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# Animal Models of Human Viral Diseases

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## INTRODUCTION

Well-developed animal models are necessary to understand the disease progression, pathogenesis, and immunologic responses in humans. Furthermore, to test vaccines and medical countermeasures, well-developed animal models are essential for preclinical studies.

Ideally, an animal model of human viral infection should mimic the host–pathogen interactions and the disease progression that is seen in the natural disease. A good animal model of viral infection should allow many parameters of infection to be assayed, including clinical signs, growth of virus, clinicopathological parameters, cellular and humoral immune responses, and virus–host interactions. Furthermore, viral replication should be accompanied by measurable clinical manifestations, and pathology should resemble that of human cases such that a better understanding of the disease process in humans is attained. There is often more than one animal model that closely represents human disease for a given pathogen. Small animal models are typically used for first-line screening, and for initially testing the efficacy of vaccines or therapeutics. In contrast, nonhuman primate (NHP) models are often used for the pivotal preclinical studies. This approach is also used for basic pathogenesis studies, with most experiments performed in small animal models when possible, and NHPs only used to fill in remaining gaps in knowledge.

The advantages of using mice to develop animal models are low cost, low genetic variability in inbred strains, and abundant molecular biological and immunological reagents. Specific pathogen-free (SPF), transgenic, and knockout mice are also available. A major pitfall of mouse models is that the pathogenesis and protection afforded by vaccines and therapeutics cannot always be extrapolated. Additionally, blood volumes for sampling are limited in small animals, and viruses often need to be adapted through serial passage in the species to induce a productive infection.

The ferret's airways are anatomically and histologically similar to that of humans, and their size enables larger or more frequent blood samples to be collected, making them an ideal model for certain respiratory pathogens. Ferrets are outbred, with no standardized breeds or strains; thus, greater numbers are required in studies to achieve statistical significance and overcome the resulting variable responses. Additionally, SPF and transgenic animals are not available, and molecular biological reagents are lacking. Other caveats making ferret models more difficult to work with are their requirement for more space than mice (rabbit-style cages), and the development of aggressive behavior with repeated procedures.

NHPs are genetically the closest species to humans; thus, disease progression and host–pathogen responses to viral infections are often the most similar to that of humans. However, ethical concerns of experimentation on NHPs, along with the high cost and lack of SPF NHPs raise barriers for such studies. NHP studies should be carefully designed to ensure that the least amount of animals are used, and the studies should address the most critical questions regarding disease pathogenesis, host–pathogen responses, and protective efficacy of vaccines and therapeutics.

Well-designed experiments should carefully evaluate the choice of animal, including the strain, sex, and age. Furthermore, route of exposure and the dose should be as close as possible to the route of exposure and dose of human disease. The endpoint for these studies is also an important criterion. Depending on the desired outcome, the model system should emulate the host responses in humans when infected with the same pathogen.

In summary, small animal models are helpful for the initial screening of vaccines and therapeutics, and are also often beneficial in obtaining a basic understanding of the disease. NHP models should be used for a more detailed characterization of pathogenesis and for pivotal preclinical testing studies. Ultimately, an ideal animal model may not be available. In this case, a combination of different well-characterized animal models should be considered to understand the disease progression and to test medical countermeasures against the disease.

In this chapter, we will be reviewing the animal models for representative members of numerous virus families causing human diseases. We will focus on the viruses for each family that are the greatest concern for public health worldwide.

## PICORNAVIRIDAE

### Poliovirus

Poliovirus (PV) is an enterovirus in the picornavirus family and causes poliomyelitis.<sup>1</sup> Humans are the only natural host for the virus, but a number of NHP species are also susceptible. All three serotypes of PV cause paralytic disease, but it is relatively rare with only 1–2% of infected individuals ultimately developing paralysis. Humans typically acquire and transmit the virus by the oral–fecal route, although transmission by aerosol droplets may also be possible.<sup>2</sup> The virus replicates in the oropharyngeal and intestinal mucosa, made possible by the resistance of PV to stomach acids.<sup>3</sup> CD155 expression in Peyer's patches and M cells suggest that these cell types may be important during initiation of infection.<sup>4</sup> Replication at extraneural sites

precedes invasion into the central nervous systems (CNSs), when it occurs.

Two effective vaccines, the Salk killed polio vaccine delivered by the intramuscular route and the Sabin live attenuated polio vaccine delivered orally, have been used very successfully to eliminate the disease from most parts of the world.<sup>5</sup> The World Health Organization has led a long and hard-fought global polio eradication campaign, with much success, but full eradication has not yet been achieved. Since 2003, between 1000 and 2000 cases of PV infection are reported worldwide each year.<sup>6</sup> Thus, animal models are also needed to test new vaccine approaches that could be used toward eradication of polio in the areas where it still persists. Additionally, the recent focus of work with PV animal models has been fraught with urgency, as to gain understanding of PV pathogenesis before the eradication effort is complete and work with this virus ceases.

Animal models for the study of PV consist of NHP models and mouse models. Mice are susceptible to certain adapted PV strains: P2/Lansing, P1/LSb, and a variant of P3/Leon. Mice infected intracerebrally with P2/Lansing develop disease with some clinical and histopathological features resembling that of humans.<sup>7</sup> Wild-type mice are not susceptible to wild-type PV; however, the discovery of the PV receptor (CD155) in 1989 led to the use of hCD155 transgenic mice as a model of PV infection.<sup>8</sup> These mice are not susceptible to PV by the oral route and must be exposed intranasally or by intramuscular infection to induce paralytic disease.<sup>9</sup> Interestingly, hCD155 mice that have a disruption in the interferon (IFN)- $\alpha/\beta$  receptor gene are susceptible to oral infection.<sup>10</sup> This finding has given rise to speculation that an intact IFN- $\alpha/\beta$  response may be responsible for limiting infection in the majority of individuals exposed to PV. Thus, mouse models have proven to be very useful in gaining a better understanding of PV disease and pathogenesis.

Rhesus macaques are not susceptible to PV by the oral route, but they have been used extensively to study vaccine formulations for safety and immunogenicity, for monitoring neurovirulence of the live attenuated Sabin vaccine, and in the past for typing PV strains.<sup>11</sup> Bonnet monkeys are also susceptible to oral inoculation of PV, which results in the gastrointestinal shedding of virus for several weeks, with paralysis occurring in only a small proportion of animals. Consistent paralytic disease can be induced in Bonnet monkeys (*Macaca radiata*) through exposure to PV by infection into the right ulnar nerve (at the elbow), resulting in limb paralysis that resembles human paralytic poliomyelitis both clinically and pathologically.<sup>12</sup> As such, Bonnet monkeys can be used to study PV distribution and pathology

and the induction of paralytic poliomyelitis or provocation paralysis.<sup>13</sup>

## Hepatitis A Virus

Hepatitis A virus causes jaundice, which is a public health problem worldwide. The incubation period lasts from 15 to 45 days with an average of 28 days. Transmission between humans occurs by the oral–fecal route, person-to-person contact, or ingestion of contaminated food and water.<sup>14</sup> Hepatitis A virus causes an acute and self-limited infection of the liver with a spectrum of signs and symptoms ranging from subclinical disease, to jaundice, fulminant hepatitis, and in some cases death.<sup>15,16</sup>

The disease can be divided into four clinical phases: (1) Incubation period, during which the patient is asymptomatic but virus replicates and possibly transmits to others. (2) Prodromal period, which might last from a few days to a week with patients generally experiencing anorexia, fever (<103 °F), fatigue, malaise, myalgia, nausea, and vomiting. (3) Icteric phase, in which increased bilirubin causes characteristic dark brownish colored urine. This sign is followed by pale stool and yellowish discoloration of the mucous membranes, conjunctiva, sclera, and skin. Most patients develop an enlarged liver, and approximately 5–15% of the patients have splenomegaly. (4) Convalescent period, with resolution of the disease and recovery of the patient.

Rarely, during the icteric phase, extensive necrosis of the liver occurs. These patients show a sudden increase in body temperature, marked abdominal pain, vomiting, jaundice, and the development of hepatic encephalopathy associated with coma and seizures, all signs of fulminant hepatitis. Death occurs in 70–90% of patients with fulminant hepatitis.<sup>16</sup>

Experiments showed that hepatitis A causes disease only in humans, chimpanzees, several species of South American marmosets, stump-tailed monkeys, and owl monkeys via the oral or intravenous (IV) routes.<sup>17–20</sup> It is known that cynomolgus macaques are infected with hepatitis A virus in the wild.<sup>21</sup> Amado et al. used cynomolgus macaques (*Macaca fascicularis*) for experimental hepatitis A infections.<sup>17</sup> The animals did not exhibit clinical signs of disease, but viral shedding was observed in saliva and stool as early as 6 h postinoculation (pi) and 7 days pi, respectively. Although mild-to-moderate hepatic pathology was observed in all macaques, seroconversion and mildly increased alanine aminotransferase (ALT), an enzyme associated with liver function, were observed in some of them. Because this study had a very small group of animals (four macaques), the data should not be considered as conclusive, and more studies are needed to better define the cynomolgus macaque model.

Although hepatitis A virus is transmitted by the oral-fecal route, studies in chimpanzees and tamarins showed that the IV route was much more infectious than oral route was. There was no correlation between dose and development of clinical disease for either species or experimental routes, and similar to cynomolgus macaques, none of these species showed clinical signs of disease.<sup>20</sup>

Inoculation of common marmosets (*Callithrix jacchus*) with hepatitis A virus did not produce clinical signs of disease as seen in other NHP models.<sup>22,23</sup> Liver enzyme levels increased on day 14 pi, and monkeys had measurable antihepatitis A antibodies by day 32 pi.

An experimental study with cell culture-adapted hepatitis A virus in guinea pigs challenged by oral or intraperitoneal routes did not result in clinical disease, increase in liver enzymes, or seroconversion.<sup>24</sup> Viral load was detected in stool and serum between days 14 and 52 and 21 and 49 days, respectively. Liver pathology showed mild hepatitis. Furthermore, histopathology indicated that virus replicated in extrahepatic tissues such as spleen, regional lymph nodes, and intestinal tract.

In summary, none of the animal models for hepatitis A infection is suitable for studying pathogenesis of the virus because all clinical and most of the laboratory parameters remain within normal range or only slightly increased after the infection. One possibility is to test the safety of vaccines against hepatitis A virus in those models with demonstrable viral shedding.

## CALICIVIRIDAE

### Norwalk Virus

Noroviruses, of which Norwalk is the prototypic member, are responsible for up to 85% of reported food-borne gastroenteritis cases. In developing countries, this virus is responsible for approximately 200,000 deaths annually.<sup>25</sup> A typical disease course is self-limiting, but there have been incidences of necrotizing enterocolitis and seizures in infants.<sup>26,27</sup> Symptoms of infection include diarrhea, vomiting, nausea, abdominal cramping, dehydration, and fever. Incubation normally is for 1–3 days, with symptoms enduring for 2–3 days.<sup>28</sup> Viral shedding is indicative of immunocompromised status within an individual with the elderly and young having a prolonged state of shedding.<sup>29</sup> Transmission occurs predominately through the oral-fecal route with contaminated food and water being the major vector.<sup>30</sup>

A major hindrance to basic research into this pathogen is the lack of a cell culture system. Therefore, animal models are used not only to determine the efficacy of novel drugs and vaccines but also for understanding the pathogenesis of the virus. Therapeutic intervention consists of rehydration therapy and

antiemetic medication.<sup>31</sup> No vaccine is available, and development of one is expected to be challenging given that immunity is short lived after infection.<sup>32</sup>

NHPs including marmosets, cotton-top tamarins, and rhesus macaques infected with Norwalk virus can be monitored for the extent of viral shedding; however, no clinical disease is observed in these models. Disease progression and severity are measured exclusively by assay of viral shedding.<sup>33</sup> It was determined that more virus was needed to create an infection when challenging by the oral route than when challenging by the IV route. Chimpanzees were exposed to a clinical isolate of Norwalk virus by the IV route. Although none of the animals developed disease symptoms, viral shedding within the feces was observed within 2–5 days postinfection and lasted anywhere from 17 days to 6 weeks. Viremia never occurred, and no histopathological changes were detected. The amount and duration of viral shedding were in line with what is observed upon human infection.<sup>34</sup> A recently identified Calicivirus of rhesus origin, named Tulane virus, was used as a surrogate model of infection. Rhesus macaques exposed to Tulane virus intragastrically developed diarrhea and fever 2 days postinfection. Viral shedding was achieved for 8 days. The immune system produced antibodies that dropped in concentration within 38 days postinfection, mirroring the short-lived immunity documented in humans. The intestine developed moderate blunting of the villi as seen in human disease.<sup>35</sup>

A murine norovirus has been identified and is closely related to human Norwalk virus. However, clinically, the viruses present a different disease. The murine norovirus does not induce diarrhea nor vomiting and can develop a persistent infection in contrast to human disease.<sup>36–38</sup>

Porcine enteric caliciviruses can induce diarrheal disease in young pigs and an asymptomatic infection in adults.<sup>39</sup> Gnotobiotic pigs can successfully be infected with a passaged clinical noroviruses isolate orally. Diarrheal disease developed in 74% of the animals, and 44% were able to shed virus in their stool. No major histopathological changes or viral persistence was noted.<sup>40</sup>

Calves are naturally infected with bovine noroviruses. Experimentally challenging calves with an oral inoculation of a bovine isolate resulted in diarrheal disease 14–16 h postinfection. Recovery was achieved after 53.5 and 67 h postinfection.<sup>41</sup>

## TOGAVIRIDAE

### Eastern Equine Encephalitis Virus

Eastern Equine Encephalitis Virus (EEEV) was first documented as a disease of horses in Massachusetts when >75 horses died in three counties along the

northeast coast during the summer of 1831. Although additional horse cases were reported between 1845 and 1912 in New York, North Carolina, New Jersey, Maryland, and Virginia,<sup>42,43</sup> EEEV was not successfully isolated from the brains of infected horses until 1933. The link between equine cases and the human disease was confirmed in 1938 by observing 30 cases of fatal encephalitis in children living in the same area as the equine cases. During this outbreak, EEEV was isolated from the CNSs of these children as well as from pigeons and pheasants.<sup>44</sup>

EEEV primarily affects areas near salty marshes and can cause localized outbreaks of disease in the summer. The enzootic cycles are maintained in moist environments such as coastal areas, shaded marshy salt swamps in North America (NA), and moist forests in Central America and South America (SA).<sup>45</sup> Birds are the primary reservoir, and the virus is transmitted via mosquitoes. Furthermore, forest-dwelling rodents, bats, and marsupials frequently become infected and may provide an additional reservoir in Central America and SA. Despite known natural hosts, the transmission cycles in these animals are not well characterized.<sup>44</sup> Reptiles and amphibians have also been reported to become infected by EEEV.

EEEV pathogenesis and disease have been studied in several laboratory animals. As a natural host, birds do not generally develop encephalitis except pheasants or emus, in which EEEV causes encephalitis with 50–70% mortality.<sup>46</sup> Young chickens show signs of extensive myocarditis in early experimental infection and heart failure rather than encephalitis is the cause of death.<sup>47</sup> Besides the heart, other organs such as pancreas and kidney show multifocal necrosis. Additionally, lymphocytopenia has been observed in the thymus and spleen in birds.<sup>45</sup>

EEEV causes neuronal damage in newborn mice, and the disease progresses rapidly, resulting in death.<sup>48</sup> Similarly, EEEV produces fatal encephalitis in older mice when administered via the intracerebral route, whereas inoculation via the subcutaneous route causes a pantropic infection eventually resulting in encephalitis.<sup>49,50</sup>

Guinea pigs and hamsters have also been used as animal models for EEEV studies.<sup>51,52</sup> Guinea pigs developed neurological involvement with decreased activity, tremors, circling behavior, and coma. Neuronal necrosis was observed and resulted in brain lesions in these animals.<sup>52</sup> Subcutaneous inoculation of EEEV produced lethal biphasic disease in hamsters with severe lesions of nerve cells. The early visceral phase with viremia was followed by neuroinvasion, encephalitis and death. In addition, parenchyma necroses were observed in the liver and lymphoid organs.<sup>51</sup>

Intradermal, intramuscular, or IV inoculations of EEEV in NHPs cause disease but does not always result in

symptoms of the nervous system. Intracerebral infection of EEEV results in nervous system disease and fatality in monkeys.<sup>53</sup> The differences in these models indicate that the initial viremia and the secondary nervous system infection do not overlap in monkeys when they are infected by the peripheral route.<sup>54</sup> Intranasal and intraligular inoculations of EEEV also cause nervous system symptoms in monkeys, but less drastic than those caused by intracerebral injections.<sup>54</sup> The aerosol route of infection also progresses to uniformly lethal disease in cynomolgus macaques.<sup>55</sup> In this model, fever was followed by elevated white blood cells and liver enzymes. Neurological signs subsequently developed, and NHPs became moribund and were euthanized between days 5 and 9 days postexposure. Meningoencephalomyelitis was the main pathology observed in the brains of these animals.<sup>56</sup> Similar clinical signs and pathology were observed when common marmosets were infected with EEEV by the intranasal route.<sup>57</sup> Both aerosol and intranasal NHP models had similar disease progression and pathology as those seen in human disease.

A common marmoset model was used for comparison studies of SA and NA strains of EEEV.<sup>57</sup> Previous studies indicated that the SA strain is less virulent than NA strain for humans. Common marmosets were infected intranasally with either the NA or SA strain of EEEV. NA strain-infected animals showed signs of anorexia and neurological involvement and were euthanized 4–5 days after the challenge. Although SA strain-infected animals developed viremia, they remained healthy and survived the challenge.

## Western Equine Encephalitis Virus

Epizootics of viral encephalitis in horses were previously described in Argentina. More than 25,000 horses died from western equine encephalitis virus (WEEV) in the central plains of the United States in 1912.<sup>58</sup> WEEV was first isolated from the brains of horses during the outbreak in the San Joaquin Valley of California in 1930. Although it was suspected, the first diagnosis of WEEV as a cause of human encephalitis occurred in 1938, when the virus was recovered from the brain of a child with fatal encephalitis.<sup>44</sup> In horses, the signs of disease are fever, loss of coordination, drowsiness, and anorexia, leading to prostration, coma, and death in about 40% of affected animals.<sup>59</sup> WEEV also infects other species of birds and often causes fatal disease in sparrows.

WEEV infection occurs throughout western NA and sporadically in SA as it circulates between its mosquito vector and wild birds.<sup>44</sup> Chickens and other domestic birds, pheasants, rodents, rabbits, ungulates, tortoises, and snakes are natural reservoirs of WEEV.<sup>60,61</sup> WEEV has caused epidemics of encephalitis in humans, horses, and emus, but the fatality rate is lower than that for

EEEV.<sup>62</sup> Predominately young children and those older than 50 years demonstrate the clinical symptoms of the disease.<sup>63</sup> Severe disease, seizures, fatal encephalitis, and significant sequelae are more likely to occur in infants and young children.<sup>64,65</sup> Typically, the disease progresses asymptotically with seroprevalence in humans being fairly common in endemic areas.

Species used to develop animal models for WEEV are mice, hamsters, guinea pigs, and ponies. Studies with ponies resulted in viremia in 100% of the animals 1–5 days pi. Fever was observed in 7 of 11 animals, and six exhibited signs of encephalitis.<sup>44</sup>

After subcutaneous inoculation with WEEV, suckling mice started to show signs of disease by 24 h and died within 48 h.<sup>66</sup> In suckling mice, the heart was the only organ in which pathologic changes were observed. Conversely, adult mice exhibited signs of lethargy and ruffled fur on days 4–5 postinfection. Mice were severely ill by day 8 and appeared hunched and dehydrated. Death occurred between days 7 and 14, and both brain and mesodermal tissues such as heart, lungs, liver, and kidney were involved.<sup>66,67</sup> Intracerebral and intranasal routes of infection resulted in a fatal disease that was highly dependent on dose, while intradermal and subcutaneous inoculations caused only 50% fatality in mice regardless of the amount of virus.<sup>49</sup>

Studies demonstrated that although the length of the incubation period and the disease duration varied, WEEV infection resulted in mortality in hamsters by all routes of inoculation. Progressive lack of coordination, shivering, rapid and noisy breathing, corneal opacity, and conjunctival discharge resulting in closing of the eyelids were indicative of disease in all cases.<sup>68</sup> CNS involvement was evident with intracerebral, intraperitoneal, and intradermal inoculations.<sup>68</sup> WEEV is highly infectious to guinea pigs.<sup>69</sup> Intraperitoneal inoculation of WEEV is fatal in guinea pigs regardless of virus inoculum, with the animals exhibiting signs of illness on days 3–4, followed by death on days 5–9 (Nalca, unpublished results).

Very limited studies have been performed with NHPs. The intranasal route of infection causes severe, lethal encephalitis in rhesus macaques.<sup>54</sup> Reed et al. exposed cynomolgus macaques to low and high doses of aerosolized WEEV. The animals subsequently developed fever, increased white blood counts, and CNS involvement, demonstrating that the cynomolgus macaque model would be useful for testing of vaccines and therapeutics against WEEV.<sup>70</sup>

## Venezuelan Equine Encephalitis Virus

Venezuelan equine encephalitis virus (VEEV) is maintained in nature in a cycle between small rodents and mosquitoes.<sup>45</sup> The spread of epizootic strains of the

virus to equines leads to high viremia followed by a lethal encephalitis, and tangential spread to humans. VEEV can easily be spread by the aerosol route making it a considerable danger for laboratory exposure.

In humans, VEEV infection causes a sudden onset of malaise, fever, chills, headache, and sore throat.<sup>45,71,72</sup> Symptoms persist for 4–6 days, followed by a 2- to 3-week period of generalized weakness. Encephalitis occurs in a small percentage of adults ( $\leq 0.5\%$ ); however, the rate in children may be as high as 4%. Neurologic symptoms range from nuchal rigidity, ataxia, and convulsions to the more severe cases exhibiting coma and paralysis. The overall mortality rate in humans is  $<1\%$ .<sup>45</sup>

Laboratory animals such as mice, guinea pigs, and NHPs exhibit different pathologic responses when infected with VEEV. The lymphatic system is a general target in all animals infected with CNS involvement variable between different animal species. The disease caused by VEEV progresses very rapidly without showing signs of CNS disease in guinea pigs and hamsters. Mortality is typically observed within 2–4 days after infection and fatality is not dose dependent.<sup>44</sup> VEEV infection lasts longer in mice, which develop signs of nervous system disease in 5–6 days and death 1–2 days later. Lethal dose in mice changes depending on the age of mice and the route of exposure.<sup>56</sup> In contrast to guinea pigs and hamsters, the time of the death in mice is dose dependent. Mortality is observed generally within 2–4 days after infection and fatality is not dose dependent.

Subcutaneous/dermal infection in the mouse model results in encephalitic disease very similar to that seen in horses and humans.<sup>73</sup> Virus begins to replicate in the draining lymph nodes at 4 h pi. Eventually, virus enters the brain primarily via the olfactory system. Furthermore, aerosol exposure of mice to VEEV can result in massive infection of the olfactory neuroepithelium, olfactory nerves, and olfactory bulbs and viral spread to brain, resulting in necrotizing panencephalitis.<sup>74,75</sup> Aerosol and dermal inoculation routes cause neurological pathology in mice much faster than other routes of exposure do. The clinical signs of disease in mice infected by aerosol are ruffled fur, lethargy, and hunching progressing to death.<sup>56,74,75</sup> Intranasal challenge of C3H/HeN mice with high dose VEEV caused high morbidity and mortality.<sup>76</sup> Viral titers in brain peaked on day 4 postchallenge and stayed high until animals died on day 9–10 postchallenge. Protein cytokine array done on brains of infected mice showed elevated interleukin (IL)-1a, IL-1b, IL-6, IL-12, monocyte chemoattractant protein-1 (MCP-1), IFNg, MIP-1a, and regulated and normal T-cell expressed and secreted levels. This model was used successfully to test antivirals against VEEV.<sup>77</sup>

VEEV infection causes a typical biphasic febrile response in NHPs. Initial fever was observed at 12–72 h after infection and lasted <12 h. Secondary fever generally began on day 5 and lasted 3–4 days.<sup>78</sup> VEEV-infected NHPs exhibited mild symptoms such as anorexia, irritability, diarrhea, and tremors. Leucopenia was common in animals exhibiting fever.<sup>79</sup> Supporting the leucopenia, observed microscopic changes in lymphatic tissues such as early destruction of lymphocytes in lymph nodes and spleen, a mild lymphocytic infiltrate in the hepatic triads, focal myocardial necrosis with lymphocytic infiltration have been observed in monkeys infected with VEEV. Surprisingly, characteristic lesions of the CNS were observed histopathologically in monkeys in spite of the lack of any clinical signs of infection.<sup>78</sup> The primary lesions were lymphocytic perivascular cuffing and glial proliferation and generally observed at day 6 postinfection during the secondary febrile episode. Cynomolgus macaques develop similar clinical signs including fever, viremia, lymphopenia, and encephalitis upon aerosol exposure to VEEV.<sup>80</sup>

### Chikungunya Virus

Chikungunya virus is a member of the genus *Alpha-viruses*, specifically the Semliki Forest complex, and has been responsible for a multitude of epidemics mainly within Africa and southeast Asia.<sup>45</sup> The virus is transmitted by *Aedes* mosquitoes. Given the widespread endemicity of *Aedes* mosquitoes, chikungunya virus has the potential to spread to previously unaffected areas. This is typified by the emergence of disease for the first time in 2005 in the islands of the southwest Indian Ocean, including the French La Reunion island, and the appearance in central Italy in 2007.<sup>81,82</sup>

The incubation period after a mosquito bite is 2–5 days followed by a self-limiting acute phase that lasts 3–4 days. Symptoms during this period include fever, arthralgia, myalgia, and rash. Headache, weakness, nausea, vomiting, and polyarthralgia have all been reported.<sup>83</sup> Individuals typically develop a stooped posture due to the pain. For approximately 12% of infected individuals, joint pain can last months after resolution of primary disease, and has the possibility to relapse. Underlying health conditions, including diabetes, alcoholism, or renal disease, increase the risk of developing a severe form of disease that includes hepatitis or encephalopathy. Children between the ages of 3 and 18 years have an increased risk of developing neurological manifestations.<sup>84</sup> There is no effective vaccine or antiviral.

Wild-type C57BL/6 adult mice are not permissive to chikungunya virus infection by intradermal

inoculation. However, it was demonstrated that neonatal mice were susceptible, and severity was dependent upon age at infection. Six-day-old mice developed paralysis by day 6, and all died by day 12, whereas 50% of nine-day-old mice were able to recover from infection. By 12 days, mice were no longer permissive to disease. Infected mice developed loss of balance, hind limb dragging, and skin lesions. Neonatal mice were also used as a model for neurological complications.<sup>85,86</sup>

An adult mouse model has been developed by injection of the ventral side of the footpad of C57BL/6J mice. Viremia lasted 4–5 days accompanied by foot swelling and noted inflammation of the musculoskeletal tissue.<sup>87,88</sup> Adult IFNa/bR knockout mice also developed mild disease with symptoms including muscle weakness and lethargy, symptoms that mirrored human infection. All adult mice died within 3 days. This model was useful in identifying the viral cellular tropism for fibroblasts.<sup>85</sup> Imprinting control region (ICR) CD1 mice can also be used as a disease model. Neonatal mice subcutaneously inoculated with a passaged clinical isolate of Chikungunya virus developed lethargy, loss of balance, and difficulty in walking. Mortality was low, 17% and 8% for newborn CD1 and ICR mice, respectively. The remaining mice fully recovered within 6 weeks after infection.<sup>86</sup> A drawback of both the IFNa/bR and CD1 mice is that the disease is not a result of immunopathogenesis as occurs in human cases, given that the mice are immunocompromised.<sup>89</sup>

Long-tailed macaques challenged with a clinical isolate of the virus developed a similar clinical disease to humans. Initially, the monkeys developed high viremia with fever and rash. After this period, viremia resolved and virus could be detected in lymphoid, liver, meninges, joint, and muscle tissue. The last stage mimicked the chronic phase in which virus could be detected up to two months after infection, although no arthralgia was noted.<sup>90</sup>

## FLAVIVIRIDAE

### Dengue Virus

Dengue virus is transmitted via the mosquito vectors *Aedes aegypti* and *Aedes albopictus*.<sup>91</sup> Given the endemicity of the vectors, it is estimated that half of the world's population is at risk for exposure to Dengue virus. This results in approximately 50 million cases of dengue each year, with the burden of disease in the tropical and subtropical regions of Latin America, South Asia, and Southeast Asia.<sup>92</sup> It is estimated that there are 20,000 deaths each year caused by dengue hemorrhagic fever (DHF).<sup>93</sup>



There are four serotypes of Dengue virus, numbered 1–4, which are capable of causing a wide spectrum of disease that ranges from asymptomatic to severe with the development of DHF.<sup>94</sup> Incubation can range from 3 to 14 days, with the average being 4–7 days. The virus targets dendritic cells and macrophages after a mosquito bite.<sup>95</sup> Typical infection results in classic dengue fever (DF), which is self-limiting and has flu-like symptoms in conjunction with retroorbital pain, headache, skin rash, and bone and muscle pain. DHF can follow, with vascular leak syndrome and low platelet count, resulting in hemorrhage. In the most extreme cases, Dengue shock syndrome (DSS) develops, characterized by hypotension, shock, and circulatory failure.<sup>94</sup> Thrombocytopenia is a hallmark clinical sign of infection, and aids in differential diagnosis.<sup>96</sup>

Severe disease has a higher propensity to occur upon secondary infection with a different Dengue virus serotype.<sup>97</sup> This is hypothesized to occur due to antibody-dependent enhancement (ADE). There is no approved vaccine or drug, and hospitalized patients receive supportive care including fluid replacement. In developing an animal model, it is important to note that mosquitoes typically deposit  $10^4$ – $10^6$  pfu, and is therefore the optimal range to be used during challenge. A comprehensive review of the literature regarding animal models of Dengue infection was recently published by Zompi et al.<sup>98</sup>

Several laboratory mouse strains including A/J, BALB/c, and C57BL/6 are permissive to Dengue infection. However, the resulting disease has little resemblance to human clinical signs, and death results from paralysis.<sup>99–101</sup> A higher dose of an adapted Dengue virus strain induced DHF symptoms in both BALB/c and C57BL/6.<sup>102,103</sup> This model can also yield asymptomatic infections. A mouse-adapted (MA) strain of Dengue virus 2 introduced into AG129 mice developed vascular leak syndrome similar to the severe disease seen in humans.<sup>104</sup> Passive transfer of monoclonal Dengue antibodies within mice leads to ADE. During the course of infection, viremia was increased, and animals died due to vascular leak syndrome.<sup>105</sup> Another MA strain injected into BALB/c caused liver damage, hemorrhagic manifestations, and vascular permeability.<sup>103</sup> Intracranial injection of suckling mice with Dengue virus leads to death and has been used to test the efficacy of therapeutics.<sup>106</sup>

SCID mice engrafted with human tumor cells develop paralysis upon infection, and are thus not useful for pathogenesis studies.<sup>107,108</sup> DF symptoms developed after infection in NOD/SCID/IL2RgKO mice engrafted with CD34<sup>+</sup> human progenitor cells.<sup>109</sup> RAG-hu mice developed fever, but no other symptoms upon infection with a passaged clinical isolate and laboratory-adapted strain of Dengue virus 2.<sup>110</sup>

A passaged clinical isolate of Dengue virus type 3 was recently used to create a model in immunocompetent adult mice. Interperitoneal injection in C57BL/6j and BALB/c caused lethality by day 6–7 postinfection in a dose-dependent manner. The first indication of infection was weight loss beginning on day 4 followed by thrombocytopenia. A drop in systolic blood pressure along with noted increases in the liver enzymes, aspartate aminotransferase (AST) and ALT, were also observed. Viremia was established by day 5. This model mimicked the characteristic symptoms observed in human DHF/DSS cases.<sup>111</sup>

A novel model was developed that used infected mosquitoes as the route of transmission to hu-NSG mice. Female mosquitoes were intrathoracically inoculated with a clinical isolate of Dengue virus type 2. Infected mosquitoes then fed upon the mouse footpad to allow for the transmission of the virus via the natural route. The amount of virus detected within the mouse was directly proportional to the amount of mosquitoes it was exposed to, with four to five being optimal. Detectable viral RNA was in line with what is observed during human infection. Severe thrombocytopenia developed on day 14. This model is intriguing given that disease was enhanced with mosquito delivery of the virus in comparison to injection of the virus.<sup>112</sup>

NHP models have used a subcutaneous inoculation in an attempt to induce disease. Although the animals are permissive to viral replication, it is to a lower degree than that observed in human infection.<sup>113</sup> The immunosuppressive drug, cyclophosphamide enhances infection in rhesus macaques by allowing the virus to invade monocytes.<sup>114</sup> Throughout these preliminary studies, no clinical disease was detected. To circumvent this, a higher dose of dengue virus was used in an IV challenge of rhesus macaques. Hemorrhagic manifestations appeared by day 3 and resulted in petechiae, hematomas, and coagulopathy; however, no other symptoms developed.<sup>115</sup> Further development would allow this model to be used for testing of novel therapeutics and vaccines.

Although primates do not develop disease upon infection with Dengue, their immune system does produce antibodies similar to those observed during the course of human infection. This has been advantageous in studying ADE. Sequential infection led to a crossreactive antibody response, which has been demonstrated in both humans and mice.<sup>116</sup> This phenotype can also be seen upon passive transfer of a monoclonal antibody to Dengue and subsequent infection with the virus. Rhesus macaques exposed in this manner developed viremia that was 3- to 100-fold higher than was previously reported; however, no clinical signs were apparent.<sup>117</sup> The lack of inducible DHF or DSS

symptoms hinders further examination of pathogenesis within this model.

### Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) is a leading cause of childhood viral encephalitis in southern and eastern Asia and is a problem among military personnel and travelers to these regions. It was first isolated from the brain of a patient who died from encephalitis in Japan in 1935.<sup>118</sup> *Culex* mosquitoes, which breed in rice fields, transmit the virus from birds or mammals (mostly domestic pigs) to humans.

The disease symptoms range from a mild febrile illness to acute meningomyeloencephalitis. After an asymptomatic incubation period of 1–2 weeks, patients show signs of fever, headache, stupor, and generalized motor seizures, especially in children. The virus causes encephalitis by invading and destroying the cortical neurons. The fatality rate ranges from 10% to 50%, and most survivors have neurological and psychiatric sequelae.<sup>119,120</sup>

JEV virus causes fatality in infant mice by all routes of inoculation. Differences in pathogenesis and outcome are seen when the virus is given by intraperitoneal inoculation.<sup>121</sup> These differences depend on the amount of virus and the specific viral strains used. The biphasic viral multiplication after peripheral inoculation is observed in mice tissues. Primary virus replication occurs in the peripheral tissues and the secondary replication phase in the brain.<sup>122</sup>

Hamsters are another small animal species that are used as an animal model for JEV. Fatality was observed in hamsters inoculated intracerebrally or intranasally, while peripheral inoculation caused asymptomatic viremia. Studies with rabbits and guinea pigs showed that all routes of inoculation of JEV produce asymptomatic infection.<sup>123</sup>

Serial sampling studies with 12-day-old Wistar rats inoculated intracerebrally with JEV indicated that JEV causes the overproduction of free radicals by neurons and apoptosis of neuronal cells.<sup>124</sup> Following a study in 2010 by the same group, showed that although cytokines tumor necrosis factor (TNF)- $\alpha$ , IFN- $\gamma$ , IL-4, IL-6, IL-10, and chemokine MCP-1 increased gradually and peaked on days 10 pi with JEV in rats, the levels eventually declined, and there was no correlation with the levels of cytokines and chemokines and neuronal damage.<sup>125</sup>

Intracerebral inoculation of JEV causes severe histopathological changes in brain hemispheres of rhesus monkeys. Symptoms such as weakness, tremors, and convulsions began to appear on days 6–10, with indicative signs of encephalomyelitis occurring on days 8–12 postinfection for most of the animals followed by death occurring on days 8–12 postinfection for most of the animals followed by death.<sup>126</sup> Although intranasal

inoculation of JEV results in fatality in both rhesus and cynomolgus monkeys, peripheral inoculation causes asymptomatic viremia in these species.<sup>123,127</sup>

### West Nile Virus

West Nile virus (WNV) was first isolated from the blood of a woman in the West Nile district of Uganda in 1937.<sup>128</sup> After the initial isolation of WNV, the virus was subsequently isolated from patients, birds, and mosquitoes in Egypt in the early 1950s<sup>129,130</sup> and was shown to cause encephalitis in humans and horses. WNV is recognized as the most widespread of the flaviviruses, with a geographical distribution that includes Africa, the Middle East, western Asia, Europe, and Australia.<sup>131</sup> The virus first reached the western hemisphere in the summer of 1999, during an outbreak involving humans, horses, and birds in the New York City metropolitan area.<sup>132,133</sup> Since 1999, the range of areas affected by WNV quickly extended. Older people and children are most susceptible to WNV disease. WNV generally causes asymptomatic disease or a mild undifferentiated fever (West Nile fever), which can last from 3 to 6 days.<sup>134</sup> The mortality rate after neuroinvasive disease ranges from 4% to 11%.<sup>131,135–137</sup> The most severe complications are commonly seen in the elderly, with reported case fatality rates from 4% to 11%. Hepatitis, myocarditis, and pancreatitis are unusual, severe, nonneurologic manifestations of WNV infection.

Although many early laboratory studies of WN encephalitis were performed in NHPs, mice, rat, hamster, horse, pig, dog, and cat models were used to study the disease.<sup>138–144</sup>

Inoculation of WNV into NHPs intracerebrally resulted in the development of either encephalitis, febrile disease, or an asymptomatic infection, depending on the virus strain and dose. Viral persistence is observed in these animals regardless of the outcome of infection (i.e. asymptomatic, fever, encephalitis).<sup>141</sup> Thus, viral persistence is regarded as a typical result of NHP infection with various WNV strains. After both intracerebral and subcutaneous inoculation, the virus localizes predominantly in the brain and may also be found in the kidneys, spleen, and lymph nodes. WNV does not result in clinical disease in NHPs although the animals show a low level of viremia.<sup>145,146</sup>

WNV has also been extensively studied in small animals. All classical laboratory mouse strains are susceptible to lethal infections by the intracerebral and intraperitoneal routes, resulting in encephalitis and 100% mortality. Intradermal route pathogenesis studies indicated that Langerhans dendritic cells are the initial viral replication sites in the skin.<sup>147,148</sup> The infected Langerhans cells then migrate to lymph nodes, and the virus enters the blood through lymphatic and thoracic ducts and

disseminates to peripheral tissues for secondary viral replication. Virus eventually travels to the CNS and causes pathology that is similar to human cases.<sup>149–152</sup>

Tesh et al. developed a model for WN encephalitis using the golden hamster, *Mesocricetus auratus*. Hamsters appeared normal during the first 5 days, became lethargic at approximately day 6, and developed neurologic symptoms by days 7–10.<sup>143</sup> Many of the severely affected animals died 7–14 days after infection. Viremia was detected in the hamsters within 24 h after infection and persisted for 5–6 days. Although there were no substantial changes in internal organs, progressive pathologic differences were seen in the brain and spinal cord of infected animals. Furthermore, similar to the above-mentioned monkey experiments by Pogodina et al., persistent WNV infection was found in the brains of hamsters.

## CORONAVIRIDAE

### Severe Acute Respiratory Syndrome Coronavirus

The etiologic agent of severe acute respiratory syndrome (SARS), SARS-Coronavirus (CoV), emerged in 2002 as it spread throughout 32 countries in a period of 6 months, infecting >8000 people and causing nearly 800 deaths.<sup>153,154</sup> The main mechanism of transmission of SARS-CoV is through droplet spread, but it is also viable in dry form on surfaces for up to 6 days and can be detected in stool, suggesting other modes of transmission are also possible.<sup>155</sup>

Although other members of the family usually cause mild illness, SARS-CoV infection has a 10% case fatality with the majority of cases in people over the age of 15 years.<sup>156,157</sup> After an incubation period of 2–10 days, clinical signs of SARS include general malaise, fever,

chills, diarrhea, dyspnea, and cough.<sup>158</sup> In some SARS cases, pneumonia may develop and progress to acute respiratory distress syndrome (ARDS). Fever usually dissipates within 2 weeks and coincides with the induction of high levels of neutralizing antibodies.<sup>159</sup>

In humans, SARS-CoV replication destroys respiratory epithelium, and a great deal of the pathogenesis is due to the subsequent immune responses.<sup>160</sup> Infiltrates persisting within the lung and diffuse alveolar damage (DAD) are common sequelae of SARS-CoV infection. Virus can be isolated from secretions of the upper airways during early, but not later stages of infection as well as from other tissues.<sup>161</sup>

SARS-CoV can replicate in many species, including dogs, cats, pigs, mice, rats, ferrets, foxes, and NHPs.<sup>162</sup> Chinese palm civets, raccoon dogs, and bats are possible natural hosts. No model captures all aspects of human clinical disease (pyrexia and respiratory signs), mortality (~10%), viral replication, and pathology.<sup>163</sup> In general, the SARS-CoV disease course in the model species is much milder and of shorter duration than in humans. Viral replication in the various animal models may occur without clinical illness and/or histopathologic changes. The best-characterized models use mice, hamsters, ferrets, and NHPs (Table 38.1).

Mouse models of SARS-CoV typically are inoculated by the intranasal route under light anesthesia. Young, 6- to 8-week old BALB/c mice exposed to SARS-CoV have viral replication detected in the lungs and nasal turbinates, with a peak on day 2 and clearance by day 5 postexposure. There is also viral replication within the small intestines of young BALB/c mice. However, young mice have no clinical signs, aside from reduced weight gain, and have little to no inflammation within the lungs (pneumonitis). Intranasal SARS-CoV infection of C57BL/6 (B6) also yield reduced weight gain and viral replication in the lungs, with a peak on day 3 and clearance by day 9.<sup>171</sup> In contrast, BALB/c mice 13–14

TABLE 38.1 SARS-CoV Animal Models

Virus species	Route of exposure	Characteristics of human disease	Animal model	Route of exposure	Clinical outcome	References
SARS-CoV	Droplet	Fever, lung damage, 10% mortality	Young mouse	Intranasal	Little to no clinical signs or lung disease	164,165
			Old mouse	Intranasal	Clinical signs and lung damage, mortality	166
			Hamster	Intranasal	Reduced activity, lung damage, mortality	163
			Ferret	Intratracheal	Fever, lung damage, mortality	167
			NHP	Intranasal or intratracheal	Little to no clinical signs, variable mild lung damage	164,168–170

months of age show weight loss, hunched posture, dehydration, and ruffled fur on days 3–6 postexposure. Interstitial pneumonitis, alveolar damage, and death also occur in old mice, resembling the age-dependent virulence observed in humans. 129S mice and B6 mice show outcomes to SARS-CoV infection similar to those observed for BALB/c mice but have lower titers and less prolonged disease. One problem is that it is more difficult to obtain large numbers of mice older than 1 year.

A number of immunocompromised knockout mouse models of intranasal SARS-CoV infection have also been developed. 129SvEV mice infected with SARS-CoV by the intranasal route develop bronchiolitis, with peribronchiolar inflammatory infiltrates, and interstitial inflammation in adjacent alveolar septae.<sup>172</sup> Viral replication and disease in these mice resolve by day 14 post-exposure. Beige, CD1–/–, and RAG1–/– mice infected with SARS-CoV have similar outcomes to infected BALB/c mice with regard to viral replication, timing of viral clearance, and a lack of clinical signs. Signal transducer and activator of transcription-1 (STAT1) KO mice infected intranasally with SARS-CoV have severe disease, with weight loss, pneumonitis, interstitial pneumonia, and some deaths. The STAT1 KO mouse model is therefore useful for studies of pathogenicity, pathology, and evaluation of vaccines.

Syrian golden hamsters (strain LVG) are also susceptible to intranasal exposure of SARS-CoV. After the administration of  $10^3$  TCID<sub>50</sub> (tissue culture infective dose), along with a period of transient viremia, SARS-CoV replicates in nasal turbinates and lungs, resulting in pneumonitis. There are no obvious signs of disease, but exercise wheels can be used to monitor decrease in nighttime activity. Some mortality has been observed, but it was not dose dependent and could have more to do with genetic differences between animals because the strain is not inbred.<sup>163</sup> Damage is not observed in the liver or spleen despite detection of virus within these tissues.

Several studies have shown that intratracheal inoculation of SARS-CoV in anesthetized ferrets (*Mustela furo*) results in lethargy, fever, sneezing, and nasal discharge.<sup>167</sup> Clinical disease has been observed in several studies. SARS-CoV is detected in pharyngeal swabs, trachea, tracheobronchial lymph nodes, and high titers within the lungs. Mortality has been observed around day 4 postexposure as well as mild alveolar damage in 5–10% of the lungs, occasionally accompanied by severe pathology within the lungs.<sup>173</sup> With fever, overt respiratory signs, lung damage, and some mortality, the ferret intratracheal model of SARS-CoV infection is perhaps most similar to human SARS, albeit with a shorter time course.

SARS-CoV infection of NHPs by intranasal or intratracheal routes generally results in a very mild infection,

which resolves quickly. SARS-CoV infection of old world monkeys, such as rhesus macaques, cynomolgus macaques (cynos), and African green monkeys (AGMs) have been studied with variable results, possibly due to the outbred nature of the groups studied or previous exposure to related pathogens. Clinical illness and viral loads have not been consistent; however, replication within the lungs and DAD are features of the infections for each of the primate species. Some cynos have no illness, but others have rash, lethargy, and respiratory signs and pathology.<sup>170</sup> Rhesus have little to no disease and only have mild findings upon histopathological analysis. AGMs infected with SARS-CoV have no overt clinical signs, but DAD and pneumonitis have been documented. Viral replication has been detected for up to 10 days in the lungs of AGMs; however, the infection resolves and does not progress to fatal ARDS.

Farmed Chinese masked palm civets, sold in open markets in China, were thought to be involved in the SARS-CoV outbreak. Intratracheal and intranasal inoculation of civets with SARS-CoV results in lethargy, decreased aggressiveness, fever, diarrhea, and conjunctivitis.<sup>174</sup> Leucopenia, pneumonitis, and alveolar septal enlargement, with lesions similar to those observed in ferrets and NHPs, have also been observed in laboratory-infected civets. Common marmosets have also been shown to be susceptible to SARS-CoV infection.<sup>175</sup>

Vaccines have been developed for related animal CoVs in chickens, cattle, dogs, cats, and swine have used live-attenuated, killed, DNA and viral-vectored vaccine strategies.<sup>176</sup> An important issue to highlight from work on these vaccines is that CoV vaccines, such as those developed for cats, may induce a more severe disease.<sup>177</sup> As such, immune mice had Th2-type immunopathology upon SARS-CoV challenge.<sup>178</sup> Severe hepatitis in vaccinated ferrets with antibody enhancement in liver has been reported.<sup>179</sup> Additionally, rechallenge of AGMs showed limited viral replication but significant lung inflammation, including alveolitis and interstitial pneumonia, which persisted for long periods of time after viral clearance.<sup>180</sup>

Mouse and NHP models with increased virulence may be developed by adapting the virus by repeated passage within the species of interest. MA SARS and human ACE2 transgenic mice are available.<sup>181</sup>

## RHABDOVIRIDAE

### Rabies Virus

All mammals experimentally or naturally exposed to rabies virus have been found to be susceptible. This highly neurotropic virus is a member of the *Lyssavirus*

genus and is transmitted from the bite of an infected animal to humans.<sup>182</sup> The virus is able to replicate within the muscle cells at the site of the bite, and then travel to the CNS. Once reaching the CNS by retrograde axonal transport, the virus replicates within neurons creating inflammation and necrosis. The virus subsequently spreads throughout the body via peripheral nerves.<sup>183</sup>

A typical incubation period is 30–90 days, and is highly dependent upon the location of the bite. Proximity to the brain is a major factor for the onset of symptoms. The prodromal stage lasts from 2 to 10 days and is when the virus initially invades the CNS. Flu-like symptoms are the norm in conjunction with pain and inflammation at the site of the bite. Subsequently, there are two forms of disease that can develop. In 80% of cases, an individual develops the encephalitic or furious form. This form is marked by hyperexcitability, autonomic dysfunction, and hydrophobia. The paralytic, or dumb form, is characterized by ascending paralysis. Ultimately, both forms result in death days after the onset of symptoms. Once the symptoms develop, there is no proven effective therapy. In the developing world, death is caused by the lack of access to medical care including postexposure prophylaxis. In NA, fatal cases result because of late diagnosis.<sup>184</sup>

Syrian hamsters have been challenged with rabies virus intracerebrally, intraperitoneally, intradermally, and intranasally. All animals died as a result of the exposure, although intracerebral and intranasal inoculation led to only the furious form depicted by extreme irritability, spasms, excessive salivation, and cries. The virus used had been isolated from an infected dog brain and passaged in Swiss albino mice. Animals inoculated by intracerebral injection develop disease within 4–6 days, whereas all other routes of entry develop disease within 6–12 days.<sup>185</sup> This model has been used to study and test novel vaccine candidates.<sup>186</sup>

Mice have been extensively studied as an animal model for rabies. It was shown that Swiss Albino mice intracerebrally injected with a virus isolated from a dog developed only the paralytic form of disease 6 days after the initial challenge. BALB/c mice are universally susceptible to intracerebral injection of rabies virus within 9 days. Disease symptoms include paralysis, cachexia, and bristling appearing 1–3 days before death.<sup>187</sup> A more natural route of infection via peripheral injection into masseter muscles was tested on ICR mice. These mice developed neurological signs including limb paralysis, and all died within 6–12 days.<sup>188</sup> ICR mice have been instrumental in analyzing novel vaccines and correlates of protection.<sup>189</sup> This line of mice was also used to assess the value of ketamine treatment to induce coma during rabies infection.<sup>190</sup>

Another mouse line used is the p75 neurotrophin receptor-deficient mouse. This mouse developed a fatal encephalitis when inoculated intracerebrally with the challenge virus standard.<sup>191</sup> Bax-deficient mice have also been used to determine the role of apoptotic cell death in the brain during the course of infection.<sup>192</sup> A viral isolate from silver-haired bats can also be used in the mouse model. This strain is advantageous given that it is responsible for the majority of deaths in north NA.<sup>193</sup>

Early death phenomenon is typified by a decrease to time of death in a subset of individuals and animals that have been vaccinated and subsequently exposed to rabies.<sup>194</sup> This trend has been demonstrated experimentally in Swiss outbred mice and primates.<sup>195,196</sup>

Cynomolgus and rhesus were both infected with passaged rabies virus to create an NHP model. A high titer of virus was needed to induce disease, but exposure was found to not be universally fatal. The animals that survived beyond 4 weeks within the experiment did not develop clinical disease nor succumb to infection. Primates that did develop disease refused food and had progressively less activity until death. This lasted from 24 h up to 4 days, with all animals with symptoms dying within 2 weeks.<sup>196</sup>

Bats have been experimentally challenged with rabies. Vampire bats, *Desmodus rotundus*, intramuscularly injected with a bat viral isolate displayed clinical signs including paralysis in half of the population of the study animals. Of those who did develop disease, the duration was 2 days, and incubation period ranged from 7 to 30 days. Regardless of disease manifestation, 89% of challenged animals died.<sup>197</sup>

Skunks can be challenged intramuscularly or intranasally with either challenge virus strain or a skunk viral isolate. Interestingly, the challenge virus strain more readily produced the paralytic form, whereas the street form of rabies developed into the furious form. However, the challenge strain virus resulted in a shorter incubation period of 7–8 days in comparison to 12–14 days seen with the street virus.<sup>198</sup>

## FILOVIRIDAE

### Filoviruses

Filoviridae consists of two well-established genera, ebola virus and marburg virus (MARV) and a newly discovered group, cuevavirus<sup>199</sup> (Table 38.2). It is thought that fruit bats are the natural host reservoir for these viruses that have lethality rates from 40% to 82% in humans. MARV first emerged in 1967 in Germany when laboratory workers contracted the virus from AGMs (*Chlorocebus aethiops*) that were

TABLE 38.2 Filoviruses Causing Human Diseases

Genus	Species	Virus	Disease in humans
Marburg virus	<i>Marburg marburg virus</i>	MARV	Yes
		Ravn virus (RAVV)	Yes
Ebola virus	<i>Zaire ebola virus</i>	EBOV	Yes
	<i>Sudan ebola virus</i>	SUDV	Yes
	<i>Tai Forest ebola virus</i>	TAFV	Yes
	<i>Reston ebola virus</i>	RESTV	No
	<i>Bundibugyo ebola virus</i>	BDBV	Yes

shipped from Uganda. Ebola viruses Sudan and Zaire (SUDV and EBOV) caused nearly simultaneous outbreaks in 1976 in what is now the Democratic Republic of Congo (DRC). Bundibugyo virus (BDBV) first emerged in 2007 in Bundibugyo, Uganda with 56 confirmed cases.<sup>200</sup> Two other ebola viruses are known; Tai Forest (TAFV; previously named Cote (circumflex over the 'o') d'Ivoire) (CIEBOV) and Reston (RESTV), which have not caused major outbreaks or lethal disease in humans. The disease in humans is characterized by aberrant innate immunity and a number of clinical symptoms such as fever, nausea, vomiting, arthralgia/myalgia, headaches, sore throat, diarrhea, abdominal pain, anorexia, and numerous others.<sup>201</sup> Approximately 10% of patients develop petechia and a greater percentage, depending on the specific strain, may develop bleeding from various sites (gums, puncture sites, stools, etc.).<sup>199</sup>

Natural transmission in an epidemic is thought to be through direct contact or needle sticks in hospital settings. However, much of the research interest in filoviruses primarily stems from biodefense needs, particularly from aerosol biothreats. As such, intramuscular, intraperitoneal, and aerosol models have been developed in mice, hamsters, guinea pigs, and NHPs for the study of pathogenesis, correlates of immunity, and for testing countermeasures.<sup>202</sup> Because filoviruses have such high lethality rates in humans, scientists have looked for models that are uniformly lethal to stringently test efficacy of candidate vaccines and therapeutics.

Immunocompetent mice have not been successfully infected with wild-type filoviruses due to the control of the infection by the murine type 1 IFN response.<sup>203</sup> However, wild-type inbred mice are susceptible to filovirus that has been MA by serial passage.<sup>204</sup> BALB/c mice, which are the strain of choice for intraperitoneal inoculation of MA-EBOV, are not susceptible by the aerosol route.<sup>205</sup> For aerosol infection of

immunocompetent mice, a panel of BXD (BALB/c × DBA) recombinant inbred strains were screened, and one strain, BXD34, was shown to be particularly susceptible to airborne MA-EBOV, with 100% lethality to low or high doses (~100 or 1000 pfu). These mice developed weight loss of >15% and succumbed to infection between days 7 and 8 postexposure. The aerosol infection model uses a whole-body exposure chamber to expose mice aged 6–8 weeks to MA-EBOV aerosols with a mass median aerodynamic diameter (MMAD) of approximately 1.6 μm and a geometric standard deviation of approximately 2.0 for 10 min. Another approach uses immunodeficient mouse strains such as SCID, STAT1 KO, IFN receptor KO, or perforin KO with a wild-type EBOV inoculum by intraperitoneal or aerosol routes.<sup>206</sup> Mice are typically monitored for clinical disease “scores” based on activity and appearance, weight loss, and moribund condition (survival). Coagulopathy, a hallmark of filovirus infection in humans, has been observed, with bleeding in a subset of animals and failure of blood samples to coagulate late in infection. Liver, kidney, spleen, and lung tissue taken from moribund mice have pathology characteristic of filovirus disease in NHPs. Although most mouse studies have used MA-EBOV or EBOV, an intraperitoneal MA MARV model is also available.<sup>207</sup> MA-MARV and MA-EBOV models are particularly useful for screening novel antiviral compounds.<sup>208</sup>

Hamsters are frequently used to study cardiovascular disease, coagulation disorders, and thus serve as the basis for numerous viral hemorrhagic fever models.<sup>209</sup> An intraperitoneal MA-EBOV infection model has been developed in Syrian hamsters.<sup>210</sup> This model, which has been used to test a vesicular stomatitis virus vectored vaccine approach, uses male 5- to 6-week-old Syrian hamsters that are infected with 100 LD<sub>50</sub> of MA-EBOV. Virus is present in tissues and blood collected on day 4, and all animals succumbed to the disease by day 6. Detailed accounts of this model have been presented at international scientific meetings by Ebihara and Feldmann et al. but have not been reported in a scientific journal at the time of writing this chapter.<sup>211</sup>

Guinea pig models of filovirus infection have been developed for intraperitoneal and aerosol routes using guinea pig-adapted EBOV (GP-EBOV) and MARV (GP-MARV).<sup>212,213</sup> Guinea pigs models of filovirus infection are quite useful in that they develop fever, which can be monitored at frequent (hourly) intervals by telemetry. Additionally, the animals are large enough for regular blood sampling in which measurable coagulation defects are observed as the infection progresses. Hartley guinea pigs exposed to aerosolized GP-MARV or GP-EBOV become moribund

at times comparable to that of NHPs, generally succumbing to the infection between 7 and 12 days postexposure.

By aerosol exposure, GP-EBOV is uniformly lethal at both high and low doses (100 or 1000 pfu target doses) but lethality drops with low (<1000 pfu) presented doses of airborne GP-MARV, and more protracted disease is seen in some animals.<sup>213</sup> Weight loss of between 15% and 25% is a common finding in guinea pigs exposed to GP-EBOV or GP-MARV. Fever, which becomes apparent by day 5, occurs more rapidly in GP-EBOV exposed guinea pigs than with GP-MARV exposure. Lymphocytes and neutrophils increase during the earlier part of the disease, and platelet levels steadily drop as the disease progresses. Increases in coagulation time can be seen as early as day 6 postexposure. Blood chemistries (i.e. ALT, AST, alkaline phosphatase (ALKP), and blood urea nitrogen) indicating problems with liver and kidney function are also altered late in the disease course.

NHP models of filovirus infection are the preferred models for more advanced disease characterization and testing of countermeasures because they most closely mimic the disease and immune correlates seen in humans.<sup>214</sup> Old world primates have been primarily used for the development of intraperitoneal, intramuscular, and aerosol models of filovirus infection. Uniformly lethal filovirus models have been developed for most of the virus strains in cynomolgus macaques, rhesus macaques, and to a lesser degree, in AGMs and marmosets.<sup>215–219</sup> Low-passage human isolates that have not been passaged in animals have been sought for development of NHP models to satisfy the Food and Drug Administration (FDA) Animal Rule. Prominent features of the infections are onset of fever by day 5 postexposure, alteration in liver function enzymes (ALT, AST, and ALKP), decrease in platelets, and increased coagulation times. Clinical disease parameters may have a slightly delayed onset in aerosol models. Petichial rash is a common sign of filovirus disease and may be more frequently observed in cynomolgus macaques than in other NHP species. Dyspnea late in infection is a prominent feature of disease after aerosol exposure. A number of pronounced pathology findings include multifocal necrosis and fibrin lesions, particularly within the liver and the spleen. Lymphocytolysis and lymphoid depletion are also observed. Multilead, surgically implanted telemetry devices are useful in the continuous collection of temperature, blood pressure, heart rate, and activity levels. As such, blood pressure drops as animals become moribund and heart rate variability (standard deviation of the heart rate) is altered late in infection. The most recently developed telemetry devices can aid in plethysmography to measure respiratory

minute volume for accurate delivery of presented doses for aerosol exposure.

## PARAMYXOVIRIDAE

### Hendra and Nipah Virus

Hendra and Nipah virus are unusual within the *Paramyxoviridae* family given that they can infect a large range of mammalian hosts. Both viruses are grouped under the genus *Henipavirus*. The natural reservoirs of the viruses are the fruit bats from the genus *Pteropus*. Hendra and Nipah have the ability to cause severe disease in humans with the potential for a high case fatality rate.<sup>220</sup> Outbreaks caused by Nipah virus have been recognized in Malaysia, Singapore, Bangladesh, and India, while Hendra outbreaks have yet to be reported outside of Australia.<sup>221,222</sup>

Hendra was the first member of the genus to be identified and was initially associated with an acute respiratory disease in horses. All human cases have been linked to transmission through close contact with an infected horse. There have been no confirmed cases of direct transmission from human to human or bat to human. Nipah has the distinction of being able to be transmitted by humans, although the exact route is unknown.<sup>223</sup> The virus is susceptible to pH, temperature, and desiccation, and thus close contact is hypothesized to be needed for successful transmission.<sup>224</sup> Both viruses have a tropism for the neurological and respiratory tract.

Hendra virus incubation period is 7–17 days and is marked by a flu-like illness. Symptoms at this initial stage include myalgia, headache, lethargy, sore throat, and vomiting.<sup>225</sup> Disease progression can continue to pneumonitis or encephalitic manifestations, with the person succumbing to multiorgan failure.<sup>226</sup> Nipah virus has an incubation period of 4 days to 2 weeks.<sup>227</sup> Much like Hendra, the first signs of disease are nondescript. Severe neurological symptoms subsequently develop including encephalitis and seizures that can progress to coma within 24–48 h.<sup>228</sup> Survivors of infection typically make a full recovery; however, 22% suffer permanent sequelae, including persistent convulsions.<sup>229</sup> At this time, there is no approved vaccine or antiviral, and treatment is purely supportive. Animal models are being used to not only test novel vaccines and therapeutics, but also deduce the early events of disease because observed human cases are all at terminal stages.

The best small animal representative is the Syrian golden hamster due to their high susceptibility to both henipaviruses. Clinical signs upon infection recapitulate the disease course in humans including acute encephalitis and respiratory distress. Challenged animals died

within 4–17 days postinfection. The progression of disease and timeline are highly dependent on dose and route of infection. Intranasal inoculation leads to imbalance, limb paralysis, lethargy, and breathing difficulties whereas intraperitoneal resulted in tremors and paralysis within 24 h before death. Virus was detected in lung, brain, spleen, kidney, heart, spinal cords, and urine, while the brain was the most affected organ. This model has been used for vaccination and passive protection studies.<sup>230–232</sup>

The guinea pig model has not been widely used due to the lack of a respiratory disease upon challenge.<sup>233,234</sup> Inoculation with Hendra virus via the subcutaneous route leads to a generalized vascular disease with 20% mortality. Clinical signs were apparent 7–16 days postinfection with death occurring within 2 days of CNS involvement. Higher inocula have been associated with the development of encephalitis lesions. Intradermal and intranasal injections do not lead to disease, although the animals are able to seroconvert upon challenge. Inoculum source does not affect clinical progression. Nipah virus challenge only develops disease upon intraperitoneal injection and results in weight loss and transient fever for 5–7 days. Virus was shed through urine and found to be present in the brain, spleen, lymph nodes, ovary, uterus, and urinary bladder.<sup>235</sup>

Ferrets display the same clinical disease as seen in the hamster model and human cases.<sup>236,237</sup> Upon inoculation by the oronasal route, ferrets develop severe pulmonary and neurological disease within 6–9 days including fever, coughing, and dyspnea. Lesions do develop in the ferrets' brains, but to a lesser degree than seen in humans.

Cats have also been used as an animal model for henipaviruses. Disease symptoms are not dependent upon the route of infection. The incubation period is 4–8 days and leads to respiratory and neurological symptoms.<sup>238,239</sup> This model has proven to be useful in a vaccine challenge model.

Squirrel and AGMs are representative of the NHP models. Within the squirrel monkeys, Nipah virus is introduced by either the intranasal or IV route and subsequently leads to clinical signs similar to that in humans, although intranasal challenge results in milder disease. Upon challenge, only 50% animals develop disease manifestations including anorexia, dyspnea, and acute respiratory syndrome. Neurological involvement is characterized by uncoordinated motor skills, loss of consciousness, and coma. Viral RNA can be detected in the lung, brain, liver, kidney, spleen, and lymph nodes but is only found upon IV challenge.<sup>240</sup> AGMs have been found to be a very consistent model of both viruses. Intratracheal inoculation of the viruses results in 100% mortality, and death within 8.5 and 9–12 days postinfection for Hendra and Nipah, respectively. The animals

develop severe respiratory and neurological disease with generalized vasculitis.<sup>241,242</sup>

The reservoir of the viruses, gray-headed fruit bats, has been experimentally challenged. Due to their status as the host organism for henipaviruses, the bats do not develop clinical disease. However, Hendra virus can be detected in kidneys, heart, spleen, and fetal tissue and Nipah virus can be located in urine.<sup>243</sup>

Pigs have been investigated as a model as they develop a respiratory disease upon infection with both Nipah and Hendra.<sup>244–246</sup> Oral inoculation does not produce a clinical disease, but subcutaneous injection represents a successful route of infection. Live virus can be isolated from the oropharynx as early as 4 days postinfection. Nipah can also be transmitted between pigs. Nipah was able to induce neurological symptoms in 20% of the pigs, even though virus was present in all neurological tissues regardless of symptoms.<sup>247</sup> Within the pig model, it seemed that Nipah had a greater tropism for the respiratory tract, while Hendra for the neurological system.

Horses also are able to develop a severe respiratory tract infection accompanied with fever and general weakness upon exposure to Nipah and Hendra. Oronasal inoculation led to systemic disease with viral RNA detected in nasal swabs within 2 days.<sup>248,249</sup> Animals died within 4 days postexposure and were found to have interstitial pneumonia with necrosis of alveoli.<sup>250,251</sup> Virus could be detected in all major systems.

Mice, rats, rabbits, chickens, and dogs have been tested but found to be nonpermissive to infection.<sup>232,252</sup> Suckling BALB/c mice succumb to infection if the virus is inoculated intracranially.<sup>253</sup> Embryonated chicken eggs have been inoculated with Nipah virus leading to a universally fatal disease within 4–5 days postinfection.<sup>254</sup>

## Respiratory Syncytial Virus

Respiratory Syncytial virus is responsible for lower respiratory tract infections of 33 million children under the age of 5 years, which in turn results in three million hospitalizations and approximately 200,000 deaths.<sup>255</sup> Within the United States, hospital costs alone amount to >600 million dollars.<sup>256</sup> Outbreaks are common in the winter.<sup>257</sup> The virus is transmitted by large respiratory droplets that replicate initially within the nasopharynx and further spreads to the lower respiratory tract. Incubation for the virus is 2–8 days. Respiratory syncytial virus is highly virulent leading to very few asymptomatic infections.<sup>258</sup> Disease manifestations are highly dependent upon the age of the individual.

Primary infections in neonates produce nonspecific symptoms including the overall failure to thrive, apnea,



and feeding difficulties. Infants present with a mild upper respiratory tract disease that could develop into bronchiolitis and bronchopneumonia. Contracting the virus at this age results in an increased chance of developing childhood asthma.<sup>259</sup> Young children develop recurrent wheezing, whereas adults exacerbate previous respiratory conditions.<sup>260</sup> Common clinical symptoms are runny nose, sneezing, and coughing accompanied with fever.

Mortality rates in hospitalized children are 1–3% with the greatest burden of disease seen in 3–4-month-olds.<sup>261</sup> There is no vaccine available, and ribavirin usage is not recommended for routine treatment.<sup>262</sup> Animal models were developed in the hopes of formulating an effective and safe vaccine unlike the formalin-inactivated respiratory syncytial virus vaccine (FI-RSV). This vaccine-induced severe respiratory illness in infants who received the vaccine and were subsequently infected with live virus.<sup>263</sup>

Mice can be used to model disease, although a very high intranasal inoculation is needed to achieve clinical symptoms.<sup>264,265</sup> Strain choice is crucial to reproducing a physiological relevant response.<sup>266</sup> Age does not affect primary disease manifestations.<sup>267</sup> However, it does play a role in later sequelae showing increased airway hyperreactivity.<sup>268</sup> Primary infection produces increased breathing with airway obstruction.<sup>264,269</sup> Virus was detected as early as day 3 and reached maximum titer at day 6 postinfection. Clinical illness is defined in the mouse by weight loss and ruffled fur as opposed to runny nose, sneezing, and coughing as seen in humans.

Cotton rats are useful given that it is a small animal disease model. The virus is able to replicate to high titers within the lungs and can be detected in both the upper and lower airways after intranasal inoculation.<sup>270,271</sup> It has been reported that viral replication is 50- to 1000-fold greater in the rat model than in the mouse model.<sup>272</sup> The rats develop mild-to-moderate bronchiolitis or pneumonia.<sup>273</sup> Although age does not seem to factor in clinical outcome, it has been reported that older rats tend to take longer to achieve viral clearance. Viral loads peak by the fifth day, dropping to below the levels of detection by 8. The histopathology of the lungs seems to be similar to that in humans after infection.<sup>274</sup> This model has limited usage in modeling the human immune response to infection as challenge with the virus creates a Th2 response, whereas humans tend to skew toward Th1.<sup>275–277</sup> FI-RSV disease was recapitulated upon challenge with live virus after being vaccinated twice with FI-RSV.

Chinchillas have been challenged experimentally via intranasal inoculation. The virus was permissive within the nasopharynx and Eustachian tube. The animals displayed an acute respiratory tract infection. This model is thought to be useful in studying mucosal immunity during infection.<sup>278</sup>

Chimpanzees are permissive to replication and clinical symptoms of respiratory syncytial virus including rhinorrhea, sneezing, and coughing. Adult squirrel monkeys, newborn rhesus macaques, and infant Cebus monkeys were also challenged but did not exhibit any disease symptoms nor high levels of viral replication.<sup>279</sup> Bonnet monkeys were also tested and found to develop an inflammatory response by day 7 with viral RNA detected in both bronchial and alveolar cells.<sup>280</sup> The chimpanzee model has proven to be useful for vaccine studies.<sup>281,282</sup>

Sheep have also been challenged experimentally since they develop respiratory disease when exposed to ovine respiratory syncytial virus.<sup>283</sup> Lambs were also found to be susceptible to human respiratory syncytial infection.<sup>284,285</sup> When inoculated intratracheally, the lambs developed an upper respiratory tract infection with cough after 6 days. Some lambs went on to develop lower respiratory disease including bronchiolitis. The pneumonia resolved itself within 14 days. During the course of disease, viral replication peaked at 6 days, and rapidly declined. Studying respiratory disease in sheep is beneficial given the shared structural features between them and humans.<sup>286,287</sup>

## ORTHOMYXOVIRIDAE

### Influenza Virus

The influenza viruses consist of three types: influenza A, B, and C, based on antigenic differences. Influenza A is further classified by subtypes; 16 HA and 9 NA subtypes are known. Seasonal influenza is the most common infection and usually causes a self-limited febrile illness with upper respiratory symptoms and malaise that resolves within 10 days.<sup>288</sup> The rate of infection is estimated at 10% in the general population and can result in billions of dollars of loss annually from medical costs and reduced work-force productivity. Approximately 40,000 people in the United States die each year from seasonal influenza.<sup>289</sup> Thus, vaccines and therapeutics play a critical role in controlling infection, and development using animal models is ongoing.<sup>290</sup>

Influenza virus replicates in the upper and lower airways, peaking at approximately 48 h postexposure. Infection can be more severe in infants and in children under the age of 22 years, people over the age of 65 years, or immunocompromised individuals in whom viral pneumonitis or pneumonia can develop or bacterial superinfection resulting in pneumonia or sepsis.<sup>291</sup> Pneumonia from secondary bacterial infection, such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Neisseria meningitidis*, and more rarely, *Staphylococcus*

*aureus*, is more common than viral pneumonia from the influenza itself, accounting for approximately 27% of all influenza-associated fatalities.<sup>292</sup> Death, often due to ARDS can occur as early as 2 days after the onset of symptoms. Lung histopathology in severe cases may include DAD, alveolar edema and damage, hemorrhage, fibrosis, and inflammation.<sup>288</sup> The H5N1 avian strain of influenza has lethality rates of approximately 60% (of known cases), likely because the virus preferentially binds to the cells of the lower respiratory tract, and thus, the potential for global spread is a major concern.<sup>293</sup>

The most frequently used animal models of Influenza infection include mice, ferrets, and NHPs. A very thorough guide to working with mouse, guinea pig, ferret, and cynomolgus models was published by Kroeze et al.<sup>294</sup> Lethality rate can vary with the virus strain used (with or without adaptation), dose, route of inoculation, age, and genetic background of the animal. The various animal models can capture differing diseases caused by influenza: benign, severe, superinfection and sepsis, severe with ARDS, and neurologic manifestations.<sup>290</sup> Also, models can use seasonal or avian strains and models have been developed to study transmission, important for understanding the potential for more lethal strains such as H5N1 for spreading among humans.

Mouse models of influenza infection are very predictive for antiviral activity and tissue tropism in humans, and are useful in testing and evaluating vaccines.<sup>295</sup> Inoculation is by the intranasal route, using approximately 60  $\mu$ l of inoculum in each nare of anesthetized mice. Exposure may also be to small particle aerosols containing influenza with an MMAD of <5  $\mu$ m. Most inbred strains are susceptible, with particularly frequent use of BALB/c followed by C57BL/6J mice. Males and females have equivalent disease, but influenza is generally more infectious in younger 2- to 4-week-old (8–10 g) mice.

Mice are of somewhat limited use in characterizing the immune response to influenza. Mice lack the MxA gene, which is an important part of the human innate immune response to influenza infection. The mouse homolog to MxA, Mx1, is defective in most inbred mouse strains.<sup>296</sup>

Weight loss or reduced weight gain, decreased activity, huddling, ruffled fur, and increased respiration are the most common clinical signs. For more virulent strains, mice may require euthanasia as early as 48 h postexposure, but most mortality occurs from 5 to 12 days postexposure accompanied by decreases in rectal temperature.<sup>297</sup> Pulse oximeter readings and measurement of blood gases of oxygen saturation are also used to determine the impact of influenza infection on respiratory function.<sup>298</sup> Virus can be isolated from bronchial

lavage fluids throughout the infection and from tissues after euthanasia. For influenza strains with mild-to-moderate pathogenicity, disease is nonlethal and virus replication is detected within the lungs, but usually not other organs. Increases in serum alpha-1-acidglycoprotein and lung weight are also frequently present. However, mice infected with influenza do not develop fever, dyspnea, nasal exudates, sneezing, or coughing.

Mice can be experimentally infected with Influenza A or B, but the virus generally requires adaptation to produce clinical signs. Mice express the receptors for influenza attachment in the respiratory tract; however, the distribution varies and SA 2,3 predominates over SA 2,6 which is why H1, H2, and H3 subtypes usually need to be adapted to mice and H5N1, H2, H6, and H7 viruses do not require adaptation.<sup>299</sup> To adapt, mice are infected intratracheally or intranasally by virus isolated from the lungs, and reinfected into mice and then the process is repeated a number of times. Once adapted, influenza strains can produce severe disease, systemic spread, and neurotropism. However, H5N1 and the 1918 pandemic influenza virus can cause lethal infection without adaptation.<sup>300</sup> H5N1 infection of mice results in viremia and viral replication in multiple organ systems, severe lung pathology, fulminant diffuse interstitial pneumonia, pulmonary edema, high levels of proinflammatory cytokines, and marked lymphopenia.<sup>301</sup> As in humans, the virulence of H5N1 is attributable to damage caused by an overactive host immune response. Additionally, mice infected with the 1918 H1N1 influenza produces severe lung pathology and oxygen saturation levels that decrease with increasing pneumonia.<sup>302</sup>

In superinfection models, a sublethal dose of influenza is given to mice followed 7 days later by intranasal inoculation of a sublethal dose of a bacterial strain such as *S. pneumoniae* or *S. pyogenes*.<sup>303</sup> Morbidity, characterized by inflammation in the lungs, but not bacteremia, begins a couple of days after superinfection and may continue for up to 2 weeks. At least one transmission model has also been developed in mice. With H2N2 influenza, transmission rates of up to 60% among cage mates can be achieved after infection by the aerosol route and cocaging after 24 h.<sup>304</sup>

Domestic ferrets (*Mustela putorius furo*) are frequently the animal species of choice for influenza animal studies because the susceptibility, clinical signs, peak virus shedding, kinetics of transmission, local expression of cytokine mRNAs, and pathology resemble that of humans.<sup>305–307</sup> Ferrets also have airway morphology, respiratory cell types, and a distribution of influenza receptors (SA 2,6 and SA 2,3) within the airways similar to that of humans.<sup>308</sup> Influenza was first isolated from ferrets infected intranasally with throat

washes from humans harboring the infection and ferret models have since been used to test efficacy of vaccines and therapeutic treatments.<sup>309</sup>

When performing influenza studies in ferrets, animals should be serologically negative for circulating influenza viruses. Infected animals should be placed in a separate room from uninfected animals. If animals must be placed in the same room, uninfected ferrets should be handled before infected ferrets. Anesthetized ferrets are experimentally exposed to influenza by intranasal inoculation of 0.25–0.5 ml containing approximately  $10^4$ – $10^6$  egg ID<sub>50</sub> dropwise to each nostril.

Influenza types A and B naturally infect ferrets, resulting in an acute illness, which usually lasts 3–5 days for mildly to moderately virulent strains.<sup>310</sup> Ferrets are more susceptible to influenza A than to influenza B strains and are also susceptible to avian influenza H5N1 strains without adaptation.<sup>311</sup> Virulence and degree of pneumonitis caused by different influenza subtypes and strains vary from mild to severe and generally mirror that seen in humans. Nonadapted H1N1, H2N2, and H3N2 have mild-to-moderate virulence in ferrets.

Strains of low virulence have predominant replication in the nasal turbinates. Clinical signs and other disease indicators are similar to that of humans with a mild respiratory disease, sneezing, nasal secretions containing virus, fever, weight loss, high viral titers, and inflammatory infiltrate in the airways, bronchitis, and pneumonia.<sup>312</sup> Replication in both the upper and lower airways is associated with more severe disease and greater mortality. Additionally, increased expression of proinflammatory mediators and reduced expression of antiinflammatory mediators in the lower respiratory tract ferrets correlates with severe disease and lethal outcome. H5N1-infected ferrets develop severe lethargy, greater IFN response, transient lymphopenia, and replication in respiratory tract, brain, and other organs.<sup>313</sup>

Old and New World primates are susceptible to influenza infection and have an advantage over ferret and mouse models, which are deficient for H5N1 vaccine studies because there is a lack of correlation with hemagglutination inhibition.<sup>314</sup> Of Old World primates, cynomolgus macaque (*M. fascicularis*) are most frequently used for studies of vaccines and antiviral drug therapies.<sup>315,316</sup>

H5N1 and H1N1 1918 infections of cynos are very similar to those in humans.<sup>317</sup> Cynos develop fever and ARDS upon intranasal inoculation of H5N1 with necrotizing bronchial interstitial pneumonia.<sup>318</sup> NHPs are challenged by multiple routes (ocular, nasal, and tracheal) simultaneously  $1 \times 10^6$  pfu per site. Virus antigen is primarily localized to the tonsils and pulmonary tissues. Infection of cynos with H5N1 results in

fever, lethargy, nasal discharge, anorexia, weight loss, nasal and tracheal washes, pathologic and histopathologic changes, and alveolar and bronchial inflammation. The 1918 H1N1 caused a very high mortality rate due to an aberrant immune response and ARDs and had >50% lethality (humans only had a 1–3% lethality).

ARDS and mortality also occur with the more pathogenic strains, but NHPs show reduced susceptibility to less virulent strains such as H3N2.<sup>299</sup> Influenza-infected rhesus macaques represent a mild disease model pathogenesis for vaccine and therapeutic efficacy studies.<sup>319</sup> Other NHP models include influenza infection of pigtailed macaques as a mild disease model and infection of new world primates such as squirrel and cebus monkeys.<sup>320</sup>

Rats (F344 and SD) inoculated with rat-adapted H3N2 developed inflammatory infiltrates and cytokines in bronchoalveolar lavage fluids, but had no lethality and few histopathological changes.<sup>321</sup> Additionally, an influenza transmission model has been developed in guinea pigs as an alternative to ferrets.<sup>322</sup>

Cotton rats (*Sigmodon hispidus*) have been used to test vaccines and therapeutics in a limited number of studies.<sup>323,324</sup> Cotton rats have an advantage over mice in that the immune system is similar to humans (including the presence of the Mx gene) and influenza viruses do not have to be adapted.<sup>325,326</sup> Nasal and pulmonary tissues of cotton rats were infected with unregulated cytokines and lung viral load peaking at 24 h postexposure. Virus was cleared from the lung by day 3 and from the nares by day 66, but animals had bronchial and alveolar damage, and pneumonia for up to 3 weeks. There is also a *S. aureus* superinfection model in cotton rats.<sup>327</sup> Coinfection resulted in bacteremia, high bacterial load in lungs, peribronchiolitis, pneumonitis, alveolitis, hypothermia, and higher mortality.

Domestic pig influenza models have been developed for vaccine studies for swine flu. Pigs are susceptible in nature as natural or intermediate hosts but are not readily susceptible to H5N1.<sup>328,329</sup> Although pigs infected with influenza may have fever, anorexia, and respiratory signs such as dyspnea and cough, mortality is rare.<sup>330</sup> Size and space requirements make this animal difficult to work with, although the development of minipig (Ellegaard Gottingen) models may provide an easier-to-use alternative.

## BUNYAVIRIDAE

### Rift Valley Fever Virus

Rift Valley Fever virus (RVFV) causes epizootics and human epidemics in Africa. RVFV mainly infects livestock such as sheep, cattle, and goats. After 2- to 4-day

incubation period, animals exhibit signs of fever, hepatitis, and abortion, which is a hallmark diagnostic sign known among farmers.<sup>331</sup>

Mosquito vectors, unpasteurized milk, aerosols of infected animal's body fluids, or direct contact with infected animals are the important routes of transmission to humans.<sup>332,333</sup> After 2–6 days of incubation period, RVFV causes a wide range of signs and symptoms in humans ranging from asymptomatic to severe disease with hepatitis, vision loss, encephalitis, and hemorrhagic fever.<sup>334–336</sup> Depending on the severity of the disease when the symptoms start, 10–20% of the hospitalized patients might die in 3–6 days or 12–17 days after the disease onset.<sup>334</sup> Hepatic failure, renal failure or disseminated intravascular coagulation (DIC), and encephalitis are demonstrated within patients during postmortem examination.

Mice are one of the most susceptible animal species to RVFV infection. Subcutaneous or intraperitoneal routes of infection cause acute hepatitis and lethal encephalitis at a late stage of the disease in mice.<sup>337,338</sup> Mice start to exhibit signs of decreased activity and ruffled fur by day 2–3 postexposure. Immediately after these signs are observed, they become lethargic and generally die 3–6 days postexposure. Ocular diseases or hemorrhagic form of the disease has not been observed in mice models so far.<sup>334</sup> Increased viremia and tissue tropism were reported in mice with<sup>338</sup> increased liver enzymes and lymphopenia observed in sick mice.

Rats and gerbils are also susceptible to RVFV infection. Rats' susceptibility is dependent on the rat strain used for the challenge model. There was also noted an age dependence in susceptibility of rats. Although Wistar-Furth and Brown Norway strains and young rats are highly susceptible to RVFV infection, Fisher 344, Buffalo and Lewis strains, and old rats demonstrated resistance to infection.<sup>339,340</sup> Similar pathologic changes such as liver damage and encephalopathy were observed in both rats and mice. There was no liver involvement in the gerbil model and animals died from severe encephalitis. The mortality rate was dependent on the strain used and the dose given to gerbils.<sup>341</sup> Similar to the rat model, the susceptibility of gerbils was also dependent on age.

So far, studies showed that RVFV does not cause uniform lethality in an NHP model. Intraperitoneal, intranasal, IV, and aerosol routes have been used to develop the NHP model. Rhesus macaques, cynomolgus macaques, African monkeys, and South American monkeys were some of the NHP species used for this effort.<sup>342</sup> Monkeys showed a variety of signs ranging from febrile disease to hemorrhagic disease and mortality. Temporal viremia, increased coagulation parameters (PT, APTT), and decreased platelets were some other signs observed in NHPs. Animals that

succumbed to disease showed very similar pathogenesis to those seen in humans such as pathological changes in liver and hemorrhagic disease. There was no ocular involvement in this model.

Recently, Smith et al. compared IV, intranasal, and subcutaneous routes of infection in common marmosets and rhesus macaques.<sup>343</sup> Marmosets were more susceptible to RVFV infection than were rhesus macaques with marked viremia, acute hepatitis, and late onset of encephalitis. Increased liver enzymes were observed in both species. Necropsy results showed enlarged livers in the marmosets exposed by IV or subcutaneous routes. Although there were no gross lesions in the brains of marmosets, histopathology showed encephalitis in the brains of intranasally challenged marmosets.

### Crimean–Congo Hemorrhagic Fever Virus

Crimean–Congo hemorrhagic fever virus (CCHFV) generally circulates in nature unnoticed in an enzootic tick–vertebrate–tick cycle and similar to other zoonotic agents, seems to produce little or no disease in its natural hosts, but causes severe disease in humans.

CCHFV transmits to humans by ixodid ticks, direct contact with sick animals/humans, or body fluids of animals/humans.<sup>344</sup> Incubation, prehemorrhagic, hemorrhagic, and convalescence are the four phases of the disease seen in humans. The incubation period lasts 1–9 days. During the prehemorrhagic phase, patients show signs of nonspecific flu-like disease for approximately a week. The hemorrhagic period results in circulatory shock and DIC in some patients.<sup>345,346</sup>

Over the years, several attempts have been made to establish an animal model for CCHF in adult mice, guinea pigs, hamsters, rats, rabbits, sheep, NHPs, etc.<sup>347–350</sup> Until recently, the only animal that manifests disease is the newborn mouse. Infant mice infected with CCHFV intraperitoneally caused fatality around day 8 postinfection.<sup>351</sup> Pathogenesis studies showed that virus replication was first detected in the liver, with subsequent spread to the blood (serum). Virus was detected very late during the disease course in other tissues including the heart (day 6) and the brain (day 7).

The recent studies using knockout adult mice were successful to develop a lethal small animal model for CCHFV infection.<sup>352,353</sup> Bente et al. infected STAT1 knockout mice by the intraperitoneal route. In this model, after the signs of fever, leucopenia, thrombocytopenia, viremia, elevated liver enzymes, and proinflammatory cytokines, mice were moribund and succumbed to disease in 3–5 days of postexposure. The second model was developed by using IFN- $\alpha$ / $\beta$  receptor knockout mice.<sup>353</sup> Similar observations were made in this model as in the STAT1 knockout mouse model. The animals were moribund and died 2–4

days after exposure with high viremia levels in the liver and spleen.

Other laboratory animals, including NHPs, show little or no signs of infection or disease when infected with CCHFV.<sup>348</sup> Butenko et al. used AGMs (*Cercopithecus aethiops*) for experimental CCHFV infections. Except one monkey with a fever on day 4 postinfection, the animals did not exhibit signs of disease. Antibodies to the virus were detected in three out of five monkeys, including the one with fever. In 1975, Fagbami et al. infected two Patas monkeys (*Cercopithecus erythrocebuserythrocebus*) and one Guinea baboon (*Papio papio*) with CCHFV.<sup>347</sup> Although all three animals had low level viremia between days 1 and 5 after inoculation, only the baboon serum had neutralizing antibody activity on day 137 postinfection.

Similar results were obtained when horses and donkeys have been used for experimental CCHFV infections. Donkeys develop a low-level viremia,<sup>354</sup> and horses developed little or no viremia, but high levels of virus-neutralizing antibodies, which remained stable for at least 3 months. These studies suggest that horses may be useful in the laboratory to obtain serum for diagnostic and possible therapeutic purposes.<sup>355</sup>

Shepherd et al. infected 11 species of small African wild mammals and laboratory rabbits, guinea pigs, and Syrian hamsters with CCHFV.<sup>349</sup> Although scrub hares (*Lepus saxatilis*), cape ground squirrels (*Xerus inauris*), red veld rats (*Aethomys chrysophilus*), white-tailed rats (*Mystromys pumilio*), and guinea pigs had viremia; South African hedgehogs (*Atelerix frontalis*), highveld gerbils (*Tatera brantsii*), Namaqua gerbils (*Desmodillus auricularis*), two species of multimammate mouse (*Mastomys natalensis* and *M. coucha*), and Syrian hamsters were negative. All species regardless of viremia levels developed antibody responses against CCHFV. IV and intracranially infected animals showed the onset of viremia earlier than those infected by the subcutaneous or intraperitoneal routes.

## Hantaan Virus

The genus *Hantavirus* is unique among the family Bunyaviridae in that it is not transmitted by an arthropod vector, but rather by rodents.<sup>356</sup> Rodents of the family Muridae are the primary reservoir for hantaviruses. Infected host animals develop a persistent infection that is typically asymptomatic. Transmission is achieved by the inhalation of infected rodent saliva, feces, and urine.<sup>357</sup> Human infections can normally be traced to a rural setting with activities such as farming, land development, hunting, and camping as possible sites of transmission. Rodent control is the primary route of prevention.<sup>358</sup>

The viruses have a tropism for endothelial cells within the microvasculature of the lungs.<sup>359</sup> There are two

distinct clinical diseases that infection can yield; hemorrhagic fever with renal syndrome (HFRS) due to infection with Old World hantaviruses or hantavirus pulmonary syndrome (HPS) caused by New World hantaviruses.<sup>360</sup> HFRS is mainly seen outside of the Americas and is associated with the hantaviruses Dobrava–Belgrade (also known as Dobrava), Hantaan, Puumala, and Seoul.<sup>358</sup> Incubation lasts two to three weeks and presents as flu-like in the initial stages that can further develop into hemorrhagic manifestations and ultimately renal failure. Thrombocytopenia subsequently develops, which can further progress to shock in approximately 15% patients. The overall mortality rate is 7%. Infection with Dobrava and Hantaan viruses are typically linked to the development of severe disease.

HPS was first diagnosed in 1993 within the southwestern United States when healthy young adults became suddenly ill, progressing to severe respiratory distress and shock. The etiological agent responsible for this outbreak was identified as Sin Nombre virus.<sup>361</sup> This virus is still the leading cause within NA of HPS. HPS due to other hantaviruses has been reported in Argentina, Bolivia, Brazil, Canada, Chile, French Guiana, Panama, Paraguay, and Uruguay.<sup>362,363</sup> The first report of HPS in Maine was recently documented.<sup>361</sup> Andes virus was first identified in outbreaks in Chile and Argentina. This hantavirus is distinct in that it can be transmitted between humans.<sup>364</sup> The fulminant disease is more lethal than that observed for HFRS with a mortality rate of 40%.

There are four phases of disease including prodromal, pulmonary, cardiac depression, and hematologic manifestation.<sup>365</sup> Incubation typically occurs 14–17 days after exposure.<sup>366</sup> Unlike HFRS, renal failure is not a major contributing factor to the disease. There is a short prodromal phase that gives way to cardiopulmonary involvement accompanied by cough and gastrointestinal symptoms. It is at this point that individuals are typically admitted to the hospital. Pulmonary function is hindered and continues to suffer within 48 h after cardiopulmonary involvement. Interstitial edema and air-space disease normally follow. In fatal cases, cardiogenic shock has been noted.<sup>367</sup> Vaccine development has been hampered by the vast diversity of hantaviruses and the limited number of outbreaks.<sup>368</sup>

Syrian Golden hamsters are the most widely used small animal models for hantavirus infection. Hamsters inoculated intramuscularly with a passaged Andes viral strain died within 11 days postinfection. Clinical signs did not appear until 24 h before death at which point the hamsters were moribund and in respiratory distress. Mortality was dose dependent, with high inoculums leading to a shorter incubation before death. During the same study, hamsters were inoculated with a passaged Sin Nombre isolate. No hamsters developed

any symptoms during the course of observation. Although an antibody response to the virus that was not dose dependent was determined via an enzyme-linked immunosorbent assay. Hamsters infected with Andes virus were found to have significant histopathological changes to their lung, liver, and spleen. All had an interstitial pneumonia with intraalveolar edema. Infectious virus could be recovered from these organs. Viremia began on day 8 and lasted up to 12 days postinfection. Infection of hamsters with Andes virus yielded a similar clinical disease progression as is seen in human HPS including rapid progression to death, fluid in the pleural cavity, and significant histopathological changes to the lungs and spleen. A major deviation in the hamster model is the detection of infectious virus within the liver.<sup>369</sup>

Lethal disease can be induced in newborn mice but does not recapitulate the clinical symptoms observed in human disease.<sup>370</sup> Adult mice exposed to Hantaan virus leads to a fatal disease dependent upon viral strain and route of infection. The disease progression is marked by neurological or pulmonary manifestations that do not mirror human disease.<sup>371,372</sup> Knockout mice lacking IFN- $\alpha/\beta$  were found to be highly susceptible to Hantaan virus infection.<sup>373</sup> In a study looking at a panel of laboratory strains of mice, C57BL/6 mice were found to be most susceptible to a passaged Hantaan viral strain injected intraperitoneally. Animals progressed to neurological manifestation including paralysis and convulsions and succumbed to infection within 24–36 h postinfection. Clinical disease was markedly different than that observed in human cases.<sup>372</sup>

NHPs have been challenged with New World hantaviruses; however, no clinical signs were reported.<sup>374,375</sup> Cynomolgus monkeys challenged with a clinical isolate of Puumala virus developed a mild disease.<sup>376,377</sup> Challenge with Andes virus to cynomolgus macaques by both IV and aerosol exposure led to no signs of disease. All animals did display a drop in total lymphocytes within 5 days postinfection. Aerosol exposure led to 4 of 6 monkeys and 8 of 11 IV injected monkeys developed viremia. Infectious virus could not be isolated from any of the animals.

## ARENAVIRIDAE

### Lassa Fever Virus

The family Arenaviridae is composed of two serogroups: Old World Arenaviruses including Lassa fever virus and lymphocytic choriomeningitis virus and the New World viruses of Pichinde virus and Junin virus. All these viruses share common clinical manifestations.<sup>378</sup> Lassa fever virus is endemic in parts of west

Africa and outbreaks are typically seen in the dry season between January and April.<sup>379</sup> This virus is responsible for 100,000–500,000 infections per year, leading to approximately 5,000 deaths.<sup>380</sup> Outbreaks have been reported in Guinea, Sierra Leone, Liberia, Nigeria, and Central African Republic. However, cases sprung up in Germany, The Netherlands, the United Kingdom, and the United States due to transmission to travelers on commercial airlines.<sup>381</sup>

Transmission of this virus typically occurs via rodents, in particular the multimammate rat, *Mastomys* species complex.<sup>379</sup> Humans become infected by inhaling the aerosolized virus or eating contaminated food. There has also been noted human-to-human transmission by direct contact with infected secretions or needle-stick injuries. The majority of infections are asymptomatic; however, severe disease can occur in 20% of individuals. The incubation period is from 5 to 21 days, and the initial onset is characterized by flu-like illness. This is followed by diarrheal disease that can progress to hemorrhagic symptoms including encephalopathy, encephalitis, and meningitis. A third of patients develop deafness in the early phase of disease, which is permanent for a third of those affected. The overall fatality is about 1%; however, of those admitted to the hospital, it is between 15% and 25%. There is no approved vaccine, and besides supportive measures, ribavirin is effective only if started within 7 days.<sup>382,383</sup>

The primary animal model used to study Lassa fever is the rhesus macaque.<sup>384</sup> Aerosolized infection of lymphocytic choriomeningitis virus has been a useful model for Lassa fever. Both rhesus and cynomolgus monkeys exposed to the virus developed disease, but rhesus more closely mirrored the disease course and histopathology observed in human infection.<sup>385</sup> IV or intragastric inoculation of the virus led to severe dehydration, erythematous skin, submucosal edema, necrotic foci in the buccal cavity, and respiratory distress. The liver was severely affected by the virus as depicted by measuring the liver enzymes AST and ALT.<sup>386</sup> Disease was dose-dependent with IV, intramuscular, and subcutaneous inoculation requiring the least amount of virus to induce disease. Aerosol infections and eating contaminated food could also be used, and mimic a more natural route of infection.<sup>387</sup> Within this model, the NHP becomes viremic after 4–6 days. Clinical manifestations were present by day 7, and death typically occurred within 10–14 days.<sup>388,389</sup> Intramuscular injection of Lassa virus into cynomolgus monkeys also produced a neurological disease due to lesions within the CNS.<sup>390</sup> This pathogenicity is seen in select cases of human Lassa fever.<sup>391,392</sup>

A marmoset model has recently been defined using a subcutaneous injection of Lassa fever virus. Virus

was initially detected by day 8 and viremia achieved by day 14. Liver enzymes were elevated, and an enlarged liver was noted upon autopsy. There was a gradual reduction in platelets and interstitial pneumonitis diagnosed in a minority of animals. The physiological signs were the same as seen in fatal human cases.<sup>393</sup>

Mice develop a fatal neurological disorder upon intracerebral inoculation with Lassa, although the outcome of infection is completely dependent upon the major histocompatibility complex (MHC) background and age of animal along with the route of inoculation.<sup>394</sup> Guinea pig inbred strain 13 was found to be highly susceptible to Lassa virus infection. The outbreed Hartley strain was less susceptible, and thus, strain 13 has been the preferred model given its assured lethality. The clinical manifestations mirror those seen in humans and rhesus.<sup>395</sup> Infection with Pichinde virus that has been passaged in guinea pigs has also been used. Disease signs include fever, weight loss, vascular collapse, and eventual death.<sup>396,397</sup> The guinea pig is an excellent model given that it not only results in similar disease pattern as humans but also the viral distribution is similar along with the histopathology and immune response.<sup>398,399</sup>

Infection of hamsters with a cotton rat isolate of Pirital virus is similar to what is characterized in humans, and the NHP and guinea pig model. The virus was injected intraperitoneally resulting in the animals becoming lethargic and anorexic within 6–7 days. Virus was first detected at 3 days and reached maximum titers within 5 days. Neurological symptoms began to appear at the same time, and all the animals died by day 9. Pneumonitis, pulmonary hemorrhage, and edema were also present.<sup>400</sup> These results were recapitulated with a nonadapted Pichinde virus.<sup>401–403</sup>

## REOVIRIDAE

### Rotavirus

Globally, diarrheal disease is the leading cause of death with rotavirus being one of the main etiological agents responsible. According to the World Health Organization, rotavirus alone is responsible for a third of all hospitalization related to diarrhea and 500,000–600,000 deaths per year.<sup>404</sup> The virus is very stable due to its three-layer capsid, which allows it to be transmitted via the oral–fecal route, depositing itself in the small intestine. Rotavirus is highly contagious, and only 10 viruses are needed to cause symptomatic disease.<sup>405</sup>

The host determinant with the greatest influence on clinical outcome is age. Neonates typically are asymptomatic, which is suggested to be due to the existence of maternal antibodies. Hence, the most susceptible

age group is 3 months to 2 years, coinciding with a drop in these protective antibodies.<sup>406</sup> Within this age range, children will develop noninflammatory diarrhea. Virus replicates in the intestinal villus enterocytes resulting in their destruction and malabsorption of needed electrolytes and nutrients. Symptoms of disease include watery, nonbloody diarrhea with vomiting, fever, and potentially dehydration that lasts up to a week.<sup>407</sup> There is a short episode of viremia during the course of infection.<sup>408</sup>

Mice can be used as both an infection and disease model depending upon age at challenge. Mice <14 days old develop disease, whereas older mice are able to clear the infection before the onset of symptoms.<sup>409</sup> This halts the study of active vaccination against disease in the infection model. In the adult mouse model, the course of the infection is monitored via viral shedding within the stool.<sup>410</sup> Infant mice, specifically BALB/c, receiving an oral inoculation of a clinical strain of virus developed diarrhea within 24 h postinfection, and 95% of those exposed developed symptoms within 72 h postinfection. Symptoms lasted from 2 to 4 days with no mortality. Viral shedding was at its peak at 24 h and lasted up to 5 days. There were noted histopathological changes within the small intestine localized to the villi that was reversible.<sup>407</sup> Within the adult mouse model, oral inoculation of a mouse rotavirus strain showed viral shedding by 3 days lasting up until 6 days postinfection.<sup>410</sup> These mouse models have been used to study correlates of protection and therapeutic efficacy including Gastro-gard®.<sup>409,411,412</sup>

Rats can also be used as disease models depending upon the strain of rat.<sup>413,414</sup> Suckling Fischer 344 rats were exposed to a simian strain of rotavirus orally. The rats were susceptible to diarrheal disease till they were 8 days old with age determining the length of viral shedding.<sup>415</sup> Rats have mainly been used to study the correct formulation for oral rehydration. These rodents are large enough to perform in situ intestinal perfusions. Within these studies, 8-day-old rats were infected with a rat strain of rotavirus by orogastric intubation. Within 24 h postinfection, the rats developed diarrhea, at which point the small intestine was perfused to compare differing solutions of oral rehydration.<sup>416</sup>

Gnotobiotic pigs are also used given that they can be infected with both porcine and human strains.<sup>417</sup> They are susceptible to developing clinical disease from human strains up to 6 weeks of age. They allow for the analysis of the primary immune response to the virus given that they do not receive transplacental maternal antibodies and are immune competent at birth.<sup>418</sup> Another advantage of this model is that the gastrointestinal physiology and mucosal immune system closely resemble that of humans.<sup>419</sup> This model has been useful in studying correlates of protection.

Gnotobiotic and colostrum-deprived calves have also been used as an experimental model of rotavirus infection. They are able to develop diarrhea and shed live virus.<sup>420</sup> Gnotobiotic lambs can also develop clinical disease upon oral inoculation with clinical strains.<sup>421</sup> Infant baboons, AGMs, and rhesus macaques have all proven to be infection models with severity measure by viral shedding.<sup>422,423</sup>

## RETROVIRIDAE

### Human Immunodeficiency Virus Type 1

The *Lentiviruses* are a subfamily of *Retroviridae*, which includes human immunodeficiency virus (HIV), a virus that infects 0.6% of the world's population. A greater proportion of infections and deaths occur in sub-Saharan Africa. Worldwide, there are approximately 1.8 million deaths per year with >260,000 being children. Transmission of HIV occurs by exposure to infectious body fluids. There are two species, HIV-1 and HIV-2, with HIV-2 having lower infectivity and virulence (confined mostly to west Africa). The vast majority of cases worldwide are HIV-1.<sup>424</sup>

HIV targets T-helper cells (CD4+), macrophages, and dendritic cells.<sup>425</sup> Acute infection occurs 2–4 weeks after exposure, with flu-like symptoms and viremia followed by chronic infection. Symptoms in the acute phase may include fever, body aches, nausea, vomiting, headache, lymphadenopathy, pharyngitis, rash, and sores in the mouth or esophagus. CD8+ T-cells are activated which kill HIV-infected cells, and are responsible for antibody production and seroconversion. Acquired immune deficiency syndrome (AIDS) develops when CD4+ T-cells decline to <200 cells per microliter; thus, cell-mediated immunity becomes impaired, and the person is more susceptible to opportunistic infections and certain cancers.

Humanized mice, created by engrafting human cells and tissues into SCID mice, have been critical for the development of mouse models for the study of HIV infection. A number of different humanized mouse models allow for the study of HIV infection in the context of intact and functional human innate and adaptive immune responses.<sup>426</sup> The SCIDHu HIV infection model has proven to be useful, particularly in screening antivirals and therapeutics.<sup>427</sup> A number of different humanized mouse models have been developed for the study of HIV, including Rag1<sup>-/-</sup>γc<sup>-/-</sup>, Rag2<sup>-/-</sup>γc<sup>-/-</sup>, NOD/SCIDγc<sup>-/-</sup> (hNOG), NOD/SCIDγc<sup>-/-</sup> (hNSG), NOD/SCID BLT, and NOD/SCIDγc<sup>-/-</sup> (hNSG) BLT. CD34+ human stem cells derived from the umbilical cord blood or fetal liver are used for humanization.<sup>428</sup> HIV-1 infection by

intraperitoneal injection can be successful with as little as 5% peripheral blood engraftment.<sup>429</sup> Vaginal and rectal transmission models have been developed in BLT SCIDHu mice in which mice harbor human bone marrow, liver, and thymus tissue. HIV-1 viremia occurs within approximately 7 days pi.<sup>430</sup> In many of these models, spleen, lymph nodes, and thymus tissues are highly positive for virus, similar to humans.<sup>431</sup> Importantly, depletion of human T-cells can be observed in blood and lymphoid tissues of HIV-infected humanized mice, and at least some mechanisms of pathogenesis that occur in HIV-infected humans also occur in the HIV-infected humanized mouse models.<sup>432</sup> The advantage of these models is that these mice are susceptible to HIV infection, and thus, the impact of drugs on the intended viral targets can be tested. One caveat is that although mice have a “common mucosal immune system,” humans do not, due to the differences in the distribution of addressins.<sup>433</sup> Thus, murine mucosal immune responses to HIV do not reflect those of humans.

There are a number of important NHP models for human HIV infection. Simian immunodeficiency virus (SIV) infection of macaques is widely considered to be the best platform for modeling HIV infection of humans. Importantly, NHPs have similar, pharmacokinetics, metabolism, mucosal T-cell homing receptors, and vascular addressins to those of humans. Thus, although the correlates of protection against HIV are still not completely known, immune responses to HIV infection and vaccination are likely comparable. These models mimic infection through the use of contaminated needles (IV), sexual transmission (vaginal or rectal), and maternal transmission in utero, or through breast milk.<sup>434–436</sup> There are also macaque models to study the emergence and clinical implications of HIV drug resistance.<sup>437</sup>

These models most routinely use rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and pigtailed macaque (*Macaca nemestrina*). Animals of all ages are used, depending on the needs of the study. For instance, the use of newborn macaques may be more practical for evaluating the effect of prolonged drug therapy on disease progression; however, adult NHPs are more frequently used. Studies are performed in BSL-2 animal laboratories, and NHPs must be of Simian type-D retrovirus free and SIV seronegative. SIV infection of pigtailed macaques is a useful model for HIV peripheral nervous system pathology, wherein an axotomy is performed and regeneration of axons is studied.<sup>438</sup>

Challenges may be through a single high dose. IV infection of rhesus macaques with 100 TCID<sub>50</sub> of the highly pathogenic SIV/DeltaB670 induces AIDS in most macaques within 5–17 months (mean of 11



months).<sup>439</sup> Peak viremia occurs around week 4. AIDS in such models is often defined as CD4+ T-cells that have dropped to <50% of the baseline values. Alternatively, repeated low-dose challenges are often used, depending on the requirements of the model.<sup>440,441</sup>

Because NHPs infected with HIV do not develop an infection with a clinical disease course similar to that in humans, SIV or SIV/HIV-1 laboratory-engineered chimeric viruses (simian-human immunodeficiency virus or SHIV) are used as surrogates. NHPs infected with pathogenic SIV may develop clinical disease, which progresses to AIDS and are thus useful pathogenesis models. A disadvantage is that SIV is not identical to HIV-1 and is more closely related to HIV-2. However, the polymerase region of SIV is 60% homologous to that of HIV-1, and it is susceptible to many reverse transcriptase (RT) and protease inhibitors. SIV is generally not susceptible to nonnucleoside inhibitors; thus, HIV-1 RT is usually put into SIV for such studies.<sup>442</sup>

SIVmac239 is similar to HIV in the polymerase region and is therefore susceptible to nucleoside, RT or integrase inhibition.<sup>443</sup> NHPs infected with SIVmac239 have an asymptomatic period and disease progression resembling AIDS in humans, characterized by weight loss/wasting, CD4+ T-Cell depletion. Additionally, SIVmac239 uses the CXCR5 chemokine receptor as a coreceptor, similar to HIV, which is important for drugs that target entry.<sup>444</sup>

NHPs infected with SHIV strains may not develop AIDS, but these models are useful in testing vaccine efficacy. For example, RT-SHIVs and env-SHIVs are useful for the testing and evaluation of drugs that may target the envelope or RT, respectively.<sup>442</sup> One disadvantage of the highly virulent env-SHIV (SHIV-89.6 P) is that it uses the CXCR4 coreceptor. Of note, env-SHIVs that do use the CXCR5 coreceptor are less virulent; viremia develops and then resolves without further disease progression.<sup>445</sup>

Simian-tropic (st) HIV-1 contains the Vif gene from SIV. Infection of pigtailed macaques with this virus results in viremia, which can be detected for three months, followed by clearance.<sup>446</sup>

A number of routes are used for SIV or SHIV infection of NHPs, with IV inoculation being the most common route. Mucosal routes include vaginal, rectal, and intracolonic. Mucosal routes require a higher one-time dose than does the IV route for infection. For the vaginal route, female macaques are treated with Depo-Provera (estrogen) one month before infection to synchronize the menstrual cycle, thin the epithelial lining of the vagina, and increase the susceptibility to infection by atraumatic vaginal instillation.<sup>447</sup> Upon vaginal instillation of 500 TCID<sub>50</sub> of SHIV-162P3, peak viremia was seen around 12 days postexposure with >10<sup>7</sup> copies per milliliter and dropping thereafter to a constant level of 10<sup>4</sup> RNA copies per milliliter at 60 days and beyond.

In another example, in an investigation of the effect of vaccine plus vaginal microbicide on preventing infection, rhesus macaques were vaginally infected with a high dose of SIVmac251.<sup>448</sup> An example of an intrarectal model used juvenile (2-year-old) pigtailed macaques, challenged intrarectally with 10<sup>4</sup> TCID<sub>50s</sub> of SIV<sub>mne027</sub> to study the pathogenesis related to the virulence factor, Vpx.<sup>449</sup> Here, viremia peaked at approximately 10 days with >10<sup>8</sup> copies per milliliter. Viral RNA was expressed in the cells of the mesenteric lymph nodes.

The male genital tract is seen as a viral sanctuary with persistently high levels of HIV shedding even with antiretroviral therapy. To better understand the effect of highly active antiretroviral therapy on virus and T-cells in the male genital tract, adult (3- to 4-year old) male cynomolgus macaques were intravenously inoculated with 50 AID<sub>50s</sub> of SIVmac251, and the male genital tract tissues were tested after euthanasia by PCR, IHC, and in situ hybridization.<sup>450</sup>

Pediatric models have been developed in infant rhesus macaques through the infection of SIV, allowing for the study of the impact of developmental and immunological differences on the disease course.<sup>451</sup> Importantly, mother-to-infant transmission models have also been developed.<sup>452</sup> Pregnant female pigtailed macaques were infected during the second trimester with 100 MID<sub>50</sub> SHIV-SF162P3 by the IV route. Four of nine infants were infected; one in utero and three either in partum or immediately postpartum through nursing. This model is useful for the study of factors involved in transmission and the underlying immunology.

NHPs infected with SIV or SHIV are routinely evaluated for weight loss, activity level, stool consistency, appetite, virus levels in blood, and T-cell populations. Cytokine and chemokine levels, antibody responses, and cytotoxic T-lymphocyte responses may also be evaluated.

The ultimate goal of an HIV vaccine is sterilizing immunity (preventing infection). However, a more realistic result may be to reduce severity of infection and permanently prevent progression. Strategies have included live attenuated, nonreplicating and subunit vaccines. These have variable efficacy in NHPs due to the genetics of the host (MHC and terminal-repeat retrotransposon in miniature (TRIM) alleles), differences between challenge strains, and challenge routes.<sup>453</sup> NHP models have led to the development of antiviral treatments that are effective at reducing viral load and indeed transmission of HIV among humans. One preferred variation on the models for testing the long-term clinical consequences of antiviral treatment is to use newborn macaques and treat from birth onward, in some cases more than a decade.<sup>454</sup> Unfortunately, however, successes in NHP studies do not always translate to success in humans, as seen with the recent

STEP study that used an adenovirus-based vaccine approach.<sup>455</sup> Vaccinated humans were not protected and may have even been more susceptible to HIV, viremia was not reduced, and the infections were not attenuated as hoped. With regard to challenge route, IV is more difficult to protect than mucosal and is used as a “worst-case scenario.” However, efficacy at one mucosal route is usually comparable to that at other mucosal routes.

## PAPILLOMAVIRIDAE

### Papillomavirus

Human and animal papillomaviruses cause benign epithelial proliferations (warts) and malignant tumors of the various tissues that they infect.<sup>456</sup> There are >100 human papillomaviruses (HPVs), with different strains causing warts on the skin, oropharynx, nasopharynx, larynx, and anogenital tissues. Approximately a third of these are transmitted sexually. Of these, virulent subtypes such as HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45 place individuals at high risk for cervical and other cancers. Major challenges in the study of these viruses are that papillomaviruses generally do not infect any other species outside of the natural hosts and can cause a very large spectrum of severity. Thus, no animal models have been identified that are susceptible to HPV. However, a number of useful surrogate models exist that use animal papillomaviruses in their natural host, or a very closely related species.<sup>457,458</sup> These models have facilitated the recent development of useful and highly effective prophylactic HPV vaccines.<sup>459</sup>

Wild cottontail rabbits (*Sylvilagus floridanus*) are the natural host for cottontail rabbit papillomavirus (CRPV), but this virus also infects domestic rabbits (*Oryctolagus cuniculus*), which are a very closely related species.<sup>460</sup> In this model, papillomas can range from cutaneous squamous cell carcinomas on the one end of the spectrum, and spontaneous regression on the other. Lesions resulting from CRPV in domestic rabbits do not typically contain infectious virus.

Canine oral papillomavirus (COPV) cause florid warty lesions in the mucosa of the oral cavity within 4–8 weeks postexposure in experimental settings.<sup>461</sup> The mucosotropic nature of these viruses and the resulting oropharyngeal papillomas that are morphologically similar to human vaginal papillomas caused by HPV-6 and HPV-11 make this a useful model.<sup>462</sup> These lesions typically spontaneously regress 4–8 weeks after appearing; this model is therefore useful in understanding the interplay between the host immune defense and viral pathogenesis. Male and female beagles, aged 10 weeks to 2 years, with no history of COPV, are typically used for these studies. Infection is

achieved by the application of a 10 µl droplet of virus extract to multiple 0.5 cm<sup>2</sup> scarified areas within the mucosa of the upper lip of anesthetized beagles.<sup>463</sup>

Bovine papillomavirus (BPV) has a wider host range than do most papillomaviruses, infecting the fibroblasts cells of numerous ungulates.<sup>458</sup> BPV-4 infection of cattle feeding on bracken fern, which is carcinogenic, can result in lesions of the oral and esophageal mucosa that lack detectable viral DNA. BPV infections in cattle can result in a range of diseases such as skin warts, cancer of the upper gastrointestinal tract and urinary bladder, and papillomatosis of the penis, teats, and udder.

Finally, sexually transmitted papillomaviruses in rhesus macaques and cynomolgus macaques, rhesus papillomavirus, is very similar to HPV-16 and is associated with the development of cervical cancer.<sup>464</sup>

Mice cannot be used to study disease caused by papillomaviruses unless they are engrafted with relevant tissue, but they are often used to look at immunogenicity of vaccines.<sup>465,466</sup>

*Herpesviridae* Please see Chapter 25.

## POXVIRIDAE

### Monkeypox Virus

Monkeypox virus (MPXV) causes disease in both animals and humans. Human monkeypox, which is clinically almost identical to ordinary smallpox, occurs mostly in the rainforest of central and western Africa. The virus is maintained in nature in rodent reservoirs including squirrels.<sup>467,468</sup> MPXV was discovered during the pox-like disease outbreak among laboratory Java macaques in Denmark in 1958. No human cases were observed during this outbreak. The first human case was not recognized as a distinct disease until 1970 in Zaire (the present DRC) with the continued occurrence of a smallpox-like illness despite eradication efforts of smallpox in this area.

During the global eradication campaign, extensive vaccination in central Africa decreased the incidence of human monkeypox, but the absence of immunity in the generation born since that time and increased dependence on bush meat have resulted in renewed emergence of the disease.

In the summer of 2003, a well-known outbreak in the Midwest was the first occurrence of monkeypox disease in the United States and the western hemisphere. Among 72 reported cases, 37 human cases were laboratory confirmed during an outbreak.<sup>469,470</sup> It was determined that native prairie dogs (*Cynomys* sp.) housed with rodents imported from Ghana in West Africa were the primary source of outbreak.

The virus is mainly transmitted to humans while handling infected animals or by direct contact with the infected animal's body fluids, or lesions. Person-to-person spread occurs by large respiratory droplets or direct contact.<sup>471</sup> Most of the clinical features of human monkeypox are very similar to those of ordinary smallpox.<sup>472</sup> After a 7- to 21-day incubation period, the disease begins with fever, malaise, headache, sore throat, and cough. The main sign of the disease that distinguishes monkeypox from smallpox is swollen lymph nodes (lymphadenitis), which is observed in most of the patients before the development of rash.<sup>471,473</sup> Typical maculopapular rash follows the prodromal period generally lasting 1–3 days. The average size of the skin lesions is 0.5–1 cm, and the progress of lesions follows the order macules through papules, vesicles, pustules, umbilication then scab and desquamation and lasts typically 2–4 weeks. Fatality

rate is 10% among the unvaccinated population and death generally occurs during the second week of the disease.<sup>469,471</sup>

MPXV is highly pathogenic for a variety of laboratory animals, and so far, many animal models have been developed by using different species and different routes of exposure (Table 38.3). Because of the unavailability of variola virus to develop animal models and resulting disease manifestations in humans that are similar, MPXV is one of the pox viruses that are used very heavily to develop a number of small animal models via different routes of exposure. Wild-derived inbred mouse, STAT1-deficient C57BL/6 mouse, prairie dogs, African dormice, ground squirrels are highly susceptible to MPXV by different exposure routes.<sup>474–481</sup>

CAST/EiJ mice, one of the 38 inbred mouse strains tested for susceptibility to MPXV, showed weight loss and dose-dependent mortality after intranasal exposure

**TABLE 38.3** MPXV Animal Models

Virus species	Route of exposure	Characteristics of human disease	Animal model	Route of exposure	Clinical outcome in animals	References
MPXV	Direct contact with body fluids or lesions of infected person or animal and/or aerosol	Fever, malaise, lymphadenopathy, rash	Mice	Intranasal	Weight loss, viremia, mortality	474
				Intraperitoneal	Weight loss, viremia, mortality	474,476
			Prairie dogs	Intraperitoneal	Rash, viremia, mortality	481
				Intranasal	Rash, viremia	475,481
				Intradermal	Rash, viremia	475
			Ground squirrels	Intraperitoneal	Anorexia, lethargy, viremia, mortality	480
				Intranasal	Anorexia, lethargy, viremia, mortality	480
				Subcutaneous	Anorexia, lethargy, viremia, mortality	477
			Dormice	Intranasal	Weight loss, viremia, hemorrhage in internal organs	478
			NHP	Aerosol	Fever, lymphadenopathy, rash (-/+), bronchopneumoni, viremia	482,483
				IV	Fever, lymphadenopathy, vesiculopustular rash, viremia,	484–486
				Intranasal	Fever, weight loss, rash, viremia	487
				Intratracheal	Fever, weight loss, lymphadenopathy, rash, viremia	488,489
			Intrabronchial	Fever, rash, viremia	490	

to MPXV. Studies with the intraperitoneal route of challenge indicated a higher susceptibility to MPXV with an almost 50-fold less LD<sub>50</sub> when compared to the intranasal route.<sup>474</sup> SCID-BALB/c mice were also susceptible to the intraperitoneal challenge route, and the disease resulted in mortality day 9 postinfection.<sup>476</sup> Similarly C57BL/6 STAT1<sup>-/-</sup> mice were infected intranasally with MPXV, and the infection resulted in weight loss and mortality 10 days postexposure. Mice models mentioned here are very promising for screening of therapeutics against pox viruses, but testing in additional models will be required for advanced development.

High doses of MPXV by intraperitoneal or intranasal route caused 100% mortality in 6 days postexposure and 8 days postexposure, respectively, in ground squirrels.<sup>480</sup> The disease progressed very quickly, and most of the animals were lethargic and moribund by day 5 postexposure without any pox lesions or respiratory changes. A comparison study of USA MPXV and central African strain of MPXV in ground squirrels by subcutaneous route resulted in systemic disease and the mortality in 6–11 days postexposure. The disease resembles hemorrhagic smallpox with nose bleeds, impaired coagulation parameters, and hemorrhage in the lungs of the animals.

Because in the US outbreak the virus was transmitted by infected prairie dogs, this animal model has recently been studied much further and used to test therapeutics and vaccines compared to other small animal models.<sup>475,481,491,492</sup> Studies using intranasal, intraperitoneal, and intradermal routes of exposure showed that MPXV was highly infectious to prairie dogs. By using the west African MPXV strain, the intraperitoneal route caused a more severe disease and 100% mortality than challenge by the intranasal route. Anorexia and lethargy were common signs of the disease for both exposure routes. In contrast to the intraperitoneal route, the intranasal route of exposure caused severe pulmonary edema and necrosis of lungs in prairie dogs, while splenic necrosis and hepatic lesions were observed in intraperitoneally infected animals.<sup>481</sup> Recent studies by Hutson et al. used intranasal and intradermal infections with west African and Congo basin strains and showed that both strains and routes caused smallpox-like disease with longer incubation periods and generalized pox lesions.<sup>475</sup> Therefore, this model can be used for testing therapeutics and vaccines against pox viruses.

The African dormouse is susceptible to MPXV by the foodpad injection route or intranasal route.<sup>478</sup> Mice exhibited decreased activity, hunched posture, dehydration, conjunctivitis, and weight loss. Viral doses of 200 and 2000 pfu provided 100% mortality with a mean time to death of 8 days. Upper gastrointestinal hemorrhage, hepatomegaly, lymphadenopathy, and hemorrhage in lungs were observed during necropsy. With

the hemorrhage in several organs, this model resembles hemorrhagic smallpox.

Considering the limited availability of ground squirrels and African dormice, lack of reagents to these species, and resemblance to hemorrhagic smallpox disease, these models are not very attractive for further characterization and vaccine and countermeasure testing studies.

NHPs were exposed to MPXV by several different routes to develop animal models for MPXV.<sup>482,483,485,489,490</sup> During our studies by using an aerosol route of exposure, we observed that macaques had mild anorexia, depression, fever, and lymphadenopathy on day 6 postexposure.<sup>482</sup> Complete blood count and clinical chemistries showed abnormalities similar to those of human monkeypox cases with leukocytosis and thrombocytopenia.<sup>493</sup> Whole-blood and throat swabs had viral loads peak around day 10, and in survivors, gradually decrease until day 28 postexposure. Because doses of  $4 \times 10^4$ ,  $1 \times 10^5$ , or  $1 \times 10^6$  pfu resulted in lethality for 70% of the animals, whereas a dose of  $4 \times 10^5$  pfu resulted in 85% lethality, survival was not dose dependent. The main pitfall of this model was the lack of pox lesions. With the high dose, before animals can develop pox lesions, they succumbed to disease. With the low challenge dose, pox lesions were observed, but they were few in comparison to the IV model.

MPXV causes dose-dependent disease in NHPs when given by the IV route.<sup>490</sup> Studies showed that with  $1 \times 10^7$  pfu IV, challenge results in systemic disease with fever, lymphadenopathy, macula-papular rash, and mortality.

An intratracheal infection model deposits virus into the trachea, delivering directly to the airways without regard to particle size and the physiological deposition that occurs during the process of inhalation by skipping the upper respiratory system. Fibrinonecrotic bronchopneumonia was described in animals that received  $10^7$  pfu of MPXV intratracheally.<sup>489</sup> Although a similar challenge dose of intratracheal MPXV infection resulted in a similar viremia in NHPs than with the aerosol route of infection, the timing of the first peak was delayed by 5 days in intratracheally exposed macaques compared to aerosol infection, and the amount of virus detected by qPCR was approximately 100-fold lower. This suggests that local replication is more prominent after aerosol delivery compared to that after intratracheal delivery.

An intrabronchial route of exposure resulted in pneumonia in NHPs.<sup>490</sup> Delayed onset of clinical signs and viremia were observed during the disease progression. In this model, similar to aerosol and the intratracheal route of infection models, the number of pox lesions was much less than in the IV route of the infection model.

A major downside of the IV, intratracheal and intra-bronchial models is that the initial infection of respiratory tissue, incubation, and prodromal phases are circumvented with the direct inoculation of virus into the blood stream or into the lung. This is an important limitation when the utility of these models is to test possible vaccines and treatments in which the efficacy may depend on protecting the respiratory mucosa and targeting subsequent early stages of the infection, which are not represented in these challenge models. Although the aerosol model is the natural route of transmission for human variola virus (VARV) infections and a secondary route for human MPXV infections, the lack of pox lesions is the main drawback of this model. Therefore, when this model is decided to be used to test medical countermeasures, the endpoints and the biomarkers to initiate treatment should be chosen carefully.

## HEPADNAVIRIDAE

### Hepatitis B

Hepatitis B is one of the most common infections worldwide with >400 million people chronically infected and 316,000 cases per year of liver cancer due to infection.<sup>494</sup> The virus can naturally infect both humans and chimpanzees.<sup>495</sup> Hepatitis B is transmitted parenterally or postnatally from infected mothers. It can also be transmitted by sexual contact, IV drug use, blood transfusion, and acupuncture.<sup>496</sup> The age at which one is infected dictates the risk of developing chronic disease.<sup>497</sup>

Acute infection during adulthood is self-limiting and results in flu-like symptoms that can progress to hepatocellular involvement as observed with the development of jaundice. The clinical symptoms last for a few weeks before resolving.<sup>498</sup> After this acute phase, life time immunity is achieved.<sup>499</sup> Of those infected, <5% will develop the chronic form of disease. Chronicity is the most serious outcome of disease as it can result in cirrhosis or liver cancer. Hepatocellular carcinoma is 100 times more likely to develop in a chronically infected individual than in a noncarrier.<sup>500</sup> The viral determinant for cellular transformation has yet to be determined, although studies involving the woodchuck hepadna virus suggest that X protein may be responsible.<sup>501</sup> Many individuals are asymptomatic until complications emerge related to chronic carriage.

Chimpanzees have a unique strain that circulates within the population.<sup>502,503</sup> It was found that 3–6% of all wild-caught animals from Africa are positive for hepatitis B antigen.<sup>504</sup> Natural and experimental challenge with the virus follows the same course as human disease; however, this is only an acute model of

disease.<sup>505</sup> To date, the use of chimpanzees provides the only reliable method to ensure that plasma vaccines are free from infectious particles.<sup>506</sup> This animal model has been used to study new therapeutics and vaccines. Chimpanzees are especially attuned to these studies given that their immune response to infection directly mirrors humans.<sup>507</sup> Other NHPs that have been evaluated are gibbons, orangutans, and rhesus monkeys. Although these animals can be infected with hepatitis B, none develop hepatic lesions or liver damage as noted by monitoring of liver enzymes.<sup>508</sup>

Mice are not permissible to infection, and thus, numerous transgenic and humanized lines that express hepatitis B proteins have been created to facilitate their usage as an animal model. These include both immunocompetent and immunosuppressed hosts. The caveat to all of these mouse lines is that they reproduce only the acute form of disease.<sup>495</sup> Recently, the entire genome of hepatitis B was transferred to an immunocompetent mouse line via adenovirus. This provides a model for persistent infection.<sup>509</sup>

Hepatitis B can also be studied using surrogate viruses, naturally occurring mammalian hepadna viruses.<sup>510</sup> The woodchuck hepatitis virus was found to induce hepatocellular carcinoma.<sup>511</sup> Within a population, 65–75% of all neonatal woodchucks are susceptible to chronic infection.<sup>512</sup> A major difference between the two hepatitis isolates is the rate at which they induce cancer; almost all chronic carriers developed hepatocellular carcinoma within 3 years of the initial infection in woodchucks, whereas human infection takes much longer.<sup>513</sup> The acute infection strongly resembles what occurs during the course of disease in humans. There is a self-limiting acute phase resulting in a transient viremia that has the potential of chronic carriage.<sup>514</sup> Challenge with virus in neonates leads to a chronic infection, while adults only develop the acute phase of disease.<sup>515</sup> A closely related species to the woodchuck is the *Marmota Himalayan*. This animal is also susceptible to the woodchuck hepadna virus upon IV injection. It was found to develop an acute hepatitis with a productive infection.<sup>516</sup>

Hepatitis D is dependent upon hepatitis B to undergo replication and successful infection in its human host.<sup>517</sup> There are two modes of infection possible between the viruses: coinfection in which a person is simultaneously infected or superinfection in which a chronic carrier of hepatitis B is subsequently infected with hepatitis D.<sup>518</sup> Coinfection leads to a similar disease as seen with hepatitis B alone; however, superinfection can result in chronic hepatitis D infection and severe liver damage.<sup>519</sup> Both coinfection and superinfection can be demonstrated within the chimpanzee and woodchuck by inoculation of human hepatitis D.<sup>520</sup> A recently published report demonstrated the use of a humanized chimeric

mouse to study the interactions between the two viruses and drug testing.<sup>521</sup>

## CONCLUSIONS

The ideal animal model for human viral disease should closely recapitulate the spectrum of clinical symptoms and pathogenesis observed during the course of human infection. Whenever feasible, the model should use the same virus and strain that infects humans. It is also preferable that the virus be a low passage clinical isolate; thus, animal passage or adaptation should be avoided if model species can be identified that are susceptible. Ideally, the experimental route of infection would mirror that which occurs in natural disease. To understand the interplay and contribution of the immune system during infection, an immunocompetent animal should be used. The above characteristics cannot always be satisfied, however, and often virus must be adapted, knockout mice must be used, and/or the disease is not perfectly mimicked in the animal model.

Well-characterized animal models are critical for licensure to satisfy the Food and FDA's "Animal Rule." This rule applies to situations in which vaccines and therapeutics cannot safely or ethically be tested in humans; thus, licensure will come only after preclinical tests are performed in animal models. Many fields in virology are moving toward standardized models that can be used across institutions to test vaccines and therapeutics. A current example of such an effort is within the filovirus community, where animal models, euthanasia criteria, assays, and virus strains are in the process of being standardized. The hope is that these efforts will enable results of efficacy tests on medical countermeasures to be compared across institutions. This chapter has summarized the best models available for each of the viruses described.

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