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Vaccines for Viral Hemorrhagic Fevers – Progress and Shortcomings

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Abstract

With a few exceptions, vaccines for viruses that cause hemorrhagic fever remain unavailable or lack well-documented efficacy. In the past decade this has not been due to a lack of the ability to develop vaccine platforms against highly pathogenic viruses, but rather the lack of will/interest to invest in platforms that have the potential to become successful vaccines. The two exceptions to this are vaccines against Dengue virus and Rift Valley Fever virus, which recently have seen significant progress in putting forward new and improved vaccines, respectively. Experimental vaccines for filoviruses and Lassa virus do exist but are hindered by a lack of financial interest and only partially or ill-defined correlates/mechanisms of protection that could be assessed in clinical trials.

Introduction

Several families of RNA viruses have members that can cause viral hemorrhagic fever (VHF) in humans: Arenaviruses, Bunyaviruses, Filoviruses, Flaviviruses and possibly a newly discovered not yet isolated Rhabdovirus [1]. Live-attenuated and inactivated whole virus vaccines are available for some VHFs and in some cases these vaccines are highly effective and in widespread use within specific countries. However, regulatory procedures usually mean they are unavailable outside of the source country as they often cannot meet the requirements to proceed to either clinical trials or licensing in the majority of western countries. Globalization, international travel and climate change are increasing the number of individuals at risk for VHFs, suggesting that at some point the mechanisms for moving vaccines against VHFs to clinical use are going to have to change.

While most VHFs can be considered neglected tropical diseases, the combined public health impact of all VHFs combined is substantial. While the total number of lab confirmed VHF cases is relatively small, there are an estimated 100 million cases of Dengue virus (DENV) infection per year, with approximately 500,000 infections from all other VHFs combined (Figure 1). Moreover, more than a third of the world's population lives in areas that are at risk for VHFs (Figure 2). In the case of tick-transmitted viruses, incidence levels are less likely to increase quickly; however, the identification of severe fever with thrombocytopenia syndrome virus (SFTSV) in China in 2009 and its subsequent identification in Japan in 2012

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serves as a reminder that novel viruses continue to emerge [2]. With the expansion of tick ranges due to climate change further spread of viruses and the emergence of novel viruses is possible. More concerning is the spread of mosquitos that are capable of transmitting DENV, yellow fever virus (YFV) and rift valley fever virus (RVFV). Given their already significant public and animal health impact and their potential for spread, more resources should be devoted to pushing proven experimental vaccines into clinical trials.

Arenaviruses

Old world arenaviruses (OWA) that result in VHF include Lassa virus (LASV) and Lujo virus. Lujo virus has recently been identified as a new genetically distinct OWA; however, no vaccines have been developed to date [3]. LASV remains one of the most neglected of the tropical viral diseases and next to DENV and YFV has the most significant impact on human health. LASV is endemic to West Africa with an estimated 300,000 infections per year and fatality rate of approximately 2% [4]. It is transmitted to humans via its rodent reservoir *Mastomys natalensis* through inhalation of contaminated droplets/dust or ingestion of contaminated food (Table 1) [4]. Currently there are no licensed vaccines for the prevention of LASV. A single-dose vaccine would be ideal for use in endemic areas as the infrastructure in these regions is limited [5]. For LASV it is thought that cell-mediated immunity plays a major role in recovery and protection, thus favoring the development of live-attenuated vaccines [5].

While inactivated, peptide epitope and alphavirus replicon-based vaccines have been generated for LASV; the utility of these in nonhuman primate model is lacking [5]. A live-attenuated vaccine based on recombinant vesicular stomatitis virus (rVSV) expressing the glycoprotein was protective in cynomolgus macaques; however, the correlates of protection have not been established [6]. Virus-like particles containing the glycoprotein, nucleoprotein and Z matrix protein were immunogenic in mice but efficacy data are not available [7].

A LASV/Mopeia virus reassortant (ML29) containing the glycoprotein and nucleoprotein of LASV and the RNA polymerase and zinc-binding protein of Mopeia virus, a related but apathogenic arenavirus, was protective in marmosets [8]. In guinea pigs, ML29 provided protection against challenge from genetically diverse LASV isolates and also provided 80% protection when administered 48h post-infection [5]. Furthermore, ML29 has recently been shown not to cause disease in Simian immunodeficiency virus-infected rhesus macaques, supporting its safety [9]. The YFV vaccine strain 17D has also been genetically manipulated to express the LASV glycoprotein as a soluble product; and while it protected 80% of guinea pigs [10] it completely failed to protect marmosets and is genetically unstable [5].

New world arenaviruses (NWA) that result in VHF include the following viruses and their respective disease: Junín virus (Argentine HF), Machupo virus (Bolivian HF), Guanarito virus (Venezuelan HF), Sabia virus (Brazilian HF) and Chapare virus (not defined). Each virus has its own unique rodent reservoir (Table 1). Together they cause thousands of cases per year with up to a 20% case-fatality rate (Figure 1) [11]. A live-attenuated version of Junín virus, called Candid#1, is used in Argentina but is not recommended for use in pregnant women and children [12]. This vaccine represents a good example of a successful national/local vaccine that has not been approved for use in other countries. Molecular characterization into the attenuation of Candid#1 has indicated that mutations in glycoprotein G2 are responsible for its attenuation in mice [13]. An alphavirus replicon expressing the Junín glycoprotein provided protection following two immunizations in guinea pigs [14]. Junín virus-like particles (VLPs) containing Z protein were immunogenic in mice, but have been used to demonstrate efficacy from challenge [15]. Experimental

vaccines for the other NWA are not available and partial cross-protection with Candid#1 has only been reported for Machupo virus but need further evaluation [12].

Bunyaviruses

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne Nairovirus that is distributed throughout Asia, the Middle East, south-eastern Europe, the Balkans and Africa (Figure 2). It can be transmitted directly through tick bites (main vector *Hyalomma sp.*) or through contact with tissues or blood from infected animal and patients (Table 1). Cattle, sheep, horses, goats and swine are susceptible to CCHFV as are small wild-life species such as hedgehogs and hares. Despite its wide distribution, historically CCHFV has caused only small outbreaks [16]; however, outbreaks have been occurring with increasing frequency and size in the past decade especially in Turkey (>5000 confirmed cases since 2002), Iran and the Balkans (Figures 1 & 2) [17].

Currently there is a chloroform/heat inactivated suckling mouse brain derived vaccine. It is used exclusively in Bulgaria in higher risk individuals where it has resulted in a four-fold reduction in the number of reported CCHFV cases [18]. This vaccine is not approved in other countries. Recently, it has been shown that vaccination with the CCHFV glycoproteins Gn and Gc in either a DNA vaccine [19] or purified from transgenic plants induced antibody responses in mice [20]; however, due to a lack of an animal model protection could not be determined. This limitation has recently been overcome with the development of two immunocompromised adult mouse models (STAT-1$^{-/-}$ and IFNAR$^{-/-}$).

Old world hantavirus (HFRS/NE)

The old world hantaviruses (Hantaan, Seoul, Puumala and Dobrava viruses) are the causative agent of hemorrhagic fever with renal syndrome (HFRS). While hantavirus distribution is considered to be worldwide, HFRS-causing hantaviruses appear to be restricted to Asia and Eastern Russia, although less severe forms termed nephropathia epidemica (NE) are found in Europe. China alone has recorded over 1.5 million cases of HFRS with 46,000 deaths in the last 60 years (Figure 1 & 2) [21]. In contrast to the other bunyaviruses that cause hemorrhagic fever, these viruses are rodent-borne and transmission is through aerosol exposure to urine, feces or saliva (Table 1) [22].

There are multiple different inactivated vaccines that are currently in use. A formalin-inactivated Korean Hantaan virus derived from suckling mouse brain (Hantavax) elicits a good humoral immune response, but the protective efficacy has not been established despite wide-spread use [23]. Cell culture derived inactivated Hantaan or Seoul virus vaccines have been used in Korea, North Korea and China [24]. A formalin-inactivated bivalent vaccine containing both Hantaan and Seoul viruses derived from Syrian Golden hamster kidney cells has also been produced and used in China [25]. Hantavax and the bivalent vaccine elicited positive antibody response in 97% of individuals one month after booster (75% of individuals have neutralizing response). This response waned over one year to approximately 40% with a positive antibody response which was returned to near 100% following a booster [26]. The vaccines used in Korea and China appear to have reduced the number of HFRS cases since implementation [21,26,27]. DNA vaccines for Hantaan and Puumala have also been tested in human trials and elicited good antibody responses in approximately 50% of recipients [28].

Rift Valley Fever virus (RVFV) is a mosquito-borne Phlebovirus that has spread across most of Africa and into the Arabian Peninsula (Figure 2) [29]. Given the ability of RVFV to use multiple mosquito vectors (*Aedes*, *Culex*, *Anopheles sp.* and other species), some of which are present in Europe and North America, and its wide host range (sheep, cattle,

goats, water buffalo and humans) further spread out of Africa/Arabia is certainly possible (Table 1) [30]. Outbreaks have resulted in tens to hundreds of thousands of human cases (Figure 1) and affected millions of livestock. Ruminant livestock, especially sheep and cattle, can have up to 70% neonatal mortality and 20-30% adult mortality [29]. Human cases are typically self-limiting, but 1-2% of cases involve more serious syndromes with a case-fatality rate of 10-20% in these individuals [29]. Contact with infected animal tissue and fluids, is thought to be a significant risk factor for severe and fatal human infections. Therefore, vaccination of livestock appears to be an ideal intervention point for preventing human disease and reducing the economic impact of RVFV.

Currently, there are no approved vaccines against RVFV for general use in humans, although the live-attenuated MP-12 vaccine has been used [31]; however, there are multiple livestock vaccines that are used in endemic regions and during outbreaks [29]. In order to be able to export animals it is essential to be able to serologically differentiate between infected and vaccinated animals (DIVA). This has further complicated vaccine development, in addition to the limitations of currently used vaccines including requirement for multiple doses, difficulties in manufacturing or post-vaccination abortion and teratogenicity [31]. Multiple live-attenuated vaccines are currently under development for use in livestock. A derivative of Clone 13 named R566, which contains the S segment of Clone 13 and the M and L segments of MP-12, has been developed; however, it may have reduced efficacy compared to Clone 13 [32]. Recombinant strain ZH501, lacking both NSs and NSm, was also protective, did not appear to be teratogenic and fulfills DIVA [33].

Alphavirus-based vaccines have also been developed using Sindbis and Venezuelan equine encephalitis virus replicons expressing Gn or Gn/Gc [34-36]. Other attenuated virus vectors including vaccinia [37] and Newcastle Disease virus [38] expressing Gn/Gc provided protection in mice and sheep, respectively. Sheep were protected against both Lumpy skin disease virus and RVFV challenge when vaccinated with an attenuated Lumpy skin disease virus strain of Capripoxvirus expressing Gn and Gc or RVFV [39,40]. VLPs with [41,42] or without the nucleocapsid [43] protect mice but require multiple doses. A subunit vaccine containing purified Gn ectodomain was produced that has been tested in mice and lambs with success [38]. Vaccine induced neutralizing antibody responses (against Gn/Gc) appear to be of primary importance for protection against subsequent RVFV challenge [38].

Filoviruses

Ebola virus (EBOV) and Marburg virus (MARV) cause unpredictable outbreaks of severe VHF in humans and non-human primates in equatorial Africa (Figures 1 & 2) [44,45]. Transmission is typically due to direct contact with blood, secretions or tissues from infected patients or animals; although fruit bats are suspected to be the reservoir [44,45]. Case-fatality rates vary by virus species and strain but can be as high as 90% (Table 1) [44,45]. Multiple experimental vaccine platforms have demonstrated efficacy in the gold-standard macaque models following homologous challenge. This has included DNA/recombinant Adenovirus-5 (rAd5) [46], rVSV [47] and recombinant human parainfluenza virus-3 (rHPIV3) [48] all expressing the glycoprotein. rVSV has also demonstrated efficacy when used up to 48 hours post-infection [49]. With this success, the focus has shifted towards delineating the correlates/mechanisms of protection and generating multivalent vaccines against the most relevant species.

Currently, there are four human pathogenic EBOV and one MARV species. Generally, there is no cross-protection between species following vaccination; however, there is cross-protection within a species. As the individual filovirus species are not geographically contained, attempts to generate a single vaccine that would be cross-protective against

multiple species are being developed [50]. A single-injection blended vaccine composed of multiple rVSV vaccines expressing different filovirus glycoproteins independently, protected macaques from challenge against any of the species included in the vaccine [49]. A two injection pan-filovirus blended complex adenovirus (CAdVax) expressing multiple glycoproteins and nucleoproteins was also protective against challenge from the included viruses [51]. Similarly, a multivalent vaccine candidate (EBO7) expressing the glycoproteins of two EBOV species in CAdVax provided protection against challenge with either species [52].

Both a blended DNA vaccine containing glycoproteins from two EBOV species and the glycoprotein and nucleoprotein form a single species and the rAd5-ZEBOV GP vaccine were shown to be safe in clinical trials [53]. For the rAd5 vaccine, where T-cell responses have been reported as the mechanism of protection [54], less than half of individuals elicited a desirable immune response [55]. Pre-existing immunity has been a concern for the rAd5 and rHPIV3 platforms; however, airway delivery of rAd5 ZEBOV GP can circumvent pre-existing immunity and confer complete protection in macaques [56]. Despite presumed safety concerns, the rVSV-based vaccine has been shown to cause not be neurovirulent [57], nor side effects in immunocompromised simian-human immunodeficiency virus (SHIV)-infected macaques [49]. Moreover, 67% of SHIV-infected macaques were protected from subsequent challenge, indicating rVSV should be safe and could even provide protection in immunocompromised individuals. For the rAd5, rVSV and rHPIV3 platforms, virus glycoprotein-specific total IgG response appears to correlate with protection in survivors [46-49]. IgG titers are predictive for survival in the rAd5 platform, despite data showing that T cell subsets (CD8+) were required for the mechanism of immunity [54,58]. Furthermore, antibodies were shown to play a critical role in protection for the rVSV vaccine [59].

Flaviviruses

Among the VHFs, **Dengue Virus (DENV)** has the single largest impact on public health, causing an estimated 50-100 million infections per year in over 100 countries with 500,000 people requiring hospitalization resulting in 12,500 deaths for severe dengue (Table 1; Figures 1 & 2). It is currently estimated that 50% of the world's population is at risk for infection with DENV. Vaccines for DENV have seen a recent surge in potential intervention strategies. Multiple vaccines for DENV are currently under investigation but due to the extensiveness of this effort these are reviewed elsewhere (cross-reference to DENV review). Live-attenuated DENV has been found to be genetically unstable and frequently causes dengue-like syndromes, recombinant virus vectors expressing dengue envelope proteins, purified inactivated viruses, recombinant subunit vaccines, VLPs and DNA vaccines have all been attempted [60,61].

A live-attenuated tetravalent vaccine that expresses the pre-membrane and envelope genes of each of the four DENV serotypes [62] within the 17D YFV vaccine has recently been tested in a clinical trial in healthy Thai children with an overall efficacy of ~30% [63]. This poor finding was a result of very low efficacy against DENV 2, which was also the prevalent serotype during the study. Encouragingly, despite the concern over incomplete immune response against all four serotypes leading to disease enhancement; this was not observed, even with the incomplete protection against DENV 2. The lack of efficacy against DENV 2 has yet to be explained given that there was a satisfactory immune response to the serotype. The brings in to question whether a balanced immune response to all four DENV serotypes as assessed by neutralizing antibodies is a correct assumption for protection.

Kyasanur forest disease virus (KFDV) and **Omsk hemorrhagic fever virus (OHFV)** are related to the tick-borne encephalitis (TBE) serocomplex of viruses; however, infection in

humans tends to be characterized by hemorrhagic syndrome whereas other members of the TBE complex result in primarily neurological manifestations. There are an estimated 400-500 cases per year of KFDV in India with a case-fatality rate of 1-3% (Figures 1 & 2) [64]. OHFV occurs in some regions of western Siberia in Russia with case-fatality rates between 0.4 and 2.5% (Figures 1 & 2) [65]. The probable tick vector for KFDV has been identified as *Hemaphysalis sp.*; however, other species have been demonstrated to be capable of KFDV transmission (Table 1). KFDV was thought to be localized to Karnataka State, but serosurveys have suggested cases also occur in other areas of India and the Andaman Islands. Moreover, variants of KFDV have been reported to cause disease in China, while the closely related Alkhurma hemorrhagic fever virus (AHFV) has also been reported in Saudi Arabia, Egypt and Sudan. Transmission of OHFV is mainly via *Dermacentor reticulatus*; however, humans are mainly infected following contact with infected muskrats (*Ondatra zibethicus*) (Table 1). Person-to-person transmission has not been noted [64,65].

Currently a formalin-inactivated chick embryo fibroblast-derived vaccine against KFDV is used on a two-dose schedule with boosts at 6-9 months and then every 5 years. The vaccine appears to be well-tolerated and provides a good level of protection (0.027% vs. 0.86% incidence) [64]. The TBEV vaccines used in Europe and Russia may provide protection against KFDV; however, they have not been tested in KFDV regions. Recent data supports that humans vaccinated with the TBEV vaccine FSME-IMMUN have cross-reactive neutralizing antibody responses against OHFV, albeit at a lower titer than against related TBEV viruses [66]. Data from mouse and African green monkeys found that, while the TBEV vaccine did not prevent OHFV infection, it reduced the viral spread and alterations in the blood [67], suggesting that this widely used vaccine may be useful against OHFV. Further studies would be necessary to demonstrate this, but given the safety profile of the TBEV vaccine there does not appear to any reason why this vaccine could not be used in the event of an OHFV outbreak [68].

Yellow fever virus (YFV) is a mosquito-borne flavivirus that is endemic and epidemic in South America and Sub-Saharan Africa (Table 1; Figure 2). It is a bi-phasic disease that initially causes flu-like symptoms which can progress to a toxic phase with increased bleeding tendency, liver damage and jaundice. There are approximately 1500 reported cases annually with a 20-50% case-fatality rate, but it is estimated that up to 200,000 cases may occur annually with a 15% case-fatality rate (Figure 1) [69,70]. Highly efficacious, live-attenuated strains of YFV, known as 17D-204 or 17DD, both of which are produced in eggs, are currently used worldwide for vaccination. Its use however, is contraindicated in persons who are immunocompromised, infants under 6 months and persons with allergies to eggs. The vaccine can cause both neurotropic and viscerotropic disease, which generally occurs after the first dose. The viscerotropic form of disease has a case-fatality rate of 65% with the incidence rising with increasing age of the vaccinee [71]. The reported rate of adverse effect of the 17D vaccine is higher (0.4−0.8/100,000) than for both the smallpox (0.29/100,000) and the oral polio (0.11/100,000), which are no longer widely used due to safety concerns [71].

For YFV, the effectiveness of the current vaccine is not in question but the development of vaccine with a better safety profile is currently the goal. The prME gene which encodes the membrane and envelope proteins from 17D was inserted into non-replicating modified vaccinia virus Ankara and the D4R-defective vaccinia virus and subsequently shown to provide protection in a mouse model of YFV [72]. A β-propiolactone-inactivated whole YFV vaccine (XRX-001) produced in Vero cells has shown to be efficacious in animal models and humans [74,75]. Given the large population that requires vaccination against

YFV, transitioning to a safer vaccine, especially in groups that are at higher risk for complications should be considered.

Summary

Given the combined global impact of all VHFs, the lack of urgency to develop vaccines to these viruses is somewhat surprising. Global travel and expanding vector ranges due to climate change are driving expansion of the range of some of these viruses. Experimental vaccine platforms that have extensive evaluation in animal models certainly exist for several VHFs, but are not close to being used in clinical trials (i.e. LASV, EBOV and MARV). Current clinical and field trials of DENV and RVFV vaccines are exciting and demonstrate that when a large economic need is present vaccine trials are possible. A mechanism to address vaccines that have less of an immediate economic impact needs to be developed. The success of the Candid#1 as a vaccine for Argentine HF is the example of how a vaccine of limited utility can be generated with governmental support. As the correlate/mechanisms of protection become better defined for the different VHF vaccine approaches establishing clinical trials (especially phase II and III) that meet current standards should be easier and less controversial.

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Highlights

- Dengue vaccine trial shows limited protection against Dengue 2 but no safety concerns
- Development of Rift Valley Fever vaccines that are safe in pregnant livestock
- Defined correlates of protection for Ebola vaccine platforms could allow clinical trials

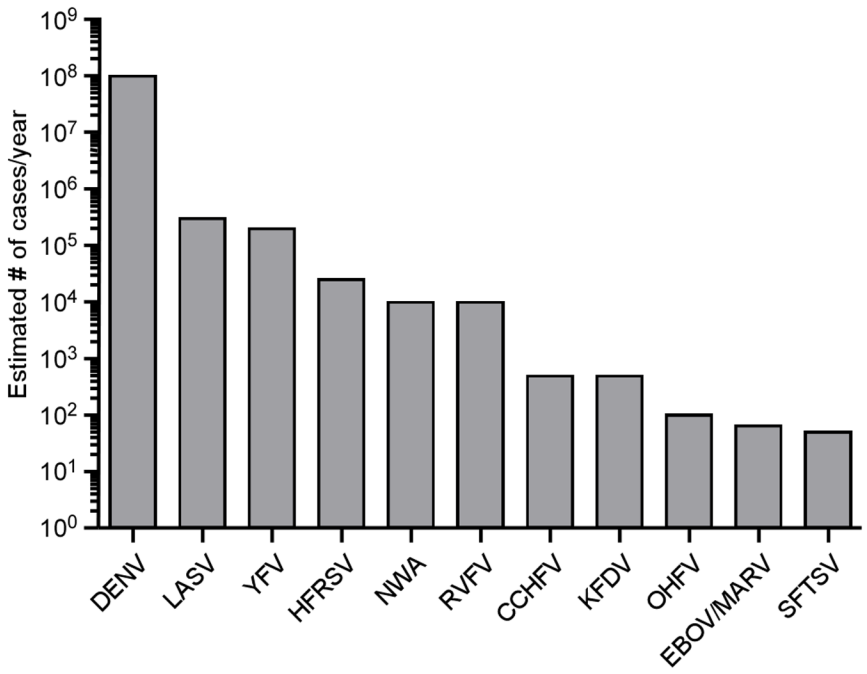

Figure 1. Estimated global burden of viral hemorrhagic fevers

The number of estimated cases per year of Dengue virus (DENV), Lassa virus (LASV), Yellow fever virus (YFV), hemorrhagic fever with renal syndrome viruses (HFRSV), New-world arenaviruses (NWA), Rift Valley fever virus (RVFV), Crimean-Congo hemorrhagic fever virus (CCHFV), Kyasanur Forest disease virus (KFDV), Omsk hemorrhagic fever virus (OHFV), Ebola (EBOV) and Marburg virus (MARV), and Severe fever with thrombocytopenia syndrome virus (SFTSV).

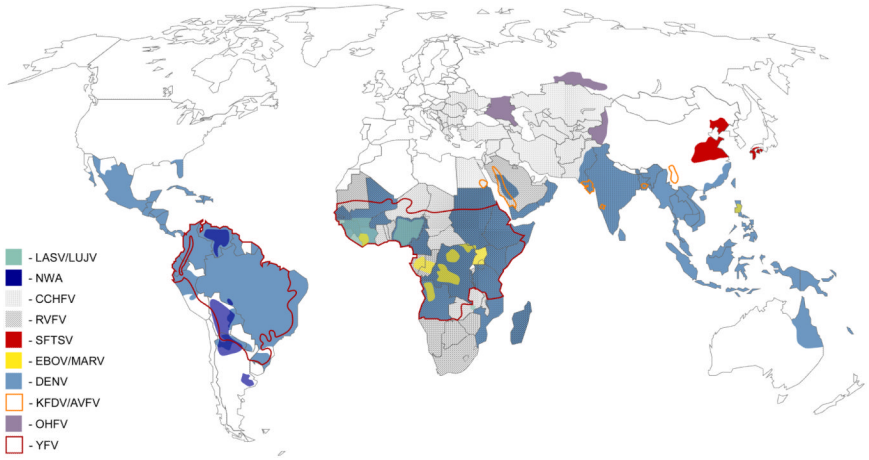

Figure 2. Risk zones for hemorrhagic fever viruses

Regions with current risk or past occurrence of the following viral hemorrhagic fevers: Dengue hemorrhagic fever (DHF), Crimean-Congo hemorrhagic fever (CCHF), Omsk hemorrhagic fever (OHF), Rift Valley hemorrhagic fever (RVF), Yellow fever (YF), Severe fever with thrombocytopenia syndrome (SFTS), Kyasanur Forest disease (KFD).

Table 1

Summary of hemorrhagic fever viruses and the status of available vaccines (in use, clinical trial or experimental).

	Hemorrhagic Fever Viruses	Vector/Host Species	Vaccine in Use or [Clinical trial]	Experimental Vaccine
Arenavirus	Chapare virus	Unknown	No	No
	Guanarito virus	*Sigmodon alstoni, Zygodontomys brevicauda*	No	No
	Junín virus	*Calomys musculinus*	Candid#1	Yes
	Lassa virus	*Mastomys sp.*	No	Yes
	Lujo virus	Unknown	No	No
	Machupo virus	*C. callosus*	No	No
	Sabia virus	*Huesped desconocido*	No	No
Bunyavirus	Crimean-Congo Hemorrhagic Fever virus	*Hyalomma sp.*	Inactivated	Yes
	HFRS viruses	*Apodemus sp., Rattus norvegicus, Clethrionomys glareolus*	Hantavax	Yes
	Rift Valley virus	*Aedes sp., Culex sp., Anopheles sp.*	Inactivated, MP-12, Smithburn, Clone13	Yes
	Severe Fever with Thrombocytopenia Syndrome virus	*Haemaphysalis longicornis*	No	No
Filovirus	Ebola virus	*[*]Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata, Micropteropus pusillus, Mops condylurus, Rousettus aegyptiacus*	[DNA, Adenovirus 5]	Yes
	Marburg virus	*R.aegyptiacus, H. monstrosus*	No	Yes
Flavivirus	Dengue virus	*Aedes sp.*	[tetravalent YFV17D-based]	Yes
	Kyasanur Forest	*Hemaphysalis sp.*	Inactivated	No
	Disease virus Omsk Hemorrhagic fever virus	*Dermacentor reticulatus*	Inactivated (discont.), [FSME-IMMUN (TBEV vaccine)]	No
	Yellow fever virus	*Aedes sp.*	17D, 17DD	Yes

[*] Vector species have only been identified for the Zaire species of Ebola virus.