of organ tissues (Hensley et al., 2011). The virus or viral antigen was detected in liver, lymph nodes, spleen, adrenal grand, kidney, and blood in infected cynomolgus macaques. Onset of viremia occurred on Day 3, and in cynomolgus macaques and African green monkeys the maximum titer was 10^{7-8} pfu/ml on Day 8 after infection (Hass and Maass, 1971; Hensley et al., 2011).

Under experimental conditions, the possibility of aerosol transmission of MARV was shown in macaque models, although such a transmission route has not been described in human outbreaks (Pokhodiaev et al., 1991; Alves et al., 2010).

EBOLAVIRUS INFECTION IN NHPs

African green monkeys, cynomolgus macaques, rhesus macaques, and hamadryas baboons (*Papio hamadryas*) have been employed as a model of EBOV infection (Baskerville et al., 1978, 1985; Bowen et al., 1978, 1980; Ellis et al., 1978; Fisher-Hoch et al., 1985, 1992; Johnson et al., 1995; Jaax et al., 1996; Jahrling et al., 1996; Davis et al., 1997; Ryabchikova et al., 1999; Ignatiev et al., 2000; Geisbert et al., 2003b,e). The monkeys infected with EBOV became febrile 3 days after infection with temperatures above 40°C. Pyrexia usually persisted throughout the course of the disease, which usually ended in a decrease in temperature followed by death, which occurred within 5–8 days after infection (Baskerville et al., 1978; Bowen et al., 1978, 1980; Ellis et al., 1978; Fisher-Hoch et al., 1985; Luchko et al., 1995). By Day 4, anorexia developed with a loss of drinking ability, causing severe weight loss and dehydration. Some monkeys that survived until Day 5 had diarrhea, rectal bleeding, and/or intermittent melena. Petechial skin rashes appeared on the forehead, fore and hind limbs, and chest 4–5 days post-infection in macaques, but on Day 7 in baboons (Bowen et al., 1978; Ellis et al., 1978; Luchko et al., 1995; Ignatiev et al., 2000; Geisbert et al., 2003b). African green monkeys did not develop the cutaneous rash as demonstrated in monkeys infected with MARV (Simpson, 1969; Baskerville et al., 1978). Viremia became detectable within 3 days after infection with the maximum virus titer at the level of $10^{6.5-7}$ pfu/ml on Day 4–5 (Bowen et al., 1978; Fisher-Hoch et al., 1992; Jahrling et al., 1996; Geisbert et al., 2003b). The virus was positive in liver, spleen and lung on Day 4 and also appeared to have lower affinity for kidney, adrenal, lung, testis, lymph node, and pancreas (Baskerville et al., 1978, 1985; Bowen et al., 1978; Geisbert et al., 2003b). Mean virus titers in these organs increased progressively and reached the highest level of $10^{5.5-8.6}$ pfu/g on Day 6 (Geisbert et al., 2003b).

Total blood cell counts revealed marked neutrophilia and lymphopenia in the monkeys. Neutrophils and immature neutrophils increased remarkably by Day 4 (Fisher-Hoch et al., 1985; Geisbert et al., 2003b; Ebihara et al., 2011). Coincident with this process, severe lymphopenia due to lymphocyte apoptosis developed by Day 3 (Fisher-Hoch et al., 1985; Geisbert et al., 2003b). Extensive lymphocyte apoptosis, both in the vasculature and in lymphoid tissue, appears to be critical to the pathogenesis of EHF. Especially within the $CD8^+$ subset, the NK cell population dropped dramatically in the early stage of infection (Geisbert et al., 2003b). Lymphocytes were not productively infected and the apoptosis was not associated with direct viral infection (Geisbert et al., 2000). However, the mechanism underlying such apoptosis is unclear. Another characteristic feature was the abnormality of platelet function preceding thrombocytopenia (Fisher-Hoch et al., 1985; Geisbert et al., 2003b). Thrombocytopenia developed between 3 and 4 days and abnormalities in coagulation parameters, including prolonged PT, aPTT, and TT appeared (Geisbert et al., 2003b; Ebihara et al., 2011). Examination of coagulation parameters revealed that decreased protein C coagulation inhibitor activity due to excessive consumption triggered severe coagulopathy as indicated by prolonged coagulation times and decreased fibrinogen levels (Ebihara et al., 2011).

The NHP model has been proven to be valuable in providing new information regarding filoviral pathogenesis. EBOV spreads from the initial infection site via monocytes/macrophages and DCs to regional lymph nodes, likely via lymphatics, and to liver and spleen through the blood stream. Tissue macrophages, including Kupffer cells, DCs, and fibroblastic reticular cells become infected with EBOV at this stage. EBOV activates DCs by upregulating expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is expressed on DCs and mediates their cytotoxic activity (Geisbert et al., 2003b). Such overexpression of TRAIL is enhanced by overexpression of IFN-α in NHPs infected with EBOV and triggers lymphocyte apoptosis. Monocytes/macrophages infected with EBOV release various soluble factors including proinflammatory cytokines to recruit additional target cells to areas of infection. As disease progresses, increased levels of oxygen free radicals (e.g., nitric oxide) released from virus-infecting macrophages at the inflammatory sites trigger apoptosis of NK cells. The lymphocyte apoptosis caused by TRAIL and nitric oxide interferes with the innate immune response, resulting in escape of EBOV infection from mounting an adaptive response. Coagulation abnormalities are not the direct result of EBOV replication-induced cytolysis of endothelial cells, but are likely triggered by immune-mediated mechanisms (Geisbert et al., 2003d,e). Extensive viral replication leads to increased levels of additional proinflammatory cytokines, notably IL-6, which triggers the coagulation irregularities. This is probably through upregulation of tissue factor expression/release from virus-infected monocytes/macrophages. Tissue factor works as the primary cellular inhibitor of coagulation protease cascades. Activation of the coagulation cascade induces the fibrinogenic and fibrinolytic pathways and finally leads to DIC, hemorrhagic shock, thrombosis-related organ failure and death (Arai et al., 2000).

Monkey species-specific disease features of the pathogenesis of EBOV infection were observed, not only in the development of cutaneous rash but also in the impairment of the clotting systems of African green monkeys and baboons infected with EBOV. In African green monkeys, fibrin thrombosis was generalized in all visceral organs, while in baboons hemorrhages were prominent in visceral organs, most notably in the liver and spleen (Ryabchikova et al., 1999; Ignatiev et al., 2000). Genetic differences, even among the same animal species, and the origin of a species may influence disease presentation and progression.

The dose and species of challenge virus affects the progression of disease. Intramuscular inoculation of cynomolgus macaques with 10^3 pfu of EBOV produced a 100% lethal infection, with deaths occurring 6–7 days post-infection (Geisbert et al., 2002). When the challenge dose was lowered to 10 pfu, uniform lethality was still achieved, but deaths occurred 9–12 days post-infection (Geisbert et al., 2003c). Viremia was demonstrated as early as 24 h after subcutaneous infection of rhesus macaques with a high infectious dose (10^5 pfu) of EBOV. In rhesus and cynomolgus macaques infected with 10^3 pfu of EBOV, viremia is first detected by Day 3 after infection. SUDV causes slower disease progression than EBOV and it has been reported that some monkeys infected with SUDV did not die and recovered from the illness (Ellis et al., 1978; Bowen et al., 1980; Fisher-Hoch et al., 1992). One of four rhesus macaques infected with SUDV died on Day 12 but the other macaques survived and remained normal thereafter (Ellis et al., 1978). The dead macaque had small numbers of virus particles in the liver but no virus particles were found in the kidney, spleen, heart, lung, and brain. The liver, lung, and spleen from EBOV infected macaques, which were moribund and killed on Day 6 contained large numbers of virions. The apparent limitation of viral replication in the liver of SUDV-infected host and the contrasting widespread involvement of liver and other organs such as the spleen and lung of EBOV-infected host are similar in patients and macaques. RESTV, which is considered not to be virulent to humans, is clearly less pathogenic than EBOV and SUDV in African green monkeys and cynomolgus macaques (Fisher-Hoch et al., 1992; Jahrling et al., 1996). Only 5 of 16 monkeys infected with EBOV or SUDV survived, whereas 11 of 15 monkeys infected with RESTV survived (Fisher-Hoch et al., 1992). Viremia, clinical signs (temperature rise, anorexia, depression, or evidence of disturbed hemostasis), serum chemistry changes (elevated aspartate aminotransferase and lactate dehydrogenase activities) and pathological changes (necrosis of hepatocytes, adrenalcytes, and lymphoid elements of the spleen and prominent fibrin thrombi and fibrin precipitation) in RESTV infected monkeys developed slower and/or milder than those observed in monkeys infected with EBOV and/or SUDV.

Most human cases are thought to occur by direct contact with blood and/or body secretions from patients or animal cadavers. Aerosol transmission among humans has not been reported. However, evidence of intercage transmission of RESTV was observed in the 1989–1990 epizootic cases of RESTV in the Hazleton facility in Reston, Virginia, and demonstration of high concentrations of ebolavirus in nasal secretions and alveoli in experimental infection implicated the potency of aerosol transmission of ebolavirus (Baskerville et al., 1978, 1985; Bowen et al., 1978; Jahrling et al., 1990, 1996; Dalgard et al., 1992; Jaax et al., 1995; Miranda et al., 1999, 2002). Furthermore, the rhesus macaques experimentally challenged with aerosolized EBOV developed the same disease as macaques infected parenterally (Johnson et al., 1995). Regardless of the route of infection (intramuscular, subcutaneous, conjunctival, and aerosol injections), NHPs are highly susceptible to EBOV infection (Baskerville et al., 1978; Bowen et al., 1978, 1980; Ellis et al., 1978; Baskerville et al., 1985; Fisher-Hoch et al., 1985, 1992; Johnson et al., 1995; Jaax et al., 1996; Jahrling et al., 1996; Davis et al., 1997; Ryabchikova et al., 1999; Ignatiev et al., 2000; Geisbert et al., 2003b,e).

VACCINES

INACTIVATED WHOLE VIRION

The development of filovirus vaccines has been performed based on inactivated whole virion preparations. About half of the rhesus macaques or African green monkeys treated were protected against homologous MARV challenge, when formalin- or gamma-inactivated whole MARV virions were used as vaccine candidates (Ignat'ev et al., 1991; Ignatyev et al., 1996). Vaccination with formalin-inactivated EBOV virions protected 4 of 5 hamadryas baboons (Mikhailov et al., 1994), while other studies suggested that inactivated virus did not induce sufficient immunity to protect baboons against a lethal challenge (Chupurnov et al., 1995). Furthermore, vaccination with gamma-irradiated EBOV virions alone or in a form of liposomes containing lipid A failed to protect cynomolgus macaques against lethal infection (Geisbert et al., 2002). Overall, these vaccine candidates based on inactivated virions did not confer sufficient protection in NHP models. Furthermore, these vaccines are unlikely to be used in humans due to safety risk of incomplete inactivation. However, these results promoted the development of an alternative vaccine platform, such as DNA-based vaccines, recombinant viral vector, or virus-like particles as described below and **Table 2**.

VENEZUELAN EQUINE ENCEPHALITIS VIRUS REPLICON

Venezuelan equine encephalitis virus (VEEV) replicons that express either GP or NP of MARV Musoke protected guinea pigs from viremia and death caused by GP-adapted MARV challenge (Hevey et al., 1998). Cynomolgus macaques administrated with MARV Musoke GP-expressing VEEV replicons alone or in combination with NP were also protected from lethal infection with the homologous Musoke strain, but not from heterologous RAVV (Hevey et al., 1998; Falzarano et al., 2011). additionally, vaccination with NP alone prevented death but not disease onset in two of three monkeys and allowed all animals to become viremic. For EBOV, EBOV GP-expressing VEEV replicons, alone or in combination with EBOV NP-expressing VEEV replicons, protected mice, and guinea pigs from lethal infection, whereas immunization with EBOV NP-expressing VEEV replicons alone protected mice but not guinea pigs (Pushko et al., 2001). Furthermore, vaccination with recombinant VEEV, expressing EBOV GP, NP, or both GP and NP, failed to protect cynomolgus macaques from a lethal EBOV infection (Geisbert et al., 2002). One recent study produced different results, whereby a VEEV-based vaccine was fully protective in cynomolgus macaques against EBOV, SUDV, and MARV (Friedrich et al., 2012). The results obtained from these studies are inconsistent, suggesting that VEEV-based vaccine may be promising although further research is needed.

ADENOVIRUS-BASED VACCINES

Adenovirus (AdV) vectors commonly used are based on serotype 5 (AdV5). A single infection of the recombinant MARV Angola

Table 2 | Efficacy of vaccines in animal models of filovirus infection.

Vaccine	Viral protein including vaccines	Species tested	Strategy		Challenge virus	Survival rate (%)	References
			Dose/Schedule	Route			
VEEV	MARV Musoke GP or NP	Guinea pig	10^6 FFU, 2 or 3 doses	sc	GP-adapted MARV Musoke	100	Hevey et al., 1998
	MARV Musoke GP or GP + NP	NHP	10^7 FFU, 3 doses		MARV Musoke	100	
	MARV Musoke NP					67	
	EBOV NP or GP + NP EBOV GP	Mouse	2×10^6 IU, 2 doses		mouse-adapted EBOV	100 90	Pushko et al., 2001
	EBOV GP or GP + NP EBOV NP	Guinea pig	10^7 IU, 3 doses		GP-adapted EBOV	100 20	
	EBOV GP, NP or GP + NP	NHP	10^7 FFU, 3 doses		EBOV	0	Geisbert et al., 2002
AdV	GPs of MARV (Musoke and Ci67) and RAVV	Guinea pig	$5 \times 10^{7-8}$ PFU, 2 doses	sc	MARV (Musoke or Ci67) or RAVV	100	Wang et al., 2006a
	MARV Angola GP	NHP	10^{11} PU, 1dose	im	MARV Angola	100	Geisbert et al., 2010a
	EBOV GP	Mouse	10^8 PFU, 2 doses	sc	mouse-adapted EBOV	100	Wang et al., 2006b
	EBOV GP + NP	NHP	2×10^{12} particles, 1 or 2 doses	im	EBOV	100	Sullivan et al., 2003
	GPs of EBOV and SUDV, MARV (Musoke and Ci67) and RAVV + NP of EBOV and MARV Musoke		4×10^{10} PFU, 2 doses		EBOV, SUDV or MARV (Musoke or Ci67)	100	Swenson et al., 2008a
DNA	MARV Musoke or RAVV GP	Guinea pig	10 μg, 3 or 4 doses with RIBI aadjuvand	sc	GP-adapted MARV Musoke	100	Riemenschneider et al., 2003
	MARV Musoke GP	NHP	20 μg, 3 doses		MARV Musoke	67	
	MARV Angola GP		4 mg, 4 doses	im	MARV Angola	100	Geisbert et al., 2010a
	EBOV GP	Mouse	0.5 μg, 4 doses 0.5 μg, 1 dose and 1.5 μg, 3 or 4 doses		mouse-adapted EBOV	78 100	Vanderzanden et al., 1998
	EBOV GP or NP	Guinea pig	500 μg, 4 doses		GP-adapted EBOV	100	Xu et al., 1998
DNA + AdV	DNA: GPs of EBOV, SUDV and TAFV + EBOV NP AdV: EBOV GP	NHP	4 mg of DNA, 3 doses and boosted with 10^{10} PFU of AdV	im	EBOV	100	Sullivan et al., 2000
	DNA: MARV Angola GP AdV: MARV Angola GP		4 mg of DNA, 3 doses and boosted with 10^{11} PU of AdV		MARV Angola	100	Geisbert et al., 2010a

(Continued)

Table 2 | Continued

Vaccine	Viral protein including vaccines	Species tested	Strategy			Challenge virus	Survival rate (%)	References
			Dose/Schedule		Route			
HPIV3	EBOV GP or GP + NP	Guinea pig	$10^{5.3}$ PFU		in	GP-adapted EBOV	100	Bukreyev et al., 2006
	EBOV GP, GP + NP, or GP + GM-CSF	NHP	4×10^6 TCID50, 1 dose		in and intracheally	EBOV	83	Bukreyev et al., 2007
	EBOV GP		2×10^7 TCID50, 2 doses				100	
HPIV3/ ΔHN-F	EBOV GP	Guinea pig	4×10^5 PFU, 1 dose		in	GP-adapted EBOV	100	Bukreyev et al., 2009
VSV	MARV Musoke GP	NHP	2×10^7 PFU, 1 dose	28 day before infection	im	MARV (Musoke or Angola) or RAVV	100	Daddario-Dicaprio et al., 2006a
			10^7 PFU, 1 dose	28 or 141 d before infection[a]		MARV Musoke and Popp	100	Jones et al., 2005
	EBOV GP	Mouse	2×10^5 PFU, 1 dose	24 h before infection	ip	mouse-adapted EBOV	100	Feldmann et al., 2007
				30 mpi			100	
				24 hpi			100	
		Guinea pig		24 h before infection		GP-adapted EBOV	67	
				1 hpi			83	
				24 hpi			50	
		NHP	10^7 PFU, 1 dose	28 day before infection	im	EBOV	100	Jones et al., 2005
			10^7 PFU, 1 dose	262 day before infection[b]		SUDV	25	
	EBOV GP + SUDV GP + Musoke GP		3×10^7 PFU, 1 dose	28 day before infection		EBOV, SUDV, TAFV or MARV Musoke	100	Geisbert et al., 2009
	MARV Musoke GP		2×10^7 PFU, 1 dose	24 hpi		MARV Musoke	83	Geisbert et al., 2010c
				48 hpi			33	
			1×10^7 PFU, 1 dose	20–30 mpi			100	Daddario-Dicaprio et al., 2006b
	EBOV GP		2×10^7 PFU, 1 dose, 20–30 mpi			EBOV	50	Feldmann et al., 2007
	SUDV GP					SUDV	100	Geisbert et al., 2008

(Continued)

Table 2 | Continued

Vaccine	Viral protein including vaccines	Species tested	Strategy		Challenge virus	Survival rate (%)	References
			Dose/Schedule	Route			
VLP	MARV Musoke GP + VP40 produced in 293T[c]	Guinea pig	50 μg, 3 doses with RIBI adjuvant	im	GP-adapted MARV (Musoke or Ci67) or RAVV	100	Swenson et al., 2008b
		NHP	1 mg, 3 doses with QS-21 adjuvant		MARV (Musoke or Ci67) or RAVV	100	
	EBOV GP + VP40 + NP produced in 293T[c]	Mouse	50 μg, 2 doses, with QS-21 adjuvant		Mouse-adapted-EBOV	100	Warfield et al., 2007a
	EBOV GP + VP40 produced in 293T[c]		10 μg, 3 doses	im or ip		100	Warfield et al., 2003
	EBOV GP + NP + VP40 produced in 293T[c]	NHP	250 μg, 3 doses, with RIBI adjuvant	im	EBOV	100	Warfield et al., 2007b
	EBOV GP + VP40 produced in insect cells[d]	mouse	50 μg, 2 doses		Mouse-adapted-EBOV	100	Sun et al., 2009
			10 μg, 3 doses			83	
	EBOV GP + VP40 + NP produced in insect cells[d]		10-50 μg, 2 doses, with QS-21 adjuvant			100	Warfield et al., 2007a

[a] Cynomolgus macaques were immunized by intramuscular injection with a single dose of VSVΔG expressing MARV Musoke GP and subsequently challenged on Day 28 after immunization by intramuscular injection with MARV Musoke strain. The immunized macaques, which were protected from the lethal MARV challenge, were rechallenged with MARV Popp strain 113 days after initial challenge (141 days after immunization).

[b] Cynomolgus macaques were immunized by intramuscular injection with a single dose of VSVΔG expressing EBOV GP and subsequently challenged on Day 28 after immunization by intramuscular injection with EBOV. The macaques protected from the lethal EBOV challenge were rechallenged with SUDV 234 days after initial challenge (262 days after immunization).

[c] 293T cells were cotransfected with plasmid vectors encoding GP and VP40 (and NP) of EBOV or MARV. The VLPs were collected and purified from the cell supernatants.

[d] The VLPs were produced by use of recombinant baculovirus constructs expressing GP and VP (and NP) of EBOV or MARV from coinfected insect cells.

Abbreviations; FFU, focus-forming unit; GP-adapted, guinea pig-adapted; IU, infectious unit; PFU, plaque-forming units; PU, particle units; sc, subcutaneously; im, intramuscularly; in, intranasally; ip, intrapenitoneally; mpi, minutes post-infection; hpi, hours post-infection; dpi, days post-infection.

GP-expressing AdV5 resulted in complete protection of cynomolgus macaques from illness and death by challenge with homologous virus (Geisbert et al., 2010a). Vaccination with a mixture of EBOV GP—expressing AdV5 and EBOV NP—expressing AdV5 have demonstrated 100% protection in cynomolgus macaques against homologous virus challenge (Sullivan et al., 2003). However, the genome insert size in this first generation AdV vector was restricted to as little as a single filovirus GP gene. The second generation AdV vector has the advantage of being able to express multiple antigens in a single construct over the first generation vector. The second generation bivalent AdV vector expressing GPs of EBOV and SUDV led to efficient induction of antibodies specific to EBOV and SUDV (Wang et al., 2006b). A trivalent AdV vector expressing MARV GPs of Ci67, Musoke and RAVV efficiently led to MARV-specific antibodies in mice and guinea pigs and showed complete protection of guinea pigs against MARV and RAVV infections (Wang et al., 2006a). Additionally, vaccination of cynomolgus macaques with second generation AdV vectors, which expressed multiple filovirus GPs of EBOV, SUDV, Ci67, Musoke, and RAVV, induced 100% protection against challenge with EBOV and SUDV and two different strains of MARV (Ci67 and Musoke; Swenson et al., 2008a). Although the AdV-based vaccines showed efficacy, the vaccines have a major obstacle: the prevalence of pre-existing immunity to AdV that may substantially limit their immunogenicity and clinical utility. It is estimated that the prevalence of antibody to AdV5 is up to 60% in the general human population and up to 85% in Africa (Schulick et al., 1997; Piedra et al., 1998). Indeed, macaques pre-immunized against AdV5 and vaccinated with EBOV GP-expressing AdV5 were not protected from lethal challenge with EBOV infection (Geisbert et al., 2010b). AdV serotype 26 and 35 segregated genetically from AdV5 exhibit lower seroprevalence in humans (Vogels et al., 2003; Abbink et al., 2007; Mast et al., 2010). Therefore, AdV serotype 26 and 35 vectors with expression of EBOV or SUDV GPs have been generated and the protective efficacy examined by using the NHP model, but these vectors failed to protect cynomolgus macaques against lethal EBOV challenge (Geisbert et al., 2011).

DNA

The plasmid coding the DNA of GP from MARV Musoke or RAVV demonstrated efficacy in protection of guinea pigs and cynomolgus macaques against lethal infection of each homologous strain (Riemenschneider et al., 2003). All of the guinea pigs vaccinated three or four times with DNA vaccines were aviremic and appeared healthy. In cynomolgus macaques, four of six monkeys immunized with 3 doses of DNA vaccine encoding Musoke GP were protected from homologous challenge with MARV Musoke (Riemenschneider et al., 2003). In a report of DNA vaccines encoding GP of MARV Angola strain, the 4 vaccination doses resulted in protection of all four vaccinated monkeys, but three of the four monkeys showed symptoms and/or lymphopenia (Geisbert et al., 2010a). A combination vaccine regimen (3 times injection with DNA and boost with recombinant Angola GP-expressing AdV vector) protected the monkeys from lethal infections but two of the four monkeys showed rash or lymphopenia. A single inoculation with AdV vaccine induced optimal immune responses to eliminate symptoms and death by itself. These data suggest that DNA vaccines do not optimally control MARV infection (Geisbert et al., 2010a). However, three-plasmid DNA vaccines encoding EBOV GP, SUDV GP, and EBOV NP were evaluated in a phase I trial as safe and immunogenic in humans (Martin et al., 2006). The EBOV DNA vaccine also protected mice and guinea pigs against a lethal challenge (Vanderzanden et al., 1998; Xu et al., 1998; Martin et al., 2006). In one study, cynomolgus macaques, which received 3 injections of DNA vectors encoding EBOV GP, SUDV GP, TAFV GP, and EBOV NP, were boosted with recombinant EBOV GP-expressing AdV (Sullivan et al., 2000). All four monkeys vaccinated survived and showed no symptoms of EBOV infection. This prime-boost strategy provided a sufficient immune response to clear the virus efficiently.

HUMAN PARAINFLUENZA VIRUS

In an outbreak of RESTV in the Hazleton facility in Reston, Virginia, aerosol transmission between NHPs may have occurred (Jahrling et al., 1990; Dalgard et al., 1992; Miranda et al., 1999, 2002). To address the assumed aerosol transmission of filovirus, a vaccine that induces a strong immune response in the respiratory tract was developed. Human parainfluenza virus type 3 (HPIV3), a common respiratory virus, was modified as a form of vaccine vector and used for development of a vaccine against EBOV. The HPIV3 vectors, which express EBOV GP or EBOV GP together with NP, protected guinea pigs and rhesus macaques against EBOV challenge (Bukreyev et al., 2006, 2007). In guinea pigs, a single intranasal inoculation with HPIV3 expressing EBOV GP or both GP and NP showed complete protection against signs of illness and death (Bukreyev et al., 2006). The rhesus macaques were immunized with a single dose of EBOV GP-expressing HPIV3, or EBOV GP and NP-expressing HPIV3, through a combined intranasal and intratracheal inoculation. Five of six monkeys immunized with the HPIV3 based vaccine survived and four of six monkeys did not show any clinical illness (Bukreyev et al., 2007). Two doses of intranasal immunizations showed greater efficacy, including complete protection of all three rhesus macaques against clinical illness and death. However, HPIV3 may not be effective as a vaccine vectors in humans, since HPIV3 is a common childhood pathogen and the majority of the population have pre-existing immunity to HPIV3. To overcome the problem of pre-existing immunity, a chimeric HPIV3, where both HPIV3 surface proteins, HN and F, were deleted and replaced with EBOV GP was developed (Bukreyev et al., 2009). A single immunization with the vaccine completely protected guinea pigs against a lethal infection. It was shown that the HPIV3 based vaccine, which expressed EBOV GP, was immunogenic equally among HPIV3-naïve and HPIV3 antibody-positive subjects and effective when vaccinated twice. However, pre-existing HPIV3-specific immunity in rhesus macaques reduced the replicative capacity of the HPIV3-based vaccine in the respiratory tract (Bukreyev et al., 2010). Nevertheless, this study indicated that the vaccination induced an appropriate antibody response.

VESICULAR STOMATITIS VIRUS

A vaccine to resolve the problem of pre-existing immunity utilized the recombinant vesicular stomatitis virus (VSV) vector, which expresses filovirus GP. VSV is mainly a veterinary pathogen and human infection with VSV is rare and not associated with disease in humans. A single intramuscular vaccination of cynomolgus macaques with recombinant VSV with expression of MARV Musoke GP elicited complete protection against a high dose (10^3 pfu) intramuscular challenge with both homologous Musoke strain and heterologous Popp strain, Angola strain, and RAVV (Jones et al., 2005; Daddario-Dicaprio et al., 2006a). For EBOV, a single immunization of cynomolgus macaques with recombinant VSV vector, which expresses EBOV GP, also elicited complete protection against EBOV challenge (Jones et al., 2005). The surviving macaques from lethal EBOV infection were re-challenged with heterologous SUDV, but the cross-protection was not observed (Jones et al., 2005). Administration of the EBOV GP-expressing VSV vaccine through the oral or intranasal route completely protected cynomolgus macaques from EBOV challenge (Qiu et al., 2009). A blended vaccine consisting of equal amounts of 3 different VSV vectors, which expresses GP of each of EBOV, SUDV, and MARV, generated complete protection of cynomolgus macaques against challenges with EBOV, TAFV, and MARV (Geisbert et al., 2009). Macaques vaccinated with the blended vaccine followed by challenge with SUDV showed mild clinical sign of illness including fever, lymphopenia, and mild anorexia, and the macaques recovered from illness. Importantly, none of the macaques vaccinated with the blended vaccine succumbed to a filovirus challenge. The efficacy of the recombinant VSV vaccine has been evaluated as a post-exposure prophylaxis for filovirus infections. Administration of recombinant VSV with MARV Musoke GP expression to rhesus macaques shortly after a homologous high-dose MARV challenge resulted in complete protection of all subjects from clinical illness and death (Daddario-Dicaprio et al., 2006b). Furthermore, administration of recombinant MARV Musoke GP-expressing VSV at 24 and 48 h following infection resulted in protection of 83 and 33% of rhesus macaques, respectively (Geisbert et al., 2010c). When recombinant EBOV GP-expressing VSV were administrated to mice 24 h prior to challenge, and 1 and 24 h post-challenge, all treated mice survived (Feldmann et al., 2007). In guinea pigs treated with EBOV GP-expressing VSV at 24 h prior to challenge, and 1 or 24 h post-challenge, the survival rates were 67, 83, and 50%, respectively. It was also demonstrated that post-exposure vaccination with the recombinant VSV GP vectors for EBOV and SUDV in rhesus macaques was effective against challenge with homologous viruses, although the protection rate was dependent on the species of ebolavirus. The survival rates of the EBOV- or SUDV-infected monkeys were 50 and 100%, respectively (Feldmann et al., 2007; Geisbert et al., 2008).

VIRUS-LIKE PARTICLE

Virus-like particle (VLPs), which mimic authentic virions structurally but do not contain infectious genetic material, are non-infectious and safer than replicating vaccines. The efficiency of a MARV vaccine consisting of VLPs with MARV Musoke GP and VP40 was assessed in guinea pig and cynomolgus macaque models (Swenson et al., 2008b). The guinea pigs and monkeys immunized three times with MARV-Musoke VLPs with RIBI or QS-21 adjuvant were challenged with Musoke strain, Ci67 strain, or RAVV. All guinea pigs and eight monkeys were protected from death and clinical illness following the lethal challenge, except for a single monkey. The monkey challenged with RAVV, which is the most genetically distinct strain of marburgvirus, developed minor signs of disease without detectable viremia. For ebolavirus, mice vaccinated with EBOV VLP in the presence or absence of adjuvant were protected from lethal EBOV infection in a dose-dependent manner (Warfield et al., 2003, 2007a; Sun et al., 2009). Furthermore, the efficacy of the EBOV VLP, which consists of EBOV GP, NP, and VP40 was evaluated in cynomolgus macaques (Warfield et al., 2007b). All five monkeys that received three injections of the EBOV VLPs with RIBI adjuvant were completely protected against EBOV challenge.

There are some other vaccine candidates, including an EBOV lacking VP30 (which encodes the essential transcription factor), an Fc portion of a human IgG fused to EBOV-GP, a bean yellow dwarf virus-derived replicon system, and a cytomegalovirus-based vaccine encoding an EBOV NP CTL epitope (Halfmann et al., 2009; Konduru et al., 2011; Phoolcharoen et al., 2011; Tsuda et al., 2011a). However, the immunogenic efficacy of these vaccines has only been confirmed in the rodent models and further studies are needed to evaluate the protective efficacy and safety in NHPs.

TREATMENTS

RECOMBINANT NEMATODE ANTICOAGULANT PROTEIN C2

Coagulation abnormalities are one of the most prominent hallmarks of filovirus infection. It has been suggested that tissue factor plays an important role in triggering the hemorrhagic complications in NHPs infected with filoviruses (Geisbert et al., 2003d). Overexpression of tissue factor that performs as the primary cellular inhibitor of the coagulation protease cascades is one of the causes of DIC and thrombosis-related organ failure. The effect of blocking the pathway leading from the complex of activated factor VII and tissue factor to thrombin was examined in filovirus infection. Recombinant nematode anticoagulant protein c2 (rNAPc2), which directly inhibits factor VII and tissue factor, provided partial post-exposure protection to rhesus macaques infected with filovirus (Geisbert et al., 2003a, 2009). In rNAPc2-treated rhesus macaques, the mean survival time (11.7 days) was longer than that in untreated control monkeys (8.3 days) and 33% of EBOV-infected macaques survived. In MARV Angola-infected rhesus macaques treated with rNAPc2, 1 of 6 (17%) monkeys survived and the mean survival time for the five dead monkeys was significantly prolonged compared with that of the untreated control monkeys. rNAPc2 demonstrated a clear improvement in terms of survival rate and an increase in mean survival time in a normally 100% lethal model of filovirus infection.

RECOMBINANT HUMAN ACTIVATED PROTEIN C

Activated protein C (APC) is generated from the protein C, which is a vitamin K-dependent plasma protein and inactivates factors V and VIII to down-regulate thrombin generation. It has

Table 3 | Efficacy of post-exposure treatment in animal models of filovirus infection.

Treatment	Mechanism/target viral protein	Species tested	Strategy			Challenge virus	Survival rate (%)	References
			Dose	Route	Dose schedule			
rNAPc2	Blocks TF: FVIIa mediated activation of factor X	NHP	30 μg/kg bw	sc	10 mpi and administraion daily for 14 days	EBOV	33	Geisbert et al., 2003a
					24 hpi and administraion daily for 8 days		33	
					10 mpi and administraion daily for 14 days	MARV Angola	17	Geisbert et al., 2007
APC	Anti-thrombotic: cleaves and inhibits coagulation cofactors FVIIIa and Fva	NHP	2 mg/m^2/h	iv	30–60 mpi and administration for 7 days	EBOV	18	Hensley et al., 2007
PMO	Targets viral mRNA to block transcription EBOV VP24,VP35 and L	Mouse	500 μg	ip	twice at 24 h and 4 h before infection	mouse-adapted EBOV	100	Warfield et al., 2006
		Guinea pig			single dose at 24 hpi	GP-adapted EBOV	100	
					single dose 24 h before infection		>25	
					single dose 24 hpi		25–50	
					single dose 96 hpi		50–75	
		NHP	12.5–200 mg	im	2 day before challenge and administration for 9 days	EBOV	75	
PMO plus	EBOV VP24 and VP35 (AVI-6002)	NHP	40 mg/kg bw	sc and ip	30–60 mpi and administration daily for 10 or 14 days	EBOV	63	Warren et al., 2010
			28 or 40 mg/kg bw	iv	30-60 mpi and administration daily for 14 dpi		60	
			4 mg/kg bw				0	
			16 mg/kg bw				20	

(Continued)

Table 3 | Continued

Treatment	Mechanism/target viral protein	Species tested	Strategy			Challenge virus	Survival rate (%)	References
			Dose	Route	Dose schedule			
	MARV Musoke VP24 and NP (AVI-6003)	NHP	30 or 40 mg/kg bw	sc and ip		MARV Musoke	100	
			40 mg/kg	sc or iv			100	
			30 mg/kg	iv			100	
			7.5 or 15 mg/kg				60	
siRNA	Targets viral mRNA to block transcription	EBOV L	guinea pig	PEI-mixed, 8 mg/kg	ip	3 h before infection and 24, 48 and 96 hpi	GP-adapted EBOV	25	Geisbert et al., 2006
				SNALP-formulated, 1 mg/kg		1, 24, 48, 72, 96, 120 and 144 hpi		60[a]	
				SNALP-formulated, 0.75 mg/kg				100	
		EBOV L, VP24 and VP35	NHP	SNALP-formulated, 2 mg/kg	iv	30 mpi, 1, 3 and 5 dpi	EBOV	66	Geisbert et al., 2010d
		EBOV L, VP24 and VP35				30 mpi, 1, 2, 3, 4, 5 and 6 dpi		100	

[a] *Two of five guinea pigs received the siRNAs using the SNALP delivery systems died but the death could not be attributed to viral replication.*

Abbreviation; bw, bodyweight; sc, subcutaneously; iv, intravenously; ip, intrapenitoneally; im, intramuscularly; mpi, minutes post-infection; hpi, hours post-infection; dpi, days post-infection.

been reported that circulating levels of protein C were rapidly and significantly reduced in cynomolgus macaques and rhesus macaques during EBOV infections, because the protein C might be produced in the liver, which is a main target of filovirus infection (Geisbert et al., 2003a). In rhesus macaque models, administration of recombinant human APC (rhAPC) at 30–60 min after challenge and continuing for 7 days, protected 2 of 11 (18%) monkeys against lethal EBOV infection (Hensley et al., 2007). The mean survival time in the rhAPC-treated monkeys was prolonged compared with the untreated monkeys (Hensley et al., 2007).

PHOSPHORODIAMIDATE MORPHOLINO OLIGOMER

Phosphorodiamidate morpholino oligomers (PMOs) inhibit targeted gene translation by steric blockage of ribosomal assembly. A combination of EBOV-specific PMOs targeting sequences of viral mRNAs for the VP24, VP35, and RNA polymerase L protected rodents in both pre- and post-exposure therapeutic regimens (Warfield et al., 2006). In rhesus macaque models, treatment with a combination of the PMOs of VP24, VP35, and L from 2 days prior to EBOV challenge through Day 9 of the infection protected 3 of 4 (75%) rhesus macaques against lethal infection (Warfield et al., 2006). Furthermore, it was demonstrated that the antiviral potency of PMOs could be enhanced by chemical modification, either by conjugating PMOs with peptides or by introducing positively charge to the PMOs (PMO*plus*™, Avi BioPharma, Inc.; Swenson et al., 2009). Subsequently, PMO*plus* targeting EBOV VP24 and VP35 or MARV Musoke VP24 and NP showed significant protection of mice and guinea pigs against lethal challenge with EBOV and MARV Musoke, respectively (Warren et al., 2010). AVI-6002 PMO*plus* against both EBOV VP24 and VP35, and AVI-6003 PMO*plus* against MARV VP24 and NP, were developed and tested for treatment efficacy using NHP models. These PMOs, delivered 30–60 min post-exposure, protected 62.5% of rhesus macaques against lethal EBOV infection and 100% of cynomolgus macaques against MARV Musoke infection (Warren et al., 2010). AVI-6002 and AVI-6003 are currently in phase I clinical trials.

RNA INTERFERENCE

RNA interference (RNAi) inhibits gene expression to the extent that their function is abrogated through a highly regulated enzyme-mediated process. It was demonstrated that small-interfering RNA (siRNA) down-regulated various MARV mRNA transcripts, resulting in a significant decrease in viral protein production and subsequent viral release *in vitro* (Fowler et al., 2005). Furthermore, siRNA targeting the EBOV RNA polymerase L protein formulated in stable nucleic acid-lipid particles (SNALPs) completely protected guinea pigs when administered shortly after a lethal EBOV infection (Geisbert et al., 2006). In rhesus macaques, a combination of siRNA targeting the EBOV L, VP24, and VP35 were formulated in SNALPs and administrated to the monkeys. Two of three monkeys, which were treated four times with siRNA at 30 min, 1, 3, and 5 days after challenge, survived lethal infection. Furthermore, all four monkeys treated seven times at 30 min, 1–6 days after challenge survived (Geisbert et al., 2010d).

THERAPEUTIC EFFICACY IN THE MOUSE MODEL AND *in vitro*

In the mouse model, administration of recombinant mannose-binding lectin and hexaamminecobalt (III) chloride showed efficacy in protecting against EBOV infections (Michelow et al., 2011). Mannose-binding lectin targets diverse microorganisms for phagocytosis and complement-mediated lysis by binding specific surface glycans. Hexaamminecobalt (III) chloride is a complex of a cobalt (III) ion surrounded by six ammonia ligands in a full octahedral coordination. Furthermore, by high-throughput screening, some compounds such as FGI-103, FGI-106, and NSC 62914 (a reactive oxygen species scavenger), were identified to have high antiviral activity against filoviruses (Aman et al., 2009; Warren et al., 2010). Some other substances, for example inhibitors of heat-shock protein 90 and Niemann-Pick C1, showed antiviral activity *in vitro* (Smith et al., 2010; Cote et al., 2011). As mentioned above (Table 3), several candidates are discussed as therapeutic agents for Ebola and Marburg HFs, but no licensed therapeutics are yet available (Friedrich et al., 2012).

CONCLUSIONS

Significant progress has been made in developing animal models, including mice, guinea pigs, hamsters and NHPs, for EHF and MHF. The NHPs are the most feasible model, because they are the only animals that are lethally infected with non-adapted virus isolates and the pathophysiology is close to that demonstrated in patients. The rodent models need serial passages of original filoviruses in rodents for acquiring lethal infection capacity and they have limited value, because the disease course in rodents differs from that demonstrated in humans and NHPs. However, the rodent models are the first choice for preliminary studies to explore vaccines and therapeutic agents, because of their ease to handling. The newly developed Golden hamster model will also be used for studies on pathogenesis and evaluation of efficacy of candidate vaccines and therapeutics because they show manifestations similar to those of patients and NHPs, including severity of coagulopathy that is lacking in mouse and guinea pig models. Among the candidate vaccines so far developed, recombinant VSV-based vaccines against EHF and MHF are confirmed to be effective in mouse, guinea pig, Golden hamster, and NHP models, and are the only platform with the potential to prevent lethal infection, especially via both vaccine and post-exposure treatment (Jones et al., 2005; Daddario-Dicaprio et al., 2006b; Feldmann et al., 2007; Geisbert et al., 2008, 2009, 2010c; Qiu et al., 2009; Tsuda et al., 2011b). Furthermore, the VSV have been used as a treatment following a recent laboratory exposure (Tuffs, 2009). Further research is needed to develop vaccines with sufficient long-term efficacy by single-dose vaccination, because expensive and time-consuming vaccinations may pose difficulties due to logistical and financial problems in developing countries, where EHF and MHF are endemic. Neither licensed vaccines nor therapeutic agents are available so far. The development of vaccines and therapeutic testing using the animal models has only recently begun to progress. We hope that further research facilitates progress toward elucidating the disease pathophysiology and developing prophylactic and therapeutic measures against EHF and MHF.

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Novel Bat-borne Hantavirus, Vietnam

To the Editor: Compelling evidence of genetically distinct hantaviruses (family *Bunyaviridae*) in multiple species of shrews and moles (order Soricomorpha, families Soricidae and Talpidae) across 4 continents (*1–7*) suggests that soricomorphs, rather than rodents (order Rodentia, families *Muridae* and *Cricetidae*), might be the primordial hosts (*6,7*). Recently, the host range of hantaviruses has been further expanded by the discovery that insectivorous bats (order Chiroptera) also serve as reservoirs (*8,9*). Conjecturing that Mouyassué virus in the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire (*8*) and Magboi virus (MGBV) in the hairy split-faced bat (*Nycteris hispida*) in Sierra Leone (*9*) represent a much broader geographic distribution of bat-borne hantaviruses, we analyzed tissues from bats captured in Mongolia and Vietnam.

Total RNA was extracted from 51 lung tissues, collected in RNAlater Stabilization Reagent (QIAGEN, Valencia, CA, USA), from insectivorous bats, representing 7 genera and 12 species, captured in Mongolia and Vietnam. cDNA was then prepared by using PrimeScript II 1st strand cDNA Synthesis Kit (Takara Bio, Otsu, Shiga, Japan) for reverse transcription PCR (RT-PCR), and using oligonucleotide primers previously designed for amplification of soricid- and talpid-borne hantaviruses (*1–7*).

A novel hantavirus, designated Xuan Son virus (XSV), was detected in 1 of 5 Pomona roundleaf bats (*Hipposideros pomona*) by using a heminested large (L)–segment primer set (outer: HNL-2111F, 5′-CARTCWACWGTIGGIGCIAGTGG-3′, and HAN-L-R1, 5′-AACCADTCWGTYCCRTCATC-3′; inner: HNL-2111F and HAN-L-R2, 5′-GCRTCRTCWGARTGRTGDGCAA-3′) and a nested small (S)–segment primer set (outer: OS-M55F, 5′-TAGTAGTAGACTCC-3′, and XSV-S6R, 5′-AGITCIGGRTCCATRTCRTCICC-3′; inner: Cro-2F, 5′-AGYCCIGTIATGRGWGTIRTYGG-3′, and JJUVS-1233R, 5′-TCACCMAGRTGRAAGTGRTCIAC-3. The bat was captured during July 2012 in Xuan Son National Park, a nature reserve in Thanh Sơn District, Phu Tho Province, ≈100 km west of Hanoi (21°07′26.75′N, 104°57′29.98″E).

For confirmation, RNA extraction and RT-PCR were performed independently in a laboratory in which hantaviruses had never been handled. After initial detection, the L-segment sequence was extended by using another primer set (PHL-173F: 5′-GATWAAGCATGAYTGGTCTGA-3′; and TNL-5084R: 5′-GATCCTGAARTACAATGTGCTGG-3′). To calculate the number of virus copies in tissues by real-time RT-PCR, we used a virus-specific primer set (XSV-F: 5′-GTTGCACAGCTTGGTATTGG-3′; and XSV-R: 5′-TTAGCACCCAAACCTCCAAG-3′) and probe (XSV-Probe: 5′-ACAGCTCCTGGCATGGTAAATTCTCC-3′).

Pairwise alignment and comparison (with ClustalW, www.clustal.org) of a 4,582-nt (1,527 aa) region of the RNA-dependent RNA polymerase–encoding L segment indicated sequence similarities of 71.4%–71.5% and 75.9%–78.7% at the nucleotide and amino acid levels, respectively, between XSV and Mouyassué virus and MGBV. Sequence analysis of a 499-nt (166 aa) region of the nucleocapsid-encoding S segment showed that XSV differed by 42.8%–58.3% from representative hantaviruses harbored by rodents and most soricomorphs. XSV sequences were identical in lung, liver, kidney, and spleen; and the highest number of virus copies (7.6×10^1) was in lung tissue, determined by real-time RT-PCR. No additional hantavirus-infected Pomona roundleaf bats were found by RT-PCR that used XSV-specific primers.

Phylogenetic analyses was performed with maximum-likelihood and Bayesian methods, and we used the GTR+I+Γ model of evolution, as selected by the hierarchical likelihood-ratio test in MrModel-test version 2.3 and jModelTest version 0.1 (*10*), partitioned by codon position. Results indicated 4 distinct phylogroups, with XSV sharing a common ancestry with MGBV (Figure). Similar topologies, supported by high bootstrap (>70%) and posterior node (>0.70) probabilities, were consistently derived when various algorithms and different taxa and combinations of taxa were used. Moreover, as we reported previously, the incongruence between some hantaviruses and their reservoir hosts might be indicative of host-switching events (*5–7*).

The striking sequence divergence of XSV presented considerable challenges for designing suitable primers for RT-PCR and sequencing. Also, sequencing efforts were constrained by the limited availability of tissues and concurrent virus isolation attempts. Consequently, we were unable to obtain the full-length sequence of XSV. Similarly, the inability to detect hantavirus RNA in tissues from other species of bats in this study might be attributed to several factors, including the highly focal nature of hantavirus infection, small sample sizes of bats of any given species, primer mismatches, and suboptimal cycling conditions.

Bats of the genus *Hipposideros,* family Hipposideridae, are among the most speciose insectivorous bats; ≈70 species are distributed across Africa, Europe, Asia, and Australia. Pomona roundleaf bats are frequently found in or near limestone or sandstone caves. Their colony sizes vary from few to many hundreds of individuals. The vast geographic distribution of the Pomona roundleaf bat throughout

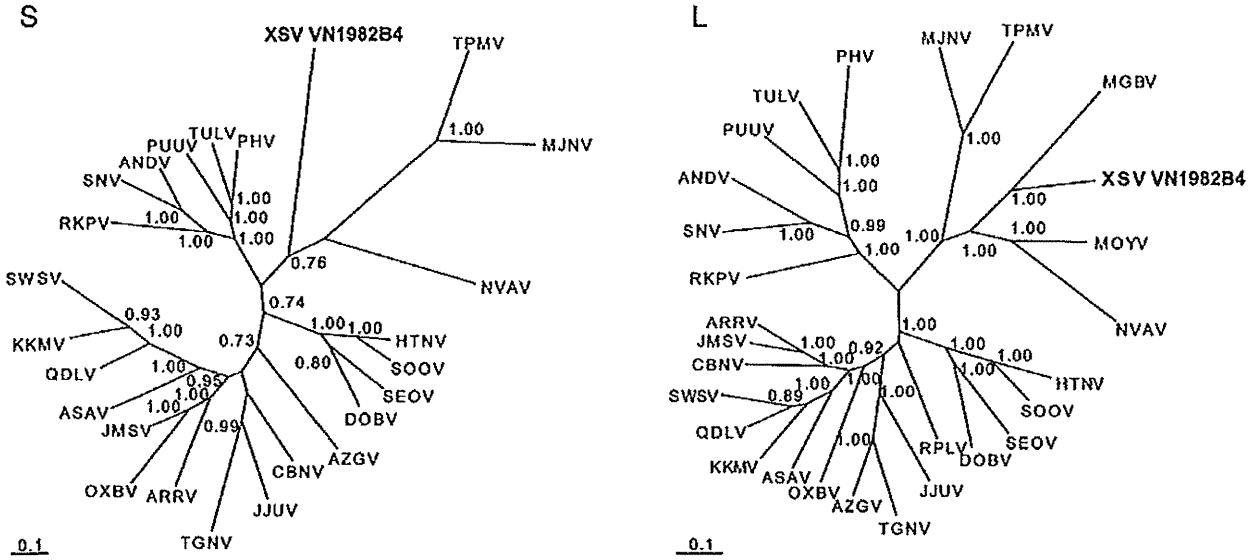

Figure. Phylogenetic trees, based on 499-nt and 4,582-nt regions of the small (S) and large (L) genomic segments, respectively, of Xuan Son virus (XSV VN1982B4) (GenBank accession nos. S: KC688335, L: JX912953), generated by the maximum-likelihood and Bayesian Markov chain Monte Carlo estimation methods, under the GTR+I+Γ model of evolution. Because tree topologies were similar when RAxML and MrBayes were used, the tree generated by MrBayes was displayed. The phylogenetic position of XSV is shown in relation to chiropteran-borne hantaviruses, Mouyassué virus ([MOYV] JQ287716) from the banana pipistrelle and Magboi virus ([MGBV] JN037851) from the hairy slit-faced bat. The taxonomic identity of the XSV-infected Pomona roundleaf bat was confirmed by mitochondrial DNA analysis (GenBank accession no. JX912954). The numbers at each node are Bayesian posterior probabilities (>0.7), and the scale bars indicate nucleotide substitutions per site. **Boldface** indicates the Xuan Son virus detected in Pomona roundleaf bat, Vietnam. Representative soricomorph-borne hantaviruses include Thottapalayam virus ([TPMV] AY526097, EU001330) from the Asian house shrew; Imjin virus ([MJNV] EF641804, EF641806) from the Ussuri white-toothed shrew; Jeju virus ([JJUV] HQ663933, HQ663935) from the Asian lesser white-toothed shrew; Tanganya virus ([TGNV] EF050455, EF050454) from the Therese's shrew; Azagny virus ([AZGV] JF276226, JF276228) from the West African pygmy shrew; Cao Bang virus ([CBNV] EF543524, EF543525) from the Chinese mole shrew; Ash River virus ([ARRV] EF650086, EF619961) from the masked shrew; Jemez Springs virus ([JMSV] FJ593499, FJ593501) from the dusky shrew; Seewis virus ([SWSV] EF636024, EF636026) from the Eurasian common shrew; Kenkeme virus ([KKMV] GQ306148, GQ306150) from the flat-skulled shrew; Qiandao Lake virus ([QDLV] GU566023, GU566021) from the stripe-backed shrew; Camp Ripley virus ([RPLV] EF540771) from the northern short-tailed shrew; Asama virus ([ASAV] EU929072, EU929078) from the Japanese shrew mole; Oxbow virus ([OXBV] FJ539166, FJ593497) from the American shrew mole; Rockport virus ([RKPV] HM015223, HM015221) from the eastern mole; and Nova virus ([NVAV] FJ539168, FJ593498) from the European common mole. Also shown are representative rodent-borne hantaviruses, including Hantaan virus ([HTNV] NC_005218, NC_005222), Soochong virus ([SOOV] AY675349, DQ562292), Dobrava-Belgrade virus ([DOBV] NC_005233, NC_005235), Seoul virus ([SEOV] NC_005236, NC_005238), Tula virus ([TULV] NC_005227, NC_005226), Puumala virus ([PUUV] NC_005224, NC_005225), Prospect Hill virus ([PHV] Z49098, EF646763), Andes virus ([ANDV] NC_003466, NC_003468), and Sin Nombre virus ([SNV] NC_005216, NC_005217).

Vietnam and in Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Nepal, and Thailand, provides opportunities to ascertain the genetic diversity and phylogeography of XSV and XSV-related hantaviruses. In this regard, although hantavirus RNA was not detected in archival tissues from bats of ≈20 genera, including several other *Hipposideros* species (*8*,*9*), many more genetically divergent hantavirus species are probably harbored by insectivorous bats. Not all orphan viruses warrant intensive study at the time of their discovery. However, insights into the ecology and transmission dynamics of newfound bat-borne hantaviruses might prepare us to more rapidly diagnose future outbreaks caused by emerging hantaviruses.

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Possible Cause of Liver Failure in Patient with Dengue Shock Syndrome

To the Editor: We report a rare hepatic ultrasonograph finding for a patient with liver failure associated with dengue virus (DENV) infection. This finding might shed light on the pathogenesis of liver involvement in this disease.

In March 2006, a 10-year-old previously healthy boy was hospitalized for a 3-day history of fever, headache, and nausea/vomiting. Fever subsided on the day of admission, but the patient was in shock (blood pressure 80/40 mm Hg) and had gastrointestinal bleeding and hematuria. Physical examination showed an obese, confused patient with generalized petechiae and hepatomegaly. The initial diagnosis was dengue shock syndrome (DSS). The patient was intubated and received intravenous fluid infusion, packed red blood cells, ceftriaxone, sodium bicarbonate, and ranitidine before being transferred to King Chulalongkorn Memorial Hospital in Bangkok. The patient's blood pressure increased to 130/90 mm Hg after the initial fluid resuscitation (28 mL/kg free flow), and systolic pressure remained at ≈130 mm Hg until transfer.

Laboratory examinations found 14,930 leukocytes/mm^3, hemoglobin 16.4 g/dL, hematocrit 48.2%, platelet 18,000/mm^3, blood urea nitrogen 33 mg/dL, creatinine 1 mg/dL, sodium 128 mEq/L, potassium 6.2 mEq/L, chloride 91 mEq/L, total CO_2 5 mEq/L, total bilirubin 6.9 mg/dL, direct bilirubin 3.9 mg/dL, aspartate transaminase 3,507 IU/L, alanine transaminase 2,775 IU/L, prothrombin time 43 seconds (international normalized ratio 3.4), and partial thromboplastin time 93.5 s (control 28.7 s). Blood and urine cultures showed negative results. Serum was positive for IgM against DENV. Unfortunately, we did not investigate other viral causes of liver failure.

DSS with liver failure was diagnosed and treated with intravenous fluid, sodium bicarbonate, omeprazole, fresh frozen plasma, platelet transfusion, vitamin K, and recombinant factor VIIa concentrate (NovoSeven; Novo Nordisk, Bagsvaerd, Denmark). Despite stable blood pressure over the next 6 days, liver enzymes continued to rise with progressive jaundice (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/7/12-1820-Techapp1.pdf). Hepatic ultrasonograph on the second

平成２５年度厚生労働科学研究費補助金
新型インフルエンザ等新興・再興感染症研究事業

我が国における一類感染症の患者発生時に備えた
診断・治療・予防等の臨床的対応及び積極的疫学調査に関する研究

第３回

一類感染症ワークショップ

会期：平成２５年７月２７日(土)・２８日(日)

会場：独立行政法人国立国際医療研究センター

東京都新宿区戸山１−２１−１

平成25年度厚生労働科学研究費補助金新型インフルエンザ等新興・再興感染症研究事業
我が国における一類感染症の患者発生時に備えた診断・治療・予防等の臨床的対応
及び積極的疫学調査に関する研究

第3回　一類感染症ワークショップ

【目的】一類感染症（とくにウイルス性出血熱）の患者発生時に、職業感染のリスクを最低限に抑えつつ、診療やケアを遂行するための基本的な知識とスキルを習得する

【対象】第一種感染症指定医療機関において一類感染症の実地診療に関わると予想される医師、看護師

【日時】平成25年7月27日（土曜日）～ 7月28日(日曜日)

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