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Barbara Zawilińska[1], Magdalena Kosz-Vnenchak[1]

GENERAL INTRODUCTION INTO THE EBOLA VIRUS BIOLOGY AND DISEASE

Abstract: Epidemic of Ebola hemorrhagic fever which appeared in the countries of West Africa in 2014, is the largest outbreak which occurred so far. The virus causing this epidemic, Zaire Ebolavirus (ZEBOV), along with four other species of Ebolaviruses is classified to the genus *Ebolavirus* in the family *Filoviridae*. ZEBOV is one of the most virulent pathogens among the viral haemorrhagic fevers, and case fatality rates up to 90% have been reported. Mortality is the result of multi-organ failure and severe bleeding complications. The aim of this review is to present the general characteristics of the virus and its biological properties, pathogenicity and epidemiology, with a focus on laboratory methods used in the diagnosis of these infections.

Key words: Ebola virus properties and structure, taxonomy, Ebola virus pathogenicity and epidemiology, Ebola hemorrhagic fever diagnosis.

INTRODUCTION

The epidemic of Ebola haemorrhagic fever (now called Ebola virus disease) observed in 2014 in West Africa is the largest outbreak which occurred since the first case of this disease in 1976. The number of cases and deaths has already exceeded the number of recorded cases in all previous epidemics together. According to the WHO report of November 21, 2014, the total number of confirmed or suspected cases in the current outbreak is 15,351, with 5,459 reported deaths. Guinea, Liberia and Sierra Leone are the most affected countries [1]. The aim of the review is to present the characteristics and biology of the virus responsible for this haemorrhagic fever, the sources of infection and clinical feature of disease, as well as a possible methods of diagnosis.

TAXONOMY

According to the recently announced taxonomy of viruses, Ebolavirus belongs to order — *Mononegavirales*, family — *Filoviridae*, and genus — *Ebolavirus* with

5 identified species: *Bundibugyo Ebolavirus* (BEBOV), *Reston Ebolavirus* (REBOV), *Sudan Ebolavirus* (SEBOV), *Tai Forest Ebolavirus* (formerly *Ivory Coast Ebolavirus*, ICEBOV), *Zaire Ebolavirus* (ZEBOV).

In addition to the genus *Ebolavirus*, family *Filoviridae* includes two other genera: *Cuevavirus* and *Marburgvirus* [2]. The filoviruses are primarily African in origin, with the exception of Reston virus and recently identified in Spanish bats *Lloviu virus*, which represents distinct *Cuevavirus* genus.

Virus name is derived from the Ebola River in the Democratic Republic of Congo (formerly Zaire), where the first cases of haemorrhagic fever were recorded in 1976. The disease was observed simultaneously in southern Sudan, and in the northern Zaire, and now it is known that it was caused by two different species SEBOV and ZEBOV, respectively. The name of the family *Filoviridae* comes from the Latin word "*filum*" or thread, because the virion shape resembles a twisted thread when viewed under an electron microscope [3, 4].

Among the viruses of the *Ebolavirus* genus, ZEBOV has the highest fatality rate (up to 90%). In case of infection with other species this ratio is significantly lower (approx. 53% and 25%, respectively for SEBOV and BEBOV). Sick people or contact with human corpses are the main sources of infection during an epidemic, but the natural reservoir of the virus are probably asymptomatic infected fruit bats, whose isolates of filoviruses are characterized by very high genetic diversity [5, 6]. Phylogenetic analyses of *Marburg virus* RNA sequences derived from both, people and bats, suggest that the virus spread from bats might generate an epidemic in humans [7]. In addition, macaques, chimpanzees, antelopes, rodents and other so far unidentified species may represent a significant source of infection for humans.

Sudan Ebolavirus (SEBOV) isolated from humans in Nzara in southern Sudan most likely does not have animal reservoir. This assumption is justified by the territorially limited occurrence and genetic stability of strains originated from neighboring Sudan and Uganda for almost 30 years [6]. In 1994, *Tai Forest Ebolavirus* (TAFV) was isolated in the Ivory Coast from an ethnologist making the autopsy of chimpanzee from the Tai National Park reserve. Because it has been described only in a single nonfatal human case, therefore was assumed to infect mainly chimpanzees.

Ebola-Bundibugyo virus (BEV) appeared in 2007. The name *Bundibugyo virus* comes from the town in Uganda. BEV infection cannot be differentiated from other ebolaviruses by clinical symptoms. Further, the *Reston Ebolavirus* (REBOV) originates from the Philippines. It was first detected in Reston, VA, USA, in macaques imported from the Philippines and housed at a quarantine facility. This species caused in nonhuman primates haemorrhagic fever with high mortality. It also emerged in Philippines pigs, usually co-infected with porcine respiratory and reproductive virus (PRRS), but the actual pathogenic potential of REBOV in pigs remains unclear. It is known to be non-pathogenic to humans, although the

presence of specific antibodies in humans suggests the possibility of its transfer from infected animals [4].

SENSITIVITY TO PHYSICAL AND CHEMICAL AGENTS

Ebola viruses are classified as both biosafety level 4 and category A list pathogens. Currently, there are no approved options available for either treatment or postexposure prophylaxis. Filoviruses infectivity is quite stable at room temperature, but is largely inactivated by 30 minute incubation at 60°C. Current methods inactivating filoviruses are limited to high doses of ultraviolet light and gamma irradiation, formalin treatment, lipid solvents, β-propiolactone, photo-induced alkylating probe 1,5-iodonaphthylazide, and commercial hypochloride and phenolic disinfectants [8, 9].

VIRION STRUCTURE

The virion has a uniform diameter of about 80 nm, and the length from 970 to 1200 nm. When propagated in cell cultures it is characterized by a significant pleomorphism and its length can then increase up to 14,000 nm [10]. The virion core contains one molecule of linear, non-segmented, single-stranded, negative-sense RNA. The RNA is helically wound and complexed with the NP, VP35, VP30, and L proteins. The helical nucleocapsid is surrounded by an outer envelope with anchored specific glycoprotein (GP) spikes, of about 10 nm length. These glycoproteins play a key role in the pathogenesis, due to the role in virus entry and its immunogenicity. Glycoproteins are targets for the immune cells, and thus are taken into account in the development of vaccines. Viral matrix protein, VP40 and VP24, are located between the nucleocapsid and the outer envelope (derived from the host cytoplasmic membrane). The viral genome, approximately 19 kb in length, is the longest among all viruses belonging to the order *Mononegavirales*. Sequentially arranged genes encode seven structural proteins, respectively 3'-nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), protein VP30, matrix protein (VP24), RNA-dependent RNA polymerase (L) and one non-structural small glicoprotein — sGP, the function of which is not yet completely understood. This small glycoprotein is not a part of the virion, but is excreted from the infected cell in large quantities. It may play a role in confounding the immune system to prevent the marshaling of an effective immune response. Moreover, viral proteins VP24 and VP35 are also important virulence factors, because they act as a type I interferon (IFN) antagonists [4, 11].

The 3' terminus of viral genome is not polyadenylated and the 5' end is not capped. The leader and trailer at the 3'- and 5'- ends are non-transcribed regions,

but they carry important signals to control transcription, replication, and packaging of the viral genomes into new capsids. Sequences encoding the viral structural proteins contain open reading frames and are flanked by the non-translated intergenic regions.

REPLICATION

The first step in viral replication is attachment to the host cell membrane and penetration into the cell. This process is not completely understood, but it is known that glycoprotein (GP) spikes are involved in entry of virions into the host cell and are used in the mechanisms similar to macropinocytosis [12]. The other proposed mechanisms of cell entry include: clathrin-mediated endocytosis or glycoprotein-facilitated receptor binding. The glycoprotein is posttranscriptionally cut into GP1 and GP2 proteins. The GP1 takes part in the attachment of the virus particle to the cell membrane, while the GP2 participates in viral fusion with the cell membrane. Viral membrane fuses with cell vesicle membrane to allow the release of the nucleocapsid into the cytoplasm. It is believed that the further steps of replication occur in the cytoplasm, analogously to paramyxoviruses and rhabdoviruses. Encapsidated, genomic RNA is used then as a template for transcription into seven polyadenylated, monocistronic mRNAs and translated by the cellular translation machinery into individual viral proteins. Transcription is regulated by conserved transcription start and stop signals located at the viral gene borders. The gene start signals are parts of RNA secondary structures, and it has been proposed that VP30 binds to the RNA at the first gene start signal to initiate transcription. In addition, VP30 was shown to be important for reinitiation of transcription of subsequent genes [13]. VP30 activity is regulated via its phosphorylation state: phosphorylation of VP30 inhibits viral transcription while viral replication is increased. Because of its essential function in these processes, VP30 is a potential interesting candidate as antiviral therapy target [3].

Subsequently, when a positive-sense full-length genome is replicated, it is concomitantly encapsidated by newly synthesized NP molecules. Other structural nucleocapsid proteins (polymerase cofactor — VP35, and the viral RNA polymerase L) participate in the synthesis of the viral genome. The presence of matrix VP24 together with NP and VP35 is required for assembly of viral nucleocapsids, and silencing of VP24 expression prevents the release of viruses. Moreover, in the VP24-deficient viral particles VP30 transcription and translation are diminished [14]. Further, the most abundantly expressed matrix protein VP40 plays an important role in the formation of new virus particles, and is associated with the endosomal pathway and virus budding from the cell [15]. The mechanisms of this process are not fully understood but it is known that mutations in the sequences encoding the VP40 leads to inhibition of virus release from the infected cell.

CLINICAL MANIFESTATION AND PATHOGENESIS OF EBOLA VIRUS HAEMORRHAGIC FEVER

All filovirus infections of human are characterized by a similar disease, which severity and fatality rate depends on infecting viral species. Pathomechanism of Ebolavirus infection is complex. It involves phagocytic cells, released proinflammatory cytokines, chemokines, and growth factors, endothelial cell dysfunction and triggering of coagulopathy, direct damage of cells by viral replication as well as suppression of innate and adoptive immune response [16].

The symptoms of the disease appear after 4–10 days of incubation period (range 2–21 days, depending on the infective dose). Typical is the sudden onset of flu-like symptoms, vomiting and diarrhea. The patient's condition deteriorates rapidly and the next phase of the disease results from external and internal bleeding complications, hypotension and coagulation disorders, and often leads to fulminant shock and subsequent multi-organ failure. Fully symptomatic patients usually die between 6–16 days after onset of symptoms. In cases of recovery, the patient's clinical condition was improved with the appearence of specific antibodies. However, long-term consequences of infection may persist as a recurrent hepatitis, spinal cord injury, uveitis, psychosis or hair loss [17, 18].

Existing data on the pathological mechanism involved in the disease have been obtained by experimental infection of non-human primates and rodents as well as by clinical and laboratory observation collected during the human outbreaks.

The virus enters the body parenterally, through the skin and mucous membranes. It exhibits a broad tropism to different cells. This apparent lack of target specificity is probably due to the wide distribution of cell-surface lectins involved in the binding of the viral surface GPs. Macrophages, monocytes, and dendritic cells are the early targets of viral infection. Due to their high migratory activity these cells play a key role in the spread of the virus from the initial site of infection to the regional lymph nodes, liver, spleen and adrenal glands.

Virus utilize multiple mechanisms to evade detection and undermine innate immune responses. The mentioned structural proteins VP24 and VP35 are crucial to evade host innate immunity and inhibit type I interferon responses [19]. The sGP protein inhibits the migration of leukocytes. Viral infection is accompanied by a massive release of proinflammatory mediators and vasoactive substances, which promotes inflammation and coagulation, but at the same time renders the immune system unable to effectively prevent systemic spread of the virus.

Haemorrhagic effects of Ebolavirus infection in endothelial cells are likely triggered by immune-mediated mechanisms. It has been hypothesized that Ebolavirus VP40, GP1, and GP2 were able to activate endothelial cells and significantly impair the barrier function [3].

The coagulopathy is multifactorial and appears to be caused by a combination of activation of several factors, such as: the mononuclear phagocytic system, platelet

aggregation and consumption, activation of the coagulation cascade, deficiency of coagulation factors due to liver damage, and endothelial damage. Therefore, the cause of death may be either bleeding or blood clotting, frequently with multiple organ failure.

EPIDEMIOLOGY

Ebola virus disease is a zoonotic disease and each outbreak in the human population is initiated by an introduction from an animal reservoir (e.g. due to hunting, direct contact with infected live or dead animals, consuming of bush meat). The spread of sporadic cases appearing among the rural population living near the rainforest, to the large urban agglomerations is responsible for the development of a new epidemic. A leading source of infection is direct contact with a sick person (particularly in the late stages of infection, when viral loads are the highest) or contaminated objects used by the patient. Infection of healthcare workers or those taking care of sick persons, in the absence of appropriate personal protective equipment, and during the funeral ritual are an important element in the epidemiological chain. Sexual transmission during convalescence stage was also described. Body fluids (mainly blood) and secretions (saliva, urine, vomit, feces, semen) are infectious. Filoviruses enter the host through mucosal surfaces, breaks, and abrasions in the skin, or by accidental injection. In hospital environment infection through the aerosols is also possible (e.g. during intubation, bronchoscopy), although there is no clear evidence that airborne spread from person to person occurs. In an animal model it was confirmed that in the case of needle-stick injury the risk of infection and disease progression is significantly greater than after administration of a similar dose by aerosol [20].

The current epidemic in West Africa, which was announced in March 2014, was preceded by the incidences already recorded in December 2013 in Forested Guinea. The causative agent has been identified as an outlier strain of Zaire Ebola virus [21]. Characteristic of the current ongoing epidemic mortality rate is much lower than that observed in previous outbreaks.

LABORATORY DIAGNOSTIC METHODS

Since the Ebola virus has been classified by the CDC as a pathogen of category A, the category that includes most dangerous pathogens causing diseases with high morbidity and mortality, viral diagnosis should be performed only in specialized laboratories with the highest level of biosafety, i.e. BSL-4.

It should be fast, sensitive and specific, and the methodology used should greatly limit the possibility of exposure of a person engaged in the study to the

risk of the laboratory infection. If the diagnostic methodology allows, chemical or radiation inactivation of clinical material should be used before testing. The selection of the tests is also dependent on the capabilities of performers being under different conditions, even at the site of the epidemic outbreaks.

Currently, real time RT-PCR is considered as the most sensitive method, which allows for detection of the number of viral copies in specimen. Although there are other methods of virus identification. But the presence of viral RNA can be detected by polymerase chain reaction with reverse transcription (RT-PCR) even after 48 hours post onset. However, it should be emphasized that due to continuous virus mutations RT-PCR method may be unreliable and results should be confirmed by other assay. One-tube real-time RT-PCR assay was developed for identification of ZEBOV and SEBOV. To distinguish between Ebolavirus species and strains sequencing of amplified genomic RNA can also be used [22].

The enzyme-linked immunosorbent assay (ELISA) may be used to detect both antibodies as well as virus-specific antigens. Assays for the detection of antibodies are less useful because the patient often dies before the formation of a specific antibodies. Therefore, they are carried out mainly for epidemiological purpose, for patients who survived this terrible disease. Positive results obtained by the ELISA can be confirmed by Western blot. Sometimes only IgM antibodies are detectable in specimen of sick person. They appear after 2 days of symptoms onset and may last for 30–168 days. In contrast, IgG antibodies usually are detected between day 6 and 18 post onset of illness and persist for years. The antibody profile of the sera is significantly different in patients with lethal disease as compared to those that survived. This difference can serve as a prognostic marker for the management of the patient. It has been shown that deceased patients show a much lower or even absent antibody response compared with survivors [18]. During outbreaks, ELISA methods for the detection of specific viral antigens are highly useful and frequently applied. Because high titers of filovirus particles are present in the blood and tissues of patients at the early stage of illness, several ELISA systems have been developed to diagnose an acute infection. Currently applied assays use monoclonal antibodies against different viral proteins, e.g. VP40. These systems are able to provide the results within 30 min, without a need for electricity or sophisticated equipment.

Virus isolation in cell cultures is one of the very sensitive methods. Acute phase patient sera or postmortem tissue samples may be appropriate material for the virus isolation. Ebola virus is able to replicate in numerous cell lines and virus growth can be detected by cytopathic effect. Vero or Vero E6 cells have been commonly used for this purpose. Additionally it is also possible to use a fluorescently-labeled specific antibodies for confirmation of antigens in infected cells.

Electron microscopy has also been useful in identification and detection of viral infections [23]. Due to the very characteristic shapes of virus particles this

method is specific and rapid, but requires a large number of virus particles in a sample, specific and expensive equipment and trained personnel respectively.

The paraffin sections of autopsy material, particularly from the liver and spleen, due to the high condensation of antigens and viral particles, are useful in immunohistochemical assays with the use of specific polyclonal or monoclonal antibodies. Formalin-fixed specimens are not infectious and may be sent without special precautions or refrigeration.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

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[1] Department of Virology, Chair of Microbiology
Jagiellonian University Medical College
Kraków, Poland

Corresponding author:
Dr hab. n. med. Barbara Zawilińska
Department of Virology, Chair of Microbiology
Jagiellonian University Medical College,
ul. Czysta 18, 31-121 Kraków, Poland
Phone: +48 12 634 54 00
E-mail: mbzawili@cm-uj.krakow.pl