

SYLVATIC TRANSMISSION OF ARBOVIRUSES AMONG BORNEAN ORANGUTANS

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Abstract. Wild populations of nonhuman primates live in regions of sylvatic arbovirus transmission. To assess the status of arbovirus transmission in Bornean forests and the susceptibility of wild orangutans to arboviral infection, blood samples of wild orangutans, semi-captive orangutans, and humans were examined. Samples were tested by plaque reduction neutralization test for antibodies to viruses representing three families (Flaviviridae, Alphaviridae, and Bunyaviridae), including dengue-2, Japanese encephalitis, Zika, Langat, Tembusu, Sindbis, Chikungunya, and Batai viruses. Both wild and semi-captive orangutan groups as well as local human populations showed serologic evidence of arbovirus infection. The presence of neutralizing antibodies among wild orangutans strongly suggests the existence of sylvatic cycles for dengue, Japanese encephalitis, and sindbis viruses in North Borneo. The present study demonstrates that orangutans are susceptible to arboviral infections in the wild, although the impact of arboviral infections on this endangered ape remain unknown.

INTRODUCTION

Most arboviruses are perpetuated in transmission cycles independent of human hosts.¹ Those that exist in sylvatic cycles often infect people who accidentally intrude on these cycles. Less frequently, arboviruses may make the jump from a sylvatic transmission cycle to a mainly human-mosquito transmission cycle, as has presumably happened for Chikungunya virus.² In other cases, humans are dead-end hosts in complex transmission cycles involving multiple wild and domestic vertebrate hosts, as in Japanese encephalitis virus.³ Understanding the emergence of human arboviral disease and developing surveillance methods to predict its occurrence depends upon an understanding of the ecology of individual arboviruses and their life-cycles.⁴

Traditionally, mosquito sampling and direct sampling of forest-dwelling humans have been used to assess the presence of sylvatic cycles. While viruses are more easily isolated from mosquitoes than from their vertebrate hosts, mosquito based surveillance does not distinguish well between viruses likely to be able to infect humans and those that may be restricted to taxonomically distinct hosts. Direct human sampling is likely to identify relevant arboviruses, but due to dwindling numbers of forest-dwelling humans, adequate samples are often difficult to obtain. Wild populations of nonhuman primates have a number of features that make them useful sentinels for identifying sylvatic transmission of arboviruses relevant to humans.⁵ Human and nonhuman primates share susceptibility to a wide range of pathogenic microorganisms.⁶ Nonhuman primates live primarily in forested environments.⁷ They can have large body sizes and are often social, characteristics that attract vectors.⁸ Nonhuman primates are also generally mobile, a trait that may increase exposure to pathogens.^{9,10}

Nonhuman primates are also thought to play an important role in the sylvatic maintenance cycles of certain flaviviruses, including yellow fever,¹¹ dengue,^{12–14} and Zika,¹⁵ and alphaviruses including, Chikungunya.^{16,17} While definitive proof that a particular nonhuman primate species is required for maintenance of any sylvatic cycle does not exist, the role that wild populations have played as sentinels for arbovirus-

es is clear. The deaths of New World primates, particularly Howler monkeys (*Alouatta* sp.), have been used to accurately signal yellow fever outbreaks.¹¹ Furthermore, the examination of wild primate deaths has led to the identification of novel arboviruses as in the case of the tick-borne flavivirus, Kyasanur Forest disease virus, which was discovered following a die-off of bonnet macaques (*Macaca radiata*) and human langurs (*Presbytis entellus*) in the Kyasanur Forest of India. Kyasanur Forest disease was retrospectively determined to be a source of human illness in the area.¹⁸ While work with wild primate populations is limited by the many logistical problems associated with safely and ethically sampling from endangered and often remotely located populations, a recent conservation project provided the opportunity to collect blood samples from wild and semi-captive orangutans, endangered apes that are among our closest living relatives. These were tested serologically for arboviruses.

MATERIALS AND METHODS

Study site and population. During 1996 and 1997, wild orangutans were translocated as part of a conservation project conducted by the Sabah Wildlife Department and the Wildlife Conservation Society. Wild individuals were transferred from forest fragments in Eastern Sabah to the Tabin Wildlife Reserve.¹⁹ All forest fragments were within 200 km of the Tabin Wildlife Reserve. Forest fragments varied in size and were surrounded by oil palm plantations. The Tabin Wildlife Reserve consists of a 1,600 km² tract of protected primary and secondary lowland tropical rain forest. Wild orangutans were captured using anaesthetic darts for translocation and were subjected to a venous blood draw at that time.

Orangutan samples were also collected from semi-captive individuals at the Sepilok Orangutan Rehabilitation Center (SOURC), located on the Northern edge of the Sepilok Forest Reserve, a 400 km² tract of protected primary and secondary forest outside of Sandakan, Sabah. Semi-captive individuals arrive at the SOURC following some level of contact with people. Upon arriving at the SOURC individuals are placed in quarantine for six months, after which they are

TABLE 1
Characteristics of viruses examined

Virus name (and code)	Family, Genus	Vector*	Presumed reservoir host	From wild NHPs†	Distribution	Symptoms
Dengue-2	Flaviviridae, <i>Flavivirus</i>	Culicine mosquitoes	Humans and other Primates	Africa, Asia	Global Tropics	Fever, hemorrhage, rash
Japanese encephalitis	Flaviviridae, <i>Flavivirus</i>	Anopheline and Culicine mosquitoes	Birds	Asia	Asia	Encephalitis, fever
Zika	Flaviviridae, <i>Flavivirus</i>	Culicine mosquitoes	Primates	Africa	Africa, Asia	Fever, rash
Langat	Flaviviridae, <i>Flavivirus</i>	Ixodid ticks	Rodents		Asia	Fever
Tembusu	Flaviviridae, <i>Flavivirus</i>	Anopheline and Culicine mosquitoes	Birds		Asia, Australasia	Unknown, encephalitis may be possible
Chikungunya	Togaviridae, <i>Alphavirus</i>	Culicine mosquitoes and Argasid ticks	Humans and other primates	Africa	Africa, Asia	Fever, arthritis, rash
Sindbis	Togaviridae, <i>Alphavirus</i>	Culicine and Anopheline mosquitoes and Ixodid ticks	Birds		Africa, Asia, Europe, Australasia	Fever, arthritis, rash
Batai	Bunyaviridae, <i>Bunyavirus</i>	Culicine and Anopheline mosquitoes	Unknown		Asia, Europe	Unknown

* Based on vectors from which virus has been isolated (from Karabastos¹).

† NHPs = nonhuman primates.

held in a range of conditions from captivity to completely free-ranging. Orangutans at SOURC are provisioned daily with bananas and milk.

From experience with semi-captive individuals of known age at the SOURC, wild and semi-captive individuals were categorized into four classes based on weight and dental morphology. These categories correspond to approximate age groups: infant, < 1 year; juvenile, 1–5 years; subadult, 6–10 years; and adult, > 10 years.

The study was conducted with the approval of the directors of the Sabah Health Department and the Sabah Wildlife Department. Ethical review and approval of the protocol was conducted by the National Research Review Committee of the Economic Planning Unit (EPU). The protocol was also reviewed and approved in the ethical review processes of the Institute of Medical Research (IMR), Kuala Lumpur, and the Sabah Health Department. Following informed consent, samples were also collected from adult humans, classified as either native-born Bornean or non-native-born Bornean (migrants), who lived or worked on the boundaries of the Sepilok Forest Reserve. Following venipuncture, human and orangutan blood were processed identically.

Blood sampling and serology. Prior to blood sampling, orangutans were immobilized using anaesthetic darts. Blood was collected in EDTA, centrifuged, and the plasma was frozen in liquid nitrogen. Plasma samples were later tested for neutralizing antibody to eight viruses, including five flaviviruses, two alphaviruses, and one bunyavirus (Table 1) at the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention laboratory in Fort Collins, Colorado.²⁰ Sera were screened at a dilution of 1:10, and endpoint titrations were conducted for samples that tested positive (i.e., titer > 10) in the screening test.

RESULTS

A total of 185 individuals were sampled, including 40 wild orangutans, 31 semi-captive orangutans, and 114 humans. Of the 114 humans sampled, 31 were native-born Borneans and 83 were migrants who had migrated to North Borneo from other islands in the southern Philippines and Indonesian archipelago. The orangutan samples were unevenly distributed with respect to age; the semi-captive population consisted primarily of juveniles, while the wild population consisted primarily of adults. The human samples were unevenly distributed with respect to age and sex, and consisted primarily of adult males.

Neutralizing antibody titers of greater than or equal to 20 were considered positive, a likely result of exposure to the tested virus or a very closely-related strain (Table 2). The results of endpoint titrations suggest that wild orangutans were infected with dengue-2 (DEN-2), Japanese encephalitis (JE), Zika, Tembusu (TMU), and Sindbis (SIN) viruses; semi-captive orangutans were infected with DEN-2, Zika, JE, Langat (LAN), and TMU viruses; humans living along the boundary of the Sepilok Forest Reserve were infected with DEN-2, JE, Zika, LAN, TMU, Chikungunya (CHIK), and Batai (BAT) viruses. Of these, LAN and CHIK viruses were found only among human migrants.

At least one human and one orangutan were positive for each of the five flaviviruses examined (Table 3). Among humans, native-Bornean and migrant populations differed with respect to frequency of DEN-2 virus infection with 37% of native Borneans positive versus 74% of migrants positive (G-test of independence [G] = 6.21, $P < 0.05$). There were no significant associations between flavivirus seropositivity and orangutan group status (i.e., wild or semi-captive) for any flavivirus. For viruses that showed no differences within

TABLE 2
Examples of serologic responses to one or more viruses*

Serum code	Titer against							
	DEN-2	JE	Zika	TMU	LAN	CHIK	SIN	BAT
Wild02	80	160	—	—	—	—	—	—
Wild16	—	40	—	—	—	—	40	—
Wild20	(10)	>320	—	—	—	—	—	—
Wild23	20	80	(10)	20	—	—	—	—
Wild27	(10)	>320	—	—	(10)	—	—	—
Wild39	160	—	>320	—	—	—	—	—
Wild43	160	—	—	—	—	—	—	—
Semi245	20	320	80	—	40	—	—	—
Semi399	160	80	—	—	—	—	—	—
Semi304	20	320	—	80	—	—	—	—
Human07†	20	—	>320	—	40	—	—	—
Human33†	80	160	40	—	—	160	—	—
Human39	40	—	—	—	—	—	—	40
Human59	160	320	—	—	—	—	—	—
Human91	320	320	40	160	—	(10)	—	—

* Titers of 10 are indicated in parentheses and are not considered positive responses. DEN-2 = dengue-2; JE = Japanese encephalitis; TMU = Tembusu; LAN = Langat; CHIK = Chikungunya; SIN = Sindbis; BAT = Batai.

† Samples obtained from non-native Borneans.

human or orangutan sub-groups, these groups were combined for overall analyses comparing humans and orangutans. Humans, overall, were more frequently infected (44%) with Zika virus than were orangutans (8%) ($G = 14.6$, $P < 0.001$).

Of the two alphaviruses and one bunyavirus examined, humans were infected with all three, while orangutans were only infected with SIN virus (Table 4). CHIK virus was seen only among migrants and not among native-born Borneans or in either orangutan group. SIN virus was seen only among wild orangutans and not among semi-captive orangutans or humans. BAT virus was seen in only one human, a 43-year-old native-born Bornean man.

Orangutan and human groups were also examined for seropositivity by age (Tables 5–7). The G-test of independence was used to assess possible associations between age grouping and seropositivity for each virus. Age grouping and seropositivity were independent for all viruses in both orangutan and human populations.

DISCUSSION

A number of arboviruses in the Indo-Australian archipelago exhibit a pattern of geographic distribution corresponding to the geographical deep-sea barriers of Wallace's line which runs east of Borneo and south through to the east of

Bali, and Weber's line which runs west of Papua New Guinea and Australia (Figure 1). In the past, antibodies to JE virus were common among humans to the west of Wallace's line while prevalence virtually disappeared to the east of Wallace's line.²¹ In recent years, however, JE outbreaks have been documented in Papua New Guinea and the Torres Straits of Australia.^{22,23} Zika virus appears to occur only west of Wallace's line,¹ while CHIK virus exists mainly to the west of Weber's line, but is also present in lower frequencies in Papua New Guinea.²⁴ Conversely, Ross River virus and Murray Valley encephalitis virus are present exclusively in Australia and Papua New Guinea, and have not been documented west of Weber's line.²⁵ Other viruses, like DEN and SIN have distributions that do not correspond well to these geographic barriers, existing in all regions. It is likely that JE virus from the Oriental zoogeographic region and Murray Valley encephalitis and Ross River viruses from the Australasian zoogeographic region have been geographically separated for thousands or millions of years. It is only relatively recently, with the movements of humans and their domestic animals, that JE virus has had the opportunity to bridge this gap. For other viruses such as SIN and DEN, it remains unclear if their biohistorical distributions includes all regions or if human migrations have contributed to current distributions. However, evidence suggests that the DEN viruses evolved in Asia and spread around the world as commerce developed in the 18th and 19th centuries.²⁶

A series of studies during the 1960s and early 1970s examined arbovirus infections in Sarawak, Malaysian West Borneo. Using virus isolation from mosquitoes this group demonstrated the presence of enzootic JE, TMU, and SIN viruses in areas of rice padi and JE and SIN viruses in inland forest regions,²⁷ as well as the presence of a Bunyamwera virus, isolated from a padi region. Serologic studies in humans in Sarawak showed neutralizing antibody to DEN-2 (50%), JE (57%), TMU (22%), West Nile, and Getah viruses.²⁸ Bowen and others²⁸ did not present neutralization test results for CHIK virus, but based on hemagglutination-inhibition (HI) results concludes that it is common among humans in Sarawak, a finding that may be premature due to the low specificity of the HI test. No evidence of SIN neutralizing antibodies was found among humans.²⁸ Other studies by the group found neutralizing antibodies to JE virus among dogs (84%), ducks (19%), wild birds (18%), and wild bats (2%);²⁹ to SIN virus among chickens (56%);²⁸ and to TMU virus among chickens (72%).²⁸ Studies conducted by other groups have also shown the presence of antibodies to

TABLE 3
Seroprevalence to flaviviruses

	Percent and number positive to									
	Dengue-2	Japanese encephalitis		Zika	Tembusu		Langat			
Orangutans										
Wild	28%	11/40	38%	15/40	13%	5/40	3%	1/39	0%	0/40
Semi-captive	32%	10/31	16%	5/31	3%	1/31	3%	1/29	3%	1/31
Humans										
Native Bornean	37%	11/30	40%	12/30	30%	9/30	4%	1/25	0%	0/31
Migrants	74%	59/80	29%	24/83	49%	40/81	4%	3/73	1%	1/83
Total	50%	91/181	30%	56/184	30%	55/182	4%	6/166	1%	2/184

TABLE 4
Seroprevalence to alphaviruses Chikungunya and Sindbis and to a bunyavirus, Batai

	Percent and number positive to					
	Chikungunya		Sindbis		Batai	
Orangutans						
Wild	0%	0/40	10%	4/40	0%	0/40
Semi-captive	0%	0/31	0%	0/31	0%	0/31
Humans						
Native Bornean	0%	0/31	0%	0/31	3%	1/31
Migrants	28%	23/82	0%	0/81	0%	0/83
Total	13%	23/184	2%	4/183	1%	1/185

Zika (18%)¹ and CHIK (14%)²⁴ viruses among humans elsewhere in Borneo.

Of the five flaviviruses examined in the present study, wild orangutans were infected with DEN, JE, Zika, and TMU viruses. Semi-captive orangutans were infected with each of these four viruses as well as LAN, a tick-borne flavivirus. Both DEN and Zika viruses have been previously shown to have sylvatic cycles and infect wild nonhuman primates. Research on DEN virus has shown sylvatic cycles that infect wild nonhuman primates both in Africa¹³ and mainland Southeast Asia.¹² For Zika virus, infection of wild nonhuman primates has been shown in Africa;¹⁵ the presence of antibody to Zika virus among Asian nonhuman primates has not previously been examined. Nonhuman primates in continental Southeast Asia have also been shown to have neutralizing antibodies to JE virus.³⁰ There has been limited research on the forest ecology of either LAN or TMU viruses.

The present study shows that wild orangutans may be infected with DEN, JE, and Zika viruses and strongly suggests the presence of sylvatic transmission of DEN and JE viruses in forests in Borneo. The wild orangutans studied were free-ranging individuals living in forest regions within their natural geographic range. While it is possible that wild orangutans were infected through exposure to mosquitoes fed on domestic animals, or viremic agricultural or timber workers, it is unlikely. North Borneo has a low human population density and forests in the study were surrounded by oil palm plantations and not near human settlements. While the results of the present study strongly suggest current evidence of sylvatic transmission of DEN and JE viruses in North Borneo, they cannot distinguish between long-term biohistorical presence of sylvatic cycles versus more recent human introduction of viruses into sylvatic cycles. Only one wild and one semi-captive orangutan showed evidence of infection with TMU virus and only one semi-captive individual

had neutralizing antibodies to LAN virus. While it is difficult to form conclusions for TMU and LAN viruses based on three seropositive animals, it is interesting to note that semi-captive individuals spend considerably more time in a terrestrial habitat, perhaps making them more likely to be infected with a tick-borne virus such as LAN.

Humans were also infected with all five flaviviruses examined in this study, although native-born Borneans showed no evidence of infection with LAN virus. Migrants were more frequently infected with DEN virus than native Borneans. The present study only examined neutralizing antibody to DEN-2 virus, so a more detailed study using multiple dengue strains might help to determine if dengue among native-born Borneans results from exposure to recently arrived migrants or from locally present sylvatic strains. People were more frequently infected with Zika virus than orangutans. A greater infection rate of humans with Zika virus indicates the possibility that Zika virus among orangutans may result from incidental infection through contact with mosquitoes infected by viremic humans or from recently established sylvatic cycles. Another possibility is that sylvatic Zika virus in Borneo has a more limited distribution or an ecology that does not lead to frequent exposure by orangutans.

Of the alphaviruses and the bunyavirus examined, orangutans were only infected with SIN virus, and then only in the wild population. Humans native-born to Borneo showed no evidence of infection with the two alphaviruses, while a number of migrants were infected with CHIK virus. The conventional wisdom is that Asian CHIK virus has a primarily human-mosquito transmission cycle, which periodically emerges in the urban centers of Asia to cause epidemics.² African CHIK virus has a known sylvatic cycle, thought by some to depend upon nonhuman primate reservoirs.^{16,17} The lack of antibody to CHIK virus in orangutans suggests that this virus is not enzootic in Borneo, but it is premature

TABLE 5
Seroprevalence in different age classes for all orangutans combined

Age class	Percent and number positive to											
	Dengue-2		Japanese encephalitis		Zika	Tembusu		Langat		Sindbis		
Adult	39%	13/33	48%	16/33	15%	5/33	3%	1/32	3%	1/30	12%	4/33
Sub-adult	17%	1/6	33%	2/6	17%	1/6	17%	1/6	0%	0/6	0%	0/6
Juvenile	23%	7/31	6%	2/31	0%	0/31	0%	0/29	0%	0/31	0%	0/31
Infant	0%	0/1	0%	0/1	0%	0/1	0%	0/1	0%	0/1	0%	0/1
Total	30%	21/71	28%	20/71	8%	6/71	3%	2/68	1%	1/68	6%	4/71

TABLE 6
Seroprevalence in different age classes for native-born Borneans

Age range (yr)	Percent and number positive to									
	Dengue-2		Japanese encephalitis		Zika		Tembusu		Batai	
21–30	33%	1/3	67%	2/3	33%	1/3	0%	0/3	0%	0/3
31–40	24%	4/17	41%	7/17	35%	6/17	7%	1/14	0%	0/18
≥41	60%	6/10	30%	3/10	20%	2/10	0%	0/8	10%	1/10
Total	37%	11/30	40%	12/30	30%	9/30	4%	1/25	3%	1/31

to draw such a conclusion for all of Asia based on the examination of a single Asian primate species. The SIN virus infections among wild orangutans is the first evidence of natural nonhuman primate infection with this virus. While SIN virus is thought to be maintained primarily by birds, the presence of monotypic responses to this virus in four wild orangutans shows that nonhuman primates can be infected in natural settings. Since orangutans are primarily arboreal and Borneo has a diverse arboreal avian fauna, vector mediated transmission from birds to orangutans seems to be the most likely explanation for this finding. For BAT virus, only a single native-born human Bornean was infected. While the relationship of BAT virus to an earlier Bunyamwera-group virus isolated from mosquitoes in Sarawak²⁷ remains unclear, the current result suggests that BAT virus or a closely related Bunyamwera-group virus may occasionally be transmitted to humans in Borneo.

The extent to which orangutans play a role in the maintenance of sylvatic cycles of arboviruses remains unknown. Wild orangutans have a population density of approximately two individuals/km². Due to this very low population density, it is unlikely that orangutans play a necessary role in the sylvatic cycles of arboviruses. In the case of JE virus, domestic pigs and wild Ardeid birds are the primary suspects for virus maintenance, although snakes and bats may play a role.³ A wild bearded pig (*Sus barbatus*)³¹ and a diverse group of wild Ardeid birds³² inhabit lowland forest in Borneo, and they may play a role in maintaining and/or amplifying sylvatic JE virus. While sylvatic DEN virus is thought to be maintained primarily by nonhuman primates, the existence of 12 species of forest-dwelling nonhuman primates in Borneo³¹ makes results from a single host species insufficient for identification of a maintenance host. One possibility, rarely discussed, is that arboviruses in sylvatic cycles may be maintained by a broad assemblage of host species. An arbovirus with a broad host-range might have selective advantages over one capable of causing productive viremia in only a single host species. Intergroup competition and territoriality in tropical forests often leads to the presence of

groups of overlapping, or sympatric, host species. Generalizing to a broader assembly of hosts may increase the probability of secondary infections in a region of low host densities. A study on a range of intestinal parasites among groups of wild primates in the Kibale Forest in Uganda indicated that the presence of intestinal parasites was best predicted by membership in local multi-species primate groups and was not well predicted by host species.³³ As a number of arboviruses, including DEN virus, are known to have the capacity for trans-ovarial or vertical transmission from infected female mosquitoes to their offspring, transmission may also play a role in viral maintenance in regions with low host densities.³⁴ Further research will be necessary to assess the importance of individual host species compared to multiple-host assemblages in the sylvatic maintenance of arboviruses.

The public health implications of the presence of sylvatic cycles remains complex. Most discussion of sylvatic cycles focuses on their negative implications. Tropical lowland forests, as regions of exceptionally high plant and animal biodiversity, are likely to house high levels of microorganism biodiversity and large numbers of unknown hazardous pathogens capable of infecting humans. Identification of these pathogens in forests may assist in the identification of undiagnosed illness, as in the case of the Kyasanur Forest disease virus,¹⁸ as well as predict potential emergence of novel pathogens or localized epidemics, such as yellow fever.¹¹ While rarely emphasized, the presence of sylvatic cycles may also have positive health consequences, as illustrated by the well-studied case of dengue. It has long been suggested that sylvatic cycles of dengue and their associated human infections are less virulent than those resulting from primarily human-oriented transmission. Studies of dengue-1 and dengue-2 virus molecular evolution³⁵ suggest that sylvatic strains of dengue are genetically distinct and have decreased potential for transmission and/or virulence. The high endemicity of dengue in Asia may also help to explain the lack of yellow fever in Asia.¹¹ The presence of antibodies to dengue virus among wild animals may have led to cross-

TABLE 7
Seroprevalence in different age classes for migrants to Borneo

Age range (yr)	Percent and number positive to											
	Dengue-2		Japanese encephalitis		Zika		Tembusu		Langat		Chikungunya	
10–20	64%	9/14	21%	3/14	14%	2/14	0%	0/14	0%	0/14	12%	3/25
21–30	77%	27/35	36%	13/36	49%	17/35	6%	2/32	0%	0/36	36%	13/36
31–40	71%	17/24	19%	5/26	60%	15/25	5%	1/21	4%	1/26	32%	8/25
≥41	86%	6/7	43%	3/7	86%	6/7	0%	0/6	0%	0/7	0%	0/7
Total	74%	59/80	29%	24/83	49%	40/81	4%	3/73	1%	1/83	26%	24/93



FIGURE 1. Southeast Asia with Wallace's and Weber's Lines, showing the major continental shelves. Reproduced with permission from Whitmore.³⁷

reactive immunity preventing yellow fever from establishing a stable sylvatic cycle in Asia.

As endangered primates, and one of our closest living relatives, orangutans and their habitat have become a conservation priority. The study of the infectious diseases of wild primate populations has the potential to assist wildlife conservation.³⁶ Arboviral infection can play an important role in the mortality and morbidity of wild primate populations. Yellow fever introduction on Barro Colorado Island led to the death of a number of mantled howler monkeys (*Alouatta villosa*) and the Kyasanur Forest virus was responsible for the deaths of macaques and leaf monkeys as cited earlier. While earlier arbovirus research involved the hunting of wild primates, the increasingly vulnerable and endangered status of nonhuman primate populations worldwide makes this method untenable. Nevertheless, particularly in concert with conservation efforts, there are occasional opportunities to sample from wild primates in a way that improves the understanding of pathogen ecology while complementing animal conservation goals.

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