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Review Article

An update on Ebola virus epidemiology and experimental modalities

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Abstract

Ebola virus disease (EVD), formerly known as Ebola hemorrhagic fever, is a re-emerging zoonotic disease that has caused periodic outbreaks since the first reported outbreak in 1976. In 2014, in West Africa, the largest outbreak of Ebola virus in history emerged, affecting close to 30,000 people; more than 10,000 of these individuals died. This virus, which causes fatal hemorrhagic fever in humans and non-human primates, has the highest mortality rate (25-90%) among the viral hemorrhagic fever diseases. Ebola virus is transmitted through the blood and body fluids of infected animals and humans, as well as through contaminated materials. The virus is often spread in the hospital setting, and it is classified as a biosafety level 4 agent. Accordingly, diagnostic procedures and infected patient care should be performed at the highest safety levels. The potential therapies and vaccines against EVD have continued developing or been used in emergency situations during last outbreak. Some of these include the chimpanzee adenovirus type-3 and recombinant vesicular stomatitis virus-Ebola vaccines and new treatment modalities, such as passive immunization, brincidofovir, and ZMapp. Although these trials have shown different levels of success, there are no standardized treatments or vaccines that have been approved for humans. Such prevention is the only way to reduce human infection and the spread of outbreaks and is primarily based on personal protection measures and raising awareness of the risk factors.

Ebola Virus

Ebola virus was detected, in 1976, during the first documented outbreaks in Zaire and Sudan; its name is derived from a river near Yambuku in Zaire (now the Democratic Republic of the Congo; DRC) (1,2). Ebola virus is an enveloped, non-segmented, negative-stranded RNA virus, and its characteristic filamentous structure led to give the family name (3). Ebola virus particles have a uniform diameter of 80 nm and a typical length of 800-900 nm, but sometimes up to 1400 nm long (3,4). The viral genome is 19 kb in length, and it encodes the following seven proteins: Nucleoprotein (NP), glycoprotein (GP), RNA-dependent RNA polymerase (L), and four structural proteins: Termed VP24, VP30, VP35, and VP40 (3,5).

According to the latest classification of International Committee on Taxonomy of Viruses in 2010, the Ebola virus genus, which is one of three genera belonging to the Filoviridae family, consists of five species, as follows *Bundibugyo ebolavirus* (BuEV), *Reston ebolavirus* (ReEV), *Sudan ebolavirus* (SuEV), *Taï Forest ebolavirus* (TFEV), and *Zaire ebolavirus* (ZaEV) (6,7). The BuEV, ZaEV, and SuEV species have been detected in high-fatality epidemics in Africa (8) (Figure 1). TFEV occurs less frequently, and it was first identified from a patient and a chimpanzee, in 1994, in the Ivory Coast (8,9). ReEV is different from the other four species in that it has not been found in Africa; it was first detected in macaques imported into the

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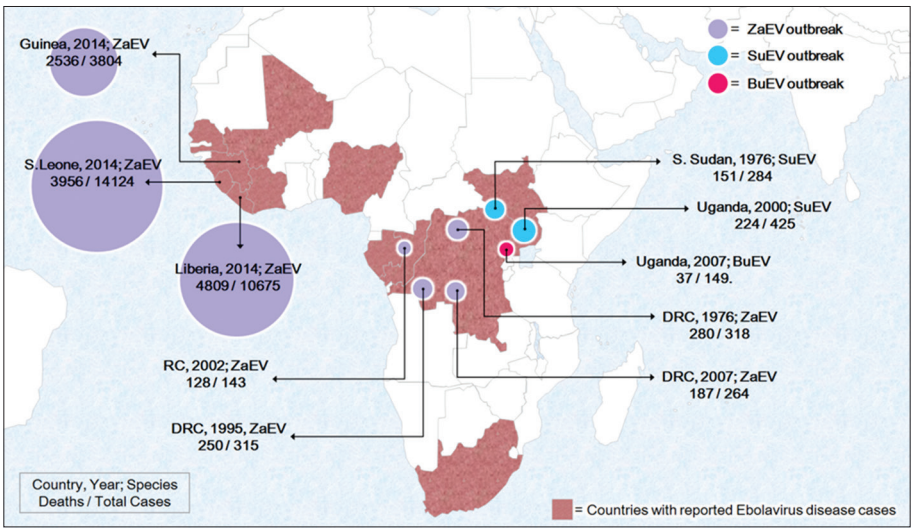

Figure 1: Ebola virus outbreaks from its discovery until the present (the outbreaks with more than 100 cases are shown only, according to case numbers) (8,17). DRC: Democratic Republic of the Congo, RC: Republic of the Congo

United States from the Philippines (10). Serological analyzes have shown that humans can be infected with ReEV, but transmission to humans has been infrequent and has not resulted in any reported illness (10). Ebola virus can infect a variety of other animals, including chimpanzees, gorillas, pigs, rodents, bats, and antelopes (3,11,12).

Immunopathogenesis

Ebola virus infects many different cell types including monocytes, macrophages, dendritic cells, adrenal cortical cells, fibroblasts, hepatocytes, and several types of epithelial cells (13-15). The early targets of the virus are monocytes, macrophages, and dendritic cells in the host immune system (13). After replication in these cells, the virus disseminates to regional lymph nodes and then to the liver and spleen (14). Infected cells cause the release of pro-inflammatory and antiviral cytokines (interferon [IFN]-alpha; interleukin-1, 6, 8, and 12; tumor necrosis factor; and coagulations factors), and severe dysregulation of the vascular and inflammatory response plays a key role in EVD manifestations (14). Different mechanisms interfere with the host immune system in Ebola virus. Inhibition of the type-1 IFN response seems to be a key feature of EVD pathogenesis (3). VP35 protein is a type-1 IFN antagonist, and VP24 interferes with type-1 IFN signaling (3). In addition, the Ebola virus envelope is heavily glycosylated, and this structural characteristic and induction of cell-cell fusion by virus GP contribute to the ability of the virus to escape immune mechanisms (5,16).

Epidemiology

Ebola virus was first identified in two simultaneous outbreaks in Zaire (now DRC) and South Sudan in 1976 (1). In these outbreaks, a total of 602 people were infected with Ebola virus, and the mortality rate was observed to be 71.59% (8). In 1977-1979, sporadic infections and cumulative cases were observed in both countries, and a total of 35 people were infected with the virus (8). After 15 years of epidemiological silence (1979-1994), in following two decades, many epidemics and sporadic cases were reported in Gabon, Republic of Congo, DRC, South Africa, Uganda, South Sudan, and Ivory Coast (8,11). In addition, until the present, sporadic infections and cumulative cases have also been reported in countries outside of Africa including the United States, Russia, Italy, United Kingdom, Spain, and the Philippines, as well as in other African countries, including Nigeria, Senegal, and Mali (3,10,17).

On 23 March 2014, the West African outbreak exploded, representing the first global threat caused by Ebola virus (12,13). A few months later, the World Health Organization (WHO) announced that this outbreak was an international "public health emergency" (18). Liberia, Guinea, and Sierra Leone were the three countries that were most affected by this epidemic (Figure 1); 28,603 EVD cases were reported in these countries, with 11,301 deaths (the mortality rate was 39.5%) (17) (Figure 1).

Transmission

The natural reservoir of the disease remains unclear but after discovering long-term survival of seropositive

bats, it has been claimed that bats play a major role in the transmission of EVD (3,12,13,19). There is no evidence that mosquitoes or other insects can transmit Ebola virus (13). Previous studies revealed that Ebola virus can be replicated in primate cells, but arthropod cells and arthropods themselves are not readily infected with the virus (20,21). Latent infection has not been determined in non-human primates, but animals that have direct or indirect contact the source (fruit bats), such as chimpanzees, gorillas, cynomolgus monkeys, rodents, and forest antelope, have been reported as possible vectors in the transmission of infection to humans (3,11,15). A piece of evidence supporting this suggestion is that Ebola virus species have been responsible for deaths in wild populations of gorillas and chimpanzees, and these deaths have been closely associated with human outbreaks (22).

It is known that transmission may occur following contact with the tissue, blood, and other body fluids of infected organisms; contaminated environmental material and medical devices (needle stick, reuse of contaminated needles); butchering of a chimpanzee for food; contact during unhygienic applications, such as cleaning an infected dead body; or during traditional burial practices (3,11). The route of infection seems to affect the disease course and outcome. The virus is not transmitted via air or water or by food in general, but aerosol transmission, mucous membrane exposure, pharyngeal contamination, conjunctival and oral routes of infection to be possible according to non-human primate studies (3,15).

Infected persons are not infectious before the appearance of clinical symptoms; they become infectious to others after developing the early signs and symptoms of EVD such as fever and headache (13,23). However, low levels of viremia continue after the regression of clinical findings in recovering patients, and the virus is present in their body fluids (3,13). Therefore, infected patient care and diagnostic procedures should be performed at the highest safety levels.

Clinical Presentation

Ebola hemorrhagic fever disease began to be called as EVD after a lack of hemorrhagic symptoms was observed in more than half of the cases in the outbreak in West Africa in 2014 (24). EVD is an acute severe illness with a high-fatality rate caused by the pathogenic Ebola virus species. The fatality rate has varied from 25% to 90% in several outbreaks of the disease over 40 years (Table 1) (8,17).

Table 1: An overview on case counts of Ebola virus outbreaks and mortality rates (8,17)

Species*	Period	Different outbreaks	Total cases	Fatal cases	Estimated mortality rate (%)
ZaEV	1976-2008	14	1398	1091	78 (41.2-89.5)
SuEV	1976-2012	6	761	405	53.2 (36.3-64.7)
BuEV	2007-2012	2	185	50	27 (24.8-36.1)
TFEV	1994	1	1	0	-
ReEV	-	-	-	-	-
ZaEV	2014-2016	1**	28,639	11,316	39.5
ZaEV	2014	1	66	49	74.2
Total	1976-2016	25	31,050	12,911	41.5 (24.8-89.5)

*ZaEV and SuEV were discovered in 1976, ReEV was discovered in 1989, TFEV was discovered in 1994, and BuEV was discovered in 2007 (7). **West Africa outbreak and associated cases. ZaEV: *Zaire ebolavirus*, SuEV: *Sudan ebolavirus*, BuEV: *Bundibugyo ebolavirus*, TFEV: *Taï Forest ebolavirus*, ReEV: *Reston ebolavirus*

The disease is characterized by sudden onset of symptoms and signs after an incubation period of 2–21 days (mean 4-10) (3). Usually, the initial symptoms are high fever of 38.5-39°C or more (23,24). Other common symptoms are a headache, muscle and joint pain, fatigue, diarrhea, vomiting, stomach pain, and a loss of appetite (3,11). These symptoms are followed by chest pain, shortness of breath, cough, nasal discharge, fast and severe weight loss, and severe disease symptoms (3,23). A pruritic, generalized maculopapular rash can often be noted by day 5-7 of the illness (3,23). In more severe cases, bleeding (cutaneous, mucosal, and tissue–organ), shock, and other neurological symptoms may develop (3,9,11,13). Bleeding is sometimes limited to the gastrointestinal tract (3). In fatal cases, death typically occurs on day 6-16 (generally day 8 or 9) after the onset of symptoms because of septic shock, multiple organ failures, and disseminated intravascular coagulation (12,25).

Diagnosis

The initial diagnosis of Ebola virus infection is based on clinical assessment, but it is often difficult because of the non-specific symptoms of the disease, especially in the early stages of EVD (3). Other viral hemorrhagic diseases and other infectious diseases, including malaria, shigellosis, cholera, leptospirosis, plague, rickettsiosis, typhoid fever, meningococcal meningitis, anthrax, relapsing fever, and fulminant viral hepatitis, should be considered in the differential diagnosis (3,11,13).

Laboratory diagnosis can be carried out via examination of blood, serum, tissue, body fluids, and other clinical samples obtained from suspected animals and

humans within the appropriate biosafety conditions using enzyme-linked immunosorbent assay (ELISA), antigen detection tests, the serum neutralization test, reverse transcription-polymerase chain reaction (RT-PCR), and virus isolation by cell culture (e.g., Vero cells) (5,9,11,13,14,23). RT-PCR and antigen detection ELISA are the primary assays used to diagnosis of acute infections (3). The virus can be isolated from blood from day 3 up to day 7-16 after the onset of symptoms of the disease (3). Immunoglobulin M (IgM) and G (IgG) antibodies can be measured using immunofluorescence assay (IFA), immunoblotting tests, or ELISA (3,23). While IgM antibodies can be detected 2 days after the onset of symptoms and disappear between 30 and 168 days, IgG antibodies can be detected 6 and 18 days after onset of symptoms and continue be detectable for many years (3). The viral antigen and genomic RNA may also be detected using the immunohistochemistry, IFA, ELISA, and RT-PCR techniques. In addition, direct detection of virus particles may be undertaken using electron microscopy (23).

Ebola virus is classified as a biosafety level 4 agent in the biosecurity risk group; when working in the laboratory, the highest level of biocontaminant facilities are needed (13,23). Accordingly, laboratory personnel working in diagnosis and analysis setting must be have detailed information about highly infectious diseases such as Ebola and sufficient equipment.

Treatment and Vaccination

Currently, there are no approved vaccines or standard treatments for EVD that have been validated in humans (25). The main treatment strategies include symptomatic and supportive palliative care, such as hydration and electrolyte replacements, nutritional support, maintaining oxygen status, transfusion of blood components, and treating other infections (11-13). However, different potential therapies and vaccines have been used experimentally for EVD; some of these including new vaccines, passive immunization, ZMapp, and some antiviral agents have shown different levels of success. A list of potential therapeutic modalities and vaccines is listed in Tables 2 and 3.

The WHO has previously stated that whole blood and plasma transfusions from convalescent patients may be used to treat of EVD (25). In a previous study, eight patients in Kikwit, DRC, were treated with blood transfusions from five convalescent patients (26). Only one of these patients was died. This is notable because the high-fatality rate of EVD. Transfusion therapy has also been successfully used in the United States, and more comprehensive trials using convalescent plasma are underway in Liberia, Sierra Leone, and Guinea (25-28). A recent large-scale convalescent blood transfusion campaign was done in Liberia, and the results of the study showed that convalescent therapy is reduced the case fatality rate in relation to the rate of hospitalization (29). However, convalescent therapy is problematic in some situations for example: (i) The donor and recipient must be of the same blood group, (ii) convalescent plasma should be collected and screened for other infectious agents such as hepatitis B and human immunodeficiency virus infection, and (iii) all procedures should be carried out safely and properly, but it is difficult in the conditions and facilities in West Africa where the majority of Ebola epidemics occur (25,26).

ZMapp is a cocktail of three humanized Ebola virus-specific recombinant monoclonal antibodies manufactured in tobacco plants, which targets Ebola virus GP (25). ZMapp is effective in non-human primate models and has been used under emergency treatment protocols in human patients (30,31). Recently, ZMapp has been used to treat seven patients and five of those survived (32). However, these cases are too few to permit any conclusion regarding safety and efficacy of the drug (28). The major problem of this therapy is that expanding ZMapp production is difficult, although government supporting (25,32).

Certain agents have demonstrated *in vitro* efficacy against Ebola virus (25). Brincidofovir, which is one of these agents, was approved by the United States Food and Drug Administration (FDA) for emergency use in October 2014 (25). TKM-Ebola is second FDA approved drug for emergency use in EVD. Brincidofovir and TKM-Ebola are small molecules; therefore, their production easier than monoclonal antibodies. Favipiravir, lamivudine, AVI-7537, BCX-4430, and IFN are other antiviral agent used in the experimental treatment of EVD (Table 2) (25).

In addition, several molecules have been shown to have *in vitro* activity against Ebola virus or have been proposed as a treatment option; these include chloroquine and amodiaquine (antimalarial drugs), cationic amphiphiles (e.g., amiodarone, clomiphene, and toremifene; these prevent Ebola virus from entering

Table 2: Experimental therapeutic strategies for EVD (13,25,28,33,35)

Immunotherapy (antibody therapy)		
Convalescent therapy	• Blood or plasma from recovered Ebola patients (passive immunization)	• Phase 2/3 clinical trials in Guinea, Sierra Leone, and Liberia
		• Protective effect *in vitro*
		• Therapeutic effect in rodents
ZMapp	• Cocktail of three monoclonal antibodies; targeting of Ebola virus GP; binds and inactivates virus	• Treats 100% of infected non-human primates (monkeys)
	• Plant-derived (tobacco plants)	• Phase 2 in Liberia, Sierra Leone, and the United States of America
Antiviral therapy		
Brincidofovir	• Inhibition of DNA synthesis, an antiviral used to treat CMV	• Phase 2 (Ebola) in Liberia; phase 3 (some DNA viruses)
	• An orally available lipid conjugate of cidofovir	• Fewer renal side effects compared with cidofovir
Favipiravir	• Oral nucleotide analog that inhibits viral RNA polymerase	• Promising results in cell culture models and mice
		• Phase 3 trials in humans with influenza
		• Phase 2 trials for Ebola virus in Guinea
TKM-Ebola (TKM-100802)	• A siRNA; interferes with proteins L, VP24, and VP35	• Phase 2 trials in Sierra Leone
	• It cleaves Ebola RNA in cells and prevents virus multiplication	• Authorized for emergency use (TKM-Ebola) by the FDA
		• Treats 100% of infected monkeys
AVI-7537	• Inhibition of protein VP24 of Ebola virus	• Phase 1 trials
		• Significant improvement in mortality after a lethal dose of Ebola virus in rhesus macaques
BCX-4430	• A novel broad-spectrum nucleoside (adenosine) analog	• Phase I safety trial is underway
	• Indirectly inhibits RNA polymerase activity	• Conferred protection up to 96 hours after exposure to a fatal dose in a mouse model
Lamivudine	• Lamivudine is a cytosine analog	• One Liberian doctor has reported that of the 15 patients he has treated with lamivudine, 13 have survived
	• Inhibits RNA and DNA polymerase activity of reverse transcriptase	
Interferons	• Approved for treatment of hepatitis B and C and multiple sclerosis	• Phase 2 trials for Ebola virus in Guinea
Non-antiviral drugs with *in vitro* activity against Ebola virus		
Chloroquine	• An antimalarial drug that may inhibit Ebola virus entry into host cells	• It was studied in various cellular and small animal models
		• It appears that the benefit of chloroquine would be most evident when used as a prophylactic agent
Amiodarone	• A cationic amphiphile	• Observational in Sierra Leone
	• Used to treat cardiac dysrhythmia	• Reportedly reduced case fatality ratio when compared with local historical norms in 80 patients

EVD: Ebola virus disease, GP: Glycoprotein, CMV: Cytomegalovirus, siRNA: Small interfering RNA, FDA: Food and Drug Administration

cells), melatonin (a powerful immune regulator), statins (anti-inflammatory and immunomodulatory effects), IFN, neplanocin A and 3-deazaneplanocin A (S-adenosyl-L-homocysteine hydrolase inhibitor), lectins (viral entry), endoplasmic reticulum glucosidase inhibitors (viral entry), CMLDBU3402 (RNA transcription inhibitor), ion channel blockers (viral entry), FX06 (peptide for use in treating vascular leakage), and more (25,33,34).

Table 3: Ebola virus vaccine trials (13,25,28,33,35,36,39)

Vaccines		
cAd3+ZaEV-GP	• Recombinant technology is based on chimpanzee adenovirus serotype 3	• Phase 1/2a clinical trials completed
		• Phase 2/3 clinical trials planned-continued in several Central Africa countries
rVSV+ZaEV-GP	• A gene from Ebola loaded in a weakened version of vesicular stomatitis virus	• Phase 2 and 3 clinical trials underway in Guinea and Sierra Leone
		• Prevents lethal infection in non-human primates
		• Vaccines might also offer benefit in patients already exposed to Ebola virus
		• Suited for outbreak response, including post-exposure prophylaxis
rRABV+ZaEV-GP	• A dual vaccine against Rabies virus and ZaEV	• Prevents lethal infection in non-human primates and mice
	• Recombinant RABV vaccine vectors expressing ZaEV-GP	• Suited for human and wildlife immunizations vaccination
rAd5+ZaEV-GP	• Possible interference with preexisting immunity to Ad5	• Phase 1 clinical trials
VLP+ZaEV-GP	• VLP can be produced in insect cells (suitable for large-scale production)	• Prevents lethal infection in non-human primates
rHPIV3+ZaEV-GP	• Chimeric human parainfluenza virus bearing the ZaEV-GP	• Prevents lethal infection in non-human primates and guinea pigs
		• Potential for needle-free application
rCMV+ZaEV-NP	• A recombinant murine CMV was genetically engineered to express a CTL epitope located on ZaEV-NP by fusing it to the ie2 gene	• Prevents lethal infection in mice
		• Suited for wildlife vaccination (great ape) in Ebola-endemic areas
		• Highly species-specific
rEV subunit vaccine+TLR agonist	• VLPs consist of the ZaEV matrix protein VP40 and ZaEV-GP,	• Prevents lethal infection in mice
	• In some cases, ZaEV-NP is also present	• Stable for storage and delivery at ambient temperatures

cAd3: Chimpanzee adenovirus type-3, rVSV: Recombinant vesicular stomatitis virus, rRABV: Recombinant rabies virus, rAd5: Recombinant adenovirus serotype 5, VLP: Virus-like particles, rHPIV3: Recombinant human parainfluenza virus type 3, rCMV: Recombinant cytomegalovirus, rEV: Recombinant Ebola virus, TLR: Toll-like receptor, ZaEV-GP: Zaire Ebola virus surface glycoprotein, ZaEV-NP: Zaire Ebola virus nucleoprotein

Vaccine developing efforts are one of the stand out approaches among preventive strategies for EVD (33,35,36). Vaccine trials include preparedness strategies for health-care workers and high-risk populations (36), as well wildlife vaccination in Ebola-endemic areas to prevent the spread of disease. Non-human primates serve as the gold standard for animal models of Ebola infection and vaccine trials (37), and also mice and guinea pigs are other animal models in some Ebola vaccine trials (36).

Ebola virus vaccine trials include different technical methodologies such as using inactivated virus and subunit vaccines, DNA vaccines, non-replicating vaccine vectors, and replication-competent vaccine vectors (28). Ebola virus GP spikes, help in anchorage and membrane fusion of the virus to the host cell, and are a crucial factor for pathogenicity and the majority of vaccine studies focused on recombinant technology based on ZaEV-GP (Table 3) (38). Vesicular stomatitis virus, rabies virus, adenovirus serotype 5, human parainfluenza virus type-3, chimpanzee adenovirus type-3 (cAd3), and virus-like particles have been used as vectors for ZaEV-GP recombinant vaccines (33,36). Cytomegalovirus based ZaEV-NP vaccine and Ebola virus subunit vaccine strengthened with toll-like receptor agonist are other important recombinant vaccine models (33,36).

Recently, in Switzerland, phase 1/2a trial was completed for a monovalent, recombinant, cAd3 vector-based ZaEV-GP vaccine (35). According to the results of this randomized, double-blind, placebo-controlled, and dose-finding study; the new developed, safe, and well-tolerated vaccine provides antibody response with protective level independently from dosage. In addition, it

was also shown that the antibody responses were still significantly present at 6 months. Following this study carried out on volunteers, phase 2 and phase 3 efficacy trials planned to be performed in Africa.

Prevention

As neither treatments nor vaccines are available, prevention based on personal protection measures and raising awareness of the risk factors of Ebola infection are the only ways to reduce human infection and death (11). Health-care workers should always use standard precautions. For instance, regular hand washing is required after visiting sick relatives in hospital; moreover, liquid-tight gowns covering the entire surface of the body, double gloves, face visors-masks, head coverings, high-quality and sterile gloves, long-sleeved coats, protective shoes, and goggles should be worn, and disposable tools and equipment should be used (11,14,40).

Avoiding contact with infected material (possibly infected bats and non-human primates, their blood and other body fluids, raw meat prepared from these animals, and infected human materials and other contaminated environmental materials or devices) and establishing patient isolation units at the hospitals or other health-care settings are still the best ways to prevent the spread of the outbreak and the transmission of infection (13,40). The virus loses infectivity through treatment with sodium hypochlorite, calcium hypochlorite, 3% acetic acid, 1% glutaraldehyde, and phenolic disinfectants; heating for 30-60 minutes at 60°C, and exposure to gamma irradiation (2). Finally, the dead must be buried immediately to prevent transmission of the virus to other susceptible individuals (11,23).

Conclusion

Although improved diagnostic, preventive, and treatment strategies have been developed in the medical world, viral infections such as Ebola virus continue to challenge humanity by forcing the limits of science. While new experiences gained in this fight and new discoveries are in progress toward treatment and prevention, so far, many health workers have lost their lives in this struggle. Thus, it is important to make efforts to generate new discoveries in this field due to the serious risks it poses to humankind; moreover, it is necessary to rectify poor living conditions and improper behaviors because these are major sources of infection.

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