Arboviruse
Smallpox
Adenoviruses

By Dr. Ekram Abd El Rahman

Arthropod borne and rodent borne viruses

- **Arboviruses** = arthropod-borne viruses

viral diseases are transmitted by the bite of an arthropod vector.

- **Roboviruses =** Rodent-borne viruses

Are transmitted directly from rodents to humans without an arthropod vector by contact with rodent's body fluids or inhalation of their dry excreta. Major rodent-borne viral diseases are; Hantavirus infections and lassa fever, these cause haemorrhagic fevers.

Commenest causes of haemorrhagic fevers

Arboviruses:

yellow fever, dengue fever and Rift Valley Fever

Roboviruses:

Hantavirus infections and lassa fever

Filoviruses:

Marburg and Ebola viruses

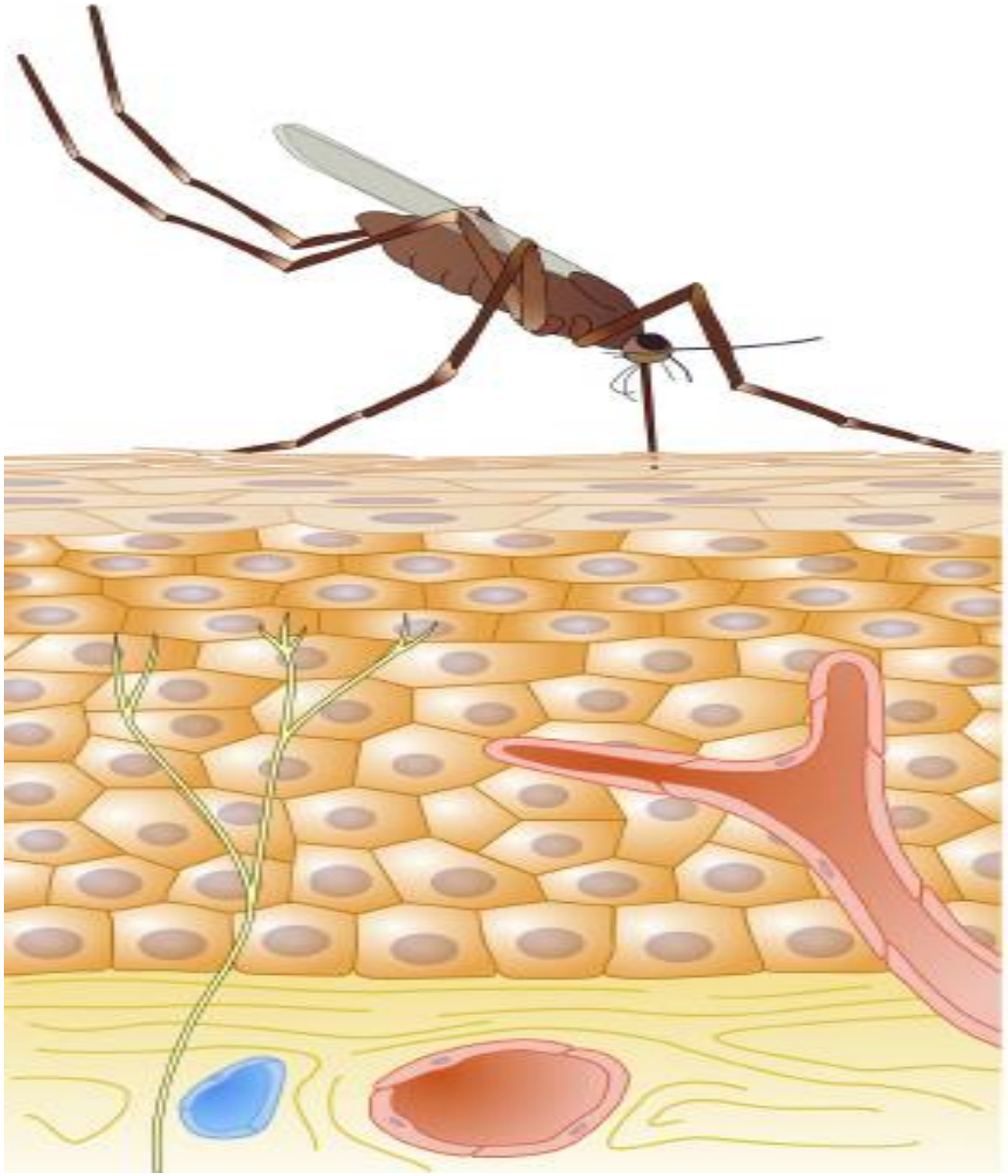
Arthropod born viruses (Arboviruses)

- Arboviruses are extremely numerous and include many unrelated viruses belonging to different genera, some of which are mentioned below:

Family	Genus	Important members	Characters
Togaviridae	Genus alphavirus (About 30 viruses).	*Mosquito-borne encephalitis causing viruses e.g. Eastern, Western and Venezuelan equine encephalitis viruses and Sindbis virus.	*Icosahedral, enveloped (+) ss RNA viruses. * All alphavirus are serologically related (cross reaction).

Family	Genus	members	Characters
Flaviviridae (About 70 viruses).	Genus flaviviruses	Tick-borne: (encephalitis viruses) *Brazilian encephalitis virus *Japanese B encephalitis virus *Mosquito-borne: *Yellow fever virus. *Dengue fever virus *West Nile fever virus	*Icosahedral, enveloped (+) ss RNA viruses. *all flaviviridae are serologically related (cross reaction).

Family	Genus	Important members	Characters
Bunyaviridae	Genus phlebovirus	*Sandfly fever virus *Rift Valley fever virus.	Helical, enveloped (-) ssRNA viruses



West Nile Virus Host Factors

Mosquito Factors

Pro-viral:	CPIJ010995
	AAEL000563
	AAEL013105
Anti-viral:	CQG12A2
	AAEL003012
	AAEL011045
	AAEL001704
	AAEL001022
	AAEL003881

Mammalian Factors

Anti-viral:	RIG-I
	MDA5
	PKR
	RNaseL
	IFNα
	CXCL10
	CCL2
	IFITM
	MCT4
Pro-viral:	eEF1A
	vATPase
	c-Yes
	HMGCR
	TIAR
	CBLL1
	ERAD
	UBE21

Tick

Pathogenesis of arboviruses infections:

- Arboviruses are infectious agents transmitted by blood-sucking arthropods e.g. mosquitoes, ticks, or sand flies. Arthropods become infected by feeding on blood of animals or man during the viraemic stage. The virus multiplies in the body of the insect without causing disease and is excreted in the saliva whereby the insect becomes infective. Various animals, rodents and birds act as reservoirs of infection.

Diseases caused by arboviruses:

- The clinical picture varies, but usually present in one or more of the following pictures:
- **1-** Encephalitis which is fatal.
- **2-** Hemorrhagic fevers, frequently severe and fatal.
- **3-** Fever with myalgias, arthralgias, and non-hemorrhagic rash. Lymphadenopathy may occur in some cases.

Diagnosis of diseases caused by arboviruses:

- **A- Non specific laboratory tests:**
- Disturbed liver function tests due to liver invasion.
- Albuminuria due to renal invasion.
- Hematological disorders due to bone marrow invasion.

- **B- Specific laboratory tests:**
- 1- PCR assays are available for direct detection of viral RNA in clinical specimens for some arboviruses.

- 2- Serologic diagnosis by ELISA for detection of specific IgM or a rising titer of IgG has largely replaced the classic methods, i.e. haemagglutination inhibition, complement fixation and neutralization of virus infectivity.

- 3- The use of virus specific monoclonal antibodies in IF assays to allow rapid virus identification in clinical samples.

- 4- Isolation of the virus (difficult and requires appropriate safety precautions) from the blood during the acute phase or from CSF, skin, and tissue biopsy depending on the agent. Several cell lines are used including mosquito cell lines and intracerebral inoculation of suckling mice.

Arbovirus encephalitis

- Different encephalitis arboviruses in the togavirus and flavivirus groups are endemic in many parts of the world. In Egypt, two such viruses exist; the West Nile virus and the Sindbis virus which are transmitted by mosquitoes. The reservoir of infection is various animals, rodents and birds.

- **Clinical pictures:**
- fever, which is associated with generalized lymphadenopathy and maculopapular rash. Most infections are subclinical without affection of CNS.
- Invasion of CNS leads to disseminated encephalitis, meningitis and myelitis. West Nile and Sindbis viruses cause mild meningitic disease.

Yellow Fever

- It is an acute febrile life threating, mosquito-borne disease characterized by fever, jaundice, hemorrhages and albuminuria.
- Caused by a flavivirus (icosahedral, enveloped, + ss RNA virus).

- **Epidemiology:**
- Yellow fever has 2 distinct cycles:
- **1-Jungle yellow fever (a disease of monkey)**
- **2-Urban yellow fever (a disease of man)**

- **1-Jungle yellow fever (a disease of monkey):**
- The disease is endemic in equatorial countries of Africa and South America. The main reservoir is monkey. Monkey to monkey transmission occurs by the mosquito *Aedes africanus* in Africa and Haemagogus in South America. Man becomes infected if he visits the jungle for cutting trees, picking nuts, road constructing or hunting.

the mosquito *Aedes africanus*

the mosquito Haemagogus

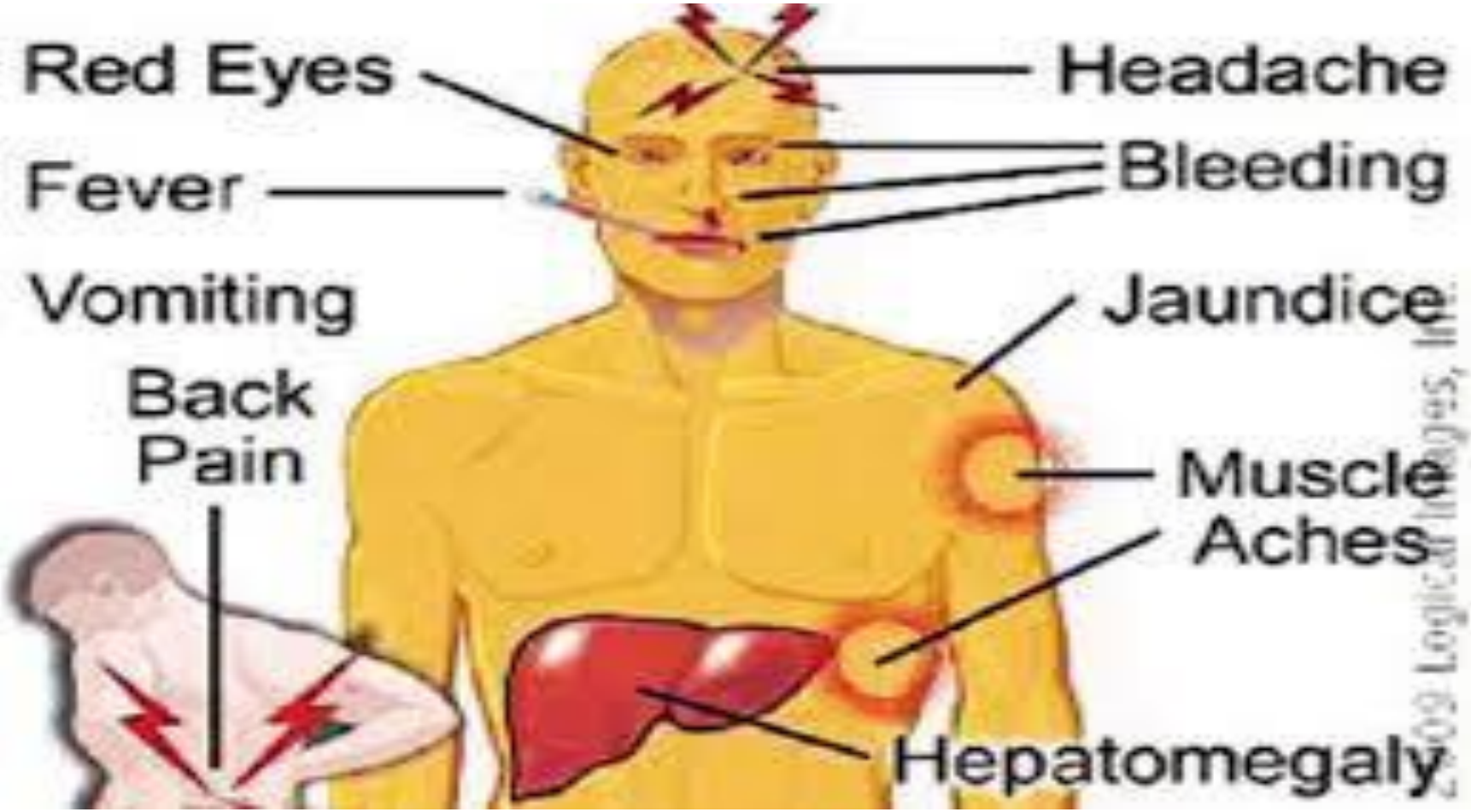
- **2-Urban yellow fever (a disease of man):**
- When infected persons return to urban areas, man to man transmission occurs by the mosquito *Aedes aegypti*.

Aedes aegypti.

- **Pathogenesis and clinical picture:**
- The virus is introduced by the bite of infective mosquito. It multiplies in the lymph nodes& spreads to the blood; this coincides with sudden fever, headache, myalgia & photophobia. Some patients recover at this point.

- The virus may reach the liver, spleen, kidney& bone marrow leading to their destruction. This results in jaundice, hemorrhages& proteinuria. It may affect the heart or GIT leading to shock, prostration haematemesis (black vomit). Death (> 50%) results from kidney or liver failure.

Symptoms of Yellow fever



- **Diagnosis:**
- **<u>1-Non specific:</u>**
- **1-hematological abnormalities (due to invasion of bone marrow):**
- Leucopenia, thrombocytopenia and prolongation of clotting, prothrombin PT and partial thromboplastin PTT times are detected.
- **2-Disturbed liver functions** (due to liver invasion):
- Elevated serum bilirubin & serum transaminases SGOT.
- **3- Albuminuria** (due to kidney invasion).

- **2-Specific:**
- 1- RT-PCR testing of genomic RNA or viral m RNA in blood or other samples.
- 2- Serology: ELISA is used to detect the presence of specific IgM or 4fold ↑ between acute and convalescent sera. The serologic cross reactivity among viruses limits distinction of the actual viral species in many cases.

- 3- The use of virus-specific monoclonal antibodies in IF assays affords rapid virus identification in clinical samples.
- 4- Isolation of the virus (difficult) from the blood in tissue culture (mouse embryonic cells / mosquito cell lines) or by Intracerebral inoculation of suckling mice leading to encephalitis and intranuclear inclusion bodies **(Torr's bodies)** can be detected.
- 5-Cytopathologic examination of liver cells to detect Torr's bodies.

- **Control& prophylaxis:**
- 1- Eradication of mosquitoes.
- 2- Vaccination using 17-D vaccine given S.C. and gives immunity for 10 years.
- It is a living attenuated vaccine
- The vaccine is contraindicated for infants less than 9 months of age, during pregnancy and immuno-suppressed patients e.g. HIV or organ transplant patients.

Dengue (Break bone Fever)

- Dengue occurs in the tropical and subtropical areas where the mosquito vector *Aedes aegypti* prevails. The virus is flavivirus
- 4 antigenic types are known.

Clinical picture:

Classic dengue:

- It begins suddenly with influenza-like syndromes consisting of fever, malaise, cough, headache, severe muscle and bone pain. nausea vomiting & eye pain. Enlarged lymph nodes & maculopapular rash may occur. This form of disease is rarely fatal and recovery occurs after few weeks.

Hemorrhagic dengue fever:

- It is a severe form of dengue which is fatal. The initial picture is the same as classic dengue, but then shock, and hemorrhage, especially into the GIT and skin develop.

Symptoms:

Sudden onset of fever for 2 to 7 days

Severe headache with retro-orbital (behind the eye) pain

Joint and muscle pain

Skin rashes

Nausea and vomiting

Bleed from the nose

Please refer to our friendly staff at **Guardian** to get the relevant dengue related products.

- **Pathogenesis:**
- The patient recovers from classic dengue caused by one of the 4 types and antibodies are produced. When the patient is infected with another serotype an anamnestic response occurs and large amounts of cross-reacting antibodies to the 1st type are produced. It is postulated that the virus-antibody complexes are formed within few days of the 2nd infection; these activate the complement causing increased vascular permeability and thrombocytopenia. It is also postulated that the antibodies increase the entry of virus into monocytes & macrophages with the release of large amounts of cytokines causing DIC, shock and hemorrhages.

- **Diagnosis:**
- (Blood sample is used. The steps are the same as mentioned for all arboviruses but no virus isolation).
- **Prevention:**
- Mosquito control. No vaccine is available.

There is no vaccine available against dengue, and there are no specific medications to treat a dengue infection. This makes prevention as the most important step.

Prevention Tips:

Use mosquito repellent products

Wear long sleeves and pants in the evening as additional protection

Make sure no stagnant water at home or premises around you

Spray insecticide in dark corners of your home (e.g. under the bed and sofa and behind curtains)

Increase water intake to control body temperature

II- Poxviridae

- **Classification:**
- Poxviruses are the largest and most complex viruses. The group includes:
- A- 3 antigenically related viruses:
- Variola virus; the etiologic agent of small pox.
- Vaccinia virus.
- Cow pox virus.

- B- Three viruses antigenically unrelated to each others or to variloa virus:
- Molluscum contagiosum which causes human warts (benign skin nodules).
- Monkey pox virus.
- buffalo pox virus.

- **Vaccinia virus**

is important as

❖it is the agent used for preparation of small pox vaccine.

❖It is also used as a vector to carry immunizing genes for several viruses to be used in preparation of a polyvalent live virus vaccine.

❖ It is also used as a vector in many gene therapy experiments.

Smallpox

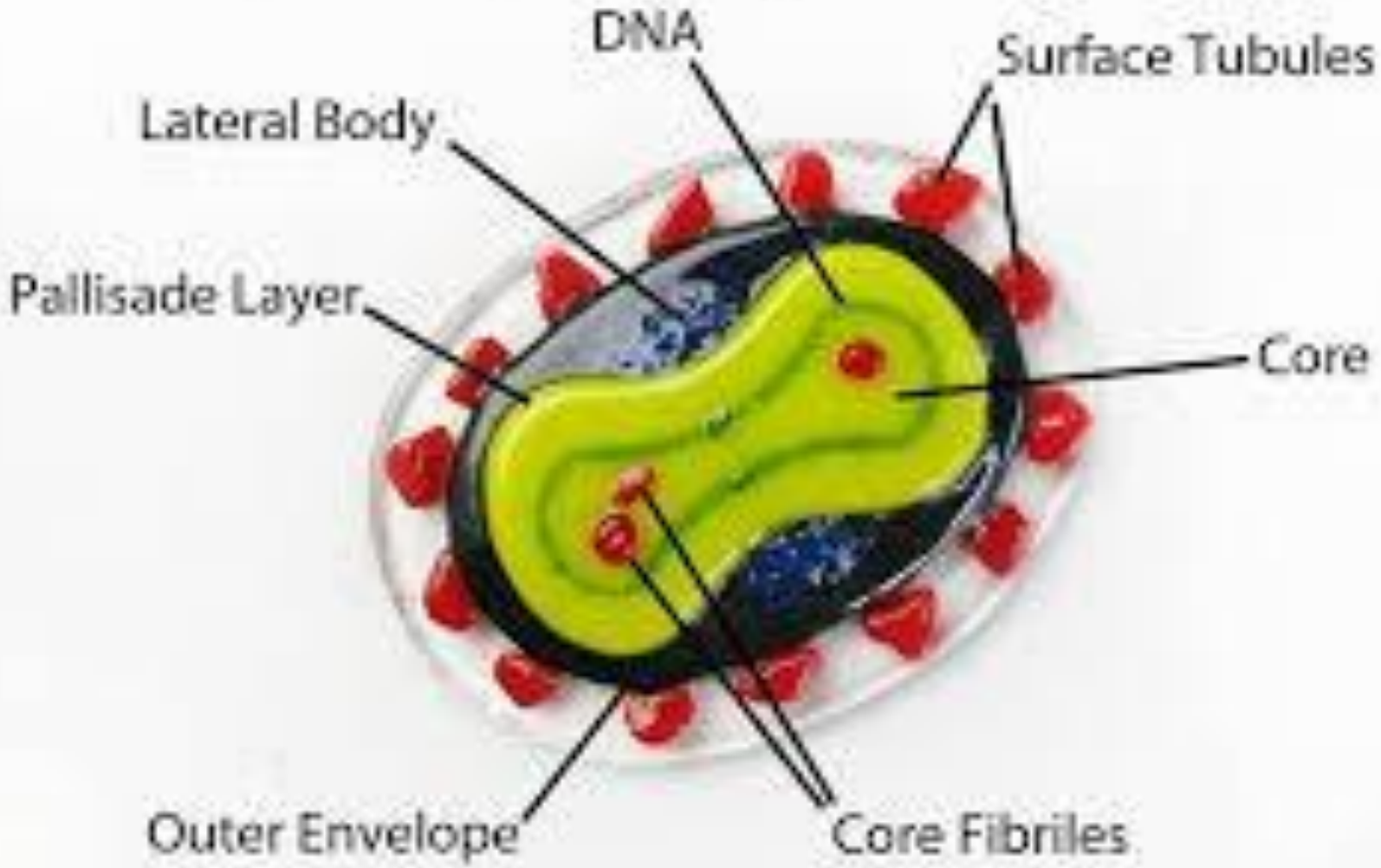
- Smallpox is the first disease to be controlled by immunization and the first to be eradicated.
- A brief description should be included for several reasons:
- 1- Differentiation from similar clinical conditions e.g. chickenpox, monkeypox, pustular acne, meningococcaemia and drug rash.

- 2- The vaccine is still used on a small scale for military personnel and laboratory workers in contact with virus, and may cause complications.

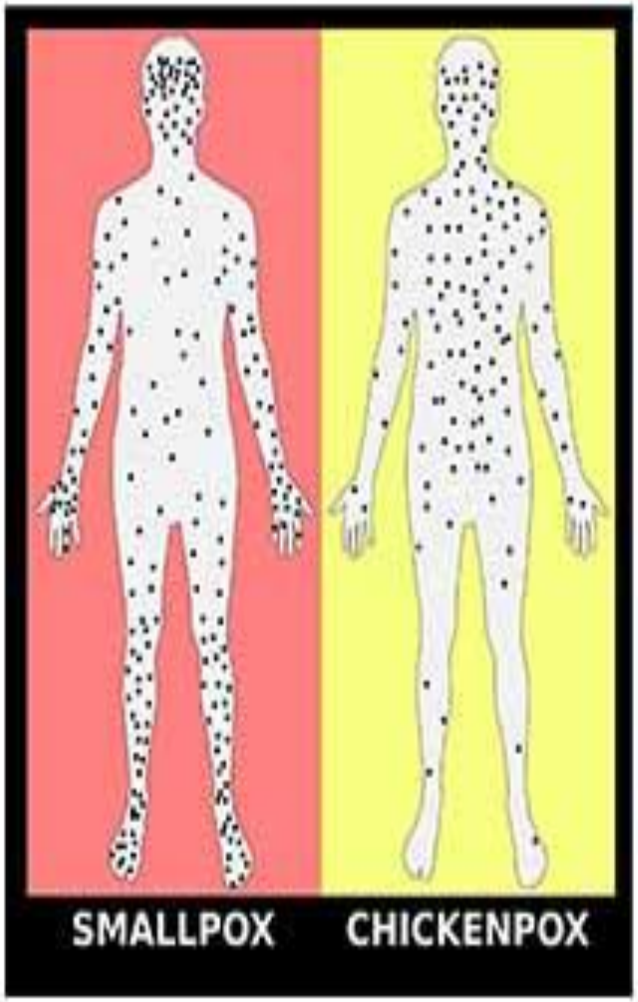
- 3- There is concern that the virus could be reintroduced as a **biologic weapon.**

- It is caused by **variola virus** which:
- Is a large ds DNA brick shaped virus 230 nm diameter x 400 nm length.
- Can be seen by the light microscope.
- Grows on the chorioallantoic membrane of chick embryo producing characteristic lesions called "pocks".
- Is one of biological weapons.

Variola (Smallpox Virus)



Differences Between Chickenpox and Smallpox



Chickenpox rash

Smallpox rash

- **Pathogenesis:**

* The virus enters by the respiratory route or by direct contact with skin lesions or contaminated fomities.

* It is a systemic disease with a viraemic stage and final localization in the skin causing the rash.

* Incubation period is 12 days followed by fever for 1-5 days followed by the appearance of rash which is first maculopapular (1-4 days) turning to vesicles (1-4 days), to pustules (2-6 days) forming crusts which fall of after 2-5 weeks leaving scarred area.

* All stages of the rash are infectious as well as discharges and saliva.

* Immunity following disease is life-long.

- **Replication:**
- Inspite poxviruses are double-stranded DNA genome (dsDNA) their replication occurs in the cytoplasm because poxviruses encode thier own machinery for genome transcription; a DNA dependent RNA polymerase, which makes replication in the cytoplasm possible while most dsDNA viruses require the host cell's enzyme to perform transcription which is present within the host cell's nucleus.
- Replication of the poxvirus involves the same steps of replication as any ds DNA virus and the progeny virions are released by lysis of host cells.

- **Diagnosis:**

1-Material collected from skin lesions is used for:

❖Detection of virus by electron microscopy.

❖Detection of viral antigens by IF.

❖Isolation of the virus using chick embryo or tissue culture.

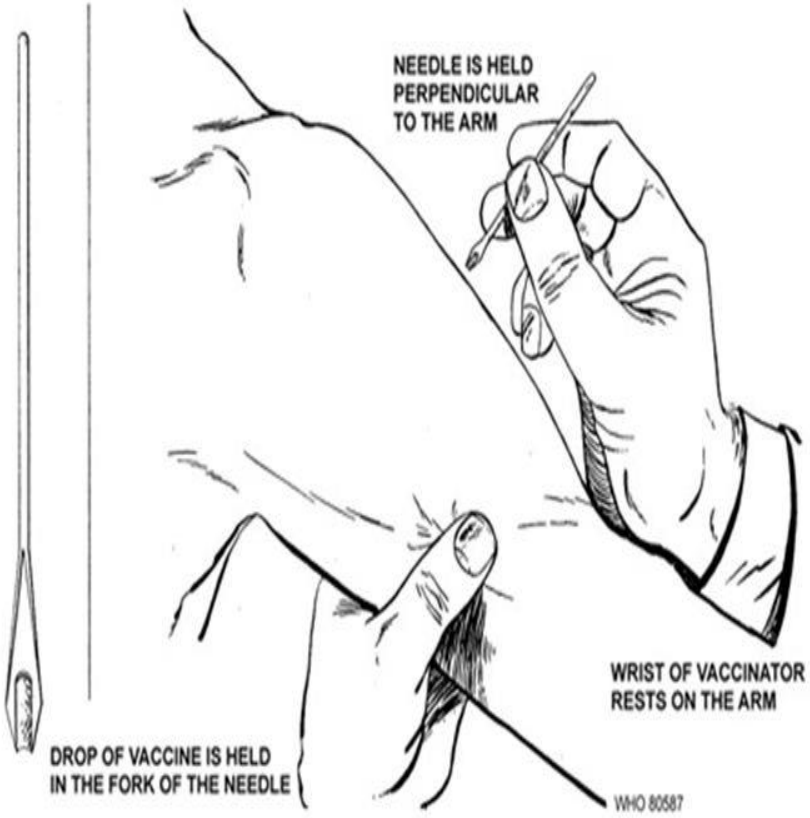
❖PCR for detection of viral genome.

2-Detection of serum antibodies by neutralization tests, ELISA or RIA.

- **Vaccination:** by the live vaccinia virus:
- The vaccinia virus is naturally attenuated for man giving rise only local lesions (vesicles that ulcerate and heal by scarring) that is followed by humeral and cell mediated immunity to small pox (as both are antigenically related).

- 1- Calf lymph vaccine is prepared by inoculation of vaccinia virus on shaved skin of calves. The fluid of the resulting vesicles is collected; to which is added 40% glycerol to stabilize the virus and 0.4% phenol which destroy contaminating bacteria.
- 2- Lyophilized vaccine prepared from infected embryonated eggs is also used. Both vaccines are given intradermally by multiple punctures

Multipuncture vaccination by bifurcated needle



- **Complications of Vaccination:**

(Treated by methisazone or cidofovir and vaccinia immunoglobulins)

- ❖Bacterial infection of the vaccination site.
- ❖Generalized vaccinia; spread of the virus through the blood causing generalized skin lesions. It occurs in immuno-suppressed children.
- ❖Post vaccination encephalitis is rare.
- ❖Eczema following vaccination.

- Vaccination is no longer recommended due to eradication of small pox since 1977. Small pox was officially declared eliminated in 1980. But now vaccination is recommended in a narrow scale for fear of bioterrorist attack starting with health care workers as they will be the first to be exposed to small pox cases.

- **Causes of Successful Eradication:**

❖Man is the only host, there is no animal reservoir.

❖There is only one stable serotype of the virus.

❖There is no carrier state or subclinical infections or chronic cases.

❖Effective vaccine that is highly immunogenic which was used worldwide.

❖A surveillance-containment program was used by the WHO. Smallpox cases are easily recognized clinically. Cases were traced and all susceptible contacts were identified and vaccinated. The vaccine is protective if given <u>within 4 days after exposure.</u>

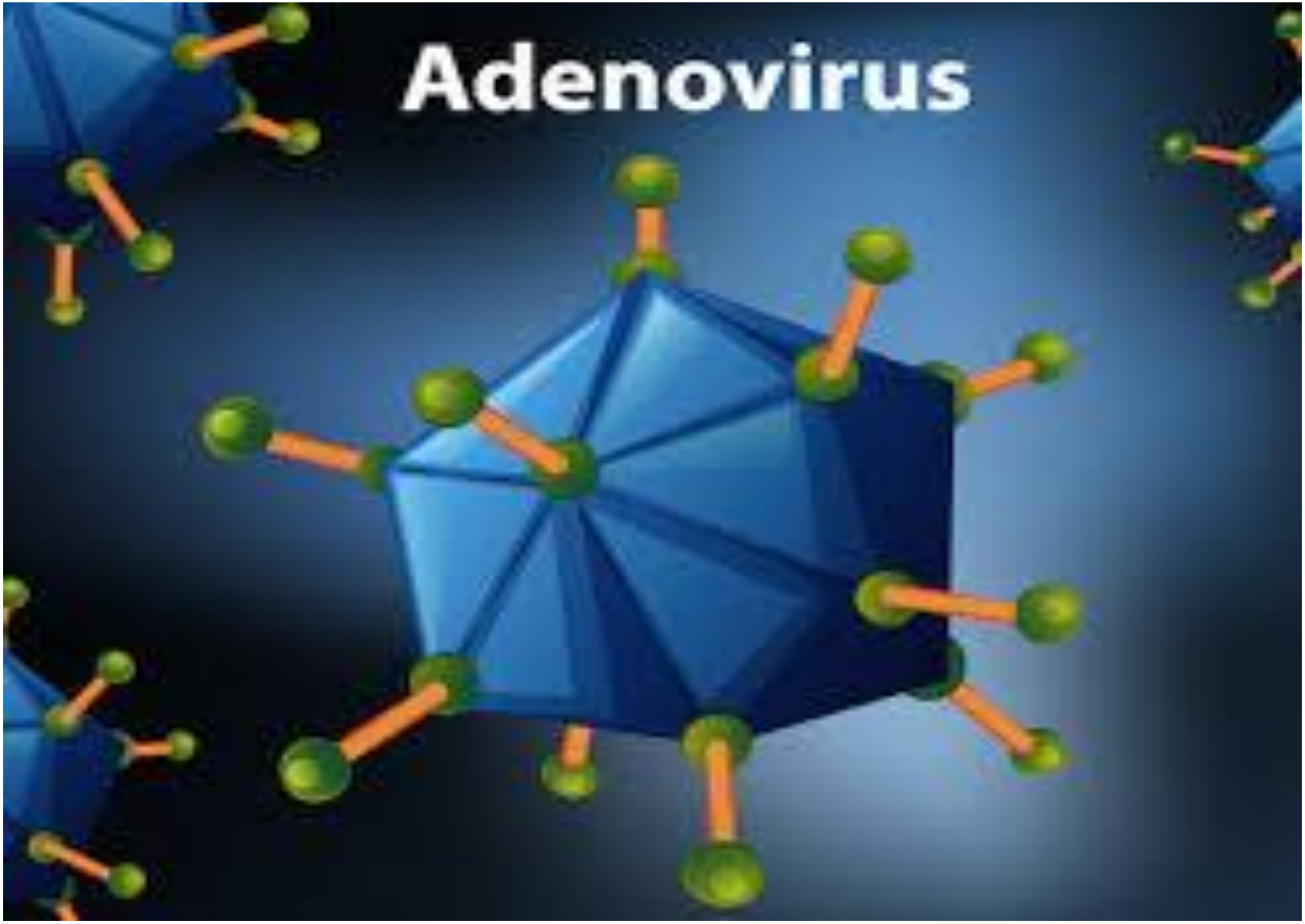
Molluscum contagiosum virus

- It causes human warts (benign skin nodules) usually on arms, face and genitalia.
- It's transmitted by close contact and sexually.
- It's common in children and immunosuppressed people.
- The virus resembles smallpox in morphology but is not antigenically related.
- Diagnosis is clinical however PCR or electron microscopy may be used.
- **Treatment** is by surgical removal.

warts

ADENOVIRUSES

- It was first isolated in the human adenoids cell culture, hence the name.
- **Structure:**
- They are dsDNA, icosahedral, non-enveloped viruses.
- They are the only viruses with a **fiber** protruding from each of the 12 vertices of the capsid. The fiber is the organ of attachment and is a haemagglutinin. There are about 51 human antigenic types. The fiber protein is the type specific protein.


Adenovirus

- **Pathogenicity:**
- In humans, adenoviruses can cause disease in several organs. The virus causes **latent infection** in the adenoids, tonsils and lymphoid tissues.
- Types 12, 18 and 31 induce tumors in newborn hamsters. Some adenoviruses can cause transformation of cells in culture. However, adenovirus oncogenesis has never been observed in humans.
- No apparent animal reservoirs for the virus.

Diseases:

* Infection is acquired by respiratory droplets, feco-oral route and by direct contact.

* Most infections resolve spontaneously, 50% are asymptomatic or mild and induce life-long type specific immunity against re-infection.

They may cause:

- Respiratory disease e.g. acute febrile pharyngitis and pneumonia, common in children & military camps by types 3, 4, 7 and 21.
- Pharyngoconjuntival fever (swimming pool conjunctivitis) occur in outbreaks in summer camps by types 3, 4, 7).
- Conjunctivitis and keratoconjunctivitis by types 8, 19 and 37.

- Gastroenteritis and intussusceptions in infants by types 40 and 41.
- Acute haemorrhagic cystitis in children by types 11 and 21.
- Severe GIT infections in immunocompromised patients.
- Transplant patients may suffer severe infections due to reactivation of a latent virus.

- **Diagnosis:**

1- Body secretions are examined by:

❖E.M (especially for fastidious enteric adenovirus).

❖Immunoflourescence to detect clustering of cells and inclusion bodies.

❖PCR.

❖Isolation of the virus in cell culture.

❖Antigen detection by ELISA.

2- Detection of specific antibodies, 4-fold rise of antibody titre is a good evidence of infection.

- **Prevention:**
- Live vaccine in form of enteric-coated capsules containing 4, 7 and 21 types are given orally to military camps.
- Sterilize eye instruments used in examination.
- Good chlorination of swimming pools.
- No approved treatment.

Adenoviruses and Gene Therapy

- Adenoviruses are used as gene delivery vehicles by coupling the DNA of interest with the virus particles. So adenoviruses can be used for cancer therapy, gene therapy of several human diseases as immune deficiencies and cystic fibrosis and for genetic immunization studies.


Thank you !!!