Ebolavirus: Infection, Vaccination and Control

**Abdul Ahad[a], Sabahat Mahnoor[a], Muhammad Zaid[a],
Muhammad Ali[a], *, and Muhammad Sohail Afzal[a]**

[a] *Department of Life Sciences, School of Science, University of Management and Technology (UMT),
C-ll Johar Town, Lahore, 54770 Pakistan*
*e-mail: ali4982@gmail.com

Abstract—Members of the genus *Ebolavirus* (family Filoviridae) are among the deadliest viral pathogens spread throughout the world with severe rate of mortality, at least 90% in some outbreaks. Their virions are filamentous and enveloped with enclosed negative-sense single-stranded RNA genome. The genome potentially expresses seven structural and nonstructural proteins. The replication cycle is complex consisting of multiple molecular processes and interactions with human-host factors and proteins. Due to high mortality rate of infection, the studies regarding cure is still infancy. This review covers the current understanding of the virus replication cycle and vaccine development, and herbal treatments to control Ebola covering the available literature on the subject.

INTRODUCTION

Ebolavirus disease (EVD) is among the world's life-threatening infections repeatedly re-emerging specifically in Africa from the last 50 yr [1]. EVD named after Ebola River that passes close to the Yambuku town, the place where first outbreak has occurred. Ebola outbreak in 1976 caused the death of 151 patients (out of 281) in Sudan. They were the vaccine workers dealing with the specimen of African green monkey. In Yambuku, 280 individuals died in 318 infected individuals [2]. In December 2013, the world's largest Ebola outbreak occurred in Guinea, Liberia, and Sierra Leone [3]. It was the first outbreak outside the Central Africa where *Zaire ebolavirus* was the causal virus [4]. The World Health Organization (WHO), in August 2014, nominated the West African flare-up as a Public Health Emergency of International Concern [5]. Whereas, WHO reported 10 141 cases and 4922 deaths in October 2014 [6]. Recently, an epidemic has been reported from Congo with some 2000 deaths (Table 1). Ebola hemorrhagic fever is zoonotic viral disease that initially happened in 1976 in the provincial territories of Central Africa [7]. The non-specific symptoms that can be observed in an Ebola infected individual may be fever, abdominal pain, vomiting, diarrhea, sore throat, muscle pain, cough, headache, nausea, bleeding, rashes, organ failure, and arthralgia [8]. Upon contamination, the infection moves through direct contact with body liquids like blood etc. Along with this, the virus was detected in individual's body fluids like tears, sweat, saliva, breast milk, semen, urine [9].

Amazingly, the infected dead-body may also transmit the disease. Ebola is among those pathogens that may replicate in a dead body and may release with the infectious fluid [10]. This review is an effort to encompass EVD in context with the literature and knowledge available on the viral physiology, pathology, pathogenesis and the host immune responses.

Despite the fact that there is no cure or commercially approved vaccine for *Ebolavirus*, the recombinant vesicular stomatitis virus−*Zaire ebolavirus* (VSV-EBOV) immunization was first utilized in Guinea in 2016, and the Democratic Republic of Congo in 2017−2018 [11]. Some other treatments and vaccines are monoclonal antibody cocktail, "Zmapp", and a chimpanzee adenovirus-derived vaccine, "cAd3-ZEBOV", respectively [12]. Clinical trials are summarized in Table 2. Natural reservoir hosts for EBOV is yet to be confirmed. Studies showed that the fruit bats may be associated with it. EBOV outbreaks among humans is associated with their direct contact to fruit bats. Epidemiological studies also indicated that fruit bat could be the intermediate host. Studies also showed that EBOV outbreaks could link drastic shift from dry to wet condition. Further studies indicated that EBOV outbreaks could be linked with higher humidity and lower temperature. EBOV infection transmission is possible from human to human through air/space, touching/bath and sex/blood. The replication cycle and pathogenesis of *Ebolavirus* is complex that involve binding of viral proteins with the host factors.

Table 1. Reports on epidemics of *Ebolavirus* disease in the world

Year	Country	Reported cases
2019	Democratic Republic of the Congo	2000 deaths
2018	Democratic Republic of the Congo (Ituri and North Kivu)	1459 deaths (2181 cases)
2018	Équateur Province, Democratic Republic of the Congo	33 deaths (54 cases)
2014–2016	West Africa	11 310 deaths (28, 616 cases)
2014	Sierra Leone (Rural West Africa)	259 deaths (489 cases)
2014	Nigeria	8 deaths (20 cases)
2014	Central Africa (Guinea, Liberia, and Sierra Leone)	4922 deaths (10, 141 cases)
2000–2001	Uganda	224 deaths (425 cases)
2000	Yambuku (Northern Democratic Republic of the Congo)	280 deaths (318 cases)
1976	Sudan	151 deaths (281 cases)

Ebolavirus spike glycoprotein (GP) accomplishes entry into the host cell for replication that might also trigger micropinocytosis pathway. The host *cathepsin L* and *cathepsin B* process GP into GP1. However, cathepsin L and B are not always required for EBOV replication. The GP1 then interacts with Niemann-pick C-1 (NPC-1) in the late endosome followed by its fusion with the vesicle-membrane. As a result, the virion contents (including the virus-encoded polymerase) are released in the cytoplasm. Viral mRNAs are transcribed using the polymerase and translated using host translation machinery. Viral genomic-RNA replication commences once enough nucleoprotein are expressed to encapsulate the newly synthesized negative-sense (−ve) single-stranded (ss) RNA genome. Finally, *VP40* helps in the assembly of virion particles and in budding. The *VP24* protein suppresses host immune system thereby inhibiting interferon signaling (Fig. 1).

Ebolaviruses (members of the genus *Ebolavirus*) belongs to the family Filoviridae, order Mononegavirales, class Monjiviricitae, subphylum Haploviricotina, phylum Negarnaviricotina, Realm Riboviria. They can circulate among humans, non-human hosts and non-human primates (NHP) (African fruit bats, for instance) in the nature. Filovirid virions are filamentous and enveloped. The virions are about 920 nm long and 80 nm wide containing 19 kb −ve ssRNA genome. Ebola expresses seven structural proteins, NP (Nucleoprotein), VP35 (RNA-dependent RNA polymerase cofactor), VP40 (Primary matrix protein), GP1,2 (Spike glycoprotein), VP30 (Transcriptional activator), VP24 (Secondary matrix protein), L (RNA-dependent RNA polymerase) (Table 3, Fig. 2). These proteins are transcribed in an order of 3'-NP-VP35-VP40-GP-VP30-VP24-L-5' (Fig. 1). Species in the genus *Ebolavirus* includes *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, *Tai Forest ebolavirus*, and *Zaire ebolavirus* (type species).

CONTROL STRATEGIES AND VACCINES

Even though there is no exact treatment for *Ebolavirus* disease, some precautionary and control measures must considered. They include, (1) administer intravenous fluid to avoid dehydration, (2) maintain normal blood pressure, (3) blood clotting through medications, (4) maintain the cleanliness around the patient, (5) use of breathing devices and drugs to control fever, (6) avoid contact with healthy individuals, however, if necessary, personal protective equipment must be worn [13]. DNA vaccines or DNA immunization in comparison with live/attenuated vaccines has a number of advantages, where plasmids can be used to induce antigenic response. They are non-infectious and easily adaptable for evolving pathogens. They can easily be produced in large amounts. However, booster doses are required for immunity. They can induce cell-mediated response as well as humoral response. One major advantage is that the existing immunity may not be a problem with DNA vaccines [14]. First successful vaccination was done in 1998 on mice in which four doses having plasmids encoding *Zaire ebolavirus* Glycoprotein (ZEBOV-GP) and Zaire *Ebolavirus* Nucleoprotein (ZEBOV-NP) were used in the mice [15]. Unfortunately, there is no data available for DNA vaccine for Non-Human Primates alone. Studies have shown that DNA vaccine combined with recombinant vectors like adenovirus encoding GP protected the cynomolgus macaques from lethal EBOV challenge [16]. A clinical trial was conduct in which 3 doses of DNA vaccine were given to the 20 participants encoding ZEBOV-GP, NP and SEBOV-GP. No side effects were found in participants and induction of antibodies and CD4* T-cells were observed. CD8* T-cells were also detected in 8 of the participants [17].

Another DNA vaccine was developed having two plasmids encoding the wild type glycoproteins of EBOV and SEBOV. It was experimented on 10 healthy persons in the US. A dose of 4 mg of dose was administered thrice during 4-weeks period followed by a booster dose after 32 weeks. As the T-cell response was

Table 2. Clinical trials of *Ebolavirus* vaccines

Vaccination study year and month	Vaccine type	Vaccine constituents	Dose	Country	Trial number**
November 2003	DNA vaccine	Transmembrane deleted EBOV GP, SUDV GP	8 or 4 or 2 mg	United States, MD	NCT00072605
September 2006	Replication deficient EBOV	rAdHu5 EBOV and SUDV GP (mutated GP)	2×10^9 or 2×10^{10} vp	United States, MD	NCT00374309
January 2008	DNA vaccine	SUDV and EBOV (plasmid) GPs with a Marburg DNA vaccine	4 mg	United States, MD	NCT00605514
November 2009	DNA vaccine	SUDV and EBOV (plasmid) GPs with a Marburg DNA vaccine	4 mg	Uganda	NCT00997607
September 2014	Replication deficient EBOV	SUDV GP and ChAd3	1×10^{10} or 1×10^{11} vp	United States, MD	NCT02231866
September 2014	Replication deficient EBOV	ChAd3 (EBOV GP)	1×10^{10} or 2.5×10^{10} or 5×10^{10} vp	United Kingdom, Oxford	NCT02240875
September 2014	Replication deficient EBOV	MVA BN Filo, ChAd3 (EBOV GP)	ChAd3: 1×10^{10} or 2.5×10^{10} or 5×10^{10} vp MVA: 1.5×10^8 or 3×10^8 pfu	United Kingdom, Oxford	NCT0224087
October 2014	Live replicating EBOV	rVSV (ZEBOV)	3×10^6 or 2×10^7 pfu	United States	NCT02269423 NCT02280408
October 2014	Replication deficient EBOV	ChAd3 (EBOV GP)	2.5×10^{10} or 5×10^{10} vp	Switzerland	NCT02289027
October 2014	Replication deficient EBOV	ChAd3 (EBOV GP)	1×10^{10} or 2.5×10^{10} or 5×10^{10} or 1×10^{11} vp	Mali	NCT02267109
November 2014	Live replicating EBOV	rVSV (ZEBOV)	3×10^5 or 3×10^6 or 1×10^7 or 2×10^7 or 5×10^7 pfu	Kenya, Germany, Switzerland	NCT02296983 NCT02283099 NCT02287480
December 2014	Replication deficient EBOV	MVA-BN Filo and AdHu 26 EBOV GP	MVA: 1×10^8 and ChAd3: 5×10^{10} vp	United Kingdom, Oxford	NCT02313077
December 2014	Replication deficient EBOV	rAdHu5 encoding EBOV GP	4×10^{10} or 1.6×10^{11} vp	China	NCT02326194

Table 2. (Contd.)

Vaccination study year and month	Vaccine type	Vaccine constituents	Dose	Country	Trial number**
January 2015	Live replicating EBOV	rVSV (ZEBOV)	3×10^5 pfu	Switzerland	NCT02287480
February 2017	Heterologous based vaccine	Heterologous VSV and Ad-5 vectored based encoding EBOLA glycoproteins	Full dose of VSV: 2.5×10^7, Full dose of Ad5: 2.5×10^{11} vp	Republic of Guinea	NCT03072030
March 2017	Ad26 ZEBOV and MVA BN-Filo	GP from Ebola, Sudan, Marburg and Tai Forest viruses nucleoprotein	Ad26: 5×10^{10} and MVA: 1×10^8 vp	United Kingdom	NCT02313077

  * vp: Viral particles; pfu: Plaque forming unit; 79.
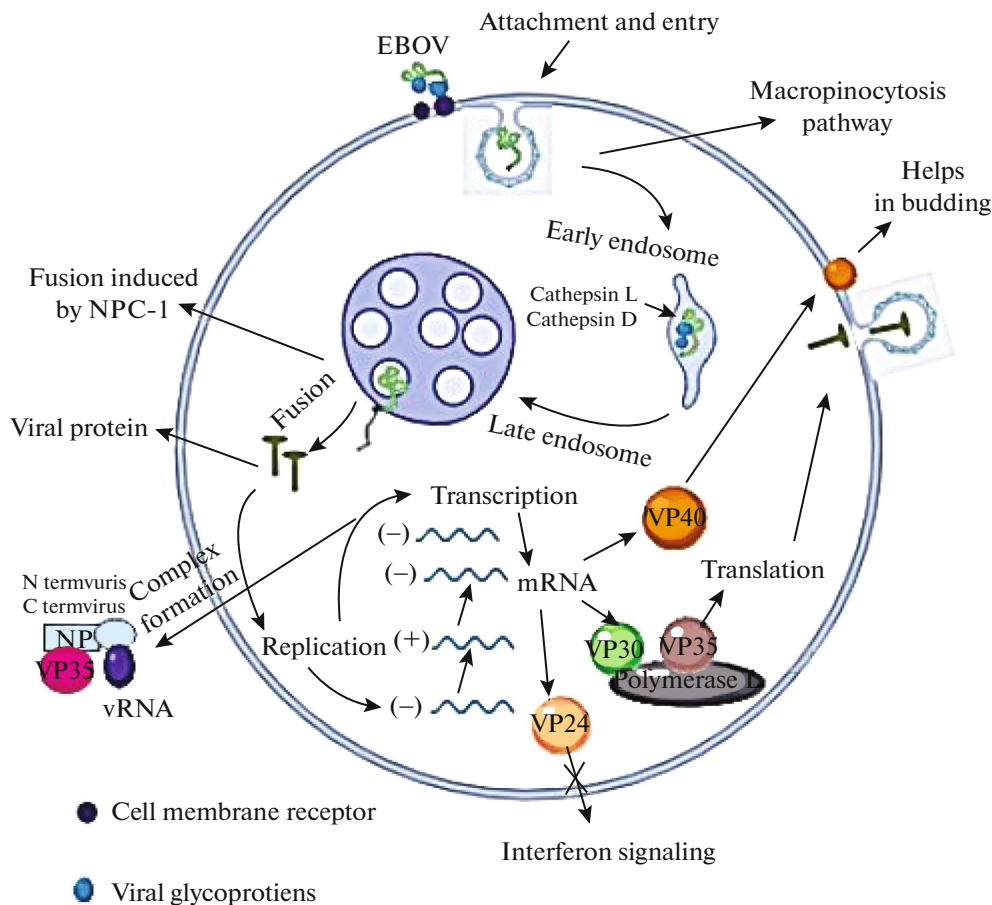** ClinicalTrials. gov Identifier.

Fig. 1. *Ebolavirus* entry is accomplished by viral spike glycoproteins (GP) and it triggers the macropinocytosis pathway. As a result GP are processed by the host Cathepsin L and cathepsin B into GP1, it is also has been confirmed Cathepsin L and B are not always required for EBOV replication in some cells or all species of EBOV. Then this GP1 interacts with the NPC-1 (niemann-pick C-1) in late endosome and causes fusion of virus membrane with the vesicle membrane, as result ribonucleocapsid is released into cytoplasm. Viral mRNAs transcribed by viral polymerase and viral proteins are translated. Replication starts when enough nucleoprotein present to encapsulate neo synthesized anti-genomes and genomes. VP40 helps in assembly of viral particles and in budding. VP24 suppresses the host immune system by inhibiting interferon signaling.

Table 3. Viral proteins, their possible functions and known interaction with the viral host system

SN	Viral proteins	Functions	Host interaction
1.	NP	Essential component of nucleocapsid	–
2.	P35	Also component of nucleocapsid acts as L polymerase cofactor helps in ssRNA packaging	Target the innate immune response of host. Binds with the cellular kinases
3.	VP40	Maintains the structural integrity of virus helps in cellular egress of virus	Form cluster at the plasma membrane of host and cause bud formation
4.	GP	Two types: GP and sGP GP: Helps in the formation of spikes on outer surface of virus, targeting, viral cellular entry, cell fusion sGP: secreted from host cells	GP: Binds cells lectin and interact with the host-encoded Niemann Pick C1 (NPC1), a cholesterol transporter protein and TIM-1 (aka HAVCR1) sGP: Droning of host immunity
5.	VP30	Transcription activator	
6.	VP24	Fully functional nucleocapsid	Binds with endosomal trafficking protein immune dysregulation Inhibits the IFN-α/β + IFN-γ signaling
7.	L	Multifunctional protein 2210 amino acid helps in mRNA transcription, genome replication, capping, methylation, polyadenylation	–

poor, a booster dose was therefore, needed for the longevity of vaccination [18]. Combination of different plasmids encoding ZEBOV-GP, SEBOV-GP and *Marburgvirus* glycoprotein (MARV-GP) was proved effective to protect guinea pigs from lethal ZEBOV challenge. Studies have shown that induction of antibodies and T-cells were observed in mouse model and was 100% protected from lethal ZEBOV challenge [19]. Further experimentation required to analyses the effectiveness of this vaccine in the NHP. Main requirement of DNA vaccine is the need of multiple/booster doses for the longevity of vaccination.

The use of replication deficient EBV was one of the earliest strategies used to create vaccine. The mutant EBOVAVP30 (deletion of VP30 gene) make the virus unable to replicate. VP30 is necessary for the reasonability of the infection and for replication along these lines encoding viral polymerase cofactors. It was considered a key step to use a whole virus for vaccination as it could provide a better protection against *Ebolavirus* by triggering the immune system using the complete viral protein and the genetic material. To check the safety assessment EBOVAVP30 was introduced in immunocompromised mice (STAT-1 double knockout mice) and surprisingly mice showed no clinical signs or viremia. On further investigation, it was found that EBOVAVP30 is completely safe for mice and guinea pigs. EBOVAVP30 proved to be very effective because it is genetically stable even after serial passage in Vero cells expressing VP30 [20]. Later on EBOVAVP30 proved 100% safe and protective in

NHP when one or two doses of 10^7 infectious unit was given to them. The subjects were fully protected against lethal EBOV infection. Some of them had shown little signs of viremia but they eventually clear the virus. To make vaccine more protective, it was treated with hydrogen peroxide but the immune response of vaccine that was treated with hydrogen peroxide was slightly lower as compared to non-treated vaccine but it was considered sufficient [21].

Adenovirus family based vaccines have been proven effective against many infections. AdHu5 (adenovirus serotype (5) is an attractive choice as vaccine vector due to its potential to induce effective immune response. Deletion of E1 gene makes them unable to replicate and further E3 and E4 deletion increased the size of vector [22]. A single dose of AdHU5 expressing EBOV GP and NP presented a total protection in NHP against ZEBOV challenge. The issues with AdHu5 is the pre-existing immunity, it is estimated that 30 to 50% of the population in North America and more than 90% of the population in developing countries have pre-existing immunity to this vector, making it less applicable.

Another adenovirus vector rAd5 with EBOV GP provide 100% protection against EBOV lethal challenge in NHP. The higher amount of rAd5 may be toxic but the non-replicating behavior make them safe to use. The vector Ad5 having codon optimized ZEBOV GP that was tested on rodent models with much lower dose successfully survived EBOV lethal challenge [23]. Again the main problem associated
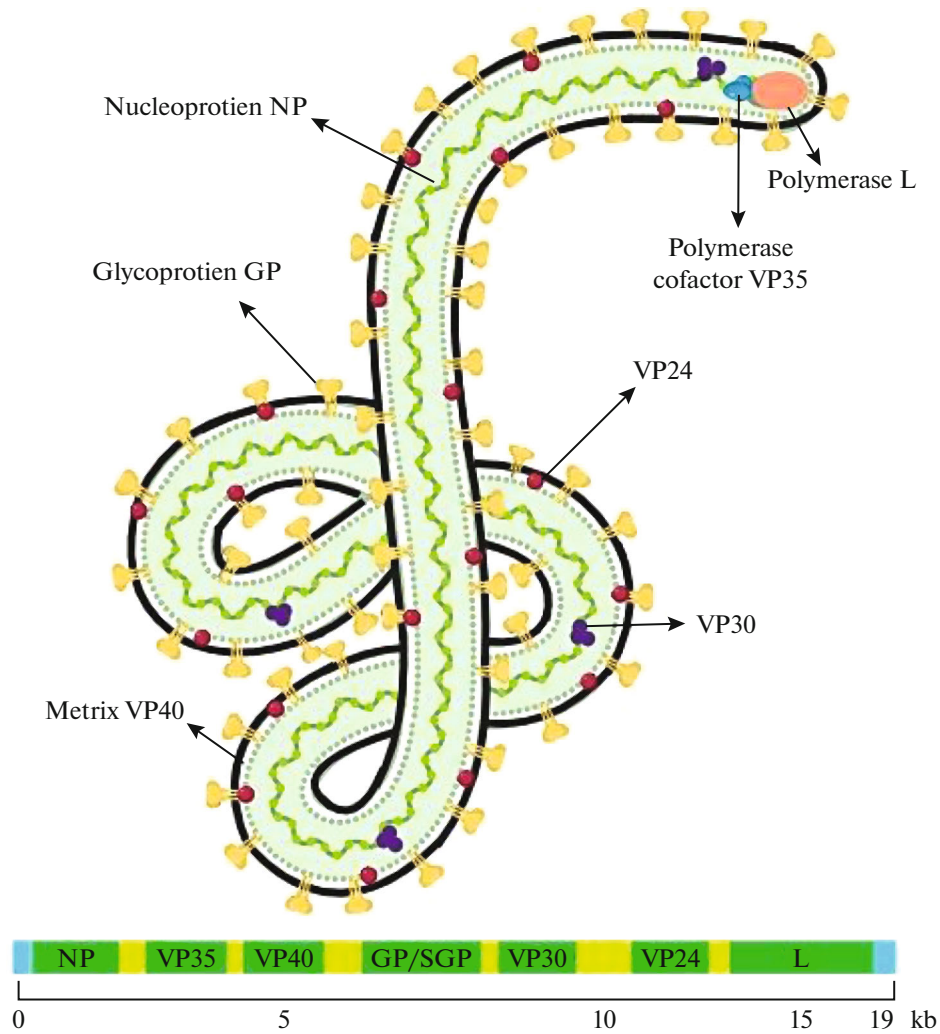
Fig. 2. Non-segmented (19 kb) negative strand RNA genome contains 7 genes. VP40 and VP30 make inner matrix, other nucleoprotein (NP), VP30, VP35 and RNA polymerase L comprise the ribonucleoprotein complex. Open reading frames are shown in dark green color and non-coding shown yellow color. The minimal machinery for replication required NP, VP30, VP35 and L. NP and VP40 can form virus like particles.

with Ad5 is the pre-existing immunity in certain population, varying 60 to 90% but high dose of rAd5 may overcome the problem. The dose required is about 1.6×10^{11} vp. To avoid preexisting immunity adenovirus serotypes having a very low seroprevalence such as Ad26, chimpanzee CHAd3 and Ad35 were used to substitute Ad5. However, there is not much data available of EBOV challenges for these vaccines but studies have shown that they can induce antigen-specific antibodies and T cell response in NHP. Ad26-EBOV that was tested on NHP provided only partial protection. One month later after providing heterologous Ad35-EBOV complete protection was witnessed [24].

Vesiculovirus stomatitis virus (VSV) is a negative stranded RNA infection belonging to Rhabdoviridae family that can infect cattle and in humans it can cause little illness. VSV can be utilized in replication competent form so it can influence a high humoral and cellular response in humans. VSV expressing *Ebolavirus* glycoproteins conferred a complete protection in mice against ZEBOV challenge [25]. After successful vaccination in mice, it was tested on NHP thereby conferring complete protection against ZEBOV challenge. VSV also can give cross protection against different species as well. Recombinant VSV expressing SEBOV GP, ZEBOV GP, ICEBOV GP in equal amount administered to NHP showed 100% survival and protection against viral challenges. In another studies to measure the multivalent protective ability of this vaccine rVSV encoding MARV GP, TEBOV GP, SEBOV GP, EBOV GP was tested and the results showed that cross protection is possible against all these viruses. Another study has shown that this vaccine can be safe for immunocompromised people as well [26]. In 2014 initial trials were setup to analyze the safety and side

effects of the VSV base vaccines. Phase 1 clinical trials were conduct in which 158 adult healthy volunteers were given a single IM injection of VSV-EBOV at different doses in escalating manner (3×10^5, 3×10^6, 1×10^7, 2×10^7 and 5×10^7 pfu). Viremia was noted in all the individuals as confirmed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Approximately 90% vaccine recipient's experiences different adverse effects such as pain at the site of injection, fatigue and myalgia. Some participants developed arthritis in Geneva, Kenya and Germany but no viremia was detected. After immunization within 24 to 36 h all the symptoms were resolved. It was observed that higher (3×10^6) dose of VSV-EBOV induce a strong immune response than the lower dose (3×10^5) but antibodies response was not significantly different [27].

In 2016, a clinical trial was conducted in Guinea to analyze the efficiency of heterozygous based vaccine combining two components—the recombinant vesicular stomatitis virus (VSV) and the adenovirus serotype-5 (Ad-5). Both components were genetically altered expressing Ebola virus glycoproteins. Seroconversion was observed in all patients with minimal adverse effects. The most common adverse effect observed was pain at the site of injection. Other than this, no significant adverse reactions was found. Two different doses were given to the subjects. The first group was given half doses of VSV and Ad-5 and the other group was given full doses. On day 42, all participants (including both groups) were detected with glycoprotein specific antibodies. This study demonstrates that the vaccine was completely safe and could induce high cellular and humoral response [28].

Vaccination providing protection may be classified into two different categories. Mechanistic that provide main protection against the virus and non-mechanistic that may participate in the protection but is not much effective. Pre-clinical studies on NHP showed that VSV-EBOV could induce GP-specific antibodies with levels correlating protection. Further studies showed that cell-mediated immunity does not play important role in VSV-EBOV vaccine mediated protection because lethal challenge was not affected by the depletion of CD8$^+$ T cells or CD4$^+$ T cells. It is evident that CD8$^+$ T cells play regulatory role in VSV-EBOV mediated protection. CD8$^+$ T cells play important role in the protection given by Venezuelan equine encephalitis virus (VEEV) based replicon vaccine. Protection conferred by VLP vaccine requires GP and specific CD8$^+$ T cells. EBOV vaccine platforms elicit distinct immune profiles thereby considering different strategies of protection (Table 4).

HERBAL AND OTHER REMEDIES

Many diseases like Alzheimer, diabetes mellitus, cardiovascular disorder have reached to endemic proportion. Whereas, the emergence of some deadly diseases like HIV, bird flu, rabies, EBOV have increased the mortality rate. The origin of the virus plays a key role to control epidemics and mortality rate [13]. Still there is no proven treatment for Ebola. However, some clinical-, herbal- and immune-therapies are used to suppress the adverse effects. Some natural cures have enlisted in (Table 5).

Since centuries, plants are used as therapeutic agents treating human and animal diseases. Plants produce secondary metabolites, which have a diverse range of pharmacological activities to fight against certain diseases. These plant-derived remedies show some significant treatment of Ebola. Some chief drugs like Belladonna, Arsenic, Bryonia, Aconite and Gelcemium have shown symptomatic treatment of Ebola. Belladonna herb also known as deadly nightshade leaf. It is originated and cultivated in England and European countries. The herb is also found in India and in the forests of Jammu. Belladonna showed significant cure in fever, brain infections, cough, measles, menstrual irregularity, pregnancy defects, whooping cough, uterine infections and abdominal related issues. Aconite herb was used as a strong poison in the past some decades. Now a days, it is also used to fight against many infections like vertigo, dropsy, chicken pox, asthma, fever and headache disorders. However, it causes many adverse and intense effects (pupil contraction, anxiety attacks and short of breath) in patients. It is, therefore, not recommended to the patients who suffer from some cardiovascular and nervous sensitivity disorders. Bryonia is originated and cultivated in the western Eurasia, North Africa, South Asia and Canary Island. It is found to be effective in the cure of breast infection, respiratory disorders, whooping cough, fever, constipation, gastric disorders and meningitis. Gelcemium, poisonous flowering plant family, initially was originated and cultivated in China, North America and Southeast Asia. The plant shows some significant effects in the treatment of many diseases like cerebral disorders, dengue fever, locomotors Ataxia, fever, cardiovascular disorders and eye infections. It was found that Belladonna, Aconite and Bryonia treats all the infections of Ebola except blindness, red and itchy eyes and blood vomiting respectively. While Gelcemium treats all the significant symptoms of Ebola (Table 5).

Berries are powerful antioxidants and found in red, blue and black colors. They are originated and cultivated in Europe. They provide an effective coverage from the bacterial and viral infections. Green tea is native to China, where it is cultivated and exported to other countries. It contains flavonoid known as epigallocatechin gallate, which acts as a protective shield in body against infections and involves in the boosting of immune system. *Phyllanthus amarus* is a naturally occurring herb cultivated in America. It also is used to treat cold and other bacterial infections. Chili pepper originated in Mexico contains capsaicin, which helps

Table 4. Protection studies in pre-clinical *Ebolavirus* vaccines

Vaccine type	Animal model	Administration route	Specific binding antibodies (GP)	Antibodies (neutralizing)	Protection	Mechanistic correlate
VSV EBOV	NHP	IM	+++	++	Production of EBOV GP-specific antibodies	Yes
VSV EBOV	Mouse	IP	+++	+/–	Production of EBOV GP-specific antibodies	Yes
Ad5	NHP	IM	++/+++	NA	Production of EBOV GP-specific CD8$^+$ T cells	Yes
Ad5	NHP	IM	++	+/–	Production EBOV GP-specific IgG	No
HPIV3	NHP	Aerosol	+++	+++	EBOV GP-specific mucosal and systemic IgG, IgA and neutralizing anti-bodies	No
RhCMV	NHP	SC	+++	–	Production of EBOV GP-specific IgG	No
ChAd3	NHP	IM	++/+++	NA	EBOV GP-specific IgG for a very short term protection	No
ChAd3	NHP	IM	+++	NA	Production of EBOV GP-specific CD8$^+$ T cell immunity for long term protection	No
RABV	NHP	IM	+	NA	EBOV GP-specific (IgG1 : IgG2 ratio)	No
VLP	Mouse	IM	++/+++	NA	EBOV GP-specific CD8$^+$ T cells and B cells	Yes
VEEV based replicon	Mouse	Subcutaneous	NA	NA	EBOV-specific CD8$^+$ T cells	Yes
GP/VSVΔG pseudovirions	Mouse	IM	++/+++	–	EBOV GP-specific anti-bodies	Yes

*IP: intraperitoneal; IM: intramuscular; NA: not available; SC: subcutaneous; NHP: nonhuman primate; VLP: virus-like particles; VSV: vesicular stomatitis virus; Ad5: human adenovirus 5; HPIV3: human parainfluenza virus type 3; RhCMV: rhesus cytomegalovirus; ChAd3: chimp adenovirus type 3; RABV: rabies virus; VEEV: venezuelan equine encephalitis virus; GP: glycoprotein.

in the boosting of immune system. A necessary vita-min, beta-carotene also found in chili pepper, which helps in the maintenance of immune system. Ginger originated and cultivated in Island Southeast Asia. It acts as a strong antioxidant, antimicrobial, anti-inflammatory, antibiotic and antiseptic. Mushrooms originated and cultivated in Paris. They are rich source of antioxidant and minerals (selenium & copper) assist in activating the immune system. Brazil nuts, origi-nated from Brazil, eastern Colombia, eastern Peru and eastern Bolivia, are rich source of selenium. Two Bra-zil nuts daily help preventing from cold and influenza.

Turmeric, originated and cultivated in India, has an anti-microbial activity. It helps to prevent cold and influenza. Garlic, originated and cultivated in Central Asia, South Asia or Southwestern Siberia, contains antifungal, antiviral and antibiotic activity to fight against the infections. Eucalyptus oil helps to relieve headaches and in soothing of throat (Table 5).

Arsenic is a semi-metallic element found in the earth crust with atomic weight of 74.9 g. Its pure form exists in three forms alpha (yellow), beta (black) and gamma (grey). It is found to be effective in the treat-ment of alcoholism, cancer infection, cholera, kidney

Table 5. Natural/herbal remedies for *Ebolavirus* treatment

Plant	Activity	Native	Phytochemical
Belladonna	Anti-microbial & anti-inflammatory	England	Belldonic, hyoscine
Bryonia	Anti-microbial & anti-inflammatory	Western Eurasia	*B. dioica*
Gelcemium	Anti-microbial & anti-inflammatory	North America, China, Southeast Asia	—
Aconite	Cardiovascular and nervous disorders	Europe and Asia	—
Berries	Anti-microbial	Europe	—
Chilli pepper	Maintenance of immune system	Mexico	—
Mushrooms	Maintenance of immune system	Paris	—
Green Tea	Anti-microbial and boosting immune system	China	—
Garlic	Anti-microbial and immune booster	Central Asia, South Asia or Southwestern Siberia	—
Tumeric	Anti-microbial	India	—
Brazil Nuts	Anti-microbial and immune booster	Guianas, Venezuela, Brazil, eastern Colombia, eastern Peru, and eastern Bolivia	—
Phyllanthus amarus	Cold treatment	America	—
Ginger	Anti-microbial, antiseptic	Island Southeast Asia	—
TCM	Anti-microbial and anti-viral	China	—
—	Anti-microbial & anti-inflammatory	Earth crust	Arsenic

*TCM: Traditional Chinese Medicine.

infection, vomiting, pleurisy and rickets. Another drug known as TCM (Traditional Chinese Medicine), widely used in China to cure the deadly diseases. TCM approach rely on the belief that virus-induced fever causes a deficiency of energy in the body. Soups of cheap and effective herbs are, therefore, used to recover the depleted energy. Master Zhang include "Ma-Huang Soup," "Da-Qing-Long Soup," "Gui-Zhi Soup," and "Ge-Geng Soup." The effective symptomatic control of TCM has been observed against the Zika virus infection. In Ebola infection, hemorrhage is a lethal issue, while the herbs (sanqi, chuanxiong, danshen) present in TCM options have the potential to regulate the circulatory system (Table 5).

EXECUTIVE SUMMARY

Ebolavirus (EBOV) has the ability to invade the adaptive and innate immunity and can inhibit both type-I IFNs synthesis and response. Some more facts are still needed to explore the replication mechanism of EBOV in the host. Vaccination against EBOV is still under clinical trials. Vesicular stomatitis virus (VSV)-based vaccines showed some real protection against EBOV but still more clinical trials are needed to analyze the protection and effectiveness of the vaccine. The main problem associated with VSV-based vaccine is the adverse effects that need to be addressed. Future research will unravel the mechanisms associated with

the infection cycle and the associated high mortality rate. The protection approaches like the use of herbs, specifically the TCM has the potential to be used as cure against EBOV disease.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans. All applicable international, national, and/or institutional guidelines were followed.

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