Am. J. Trop. Med. Hyg., 95(5), 2016, pp. 993–996
doi:10.4269/ajtmh.16-0625

Review Article

Flavivirus Infections of Bats: Potential Role in Zika Virus Ecology

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Abstract. Understanding the vector and nonhuman vertebrate species contributing to Zika virus (ZIKAV) transmission is critical to understanding the ecology of this emerging arbovirus and its potential to establish in new geographic areas. This minireview summarizes what is known regarding the association of bats with flaviviruses (*Flaviviridae*: *Flavivirus*) with a particular emphasis on the potential role of bats in the sylvatic transmission of ZIKAV. Key research directions that remain to be addressed are also discussed.

INTRODUCTION

Zika virus (ZIKAV) has emerged from a relatively obscure mosquito-transmitted flavivirus (*Flaviviridae*: *Flavivirus*) among nonhuman primates in Uganda[1,2] to a major pandemic. Information regarding the natural history of ZIKAV is limited, and a greater understanding of the vector and nonhuman vertebrate species contributing to ZIKAV transmission is paramount to understanding the ecology of this emerging arbovirus and its potential to establish and sustain circulation in areas of introduction. The purpose of this minireview is to examine what is presently known regarding the potential role of bats in the sylvatic circulation of ZIKAV and identify key research directions that remain to be addressed.

ZIKAV was first isolated from a sentinel rhesus monkey in the Zika forest, Uganda.[1] The first isolate (strain MR766), was obtained in 1947 from a caged sentinel animal positioned in the forest canopy. The importance of canopy-biting mosquitoes was recognized in the context of yellow fever virus (YFV) transmission among nonhuman primates, and Haddow and others[2] reported numerous isolations of ZIKAV from the arboreal sylvan mosquito, *Aedes* (*Stegomyia*) *africanus* (Theobald). At that time, attention was focused on nonhuman primates as reservoirs for sylvatic YFV and ZIKAV, but research in Uganda shortly thereafter examined bats as potential reservoirs of arboviruses, as these small mammals also would be exposed to host-seeking mosquitoes in upper forest stratifications.[3]

BATS AND FLAVIVIRUSES

Many flaviviruses have been isolated from naturally infected bats (Table 1).[4–24] Some of these viruses belong to the clade of the *Flaviviridae* that have no known arthropod vector, including Carey Island virus, Montana myotis leucoencephalitis virus, Rio Bravo virus (RBV), Dakar bat virus (DBV), Bukalasa bat virus (BBV), and Phnom-Penh bat virus.[25] Isolates of RBV, DBV, and BBV were made from bat salivary glands, suggesting transmission by bite.[4–6,15] However, some flaviviruses that have been isolated from bats are medically important mosquito-transmitted arboviruses such as St. Louis encephalitis virus (SLEV) and Japanese encephalitis virus (JEV). Other flaviviruses isolated from bats are grouped phylogenetically within the mosquito-transmitted clade, but have not yet been isolated from field-collected mosquitoes (Entebbe bat virus [ENTV], Yokose virus [YOKV]).[16,25,26] These observations suggest that bats may be competent amplifying hosts for arthropod-borne flaviviruses as well. Liu and others[13] isolated JEV from five species of bats in China: the Lechenault's rousette (*Rousettus leschenaultia*), little tube-nosed bat (*Murina aurata*), Rickett's big-footed bat (*Myotis ricketti*), common bent-winged bat (*Miniopterus schreibersii*), and intermediate horse-shoe bat (*Rhinolophus affinis*). All isolates were phylogenetically similar to isolates from mosquitoes and humans, suggesting a potential role for bats in the natural cycle of JEV.[13]

In support of these field studies, the competence of bats as amplifying hosts for flaviviruses has been evaluated in the laboratory. Significant viremia of more than 6 days' duration was produced in bats infected with JEV. Bats maintained a latent JEV infection during simulated hibernation, and detectable viremia was induced after as long as 107 days.[27] A bat-mosquito-bat cycle of infection was also established in the laboratory.[27] Herbold and others[28] demonstrated that big brown bats (*Eptesicus fuscus*) inoculated with SLEV also maintained virus circulation through a 70-day hibernation, and the bats developed a viremia within 4 days of arousal from hibernation, 105 days after inoculation. Sulkin and others[29] studied the susceptibility of three species of bats to experimental inoculation with JEV and SLEV and found that the characteristics of experimental infection included persistent viremia for 15–30 days, and viral invasion of and replication in a variety of tissues. Additional experimental work by Sulkin and others demonstrated evidence for spontaneous recurrent viremia of JEV and susceptibility to reinfection in bats held in the laboratory for 2–3 years.[30] Pregnancy did not alter the susceptibility of Brazilian free-tailed bats (*Tadarida brasiliensis*) to infection with JEV or SLEV; however, JEV was found to cross the placenta during all stages of pregnancy, whereas transplacental transmission by SLEV was rare.[31] Perea-Martinez and others[32] performed experimental infections of great fruit-eating bats (*Artibeus intermedius*) with serotype-2 dengue virus (DENV-2). Twenty-three bats were intraperitoneally inoculated with DENV-2. Histological analyses showed evidence of viral infection in the spleen and bleeding in the liver and intestines, but virus was only found in the kidneys of one bat by seminested reverse transcription polymerase chain reaction. Low levels of circulating viral RNA was detected in sera from seven of eight (87.5%) bats, and only two (25%) bats seroconverted. The authors concluded that these bats were not suitable hosts for DENV-2.[32] Simpson and O'Sullivan[33] inoculated Egyptian fruit

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TABLE 1
Flaviviruses isolated from naturally infected bats

Virus	Bat species	References
Bukalasa bat virus*	Little free-tailed bat (*Chaerephon pumilus*), Angola free-tailed bat (*Mops condylurus*)	9,14,15
Carey Island virus*	Lesser short-nosed fruit bat (*Cynopterus brachiotis*), lesser long-tongued fruit bat (*Macroglossus minimus*)	14,21
Dakar bat virus*‡	Little free-tailed bat (*Chaerephon pumilus*), Angola free-tailed bat (*Mops condylurus*), Egyptian tomb bat (*Taphozous perforatus*), *Scotophilus* sp.,	9,14,15,18,21
Entebbe bat virus*	Little free-tailed bat (*Chaerephon pumilus*), Angola free-tailed bat (*Mops condylurus*)	4,8,14,16
Japanese encephalitis virus†‡	Great roundleaf bat (*Hipposideros armiger terasensis*), Schreibers' long-fingered bat (*Miniopterus schreibersii*), little Japanese horseshoe bat (*Rhinolophus cornutus*)	13,14,21
Jugra virus†	Lesser short-nosed fruit bat (*Cynopterus brachiotis*)	14,21
Kyasanur Forest disease virus‡§	Rufous horseshoe bat (*Rhinolophus rouxi*), greater short-nosed fruit bat (*Cynopterus sphinx*)	14,22,23
Montana myotis leucoencephalitis virus*	Little brown bat (*Myotis lucifugus*)	11,14
Phnom-Penh bat virus*	Lesser dawn bat (*Eonycteris spelaea*), lesser short-nosed fruit bat (*Cynopterus brachyotis*)	14,19
Rio Bravo virus*‡	Mexican free-tailed bat (*Tadarida brasiliensis mexicana*), big brown bat (*Eptesicus fuscus*)	5–7,14,17,21
St. Louis encephalitis virus†‡	Mexican free-tailed bat (*Tadarida brasiliensis mexicana*)	10,14,21
Saboya virus§	Gambian slit-faced bat (*Nycteris gambiensis*)	14,21,24
Sokuluk virus*	Common pipistrelle (*Vespertilio pipistrellus*)	14,20
Tamana bat virus*‡¶	Parnell's mustached bat (*Pteronotus parnellii*)	14,17
Uganda S virus†	*Rousettus* sp., *Tadarida* sp.	14,21
Yokose virus*	Eastern bent-winged bat (*Miniopterus fuliginosus*)	12,14

*No known arthropod vector.
†Mosquito vector.
‡Human pathogen.
§Tick vector.
¶Serological evidence for human exposure, but disease unclear.

bats (*Rousettus aegyptiacus*), African straw-colored fruit bats (*Eidolon helvum*), and Angolan free-tailed bats (*Mops condylurus*) with YFV and found that fruit bat species circulated detectable levels of virus early after infection, but the free-tailed bat did not. Finally, Davis and others[34] inoculated big brown and Brazilian free-tailed bats with the New York 99 strain of West Nile virus (WNV) to assess their potential to serve as amplifying hosts and determine the clinical effect of infection. Virus was isolated each day from one or more big brown bats between days 2 and 6 postinfection; however, titers were low (10–180 plaque-forming units per milliliter of serum). Virus was not isolated from any of the serum samples collected from free-tailed bats, and no bats from either species showed clinical signs.[34] From the relatively limited data available on experimental infection of bats with flaviviruses, the susceptibility and reservoir competency of bats for mosquito-borne flaviviruses appear to be bat species and flavivirus specific. Further, although the reservoir competency of bats for flaviviruses is difficult to assess, it is likely low based on generally low viremia detected in experimental studies.

Surveys have also been conducted to assess the exposure and seroprevalence of bats to flaviviruses in the field. Allen and others[10] surveyed Brazilian free-tailed bats in south Texas for evidence of infection with SLEV. Twenty-six strains of the virus were isolated from the blood or spleen tissue of 1,649 bats, and neutralizing antibodies against SLEV were detected in 25 of 275 (9%) plasma samples from bats netted near Houston and in 108 of 388 (28%) plasma samples from bats collected in Corpus Christi. Herbold and others[28] surveyed big brown bats and little brown bats (*Myotis lucifugus*) in five regions of Ohio between 1979 and 1981, and found a 9% neutralizing antibody prevalence against SLEV. The authors also documented cohabitation of natural caves and abandoned mineshafts by *Culex pipiens* complex mosquitoes and both bat species. Serosurveys of insectivorous bats for WNV in the United States have found bats with WNV-neutralizing antibodies, but at a low seroprevalence.[35,36] Flavivirus-neutralizing antibodies, possibly due to DENV infection, were detected in 26/140 bats (19%) captured on the Yucatan Peninsula, Mexico. The antibody-positive bats belonged to three species: the Pallas's long-tongued bat (*Glossophaga soricina*) (33%), Jamaican fruit bat (*Artibeus jamaicensis*) (24%), and great fruit-eating bat (*Artibeus literatus*) (9%).[37] On the Caribbean island of Trinidad, Thompson and others[38] found antibodies to Tamana bat virus (TABV) and RBV in 47 (15.3%) and three (1.0%) bats, respectively, using hemagglutination inhibition assay (HAI). Their results also suggested the presence of antibodies to an undetermined flavivirus(es) in eight (2.6%) bats.[38] Generally, bats appear to be frequently infected with flaviviruses in the field, although the relative importance of bats to mosquito-borne flavivirus transmission cycles is unclear.

SEROLOGICAL EVIDENCE FOR NATURAL CIRCULATION OF ZIKAV IN FIELD-COLLECTED BATS

Shepherd and Williams[3] examined the sera of 172 bats comprising both fruit and insectivorous bat species, captured from around Entebbe and the slopes of Mount Elgon in eastern Uganda. Using HAI, they reported antibodies reactive to ZIKAV at a high seroprevalence among Angolan free-tailed bats (26/36) and little free-tailed bats (*Chaerephon pumilus*) (16/44). The presence of ZIKAV-reactive antibodies in Ugandan bats was also found by Simpson and others,[15] who

reported antibodies to ZIKAV in Angolan free-tailed bats (3/132), as well as the African straw-colored fruit bats (7/31), and Old World fruit bats of the genus *Rousettus* (1/25). However, a major weakness of these early studies is the possible lack of differentiation amongst flaviviruses due to the testing method. The bats with antibodies reactive to ZIKAV also were reactive to YFV, WNV, ENTV, DBV, BBV, Usutu virus, and Ntaya virus.[3,15] Still, the results indicated a high degree of exposure of Ugandan bats to flaviviruses.

EXPERIMENTAL INFECTIONS OF ZIKAV IN BATS

In addition to evidence for natural infection, some African bat species supported replication of ZIKAV in the laboratory. Shepherd and Williams[3] inoculated one Angolan fruit bat (*Lissonycteris angolensis*) with 3.8 log LD_{50} ZIKAV strain MR766. Bats were bled on days 2, 4, 6, 8, 9, 10, and 14 postinoculation. When serum was drawn on days 2–6 postinfection and inoculated into neonatal mice, the mice died, indicating that the bat was circulating a low level of infectious virus. Simpson and O'Sullivan[33] inoculated additional species of bats with ZIKAV and found detectable virus titers (approximately 1.7–2.5 log LD_{50} per 0.02 mL) produced by African straw-colored fruit bats and Egyptian fruit bats during the first few days after infection. In earlier work, Regan and others[39] experimentally inoculated little brown bats with ZIKAV, and found that intracerebral, intraperitoneal, intradermal, or intrarectal inoculations resulted in severe neurological disease, whereas intranasal inoculation failed to cause infection. Unfortunately, no other laboratory work has been performed to substantiate this work or expand its findings.

A strength of these studies was the foresight of the authors to test local bat species for ZIKAV susceptibility and conduct pilot studies on the reservoir competence of bats for ZIKAV replication. These studies form a foundation for the potential of bats to serve as reservoirs for mosquito-borne flaviviruses, but much work in this area remains to be performed.

MOSQUITO FEEDING ON BATS

Do mosquitoes use bats as blood hosts? Evidence exists for mosquitoes feeding on bats, although the frequency with which this happens in the field is not well understood and difficult to study. In a study focused on the blood hosts of two species of *Culiseta* mosquitoes in New York with respect to transmission of eastern equine encephalitis virus, Molaei and others[40] catalogued one *Culiseta morsitans* Theobald blood meal from an eastern pipistrelle (*Pipistrellus subflavus*) bat. This observation comprised less than 1% of the total number of blood meals analyzed from this mosquito species. Among blood meals from *Anopheles punctulatus* s.l. mosquitoes in Papua New Guinea, Logue and others[41] identified one blood meal from each of two fruit bat species: *Dobsonia moluccensis* and *Dobsonia praedatrix*. In Uganda, Crabtree and others[42] identified blood meals from multiple species of fruit bats in *Culex neavei* Theobald, *Culex perfuscus* Edwards, *Culex decens* group, and *Coquillettidia fuscopennata* Theobald mosquitoes. Several human arbovirus pathogens have been identified from field collections of each of these mosquito species, including Spondweni virus and ZIKAV from *Cx. neavei*.[43,44] Given the number of flaviviruses isolated from bats (Table 1) and the high seroprevalence of flaviviruses in bats, the circulation of mosquito-borne flaviviruses, including ZIKAV, among mosquitoes and bats, requires further study.

CONCLUSIONS AND FUTURE DIRECTIONS

ZIKAV has likely been infecting bats in Africa for a substantial amount of time. However, with its introduction to the New World, it is unknown what effects the virus may have on natural populations of bats. Does ZIKAV infect any New World bat species? If so, can it cause disease or teratogenic effects (e.g., microcephaly)? Can it be transmitted between bats without a vector (e.g., sexually)? Because experimentally inoculated North American little brown bats are susceptible to ZIKAV infection,[39] the virus may be of wildlife disease concern. However, it is unclear how ZIKAV could circulate in bat populations.

There is limited information about ZIKAV viremia in bats. In one experimental inoculation of a fruit bat, there was sufficient viremia to cause paralysis in mice inoculated with sera collected on days 2, 4, and 6 but not thereafter.[3] In humans, it is evident that viremia is very low or not detectable. However, viruria occurs in human infections;[45,46] thus, it is possible that ZIKAV is shed in the urine of bats as well. Because of the high population densities of bat colonies, this could provide a transmission mechanism between bats, but is unlikely to result in transmission to humans.

Although our knowledge about the virology and biology of ZIKAV is growing rapidly, virtually nothing is known about what impacts this virus may have on wild bat populations. Collection of additional field samples from bats could help determine whether ZIKAV infects bats. Serology can provide evidence of past infection, but is complicated by the potential for cross-reactivity with other flaviviruses; thus, its interpretations must be carefully considered. An important consideration is that if ZIKAV is highly fatal to certain species of bats, field samples may not permit identification of those species. Detection of viral RNA or isolation of infectious virus in field samples will also be useful for determining natural exposure of bats to ZIKAV and may identify which species are more susceptible.

An important complement to fieldwork is experimental infections of bats. However, few bat colonies are available for such work, and no bats species is a model organism. Thus, many contributory factors will make experimental infections difficult. Despite these limitations, some experimental work could be done to better gauge the susceptibility and pathogenesis of ZIKAV in this taxon.

Received August 1, 2016. Accepted for publication August 22, 2016.

Published online September 19, 2016.

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