RNA and DNA viruses

Petr Hubáček
Dept. of Medical Microbiology and Paediatric Haematology and Oncology
2nd Medical Faculty of Charles University and Motol University Hospital

DNA viruses

ds DNA

- *Herpesviridae* → α HSV, VZV; β CMV, HHV-6 a 7; γ EBV, HHV8
- *Adenoviridae* → Adenoviruses (group A-F)
- *Polyomaviridae* → BKV, JCV, WUV, KIV, SV40...
- *Papillomaviridae* → Papillomaviry
- *Poxviridae* → Variolla virus, vaccinia, molluscum contagiosum
- *Hepadna* → HBV

ss DNA

- *Parvoviridae* → Parvovirus B19, lidský bocavirus (HBoV)
- *Anelloviridae* → TTV, TTMV, TTMDV

RNA viruses

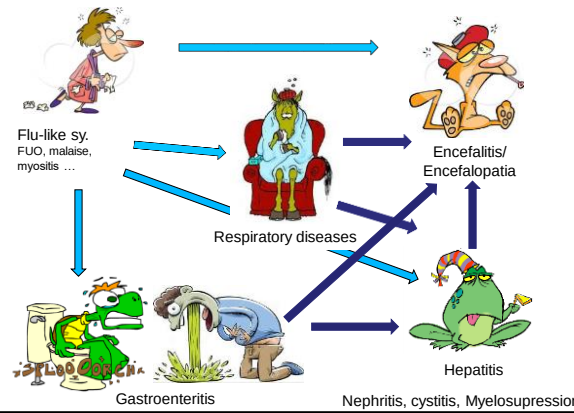
ss RNA

- *Ortomyxoviridae* → Influenza A-C
- *Paramyxoviridae* → Paramyxovirus → PIV 1-4; Morbillivirus; Pneumovirus → RSV, hMPV
- *Coronaviridae* → HCoV (229E, NL63, OC43, HKU1, MERS, SARS…)
- *Picornaviridae* → Enteroviruses; Rhinovirus → HRV
- *Flaviviridae* → HCV, Yellow fever Virus, WNV, Denque v…
- *Caliciviridae* → Human caliciviruses – Norovirus, Sapovirus
- *Astroviridae* → Astrovirus
- *Rhadboviridae* → Lyssa virus

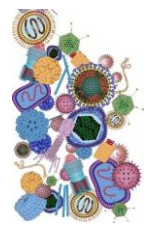
ds RNA

- *Reoviridae* → Rotavirus; Orbivirus

Clinical consequences



Balance in the (immunocompromised) patient



Immune system
(immunocompromised treatment, chemotherapy, …)

Pathogens
Lymfocyte regulated – viruses, mycoses

Methods of the viral detection

Detection methods in virology

- Microscopic — **Direct detection**
- Cultivation
- Detection of the antigen
- Detection of the nucleic acid
- Detection of the antibodies
- (Signs of disease)

Indirect detection

Methods of the viral detection - INDIRECT

Signs of the disease

Clinical signs of disease leading to suspition of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

- varicella
- zoster
- fully deleloped IM
- papillomaviral infection (wart)
- also in HHV-8 and other viral infections

Group of disease related to RNA viral infections

Respiratory tract infections – influenza, PIV, RSV, hantaviruses…

CNS infections – enteroviruses, parechoviruses, flaviviruses (WNV), TBE,…

Liver infections – picornaviruses (HAV), flaviviruses (HCV, Yellow fever..)…

Kidney infections – hantaviruses,…

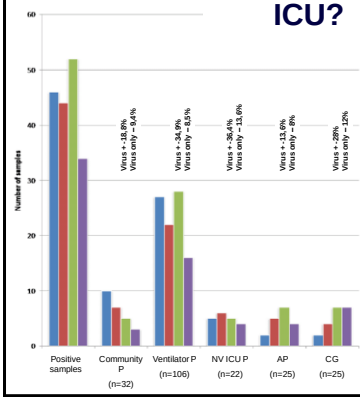
Immune related infections – HIV

GIT infections – astroviruses, caliciviruses, rotaviruses

Haemorhagic fevers – Lassa virus, Ebola virus, Marburg virus…

Exanthematic diseases – Mumps virus, Rubella, Dengue…

How often do we detect viruses at ICU?



Detected	Pneumonia (n=185)	Control group (n=25)
HSV	51 (27,5%)	7(28%)
CMV	31 (16,8%)	3 (12%)
PIV-1	3 (1,6%)	1 (4%)

Bousbia et al. PloS 2012

BMC Pulmonary Medicine 2009, **9**:22 http://www.biomedcentral.com/1471-2466/9/22

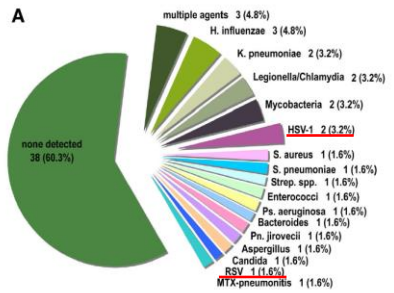


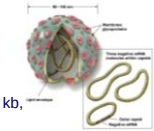
Figure 3
A: Detected primary responsible (leading) infectious agents in 63 patients with ambulatory-acquired pneumonia/pneumonitis and autoimmune disease. RSV = respiratoy syncytial virus. B: Immunosuppression scores were significantly more severe in the 6 patients with HSV-1 detection in BAL than in those subjects without clinical or laboratory evidence for HSV-1 (as assessed for 56/63 patients with reliable information on immunosuppressive regimens available; ** $p < 0.01$, Mann-Whitney two-sided test).

How often do we detect viruses at ICU?

Hematooncological patients

- **RSV**
  - **in 0.3% - 2.2% of paediatric pts with AML and 1%-12% adult HSCT pts**
  - UTRI to LRTI progression in 20-68% pts.
  - RSV related mortality 17-70%
- **PIV**
  - PIV causes URTI during year from laryngotracheitis, bronchiolitis to pneumonii in **15% of children from autumn to spring**
  - In patients after **HSCT in 2% - 7% symptomatically**, when asymptomatic patients are included up to 18%
  - Long lasting expression can lead to nosocomial epidemy.
  - PIV-3 is after HSCT most frequently (up to 90% of cases) later PIV-1 a -2
  - URTI decrease of ventilation up to 40%, infection progress to LRTI in 13-37% with fatal end 10-30%.
- **hMPV**
  - Related to RSV causing **5%-20% of URTI** and tracheobronchitis in children and adults during winter
  - At HSCT patients described in **5%-9% during first 2 years after HSCT**.
- **Coronaviry**
  - In pts. after **HSCT detected in 6.7% - 15.4%**, asymptomatic shedding in 41%..
  - In symptomatic pts. often coinfections
- **HRhV**
  - HRhVs most frequent viral cause of CARI with cumulative incidence up to **22.3% at D+100**.
  - Asymptomatic in 13% of HSCT patientů, detection with other CARI viruses in 19%
  - LRTI in allogeneic HSCT rare (<10%), might be associated with bad outcome in less then 10%

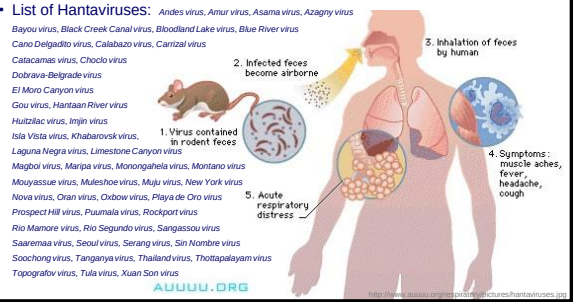
Hantaviruses



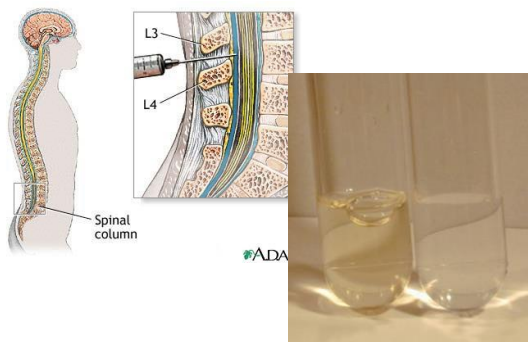
- Bunyaviridae
- ss(-) RNA - 3 segments (small ~ 1.7-2 kb, medium ± 3.7 kb, large ± 6.5 kb)
- enveloped 120-160 nm in diameter
- Incubation period – 2-4 weeks
- The described in 1951, where a hantavirus caused hemorrhagic fever with renal syndrome (HFRS) in North and South Korea.
- Transmitted from rodens, even pet rodens.
- The viruses that caused HFRS in Asia were later grouped as Old World Hantaviruses.
- In 1993 (southwestern USA) was described hantavirus pulmonary syndrome (HPS) - Sin Nombre.
- Hantavirus strains that occur globally – affecting kidneys and lungs mainly.
- Airborne transmission
- Underdiagnosed diseases.

Hantaviruses

- HFRS – viruses - Dobrava, Hantaan, Puumala a Seoul. Mortality is highest in Hantaan virus – 5–15 %; Puumala and Seoul virus about 1%.
- HPS (Sin Nombre) rare 534 case (1993-2009) – mortality rate 36%.
- List of Hantaviruses: *Andes virus, Amur virus, Asama virus, Azagny virus Bayou virus, Black Creek Canal virus, Bloodland Lake virus, Blue River virus Cano Delgadito virus, Calabazo virus, Carrizal virus Catacamas virus, Choclo virus Dobrava-Belgrade virus El Moro Canyon virus Gou virus, Hantaan River virus Huitzilac virus, Imjin virus Isla Vista virus, Khabarovsk virus, Laguna Negra virus, Limestone Canyon virus Magboi virus, Maripa virus, Monongahela virus, Montano virus Mouyassue virus, Muleshoe virus, Muju virus, New York virus Nova virus, Oran virus, Oxbow virus, Playa de Oro virus Prospect Hill virus, Puumala virus, Rockport virus Rio Mamore virus, Rio Segundo virus, Sangassou virus Saaremaa virus, Seoul virus, Serang virus, Sin Nombre virus Soochong virus, Tanganya virus, Thailand virus, Thottapalayam virus Topografov virus, Tula virus, Xuan Son virus*



CSF



Neurotropic viruses

- Neurotropismus (encefalitis)
  - Coronaviridae -
  - Flaviviridae – e.g. West Nile virus (WNV), Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV), St. Louis encephalitis virus (SLEV), tick-borne encephalitis virus (TBEV)
  - Lentiviridae - HIV
  - Herpesviridae – HSV-1, 2, CMV, HHV-6, HHV-7, EBV (?)
  - Paramyxoviridae – Morbilivirus, Hedra a Nipah virus
  - Picornaviridae - enteroviry
  - Rhabdoviridae – Lyssa
  - Polyomaviridae – JCV (PML)

Symptoms associated with CNS disease

Observed -- Rare ++ Often

Clinical symptoms	***Encefalopathy***	***Encefalitis***
Fever	--	++
Head ache	--	++
Decrease of the mental status	Stabil worsening	Status fluctuation
Focal neurological symptoms	--	++
Seisures	Generalized	Generalized and focal
Lab.-Blood	Leukocytosis --	Leukocytosis ++
Lab.-CSF	Pleocytosis --	Pleocytosis ++
Lab.-EEG	Diffuse decrease of waves	Diffuse decrease of waves and focal abnor.
Lab.-MRI	Often normal	Focal abnormalities

Kennedy J Neurol Neurosurg Psychiatry 2004;75 (Suppl I).

Differential diagnosis of encephalitis

ADEM – acute disseminated encefalomyelitis

CNS vasculitis (including VZV)

Non-virus associated infectious ~~encefalitis~~

Encephalopathy

- Anoxic/ischaemic
- Metabolic
- Nutritional deficiency
- Toxic
- Systemic infections
- Critical illness
- Malignant hypertension
- Mitochondrial cytopathy (Reye's and MELAS syndromes)
- Hashimoto's encephalopathy
- Paraneoplastic
- Neuroleptic malignant syndrome
- Traumatic brain injury
- Epileptic (non-convulsive status)

Bacterial
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Listeria monocytogenes
Borrelia burgdorferi
Leptospirosis
Brucellosis
Leptospirosis
Legionella
Tropheryma whippelli (Whipple's disease)
Nocardia actinomyces
Treponema pallidum
Salmonella typhi
All causes of pyogenic meningitis
Rickettsial
Rickettsia rickettsii (Rocky Mountain spotted fever)
Rickettsia typhi (endemic typhus)
Rickettsia prowazekii (epidemic typhus)
Coxiella burnetti (Q fever)
Ehrlichiosis (*Ehrlichia chaffeensis* – human monocytic ehrlichiosis)
Fungal
Cryptococcus
Aspergillosis
Candidosis
Coccidiomycosis
Histoplasmosis
North American blastomycosis
Parasitic
Human African trypanosomiasis (sleeping sickness)
Cerebral malaria
Toxoplasma gondii
Echinococcus granulosus
Schistosomiasis

Kennedy J Neurol Neurosurg Psychiatry 2004;75 (Suppl I).

Most frequently detected viruses according the risk factors

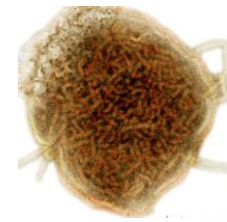
Risk factor	Possible aetiological agent
Unvaccinated status	Polio, measles, mumps, rubella viruses
Animal contact	Rabies virus, cat scratch disease, Hendra virus, Q fever
Bird contact	WNV, Japanese encephalitis, *Cryptococcus neoformans*
Insect contact	Malaria, WNV, tick-borne encephalitis virus, typhus, Lyme disease, trypanosomiasis
Ingested meat/unpasteurised milk	Toxoplasmosis, listeria, Q fever
Sexual contact	HIV, syphilis
Swimming	Enteroviruses, *Naegleria fowleri*
Camping/hunting	Malaria, tick-borne encephalitis virus, typhus

Virus vztekliny

Thompson et al. Arch Dis Child 2012;97:150-161.

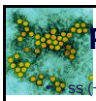
Most frequently detected viruses according to the clinical symptoms

Clinical presentation	Possible aetiological agent
Cranial nerve abnormalities	HSV, EBV, listeria, tuberculous meningitis, syphilis, Lyme disease, *Cryptococcus neoformans*
Cerebellar ataxia	VZV, EBV, mumps virus, trypanosomiasis
Dementia	HIV, measles virus, syphilis, human transmissible spongiform encephalopathies
Poliomyelitis-like flaccid paralysis	JEV, poliovirus, enteroviruses, WNV, tick-borne encephalitis virus
Parkinsonism	JEV, WNV, Nipah virus
Retinitis	CMV, WNV, cat scratch disease, syphilis
Rash	VZV, HHV-6, rubella virus, typhus, syphilis, Lyme disease, WNV, HIV, enteroviruses, *Mycoplasma pneumoniae*
Respiratory tract findings	Flu virus, adenovirus, *M pneumoniae*, *Mycobacterium tuberculosis*, Q fever
Parotitis	Mumps virus
Lymphadenopathy	HIV, EBV, CMV, measles virus, rubella virus, WNV, syphilis, cat scratch disease, tuberculous meningitis, toxoplasmosis, trypanosomiasis
Hepatitis	Q fever



Parotitis virus

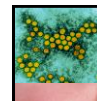
Thompson et al. Arch Dis Child 2012;97:150-161.



Picornaviridae - Enteroviruses

ss(+) RNA, genome 7.2-8.5 kb

- Most frequent cause of encephalitis/meningoencephalitis (90%)
- Different serotypes (dividing to) – Polioviruses
  - Coxackieviruses (e.g. Myocarditis, Hand Foot Mouth disease…)
  - Echoviruses
  - Other… (e.g. Enterovirus 71, human rhinoviruses, HAV)
- Symptoms – very different – conjunctivitis, hepangine, start of T1DM, exanthema, neonatal sepsis, pleurodynia…
- Encephalitis/myeloencephalitis
  - Prodromal symptoms - fever, chills, headache, photophobia and nuchal rigidity; rash and upper respiratory symptoms
  - fever and meningeal signs subside within 2-7 days
  - Most frequent - Coxsackievirus B, echoviruses
  - EV-71 particularly aggressive CNS infection



Hand Food & Mouth Disease

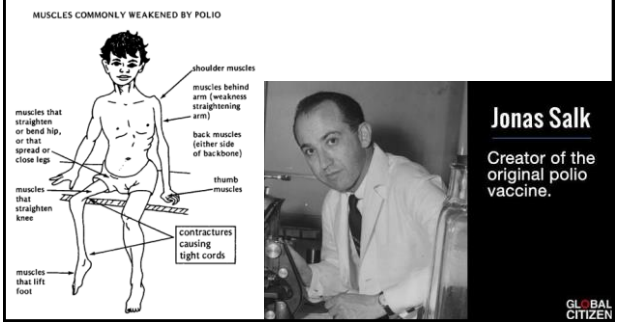
http://healthosphere.com/wp-content/uploads/2012/02/Hand-Foot-and-Mouth-Disease1.jpg

Picornaviridae – Enteroviruses - Polio

Through early morning fog I see, visions of the things to be,
the pains that are withheld for me, I realize and I can see...

Picornaviridae - Enteroviruses

- Salk vaccine - first tested in 1952 – injected inactivated (dead) poliovirus
- Sabine vaccine - oral attenuated poliovirus – trials began in 1957, licensed in 1962

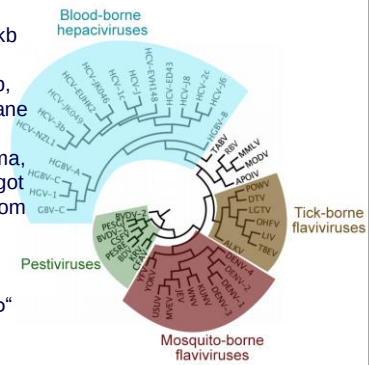


Picornaviridae - Enteroviruses

- Vaccines eradicated polio from most countries in the world, and reduced the worldwide incidence from an estimated 350,000 cases in 1988 to just 223 cases in 2012.
- In November 2013, the WHO announced a polio outbreak in Syria.

Flaviviridae

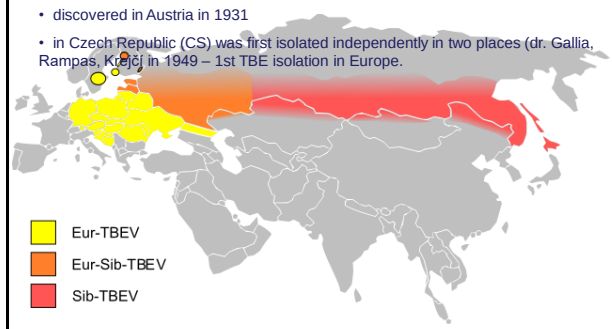
- avr. 40-60 nm
- ss (+)RNA approx. 11 kb
- virions 3 structural proteins – env. gp, core and membrane protein
- replication in cytoplasma, lipid envelope is got during budding from cytoplasmatic vesicules
- disease has often „two“ waves of clinical symptoms



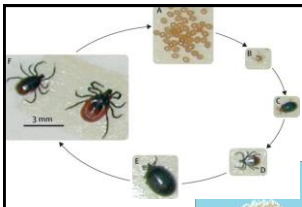
https://www.utmb.edu/discoveringdenguedrugs-together/images/Flaviviridae_adj_LG.jpg

Tick Borne Encephalitis – TBE
geographical distribution

- not west from Austria
- discovered in Austria in 1931
- in Czech Republic (CS) was first isolated independently in two places (dr. Gallia, Rampas, Křečí in 1949 – 1st TBE isolation in Europe.

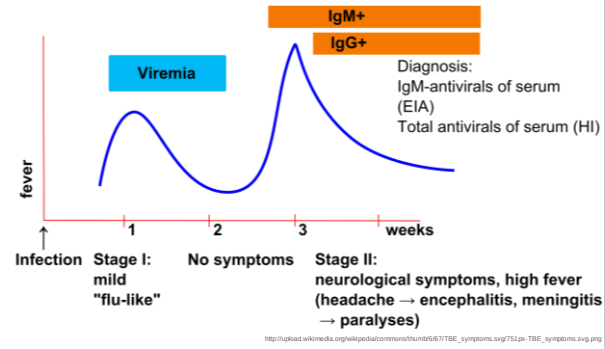


TBE
Vector



- in 1937 rusian scientest L. A. Zilber proved transmission with tick (in russian spring-summer encefalitis)

Tick Borne Encephalitis – TBE
symptoms and diagnosis

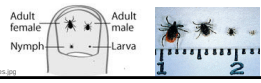


- Vaccination - inactivated virus

Tick Borne Encephalitis – TBE
symptoms

- 2/3 of infections asymptomatic
- Incubation period - 8 days (range 4–28 days)
- I: nonspecific febrile illness, headache, myalgia and fatigue. - Up to 2/3 of patients may recover without any further illness.
- II: CNS - aseptic meningitis, encephalitis, or myelitis. Disease severity increases with age.

- The European subtype - milder disease, a case-fatality ratio of <2%, and neurologic sequelae in up to 30% of patients.
- The Far Eastern subtype – often more severe disease course, a case-fatality ratio of 20%–40% and higher rates of severe neurologic sequelae.
- The Siberian subtype - more frequently chronic or progressive disease and has a case-fatality ratio of 2%–3%.



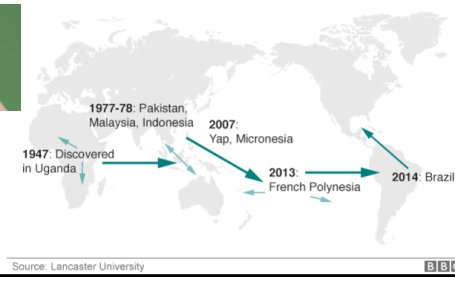
Vaccination - inactivated virus

Flaviviridae

Zika virus

- Described in apes (Makak rhesus) in Uganda during monitoring of the yellow fever in 1947.
- In humans described for the first time in Uganda and Tanzania in 1952 v Ugandě. Subsequently recognised in Africa, Asia, and Pacific (2007-2013) and America (2015 – Brazilia and Columbia).

How Zika virus spread from Africa

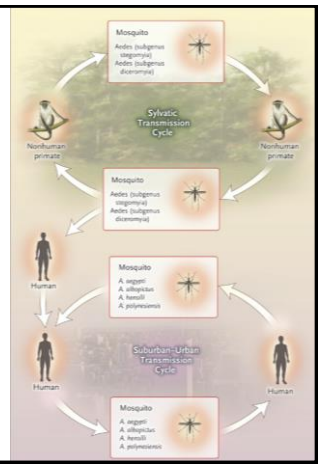


Flaviviridae

Zika virus

- Transmitted by mosquitos genus Aedes (especially A. aegypti) by blood.
- Transmission is described also by blood directly, perinatal transmission, amnionic fluid, CSF and sperm.

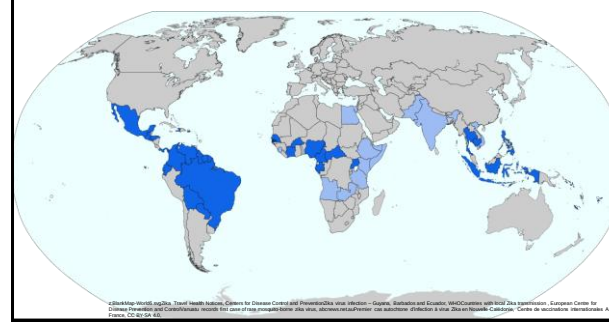
  (However, there are doubts about real presence of the virus in the sperm, or blood contamination).



Flaviviridae

Zika virus

- Incubation period 3-12 days
- Zika fever is presented with fever, conjunctivatis, rash, pain of muscles, joints, and head, malaise lasting for about 2-7 days.



Flaviviridae

Zika virus

Microcephaly was described in infection during pregnancy during outbreak in Brasil in 2015.

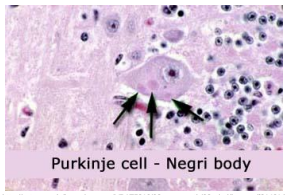
Risk of microcephaly in retrospective study from French polymesia
95 (34–191)/ 10 000 women +- 0,95%
In Brasil 29%.
(NEJM, Lancet 2016)

Described as causal pathogen in myelitis and Guillain–Barré syndrome.
(NEJM 2016)

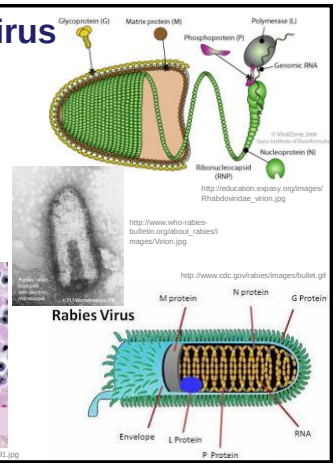
Rhabdoviridae

Lyssavirus

- ss (-) RNA; genome 11 kb
- enveloped
- 75 nm wide and 180 nm long
- cellular receptor: acetylcholine receptor
- Transmission: mainly from infected animals by saliva
- Clathrin mediated endocytosis
- Cytoplasmatic proliferation – **Negri bodies**



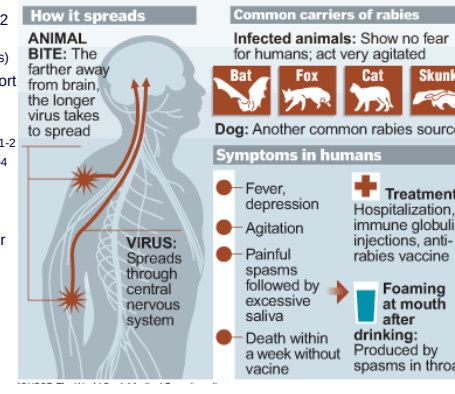
http://vet.uga.edu/vcm/courses/VPATS316/02_neuropath/09_viral/images/f21491.jpg



Rhabdoviridae

Lyssavirus - Rabies

- Incubation: av. 3-12 weeks
  (1 week to 15 months)
- Retrograde transport from periphery to CNS
- Prodromal phase (1-2 days), symptoms (3-4 days) after 5 days encephalitis and paralysis
- Encephalitis and/or myelitis
  (in fully developed 100%)

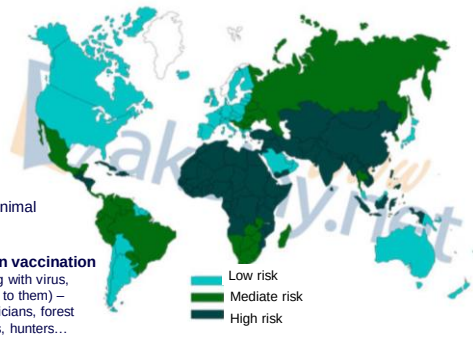


http://peterandmorrisonrabies.weebly.com/uploads/5/3/5/7/53574157/807037792.png

Rhabdoviridae

Lyssavirus - Rabies

Risk of the lyssavirus exposition in the world (WHO 2013)



- Prevention – animal vaccination
- **Pre-exposition vaccination** (persons working with virus, animals or close to them) – laboratory technicians, forest workers, rangers, hunters…
- **Post-exposition vaccination**

Zdroj: www.vakciny.net

Stool samples

Most frequent viral pathogens

- Astroviruses
- Norovirus
- Rotavirus
- Adenovirus

- And others
  - Enteroviruses
  - Influenza…

- „All“ are agens of watery diarrhea together with vomitting
- Incubation period 1-4 (9) days
- Lasting 2-8 days
- Highly infectious

  (norovirus 1-10 particles)

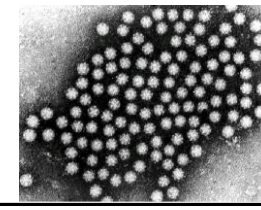
MAJOR ARTICLE

Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients

Julianne R. Brown,[1,2] Sofia Morfopoulou,[3] Jonathan Hubb,[4] Warren A. Emmett,[3] Winnie Ip,[5] Divya Shah,[2] Tony Brooks,[6] Simon M. L. Paine,[7,8] Glenn Anderson,[7] Alex Virasami,[2] C. Y. William Tong,[4] Duncan A. Clark,[4] Vincent Plagnol,[3] Thomas S. Jacques,[7,9] Waseem Qasim,[5] Mike Hubank,[6] and Judith Breuer[1,8]

[1]Virology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, [2]NIHR Biomedical Research Centre, Great Ormond Street Hospital for Children NHS Foundation Trust and University College London, [3]UCL Genetics Institute, University College London, [4]Virology Department, Barts Health NHS Trust, [5]Molecular and Cellular Immunology, [6]Molecular Haematology and Cancer Biology Unit, Institute of Child Health, University College London, [7]Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, [8]Department of Infection and Immunity, and [9]Birth Defects Research Centre, Institute of Child Health, University College London, United Kingdom

Neurotropic Pathogen HAstV VA1/HMO-C • CID 2015:60 (15 March) • 881



DNA viruses

Indicative disease for HIV re-classification to AIDS stage (WHO criteria):

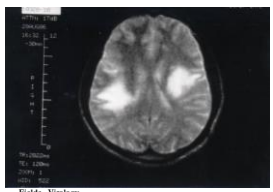
1. pneumocystis pneumonia
2. toxoplasma encefalitis
3. esofageal, tracheal, bronchial or lung candidiasis
4. **Chronic anal herpes simplex or herpetic bronchitis, pneumonia or esofagitis**
5. **CMV retinitis**
6. **generalized CMV infevtion (excluding liver and spleen)**
7. **progresive multifocal leukoencefalopatia**
8. repeating salmonela bacteriemia
9. repeating pneumonia within 1 year
10. chronic intestinal cryptosporidiosis
11. chronic intestinal isosporosa
12. extrapulmonary cryptococcus infection
13. Disseminated or extrapulmonary histoplasmosis
14. disseminated coccidioidomycosis
15. tuberkulosis
16. disseminated or extrapulmonary atypic mycobacteriosis
17. **Kaposhi sarkoma**
18. **malignant lymfoma (Burkitt‘s lymfoma, imunoblastic and primary cerebelar lymfoma)**
19. Invasi carcinoma of cervix
20. HIV encefalopatia
21. wasting syndrom

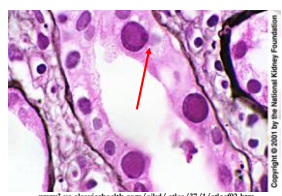
Polyomaviruses

- small ds DNA viruses with circular NA
- Capsid diameter 42-45 nm, genome: 5 kbp
- Transmission by fecal-oral route

- **JC virus** – progressive multifocal leukoencephalopaty PML
- **BK virus** – hemorrhagic cystitis, nephropathy (graft rejection in kidney transplant)
- **WUV and KIV** – respiratory infections
- **MCV** – Merkel cell carcinoma virus (rare skin carcinoma)
- **HPyV 7-12** (Human Polyomavirus) – mainly skin viruses

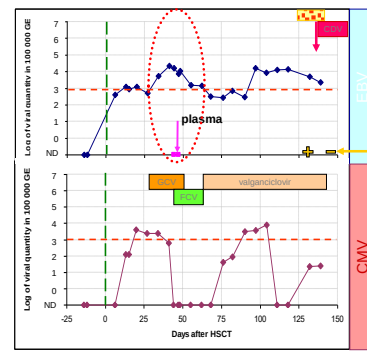
- Potencially treatable with **cidofovir**





Patient 4

BKV – haemorrhagic cystitis

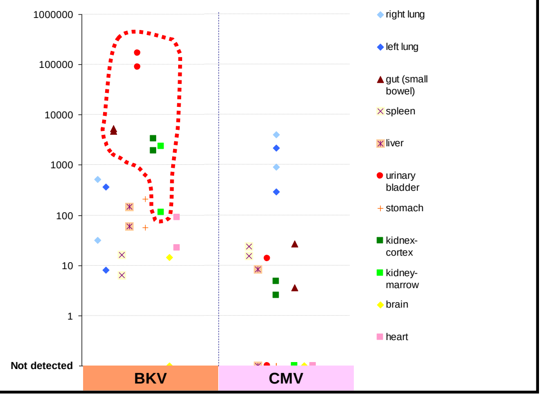


BKV - urine

Age at HSCT.:18 let, Fanconi anemia, MUD 9/10, BM, aGvHD grade I.

Normalised viral load in 10,000 g.e. of the tissue

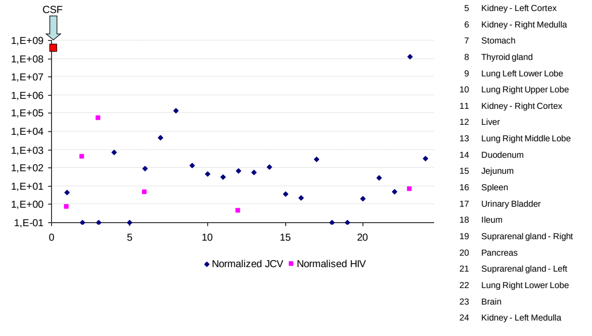
tissue specificity



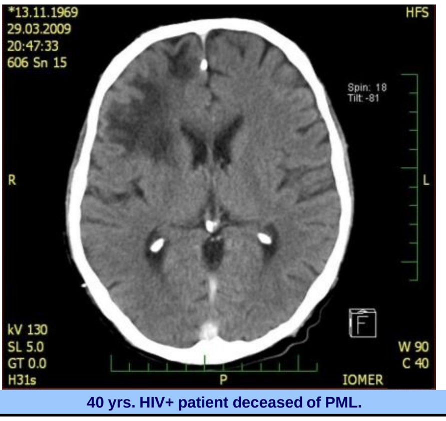
Patient 5

JCV

Pacient J.Z. (HIV+)


CSF
1,E+09
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
1,E-01
0
5
10
15
20
Normalized JCV
Normalised HIV
1 Colon
2 Lung Left Upper Lobe
3 Myocard
4 Testis
5 Kidney - Left Cortex
6 Kidney - Right Medulla
7 Stomach
8 Thyroid gland
9 Lung Left Lower Lobe
10 Lung Right Upper Lobe
11 Kidney - Right Cortex
12 Liver
13 Lung Right Middle Lobe
14 Duodenum
15 Jejunum
16 Spleen
17 Urinary Bladder
18 Ileum
19 Suprarenal gland - Right
20 Pancreas
21 Suprarenal gland - Left
22 Lung Right Lower Lobe
23 Brain
24 Kidney - Left Medulla

JCV



40 yrs. HIV+ patient deceased of PML.

Papilomavires

- ds DNA virus
- DNA lenght approx. 8 kb
- > 100 serotypes
- causing – warts
  - Condylomata accuminata
  - Epitelial carcinoma
    - cervix
    - larynx
    - penis …
- genital warts around 30 types
- most of the people gets infected in first 2-3 years of sexual activity (2/3 within 1st 3 months)


Common Wart
Common on hand and knuckles but may appear anywhere
Plantar Wart
On soles heel or toes of feet
Flat Wart
Usually on leg hand face
Filiform Wart
Usually on face near eyelids lips
Mosaic Wart
Group of tightly clustered wart
Periungual Wart
Wart around finger nail toe nail
Oral Wart
Within oral cavity
Genital Wart
Located near or in genital areas

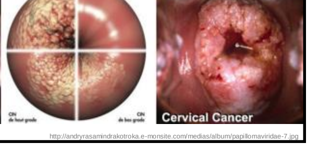
http://www.healthyeatingandyou.com/wp-content/uploads/2016/02/types-of-warts.jpg

Papilomaviruses

HPV-LR low risk
6, 11, 40, 42, 43, 44, 54, 61, 70, 72 a 82
(condylomata accuminata, ca.)
- 2-8 months after infection is neccesary for lesion development on 50% of infected women
- non-oncogenic
- devected ussually around 25 yrs.

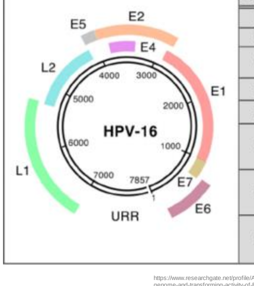
HPV-HR high risk
High risk: 16, 18, 31, 33, 35, 39, 45,51, 52, 56 a 86
- unifocal lesion (CIN 1–3 a ca.)
- transmission by sex. contact
- highly protective specific immunity
- in 35 years (CIN 3) and 45 yrs. (ca.)
- CIN 3 after 18M-5 yrs. after infection
- 80–90 % of women eliminate virus spontanuously within 8–16 months
- from 10–20 % of women with lasting infection :
  - 20 % develops CIN 3 within 5 yrs.
  - 5 % develops ca. until 15–20 yrs.
  (in women with regular preventive testing only 1 % really develops ca.)

http://images.medicinenet.com/images/slideshow/stds-s1-photo-of-genital-warts.jpg



http://andryrasamindrakotroka.e-monsite.com/medias/album/papillomaviridae-7.jpg

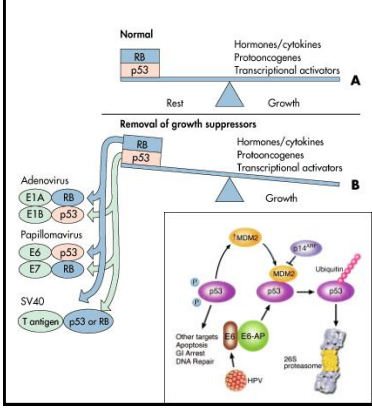
Papilomaviruses – genome

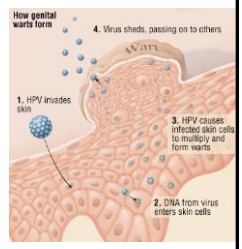


Gene	Function
L1	Major capsid protein.
L2	Minor capsid protein.
E1	Transcription factor, helicase activity. Mediates episomal DNA replication.
E2	Transcription factor. Regulates viral copy number.
E4	Facilitates virion release.
E5	Stimulates cell proliferation and prevents differentiation. Downregulates surface MHC class I expression.
E6	Deregulates cell cycle control through p53 inactivation/degradation. Induces malignant transformation together with E7.
E7	Keeps cells active in the cell cycle through Rb inactivation. Induces malignant transformation alone and together with E6.

https://www.researchgate.net/profile/Angelika_Riemer/publication/45113419/figure/fig1/AS:307360930254856@1450291964254/FIGURE-1-HPV-16-genome-and-transforming-activity-of-E6-and-E7-The-left-panel-shows-the.png

Papilomaviruses – oncogenic potential





Ubiquitinilation and proteolysis

HPV 16 a 18

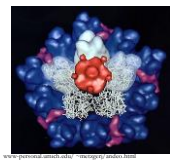
Causes up to:

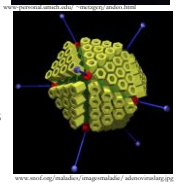
- 70% of cervical carcinoma
- 80% rectal ca.
- 60% ca. of vaginy
- 40% ca. of vulva
- 90% of genital warts

- HPV is most frequently transmitted STD in MSW adults
  (> 80% of american women got at least 1 HPV typ at the age of 50)
- 529,000 of new cervical ca. cases and 275,000 deaths/year
- **VACCINATION!!!**
  HPV vaccines: Gardasil(Silgard)
  Cervarix

Adenoviruses

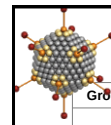
- non enveloped ds DNA viruses
  ikosahedral structure70-75 nm
  genome: 35 kbp
  according to similarity– 7 subgenes A-G
  accorging to antigenic specificity– more than 60 serotypes
- **Acute faryngitis, Faryngoconjunctivitis, Acute respiratory tract infection, Pneumonia, Acute hemorrhagic cystitis, Keratokonjunktivitis, Pertussis-like sy., Hepatitis, Gastroenteritis, Meningoencefalitis, Myokarditis**
- Persistence in BMT, patients with immunodeficiencies or immunosupression – in colon, and urinary tract





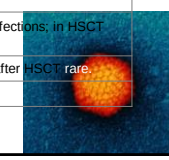
www.snof.org/maladies/imagesmaladie/adenovirusfarg.jpg

Serotypes

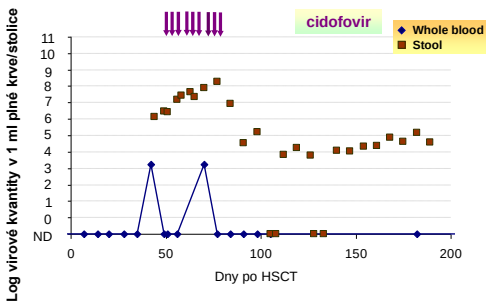


Group	Serotypes	Localisation of the infection
A	12, 18, **31**	Respiratory, urinary, GIT infections and CNS infections; in HSCT patients rare.
B	3, 7, 11, 14, 16, 21, 34, 35, 50	Respiratory, eye, urinary, GIT and CNS infections.
C	**1, 2, 5, 6**	**Respiratory, urinary and GIT infections – hepatitis too.**
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51	Eye, GIT and CNS infections; in HSCT patients rare.
E	4	Eye and respiratory tract infections; in HSCT patients rare.
F	41	GIT infections; in patients after HSCT rare.
G	52	GIT infections.

Rozdělení adenovirových infekcí do skupin (upraveno dle Fields Virology 5th edition, Kapitola 63).

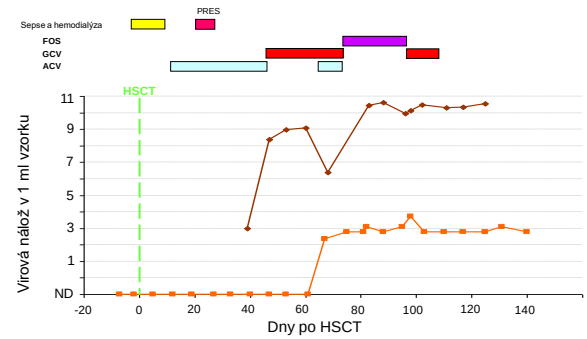


Patient 6

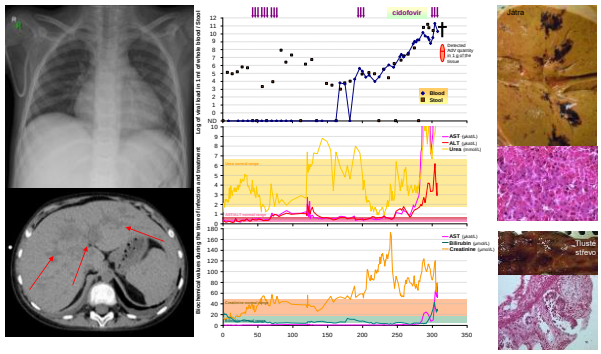


Dívka Věk při HSCT: 1 rok Dg.: ALL v CR2 Štěp: CB (5/6)
Příprava: busulfan, cyklofosfamid, melfalan a ATG Přihojení D+25.
GvHD grade II (GIT1, kůže 3) léčená kortikoidy.
Kompletní chiméra ode D+14.

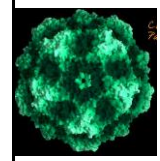
Patient 7



Patient 8

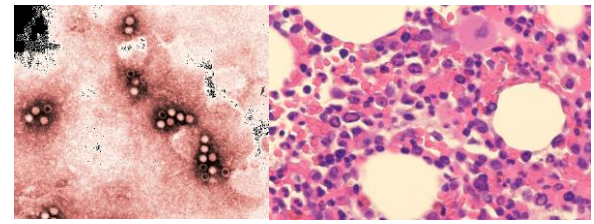


Parvovirus B19



- small non-enveloped ss DNA +/-
- capsida in diameter 20-26 nm, genome: 5 kbp
- prolipheration in erythroid progenitors – transient stop of erythrocyte production and so it leads in certain clinical situations (e.g. Hereditary erythropoiesis disorders) to anaemia.
-
- E.g. aplastic crises, Bone marrow aplasia, teratogenicity-hydrops foetalis…
- ***Fiths exanthematic disease*** (see lecture)

http://fai.unne.edu.ar/biologia/virologia/images/virulo6.jpg



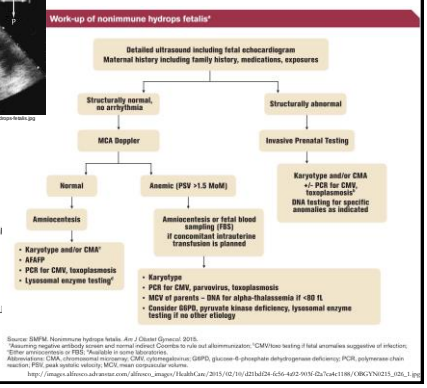
http://www.wadeworth.org/databank/hiroz/gradp2.gif

http://www.yamagiku.co.jp/pathology/image/210/1.jpg

Parvovirus B19

http://www.parsefetalmedicine.com/wp-content/uploads/2014/01/nonimmune-hydrops-fetalis.jpg

http://www.tibbijeardim.com/wp-content/uploads/non-immune-hydrops-fetalis.jpg

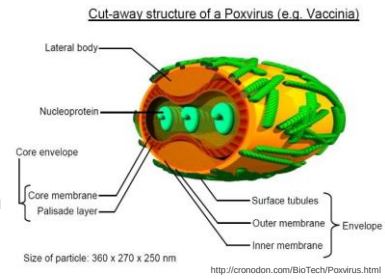


Source: SMFM. Nonimmune hydrops fetalis. *Am J Obstet Gynecol.* 2015.
*Assuming negative antibody screen and normal indirect Coombs to rule out alloimmunization; †CMV/toxo testing if fetal anomalies suggestive of infection; ‡Either amniocentesis or FBS; §Available in some laboratories.
Abbreviations: CMA, chromosomal microarray; CMV, cytomegalovirus; G6PD, glucose-6-phosphate dehydrogenase deficiency; PCR, polymerase chain reaction; PSV, peak systolic velocity; MCV, mean corpuscular volume.

http://images.alfresco.advanstar.com/alfresco_images/HealthCare/2015/02/10/d2f1bd24-fc56-4a02-915f-f2a7ea4c1188/OBGYN0215_f26_1.jpg

Poxviry

- Complex structure (symetria)
- Enveloped but resistant to inactivation
- linear ds DNA
- Genome 130–375 kb coding approx. 250 genes (>100 polypeptides-often immunogenic)
- Replication in cytoplasma
- Highly species specific
- Used for genome vector constructions
- Human pathology is associated with 4 genera:
  - Orthopoxvirus
  - Parapoxvirus
  - Yatapoxvirus
  - Molluscipoxvirus



http://cronodon.com/BioTech/Poxvirus.html

Orthopoxvirus

- Variola virus
  - Variola major (mortality 20%), variola minor (mortality 1-2%)
  - Eradicated (last diagnosed in 1977)
  - All eruptions in same status of development
  - Primary replication in air-ways
- Vaccinia virus (used for vaccination and eradication of variola)
- Cow pox virus (first vaccination against variola – Edward Jenner – 1796)

http://www.smithsonianmag.com/ist/?next=/smart-news/queen-elizabeth-1-loved-live-

http://www.wikihealth.com/wp-content/uploads/2014/07/rsz_smallpox.jpg

Parapoxvirus

- Zoonosis
- Human infections causes
  - Bovine papular stomatitis virus
  - Orf virus
  - Pseudocowpox virus
- Aftous eruptions on mucous and/or skin
  Clinically called
  -“farmyard pox“

Orf (Ecthyma contagiosum)

- **C/P:**
- Typically presents as a papule/nodule on the dorsal index finger.
- ***Progression through several stages:***
  - maculopapular
  - targetoid
  - weeping nodule
  - regenerative dry stage with black dots
  - papillomatosis
  - regression with a dry crust
- ***Other Findings;*** Ascending *lymphangitis, lymphadenopathy, malaise*, and *fever* may occur.
- Bacterial superinfection may occur.
- Erythema multiforme occasionally occurs 10 to 14 ds. later

http://www.slideshare.net/HimaFarag/viral-diseases-of-the-skin-other

Yatapoxvirus

- Yaba monkey pox virus
  - Oncogenic virus – histiocytomas (tumour from macrophages) in humans and monkeys (e.g. *Macaca fascicularis*)
  - Presence by the river Niger

https://upload.wikimedia.org/wikipedia/commons/9/9f/Macaca_fascicularis.jpg

https://en.wikipedia.org/wiki/Monkeypox_virus#/media/File:Monkeypox.gif

Molluscipoxvirus

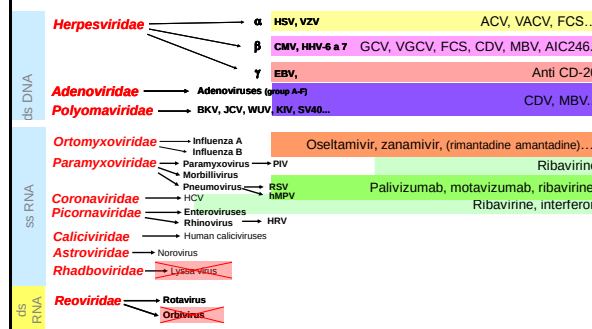
- Molluscum contagiosum
  - Viral infection of skin, rarely mucous membranes
  - Charakteristic skin lessions
  - Infection of human, primate and kangaroos
- 4 types
- Often STD (MCV 1,2)
- Incubation period – up to months

http://www.dermapics.com/molluscum%20contagiosum.html

http://www.molluscumrx.com/molluscum-contagiosum-pictures/

Therapeutical possibilities of virostatics and specific antibodies

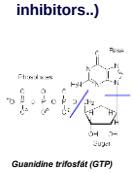
More or less specific for certain viral groups:



Virostatic drugs impact

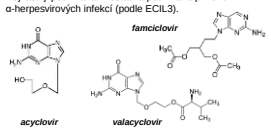
Virostatics

usually cellular nucleotides analogues blocking (more or less specifically) viral polymerase (acyklovir, ganciklovir, cidofovir…), or polymerase directly blocking drugs without similarity to nucleosides (e.g. foscarnet) or viral protein blocking drugs (neuraminidase inhibitors..)

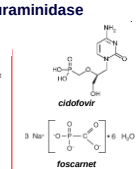


Guanidine trifosfát (GTP)

Nejčastěji používaná virostatika používaná při léčbě α-herpesvirových infekcí (podle ECIL3).



Léky první volby.



Léky používané při HSV rezistentní k léčbě.

Antibodies with virostatic effect

Neutralising antibodies against certain proteins important in pathogenesis of viral disease (F protein in RSV) or aimed against target cells (anti-CD20 in EBV).

Anti CD-20

Dosing of most frequently used virostatic drugs

- **acyclovir** (HSV, VZV)
  - ***Prophylactical dosing*** – 500 mg/m^2/dose in infusion for 60 minut twice daily with maximum 750 mg/dose
  - ***Therapeutical dosing*** – for 7–10 days
    250 mg/m^2/dose in infusion for 60 minutes á 8 hours with maximum of 500 mg/dose (resp. 10-15 mg/kg/dose)
- **ganciclovir** (CMV, HHV-6, HHV-7)
  - ***Therapeutical dosing*** – at least 3 weeks
    2 weeks 5 mg/kg/dose in infusion for 60 min á 12 hours, 2 týdny; subsequently 5 mg/kg/dose in infusion for 60 min/ day
- **foscarnet** (CMV, HHV-6, HHV-7, HSV, VZV)
  - ***Therapeutical dosing*** – for 3 weeks
    60 mg/kg/dose in infusion for 60 min (or i.v.) á 12 hours, 1- 2 weeks; subsequently 90 mg/kg/dose in infusion for 60 min (or i.v.) á 24 hours
- **cidofovir** (CMV, HHV-6, HHV-7, HSV, VZV, adenoviruses, BKV, …)
  - In case of CMV disease 5 mg/kg/dose in infusion (1/1 fysiological solution) 1x week
- **oseltamivir** (Influenza)
  - ***Prophylactical dosing*** - 30-60 mg in children younger 12 yrs. according to the weight (>15 kg - 30 mg, 15 to 23 kg - 45 mg, 23 to 40 kg – 60 mg), in patients older 13 yrs. and heavier 40 kg then 75 mg for at least 10 dní.
  - ***Therapeutical dosing*** – at least 10 days in children and adults; dvojnásobek prophylactic dosing – in adults 75 mg 2x day, in very severe cases 150 mg 2x day.

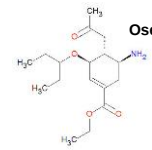
Adverse effects of the virostatic drugs

- **Acyclovir/valaciclovir**
  - ***AE usually reversible***, usually in patients with hepatopathy.
  - rarely haematopoietic and lymphatic system disorders (anaemia, leucopenia, thrombocytopenia), hepatitis, nefrotoxicity.
- **Ganciclovir/valganciclovir**
  - ***myelosupressive effects*** (neutropenia (25–40 %), thrombocytopenia (9-20 %
  - nauzea, vomiting and diarrhea, increase of the liver enzymes: confusion and seizures; renal insufficiency (rarely in patients after heart tx.); enormously rare exanthema or eosinophilia
- **Foscarnet**
  - ***Nephrotoxicity***- rarely acute renal failure (uremia and polyuria), potencially metabolic acidosis and diabetes insipidus
  - Increase of the liver enzymes, LDH, ALP and amylasis; often nauzea, vomitting nad diarrhea, rash (exanthema), tremor, muscle weakness and increase in body temperature, thrombocytopenia, hypokalemia, hypomagnezemia, hypo- or hyperfosfataemia, ***hypocalcemia*** (shortly after infusion or tonic-clonic seizures) – increased risk in CNS disorder or ciprofloxacine administration
  - Headache, tiredness, paresthesia, tremor, ataxia. Neuropathy, hypestazia, confusion, depression, psychosis, agressive reactions, psychosis, agressive reactions; changes in ECG, hyper- hypotension, rarely even chamber arythmias
  - Often Phlebitis (thrombophlebitis) in addministration of concentrated solutions (> 12 mg/ml) to peripheral vein.

Adverse effects of the virostatic drugs

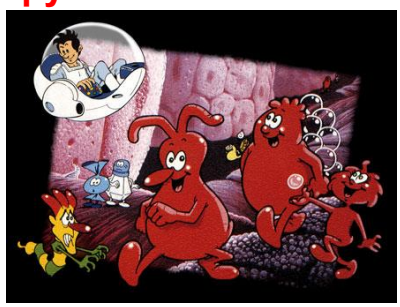
- **Cidofovir**
  - ***nephrotoxicity*** – proteinuria, creatinine increase; acute and even with delay; - good hydration, together with probenecid
  - potencially to ***chronic renal failure*** with dialysis
  - other more common neutropenia, headache, nauzea, vomitting, alopecia, rash, weakness and fever. Described also occular toxicity.
- **Oseltamivir**
  - most frequent AE are nausea, vomitting and belly pain
- **Ribavirine**
  - ***Haematopoietic disorders, depression, teratogenic effect (inhalation)*** from that reason there must not be exponed men or women about the conception. In case of higher cumulative dose risk of teratogenicity lasts for months; nausea, pain in belly….

Oseltamivir



Ribavirine

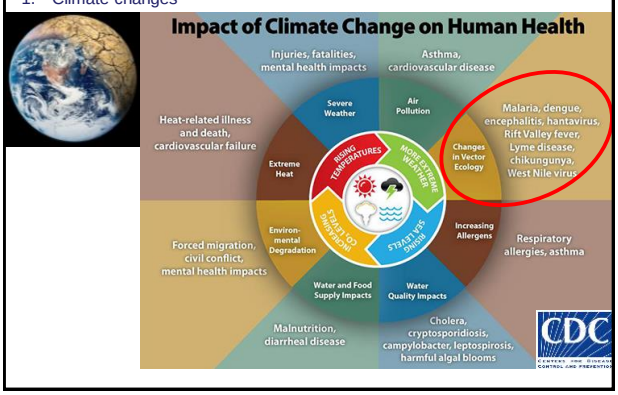
However – for success of the therapy is still crucial …



… reconstitution of immunity!

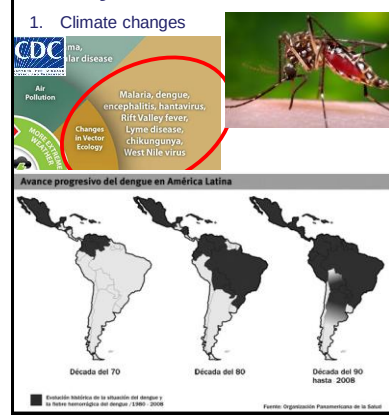
Why we observe emerging viruses?

1. Climate changes



Why we observe emerging viruses?

1. Climate changes

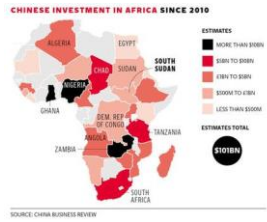


Ismaili virus, BFV
Eastern equine encephalitis virus, EEEV
Middelburg virus, MIDV
Ndumu virus, NDUV
Bebaru virus, BEBV[3]
Chikungunya virus, CHIKV[3]
Mayaro virus (–Una virus), MAYV–UNAV[3]
O'nyong'nyong virus, ONNV[3]
Ross River Virus, RRV[3]
Semliki forest virus, SFV[3]
Venezuelan Equine Encephalitis virus, VEEV[4]
Cabassou virus, CABV[4]
Everglades virus, EVEV[4]
Mosso das Pedras virus, MDPV[4]
Mucambo virus, MUCV[4]
Rio Negro virus (RNV)[4]
Western Equine Encephalitis Virus, WEEV[5]
Aura Virus, AURAV[6]
Sindbis Virus ,SINV[6]
Babanki Virus, SINV–B[2]
Kyzylagach virus, SINV–K[2]
Ockelbo Virus, SINV–O[2]
Whataroa virus, WHAV[6]
Highlands J virus, HJV[6]
Buggy Creek Virus, BCV[6]
Fort Morgan Virus, FMV[6]
Tonate virus, TONV

Why we observe emerging viruses?

2. Changes in human behaving and travelling

- E.g. expansion of Peoples Republic China activities in Africa



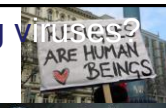
- Fly time

  Amsterdam – Sydney shortest trip 27 hours and 20 minutes – less then 2 days…

- ….

Why we observe emerging viruses?

2. Changes in behaving of the people and travelling



Refugees crisis Epidemiological diseases

- Vaccination absence, or low frequency vaccination

Polio outbreak in the Middle East - update

Ongoing transmission in the Syrian Arab Republic with international spread

As of 20 March 2014, in the Syrian Arab Republic a total of 37 WPV1 cases have been reported: 25 cases by the Syrian Arab Republic Ministry of Health, and 12 cases from contested areas (Aleppo, Edleb and Deir Al Zour) not yet reflected in official figures. The most recent case had onset of paralysis on 17 December 2013, from Edleb.

Circulating vaccine-derived poliovirus – Lao People's Democratic Republic (29.1.2014)
Circulating vaccine-derived poliovirus – Myanmar (23.12.2013)
Circulating vaccine-derived poliovirus – Lao People's Democratic Republic (15.12.2006, 26.11.2004, 12.10.2010)
Circulating vaccine-derived poliovirus – Ukraine (1.8.2013)
Poliovirus in Madagascar (9.7.2019)
Poliovirus in South Sudan and Madagascar (14.11.2014)
Poliovirus in Cameroon – update (6.9.2013)
Update on polio in Equatorial Guinea (17.7.2014)
Update on polio in central Africa (26.7.2014)
Detection of poliovirus in sewage, Brazil (23.6.2014)
Update on polio in central Africa - polio confirmed in Equatorial Guinea, linked to outbreak in Cameroon (17.4.2014)

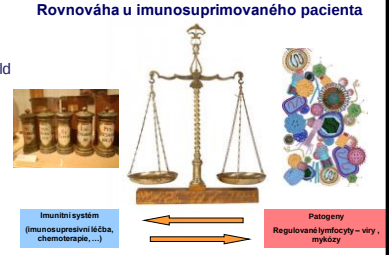
Why we observe emerging viruses?

3. More immunosupression

- from 2008 WHO recognized 100 800 solid organ transplants in 104 countries per year (approx. 90% world population).
  - 69 400 kidney (46% from living donors)
  - 20 200 liver (14.6% from living donors)
  - 5 400 heart
  - 3 400 lungs
  - 2400 pancreas

  Approx. 110 000 HSCT per year.
- More monoclonal antibodies (anti-CD20, CD52, TNF-α…) …

Rovnováha u imunosuprimovaného pacienta



Imunitní systém (imunosupresivní léčba, chemoterapie, …)

Patogeny
Regulované lymfocyty – viry , mykozy

Steroids more then > 2 mg/kg – highly lymphotoxic (used e.g. in NHL, ALL…)

Why we observe emerging viruses?

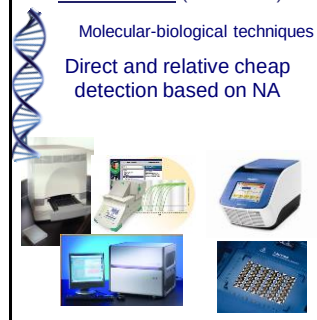
4. Better detection (even in new) – treatment – resistance

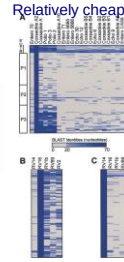
Molecular-biological techniques

Direct and relative cheap detection based on NA

Reasonable time for detection of the agens

Relatively cheap detection of new viruses

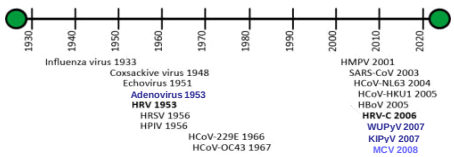


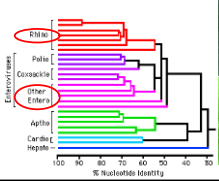


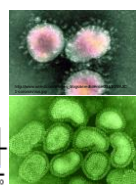
CHIP technique was used in new WUV and KIV polyomavirus detection in 2007, which were detected in respiratory tract.

Why we observe emerging viruses?

4. Better detection – treatment – resistance



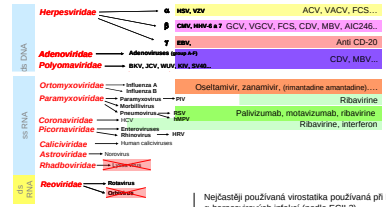




Why to act?

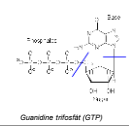
4. Better detction – treatment – resistance

Virostatic therapy



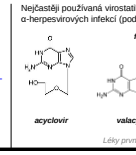
brincidofovir (CMX001)
famciclovir
penciclovir
boceprevir
telaprevir
sofosbuvir
simeprevir
ledipasvir
….. and more

Nejčastěji používaná virostatika používaná při léčbě α-herpesvirových infekcí (podle ECIL3).



Guanidine trifosfát (GTP)

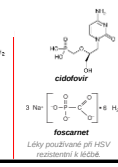
famciclovir



acyclovir

valacyclovir

Léky první volby.

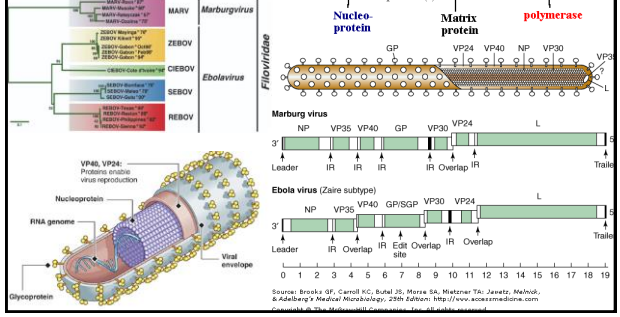


Léky používané při HSV rezistentní k léčbě

BioSafety Level 4

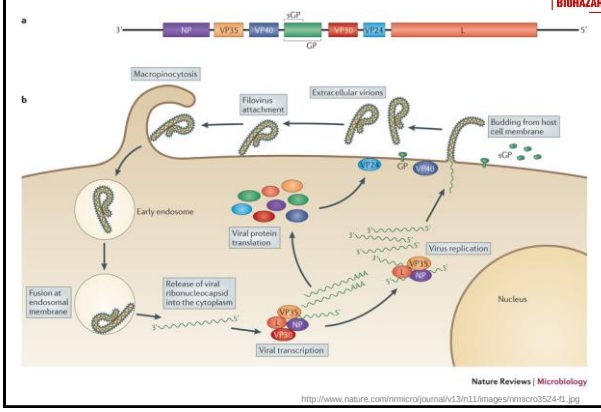
Filoviridae

- ss (-) RNA
- Helical nucleoprotein 13-20 nm wide
- Ebolavirus and Marburg virus
- highly infectious 1-10 virions
- **High mortality**



BioSafety Level 4

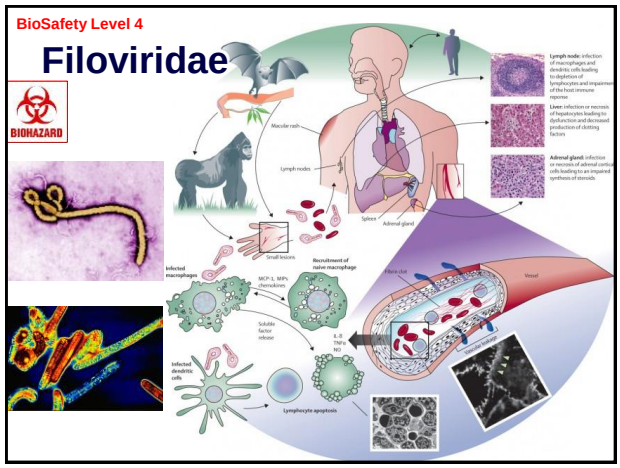
Filoviridae



http://www.nature.com/nrmicro/journal/v13/n11/images/nrmicro3524-f1.jpg

BioSafety Level 4

Filoviridae



BioSafety Level 4

Filoviridae

Ebola virus disease

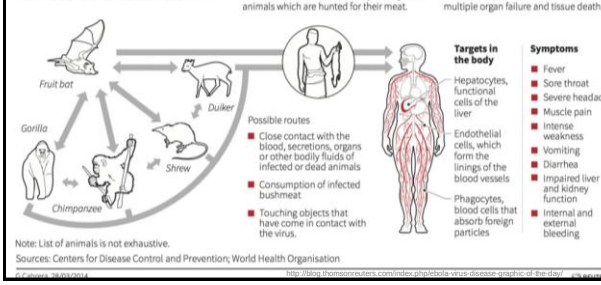
Mortality rate 25-90%

Ebola, which first appeared in outbreaks in Sudan and DR Congo in 1976, is a severe and often fatal disease with no known specific treatment or vaccine. It has since killed more than 1,500 people in parts of Africa.

SOURCE
In Africa, particular species of fruit bats are considered possible natural hosts for Ebola virus.

TRANSMISSION
Infected bats are thought to transmit the disease to humans, or indirectly through other animals which are hunted for their meat.

DAMAGE
Incubation period is from two to 21 days. Death from the disease is often caused by multiple organ failure and tissue death.



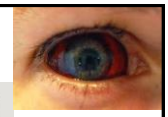
Note: List of animals is not exhaustive.

Sources: Centers for Disease Control and Prevention; World Health Organisation

http://blog.thomsonreuters.com/index.php/ebola-virus-disease-graphic-of-the-day/

BioSafety Level 4

Filoviridae



SYMPTOMS
- Early stages
- Advanced stages
- Headache
- Fever
- Fatigue
- Bleeding from eyes, nose and mouth
- Muscle pain
- Sore throat
- Impaired liver and kidney
- Diarrhoea
- Vomiting
- Rash
- Internal and external bleeding

Preventative measures
- Stop contact with infected animals and the consumption of their meat
- Isolate the sick
- Prompt disposal of victims' bodies
- Disinfect homes of dead and infected
- Protective clothing for healthcare workers

Source: WHO

Therapy:

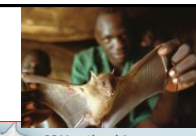
study only

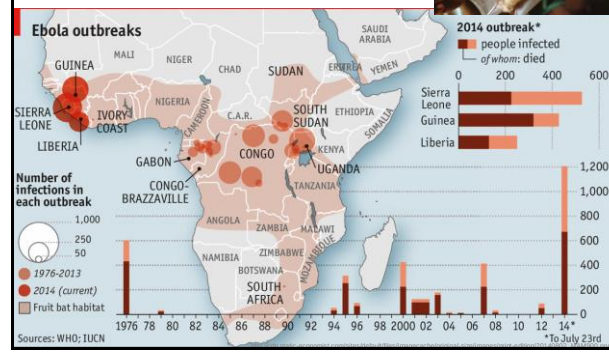
ZMapp – 3 Ab

at the moment not available!!!

BioSafety Level 4

Filoviridae





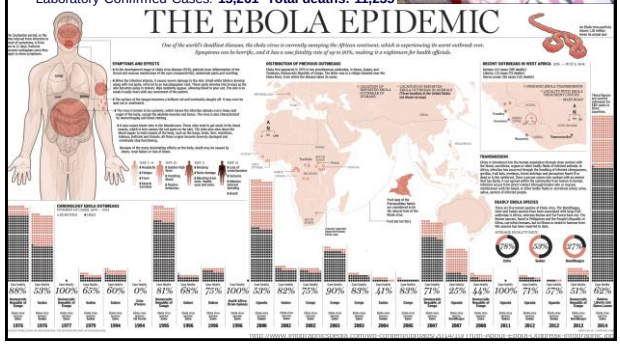
BioSafety Level 4

Filoviridae

2014 EBoV in West Africa (13th April 2016)

-Ebola outbreak: Total Cases: **28,652**

Laboratory-Confirmed Cases: **15,261** **Total deaths: 11,235**



BioSafety Level 4

Filoviridae

- Double gloves
- Boot covers that are waterproof and go to at least mid-calf or leg covers
- Single use fluid resistant or imperable gown that extends to at least mid-calf or coverall without intergraded hood.
- Respirators, including either N95 respirators or powered air purifying respirator (PAPR)
- Single-use, full-face shield that is disposable
- Surgical hoods to ensure complete coverage of the head and neck
- Apron that is waterproof and covers the torso to the level of the mid-calf should be used if Ebola patients have vomiting or diarrhea

Viral exanthematic diseases

Childhood exanthema diseases

Classical name	„systematic exant. name“	Pathogen
Measles (rubeola)	1st childhood disease	morbillivirus
Scarlet fever	2nd childhood disease	Streptococcus pyogenes
Rubella (German measles)	3rd childhood disease	Rubivirus
Filatov-Duke‘s disease (pseudoscarlantina)	4th childhood disease	Coxackie and Echoviruses
Erythema infectiosum	5th childhood disease	Parvovirus B19
Exanthema subitum – Roseola infantum	6th childhood disease	HHV-6 and HHV-7

Chicken pox - VZV