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Review Article

Animal models for some important RNA viruses of public health concern in SEARO countries: Viral hemorrhagic fever

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ABSTRACT

Viral hemorrhagic fevers (VHFs) are major public health problems in the South-East Asia Regional (SEAR) countries. VHFs are a group of illnesses; that are caused by four families of viruses, *viz. Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. All VHFs have common features: they affect several organs and damage the blood vessels. These symptoms are often accompanied by hemorrhage. To understand pathogenesis, genetic and environmental influence that increase the risk of VHFs, efficacy and safety studies on candidate vaccines and testing of various therapeutic agents, appropriate animal models are essential tools in public and animals health. In the current review, the suitable animal models for *Flavivirus* [Dengue hemorhagic fever (DHF), Kyasanur forest disease (KFD)]; *Bunyavirus* [Crimean-Congo hemorrhagic fever (CCHF), Hantavirus fever (HF)]; and *Paramyxovirus* [Nipah virus fever (NiV)] have been reviewed with specific emphasis on emerging and re-emerging viruses in SEAR countries.

Key words Animal models; Crimean-Congo hemorrhagic fever; dengue haemorrhagic fever; Hantavirus fever; Kyasanur forest disease; Nipah virus infection; viral hemorrhagic fever

INTRODUCTION

Viral hemorrhagic fever (VHF) is caused by RNA viruses belonging to families: *Flaviviridae*, *Bunyaviridae, Filoviridae* and *Arenaviridae*[1]. VHF is mild to severe and a life threatening disease in humans caused by exposure to infected animal or vector reservoir host. In the past decade, there have been sporadic outbreaks of many emerging and remerging zoonotic viral diseases in the South-East Asia Regional (SEAR) countries (Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, and Timor-Leste)[2].

Severity and clinical presentation of VHFs may significantly change according to several factors related to the causative agent, host factors and epidemiological features. Commonly, patients with VHFs exhibit fever and coagulation abnormalities that may progress towards disseminated intravascular coagulation, multiorgan failure and shock eventually leading to death[3]. It is difficult to conduct human clinical trials for therapeutic/prophylactic drugs or vaccines because of inadequate intervention strategies and relative rarity and volatility of VHF outbreaks. Therefore, animal models that can recapitulate human disease are essential for the development of effective antivirals and vaccines. Ideally, the spectrum of disease in an animal model should resemble that of the human. Reservoir hosts are generally not suitable animal models since they do not respond to their respective viral infections in a manner analogous to the human response. VHF animal model should be based on the primary infection of macrophages, monocytes and viral dissemination to other organs and tissues. Most types of VHFs are associated with significant liver disease which likely originates either from circulatory failure or through the spread of infection via the blood stream to Kupffer cells with subsequent spill-over into parenchymal cells[4].

All VHFs are major public health problems in SEAR countries which include dengue hemorrhagic fever (DHF), Kyasanur forest disease (KFD), Crimean-Congo hemorrhagic fever (CCHF), Hantavirus hemorrhagic fever (HHF) and Nipah virus (NiV) disease (Table 1). Significant efforts have been made to develop animal models to characterize disease progression, determine correlates of protection and to screen therapeutics and vaccines. The aim of the present article is to review currently available animal models for hemorrhagic fevers caused by viruses of immense public health importance in the SEAR countries.

Animal models for Flaviviral hemorrhagic fever

Dengue hemorrhagic fever (DHF): Dengue is the

Table 1. Geographical distribution of dengue hemorrhagic fever, Kyasanur forest disease, Crimean-Congo hemorrhagic fever, Hantavirus fever and Nipah virus fever in South-East Asia Regional (SEAR) countries

S. No.	SEAR countries	Viral hemorrhagic fever
1.	India	Dengue hemorrhagic fever, Kyasanur forest disease, Crimean-Congo hemorrhagic fever, Hantavirus fever, Nipah virus fever
2.	Bangladesh	Dengue hemorrhagic fever, Nipah virus fever
3.	Bhutan	Dengue hemorrhagic fever
4.	Nepal	Dengue hemorrhagic fever
5.	Indonesia	Dengue hemorrhagic fever, Hantavirus fever
6.	Maldives	Dengue hemorrhagic fever
7.	Sri Lanka	Dengue hemorrhagic fever, Hantavirus fever
8.	Myanmar	Dengue hemorrhagic fever
9.	Thailand	Dengue hemorrhagic fever, Hantavirus fever
10.	Timor-Leste	Dengue hemorrhagic fever
11.	Democratic People's Republic of Korea	Hantavirus fever

most prevalent arthropod-borne viral illness in humans and > 50 million cases of dengue are estimated each year. Dengue virus (DENV) is a member of the *Flaviviridae* family. DHF is a self-limiting, though incapacitating, febrile illness accompanied by retro-orbital pain, headache, skin rash, bone and muscle pain. In case of DHF, hemorrhagic manifestations, low platelet count and signs of vascular leakage, such as increased hematocrit level or pleural effusion have been reported[5]. Carefully controlled experiments performed in relevant animal models are needed to explore the dynamics of hematological dysfunction and other factors potentially involved in dengue disease.

Macaque model was developed by Halstead *et al*[6] by subcutaneous inoculation of DENV in rhesus monkeys. Infected macaques revealed low platelet count. Similarly, viremia-post subcutaneous (s.c.) inoculation in rhesus monkeys with wild type dengue 1, 2 and 3 was demonstrated in another study[7]. Further, Martin *et al*[8] demonstrated viremia and antibody responses in green monkeys (*Chlorocebus aethiops sabaeus*) inoculated with DENV making potential model for evaluation of novel candidates for dengue vaccines. Infection with DENV in marmosets revealed clinical signs of disease and changes in hematological and biochemical parameters[9]. DENV inoculated by intravenous (i.v.) route in *Rhesus macaques* produced hemorrhagic and coagulopathy signs, reminiscent of hemorrhagic manifestations seen in humans, making them potentially useful for pre-clinical testing of therapeutic interventions specifically targeting DENV associated coagulopathy[10]. The role of dengue virus (DV) specific cell-mediated responses in non-human primate (NHP) models has received relatively less attention, although some researchers reported recognition of non-structural proteins in addition to viral structural components by both CD4+ and CD8+ T-cells[11]. However, such responses have been difficult to detect in DNA vaccine immunized monkeys, even in those that show protection from challenge[12]. DV infection of monkeys elicits a vigorous innate response leading to activation and marked shifts in circulating subsets of T, NK, and NK-T cells in the marmoset model[13].

Dengue disease has been extensively studied in murine models. Host genetic factors and immune components influencing the susceptibility have been studied in various inbred and immunocompromised genetically modified mouse strains respectively. Besides that, viral factors were also explored in the form of attempts to adapt the virus in mouse or testing the susceptibility of different virus strains isolated from patients and mosquitoes. Inter-strain variation in manifestation of symptoms in mice has been observed depicting influence of host genetic factors on susceptibility to DENV2[14]. Studies on severe combined immunodeficient (SCID) mice reconstituted with human cells represent the most susceptible model to DENV infection. Wu *et al*[15] reported that engrafting SCID mice with human cells as targets for DENV infection, yields limited success partly because of low levels of human engraftment. The involvement of liver cells in the pathogenesis of DENV infection has been indicated by abnormal liver function, pathological findings and detection of viral antigen in hepatocytes and Kupffer cells. DENV could replicate in a human hepatocarcinoma cell line (HepG2) and infectious particles were released into the culture medium. Therefore, An *et al*[16] established an animal model for DV infection using SCID mice transplanted with a HepG2. At 7–8 wk post-transplantation with HepG2, grafted mice infected intraperitoneally with DEN2 produced clinical symptoms like thrombocytopenia, prolonged partial thromboplastin time; and increased hematocrit, blood urea nitrogen; and tumor necrosis factor-α (TNF-α) were also observed in the paralyzed mice. This model is suitable for the pathogenesis of dengue virus diseases[16]. Experimental infection of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice xenografted with human CD34+ cells led to clinical

signs of DF as seen in humans (fever, rash, and thrombocytopenia)[17–18]. Infection of humanized NOD-SCID IL2R gamma (null) mice with different strains (representing the four genotypes) of DEN2 virus could induce the development of disease similar to that observed in patients. This attribute makes this mouse model ideal for the study of dengue pathogenesis and the evaluation of antivirals and virus attenuation[19].

Significant development in dengue model research could be attributed to susceptibility of AG129 mice to DEN (lacking α/β-IFN and γ-IFN receptor genes). Intraperitoneal administration of mouse-adapted DEN2 virus was uniformly lethal in AG129 mice (which lacks α/β-IFN and γ-IFN receptor genes)[20]. Besides, the use of genetically modified mouse strains, attempts to adapt virus in mouse were successful. Generation of a mouse-adapted DENV strain enabled development of a mouse model that manifested signs of severe dengue reminiscent of human disease, including vascular leak. Thus, this model of systemic dengue disease may constitute a more satisfactory approach for testing vaccine efficacy[21].

AG129 mice, though partially immunocompromised, develop a broadly cross-reactive and long-lasting antibody response to DENV. Sequential DENV infection in this mouse model resulted in decreased viral load of the subsequently infecting serotype[22]. AG129 mice infected with DENV had high levels of circulating NS1 and anti-NS1 antibodies, making it a good model to study the role of NS1 and anti-NS1 antibodies *in vivo*[23]. AG129 mouse model is one of the models that permits infection by all four serotypes of DENV, supports replication in relevant cell and tissue types comparable to human infection and allows antibody-mediated protection and enhancement of DENV infection[24]. The AG129 model has proven to be useful in the testing of a live-attenuated vaccine and in testing the immune response to non-structural proteins in a chimeric vaccine[25]. DENV infection of AG129 mice reproduces key features of human disease. Mice depleted of T-cells developed signs of disease, but recovered after secondary DENV infection. Overall, protective cross-reactive antibodies are secreted by both long-lived plasma cells and memory B-cells, and both cross-reactive B-cells and T-cells provide protection against a secondary heterotypic DENV infection[26–27]. The attribute of differential virulence of virus strains isolated from mosquitoes and patients was explored in various inbred strains of mice in an attempt to develop models for different clinical features of DENV infection. Nonmouse-adapted DEN2 virus strain (Accession number D2Y98P) is highly infectious in AG129 mice. Infection with a high dose of D2Y98P induced cytokine storm, massive organ damage and severe vascular leakage leading to hemorrhage and rapid death of the animals at the peak of viremia. Infection with D2Y98P provides a new platform for testing of drugs and vaccines[28].

A/J strain, Balb/c or B6 mice inoculated with DEN2 virus displayed thrombocytopenia and anti-platelet antibody. These mouse models could be effectively used to study the pathogenicity of dengue virus[29]. MON501 is a dengue strain found to be neurovirulent in mice. Two genomic-length cDNA clones (TB62 and TB203) were constructed by point mutation of pDVWS501 (genomic-length cDNA clone of DEN2 virus) wherein, Lys at position 62 was mutated to Glu and Asn at position 203 mutated to Asp. The properties of these mutants demonstrated that E62 and E203 are determinants of suckling mice neurovirulence[30–32].

Balb/c mice infected with dengue virus serotype 2 (non-neuroadapted), by intraperitoneal and intravenous routes provide useful information of morphological aspects of dengue virus infection[33–34]. An inbred mouse model in which mice develop signs of human DENV-induced disease is needed to be investigated for protection or pathogenesis of DENV infections, and to test the efficacy of DENV vaccines and antiviral[35]. Balb/c mouse model developed by Paes *et al*[36] revealed DENV-specific pathogenesis, *viz*. liver damage, hyperplasia in Kupffer cells, increased white blood cell (WBC) counts, thrombocytopenia and an increase in hematocrit level reminiscent of vascular leak viremia[36–38].

Shresta *et al*[21], developed a murine model relevant to DHF. A novel DEN strain, D2S10, was generated by alternately passaging a non-mouse-adapted DEN strain between mosquito cells and mice, thereby mimicking the natural transmission cycle of the virus between mosquitoes and humans. In this model, mice infected with D2S10 showed increased serum level of tumor necrosis factor α (TNF-α); one of the key mediators of severe DEN induced disease *in vivo*. This model provides mechanistic insights into DEN-induced disease[21]. Orozco *et al*[39] developed DENV2 strain (D220) by 10 additional cycles of subcutaneous inoculation of mice with supernatant from infected mosquito cells.

AG129/Pas mice lacking interferon α/β or γ receptors were inoculated subcutaneously with DENV. Initial cellular tropism of DENV in these mice was similar to that reported in humans[40]. In dengue tropism mice model, DENV2 non-structural protein 3 (NS-3) supports roles for infected phagocytes, hepatocytes and endothelial cells in the pathogenesis of severe dengue[41]. However, the majority of responses were derived from the highly conserved non-structural proteins NS-3 and NS-5. This novel

murine model can be used for investigation of T cell-mediated immune mechanisms relevant to vaccine design[42].

Immunocompetent mice inoculated intra-dermally with DENV2 strain 16681 develop hemorrhage locally or systemically. Intra-dermally DENV2 strain 16681 injected in immunocompetent C57BL/6 mice leads to endothelium damage and hemorrhage development[43-45]. Intraperitoneal inoculation of DENV1 in immunocompetent C57BL/6 mice presented signs of dengue disease, *viz.* thrombocytopenia, spleen hemorrhage, liver damage and increase in production of IFN-γ and TNF-α cytokines[46].

Using mice deficient in iNOS, phox47 and apocynin; reactive nitrogen species (RNS) and reactive oxygen species (ROS) are important for hemorrhage development after infection by DENV. This mouse model offers the opportunity to test potential dengue vaccines and therapeutics to treat dengue hemorrhage and to test hemorrhage induction potentials of dengue viral strains[43].

Swiss albino mice were immunized with a single dose of infective DEN2 virus and different markers of both branches of the induced adaptive immunity. Immunized mice were also inoculated with the depleting monoclonal antibodies anti-CD4 or anti-CD8. Only depletion with anti-CD8 decreased the level of protection to 50% when compared to that reached in the non-depleted mice. The mouse model contributed to understand the role of cellular immune response in protection against dengue virus[47].

The virulence of DENV3 isolates in a mouse model was assessed by intracerebral (i.c.) inoculation with genotypes I and III. DENV3 has the propensity to cause neurological disease in mice while the genotype III is associated with asymptomatic infection in mice. This mouse model is a way to study the biology of DENV3 isolates and neurovirulence of the different genotypes of DENV[48]. Mice inoculated with DENV-specific antibodies can sufficiently increase severity of disease so that a mostly non-lethal illness becomes a fatal disease resembling human DHF[49–50].

Mota and Rico-Hesse[51] have developed a new model of DF in immuno-deficient mice transplanted with human stem cells from umbilical cord blood. These mice inoculated with DENV2 showed similar signs of dengue disease as found in humans (fever, viremia, erythema and thrombocytopenia). This was the first valid and relevant model for studying dengue fever pathogenesis in humans. DENV are transmitted to humans by the bite of *Aedes aegypti* or *Aedes albopictus* (Diptera: Culicidae) mosquitoes. Humanized mouse model of DEN; mice transplanted with human hematopoietic stem cells, produced signs of DENV disease. This was the first animal model useful for evaluation of human immunity to DENV infection after mosquito inoculation[52].

In DENV3 inoculated IFN-γ (-/-) mice, enhanced lethality preceded by severe disease manifestation and virus replication was seen. Lack of IFN-γ production was associated with diminished nitric oxide-synthase 2 (NOS2) expressions and higher susceptibility of NOS2 (-/-) mice to DENV3 infection[53]. Anxiety-like behaviour and expression of pro-inflammatory cytokines and pro-apoptotic caspase-3 intra-cranially was seen in C57BL/6 mice inoculated with DENV3. This model produced anxiety-like behaviour, hippocampal inflammation and neuronal apoptosis associated with DENV3 infection in the central nervous system[54].

Dengue virus strain (D2Y98P) upon intraperitoneal (i.p.) or subcutaneous (s.c.) administration in immunocompromised mice increased vascular permeability, intestine damage, liver dysfunction, transient lymphopenia, which are the hallmarks of severe DEN in patients. This novel mouse model of DEN associated vascular leakage will contribute to a better understanding of DEN pathogenesis and represents a relevant platform for testing novel therapeutic treatments and interventions[55–56]. Advantages and disadvantages of animal models for dengue are shown in Table 2.

Kyasanur forest disease (KFD)

Kyasanur forest disease virus (KFDV) was first isolated during an outbreak in 1957 in the Kyasanur forest area of the Shimoga district in Karnataka state of India[57]. Serological evidence of KFDV was found in the Andaman Island, during 1988–89 indicating the presence of KFDV or closely related virus in the area distant from the endemic foci[58]. Since 1950s, KFDV or closely related viruses could be present in other parts of India, such as parts of Kutch district in Gujarat state and forested regions of West Kolkata in West Bengal state[59–60]. KFDV causes severe to fatal disease in primates of species *Macaca radiata* and *Presbytis entellus*, often associated with the onset of outbreak in humans. Infection in primates primarily involves gastrointestinal and lymphoid tissues[61]. In other nonhuman primates (monkey) infected with KFDV, encephalitic lesions have been observed[62].

Suckling mice are commonly used for isolation and propagation of virus by intracerebral inoculation of serum or tissue suspensions from wild primates or patients[58]. Sub-adult Swiss albino mice (weanling, 21 days old) infected with KFDV via subcutaneous (s.c.) or intraperitoneal (i.p.) routes also developed the disease[63–64]. Further, studies using Swiss albino mice revealed that 2–3 days

Table 2. Advantage and disadvantages of animal models for dengue

Animal models for dengue		Advantages	Disadvantages
NHP	Route of inoculation : s.c.	Low platelet counts, viremia and neutralizing antibody response	Low level of replication restricted to lymphoid tissues; No overt clinical disease
Mice	Route of inoculation: i.v.	Typical clinical features; hemorrhages and coagulopathy	Not natural route of infection
	SCID	Possibility of studying the human immune response *in vivo*; useful model to study the role of cross-reactive T-cells in sequential infections.	These mice are unable to produce the innate immune response; Not relevant for tropism and pathogenesis studies
	AG129	AG129 mice were a promising small animal model for DEN virus vaccine trials.	
	Balb/c	The activation of innate immune response is at least partially responsible for mortality in DEN2 virus infection, and in line with this concept, anti-TNF treatment significantly reduces the mortality rates. Therefore, inbred 4-wk old Balb/c mice are useful models to study the immune activation in host during DEN2 virus infection.	

old mice injected by i.p. route succumbed to disease in 4–6 days, while 3–4 wk old mice died in 5–7 days. Unlike humans and non-human primates, adult mice infected i.c. or i.p. developed histological signs of encephalitis[65]. Suckling (2–3 days old) and weanling Swiss albino mice succumb to infection, 5–7 days post-inoculation by i.p. route. However, adult mice infected by i.c. or i.p. route develop histological signs of encephalitis without mortality. Therefore, though not ideal, this model has been used to assess KFD vaccine efficacy. Infected mice of any age do not show histological abnormalities in the liver or spleen[65]. Some wild-caught rodents may be potential natural reservoirs for the virus and might not develop disease[66].

Contrary to mouse studies, necropsy of *Macaca radiata* (bonnet macaques) infected with KFDV demonstrated KFD virus-specific gastrointestinal and lymphoid lesions. Viral antigens were found in small and large intestine, spleen and lymph nodes. Thus, *M. radiata* seems to be an excellent model to study human disease caused by KFD virus similar to infected patients[64].

Animal models for Bunyaviral hemorrhagic fever

Crimean-Congo hemorrhagic fever (CCHF): CCHF is a notifiable disease to the World Organization for Animal Health and the WHO and notification is not related to the consequences of its spread within the animal population, but rather to the risk posed by zoonotic potential[67]. CCHF is an acute, highly contagious and life-threatening disease. The virus belongs to the genus *Nairovirus* in the *Bunyaviridae* family. CCHF was described by a physician in Tajikistan in 1100 AD in a patient with hemorrhagic manifestations. Crimean hemorrhagic fever (CHF) was first described as a clinical entity in 1944–1945 when about 200 Soviet military personnel were infected in devastated Crimea after Nazi invasion[68]. Later, it became evident that the causative agent was identical to a virus isolated from a patient in Congo in 1956 and the name Crimean-Congo hemorrhagic fever (CCHF) was adopted[69]. Recently, outbreaks have been reported from India[70–71]. CCHF virus (CCHFV) circulates in nature in a tick-vertebrate-tick cycle, mainly cattle, sheep, goats and hares. The virus is transmitted to humans primarily by ticks of the genus *Hyalomma*. CCHF progresses rapidly with high fever, malaise, severe headache and gastrointestinal symptoms[69–70].

Mouse model, deficient in the STAT-1 signaling was found to be highly susceptible to infection with mortality within 3 to 5 days. After CCHFV inoculation, mice exhibited fever, leukopenia, thrombocytopenia and highly elevated liver enzymes. Rapid viremic dissemination and extensive replication in visceral organs (mainly in liver and spleen), increased pro-inflammatory cytokines in the blood, delayed immune cell activation and intensive lymphocyte depletion were observed in the mouse model. Hence, infection study in this model offers an in-depth *in vivo* analysis of CCHFV pathophysiology[72–73]. Varying levels of liver lesions are observed in CCHFV infected

patients[74]. The presence of virus in the spleen of patients is also consistent with CCHFV infected IFN-α/βR-/- mice[75]. Adult mice missing the type I interferon (IFN) receptor (IFNAR) were found susceptible to CCHFV and developed an acute disease with fatal outcome[76].

Hantaviral hemorrhagic fever

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are rodent-borne viruses. They are the causative agents for hemorrhagic fever. A mild form of HFRS (HF with renal syndrome) called nephropathia epidemica (NE) is caused by *Puumala virus* (PUUV)[77]. The term HF with renal syndrome (HFRS) is commonly used when referring to such diseases caused primarily by Hantaan (HTNV), Seoul (SEOV), Dobrava (DOBV) and Puumala (PUUV) viruses. HFRS is predominantly a Eurasian disease that varies in severity, with HTNV and DOBV infections being the most lethal and PUUV infections having the lowest mortality rates[78–79]. Old World hantaviruses cause a form of HF that is characterized by clinically significant kidney disease as well as other more variable disease signs and symptoms[80].

A parallel to the human diseases has been observed in experimental infection of hamsters with Andes virus (ANDV). This South American hantavirus, was found to be highly lethal in adult Syrian hamsters. The characteristics of the disease in hamsters closely paralleled to that observed in patients. Those included the incubation period, symptoms of rapidly progressing respiratory distress, pathological changes in lungs, in the form of pulmonary edema and pleural effusion in humans[81]. In addition to the uniform lethality of the ANDV model that makes it amenable to drug and vaccine efficacy studies, many similarities to human HPS exist including short time to death following the onset of symptoms, laboured breathing, pleural effusion, pathology of the liver and spleen, and hypotension[78, 81]. Capillary leakage is central to hantaviral diseases. *Cynomolgus macaques* infected with wild-type *Puumala hantavirus* produced viral RNA, nucleocapsid protein in kidney, spleen and liver tissues. Inflammatory cell infiltrations and tubular damage has been seen in the kidneys. Thus, this model is reliable to study hantavirus infection[76].

Animal models for Nipah virus disease

Nipah virus (NiV) infection is an emerging infectious disease of public health importance in the South-East Asia Region. NiV is enveloped, negative-sense, single-stranded RNA virus in the family *Paramyxoviridae*, genus *Henipavirus*. The name of the virus and disease caused by it is derived from the village name "Sungai Nipah" in Malaysia, where the first human case was detected. First Nipah disease outbreak occurred in Malaysia and Singapore in 1998 and 1999 respectively[82]. Subsequent outbreaks occurred in Bangladesh during winter seasons in 2001, 2003 and 2004[83–86]. During January and February 2001, an outbreak of febrile illness associated with altered sensorium occurred in Siliguri, West Bengal, India[87].

Although, small percentage of Nipah cases were found to be asymptomatic or presented mild disease most diagnosed clinical cases present with acute neurological signs. The initial symptoms are flu-like with high fever, headache and myalgia. In patients who develop encephalitis, the symptoms may include drowsiness, disorientation, convulsions and/or coma. Nausea and vomiting can also be seen. Less often, patients develop respiratory signs, which may include acute respiratory distress syndrome. Septicemia, bleeding from the gastrointestinal tract, renal impairment and other complications can occur in severely ill patients[88].

Wong *et al*[89], reported golden hamster (*Mesocricetus auratus*) model that appears to reproduce the pathology and pathogenesis of acute human Nipah infection. Hamsters infected by intranasal or intraperitoneal routes with Nipah virus died within 9 to 29 days or 5 to 9 days, respectively. Pathological lesions were most severe and extensive in the hamster brain. Vasculitis, thrombosis, multinucleated endothelial syncytia were found in blood vessels of multiple organs. Viral antigen and RNA were localized in both vascular and extravascular tissues[89]. NiV initially replicated in the upper respiratory tract epithelium. The development of neurological signs coincided with disruption of the blood brain barrier (BBB) and expression of tumor necrosis alpha (TNF-α) and interleukin 1β (IL-1β). In addition, interferon-inducible protein 10 (IP-10) was identified as playing an important role in NiV pathogenesis. This model provided novel information on the development and progressions of NiV clinical disease and identifies specific cytokines and chemokines that serve as important targets for treatment[90]. The antiviral efficacy of ribavirin and 6-aza-uridine were tested in hamsters infected with a lethal dose of Nipah virus. The activity of these small-molecule inhibitors was compared with that of the interferon inducer poly(I)-poly(C12U). Poly(I)-poly(C(12)U), (3 mg/kg of body weight) dose daily from the day of infection to 10 days post-infection, prevented mortality in 5 of 6 infected animals[91]. The combination of chloroquine with ribavirin treatment of NiV infection in golden hamster model showed antiviral activity. Ribavirin delayed death from viral disease in NiV-infected hamsters by approximately five days[92]. Nipah

virus glycoproteins (G and F) when expressed as vaccinia virus recombinants induced an immune response in hamsters which protected against a lethal challenge by Nipah virus[93]. NiV infected Syrian hamsters had accelerated virus replication, pathology and death compared to NiV infected animals. This model can be used to study NiV-pathogenesis, transmission and counter measures that could be used to control outbreaks[94].

In ferret model, a cross-reactive neutralizing human monoclonal antibody; m102.4, targeting the henipavirus G-glycoprotein was evaluated *in vivo* as a potential therapeutic agent. All ferrets that received m102.4 10 h following a high dose oral-nasal Nipah virus challenge were protected from disease while all controls died. This study was the first successful post-exposure passive antibody therapy for Nipah virus using a human monoclonal antibody[95].

Guinea pigs intraperitoneally inoculated with NiV produced a disease with considerable resemblance to the disease in humans but with reduced pulmonary involvement and marked infection of urinary bladder and the female reproductive tract[96].

Two groups of two adult cats each were inoculated subcutaneously with either 500 or 5000 tissue culture infective dose(s) ($TCID_{50}$) of NiV. Inoculated cats with both doses developed clinical disease 6 to 9 days post-infection. These results indicated that the cat provides a consistent model for acute NiV infection and associated pathogenesis[97].

Infection of squirrel monkeys by i.v. injection was followed by high death rates associated with acute neurologic and respiratory illness, and viral RNA and antigen production[98]. African green monkey (AGM) model provided reliable platform for evaluation of either passive and active immunization or therapeutic strategies for human use[99].

CONCLUSION

Many of hemorrhagic fever causing viruses are considered as potential bioweapons owing to their aerosolization capacity. No effective vaccine and specific antivirals are available to combat these infections. Animal models provide important tools for the study of *in vivo* viral replication and pathogenesis modulated by the host immunity. Animal experiments can afford us solid scientific basis for exploration of antiviral drugs and vaccines development.

In this review, we have discussed the contributions of animal models made in the study of DHF, KFD, CCHF, HHF and NiV host range and pathogenesis. It is hypothesized that the close genetic relationship between primates and humans, and the presence of a comparable immune responses make NHPs the best models for studying dengue virus. AG129 mice are highly susceptible to dengue, replicate virus to high titers and display vascular leakage. The NOD/SCID/IL-2Rγ mice reconstituted with human CD34+ cells are infrequently used but have the greatest potential as future mouse models. In KFD, non-human primate model (Bonnet macaque) is more accurate model with human disease; while rodent (mice) model do not replicate with human disease. Adult mice, rats, hamsters, guinea pigs, rabbits, cattle, sheep, goats, horses and NHPs are susceptible to CCHFV infection but do not develop sign of disease[100]. CCHF disease progression in these animals differs from human CCHF, which limits the use of these models to study CCHF disease progression. In HHF disease; adult Syrian hamster exhibit disease similar to that observed in HHF patients. Unlike the mice, hamster, cat squirrel and monkey models of NiV infection; severe respiratory pathology, neurological disease and generalized vasculitis could be manifested in NiV infected African green monkey (AGM). These attributes make AGM an accurate model for NiV infection. Goals of experimental virology research are not only to include improvement of existing screening methods but also development of new screening methods for antivirals and vaccines. We are anticipating that research on animal models of viral hemorrhagic fever will continue to provide insights into the pathology of pathogenesis, genetic and environmental influence.

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