HHS Public Access

Author manuscript

*Curr Opin Virol*. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

*Curr Opin Virol*. 2017 August ; 25: 34–40. doi:10.1016/j.coviro.2017.06.011.

# Macaque monkeys in Zika virus research: 1947-present

**Christina Newman**[a], **Thomas C. Friedrich**[b,c], and **David H. O'Connor**[a,c]

[a]University of Wisconsin-Madison Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, 3170 Medical Foundation Centennial Building (MFCB), 1685 Highland Avenue, Madison, WI 53705

[b]University of Wisconsin-Madison Department of Pathobiological Sciences, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706

[c]Wisconsin National Primate Research Center (WNPRC), 1220 Capitol Court, Madison, WI 53715

## Abstract

Zika virus was first isolated in 1947 from an exotic rhesus macaque. Nearly 70 years later, the emergence of Zika virus in the Americas and its newly described association with birth defects has motivated the development of captive macaque monkey models of human Zika virus infection. This review describes similarities between macaque and human Zika virus pathogenesis and discusses specific advantages and disadvantages of using macaques instead of other laboratory animal models. In particular, macaques provide an outstanding model for understanding in-utero Zika virus infections that are essential for evaluating preclinical interventions for use in pregnancy.

## A brief history of Zika virus

Zika virus (ZIKV) is a mosquito-borne and sexually-transmitted flavivirus that is closely related to dengue virus (DENV) [1–3]. It was first isolated in 1947 in the Zika Forest near Entebbe, Uganda, from the serum of a sentinel rhesus macaque kept there for yellow fever surveillance [1]. ZIKV was subsequently isolated from *Aedes africanus* mosquitoes in 1948 and from humans in 1952 in Uganda and Tanzania [1, 4–6]. Isolated human cases and outbreaks of ZIKV were periodically identified in Africa. In the 1970s and 1980s, ZIKV emerged in Asia [6–9]. ZIKV infection was thought to be largely asymptomatic or to cause only a mild, febrile illness characterized by fever, rash, conjunctivitis, headache, and joint pain. It was not historically associated with widespread outbreaks or epidemics [6, 7, 10].

Corresponding author: David H. O'Connor, AIDS Vaccine Research Laboratory (AVRL), 585 Science Drive, Madison, WI 53711, dhoconno@wisc.edu.



Conflicts of interest

Authors have identified no conflicts of interest associated with this publication.
The authors declare that there are no conflicts of interest.

Author declaration

All authors reviewed the final draft of this manuscript.

Then in 2007, a large ZIKV outbreak occurred on Yap Island in the South Pacific [11]. This ZIKV epidemic infected approximately 70% of residents and was the largest ZIKV outbreak ever recorded at the time [11]. Subsequently, ZIKV caused outbreaks throughout the Pacific Islands, including French Polynesia [12–15]. Throughout this period ZIKV was largely ignored by the scientific community; at the beginning of 2013 there were more articles in NCBI PubMed by authors with the surname “Zika” (65) than there were articles with “Zika virus” in the title or abstract (62).[1]

The emergence of ZIKV as a global public health emergency in 2016 [16] was therefore surprising. The FIFA Confederations Cup was hosted by Brazil in 2013 and though existing data are not definitive, retrospective phylogenetic analyses suggest that ZIKV was introduced to northeast Brazil at approximately the same time [17], perhaps from visiting players or their supporters. ZIKV spread in northern Brazil, and likely throughout the region, unnoticed for nearly two years until its presence was associated with a surge in reports of febrile rash [18]. From there, ZIKV spread rapidly throughout the Americas, with autochthonous cases identified in 48 countries and territories as of March 2017 [19].

## Zika virus Transmission

In the Americas, ZIKV is thought to be primarily transmitted between humans by *Aedes aegypti (Ae. aegypti)* mosquitoes [20–24]. This mosquito species has adapted to live in close proximity to humans and transmits a number of important human pathogens including DENV and chikungunya virus (CHIKV) [24, 25]. Rapid human population growth and unprecedented expansion of urban areas has resulted in inadequate water and sewer infrastructure, and has made controlling *Ae. aegypti* populations exceedingly difficult [24]. In addition to vector-borne and vertical transmission, ZIKV is also sexually transmitted [26]. Sexual transmission of ZIKV was first reported in 2011, but received little attention until additional cases were identified during the ongoing outbreak in the Americas [2, 26]. To date, cases of ZIKV sexual transmission have included male-to-male, male-to-female, and female-to-male transmission [2, 27–29]. It is likely that sexual transmission went previously unnoticed in areas with autochthonous transmission, as it is difficult to distinguish between sexual and vector-borne transmission. The contribution of sexual transmission to the epidemiology of ZIKV remains unclear [30]. From an epidemiological perspective, it is important to disentangle vector-borne and sexual transmission, and determine how the mode of transmission influences the pathology of ZIKV infection. To this end, developing a translatable animal model that allows recapitulation of the primary modes of ZIKV transmission is especially important.

## Congenital Zika syndrome

Congenital ZIKV infection is associated with a spectrum of adverse outcomes including fetal demise, in utero growth restriction, and a range of brain and neurological defects, including microcephaly (Table 1). Collectively, these outcomes have been designated

[1]Pubmed search parameters: ((((zika) AND ("1947/01/01"[Date - Publication] : "2013/01/01"[Date - Publication]))) AND zika[Author]) vs. ((((zika virus) AND ("1947/01/01"[Date - Publication] : "2013/01/01"[Date - Publication]))) NOT zika[Author])

"congenital Zika syndrome (CZS)" [31–33]. Currently, the prevalence of CZS is unclear. A study from Rio de Janeiro found that 42% of infants infected in utero had clinical and/or brain abnormalities [34]. In contrast, despite having thousands of women infected with ZIKV during pregnancy, Puerto Rico has reported only 16 cases of CZS as of this writing [35]. The United States Zika Pregnancy Registry reports an intermediate CZS risk of approximately 5%, with 63 birth defects in 1,311 completed pregnancies as of March 28, 2017 [36]. This discrepancy may be due to the use of different criteria for diagnosing CZS. It may also reflect genuine differences in the outcome severity of congenital ZIKV infection in different populations; for example, pre-existing immunity to DENV, which has been associated with increased ZIKV replication in vitro and more severe pathology mice [37, 38], is much more common in Brazil than in the continental United States. Nonetheless, data are concordant with respect to two key points: first, CZS is the most common pathologic consequence of ZIKV infection and second, CZS severity can range from mild to very severe, and there is currently no way to quantify or mitigate this risk.

## Animal Models of Zika virus Infection

It is difficult to study acute ZIKV infection and pathogenesis in humans because a large proportion of infections, up to 80%, are subclinical [11]. In addition, identifying past infection is hindered by serologic cross-reactivity between closely related flaviviruses; serologic tests may not differentiate between ZIKV infection and infection with related flaviviruses, such as DENV or yellow fever virus, or vaccination [39]. Animal models allow for timed infections and thorough analysis of acute viral replication kinetics, even if an infection is subclinical. Animal infections provide a framework within which neurologic complications, viral distribution in tissues, maternal-fetal transmission, and CZS can be studied in detail. To date, descriptions of CZS are limited to a relatively small number of human clinical cases, but further development of animal models may provide a more comprehensive description of the pathology and neurodevelopmental outcomes [31, 32].

ZIKV infection has been modeled in mice, neonatal pigs, chicken embryos, and nonhuman primates [40–56]. Each of these models has strengths and weaknesses making them more or less suited for one or more areas of ZIKV research. For example, mice are relatively inexpensive, have short generation times, and large litters, and may be well-suited for initial vaccine development and therapeutic evaluation studies, but may be less useful for natural history studies because most susceptible mouse strains are interferon deficient and may display exaggerated pathology [41–43]. Differences in placentation (Table 2) and the timing for brain and central nervous system (CNS) development in mice versus humans may also limit the translatability of mouse models for studies of CZS pathophysiology [57, 58]. Although these limitations exist, mouse models of ZIKV infection have been instrumental in identifying potential tissue and cellular targets of ZIKV in vivo [44, 59, 60]. However, studies of ZIKV infection in mice have also reported findings such as testicular atrophy, which has not been reported in human clinical cases to date [61]. Both the chick embryo and neonatal pig models may be very useful for testing therapeutics and/or understanding ZIKV infection outcomes in the early neonatal period respectively, and are relatively inexpensive [49, 55]. However, 119 these ZIKV infection models both bypass the maternal-fetal interface, a potentially important barrier and/or site of virus replication in congenital ZIKV

infections [49, 55, 62]. Animal models that bypass natural barriers in congenital transmission have limited translatability to human infections with regard to the epidemiologic risk of CZS and maternal-fetal transmission of ZIKV.

## Nonhuman Primate Models of Zika virus Infection

Given that ZIKV was initially isolated from a rhesus macaque, it is perhaps not surprising that macaques are susceptible to ZIKV in laboratory studies. In the past year, several groups have shown that rhesus, cynomolgus, and pig-tailed macaques can be infected with a variety of ZIKV strains, including isolates from Uganda [52], Thailand [51], French Polynesia [46] [63], Brazil [54], and Puerto Rico [51, 56]. Since African and Asian ZIKV isolates are approximately 90% nucleotide identical, and Asian/American ZIKV isolates are 99% nucleotide identical [52], it is likely that macaques are permissive for infection with all, or nearly all, global ZIKV strains. This is additionally supported by successful infection of macaques with the highly mouse-adapted MR766 ZIKV strain [52].

Zika virus can productively infect macaques via multiple routes. To mimic vector-borne transmission, our initial studies, and those of other groups, infected macaques subcutaneously with doses ranging from $10^2$ to $10^6$ PFU [46, 47, 51, 53, 63]. Intravenous inoculation has also been successfully utilized to infect macaques [54] and researchers at University of California Davis (UC-Davis) and others are exploring the use of intravaginal and intrarectal transmission to model sexual transmission [71]. Finally, intra-amniotic administration of ZIKV has been used for direct in utero infection of fetuses (Koen Van Rompay, personal communication, April 24, 2017). Thus, all three major routes of transmission -- vector, sexual, and vertical -- can be modelled efficiently in macaques. Moreover, our group recently demonstrated that macaques can also be infected via high-dose application of ZIKV directly to the tonsils, providing evidence for a theoretical risk of transmission via the oropharyngeal mucosa [64]. In addition, we have also shown that feeding ZIKV-infected mosquitoes on macaques is a reliable route of transmission associated with delayed, and modestly higher, peak viremia than needle inoculation in three of four animals [65]. Mosquito challenges may be more widely used in the future in laboratories that have sufficient expertise in mosquito husbandry and containment because it most closely mimics the epidemiology and physiology of human infections. In addition, data from studies of other flaviviruses suggest that vector transmission may differ, sometimes significantly, from experimental modes of transmission [66]. In particular, vector saliva has immune modulatory effects that may influence the progression and pathology of infections [67–69].

Macaques and humans infected with ZIKV are virologically and clinically similar. Plasma viremia typically peaks within the first five days of infection and resolves within 10–14 days. Viral RNA can be detected in lymph nodes after resolution of viremia, though this does not necessarily imply that lymph nodes are sanctuaries for replication-competent virus [54]. Lower titers of viral RNA are detected in other fluids, including urine, saliva, and cerebrospinal fluid. Persistence of ZIKV RNA in semen has not been evaluated in macaques, though in people viral RNA can be detected months after infection [70]. Like most infected people [11], macaques infected with ZIKV do not typically exhibit febrile rash or other

clinical symptoms such as joint pain or swelling, or muscle pain [46]. However, inoculation site rash and joint swelling have been reported by some investigators [56]. Immune responses to ZIKV are robust; rechallenge with homologous or heterologous ZIKV does not cause recrudescent viremia [52]. We are not aware of any ZIKV-associated GBS in macaques, though this is not surprising given the rarity of GBS in people. In addition to natural history studies, nonpregnant macaques have been used to model sexual transmission of ZIKV, study tropism and persistence of ZIKV, characterize clinical isolates of ZIKV, and test ZIKV vaccine platforms [47, 50, 54, 56, 71].

## Understanding congenital Zika syndrome in macaques

The similarities between human and macaque ZIKV infection are useful for studying maternal-fetal transmission and CZS. Macaques have been used as models for understanding human pregnancy and disease for decades. This is due, in part, to the 1977 ban on the export of rhesus macaques from India [72]. The vital need for these animals in biomedical research [73] motivated the establishment and expansion of rhesus macaque breeding colonies in the United States. Thousands of macaque pregnancies have been carefully monitored in recent decades, generating a wealth of husbandry, reproductive, and developmental biology knowledge. Macaque gestation is similar to that of humans in its division into trimesters, placentation and blood supply remodeling, and fetal developmental trajectory (Table 2) [57].

To date, two studies have investigated the impact of ZIKV infection on fetal development and outcomes [48, 63]. One of these studies, led by our group, aimed to mimic vector transmitted ZIKV infection during pregnancy using a subcutaneous dose of $10^4$ PFU. Two macaques each were infected in the first and third trimesters of pregnancy. Ten days before full gestation, the neonates were delivered by cesarean section and analyzed for fetal injury, growth restriction, and ZIKV infection. All four fetal macaques had pathological abnormalities; both of the first trimester infections exhibited significant injury to the visual system. Ocular pathology correlated with the presence of viral RNA in the optic nerve, among other tissues. Notably, the detection of viral RNA in all four fetuses provides unequivocal evidence of 100% vertical transmission efficiency. While none of the four neonates had microcephaly, all four had below average head circumference and reduced head growth velocity in the last month of pregnancy. These results mirror observational cohorts of human neonates, where microcephaly is rare but other manifestations of CZS are common [34, 74]. This suggests that ZIKV infection of macaques, particularly in the first trimester, consistently causes fetal injury and provides a model for evaluating ZIKV pathogenesis, studying potential cofactors that may increase the severity of in utero ZIKV infection (e.g., preexisting DENV immunity), and evaluating the safety and efficacy of interventions to mitigate fetal pathology (e.g., hyperimmune globulin or monoclonal antibody treatments).

One remarkable feature of ZIKV infections in pregnant macaques is the observation of a prolonged period of detection of plasma viremia that parallels observations that have been made in some women, including an asymptomatic case [75–77]. In nonpregnant humans and monkeys, ZIKV plasma viremia becomes undetectable in approximately two weeks, but in pregnant women, persistent detection of serum or plasma viremia has been described for

more than 10 weeks after clinical symptom onset [77]. Three of four pregnant macaques had prolonged viremia, including one animal who had detectable ZIKV RNA in her plasma for 10 weeks [63]. Additional work is needed to determine the anatomic source of replicating virus during sustained viremia and to assess whether the risk of fetal injury is correlated to the duration of viremia. If it is, longitudinal blood sampling of pregnant women might provide a non-invasive biomarker for fetal CZS risks that cannot be easily visualized via ultrasound.

A second study infected a single pregnant pigtailed macaque at 119 days gestation with a Cambodian ZIKV isolate administered five times at a dose of $10^7$ plaque forming units (PFU) per inoculation [48]. This supraphysiologic dose is approximately 5,000 times higher than the dose used in the study described above and is likely much higher than women would encounter naturally. While the dam showed no signs of clinical illness, the fetus developed periventricular lesions and occipital-parietal asymmetry by 10 days post infection (dpi) [48]. At the time of the fetal necropsy that was performed at 162 days' gestation, cerebral white matter hypoplasia, periventricular white matter gliosis, and axonal and ependymal injury were diagnosed [48]. This study demonstrates how high ZIKV challenge dose might be used to evaluate extreme phenotypes including severe neuropathology and microcephaly, however, more data are needed to understand how consistently these phenotypes are observed under experimental conditions.

Although to date, the number of macaque studies (n = 2) that have investigated ZIKV infection during pregnancy and CZS are small, the findings in macaques have been similar to findings in human clinical case reports (Table 1) in terms of identification of abnormalities in the ocular system and reduction in head growth trajectory or microcephaly [48] [63]. Further development of the macaque model of ZIKV infection during pregnancy may yield additional similarities to humans in terms of fetal outcomes.

## Challenges and opportunities

Zika virus is poised to threaten a generation of pregnancies in the Americas, particularly in regions where its mosquito vectors are abundant. Fortunately, protective immunity can be elicited by natural infection and by vaccination [47, 51, 52]. Within a decade, it is likely that a safe and effective vaccine that protects women from ZIKV will be available. Until such a vaccine is available, it is important to develop a better understanding of ZIKV pathogenesis and countermeasures to protect pregnant women and their babies. The similarities between macaque and human pregnancies and the clinical course of ZIKV provides an opportunity to perform timed infections, ask questions that require invasive sampling, and test high-risk, high reward interventions. The macaque model, however, is not perfect. Studies that require impregnating macaques and intensive follow-up are necessarily very expensive and only possible at facilities with extensive experience and infrastructure to support macaque husbandry. Demand and cost limit the number of animals available for studies, limiting statistical power. For this reason, many aspects of the natural history of ZIKV in pregnancy will likely be resolved more effectively using large observational cohorts of pregnant women than small numbers of pregnant macaques. Nonetheless, the NHP model allows for detailed analyses that are not possible in human infections. NHP studies will be indispensable for

understanding the mechanisms of CZS and for evaluating measures to reduce and eliminate the impacts of this newly recognized disease.

## Acknowledgments

Financial support

Financial support for this work was provided by the DHHS/PHS/NIH (R01Al116382-01A1 to DHO) and P51OD011106 awarded to the WNPRC, Madison-Wisconsin.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952; 46:509–520. [PubMed: 12995440]
2. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, Lanciotti RS, Tesh RB. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011; 17:880–882. [PubMed: 21529401]
3. Moureau G, Cook S, Lemey P, Nougairede A, Forrester NL, Khasnatinov M, Charrel RN, Firth AE, Gould EA, de Lamballerie X. New insights into flavivirus evolution, taxonomy and biogeographic history, extended by analysis of canonical and alternative coding sequences. PLoS One. 2015; 10:e0117849. [PubMed: 25719412]
4. Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952; 46:521–534. [PubMed: 12995441]
5. Smithburn KC. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. J Immunol. 1952; 69:223–234. [PubMed: 14946416]
6. Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg. 1954; 48:139–145. [PubMed: 13157159]
7. Simpson DI. ZIKA VIRUS INFECTION IN MAN. Trans R Soc Trop Med Hyg. 1964; 58:335–338. [PubMed: 14175744]
8. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, Guzman H, Tesh RB, Weaver SC. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis. 2012; 6:e1477. [PubMed: 22389730]
9. Olson JG, Ksiazek TG, Suhandiman, Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. Trans R Soc Trop Med Hyg. 1981; 75:389–393. [PubMed: 6275577]
10. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. J Hyg (Lond). 1979; 83:213–219. [PubMed: 489960]
11. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009; 360:2536–2543. [PubMed: 19516034]
12. Wæhre T, A Maagard, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. Emerg Infect Dis. 2014; 20:1412–1414. [PubMed: 25062427]
13. Nishiura H, Kinoshita R, Mizumoto K, Yasuda Y, Nah K. Transmission potential of Zika virus infection in the South Pacific. Int J Infect Dis. 2016; 45:95–97. [PubMed: 26923081]
14. Kucharski AJ, Funk S, Eggo RM, Mallet HP, Edmunds WJ, Nilles EJ. Transmission Dynamics of Zika Virus in Island Populations: A Modelling Analysis of the 2013–14 French Polynesia Outbreak. PLoS Negl Trop Dis. 2016; 10:e0004726. [PubMed: 27186984]
15. Bierlaire D, Mauguin S, Broult J, Musso D. Zika virus and blood transfusion: the experience of French Polynesia. Transfusion. 2017; 57:729–733. [PubMed: 28185278]

16. Gulland A. Zika virus is a global public health emergency, declares WHO. BMJ. 2016; 352:i657. [PubMed: 26839247]

17. Faria NR, Azevedo RS, Kraemer MU, Souza R, Cunha MS, Hill SC, Thézé J, Bonsall MB, Bowden TA, Rissanen I, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science. 2016; 352:345–349. [PubMed: 27013429]

18. Globo Comunicação e Participações S.A., Bahia. Identificado vírus causador de doença misteriosa em Salvador e RMS. 2015. http://g1.globo.com/bahia/noticia/2015/04/identificado-virus-causador-de-doenca-misteriosa-em-salvador-e-rms.html

19. Pan American Health Organization. Regional Zika virus epidemiological update (Americas). 2017. http://www2.paho.org/hq/index.php?option=com_content&id=11599&Itemid=41691

20. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. Am J Trop Med Hyg. 1969; 18:411–415. [PubMed: 4976739]

21. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 2013; 7:e2348. [PubMed: 23936579]

22. Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, Fontenille D, Paupy C, Leroy EM. Zika virus in Gabon (Central Africa)--2007: a new threat from Aedes albopictus. PLoS Negl Trop Dis. 2014; 8:e2681. [PubMed: 24516683]

23. Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015; 110:569–572. [PubMed: 26061233]

24. Marcondes CB, Ximenes MF. Zika virus in Brazil and the danger of infestation by Aedes (Stegomyia) mosquitoes. Rev Soc Bras Med Trop. 2016; 49:4–10. [PubMed: 26689277]

25. Powell JR, Tabachnick WJ. History of domestication and spread of Aedes aegypti--a review. Mem Inst Oswaldo Cruz. 2013; 108(Suppl 1):11–17. [PubMed: 24473798]

26. D'Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, Descamps D, Damond F, Leparc-Goffart I. Evidence of Sexual Transmission of Zika Virus. N Engl J Med. 2016; 374:2195–2198. [PubMed: 27074370]

27. Deckard DT, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, Kwit N, Mead P. Male-to-Male Sexual Transmission of Zika Virus--Texas, January 2016. MMWR Morb Mortal Wkly Rep. 2016; 65:372–374. [PubMed: 27078057]

28•. Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. Clin Microbiol Infect. 2017:1–10. [PubMed: 27908744]

29. Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. Suspected Female-to-Male Sexual Transmission of Zika Virus - New York City, 2016. MMWR Morb Mortal Wkly Rep. 2016; 65:716–717. [PubMed: 27442327]

30. Grischott F, Puhan M, Hatz C, Schlagenhauf P. Non-vector-borne transmission of Zika virus: A systematic review. Travel Med Infect Dis. 2016; 14:313–330. [PubMed: 27425793]

31•. Miranda-Filho DB, Martelli CM, Ximenes RA, Araújo TV, Rocha MA, Ramos RC, Dhalia R, França RF, Marques Júnior ET, Rodrigues LC. Initial Description of the Presumed Congenital Zika Syndrome. Am J Public Health. 2016; 106:598–600. [PubMed: 26959258]

32. França GV, Schuler-Faccini L, Oliveira WK, Henriques CM, Carmo EH, Pedi VD, Nunes ML, Castro MC, Serruya S, Silveira MF, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. Lancet. 2016; 388:891–897. [PubMed: 27372398]

33••. Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Fonseca EB, Ribeiro EM, Ventura LO, Neto NN, Arena JF, Rasmussen SA. Characterizing the Pattern of Anomalies in Congenital Zika Syndrome for Pediatric Clinicians. JAMA Pediatr. 2017; 171:288–295. [PubMed: 27812690]

34. Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, Rabello RS, Valderramos SG, Halai UA, Salles TS, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro. N Engl J Med. 2016; 375:2321–2334. [PubMed: 26943629]

35. Branswell, H. 'They're just hiding': Experts say Puerto Rico may be underreporting Zika-affected births. 2017. https://www.statnews.com/2017/04/18/zika-virus-puerto-rico-pregnancies/

36. Centers for Disease Control and Prevention. Outcomes of Pregnancies with Laboratory Evidence of Possible Zika virus Infection in the United States and District of Columbia, 2016–2017. 2017. https://www.cdc.gov/zika/geo/pregnancy-outcomes.html

37. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, Sakuntabhai A, Cao-Lormeau VM, Malasit P, Rey FA, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. Nat Immunol. 2016; 17:1102–1108. [PubMed: 27339099]

38. Bardina SV, Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown JA, Nachbagauer R, Foster GA, Krysztof D, Tortorella D, et al. Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity. Science. 2017; 356:175–180. [PubMed: 28360135]

39. Rabe IB, Staples JE, Villanueva J, Hummel KB, Johnson JA, Rose L, MTS, Hills S, Wasley A, Fischer M, Powers AM. Interim Guidance for Interpretation of Zika Virus Antibody Test Results. MMWR Morb Mortal Wkly Rep. 2016; 65:543–546. [PubMed: 27254248]

40••. Rossi SL, Tesh RB, Azar SR, Muruato AE, Hanley KA, Auguste AJ, Langsjoen RM, Paessler S, Vasilakis N, Weaver SC. Characterization of a Novel Murine Model to Study Zika Virus. Am J Trop Med Hyg. 2016; 94:1362–1369. [PubMed: 27022155]

41. Lazear HM, Govero J, Smith AM, Platt DJ, Fernandez E, Miner JJ, Diamond MS. A Mouse Model of Zika Virus Pathogenesis. Cell Host Microbe. 2016; 19:720–730. [PubMed: 27066744]

42. Aliota MT, Caine EA, Walker EC, Larkin KE, Camacho E, Osorio JE. Characterization of Lethal Zika Virus Infection in AG129 Mice. PLoS Negl Trop Dis. 2016; 10:e0004682. [PubMed: 27093158]

43. Dowall SD, Graham VA, Rayner E, Atkinson B, Hall G, Watson RJ, Bosworth A, Bonney LC, Kitchen S, Hewson R. A Susceptible Mouse Model for Zika Virus Infection. PLoS Negl Trop Dis. 2016; 10:e0004658. [PubMed: 27149521]

44•. Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, Zhang N, Shi L, Qin CF, Xu Z. Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. Cell Stem Cell. 2016; 19:120–126. [PubMed: 27179424]

45. Miner JJ, Cao B, Govero J, Smith AM, Fernandez E, Cabrera OH, Garber C, Noll M, Klein RS, Noguchi KK, et al. Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise. Cell. 2016; 165:1081–1091. [PubMed: 27180225]

46•. Dudley DM, Aliota MT, Mohr EL, Weiler AM, Lehrer-Brey G, Weisgrau KL, Mohns MS, Breitbach ME, Rasheed MN, Newman CM, et al. A rhesus macaque model of Asian-lineage Zika virus infection. Nat Commun. 2016; 7:12204. [PubMed: 27352279]

47. Abbink P, Larocca RA, De La Barrera RA, Bricault CA, Moseley ET, Boyd M, Kirilova M, Li Z, Ng'ang'a D, Nanayakkara O, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science. 2016; 353:1129–1132. [PubMed: 27492477]

48••. Adams Waldorf KM, Stencel-Baerenwald JE, Kapur RP, Studholme C, Boldenow E, Vornhagen J, Baldessari A, Dighe MK, Thiel J, Merillat S, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. Nat Med. 2016; 22:1256–1259. [PubMed: 27618651]

49. Goodfellow FT, Tesla B, Simchick G, Zhao Q, Hodge T, Brindley MA, Stice SL. Zika Virus Induced Mortality and Microcephaly in Chicken Embryos. Stem Cells Dev. 2016; 25:1691–1697. [PubMed: 27627457]

50. Li XF, Dong HL, Huang XY, Qiu YF, Wang HJ, Deng YQ, Zhang NN, Ye Q, Zhao H, Liu ZY, et al. Characterization of a 2016 Clinical Isolate of Zika Virus in Non-human Primates. EBioMedicine. 2016; 12:170–177. [PubMed: 27693104]

51. Osuna CE, Lim SY, Deleage C, Griffin BD, Stein D, Schroeder LT, Omange R, Best K, Luo M, Hraber PT, et al. Zika viral dynamics and shedding in rhesus and cynomolgus macaques. Nat Med. 2016; 22:1448–1455. [PubMed: 27694931]

52. Aliota MT, Dudley DM, Newman CM, Mohr EL, Gellerup DD, Breitbach ME, Buechler CR, Rasheed MN, Mohns MS, Weiler AM, et al. Heterologous Protection against Asian Zika Virus Challenge in Rhesus Macaques. PLoS Negl Trop Dis. 2016; 10:e0005168. [PubMed: 27911897]

53. Koide F, Goebel S, Snyder B, Walters KB, Gast A, Hagelin K, Kalkeri R, Rayner J. Development of a Zika Virus Infection Model in Cynomolgus Macaques. Front Microbiol. 2016; 7:2028. [PubMed: 28066354]

54. Coffey LL, Pesavento PA, Keesler RI, Singapuri A, Watanabe J, Watanabe R, Yee J, Bliss-Moreau E, Cruzen C, Christe KL, et al. Zika Virus Tissue and Blood Compartmentalization in Acute Infection of Rhesus Macaques. PLoS One. 2017; 12:e0171148. [PubMed: 28141843]

55. Darbellay J, Lai K, Babiuk S, Berhane Y, Ambagala A, Wheler C, Wilson D, Walker S, Potter A, Gilmour M, et al. Neonatal pigs are susceptible to experimental Zika virus infection. Emerg Microbes Infect. 2017; 6:e6. [PubMed: 28196970]

56. Hirsch AJ, Smith JL, Haese NN, Broeckel RM, Parkins CJ, Kreklywich C, DeFilippis VR, Denton M, Smith PP, Messer WB, et al. Zika Virus infection of rhesus macaques leads to viral persistence in multiple tissues. PLoS Pathog. 2017; 13:e1006219. [PubMed: 28278237]

57. Carter AM. Animal models of human placentation--a review. Placenta. 2007; 28(Suppl A):S41–7. [PubMed: 17196252]

58. Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. Neurotoxicology. 2007; 28:931–937. [PubMed: 17368774]

59. van den Pol AN, Mao G, Yang Y, Ornaghi S, Davis JN. Zika Virus Targeting in the Developing Brain. J Neurosci. 2017; 37:2161–2175. [PubMed: 28123079]

60. Kawiecki AB, Mayton EH, Dutuze MF, Goupil BA, Langohr IM, Del Piero F, Christofferson RC. Tissue tropisms, infection kinetics, histologic lesions, and antibody response of the MR766 strain of Zika virus in a murine model. Virol J. 2017; 14:82. [PubMed: 28420392]

61. Uraki R, Hwang J, Jurado KA, Householder S, Yockey LJ, Hastings AK, Homer RJ, Iwasaki A, Fikrig E. Zika virus causes testicular atrophy. Sci Adv. 2017; 3:e1602899. [PubMed: 28261663]

62. Schwartz DA. Viral infection, proliferation, and hyperplasia of Hofbauer cells and absence of inflammation characterize the placental pathology of fetuses with congenital Zika virus infection. Arch Gynecol Obstet. 2017; 295:1361–1368. [PubMed: 28396992]

63••. Nguyen SM, Antony KM, Dudley, S Kohn DM, Simmons HA, Wolfe B, Salamat MS, Teixeira LBC, Wiepz GJ, Thoong TH, et al. Highly efficient maternal-fetal Zika virus transmission in pregnant rhesus macaques. PLoS Pathog. 2017; 13:e1006378. [PubMed: 28542585]

64. Newman, CM., Dudley, DM., Aliota, MT., Weiler, AM., Barry, GL., Mohns, MS., Breitbach, ME., Stewart, LM., Buechler, CR., Graham, ME., et al. Oropharyngeal mucosal transmission of Zika virus in rhesus macaques. BioRxiv. 2017. doi: http://doi.org/10.1101/107128

65. Zika Experimental Science Team (ZEST). ZIKV-023. 2017. https://zika.labkey.com/project/OConnor/ZIKV-023/begin.view?

66. Cox J, Mota J, Sukupolvi-Petty S, Diamond MS, Rico-Hesse R. Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. J Virol. 2012; 86:7637–7649. [PubMed: 22573866]

67. Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. Trans R Soc Trop Med Hyg. 2008; 102:400–408. [PubMed: 18342898]

68. Ockenfels B, Michael E, McDowell MA. Meta-analysis of the effects of insect vector saliva on host immune responses and infection of vector-transmitted pathogens: a focus on leishmaniasis. PLoS Negl Trop Dis. 2014; 8:e3197. [PubMed: 25275509]

69. Pingen M, Bryden SR, Pondeville E, Schnettler E, Kohl A, Merits A, Fazakerley JK, Graham GJ, McKimmie CS. Host Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection. Immunity. 2016; 44:1455–1469. [PubMed: 27332734]

70. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, Perez-Padilla J, Medina FA, Waterman SH, Gubern CG, et al. Persistence of Zika Virus in Body Fluids - Preliminary Report. N Engl J Med. 2017; doi: 10.1056/NEJMoa1613108

71•. Haddow AD, Nalca A, Rossi FD, Miller LJ, Wiley MR, Perez-Sautu U, Washington SC, Norris SL, Wollen-Roberts SE, Shamblin JD, et al. High Infection Rates for Adult Macaques after Intravaginal or Intrarectal Inoculation with Zika Virus. Emerg Infect Dis. 2017; 23

72. National Academy of Sciences. National Research Council (US) Institute for Laboratory Animal Research. Washington DC: National Academies Press; 2003. International Perspectives: The Future of Nonhuman Primate Resources.

73. Gardner MB, Luciw PA. Macaque models of human infectious disease. ILAR J. 2008; 49:220–255. [PubMed: 18323583]

74. Melo AS, Aguiar RS, Amorim MM, Arruda MB, Melo FO, Ribeiro ST, Batista AG, Ferreira T, Dos Santos MP, Sampaio VV, et al. Congenital Zika Virus Infection: Beyond Neonatal Microcephaly. JAMA Neurol. 2016; 73:1407–1416. [PubMed: 27695855]

75•. Driggers RW, Ho CY, Korhonen EM, Kuivanen S, Jääskeläinen AJ, Smura T, Rosenberg A, Hill DA, DeBiasi RL, Vezina G, et al. Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. N Engl J Med. 2016; 374:2142–2151. [PubMed: 27028667]

76. Meaney-Delman D, Oduyebo T, Polen KN, White JL, Bingham AM, Slavinski SA, Heberlein-Larson L, St George K, Rakeman JL, Hills S, et al. Prolonged Detection of Zika Virus RNA in Pregnant Women. Obstet Gynecol. 2016; 128:724–730. [PubMed: 27479770]

77. Suy A, Sulleiro E, Rodó C, Vázquez É, Bocanegra C, Molina I, Esperalba J, Sánchez-Seco MP, Boix H, Pumarola T, Carreras E. Prolonged Zika Virus Viremia during Pregnancy. N Engl J Med. 2016; 375:2611–2613.

78. Moura da Silva AA, Ganz JS, Sousa PD, Doriqui MJ, Ribeiro MR, Branco MD, Queiroz RC, Pacheco MJ, Vieira da Costa FR, Silva FS, et al. Early Growth and Neurologic Outcomes of Infants with Probable Congenital Zika Virus Syndrome. Emerg Infect Dis. 2016; 22:1953–1956. [PubMed: 27767931]

79. Ventura CV, Fernandez MP, Gonzalez IA, Rivera-Hernandez DM, Lopez-Alberola R, Peinado M, Floren AA, Rodriguez PA, Williams BK, de la Vega Muns G, et al. First Travel-Associated Congenital Zika Syndrome in the US: Ocular and Neurological Findings in the Absence of Microcephaly. Ophthalmic Surg Lasers Imaging Retina. 2016; 47:952–955. [PubMed: 27759862]

80. van der Linden V, Filho EL, Lins OG, van der Linden A, Aragão MF, Brainer-Lima AM, Cruz DD, Rocha MA, Sobral da Silva PF, Carvalho MD, et al. Congenital Zika syndrome with arthrogryposis: retrospective case series study. BMJ. 2016; 354:i3899. [PubMed: 27509902]

81. Leal MC, Muniz LF, Ferreira TS, Santos CM, Almeida LC, Van Der Linden V, Ramos RC, Rodrigues LC, Neto SS. Hearing Loss in Infants with Microcephaly and Evidence of Congenital Zika Virus Infection - Brazil, November 2015-May 2016. MMWR Morb Mortal Wkly Rep. 2016; 65:917–919. [PubMed: 27585248]

82. Pacheco O, Beltrán M, Nelson CA, Valencia D, Tolosa N, Farr SL, Padilla AV, Tong VT, Cuevas EL, Espinosa-Bode A, et al. Zika Virus Disease in Colombia - Preliminary Report. N Engl J Med. 2016; doi: 10.1056/NEJMoa1604037

83. Chez RA, Schlesselman JJ, Salazar H, Fox R. Single placentas in the rhesus monkey. J Med Primatol. 1972:1230–240.

84•. Grigsby PL. Animal Models to Study Placental Development and Function throughout Normal and Dysfunctional Human Pregnancy. Semin Reprod Med. 2016; 34:11–16. [PubMed: 26752715]

85. Barry PA, Lockridge KM, Salamat S, Tinling SP, Yue Y, Zhou SS, Gospe SM Jr, Britt WJ, Tarantal AF. Nonhuman primate models of intrauterine cytomegalovirus infection. ILAR J. 2006; 47:49–64. [PubMed: 16391431]

86. Patten AR, Fontaine CJ, Christie BR. A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. Front Pediatr. 2014; 2:93. [PubMed: 25232537]

87. Furukawa S, Kuroda Y, Sugiyama A. A comparison of the histological structure of the placenta in experimental animals. J Toxicol Pathol. 2014; 27:11–18. [PubMed: 24791062]

88. Blankenship TN, Enders AC, King BF. Trophoblastic invasion and the development of uteroplacental arteries in the macaque: immunohistochemical localization of cytokeratins, desmin, type IV collagen, laminin, and fibronectin. Cell Tissue Res. 1993; 272:227–236. [PubMed: 7685655]

89. Carter AM. Animal models of human placentation-- A review. Placenta. 2007; 28:S41–S47. [PubMed: 17196252]

## Highlights

- Multiple macaque species are susceptible to Zika virus.
- A number of Zika virus strains have been used to infect macaques.
- Different routes of inoculation have all led to productive infections in macaques.
- Zika virus infection has been examined in pregnant and nonpregnant macaques.

**Table 1**

Clinical features of congenital Zika syndrome (CZS).

| Clinical finding | Description | References |
|---|---|---|
| abnormal cranial morphology | microcephaly, overlapping cranial sutures, prominent occipital bone, redundant scalp skin, facial disproportion, premature closure of the anterior fontanelle | [31, 33, 74, 78] |
| brain anomalies | intracranial calcifications, increased fluid spaces, cortical thinning, hypoplasia or absence of the corpus callosum, cerebellar hypoplasia, ventriculomegaly | [31, 33, 78] |
| ocular anomalies | microphthalmia, coloboma, cataracts, intraocular calcifications, chorioretinal atrophy, optic nerve atrophy, retinal lesions | [31, 33, 79] |
| congenital contractures | arthrogryposis of one or more joints | [31, 79, 80] |
| hearing anomalies | sensorineural hearing loss | [31, 33, 81, 82] |

## Table 2

Similarities and differences between human, macaque, and mouse gestation.

| Feature | Humans | Macaques | Mice | References |
|---|---|---|---|---|
| gestation easily divided into trimesters | yes | yes | no | [83–86] |
| hemochorial placentation | yes | yes | yes | [84] |
| discoid placenta | yes | yes[a] | yes | [83, 84, 87] |
| trophoblast invasion | yes[b] | yes[c] | no | [84, 88] |
| remodeling of decidual spiral arteries | yes | yes | no | [84, 88, 89] |
| unicornuate uterus | yes | yes | no | [84] |
| primarily singleton gestation | yes | yes | no | [84, 89] |
| long gestational length[d] | yes | yes | no | [84, 89] |

[a] bi-discoid placenta is common, but discoid may also occur.

[b] extensive invasion.

[c] invasion is less extensive than in humans.

[d] gestational length >150 days.