

REVIEW

Applications and advancements in animal models for antiviral research on mosquito-borne arboviruses

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Abstract

Vector-borne diseases caused by arthropod-borne viruses (arboviruses) are a considerable challenge to public health globally. Mosquito-borne arboviruses, such as Chikungunya, Dengue, and Zika viruses, cause a range of human illnesses and may be fatal. Currently, efforts to control these diseases still face challenges due to growing vector resistance towards insecticides, urbanization, and limited effective antiviral treatments and vaccines. Animal models are crucial in antiviral research on mosquito-borne arboviruses, playing a role in understanding disease mechanisms, vaccine development, and toxicity testing, but the application of animal models still faces the challenges of ethical considerations and animal-to-human translational success. Genetically engineered mouse models, hamster models and non-human primate (NHP) are currently used in arbovirus research, but new models such as tree shrews and novel humanized mice are emerging. In the context of Malaysian research, the use of long-tailed macaques as potential NHP models for arbovirus research is possible; however, it faces the ethical dilemma of using an endangered species for scientific purposes. Overall, animal models play a crucial role in advancing infectious disease research, but a balance between medical research and species conservation must be upheld.

KEYWORDS

animal models, arbovirus, biomedical research, ethics, infectious diseases

1 | INTRODUCTION

Globally, vector-borne diseases (VBDs) account for 17% of all infectious diseases worldwide and are reported to cause up to 700 000 deaths annually.¹ Furthermore, there has been a significant rise in the emergence, re-emergence, and prevalence of arthropod-borne viruses (arboviruses) worldwide in the past 20 years, presenting a substantial public health challenge.² Arboviruses are a diverse group of RNA viruses primarily classified within the *Flaviviridae*, *Togaviridae*, *Reoviridae*, and other families within the Bunyavirales

order.^{2,3} Transmission of the arboviruses to vertebrate hosts occurs when the arthropod vectors (mostly mosquitoes, flies and ticks) take a blood meal, causing the infected individual to develop an array of symptoms including arthritis, biphasic fever, haemorrhagic fever, encephalitis, and meningitis that may result in death.²⁻⁴

Mosquito-borne arboviruses are explicitly transmitted by mosquitoes. The vectors are infected when they take a blood meal from viraemic vertebrate hosts. The arbovirus then replicates considerably within the vector, which facilitates its dissemination to new vertebrate hosts during their next blood meal.^{2,5} Typically, the vectors

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are unharmed as they are crucial for the long-term survival of the arbovirus, but the virus causes an acute, highly pathogenic disease in the vertebrate hosts.² Some well-known mosquito-borne arboviruses include Chikungunya virus (CHIKV), Dengue virus (DENV), and Zika virus (ZIKV).^{2,3} DENV, transmitted by *Aedes* mosquitoes, is endemic to more than 100 countries within tropical and subtropical areas.^{2,3,6} In Malaysia, dengue incurs a yearly infection burden in the population, with 130 101 dengue cases in 2019 alone.⁶ CHIKV and ZIKV, also transmitted by *Aedes* mosquitoes, are also emerging as a major public health concern.^{2,3} Traditionally confined to remote African regions, these viruses have disseminated into tropical areas due to urban transmission cycles, causing human diseases.² To illustrate and compare the impacts and epidemiology of these mosquito-borne viruses, [Table 1](#) briefly highlights recent updates regarding these viruses and their associated diseases.

Efforts to control arboviral diseases include vector surveillance, control programs, and vaccination, but they face challenges such as vector resistance to insecticides, expanding mosquito habitats, and increasing urbanization.² Currently, vaccines are only available for certain mosquito-borne arboviruses like yellow fever virus (YFV) and Japanese encephalitis virus (JEV); most other mosquito-borne arboviruses lack effective antiviral treatments and vaccines.^{2,3} As a result of the lack of targeted antiviral drugs, case management for mosquito-borne arboviral diseases is focused on symptomatic treatment and surveillance of disease severity.³¹ Therefore, understanding the mechanisms behind the circulation of these viruses between vertebrates and mosquitoes holds the potential for identifying drug-gable targets for antiviral interventions and improving disease control strategies.^{2,3}

2 | ANIMAL MODELS IN ANTIVIRAL RESEARCH—IMPORTANCE AND CHALLENGES

In any discussion of the importance of animal models in antiviral research, the transmission of a mosquito-borne arbovirus from vector to vertebrate host must be understood. Fundamentally, an infected mosquito vector uses its maxillae, two thin serrated cutting projections, to penetrate a vertebrate host's dermis layer and inject protein-rich saliva, which aids in extracting blood from the host.³² The virus enters the host via the injected vector saliva, promoting viral dissemination and enhancing viral pathogenesis.³²

Antiviral research *in vitro* involves infecting mammalian cell lines with the virus before treating them with a potential antiviral compound to assess its ability to inhibit viral replication. This acts as a screening process for the antiviral compound to be further developed as a viable antiviral treatment. However, to initiate that progress, antiviral testing on animal models cannot be avoided as animals act as the intermediary between initial screening and approval for human consumption.³³ When conducting antiviral research on mosquito-borne arboviruses specifically, it is particularly important that the animal model mimics human infection whereby

mosquito vectors inoculate the host with the virus via their blood meals. The animal model should also present comparable genetic similarities, physiology, and known immunological behavior to the human body.³³

Only after an established infection in a compatible animal model will the potential therapeutic be administered to the test group as a preclinical experiment. The blood-meal infection mimicry is crucial as it recreates human immune responses in animal models, allowing researchers to identify the safety and efficacy of a potential drug for human administration.³⁴ Ergo, animal models are essential tools in scientific research to help in our understanding of various biological processes, disease mechanisms, therapies, and toxicology. While mice and rats have been long-standing choices for research, advances in technology have led to the creation of new animal models.³⁵ Techniques like somatic cell nuclear transfer and genome editing have broadened the range of available animal models, including transgenic large animal models, which are advantageous for tasks such as imaging, pathology, and surgical approaches.³⁵ These models continue to evolve and diversify, complementing traditional mouse models and have the potential to significantly enhance the impact of scientific research. Furthermore, preclinical tests on animal models also allow researchers to identify potentially undesired or dangerous side effects if administered to humans, such as carcinogenic effects, congenital disabilities, liver damage, toxicity, and infertility.³⁴ Consequently, animal models are an essential stepping stone in antiviral research for drug development, ensuring that the potential therapeutic does not present any undesired side effects in animal models before progressing to clinical trials.

Nonetheless, conducting preclinical studies on animal models has its challenges. It has been reported that many potential drugs show tremendously promising efficacy in preclinical models but fail in human clinical trials.³³ To illustrate this, [Figure 1](#) presents the percentages of preclinical and clinical trial failures and successes. Only 12% of drugs tested in preclinical trials advance to the clinical trial stage, and from that 12%, only 11.7% succeed in human clinical trials. Overall, only approximately 1% of drugs tested in preclinical trials display promising results in clinical trials. Furthermore, it has been stated that “the poor correlation of animal studies to human toxicity and efficacy have led many developers to question the value of requiring animal studies in determining which drugs should enter in-human trials.”³⁷

Conversely, the opposite is also possible—animal models can falsely identify a safe potential therapeutic as unsafe and ‘toxic’, leading to a halt in drug development. The number of potential drugs that have ceased development due to false toxicity in animal model testing is unknown because animal testing is a compulsory step in the drug development process.³⁶ Many vital drugs developed before the animal testing requirement, for example, aspirin and penicillin, wouldn't exist today because they show toxicity in preclinical tests on animal models.³⁶ Unfortunately, at the present time avoiding animal testing antiviral drug development is impossible as drug regulatory authorities do not recognize *in vitro* models as a viable replacement for drug safety and efficacy studies.³³

TABLE 1 Common mosquito-borne arboviruses and their epidemiology, and recent antiviral research attempts.

Mosquito-borne arbovirus	Epidemiology highlights	Recent antiviral research (in vitro/in vivo)
Chikungunya virus (CHIKV)	<ul style="list-style-type: none"> • Largely confined to Africa and Asia, causing occasional outbreaks.⁷ • Globalization and climate change have contributed to its global spread, reaching more than 60 countries in Asia, Africa, Europe, and the Americas.^{7,8} 	<ul style="list-style-type: none"> • α-Mangostin tested in vitro and in vivo⁹ <ul style="list-style-type: none"> • Cotreatment conditions (in vitro): 8 μM of the compound completely inhibited CHIKV infectivity • The compound significantly reduced viral replication in CHIKV infected C57BL/6 mice
Dengue virus (DENV)	<ul style="list-style-type: none"> • Encompasses four different serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) based on their unique structural and non-structural viral proteins.¹⁰ • Globally, dengue incidence has surged drastically in recent decades, increasing from 505 430 cases in 2000 to 5.2 million in 2019, with Asia shouldering approximately 70% of the global disease burden.¹¹ 	<ul style="list-style-type: none"> • JNJ-1802 tested in vivo on non-human primates (NHPs)¹² <ul style="list-style-type: none"> • The compound was found to be safe and well tolerated in healthy volunteers, successfully completing its phase I human clinical study
Zika virus (ZIKV)	<ul style="list-style-type: none"> • Can be divided into Asian and African lineages based on phylogenetic analysis, both originating in East Africa during the late 1800s or early 1900s, with the Asian lineage emerging during its migration to Southeast Asia and the first detection in Malaysia.¹³ • Unlike other mosquito-borne arboviruses, ZIKV is known to be transmitted sexually <ul style="list-style-type: none"> • There was an increase in sexually acquired ZIKV cases in non-endemic regions, often involving individuals who had sexual contact with partners returning from endemic areas.¹³ • In 2019, Europe reported its first instances of locally transmitted ZIKV disease through mosquitoes, and in 2021, an outbreak of ZIKV activity was identified in India.¹⁴ 	<ul style="list-style-type: none"> • Pinoцембрin tested in vitro¹⁵ <ul style="list-style-type: none"> • The compound was found to inhibit ZIKV in its post-adsorption stages
Mayaro virus (MAYV)	<ul style="list-style-type: none"> • Endemic to tropical forests within the Amazon basin.¹⁶ • Exhibits serological cross-reactivity with other alphaviruses, implying that infection with another alphavirus (such as CHIKV) can cause a rise in MAYV antibodies despite no prior infection with MAYV.¹⁶ 	<ul style="list-style-type: none"> • Microalgae extract (<i>Arthrospira maxima</i>) tested in vitro¹⁷ <ul style="list-style-type: none"> • Extract was shown to exhibit higher MAYV inactivation compared to antiviral drug ribavirin
Japanese Encephalitis virus (JEV)	<ul style="list-style-type: none"> • Can be divided into five genotypes (G1, G2, G3, G4, and G5) with unique distribution patterns. Currently, the dominant genotypes¹⁸ in different regions are as follows: <ul style="list-style-type: none"> • G1—Asia • G2—Australia • G3—Africa and Europe • G4—Uncommon, previously reported in Papua New Guinea and Indonesia • G5—China and South Korea • Primary causative agent of viral encephalitis in many Asian countries, causing approximately 68 000 clinical cases annually¹⁹ 	<ul style="list-style-type: none"> • Luteolin tested in vitro²⁰ <ul style="list-style-type: none"> • The compound was able to inhibit JEV in its post-entry stages, and was found to exhibit virucidal effects against JEV extracellularly
Yellow Fever virus (YFV)	<ul style="list-style-type: none"> • Endemic to tropical and subtropical parts of Africa and Central South America²¹ • Can be divided into seven genotypes,^{22,23} as follows: <ul style="list-style-type: none"> • West Africa genotype I • West Africa genotype II • East and Central African genotype • East African genotype • Angola genotype • South America genotype I • South America genotype II 	<ul style="list-style-type: none"> • Remdesivir tested in vivo hamster model²⁴ <ul style="list-style-type: none"> • Remdesivir significantly improved the survival of Syrian hamsters up to 4 days post-YFV infection

(Continues)

TABLE 1 (Continued)

Mosquito-borne arbovirus	Epidemiology highlights	Recent antiviral research (in vitro/in vivo)
West Nile virus (WNV)	<ul style="list-style-type: none"> • Endemic in various regions, including Asia, Africa, Australia, Europe, and the Middle East²⁵ • Can cause lethal neuroinvasive disease in birds, humans, and horses, and other mammal species²⁵ 	<ul style="list-style-type: none"> • Cilnidipine, mycophenolate mofetil, nitazoxanide, and teriflunomide tested in vitro²⁶ • All four compounds were found to efficiently inhibit WNV in Vero cells and SH-SY5Y human neuroblastoma cells
La Crosse encephalitis virus (LACV)	<ul style="list-style-type: none"> • Uncommon mosquito-borne disease • 787 reported cases and 11 deaths in the United States of America between 2004 and 2013²⁷ • Primarily confined to the United States of America²⁷ 	<ul style="list-style-type: none"> • Rottlerin tested in vivo²⁸ • In MAFIA mice (a strain of C57BL/6 mice) • The compound effectively reduced LACV disease development by 30 to 50% in infected mice
St Louis Encephalitis virus (SLEV)	<ul style="list-style-type: none"> • Causative agent for encephalitis outbreaks in the Americas²⁹ • Remains endemic throughout the United States of America²⁹ • Can be divided into eight genotypes—genotypes I to VIII²⁹ 	<ul style="list-style-type: none"> • No recent successful antiviral research performed • From the year 2012: <i>Heterophyllaea pustulata</i> extracts tested in vitro³⁰ • The extract was not found to inhibit SLEV

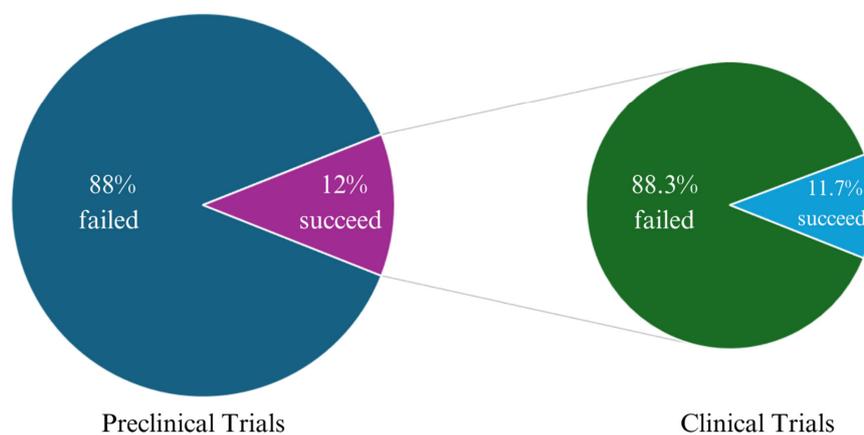


FIGURE 1 Pie chart depicting the percentages of failures and successes of potential therapeutic candidates in preclinical and clinical trials, adapted based on data from Van Norman.³⁶

Additionally, research on animal models is also subjected to challenges regarding ethics and legislations. As explored by Kiani et al.³⁴ and Shim and Kim,³⁸ animal research ethics are deeply rooted in considerations of moral acceptability and the prevention of undue suffering in experimental animals. These ethical principles are vital to ensure the quality and reproducibility of scientific results, as neglecting the ethical treatment of animals can compromise the outcomes of experiments and hinder the reproducibility of research.³⁴

The 4Rs, as presented by Kiani et al.³⁴ are fundamental in shaping ethical guidelines for animal experimentation. Reduction, the first 'R', focuses on minimizing the number of experimental animals through advanced experimental design, literature review, and advanced statistical analysis, emphasizing the importance of reducing animal use. Refinement, the second 'R', seeks to enhance animal welfare by reducing harm, including pain and suffering, through improving living conditions, proper training, anesthesia, and humane euthanasia.³⁴ Replacement, the third 'R', advocates for exploring alternative methods to replace experimental animals,

such as in silico and in vitro techniques, and using invertebrates instead of vertebrates.³⁴ Responsibility, the fourth 'R', centers on promoting animal welfare, advancing scientific understanding of animal experiences, and engaging in public discourse on animal ethics.³⁴

Several organizations, including the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) and the European Centre for the Validation of Alternative Methods (ECVAM), are dedicated to developing alternatives to animal experimentation.³⁴ Despite laws and guidelines, discrepancies still exist, with some species receiving more protection than others under different regulations. Shim and Kim³⁸ emphasized that the ethical considerations surrounding animal experimentation have grown significantly over time, reflecting the increasing importance placed on animal welfare in research. These ethical guidelines ensure that research is conducted responsibly and ethically, ultimately leading to reliable and convincing results.³⁸ Evidently, aligning with these ethical principles is essential to meet societal expectations and uphold the welfare of experimental animals.

3 | TYPES OF ANIMAL MODELS IN ANTIVIRAL RESEARCH

Mosquito-borne arboviruses have drastic global impacts, and it is vitally important to develop antiviral therapeutics for these viruses. As animal models are a requirement in modern-day drug development, the types of animal models available for antiviral research must be well understood. In this review, the application of small animal models (mice and hamster) and non-human primate (NHP) models will be further explored.

3.1 | Small-animal models

3.1.1 | Mouse model

Mice models are a popular small animal model in infectious disease research.^{39,40} Immunocompetent, wild-type mice are typically resistant to arbovirus infection, which limits the availability of appropriate animal models that can mimic the natural cycle of transmission and pathogenesis of arboviruses.³⁹ To overcome this, the mouse genome may be altered to develop traits that facilitate the study of pathogenesis, antivirals, vaccines, immunity, and virus-host interactions in murine models.⁴¹ Alternatively, the arbovirus strains can also be adapted to infect mice to facilitate *in vitro* and *in vivo* studies. In this section, we will explore the contribution of syngeneic mice models, transgenic and transduced mice models in addition to mice-adapted virus strains in arbovirus research.

Syngeneic mouse models

Syngeneic mice models are immunocompetent mice implanted with immortalized cancer cell lines of the same mouse strain to create a range of clinically relevant mutations for immunotherapy research.^{42,43} Additionally, the models also prevent immediate immune rejection of the implanted tumors within the animal model itself.⁴³ These mice models have been employed in antiviral studies, such as those against DENV,^{44,45} influenza A viruses,⁴⁶ and MAYV.⁴⁷ Their great popularity in laboratory settings may be attributed to their cost-effectiveness, ease of maintenance, and similar genetic backgrounds, conferring a reduced genetic variability between individuals and enhanced consistency in experimental outcomes.²⁴

The C57BL/6 and BALB/c mice are prominent examples of syngeneic mice models used in arbovirus research. In fact, these mice models are usually considered the most viable DENV-2 experimental infection models.⁴⁵ In various studies, arbovirus-infected strains are used to investigate the *in vivo* efficacy and pathological effects of potential antiviral compounds. In CHIKV and MAYV-infected mice (tested with ixazomib and favipiravir, respectively), the C57BL/6 model was used to obtain information on the viral load, degree of footpad swelling (a clinical manifestation in mice models), biomarkers of liver damage (characteristic of CHIKV and MAYV infection), and most importantly the effect on proinflammatory cytokines and chemokines.^{48,49} Similarly, BALB/c mice infected with DENV-2 and

MAYV have also been used to study the therapeutic properties of geraniin and silymarin, respectively, providing significant data on any histopathological changes with established parallels to human disease, the viral load, hematological profiles, and the levels of proinflammatory cytokines.^{45,47} Notably, the immunocompetence of these mice models optimizes its suitability in antiviral investigations.

Transgenic mouse models

Many viruses with disease outbreak potential cannot naturally infect immunocompetent wild-type mice, which undoubtedly limits the study of infectious diseases in wild-type mouse models. Thus, transgenic mice have been generated by using molecular genetic techniques to manipulate specific genes (overexpression/knock-out) to produce a model that resembles human disease.^{50,51} The tissue-specific expression of transgenes in these models allows the visualization of relevant pathways and/or the regulation of the disease-associated gene.

The genome of mice models is easily manipulated to create mutant strains that facilitate pathological and physiological investigations of proteins in virus infection.²⁵ Various knockout strains of immunocompromised syngeneic mice have been specifically developed for antiviral research on arboviruses, including IFN α , β , γ R^{-/-}, IFN α , β , R^{-/-}, IFN γ R^{-/-}, IRF3^{-/-}, IRF7^{-/-}, MAVS^{-/-}, STAT1^{-/-}, STAT2^{-/-}.³⁹

A prominent example is the AG129 mouse model strain, which is a double-knockout mouse lacking alpha/beta and gamma interferon receptors (IFN α , β , γ R^{-/-}). In a study by Baldon et al.³⁹ the AG129 mice were susceptible to CHIKV, DENV, and ZIKV, displaying high viraemia levels and mortality rates, and could transmit the virus to mosquito vectors. Another study by Aliota et al.⁵² also described rapid viraemic dissemination in AG129 mice when infected with ZIKV. The successful arboviral infections established in AG129 mice by these researchers show that AG129 is a viable murine model to study potential antiviral therapeutics and develop a cure for these mosquito-borne arboviruses.

The IRF3/7^{-/-} mouse model strain has a diminished type I interferon response due to gene deficiencies in its interferon regulatory factors (IRF) 3 and 7, and when infected with a non-mouse-adapted DENV-2 strain, is competent at DENV replication and subsequent transmission to mosquitoes, indicating successful replication of the DENV natural transmission cycle.⁵³ Concurrently, another study on CHIKV using the IRF3/7^{-/-} mouse model reported that the mice displayed CHIKV-induced hemorrhagic shock, febrility, hypothermia, as well as high viraemia.⁵⁴ Thus, these successful infections in the IRF3/7^{-/-} mouse model on CHIKV and DENV establish its viability as an animal model in antiviral research on arboviruses.

Transduced mouse models

Transduced mice models, which involve the introduction of a transgene into mice through viral vectors, aim to overcome several issues associated with transgenic mice. Despite the versatile applications of transgenic mice, creating transgenic mice is costly, laborious, and must be generated based on the gene under study.⁵¹ A

commonly used viral vector for genetic manipulation of mammalian cells is the adenovirus vector (AdV), especially since it can transiently express the transgene without integrating into the host chromosomes of mammalian cells.⁵⁵ Several recent studies have reportedly established transduced mice models for SARS-CoV-2 infection by intranasally administering adenovirus vectors carrying the human ACE2 gene (hACE2) into BALB/c mice, generating a transduced model of SARS-CoV-2 infection to evaluate the virus variants for their virulence and prospective interventions.^{56,57} For arboviruses, a study reported the use of a transduced mice model to investigate the prophylactic therapeutic efficiency of IFN- α against a lethal dose of CHIKV.⁵⁸ Specifically, the CHIKV-permissible BALB/c mice were intranasally administered with an AdV expressing the mouse IFN- α gene (mDEF201), which allowed the researchers to determine the clinical potential of mDEV201 based on its prophylactic properties and therapeutic activity within 6 h post infection.⁵⁸ Undoubtedly, this paves the way for future studies on arboviruses using AdV-transduced mice models.

Mice-adapted virus strains

Various adaptations have been performed in mice to facilitate arboviral research; however, the virus can also be modified to be effective in murine cells. Note that mice-adapted arboviruses are arboviruses that are specifically adapted to infect and replicate in mice, which are not natural hosts for arboviruses.

The mouse-adapted DENV-2 strain, D220, stands out as a recent and relevant example of a mouse-adapted arbovirus. As compared to the parental strain DS210, the D220 was created to be more infectious and virulent, allowing it to infect less immunocompromised mice with and without antibody-enhanced environments.⁵⁹ This was successfully achieved by passaging through C6/36 mosquito cells and AG129 mice (lacking IFN- α/β and γ receptors), which allowed it to cause significant morbidity and mortality in mice lacking IFN- α/β only. Since then, the D220 has been used in studying DENV immunopathogenesis in *Ifnar*^{-/-} (knockout) type 1 IFN receptor mice models⁶⁰ in addition to testing prospective DENV vaccines in AG129, C57BL/6 and *Ifnar*^{-/-} mice.⁶¹

Undoubtedly, mouse-adapted strains of viruses are a valuable tool in facilitating infectious disease studies of various commercially available mice models.⁶² Additionally, virus modification is unequivocally less of an ethical concern compared to modifying animals in infectious disease research. However, adapting an arbovirus to a non-natural host such as mice could alter its characteristics and resulting outcomes. Ultimately, it may reduce transferability of data such as disease pathogenesis compared to non-adapted viruses.⁶³ Nevertheless, modified virus strains provide a significant contribution to the field of arbovirus research.

3.1.2 | Syrian hamster model

The Syrian hamster is a highly valuable animal model in human disease studies, and its application can be traced back over 60 years.⁶⁴

Hamster models are preferred to murine models in virus infection analysis, as they are more similar to humans in terms of their clinical signs, disease pathogenesis, and immunological responses, while possessing similar advantages to murine models.⁶⁴ Hamster models are also easy to handle, reproduce quickly, and can be genetically manipulated.^{64,65}

Multiple studies on arboviruses, including JEV, YFV, ZIKV, and WNV, have been performed in hamster models.^{64,66-70} For example, the identification of virulence in YFV by isolating residues contributing to disease phenotypes in infected hamster models assisted future *in vivo* investigations on antiviral strategies.⁷¹ A study on WNV also found that the disease severity was dependent on the age of the hamsters used, which assists in optimizing future *in vivo* studies.⁷² In addition, Syrian hamster strains were used to investigate remdesivir as a potential therapeutic and prophylactic against YFV.²⁴ Hamster models are also used in immunization studies, where the candidate MVA-BN YF and YF-SO vaccines successfully induced protective levels of neutralizing antibodies against YFVs.^{24,73} Although the hamster model has also been suggested for anti-CHIKV assays because it supports CHIKV replication, its use is limited due to lack of clinical presentations.⁶⁶ Overall, the hamster model proves to be a noteworthy model for arboviral research.

3.2 | Non-human primate models

NHP models play a vital role in antiviral research because of their close genetic relationship to humans, resulting in similarities in their immune systems and responses.⁷⁴ This significance is underscored by NHPs' susceptibility to various human viruses, including mosquito-borne arboviruses, for which they serve as reservoirs. Furthermore, many reagents designed to investigate human immune responses can be applied to NHP systems due to cross-reactivity.⁷⁴ Consequently, NHPs are frequently used as models to assess potential antiviral therapies' safety and efficacy and gain insights into viral pathogenesis. Specifically, the rhesus macaque has long been regarded as the leading animal model for human research, having been applied since the 1960s.⁷⁵ Notably, the immune system of rhesus monkeys bears greater resemblance to that of humans than rodents, making them the preferred choice for evaluating and drawing connections between immunological responses and central nervous system disorders.⁷⁵

While mice can be infected with some mosquito-borne arboviruses, they are highly sensitive to type I interferons. As a result, mouse models often rely on mice deficient in type I interferon signaling, which may not fully replicate the virus-host interactions seen in humans.⁷⁴ Therefore, NHPs have become essential for studying these viral infections. DENV, with its four serotypes and cross-reacting antibodies, presents a challenge for NHP modeling. Various NHP species, including macaques, patas monkeys, and African green monkeys, have been tested for DENV infection.⁷⁴ While they can replicate the virus and exhibit viraemia, NHPs do not manifest the same symptoms as infected humans.⁷⁴ However, NHPs

have successfully been used to evaluate vaccine-induced immunity against DENVs, confirming that attenuated viral vaccines do not replicate at high levels in live subjects. Importantly, numerous vaccine approaches, proven safe in NHPs and capable of eliciting high levels of neutralizing antibodies against DENV, have progressed to human clinical trials.⁷⁴

ZIKV has also been studied in NHPs, including cynomolgus macaques, rhesus macaques, and pigtail macaques.⁷⁴ Infection through subcutaneous injection replicates human infection features like fever, mild weight loss, and elevated liver enzymes.⁷⁴ Viraemia is detected in NHPs, and the virus can be found in various bodily fluids and tissues. NHPs mount immune responses and can be used to study vaccine-mediated protection. To illustrate, Haese et al.⁷⁶ describe the importance of NHP in understanding congenital ZIKV infection, which has been extensively studied in various NHP species. In adult macaques, ZIKV infection was reported to cause transient symptoms such as rash, conjunctivitis, and fever.⁷⁶ Moreover, high viremia can be observed, where the virus can be found in urine, saliva, lacrimal fluid, cerebrospinal fluid, semen, vaginal swabs, and various tissues, indicating its ability to establish a widespread infection.⁷⁶

Overall, NHPs play a crucial role in understanding and developing interventions for mosquito-borne arbovirus infections, allowing progressive research that complements *in vitro* systems and supplements research from small animal models (Table 2).

4 | ADVANCEMENTS IN ANIMAL MODELS FOR ANTIVIRAL RESEARCH—NEW TECHNOLOGIES AND ANIMALS

Advances in developing animal models for antiviral research could potentially help researchers spearhead their progress with fewer ethical concerns and lower overall costs. This section will discuss advances in the standard animal model systems used in antiviral research on mosquito-borne arboviruses and some lesser-known models, including their limitations.

TABLE 2 Examples of animal models used for antiviral research for respective arboviruses and their primary findings.

Type of research	Animal model used	Primary findings
Antiviral potential of favipiravir against ZIKV ⁷⁷	Cynomolgus macaques (NHP)	Favipiravir significantly reduces ZIKV viral load in plasma compared to the untreated groups
Antiviral potential of favipiravir against MAYV ⁴⁸	C57BL/6 mice (syngeneic mice)	Pre-treated and concurrently treated MAYV-infected mice displayed significantly reduced infectious viral particles and viral RNA transcripts in their tissues and blood
Antiviral efficacy of remdesivir against YFV ²⁴	Syrian hamster	Remdesivir significantly improved the survival of Syrian hamsters up to 4 days post-YFV infection

4.1 | Small-animal models

4.1.1 | Mouse model

According to Douam and Ploss,⁷⁸ neutrophils are essential in the human immune response to viral infection, and mice have different granulocyte colony-stimulating factors (G-CSF) compared to humans. Therefore, Douam and Ploss⁷⁸ modified the mice's genetic makeup to humanize the G-CSF and genetically remove the mouse G-CSF receptor; this mouse model was named the MISTRGGR model. This modification increased the production of neutrophils in the mice's bone marrow while preventing neutrophil differentiation competition between the human and mouse G-CSF receptors, allowing the human-like neutrophils to proliferate. These neutrophils exhibited similar functions to those found in healthy humans, including the ability to phagocytose pathogens and respond to inflammation by producing reactive oxygen species (ROS) and expressing specific receptors.⁷⁸ Additionally, these humanized mice displayed improved neutrophil recruitment to various tissues upon inflammation, which is crucial for studying human immune responses.⁷⁸ However, this model has limitations, including susceptibility to opportunistic infections and a relatively short lifespan.⁷⁸ While the MISTRGGR model represents a significant advancement in modeling human immune responses involving neutrophils, it still has some challenges to overcome. Nonetheless, it holds tremendous potential for advancing our understanding of neutrophil functions in various diseases and immunological contexts.

4.1.2 | Syrian hamster model

A study on encephalitic alphaviruses in a Syrian hamster model found that the lethality, pathology, and viremia observed in infected hamsters differed from those observed in humans and NHP models, indicating that hamsters might not be a suitable animal model to study antiviral therapies for encephalitic alphaviruses.⁷⁹ Recently, transgenic hamster models have been used for antiviral research but have not

been explored for research into mosquito-borne arboviruses. When hACE2 hamsters were used to study SARS-CoV-2, the infected transgenic hamsters developed lethal and severe disease, eventually succumbing to SARS-CoV-2 by the fifth day post-infection.⁸⁰ It was also reported that the viral infection observed in hACE2 hamsters were more extensive than that of in wild type (WT) Syrian hamsters, with more lethal outcomes observed.⁸⁰ However, as the transgenic model has not yet been studied in arboviruses, its efficacy and applicability cannot be guaranteed, and further studies should be performed using the hACE2 transgenic hamster model to determine its suitability in studying mosquito-borne arboviruses compared to WT hamsters.

4.1.3 | Tree shrew model

Subsequently, Jiang et al.⁸¹ and Kayesh et al.⁸² have reported the tree shrew as an emerging murine small-animal model for human viral infections, including mosquito-borne arboviruses such as DENV and ZIKV. The term 'tree shrew' usually denotes *Tupaia belangeri* (northern tree shrew) or *T. belangeri chinensis* (Chinese tree shrew). These species belong to the order Scandentia, which is divided into two families: *Tupaiaidae* and *Ptilocercidae*.⁴³ Furthermore, phylogenetic analysis of the tree shrew provides evidence for its close evolutionary relationship to primates; this animal has greater genomic similarities to humans than murine species such as marmots, mice, and rats.⁸¹ When subcutaneously infected with ZIKV, the tree shrew displayed similar responses as humans, such as manifestation of skin rashes, cutaneous inflammation, and transient viraemia; no febrility was observed.⁸² Importantly, adult tree shrews produced an antibody response that prevented reinfection with a homologous virus upon ZIKV infection, indicating that the tree shrew model is a viable candidate for vaccine screenings.⁸² In addition, when infected with DENV-2 and DENV-3 (intravenous or subcutaneous), tree shrews displayed symptoms of dengue similar to humans, such as increased body temperature (febrility) and proliferation of DENV in the brain. Interestingly, the model exhibited low viraemia and thus did not display any symptoms of severe dengue.^{81,82} While tree shrews show promise as animal models for studying arboviral infections, more research into the tree shrew model is required to develop a standardized transgenic model and remove genetic variability.

4.2 | NHP models—A Malaysian perspective

Rhesus macaques are not readily accessible in mosquito-borne arbovirus endemic countries such as Malaysia and their use in research would incur high costs, resulting in low sample sizes. Long-tailed macaques (*Macaca fascicularis*) are a more accessible alternative NHP model for antiviral research on mosquito-borne arboviruses in Malaysia. According to a population study by Karuppanan et al.⁸³ in 2014, it was estimated that there were 133 403 long-tailed macaque individuals in Malaysia that year. There have been no additional population studies on long-tailed macaques in Malaysia since the 2014 study. Therefore, the species' current population size can only

be estimated using the established population growth rate. Given the species' population growth rate of 5% annually,⁸³ the estimated population size in 2023 could be 206 951. Officially, long-tailed macaques are considered pests in Malaysia due to their food-motivated behavior leading to human-wildlife conflict. As Choong et al.⁸⁴ illustrated, long-tailed macaques have successfully adapted to urban areas, and are known to cause bodily harm to humans and damage to property during conflict. Furthermore, local authorities have resorted to culling to control the macaque population, with the goal of mitigating this long-term issue.⁸⁵ For these reasons, this review suggests enlisting this pest species as an animal model candidate for antiviral research on mosquito-borne arboviruses, precluding the need for culling by local authorities.

Based on a report by the National Primate Research Centers (NPRC), long-tailed macaques are one of the most used NHP models in biomedical research, including in toxicology and pharmaceutical research, and research into infectious diseases such as Ebola virus and SARS-CoV, as well as SARS-CoV-2.⁸⁶ Despite its significant contributions in these fields, this animal model has yet to be utilized in antiviral research on mosquito-borne arboviruses. Existing studies on the seroprevalence of mosquito-borne arboviruses reported that a low percentage of long-tailed macaques sampled across the studies displayed seropositivity for CHIKV, WNV, and ZIKV, indicating prior exposure to the arbovirus.⁸⁷⁻⁸⁹ This indicates that mosquito-borne arboviruses indeed infect long-tailed macaques; however, no research has been done to test potential antiviral therapeutics on these species.

There are also concerns regarding using long-tailed macaques in antiviral research on arboviruses. Despite their negative connotation as pests in Malaysia, long-tailed macaques are actually listed as endangered on the IUCN Red List, with a decreasing global population trend.⁹⁰ From that perspective, Choong et al.⁸⁴ add that long-tailed macaques have become rampant pests involved in human-wildlife conflict because their habitats are diminishing. Reportedly, Malaysia's forest cover has declined from 91.9% in 2011 to 83% in 2020, driving the long-tailed macaques into urban areas to obtain food.⁸⁴ At the same time, Inglis⁹¹ states that the invasiveness status of a species does not excuse its killing, calling it an ethically contentious act; efforts to conserve the long-tailed macaque population are still warranted. Does the significant need to discover a therapeutic for arboviruses that affect millions of people annually outweigh the need to conserve an endangered species? Does the pest status of long-tailed macaques in Malaysia justify their use in animal testing, placing a lower value on their lives? Using these species as a potential animal models for arbovirus research requires careful consideration. Aside from strict ethical considerations and adherence to animal testing legislation, their status as an endangered species should also be considered. When these questions have been answered and a decision can be made, more studies can be conducted on developing long-tailed macaques as an animal model for arbovirus research. In summary, long-tailed macaques in Malaysia show promise as models for arbovirus research, but ethical considerations, coupled with their endangered status, must be carefully weighed against the urgent

TABLE 3 Summary of advantages and disadvantages of small animal models and NHP models discussed in the previous section.

	Small animal model	Non-human primate (NHP) model
Advantages	<ul style="list-style-type: none"> • Cost effective—inexpensive to obtain. • Can be easily genetically manipulated to simulate human responses. • Rapid reproduction cycles. • Easy to handle in laboratory settings. 	<ul style="list-style-type: none"> • Close genetic relationship to humans—similar immune systems and responses. • NHPs are crucial for evaluating vaccine-induced immunity and safety against arboviruses.
Disadvantages	<ul style="list-style-type: none"> • Low genetic variability limits applications of results to diverse populations. • Hamster models which are less developed are known to present limited clinical manifestations. • Small animal models may not fully replicate human immune responses and disease manifestations. 	<ul style="list-style-type: none"> • NHPs raise high ethical concerns due to their close genetic relationship to humans. • NHPs can be expensive to acquire and maintain. • Longer reproductive cycles, which lead to longer times required to produce large sample sizes.

need for antiviral solutions. Striking a balance between medical research and species conservation remains a critical challenge.

4.3 | Alpaca model

In the year 2022, a rare case of JEV was reported in an alpaca from South Australia, the first known report of JEV in the species.⁹² The application of an alpaca model in mosquito-borne arbovirus research has not been reported. Nevertheless, a novel alpaca model has been used in a study of MERS-CoV infection of ex vivo alpaca tracheal explants.⁹³ Although there is insufficient literature available to determine the applicability of the alpaca as an animal model for mosquito-borne arbovirus research, the report of the rare JEV-infected alpaca indicates that this animal model could be used in future studies and the discovery of new technologies.

5 | SELECTING THE RIGHT ANIMAL MODEL

Research animal models must be selected based on their characteristics, and each model, from small animal (mice and hamster) models to NHP models, carries its own advantages and disadvantages when it comes to applying the model to antiviral research. The advantages and disadvantages of each animal model discussed are presented in [Table 3](#).

6 | CONCLUSIONS

The emergence and prevalence of mosquito-borne arboviruses continue to challenge global public health. Ergo, understanding these viruses and developing effective antiviral strategies requires the

application of animal models to ensure drug safety and efficacy. Mouse models with humanized genetic modifications and tree shrews have shown promise in replicating human immune responses to arboviruses, while NHP models, particularly rhesus macaques, have provided valuable insights into vaccine development and disease pathogenesis. However, the choice of animal models for research raises ethical concerns, with the 4Rs guiding responsible animal experimentation. This review introduced the use of long-tailed macaques in Malaysia as potential animal models for arbovirus research. This suggestion confronts a complex ethical dilemma, balancing the urgent need for antiviral solutions against the conservation of the long-tailed macaque as an endangered species. Ultimately, the decision to use long-tailed macaques in research should take account of strict ethical guidelines and the endangered status of these animals. In conclusion, animal models are invaluable in advancing our understanding of mosquito-borne arboviruses and developing antiviral interventions. However, ethical considerations and conservation efforts should guide the responsible use of these models in scientific research. Achieving this balance is a critical challenge as we work towards improving global health and safeguarding biodiversity. To make progress in the search for the most suitable animal model in antiviral research for mosquito-borne arboviruses, priority should be given to determining the efficacy and suitability of new animal models and technologies, such as the transgenic hamster models, alpaca model, and tree shrew model discussed in this review. The data obtained from these studies can be used as a benchmark in establishing the ideal animal model for mosquito-borne arbovirus research.

AUTHOR CONTRIBUTIONS

Megan Caifeng Tang: Conceptualization (lead); writing-original draft (lead); investigation (lead); writing-review and editing (equal); **Ka Heng Wong:** writing-review and editing (supporting), **Adzzie Shazleen Azman:** Supervision (lead); Conceptualization (equal); writing-review and editing (equal). **Rafidah Lani:** Supervision (lead); Conceptualization (equal); writing-review and editing (equal).

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None declared.

ETHICS STATEMENT

Not applicable.

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