

FACULTÉ DE PHARMACIE

#### Arboviruses and tropical diseases

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#### Content

#### **Arboviruses**

- vectors
- Flaviviruses: Yellow fever, Dengue, Zika
- Chikungunya

#### Hemorrhagic fever

- Ebola

#### **Neglected tropical diseases**

- Rabies

#### ARBOVIRUSES

#### Arboviruses

- Arboviruses belongs to different viral families: *Flaviviridae, Togaviridae, Rhabdoviridae, Bunyavirales (Phenuiviridae, Nairoviridae...)*
- **AR**thropod-**BO**rne Viruses : transmission by hematophagous arthropods
- Vectors: insects (mosquitoes, midges) and ticks



Weaver et al, Nat Rev Microbio, 2021<sup>4</sup>

#### Arbovirus transmission cycles and emergence

Enzootic: Ancestral, often continuous, transmission cycles of zoonotic arboviruses involving wild animals serving as amplifying and/or reservoir hosts. Often called the sylvatic cycle Human-vector-human (also Direct spillover from Secondary amplification in domesticated animals sexual.vertical human-human enzootic cycles transmission for ZIKV) Human-amplified cycle Enzootic cycle Epizootic cycle A aegypti A albopictus CHIKV, DENV, ZIKV, YFV VEEV WNV. WEEV 5 Weaver et al, Nat Rev Microbio, 2021

West Nile virus (WNV) Western equine encephalitis virus (WEEV) Venezuelian equine encephalitis virus (VEEV)

#### Emergence/re-emergence of arboviruses: why now?

For ~40years: resurgence of several arboviruses

- genetic changes in the viruses: RNA viruses
- adaptation to new vectors: Ae. aegypti and Ae. albopictus
- adaptation to new reservoir/amplification hosts
- expansion of the geographic distribution of mosquito vectors
- climate change
- resistance to insecticides due to uncontrolled use
- global growth of human populations and unplanned urbanization
- increased travel

#### Flaviriruses

#### Flaviviridae

- Genus *Flavivirus*
- Enveloped
- icosahedral capsid
- Genome ssRNA (+),10kb
- More than 70 viruses





#### Flavivirus replication cycle



#### Flaviviruses and human diseases

Virus	Vector(s)	Zoonotic reservoir
Dengue	<i>Aedes</i> mosquitoes ( <i>Aegypti</i> and <i>Albopictus</i> )	Non-human primates
Zika	A. Aegypti and A. Albopictus	Non-human primates
Yellow fever	A. Aegypti	Non-human primates
West nile	Culex mosquitoes	Birds
Japanese encephalitis	C. pipiens	Birds, pigs
Tick-borne encephalitis	Ixodes ticks	Small rodents,
Usutu	C. pipiens	Birds

50-80% of flavivirus infection = asymptomatic

clinical presentation includes mild illness (fever), flu-like symptoms, and more severe diseases (hemorrhagic fever, encephalitis, congenital defects, hepatic failure...)

### Yellow fever virus (YFV)

- First human virus isolated (1927)
- Outbreaks documented in the Americas since the 17th century (Caribbean) = originates from Africa, spread to the New World and Europe because of trade



- 1 serotype, 5 genotypes
- Endemic regions in 47 countries in Africa and central and south America
- >100 000 severe cases/year
- Eliminate Yellow fever Epidemics (EYE) strategy launched in 2017

Monath, The Lancet 2001

#### Yellow fever

- Incubation 3-6 days
- Asymptomatic forms
- Most common symptoms: fever, headache, muscle pain, nausea/vomiting, fatigue, lost of appetite. Last 3-4 days



Severe forms develop after a « remission » period (24h)

- Hemorrhagic disease
- Organ affected (liver and kidney)
- ➤ 50% mortality



#### Yellow fever



Monath, The Lancet 2001

### Dengue virus (DENV)

 Transmission by mosquitoes: Aedes aegypti and Aedes albopictus



• 4 serotypes : DEN-1, DEN-2, DEN-3 and DEN-4

# DENV: epidemiology

- increased incidence: WHO estimates around 390 million of dengue cases per years
- 5.2 million cases reported in 2019 (8-fold increase in 20 years), increased since 2023 (14 million cases in 2024, Americas +++)
- Spreading in new zones: endemic in more than 100 countries. First report of local transmission in Europe in 2010 (France and Croatia)
- 1/2 of the world population is at risk
- 10 000 deaths in 2024

#### Dengue: a globally emerging disease

Americas, South-East Asia and Western Pacific regions are the most affected regions



Number of suspected or laboratory-confirmed dengue cases notified to WHO, 1990–2015 (Americas, South-East Asia and Western Pacific regions)

#### Global distribution of Dengue cases (2024)



Note: Data refer to Dengue virus cases reported in the last 12 months (January 2024-December 2024) [Data collection: January 2025]. Case numbers are collected from both official public health authorities and non-official sources, such as news media, and depending on the source, autochthonous and non-autochthonous cases may be included. Administrative boundaries: © EuroGeographics

The boundaries and names shown on this map do not imply official endorsement or acceptance by the European Union. ECDC. Map produced on 23 January 2025

### **Dengue in France**

- Aedes aegypti present in French Antilles, in French Guyana and Mayotte
- Aedes albopictus present on Réunion Island and now several departments in metropolitan France (78)
- Outbreaks :
- Ongoing outbreaks in Martinique and Guadeloupe (DENV-3)
- ✤ Reunion in 2021: around 60 000 cases (30 000 confirmed), 33 death.



Metropole: 13 autochthonous cases in 2020 (834 travel-associated cases), 1 in 2021 but... 65 in 2022, 68 in 2024 and more than 1500 travel associated cases

# Dengue: Pathology

- Asymptomatic in 50 to 90% of cases
- Incubation 4-7 days
- Classic dengue:
- starts with a high fever (40°C)
- symptoms during febril phase: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands, rash
- Severe Dengue: 1-5% of symptomatic cases
- Caracterized by
- plasma leakage
- bleeding
- organ impairment
- warning signs: before shock, start at the end of the febrile phase 18

#### Severe dengue: warning signals



#### Suggested dengue case classification and levels of severity

# DENGUE ± WARNING SIGNS SEVERE DENGUE with warning signs 1. Severe plasma leakage without 2. Severe haemorrhage 3. Severe organ impairment

#### CRITERIA FOR DENGUE ± WARNING SIGNS

#### Probable dengue

live in /travel to dengue endemic area. Fever and 2 of the following criteria:

- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

#### Laboratory-confirmed dengue

(important when no sign of plasma leakage)

#### Warning signs\*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count
- \*(requiring strict observation and medical intervention)

#### CRITERIA FOR SEVERE DENGUE

#### Severe plasma leakage

leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

#### Severe bleeding

as evaluated by clinician

#### Severe organ involvement

- Liver: AST or ALT >=1000
- CNS: Impaired consciousness
- Heart and other organs

#### The course of severe dengue



dengue guidelines for diagnosis, treatment, prevention and control https://www.who.int/neglected\_diseases/resources/9789241547871/en/



- viral tropism:
- cells from the myeloid lineage:
- dendritic cells, circulating monocytes macrophages
- also megakaryocytes and platelets
- virus also detected in endothelial cells in patients with severe dengue
- Establishment of systemic infection via lymph nodes
- Viremia 24-48h post inoculation
- Disseminates in different tissues

- primary dengue: first infection with one serotype of DENV
- after primary dengue, development of immunity against this serotype
- possibility of <u>secondary dengue</u>: infection by another serotype
- The risk of severe dengue is more important during secondary infection
- homologous antibody: neutralizing
- heterologous antibody: facilitating
- → antibody-dependent enhancement (ADE)
- Infection of cells expressing Fc receptors (target of dengue virus)
- « extrinsic » ADE = **7** of the number of infected cells
- « intrinsic » ADE =  $\blacksquare$  of viral replication in this cells

plasma leakage:

オ increased endothelium permeability

dysfunction of the endothelial barrier



#### **Bleedings:**

- mostly mild hemorrhagic manifestation on skin and mucosa: petechiae, purpura, epistaxis, and gingival bleeding
- more severe hemorrhage are possible, especially gastrointestinal bleedings

Petechiae

#### Causes:

- thrombocytopenia
- coagulation abnormalities
- disseminated intravascular coagulation



# Dengue prevention

- Vector control
- Personal protection
- Vaccination : first vaccine available since 2015 (Dengvaxia ®, Sanofi)
- for people aged 9-45 years in endemic areas
- WHO recommands a pre-vaccination screening

New vaccine: Qdenga® (Takeda)



#### **Fast Facts**





Dengue is a viral infection transmitted by the bite of an infected female Aedes mosquito One bite is enough to infect a person after which symptoms may appear after 5-6 days

anyone, and can be more severe in those with compromised immune system

#### **Preventing Dengue**



For more information: 🗗 🗐 🛇 123

# Zika virus (ZIKV) emergence

declared a Public Health Emergency of International Concern in 2016 >80 countries in Africa, Asia, the Americas and Pacific have reported the presence of Zika



Gutiérrez-Bugallo et al, Nature Ecology & Evolution 2019

### ZIKV: course of infection

- transmission:
- mosquitoe bites
- sexual (male-to-female ++)
- blood transfusion
- Vertical
- incubation 3-14 days
- Viral tropism



# ZIKV: pathology

- 50-80% asymtomatic
- usuelly mild
- symptoms last 2-7 days

- Complications (<1%)</li>
- neurological: Guillain-Barré syndrome
- congenital zika syndrome
- Thrombocytopenia



# ZIKV neurological complications

- Guillain-Barré syndrome
- 2 to 3 cases per 10,000 ZIKV infections
- auto-immune disease that attacks peripheral nervous system
- progressive weakness in the extremities (legs ++)
- usually: full recovery
- in severe cases can progress to paralysis and death



Willison et al, The Lancet 2016

# ZIKV complications during pregnancy

- Vertical transmission can happen in all trimesters of pregnancy, either if the mother is symptomatic or not
- transmission during breastfeeding  $\rightarrow$  requires more confirmation/studies



# Chikungunya (CHIKV)

- Family: Togaviridae
- genus: Alphavirus

- Enveloped, icosahedral capsid
- ssRNA(+) of 11-12kb
- other alphaviruses:
- Sindbis virus
- Semliki forest virus



### CHIKV: epidemiology



ECSA: eastern, central and southern African, IOL: Indian Ocean lineage

33 Weaver SC, Lecuit M. N Engl J Med 2015;372:1231-1239.

# CHIKV: epidemiology

- Globally:
- > 2 million cases reported since 2005
- Mostly in Africa, Asia, India
- More recently: outbreaks in the Americas
- 620 000 cases in 2024 : America (Brazil), India, Pakistan...
- Europe: outbreak in Italy (200 cases, 2007)
- France:
- La Réunion: after outbreak in Kenya, 270 000 cases reported (2005-2006) ~40% of the population!
- metropolitan: 6 autochthonous cases in 2020, 1 in 2024
- Ongoing reemergence in la reunion? : 1069 cases (since 08/2024)

### CHIKV: replication cycle

8 Mature virion identified receptors: **Budding particles DC-SIGN** Plasma Heparan sulfate membrane 8008 00 0 0 0000 integrins Cytoplasm Receptormediated . . . endocytosis 00000 -Endosome Nucleocapsid Processed core assembly glycoproteins El peptide and genomic transported exposed Release of RNApackaging to plasma nucleocapsid core membrane and viral genome 49S RNA genome 5'm<sup>7</sup>G 495 RNA genome Golgi A\_3' furin cleavage of pE2 (p62) = Processing and Translation and 8 Replication maturation of E2 and E3 Replication processing glycoprotein - U\_5' Minus strand RNA 2 180 Replication ER 5'26S mRNA complex - A\_3' Nucleus Translation . Processing 35 Capsid pE2 E1 C-pE2-6K-E1 Schwartz et al, Nat Rev Microbiol 2010

# Chikungunya: pathology

- Incubation 2-6 days
- acute phase: high fever, rigors, headache, photophobia and rash + severe joint pain
- last a few days 2 weeks
- 30-40%: chronic phase with recurrent joint pain



# Chikungunya: pathology

Sym	ptoms		_			
		Fever, usually lasts about 1 week (90% of patients)				
	Myalgia, usually lasts 7–10 days (90% of patients)					
	Polyarthralgia, polyarthritis, or both, can last weeks to months (95% of patients)					
	Rash, lasts about 1 week (40–50% of patients)					
Infection						
	2.6.1					
	<b>2–6 days</b> Incubation period	Approximately 1 week	Weeks to months	Years		
	<b>2–6 days</b> Incubation period	Approximately 1 week /iremia, usually lasts 5–7 days	Weeks to months	Years		
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Burt et al, Lancet Infect Dis 2017 Miner JJ, et al, Arthritis Rheumatol 2015

### Testing for DENV ZIKV and CHIKV

Collect bloo	d and urine	
(keep a portion of blood as the f	If sequential testing is adopted, prioritize testing to the epidemiological context	
RT-PCRs for DENV, CHIK, ZIKV sequential or parallel testing		of circulating flaviviruses
Result for multiplex reactions	Interpretation	
ZIKV pos, DENV neg, CHIK neg	ZIKV confirmed case	
ZIKV neg, DENV pos, CHIK neg	DENV confirmed case	
ZIKV neg, DENV neg, CHIK pos	CHIK confirmed case	
ZIKV pos and/or DENV pos or CHIK pos	Co-detection and ZIKV confirmed case	

Figure 1. Proposed testing algorithm for suspected cases of arbovirus infection identified within seven days of onset of symptoms

#### Testing for DENV ZIKV and CHIKV



#### Figure 2. Proposed testing algorithm for suspected cases of arbovirus infection more than one week after onset of symptoms

#### Prevention of arboviruses infection

- Vaccines available for several arboviruses: YFV, Japanese encephalitis virus, DENV, TBEV and CHIKV
- Personal protection against mosquitoes and ticks
- Vector control: global response is required
- Better development of urban centres
- Development of new tools and technologies

#### **HEMORRHAGIC FEVER**

### Hemorrhagic fevers

Famille	Virus
Arenaviridae	Lassa Junin Machupo
Phenuiviridae	Rift valley fever
Nairoviridae	Crimean Congo hemorrhagic fever (CCHF)
Filoviridae	Ebola Marburg
Flaviviridae	Dengue Yellow fever

# Ebola virus (EBOV)

- family: *Filoviridae*
- genus: Ebolavirus
- filamentous enveloped virus : 970 nm long
- Capsid: nucleoprotein N
- Matrice
- genome: ssRNA(-),18-19 kb
- 7 structural proteins
- 7 filoviruses detected in humans or primate, from the *Ebolavirus* (6) or *Marburgvirus* genus (1)
- Zaire and Sudan ebolavirus ++



# Ebola: epidemiology

- filoviruses: zoonotic pathogens, maintained in reservoir(s)
- epizootic, not considered as endemic



Jacob et al, Nat Rev Dis Primers,2020

- transmitted to human from wild animals: contact with infected fluids from fruit bats, chimpanzees, gorillas, monkeys, forest antelope or porcupines
- human to human transmission by direct contact, or indirect

#### Ebola: outbreaks

- Discovered in 1976 in the Democratic Republic of the Congo
- until 2013, outbreaks in central africa
- 2013-2016: 28 600 cases, 11 325 deaths
- outbreaks in DRC 2018-2020
- 2021: cases reported in Guinea and in RDC
- 2022 and currently : in Uganda (Sudan ebolavirus)





#### **EBOV:** replication cycle



# Ebola virus disease pathophysiology



Baseler L, et al. 2017. Annu. Rev. Pathol. Mech. Dis. 12:387–418

- inoculation onto mucous membranes or skin wounds
- Incubation 9-10 days
- infect dendritic cells
- replication and dissemination of the virus (lymph nods)
- viremia with widespread viral dissemination
- tissue and vascular damage
- multiple cell types can be infected within organs
- tissue damage due to viral-induced cytopathic effects and indirect organ injury (host inflammatory responses, endothelial dysfunction, and disordered coagulation)

### Ebola virus disease pathology



- incubation followed by nonspecific symptoms: malaise, fatigue, and muscle weakness and/or myalgia, before or associated with fever
- progression to nausea, vomiting and profuse diarrhea, with profound fatigue
- peak of illness: 2<sup>nd</sup> week. Sequential organ failure
- fatality rate ~50%

#### Time course of EBOV in body fluids and testing



WHO recommendation for testing:

- nucleic acid detection when possible
- rapid antigen detection tests (need confirmation)
- specimens: blood (live patients) or other body fluid (dead patients)

#### Ebola virus prevention and control

- reduce the risk of transmission from wild animal to human
- reduce the risk of human-to human transmission
- containment mesure during outbreaks
- reduce the risk of sexual and vertical transmission
- vaccines



### RABIES VIRUS: A NEGLECTED TROPICAL DISEASE

#### Neglected tropical diseases

- "Neglected tropical diseases (NTDs) are ancient diseases of poverty that impose a devastating human, social and economic burden on more than 1 billion people worldwide, predominantly in tropical and subtropical areas among the most vulnerable, marginalized populations." WHO
- 20 diseases among which dengue and rabies
- For rabies, WHO goals: to eliminate dog-mediated human rabies

### Rabies virus

- Rhabdoviridae
- Lyssavirus
- enveloped, bullet-shaped
- ssRNA(-) genome, 11kb







### Rabies epidemiology

- Rabies is a zoonotic diseases, mainly transmitted by dogs to human
- · Tens of thousands of deaths/year
- Present on all continents but causes death mainly in Asia and Africa





# Rabies: pathology and diagnosis



• clinical diagnosis (+ animal contact)

Nature Reviews | Disease Primers

- ante-mortem diagnosis : nuchal skin biopsies and saliva (antigen detection, virus isolation, RT-PCR)
- post-mortem (brain tissue)

#### Rabies: prevention



- rabies vaccination recommended for travelers to endemic regions
- secondary prevention: wound care and post-exposure prophylaxis (rabies immunoglobulin and vaccination)