pregnancy. There is no suggestion that pregnant women are more susceptible to ZIKV infection and there is no evidence of greater severity of this infection during pregnancy.

20-30% of fetuses and neonates will become infected when mothers are infected during pregnancy. This will lead to foetal loss in 14%, to congenital Zika syndrome in 21% with microcephaly in about half the cases. 80-90% of all fetuses exposed to Zika (not necessarily vertically infected) will be asymptomatic during the first weeks of life. Follow-up is needed to know whether longer term sequelae (learning difficulties, ...) in this last group will occur. Not just the brain that is affected in the congenital zika syndrome: infants from ZIKV infected mothers frequently show retinal defects, such as chorioretinal atrophy surrounded by a hyperpigmented halo and hyperpigmented mottling. Hence, the
neurodevelopmental disorders observed in neonates and children after ZIKV infection of the mother can be referred to as Zika virus congenital syndrome.

**Significance of asymptomatic ZIKV infections and sexual transmission**

At present, approximately 80% of ZIKV infected patients are thought to have no clinical manifestations of infection. In areas where suitable mosquito vectors are present, these patients will add to the reservoir and fuel the epidemic. It is estimated that 1% of ZIKV infections reported in Europe and the United States were acquired through sexual transmission. ZIKV RNA is detected up to 3±0 days after onset of symptoms, but shedding of infective virus is unlikely to occur after 30 days from the onset of illness.

**Diagnosis**

Laboratory diagnosis is needed to confirm the diagnosis of ZIKV infection.

Specific laboratory diagnosis is based on detection of viral RNA from clinical specimens by RT-PCR. The window of detection in blood samples is a period of 1–5 days after the start of symptoms. However, the sensitivity of RT-PCR is estimated to be 40%. Because of the longer persistence of the virus in urine, RT-PCR on urine can be attempted up to the 15th day after the start of symptoms. Seroconversion (detection of anti-ZIKV IgM antibodies) is thought to occur from the 4th day after infection and IgG a little later. Seroconversion occurs on average at 9 days and 95% by 14 days.
As with other serological tests for flavivirus infections, cross-reactivity of ZIKV antibody detection assays can yield false positive results; in endemic areas this may be a significant problem, because of possible simultaneous or previous circulation of other flaviviruses. Virus neutralization tests can be used to increase specificity.

**Treatment**

**General**

There is no specific antiviral treatment for treating ZIKV. Antipyretics or analgesics can be used for symptom relief. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) should not be used until dengue has been ruled out. NSAIDs should not be used in pregnant women beyond the 32nd week of gestation because of the risk of early closure of the arterial duct.

**Management of pregnant women**

Current CDC recommendations for the management of pregnant women with ZIKV infection include:
• Use of serial ultrasound examinations.
• In case of a confirmed diagnosis of fetal microcephaly, amniocentesis should be considered from the 15th week of pregnancy onwards.

**Management of microcephaly/ ZIKV congenital syndrome**

There is no specific treatment for microcephaly. Microcephaly may be accompanied by epilepsy, cerebral palsy, delayed cognitive, motor and speech development and hearing and eyesight problems. Since each child develops complications of different type and severity (eg. respiratory, neurological and motor problems), follow-up by specialists in different fields is warranted.

**Guillain Barré Syndrome**

Treatment of GBS in the acute phase consists of immunotherapy, such as plasmapheresis or application of human immunoglobulin (IVIG, dose: 400 mg/ kg of body weight per day, for a period of 5 days). IVIG is relatively simple to administer, however expensive and can be difficult to obtain. The best results of IVIG or plasmapheresis are obtained when it is started within the first 2 weeks after the onset of neurological symptoms. Use of corticosteroids as a stand-alone treatment does not accelerate the recovery or alter the long-term result.

**Prevention**

A vaccine is not yet available.

LAST UPDATED BY ADMIN ON JULY 14TH, 2022

**Yellow fever**

**Summary**

• Flavivirus, prototype
• Zoonosis
• Endemic and epidemics in Africa, South America.
• Vector: mosquito, Aedes species
• Main clinical presentations: Fever, haemorrhagic syndrome (FD, HS), hepatitis
Effective vaccine available

**Virus**

The Yellow Fever virus (YFV) is the prototype virus of the family Flaviviridae, a group that also includes the epidemic arthropod-borne viruses causing dengue, Japanese encephalitis (JE), and Zika. It is an enveloped positive-sense, single-stranded RNA virus. The genome presents a single open reading frame encoding a polyprotein. Host proteases cut this polyprotein into 3 structural (C, prM, E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).

**Transmission**

Yellow fever is a zoonosis, caused by infection with Yellow Fever Virus (YFV). Yellow fever causes 200,000 infections and 30,000 deaths every year with nearly 90% of these occurring in Africa. It is endemic to large parts of Africa and South America. Its vectors are mosquitoes belonging to the *Aedes* genus. YFV maintains a sylvatic cycle mosquito-monkey-mosquito. In monkeys, viraemia lasts 2-9 days. African monkeys do not die from the infection. Once infected African monkeys develop a lifelong immunity. In South American monkeys, the infection is often fatal. Sometimes large numbers of animals die.

Humans can be infected when they enter this biotope during the day, resulting in sporadic cases of yellow fever (sylvatic or ‘jungle’ transmission). Upon returning in their communities, infected persons may infect peridomestically living *Aedes* mosquitoes (notably *Aedes aegypti*). Subsequent transmission by peridomestic mosquitoes can take on epidemic proportions (epidemic or urban yellow fever).

Large outbreaks occurred Ethiopia (1960-62, 30,000 to 100,000 deaths), Senegal (1965, 2000 to 20,000 cases), Nigeria (1969, 1986 and 1988-1990), Uganda (2010), and Sudan (2003, 2005, 2012-2013) and Ethiopia (2012–2013). In 2016, Angola suffered an outbreak of yellow fever. Authorities have reported at least 3,867 suspected and confirmed cases nationally, including 369 deaths. There are frequently small outbreaks. The southern part and the east coast of Africa are relatively free of the disease. In South America, recent outbreaks affected southern Brazil, Paraguay and Argentina (2007-2009).

Early 2016, sporadic yellow fever cases were introduced into China from Angola, where a large Chinese workforce is present. Since suitable vector species are also widespread in Asia, the prospect of sustained introduction of viraemic travellers raises the possibility of a yellow fever epidemic in Asia.
Urban yellow fever transmission in an unimmunized population is a major public health concern.

In order to prevent yellow fever from being imported, many countries where yellow fever does not occur require proof of vaccination following a recent visit to an endemic country.

Clinical features

Yellow fever begins after an incubation period of 3–6 days. It presents as a flu-like syndrome, with fever, chills, headache, backache, muscle aches, fatigue and vomiting. This phase lasts 3-4 days. A second febrile episode develops in 15% of infected persons (biphasic fever), characterised by mild jaundice (yellow or toxic phase). Liver (transaminases up to 15,000-40,000 IU/l) and kidney failure occurs. There is no splenomegaly. The patient’s general condition then deteriorates dramatically, with haemorrhaging (skin, mucosa, uterus, intestines), hypotension and shock. Gastric bleeding (“Vomito Negro”) is an indication of an extremely poor prognosis. There is considerable kidney involvement (proteinuria, oliguria). There is no real encephalitis but neurological signs such as convulsions can occur due to cerebral bleeding as well as hepatic encephalopathy.

Death occurs mainly between 7-10 days. If the patient survives after 12 days, complete recovery can be expected. Surviving the infection results in lifelong immunity and normally there is no permanent organ damage.

The toxic phase is fatal in 20 – 50 % of cases, resulting in an overall fatality rate for yellow fever of 3.0 to 7.5 %. Case fatality appears lower in Africa (20%) than in South America (40–60%); this suggests that genetic factors determine lethality of the infection.

The differential diagnosis of any case of fulminating hepatitis in an endemic area should include yellow fever, particularly if there is haemorrhaging and kidney involvement. If confirmed, the authorities must be made aware of it and the WHO notified.

Diagnosis

A presumptive diagnosis of yellow fever is often based on the patient’s clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where the presumed infection occurred.

Laboratory diagnosis of YFV faces several challenges, such as a lack of commercial test kits, a lack of biosafety level 3 (BSL3) laboratories for virus isolation and the presence of serological cross-reactivity
with other flavivirus infections. Current WHO recommendations for laboratory confirmation of YFV entail testing for specific IgM antibodies and/or a ≥4-fold increase in the specific serum IgG level when other flaviviruses are ruled out.

Antibody detection assays (IgM antibody capture by enzyme-linked immunosorbent assay (MAC-ELISA), hemagglutination inhibition (HI), complement fixation (CF) and virus neutralization tests (VNT)) can be used for the diagnosis of YFV. However, anti-YFV IgM is detectable only from 5 days after the onset of symptoms, when the severity increases. There is cross-reactivity with other flaviviruses.

Yellow fever may be diagnosed on samples obtained during acute illness by the isolation of the virus in mosquito cell lines or by genome detection through PCR-based methods. A negative test result does not rule out infection.

Antigen detection: Antigen detection is only positive in serum during the first 3 days of illness. Monoclonal antibody-based antigen detection by ELISA are being developed, but they are currently not commercially available. Immunohistochemical detection of YFV antigen is performed on tissues in reference laboratories for post-hoc diagnosis.

**Treatment**

No anti-viral treatment is available for the treatment of YFV infection. Ribavirin reduced mortality and hepatocellular dysfunction in a hamster model, but was not effective in non-human primates.

Supportive treatment reduces mortality. This requires hospitalization and close monitoring of vital functions and fluid balance. Hypotension, hypoxaemia and hypoglycaemia must be prevented or corrected.

Kidney failure often has a combined aetiology here. Pre-renal failure can be corrected by giving fluid. Renal replacement therapy might be indicated for patients with acute tubular necrosis.

**Prevention**

There are 3 main strategies for preventing Yellow Fever virus infections.

1. Vaccination
2. Isolation of patients
3. Vector control

**Vaccination**

There is a very efficient vaccine. This consists of a live attenuated virus (17D strain). It is cultured on embryonated chicken eggs and is stored in freeze-dried form. After adding solvent the reconstituted vaccine is administered subcutaneously. A single vaccination offers lifelong protection in immunocompetent persons from 10 days after the injection. In rare cases post-vaccination encephalitis has been reported in babies (younger than 4 months) and the vaccine is therefore not recommended for children under 9 months of age. Other contra-indications to vaccination are pregnancy (except during a yellow fever outbreak); severe allergies to egg protein; and people with severe immunodeficiency.

Routine vaccination is part of the Extended Programme of Immunisation (EPI) of in number of endemic African countries. In the event of an epidemic vector control and a mass vaccination campaign is essential. The WHO keeps a special stock of yellow fever vaccine available to combat epidemics. During a big Yellow Fever outbreak in 2016, millions of people were vaccinated and there was a threat for an international stock rupture. WHO authorized the use of fractional dose (one-fifth the usual dose) during the outbreak. A follow-up study showed that 98% of people had developed sufficient antibodies.

**Isolation**

During outbreaks; patients should be isolated in mosquito-free rooms. Medical staff should take personal protective measures: blood and body fluids of patients are infectious during the first few days. Staff and family members must be vaccinated. Suspected cases should be held in quarantine for the duration of the maximum incubation period, which is 6 days.

**Vector control**

Vector control efforts should target both peridomestic and sylvatic vectors: improving basic sanitation, improving the water supply and destroying breeding grounds. Sylvatic vectors have to be combated with appropriate agents.
Japanese encephalitis

Summary

- Flavivirus, belongs to JEV serogroup
- Vector: mosquito, Culex species
- Main clinical presentation: Febrile disease, neurological syndrome (FD, NS)
- Vaccine available.

Virus

JEV is the prototype virus of the JE serogroup Flaviviruses, which also includes several medically important etiological agents of encephalitis (see below). Taxonomically, JEV is closely related to other clinically important flaviviruses, including yellow fever virus (YFV), dengue virus, and tick-borne encephalitis virus. Like all flaviviruses, JEV is a small enveloped virus, with a single-stranded positive-sense RNA genome. The genome encodes a single long open reading frame (ORF) flanked by 2 short non-coding regions (NCRs) at the 5′ and 3′ ends.

The Japanese encephalitis serological group of flaviviruses counts 8 virus species and 2 subtype viruses with an extensive geographic distribution (Figure 11):

Japanese encephalitis virus (JEV) in South-east Asia, Papua New Guinea and the Torres Strait of northern Australia.

West Nile virus (WNV) in Africa, southern and central Europe, India, the Middle East and North America.

Kunjin virus (a subtype of WNV) in Australia and Papua New Guinea.

Murray Valley encephalitis virus (MVEV) in Australia, Papua New Guinea and the western Indonesian archipelago

St. Louis encephalitis virus (SLEV) in North and South America.

Other minor members of the group are Usutu (USUV), Koutango and Yaounde viruses in Africa; Cacipacore virus in South America; and Alfuy, a subtype of MVEV, in Australia. Most members have
avian vertebrate hosts and are vectored primarily by Culex spp. mosquitoes.

Figure 11 Global distribution of Japanese Encephalitis serogroup flaviviruses (Mackenzie et al, Nat Med)

**Transmission**

JEV is the most important cause of viral encephalitis SEA, with 30,000–50,000 cases reported annually, although this may be a considerable underestimate because of inadequate surveillance and reporting. JEV is amplified in an enzootic cycle that involves mosquito vectors (mainly Culex species) and vertebrate hosts (primarily pigs and birds) (Figure 12). JEV is occasionally transmitted to dead-end hosts, such as humans and horses.
Clinical features

The incubation period for JEV is 5-15 days. Most infections remain asymptomatic, with estimates of the ratio of symptomatic to asymptomatic infection from 1 in 25 or lower. Sero-surveys in JEV endemic areas have shown that the majority of adults have been exposed to JEV. As with other flaviviruses, the determinants of clinical disease manifestation are ill understood, but are likely to include endemicity, exposure to mosquitoes, pre-existing antibodies to flaviviruses and virus strain differences. Clinical disease often starts with unspecific febrile illness. In neuroinvasive JEV infections, patients usually seek consultation a couple of days after a prodromal syndrome, when meningeal irritation, headache, stupor, coma and convulsions occur. Classical description of Japanese encephalitis includes a Parkinsonian syndrome with a mask-like face, wide unblinking eyes, tremor, generalized hypertonia, cogwheel rigidity and other abnormalities of movement. Along with upper motor neuron signs, cerebellar signs and cranial nerve palsies may occur. Paralysis of the upper extremities is more common than that of the legs. Persistent motor deficits are common (30%), as are severe cognitive and language impairment (20%).
When performing lumbar puncture, CSF opening pressure is increased in about 50% of patients. High pressures (>250 mm) are associated with a poor outcome. Typically, there is a moderate CSF pleocytosis (10–100 cells/mm³), with predominant lymphocytes, mildly increased protein (50–200 mg%) and a normal glucose ratio. However, polymorphonuclear cells may predominate early in the disease, or there may be no CSF pleocytosis.

In about 50% of patients CT shows bilateral non-enhancing low-density areas in one or more of the thalamus, basal ganglia, midbrain, pons and medulla. Magnetic resonance imaging is more sensitive, typically demonstrating more extensive lesions, (typically high signal intensity on T2 weighted images) of the thalamus, cerebral hemispheres, and cerebellum. Thalamic lesions of mixed intensity may also be seen on T1 and T2 weighted scans suggesting haemorrhage.

Encephalitis has a high mortality rate (25-30%). Pregnant women are at risk of intra-uterine infection and death of the foetus during the first two trimesters.

**Diagnosis**

Anti-JEV immunoglobulin M (IgM) is produced soon after infection and is detectable in 90% of cases in cerebrospinal fluid (CSF) by 4 days and in serum by 7–9 days following the development of clinical illness. Anti-JEV IgM is less cross-reactive and therefore more specific than IgG. WHO recommends JEV-specific IgM antibody capture ELISA (MAC ELISA) as the first-line serological assay to diagnose acute JEV infection. However Serology MAC ELISA underestimates recent infection with Japanese encephalitis virus, in comparison to real time reverse transcriptase PCR

The diagnosis can be made by isolating the virus from the cerebrospinal fluid early in the disease or by serology, but it is not a sensitive method of laboratory diagnosis in clinical specimens because the low-level transient viremia is cleared soon after onset of illness.

**Treatment**

As with other flaviviruses, treatment for Japanese encephalitis is supportive. Convulsions and raised intracranial pressure should be treated when they occur. Randomized controlled trials failed to show benefit for the use of corticosteroids, interferon-alpha-2a or ribavirin. Intravenous Immunoglobulins (IVIG) produced in countries where flaviviruses are endemic contains high titres of specific neutralizing antibody, because most of the population have been exposed to the virus. A recent pilot study (2016) cleared the way for a phase III trial of treatment of JEV with IVIG in Nepal.
Prevention

Given its endozootic life cycle JEV cannot be eradicated. In absence of effective antiviral therapy, vaccination is the most important tool to control human JEV infections.

Vaccination

Four different vaccines are available, but all induce only short-term immunity. The vaccine IXIARO® (2 injections, on day 1 & 28) is approved in Europe for people aged 18 years and older. Indications for vaccination in travellers include people who travel at least 3-4 weeks in a rural endemic area or who intend to live in these areas for longer periods even in an urban environment. After a 2-dose primary immunization schedule (0-28 days), the seroprotection rate declines from 8% at 1 month to 48% at 24 months but reconversion is complete with a booster after 1 or 2 years. In older people the vaccine can be given safely, but a 3rd dose may be needed at primary immunization.

Vector control

In addition to vaccination, Japanese Encephalitis can be prevented by vector control measures, see also general section.

West Nile virus

Summary

- Flavivirus, belongs to JEV serogroup
- Vector: mosquito, Culex species
- WNV neuroinvasive disease is an important clinical syndrome with up to 15% mortality and frequently accompanied by long-term sequelae

Virus

West Nile Virus (WNV) was originally discovered in 1937 in the West Nile district of the Northern Province of Uganda. It belongs to the Flaviridae. It belongs to the Japanese Encephalitis group
flaviviruses (see section on Japanese Encephalitis). Kunjin virus is regarded as a variant of West Nile Fever Virus. There is a considerable variation between different strains (isolates).

Transmission

WNV is transmitted by mosquitoes, mainly Culex species. Culex univittatus and C. pipiens are the main vectors in Africa and the Middle East. WNV has also been known to circulate Southern Europe (Romania, southern France, Spain), Israel, Asia, the Ukraine and Southern Russia. The main reservoir is probably formed by viraemic birds and a zoonotic mosquito-bird-mosquito cycle is assumed. Many bird varieties can be infected and can be viraemic for a long time (amplifying host). Since 1999 WNV has become endemic in the USA and Canada, where it demonstrates seasonality: 90% of infections occur in August and September.

One of the reasons why West Nile virus has spread so rapidly in the United States, is due to a hybrid mosquito species (Culex pipiens s.s. X Culex pipiens molestus), which bites both birds (ornithophilic) and man (anthropophilic). The infection usually takes a subclinical course in birds, but in an outbreak of West Nile Fever-like virus (afterwards confirmed as being West Nile Fever virus) in Queens in New York in the autumn of 1999 hundreds of birds in this city died from the infection (mainly crows, magpies and a few flamingos in the Bronx zoo). Prior to this the virus was unknown in the New World.

The infection is not transmitted directly from man-to-man, but it can be transmitted by blood transfusion, organ transplantation and breast feeding.

Kunjin virus occurs in Australia, Papua New Guinea (including Saibai island in the Torres Strait) and Borneo.

Geographical distribution

Past epidemics occurred in South Africa in 1974 (with more than 3000 clinical cases), the Camargue (France) and the Ebro delta (Spain). From 1999 through 2010, 3 million WNV infections are thought to have occurred in the USA, resulting in 780 000 clinical illnesses. From 1999-2012 the USA have recorded 16,196 patients with WNV neuroinvasive disease and 1549 deaths.

Clinical aspects

Incubation period varies from 3-15 days. Many infected patients experience a subclinical infection or a mild flu-like syndrome. Symptomatic patients present with headache, generalized weakness, morbilliform or maculopapular rash (often at time of defervescence), fever (often low grade, lasting 5
days on average), myalgia. Less commonly reported symptoms are joint pains, chills, painful eyes, vomiting or diarrhoea and lymphadenopathy.

**Table: Symptoms experienced by WNV viraemic blood donors in 14 days preceding donation**
*(Zou et al, J Infect Dis)*
Neuroinvasive disease occurs in less than 1% of those infected by a mosquito bite and appears more frequent in elderly persons. The risk may approach 1 in 50 among persons aged at least 65 years, a rate 16 times higher than that for persons aged 16 to 24 years. In addition, a history of cancer,

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. (%) of donors with symptom</th>
</tr>
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<tbody>
<tr>
<td>Headache&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125 (75)</td>
</tr>
<tr>
<td>Generalized weakness&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125 (75)</td>
</tr>
<tr>
<td>New rash&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97 (58)</td>
</tr>
<tr>
<td>Fever&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94 (56)</td>
</tr>
<tr>
<td>Severe muscle pain&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90 (54)</td>
</tr>
<tr>
<td>Joint pain&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81 (49)</td>
</tr>
<tr>
<td>Chills&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79 (47)</td>
</tr>
<tr>
<td>Painful eyes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67 (40)</td>
</tr>
<tr>
<td>Vomiting or diarrhea</td>
<td>45 (27)</td>
</tr>
<tr>
<td>Swollen glands</td>
<td>36 (22)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>31 (19)</td>
</tr>
<tr>
<td>New difficulty thinking</td>
<td>29 (17)</td>
</tr>
<tr>
<td>Bone pain</td>
<td>27 (16)</td>
</tr>
<tr>
<td>Tremor</td>
<td>4 (2)</td>
</tr>
</tbody>
</table>
diabetes, hypertension, alcohol abuse, or renal disease also increases the risk.

Other host factors associated with an increased risk of neuroinvasive disease are and chemokinereceptor CCR5 deficiency (which diminishes the risk for HIV infection) as well as male sex.

Figure: Potential mechanisms for neuroinvasion of West Nile virus (Petersen et al, JAMA)

**Mechanism of neuro invasion**

Neuroinvasive disease occurs in less than 1% of those infected by a mosquito bite and appears more frequent in elderly persons. Potential mechanisms for neuroinvasion of West Nile virus include (1) direct infection of the vascular endothelium and subsequent entry to the central nervous system, (2) viral passage through the vascular endothelium due to disruption of the blood-brain barrier integrity by vasoactive cytokines, (3) a Trojan horse mechanism through which infected monocytes are trafficked into the central nervous system, or (4) retrograde axonal transport to the central nervous system following infection of peripheral neurons.

Reported clinical syndromes of WNV neuro-invasive disease are:
Meningitis, characterized by clinical signs of meningeal inflammation, including nuchal rigidity, Kernig or Brudzinski sign, or photo- or phonophobia.

Encephalitis characterized by depressed or altered level of consciousness, lethargy or personality change lasting more than 24 hours.

Acute flaccid paralysis, characterized by acute onset of limb weakness with marked progression over 48 hours, which is usually asymmetric, areflexic or hyporeflexic, and without sensory abnormalities. 80% of acute flaccid paralysis cases occur in conjunction with encephalitis or meningitis.

Examination of CSF of patients with neuroinvasive disease shows normal glucose, elevated protein (generally <150 mg/dL) and moderate pleocytosis (generally <500 cells/μL) usually with a predominance of lymphocytes; however, neutrophils may predominate in early infection.

Imaging studies are usually normal, but focal lesions in the pons, basal ganglia, thalamus and anterior horns, and enhancement of the leptomeninges, the periventricular areas or both are occasionally seen. These lesions may appear hyperintense on T2-weighted magnetic resonance and fluid attenuated inversion recovery images.

The duration of WNV neuroinvasive disease is weeks to months; long-term functional and cognitive difficulties are common in these patients, but the number of quality studies (with adequate control groups) is low. The mortality rate is 0% in the aspecific flu-like syndrome, 2% in meningitis and up to 15% in the case of encephalitis.

**Diagnosis**

West Nile virus is mostly diagnosed by detection of IgM antibody in serum or cerebrospinal fluid (CSF) by IgM antibody-capture ELISA (MAC-ELISA). Presence of anti-WNV IgM in CSF indicates CNS infection; it is found in 90% of patients with neuro-invasive disease within 8 days of symptom onset. However, anti-WNV IgM may not be detected in serum at clinical presentation. Demonstration of seroconversion in a convalescent sample will provide a definitive diagnosis. Testing for IgG antibodies has no utility in the acute clinical diagnostic setting. Cross-reactivity with other flaviviruses can be distinguished by performing a plaque-reduction neutralization test (PRNT), but the test is only available in reference laboratories.

Nucleic acid amplification testing (eg. RT-PCR) is used in blood donor screening in the United States and Canada has nearly eliminated the risk of West Nile virus transfusion transmission. It also has utility in the diagnosis of WNV in symptomatic patients as an adjunct to MAC-ELISA. In a study of 276
WNV cases, 191 were tested by both serology and NAAT. Of these, 86 (45.0%), 111 (58.1%), and 180 (94.2%) were detected by NAAT, serology, and combined NAAT and serology, respectively. RT-PCR may prove useful to diagnose WNV in immunocompromised patients when antibody development is delayed or absent.

**Treatment**

No antiviral treatment is available. Intravenous immunoglobulin (IVIG), West Nile virus–specific neutralizing monoclonal antibodies, corticosteroids, ribavirin, interferon α-2b, and antisense oligomers were not effective.

**Prevention**

**Vaccination**

In spite of 4 licensed equine vaccines and promising preliminary results from several phase 1 and 2 human vaccine candidates, phase 3 efficacy trials have not been attempted, probably because universal vaccination against WNV disease is unlikely to be cost-effective unless disease incidence increases substantially.

**Surveillance**

Potentially epidemic conditions due to increased virus transmission can be monitored by regularly testing the blood of birds for the presence of the virus or antibodies. So-called “sentinel birds” are used for this. Crows are very sensitive to infection. Analysis of samples of dead crows is useful in the New World.

**Personal protection**

The risk can be limited by reducing contact with mosquitoes. When there is an outbreak it is recommended that covering clothing is worn and that mosquito repellents are used. Insecticide can also be sprayed indoors. In the case of large epidemics, outdoor vector control is also important (larvicides and adulticides).
Rift Valley Fever

Summary

- Main clinical features: fever, haemorrhagic disease and neurological symptoms (FD, HS, NS), hepatitis
- Acute disease mainly of African domesticated ruminants, sometimes humans
- Transmission via mosquitoes and direct contact with infected animals
- Intermittent but severe epidemics

Virus

The virus which causes Rift Valley Fever (RVF) is a Phlebovirus and belongs to the Bunyaviridae family. There are several subtypes with each apparently having their own pathogenic capability. Zinga virus is currently regarded as a variant of the RVF virus. It is possibly identical.

Transmission

Between the epidemics it has never been possible to demonstrate a sylvatic vertebrate reservoir, but RVFV has been isolated from over 30 species of mosquitoes in six genera. The virus is passed from generation to generation of mosquito via the transovarial route. Mechanical transmission by arthropods is also documented.

The disease is primarily a zoonosis which affects sheep, goats, cattle and buffalo. Rodents are highly susceptible, although subclinical infections do occur. Birds, reptiles and amphibians are refractory. An epidemic in animals is called an epizootic. In animals the virus causes a severe infection with high mortality, mainly in newborn lambs. Adult pregnant animals often abort. A subclinical infection may occur in dogs, cats and camels (can abort). Horses and pigs are resistant.

The disease was first described in detail by Daubney, in Kenya in 1931 (epidemic in sheep on a farm near Lake Naivasha, one of the lakes in the Rift Valley). Until 1977 it was assumed that the illness only occurred in sub-Saharan Africa and Madagascar, but in 1977-78 there was a great epidemic in Egypt so that the area of distribution was found to be more extensive. Other important epidemics occurred in 1950-51 in South Africa (sheep: an estimated 100,000 dead and 500,000 abortions), in the river basin of the Senegal river in Senegal and southern Mauritania (1987) and in Kenya-Somalia (1997-1998). In 2000 numerous cases were reported from Saudi Arabia and the neighbouring Yemen. More than 200 people died. It was the first time the virus was detected outside Africa.
Figure: Rift Valley Fever epidemics (Nanyingi et al, Infect Ecol Epidemiol)

Rift Valley Fever occurs in intermittent epidemics with intervals of 10 to 15 years, mainly after periods
of exceptionally heavy rainfall. It has been proposed that factors such as rainfall, ocean temperature and climate change all play roles in determining the likelihood of an epidemic.

Transmission of Rift Valley Fever to man can occur either via direct contact with the blood of a viraemic animal (e.g. in slaughterhouses, farmers, butchers, ranchers, veterinary surgeons, herdsmen, etc.), possibly via the milk of an infected animal or via a bite from an infected insect. There are numerous types of mosquitoes which can transmit the virus. Aedes sp. are usually the most important but Anopheles, Culex, Eratmopodites, Mansonia, Mansonoides and Coquillettidiae mosquitoes also play a role. The virus can be transmitted transovarially in Aedes mcintoshi (= Aedes lineatopennis sl.) and can survive for a long time (years) in a mosquito egg. In heavy rainfall, floods etc. numerous infected mosquito eggs will simultaneously hatch due to the rising water level and moistening of the eggs. Rift Valley Fever virus can also be transmitted by mechanical vectors such as stomoxys, phlebotomes, simulids and Culicoides sp. Infected insects can be carried over large distances by the prevailing winds such as the north and south trade winds. Transporting infected cattle to a non-epidemic area is an important factor in the epidemiology.

Clinical aspects

The incubation period of Rift Valley fever is 3 to 7 days. Clinically the disease can provoke a non-specific flu-like syndrome, sometimes with biphasic fever. Fever develops together with muscle and joint pain, anorexia, diarrhoea, vomiting, headache and sometimes photophobia and retro-orbital pain. Sometimes there is petechial rash. The acute phase of the disease lasts 4-7 days. Complications occur in fewer than 5% of cases. In case of a haemorrhagic form, diffuse intravascular coagulation, bleeding (epistaxis, melena, haematemesis, seeping of blood at infusion and needle prick sites) and jaundice predominate such that the disease resembles yellow fever. Pneumonitis, shock, hepatic failure and renal failure with proteinuria and shock can occur. Sometimes bilateral vision disturbances occur about a week after the start of the fever. These are the result of vasculitis of the retina with arteriolar thrombosis, retinitis, retinal ischaemia, bleeding and detachment of the retina. The macular and peri-macular areas are affected preferentially. The lesions can result in permanent blindness or slowly improve over the course of the following weeks. Neurological complications also occur (< 1%): meningeal signs, dizziness, confusion, hallucinations, hypersalivation, grinding of teeth, chorea, convulsions and other signs of encephalitis. Coma, with or without decerebration, can occur in the terminal stage. In the complicated forms mortality is high.
Diagnosis

The disease may be suspected if large numbers of young lambs and goats die, with or without epidemic abortion among the animals and when at the same time multiple human cases with fever and haemorrhagic or neurological symptoms occur in an endemic area. In animals there is congestion in the liver, with small haemorrhagic areas and necrotic foci. The bile may be dark, almost black, and may contain blood. Tissue biopsies of animals can be used for anatomopathology, immunoperoxidase techniques for detecting the virus and of course virus isolation. Confirmation of the diagnosis in man is based on serology (IgM antibodies, including in the cerebrospinal fluid) and on virus isolation. Definitive identification is based on neutralisation tests with reference sera. Initially there is leukocytosis, then leukopenia and thrombocytopenia. Schistocytes may be found. With neurological symptoms lymphocytes predominate in the cerebrospinal fluid.

Treatment

There is no specific treatment. Symptomatic therapy is essential and occasionally requires intensive level care. There are insufficient data about the use of ribavirin and/or of convalescent plasma. Ribavirin inhibits virus replication in cell culture. Ribavirin is a ribonucleoside analogue that induces lethal mutagenesis of RNA viral genomes. The possible therapeutic place of interferon is not clear yet. Hepatotoxic medication as well as aspirin and NSAIDs should be avoided during the acute disease.

Prevention

Vaccination

A live attenuated strain (also known as the Smithburn strain) has shown to be potent in inducing protection from viral infection, and it is used as a vaccine for livestock. However, its ability to induce abortions and exhibit pathogenicity in European cattle has limited its use to areas threatened by an imminent outbreak. Studies on new vaccines are ongoing. These candidate vaccines can be classified into four groups: live attenuated, inactivated, viral-recombinant, and DNA vaccines. There is still no commercial vaccine available for humans.

Vector control

The transport of animals should be limited. In epidemics the transporting of cattle should be prohibited, or the animals must be quarantined. Contact with sick or dead animals must be avoided. Cattle can be vaccinated. If the epidemic has already started it is usually too late to employ with
vaccination as a control strategy. Thus in sheep-farming areas it is advised that the animals be vaccinated regularly either with the live Smithburn vaccine (single dose, life-long protection), or vaccination with the formol-inactivated vaccine (boosters needed).

Because of the variety of vectors, it is difficult to control insects breeding sites. Sometimes in epidemics insecticides are used on a large scale. For personal protection covering clothing (long sleeves, long trousers), insect repellents (best with DEET) and impregnated mosquito nets are adequate in normal situations. Barrier-nursing is indicated in the care of patients.

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Crimean Congo Haemorrhagic fever

Summary

- Main clinical presentation: Febrile disease, haemorrhagic symptoms, neurological syndrome (FD, HS (NS))
- Transmission via ticks (esp. Hyalomma marginatum marginatum) and direct contact with infected animals
- Human to human transmission occurs; CAVE nosocomial infection
- High mortality (up to 40%)

Transmission

Crimean-Congo Haemorrhagic Fever (CCHF) occurs throughout Africa, in Asia, in the former USSR and in Eastern Europe, the Balkans (Kosovo, Albania), the Middle East (including Oman and the United Emirates), Pakistan and the Maghreb, including Egypt. The virus was originally isolated in 1944-45 in the Crimean Peninsula in the north of the Black Sea, during an outbreak in Soviet military personnel. In 1956 it was found in Kinshasa, Congo, first in a patient and shortly afterwards in a scientist who acquired a subsequent laboratory infection. In 1967 it was shown by Chumakov and Casals that both viruses were virtually identical, so now it is referred to as Crimean-Congo Haemorrhagic Fever Virus.
Man can be infected by the bite of ticks, especially H. tick species are involved. The virus can survive in a tick population because it is transmitted both by the transovarial and the transstadial route. The larvae and nymphs of the ticks become infected when they suck blood from viremic small mammals and birds. Adult ticks infect themselves through the blood of infected wild or domesticated ruminants. Man can be also infected by direct contact with infected animal tissue or blood (goats, cattle, sheep, hares, ostriches) and during shearing of tick-
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Infested sheep. In sheep and goats, the viraemia lasts a week. When these animals are slaughtered or die from their babesiosis/anaplasmosis, they can still be viraemic. The people who look after the animal or deals with the carcass can therefore become infected. Herdsmen, farmers, veterinary surgeons and slaughterhouse workers have an increased risk of infection. Human to human transmission is well documented, and nosocomial transmission also occurs. A classic scenario is a patient with bleeding who requires surgery after which the virus spreads to medical staff and/or members of the family.

**Virus**

CCHF virus that causes Crimean-Congo Haemorrhagic Fever belongs to the family of the Bunyaviridae, genus Nairovirus. Other genera within the family include Orthobunyavirus, Hantavirus, Phlebovirus, and Tospovirus. CCHF is a tripartite, negative-sense, single-stranded RNA genome that comprises Large (L), Medium (M) and Small (S) segments. The three genome segments encode four structural proteins—the RNA-dependent RNA polymerase (L protein) is encoded by the large (L) segment, the glycoproteins (GN and GC) are encoded by the medium (M) segment, and the nucleocapsid protein (N) is encoded by the small (S) segment.

**Clinical aspects**

After an incubation period of 3 days after a tick bite and up to 6 days after contact with infected animal tissues, the disease starts with a sudden onset of fever. Clinical features commonly show a dramatic progression characterised by haemorrhage and myalgia, headache and vomiting. A discrete exanthema/enanthema can be seen, mainly on the palate. On the 4th day petechiae and extensive ecchymoses appear, followed by severe systemic bleeding including melaena, haematemesis, epistaxis and haematuria. There is no direct effect on the central nervous system, although confusion, lethargy and aggressive behaviour can occur.
Haematology results frequently show leukopenia and thrombocytopenia. The levels of liver enzymes, creatinine phosphokinase, and lactate dehydrogenase are raised and coagulation markers are prolonged. Infection of the endothelium has a major pathogenic role. Besides direct infection of the endothelium, indirect damage by viral factors or virus-mediated host-derived soluble factors that cause endothelial activations and dysfunction are thought to occur.

Mortality is high (15-40%), especially during an epidemic but mild cases and spontaneous recovery also occurs.
Diagnosis

Early diagnosis is critical both for patient management and for the prevention of human to human transmission. The diagnosis is made by demonstrating the presence of the virus in viraemic phase plasma either by culturing or RT-PCR or by detecting a seroconversion. RT-PCR is highly sensitive and specific.

IgM and IgG antibodies are detectable by ELISA and immunofluorescence assays from about 7 days after the onset of disease. Specific IgM declines to undetectable levels by 4 months post-infection, but IgG remains detectable for at least 5 years.

Treatment

General supportive measures and symptomatic therapy. People who are infected should be treated in strict isolation since airborne transmission can occur. Barrier-Nursing should be in place for infection control.

Ribavirin (Virazole®) was used to treat CCHF. There is no evidence from randomised clinical trials for the use of ribavirin to treat human CCHF — its effectiveness has only been described in observational studies. Patients should be treated for 10 days (30 mg/kg as an initial loading dose, then 15 mg/kg every 6 hours for 4 days, and then 7·5 mg/kg every 8 hours for 6 days).

Another study suggested treatment using passive immunotherapy, transferring the plasma of convalescing survivors to infected patients. However, the study had no control groups and was limited to seven patients, therefore conclusions cannot be made.

Prevention

Vector control

In endemic areas ticks should be eliminated from animals two weeks before they are slaughtered (e.g. with a pyrethroid acaricide). The virus is sensitive to heat and is not resistant to an acid environment. This explains why transmission by eating infected meat is rare.

Vaccination

There is no commercial vaccine.
Kyasanur Forest disease

Kyasanur forest disease is caused by Kyasanur forest disease virus, a flavivirus. It occurs principally in the Shimoga and Kanara district of Karnataka (formerly Mysore), India. The geographic distribution of this virus is not restricted to Karnataka, e.g. 22 percent of persons living in the Andaman and Nicobar Islands were found to be seropositive for KFD in 2002. Human infection by closely related viruses is known in Saudi Arabia (Alkhurma virus) and China (Nanjianyin virus).

The virus was identified in 1957 when it was isolated from a sick monkey from the Kyasanur forest in Karnataka state. This happened during a fatal epizootic among wild monkeys. The main hosts of this virus are small rodents, but shrews, bats, and monkeys may also carry the virus. Transmission is via the bite of an infected tick, mainly Haemaphysalis spinigera. Apart from tick bite, humans can also get infected by contact with an infected animal, such as a sick or recently dead monkey. Goats, cows, and sheep may become infected with KFD, but they do not have a role in the transmission of the disease. There is no evidence of the disease being transmitted via the unpasteurized milk of any of these animals.

The incubation period is not well known, some state 3-8 days, others 1-2 weeks. The patient develops sudden onset fever, severe headache, followed by back pain, muscle pain in the extremities, inflammation of the eyes, dehydration, gastrointestinal symptoms with or without gastrointestinal bleeding. Hypotension and pancytopenia can ensue. Some patients develop cough due to bronchopneumonia prior to coma and death. Some patients recover without complications after this first phase. However in most patients, the illness is biphasic, and the patient begins experiencing a 2nd wave of symptoms at the beginning of the 3rd week. These symptoms include fever and signs of encephalitis. The diagnosis is made by virus isolation from blood or by serologic testing using ELISA. There are approximately 400-500 symptomatic cases of KFD per year with a case fatality rate of 3-5 percent.

There was an important outbreak in May and June 2003. Forest workers are particularly at risk. There is a safe, effective formalin-inactivated vaccine available for control of Kyasanur Forest disease since 1990. More than 80,000 people were immunized in trials during 1990 to 1992, with no report of adverse effects. The vaccine is prepared from tissue culture and administered at a dose of 1.0 ml subcutaneously (0.5 ml below age 6), with a booster dose after 4 weeks.
Nipah virus

Nipah virus is at present not considered to be an arbovirus but is included in this chapter because of its close resemblance to Japanese Encephalitis.

From September ‘98 to March ‘99 a new paramyxovirus appeared in Malaysia. It was given the name Nipah virus and is related to Hendra virus which in 1994 caused fatal infections in horses and people in Australia. Nipah virus causes an encephalitis that clinically is indistinguishable from Japanese Encephalitis. An incubation period of 4-18 days is followed by 3-14 days of fever, headache, vomiting, reduced consciousness, meningism, myoclonus, convulsions, areflexia and hypotonia, tachycardia, abnormal pupils, nystagmus. There is often a considerable effect on the brain stem, often resulting in an abnormal oculovestibular reflex (abnormal “doll’s eye reflex”). Sometimes there is a non-productive cough. In man the mortality rate is high. During the first epidemic more adults than children were affected, mainly those who were working as pig-farmers. Pigs can be infected and develop a cough. In animals infection often results in death, unlike with Japanese Encephalitis. Flying foxes (large bats, including *Pteropus hypomelanus*) are thought to be the reservoir. The virus has been isolated from their urine and saliva.

Bats and zoonoses

There are multiple reasons why several zoonotic diseases originate in bats (rabies, Nipah virus, Hendra virus, SARS-CoV, Marburg, …). About a quarter of all mammal species on the planet are bats. The genetic diversity among the more than 1000 species of bats creates numerous niches for viruses. Bats live from 5 to 50 years, which is much longer than most small mammals. This could be useful for viruses seeking stable reservoirs. Many species roost packed together in large clusters, making it easy for a virus to spread through a colony. Cave-sharing among different species also facilitates infection across species, which in turn increases the chances of viral recombination. Some bats can fly up to 20 km a day, foraging, and some species are migratory. Such animals have the capacity of widely transporting a pathogen over a relatively short period. Some bats seem to be able to carry and shed a virus for a long time without getting sick and without clearing the infection, but more study is required.
Venezuelan Equine Encephalitis

Summary

- New World arboviral infection with mainly neurological symptoms
- Transmission via mosquitoes
- No vaccine available for people

General

The virus only occurs in the New World. It belongs to the Alphaviridae. The virus is normally maintained enzootically in a cycle between small mammals and Culex mosquitoes, mainly those belonging to the subgenus Melanoconion. Rodents form the reservoir. This is an acute viral disease that is transferred from horses to man by various mosquitoes (Aedes and Culex sp.). In a minority of those infected this leads to a serious and sometimes fatal encephalitis. It is the main arbovirus (together with dengue) in (sub)tropical America. The infection occurs in Central America and in a sickle-shaped area in the north of South America. There are regular outbreaks and epidemics, such as in Mexico in ‘93 and ‘96. In 1995 there was an outbreak in Colombia with ± 75,000 cases (3,000 with neurological complications). Epidemics in man are always preceded by epidemics in horses. Encephalitis occurs in 90% of infected horses, 50% of which die.

Clinical aspects

Asymptomatic infections are rare in man. Usually there is a flu-like syndrome lasting for 3 days. Fever, myalgia, headache, vomiting and diarrhoea are frequent and for this reason the disease is often assumed to be dengue. In a minority of the symptomatic patients (4% in children) this develops into encephalitis with various neurological symptoms. Confusion, stupor and convulsions can follow. There is leukopenia as well as an increased level of proteins and an increased number of lymphocytes in the cerebrospinal fluid. Sequelae are more frequent in children than in adults. Abortion is frequent in infected pregnant women. Diagnosis is clinical, epidemiological and serological.

Prevention

In the case of an epidemic of VEE, horses should be vaccinated, and the vector should be controlled.
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(insecticides). Most horses are not vaccinated because the vaccine is expensive. The vaccine is not in general use or readily available for man. For other encephalitis viruses such as Eastern Equine Encephalitis, Western Equine Encephalitis, St. Louis encephalitis, La Crosse encephalitis, California Encephalitis, Jamestown Canyon and Cache Valley (now West Nile as well) surveillance is carried out in North America. This involves, among other things, using birds such as chickens or pheasants because the vectors preferably bite birds. Sera are taken from these sentinels every two weeks and tested for antibodies to VEE. Surveillance can also be carried out by catching mosquitoes. After the catch has been sorted, virus culture or PCR is then carried out on the insect collections. If the virus becomes too frequent, insecticides can be sprayed. If a sudden increase in mosquitoes is anticipated, such as after a severe rainstorm or hurricane, surveillance is increased.

Eastern and Western Equine Encephalitis

Alphaviruses that are related to VEE include the viruses that cause Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE). EEE is a very serious, but quite rare arbovirosis in the east of the USA, but also occurs sporadically in Central and South America. An incubation period of 7-10 days, fever, meningism, severe encephalitis and a mortality rate that can be as high as 50% characterise the disease. The epidemic potential became evident in 1938. After a severe storm in Boston, Massachusetts there was a major outbreak with a high mortality rate. What was the connection between the storm and the disease? Birds form the reservoir. The virus is transmitted between birds by mosquitoes such as Culiseta melanura. This mosquito lays its eggs in dark underground hollows in an acid soil, such as root hollows in marsh cypresses or red maple trees. It is an unusual habitat for oviposition (egg-laying). The larvae are not in open water and are not easy to find. Such places easily become water-logged after heavy rainfall. In this way huge numbers of mosquitoes can appear simultaneously. Transmission between birds then increases. More than 75 different types of bird can be infected. When mosquitoes that bite both birds and man are infected (such as Aedes vexans, Coquilletidia perturbans), the infection can be transmitted to man. Culex tarsalis is also important in transmission. Horses and donkeys can be infected. In these animals the course of the infection is often dramatic and death among horses can precede an epidemic. Surveillance is carried out with sentinel birds. If there is a threat of an epidemic, insecticides are sprayed, e.g. by ULV (ultra low volume spraying).

In the west of the USA WEE occurs sporadically in man and animals. In other regions of the USA and South America WEE also occurs, but until now apparently only in animals. It is not known whether this...
is to do with the different antigenic types in North and South America. Most of the infections in adults are pauci- or asymptomatic. After an incubation period of 5-10 days there is a gradual onset of fever, malaise, headache, neck stiffness and dizziness. In serious cases this develops into stupor, coma, flaccid and spastic paralysis. There is pleocytosis in the cerebrospinal fluid as well as an increase in the protein content. Children often have permanent neurological sequelae. The mortality rate among symptomatic patients is about 10%.

Tick-borne encephalitis

Summary

- Flavivirus, 3 subtypes
- Vector: Ticks, Ixodes species
- Main clinical presentation: Febrile disease, neurological syndrome (FD, NS)
- Effective vaccine is available

Virus

Tick-borne encephalitis (TBE) is caused by 3 closely related flaviviruses. These as known at present are European, Siberian, and Far Eastern strains.

Transmission

Tick-borne encephalitis (TBE) is also called Frühsommer Meningo-Enzephalitis (Early Summer Meningo-Encephalitis). This name is a misnomer, since transmission lasts well into autumn (April till October). TBE refers to both Central European encephalitis (CEE, syn. FSME) and Russian spring-summer encephalitis (RSSE). TBE is transmitted to humans usually by the bite of a tick (either Ixodes persulcatus or Ixodes ricinus). In contrast with Lyme disease, transmission of the TBE virus occurs immediately after the tick bite, hence tick removal will not prevent the disease. Occasionally, cases occur following consumption of infected unpasteurized milk.

All 3 subtypes co-circulate throughout most of the TBEV endemic areas. However, currently the Siberian subtype dominates in many endemic regions from Eastern Europe to Eastern Siberia. The geographical distribution of TBE is from eastern France, over South Germany, Switzerland, Austria,
the previous East Block countries via Russia to northern Japan, and from Scandinavia (Sweden) and the Baltic states to Croatia and northern Italy. In Europe and Asia between 10000 and 15000 TBE cases are reported annually. The number is very likely underestimated because in many countries notification of the disease is not mandatory and only in a subset of the countries TBE case definition is in place. TBE is endemic in 27 European countries, and is a reportable disease in only 16 countries.

Vertical transmission in laboratory animals has been demonstrated to be widespread.

**Accidental hosts**

**Normal cycle of transmission**

![Transmission cycle of TBE](image)

Figure: Transmission cycle of TBE (Dumpis et al, Clin Infect Dis)

**Clinical aspects**

The incubation period of TBE ranges from 2 to 28 days (7-14 days). After alimentary TBEV transmission the incubation period is generally 3 to 4 days. published data suggest that the ratio of asymptomatic infections is between 70% and 98%. However the proportion of asymptomatic cases is hard to ascertain because patients with mild clinical signs and symptoms may remain undiagnosed.
The initial phase correlates with viremia and like in other neurotropic flaviviruses, it presents with aspecific flulike symptoms (moderate fever, headache, body pain (myalgia and arthralgia), fatigue, general malaise, anorexia, nausea).

This phase lasts for 2 to 7 d and is followed by amelioration or even an asymptomatic interval that usually lasts for about 1 wk (1-21 d). Then the second phase appears: in approximately 50% of adult patients it presents as meningitis, in about 40% as meningoencephalitis and in around 10% as meningoencephalomyelitis.

The severity of TBE increases with age; in children and adolescents, meningitis is the predominant form of the disease. The long-term prognosis is unfavourable in about 40% to 50% of patients who sustain sequelae (pareies, ataxia, and other gait disturbances) for months to years, and severity of TBE-related sequelae also seems age-related.

Classification of sequelae:

- **Mild** - without any real impact on quality of life.
- **Moderate** - residual symptoms or signs that affected quality of life but that did not require
adjustments of daily activities.

- Severe symptoms or signs that led to an inability to continue previous activities or that required adjustments of daily activities.

In general the case fatality rate is approximately 1–2% following European subtype infection but can be as high as 20–40% following infection with a far-eastern subtype. Infection with the Siberian subtype produces a mortality rate of 2–3%. However it is possible that the high mortality figures for the far-eastern subtype may be due to the lack of detection of mild cases therefore skewing the mortality data.

**Diagnosis**

As a rule, anti-TBEV- IgM and usually TBEV-IgG antibodies are present in the first serum samples taken when CNS symptoms manifest in the second phase of the disease. In the first phase of illness, the virus can be isolated or detected by RT-PCR from blood, but only rarely is TBEV detected at the beginning of the second phase in CSF and occasionally in cases of progressive disease. Intrathecal IgM and IgG antibody response can be detectable in CSF, but several days later than in serum, and in all cases by day 10.

Enzyme immunoassays are usually used for specific sero-diagnosis; these assays could be based on either purified virions or recombinant virus-like particles obtained by expression of prM and E proteins. ELISA for serum and/or CSF IgM antibodies to TBEV has been shown to be the most reliable serological test. Haemagglutination inhibition is also widely used but measures all antibody classes and needs a rise in antibody titre for definitive diagnosis. High cross-reactivity of the antigenic structure in the flavivirus may reduce specificity.

**Treatment**

There is no specific antiviral treatment for TBE. Patients as a rule need hospitalization and supportive care based on the severity of signs/symptoms, and usually encompasses administration of antipyretics, analgesics, antiemetics, maintenance of water and electrolyte balance and if necessary administration of anticonvulsive agents. In patients with neuromuscular paralysis leading to respiratory failure, intubation and ventilatory support are necessary.

**Prevention**
**Personal protection**

Personal protective measures help in prevention of tick bites (repellents like DEET being less effective than against mosquitoes) and protective clothing.

**Vaccination**

In Europe two vaccines are licensed: FSME immun® (from Baxter) and Encephur® (from Chiron Behring). 14 days after the second dose of basic vaccination protective antibodies develop in about 85% of the subjects, while after three doses more than 98% of persons with normal immunity are protected.

In some countries, such as Austria, vaccination coverage is very high. Other areas where the cost of vaccination is prohibitive lag behind.

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**Arenaviruses**

**Summary**

- Arenaviruses: Zoonotic viruses transmitted via rodents mainly, but for some also via secondary person-to-person transmission and nosocomial infection
- Clinically atypical febrile, haemorrhagic, neurological or pulmonary syndrome.
- Ribavirin is used in Lassa fever

**General**
The name of arenaviruses refers to their granular appearance under an electron microscope (L. arena = sand). This structure is created by the inclusion of electron dense host cell ribosomes in the viral envelope. They are RNA viruses, of which the genome consists of a short and a long RNA fragment. Some viruses from this group are pathogenic for humans. Our knowledge concerning these viruses is clearly incomplete. Most arenaviruses have a rodent reservoir. The rodent hosts are chronically infected with the virus, without causing them an obvious illness. Human infection occurs when a
person comes into contact with excretions or other materials contaminated with excretions of the infected rodent via ingestion, via direct contact through broken skin/mucosa or via aerosol transmission. Taracibe virus was isolated from fruit-eating bats.

**Known pathogenic arenaviruses:**

1. Lymphocytic choriomeningitis virus
2. Lassa virus (with substrains Josiah, Nigeria, LP, AV)
3. Junin virus
4. Machupo virus
5. Lujo virus

**Non-pathogenic arenaviruses** and viruses with unknown pathogenicity:

1. Old World: Mopeia, Mobala, Ippy, Acar
2. New World: Tacaribe, Tamiami, Parana, Amapari, Flexal, Pichende, Latino, Oliveros

**Incubation time**

Nosocomial transmission and transmission via infected body fluids are known for Lassa fever, Ebola and Marburg virus as well as other non-arboviral haemorrhagic fevers. The Bunya-, Filo- and Flaviviruses are cytolytic. They destroy cells particularly endothelial cells. The incubation time is usually less than one week.

Arenaviruses are not cytolytic. They act indirectly by forming antigen-antibody complexes and activating complement. The incubation time tends to be longer than in the other groups.

**New Arenaviruses**

It is very likely that new viruses will be discovered in the future. An example is Lujo virus, a new member of the family Arenaviridae. This haemorrhagic fever virus was discovered in 2008, when it was responsible for an outbreak in South Africa (the index patient came from Zambia, 5 cases in total). Human disease is characterized by nosocomial transmission and a very high case fatality rate of 80 percent.
Lymphocytic choriomeningitis virus

The first arenavirus to be isolated was lymphocytic choriomeningitis virus (LCM). It was discovered in 1933 during an epidemic of St Louis encephalitis in the USA. The virus can infect mice. Neonatally infected mice become chronic carriers and excrete the virus for a long time in their urine. The course of the infection is determined by age, immunological resistance, the virus strain and the genetic makeup of the rodent. Both *Mus musculus* and *Mus domesticus* (the common house mouse) can be infected. Other rodents, such as hamsters, which are sometimes kept as pets, can also become infected and can be responsible for transmission. Lymphocytic choriomeningitis virus can also be transmitted via organ transplantation.

In humans it is mainly known for causing an “aseptic” meningitis, with or without fever about 10 days before the meningeal signs appear, though infection is more often without symptoms or a mild febrile illness. LCMV infection in immune compromised patients tends to be severe. Sometimes there is severe damage to the central nervous system. Transient hydrocephalus has been described. Chorioretinitis and congenital hydrocephalus may occur in foetal infection. The cerebrospinal fluid exhibits lymphocytic pleocytosis, an elevated protein content and in 25% of patients there is also reduced sugar. Rarely transverse myelitis, ascending myelitis or bulbar paralysis occur. Some cases of residual deafness have been described after LCM infection. At present, a significant fraction of cases of neonatal mental retardation and blindness remain unexplained. Congenital LCMV infection is an understudied potential cause of a portion of these cases.

There is no specific treatment. There is no vaccine. In general, mortality is less than 1%.

Lassa fever

Lassa virus

Lassa virus is an arenavirus. There are some subtypes, such as the Josiah, Nigeria, LP and AV strains. The disease “Lassa fever” takes its name from a small town in Nigeria. The disease occurs, endemically, in West Africa: Sierra Leone, Guinea, Liberia and Nigeria, but probably also outside these countries, based on case reports and serosurveys in humans and animals (Ghana, Ivory Coast, Burkina Faso, Senegal, Mali, Central African Republic). The total number of annual cases is estimated between 100,000 and 300,000 case with 5000 deaths.
Transmission is via ingestion of food infected with urine or faeces of infected peridomestic rats (*Mastomys natalensis* = *Praomys natalensis*). The rat itself exhibits no symptoms. There are many morphologically similar rodents, which differ in karyotype. Transmission via aerosol has been demonstrated in the laboratory. Person-to-person transmission occurs, as does nosocomial transmission, including due to re-use of needles. Transmission may also occur via sexual intercourse (Lassa virus has been isolated from semen up to 6 weeks after the acute stage).

Isolation and strict barrier nursing are sufficient to prevent transmission in the hospital. Avoidance of contact with rodents is important (especially of food storage areas where these rodents are common). From time to time there are imported cases in Europe and North America.

**Clinical aspects**

In about 80% of patients, the disease has a mild course. After an incubation period of 7-18 days, infected persons gradually develop a sore throat with an inflammatory exudative pharyngitis, fever, malaise and myalgia, conjunctivitis and swollen eyelids, abdominal pain with or without nausea, vomiting and diarrhoea, cough, dyspnoea and tachypnoea, thoracic pain, pleural fluid and pain in the joints and loins. Oedema of the face may occur. Patients do not die with a clinical picture of DIC [diffuse intravascular coagulation], but with liver necrosis, haemorrhage, shock and pulmonary oedema. Icterus occurs rarely. Diffuse haemorrhages and swelling of the head and neck indicate increased vascular permeability and a poor prognosis. The cerebrospinal fluid is usually normal. After a few weeks pericarditis and/or cerebellar ataxia occur. There is moderate thrombocytopenia, but there is significant and pronounced blood platelet and endothelium dysfunction. Proteinuria is common. Death results from multi-organ failure in about 20% of those hospitalized. In those surviving there is often sensorineural deafness (25%). ARDS is a frequent cause of death in Lassa fever. Spontaneous abortion is a possible complication in pregnancy.

**Diagnosis**

Diagnosis is suggested via clinical symptoms in West Africa (thoracic pain, fever, haemorrhage, pharyngitis). In Lassa fever, the white cell count tends to be normal. In severe cases, lymphopenia with neutrophilia as well as haemoconcentration can occur. Mild thrombocytopenia can be expected. Confirmation will be obtained via serology (ELISA IgM and/or seroconversion IgG), virus isolation or RT-PCR [reverse transcriptase polymerase chain reaction] for viral RNA in a high-containment laboratory (urine, blood, throat swab). IFA (indirect fluorescence assay) can be done on serum using a fluorescence microscope using anti-Lassa monoclonal antibodies. Immunoblotting with gel
electrophoresis can detect Lassa proteins using specifically labelled antibodies.

**Treatment**

Patients should be isolated in an intensive care unit. Ribavirin (Virazole®, Rebetol® – caps. 200 mg), a guanosine analogue, administered during the first 6 days of the disease, is effective (30 mg/kg IV loading dose; then 16 mg/kg IV every 6 hours for 4 days, then 8 mg/kg IV every 8 hours for 6 days). Probably it is also beneficial as chemoprophylaxis (direct contacts PO 500 mg QID for 7 days). In practice ribavirin will often not be available. In the West this drug is used as an aerosol for the treatment of severe pulmonary infection with RSV (respiratory syncytial virus). In China it is used in hantavirus epidemics.

**Prevention**

Contact with rodents and their excreta (especially urine) should be limited as far as possible. Infected patients should be cared for and treated with the necessary caution (barrier nursing) to avoid nosocomial transmission. In experiments it has been possible to protect primates with a vaccinia virus-expressed Lassa virus vaccine. However, vaccines based upon vaccinia constructs might be dangerous in a population with a high seroprevalence of HIV infection. A recombinant vesicular stomatitis virus-based vaccine protected primates from lethal Lassa virus infection. There is no commercial vaccine for humans available.

**New World arenaviruses**

**General**

There are at least 16 arenaviruses in the New World, but most of these are not pathogenic for humans. Junin and Machupo virus occur in South America. The viruses were named after places in Argentina and Bolivia. Guanarito virus causes Venezuelan haemorrhagic fever. Sabia virus causes Brazilian haemorrhagic fever. In North America in 1970 the apathogenic Tamiami virus was found in cotton rats in Florida, but otherwise it was thought that arenaviruses did not occur in North America. In 1996 Whitewater Arroyo virus was identified in the USA. The name refers to a place in the state of New Mexico. It was not known at the time whether this virus was pathogenic or not. In 2000 several people became infected with this virus, with serious consequences. Bear Canyon virus is a third North American arenavirus, the pathogenic capacity of which is to date still unknown.
Transmission

Transmission of Junin and Machupo virus is via rodents (*Calomys musculinus* and *Calomys callosus* respectively) which live in the fields (not peridomestic). Female rodents infected neonatally with Junin or Machupo virus are subfertile. Infection is via inhalation of swirling dust containing dried rodent urine (aerogenic transmission). Infection with Junin virus is seasonal and shows a peak during the harvest in autumn. *Calomys musculinus* has a preference for linear habitats, e.g. hedges and roadsides. *Calomys callosus* prefers to live in open fields. An outbreak of 1963-64 with 637 cases and 113 deaths was due to a proliferation of the rodents in a Bolivian town. Transmission was stopped by catching or killing the rodents. Many children all over the country gave their pet cats in an emotional gesture to help catch the rodents.
Clinical aspect

Machupo and Junin viruses cause similar clinical pictures. Initially there is a rather slow onset of aspecific malaise and fever, muscle pain, conjunctivitis, nausea, vomiting and sometimes photophobia. Unlike Lassa fever, pharyngitis is not pronounced. Enlarged lymph nodes and pronounced erythema of the face, neck and thorax are common. Thrombocytopenia, leukopenia and albuminuria are generally present. Chest X-ray is usually normal. Machupo and Guanarito virus infections often cause neurological symptoms. Haemorrhage and shock herald a poor prognosis. Whitewater Arroyo virus causes high fever, liver problems, internal haemorrhage and possibly death. Only a few cases of Sabia virus infection have been documented.

Treatment

Physical protection of doctors and nurses is necessary (barrier nursing). Good results have been described with convalescent plasma from survivors, especially if this is administered early. Ribavirin is active in vitro against all arenaviruses. The penetration of ribavirin into the cerebrospinal fluid is very low. Salicylates and intramuscular injections should be avoided. Thrombocytes should be transfused in case of severe thrombocytopenia. In view of the heightened vascular permeability, caution is advised with IV fluid (risk of pulmonary oedema).

Prevention

Sometimes high-risk persons are given ribavirin preventively for two weeks (1.2 g daily PO).

Hantaviruses

Summary

- Hantaviruses are enzootic viruses transmitted to humans by rodents.
- Symptoms: fever, flu-like syndrome followed by nephropathy, haemorrhage, hyper acute pulmonary syndrome
- A febrile patient with acute respiratory distress due to pulmonary oedema who has a combination of bandemia (left shift), atypical lymphocytosis with possible lymphoblasts, hemoconcentration, thrombocytopenia is highly suspect for infection with Sin Nombre virus (North America) or Andes
virus (South America) if he recently visited a transmission area.

**General**

Hantaviruses belong to the Bunyaviridae family. They are spread by rodents and rarely by insectivores. There are several viruses named for instance, Hantaan, Dobrava, Seoul, Puumala, Andes and Sin Nombre.

**Transmission**

The viruses are transmitted to man mainly via the inhalation of infected particles and more rarely via ingestion of *food contaminated* with urine, saliva or faeces of rodents. Once infected, these animals excrete the virus for a long time. In the case of some of the South American viruses, it is thought that occasionally they can be transmitted from human to human. There is a close connection between the specific virus and the rodent species that forms the reservoir.

**Geographical distribution**

![Map Hantaviruses. Copyright ITM](image)
Infections occur worldwide, however each serotype has its own geographical range. During the Korean war, approximately 3000 UN soldiers were infected with Hantaan virus which is very virulent, producing Korean Hemorrhagic Fever. The virus derives its name from a river in Korea.

In 1993, a previously unknown serotype emerged in the USA. The virus responsible was initially called the Four Corners virus, then Muerto Canyon, and finally the name Sin Nombre was adopted. Pulmonary Hantavirus syndrome is also seen in South America.

Hantavirus infection also occurs in Belgium (especially Wallonia) and the Netherlands, as so-called epidemic nephropathy. At first the disease was called “muroid virus nephropathy”, assuming that rats or mice were involved but this nomenclature has now been abandoned.

A serious form with renal involvement, caused by the Dobrava serotype occurs in the Balkans (in Bosnia, among others).

A mild form, caused by the Puumala serotype, occurs in Scandinavia. A large outbreak of nephropathia epidemica occurred in North Sweden in 2007.

Infections with Seoul virus occur worldwide because the normal host (rat) is distributed worldwide.

**Clinical aspects**

Depending on the hantavirus serotype and the host, the course of the disease varies from benign to lethal. In humans the incubation period is approximately 1 to 6 weeks. Initially, there is an acute non-specific flu-like syndrome with fever, headache, asthenia, muscle pain, abdominal pain, sometimes some discomfort in the eyes with blurred vision and red conjunctivae. The benign form (Puumala) has a low mortality rate (0-0.2%) and the serious form a high mortality rate (up to 40% in the case of Sin Nombre).

In Puumala and Dobrova virus infection lumbar pain and oliguria can be expected about 4-10 days after onset. The urine contains protein and blood and interstitial nephritis is present. The creatinine and urea levels increase. In severe forms, kidney failure can be fatal, but if the patient survives, after a polyuric phase, kidney function returns to normal within two to six weeks. In approximately three quarters of cases, thrombocytopenia is present. The leukocyte count is either normal or raised.

Haemorrhages can occur.

The Sin Nombre virus often leads to hantavirus pulmonary syndrome with development of
tachycardia, hypotension or shock and acute pulmonary oedema with tachypnoea. The fulminant pulmonary oedema is initially non-cardiogenic and is based on a capillary leakage syndrome. Most deaths are caused by myocardial dysfunction (cardiogenic shock) and hypoperfusion rather than hypoxia. This led to the use of the term “hantavirus cardiopulmonary syndrome” (HCPS) rather than the name “hantavirus pulmonary syndrome”.

Note. Infection with Junin, Machupo, Sabia and Guanarito virus, which are New World arenaviruses transmitted through rodents, produce similar clinical syndromes with haemorrhagic tendency and sometimes neurological signs: absence of tendon reflexes, tremor, ataxia, confusion, delirium and convulsions can occur.

**Diagnosis**

The combination of thrombocytopenia, leucocytosis (often with left shift), elevated haematocrit, and presence of immunoblasts in peripheral blood smear is a sensitive and specific early clue to the diagnosis of pulmonary Hantavirus syndrome. These findings in a patient with rapid onset of respiratory insufficiency should suggest the diagnosis.

The diagnosis is confirmed via serology (seroconversion, IgM), RT-PCR and immunohistochemistry. The latter can be carried out on tissue biopsies, which are stored in formalin. Viral RNA can be detected via reverse transcriptase PCR. Due to the extreme sensitivity of this technique, laboratory contamination is a considerable problem. Virus culture is possible but is rarely performed.

The differential diagnosis of pulmonary Hantavirus syndrome encompasses septic shock, leptospirosis, meningococcal septicaemia, plague, tularemia, severe influenza, SARS, myocardial infarction and fulminant pneumonia due to other causes.

The diagnosis of the other hantaviral infections will be laboratory based. Patients with acute renal failure and interstitial nephritis with or without haemorrhagic symptoms will be tested.

**Treatment**

In the acute phase, it is necessary to treat severe cases in an intensive care unit. Ribavirin may have in vitro activity against some viral strains but showed no benefit against Sin Nombre virus in a clinical study. Symptomatic treatment and supportive measures are essential (haemodialysis, treatment of pulmonary oedema, extra-corporeal membrane oxygenation). A great deal of attention goes to proper oxygenation, fluid balance and blood pressure control. Mechanical ventilation, extra oxygen, IV
Viruses fluid and inotropic drugs should be used when needed.

Isolation of the patient is not needed.

**Prevention**

There is still no vaccine for most Hantaviruses. Hantavax® is a vaccine which can be used in the Far East against Seoul and Hantaan virus. Booster injections are necessary. This vaccine is not available in Europe.

Contact with rodents and their excretion products must be avoided. Places where there have been rats are best decontaminated with bleach and ventilated (do not brush away the dry dust: airborne particles!). Attracting rodents must be avoided by careful monitoring of potential food sources and hiding places. Rodent control: see further.

### Rodents

**Medical significance**

Most medically significant rodents belong to the Muridae and the Cricetidae. Rodents play a part in many diseases, such as plague, transmitted by the rat flea *Xenopsylla cheopis* and Weil’s disease, a severe form of leptospirosis transmitted via infected rat urine. Rodents play a part in conditions such as echinococcosis (*E. multilocularis*), trichinellosis, Lyme borreliosis, recurrent fever (*Borrelia recurrentis*), salmonellosis, rat bite fever, tularemia, lymphocytic choriomeningitis, *Hymenolepis diminuta* and rickettsioses such as RMSF, scrub typhus and murine typhus. Haemorrhagic fevers that are transmitted by rodents (“rodent-borne”) include Hantaviruses and Arenaviruses such as Junin, Machupo and Lassa fever. Infection with *Talaromyces marneffei* is essentially a disease of rodents but can occur in AIDS patients in Southeast Asia. In 2003 an imported and infected Gambian giant rat spread monkeypox virus in the USA, a country where there had been no cases until that moment.

**Importance in research**

Numerous laboratories use mice and rats as experimental animals, to gain knowledge which would otherwise be impossible or very difficult to obtain. Today, working with experimental animals is avoided as much as possible, but alternative in vitro experiments are not always available. Rodent
strains have been bred to provide experimental models for, e.g. immune deficiency, increased likelihood of forming tumours or hypertension, etc. These strains are maintained by inbreeding.

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Filoviruses

General

These viruses are filamentous in structure and are therefore known as filoviruses. Marburg virus and Ebola virus belong to this group. Infections with some of these viruses have a very high case-fatality ratio (e.g. Zaire ebolavirus), other are seemingly non-pathogenic (e.g. Reston ebolavirus). Epidemics with human pathogenic filoviruses have become more common in the beginning of the 21st Century and the risk is not negligible that the infections become endemic, at least in Central Africa.

In the last years, several new filoviruses were detected in bat and fish species. Lloviu virus was discovered in 2010 in Schreiber’s long fingered bats (Miniopterus schreibersii) found dead in a cave (the dead bat was already found in 2002), the so-called Cueva del Lloviu, Asturias, northern Spain. Later similar discoveries were made in caves in France, Portugal and Hungary. In 2018, Bombali virus sequences were discovers in bats from Sierra Leone, Guinea and Kenya and the virus is considered to be a new ebolavirus species. Měnglà dianlovirus (diān is the Chinese abbreviation for Yunnan) was found in Rousettus bat in Mengla County, Yunnan province in China in January 2019. Fish-derived filoviruses constitute members of two new genera: striavirus and thamnovirus. At present it is uncertain if these new viruses are pathogenic for the concerned animal species. These new filoviruses have not been cultured yet, only their RNA genome has been sequenced. No human infections or human disease have been detected (yet) and since no isolates are available, their zoonotic or pathogenic potential cannot be tested.

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Marburg virus

In 1967 there was an epidemic of Marburg virus infection among laboratory staff in Marburg, Germany. These people worked with African green monkeys (Cercopithecus aethiops), imported from
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Uganda. Some people in Frankfurt and Belgrade, Yugoslavia, who encountered the same batch of animals also fell ill. In all, 32 people were affected: 26 primary infections and 6 secondary infections. The mortality rate of the primary infections was 25%. In the next few years a few sporadic cases were seen in Zimbabwe (’75), Kenya (’80 and ’87) and a laboratory infection in Russia (’87). In 1999 and 2000 multiple cases were diagnosed in the north east of Congo, in the area of Watsa and Durba. Infection occurred mainly in gold miners, working in very primitive conditions in old mines. There had probably already been a low level of transmission in this area for some considerable time (maybe even years). Social unrest and armed conflicts in the area hindered local research. The end of the epidemic coincided with the flooding of the mine.

Early 2005 there was a large epidemic in Uige, Northern Angola, with 374 cases (initial case fatality rate 92%). It was the largest Marburg epidemic to date (the initial estimate was above 400 cases). Two viral subtypes are responsible for all described outbreaks: MARV (Marburg virus), RAVV (Ravn virus) which both diverge from the prototype Marburg virus variant Musoke (MARV/Mus) by < 10% at nucleotide level. There are very probably other subtypes as well. In 2007, it was found that certain fruit bats (Rousettus aegyptiacus) were carrying Marburg viral RNA as well as antibodies against the virus. It seems more and more likely that bats form the reservoir, although more research is needed. In 2009, the successful isolation of infectious Marburg virus was reported from caught healthy Egyptian rousettes (Rousettus aegyptiacus). This isolation strongly suggests that Old World fruit bats are involved in the natural maintenance of marburgviruses and makes bats the prime suspect as reservoir for Ebola virus, though the latter has never been cultured from bats. Further studies are necessary to establish whether Egyptian rousettes are the actual hosts of MARV and RAVV or whether they get infected via contact with another animal and therefore serve only as intermediate hosts. Experimentally infected bats developed relatively low viremia lasting at least 5 days but remained healthy and didn’t develop any notable gross pathology. The virus also replicated to high titers in major organs (liver and spleen) and organs that might possibly be involved in virus transmission.

In 2008 a Dutch tourist became infected after visiting a cave in Uganda. She became sick after her return home and subsequently died in the Netherlands. Also, in 2008 an American tourist developed chills and diarrhoea, severe leukopenia, massively elevated transaminases, coagulation problems, pancreatitis and renal failure after a similar voyage. The diagnosis of Marburg infection was obtained in retrospect, when she was informed of the death of the above-mentioned Dutch tourist. In October 2012, the disease flared-up in Uganda, short after an outbreak of Ebola virus. The clinical signs and symptoms are similar to Ebola (see further). There is no effective treatment.

At present, an experimental vaccine against Marburg has been developed. It is based on a live attenuated recombinant vesicular stomatitis virus, a well-known pathogen of horses, bovines and
pigs. The gene coding for Marburg glycoprotein was inserted into the viral genome (similar work was performed with the Ebola Zaire virus). Experiments in monkeys showed a good humoral and cellular immune response and protection against infection with wild type virus. There was no cross-protection against other filoviruses, such as Ebola virus. So far, the vaccine has shown no evidence of pathogenicity in four species of animals (mouse, guinea pig, goat, monkey).

In 2012 it was demonstrated that macaque monkeys could be protected from Marburg virus disease by post-exposure treatment with hyperimmune serum (Marburg virus-specific IgG). No clinical human trials have been performed to date. On the basis of efficacy in nonhuman primates and pharmacokinetic data in humans, AVI-7288 – a phosphorodiamidate morpholino oligomer with positive charges that targets the viral messenger RNA that encodes Marburg virus (MARV) nucleoprotein – has potential as postexposure prophylaxis for MARV infection in humans.

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**Ebola virus**

**General**

Ebola virus is a member of the Filoviridae family (Mononegavirales order). It is a enveloped filamentous particle with a non-segmented, negative-sense RNA genome. The viral spike on the viral envelope is formed by the sole trimeric transmembrane glycoprotein and mediates viral entry; this spike is a target for the host immune response and for vaccine development. EBOV or Ebola virus refers to the Zaire ebolavirus in the genus ebolavirus. The other known species within the genus are Bundibugyo ebolavirus (Bundibugyo virus), Reston ebolavirus (Reston virus), Sudan ebolavirus (Sudan virus), Taï Forest ebolavirus (Taï Forest virus), and Bombali virus. Only Bundibugyo, Sudan, and Ebola viruses have been associated with disease outbreaks in humans. Ebola virus disease (EVD) refers to a disease caused by four of five viruses of the genus Ebola virus: BDBV, SUDV, TAFV and EBOV.
Ebola-Zaire (EBOV) and Ebola-Sudan (SUDV)

In 1976 there was a sudden large-scale epidemic of 2 different Ebola viruses in Maridi (South Sudan) and in Yambuku, on the Ebola river in North Congo. The mortality rate in Yambuku was very high (280 deaths out of 318 cases = 88%) and slightly lower in Sudan (53%). In 1977 there was one fatal case in Tandala, North Congo. New major outbreaks occurred in 1979 in Nzara (South Sudan), in 1995 in Kikwit, Congo and in 2003 in Kelle, Congo Brazzaville. The virus, which emerged in Kikwit, very closely resembled that in Yambuku (less than 1.6% difference in RNA). This is a sign of a genome, which is not under selection pressure, suggesting a stable ecological niche between epidemics. The Sudanese virus isolates of 1976 and 1979 were also almost identical.
From 1994 till 2012, several small epidemics occurred with the number of infections never exceeding 500, with the case fatality rate varying between 41 and 100% (100% was 4 times due to a single case) (see table below). During an outbreak in October 1996, an infected doctor was flown over to South Africa and there caused a fatal secondary case in a nurse. This illustrates how easily pathogenic organisms can be spread in this age of long-distance transport. Early in 2003, a large-scale epidemic occurred in Mbomo and Kelle, a very remote and rural area of Congo Brazzaville, just south of Odzala National Park. It started by a large-scale die-off among the lowland gorillas in the park. The disease flared up again in the same area, in November the same year, but was contained before New Year 2004.

**Ebola outbreak in West-Africa**

See also: https://www.who.int/features/ebola/storymap/en/

On 14 March 2014, rumours of a ‘mysterious disease’ were reported by the Ministry of Health in Guinea. Several health staff taking care of the sick had died and mortality was very high. Suspicion of Lassa viral haemorrhagic fever rose, but what jumped out were the hiccups, a typical symptom associated with Ebola. 28 March 2014, the World Health Organization was notified of an outbreak of a communicable disease characterized by fever, severe diarrhoea, vomiting, and a high fatality rate in Guinea. Virologic investigation identified *Zaire ebolavirus* (EBOV) as the causative agent. Full-length genome sequencing and phylogenetic analysis showed that EBOV from Guinea forms a separate clade in relationship to the known EBOV strains from the Democratic Republic of Congo and Gabon. the suspected first case of the outbreak was a 2-year-old child who died in Meliandou in Gu Gundou prefecture on December 6, 2013. A health care worker from Guom Gu with suspected disease, seems to have triggered the spread of the virus to Macenta, Nzcenta, and Kissidougou in February 2014. The initial case fatality rate was 86% (12/14 patients). What followed was an unprecedented outbreak going from bad to worse. Ebola had been stealthily spreading undetected for more than three months. It is not unusual for Ebola to go undiagnosed for a substantial period of time; the past eight Ebola outbreaks each took two months on average to be discovered and investigated. Ebola’s symptoms are easily confused with other diseases, such as cholera and malaria, and experts trained to recognise it are rare. However, past outbreaks took place mostly in remote villages in central and eastern Africa, where they were more easily contained. In a twist of geographic fate, Ebola erupted at the junction of Guinea, Liberia and Sierra Leone, where people regularly move across the porous borders. Fear and suspicion of the unknown virus, unsafe burial practices, mistrust in politicians, the hiding of cases, and a weak public health system, which lacked the resources to recognise and efficiently respond to Ebola, all contributed to
the virus surging through the region. For months, the epidemic spread faster than the international community’s response. The Ebola virus was introduced into Nigeria on 20 July 2014 when an infected Liberian man arrived by airplane into Lagos, Africa’s most populous city. The man, who died in hospital 5 days later, set off a chain of transmission that infected a total of 19 people, of whom 7 died.

On August 8 2014, the WHO declared the epidemic to be an emergency of international concern. In Mali 8 people were infected of whom 6 died and 1 case was detected in Senegal. On 6 October 2014, the World Health Organization (WHO) was informed of the first confirmed autochthonous case of Ebola virus disease in Spain. This case represents the first human-to-human transmission of EVD outside Africa. The case is a female healthcare worker with no travel history to West Africa but who participated in the medical care of an EVD case in a Spanish citizen, who had been infected in Sierra Leone and evacuated to Madrid, Spain on 22 September 2014 and who died on 25 September 2014. She was in contact with the repatriated EVD case twice; on 24 and 25 September 2014. On both occasions she is reported to have worn appropriate personal protection equipment (PPE). Following the Spanish national protocol for EVD cases, the healthcare worker was considered a low risk contact and monitored accordingly. The female case developed a fever on 29 September 2014 and was admitted into isolation on 6 October 2014 where she tested positive for Ebola.

In total 28,652 Ebola cases are recorded during the 2013-2016 epidemic with a death toll of 11,325. Ebola has destroyed lives and families, left deep scars, and ripped at the social and economic fabric of Guinea, Liberia and Sierra Leone. The virus cut a vast swathe through the three countries, in a cross-border geographical spread never seen before. Fear and panic set in, the sick and their families were desperate, and national health workers and MSF teams were overwhelmed and exhausted. Medical workers are not trained to deal with at least 50 percent of their patients dying from a disease for which no treatments exist. Nevertheless, the world at first ignored the calls for help and then belatedly decided to act. Meanwhile, months were wasted and lives were lost. No one knows the true number of deaths the epidemic will have ultimately caused. Across the three countries, local healthcare workers were tragically dying by the dozens. In Ebola outbreaks, health facilities without proper infection control often act as multiplying chambers for the virus, become dangerous places for both health workers and patients. This outbreak was no different, but it happened on a massive scale. The resulting collapse of health services means that untreated malaria, complicated deliveries and car crashes will have multiplied the direct Ebola deaths many times over. Why was the world so slow to wake up to its severity and respond? Was it due to fear, lack of political will, lack of expertise, or a perfect storm of all three?
### Viruses

**Ebola cases in 3 countries with widespread transmission during the 2013-2016 epidemic. Source: CDC**

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Cases (Suspected, Probable, and Confirmed)</th>
<th>Laboratory-Confirmed Cases</th>
<th>Total Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea</td>
<td>3814</td>
<td>3358</td>
<td>2544</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>14124</td>
<td>8706</td>
<td>3956</td>
</tr>
<tr>
<td>Liberia</td>
<td>10678</td>
<td>3163</td>
<td>4810</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>28616</strong></td>
<td><strong>15227</strong></td>
<td><strong>11310</strong></td>
</tr>
</tbody>
</table>

**Countries with lower case load during the 2013-2016 epidemic. Source: CDC**

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Cases (Suspected, Probable, and Confirmed)</th>
<th>Laboratory-Confirmed Cases</th>
<th>Total Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria</td>
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<td>8</td>
</tr>
<tr>
<td>Senegal</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
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<td>1</td>
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<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Mali</td>
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<td>7</td>
<td>6</td>
</tr>
<tr>
<td>United Kingdom</td>
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<td>0</td>
</tr>
<tr>
<td>Italy</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>34</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>
**Ebola outside Africa**

During the West-African outbreak, 17 persons with EVD disease have been cared for outside Africa of which three persons have contracted Ebola outside Africa. In the United States eleven cases of EVD have been reported: nine of them contracted the disease outside the US and travelled into the country, either as regular airline passengers or as medical evacuees; of those nine, two died. Two nurses have contracted Ebola in the United States, both treating an Ebola patient; both have recovered. Of the eleven cases, four have been diagnosed within the US: the two above mentioned nurses and two travellers that became ill in the US. Only 6 cases of Ebola have been diagnosed in Europe, all in connection with the Ebola outbreak in West Africa: one in Italy, one in Spain and three in the United Kingdom and one locally acquired in a health care worker in caring for an evacuated Ebola patient in Spain.

In August 2018, the Democratic Republic of Congo MOH tested 4 individuals positive for the Ebola virus in North Kivu. In this war-ravaged province in which there is mistrust of the government and mistrust of the Ebola response, the outbreak became the second largest ever recorded with a total of 3406 cases (3262 confirmed and 144 probable) and 2243 deaths, corresponding with a mortality rate of 65.9% which is significantly higher than in the West-African epidemic (39.5%).

**Ebola Ivory Coast (Thaï Forest virus, TAFV)**

In 1994 many chimpanzees died following an Ebola epidemic in the Tai nature reserve in Côte d’Ivoire on the border with Liberia. Here one person was infected during an autopsy on a chimpanzee that had died. She was evacuated to Switzerland where she was treated. The causative agent turned out to be a new genetic subtype of Ebola virus. In late 1995 another (unconfirmed) case occurred in the same area (Plibo) in a Liberian refugee.

**Reston virus (RESTV)**

In 1989 an epidemic of another Ebola virus occurred in a primate centre in the USA in Reston, a town near Washington D.C. A number of people were infected but without any illness both in Reston (4) and in the Philippines (12) where the monkeys came from. Unlike the case of Ebola-Zaire, there were arguments here for aerogenic transmission. Research was complicated by the fact that another haemorrhagic fever virus epidemic was taking place at the same time among the monkeys (Simian Haemorrhagic Fever Virus). Late 2008 a Philippino farm worker was found infected by the Ebola-Reston virus that was discovered in pigs at 2 farms north of Manila. It was the 1st time Ebola-Reston
was found outside monkeys. The infected man had not shown any symptoms and was healthy. Later 5 more persons were found to have been infected, all were asymptomatic. RESTV sequences have been found in Chines pigs, raising fear about food safety.

**Bundibudyo virus (BDBV)**

Bundibudyo was the region in Uganda where the 2007 Ebola epidemic was centred. The epidemic in Uganda was caused by a fifth viral species. The genome differs by about 32% of its nucleotides, compared with the other Ebola strains. This may complicate efforts to produce a universal vaccine. Fifty-six cases of Bundibugyo Ebola virus infection were laboratory confirmed during the first epidemic. Signs and symptoms were largely nonspecific. The proportion of deaths among those infected was about 40%. A new outbreak occurred in August and September 2012, centred on Isiro and Viadana, Haut-Uele district.

**Bombali virus (BOMV)**

In 2018 a new ebolavirus – Bombali virus was detected in free-tailed bats in Sierra Leone: little free-tailed (*Chaerephon pumilus*) and Angolan free-tailed (*Mops condylurus*) bats. The bats were found resting inside houses but it is not known whether human exposure has occurred or if BOMV is pathogenic in humans.

**Summary of known human Ebola Disease cases**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>1 non-fatal case (retrospective diagnosis)</td>
<td></td>
<td>Tandala, DRC (not confirmed)</td>
</tr>
<tr>
<td>1976</td>
<td>318 cases, 280 deaths</td>
<td></td>
<td>Yambuku, DRC (discovery of the virus)</td>
</tr>
<tr>
<td>1976</td>
<td>284 cases, 151 deaths</td>
<td></td>
<td>Nzara, Maridi, Tembura and Juba, Sudan</td>
</tr>
<tr>
<td>1977</td>
<td>1 fatal case</td>
<td></td>
<td>Tandala, DRC</td>
</tr>
<tr>
<td>1979</td>
<td>34 cases with 22 deaths</td>
<td></td>
<td>Nzara and Yambio, Sudan</td>
</tr>
<tr>
<td>1980</td>
<td>1 suspected case</td>
<td></td>
<td>Kenya (not confirmed)</td>
</tr>
<tr>
<td>1994</td>
<td>44 cases, 28 deaths</td>
<td></td>
<td>Minkouka, Gabon</td>
</tr>
<tr>
<td>1994</td>
<td>1 non-fatal case</td>
<td></td>
<td>Tai Park, Côte d'Ivoire</td>
</tr>
<tr>
<td>1995</td>
<td>315 cases, 255 deaths</td>
<td></td>
<td>Kikwit, DRC</td>
</tr>
<tr>
<td>Year</td>
<td>Cases/Deaths</td>
<td>Location/Details</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>1 non-fatal case</td>
<td>Plibo, Liberia (not confirmed)</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>37 cases with 21 deaths</td>
<td>Mayibout and Makokou, Gabon</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>60 cases with 45 deaths</td>
<td>Booué, Gabon. One exported case in South Africa with one fatal secondary case.</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>425 cases with 224 deaths</td>
<td>Gulu, Masindi, Mbarara (Uganda)</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>43 deaths in Congo, 53 deaths in Gabon</td>
<td>Gabon – DRC</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>No reliable numbers available</td>
<td>Mbomo, DRC</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>About 140 cases with about 120 deaths (February-March). Flare-up in November-December, with 35 cases (29 deaths).</td>
<td>Mbomo, DRC</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>25 cases with 6 deaths</td>
<td>Mbomo and Mbandza, Congo Brazzaville</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>About 10 cases</td>
<td>Etoumbi, DRC</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>About 187 cases About &gt; 90 cases New epidemic in Congo, lasting till early 2009. Number of cases unclear In March 2009, accidental needle stick injury in Hamburg (virologist) Isolated case (May 2011) in Uganda Number of cases unclear 66 cases, 35 deaths</td>
<td>Kampungu, Mweka, Luebo, DRC (Western Kasai) Western Uganda November 2009, outbreak in Mweka, DRC Germany, the first time that vesicular stomatitis virus-based vaccine is used in a human (post-exposure) July 2012, outbreak in Kibaale, Uganda and quasi simultaneous in August 2012 outbreak is Isiro and Viadana, Haut-Uele, Congo</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td></td>
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<td></td>
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<td>2008-2009</td>
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<tr>
<td>2009</td>
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<td>2011</td>
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<td></td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013-2016</td>
<td>28.652 cases with 11.325 deaths</td>
<td>Guinea, Sierra Leone, Liberia, Nigeria, Malia, Senegal, USA, Spain</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>66 cases, 49 deaths</td>
<td>Équateur province, DRC</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Cases</td>
<td>Deaths</td>
<td>Location</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>2017</td>
<td>8 cases</td>
<td>4</td>
<td>Likati, DRC</td>
</tr>
<tr>
<td>2018</td>
<td>54 cases</td>
<td>33</td>
<td>Bikoko, Mbandaka, DRC</td>
</tr>
<tr>
<td>2018-2020</td>
<td>3470</td>
<td>2287</td>
<td>North-Kivu and Ituri province, DRC</td>
</tr>
</tbody>
</table>

**Epidemiologic and ecologic features**

Ebola epidemic in Kikwit, Congo 1995. Small animal trapping and study, as a part of the search for the reservoir of this virus. Notice the protective gear of the researchers. Copyright ITM

Today, neither EBOV nor other filoviruses are endemic anywhere, but the discovery of persistent virus in humans after infection during the 2013-2016 epidemic, indicates that the virus can temporarily circulate in persons. The natural reservoir of these viruses remains unconfirmed, nevertheless bats are the prime suspects. To date the analysis of the numerous arthropods and living vertebrates has not produced a single positive viral isolate, although Ebola virus was demonstrated in several
carcasses in the Central African rainforest, esp. primates. Analysis of 98 animal carcasses in Gabon - Congo (study period 2001-2003) showed on 10 Ebola-positive gorillas out of a 50 gorilla carcasses, 3 positive chimpanzees out of 15, and 1 positive duiker (Cephalophus) out of 14. The monkey species, which have been studied thus far, all die from the infection and therefore cannot form the natural reservoir. Filoviruses are considered regionally epizootic. Contact with infected monkeys plays a role in the beginning of an epidemic but how these animals are initially infected is not known. The epidemic, which started in November 2003 in Mbomo, Congo Brazzaville, was rumoured to have started after villagers found a dead wild pig in the forest and ate its meat. This would be the first case that such an animal would be implicated. Certain frugivorous and insectivorous bats can be experimentally infected and certain species are seropositive in nature. In 3 bat species (Epomops franqueti, Hypsignathus monstrosus and Myonycteris torquata) Ebola RNA sequences have been detected. These animals usually develop an asymptomatic infection. To date, ebolaviruses surprisingly were never isolated in a bat, which might be explained by low viral loads or inhibitors in bat tissue. Epidemics may start after spillover events from bats to humans and other mammals that serve as end-, intermediate- or amplifying hosts. These animals are often shot and eaten as “bush meat”. The Zaire strain of Ebola virus can also replicate in pigs. Infected animals develop severe lung disease. They shed large numbers of virions in the respiratory tract. Shedding continues for up to 2 weeks after infection. Infected animals can transmit the infection to non-infected pigs and possibly to humans.

Pathophysiology

Transmission takes place through direct contact with infected body fluids (including sexual contact) and nosocomial through infected needles and contact with infected blood. Sexual transmission is described up to 6 months after survival. Aerogenic transmission of Ebola has been demonstrated in the laboratory in Rhesus monkeys, thought this is never described in humans. Viral particles land on mucous membranes or occasionally enter percutaneously. Filoviruses replicate in the cytoplasm of their target cells, which are initially dendritic cells and macrophages and potently shut down early innate immune responses by blocking interferon production. Later, dendritic cells migrate to lymphoid tissues and the virus is released in the circulation with spread to the liver, spleen and other tissues. Disease is caused by the cytopathogenic effects of the virus itself leading to cell lysis, but also by an exaggerated host immune response inducing a cytokine storm causing a septic shock. Several cytokines (IL-1ß, IL-6 and TNF) and chemokines cause T-cell activation, which is rendered ineffective in severe or fatal cases due to T-cell exhaustion followed by an impaired adaptive immune response. Endothelial-cell dysfunction is caused by inflammatory mediators triggering vascular permeability and fluid extravasation. Tissue factor is produced by infected macrophages and lead to fibrin deposition in the spleen, lymphoid tissues, glomeruli and renal proximal tubules. Diffuse intravascular coagulation
arises due to consumption of clotting factors, endothelial dysfunction and platelet dysfunction with coagulopathy and bleeding as a consequence. Multiple organ failure (MOF) with tissue hypoperfusion develops due to microvascular anomalies and hypovolemia due to gastro-intestinal fluid losses. Bacterial translocation can be a consequence of the disrupted gut mucosa triggering bacteraemia and bacterial septic shock. Fatal cases are associated with defective immune responses and high viremia. Survivors have early and vigorous cellular as well as humoral immune responses. The immunological course early in the infection determines how quickly the Ebola virus replicates and whether the host will die or recover. Surviving an infection is linked to an early appearance of IgM and IgG, followed by the activation of cytotoxic cells.

**Clinical aspects**
The clinical disease is not called Ebola haemorrhagic fever anymore but Ebola virus disease (EVD) which downplays bleeding as a clinical hallmark and stresses the great variability in symptoms. After an incubation period of 2 to 21 days (average 7 days) infection often leads to multiple organ failure, with death occurring on average 6 to 9 days after the onset of symptoms. But asymptomatic infection with Ebola can occur. People infected with Ebola virus initially present with nonspecific febrile illness with malaise, fatigue and myalgia. In a second stage, gastro-intestinal symptoms with anorexia, nausea, abdominal pain, vomiting and diarrhoea develop. Patient can lose up to 10 liters per day and severe electrolyte disturbances rise: hypokalaemia, hyponatremia, hypomagnesemia, … Dysphagia,
headache, conjunctival injections, maculopapular rash and joint pain are other common symptoms. Hiccups can be caused by uncontrolled diaphragm contractions due to viral invasion of the CNS that controls the diaphragm. Hiccups were a clue that led researchers to suspect that the West-African epidemic was not caused by Lassa virus but possibly by Ebola virus. One should not focus too much on bleeding as a presenting symptom as this is a late symptom and cases will be missed. Even in end-stage disease patients, bleeding abnormalities occur in less than half of them. Bleeding from gums, petechiae, persistent oozing from venepuncture sites, subconjunctival haemorrhage, haematemesis and bloody diarrhoea can be present. Hepatitis arises due to lysis of hepatocytes and liver hypoperfusion. Once kidney failure sets in, the fluid management becomes very difficult with the risk of fluid overload, pulmonary oedema and difficult to manage hypo-/hyperkalemia. The occurrence of renal failure almost universally leads to death if renal replacement therapy is not available. Neurological complications have a multifactorial aetiology: hypoglycaemia, viral meningoencephalitis, intracranial haemorrhage, hepatic encephalopathy, delirium, ...

Healthcare providers should not minimize the psychological impact of receiving the diagnosis ‘Ebola’ on a patient, knowing that the mortality rate in some epidemics surpasses 60 percent. Anxiety and depression are common symptoms and psychological support to help patients cope with their fears is part of good patient care.

The post-Ebola syndrome refers to musculoskeletal pain, headache, encephalitis and ocular problems (uveitis) that were frequently noted in thousands of EVD survivors of the 2013-2016 EBOV epidemic. The mental health effects on survivors, their family and community are considerable.

**Diagnosis**

Diagnosis during an epidemic is based on clinical suspicion, with serum PCR as confirmation. The viral RNA can be detected via a quantitative reverse transcriptase PCR on a blood sample (qRT-PCR). Results are expressed in Cycle Threshold (Cₜ) levels: low (< 20) Cₜ levels indicate detection of the virus after a low number of cycles required for the fluorescent signal to cross the detection threshold, hence a high viral load translating in a poor prognosis. Diagnostic studies during the 2013-2016 outbreak have mainly relied on molecular diagnostic platforms. In general these tests are highly sensitive and specific. Various assays are currently available & FDA approved including an Xpert-based machine (Xpert Ebola Assay), which is a fully automated and closed device now rolled out for tuberculosis diagnosis. If this assay is installed within an Ebola treatment unit, time between sample collection and result was 2.5-3 hours in a study by MSF. The assays itself runs over around 90 minutes and cartridges specific for the EBOV Zaire strain were developed to target highly conserved sequences in the nucleocapsid protein (NP) and glycoprotein (GP) genes. Results can come out
positive or negative and a cycle threshold ($C_t$) for both gene targets is given. These molecular assays may be negative early in the disease course, warranting follow-up testing in patient with recent onset symptoms. If the initial PCR test is negative and the patient has symptoms that started less than 48 hours previously, a second sample must be taken at 72 hours of illness (after another 24-48 hours). Simple bedside antigen-based tests have become available, but their sensitivity and thus negative predictive value is lower than PCR. These tests can thus be used for quick confirmation, but not to exclude the infection. Virus can be cultured in a few BSL-4 laboratories (e.g. on Vero cells). Serological testing has no place in the diagnosis of an acute ill patient, but can be used for epidemiological research. It is worth knowing that each of the various geographical isolates have their own antigenic structure and therefore problems can arise with serological testing.

Other laboratory findings are elevated transaminases linked with hepatitis and creatinine kinase due to myositis. Consumption of clotting factors due to DIC leads to disturbed coagulation tests (PTT, D-dimers, fibrinogen). Thrombocytopenia is present in most patients and initially there is lymphocytopenia and later neutrophilia. Histologically there is focal necrosis in various organs (testes, kidneys, liver, etc.). Lower baseline viral load, creatinine and aminotransferase levels correlate with improved survival.

Patients can be safely discharged from Ebola treatment units when two sequential tests come back negative ($C_t > 40$) in a patient that has clinically improved.

**Treatment**

Early diagnosis and prompt initiation of care increase survival ratios. Paediatric patients and elderly are at higher risk of dying (however in the 2018-2020 epidemic in Eastern DRC extremes of age were not associated with poorer outcomes) as well as patients with a high viral load. During epidemics, good patient care may lower the mortality. Care for EVD patients is based on three pillars: supportive care to restore normal physiology, management of discomfort or distress and presumptive treatment of concurrent infections. In all epidemics so far, treatment was mostly done in very basic field conditions and treatment in an intensive care unit was rarely possible. Throughout the experiences gained in the recent epidemics, Ebola treatment Centres (ETC) in the field have more and more evolved towards provision of individualized care, with advances in laboratory and technical support. Staffing ratios of 1 or more more clinicians for four patients, and assessments (evaluation of each patient) performed at least three times per 24 hours are recommended. A comprehensive guidance “Optimized Supportive Care for Ebola Virus Disease” has been published by the World Health Organisation (https://www.who.int/publications-detail-redirect/optimized-supportive-care-for-ebola-virus-disease).
Gastro-intestinal symptoms can be controlled with metoclopramide (Primperan®) or domperidone (Motilium®) against vomiting and loperamide against diarrhoea. Omprazole is given as stress ulcer prophylaxis. Prevention intravascular volume depletion and avoidance of organ hypoperfusion is critical. Fluid losses from vomiting, diarrhoea and vascular leakage may require more than five liters per day of crystalloid solution intravenously if the patient is unable to compensate the losses with oral rehydration. In the last epidemics, the use of point-of-care ultrasound has been a useful addition to estimate fluid status and has the potential to increase diagnostic capacity and individually tailored patient care. On-site biochemical testing was often available, permitting correction of electrolyte abnormalities (hyponatremia, hypo-/hyperkalaemia, hypomagnesemia and hypocalcaemia) and hypoglycaemia. Oral nutrition should be encouraged, ideally guided by a nutritionist. If necessary nasogastric tube can considered. High calorie liquid food is easier to swallow than solid food, since many patients suffer from severe throat pain. Antipyretic agents as paracetamol are given to manage pain and to decrease fever. Stronger pain killers (tramadol, morphine) might be needed, but NSAID’s should be avoided to minimize the risk of renal failure and to decrease the risk of bleeding. Chlorpromazine and even haloperidol might be considered in case of agitation and confusion. Seizures are treated with diazepam.

Since coinfections are often difficult to diagnose in low resource settings with blood cultures rarely available, presumptive treatment with broad-spectrum antibiotics, in the form of a third generation cephalosporin, are usually part of the initial standard treatment. It is not unusual for EVD patients to develop new-onset fever that may be associated with leukocytosis in the second or third week of the hospital course, often despite initial improvement in the presenting symptoms and the viral load. In this setting, the development of ETU-acquired secondary infections while on broad spectrum antibiotics, and the development of resistant gram-negative bacteremia or Clostridium difficile infection, should be considered. Antibiotic management, including drug choice as well as doses, should be adjusted accordingly. In malaria endemic regions, anti-malarial treatment is sometimes added for all admitted patients, but robust data justifying this approach are lacking. A natural experiment due to a 12-day stock rupture of artesunate-lumefantrine in a Liberian MSF Ebola treatment center, noticed a lower risk of death in patients prescribed artesunate-amodiaquine compared with patients that received artesunate-lumefantrine. Amodiaquine is a compound with anti-Ebola activity in vitro. It is however not excluded that artemether-lumefantrine is associated with an increased risk of death due to torsades de points with fatal arrhythmias in patients with a long QTc interval especially when combined with hypokalemia/hypomagnesemia and/or ciprofloxacin and/or metoclopramide. Another explanation is that artesunate-amodiaquine use was associated with unmeasured patient characteristics that altered the risk of death (e.g. effective malaria infection, higher viral loads, age, patients admitted during busier periods, patients in need of parenteral treatment with worse prognosis, ...).
In the rare events when parenteral nutrition, renal-replacement therapy and mechanical ventilation where available, these treatments probably had a lifesaving impact.

Survivors of EVD need comprehensive follow-up care, including rheumatological, auditory, and ocular function with special attention to visual acuity deficits or raised intraocular pressure. Appropriate psychological and social support should be offered after mental health screening examinations.

Aside from good supportive care, several investigation treatments with anti-EBOV activity exist. The main categories are antibodies (plasma from convalescent patients, whole blood or monoclonal antibodies) and antivirals. A study with convalescent plasma in Guinea did not show sufficient mortality benefit, neither did a study with whole blood transfusion. A phase II study with the antiviral favipiravir only decreased case fatality rate in patients with a low viral load and seemed to increment mortality in patients with higher viral loads. At the end of the 2013-2016 outbreak in West-Africa, a randomized clinical trial (PREVAIL II) with ZMapp – a cocktail of three potent monoclonal antibodies – showed a fatality rate of 37% (13 of 35 patients) in those receiving the standard of care and a fatality rate of 22% (8 of 36 patients) in those receiving standard of care together with ZMapp. Although this result seems beneficial, the decline in the epidemic reduced participant enrolment hence results did not reach the statistical threshold for efficacy.

During the 2018-2020 outbreak in DRC, the Pamoja Tulinde Maisha (PALM “Together Save Lives” in Swahili) trial compared ZMapp with three newer agents: mAb114 (Ridgeback Biotherapeutics) which is a single monoclonal antibody derived from the memory B cells from a survivor of the Kikwit EVD epidemic, REGN-EB3 (Regeneron Pharmaceuticals) combining three triple monoclonal antibodies obtained by immunizing mice and the antiviral remdesivir (Gilead), a prodrug nucleotide analogue. mAb114 and REGN-EB3 have the advantage over ZMapp that they are given as a single dose whereas ZMapp is given in 3 doses, spaced 3 days apart. Remdesivir is given in daily doses for at least 10 days. An interim analysis showed superiority of Mab114 and REGN-EB3 to ZMapp and remdesivir with respect to mortality. In the mAb114 and REGN-EB3 group mortality was 35% and 33% as compared with 50% in the ZMapp and 53% in the remdesivir group. Shorter duration of symptoms before admission with earlier treatment initiation improved survival, which had not been the case in previous epidemics. Surprisingly, mortality with ZMapp in the PALM trial was 50% compared to 22% in the above mentioned PREVAIL II trial. The reasons remain unclear and subgroup analysis is ongoing to shed more light on potential differences among treatment groups. After the interim analysis patients were randomized to receive mAb114 or REGN-EB3, dropping the ZMapp and remdesivir study arm. Final results of this trial are still pending.

Hundreds of patients in the recent outbreak in Eastern DRC that were not included in the PALM trial still received above mentioned investigations drugs under the Monitored Emergency Use of
Unregistered and Investigational Interventions (MEURI) framework. Despite the lack of randomization, an analysis of patients receiving drugs under MEURI showed remarkably similar results for the same therapeutics that were provided in the PALM trial.

It is important to notice that patients developing EVD despite previous vaccination for EBOV had much better outcomes.

Future research might focus on combination therapy considering the possible synergistic effect of remdesivir – that has a delayed onset of action as compared with a antibodies – combined with antibodies. A next generation human antibodies (i.e. MBP134, FVM04 and CA45) have shown protection against EBOV, SUDV and BDBV, whereas ZMapp, REGN-EB3 and Mab114 only protect against EBOV. Nevertheless, these products will first have to prove their non-inferiority in a well-designed future trial.

<table>
<thead>
<tr>
<th>Treatment and Study Design (Country)</th>
<th>Filovirus Species (Strain)</th>
<th>Dose</th>
<th>Regimen</th>
<th>No. of Patients and Outcome</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaccine</strong></td>
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</tr>
<tr>
<td>IVV(ZEOV); open-label, cluster, randomized trial of ring vaccination (Guinea)</td>
<td>Ebola (Makona)</td>
<td>$2 \times 10^6$ PFU</td>
<td>Single injection (IM)</td>
<td>58 of 17 vaccinated; estimated efficacy, 100% (95% CI, 79.3–100.0)</td>
<td>Henao-Restrepo et al.</td>
</tr>
<tr>
<td>IVV(ZEOV); randomized, placebo-controlled phase 2–3 trial (Liberia)</td>
<td>Ebola (Makona)</td>
<td>$2 \times 10^6$ PFU</td>
<td>Single injection (IM)</td>
<td>509 vaccinated; phase 3 eliminated because of decline of EBOV in Liberia</td>
<td>Kennedy et al.</td>
</tr>
<tr>
<td>IVV(ZEOV); open-label, cluster, randomized trial of ring vaccination (DRC)</td>
<td>Ebola (Kivu)</td>
<td>$2 \times 10^6$ PFU</td>
<td>Single injection (IM)</td>
<td>93,945 vaccinated; efficacy, 97.3% (95% CI, 95.3–98.3)</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ChAd2-zEOV-Z; randomized, placebo-controlled phase 2–3 trial (Zaire)</td>
<td>Ebola (Makona)</td>
<td>$2 \times 10^{11}$ particle units</td>
<td>Single injection (IM)</td>
<td>500 vaccinated; phase 3 eliminated because of decline of EBOV in Liberia</td>
<td>Kennedy et al.</td>
</tr>
<tr>
<td><strong>Antibival Therapy</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Conventional plasma, nonrandomized comparative study</td>
<td>Ebola (Makona)</td>
<td>Unknown</td>
<td>Two consecutive IV transfusions of 200–750 ml each</td>
<td>84 enrolled; no significant survival benefit</td>
<td>van Griensven et al.</td>
</tr>
<tr>
<td>Conventional blood, nonrandomized comparative study</td>
<td>Ebola (Makona)</td>
<td>Unknown</td>
<td>One IV transfusion of 450 ml given over a period of 1–4 hr</td>
<td>49 enrolled; no significant survival benefit</td>
<td>Safr et al.</td>
</tr>
<tr>
<td>ZMapp; phase 2–3 trial (Liberia, Sierra Leone, Guinea, United States)</td>
<td>Ebola (Makona)</td>
<td>50 mg/kg</td>
<td>One dose every 3 days (IV) for a total of three doses</td>
<td>36 enrolled; 28 survived (77.8% survival rate)</td>
<td>PREVAIL II Writing Group</td>
</tr>
<tr>
<td>MA114; PALM trial (DRC)</td>
<td>Ebola (Kivu)</td>
<td>50 mg/kg</td>
<td>One dose (IV)</td>
<td>321 enrolled; 160 survived (49.5% survival rate)</td>
<td>Mulangu et al.</td>
</tr>
<tr>
<td>REGN-EB3; PALM trial (DRC)</td>
<td>Ebola (Kivu)</td>
<td>150 mg/kg</td>
<td>One dose (IV)</td>
<td>374 enrolled; 113 survived (64.6% survival rate)</td>
<td>Mulangu et al.</td>
</tr>
<tr>
<td>Remdesivir (GS-5734); double-blind, placebo-controlled, natural history trial (Liberia)</td>
<td>Ebola (Makona)</td>
<td>100 mg</td>
<td>Once daily for 5 days (IV)</td>
<td>Ongoing, with planned enrollment of 90 survivors to assess viral shedding in serum</td>
<td>Sigal et al.</td>
</tr>
<tr>
<td>Remdesivir (GS-5734); PALM trial (DRC)</td>
<td>Ebola (Kivu)</td>
<td>200 mg loading dose, 100 mg thereafter</td>
<td>Once daily for 5–13 days (IV)</td>
<td>175 enrolled; 82 survivors (46.0% survival rate)</td>
<td>Mulangu et al.</td>
</tr>
<tr>
<td>Favipiravir (T-705); single-group trial with historical controls (Guinea)</td>
<td>Ebola (Makona)</td>
<td>6000 mg loading dose, 2400 mg thereafter</td>
<td>Once daily for 2–12 days (oral)</td>
<td>124 enrolled; no significant survival benefit</td>
<td>Sankala et al.</td>
</tr>
<tr>
<td>TKM-386031; single-group, phase 2 trial with historical controls (Sierra Leone)</td>
<td>Ebola (Makona)</td>
<td>0.1 mg/kg</td>
<td>Once daily for up to 7 days (IV)</td>
<td>12 enrolled; no significant survival benefit</td>
<td>Denning et al.</td>
</tr>
</tbody>
</table>
Prevention

Prior to the 2013-2015 Ebola outbreak, no effective vaccine was commercially available nevertheless, such a vaccine was explored with several purposes, such as after lab-accidents, during epidemics, and probably in the stockpile for biowarfare defence.

Clinical development of several vaccines has advanced substantially during the 2013-2015 EBOV outbreak. Successful vaccination relies on the development of an immune response against the viral glycoprotein (GP), which is critically involved in cell attachment, fusion and cell entry. While assumed that the protection against EVD predominantly relies on the development of anti-GP antibodies, the role of the cellular immune response in vaccine protection remains to be defined. There is indeed not yet a well-defined immune marker (biomarker) that correlates with protection against EVD after vaccination. Currently, the level of IgG antibodies against the EBOV GP is the most commonly used measure of immunogenicity in vaccine trials.

The vesicular stomatitis virus (VSV)-based vaccine (rVSV-ZEBOV), a live (replication-competent) vaccine expressing the Zaire ebolavirus glycoprotein has shown limited reactogenicity (“toxicity”) and good immunogenicity in phase I/II studies, although arthritis was documented in some. This vaccine was subsequently evaluated in a phase III trial in Guinea using a ring vaccination strategy, as previously successfully employed during the eradication of small pox. In principle, ring vaccination represents a strategy of targeted vaccination (in contrast with vaccination in the general population), targeting risk individuals at high risk of exposure to and development of EVD. After the identification of a new EVD case (‘index’) case, his/her contacts, and the contacts of these contacts are eligible for vaccination. The trial compared clusters with immediate vaccination with clusters with delayed vaccination 21 days later. As cases occurring early after vaccination might have been infected before the vaccination, and since it takes some time for the vaccine to induce immunity, only cases occurring ten days or more after vaccination were taken into account. No EVD cases were seen after this ten day period in the immediate vaccination group, whereas cases continued to accrue with delayed vaccination. This yielded a vaccine efficacy of 100% although with a wide confidence interval (74.7%-100%). The vaccine effectiveness, taking into account all individuals that could/should have been vaccinated was 76.3% (95% CI -15.5% to 95.1%).
The rVSV-ZEBOV has also been used in front-line workers in Guinea and Sierra Leone. During the latest epidemic in eastern DRC, more than 265,000 people have received it as part of a ring vaccination strategy (with ring vaccination not only offered to 1st, but also to 2nd and 3rd generation contacts) with an efficacy of 97.5% for vaccines with an onset of illness more than 10 days after vaccination, and 88.1% for all those with EVD regardless of the timing of illness onset. The rVSV-ZEBOV vaccine is now approved for use by WHO during epidemics and is approved for use in Europe and the United States, mainly to protect international healthcare workers that will work in Ebola treatment units.

Since November 2019, a second vaccine Ad26.ZEBOV/MVA-BN was used to complement the ongoing
ring vaccination with rVSV-ZEBOV. This vaccine is given in two doses, 2 months apart: the first dose consists of a recombinant human adenovirus 26 encoding the Zaire ebolavirus glycoprotein, while the second dose is a modified vaccinia Ankara virus (MVA) containing glycoproteins of Zaire and Sudan ebolavirus and Marburg Musoke virus as well as the nucleoprotein of the Tai Forest ebolavirus. The 2 dose prime-boost regimen is expected to give longer protection and therefore the vaccine is given to at-risk populations neighbouring an Ebola epidemic regions where there is no active transmission yet and to health care workers. It is not part of the ring vaccination strategy when rapid immunity is needed since the single-shot rVSV-ZEBOV appears to induce a quicker immune response. Future work on vaccine efficacy, stability, storage, transport and administration as well as supply adequacy are needed.

Research & Development (R&D) during the 2013-2015 outbreak

Clinical research initiatives started only late during this EVD outbreak. Only in August 2014, WHO declared the outbreak as a “public health emergency of international concern” and funding became available from the main funding organizations from September on, in part driven by EVD infections of health care workers from international healthcare workers with the threat of EVD spreading to the US and Europe. WHO developed an inventory of vaccines and therapeutics in the pipeline. However as many had often not undergone clinical evaluation, there was a lot of discussion whether it was ethical to use/evaluate these interventions during the 2014-2015 outbreak. In September 2014, the WHO Ethics Working Group released a statement recommending that “investigational drugs or vaccines that have shown promising results in the laboratory or in animal models be urgently tested in humans by scientifically sound, rigorous methods”. Since then, many clinical studies have been launched in a relatively short time span, complemented with studies in non-human primates. A process that would otherwise take several years now had to be done in months.

Ebola outbreak management

Recognition

The very first step is to recognize possible clinical cases, which is why case definitions must be determined and widely distributed. The current case definition used by WHO for Ebola Virus Disease is: a patient with any ONE of the following:

- Sudden onset of fever (≥38°C) AND contact with confirmed or probable Ebola case or dead or sick animal; OR
• Sudden onset of fever (≥38°C) AND ≥3 symptoms (Headache, vomiting, diarrhoea, anorexia/loss of appetite, lethargy, stomach pain, myalgia, dysphagia, breathing difficulties, or hiccups); OR
• Contact AND ≥3 symptoms; OR
• Unexplained bleeding or miscarriage; OR
• Sudden unexplained death.

This generic suspect case definition may be adapted to local circumstances (clinical presentation, mode of transmission). During outbreaks, expanding the suspect case definition to include patients with mild symptoms increases sensitivity, but increases the case load in triage centres. The performance of the case definition during outbreaks should be assessed.

Steps should be taken to identify and type the virus (send a blood sample safely to a well-equipped laboratory). In a laboratory which is protected and equipped to work with dangerous pathogens (biosafety level 4), an attempt will be made to detect viral antigen, antibodies and viral RNA (reverse transcriptase PCR) and carry out an analysis of the genome in order to establish which Ebola subtype is involved.

Central organization

If it is established that it really is Ebola, the government will be notified. Central control, registration and coordination is essential for combating an epidemic. WHO and CDC will be notified. Groups specifically responsible for a certain part of the campaign will be set up: clinical care, surveillance in the community, logistics, collecting the dead and safe burials, investigating rumours, informing the population, epidemiological study, research, reception center, etc. These days it is also useful to appoint someone who can handle the press correctly. Every day information will be exchanged between the various teams and the latest developments will be reported to the WHO in Geneva.

Vaccination

In the most recent and future epidemics, vaccination of health care workers, ring vaccination and vaccination of populations at risk play a much bigger role than in earlier epidemics (cfr. above). More info can be found in the Strategic Advisory Group of Experts (SAGE) on Immunization document by WHO:
https://www.who.int/immunization/policy/position_papers/interim_ebola_recommendations_may_2019.pdf?ua=1
Isolating patients

The patients’ movements should be limited. They should be isolated (no direct physical contact with patients, blood, excreta etc.). Any new patient must be directed to a triage zone. Here, based on the history (contact with Ebola patients, fever, symptoms), patients must be divided into Ebola suspects and non-Ebola patients according to the predefined case definitions. Ebola suspects must be kept in isolation, awaiting results of their PCR. If fever came up less than 3 days ago and the PCR result is negative, the PCR test will be repeated after 2 days before a patient is considered definitely negative. Often contact with Ebola will not be reported due to superstition, fear of stigmatization or if there was sexual contact with a person who subsequently developed Ebola infection. The absence of a lab facilities on-site can be a practical problem for the clinicians working in the field.

Barrier nursing

During an outbreak, there is a crucial need to protect health care workers. The small inoculum and the high mortality rates despite (investigational) treatments impose a zero-tolerance practice. Personal protection (masks, goggles, aprons, boots, disinfection supplies) for medical staff and for people who care for the sick person in case of refusal to admission to an ETC (often family) is necessary. Demonstration of how to use the protective equipment and proper explanation are imperative. Donning and doffing is done through standard operational procedures and under direct supervision of a team member. Personal protective equipment has many inconveniences, but none greater than heat stress, limiting the time that can be spent for caring patients under tropical conditions.

Reusable equipment should be disinfected rigorously with, for example, bleach (hypochlorite solution). Objects, which cannot be sterilized, must be burnt under supervision. People who are suspected of being infected with the Ebola virus should be cared for by people who understand and use personal protection. Basic needs (drink, food, pain-relief, hygiene, etc.) have to be met. Vomitus, sputum, faeces and urine must be collected in a plastic bucket and mixed with strong bleach before disposal.

Centers with a poor medical infrastructure and with a high risk of nosocomial transmission must be closed down temporarily. This applies both to large hospitals and small one-person clinics with only a few needles and syringes. Strict guidelines have to be issued to centers which continue functioning, particularly with regards to triaging of suspect cases, disinfection, the use of needles and syringes, vaccinations and surgical procedures. In many places non-qualified private individuals have only a few (non-sterile) needles and syringes, which they use for all injections.
Cfr. section on treatment above.

**Surveillance and contact tracing**

The goals of Ebola virus disease (EVD) surveillance are to promptly detect new, suspected EVD cases and deaths so as to trigger an appropriate response. Communities and local authorities should always report all deaths. In past epidemics a system of alerts was put in place. An alert is a condition that meets a very broad (sensitive) definition that aims to identify all signals that could potentially be an EVD case or death. Alerts can be generated by the community, at health facilities, or picked-up in the media. Often checkpoints are put up at so called points of entry and exit. Alerts are reported to those in charge of surveillance through various means, e.g. a telephone hotline. If an alert is validated and a new case identified, it is primordial to establish the chain of transmission.

People who have recently had contact with Ebola patients but do not display symptoms have to be placed under supervision (surveillance) for 3 weeks, the maximum incubation period. A contact is defined as: any person who has been exposed to a suspected, probable, or confirmed case of EVD in at least one of the following ways:

- has slept in the same household as a case
- has had direct physical contact with the case (alive or dead) during the illness
- has had direct physical contact with the (deceased) case at a funeral or during burial preparation rituals
- has touched the blood or body fluids (including urine, faeces, vomit, tears, or sweat) of a case during their illness
- has touched the clothes or linens of a case
- a baby who has been breastfed by the case

Note: This should include health workers (including those involved in cleaning, waste management, laboratory technicians, nursing, etc.)

If symptoms arise, immediate investigations should be carried out. Each diagnosed patient has on average 10 to 15 contacts which are to be monitored daily for 21 days. In large epidemics, contact tracing and follow-up can be a vastly resource-intensive activity.
Convalescent patients

Ebola virus might persist up to several months in selected immunologically privileged body sites of survivors. Sexual transmission is possible up to 6 months or longer after clinical recovery. Male survivors and their partners should be counselled on safe sex practices for 6 months or until their seminal fluid is free of viral RNA.

Convalescent serum can be stored if necessary, even though studies with convalescent serum so far failed to prove survival benefit. This serum has the possibility to produce monoclonal antibodies as was the case in mAb114 development.

Information

Nothing may be as important as community engagement and public perception. A part from education about the disease and control measures, populations should be encouraged to quickly alert authorities about febrile cases and unexplained deaths. Transmission of the disease will only stop when the community is no longer caring for the sick in unprotected settings and burying the dead in an unsafe manner. Trust is not always a given in an epidemic, which was clearly shown in the 2018-2020 DRC outbreak, with several structures of the outbreak response attacked. A general large-scale information campaign with adequate and practical information for the population should be started. If this results in many questions and tips, a permanent center can be set up where information about possible new cases can be examined. In view of the extreme virulence, the incomplete knowledge about these pathogens and memories of the impact of the earlier plague and yellow fever epidemics, these pathogens can capture the imagination of the general public. Superstition and belief in witchcraft can lead to misunderstandings and violence. In an environment of mistrust towards the national or local government and towards international organizations, experimental countermeasures as vaccination and experimental drugs can fuel rumours of unsavoury experimentation. Crystal clear communication about the ongoing interventions with transparent answers to questions are a prerequisite for the interventions to be successful.

Burials

The deceased should not be washed, and the bodies have to be isolated and buried as quickly as possible and reasonable. This sometimes causes problems with the family and acquaintances of the deceased because of the disruption of traditional rituals. The government has a role to play here in law enforcement avoiding the disrespect of cultural values as much as possible.
**Social impact**

Caring for orphans in the community should be organized if this does not take place through the traditional system of the extended family. The latter sometimes does not work because of fear, prejudice and practical problems.

**Logistics**

Logistics play a very important role and include, among other things, infection control, equipment and materials, administrative support, accommodation, money and wages, communication, transport, fuel, safety and stock management. Good management is essential and has to be entrusted to reliable people. The NGO Médecins Sans Frontières and Alima (Alliance for International Medical Action), an international non-profit medical organization have a lot of experience in handling the logistics of such operations. Specific “Ebola kits” of different sizes have been prepared and are kept in stock, ready to be used in emergency situations.

**Personnel**

Experts in various areas cannot, in most cases, make themselves available quickly for a long time and a rotation system should be organized. It is best if (international) staff do not change too frequently in order to achieve a minimal continuity locally. Realistic guidelines for cases in which medical personnel are infected accidentally must be drawn up. According to the scale of the outbreak, the need for formation of national and/or international staff should be assessed.

**Epidemiology**

Epidemiological research should attempt to identify transmission routes and secondary cases. Risk factors for infection should be identified: unsafe burials, screening systems in health facilities, fear/mistrust in the community. An attempt will also be made to trace the first case in order to understand how the chain of infection started. However this person may well have died. Several people, such as customers, work colleagues, neighbours, family and friends may be able to provide useful information. A reminder of the terminology: the index case is the patient in whom the disease first indicated the existence of an outbreak. The index patient always remains the same person irrespective of whether earlier cases are discovered later. The very first case is called the primary case, not the index case. Later secondary, tertiary, etc. cases can follow. The first case might change over time with incoming retrospective data, whilst the index case of an epidemic will never change in
the future.

**Reservoir**

Because an animal reservoir is assumed to exist where the virus “hides” between epidemics, extensive attempts have been and are being made to identify this. An “ecological” team should be exclusively involved in this and will study different animals in the vicinity. An investigation should also be carried out into whether the virus is “exported” from the isolation units in the hospital to the environment. In addition to the fieldwork itself, there then follows the tedious analysis of the various potential hosts, both for the presence of the virus and their taxonomic identification.

**Laboratory**

Rapid sample analysis (blood samples of patients, samples of other body liquids, etc.) and rapid transmission of the results is recommended. Logistical problems can hinder this. Investment in research and cooperation will pay dividends.

**Looking for isolated cases**

The maximum known incubation period is 21 days. After the end of the epidemic (no more cases for a minimum of 6 weeks), surveillance can be carried out locally. It is possible that isolated cases and limited outbreaks occur. In order to obtain a better understanding of this disease, long-term surveillance is necessary. Regular flare-ups of the disease in the aftermath of the devastating epidemic in Guinea, Sierra Leone and Liberia, were seen due to sexual transmission, even several months after the declaration of the end of the epidemic.

**Future prevention**

We do not know how all epidemics started, but several followed the consumption of infected apes. The risk of nosocomial transmission is clear. Owing to modern rapid means of transportations, Ebola fever can emerge anywhere in the world. Naturally this does not only apply to Ebola, but to the whole spectrum of communicable diseases.

LAST UPDATED BY ADMIN ON JULY 14TH, 2022
Poliomyelitis

Summary

- Poliomyelitis: enteroviral infection with fecal-oral transmission
- There are three related polioviruses, but no immunological cross-protection
- Often asymptomatic infections
- Sometimes flu-like syndrome with muscle pain and fever
- In a minority of patients flaccid paralysis with sensation intact follows
- Vaccination is very effective as prevention
- Anno 2024 the Global Polio Eradication Programme has not yet achieved the final goal or eradication
- Emergence of vaccine-derived (reverse mutation) pathogenic viral strains

General

Poliomyelitis is a disease caused by a picornavirus (family enteroviruses). There are 3 different polioviruses, i.e. types 1, 2 and 3. They do not exhibit immunological cross-reactions. Chimpanzees, Rhesus monkeys and cynomolgus monkeys (syn. *Macaca fascicularis*) can be infected orally and suffer paralysis as a result, but in practice man is the only reservoir. Chronic latent carriers are very rare, but healthy and immune-compromised long-term virus shedders were identified, excreting poliovirus for more than 20 years. The epidemiological importance of these people is not yet known. The virus does not survive in the wild—ex. sewage and surface water—for more than a few months, and then only under conditions of low temperatures and high humidity.

Historical Note

Poliomyelitis has been known since ancient times. An Egyptian carving from 1580 BC shows typical sequelae of poliomyelitis. At present, it is in the Carlsberg Glyptothek Museum in Copenhagen, Denmark. A 3,500-year-old Egyptian limestone funeral stela represents a man called Roma, giving offerings to the Goddess Astarte. He was a doorkeeper of the Eighteenth or Nineteenth Dynasty. He is portrayed with a wasted and shortened leg accompanied by an equinus deformity of the foot. The most likely diagnosis is that these are sequelae from poliomyelitis acquired in childhood. The person appears with a stick which could be used as a crutch. His disability had clearly not prevented his attaining high office, marrying and having at least one child.
In 1916 there was a polio epidemic in the USA with more than 10,000 cases. It was noticeable that most victims with paralysis were found among those groups of the population that observed the greatest possible hygiene precautions. It was referred to as a disease of cleanliness. At that time the washing of the whole body (rather than just the face and hands) and the installation of bathrooms in the houses of wealthy citizens was greatly on the increase. The improved hygiene ensured that the infection did not develop at a very youthful age. The disease follows a more serious course when it occurs at a more advanced age. Under poor hygiene conditions, children are infected at a very young age, a small proportion of whom will develop paralysis (“infantile paralysis”). In 1952-53 Europe experienced a very severe epidemic. North America also was not spared, with 55,000 cases in 1953.

In the early 1950s, Jonas Salk developed the first formalin-inactivated injectable vaccine. This became available in the USA from April 1955. This was then followed in 1960 by the oral vaccine developed by the Polish clinician Albert Sabin. Important work was also undertaken by Dr Hilary Koprowski, head of the Wistar Institute in Philadelphia, in relation to an experimental oral poliomyelitis vaccine (“CHAT” vaccine). The incidence of the disease has declined very markedly since the introduction of vaccination. Poliomyelitis at present still exists in developing countries, particularly in young children. Because the only reservoir is the human being with no persisting infection and because of the efficiency of vaccination, it is an eradicable disease. Though rare persistent carriers exist mostly due to an underlying immune defect. In May 1988, the WHO adopted a resolution to eliminate poliomyelitis (“Global Polio Eradication Initiative”).

The hope that it would be possible to achieve the total eradication of poliomyelitis by the turn of the century has not become a reality. As long as a single person remains carrier of poliovirus, children in all countries are at risk of contracting the disease. The poliovirus can easily be imported into a polio-free country and can spread rapidly amongst unimmunized populations. In 1988, 350,000 cases (1000 children per day) were officially reported in 125 countries. The number is steadily declining. In 1996, the figure was still 4,000 and there were less than 2,000 registered cases in 2002. The last countries in which wild type poliovirus type 2 occurred were Afghanistan in 1997, Nigeria in 1998 and India in 1999. The last known reservoirs of type 2 were in Bihar, Uttar Pradesh and West Bengal. In 2001 the WHO reported that wild type poliovirus type 2 had been eradicated around the world.

In late 2005 however, **vaccine-derived polio virus type 2** reappeared in Nigeria. It was not wild type virus which reappeared but a pathogenic reverse mutant from oral polio vaccine. The emergence of serotype 2 circulating vaccine-derived poliovirus (cVDPV) has complicated the epidemiology of
polio. The type 2 component in trivalent OPV accounts for more than 90% of all cVDPV cases. By 2020, 24 cVDPV outbreaks have occurred in 21 countries, resulting in more than 750 cases of paralytic polio. The biggest risk factor for cVDPV emergence is low vaccination coverage. It will take many months for a cVDPV to emerge and cause and to cause an outbreak. These outbreak can cVDPV to become endemic and to spread further in under vaccinated communities and even to other countries.

Of note: **cVDPV** (circulating vaccine-derived polio virus) is not the same as **VAPP** (vaccine-associated paralytic polio). The latter is caused by a strain of poliovirus that reverts to a neurovirulent variant following OPV administration. This causes a paralysis clinically indistinguishable from poliomyelitis caused by the wild type poliovirus. VAPP occurs in recently vaccinated patients and sometimes in close contacts of recently vaccinated persons (contact VAPP). The weakened virus in VAPP does not cause outbreaks. It is estimated to occur in 1 out of 2.34 million administered first doses of OPV.

Source: WHO, Polio Global Eradication Initiative

A recent successful event in the polio eradication programme was the declaration of a world free of
wild type poliovirus type 3 in October 2019. Since 2012, the only wild type polio virus is type I and today it is only circulating in Afghanistan and Pakistan. In 2019 176 WPV1 infections were diagnosed in Afghanistan and Pakistan and 365 cases of cVDPV were diagnosed worldwide of which 40 in Afghanistan and Pakistan and 325 in non-endemic countries, mainly in sub-Saharan Africa. The militant Taliban claim that oral polio vaccination is a Western plot to sterilise Muslim children shows that success can be hampered if the 2019 numbers are compared with 2017: that year only 22 WPV1 cases were reported.

Pathophysiology

Polioviruses can attack motor neurons in the anterior horn of the spinal cord (shown) and in the bulbar area.

Transmission is faecal-oral by ingestion of contaminated food or water. The viruses proliferate in the
intestinal mucosa. The virus can still be found in the faeces up to a few months after infection. The virus does not cause diarrhoea. During the acute phase, it is also found in the throat. After passing into the body from the intestinal tract, the virus becomes localized in various tissues, such as the lymph nodes. On the cell membrane, the virus attaches itself to a specific protein: human poliovirus receptor (CD155). This protein belongs to the immunoglobulin superfamily and occurs in several tissues (brain, spinal cord, kidneys, heart, etc.). However, some cells that express the receptor appear not to suffer any adverse effect, probably because one of the subsequent stages in the intracellular replication of the poliovirus is blocked.

In a small percentage of cases, dissemination occurs to the central nervous system (it is “neurotropic”) where it can cause an non-paralytic aseptic meningitis. In about 1 percent of infections, the virus spreads to the grey matter in the ventral spinal cord (”polios” = grey, “myelon” = marrow) where the motor neurons are found. These are nerve cells that transmit impulses to the muscles. These cells become damaged or destroyed. Damage often occurs at other sites, but is usually less pronounced (medulla, cerebellar vermis, midbrain, thalamus, cerebral motor cortex).

**Clinical aspects**
Poliomyelitis, atrophy of a leg. Such lesions tend to be asymmetric. Copyright ITM

The incubation period is 9 to 12 days (rare extremes 3-35 days). The infection can follow a variety of courses: asymptomatic, flu-like syndrome, aseptic meningitis or paralytic. The most common is an asymptomatic infection (95%). Some people will develop a short-lasting flu-like syndrome with slight muscle pain. In a small minority of cases (1%) meningitis occurs after this phase of minor signs and symptoms. Fever and muscle weakness can occur, initially sometimes with severe muscle pain. Highly characteristic is the fact that the muscle pain improves on movement. The reason is unclear. 0.1% Of the total number of those infected will subsequently progress rapidly to paralysis, sometimes within a few hours: “morning paralysis”. This is an asymmetrical, flaccid (no tendon reflexes) and often ascending paralysis (exacerbation over a few days). Sensation is not affected. Sometimes the lesions may be localized at a higher level from the onset. Bulbar involvement (10% of the total number of those paralyzed) damages cranial motor nerves such as the glossopharyngeal nerve (9th cranial nerve, palate, swallowing problems), the vagal nerve (10th cranial nerve, including the recurrent laryngeal nerve) and the facial nerve (7th cranial nerve). Occasionally the ocular muscle nerves - the 3rd, 4th and/or 6th cranial nerves - are involved. Hypoxia can occur as a result of involvement of the diaphragm and intercostal muscles. Even though the virus may affect muscles on both body sides, the paralysis is usually asymmetrical. Cerebrospinal fluid contains an increased number of lymphocytes. Recovery can take months, in the course of which marked hypotrophy of the muscles occurs. Mortality during the acute disease can reach 10% due to respiratory paralysis. The sequelae of spinal polio are often permanent if the affected nerve cells are completely destroyed leading to severe disability in a quarter of patients and mild disability in another quarter. In half the patients with spinal polio, cells are not completely destroyed and full recovery will take place with maximum possible improvement occurring within 6 months.

**Bulbar versus pseudobulbar paralysis**

Bulbar paralysis is caused by injury to the lower motor neurons (motor nuclei of the throat muscles), for example due to poliomyelitis or Guillain-Barré syndrome. Speech is nasal and the tongue is flaccid, atrophic and exhibits fasciculations. The masseter reflex is normal or absent. In pseudobulbar paralysis, there is damage to the higher motor neurons. Speech is monotonous and contains many high tones: spastic dysarthria (“Donald Duck” speech). The tongue is spastic and the masseter reflex increased. There is no atrophy of the tongue.
Dysphasia versus dysarthria

In the case of dysphasia, speech is abnormal in content, usually as a result of a cerebrovascular accident. The disorder may be motor-related in the event of injury to the posterior-interior part of the dominant frontal lobe (Broca aphasia: the patient recognises an object but cannot say its name) or sensory as a result of damage to the temporal lobe (Wernicke aphasia: the patient does not understand the meaning of words).

In dysarthria there are difficulties of articulation, but the content of the speech is correct. Injuries to the 9th, 10th and 12th cranial nerves can result in dysarthria, dysphagia and nasal regurgitation.

Post-poliomyelitis syndrome

In some patients, progressive muscle weakness recurs several years after acute paralytic poliomyelitis in the muscle groups involved in the previous episode. Rapid fatigability, swallowing disorders, respiratory difficulties, muscle atrophy, discomfort and pain in muscles and joints can occur. This post-poliomyelitis syndrome affects one in three people who suffered paralytic poliomyelitis forty or fifty years earlier. It is apparently not caused by reactivation of dormant polioviruses. The exact aetiology is unknown. One hypothesis is that the symptoms are due to natural progressive deterioration of the remaining motor neurons. An as of yet unelucidated immunological mechanism might play a role. There is no specific therapy apart from muscle-strengthening exercises. Evidently, further study is necessary to understand this condition better.

Diagnosis

Most victims are children less than 5-years old. The diagnosis is clinical. A predominantly asymmetrical flaccid paralysis of sudden onset with decreased or absent tendon reflexes with normal sensation, normal level of consciousness and preceded by muscle pain is suggestive. Often there is fever and meningeal irritation. Routine laboratory tests show few abnormalities. Lumbar puncture suggests viral meningitis (lymphocytic pleocytosis). In a patients with symptoms suspect for poliomyelitis two stool samples and thwo oropharyngeal swabs should be obtained at least 24 hours apart in the two weeks after symptom onset. Reverse-transcriptase polymerase chain reaction (RT-PCR) for polio and nonpolio enteroviruses will be performed. Cell cultures will also be done if available. Serological tests with type-specific IgM on serum or on liquor can be performed but, like viral culture, are rarely available. WHO installed worldwide equipped labs for the investigation of acute flaccid paralysis cases (Collaborating Center for Reference and Research on Poliomyelitis). Confirmed cases are usually thoroughly examined with PCR to establish whether it involves a “wild type” virus or
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whether it is a reverse mutation of a vaccine strain. For every diagnosed wild type poliovirus infection, about 2000-3000 asymptomatic carriers exist, underlining the importance to determine the source of the ‘outbreak’.

**Initial differential diagnosis includes:**

1. **Guillain-Barré** syndrome or acute inflammatory demyelinating polyneuropathy is characterized by an ascending symmetrical paralysis with sensory involvement (reduced sensation and paresthesia). A form predominantly affecting the cranial nerves also exists (Miller-Fisher syndrome). Respiration becomes affected in the late stages. Determination of the vital capacity of the lungs is important. In Guillain-Barré syndrome, the cerebrospinal fluid is very typical: large quantities of protein and only a slight increase in cell count. In early stages, the CSF can still be normal. In such cases, repeated lumbar puncture is essential one week later. In the case of poliomyelitis, there is pleocytosis (large numbers of lymphocytes) in the cerebrospinal fluid and a slight elevation of protein. With poliomyelitis there is usually no progression of the paralysis after the 4th day, in contrast to Guillain-Barré.

2. **Tick paralysis**: some ticks secrete a paralytic substance in their saliva. This causes an ascending symmetrical paralysis with paresthesia, often around the mouth. The paralysis gradually increases over 5 to 6 days. Removal of the tick produces a dramatic improvement within a few hours or days. There is no fever or pain.

3. **Paralytic rabies**: previous history of infected bite (sometimes the victim is unaware of the bite: role of bats in South America). Ascending flaccid paralysis with moderate sensory disorders and with a fatal outcome.

4. **Acute transverse myelitis**: transverse lesion with bilateral sensory and motor disorders below a certain level (spinal cord segment) with back pain and flaccid paralysis initially, and subsequently spastic paralysis and major sphincter disorders. The cerebrospinal fluid often exhibits pleocytosis and a raised protein content. Consideration should also be given to trauma, herniated disk, acute schistosomiasis, compression by a spinal abscess or tumour (possibly acute symptoms from bleeding in a tumour), complications of brucellosis and Pott’s disease.

5. **Diphtheria**: Caused by toxins secreted by Corynebacterium diphtheriae. Aerogenic transmission. Incubation period 2 to 5 days. Mostly throat infection, often with pseudomembranes, dysphagia, airways obstruction, markedly enlarged cervical lymph nodes and leukocytosis. This is followed by heart failure (myocarditis) around the 2nd week and sometimes peripheral paralysis (neuritis) around the 3rd to 6th weeks. The nerve damage often occurs first in the throat, palate and the ocular muscles and may subsequently become generalised. Sometimes the infection is localised in the nose or skin, which then tends to follow a less aggressive course. Untreated patients remain infectious for 2 to 3 weeks. Treatment consists of antibiotics [(neo)macrolides or penicillins] and
diphtheria antitoxin.

6. **Buckthorn poisoning.** The clinical picture which follows ingestion of the berries of buckthorn (*Karwinskia humboldtiana* and *K. calderoni*) resembles poliomyelitis and Guillain-Barré syndrome. The neurotoxic effects of this plant are well known in Mexico and Central America (Nicaragua!) and consist of an ascending symmetric flaccid paralysis, often leading to bulbar paralysis and death.

7. **Botulism:** Intoxication by neurotoxins (type A, B or E) secreted by a Gram-positive anaerobic bacterium *Clostridium botulinum*. The toxins (zinc endopeptidases, cf. tetanus) bind presynaptically, interfere with the neurotransmitter vesicles and thus prevent the release of acetylcholine. Botulinum toxins B, D, F and G cleave synaptobrevin. Botulinum toxins A and E cleave SNAP-25 and botulinum toxin C1 cleaves syntaxin (all vesicle-associated proteins). The organism (or heat-resistant spore) can be found in a wound, the colon or in food. The role of food is reflected in the name of the disease: “Botulus” = sausage, after an incident in the 18th century in southern Germany. Bacteria can proliferate in anaerobic conditions and secrete toxins. If this happens and people eat contaminated food, disease will follow. After 12-36 hours a bilateral symmetrical and descending flaccid paralysis occurs with hypotension, dry mouth, ptosis, diplopia with dilated pupils and no light reflex, constipation and often distended abdomen (ileus) and urinary retention. Bulbar paresis with dysarthria and dysphagia may be particularly apparent and result in aspiration pneumonia. Respiratory paralysis can follow. Tendon reflexes are impaired or absent. There is normal sensation, no fever, normal consciousness, normal cerebrospinal fluid. There will be normal base line laboratory test results. Wound botulism causes no intestinal symptoms. A rapid improvement of the symptoms is obtained within a few hours with anti-ABE antitoxin (horse serum; 1 ampoule IV and 1 IM, repeated if no improvement after 2-4 hours), but complete recovery is usually very slow (weeks to months). It is a rare condition and it is difficult to diagnose. Specific EMG patterns can be detected (including a reduction in muscle action potentials at low frequency stimulation and post-tetanic potentiation, i.e. increase in muscle action potentials after high frequency stimulation or maximum voluntary muscle contraction for 30-60 seconds). An EMG as well as specific bacterial cultures and bioassays in mice to detect toxins are in general not available in developing countries. Even in well-equipped medical centres, confirmation of botulism is difficult. Botulinum toxin is used in medicine in a number of different indications, such as for the control of spasms in superficial muscles (e.g. around the eye, blepharospasms), in focal dystonia, in chronic anal fissures, in achalasia and Chagas’ disease and even in axillary hyperhidrosis. In this last case, injections of botulinum toxin A are used. The toxin blocks the release of acetylcholine at the neuromuscular junction but also at the cholinergic autonomic nerve endings (reduced sweat production as a result).

8. **Myasthenia gravis and Lambert-Eaton** myasthenia syndrome (e.g. in bronchial carcinoma) can also cause paralysis of the ocular muscles, ptosis, facial muscle weakness and swallowing difficulties, but the course is slower. If available, an EMG and an edrophonium test (Tensilon®) are
9. **Viral meningitis.** If the clinical presentation is that of acute viral meningitis, mumps, Coxsackie A7, Enterovirus 71 and echoviruses should be considered in addition to infection with poliovirus. Naturally this can only be established by a sophisticated laboratory.

10. **Enterovirus 71** deserves some additional comment. It was first isolated in a cell culture from a child with encephalitis in California in 1969. It is easily transmissible, which is important for contact persons within a family. The virus can be isolated from stools. There were large epidemics in Eastern Europe in 1975 and 1978 and in Southeast Asia (Malaysia, Singapore, Taiwan) between 1997 and 2000. In most outbreaks hand, foot and mouth disease has been the dominant clinical presentation, although herpangina, interstitial pneumonia, myocarditis, intrauterine infection and neonatal hepatic necrosis occur sporadically. The virus is also neurotropic. In contrast to other enteroviruses, it can invade the ventral brainstem, cerebellum and spinal cord. This results in a spectrum of serious neurological syndromes, ranging from acute flaccid paralysis of one or more extremities, to cranial nerve paresis, tremors, myoclonus and ataxia. Acute pulmonary oedema is thought to result from the destruction of medullary respiratory and vasomotor centres, leading to central sympathetic activation with severe systemic vasoconstriction and pulmonary vascular overload. The nervous damage is due to direct invasion of the neurons by the virus, as well hypoxia. Children who had encephalitis and cardiopulmonary failure have a high risk of poor neurodevelopment and cognitive outcome. Nowadays epidemic paralytic disease is more likely to be caused by EV-71 than by polioviruses.

11. **Acute beriberi** (thiamine deficiency). Develops more slowly with muscle weakness and often also heart failure. Good response to thiamine. See chapter on nutrient deficiencies.

**Differential diagnosis of Acute Flaccid paralysis, summary**

1. Polio : CSF, lymphocytic meningitis, PCR, viral culture
2. West Nile Fever virus polio-like : CSF, lymphocytic meningitis, serology, PCR, viral culture
3. Enterovirus 71 polio-like : CSF, lymphocytic meningitis, PCR, viral culture
4. Guillain-Barré / Fischer : CSF, protein-cellular dissociation
5. Diphtheria : inflammation throat or nose, LN, cardiomyopathy
6. Botulism : dry mouth, constipation, blurred vision, mydriasis, toxin assay
7. Rabies, paralytic : CSF variable, consciousness variable, agitation, relentless progression till death, IF, PCR
8. Myasthenia crisis, Eaton : Tensilon (edrophonium) or neostigmine test, EMG, anti-acetylcholine-receptor antibodies
9. Beriberi : cardiac, subacute, poor nutrition, vomiting, ethanol, thiaminases
10. Periodic paralysis : recurrent, induced by effort or sugars, K⁺ concentrations variable
11. Hypophosphatemia: alcoholism or rapid feeding after starvation, TPN
12. Tick paralysis: specific hard tick present, better after removal
13. Neurotoxic snake: history, ptosis, dysphagia, bite wound
14. Organophosphates: hypersalivation, cramps, diarrhoea, sweat, miosis, wheezing, bradycardia
15. Shellfish poisoning (PSP, NSP): ingestion shellfish, paresthesia, diarrhoea, ataxia, mydriasis
16. Buckthorn poisoning: ingestion *Karwinskia* berries, New World
17. Curare poisoning (medication): peracute, only parenteral, New World, consciousness OK
18. Fugu poisoning (TTX = tetrodotoxin): ingestion fish, Asia; paresthesiae lips, peracute paralysis, consciousness OK
19. TTX, other source: e.g. bite of blue-ring octopus, see fugu poisoning
20. Thallium poisoning: gastro-intestinal, painful polyneuritis (esp hands and feet), diplopia

**Treatment**

There is no specific treatment for poliomyelitis. Symptomatic and supportive measures are necessary. Bed rest is compulsory at the beginning of the disease as physical activity may aggravate the nerve damage. Moist heat packs relieve muscle pain. Attention should be paid to the possibility of urinary retention due to bladder paralysis. Physiotherapy should be instituted 3 to 4 days after the regression of the fever and if there is no further progression of the paralysis. Physiotherapy does not prevent the muscular atrophy that occurs as a result of denervation (destruction of the motor neurons). It does, however, maintain the muscles in a good state for the few regenerating neurons. In 1927, Philip Drinker and Louis Agassiz Shaw Jr invented the iron lung at the Harvard School of Public Health. This is a respirator that allowed care for patients with paralysis of the respiratory muscles. It allowed for longer survival of certain patients, but also led to dramatic situations, where paralyzed people had to remain immobile inside the respirator for the rest of their lives.

**Prevention**

**General**

In the first half of the 20th century, poliomyelitis was a major problem in the West. The development of vaccines had a dramatic effect in a very short space of time. In many countries, it has proved possible to eliminate poliomyelitis by means of a routine vaccination program. Within a generation, the disease has virtually disappeared in the developed countries. In some countries where poliomyelitis still occurs, annual national vaccination days are held in addition to the usual vaccination programs. In 1988, the Global Polio Eradication Initiative was launched and is led by five organizations: the WHO, the United States Centers for Disease Control and Prevention, the UN
Children’s Fund, Rotary International and the Bill and Melinda Gates Foundations. Four pillars form the global eradication program:

- Routine infant vaccination
- Supplementary immunization activities in at-risk middle and low-income countries: door-to-door campaigns, eg, a national campaign targeting all <5 years without regard to prior OPV immunization status
- Surveillance for acute flaccid paralysis (AFP)
- Mop-up campaigns: if a poliomyelitis patient or circulation of wild-type virus of cVDPV is found, house-to-house vaccination (“mopping-up” vaccination) is conducted over a large area.

As poliomyelitis is becoming increasingly rare, the importance of good surveillance is increasing. Patients with acute flaccid paralysis form the basis for the detection of “possible poliomyelitis”. Acute flaccid paralysis surveillance is the gold standard for surveillance in the polio eradication initiative. Cases of acute flaccid paralysis (AFP) must be officially reported. It may be assumed that every year in a population approximately 1/100,000 persons under 15 years of age will develop an acute flaccid paralysis (non-poliomyelitis). Where possible a stool specimen should be obtained for virus isolation in a regional Global Polio Laboratory Network laboratory. On top of AFP investigation, environmental surveillance sampling sewage effluents in high-risk areas complements AFP surveillance. Organisms that are found here (e.g. mutated vaccine strains) can be tested for neurovirulence in transgenic mice that are carriers of the human poliovirus receptor. The rationale for environment surveillance is based on the characteristic poliovirus excretion pattern. Infected individuals excrete poliovirus in faeces for periods up to several weeks, whether or not they are symptomatic. Occasionally very-long-term excretors will be encountered. As fewer AFP cases are to be expected, environmental surveillance may become more important to ensure early response, even before clinical cases re-occur.

In 1995, the “Global Commission for the Certification of the Eradication of Poliomyelitis” was established. This commission defined the principles, criteria and the process by which certification is to take place. All countries have to be able to show that they have stopped circulation of wild type virus. Certification, cannot be granted in less than three years from the last report of poliovirus. Each country should set up a national certification commission which should collect the necessary documentation. The national commission is not authorized to declare its own country poliomyelitis-free.
Vaccines

There are two types of vaccine:

Inactivated Polio Vaccine

The injectable Salk vaccine (1955) or IPV (Inactivated Polio Vaccine) can be administered IM or SC and has the advantage of being heat-stable, which is important under field conditions. It can also be given to immune depressed patients. It is more expensive than the Sabin vaccine. These vaccines protect a person against paralytic poliomyelitis but will not combat an asymptomatic infection in the intestinal tract. Vaccinees are still able to pass on the wild type virus to their environment. With the killed vaccine, no reverse mutations can occur. In most high-resource countries, IPV is integrated in the routine childhood vaccination programme.

Sabin vaccine

The oral Sabin vaccine (1960, OPV = oral polio vaccine) contains live attenuated viruses. It should be stored in a refrigerator. It is very efficient and cheap. It has the enormous advantage of being able to be administered without a syringe. If there is an intestinal infection (diarrhoea) during the vaccination, the vaccine has less chance of success. The first dose may be given immediately after birth. This is then followed by three doses at intervals of one to two months. A booster at the age of 18 months and 5 years is indicated. A return to neurovirulence is possible in case of specific reverse mutations. After vaccination with Sabin vaccine, the viruses can be excreted for a while. As an anecdote, poliomyelitis virus was isolated from the preflight stools of the Apollo 11 crewmen after the crewmen had been given poliomyelitis boosters.

Risks of vaccination vs non-vaccination

Questions are raised about the putative risks of poliomyelitis vaccination. In the 1950s there was the so-called “Cutter” incident (named after the Cutter laboratory). Children who received the supposedly inactivated vaccine subsequently developed the disease. This was due to a problem in the filtration process in the preparation of the vaccine. Aggregates which still contained pathogenic virus were not inactivated by the formalin and were not filtered out. This problem has obviously now long been corrected.

Between 1957 and 1960 the oral “CHAT” vaccine was used, particularly in Central Africa. The
developer of this vaccine was accused of having used contaminated chimpanzee kidneys for this vaccine and therefore of having started the AIDS epidemic. This however has never been formally confirmed and this hypothesis has been rejected. Strains that were preserved from that time have been studied for the presence of HIV and all found to be negative.

The live viruses in the Sabin vaccine differ from the pathogenic viruses through a small number of mutations. Occasionally reverse mutations occur, as a result of which a vaccine virus can recover its pathogenicity (“vaccine derived polio viruses”).

Trivalent oral polio vaccine is highly protective against cVDPV. The only way to remove the risk of future similar mutations and clinical disease is switching to the less effective and more expensive dead injectable vaccine (estimated cost/dose for oral vaccine 0,15 US$ versus 3 US$ for injectable vaccine).

The prospects for eradication are good but setbacks do occur. In the autumn of 2003, there was a dramatic increase in poliomyelitis cases in Kano, Nigeria. Early November 2003, there were already 217 cases on a global total of 491. Vaccination had been stopped due to false rumours, fear and mistrust between the Muslim population and the government. This created a very dangerous situation, even threatening the accomplishments of the global eradication campaign. Over a year time, the virus spread far and wide to 20 different countries, from countries bordering Nigeria to even Indonesia. As an example, Somalia reported 73 cases in December 2005, after a three year period where not a single case was detected. Later it became clear that a small number of cases in Nigeria were due to the spread of a mutated vaccine strain. The financial price to quell this outbreak was very high, but we have to be prepared to go all the way to eradicate this disease from our planet.

Details on the Polio Eradication and Endgame Strategic Plan can be found at:
http://polioeradication.org/who-we-are/

**Switch from tOPV to bOPV and the future of OPV**

Wild type 2 poliovirus disappeared in 1999. Nearly all vaccine-derived polioviruses in circulation are of type 2. Administration of the trivalent oral polio vaccine (tOPV, containing the 3 living vaccine strains) led mainly to an immune response against type 2. In April 2016 a new bivalent oral poliovaccine (bOPV) has replace tOPV in all OPV-using countries, which will decrease cVDPV cases and VAPP cases due to type 2 poliovirus. The main reason to continue using the oral living vaccine is cost.

Given the WPV1 and WPV3 eradication, the use of all OPV in routine immunizations should be stopped
and all countries will rely on IPV alone to prevent poliovirus after OPV withdrawal. As an intermediate step, high-risk countries can give ≥1 IPV dose in the routine immunization programme on top of the bOPV immunization, to maintain immunity levels to type 2 polio.

At the same time stockpiles of monovalent OPV type 2 are maintained to respond to future cVDPV2 outbreaks. Of course, the use of mOPV2 in response to VDPV2 outbreaks increases the risk of seeding new VDPV2 outbreaks.

**Prevention, comparison of vaccines**

**Table: Advantages and disadvantages of dead and live attenuated poliomyelitis vaccine**

<table>
<thead>
<tr>
<th>Dead vaccine (Salk)</th>
<th>Live vaccine (Sabin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies in the blood</td>
<td>Antibodies in the intestinal tract and blood</td>
</tr>
<tr>
<td>Since there is no immunity in the intestinal tract, wild type virus can still be transmitted faecal-oraly</td>
<td>Vaccine virus can spread to the family: beneficial for immunity. Sometimes transmission of virulent mutant.</td>
</tr>
<tr>
<td>No mutations towards new virulence possible</td>
<td>Vaccine virus can mutate to neurovirulent type.</td>
</tr>
<tr>
<td>Use permitted in immune deficiency</td>
<td>Possibly dangerous for immune deficient subjects</td>
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<tr>
<td>Injection necessary</td>
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<td>Booster vaccinations necessary for long-term immunity</td>
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<tr>
<td>Higher seroconversion rates than OPV in low-income settings where enteric pathogens/pathology reduces the OPV efficacy</td>
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</tbody>
</table>

**Future**

If poliomyelitis is successfully eradicated in the early 21st century, no wild type virus will then be present anywhere on the planet (except possibly for a few stocks in protected laboratories). However, Sabin strains can revert to the wild type form. Will the oral poliomyelitis vaccine ultimately have to be
destroyed? For how much longer should the injectable, dead vaccine be used and/or kept? Will silent long-term excretors start an epidemic decades after stopping vaccination? Some immunocompromised people excrete wild type or vaccine-type poliovirus for years (possible for life?). As long as this situation exists, it cannot be stated that the battle against polio has been won.

Rabies

Summary

- Rabies is caused by several closely related Rhabdoviruses
- Infection of the central nervous system: meningo-encephalitis
- Zoonosis
- Transmission via saliva of infected animals, very rarely aerogenic transmission
- Often long incubation time, usually 20-90 days, leaving a window for curative vaccination
- Muscle spasms, salivation, intermittent delirium, fever, hydrophobia
- If symptoms, 100% fatal
- Prevented by wound cleaning, antiserum and vaccination

General

The genus of Lyssaviruses (Gr. “lyssa” = madness) includes rabies virus and a few other related viruses (Mokola, Duvenhage, Lagos bat virus, European bat lyssavirus 1 and 2, Australian bat lyssavirus, Irkut virus, Aravan virus, West Caucasian Bat virus, Bokelo bat lyssavirus, Ikoma Lyssavirus, rabies virus, Shimoni bat virus). Mokola virus was originally found in Nigeria in various shrews (Crocidura). Duvenhage virus was isolated from insectivorous bats in South Africa and Zimbabwe. They are all rhabdoviruses (Gr. “rhabdos” = rod, hence “rod-shaped viruses”), a term which is derived from their cylindrical, bullet shape under electron microscopy. Various subtypes can be distinguished with monoclonal antibodies and so the source of an infection can sometimes be traced.

Rabies is a viral infection that affects the central nervous system. It is a zoonosis affecting many mammals (dog, cat, fox, squirrel, ferret, skunk, raccoon, sheep, cattle, bat, etc.). Those which are mainly responsible for transmission to humans are dogs (Africa, Asia) and vampire bats (Central and South America). The latter feed mainly on cattle blood. Rabies infection produces a form of paralysis.
in cattle, sometimes leading to large losses of livestock. The virus is distributed world-wide except in New Zealand, West Malaysia and a number of islands such as Borneo, New-Guinea, Bali, Hawaii and Great Britain (although there was an endemic case in the UK in the early 21st century). Australia was rabies-free until 1995, when a rabies virus was discovered in flying foxes in that country. The number of cases of rabies is estimated as >50,000 per year. Four million people annually receive post-exposure prophylaxis.

**Transmission**

Transmission occurs via the saliva of an infected animal as a result of a bite or of licking damaged skin or mucous membrane. Infected dogs, raccoons, cats, etc., can be responsible for transmission. In dogs, the saliva becomes infectious at least 2 days before symptoms of the disease appear. In extremely rare cases, asymptomatic dogs can excrete the virus for years. In principle, infection can also occur via aerosol, but this is rare (limited to bat-infested caves, laboratories). Transmission by eating contaminated meat has been described in animals but is not (yet) known in humans. To date, a few cases of human-to-human transmission have been described, 1 ascertained bite transmission. Transmission via corneal transplantation and organ transplantation has occurred. Successful transmission experiments were conducted as early as the beginning of the 19th-century, when saliva from a person with rabies was introduced into a healthy dog. Rabbits primarily exhibit paralytic rabies, which was of importance in the search for a vaccine (these animals were easier to study than convulsing rabid dogs).

**The type of exposure according to WHO**

Category 1 exposure: touching, stroking or feeding a suspect or sick animal or being licked on intact skin

Category 2 exposure: nibbling of unprotected skin, minor scratches without bleeding, being licked on damaged skin

Category 3 exposure: transdermal bites or scratches, exposure of mucous membrane to saliva; exposure to bat bites or scratches

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**Vampires**

Bats are classified in their own order within the mammals. The Chiroptera (Gr. “cheiro” hand;
“pteron” wing) includes approximately 900 species. An animal requires ± 20 ml of blood each day (half its body weight). In the wild, a vampire lives on average 10 years. Like its mythical human counterpart, the animal hunts at night. It lands in the vicinity of its victim (usually a cow) and then carefully creeps up close. The bat licks the hair of the pelt of the cow and then cuts away a small area with its teeth. It makes a 5 mm shallow wound with its razor-sharp, self-sharpening incisors (only in Hollywood do vampires bite with their canines). The animal licks up the blood that is discharged. The saliva contains anticoagulants (including a plasminogen activator that is being studied as a new thrombolytic). Rabies virus may be found in the saliva. *Desmodus rotundus* can also sometimes drink blood from humans and then usually bites the nose, ears or lips. While feeding, the bat urinates on its prey. This probably helps it to find the animal again the following night. Vampires must have blood very regularly. They die if they cannot eat for two or three days. Vampires, are social animals and often regurgitate and share blood meal with their young and with fellow members of the same colony that have been unable to find any prey. As a result of this social behaviour, rabies virus spreads to other animals within the colony. Rabid bats can attack other animals without provocation, including other bats and humans. Bats can be asymptomatic carriers or become ill, exhibit aberrant behaviour and die from the infection. Bats can also spread histoplasmosis through their faeces.

In addition to their known role as biologic vectors of rabies to humans and domestic animals and surra (*Trypanosoma evansi*) to horses and cattle, vampire bats can also be temporary biologic as well as mechanical vectors of Venezuelan equine encephalitis virus and foot-and-mouth disease. They are likely to be effective mechanical vectors if not biologic vectors of any bloodborne pathogen, including HIV. Besides rabies virus, other viruses ascribed to bats have proven pathogenic or fatal to people and domestic animals. Four species of Australian *Pteropus* bats in Queensland carry Hendra virus without developing symptoms. These bats disseminate virus in urine or amniotic fluid during birthing, and the virus is later ingested by pregnant horses that amplify the virus, which then spreads to people and causes a fatal pneumonia (13/20 horses were infected in a 1994 outbreak, which resulted in two human deaths).

Nipah virus, identified in urine and saliva of *Pteropus* bats in Malaysia, spreads the virus to pigs and destroyed that country’s swine industry in 1998. The virus spread from pigs to hundreds of industry workers; approximately 40% of these workers died of severe viral encephalitis caused by the agent. The symptoms are similar to Japanese encephalitis.
Pseudorabies

In veterinary medicine, rabies should not be confused with pseudorabies or Aujeszky’s disease. This viral condition (Suid herpes virus) predominantly attacks pigs (the only natural host), but can occasionally affect cattle, sheep, goats, dogs, cats and wild animals. Infections can be latent or clinically manifest, including involvement of the central nervous system with symptoms that include abnormal gait, intense scratching, self-mutilation, convulsions and death.

Pathogenesis

After the virus has entered the body, it multiplies locally in myocytes and afterwards crosses the neuromuscular junction to penetrate a peripheral nerve. The virus enters nerve cells through nerve spindles of sensory nerves or neuromuscular junctions of motor nerves. Subsequently it spreads along the nerve by retro-axonal flow to the spinal cord and brain. In the central nervous system, the virus proliferates further. The nucleocapsid of the virus can be detected by microscopy in some neurons as spherical inclusions in the cytoplasm: Negri bodies [Adelchi Negri 1876-1912, assistant to Camillo Golgi in 1900]. From there, the virus again spreads to almost all organs in the body. The saliva contains high concentrations of infectious virus.

The patient develops a very aggressive viral encephalitis. This is supposed to be 100% lethal, although there may be some exceptions. Very occasional cases of rabies survivors –with and without treatment according to the Milwaukee protocol- have been published, be it with severe sequelae. In 2012, it was reported that in a remote part of the Peruvian Amazon where rabies secondary to vampire bats is common, unvaccinated people had antirabies antibodies in their blood. It is still unclear what this finding means exactly. It is not known if these people developed minor symptoms and recovered, asymptptomatically seroconverted after a very small inoculum with or without the help of bat oral flora, specific genetics in an isolated community, natural immunity, cross-reactivity with other germs or still something else.
Rabies. Some neurons contain intraneuronal inclusions which consist of viral nucleocapsid. These inclusions are known as Negri bodies.

**Clinical aspects**

**General**

Incubation lasts 20 to 90 days (extremes of 4 days and 6 years have been described). Bites close to the face and with a large inoculum (severe wounds) are associated with the shortest incubation times. A prodromal phase lasting 2 to 10 days then follows. The first symptom is an influenza-like syndrome with moderate fever and malaise lasting a few days. This can be associated with severe local pruritus leading to scratching and excoriations, headache, pain or paraesthesia at the site of the bite. Sometimes there is moderate muscle weakness. Local myxedema after muscle percussion can occur. Agitation and insomnia can occur at a very early stage. Afterwards the disease can take two different courses, depending on which features predominate: furious rabies on the one hand (more involvement of the brain) and paralytic rabies (extensive involvement of the spinal cord) on the other.
**Furious rabies**

This form is more common accounting for about two thirds of the cases. There is increasing anxiety, excitation, hyperactivity, hyperventilation, disorientation and/or hallucinations. Symptoms occur intermittently and persist for 1 to 5 min, followed by a period of mental calm. Hyperstimulation occurs as a result of destruction of inhibitory centers in the brain stem. In approximately half the patients, painful spasms of the larynx and throat muscles occur (swallowing and vocal cord spasms). These are triggered for instance by seeing or wanting to drink a glass of water. This is associated with painful convulsive contractions of the respiratory muscles. The patient is therefore afraid of this situation (hydrophobia or fear of water). The spasms can also be induced by blowing air over the face (aerophobia) or by other, often minor, stimuli (compare with tetanus). The spasms develop into generalized convulsions. There is no trismus or muscle rigidity between convulsions (in contrast to tetanus). Neck stiffness can occur but is usually not pronounced. There is profound dysautonomia. The patient may sweat and weep profusely, as well as displaying hypersalivation, hypothermia, hypertension and tachycardia (involvement of the autonomic nervous system). Fever can occur. There is a pronounced thirst. The patient is in agony. Hypothalamic involvement can result in diabetes insipidus (insufficient ADH) or hypersecretion of antidiuretic hormone (SIADH). Myocarditis can cause cardiac arrhythmias. Coma follows within 10 days after the onset of the acute neurological symptoms and can persist for hours to months (mostly short-lasting). Finally, cardiac and respiratory arrest follow. Death occurs in nearly 100% of cases, in general 2-7 days after the onset of the disease. Medical management can prolong survival up to 133 days.

In the whole of the medical literature (up to 2016), about 15 people have been described who survived clinical rabies. Of these survivors, several received immune prophylaxis. In 2004, a 15-year-old girl who was bitten by a bat in Wisconsin survived rabies after treatment with coma induction, ketamine, midazolam, ribavirin, and amantadine. Later on, several patients have recovered with this regimen, but many failures of this new regimen have also been seen.

An immune response is essential for recovery from rabies, although vaccine would not need to be given if at the time of diagnosis- a patient had developed already rabies virus-specific antibody (controversial).
Rabies. Once symptoms have started, death is certain. The number of people who survived rabies is extremely low. Photo copyright Cochabamba, Bolivia

**Differential diagnosis of furious rabies:**

**Delirium tremens:** chronic alcohol misuse and sudden abstinence, signs of hepatic injury (spider naevi, flapping tremor, gynaecomastia, collateral circulation, etc).

**Reaction to some hard drugs** (crack, speed). This occurs more often in some large cities.

**Strychnine poisoning.** This plant product suppresses nerve impulse inhibition and thus causes convulsions. All types of sensory stimuli can cause convulsions. Consciousness is clear if no asphyxia has occurred. It is sometimes used as a rodent poison. If the patient survives the first 24-hours, the prognosis is good. In the event of death, the rapid onset of rigor mortis is characteristic.
**Acute psychosis and hysteria.** Very common in developing countries. Hysteria: no hydrophobia if the patient is unaware of the existence of this sign.

**Tetanus:** portal of entry, trismus, muscle stiffness, convulsions on sudden stimulus, clear consciousness, mostly shorter incubation, no encephalitis, clear CSF.

**Bacterial meningitis:** lumbar puncture. Note that several organisms can cause lymphocytic pleiocytosis (*Brucella, Listeria, Treponema pallidum* (syphilis), *Borrelia*, tuberculosis, *Coxiella burnetii*, various rickettsiae, etc.). Various systemic fungal infections, sarcoidosis, auto-immune diseases (S.L.E.) with cerebral vasculitis etc. can produce abnormal cerebrospinal fluid.

**Cerebral abscess.** As a result of septic emboli (subacute bacterial endocarditis) or from penetration of a collection of pus (sinus, middle ear, etc.). Cerebral toxoplasmosis is common in AIDS.

**Viral encephalitis** due to herpes simplex or an arbovirosis such as Japanese encephalitis, West Nile fever, tick-borne encephalitis or Venezuelan equine encephalitis. Often no virus can be found. There are no lucid periods and no typical spasms. For arboviral infections, serology is important. Infections with Herpes virus B (*Herpes simiae* virus) are rare. This virus can be transmitted via a bite, scratch or via body fluids from an infected monkey. Mucocutaneous lesions and encephalitis can follow inoculation. (Val-)acyclovir or ganciclovir can be tried in treatment, but the infection provokes dramatic neurological symptoms.

**Cerebral malaria** (*Plasmodium falciparum*)

**Post rabies-vaccination encephalitis** if vaccination has been given with the old nerve tissue based vaccines.

**Bite of a cobra or other elapid** snake: saliva will dribble out of the mouth as a result of throat paralysis (not from spasms). Ptosis, swelling, pain and tissue injury at the site of the bite.

**Paralytic rabies**

This is the most frequent form after a vampire bite (South America). There is a flaccid paralysis (no tendon reflexes). There are often mild sensory disorders. The paralysis often begins in the bitten part of the body and then ascends further. Death follows from general paralysis. The course is less rapid than in the furious form.
**Differential diagnosis paralytic rabies:**

**Polio**: initially fever and muscle pain, asymmetrical paralysis, clear consciousness.

**Guillain-Barre syndrome**: ascending symmetrical paralysis, typical cerebrospinal fluid with large amount of protein but few cells. Early in this syndrome, the CSF might still be normal. Control lumbar puncture some days (up to a week) later then shows the albuminocytological dissociation. There are variants in which the cranial nerves are primarily affected (Fischer syndrome). It should be noted that initially the cerebrospinal fluid can be normal, but very quickly the protein level in the CSF will raise substantially. Often the syndrome follows one or more weeks after *Campylobacter* enteritis or another infection.

**Botulism**: descending paralysis (ocular muscles, throat muscles, neck, other muscles, progressive respiratory paralysis), no fever, dry mucous membranes, large pupils. Is caused by toxins produced by a specific bacterium (*Clostridium botulinum*), related to the organism that causes tetanus. The organism can be found in a wound or more often in spoilt food.

**Diphtheria**: is rare but poses few diagnostic problems in general in case of throat, nose or laryngeal infection. Extensive membrane-like coating in the throat (“diphthera” = leather) with marked cervical lymph node enlargement. This is followed 1 to 2 weeks later by carditis and progressive paralysis, sometimes also with sensory disorders (peripheral neuropathy). Cutaneous diphtheria produces painful wounds but rarely paralysis.

**Bite of an elapid snake** (e.g. cobra): rapidly occurring descending paralysis + local reaction at the site of the bite.

**Metabolic / hypoxic / toxic encephalopathy**

**Reye syndrome**: sudden onset, often after an initial viral syndrome. Vomiting is frequent. There is hepatomegaly in 40% of cases and liver function tests are abnormal. A liver biopsy is diagnostic.

**Differential diagnosis of dysphagia:**

Determine whether there is fever, whether pain occurs on swallowing and whether the dysphagia is high (throat area) or low (retrosternal). Include visual mouth examination.

**Foreign body in the throat or oesophagus**: sudden onset, history of swallowing fishbone, chicken
bone or hard piece of food, feeling that “something is stuck in the throat”, no fever if no complications. Beware of perforation of the oesophagus by sharp objects such as toothpicks. Mediastinitis can follow.

**Neurotoxic snake bite**: progressively worse, ptosis, cough and speaking becomes difficult, history of snake bite, often pain and swelling at the site of the bite. No fever if no complications.

**Infection of the mouth or throat** *(viral, *Candida*, streptococci, abscess, aphthous stomatitis, etc.)*: painful, acute, visibly red throat/tonsils/abscess, fever, lymphadenopathy.

**Diphtheria** *(is reported separately because of its important nature)*: inflamed throat with grey membranes (sometimes skin wound), lymphadenopathy, neuritis, sometimes with regurgitation of drink or food through nose, visual problems, paralysis and/or carditis, often no history of immunization.

**Rabies**: history of animal bite, signs of encephalitis with episodic hyperactivity and paralysis, sometimes hydrophobia. Fluctuating consciousness.

**Tetanus**: begins over the course of several days, often recent wound, no immunization, the mouth cannot be opened wide, muscle spasms over the rest of the body. Temperature fluctuating. Normal consciousness.

**Neurological disorders with paralysis of the palate** *(e.g. bulbar poliomyelitis, bulbar tumour)*.

**HIV with candidiasis of the oesophagus**: check other clinical indicators, positive HIV test.

Oesophageal disorders such as **Chagas disease, stenosis, achalasia, tumours**.

**Diagnosis**

The diagnosis is clinical. Rabies must be suspected in someone who develops neurological symptoms a week or more after an animal bite. The number of white blood cells in the peripheral blood is normal or slightly raised, with a slight elevation of monocytes. Albuminuria can occur. An EEG shows abnormalities consistent with encephalitis. A CT scan or NMR scan of the brain can show surprisingly few abnormalities. Hydrophobia occurs in approximately half the patients and is pathognomonic (i.e.: highly specific). Investigation of contact with animals is important, but no history of exposure can be found in 20% of patients. The protein content in the cerebrospinal fluid is usually normal and in the
first week of the disease the white blood cell count in the CSF is raised in 70% of cases (highly fluctuating differential count). Antibodies in serum and cerebrospinal fluid cannot be detected before there are symptoms. Antibodies against rabies virus cannot be detected in most laboratories in the tropics. The virus may be detected in corneal smears. The test is highly specific, but there are many false negatives and in most cases the technique is not available (fluorescein-conjugated antirabies serum). The virus is sometimes detectable by immunofluorescence in a skin biopsy, which is best taken from the neck (many hair follicles surrounded by nerve endings). Isolation of the virus from saliva, urine and cerebrospinal fluid (not from blood) is possible, but in tropical practice not feasible. The best technique is reverse transcriptase PCR on saliva (detection of rabies RNA). After death, the diagnosis can be established retrospectively by a brain biopsy. Negri bodies (intraneuronal inclusions consisting of viral nucleocapsid) are detectable in 80% of patients. All in all, rabies is a clinical diagnosis, but this has to be supported with arguments, such as:

1. RT-PCR on saliva for rabies RNA
2. Virus isolation from saliva or cerebrospinal fluid
3. Corneal smear for rabies virus antigen
4. Antibodies in serum
5. Skin biopsy for immunofluorescence

**Rabies in the animal**

The incubation period in dogs is 2 weeks to 6 months. Rabies in dogs (and also in cats and horses) leads to changes in behaviour, aggressiveness, running away from home, difficulty in swallowing with hypersalivation, and convulsions. The animal can exhibit a more paralytic presentation with dysphagia and a drooping lower jaw (more so in the fox and cattle). Sometimes the hind legs give way. The animal usually dies within 7-10 days. Rabies in animals is not universally fatal. In case of a bite from a dog suspected of rabies, the dog should be observed for ten days. If it exhibits abnormal behaviour, the animal’s brain can be analyzed. Negri bodies will only be present if the animal has shown clear signs of rabies. If the animal is killed immediately, the opportunity of making or excluding a diagnosis via this method is lost. If state-of-the-art technical facilities are available, however, it is better to kill the animal immediately and to detect rabies virus in the spinal cord or brain. This will rarely be possible in tropical regions. If the animal cannot be found (e.g. after a scratch by a bat), treatment of the human victim should follow on the assumption that it was infected.
Treatment

Since there is quasi 100% mortality once symptoms have occurred, only palliative therapy can be given at that time: pain relief (morphine) and reduction of spasms (myorelaxants e.g. diazepam). In most cases, barbiturates and chlorpromazine are also given. Although no case of transmission from patient to medical personnel has yet been described, it is recommended that the patient should be isolated and staff should wear masks, goggles and gloves during the provision of care. Staff should also preferably be vaccinated, but that is not obligatory.

In 2004, Jeanna Giese became the first patient ever to recover from rabies without the vaccine. She survived with a treatment based on a chemical-induced coma and intense anti-excitotoxic strategy combined with antivirals. The treatment –called the “Milwaukee protocol”- comprised ketamine-induced coma, together with high doses of benzodiazepines (midazolam) and supplemental barbiturates, ribavirin and amantadine. Normally, ribavirin penetrates little into the cerebrospinal fluid, but in rabies the permeability of the blood-brain barrier is higher. Ribavirin might also protect against rabies myocarditis. Amantadine (200 mg/day) has in vitro activity against rabies virus, and
has intrinsic anti-excitotoxic properties. Ketamine is a non-competitive N-methyl-D-aspartate (NMDA)-receptor antagonist with specific activity against rabies in animal models. It is possible that the NMDA-receptor may be one of the rabies virus receptors. Ketamine inhibited the genome transcription of rabies virus and restricted viral spread in an experimental rat model. Benzodiazepines and barbiturates are gamma-aminobutyric acid (GABA)-receptor agonists.

Unfortunately, attempts to replicate the successful (modified) Milwaukee protocol have been discouraging. In 2019, about 14 adequately documented survivors of rabies have been reported worldwide, five of them from India. Most survivors had received at least 3 anti-rabies vaccines initiated on the day of the bite. Almost all survivors have moderate to severe neurological sequelae with poor functional outcomes. The focus on treatment and management of rabies should not draw away attention from the core objective, which is unarguably the “prevention” of human rabies.

**What to do after a potentially infected bite?**

**Clean the wound with a detergent (soap).** It is extremely important to wash the wound immediately with soap and water for 15 minutes, because the virus is very sensitive to cleaning agents. Afterwards the should be disinfected with iodine/isobetadine or 60-80% ethanol. Oxygenated water or mercurochrome are not indicated. Leave the wound open afterwards (no primary closure of bite wounds).

**Tetanus vaccination** status should be checked and tetanus vaccination +/- immunoglobulins should be given if needed.

**Antibiotics:** All bites are by definition bacterially contaminated but do not always become infected. Wound infection with *Pasteurella multocida* or *Capnocytophaga canimorsus* is frequent after dog or cat bites. Routine administration of antibiotics after bite wound is not recommended. Antibiotics should be given if there are clinical signs of surinfection (dolor, tumor, calor, rubor) or in severely contaminated wounds.

**Evaluation of the contact of the animal with the skin**

The measures to be taken following a contact with a possible rabid animal depend on the type of contact, the immune status of the patient, the endemicity of the region and the type animal.
### WHO EXPOSURE RISK CATEGORIES

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| Category I | • Tactile contact (stroking) or feeding the animal  
             • Licking of the intact skin  
             *In other words: no exposure* |
| Category II| • Gnawing the uncovered and originally intact skin  
             • Superficial lesions from scratches or grazes, without bleeding.  
             • Licking of non-intact skin |
| Category III| • Single or multiple bites or scratches that penetrate the dermis  
             • Contact with the mucous membranes via the saliva after licking  
             • Licking a grazed or broken skin  
             • (Possible) scratches and bites of bats: often no visible lesion or the feeling of a bite |
### Viruses

#### ANIMALS

<table>
<thead>
<tr>
<th></th>
<th>CATEGORY I</th>
<th>CATEGORY II</th>
<th>CATEGORY III</th>
<th>IMMUNE SUPPRESSION CATEGORIES II and III</th>
<th>Rabies-PrEP in good order</th>
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- Schedule 1: vaccination Day 0 and Day 3 or intradermal vaccination (4x 0.1 ml) on Day 0
- Schedule 2: vaccination Day 0 (2x), Day 7, Day 21
- Schedule 3: RIG Day 0 + vaccination Day 0, 3, 7, 14 and 28
- Rabies PrEP in good order: rabies pre-exposition prophylaxis before the bite (Day 0 and 7)

**Human anti-rabies immunoglobulins** (RIG (Rabies Immune Globulin) - e.g. Berirab®, Imogam Rabies-HT®) are administered as soon as possible after the bite, whereby the largest possible amount is administered via a deep local injection in and around the bite with the aim of locally neutralizing the virus. The amount of MARIG depends on the anatomical location or locations of the injury and the
size of the lesions, with a maximum dose based on body weight (20 IU/kg). MARIG administration at an anatomical site different from the site of the bite is no longer recommended. Following mucosal contact with saliva of a potentially rabid animal without injury (category III), MARIG is no longer indicated.

**Vaccination** in humans was first carried out by Louis Pasteur in 1885. Vaccination is possible in view of the long incubation time. Antibodies are present after 7-14 days. There are several vaccines and therapeutic post-exposure vaccination regimens. WHO currently strongly recommends the safer modern cell-culture or embryonated-egg vaccines (CCEEV’s). CCEEVs contain inactivated rabies virus that has been grown in embryonated duck or chicken eggs or in cell culture (e.g. primary chick embryo cells, Vero cells or human diploid cells). The viral harvest is concentrated, purified, inactivated and lyophilized. In some CCEEVs, human albumin or processed gelatine is used as a stabilizer.

If rabies immunoglobulins are not available, schedule 2 is always the preferred schedule (2 injections on day 0, 1 injection day 7 and 21). If it is not possible to complete the full schedule with the same brand of vaccine, another brand may be used. The WHO has documented the interchangeability of Verorab®, Rabipur®, HDCV Rabiës®.

Because preventive vaccination (pre-exposure prophylaxis) does not provide complete protection, PEP booster vaccinations are still necessary for vaccinated individuals following a type II or III contact: rabies PEP schedule 1.

**Prevention**

Do not touch any sick, paralyzed animals, or better still: simply never touch animals in the wild.

Kill stray dogs (sometimes problematical in Buddhist countries).

Vaccinate dogs (pets).

Vaccination of wild animals: for example in Switzerland and Germany foxes are vaccinated with oral live vaccine incorporated in fishmeal pellets or other bait. Vampire bats can be vaccinated by catching some and applying the live vaccine to the skin. The animals often lick one another and it would be possible in this way to vaccinate a colony of animals. The vaccine could also be applied to cattle.

Persons in high-risk occupations (e.g. veterinarians, certain laboratory personnel, medical personnel
in the infectious diseases departments of hospitals), and certain travellers to high endemic regions) should receive PrEP vaccination. It could also be considered in populations living in rabies endemic areas, where the dog bite incidence is high. WHO currently recommends a 2-day intradermal regimen at Day 0 and Day 7.

LAST UPDATED BY ADMIN ON JULY 14TH, 2022

**HTLV-1 Infection**

**Summary**

- HTLV-1: chronic retroviral infection
- Importance in certain geographical foci.
- Transmission from mother-to-child (breast feeding), sexually or via blood
- Clinical aspects: 95% of infected people remain asymptomatic, 5% become symptomatic
- Opportunistic infections: Norwegian scabies, tuberculosis, *Strongyloides stercoralis*, etc.
- Inflammatory syndromes: uveitis
- Evolution to neoplastic diseases in a minority of patients: adult T-cell leukaemia / lymphoma
- Neurological syndrome with abnormal gait pattern and urinary incontinence (HAM/TSP)

**General**

The name retroviruses refers to the unique manner in which these viruses reproduce. Their genetic information is coded in RNA. This is not in itself unusual. However, they also possess an RNA-dependent DNA-polymerase (reverse transcriptase) which produces DNA from the RNA strand. This DNA can be integrated at random into the host genome. Using this new DNA as template, mRNA can be transcribed and then translated into viral proteins. Such a flow of genetic information (from RNA to DNA) does not occur in other organisms so far as is known.

The family of Retroviridae contains three subfamilies: the Oncovirinae (with HTLV-1 as the most important representative), the Lentivirinae (with HIV as the most important virus) and the Spumavirinae (“foamy viruses”). The group of viruses known as the Primate T-lymphotrophic viruses (PTLVs) is composed of simian and human T-lymphotrophic retroviruses (SLTVs and HTLVs respectively). The viruses are genetically closely related. It has been shown that hunter-gatherers, when hunting monkeys and/or apes, are regularly exposed to the simian viruses. In urban bushmeat markets in Cameroon, about 10% seroprevalence was found in the hunted wild monkeys. Studies
have shown that the diversity of HTLVs is directly related to the genetic diversity of the STLVs from which the primary zoonotic infection originated. The ease with which STLVs seems to be able to cross species barriers warrants increased surveillance of these viruses. HTLV-1 has spread to many parts of the globe and is associated with adult T-cell leukaemia and myelopathy/tropical spastic paraparesis. In 1982 HTLV-2 was isolated in a patient with hairy cell leukaemia. HTLV-2 is less pathogenic than HTLV-1. More than 99% of infected individuals will remain asymptomatic but a minority will develop myelopathy/tropical spastic paraparesis. HTLV-3 and HIV turned out to be the same virus. Less is known about HTLV-4, which was identified in 2005 in Cameroon.

HTLV-1 is genetically very stable. This stands in marked contrast with HIV which is so variable that it is sometimes called a quasi-species.

**Epidemiology**

HTLV-1 was first isolated in 1980 from a T-cell lymphoma cell line, originating from a patient with adult T-cell leukaemia/lymphoma. It was the first time human retroviruses were shown to exist. Africa is the only continent where all different primate T-cell lymphocytotropic viruses have been found, from HTLV types 1 to 4, and the simian retroviruses STLV types 1 to 3. This suggests that the virus spread from Africa to the rest of the world. On the other hand, HTLV-1 genetic sequences have been found in a 1500-old Chilean mummy.

The seroprevalence fluctuates widely from region to region. HTLV-1 is endemic in certain geographical areas, such as Taiwan and the Southwest of Japan, Okinawa, Papua New Guinea, Melanesia (Solomon Islands, Vanuatu), Australia (in Aboriginais), the Caribbean, West and Central Africa and the northeast of South America including Peru. In certain endemic areas, more than 1% of the population can be infected (e.g. Togo, Guinea-Bissau, the southern part of Cameroun). The highest prevalence is found in Southern Japan (up to 10%). Apart from Japan, Taiwan, a Chinese mainland province near Taiwan and Iran (seroprevalence 0.1 to 1%), the infection seems to be rare in other parts of Asia, although more study is needed. For many African regions, there are no good prevalence data available at present. The virus also occurs in some other regions such as Italy, Romania, Israel and the Arctic, but is less common there. It is rare in the rest of Europe and North America, although there are some small foci among Native Americans. In the first decade of the 21st century, it is estimated that 10 to 20 million people are infected worldwide. However, few population-based studies have been performed therefore prevalence data may be lacking. Studying the prevalence in blood donors, pregnant women or other groups might bias the data. One also must check the diagnostic criteria. Different techniques and strategies can give rise to different results.
The virus

HTLV-I is a round, enveloped retrovirus which contains reverse transcriptase and integrase. Its genome is composed of positive single-stranded RNA. As with all retroviruses, this is converted to double-stranded DNA which is integrated into the host cell chromosomes. The virus exists predominantly as a cell-associated provirus and is transmitted as such. The plasma viral load is therefore often undetectable. Cytotoxic T-cells destroy infected cells by lysis. This results in the simultaneous production of inflammatory cytokines. The balance between these two processes leads to a more-or-less steady state in any given individual. HTLV-1 does not contain oncogenes. However, one of the viral encoded proteins induces abnormal cell growth. It blocks transcription of certain genes that are important for the control of the cell cycle, apoptosis and DNA repair. This results in mitosis without checking for chromosomal abnormalities. Genetically damaged cells with unstable chromosomes will not apoptose helping clonal outgrowth of these cells.

Transmission

The virus is transmitted by at least three different mechanisms:

From mother-to-child. Transmission via breastmilk is the major route. The infection is transmitted via infected lymphocytes in the milk. Intra-uterine and peripartum transmission appears to be rare (less than 5% of children with infected mothers). Children of seropositive mothers have an approximately 15 to 20% risk of infection if they receive long-term breastfeeding, as is normal in many regions. Via sexual intercourse. This is bi-directional, yet transmission from man to woman is much more common than the reverse. After 10 years of sexual intercourse with an infected man, a woman has a 60% risk of becoming infected herself. The risk in the reverse situation is only 0.4%. The presence of genital ulcers increases the risk. The risk for men who have sex with men increases greatly depending on the number of years that there has been sexual contact and on condom use.

Via infected blood transfusions or infected medical material, chiefly when cellular elements are present (plasma-derived products do not represent a risk). The infectious titre in the cell-free plasma is very low. Blood for transfusion which has been stored for longer than one week has nearly zero percent chance of transmitting infection, due to the lack of viable T-cells. Transfusion of contaminated blood results in seroconversion in more than 40% of patients. In endemic areas, candidate blood donors are screened for HTLV-1 antibodies.

Via contaminated syringes and needles. HTLV-1 infections are common among intravenous drug users in Brazil and New York, although in other North American and European IV drug users, HTLV-2 is
Clinical aspects

General

Several cell types may be infected, but T-cells are the most important of these. After infection various scenarios are possible:

1. latent infection without symptoms (the most common)
2. evolution to lymphoma / leukaemia
3. neurological syndrome with abnormal gait pattern and urinary incontinence
4. dermatitis
5. uveitis, arthropathy and other inflammatory processes, possibly with an auto-immune component
6. opportunistic infections

Latent infection

Infection may leave a person with a latent disease. He or she is infectious to others, but exhibits no symptoms or problems. During his or her life there is an approximately 90 to 95% chance that no complications will arise. If not tested specifically for this virus, the person in question will have no idea that he or she is infected. A number of seropositive individuals will be found by chance e.g. during the control of donor blood. There are some preliminary data suggesting that infection with HTLV-1 is associated with a lower risk for development of stomach carcinoma in Japanese patients.

Lymphoma / leukaemia

ATL (adult T-cell leukaemia/lymphoma). There are several histological subtypes, but the diffuse large cell lymphoma is the most common. The lifetime cumulative risk is roughly 2% (1 to 5%). The tumours consist of monoclonal proliferation of CD4-positive T-cells. The clinical course may be acute, lymphomatous, chronic or smouldering. A fifth form, primary cutaneous tumoral ATL, has also been described.

If the course is acute and aggressive, nearly all patients will have lymphadenopathy and 50% will have hepatosplenomegaly. Skin lesions can resemble those of mycosis fungoides (cutaneous T-cell lymphoma). The dermal abnormalities include nodules, papules and diffuse infiltrative lesions. About
70% of patients develop hypercalcemia and osteolytic bone lesions. Approximately 10% exhibit involvement of the cerebral meninges resulting in muscle weakness, disturbed behaviour and/or headache. Oddly enough the protein content in the cerebrospinal fluid may still be normal, while at the same time containing ATL cells. Peripheral blood contains pleomorphic atypical lymphoid cells with basophilic cytoplasm and convoluted nuclei (so-called flower cells). During the leukemic phase, leukocyte count may increase dramatically. Acute ATL has a poor prognosis, with a median survival time after diagnosis of 6 months.

The lymphomatous form occurs in approximately 20% of symptomatic patients. The course is the same as in the acute aggressive form. There is also lymphadenopathy. Neoplastic cells are found in the blood, yet there is no lymphocytosis. The average survival time is 10 months.

In the chronic form the disease lingers for two years on average, without bone lesions, hypercalcemia or neurological involvement. There is lymphocytosis. There may be hepatosplenomegaly, lymphadenopathy, skin and lung lesions.

In the smouldering form the disease lasts for more than 5 years. In this form skin lesions, and to a lesser extent pulmonary lesions, are prominent, while hypercalcemia, hepatosplenomegaly and lymphadenopathy are absent. Transformation from a smouldering or chronic form to an acute form may occur suddenly.

**HTLV-1 associated myelopathy**

HTLV-1 associated myelopathy (HAM) is also known as chronic progressive myelopathy or tropical spastic paraparesis (TSP). This is a progressive hypertonic and ataxic myelopathy. The cumulative risk to develop this after infection with HTLV-1 is 2%. The disorder is more common in women. The main pathological feature of this condition is chronic inflammation of the white and grey matter of the spinal cord. Mononuclear cells, mainly T-cells, cause perivascular cuffing and infiltrate the spinal cord, which in a later stage will lead to atrophy. It is possible that there is an auto-immune component in the destruction of nerve cells (cross-reactivity between HTLV-1 antigens and tissue antigens). Patients with rapidly progressing HAM/TSP have a higher proviral load than those with slow progression.

Most damage occurs in the lower thoracic spinal cord. Weakness and stiffness of the legs, back pain and urinary incontinence together with abnormal gait pattern, are characteristic of the disease. The sensory disturbances are usually limited, but there may be polyneuropathy with dysesthesia. Bladder disorders are an important cause of impairment among HAM/TSP patients. The course is progressive, so that many patients need to use a wheelchair and/or are bedridden within 10 years.
There is typical hypertonic symmetrical paraparesis or paraplegia with hyperreflexia and pronounced ankle clonus, for example when testing the Achilles tendon reflex or in sudden dorsiflexion of the foot. Babinski’s sign can be elicited (spreading and extension of the toes instead of the normal plantar flexion upon stimulation of the sole of the foot). The reaction corresponding to this in the hands is Hoffman’s sign. The cerebrospinal fluid may show an increased protein content and may contain mild pleiocytosis with “flower cells”, anti-HTLV-1 antibodies and oligoclonal bands. Definite diagnosis of HAM/TSP requires the demonstration of HTLV-1 infection and exclusion of other causes of myelopathy.

Norwegian scabies in HTLV-1 patient. Copyright Alexander von Humboldt Institute, Peru.
Clinical aspects, miscellaneous

People infected with HTLV-1 have a high risk of dermatitis, often with superinfection by Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*). Lesions are often eczematous, and tend to be localized on the scalp, face (paranasal skin), ears, eyelids, neck, axillae and groins. Infective dermatitis is a chronic relapsing syndrome that mainly affects children. Co-morbidities include glomerulonephritis, bronchiectasis, lymphocytic interstitial pneumonia and anaemia. Norwegian scabies present with massive crusted skin lesions, mainly in pressure areas. Opportunistic infections due to immunosuppression are common, including *Pneumocystis jiroveci* and systemic fungal infections. There is a risk of hyper-infection with *Strongyloides stercoralis*, especially in those who are being treated with corticosteroids. Since the larvae mechanically carry bacteria from the colon, sepsis is common. Relapse after treatment with ivermectin is common. Herpes zoster is not so common as an opportunistic infection.
People infected with HTLV-1 have an increased risk for tuberculosis, and patients tend to have more severe lesions due to tuberculosis.

In regions where HTLV-1 is endemic, various inflammatory and auto-immune disorders, including uveitis, the sicca syndrome, pneumonitis, arthropathy and thyroiditis are attributed to this virus. However, more research is needed into these matters. Patients with uveitis often present with blurred vision with floaters. Iritis and vitreous opacities are almost always present, often in association with retinal vasculitis, and sometimes with retinal exudates and haemorrhages. Bilateral lesions are as common as unilateral inflammation. The prognosis is good, since it tends to resolve spontaneously within weeks. Topical or systemic corticosteroid treatment hastens recovery. More than 90% of cases recur within 3 years. Complication include retinal degeneration, glaucoma and steroid-induced cataracts.
**Differential diagnosis:**

The differential diagnosis is broad. HAM / TSP is similar to multiple sclerosis, with a slow, gradual onset. The disorder should be differentiated from lathyrism and konzo, both of which have an acute onset and are caused by toxins in the diet. The cauda equina syndrome, various neurodegenerative disorders such as amyothrophic lateral sclerosis, as well as infections such as syphilis, HIV, neurobrucellosis and tuberculous meningitis may be included in the differential diagnosis. The skin lesions are similar to mycosis fungoides (cutaneous T-cell lymphoma), leukemic skin lesions and those of non-HTLV-1-related lymphoma.

**Diagnosis**

HTLV-1 is usually detected by carrying out serological tests because of clinical suspicion, screening at the blood bank or due to concerns by family members of HTLV-1 positive patients. Sometimes the diagnosis is made when a patient has a persistent *Strongyloides stercoralis* infection (faeces with larvae, cutaneous larva currens or signs of hyperinfection). In the family history, which is important due to the mother-to-child transmission, it is often possible to find maternal family members who suffered from lymphoma or who were wheelchair users.

The antibodies can be detected by enzyme immunosorbent assay (EIA). Polymerase chain reaction (PCR) can provide a definite diagnosis. With real-time PCR the proviral load can be quantified as the number of HTLV-1 DNA copies per fixed number of peripheral blood mononuclear cells. This is often used as a marker for prognosis and diseases progression.

The test for HTLV-1 also detects the majority of HTLV-II infections. MRI [magnetic resonance imaging] or a CT scan shows speckled white abnormalities in the spinal cord. In chronic ATL absolute lymphocytosis is found (more than $3.5 \times 10^9$/liter). In the acute form of ATL the blood count will suggest leukaemia. Blood smears may contain abnormal lymphocytes with a highly wrinkled nucleus, called “flower cells”. A skin biopsy shows the malignant lymphocytes.

**Treatment**

Oral or intravenous corticosteroids are used in the early phase of HAM/TSP, when inflammation is more prominent than demyelination. Motor disability, pain, and urinary dysfunction may be ameliorated, but improvement is not sustained in many patients. Valproic acid is an anti-epileptic with histone deacetylase inhibiting activity. It activates viral gene expression and exposes virus-infected cells to the immune system. Preliminary data show that the proviral charge initially increases, but
subsequently decreases. Its exact place in therapy needs to be further clarified. Other drugs, such as interferon-alpha, daclizumab (humanized anti-Tac), plasmapheresis and intravenous immunoglobulins (IVIG) are used or being studied. The place of nucleoside analogues is unclear.

Hypercalcemia can be treated with cortisone and antineoplastic drugs. Calcitonin, for osteoclast inhibition, and etidronate or other bisphosphonates (also osteoclast inhibitors) will not usually be available. The tumour is initially highly sensitive to chemotherapy (e.g. CHOP [cyclophosphamide, hydroxydaunomycin/doxorubicin (Adriamycin), oncovin, prednisone]), but there is a serious risk of opportunistic infections. Relapse is common. Physiotherapy is important. For the hypertonicity, tetrazepam (Myolastan®), dantrolene (Dantrium®), baclofen (Lioresal®) and/or tizanidine (Sirdalud®) may be used. However, sometimes patients use their spastic legs as crutches and are able to walk. Antispasmodics can have the undesirable effect that walking in these patients suddenly becomes more difficult. In case of bladder hypertonia the patient is advised to begin bladder training. The idea is that the patient urinates at regular times, even if at that moment there is no urge to urinate. The intervals are gradually lengthened. In this way the bladder can become accustomed to retaining ever larger amounts of urine.

**Prevention**

Breastfeeding by infected mothers should be discouraged. Blood donors should be screened for the virus. As with HIV, safe sex also has a role to play here. The repeated use and certainly sharing of needles should be avoided. Correct cleansing and sterilization of medical equipment should be obligatory.