



# The Open Virology Journal

Content list available at: [www.benthamopen.com/TOVJ/](http://www.benthamopen.com/TOVJ/)

DOI: 10.2174/1874357901610010001



## Viruses Causing Hemorrhagic Fever. Safety Laboratory Procedures

Fernando Cobo\*

*Microbiology Section (Biotechnology Area) and Tropical Medicine Unit*

**Abstract:** Viral hemorrhagic fevers are diseases caused by viruses which belong to different families, many of them causing severe diseases. These viruses may produce different symptomatology together with a severe multisystem syndrome, and the final result might be the production of hemorrhages in several sites of the body. The majority of them have no other treatment than supportive therapy, although some antiviral drugs can be used in some circumstances. Transmission of VHF has been demonstrated through contact with animal vectors or person-to-person through the contact with body fluids. No risk of transmission has been found during the incubation period, but when the viral load is high the risk of transmission is greatest. Both health care and clinical laboratory workers must safely handle patients and specimens by taking all required precautions during their management.

**Keywords:** Bleeding, laboratory, life-threatening, prophylaxis, safety, travelers, viral hemorrhagic fever.

### INTRODUCTION

Viral hemorrhagic fevers (VHFs) are diseases caused by viruses, which belong to the flavivirus, bunyavirus, arenavirus and filovirus families [1]. The majority of these entities have a zoonotic cycle; rodents and arthropods are the main reservoirs. All these families share some characteristics (enveloped RNA viruses); the distribution of these viruses is restricted to limited geographical areas; humans do not act as natural reservoir, and outbreaks of haemorrhagic fevers occur irregularly and sporadically, and with fewer exceptions, there is no specific treatment for these infections [1].

Viruses causing these diseases are transmitted to humans through contact with infected fluids from the hosts such as fecal matter, saliva, urine, other fluids, and some may be transmitted through the bites from arthropods. Moreover, Ebola and Marburg viruses could be transmitted person-to-person through direct contact with either infected body fluids or contaminated objects.

The distribution of these viruses is limited to several regions; therefore the risk of acquisition of these infections is restricted to those areas. However, some hosts have a worldwide distribution, thus VHF transmitted by these hosts can be acquired anywhere.

Occasionally, these viruses can be transmitted to other locations when patients are traveling. Due to the increase in the number of international travelers nowadays, outbreaks of these diseases could occur anywhere, thus healthcare providers should be aware of VHFs.

The recent 2014 Ebola outbreak emphasizes the need for a wider knowledge of the mechanisms of transmission, risk of exposure and preventive measures for these viruses. This review focuses on the main safety laboratory procedures for the management of samples from patients affected with these kinds of infections.

### CHARACTERISTICS OF THE MAIN HEMORRHAGIC VIRUSES

#### Ebola Hemorrhagic Fever

Ebola viruses may cause severe hemorrhagic fever outbreaks in humans with a death rate up to 90%. The two initial

\* Microbiology Section (Biotechnology Area) and Tropical Medicine Unit. Hospital de Poniente Ctra de Almerimar s/n 04700 El Ejido, Almería, Spain Phone: +34950022638 Fax: +34950022600 E-mail: [fernando.cobo.sspa@juntadeandalucia.es](mailto:fernando.cobo.sspa@juntadeandalucia.es)

outbreaks occurred in 1976 in Sudan and in the Democratic Republic of the Congo (near to the Ebola River) [2, 3].

There are five different species of the Ebola virus named Zaire ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus, Taï Forest ebolavirus (formerly Côte d'Ivoire ebolavirus) and Reston ebolavirus [4, 5]. The three first viruses are associated with several Ebola disease (EVD) outbreaks in Africa.

Although, the wildlife reservoir has not been definitively found, the possible natural hosts for Ebola virus are some species of bats.

EVD is introduced into the human population through direct contact with infected animal body fluids or vector bites. In non-endemic countries, EVD spreads through direct contact with the body fluids of infected patients. After the incubation period (2 to 21 days), EVD appears with fever and malaise, and other non-specific symptoms and signs (such as myalgia, weakness, headache, muscle pain, sore throat, diarrhea and vomiting). Rash and hemorrhagic symptoms are visible in 30-50% of patients with EVD, though multi-organ dysfunction leading to shock and death may be present in serious forms.

The Zaire Ebola virus has caused most outbreaks in Central Africa since their apparition in 1976. The 2014 Ebola outbreak is the first in West Africa. Until November 2 2015, 15246 cases have been confirmed in the laboratory and 11314 patients have died.

### **Marburg Hemorrhagic Fever**

Marburg virus, which causes Marburg hemorrhagic fever (MHF), and Ebola virus are the two members of the *Filoviridae* family that can cause outbreaks with high fatality rates [6]. This disease was first identified in 1967 after the outbreaks in Marburg, Frankfurt and Belgrade [7]. Travelers are at low risk of infection, but occasionally these viruses can be imported into non-endemic countries.

In Africa, some species of bats are considered natural hosts for Marburg virus. During the first Marburg outbreak, the etiology of human infection was traced to the laboratory handling of African green monkeys imported from Uganda. Since then, some outbreaks and sporadic cases have been reported in several countries such as Angola, Democratic Republic of the Congo, Kenya, South Africa and Uganda [7]. In 2008, two cases of MHF were reported in travelers; the infection was acquired as a result of exposure to caves inhabited by bats in Uganda. Transmission is mainly caused through direct contact with body fluids of infected persons. Also, transmission by infected semen can occur as well as transmission to health care workers while administering treatments to infected patients.

After the incubation period (which ranges from 2 to 21 days), MHF begins suddenly with fever, headache and malaise, myalgia, arthralgia, abdominal pain, diarrhea, nausea and vomiting, conjunctiva injection and a relative bradycardia. Most of the patients develop severe hemorrhagic features in multiple body parts between 5-7 days. Around the fifth day, a maculo-papular rash could also be seen. Involvement of the CNS can result in irritability and confusion.

### **Yellow Fever**

Yellow fever (YF) is a viral hemorrhagic disease caused by a flavivirus and transmitted by infected mosquitoes [8]. The term "yellow" refers to the jaundice that can be seen in some persons, and up to 50% of the infected patients could die without due treatments. This infection is endemic in Africa and South America; outbreaks have been identified more frequently in Western Africa. In the jungle cycle, the main hosts of this virus are monkeys, whereas in the urban cycle humans are the reservoirs. *Aedes* mosquitos' species can serve as vectors of YF in Africa, and the species involved depends on the geographical location and type of transmission cycle. Nine hundred million persons from 44 endemic countries in Africa and Latin America are at risk of acquiring this infection. Each year, an estimated 200,000 new cases of YF worldwide are diagnosed causing 30,000 deaths. Small numbers of imported cases occur every year in non-endemic countries. In December 2010, 190 cases and 48 deaths were reported in Northern Uganda, affecting mainly males aged 20 to 34 years.

YF should be suspected in persons without vaccination who have recently traveled to endemic areas [9]. International regulations require vaccination against YF, but some non-vaccinated travelers in endemic areas are able to evade controls.

After the incubation period (3 to 6 days), the infection can develop in two phases. The first phase is an acute illness presented with fever, muscular pain and loss of appetite, nausea, vomiting, shivers, backache, and headache. Most patients improve at this stage; the symptoms disappear after 3-4 days. The second phase is developed in 15% of the

infected persons with symptomatology of kidney failure such as jaundice, abdominal pain, and vomiting. Also, bleeding can be present in several locations; 50% of the patients may die within 10-14 days.

### Dengue

Dengue is a viral infection caused by viruses of the *Flaviviridae* family and endemic in tropical and sub-tropical areas. Dengue was first found in 1950 in the Philippines and Thailand. Four different serotypes of the virus (namely, DEN-1, DEN-2, DEN-3 and DEN-4) have been identified. Whenever dengue infection is developed, lifelong immunity against a specific serotype is produced, though cross-immunity to the other serotypes may be demonstrated (this is temporary and partial). Severe dengue is produced when further infections by other serotypes occur.

Dengue disease is transmitted through bites of *Aedes* mosquitoes (e.g., *Aedes aegypti*, *Aedes albopictus*); it is widely spread in Africa, America, Asia, the Pacific and the Caribbean regions. Last years, the incidence of dengue increased quickly worldwide; the transmission has grown above all in urban and semi-urban areas. Currently, dengue is a major international public health problem because over 40% of the world's population is at risk of infection. This disease is endemic in more than 100 countries; the WHO estimates that 50-100 million of dengue infections occur every year worldwide. Data from 2010 reported 1.6 million cases of dengue in American countries (49,000 of them are severe). In 2010, in Europe, 1622 cases of dengue were reported, particularly in Germany, Sweden, France, and Belgium, and local transmission was reported in France and Croatia [10, 11]. In 2012, an outbreak of dengue occurred in Madeira (Portugal) resulting in approximately 2000 cases with imported cases being detected in 10 other European countries. In 2013, some cases occurred in Florida (U.S.) and the Yunnan province of China. Each year, an estimated 500,000 people with severe dengue require hospitalization and about 2.5% of those affected persons die.

The incubation period is about 4-10 days; the disease starts with high fever (40°C), severe headache, eyes pain, muscle and joint pains, nausea, vomiting, swollen glands and rash. The prognosis of severe dengue can be fatal due to severe bleeding, respiratory distress, fluid accumulation, plasma leaking, or organ failure. Severe symptoms can include severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, gastrointestinal bleeding, restlessness and extreme fatigue.

### Lassa Fever

Lassa fever (LF) is a disease caused by a member of the Arenavirus family of viruses called Lassa virus. This virus is transmitted to humans through direct contact with food or objects contaminated with rodent excreta, though human-to-human infections and laboratory transmission could also occur, primarily through direct contact with body fluids.

LF is an endemic disease in West Africa, highly prevalent in Nigeria, Sierra Leone, and Liberia. The name "Lassa" came to existence in Nigeria, where LF was first recognized, in 1969 [12]. The majority of persons at risk are those living in rural areas of the West African countries [13]; though sporadic cases have been reported in the Netherlands, the U.S., Japan and Canada [14, 15].

The majority of patients are asymptomatic, but 20% may develop serious multi-system disease, and up to 15% of them could die as a consequence of this disease. The main symptoms and signs of LF are fever, headache, sore throat, myalgia, dysphagia, dry cough, chest pain and abdominal pain. Complications can occur with severe symptoms such as face and neck edema, pleural and pericardial effusion, respiratory distress, encephalopathy and hemorrhage in several locations. LF is more severe during pregnancy.

### Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF) is a disease caused by *Nairovirus*, which belongs to the *Bunyaviridae* family. This disease was first noticed in Russia as Crimean hemorrhagic fever in 1945 and in the Democratic Republic of the Congo as Congo virus infection in 1956. The virus causes serious hemorrhagic fever outbreaks with a case fatality rate that ranges from 10 to 40%.

CCHF is endemic in Africa, the Balkans, the Middle East and Asian countries, and can occur in large areas of Eastern Europe, the Mediterranean region, Western Asia and Africa [16, 17]. In Europe, some cases of human infections have been found in Albania, Armenia, Bulgaria, Serbia, Turkey and other countries. Two confirmed cases of CCHF were reported in 2010 in Bulgaria. In 2010, the CCHF viral genome was identified in Hyalomma ticks collected in Cáceres (Spain), which suggests that the virus might produce disease in other regions than those considered as endemic in Europe [18].

The hosts of this virus include both wild and domestic animals such as sheep, goats, and cattle. Animals can be infected by the bites of infected ticks, above all those of the genus *Hyalomma*. The main modes of transmission from animals to humans include direct contact with blood or tissues of infected animals, as well as inoculation of the virus through the bites of infected ticks. Person-to-person transmission may also occur from direct contact with body fluids of infected patients.

The incubation period ranges from 2 to 9 days; the main symptoms and signs are fever, myalgia (backache, headache, and muscle pain), dizziness, sore eyes, and photophobia. Other symptoms include nausea, vomiting, diarrhea, and abdominal pain. There may also be meningeal signs with confusion and aggression, and after 2-4 days of sleepiness, depression and lassitude may appear.

Other clinical signs include tachycardia, hepatomegaly, and lymphadenopathy. Petechial rash, ecchymoses, epistaxis or bleeding in other locations could also be present. The mortality rate is approximately 30%.

### **Rift Valley Fever**

Rift Valley fever is an acute viral zoonosis that affects mainly sheep, goats and cattle and may also affect humans. RVF virus is a member of the *Phlebovirus* genus (family *Bunyaviridae*). In 1930, this virus was first identified in Kenya. In 1997-1998, the last big RVF outbreak was reported in Kenya, Somalia and Tanzania [19]. In 2000, some cases of RVF were also reported in Saudi Arabia and Yemen [20]. However, an RVF outbreak in South Africa, which started in 2008-2009 and continued during the first half of 2010, resulted in a total of 236 human cases [21]. Mauritania also reported human cases in 2010.

RVF may be an occupational disease acquired through direct contact with infected animals and their products. RVF may also be transmitted to humans by *Aedes* bites and by ingesting unpasteurized and uncooked milk from infected animals. No person-to-person transmission has been documented to date and there is no evidence of outbreaks of RVF in urban areas.

This disease is usually found in Eastern and Southern Africa, but also in most countries of sub-Saharan Africa, Madagascar, Saudi Arabia and Yemen.

The incubation period for RVF ranges from 2 to 6 days and asymptomatic or mild infections are common. In symptomatic cases, the disease is characterized by fever, muscle and joint pain, and headache. Some patients also develop photophobia, retro-orbital pain, loss of appetite, nausea and vomiting, and maculopapular rash. However, less than 5% of the patients can develop a severe form of the disease characterized by three different syndromes involving meningo-encephalitis (< 1%), ocular disease (0.5-2%) or hemorrhagic fever (< 1%).

### **LABORATORY DIAGNOSIS**

VHFs have no specific symptoms that distinguishes them from other infectious diseases caused by other pathogens. Thus, the confirmation of VHFs must solely rely on laboratory diagnosis of particular pathogen components (nucleic acids and/or proteins) or specific antibodies rather than clinical diagnosis.

Laboratory methods to confirm the diagnosis include the study of different clinical samples such as blood, urine, throat washings, and post-mortem tissue specimens. Laboratory diagnosis of VHF is performed mainly by reverse transcription-polymerase chain reaction (RT-PCR) currently, although serological tests could represent good approaches for the diagnosis. Virus isolation by cell culture and electron microscopy could also be used, though viral isolation must only be carried out in biosafety level 4 laboratories. With regard to serological tests, a wide variety of tests are available such as enzyme-linked immunosorbent assays (ELISA), antigen detection assays, and serum neutralization tests. Moreover, an immunohistochemical assay in formalin-fixed skin biopsy samples to detect Ebola virus has been recently developed [22].

### **PROPHYLAXIS AND TREATMENT**

For most of these diseases, the main preventive measures are based on avoiding contact with host species and on preventing further transmission from human-to-human. The main efforts should be concentrated on controlling rodents and other animal populations as well as arthropod vectors (such as mosquitoes and ticks), and using insect repellent, proper clothing, bed nets, window screens, and other insect barriers. People are also encouraged to avoid direct physical contact with infected individuals and their body fluids.

However, YF is a preventable disease by means of a vaccine. The YF vaccine is safe and provides effective immunity against this disease within 7-10 days for 95% of vaccinated persons. This vaccine is composed of a live attenuated 17D strain of the virus. A single dose is sufficient to confer immunity and life-long protection; severe side effects are rare. The vaccine is usually not administered to pregnant women, immune-compromised persons and children. An inactivated vaccine has been recently developed for human use in RVF, but not licensed and not commercially available. VHF vaccine is currently a mandatory item for international travelers and could also be indicated for persons traveling as part of international outbreak investigations. Furthermore, travelers arriving from Africa or Latin America must have a certificate of YF vaccination.

There is no specific treatment in the majority of VHFs. Usually, treatment is mainly supportive, and patients frequently require intravenous fluid or oral rehydration solutions containing electrolytes. CCHF has been effectively treated with ribavirin (with both oral and intravenous formulations) [23]. Also, treatment with ribavirin, if started early, has significantly reduced the mortality rate associated with LF [24]. The treatment regimen using ribavirin includes a 30 mg/kg intravenously loading dose, followed by 16 mg/kg intravenously every 6 hours for 4 days, and then 8 mg/kg intravenously every 8 hours for 6 days.

Until now, the treatment of Ebola virus infections has been mainly supportive but recent studies suggest that some nucleoside analogs could inhibit the replication of these viruses [25]. Currently, owing to the special virulent outbreak in Western Africa, many patients are being treated with hyperimmune serum obtained from patients with favorable outcome and with an experimental drug called ZMapp. This medicine was firstly used with excellent results in nonhuman primates [26].

Recently, an rVSV-vectored vaccine has demonstrated preliminary safety and effectiveness in patients from Guinea, West Africa [27]

## CARE OF PATIENTS AND INFECTION CONTROL

VHF should be mainly suspected in patients with fever who, within the incubation period, have had direct contact with blood and other body fluids; have been in the region where VHF has recently occurred (above all in rural areas); or have been possibly exposed in a laboratory.

Patients should be treated at the nearest hospital or primary care unit in order to decrease the risk of secondary transmission. If this is not possible, patients should be moved to another local reference hospital. These patients must be treated in a private room, preferably one with negative air pressure, with an adjoining room containing supplies for routine patient care (*e.g.*, gowns, gloves, masks, clothes). Containers of decontaminating solutions and hand-washing devices should be also available in this adjoining room.

Health care workers and all persons with access to the patient's room must use barrier precautions such as adequate clothes, disposable gloves, gowns, masks, caps, and shoe covers to prevent direct contact with objects or environmental surfaces.

All body fluids and secretions should be placed in disinfectant solution, and all material used for treatment should be removed, disinfected and then incinerated or autoclaved. To prevent percutaneous injuries, all disposable material used in patient care such as syringes, needles, catheters, and others should be placed in a container with disinfectant solution, and then should be disinfected and incinerated or autoclaved. If the patient requires surgical procedures, surgeons and the rest of the personnel should wear protective clothing as well as protective eye wear and double gloves.

In the case of the disinfectant solutions, these include 0.5% sodium hypochlorite (10% aqueous solution), glutaraldehyde 2% and phenolic disinfectants (0.5-3%).

Although airborne transmission has not been demonstrated, precautions to that effect should be optionally taken for patients who have lung involvement, in order to prevent possible exposure to airborne particles that may contain virus and further transmission (*e.g.*, wear a face mask).

## SAFETY LABORATORY PROCEDURES

Transmission of VHFs in hospitals or primary health care may be associated with contaminated medical material such as syringes and needles, and with the absence of appropriate barrier measures to prevent exposure to viruses [28].

The risk for person-to-person transmission of these viruses is greatest when the viral load is highest (*e.g.* latter stages of disease); however, no infection has been reported during the incubation period (2-21 days for Ebola virus and

Marburg virus). Although the airborne route has initially not been considered adequate for person-to-person transmission [28], it remains a hypothetical route of transmission during procedures that may generate aerosols.

### **Clinical Samples Handling**

The main responsibility of a Laboratory director is to determine and assess the risk. He/she must meet all requirements and establish control measures to ensure safe practices and procedures.

Potentially infectious samples (*e.g.*, blood, cerebrospinal fluid, pleural and peritoneal fluid) should be routinely handled and tested safely in the laboratory. The majority of hemorrhagic fever viruses are considered as biosafety level four (BSL-4) pathogens, except YF and Dengue. All laboratory workers and other healthcare personnel collecting or handling these samples must wear appropriate personal protective equipment (PPE) and follow all safety recommendations for handling samples. Specimen collection should be carried out by trained personnel following strict protocols adapted for each hospital or healthcare institution. These workers should wear gloves, gowns that are impermeable, eye protection (*e.g.*, full face shield), and a mask to cover all of the nose and mouth area. Shoes and leg covers could be appropriate in certain circumstances such as if specimen collection is performed in a highly contaminated room.

The essential samples for virus isolation are a specimen of venous blood, a sample of urine, and a throat specimen and should be limited to the minimum amount necessary. Venous blood samples should be collected with care avoiding needle sticks. Safe procedures should be followed: the blood should be placed in an appropriate and safe container, and needles should be immediately discarded. Urine samples should be placed in an appropriate container with buffers (*e.g.*, bovine serum albumin at 10% or human serum albumin at 1%). Finally, throat swabs should be placed in adequate containers in the same buffers as mentioned above. These specimens should be placed in double sealed plastic bags; these containers should be disinfected outside.

Clinical specimens should be placed and processed in a class II biological safety cabinet (BSC) following universal biosafety level 3 practices. It is also a good practice to pretreat serum samples with heat inactivation at 56°C and polyethylene glycol p-tert-octylphenyl ether (Triton<sup>®</sup> X-100) [29]. Blood smears should be fixed in solvents in order to eliminate the infectivity; blood cultures should be preferably prepared in a closed system. It is essential to avoid aerosol production or splashing.

Routine cleaning and disinfecting procedures should be used for automated analyzers. These should be disinfected with 500 parts per million solutions of sodium hypochlorite. However, a new approach in specimens handling in the laboratories may be the use of point-of-care (POC) devices in the private room of the patients for critical analysis. This procedure could avoid the possible contamination of automated analyzers and other devices, surfaces and other sites of the laboratory. If POC instruments are to be used, the clinical laboratory must ensure that these devices can be used in critical care units. The laboratory should establish the accuracy, sensitivity, specificity, range of test results and reference intervals, as well as a quality control program for these instruments.

### **Packaging and Transporting Specimens**

In order to send specimens to reference centers, the samples should be placed for transport in sealed containers. These should be resistant to low temperatures (-80° C). This primary container should be surrounded by absorbent material allowing absorption of the content in case the container breaks, and then should be placed in a secondary impermeable metal container with tape. Relevant information on the sample should be labeled on the outside of the secondary container. The secondary container should be placed in a definitive secure box.

The sample should be transported on dry ice *via* a carrier that should be compliant to regulations for transporting biological specimens.

### **Environmental Control Procedures**

VHF viruses are susceptible to a broad range of hospital disinfectants. Surfaces and objects contaminated with body fluids should be disinfected using standard procedures. Common hospital disinfectants or a 1:100 dilution of sodium hypochlorite could be used for disinfection. For surfaces, a 1:10 dilution of sodium hypochlorite should be used. Feces, vomitus and other medical fluids can be thrown away in the sanitary. Solid medical devices such as contaminated syringes and needles should be discarded in rigid containers and then incinerated.

## MANAGEMENT OF PATIENT CONTACTS

Persons who have been exposed to an infected patient or to an infected body fluid within the incubation period preceding the onset of illness may be classified in three groups: casual contacts, close contacts and high-risk contacts. Casual contacts include persons who had very little contact with infected patients. In these cases, no special control measure is necessary because VHF are not spread in this situation. On the other hand, close contacts include persons living with the patient, health care personnel who serve the patient when he/she was ill, and laboratory personnel who work with the patient's specimens. These persons should be placed under surveillance for 3 weeks. Finally, high-risk contacts are persons who came in contact with body fluids from the patient by different ways. These persons should also be placed under surveillance. Special attention should be paid to persons with percutaneous or mucocutaneous exposures to blood and other body fluids from a patient with suspected VHF; they should quickly wash the affected parts of the body with soap and water. Above all, mucous membranes should be washed with a generous quantity of water or eyewash solutions. If a person has had a contact and he/she develops a temperature of 38°C or higher and any other symptoms of illness, such person should be quickly isolated and treated as a VHF patient. For high-risk contacts, ribavirin should be started as a post-exposure prophylaxis above all when Lassa fever is suspected, but also if CCHF is suspected.

## CONCLUSIONS

VHFs are severe and often fatal in humans. These infections are spread by close contact with body fluids of an infected person or by contact with objects contaminated with infected body fluids or secretions. Laboratory directors should ensure proper risk assessment; they should provide safety procedures and practices to protect health care workers.

Clinical laboratories should be prepared to safely handle specimens from patients with VHF by strict adherence to established procedures. It is necessary that all laboratory workers know the safety procedures and guidelines. All persons collecting or handling samples from patients with VHF should strictly follow the established protocols. A key point is that all health care personnel should wear appropriate PPE such as adequate clothes, gloves, water-resistant gowns, full-face shield and mask.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

## ACKNOWLEDGEMENTS

Declared none.

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