МІНІСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
Харьковский национальный медицинский университет

VIRAL ENCEPHALITIS AND HEMORRHAGIC FEVERS

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, Virology and Immunology)

ВИРУСНЫЕ ЭНЦЕФАЛИТЫ И ГЕМОРРАГИЧЕСКИЕ ЛИХОРАДКИ

Методические указания по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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Compilers V. V. Minukhin,
N. I. Kovalenko

Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III-IV level accreditation.

Learning guide includes sections of taxonomy, morphology and ultrastructure of arbo- and roboviruses that cause encephalitis and hemorrhagic fevers. The most modern information on pathogenesis, epidemiology, methods of laboratory diagnosis and specific prophylaxis is represented.

Вирусные энцефалиты и геморрагические лихорадки : метод. указ. по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов мед. и стомат. фак-тов с английским языком преподавания / сост. В. В. Минухин, Н. И. Коваленко. – Харьков : ХНМУ, 2015. – 28 с.

Составители В. В. Минухин,
Н. И. Коваленко

Theme: Microbiological diagnosis of arboviruses infections.

Actuality of the theme.

Purpose: Studying of laboratory diagnosis of encephalitis and hemorrhagic fevers that are caused by arboviruses.

Concrete goals:

1. Study of biological properties and classification of Alphaviruses, Flaviviruses and Bunyaviruses.
2. Study pathogenesis and clinical manifestations of encephalitis and hemorrhagic fevers that are caused by arboviruses.
3. Study of the methods of laboratory diagnosis of encephalitis and hemorrhagic fevers that are caused by arboviruses.
4. Study of specific prophylaxis of encephalitis and hemorrhagic fevers that are caused by arboviruses.

Students should be able to:

1. Perform serological methods (CFT, PHAIT) to diagnosis of encephalitis and hemorrhagic fevers that are caused by arboviruses.

Equipment: biological preparations for laboratory diagnosis and specific prophylaxis of encephalitis and hemorrhagic fevers, tables, atlas.

Practical tasks, being carried out during practical classes:

1. Studying ultrastructure of arboviruses (in atlas).
2. Studying biological preparations for serological methods (antigens and diagnostic sera).
3. Studying the scheme of laboratory diagnosis of encephalitis and hemorrhagic fevers that are caused by arboviruses.
4. Studying vaccines for specific prophylaxis of encephalitis and hemorrhagic fevers that are caused by arboviruses.

Therminology: arboviruses, Alfaviruses, Flaviviruses, Bunyaviruses.

Theoretical questions for control:

1. Families Alfaviruses, Flaviviruses, Bunyaviruses, ultra- and antigenic structure.
2. Routes of transmission and pathogenesis of encephalitis and hemorrhagic fevers that are caused by arboviruses.
3. Laboratory diagnosis of encephalitis and hemorrhagic fevers that are caused by arboviruses.
4. Control of encephalitis and hemorrhagic fevers that are caused by arboviruses.

ALPHAVIRIDAE (TOGAVIRIDAE) and FLAVIVIRIDAE

Many virus diseases are transmitted by the bite of arthropods. The viruses are called arboviruses (arthropod-borne viruses) and multiply in the bodies of the arthropods.

At least 27 alphaviruses and 68 flaviviruses have been recognized, approximately one-third of which are medically important human pathogens. They vary widely in their basic ecology; each virus occupies a distinct ecologic niche, often with restricted geographic and biologic distribution. As shown in *Tables 1* and *2*, alphaviruses and flaviviruses can cause various syndromes, ranging from benign febrile illnesses to severe systemic diseases with hemorrhagic manifestations or major organ involvement.

Table 1 – Principal Medically Important Alphaviruses

Virus	Antigenic Clinical Syndrome	Vector / Hosts	Distribution
Eastern equine encephalitis (EEE)	Encephalitis	Mocquito / birds	Americas
Western equine encephalitis (WEE)	Encephalitis	Mocquito / birds	North America
Venezuelan equine encephalitis (VEE)	Febrile illness, encephalitis	Mosquito / rodents, horses	Americans
Chikungunya (CHIK)	Febrile illness, rash, arthralgia	Mosquito / primates, humans	Africa, India, Southeast Asia
Mayaro (MAY)	Febrile illness, rash, arthralgia	Mosquito / primates, humans	South America, Trinidad
Rose River (RR)	Febrile illness, rash, arthralgia	Mosquito / mammals, humans	Australia, Pacific
Sindbis (SIN)	Febrile illness, rash, arthralgia	Mosquito / birds	Northern Europe, Africa, Asia, Australia

All alphaviruses and flaviviruses that cause disease in humans are arthropod-borne viruses (arboviruses). Most alphaviruses and flaviviruses survive in nature by replicating alternately in a vertebrate host and a hematophagous arthropod (mosquitoes or, for some flaviviruses, ticks). Arthropod vectors acquire the viral infection by biting a viremic host, and after an extrinsic incubation period during which the virus replicates in the vector's

tissues, they transmit virus through salivary secretions to another vertebrate host. Virus replicates in the vertebrate host, causing viremia and sometimes illness. The ability to infect and replicate in both vertebrate and arthropod cells is an essential quality of alphaviruses and flaviviruses. The principal vertebrate hosts for most are various species of wild mammals or birds. The natural zoonotic cycles that maintain the virus do not usually involve humans. However, a few viruses (yellow fever virus, dengue virus types 1, 2, 3 and 4 and chikungunya virus) can be transmitted in a human-mosquito-human cycle. As a result of being pathogenic for humans and capable of transmission in heavily populated areas, these viruses can cause widespread and serious epidemics. Because of their high transmission potential, these viruses are major public health problems in many tropical and subtropical regions of the world where appropriate mosquito vectors are present.

Table 2 – Principal Medically Important Flaviviruses

Virus	Antigenic Clinical Syndrome	Vector / Hosts	Distribution
Dengue (DEN): DEN-1, -2, -3, -4	Fibrile illness, rash, hemorrhagic fever, shock syndrome	Mosquito / humans	Tropica, worldwide
Yellow fever (EF)	Hemorrhagic fever, hepatits	Mosquito / primates, humans	Africa, South America
St.Louis encephalitis (SLE)	Encephalitis	Mosquito / birds	Americas
Japanese encephalitis (JE)	Encephalitis	Mosquito / pigs, birds	India, China, Japan, South-East Asia
Murray Valley encephalitis (MVE)	Encephalitis	Mosquito / birds	Australia
West Nile (WN)	Febrile illness	Mosquito / birds	Africa, Midddle East, Europe
Tick-borne encephalitis (TBE)	Encephalitis	Tick / rodents	Europe, Asia
Omsk hemorrhagic fever (OMSK)	Hemorrhagic fever	Tick / muskrats	Siberia
Kyassanur Forest disease (KFD)	Hemorrhagic fever	Tick / rodents, primates	India

ALPHAVIRUSES

Structure. The enveloped virions are spherical, 60 to 70 nm in diameter with a positive-sense, monopartite, single-stranded RNA genome. The lipid-containing envelope has two (rarely three) surface glycoproteins that mediate attachment, fusion, and penetration. The icosohedral nucleocapsid contains capsid protein and RNA.

Classification and Antigenic Types. The 27 alphaviruses are classified on the basis of antigenic properties. All alphaviruses share antigenic sites on the capsid and at least one envelope glycoprotein, but viruses can be differentiated by several serological tests, particularly neutralization assays.

Multiplication. Alphaviruses attach to cellular receptor of many vertebrate and invertebrate cells. Viral replication occurs in the cytoplasm. Transcription of the virion RNA through a negative-strand RNA intermediate produces a positive-strand mRNA wich encodes only the strucrural proteins, as well as additional RNA, wich is incorporated into progeny virions. Envelope proteins firmed by posttranslational cleavage are translocated to the plasma membrane. Virions mature by budding through the plasma membrane.

Epidemiology. Viruses are maintained in nature by mosquito-vertebrate-mosquito cycles (*Fig. 1*). Restricted interactions between viruses, vector species, and vertebrate hosts tend to confine the geographic spread of alphaviruses. Occasionally, a virus may escape its usual ecological niche and cause widespread epizootics (Venezuelan equine encephalitis virus) or urban epidemics (chikungunya virus). Human infections are seasonal and are acquired in endemic areas.

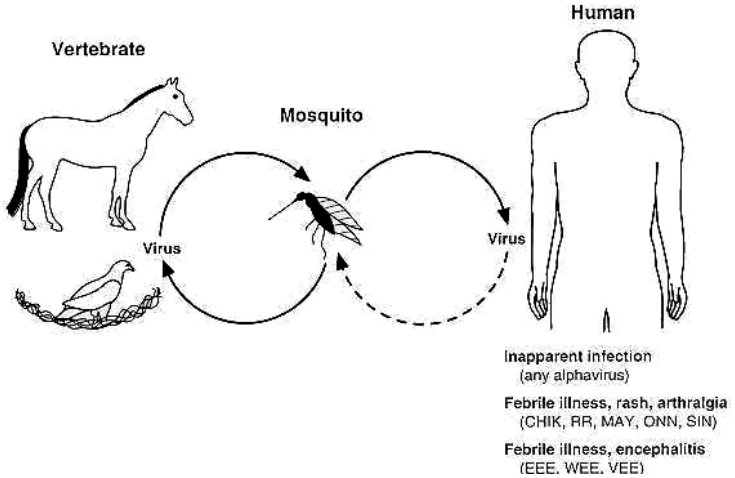

Fig. 1. Alphavirus transmission.

Pathogenesis and Clinical Manifestations. Virus introduced by the bite of an infected mosquito replicates and causes a viremia coincident with abrupt onset of fever, chills, malaise, and joint aches. The specific site of viral replication is unknown. The viremia subsides in 3 to 5 days, and antiviral antibodies appear in the blood within 1 to 4 days of the onset of symptoms. A macular-papular rash typically develops around the third to fifth day of illness. The migratory arthralgia involves mainly the small joints. In more severe cases the joints are swollen and tender, and rheumatic signs and symptoms may persist for weeks or months following the acute illness.

Disease occurs in either of two general forms, depending upon the virus: one is typified by fever, malaise, headache, and/or symptoms of encephalitis (e.g., eastern, western, or Venezuelan equine encephalitis viruses) and the other by fever, rash, and arthralgia (e.g., chikungunya, Ross River, Mayaro, and Sindbis viruses). Sindbis viruses cause diseases known as Ockelbo (in Sweden), Pogosta (Finland), or Karelian fever (Russia).

The pathogenesis of eastern and western equine encephalitis virus infection of humans (as well as of equines) similarly involves percutaneous introduction of virus by a vector (*Fig. 2*) and development of viremia; however, the majority of human infections with these viruses are either asymptomatic or present as a nonspecific febrile illness or aseptic meningitis. Symptoms usually begin with malaise, headache, and fever, followed by nausea and vomiting. Over the next few days the symptoms intensify, and somnolence or delirium may progress into coma. Seizures, impaired sensorium, and paralysis are common. All regions of the brain may be affected.

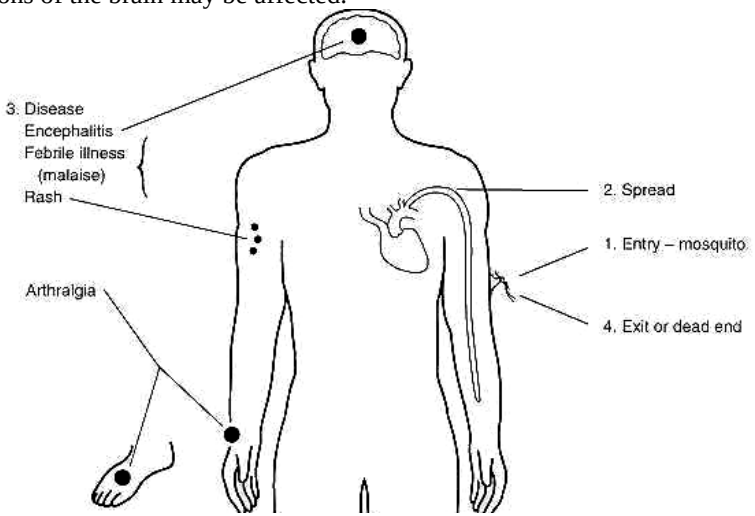

Fig. 2. Pathogenesis of alphaviruses

Venezuelan equine encephalitis virus infection in humans routinely produces an acute febrile illness with pronounced systemic symptoms, whereas the central nervous system disease occurs only infrequently and usually is much less severe than in eastern and western equine encephalitis. Following an incubation period of 2 to 6 days, patients typically develop chills, high fever, malaise, and a severe headache. A small percentage of human infections will progress to neurologic involvement with lethargy, somnolence or mild confusion. Seizures, ataxia, paralysis, or coma herald more severe central nervous system invasion.

Host Defenses. Initial resistance is conferred by nonspecific defenses such as interferon. Antibodies are important in recovery and resistance, and T-cell responses are also involved. Lasting protection is generally restricted to the same alphavirus and is associated with, but not solely attributable to, the presence of neutralizing antibodies.

Diagnosis is suggested by clinical evidence and known risk of exposure to virus. It can be confirmed only by laboratory tests. Laboratory diagnosis can be established by isolating virus from the blood during the viremic phase or by antibody determination. A variety of serologic tests, especially neutralization, but also enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition, complement fixation, and reactivities with appropriate monoclonal antibodies, are used by public health laboratories to diagnose alphavirus infections. Testing by ELISA for specific IgM is particularly useful in discriminating recent infection with one alphavirus from previous exposure to another alphavirus. The diagnosis of alphavirus infections is performed by detection of viral RNA (e.g. using polymerase chain reaction, PCR) or proteins (e.g., immunohistochemistry) in frozen or fixed tissues.

Control. Inactivated vaccines are used to protect laboratory workers from eastern, western, and Venezuelan equine encephalitis viruses. An effective live attenuated Venezuelan equine encephalitis vaccine has been employed extensively in equines as an epidemic control measure, and a similar vaccine is used to protect laboratory workers. A live attenuated chikungunya vaccine has proven safe and immunogenic in investigational human trials.

FLAVIVIRUSES

Structure. Virions are spherical and 40-50 nm in diameter with a positive-sense, nonsegmented, single-stranded RNA. The lipid-containing envelope has one surface glycoprotein that mediates attachment, fusion, and penetration, and an internal matrix protein. The nucleocapsid contains capsid protein and RNA. Virions mature at intracytoplasmic membranes.

Classification and Antigenic Types. Classification within the genus is based upon antigenic properties. Viruses are grouped into several antigenic complexes typified, for example, by dissimilar viruses such as dengue, tick-

borne encephalitis, St. Louis encephalitis, and yellow fever viruses. Although classification was not intentionally based upon vectors or diseases, the tick-borne flaviviruses important in human disease are aligned with tick-borne encephalitis virus in a single antigenic group, while several encephalitogenic mosquito-borne viruses (St. Louis encephalitis, Japanese encephalitis, Kunjin, Murray Valley encephalitis, and West Nile viruses) make up another group.

Epidemiology. The flaviviruses constitute a highly diverse genus, and their ecology is similarly varied and complex. The mosquito-borne encephalitis viruses (St. Louis encephalitis, Japanese encephalitis, Murray Valley encephalitis, and West Nile viruses) exist primarily as viruses of birds and are transmitted by *Culex* mosquitoes that feed readily on birds. Encephalitis virus it infects swine, in which it causes high viremias. Because Asian vectors of Japanese encephalitis virus feed readily on swine, these animals are an efficient amplifying host for this virus. This virus is a major public health concern in Japan, China, India, and Southeast Asia.

The tick-borne flaviviruses are maintained by tick-mammal cycles and by transovarian transmission in ticks. Humans are infected with this subgroup of flavivirus through the bite of infected ticks, and thousands of cases may occur annually in the region of the Eurasian continent between central Europe and western Siberia.

Yellow fever virus in Africa and South America has two distinct epidemiologic patterns: sylvan and urban. Sylvan (jungle) yellow fever is maintained among canopy-dwelling monkeys and tree-hole breeding mosquitoes (*Aedes* spp. in Africa, and *Haemagogus* spp. in South America). Urban yellow fever is transmitted in a highly efficient human-mosquito-human cycle by the urban mosquito *A. aegypti*. Yellow fever epidemics with high mortality rates continue to occur with alarming frequency in tropical Africa.

Dengue fever is the most common flavivirus infection among humans, with extensive distribution of virus serotypes through the tropics and warm temperate areas of Africa, Asia, Australia, Oceania, India, and the Americas.

Pathogenesis and Clinical Manifestations. Infection is initiated by the bite of an infected mosquito or tick through the skin via the saliva of an infected arthropod (*Fig. 3*). Virus replicates locally and in regional lymph nodes and results in viremia. Infection and seroconversion in the absence of apparent disease are common, but case fatality rates can be high.

In most human infections with St. Louis encephalitis (SLE) and Japanese encephalitis (JE) viruses, there is either no apparent disease or a nonspecific febrile illness with headache. The infection resolves, and lasting immunity is produced. However, central nervous system invasion may develop and present as aseptic meningitis or encephalitis. Clinical manifestations of encephalitis due to SLE, JE, or Murray Valley encephalitis (MVE) virus begin as fever, headache, and stiff neck, and progress to an altered level of consciousness and focal neurologic deficits (e.g., tremors, pathologic reflexes, ataxia). Paralysis is

more commonly seen with JE and MVE. The case fatality rate ranges from 2% in young adults to over 20% in the elderly.

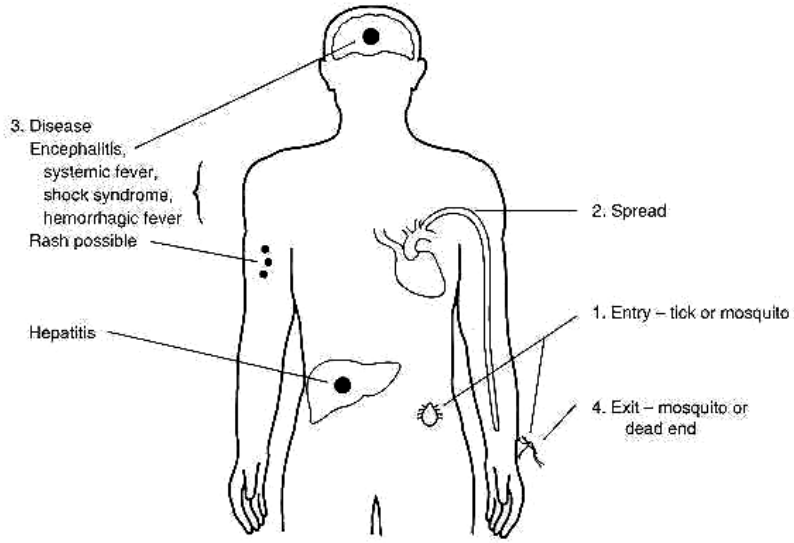

Fig. 3. Pathogenesis of flaviviruses

Yellow fever virus produces severe systemic disease with relatively high frequency. Viral replication occurs in reticuloendothelial cells in many organs and in the parenchyma of the liver, adrenal glands, heart, and kidneys. Coagulation defects, probably resulting from both liver damage and disseminated intravascular coagulation, are major manifestations and are associated with the severe gastrointestinal hemorrhages characteristic of yellow fever. The clinical course of yellow fever is that of an acute illness lasting 1 week or more, and ranges from a nonspecific, mild febrile illness to classic disease with severe hemorrhagic and hepatic involvement. The initial onset is abrupt with fever, myalgia, headache, vomiting, and minor gingival hemorrhage lasting for about 3 days. Then a brief day of improvement may precede fulminant illness manifested as severe toxicity, jaundice, extensive mucosal and gastrointestinal hemorrhage, azotemia, and shock. Death may occur within 5-10 days; case fatality rates are 10%-50%.

Dengue viruses of all four serotypes cause three distinct syndromes: classic dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. Although caused by the same viruses, dengue and dengue hemorrhagic fever are pathogenetically, clinically, and epidemiologically distinct. Dengue viruses appear to replicate in macrophages at the site of the mosquito bite, in regional

lymph nodes, and then throughout the reticuloendothelial system. Viremia is concurrent with clinical illness. Dengue fever is characterized by sudden onset of systemic toxicity, fever, headache, vomiting, and severe myalgia or bone pain. There appears a maculopapular or morbilliform rash on the trunk which spreads to the limbs and face. Dengue fever lasts 3 to 9 days, is self-limiting, and is rarely associated with serious sequelae.

In contrast, the clinical course of dengue hemorrhagic fever is characterized by an initial stage of fever, rash, and anorexia (lasting 3 to 5 days) followed by a shock phase in which hepatomegaly, hypotension, and a hemorrhagic diathesis occur.

In dengue shock syndrome, the decreased plasma volume, which results from increased vascular permeability causes clinical shock that, if uncorrected, may lead to acidosis, hyperkalemia, and death.

Host Defenses. Antiviral antibody has an important protective role in host defenses against flaviviruses. Prophylactically administered immune serum can prevent or diminish infection. Virus-specific cytotoxic and helper T cell activities are also demonstrable. Lasting protection is generally restricted to the same flavivirus, and is associated with neutralizing antibodies.

Diagnosis is suggested by clinical evidence and by known risk of exposure to virus. It is confirmed by virus isolation and identification. Virus usually can be isolated only by brain biopsy or from the brain at autopsy. Thus, serologic tests showing an antibody rise are most practical for diagnosis. In primary infections, the virus neutralization test provides virus-specific confirmation. If a patient has had previous flavivirus infections, cross-reactions make even neutralization test results difficult or impossible to interpret. Demonstration of specific IgM in the cerebrospinal fluid by antibody-capture immunoassay can be an excellent way to diagnose flaviviruses encephalitis.

In yellow fever, dengue, and dengue hemorrhagic fever, virus is present in the blood for 4 or even 5 days after onset of fever. Virus isolation in mammalian or insect cell culture is the classical method of choice for diagnosis, though genotypic detections and analyses (e.g., using PCR) provide good alternatives.

Control. A safe and effective live-attenuated 17D vaccine exists for yellow fever, and formalinized inactivated-virus vaccines are used to prevent Japanese encephalitis and tick-borne encephalitis. Live attenuated vaccines for dengue have been developed and used in Thailand. It is important that a dengue vaccine confer appropriate immunity to all four serotypes.

BUNYAVIRUSES

Bunyaviridae is a family of arthropod-borne or rodent-borne viruses. Bunyaviruses are responsible for a number of febrile diseases in humans and other vertebrates. They have either a rodent host or an arthropod vector and a vertebrate host.

Structure. Bunyaviruses are spherical, enveloped particles 90 to 100 nm in diameter. They contain three segments of antisense single-stranded RNA combined with nucleoprotein. Two external glycoproteins form surface projections. A virus-encoded transcriptase is present in the virion. The nucleocapsid is surrounded by a lipid-containing envelope. Surface spikes are composed of two glycoproteins that confer properties of neutralization of infectivity and hemagglutination of red blood cells.

Classification and Antigenic Types. There are four genera *Bunyaviruses*, *Phlebovirus*, *Nairovirus*, and *Hantavirus*, which include 35 serogroups with at least 304 types and subtypes.

Multiplication. Bunyaviruses replicate in the cytoplasm. Their RNA genome is transcribed to mRNA. The host RNA sequence in some representative viruses primes viral mRNA synthesis. Bunyaviruses mature by budding into vesicles at or near the Golgi apparatus. Reassortment of RNA segments occurs between closely related members.

Epidemiology. The distribution of each disease is determined by the distributions of the vector and vertebrate host. Except for hantaviruses, biologic transmission is by a tick, mosquito, midge, or sand fly vector. Arthropods are infected for life. Transovarial transmission is common in arthropods. Wild or domestic vertebrates usually are needed to maintain the cycle. Humans may become ill when infected, but human blood rarely infects biting arthropods in the natural cycle, therefore, humans are usually dead-end hosts for all these viruses; however, the blood of Crimean-Congo hemorrhagic fever patients may be highly infectious.

Pathogenesis Except for members of the genus *Hantavirus*, bunyaviruses replicate in arthropods. The gut of the vector is infected initially, and after a few days or weeks the virus appears in the saliva; the arthropod then remains infective for life but is not ill. When the vector takes a blood meal, the infective saliva enters the small capillaries or lymphatics of the human or other vertebrate host (*Fig. 4*).

The primary site of replication in humans is not known; it may be the vascular endothelium, the skin, or the regional lymph nodes. An incubation period of a few days ensues, after which the vertebrate host develops viremia. The infection is usually inapparent. Less often, the host becomes febrile, manifesting the more serious signs and symptoms that are characteristic of the infecting virus. Viremia subsides with the appearance of humoral antibody, and the host recovers unless a specific target organ is affected. This target organ the liver in Rift Valley fever, the brain in La Crosse encephalitis, the liver and vascular endothelium in Crimean-Congo hemorrhagic fever and hemorrhagic fever with renal syndrome, and the lung in hantavirus pulmonary syndrome is damaged, and a specific disease occurs. Although the damage in most infections is believed to result from direct invasion by the virus and not from a host-mediated antigen-antibody or antigen-lymphocyte reaction. The damage to the kidneys in hemorrhagic fever with renal syndrome and to the brain and

retina in Rift Valley fever occurs after humoral antibody is formed. These complications have been postulated to result from a host reaction.

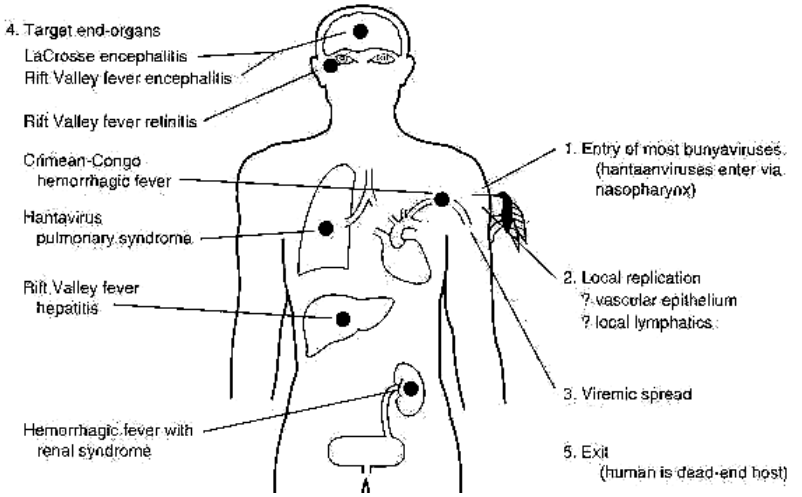

Fig. 4. Pathogenesis of bunyavirus infections

Clinical Manifestations. The Bunyaviridae are divided into arthropod-borne viruses (arboviruses) and rodent-borne viruses (roboviruses).

Bunyaviruses cause fevers sometimes with rash. Most illnesses are self-limited fevers that last 1 to 4 days and are accompanied by headache, muscle aches, nausea, conjunctival injection, and generalized weakness. A few are more serious illnesses. La Crosse encephalitis is characterized by fever, convulsions, drowsiness, and focal neurologic signs; Crimean-Congo hemorrhagic fever is characterized by headache, pain in limbs, and, in severe cases, bleeding from multiple orifices and is associated with from 2-50% case fatality rate; Rift Valley fever may mimic the febrile, encephalitic, or hemorrhagic illness of other bunyavirus infections, and the patient may also go blind as a result of retinal vasculitis.

Host Defenses. The initial response to bunyavirus infection is the production of interferon. Bunyaviruses are sensitive to the action of interferon, so this response may play a protective role. Humoral antibody is also protective. The appearance of antibody, either natural or passively administered, is associated with the disappearance of virus from the blood. The role of cell-mediated immunity has not been fully evaluated.

Diagnosis. Illnesses caused by bunyaviruses are diagnosed by isolating the virus, detecting RNA by RT-PCR, or by showing a fourfold or greater rise in

antibody titer between acute- and convalescent-phase sera. The virus can be isolated from blood (or from brain, liver, and other organs postmortem) during the viremic phase, but not usually after the third day of fever. It is propagated in baby mice or mosquitoes or in vertebrate or invertebrate tissue cultures. The RNA has been detected in lung tissue from cases of hantavirus pulmonary syndrome postmortem. Serologic tests used to diagnose bunyavirus infections include the enzyme-linked immunosorbent assay and complement fixation, fluorescent antibody, neutralization, and hemagglutination inhibition tests. The complement fixation and fluorescent antibody tests and the enzyme-linked immunosorbent assay (ELISA) are often group reactive; the neutralization and hemagglutination inhibition tests are type specific. Assessments of IgM may be especially useful in establishing an early diagnosis. Once isolated, virus is identified by the same tests with a reference immune serum.

Control. Rift Valley fever vaccines are used in Africa to immunize sheep and cattle and hence to stop the transmission cycle to humans. A human inactivated vaccine for Crimean-Congo hemorrhagic fever is used in the former Soviet Union and Bulgaria.

Medical personnel who care for viremic patients should be careful in handling needles and surgical instruments to prevent accidental transmission by blood. When hemorrhage occurs, as in Crimean-Congo hemorrhagic fever, hospital personnel should wear a gown and mask to prevent aerosol infection. For other bunyavirus infections, no quarantine, isolation, or concurrent disinfection is needed other than the precautions noted above.

Theme: Microbiological diagnosis of roboviruses infections.

Actuality of the theme.

Purpose: Studying of laboratory diagnosis of hemorrhagic fevers that are caused by roboviruses.

Concrete goals:

1. Study of biological properties and classification of Bunyaviruses, Arenaviruses and Filoviruses.
2. Study pathogenesis and clinical manifestations of hemorrhagic fevers that are caused by roboviruses.
3. Study of the methods of laboratory diagnosis of hemorrhagic fevers that are caused by roboviruses.
4. Study of specific prophylaxis of hemorrhagic fevers that are caused by roboviruses.

Students should be able to:

1. Perform serological methods (CFT, PHAIT) to diagnosis of hemorrhagic fevers that are caused by roboviruses.

Equipment: biological preparations for laboratory diagnosis and specific prophylaxis of hemorrhagic fevers, tables, atlas.

Practical tasks, being carried out during practical classes:

1. Studying ultrastructure of roboviruses (in atlas).
2. Studying biological preparations for serological methods (antigens and diagnostic sera).
3. Studying the scheme of laboratory diagnosis of hemorrhagic fevers that are caused by roboviruses.
4. Studying vaccines for specific prophylaxis of hemorrhagic fevers that are caused by roboviruses.

Therminology: roboviruses, Bunyaviruses, Arenaviruses, Filoviruses.

Theoretical questions for control:

1. Families Bunyaviruses, Arenaviruses, Filoviruses, ultra- and antigenic structure.
2. Laboratory diagnosis of hemorrhagic fevers that are caused by roboviruses.
3. Control of hemorrhagic fevers that are caused by roboviruses.

Roboviruses - viruses that are transmitted by rodents are included in three families Bunyaviridae, Arenaviridae and Filoviridae. Common morphological characteristic is presented in *table 3*.

Table 3 – Morphology of roboviruses

Envelope	Symmetry	Genome	Size
Bunyaviridae family			
yes	helical	single strand negative sense segmented	90-100 nm
Arenaviridae family			
yes	helical	single strand RNA ambisense segmented	110-130 nm
Filoviridae family			
yes	helical	single strand RNA negative sense	up to 14,000 nm in length, a diameter of 80 nm

BUNYAVIRUS FAMILY. HANTAVIRUS GENUS

The hantavirus genus differs from other members of Bunyaviridae in that members are transmitted by rodents (rather than arthropods). Each hantavirus is only transmitted by a limited number of genera/species of rodent (*Table 4*). Infected rodents can spread virus via saliva, urine (they get a viruria) or droppings. When fresh urine, droppings or recently contaminated nesting material is swept up or disturbed, the virus can be aerosolized and inhaled. Some of these viruses can cause severe disease, but even for these viruses many infections are sub-clinical, or very mild and never diagnosed.

Table 4 – Principal Medically Important Bunyaviruses

Virus and vector	Disease	Occurrence
Seoul virus - domestic rat	Korean hemorrhagic fever	Southeast Asia
Hantaan virus - field mouse	Hemorrhagic fever with renal syndrome	Southeast Asia
Dobrava virus - field mouse	Hemorrhagic fever with renal syndrome	Europe, Asia
Puumala virus - bank vole	Hemorrhagic fever with renal syndrome	Europe, Asia
Sin Nombre virus (SNV) - deer mouse	Hantavirus pulmonary syndrome (HPS)	North and South America

i) Associated with hemorrhagic fever with renal syndrome (HFRS). Korean hemorrhagic fever has a case-fatality rate of about 7%. Other members of the hantaviruses which cause HFRS (hemorrhagic fever with renal syndrome) tend to have a lower fatality rate.

ii) Associated with severe pulmonary syndrome

These are a newly recognized (1993) group of hantaviruses in North and South America that is transmitted by rodents (by inhalation or contact with excreta) and causes Hantavirus Pulmonary Syndrome (HPS) rather than hemorrhagic fever. These hantavirus pulmonary syndrome viruses have a high case fatality rate of ~36%. The viruses are widely distributed throughout the US but relatively rarely cause human disease.

Hantavirus hemorrhagic fever with renal syndrome

Hantavirus hemorrhagic fever with renal syndrome (HFRS) is a group of clinically similar illnesses caused by species of hantaviruses from the family Bunyaviridae. It is also known as Korean hemorrhagic fever, epidemic hemorrhagic fever, and nephropathis epidemica. The species that cause HFRS include Hantaan River virus, Dobrava-Belgrade, Saaremaa, Seoul, Puumala and other hantaviruses. It is found in Europe, Asia, and Africa. Of these species,

Hantaan River virus and Dobrava-Belgrade virus cause the most severe form of the syndrome and have the highest morbidity rates.

Both hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) appear to be immunopathologic, and inflammatory mediators are important in causing the clinical manifestations.

Epidemiology. HFRS is primarily a Eurasian disease, whereas HPS appears to be confined to the Americas. The geography is directly related to the indigenous rodent hosts and the viruses that coevolved with them.

Hantaan virus cycles among rodents, probably by aerosol or fomite transmission from infected rodent urine. Human infection is incidental. Transmission to humans is believed to occur via inhalation of, or contact with, rodent urine, droppings or saliva.

Clinical Manifestations. Symptoms of HFRS usually develop within 1 to 2 weeks after exposure to infectious material, but in rare cases, they may take up to 8 weeks to develop. Initial symptoms begin suddenly and include intense headaches, back and abdominal pain, fever, chills, nausea, and blurred vision. Individuals may have flushing of the face, inflammation or redness of the eyes, or a rash. Later symptoms can include low blood pressure, acute shock, vascular leakage, and acute kidney failure, which can cause severe fluid overload.

The severity of the disease varies depending upon the virus causing the infection. Hantaan and Dobrava virus infections usually cause severe symptoms, while Seoul, Saaremaa, and Puumala virus infections are usually more moderate. Complete recovery can take weeks or months.

The course of the illness can be split into five phases:

Febrile phase: Symptoms include redness of cheeks and nose, fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in the influenza virus, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.

Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.

Oliguric phase: This phase lasts for three to seven days and is characterised by the onset of renal failure and proteinuria.

Diuretic phase: This is characterized by diuresis of three to six litres per day, which can last for a couple of days up to weeks.

Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

This syndrome can also be fatal. In some cases, it has been known to cause permanent renal failure.

Hantavirus Pulmonary Syndrome (HPS)

Symptoms may develop between 1 and 5 weeks after exposure to fresh urine, droppings, or saliva of infected rodents.

Early symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. These symptoms are universal.

There may also be headaches, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain. About half of all HPS patients experience these symptoms.

Four to 10 days after the initial phase of illness, the late symptoms of HPS appear. These include coughing and shortness of breath, with the sensation of, as one survivor put it, a "...tight band around my chest and a pillow over my face" as the lungs fill with fluid.

Diagnosis. HFRS is difficult to diagnose on clinical grounds alone and serological evidence is often needed. A fourfold rise in IgG antibody titer in a 1-week interval, and the presence of the IgM type of antibodies against hantaviruses are good evidence for an acute hantavirus infection. HFRS should be suspected in patients with acute febrile flu-like illness, kidney failure of unknown origin and sometimes liver dysfunction.

Treatment. Treatment involves supportive therapy including renal dialysis. Treatment with ribavirin, administered within 7 days of onset of fever, results in a reduced mortality as well as shortened course of illness.

Control. Human inactivated vaccine is used to prevent hemorrhagic fever with renal syndrome.

Prevention. Rodent control in and around the home remains the primary prevention strategy, as well as eliminating contact with rodents in the workplace and campsite. Closed storage sheds and cabins are often ideal sites for rodent infestations. Airing out of such spaces prior to use is recommended. Avoid direct contact with rodent droppings and wear a mask to avoid inhalation of aerosolized rodent secretions.

ARENAVIRUSES

All arenaviruses have a rodent vector. The arenavirus-associated hemorrhagic fevers have a high case-fatality rate (5-35%). The hallmark of arenaviruses is their tendency to cause persistent silent infections in their natural hosts (rodents) and severe, often lethal, disseminated disease in humans. Suitable conditions for transmission of virus to humans occur in areas where humans come in contact with rodent urine that contains virus. Persistent viremia and viruria in rodents result from a slow or insufficient immune response when immunologically immature fetuses or neonates are infected. In humans, the disease is acute.

There are five pathogens of humans, who are only accidental hosts. Four cause severe hemorrhagic fever with a mortality of about 15% among hospitalized patients in circumscribed areas (Lassa virus in West Africa, Junin virus in the Argentine pampas, Machupo virus in Bolivia, and Guanarito virus

in Venezuela). The fifth, lymphocytic choriomeningitis (LCM) virus, is much more widely distributed, but causes milder infections, often neurologic (*Table 5*).

Table 5 – Principal Medically Important Arenaviruses

Virus	Disease	Occurrence
Lassa	Lassa hemorrhagic fever	Africa
Manchupo	Bolivian hemorrhagic fever	South America
Junin	Argentine hemorrhagic fever	South America
Whitewater Arroyo	Whitewater Arroyo hemorrhagic fever	Western United States
Lymphocytic choriomeningitis virus (LCMV)	Lymphocytic choriomeningitis	Widespead

Structure. The virus is round, oval, or pleomorphic, 110 to 130 nm in diameter, and enveloped. The genome consists of two distinct single-stranded viral RNA species. The arenaviruses have ambisense genomes: the 3' half is antisense, whereas the 5' half is positive-sense. During morphogenesis, sandy-appearing granules resembling ribosomes are found within the unstructured interior of nascent viruses. These particles give arenaviruses their name: arena is Latin for "sand." Highly purified arenaviruses appear to contain 18S and 28S host ribosomal RNAs. These RNAs do not seem to have a required role in virus replication.

Classification and Antigenic Types. All arenaviruses contain a set of internal cross-reacting antigens as well as species-specific envelope antigens.

Multiplication. Arenaviruses are thought to multiply like typical antisense RNA viruses (e.g. bunyaviruses).

Epidemiology. The arenaviruses exist in nature as benign infections in restricted rodent hosts (*Fig. 5*). The one common characteristic of these zoonotic infection patterns is human contact with rodent excreta.

The arenaviruses seem to establish persistent infections easily in certain rodents, which get a viremia and a viruria, and shed virus in urine, stools and saliva. Humans are thought to acquire infection from contact with contaminated materials, contaminated food, or aerosolized droppings, nesting materials, etc.

Transmission may also occur when infected urine or these other materials are directly introduced into broken skin or onto the mucous membranes of the eyes, nose, or mouth. In addition, individuals who work with live rodents can be exposed to hantaviruses through rodent bites from infected animals.

Frequent and explosive hospital-acquired infections in West Africa brought Lassa virus to the attention of the medical world 25 years ago. It is now clear that the virus is transmitted mostly at the village level and that most infections are mild or asymptomatic. For those sick enough to be admitted to the hospital,

mortality is about 15 percent. The other arenaviruses Ampari, Flexal, Ippy, Mobala, Mopeia, Latino, Parana, Pichinde, Tacaribe, and Tamiamican cause infections in laboratory personnel, especially when high concentrations of virus are being processed.

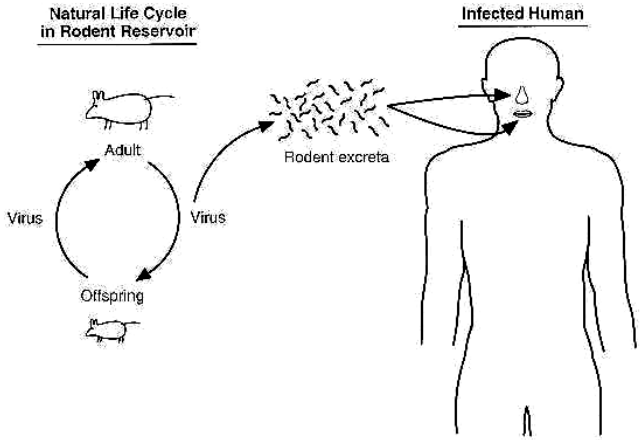

Fig. 5. Transmission of arenaviruses from rodent reservoirs to humans

Pathogenesis. The arenaviruses are not ordinarily contagious among humans, and they are nonpathogenic in their rodent hosts, but rodent-to-human infections can cause severe, sometimes fatal, disease (*Fig. 6*). This type of situation is not uncommon after interspecies transmission of viruses to humans, as with Marburg and Ebola viruses.

Infection is suspected to be caused by invasion through broken skin or the aerosol/respiratory route. Although aerosol and respiratory spread, as well as cuts and abrasions in the skin, are suspected, portal of entry of arenaviruses and time course of their systemic distribution are uncertain. The onset of the hemorrhagic fevers caused by Lassa, Junin, Machupo, and Guanarito viruses may be insidious, with the disease presentation within 7 to 14 days after infection simply as pyrexia, headache, sore throat, and myalgia. Virus can be recovered from the blood and serum for up to 3 weeks after onset of the infection, and Lassa virus can be recovered from the urine for up to 5 weeks. Hemorrhagic phenomena, heralded by unremitting high fever, can begin after day 5 of illness and are followed by dehydration and hemoconcentration, shock syndrome, hemorrhagic manifestations, and cardiovascular collapse. The pantropic nature of these viruses is revealed by their presence in various dysfunctional organs.

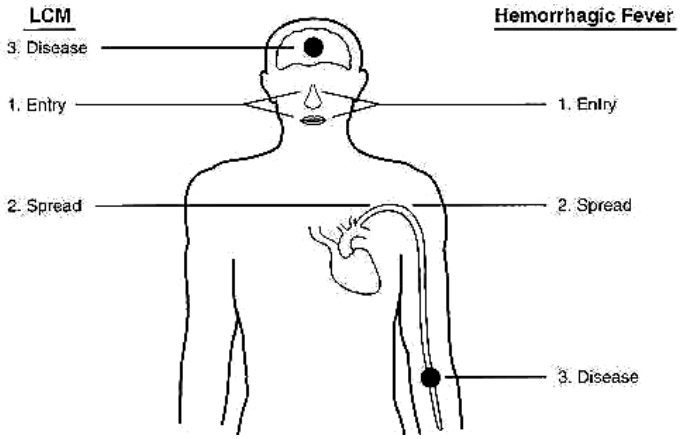

Fig. 6. Pathogenesis of lymphocytic choriomeningitis (LCM) and hemorrhagic fevers

Clinical Manifestations. Only lymphocytic choriomeningitis, Junin, Machupo, Guanarito, and Lassa viruses have demonstrated natural disease potential. About 70 percent of human lymphocytic choriomeningitis virus infections are asymptomatic or so mild that they cannot be distinguished from common respiratory or gastrointestinal illnesses. Headache, photophobia, listlessness, apathy, memory defects, confusion, and subtle mental difficulties are among the most common symptoms. Even though this infection can be temporarily debilitating, it is rarely fatal, and even when neurologic involvement occurs, complete recovery is usually seen.

The clinical presentation of Argentine hemorrhagic fever (Junin virus), Bolivian hemorrhagic fever (Machupo virus), Venezuelan hemorrhagic fever (Guanarito virus), and Lassa fever (Lassa virus) is similar in several ways, yet sufficiently different to warrant brief mention. The incubation period is probably around 10 to 14 days (as is also true for lymphocytic choriomeningitis virus infections). Disease onset usually begins with insidious progression of general malaise and fever over a 2- to 4-day period. Hepatitis is unusual or mild in the three Latin American hemorrhagic fevers, whereas it is frequent and moderately severe in Lassa fever.

Host Defenses. The humoral response is exceptionally slow. Cell-mediated immunity is probably of prime importance.

Interferon is induced by arenavirus infection, but is of questionable benefit. In general, arenaviruses are relatively resistant to the antiviral action of alpha/beta interferon. Interferon titers are significantly higher in fatal cases than in survivors (perhaps owing to higher levels of virus in the former). All evidence suggests that viral clearance in humans is complete and that chronic

infection is not established. Reinfection with Lassa virus is possible, but appears to be uncommon.

Diagnosis. Differential clinical diagnosis of the arenavirus hemorrhagic fevers is complex. The arenaviruses must be suspected if they are prevalent in geographic areas where infections have occurred and in regions known to harbor reservoir rodent species. Various diseases leading to sepsis, with disseminated intravascular coagulation and shock, can be confused with diseases caused by arenaviruses.

Differential clinical diagnosis is complex; the diagnosis is confirmed only by detecting a rise in antibody titers or by isolating the virus.

Junin and Machupo viruses are isolated primarily by intracerebral inoculation of newborn hamsters. Lassa virus is regularly isolated by inoculation of Vero cells. The most sensitive method for isolating LCM virus is intracerebral inoculation of weanling mice. If identification of specific viral antigens is the goal, antigen capture enzyme-linked immunosorbent assays (ELISA) are available. All arenaviruses appear to share antigenic determinants in the ribonucleoproteins, as well as antigenically distinct determinants in their outer glycoproteins. Positive immunofluorescent staining of acetone-fixed infected cells is definitive for more than just family identification, since with limiting dilutions of antibody, old World viruses (Lassa and lymphocytic choriomeningitis viruses) can be readily distinguished from New World viruses (Junin, Machupo, and Guanarito viruses). Arenavirus species may be identified by their unique surface glycoproteins and infectivity neutralization.

Control. Effective vaccines are becoming available. A live attenuated Junin virus vaccine has now been tested in about 100 volunteers, with a resulting humoral and cell-mediated response frequency of more than 95 percent. A Lassa virus glycoprotein gene has been cloned and expressed in vaccinia virus. This vaccine has offered a high degree of protection against disease and death in monkeys challenged with the intact Lassa virus. Plasma from convalescent patients has become the single specific therapeutic adjunct for patients severely ill with Bolivian and Argentine hemorrhagic fevers.

Early admission to the hospital, bed rest, oral hydration, sedation, and analgesia are important. In view of the frequency of Lassa virus transmission from person to person in a hospital setting, strict measures must be taken to isolate patients who have or are suspected to have the disease. Isolation of patients with the other pathogenic arenaviruses is also probably desirable.

Ribavirin has been proven to be effective against Lassa fever in humans.

FILOVIRUSES

Filoviruses belong to a virus family called *Filoviridae* and can cause severe hemorrhagic fever in humans and nonhuman primates. Two genera of the family that are commonly known are Ebola virus and Marburg virus. The Ebolavirus genus has been organized into five species: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SUDV), Reston ebolavirus (RESTV), Taï Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV).

Ebola and Marburg viruses cause hemorrhagic fevers and have a case-fatality rate which can be as high as 60-90% for certain strains of the viruses.

Structure. Filovirus virions (complete viral particles) may appear in several shapes, a biological features called pleomorphism. These shapes include long, sometimes branched filaments, as well as shorter filaments shaped like a "6", a "U", or a circle (*Fig. 7*). Viral filaments may measure up to 14,000 nanometers in length, have a uniform diameter of 80 nanometers, and are enveloped in a lipid (fatty) membrane. Each virion contains one molecule of single-stranded, negative-sense RNA. New viral particles are created by budding from the surface of their hosts' cells; however, filovirus replication strategies are not completely understood.

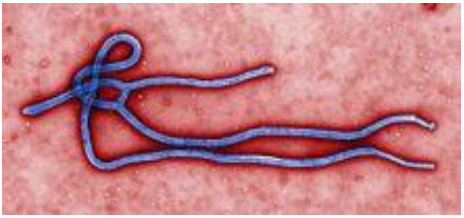

Fig. 7. Microphotograph of Ebola virus.

Filovirus history. The first Filovirus was recognized in 1967 when a number of laboratory workers in Germany and Yugoslavia, who were handling tissues from green monkeys, developed hemorrhagic fever. A total of 31 cases and 7 deaths were associated with these outbreaks. The virus was named after Marburg, Germany, the site of one of the outbreaks. In addition to the 31 reported cases, an additional primary case was retrospectively serologically diagnosed.

After this initial outbreak, the virus disappeared. It did not reemerge until 1975, when a traveler, most likely exposed in Zimbabwe, became ill in Johannesburg, South Africa. The virus was transmitted there to his traveling companion and a nurse. A few sporadic cases and 2 large epidemics (Democratic Republic of Congo in 1999 and Angola in 2005) of Marburg hemorrhagic fever (Margurg HF) have been identified since that time.

Ebolavirus was first identified in 1976 when two outbreaks of Ebola hemorrhagic fever (Ebola HF) occurred in northern Zaire (now the Democratic Republic of Congo) and southern Sudan. The outbreaks involved what eventually proved to be two different species of Ebola virus; both were named after the nations in which they were discovered. Both viruses showed themselves to be highly lethal, as 90% of the Zairian cases and 50% of the Sudanese cases resulted in death.

Since 1976, Ebolavirus have appeared sporadically in Africa, with small to midsize outbreaks confirmed between 1976 and 1979. Large epidemics of Ebola HF occurred in Kikwit, Democratic Republic of Congo in 1995, in Gulu, Uganda in 2000, in Bundibugyo, Uganda in 2008, and in Issiro, DRC in 2012.

The 2014-2015 Ebola epidemic is the largest in history, affecting multiple countries in West Africa with 12031 total laboratory-confirmed cases and 6478 total death.

Epidemiology. It appears that Filoviruses are zoonotic, that is, transmitted to humans from ongoing life cycles in animals other than humans. Despite numerous attempts to locate the natural reservoir or reservoirs of Ebolavirus and Marburgvirus species, their origins were undetermined until recently when Marburgvirus and Ebolavirus were detected in fruit bats in Africa. Marburgvirus has been isolated in several occasions from Rousettus bats in Uganda.

In an outbreak or isolated case among humans, just how the virus is transmitted from the natural reservoir to a human is unknown. Once a human is infected, however, person-to-person transmission is the means by which further infections occur. Specifically, transmission involves close personal contact between an infected individual or their body fluids, and another person. When an infection occurs in humans, the virus can be spread to others through direct contact (through broken skin or mucous membranes in, for example, the eyes, nose, or mouth) with blood or body fluids (including but not limited to urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with or has died from Ebola, objects (like needles and syringes) that have been contaminated with body fluids from a person who is sick with Ebola or the body of a person who has died from Ebola, infected fruit bats or primates (apes and monkeys), and possibly from contact with semen from a man who has recovered from Ebola (for example, by having oral, vaginal, or anal sex). There is no evidence that mosquitoes or other insects can transmit Ebola virus. Only a few species of mammals (e.g., humans, bats, monkeys, and apes) have shown the ability to become infected with and spread Ebola virus.

During recorded outbreaks of hemorrhagic fever caused by a Filovirus infection, persons who cared for (fed, washed, medicated) or worked very closely with infected individuals were especially at risk of becoming infected themselves. Nosocomial (hospital) transmission through contact with infected body fluids - via reuse of unsterilized syringes, needles, or other medical

equipment contaminated with these fluids - has also been an important factor in the spread of disease. Although in the laboratory the viruses display some capability of infection through small-particle aerosols, airborne spread among humans has not been clearly demonstrated.

During outbreaks of Ebola, the disease can spread quickly within healthcare settings. Healthcare providers caring for Ebola patients and family and friends in close contact with Ebola patients are at the highest risk of getting sick because they may come in contact with infected blood or body fluids. During outbreaks, isolation of patients and use of protective clothing and disinfection procedures (together called viral hemorrhagic fever isolation precautions or barrier nursing) has been sufficient to interrupt further transmission of Marburgvirus or Ebolavirus, and thus to control and end the outbreak.

Disseminated intravascular coagulation (DIC) leading to tissue ischemia and eventual depletion of clotting factors is a typical feature of filovirus infections.

Clinical Manifestations. *Symptoms of Ebola* include: fever, severe headache, muscle pain, weakness, fatigue, diarrhea, vomiting, abdominal (stomach) pain, unexplained hemorrhage (bleeding or bruising).

Symptoms may appear anywhere from 2 to 21 days after exposure to Ebola, but the average is 8 to 10 days.

Recovery from Ebola depends on good supportive clinical care and the patient's immune response. People who recover from Ebola infection develop antibodies that last for at least 10 years, possibly longer. It is not known if people who recover are immune for life or if they can become infected with a different species of Ebola. Some people who have recovered from Ebola have developed long-term complications, such as joint and vision problems.

Even after recovery, Ebola might be found in some body fluids, including semen for more than nine months.

Symptoms of Marburg HF. After an incubation period of 5-10 days, symptom onset is sudden and marked by fever, chills, headache, and myalgia. Around the fifth day after the onset of symptoms, a maculopapular rash, most prominent on the trunk (chest, back, stomach), may occur. Nausea, vomiting, chest pain, a sore throat, abdominal pain, and diarrhea may then appear. Symptoms become increasingly severe and can include jaundice, inflammation of the pancreas, severe weight loss, delirium, shock, liver failure, massive hemorrhaging, and multi-organ dysfunction.

Diagnosis. Diagnosing Ebola and Marburg hemorrhagic fever in a person who has been infected for only a few days is difficult because the early symptoms, such as fever, are nonspecific to Ebola infection and often are seen in patients with more common diseases, such as malaria and typhoid fever.

Ebola and Marburg HF viruses are detected in blood only after onset of symptoms, most notably fever, which accompany the rise in circulating virus

within the patient's body. It may take up to three days after symptoms start for the virus to reach detectable levels (*Table 6*).

Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, polymerase chain reaction (PCR), and IgM-capture ELISA can be used to confirm a case of Ebola and Marburg HF within a few days of symptom onset. Virus isolation may also be performed but should only be done in a high containment laboratory with good laboratory practices. The IgG-capture ELISA is appropriate for testing persons later in the course of disease or after recovery. In deceased patients, immunohistochemistry, or PCR of blood or tissue specimens may be used to diagnose Ebola and Marburg HF retrospectively.

Table 6 – Laboratory tests used in diagnosis of Ebola and Marburg HF

Timeline of Infection	Diagnostic tests available
Within a few days after symptoms begin	IgM ELISA
	PCR
	Virus isolation
Later in disease course or after recovery	IgM and IgG ELISA
Retrospectively in deceased patients	Immunohistochemistry
	PCR

Treatment. There is no specific prophylaxis and treatment for Ebola and Marburg HF. Supportive hospital therapy should be utilized, which includes balancing the patient's fluids and electrolytes, maintaining oxygen status and blood pressure, replacing lost blood and clotting factors, and treatment for any complicating infections.

REFERENCES

1. Microbiology. Handbook to laboratory classes in microbiology / A. Ya. Tsyganenko, I. L. Dikiy, V. L. Tkachenko et al. – Kharkiv : Osnova, 2005. – 210 p.
2. Tsyganenko A. Ya. Practical exercises of morphology of bacteria. Short textbook for students of high medical schools / A. Ya. Tsyganenko, V. N. Vasilchenko, N. I. Kovalenko. – Kharkiv : KSMU, 2012. – 270 p.
3. Ananthanarayan. Textbook of Microbiology. 9th Ed / Ananthanarayan, Paniker. – Orient Blackswan, 2013. – 657 p.
4. Manual of Clinical Microbiology / Karen C. Carroll et al. – 10th Edition. Vol. 2. – Washington, DC : ASM Press, 2011. – 2630 p.
5. Pfau C. J. Arenaviruses / C. J. Pfau. – Access: http://intranet.tdmu.edu.ua/data/cd/disk2/ch057.htm/

6. Schmaljohn A. L. Alphaviruses (Togaviridae) and Flaviviruses (Flaviviridae) / A. L. Schmaljohn, D. McClain. – Access: http://intranet.tdmu.edu.ua/data/cd/disk2/ch054.htm

7. Shope R. E. Bunyaviruses / R. E. Shope – Access: http://intranet.tdmu.edu.ua/data/cd/disk2/ch056.htm

ADDITIONAL REFERENCES

1. Gupte Satish. The Short Textbook of Medical Microbiology / Satish Gupte. – 9th Ed. – New Delhi : Jaypee Brothers Medical Publishers Ltd., 2006. – 509 p.

2. Manual of Clinical Microbiology / P. R. Murray et al. – 9th Ed. – Washington : ASM Press, 2007. – 2396 p.

3. CDC – Lassa fever. – Access: http://www.cdc.gov/vhf/lassa/

4. CDC – Ebola (Ebola Virus Disease). – Access: http://www.cdc.gov/vhf/ebola/index.html/

5. About Ebola Virus Disease. CDC. Retrieved 18 October 2014. – Access: http://www.cdc.gov/vhf/ebola/about.html/

6. CDC – Hantavirus Pulmonary Syndrome (HPS) - Hantavirus. Cdc.gov. 2013-02-06. Retrieved 2013-07-07. Access: http://www.cdc.gov/hantavirus/hps/

7. CDC – Marburg hemorrhagic fever (Marburg HF). Access: http://www.cdc.gov/vhf/marburg/prevention/index.html

8. Peters C. J. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome / C. J. Peters, G. L. Simpson, H. Levy // Annu Rev Med. 1999. – Vol. 50. – P. 531–545.

Учебное издание

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Методические указания по дисциплине «Микробиология, вірусологія и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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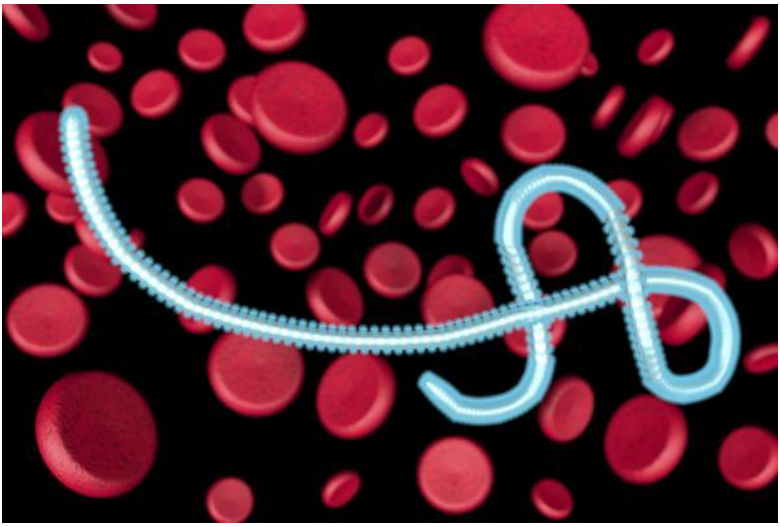

VIRAL ENCEPHALITIS AND HEMORRHAGIC FEVERS

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, Virology and Immunology)