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BRIEF REPORT

# Ebola Hemorrhagic Fever and the Current State of Vaccine Development

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**Abstract**
Current Ebola virus outbreak in West Africa already reached the total number of 1,323 including 729 deaths by July 31st. the fatality is around 55% in the south-eastern area of Guinea, Sierra Leone, Liberia, and Nigeria. The number of patients with Ebola Hemorrhagic Fever (EHF) was continuously increasing even though the any effective therapeutics or vaccines has not been developed yet. The Ebola virus in Guinea showed 98% homology with Zaire Ebola Virus.
Study of the pathogenesis of Ebola virus infection and assess of the various candidates of vaccine have been tried for a long time, especially in United States and some European countries. Even though the attenuated live vaccine and DNA vaccine containing Ebola viral genes were tested and showed efficacy in chimpanzees, those candidates still need clinical tests requiring much longer time than the preclinical development to be approved for the practical treatment.
It can be expected to eradicate Ebola virus by a safe and efficient vaccine development similar to the case of smallpox virus which was extinguished from the world by the variola vaccine.

## 1. Introduction

In February 2014, the first outbreak case of Ebola virus (Figures 1) was confirmed and registered in the region of Guinea (West-Africa) by the World Health Organization (WHO). By July 31, the total number of suspected and confirmed cases in the Ebola hemorrhagic fever (EHF) outbreak had increased to 1,323, including

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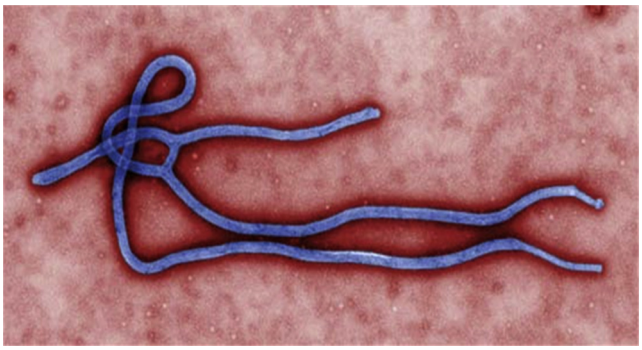

**Figure 1.** Ebola virus virion under light microscopy. (Courtesy of CDC/Cynthia Goldsmith).

729 deaths at a fatality rate of 55% in the southeastern area of Guinea, Sierra Leone, Liberia, and Nigeria [1]. The Ebola virus in Guinea showed 98% homology with the Zaire Ebola virus (ZEBOV) found in Congo and Gabon (1994−1995) [2].

Since the first outbreak of Marburg hemorrhagic fever occurred in 1967, there have been 18 reports of human outbreaks by Ebola or Marburg viruses, which has resulted in approximately 1,500 cases to date [3] (Figure 2). In 1976, the first outbreak reported in Zaire (Democratic Republic of the Congo) resulted in 318 cases and 280 deaths. Since then, Ebola virus epidemics have occurred in several countries in equatorial Africa and the strains were named after the regions of outbreak including Ebola-Zaire, Ebola-Sudan, Ebola-Tai Forest, Ebola-Bundibugyo, and Ebola-Reston. ZEBOV is the most lethal pathogen of the Ebola viruses which causes > 90% of fatalities due to hemorrhagic fever in humans and primates [4]. In 1994−1995, outbreaks in Gabon and Zaire (Democratic Republic of the Congo) also resulted in 285 deaths of 367 infected cases [5]. An epidemiological study revealed that most cases occurred after direct contact with blood, secretions, or tissue of infected patients. In 1976, 27% of death cases among 88% of death cases in Zaire were reported as being due to patients having been injected with a contaminated syringe [6]. Mortality was higher even though the percutaneous exposure was of very low Ebola virus inocula. In 1995 in Kikwit, numbers of Ebola viral particles found in human skin and lumina of sweat glands raised concern about the disease transmission by touching an infected patient or corpse [7]. The overall window period ranges from 2 to 21 days, and patients usually do not show any symptoms within the 1st week.

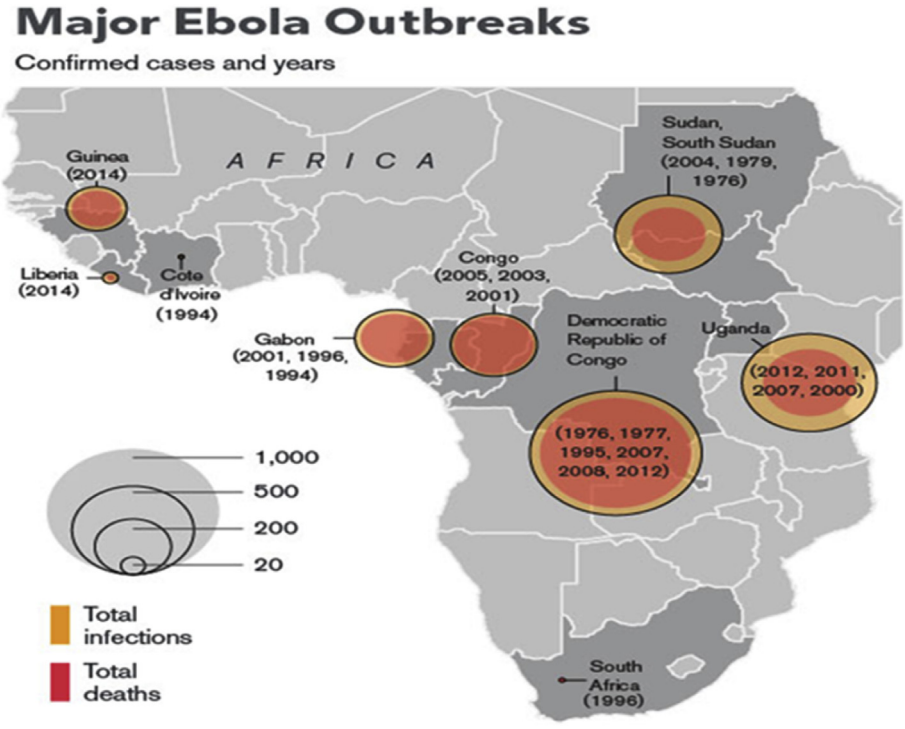


**Figure 2.** Mapping the world Ebola outbreaks. http://www.zerohedge.com/2014-04-05/mapping-workls-ebola-outbreaks.

The transmission of Ebola virus during the incubation period does not appear to be common [8]. Ebola virus is virulent in nonhuman primates and humans and could cause serious acute infection with fever and a bleeding diathesis after very short window period [9]. Usually, patients infected with the Ebola virus show acute symptoms with high fever, bleeding, disseminated intravascular coagulation, headache, high fever, myalgias, nausea, abdominal pain, nonbloody diarrhea, arthralgias, and malaise [10]. However, the ecology and the epidemiology of Ebola viruses are poorly understood.

EHF is a pathogenic symptom caused by one of four major distinct hemorrhagic fever viruses (HFVs): *Flaviviridae*, *Arenaviridae*, *Bunyaviridae*, and *Filoviridae*. HFVs belong to a group which generates febrile illnesses by the RNA viruses of this viral family [11]. Naturally, HFVs are transmitted by animal hosts or arthropod vectors, and the strains of the viruses each have characteristic features and diseases. *Flaviviridae* includes mosquito-borne yellow fever virus and Dengue virus. *Arenaviridae* is well known and includes Lassa virus and several South American HFVs transmitted by small rodent contact. *Bunyaviridae* includes Rift Valley fever (RVF) virus, Crimean-Congo hemorrhagic fever (CCHF) virus, and Hantaan virus. RVF virus is transmitted by mosquitoes and CCHF virus is transmitted by ticks and is highly pathogenic for aerosol transmission of infective particles.

The most notable HFVs, Ebola and Marburg viruses, are classified as family *Filoviridae*, but the host species in nature is still unknown [12]. Ebola and Marburg viruses give rise to a multitude of other human pathogens like measles virus and respiratory syncytial virus in *Paramyxoviridae* in structure and features; they have negative-sense RNA without a segment as a genome (approximately 15,000∼16,000 bases) and have rapid proliferation; the genome of Ebola virus is nearly 19,000 bases in length (Figure 3). This virus replicates genomes and expresses proteins by the types of host cells with an infecting capacity. Ebola virus is cytotoxic to cells and may lead to thrombocytopenia, a platelet dysfunction. The most important aspect of Ebola virus pathogenesis is how it affects the immune responses of human and nonhuman primates, which support the growth of Ebola viruses.

Common diagnostic methods include antigen detection by antigen-capture enzyme-linked immunosorbent assay (ELISA), immunoglobulin M (IgM)/IgG antibody detection by ELISA, Realtime PCR, and virus isolation. RT-PCR is the most common and useful diagnosis technique, but antibody-capture ELISA has a limited value in early diagnosis, because antibodies to these viruses usually do not appear until the onset of recovery, at 1∼2 weeks of illness [13]. Otherwise, virus isolation is also limited because it requires biosafety level 4 laboratories.

EHF is one of the aggressive infectious pathogens which has no vaccine and prophylaxis. Preparedness including vaccines, rapid treatment, and acute diagnostic methods will be helpful for decreasing and managing of infection as other infectious diseases. Regarding preparedness, development of a vaccine is the best approach to deal with the threat of high risk infectious diseases.

## 2. Ebola virus vaccine development

Study of the pathogenesis of Ebola virus infection and assessment of various candidates for a vaccine have been tried for a long time [14,15]. In the early stages of research, live attenuated vaccines were studied using guinea pigs and nonhuman primates as primary animal models, because of the similar progression of symptoms and pathogenesis to humans. However, no efficient vaccine candidates were discovered even in nonhuman primates. Recently, gene-based vaccine approaches revealed advanced results in animals by several groups; the Vaccine Research Center group of the National Institute of Health (NIH) which collaborates with research teams at the United States Army Military Research Institute for Infectious Diseases (USAMRIID), and British research groups including the Research Institute for Tropical Medicine at Cambridge University. The Cambridge University group deals with variable animal models with Ebola vaccine study in Africa, and the Whitehead Institute at Oxford University showed progression of a pathogenic characterization study of Ebola virus which gives important clues for vaccine and drug development. Early animal studies of live attenuated vaccine with guinea pigs were not

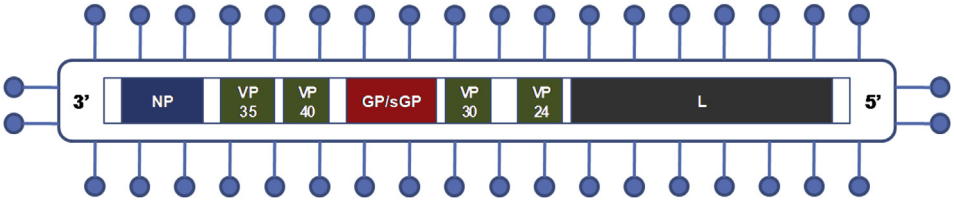


**Figure 3.** Negative-sense RNA genome of Ebola virus. Each gene transcribes functional proteins for viral replication. Some function of those genes are attenuated for live vaccine development. GP = glycoprotein; L = polymerase; NP = nucleoprotein; sGP = soluble glycoprotein; VP = viral protein such as RNA-binding protein or minor nucleoprotein.

satisfactory, but current reports from the Vaccine Research Center of NIH and Research Institute for Tropical Medicine at Cambridge University announced positive results about the efficacy of DNA vaccine against Ebola virus in mice and chimpanzees. These researches suggest a positive expectation for the development of an Ebola vaccine [16]. We will briefly look at each current candidate for an Ebola vaccine.

### 2.1. Live attenuated vaccine

In the 1990s, guinea pigs were used as an animal model in Ebola virus vaccine research, mostly using live attenuated strains. Generally, live attenuated vaccine and recombinant protein vaccine have successful effects on various pathogens, because they can be mucosally administered [17,18]. Preliminary immunization studies in mice and guinea pigs (unpublished data) indicated the usefulness of vesicular stomatitis virus (VSV) vectors for vaccine delivery against ZEBOV [19]. Based on attenuated recombinant VSV (rVSV) including expressed Ebola glycoprotein, replication-competent was developed against ZEBOV. In the first study, using a vector expressing the ZEBOV glycoprotein showed that the nonhuman primates can be protected with a single dose immunization [20]. In other cases, the utility and potential of the rabies virus vaccine (RABV) vector platform, which showed development of live and killed vaccines against ZEBOV/Marburg virus, was studied. Immunization with live RABV vaccines expressing ZEBOV glycoprotein induced humoral immunity conferred protection from lethal ZEBOV/Marburg virus in mice [21]. However, there has been no advance over two decades in the study of Ebola virus vaccine and any animals tested obtained protection by either live attenuated strains or recombinant proteins used as vaccine candidates, even though mouse models for efficacy testing were developed with mouse-adapted Ebola virus strains. There could still be difficulty for practical use, because there would be barriers in the permission process and manufacturing procedures raised by potential risks considering the extremely high fatality of Ebola virus compared to other viruses, even if a live attenuated vaccine could be developed.

### 2.2. DNA vaccine

From the late 1990s to the early 2000s, development of a DNA vaccine has been continuously attempted, which boosts immunization by delivering DNA into host cells as an Ebola virus vaccine candidate. Insertion of nucleoprotein and envelope glycoprotein for the purpose of genetic immunization with plasmid DNA (vector) as a DNA vaccine candidate was developed in some animal models using guinea pigs and mice and resulted in a high protection capacity [22,23].

In particular, DNA vaccine candidate containing glycoprotein maintained memory for the immune response for a long time to protect animals from Ebola virus infection [24]. While primary DNA vaccines have been highly effective in mice and other rodents, their efficacy in nonhuman primates or humans has been less impressive [25]. Many research groups, including the NIH Vaccine Research Center, tried to develop priming protocols of boosting immunization for the improvement of efficacy, and some of those protocols included a combination of viral vectors to enhance the immune responses in human and nonhuman primates [26].

In cases of DNA immunization followed by boosting with poxvirus vector carrying the genes for pathogen protein instead of plasmid, DNA vaccine produced highly enhanced cellular immunity, over 30-fold. Boosting with replication-defective adenovirus vectors demonstrated a strengthening of stability for both cellular and humoral immunity, enough to protect hosts from the challenge of Ebola virus at the lethal dose. This approach provides the first experimental demonstration of vaccine candidates protecting primates from the Ebola virus infection. About the same time, USAMRIID successfully induced immune responses in primates using a DNA vaccine candidate of a virus-like particle (VLP). A VLP-type DNA vaccine can contain key components of Ebola virus antigens to highly induce the immune response; this VLP should be safe due to the lack of full replication machinery.

Recently, a trial using VSV (Figure 4), designed to contain the both Ebola and Marburg virus genes, revealed regularly induced immune responses to protect hosts [16,18]. So far, adenovirus vector, VSV vector, and other DNA vaccines are in clinical trials. Not only extermination of humans, but a lethal threat from the Ebola virus to all chimpanzees, gorillas, and other nonhuman primates in Africa and worldwide is another dangerous issue of the wildlife ecosystem. A Cambridge University group lead by Peter Walsh reported perfect protection capacity of experimental vaccine using the

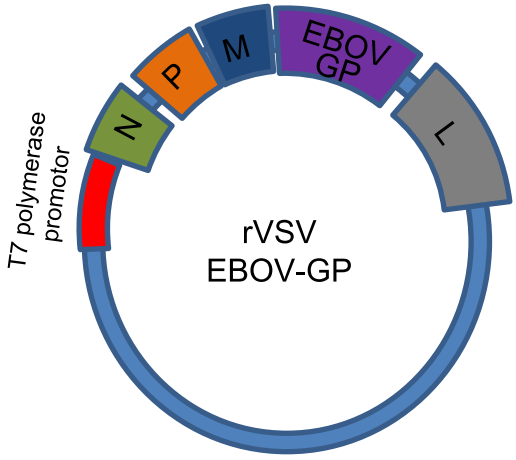


**Figure 4.** Structure of vesicular stomatitis virus (VSV)-based Ebola virus DNA vaccine. Ebola virus glycoprotein is contained in the VSV protein in the plasmid.

Ebola/Marburg gene-containing VSV in chimpanzees. Because the human immune system seems to be similar to the system of chimpanzees against Ebola virus, this type of DNA vaccine could be developed as a novel candidate for human vaccine in the future.

## 3. Conclusion

To date, no vaccines have received regulatory approval. However, there is still an outbreak and spreading of EHF in Africa. The best preparedness strategy against a crisis generated by high-risk viruses like Ebola or Marburg viruses are to save stockpiles of vaccines after development. As a warning of an outbreak of EHF which has a lethal fatality just after infection, immediate vaccination will be the most effective response for the habitants of dangerous regions against outbreaks; development of an effective vaccine is essential as an advantage in controlling the spread of disease. Above the development of this kind of vaccine against high-risk pathogens such as Ebola virus, safety is much more important than the efficacy to prevent potential accidents.

Eradication of the Ebola virus by a safe and efficient vaccine development similar to the case of the smallpox virus, which was extinguished from the world by the Variola vaccine developed by Edward Jenner, can be expected.

## Conflicts of interest

The authors declare no conflicts of interest.

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