Chapter 7 for IDCNA

Title: Viral Hemorrhagic Fevers other than Ebola and Lassa

Running title: Viral hemorrhagic fever

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Keypoints

- Viral hemorrhagic fevers represent a group of diseases caused by enveloped RNA viruses belonging to four taxonomic families: filoviruses, arenaviruses, bunyaviruses, and flaviviruses.
- Viral hemorrhagic fevers are severe febrile illnesses characterized by vascular abnormalities with plasma leakage and widespread bleeding in tissues and organs.
- Rapid identification of the viruses causing hemorrhagic fevers is fundamental for patient management, outcome improvement and limitation of disease propagation, particularly in healthcare settings.
- Treatment of viral hemorrhagic fevers is essentially supportive.

Summary/synopsis

Viral hemorrhagic fevers (VHFs) represent a group of diseases caused by enveloped RNA viruses belonging to four taxonomic families: filoviruses, arenaviruses, bunyaviruses and flaviviruse. The epidemiology of VHFs is broadly variable, ranging from geographically localized infections causing sporadic outbreaks, such as Omsk hemorrhagic fever and Kyasanur Forest disease, to more diffuse infections, such as Lassa fever in West Africa, that cause not only sporadic outbreaks but also endemic diseases. VHF viruses are considered as possible biological weapons, and are classified as category A bioweapon agents by the Centers for Disease Control and Prevention (CDC). The main characteristic of VHF is represented by severe febrile illnesses with hemorrhagic phenomena. Laboratory diagnosis of VHF take place in highly specialized reference laboratories. Mobile laboratories are under implementation to improve field diagnosis and contact tracing. Treatment of VHF is essentially supportive. In this paper we focus the attention on yellow fever and VHFs other than Ebola and Lassa virus diseases that have been described elsewhere in this publication. Yellow fever and other Flaviviruses causing VHFs (dengue, Omsk hemorrhagic fever, Kyasanur Forest disease and Alkhumra viruses

Introduction

The first definition of viral hemorrhagic fever (VHF) was given by Soviet investigators in the 1930s, while studying hantaviral hemorrhagic fever with renal syndrome.[1]

Currently, VHFs represent a group of diseases caused by enveloped single-stranded RNA viruses belonging to four taxonomic families:

- filoviruses (Ebola and Marburg);
- arenaviruses (Lassa and other Old World arenaviruses and New World arenaviruses);
- bunyaviruses (Congo-Crimean Hemorrhagic Fever, Rift Valley Fever, Huaiyangshan virus, alternatively known as severe fever with thrombocytopenia syndrome virus, and hantaviruses);
- flaviviruses (dengue, yellow fever, Omsk hemorrhagic fever, Kyasanur Forest disease, and Alkhumra viruses).[2,3]

The epidemiology of VHFs is broadly variable, ranging from geographically localized and sporadic infections to more diffuse outbreaks or endemic diseases.[1] A general overview on VHF agents, their reservoir and eventual arthropod vector is summarized in **Table 1**.

Considering that several experimental attempts demonstrated the possibility of infecting nonhuman primates through aerosolized viruses, in the 20th century different countries tried to weaponize VHF viruses.[4]

The agents of these infections are classified in risk groups 3 and 4, therefore their manipulation can be performed only in laboratories at the highest level of biocontainment (Biosafety level [BSL]-3 and -4). On the other hand, the diagnostic activities, although hampered by the scarcity of commercially available diagnostic methods, can be carried out, with due exceptions, in laboratories with lower levels of biocontainment. However, these are often complex diagnostic methods, afflicted by cross-reactivity, needing confirmation by specialized reference laboratories, especially for sporadic infections or at the beginning of an epidemic. Although molecular methods are of primary importance in laboratory diagnosis, serological methods for IgG and IgM detection assay can be helpful for the diagnosis of acute VHF, especially in case of short viremic period and viral shedding.[5]

Treatment of VHF is essentially supportive, consisting of administration of fluids, electrolytes, and blood products. Although no antiviral drugs are currently approved by United States Food and Drug Administration (FDA) for VHFs, small published trials described the use of intravenous ribavirin to treat Congo-Crimean Hemorrhagic fever and other VHFs with significant reduction in mortality.[6,7] Favipiravir, alone or in combination with ribavirin, seems to be active against different species of RNA viruses causing VHFs.[6] Treatment of VHF-infected patients should be performed maintaining appropriate barrier controls to prevent healthcare providers and laboratory personnel exposition.

For some of these infections effective human vaccines are available (such as yellow fever) and for others development and/or validation are ongoing (Dengue, Marburg), or relevant animal vaccines

have been developed (Rift Valley Fever virus). A list of recent VHF outbreaks (from January 2017 to April 2019) is summarized in **Table 2**.

In this paper we will focus the attention on yellow fever and VHFs other than Ebola and Lassa virus diseases that have been described elsewhere in this publication. ***Yellow fever and other Flaviviruses causing VHFs (dengue, Omsk hemorrhagic fever, Kyasanur Forest disease and Alkhumra viruses***

Flaviviruses are enveloped viruses characterized by a single stranded positive sense RNA molecule, which encodes for several non-structural (NS) and three structural proteins, envelope (E), precursor of membrane/membrane (prM/M) and capsid (C) protein.[8]

Among Flaviviruses, yellow fever virus (YFV) and dengue virus (DENV) can cause VHF. Besides, other flaviviruses can be responsible of hemorrhagic diseases in very limited areas of the world, such as Omsk hemorrhagic fever, Kyasanur Forest disease and Alkhumra viruses.[2]

1.1. Yellow fever

YFV is considered the prototype member of *Flavivirus* genus. YFV is endemic in tropical and subtropical regions of South America and Africa, and is transmitted by mosquitoes of the *Haemogogus*, *Sabethes*, and *Aedes* genera.[9] The virus was introduced in South America from Africa with the slave trade in the 16th century (**Figure 1**).[10]

Zoonotic cycle involving sylvatic mosquitoes (*Haemogogus* and *Sabethes* in South America, *Aedes* in Africa) and nonhuman primates occurs in tropical forests, where humans can be accidentally bitten by sylvatic mosquitoes that previously fed on a viremic monkey (jungle yellow fever). Infected humans can introduce the virus in an urban cycle, where the main vector is represented by *Aedes aegypti* (urban yellow fever). Noteworthy, in Africa YFV transmission can be sustained also by a mixed cycle (usually in the savannah), involving both sylvatic and domestic vector species and humans living or working in jungle border areas.[2,9] YFV is also maintained in mosquito populations through vertical (trans-ovarial) transmission.[9] Approximately 80.000–200.000 YFV cases are reported worldwide every year, with a case fatality rate ranging from 20% to 60%.[11]

After an incubation period of 3–6 days following the bite of an infected mosquito, yellow fever classical picture is characterized by three clinical stages. The disease begins with flulike symptoms (viremic period), lasting for 3-5 days and characterized by fever, headache, malaise, photophobia, myalgia, irritability, nausea, and vomiting. During this period, the blood is infectious to biting mosquitoes. The viremic period is followed by a remission period of 1–2 days. Subsequently, some patients (20–60%) progress to the third phase (period of intoxication) in which the patients become severely ill with signs of liver and renal failure. This phase is characterized by hemorrhages, jaundice, thrombocytopenia, and the disease can progress to more generalized multiorgan dysfunction,

vasculopathy, and even death.[12] This final stage is characterized by thrombocytopenia, coagulation abnormalities, deficiency of liver clotting factors and elevated levels of fibrin split products, which indicate disseminated intravascular coagulation (DIC).[12]

According to current models, YFV is transmitted from the mosquito salivary glands to the host's dermis, where the virus infects dendritic cells and is transported to the lymph nodes. In the lymph nodes YFV replicates and spreads through the peripheral blood, reaching the liver, where the virus infects Kupffer cells and hepatocytes, and induces apoptosis and necrosis, which result in the liver damage observed during the toxic phase of the infection.[11] Other organs can also be involved, as reported in humans[13] and in animal models.[14]

YFV pathogenesis relies on hepatocyte apoptosis induced by the virus itself and indirectly by host immunity, via an unbalanced cytokine production and CD4+ and CD8+ T lymphocytes immune response. [15] Moreover, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and transforming growth factor (TGF)-β are increased in the liver of fatal human cases, suggesting their pathogenetic role.[12,15]

Diagnostic procedures are based on IgM and IgG detection through different methods, such as ELISA, IFA and serum neutralization tests (which remains the gold standard reference for detecting specific IgM and IgG.[16] Molecular diagnostic is based on RT-PCR, which can detect YFV in clinical specimens (whole blood, serum, and also urine[17]) at a low virus concentration. New approaches are based on loop mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) assays. NS1 antigen detection by ELISA represents a promising test with high sensitivity and specificity for the diagnosis of acute yellow fever.[18] Virus isolation can be achieved with cell culture (using Vero cells or C6/36 *Aedes albopictus* cells) or by inoculation of infected samples in suckling mice or hamsters.[18,19]

No approved antiviral drugs against YFV are currently available. An effective vaccine based on the live attenuated YFV-17D virus confers long-lasting protection against the disease, in both immunocompromised and healthy individuals.[20] In 2014, the WHO indicated that a single dose of YFV-17D vaccine provides sustained immunity and lifelong protection.[21]

1.2. Dengue fever

There are four antigenically distinct DENVs, which are named DENV 1, 2, 3, and 4. Dengue infection is transmitted by *Aedes* mosquitoes in the intertropical regions worldwide.[1] In dengue-endemic countries, DENV serotypes co-circulate in the same area at the same time, causing concurrent infections.[22] Little is known about the role of possible animal reservoirs for dengue transmission.[23] Autochthonous cases of DENV infections sustained by *Aedes albopictus* have been described in Europe (France and Spain)[24] and in some states of the United States of America (Florida, Hawaii and Texas)[25] (**Figure 2**). In *Aedes* mosquitoes, natural vertical transmission of DENV, from infected adults

to some part of their offspring, has been described, representing an important phenomenon for explaining endemicity.[26]

The incidence of dengue infections is estimated to be around 400 million per year, of which about 25% are symptomatic. Asia accounts for 75% of the dengue disease burden.[27] The 2009 WHO dengue case classification identified symptomatic individuals as having dengue without major complications, or as having severe dengue if they experienced complications in any of three categories: (1) plasma leakage causing shock syndrome or respiratory distress, (2) severe bleeding, or (3) severe organ impairment.[28] Severe dengue occurs more frequently during reinfection (with a heterologous infecting serotype compared to the primary infection) and in infants from mothers who have previously had dengue during pregnancy, in the phase in which maternal antibodies wane to sub-neutralizing titers.[29,30] In hyperendemic areas, the risk for severe disease is also high in pregnant women, especially during the third trimester.[31] Additionally, in regions with low endemicity, severe clinical disease is reported more frequently among adults with underlying comorbidities.[32]

Dengue is transmitted by the bite of an infected female mosquito. Non-vector transmission is also possible, through blood transfusion, organ transplantation, needle stick injuries, and mucosal splashes.[33] Although DENV has been detected in semen[34] and vaginal secretions[35] of human beings, sexual transmission has not been reported, so far. Vertical transmission is common among mothers who are viremic at delivery; no cases of transmissions through maternal milk have been reported.[36]

Both viral and host factors contribute to dengue infection pathogenesis. Among viral factors, NS1 protein seems to play a crucial role, interacting with several host proteins, being secreted from infected cells with the function of protecting the virus from complement and lectin-mediated neutralization. Moreover, NS1 may disrupt the endothelial glycocalyx increasing vascular permeability and contributing to vascular leakage.[37] Considering host factors, the adaptive immune response after infection with any serotype of DENV provides long-term immunity to the homologous virus, but protection against heterologous DENVs is short-lived. Previous infection with one DENV serotype increases the risk of severe dengue upon secondary infection with a heterologous virus (original antigenic sin).[38] This phenomenon has been explained with the theory of the antibody-dependent enhancement (ADE): cross-reactive antibodies at sub-neutralizing concentrations bind heterologous DENV and facilitate virus entry through Fc receptors expressed on target cells.[30] However, only a fraction of infections occurring in the presence of non-neutralizing IgG progresses to severe dengue, indicating that appropriate antibody-to-virus ratios are required for ADE.[37]

DENV infections occurring in childhood are mostly asymptomatic. In adults, symptomatic dengue typically begins abruptly and follows three phases: the febrile, critical, and recovery phases. After an incubation period of 4–7 days (maximum 14 days), symptomatic infection manifests and is

characterized by a sudden onset of fever, myalgia, retroorbital headache, nausea, vomiting, conjunctival congestion, and a maculopapular rash with generalized lymphadenopathy. This phase lasts for 2 to 7 days. Complications can develop around the time of defervesence, marking the onset of the critical phase. During this phase vascular permeability is increased, and progression to dengue shock syndrome can occur. Conventionally, hemoconcentration of 20% or more marks the condition of dengue associated plasma leakage. Moreover, volume depletion causes the narrowing of the pulse pressure (PP= the difference between the systolic and diastolic blood pressure). A PP equal to or less than 20 mm Hg, defines the state of dengue shock syndrome.[37,39] During the critical phase, hemorrhagic manifestations are often observed. Hemorrhages involve the gastrointestinal mucosa, skin, pulmonary alveoli, and serosal surfaces.[39] Severe organ impairment can also be observed during the critical phase, especially in individuals with underlying diseases. Even in patients who develop complications, good supportive care can assure full recovery, within 1–2 weeks.[1,37]

During the first 5 days, DENV infection can be diagnosed by virus isolation in cell culture, detection of viral RNA by RT-PCR, or detection of viral antigens such as NS1 by ELISA or rapid tests from blood and urine. DENV-RNA amplification and sequencing allow also for serotype identification. After 4–5 days from symptom onset, specific IgM and IgG antibodies can be detected with serological assays (ELISA, IFA, hemagglutinations-inhibition test). In patients with a past dengue (or other flaviviruses) infection, dengue IgG titers rise rapidly within the first week of illness. Serological assays require paired (acute and convalescent) samples and neutralization assay to confirm specificity.[28] Currently, the combination of NS1 antigen, IgM and IgG testing at point of care has improved the diagnosis of dengue.[37]

No antiviral drugs able to reduce DENV viral load or prevent complications are currently available. Ivermectin is under evaluation as an anti-DENV molecule in a clinical trial still in progress (ClinicalTrials.gov number NCT02045069). NS4B inhibitors are under development. Steroid use is controversial.[37] In 2015, Sanofi Pasteur licensed the first recombinant, live attenuated, tetravalent vaccine, based on the yellow fever 17D backbone (CYD-TDV or Dengvaxia).[40] Post-marketing analyses revealed an excess risk of severe dengue in seronegative vaccine recipients, compared with seronegative non-vaccinated individuals.[41] In 2018 WHO recommended pre-vaccination screening in order to vaccinate only dengue-seropositive persons.[42] Two chimeric live attenuated dengue vaccines are currently in phase 3 trials.[37]

1.3. Other flaviviruses causing hemorrhagic fevers (Omsk hemorrhagic fever, Kyasanur Forest disease, and Alkhumra viruses)

Omsk hemorrhagic fever virus (OHFV) was first isolated from a patient in 1947, and later from ticks belonging to the species *Dermacentor reticulatus*, muskrats and other vertebrates and arthropods in

the rural region of Omsk (Siberia). Although antigenically and genetically distinct, OHFV is strictly related to tick born encephalitis (TBE) virus. OHFV can be found in forest–steppe of western Siberia (**Figure 3**).[43,44]

The classic route of transmission is a tick bite in the endemic regions. *D. reticulatus* is the natural reservoir, and OHFV is transmitted trans-stadially and trans-ovarially. Recently, most human cases have been related to direct contact with infected muskrats (*Ondatra zibethica*) in which the virus has been isolated from urine and feces.[43]

After an incubation period of 3-7 days, clinical manifestations of OHFV infection are characterized by fever, headache, myalgia, cough, and sometime petechial rash. This phase lasts for 5-12 days, followed by recovery or a more severe second febrile phase, during which meningeal signs can appear without neurological involvement. In this phase hemorrhagic manifestations are frequent but not severe, and are due to vascular and circulatory capillary damage. Mortality rates range from 0.5% to 3%.[43,45]

Diagnosis relies on OHFV-RNA detection through RT-PCR and specific IgM and IgG detection in patients' sera through ELISA or hemagglutinations-inhibition test.[43] There is some evidence that TBE vaccine could confer cross-protection against OHFV, although this has not yet been formally demonstrated. An OHFV formalin-inactivated vaccine was developed in 1948, but its use was abandoned because of neurological side effects.[43,45]

Kyasanur Forest disease virus (KFDV) was first isolated in the Indian state of Karnataka in 1957, after an outbreak involving monkeys in Kyasanur Forest and people living near the forest (Figure 3). Transmission of KFDV is mainly due to the bites of infected ticks from the genus *Haemaphysalis*. Natural hosts of KFDV are the Blanford rat (*Rattus blanfordi*), the striped forest squirrel (*Funambulus tristriatus tristriatus*), and the house shrew (*Suncus murinus*).[46] The annual incidence of KFD in India is estimated to be around 400–500 cases with seasonal outbreaks.[44]

The incubation period of KFDV in humans is 3-8 days. The clinical presentation of KFD is usually biphasic. In the first phase, patients usually present with sudden onset of fever, headache and generalized body pain. Conjunctivitis and gastrointestinal symptoms (vomiting, abdominal pain and diarrhea) occur in the majority of patients in this phase. Hemorrhagic manifestations may begin 3-4 days after symptom onset, and are characterized by mucosal bleeding, ocular involvement, hematemesis, epistaxis, rectal bleeding. Persistence of hemorrhagic manifestations is usually associated to poor outcome. Most patients recover in 10-14 days. Up to 20% of patients may present with biphasic illness and the second phase is characterized by neurological symptoms. Mortality rate of KFDV infection ranges from 2% to 10%.[47]

Diagnosis is based on KFDV-RNA detection through RT-PCR and specific IgM and IgG detection in patients' sera through ELISA.

A formalin-inactivated KFDV vaccine derived from infected cell cultures has been produced and used in India. However, the current vaccine protocol showed limited efficacy.[44,47]

In 1994 in Saudi Arabia a genetic variant of KFDV caused several outbreaks. This genetic variant was named **Alkhumra (or Alkurma) hemorrhagic fever virus (AHFV)** (**Figure 3**).[48] This disease was mainly found in sheep handlers, butchers and meat consumers. AHFV can be transmitted through direct contact with blood or secretions of infected animals or eventually through the bite of *Ornithodoros* soft ticks and *Hyalomma* hard ticks,[49] mosquito bites.[50] Oral transmission via camel milk has also been reported. [46]

Clinical characteristics of AHFV infection are an acute febrile flulike illness with hepatitis, hemorrhagic manifestations, and encephalitis. Mortality rate is 25%.[50,51] Geographical distribution includes Arabic peninsula and east Africa (Djibouti and the border region between Egypt and Sudan).[49] In Europe imported cases affecting travelers have been reported[52] and Alkhumra virus has been detected in ticks collected from migrating birds.[49]

2. Filoviruses: Marburg virus disease

Filoviruses show a filamentous morphology and are characterized by three distinct genera: Ebolavirus (EBOV), Marburgvirus, and Cuevavirus.[53]

Marburg virus (MARV) was first identified in 1967, when laboratory workers in Germany and Yugoslavia (now Serbia) were infected with a previously unknown infectious agent. The source of infection was traced back to African green monkeys (*Chlorocebus aethiops*) that had been imported from Uganda.[54]

Marburgvirus genus consists of a single species, *Marburg Marburgvirus,* with two variants: Marburg and Ravn virus, and causes disease in human and nonhuman primates. MARV has been isolated from Egyptian fruit bats (*Rousettus aegypticus*), which represents the major natural reservoir. Insectivorous bats and the urban-dwelling straw-colored bat may also represent viral carriers.[53] The virus is not known to be native in counties outside African continent.[53] MARV is transmitted to humans by contact with infected animals (nonhuman primates or fruit bats) or their body fluids or tissues. However, human-to-human infection occurs with direct contact with droplets or body fluids from infected persons, or contact with equipment and other objects contaminated with infectious blood or tissues.[1]

The viruses enter the body through small skin lesions or mucosal membranes. Cells of the mononuclear phagocyte system have been identified as early targets in human patients. Early sites of virus replication are the lymph nodes, liver, and spleen where the most severe necrotic lesions are

observed. Lymphatic circulation and the bloodstream contribute to the dissemination of the virus to multiple organs, resulting in a systemic infection. Hepatocytes, adrenal cortical and medullary cells and fibroblasts are permissive to MARV infection, as well as endothelial cells during late stages of MARV infection. Despite high viral load, only minor inflammation is observed in infected tissues, indicating a dysregulation in the immune response. Liver involvement is characterized by an impairment in the production of coagulation factors. Although lymphocytes are not susceptible to MARV infection, bystander lymphocyte apoptosis is a characteristic of MARV infection.[54]

Marburg disease has an incubation period ranging from 3 to 21 days (typically 5 to 10 days). The disease manifests abruptly with nonspecific flulike symptoms (chills, fever, myalgia, general malaise), followed by lethargy, nausea, vomiting, abdominal pain, anorexia, diarrhea, coughing, headache, hypotension and a maculopapular rash. Hemorrhagic manifestations do not occur in all cases and vary in severity. Early symptoms are similar in survivors and non-survivors, while blood test revealed 100- to 1000-fold higher levels of viremia in non-survivors compared to survivors. Fatal cases progress to more severe symptoms by days 7 to 14 after the onset of the disease. Survivors experience a prolonged convalescence.[54–56] Fatality rate has been estimated to be 82% in low income countries, and 24% in patients receiving care in Europe and in the United States.[1]

Diagnosis is based on RNA viral detection by RT-PCR (on blood samples and tissues) and serological assays for IgG and IgM detection. Recently an isothermal assay for RNA amplification has been developed (LAMP), with the potential of improving MARV infections diagnosis.

No approved treatments for MARV infection are available, so far. Supportive care represents the primary treatment. There are currently no licensed vaccines available against MARV, however, several vaccine obtained from different platforms have shown potential to protect nonhuman primates from MARV infection, including DNA vectors, virus like particles, recombinant adenovirus vectors and recombinant vesicular stomatitis virus vectors.[57,58]

3. New World Arenaviruses

The family *Arenaviridae* consiss of three separated genera: *Mammarenavirus*, *Reptarenavirus*, and *Hartmanivirus*. The genus *Mammarenavirus*, encompassing viruses that infect mammals, is further divided into the Old World (OW) and New World (NW) arenaviruses.[59]

All members of the family have a negative sense, bi-segmented single-strand RNA genome consisting of a large (L) and small (S) segment.[60]

The natural host for OW arenaviruses is represented by rodents belonging to the sub-family *Murinae* of the *Muridae* family of mice. OW arenavirus species include Lymphocytic choriomeningitis virus (LCMV), Lassa virus (LASV) and the newly emerged Lujo virus. The OW arenaviruses are geographically confined to the African continent, with the exception of LCMV.[61] The NW

arenaviruses are geographically distributed in South and North America (**Figure 4**). The natural host for the NW arenaviruses is the *Sigmodontinae* sub-family of *Muridae* family mice with the exception of Tacaribe virus (TCRV), which is found in *Artibeus* bats. The NW arenaviruses are further divided into four clades: A, B, C, and D (also known as A/Rec). The human hemorrhagic pathogens Junin virus (Argentine Hemorrhagic Fever), Chapare and Machupo viruses (Bolivian Hemorrhagic Fever), Guanarito virus (Venezuelan Hemorrhagic Fever), Sabia, Cupixi and Amapari viruses (Brazilian Hemorrhagic Fever), cluster in clade B together with the prototypic Tacaribe virus (causing hemorrhagic fever in Trinidad). North American viruses Whitewater Arroyo, Bear Canyon and Tamiami viruses belong to clade D (A/Rec).[61,62] Of these viruses, Junin virus (JUNV) is the most relevant pathogen, with approximately 300–1000 cases per year (before the development of the Candid#1 vaccine).[63] Humans become infected through contact with infected rodents, or inhalation of their urine or feces.[62] Although rare, human-to-human transmission of NW arenaviruses has been described and may occur via direct contact with infected body fluids of viremic patients.[64]

The clade B pathogenic NW arenaviruses use transferrin receptor 1 (TfR1) to infect human cells. TfR1 is expressed in several human cell types, thus NW arenavirus can infect a large number of target sells.[65] Studies in humans and animal models, showed that macrophages and dendritic cells represent the main targets for NW arenavirus after airborne infection, causing aberrant cytokine production and bystander effects on endothelial cells.[61–63] Specifically, NW arenaviruses proteins have been shown to block type I interferon production.[66] Moreover, patients with NW arenaviruses showed high levels TNF-α, and other inflammatory mediators that correlate with the severity of disease.[67]

Among the NW arenaviruses, Chapare virus (CHPV) and Sabia virus (SABV) infections have been identified as single cases, whilst JUNV, Machupo virus (MACV) and Guanarito virus (GTOV) have been associated to larger outbreaks.[62]

In the setting of JUNV infection and Argentine hemorrhagic fever (AHF), incubation ranges from 1 to 2 weeks. AHF manifests with fever, asthenia, muscular pain, dizziness, skin and mucosal rashes, and lymph node swelling. After 6–10 days from symptom onset, disease worsen with cardiovascular, gastrointestinal, renal and neurological involvement, associated with coagulation abnormalities and hemorrhages. Similarly, patients infected with MACV may exhibit gingival hemorrhage, nausea, gastrointestinal bleeding, thrombocytopenia, hematuria, tremor, anorexia and respiratory distress.[67] Hemorrhages observed in NW arenavirus infections are caused by coagulation abnormalities and marked thrombocytopenia, while DIC has not been observed, like in other VHF.[62,67]

Diagnosis relies on arenavirus-RNA detection by RT-PCR from serum, plasma, urine, and throat wash samples and from several human tissues. The identification of the specific arenavirus causing the

disease can be performed through viral RNA sequencing. Serological diagnosis is based on the detection of specific IgG and IgM antibodies by immunofluorescence (IF) tests and ELISA. Virus isolation can be achieved by propagation in cell culture (Vero cells).[68] NW arenaviruses are considered risk group 4 pathogens.[62]

Supportive therapy is essential during NW arenaviral infections. Ribavirin showed some efficacy in reducing fatality rates during Lassa fever and other arenaviral diseases, although few data are currently available for NW arenaviruses. New drugs are currently under development, such as polymerase and viral budding inhibitors, and small interfering RNA (siRNA). Convalescent serum from JUNV infected patients was effective in reducing fatality rate of Argentine hemorrhagic fever in a double blinded trial.[69] However, little is known about cross-protection against other NW arenaviruses.[68,70]

A live attenuated JUNV vaccine (Candid#1) is currently available. Its efficacy was proven in a double-blind trial and was able to significantly decrease the incidence of Argentine hemorrhagic fever.[62,70]

4. Bunyaviruses

Bunyaviruses belonging to *Phlebovirus*, *Nairovirus*, and *Hantavirus* genera have been associated to VHF and include Rift Valley fever virus (RVFV), Crimean-Congo hemorrhagic fever virus (CCHFV), and several hantaviral agents causing hantavirus cardiopulmonary syndrome (HCPS) and hemorrhagic fever with renal syndrome (HFRS).[71] Recently an emerging thick-born infection, due to a *Phlebovirus* known as Huaiyangshan virus, which causes severe fever with thrombocytopenia syndrome (SFTS), has been identified in China, South Korea and Japan. [3,72]

4.1. Rift Valley fever virus

Rift Valley Fever (RVF) was first described in 1930, during an outbreak characterized by high abortion rate amongst pregnant ewes associated to a high mortality rate of newborn lambs, in the Rift Valley region of Kenya (**Figure 5**). The causative agent of RVF was then identified in South Africa in 1951.[73] RVFV belongs to the *Phlebovirus* genus and its viral genome contains three single-stranded, negative-polarity RNA segments.[74]

The epidemiology of RVFV is complex and involves mosquitoes, wild animals, domesticated livestock, and humans. RVFV has been isolated from a wide range of mosquito genera (*Aedes, Culex, Anopheles, and Mansonia*).[75] *Aedes* mosquitos maintain RVFV in nature by trans-ovarial transmission.[76] RVFV alternates between mosquitoes and vertebrate hosts. Evidence of RVFV infection has been found in many wild mammalian species in Africa causing mild or inapparent illness in these species. Conversely, RVFV is highly pathogenic in domesticated ruminants, in which the virus replicates with high viral loads.[77] Humans can be infected by the bite of a mosquito, although mucous membrane exposure or inhalation of viral particles during the handling of infected

animals represent the primary means of transmission of the virus to humans. There is no documented human-to-human transmission.[77,78] Women with acute RVFV infection during pregnancy have a higher rate of miscarriage and vertical transmission has been reported.[79]

The non-structural protein encoded by the small (S) and medium (M) RNA segments of the virus, NSs and NSm, respectively, seem to be the major virulence factors, counteracting immune response and modulating host cell apoptosis.[80] The factors determining disease severity are still unknown. Genetic polymorphisms involving genes of the innate immunity may contribute to RVF severity.[65]

Comorbidities can influence disease outcome and complication onset. HIV infection appears to be associated with a higher incidence of neurological complications and death.[79]

Most infected people develop uncomplicated RVF, which is characterized by flulike symptoms, sometimes with a biphasic fever. Symptoms can be debilitating, and convalescence may take several weeks.[77] Complicated RFV usually manifests with ocular complications (up to 10% and characterized by uveitis, retinitis, vasculitis retinal hemorrhages);[81] severe hepatic disease (1-2%, jaundice and hemorrhagic manifestations, including gastrointestinal bleeding);[77,82] neurological disease, typically with a delayed-onset (severe headache, hallucination, disorientation, vertigo, excessive salivation, and weakness or partial paralysis).[82]

Blood samples from acutely infected people can be tested for the presence of RVFV-RNA by RT-PCR, multiplex PCR-based microarray assay, isothermal amplification methods (LAMP), and RPA. Antigen detection can be performed by ELISA. Isolation of live virus can be performed in suckling mice or in cell cultures. Serologic tests for the detection of specific IgG and IgM by ELISA, IFA and hemagglutinations inhibition assay are available.[73,77,82]

There are no specific treatments for RVF. Ribavirin is considered a potential antiviral drug for RVF because of its in vitro efficacy. Although no licensed vaccine preparations for use in humans are available so far, three licensed veterinary vaccines are being utilized to protect ruminant populations. Inactivated, live attenuated and innovative vaccine are currently under development.[73]

4.2. Crimean-Congo hemorrhagic fever

Crimean-Congo hemorrhagic fever virus (CCHFV) is a *Nairovirus* of the Bunyaviridae family, with a genome consisting of three negative-sense single-stranded RNA molecules.[43] It was first identified in 1944 in the Crimean Peninsula and then isolated in Congo, in 1956.[83] CCHFV is widely distributed in Africa, the Middle East and central and southwestern Asia. It has also been found in different European countries.[84] In 2017, Bulgaria reported two confirmed cases of CCHF. CCHFV is endemic in the Balkans, and Bulgaria regularly reports a small number of cases occurring every year. The United Kingdom reported one case in 2014. In 2016, for the first time, autochthonous human cases were reported in south-western Europe (Spain). The primary case most likely became infected through

contact with a tick while hiking in Ávila Province. The secondary case was a healthcare worker who looked after the patient while in intensive care (**Figure 6**).[85]

CCHF is a thick-born VHF. The virus has been isolated from at least 30 species of tick (28 *Ixodidae* and 2 *Argasidae*). However, *Argasidae* are not capable of serving as vectors. Many *Ixodidae* tick species can transmit the virus to humans, but the genus *Hyalomma* represent the most effective vector.[43] CCHFV is maintained in nature by *Ixodidae* species by trans-stadial and trans-ovarial transmission.[83] Human-to-human transmission is also reported, especially in healthcare settings.[86] CCHFV dysregulates the immune response with the consequence of a marked viral replication, vascular system alterations and lymphoid organs involvement. Infection of the endothelium plays a crucial role in CCHFV pathogenesis. The endothelium is directly targeted by viral infection and replication, and indirectly affected by host-derived soluble factors, which can cause endothelial activations and dysfunction. Endothelial damage contributes to platelet degranulation, with consequent activation of the intrinsic coagulation cascade, consumption of coagulation factors and DIC.[87,88]

After an incubation period of 2–7 days, early signs of the disease include fever, hypotension, conjunctivitis, headache, dizziness, neck pain, nuchal rigidity, photophobia, retro-orbital pain, myalgia and arthralgia, skin rash, nausea, vomiting, diarrhea and abdominal pain (pre-hemorrhagic period). Later (after 4-5 days from symptom onset) patients may develop hemorrhagic manifestations (petechial rash, ecchymoses, hematemesis and melena), DIC and circulatory shock (hemorrhagic period). Hepatomegaly and splenomegaly represent common features of CCHFV infection. Survivors enters in the convalescent period usually after 10 days from symptom onset.[88] The mortality rate of CCHF is between 3% and 30%.[43,88]

Early diagnosis is essential for patient survival and nosocomial infection prevention.[88] Classic RT-PCR is considered the method of choice for rapid laboratory diagnosis of CCHFV infection on blood and urine samples.[89] IgG and IgM antibodies can be detected with ELISA and IFA from about day 7 after the onset of the disease. Viral isolation can be achieved by intrathecal inoculation of viremic blood or urine in newborn mice or in cell culture.[90] A consensus document on laboratory management of CCHFV infection has been recently published.[91]

Ribavirin is the only available drug with a demonstrated antiviral effect against CCHFV in vitro and in animal models,[92,93] although its effectiveness in humans is controversial.[94] A vaccine for CCHFV derived from inactivated virus obtained from mouse brain, is used in Bulgaria. Its efficacy has not been well established. New potential antiviral compounds and therapeutic approach are under evaluation.[88,90]

4.3. Hantavirus cardiopulmonary syndrome and hemorrhagic fever with renal syndrome

Hantaviruses are negative-sense single-stranded RNA viruses, with a genome consisting of three distinct RNA segments.[95]

In humans, Old World hantaviruses can cause HFRS (including also a milder form, called nephropathia epidemica and caused by Puumala virus), while New World hantavirus can cause HCPS. Currently, 150.000 to 200.000 cases of hantavirus disease occur yearly, the majority being reported in Asia, with fewer infections reported in the Americas and Europe (**Figure 7**).[96]

Hantaviruses are directly transmitted to humans by small rodents, which represent the natural reservoir and influence hantavirus geographical distribution.[97,98] Transmission to humans occurs via inhalation of aerosols derived from the urine, feces or saliva of infected animals, but can also be due to infected rodent bites. In the environment, virus particles remain infectious for several weeks.[99]

Table 3 summarizes pathogenic hantaviruses and their geographical distribution.

The primary target of hantavirus infection is the endothelium of different organs and tissue, including lung and kidneys. The main endothelial receptor for pathogenic hantaviruses is β3-integrin. Although hantavirus infection of endothelial cell is non-cytolytic, this event is followed by impairment of the barrier function, fluid extravasation and organ failure. One hypothesis is that the host cellular immune response, sustained by cytotoxic CD8+ T cells, may cause endothelial disfunction.[95,100] However, animal model did not confirm this hypothesis.[101] Some studies showed higher frequency of hantavirus-specific CD8+ T cells in patients with a severe outcome, as compared with those with a mild/moderate outcome.[96,102] Host factor such as HLA B*35 have been associated to an increased risk for severe HCPS outcomes.[103] During convalescence in patients with severe HCPS viral genome can be detected up to 90 days after the onset of clinical symptoms.[96]

Hantaviruses can cause two zoonotic diseases in humans: HFRS or HCPS.[96]

For HFRS, after an incubation period ranging from 10 days to 6 weeks, clinical symptoms appear and are characterized by a first febrile phase (1-7 days) with fever accompanied by nonspecific signs (myalgia, headache, abdominal pain and malaise). Neurological, ocular, cardiovascular and gastrointestinal symptoms can also be present at this stage. Conjunctival and mucosal hemorrhages can appear. Subsequently, patients enter a hypotensive phase (1-3 days) characterized by vascular leakage, associated with thrombocytopenia, shock and mental confusion. The following oliguric phase (2-6 days) is characterized by urine output decrease, with possible hypertension, pulmonary edema and renal insufficiency. Approximately 50% of all fatalities occur in this phase. Survivors enter in the polyuric phase (lasting for several weeks), which is followed by the convalescent phase (3-6 months) characterized by residual fatigue and weakness. Severity and case fatality rates of HFRS depend on the specific causative agent.[100]

In the setting of HCPS, the incubation period is widely variable raging from 7 to 39 days.[104] Symptoms first manifest with a flulike syndrome (febrile phase), which may last up to 5 days and be characterized by fever, myalgia, headache, malaise and arthralgia. Gastrointestinal and neurological signs may also occur. Laboratory tests show thrombocytopenia, leukocytosis or leukopenia, a high hematocrit, peripheral immunoblasts, lymphocytosis, abnormal liver enzyme levels, a mild increase in creatinine, hyponatremia and proteinuria. A half of the infections evolve to the cardiopulmonary phase, characterized by capillary leakage and low cardiac output progressing to pulmonary edema with dyspnea, cough, tachycardia and hypotension. Cardiogenic shock represents the main cause of death. Thrombocytopenia and intravascular coagulation can occur in this phase and cause hemorrhagic manifestations (hematuria, intestinal bleeding, metrorrhagia). Renal failure may occur in up to 50% of the patients. Case-fatality rate ranges from 10% to 40%, according to the causative agent.[95]

IgG and IgM responses against three structural proteins of hantaviruses (Gn, Gc and N) represent the standard diagnostic tool. ELISA, IFA, immunoblot assay, focus reduction neutralization test (FRNT) represent the methods of choice. Viral genome can be detected with sensitive and specific RT-PCR in blood and urine. Viral isolation of hantaviruses is usually performed only for research purposes. Samples should be handled in a BSL-3 facility, if viral culture techniques are used.[105]

No antiviral drugs are currently available for hantaviral diseases. Data on ribavirin efficacy are controversial, considering that it seems to reduce morbidity mortality for HFRS patients,[106] but not for acute HCPS patients.[107] Passive immunotherapy could represent a promising treatment for acute HCPS. Currently, different vaccines are under development.[96,108]

5. Conclusions

VHFs represent a challenging problem for the global health, considering that effective treatments or vaccines are currently unavailable for the majority of the viruses causing VHFs. Moreover, clinical management of patients with VHF requires highly specialized personnel and in many cases specific isolation precautions to reduce the risk of human-to-human transmission of VHF in the healthcare setting, as well as accidental exposition in the diagnostic laboratories. Besides, mortality rates associated to VHFs are significantly high. Because of all these elements, WHO is currently considering VHF as a research priority. Although some viruses causing VHFs are present in high-income countries, most of them are endemic in low-income countries, thus limiting private research investments for developing new antiviral drug or vaccines. In this scenario, funding from public institutions, no-profit organizations and supranational organisms is of paramount importance to sustain the research for developing new diagnostic tools, such as rapid test for case identification and contact tracing; finding new molecules with direct antiviral activity, by using high throughput

screening methods; developing effective and safe vaccines. Considering the mobility of increasing numbers of people due to migrations, travels, climatic changes, VHFs could become a relevant health issue in larger areas of the world. For this reason, the preparedness for possible VHF outbreaks outside classical endemic areas should be reinforced. Suspect case identification, differential diagnosis and case confirmation represent the essential steps for the correct management of possible VHF cases. The knowledge of the epidemiology and clinical presentation can help in identifying patients with suspect VHF, either naturally acquired or as a consequence of bioterrorism attacks. For this reason in **Table 4** some clinical and pathological aspects of some VHFs are schematically summarized.

VHFs represent also the ideal setting for a "One Health" approach, in which optimal health outcomes can be achieved by integrating physicians, epidemiologists, virologists, veterinarians and ecologists, aiming to realize a trans-disciplinary approach. Indeed, an effective intervention for limiting or preventing VHF outbreak should take into account the population at risk, the animal reservoir, the eventual arthropod vector and the environmental factors.

References

1. Paessler S., Walker DH. Pathogenesis of the viral hemorrhagic fevers. Annu Rev Pathol 2013;8:411–40. Doi: 10.1146/annurev-pathol-020712-164041.
2. Virus Families | Viral Hemorrhagic Fevers (VHFs) | CDC. Available at: https://www.cdc.gov/vhf/virus-families/index.html. Accessed April 25, 2019.
3. Zhang Y-Z., Xu J. The emergence and cross species transmission of newly discovered tick-borne Bunyavirus in China. Curr Opin Virol 2016;16:126–31. Doi: 10.1016/j.coviro.2016.02.006.
4. Borio L., Inglesby T., Peters CJ., et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. JAMA 2002;287(18):2391–405.
5. Racsa LD., Kraft CS., Olinger GG., et al. Viral Hemorrhagic Fever Diagnostics. Clin Infect Dis Off Publ Infect Dis Soc Am 2016;62(2):214–9. Doi: 10.1093/cid/civ792.
6. Westover JB., Sefing EJ., Bailey KW., et al. Low-dose ribavirin potentiates the antiviral activity of favipiravir against hemorrhagic fever viruses. Antiviral Res 2016;126:62–8. Doi: 10.1016/j.antiviral.2015.12.006.
7. Keshtkar-Jahromi M., Kuhn JH., Christova I., et al. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res 2011;90(2):85–92. Doi: 10.1016/j.antiviral.2011.02.010.
8. Heinz FX., Stiasny K. Flaviviruses and their antigenic structure. J Clin Virol Off Publ Pan Am Soc Clin Virol 2012;55(4):289–95. Doi: 10.1016/j.jcv.2012.08.024.
9. Barrett AD., Monath TP. Epidemiology and ecology of yellow fever virus. Adv Virus Res

2003;61:291–315.

10. Bryant JE., Holmes EC., Barrett ADT. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. PLoS Pathog 2007;3(5):e75. Doi: 10.1371/journal.ppat.0030075.

11. Douam F., Ploss A. Yellow Fever Virus: Knowledge Gaps Impeding the Fight Against an Old Foe. Trends Microbiol 2018;26(11):913–28. Doi: 10.1016/j.tim.2018.05.012.

12. Quaresma JAS., Pagliari C., Medeiros DBA., et al. Immunity and immune response, pathology and pathologic changes: progress and challenges in the immunopathology of yellow fever. Rev Med Virol 2013;23(5):305–18. Doi: 10.1002/rmv.1752.

13. De Brito T., Siqueira SA., Santos RT., et al. Human fatal yellow fever. Immunohistochemical detection of viral antigens in the liver, kidney and heart. Pathol Res Pract 1992;188(1–2):177–81. Doi: 10.1016/S0344-0338(11)81176-3.

14. Meier KC., Gardner CL., Khoretonenko MV., et al. A mouse model for studying viscerotropic disease caused by yellow fever virus infection. PLoS Pathog 2009;5(10):e1000614. Doi: 10.1371/journal.ppat.1000614.

15. Quaresma JAS., Barros VLRS., Pagliari C., et al. Revisiting the liver in human yellow fever: virus-induced apoptosis in hepatocytes associated with TGF-beta, TNF-alpha and NK cells activity. Virology 2006;345(1):22–30. Doi: 10.1016/j.virol.2005.09.058.

16. Niedrig M., Kürsteiner O., Herzog C., et al. Evaluation of an indirect immunofluorescence assay for detection of immunoglobulin M (IgM) and IgG antibodies against yellow fever virus. Clin Vaccine Immunol CVI 2008;15(2):177–81. Doi: 10.1128/CVI.00078-07.

17. Reusken CBEM., Knoester M., GeurtsvanKessel C., et al. Urine as Sample Type for Molecular Diagnosis of Natural Yellow Fever Virus Infections. J Clin Microbiol 2017;55(11):3294–6. Doi: 10.1128/JCM.01113-17.

18. Domingo C., Charrel RN., Schmidt-Chanasit J., et al. Yellow fever in the diagnostics laboratory. Emerg Microbes Infect 2018;7. Doi: 10.1038/s41426-018-0128-8.

19. Monath TP., Vasconcelos PFC. Yellow fever. J Clin Virol Off Publ Pan Am Soc Clin Virol 2015;64:160–73. Doi: 10.1016/j.jcv.2014.08.030.

20. Wieten RW., Goorhuis A., Jonker EFF., et al. 17D yellow fever vaccine elicits comparable long-term immune responses in healthy individuals and immune-compromised patients. J Infect 2016;72(6):713–22. Doi: 10.1016/j.jinf.2016.02.017.

21. Yellow Fever - Chapter 3 - 2018 Yellow Book | Travelers' Health | CDC. Available at: https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/yellow-fever#9972. Accessed May 2, 2019.

22. Andrade EHP., Figueiredo LB., Vilela APP., et al. Spatial–Temporal Co-Circulation of Dengue

Virus 1, 2, 3, and 4 Associated with Coinfection Cases in a Hyperendemic Area of Brazil: A 4-Week Survey. Am J Trop Med Hyg 2016;94(5):1080–4. Doi: 10.4269/ajtmh.15-0892.

23. Thongyuan S., Kittayapong P. First evidence of dengue infection in domestic dogs living in different ecological settings in Thailand. PloS One 2017;12(8):e0180013. Doi: 10.1371/journal.pone.0180013.

24. Rapid risk assessment: Local transmission of dengue fever in France and Spain. European Centre for Disease Prevention and Control. Available at: http://ecdc.europa.eu/en/publications-data/rapid-risk-assessment-local-transmission-dengue-fever-france-and-spain. Accessed May 18, 2019.

25. Dengue in the US States and Territories | Dengue | CDC. Available at: https://www.cdc.gov/dengue/areaswithrisk/in-the-us.html. Accessed May 18, 2019.

26. Ferreira-de-Lima VH., Lima-Camara TN. Natural vertical transmission of dengue virus in Aedes aegypti and Aedes albopictus: a systematic review. Parasit Vectors 2018;11(1):77. Doi: 10.1186/s13071-018-2643-9.

27. Bhatt S., Gething PW., Brady OJ., et al. The global distribution and burden of dengue. Nature 2013;496(7446):504–7. Doi: 10.1038/nature12060.

28. Special Programme for Research and Training in Tropical Diseases., World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention, and control. New ed. Geneva: TDR : World Health Organization; 2009.

29. Halstead SB., Lan NT., Myint TT., et al. Dengue hemorrhagic fever in infants: research opportunities ignored. Emerg Infect Dis 2002;8(12):1474–9. Doi: 10.3201/eid0812.020170.

30. Katzelnick LC., Gresh L., Halloran ME., et al. Antibody-dependent enhancement of severe dengue disease in humans. Science 2017;358(6365):929–32. Doi: 10.1126/science.aan6836.

31. Nascimento LB., Siqueira CM., Coelho GE., et al. Symptomatic dengue infection during pregnancy and livebirth outcomes in Brazil, 2007-13: a retrospective observational cohort study. Lancet Infect Dis 2017. Doi: 10.1016/S1473-3099(17)30169-X.

32. Rowe EK., Leo Y-S., Wong JGX., et al. Challenges in dengue fever in the elderly: atypical presentation and risk of severe dengue and hospital-acquired infection [corrected]. PLoS Negl Trop Dis 2014;8(4):e2777. Doi: 10.1371/journal.pntd.0002777.

33. Chen LH., Wilson ME. Update on non-vector transmission of dengue: relevant studies with Zika and other flaviviruses. Trop Dis Travel Med Vaccines 2016;2:15. Doi: 10.1186/s40794-016-0032-y.

34. Lalle E., Colavita F., Iannetta M., et al. Prolonged detection of dengue virus RNA in the semen of a man returning from Thailand to Italy, January 2018. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2018;23(18). Doi: 10.2807/1560-7917.ES.2018.23.18.18-00197.

35. Iannetta M., Lalle E., Musso M., et al. Persistent detection of dengue virus RNA in vaginal secretion of a woman returning from Sri Lanka to Italy, April 2017. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2017;22(34). Doi: 10.2807/1560-7917.ES.2017.22.34.30600.
36. Arragain L., Dupont-Rouzeyrol M., O'Connor O., et al. Vertical Transmission of Dengue Virus in the Peripartum Period and Viral Kinetics in Newborns and Breast Milk: New Data. J Pediatr Infect Dis Soc 2017;6(4):324–31. Doi: 10.1093/jpids/piw058.
37. Wilder-Smith A., Ooi E-E., Horstick O., et al. Dengue. Lancet Lond Engl 2019;393(10169):350–63. Doi: 10.1016/S0140-6736(18)32560-1.
38. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol 2011;11(8):532–43. Doi: 10.1038/nri3014.
39. Morra ME., Altibi AMA., Iqtadar S., et al. Definitions for warning signs and signs of severe dengue according to the WHO 2009 classification: Systematic review of literature. Rev Med Virol 2018;28(4):e1979. Doi: 10.1002/rmv.1979.
40. Wilder-Smith A., Vannice KS., Hombach J., et al. Population Perspectives and World Health Organization Recommendations for CYD-TDV Dengue Vaccine. J Infect Dis 2016;214(12):1796–9. Doi: 10.1093/infdis/jiw341.
41. Sridhar S., Luedtke A., Langevin E., et al. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. N Engl J Med 2018;379(4):327–40. Doi: 10.1056/NEJMoa1800820.
42. Dengue vaccine: WHO position paper, September 2018 - Recommendations. Vaccine 2018. Doi: 10.1016/j.vaccine.2018.09.063.
43. Charrel RN., Attoui H., Butenko AM., et al. Tick-borne virus diseases of human interest in Europe. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2004;10(12):1040–55. Doi: 10.1111/j.1469-0691.2004.01022.x.
44. Lani R., Moghaddam E., Haghani A., et al. Tick-borne viruses: a review from the perspective of therapeutic approaches. Ticks Tick-Borne Dis 2014;5(5):457–65. Doi: 10.1016/j.ttbdis.2014.04.001.
45. Růžek D., Yakimenko VV., Karan LS., et al. Omsk haemorrhagic fever. Lancet Lond Engl 2010;376(9758):2104–13. Doi: 10.1016/S0140-6736(10)61120-8.
46. Dobler G. Zoonotic tick-borne flaviviruses. Vet Microbiol 2010;140(3–4):221–8. Doi: 10.1016/j.vetmic.2009.08.024.
47. Munivenkatappa A., Sahay RR., Yadav PD., et al. Clinical & epidemiological significance of Kyasanur forest disease. Indian J Med Res 2018;148(2):145–50. Doi: 10.4103/ijmr.IJMR_688_17.
48. Zaki AM. Isolation of a flavivirus related to the tick-borne encephalitis complex from human cases in Saudi Arabia. Trans R Soc Trop Med Hyg 1997;91(2):179–81.
49. Hoffman T., Lindeborg M., Barboutis C., et al. Alkhurma Hemorrhagic Fever Virus RNA in

Hyalomma rufipes Ticks Infesting Migratory Birds, Europe and Asia Minor. Emerg Infect Dis 2018;24(5):879–82. Doi: 10.3201/eid2405.171369.

50. Madani TA. Alkhumra virus infection, a new viral hemorrhagic fever in Saudi Arabia. J Infect 2005;51(2):91–7. Doi: 10.1016/j.jinf.2004.11.012.

51. Madani TA., Azhar EI., Abuelzein E-TME., et al. Alkhumra (Alkhurma) virus outbreak in Najran, Saudi Arabia: epidemiological, clinical, and laboratory characteristics. J Infect 2011;62(1):67–76. Doi: 10.1016/j.jinf.2010.09.032.

52. Carletti F., Castilletti C., Di Caro A., et al. Alkhurma hemorrhagic fever in travelers returning from Egypt, 2010. Emerg Infect Dis 2010;16(12):1979–82. Doi: 10.3201/eid1612.101092.

53. Emanuel J., Marzi A., Feldmann H. Filoviruses: Ecology, Molecular Biology, and Evolution. Adv Virus Res 2018;100:189–221. Doi: 10.1016/bs.aivir.2017.12.002.

54. Brauburger K., Hume AJ., Mühlberger E., et al. Forty-Five Years of Marburg Virus Research. Viruses 2012;4(10):1878–927. Doi: 10.3390/v4101878.

55. Hartman AL., Towner JS., Nichol ST. Ebola and marburg hemorrhagic fever. Clin Lab Med 2010;30(1):161–77. Doi: 10.1016/j.cll.2009.12.001.

56. Gear JS., Cassel GA., Gear AJ., et al. Outbreake of Marburg virus disease in Johannesburg. Br Med J 1975;4(5995):489–93. Doi: 10.1136/bmj.4.5995.489.

57. Reynolds P., Marzi A. Ebola and Marburg virus vaccines. Virus Genes 2017;53(4):501–15. Doi: 10.1007/s11262-017-1455-x.

58. Marzi A., Menicucci AR., Engelmann F., et al. Protection Against Marburg Virus Using a Recombinant VSV-Vaccine Depends on T and B Cell Activation. Front Immunol 2018;9:3071. Doi: 10.3389/fimmu.2018.03071.

59. Maes P., Alkhovsky SV., Bào Y., et al. Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch Virol 2018;163(8):2295–310. Doi: 10.1007/s00705-018-3843-5.

60. Morin B., Coutard B., Lelke M., et al. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. PLoS Pathog 2010;6(9):e1001038. Doi: 10.1371/journal.ppat.1001038.

61. Hallam SJ., Koma T., Maruyama J., et al. Review of Mammarenavirus Biology and Replication. Front Microbiol 2018;9:1751. Doi: 10.3389/fmicb.2018.01751.

62. Sarute N., Ross SR. New World Arenavirus Biology. Annu Rev Virol 2017;4(1):141–58. Doi: 10.1146/annurev-virology-101416-042001.

63. McLay L., Liang Y., Ly H. Comparative analysis of disease pathogenesis and molecular mechanisms of New World and Old World arenavirus infections. J Gen Virol 2014;95(Pt 1):1–15. Doi: 10.1099/vir.0.057000-0.

64. Kilgore PE., Peters CJ., Mills JN., et al. Prospects for the control of Bolivian hemorrhagic

fever. Emerg Infect Dis 1995;1(3):97–100. Doi: 10.3201/eid0103.950308.

65. Flanagan ML., Oldenburg J., Reignier T., et al. New world clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. J Virol 2008;82(2):938–48. Doi: 10.1128/JVI.01397-07.

66. Fan L., Briese T., Lipkin WI. Z proteins of New World arenaviruses bind RIG-I and interfere with type I interferon induction. J Virol 2010;84(4):1785–91. Doi: 10.1128/JVI.01362-09.

67. Moraz M-L., Kunz S. Pathogenesis of arenavirus hemorrhagic fevers. Expert Rev Anti Infect Ther 2011;9(1):49–59. Doi: 10.1586/eri.10.142.

68. Charrel RN., de Lamballerie X. Arenaviruses other than Lassa virus. Antiviral Res 2003;57(1–2):89–100.

69. Maiztegui JI., Fernandez NJ., de Damilano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. Lancet Lond Engl 1979;2(8154):1216–7.

70. Charrel RN., Coutard B., Baronti C., et al. Arenaviruses and hantaviruses: from epidemiology and genomics to antivirals. Antiviral Res 2011;90(2):102–14. Doi: 10.1016/j.antiviral.2011.02.009.

71. Gowen BB., Hickerson BT. Hemorrhagic fever of bunyavirus etiology: disease models and progress towards new therapies. J Microbiol Seoul Korea 2017;55(3):183–95. Doi: 10.1007/s12275-017-7029-8.

72. Li P., Tong Z-D., Li K-F., et al. Seroprevalence of severe fever with thrombocytopenia syndrome virus in China: A systematic review and meta-analysis. PloS One 2017;12(4):e0175592. Doi: 10.1371/journal.pone.0175592.

73. Mansfield KL., Banyard AC., McElhinney L., et al. Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. Vaccine 2015;33(42):5520–31. Doi: 10.1016/j.vaccine.2015.08.020.

74. Ferron F., Li Z., Danek EI., et al. The hexamer structure of Rift Valley fever virus nucleoprotein suggests a mechanism for its assembly into ribonucleoprotein complexes. PLoS Pathog 2011;7(5):e1002030. Doi: 10.1371/journal.ppat.1002030.

75. Linthicum KJ., Britch SC., Anyamba A. Rift Valley Fever: An Emerging Mosquito-Borne Disease. Annu Rev Entomol 2016;61:395–415. Doi: 10.1146/annurev-ento-010715-023819.

76. Linthicum KJ., Davies FG., Kairo A., et al. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J Hyg (Lond) 1985;95(1):197–209.

77. Hartman A. Rift Valley Fever. Clin Lab Med 2017;37(2):285–301. Doi: 10.1016/j.cll.2017.01.004.

78. Al-Hamdan NA., Panackal AA., Al Bassam TH., et al. The Risk of Nosocomial Transmission of Rift Valley Fever. PLoS Negl Trop Dis 2015;9(12):e0004314. Doi: 10.1371/journal.pntd.0004314.
79. McMillen CM., Hartman AL. Rift Valley fever in animals and humans: Current perspectives. Antiviral Res 2018;156:29–37. Doi: 10.1016/j.antiviral.2018.05.009.
80. Jansen van Vuren P., Shalekoff S., Grobbelaar AA., et al. Serum levels of inflammatory cytokines in Rift Valley fever patients are indicative of severe disease. Virol J 2015;12:159. Doi: 10.1186/s12985-015-0392-3.
81. Al-Hazmi A., Al-Rajhi AA., Abboud EB., et al. Ocular complications of Rift Valley fever outbreak in Saudi Arabia. Ophthalmology 2005;112(2):313–8. Doi: 10.1016/j.ophtha.2004.09.018.
82. Madani TA., Al-Mazrou YY., Al-Jeffri MH., et al. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. Clin Infect Dis Off Publ Infect Dis Soc Am 2003;37(8):1084–92. Doi: 10.1086/378747.
83. Mourya DT., Yadav PD., Shete AM., et al. Detection, isolation and confirmation of Crimean-Congo hemorrhagic fever virus in human, ticks and animals in Ahmadabad, India, 2010-2011. PLoS Negl Trop Dis 2012;6(5):e1653. Doi: 10.1371/journal.pntd.0001653.
84. Leblebicioglu H. Crimean-Congo haemorrhagic fever in Eurasia. Int J Antimicrob Agents 2010;36 Suppl 1:S43-46. Doi: 10.1016/j.ijantimicag.2010.06.020.
85. Negredo A., de la Calle-Prieto F., Palencia-Herrejón E., et al. Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. N Engl J Med 2017;377(2):154–61. Doi: 10.1056/NEJMoa1615162.
86. Leblebicioglu H., Sunbul M., Guner R., et al. Healthcare-associated Crimean-Congo haemorrhagic fever in Turkey, 2002-2014: a multicentre retrospective cross-sectional study. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2016;22(4):387.e1-387.e4. Doi: 10.1016/j.cmi.2015.11.024.
87. Schnittler H-J., Feldmann H. Viral hemorrhagic fever--a vascular disease? Thromb Haemost 2003;89(6):967–72.
88. Ergönül O. Crimean-Congo haemorrhagic fever. Lancet Infect Dis 2006;6(4):203–14. Doi: 10.1016/S1473-3099(06)70435-2.
89. Drosten C., Kümmerer BM., Schmitz H., et al. Molecular diagnostics of viral hemorrhagic fevers. Antiviral Res 2003;57(1–2):61–87.
90. Flick R., Whitehouse CA. Crimean-Congo hemorrhagic fever virus. Curr Mol Med 2005;5(8):753–60.
91. Bartolini B., Gruber CE., Koopmans M., et al. Laboratory management of Crimean-Congo haemorrhagic fever virus infections: perspectives from two European networks. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2019;24(5). Doi: 10.2807/1560-7917.ES.2019.24.5.1800093.

92. Watts DM., Ussery MA., Nash D., et al. Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. Am J Trop Med Hyg 1989;41(5):581–5.

93. Tignor GH., Hanham CA. Ribavirin efficacy in an in vivo model of Crimean-Congo hemorrhagic fever virus (CCHF) infection. Antiviral Res 1993;22(4):309–25.

94. Koksal I., Yilmaz G., Aksoy F., et al. The efficacy of ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Eastern Black Sea region in Turkey. J Clin Virol Off Publ Pan Am Soc Clin Virol 2010;47(1):65–8. Doi: 10.1016/j.jcv.2009.11.007.

95. Macneil A., Nichol ST., Spiropoulou CF. Hantavirus pulmonary syndrome. Virus Res 2011;162(1–2):138–47. Doi: 10.1016/j.virusres.2011.09.017.

96. Manigold T., Vial P. Human hantavirus infections: epidemiology, clinical features, pathogenesis and immunology. Swiss Med Wkly 2014;144:w13937. Doi: 10.4414/smw.2014.13937.

97. Kruger DH., Figueiredo LTM., Song J-W., et al. Hantaviruses--globally emerging pathogens. J Clin Virol Off Publ Pan Am Soc Clin Virol 2015;64:128–36. Doi: 10.1016/j.jcv.2014.08.033.

98. Jiang H., Zheng X., Wang L., et al. Hantavirus infection: a global zoonotic challenge. Virol Sin 2017;32(1):32–43. Doi: 10.1007/s12250-016-3899-x.

99. Hardestam J., Simon M., Hedlund KO., et al. Ex vivo stability of the rodent-borne Hantaan virus in comparison to that of arthropod-borne members of the Bunyaviridae family. Appl Environ Microbiol 2007;73(8):2547–51. Doi: 10.1128/AEM.02869-06.

100. Jiang H., Du H., Wang LM., et al. Hemorrhagic Fever with Renal Syndrome: Pathogenesis and Clinical Picture. Front Cell Infect Microbiol 2016;6:1. Doi: 10.3389/fcimb.2016.00001.

101. Prescott J., Safronetz D., Haddock E., et al. The adaptive immune response does not influence hantavirus disease or persistence in the Syrian hamster. Immunology 2013;140(2):168–78. Doi: 10.1111/imm.12116.

102. Mori M., Rothman AL., Kurane I., et al. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. J Infect Dis 1999;179(2):295–302. Doi: 10.1086/314597.

103. Borges AA., Donadi EA., Campos GM., et al. Polymorphisms in human leukocyte antigens, human platelet antigens, and cytokine genes in hantavirus cardiopulmonary syndrome patients from Ribeirão Preto, Brazil. J Med Virol 2014;86(11):1962–70. Doi: 10.1002/jmv.23836.

104. Vial PA., Valdivieso F., Mertz G., et al. Incubation period of hantavirus cardiopulmonary syndrome. Emerg Infect Dis 2006;12(8):1271–3. Doi: 10.3201/eid1208.051127.

105. Mattar S., Guzmán C., Figueiredo LT. Diagnosis of hantavirus infection in humans. Expert Rev Anti Infect Ther 2015;13(8):939–46. Doi: 10.1586/14787210.2015.1047825.

106. Huggins JW., Hsiang CM., Cosgriff TM., et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. J

Infect Dis 1991;164(6):1119–27.

107. Mertz GJ., Miedzinski L., Goade D., et al. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. Clin Infect Dis Off Publ Infect Dis Soc Am 2004;39(9):1307–13. Doi: 10.1086/425007.

108. Hjelle B. Vaccines against hantaviruses. Expert Rev Vaccines 2002;1(3):373–84. Doi: 10.1586/14760584.1.3.373.

FIGURE TITLES AND CAPTIONS

Figure 1: Countries at risk of yellow fever transmission
In the map countries at risk of yellow fever transmission are represented in shadow of yellow. Light yellow has been used for countries in which only some areas are endemic for yellow fever transmission (non-holoendemic), while dark yellow has been used for countries totally at risk of yellow fever transmission (holoendemic).

Figure 2: Countries at risk of dengue fever transmission
In the map countries at risk of dengue fever transmission are represented in shadow of blue. Light and dark blue have been used for countries at medium-low risk and high risk of dengue fever transmission, respectively.

Figure 3: Regions endemic for Omsk hemorrhagic fever, Kyasanur forest disease and Alkhumra virus disease
In the map regions endemic for Omsk hemorrhagic fever, Kyasanur Forest disease and Alkhumra virus disease are represented in blue, green and red, respectively. Some cases have also been described outside endemic regions, in travellers.

Figure 4: Regions endemic for New World arenaviruses
In the map regions endemic for New World hemorrhagic arenaviruses are represented. New world arenaviruses related diseases are: Argentine Hemorrhagic Fever, Bolivian Hemorrhagic Fever, Venezuelan Hemorrhagic Fever, Brazilian Hemorrhagic Fever, hemorrhagic fever in Trinidad and North American viruses causing outbreaks in California, Colorado, Florida, New Mexico, Oklahoma and Utah.

Figure 5: Countries reporting Rift Valley Fever cases
In the map countries reporting endemic disease and substantial outbreaks of Rift Valley Fever are represented in blue, while countries reporting few cases, periodic isolation of the virus, or serologic evidence of Rift Valley Fever virus infection are represented in green.

Figure 6: Countries reporting Crimean-Congo Hemorrhagic Fever cases
In the map countries reporting cases of Crimean-Congo Hemorrhagic Fever virus infection are represented in red.

Figure 7: Countries reporting Hantavirus related diseases

Old World hantaviruses associated to hemorrhagic fever with renal syndrome (HFRS) are represented in shadow of red, in the map. Specifically, high and low risk countries for Old World hantavirus infections are represented in dark and light red, respectively. New World hantaviruses associated to hantavirus cardiopulmonary syndrome (HCPS) are represented in shadow of blue, in the map. Specifically, high and low risk countries for New World hantavirus infections are represented in dark and light blue, respectively.