Animals in the Zika virus life cycle: what to expect from megadiverse Latin American countries.

Marina Galvão Bueno[1*]; Nádia Martinez[1]; Lívia Abdala[1], Claudia Nunes Duarte dos Santos[2]; Marcia Chame[1&]

1. Fundação Oswaldo Cruz – Programa Institucional Biodiversidade e Saúde, Av. Brasil 4036, sala 214, Rio de Janeiro - RJ - 21.040-361, Brazil.

2. Laboratório de Virologia Molecular, Instituto Carlos Chagas, Fundação Oswaldo Cruz, Rua Prof. Algacyr Munhoz Mader 3775, Curitiba – PR - 81350-010, Brazil.

*Corresponding author

Email: buenomg@gmail.com (MGB)

&Second Corresponding author

Email: mchame@fiocruz.br (MC)

ABSTRACT

Zika virus (ZIKV) was first isolated in 1947 in primates in Uganda, West Africa. The virus remained confined to the equatorial regions of Africa and Asia, cycling between infecting monkeys, arboreal mosquitoes, and occasional humans. The ZIKV Asiatic strain was probably introduced into Brazil in 2013. In the current critical human epidemic in the Americas, ZIKV is transmitted primarily by *Aedes aegypti* mosquitoes, especially where the human population density is combined with poor sanitation. Presently, ZIKV is in contact with the rich biodiversity in all Brazilian biomes, bordering on other Latin American countries. Infections in Brazilian primates have been reported recently, but the overall impact of this virus on wildlife in the Americas is still unknown. The current epidemic in the Americas requires knowledge on the role of mammals, especially non-human primates, in ZIKV transmission to humans. The article discusses the available data on ZIKV in host animals, besides issues of biodiversity, rapid environmental change, and impact on human health in megadiverse Latin American countries. The authors reviewed scientific articles and recent news stories on ZIKV in animals, showing that 47 animal species from three orders (mammals, reptiles, and birds) have been investigated for the potential to establish a sylvatic cycle. The review aims to contribute to epidemiological studies and the knowledge on the natural history of ZIKV. The article concludes with questions that require urgent attention in epidemiological studies involving wildlife in order to understand their role as ZIKV hosts and to effectively control the epidemic.

INTRODUCTION

A brief history of the Zika virus

Zika virus (ZIKV) is an emerging flavivirus from the same family as the West Nile (WNV), Japanese encephalitis (JEV), dengue (DENV), and yellow fever viruses (YFV) [1, 2]. ZIKV is an RNA virus, mostly transmitted to humans by bites from infected *Aedes* spp. mosquitoes, especially *Aedes aegypti*, which also transmits dengue and Chikungunya virus (CHIKV) in urban settings [3]. Other *Aedes* species have been implicated in ZIKV transmission, mainly in sylvatic cycles, including *A. africanus*, *A. albopictus*, *A. apicoargenteus*, and *A. furcifer* [4, 5, 6, 7].

ZIKV was first identified in 1947 in primates during a yellow fever virus study in Uganda [4]. The first reports of infected humans appeared five years later in Uganda and Tanzania [8], but the infection remained limited to equatorial regions of Africa and Asia, cycling between infective monkeys, arboreal mosquitoes, and occasional humans [9, 10]. Mosquitoes captured annually since 1965 in Senegal have shown that ZIKV amplifies cyclically every four years [11]. ZIKV outbreaks in humans occurred in 2007 on the island of Yap, Micronesia, and in Gabon [12, 5] and in 2013 in French Polynesia [13].

In the Americas, ZIKV is probably transmitted mainly by *Aedes aegypti*, a highly competent and anthropophilic vector species [14]. This mosquito, autochthonous to North Africa, spread to the Americas and Europe by the slave trade and adapted to the urban and domestic environment and, enabled the transmission of different arboviruses like DENV, YFV and CHIKV to humans, especially in areas with high population density and poor sanitation [15].

Species of the mosquito genera *Sabethes* and *Haemagogus* spp. have also been implicated in yellow fever transmission in the New World, and *Aedes albopictus*, which also occurs in the

Americas, has been incriminated to transmitt ZIKV in Gabon [16]. However, the role of these vectors in maintaining ZIKV transmission in the Americas is not known.

Recent phylogenetic and molecular studies suggests a single introduction of the ZIKV Asiatic strain into the Americas (Brazil) between May and December 2013 [17] and in February 2014 in Chile [18]. In early 2015, several patients in Northeast Brazil presented dengue-like symptoms, and molecular diagnosis revealed autochthonous ZIKV infection [19].

An undetermined percentage of individuals with ZIKV infection fail to present clinical signs, but symptomatic individuals present mild fever, rash, headache, arthralgia, myalgia, asthenia, and non-purulent conjunctivitis three to twelve days after the mosquito vector bite [10, 13]. ZIKV infection poses a public health threat in Brazil, causing fetal microcephaly and other congenital malformations, besides other neurological disorders such as Guillain-Barré syndrome in adults [3].

ZIKV has invaded the huge biodiversity of all the Brazilian biomes, bordering on other Latin American countries. Althouse et al. (2016) [16] modeled the Zika virus transmission dynamics, estimating the numbers of primates and mosquitos needed to maintain a wild ZIKV cycle. Six thousand primates and 10,000 mosquitoes are enough to support a ZIKV transmission cycle. Based on the number of Brazilian primate species, the proximity of these and other small mammal species to urban and rural areas, and the wide distribution of *A. aegypti*, *A. albopictus*, and other mosquito genera like *Haemagogus* throughout the country, ZIKV spillover to wild primates is a potentially real scenario [20]. A wildlife cycle would launch new transmissions dynamics with unknown impacts on other animal species, including humans.

This review aims to describe the available data on ZIKV infection in host animals and its relationship to biodiversity, rapid environmental changes, and the impact on human health in megadiverse Latin American countries.

METHODS

Recent advances in scientific research have emerged since ZIKV has become pandemic. We searched for scientific articles and news stories on research involving the ZIKV in animals using PubMed citation and index (http://www.ncbi.nlm.nih.gov/pubmed), the Fiocruz Library database (http://www.fiocruz.br/bibmang/cgi/cgilua.exe/sys/start.htm?tpl=home), Scopus database, (https://www.scopus.com) and websites for news stories in the mainstream lay press.

RESULTS AND DISCUSSION

Animals as ZIKV hosts

Few studies have focused on the role of animals as hosts for ZIKV. Some authors claim that there is no solid evidence of wild mammals such as non-human primates (NHP) as reservoirs for ZIKV. Meanwhile, studies have reported ZIKV antibodies in livestock like goats and sheep, rodents [21], and bats, lions, and ungulates like Artiodactyla, Perissodactyla, and Proboscidea [22]. According to a serological study in Senegal [23], monkeys from genera *Erythrocebuspatas* and *Clorocebussabeus* may act as ZIKV hosts in nature. In 1971, ZIKV antibodies were detected in primates from the Cercopithecidae family in Nigeria [24]. Several studies suggest that DENV, CHIKV and ZIKV adapted from an ancestral enzootic transmission cycle involving non-human primates and a broad spectrum of species from genus *Aedes* (*Stegomyia, aegypti*) as vectors in an urban–periurban cycle [25].

ZIKV infection has also been identified in naturally and experimentaly suscebtible other animal species (Table 1 and Fig. 1). Sera from 172 domestic animals and 157 wild rodents were tested for ZIKV in Pakistan, using the complement fixation test, showing that sheep, goats, some rodent species (*Tatera indica*, *Meriones hurrianae*, *Bandicota bengalensis)*, and one human

living in the same area tested positive for ZIKV antibodies [21]. The authors suggested the need for a better understanding of this pathogen's natural history.

A study in Kenya in 1977 focused on the potential role of livestock (goats, sheep, and cattle) and wild vertebrates (2,424 small mammals, 1,202 birds, 18 reptiles) in maintaining arbovirus transmission. Hemagglutination inhibition assays showed that domestic animals (0.4%), wild birds (0.4%), small wild mammals (5.9%), and reptiles (27.7%) tested positive for ZIKV (Table 1) [26].

So, it is noteworthy that serologic studies should be interpreted carefully in view of possible cross-reactions with other antigenic flavivirurus, despite studies suggest that plaque reduction neutralization test (PRNT) do not cross and is the most specific serological test for the proper serological identification of flaviviruses [27, 28]

Unlike humans, wild mammals with ZIKV infection display few clinical signs. In a sentinel study in Uganda in 1947, primates showed only mild pyrexia. All monkeys inoculated by different routes developed neutralizing antibodies by day 14 after inoculation [4]. In the same study, Swiss mice became ill and one animal died following intracerebral inoculation [8]. Such inoculation is not a natural transmission route, and authors point out that some species of wild and laboratory rodents are resistant to some flavivirus infections, due to genetic resistance [29].

Most primates identified as ZIKV-positive in the wild or in sentinel studies are from Old World species. Phylogenetic analysis shows that humans are more closely related to Old World primate species, especially chimpanzees and orangutans [30]. Diseases that can be transmitted between closely related species often increase the relative risk. Non-human primates thus deserve special attention because of their close relatedness to humans and potential disease exchange [31].

Favoretto et. al. (2016) [20], using RT-PCR, showed that 29% of the New World primates

Callithrix jacchus (common marmoset) *Sapajus libidinosus* (black-striped capuchin) in Ceará State in Northeast Brazil were infected with ZIKV. They also showed that the ZIKV genome sequence from monkeys was 100% similar to the ZIKV circulating in humans in South America, suggesting that primates sharing the habitat with humans could act as ZIKV reservoirs, as in the yellow fever sylvatic cycle in Brazil.

Besides the use of primates as sentinels in ZIKV studies, some experimental work has been performed with other mammals. Cotton-rats (*Sigmodon hispidus hispidus*), guinea pigs (*Cavia* sp.), and rabbits showed no clinical signs of infection after intracerebral inoculation [8]. An experiment in 1955 aimed to determine the susceptibility of cave bats (*Myotus lucifugus*) to ZIKV and showed that these bats are susceptible to ZIKV by intraperitoneal, intradermal, intracerebral, and intrarectal exposure, but not by intranasal exposure [32].

Barr et al. (2016) [33] infected cell cultures from different animal species with ZIKV and showed that 17 were susceptible to the virus, developing a cytopathic effect seven days post-infection. Some of the cell cultures were from domestic animals (dog, cats, chickens, horses, pigs, and cattle) and others from Old World wild animals (*Macaca mulatta*), while eight were from wild species found in the Americas: free-tailed bat (*Tabarida brasiliensis*), cottontail rabbit (*Sylvilagus floridanus*), gray fox (*Urocyon cinerorgeneus*), mule deer (*Odocoileus hemionus*), raccoon (*Procyon lotor*), Virginia opossum (*Didelphis virginiana*), nine-banded armadillo (*Dasypus novemcinctus*), Eastern woodchuck (*Marmota monax*), and American mink (*Neovison vison*). Most of these animals are peridomestic and sympatric to mosquito vectors. The authors also argued that with sufficiently high viremia, these animals could serve as reservoirs or hosts. However, they also indicated that the virus strain used in the experiment lacks some characteristics of the ZIKV currently circulating in the field, and that the virus in the laboratory does not mirror natural infection.

Public policy and elimination efforts in the Americas are currently based mainly on vector control and personal protection measures, so the high number of wild species with the potential to establish a sylvatic cycle makes elimination extremely difficult if not impossible [16]. We thus need studies on ZIKV in wild and domestic animals in the Americas, both to understand their potential role as hosts in the natural cycle and to target surveillance for enzootic ZIKV transmission.

Biodiversity, animal hosts, and diseases

Human health relates closely to environmental health, defined here as the relationship between the health of domestic animals, wildlife, and the environment. Most etiological agents (60.3%) circulate between animals and humans (zoonotic diseases), and 71.8% of emerging diseases are caused by pathogens originating in wildlife [34]. A recent study associated 2,107 etiological agents with diseases in humans and animals [35].

Emerging diseases often occur in areas most heavily affected by natural events and human interventions, which further exacerbates social inequalities, health care costs, which influence the quality of life [36]. Vector-borne and parasitic diseases, with the disease burden driven by changes in biodiversity, have been shown to amplify the poverty cycle in many areas [37].

Recent efforts by the Convention on Biological Diversity and the World Health Organization have addressed scientific and political discussions on the relationship between human health and biodiversity. Such relationships include global concern over the importance of emerging zoonotic diseases originating in wildlife. Environmental changes, including loss of biodiversity, can favor emerging diseases originating from wildlife and act as the source of selective forces in new genetic variations leading to spillover and infecting humans [38]. This justifies actions to improve knowledge on biodiversity and pathogens and to monitor them to

anticipate problems with installed competence. This approach has been strengthened by international and government programs that invest considerable resources in tracing pathogens worldwide. Monitoring diseases in animals poses a huge challenge for large, developing, and megadiverse countries like Brazil.

In this scenario, beyond seeking effective responses to health crises, we should implement measures that anticipate problems so that we can mitigate emerging diseases wherever possible and respond quickly when prevention and/or mitigation prove impossible or unfeasible.

The current ZIKV epidemic in Brazil requires understanding on the role of mammals, especially primates, in viral transmission to humans, especially when this interface occurs in fragmented forest areas as described by Favoretto et al. (2016) [20]. Such areas are usually bordered or surrounded by farmland and human settlements and by dense urban and unstructured areas that can increase contact between humans, wildlife, and domestic animals and occasionally promote disease spillover [39, 40]. Wild animals, especially primates, can thus be considered sentinels for pathogens of human health concern [41, 40]. ZIKV is an example of spillover, since this virus adapted from an ancestral transmission cycle involving non-human primates to an urban-periurban cycle, with humans as the main host.

Brazil is a megadiverse country with 357 million hectares of tropical forest and other highly biodiverse biomes. It is by far the world's richest country in terms of biomes. Not surprisingly, Brazil has more primate species than any other country. Its 53 species account for 27% of the world's primates. Forty of the 56 New World primate species are vulnerable, endangered, or critically endangered according to the IUCN red list of threatened species. The Atlantic Forest region is one of the highest priority areas for conservation in Brazil, since it is located in the most developed and most devastated part of the country [42], where 70% of the human population live between fragments of the natural forest [43].

Some non-human primate species occupy urban forests due to habitat fragmentation and have close contact with humans and domestic animals. Examples include primates from the Callitrichinae (*Callithrix, Leontopithecus*, and *Saguinus*), Cebinae (*Cebus*), and Atelidae families (*Alouatta* and *Brachyteles*) [44]. Favoretto et al. (2016) [20] were the first to report ZIKV in non-human primates in Northeast Brazil, highlighting that these New World primates can act as potential ZIKV reservoirs in the Americas. Many questions remain unanswered. Does ZIKV impact the health of non-human primates? Are NHPs living in urban fragments of forest more prone to ZIKV infection than those in preserved areas? Can naturally infected neotropical primates transmit ZIKV to mosquito vectors and thus help keep the virus circulating in the Americas?

Barr et al. (2016) [33] demonstrated the feasibility of infection in cell cultures from other mammalian species like carnivores, armadillos, rodents, and bats, thus raising the possibility of a transmission network shaped by biological and ecological factors. These include vector and host density and behavior, virulence, viral load, immunity, genetic variation, climate change, competition between biological communities, and anthropogenic forces like urbanization, sanitation, limited access to health services, poverty, and mistreatment of animals [29].

Considering the current epidemiological scenario with simultaneous circulation of the arboviruses ZIKV, DENV, and CHIKV and the fact that Brazil has a large non-human primate population, there is an urgent need to answer these questions to evaluate the impact of diseases like Zika on the non-human primate population in Brazil and elsewhere in the Americas. Yellow fever virus, another flavivirus that circulates in a sylvatic cycle in the Americas, has a great impact on primate populations, especially those of genus *Alouatta* [45] that exhibit disease signs after infection and act as sentinel primates for viral circulation and for implementation of control measures like human vaccination campaigns.

The pandemic ZIKV strain differs significantly from the African strain mainly in two

regions of the genome. These acquired genetic markers increase its fitness for replication in the human host [3]. Whether these mutations also alter the infectivity in non-human primates remains to be determined. The role of wild primates and other mammals in ZIKV epidemiology thus requires urgent investigation.

Another relevant issue is the development of diagnostic tests for the detection of ZIKV infection in wild mammals, enabling unequivocal results without cross-reactivity with other flavivirus infections such as dengue and yellow fever.

A major threat to biodiversity is the introduction of invasive alien species (IAS) with potential impact on human health and infectious diseases. Some pathogenic parasites like mosquito-borne West Nile (also belonging to the genus flavivirus) virus can be categorized as IAS. Some authors consider modern pandemics like HIV and SARS as microbial-level invasion [38].

IAS can involve species, sub-species, or other taxa introduced by human action outside their natural (past or present) distribution and whose introduction, establishment, and spread threaten biological diversity and ecosystem integrity [38]. ZIKV is an example of IAS, due to it allocthonous origin and wide distribution in Brazil, and its origin in Africa, as with *Aedes aegypti* [46]. However, we do not know the impact of this virus on biodiversity, as with other IAS, or the ability of ZIKV to infect native vectors and mammalian hosts in the Americas or its potential to create new and different epidemiological cycles.

Final Comments and Research Perspectives

Despite the growth of epidemiological knowledge in the last century, health interventions still mainly react to emergency events involving specific diseases in the human population, with some mitigation efforts [38]. The current ZIKV epidemic is no exception. We cannot expect to

completely block the emergence of diseases, considering vector spread due to our limited capacity to reverse climate change, the globalization of goods and people, and our mode of production and consumption of natural resources. This situation is particularly paradoxical in megadiverse countries like Brazil.

The driving forces in the spread of diseases apply to the ZIKV epidemic, including anthropogenic activities, climatic change, intense human movement, loss of biodiversity, habitat destruction, land use change, introduction of invasive species, urban development, lack of knowledge on the role of animals in maintaining the sylvatic cycle, clinical manifestations, and wildlife trafficking [38]. The latter still occurs on a wide scale in Brazil. According to a national report, Brazil accounts for 5% to 15% of all smuggled animals in the world, with the removal of 12 million specimens from nature every year. Among animals trafficked in the New World, 95% are primate species from Brazil [47]. Wild animals are also extensively displaced inside Brazil due to domestic wildlife trafficking and human interventions like the construction of hydroelectric dams and highways. Such wildlife displacement has been implicated in increasing diseases and disseminating pathogens to new areas [48].

We need to understand the diversity of pathogens in nature and correlate them with biological communities, pathogenic and genetic characteristics, and anthropic impacts in areas where transmission and diseases occur. The complex evolutionary relationships between parasites, hosts, and vectors make this a challenging but strategically important task in face of the globalization, persistent poverty, and increasing environmental change. Awareness-raising is not enough to solve this problem. We need to expand the knowledge to diverse social actors and health and environmental services. The ZIKV epidemic illustrates the importance of monitoring and predicting the pathogens arising from wild animals and biodiversity.

Based on the above and the results of other studies, we pose several questions and

hypotheses that emerge from this discussion and that require investigation:

1. What other wild animals besides primates could be infected by ZIKV in Americas? What is their role in maintaining and transmitting the virus to mosquito vectors? Which species can act as reservoirs?

2. Does the virus circulate at higher levels in wild animals inhabiting forest fragments adjacent to urban areas? What role do these animals play in maintaining the virus in areas close to humans?

3. Which wild hosts help keep the virus circulating in the Americas?

4. Do neotropical primates play a special role in the ZIKV epidemic?

5. Does the ZIKV impact wild animal populations and biodiversity? Does it cause disease and mortality in these animals?

Infectious diseases have important implications for animal and human health and biodiversity. Public health and biodiversity needs are misaligned and need to be rebalanced.

Rather than merely attacking and solving epidemic situations, as in the current ZIKV global health emergency, we need to predict and prevent future emerging diseases. Studies of wild hosts are troublesome and costly, especially when they require long-term monitoring. Funding also needs to be targeted for these studies. Future laboratory, field, and epidemiological research should focus on wildlife hosts to elucidate their role in ZIKV epidemiology in the Americas and enhance the epidemic's control.

Table 1. Chronological Zika virus natural and experimental assay (EA) infection in mammalian hosts in the world

		Mammalian hosts					
		Taxonomic group					
Date report	**Country**	**Order**	**Family**	**Common name**	**Scientific name**	**Diagnostic methods for ZIKV**	**Reference**
1947	Uganda	Primates	Cercopithecidae	Rhesus monkey (sentinela)	*Macaca mulatta*	Virus isolation	[4]
1952*	London (EA)	Rodentia	Caviidae	Guineapigs	*Cavia* sp.		[8]
1952*	London (EA)	Lagomorpha	Leporidae	Rabbit	Not mentioned		[8]
1952*	London (EA)	Rodentia	Muridae	Swiss albino mice	*Mus musculus*		[8]
1952*	London (EA)	Rodentia	Cricetidae	Cotton-rats	*Sigmodon hispidus hispidus*		[8]
1952*	London (EA)	Primates	Cercopithecidae	Rhesus monkey	*Macaca mulatta*		[8]
1952*	London (EA)	Primates	Cercopithecidae	Red-tailed monkey	*Cercopithecus ascanius schmidti*		[8]
1952*	London (EA)	Primates	Cercopithecidae	African green monkey	*Chlorocebus aethiops*		[8]
1952*	London (EA)	Primates	Cercopithecidae	Rhesus monkey	*Macaca mulatta*		[8]
1955*	EUA (EA)	Chiroptera	Pteropodidae	Cave bat	*Myotus lucifugus*		[30]
1968	Kenya	Artiodactyla	Bovidae	Gazelle	Not mentioned	HIA	[22]
1968	Kenya	Artiodactyla	Bovidae	Kongoni	*Alcelaphus buselaphus*	HIA	[22]
1968	Kenya	Carnivora	Felidae	Lion	*Panthera leo*	HIA	[22]

1968	Kenya	Artiodactyla	Bovidae	Wildebeest	*Connochaetes taurinus*	HIA	[22]
1968	Uganda	Primates	Cercopithecidae	African green monkey	*Chlorocebus aethiops*	HIA	[22]
1968	Uganda	Primates	Cercopithecidae	Red-tailed Monkey	*Cercopithecus ascanius*	HIA	[22]
1968	Uganda	Rodentia	Muridae	Abyssinian grass rat	*Arvicanthis abyssinicus*	HIA	[22]
1968	Zambia	Cetartiodactyla	Bovidae	African buffalo	*Syncerus caffer*	HIA	[22]
1968	Zambia	Artiodactyla	Hippopotamidae	Hippo	Not mentioned	HIA	[22]
1968	Zambia	Proboscidea	Elephantidae	Elephant	Not mentioned	HIA	[22]
1968	Zambia	Artiodactyla	Bovidae	Impala	*Aepyceros melampus*	HIA	[22]
1968	Kenya	Perissodactyla	Equidae	Zebra	Not mentioned	HIA	[22]
1969-1970	Uganda	Primates	Cercopithecidae	Red-tailed monkey	*Cercopithecus ascanius schmidti*	Virus isolation	[6]
1969-1970	Uganda	Primates	Cercopithecidae	Mona monkey	*Cercopithecus mona denti*	Virus isolation	[6]
1969-1970	Uganda	Primates	Cercopithecidae	Omo river guereza	*Colobus abyssinicus uellensis*	Virus isolation	[6]
1969-1970	Uganda	Primates	Cercopithecidae	White-cheeked mangabey	*Cercocebus albigena johnstoni*	Virus isolation	[6]
1969-1971	Nigeria	Primates	Cercopithecidae	African green monkey	*Chlorocebus aethiops*	HIA and SN	[24]
1971	Nigeria	Primates	Cercopithecidae	Mona Monkey	*Cercopithecus mona*	HIA and SN	[24]
1971	Nigeria	Primates	Cercopithecidae	Western Putty-nosed Monkey	*Cercopithecus nictitans martini*	HIA and SN	[24]
1971	Nigeria	Primates	Cercopithecidae	Red-capped Mangabey	*Cercopithecus torquatus*	HIA and SN	[24]
1971	Nigeria	Primates	Cercopithecidae	Olive Baboon	*Papio anubis choras*	HIA and SN	[24]

1971	Nigeria	Primates	Cercopithecidae	Wadi monkey	*Erythrocebus patas*	HIA and SN	[24]
1977	Kenya	Ciconiiformes	Threskiornithidae	African Sacred Ibis	*Threskiornis aethiopicus*	HIA	[26]
1977	Kenya	Ciconiiformes	Ardeidae	Cattle Egret	*Bubulcus ibis*	HIA	[26]
1977	Kenya	Charadriformes	Scolopacidae	Ruff	*Philomachus pugnax*	HIA	[26]
1977	Kenya	Rodentia	Muridae	African Grass Rat	*Arvicanthus niloticus*	HIA	[26]
1977	Kenya	Rodentia	Muridae	Kaiser's Rock Rat	*Aethomys kaiseri*	HIA	[26]
1977	Kenya	Rodentia	Soricidae	African giant shrew	*Crocidura occidentalis*	HIA	[26]
1977	Kenya	Squamata	Lamprophiidae	Brown House Snake	*Boaedon fuliginosus*	HIA	[26]
1977	Kenya	Squamata	Varanidae	Common Water Monitor	*Varanus niloticus*	HIA	[26]
1977	Kenya	Cetartiodactyla	Bovidae	Goat	*Capra aegagrus*	HIA	[26]
1977	Kenya	Cetartiodactyla	Bovidae	Sheep	*Ovis aries*	HIA	[26]
1977	Kenya	Cetartiodactyla	Bovidae	Cattle	*Bos taurus*	HIA	[26]
1978	Indonesia	Perissodactyla	Equidae	Horse	*Equus caballus*	HIA	[52]
1978	Indonesia	Cetartiodactyla	Bovidae	Cattle	*Bos taurus*	HIA	[52]
1978	Indonesia	Artiodactyla	Bovidae	Carabao	*Bubalus bubalis*	HIA	[52]
1978	Indonesia	Cetartiodactyla	Bovidae	Goat	*Capra aegagrus*	HIA	[52]
1978	Indonesia	Anseriformes	Anatidae	Duck	Not mentioned	HIA	[52]
1978	Indonesia	Chiroptera	Not described	Bat	Not mentioned	HIA	[52]
1983	Pakistan	Rodentia	Muridae	Antelope rat	*Tatera indica*	CTF	[21]

1983	Pakistan	Rodentia	Muridae	Indian desert jird	*Meriones hurrianae*	CTF	[21]
1983	Pakistan	Rodentia	Muridae	Sind rice	*Bandicota bengalensis*	CTF	[21]
1983	Pakistan	Cetartiodactyla	Bovidae	Sheep	*Ovis aries*	CTF	[21]
1983	Pakistan	Cetartiodactyla	Bovidae	Goat	*Capra aegagrus*	CTF	[21]
1996-1998	Malaysia	Primates	Hominidae	Western Bornean Orangutan	*Pongo pygmaeus pygmaeus*	ELISA and IFAT	[49]
2001	Malaysia	Primates	Hominidae	Bornean orangutan	*Pongo pigmaeus*	SN	[53]
2015	Indonesia	Primates	Cercopithecidae	Crab-eating macaques	*Macaca fasciculares*	PCR	[51]
2016	Brazil	Primates	Cebidae	Capuchin monkey	*Sapajus libidinosus*	RT-PCR	[20]
2016	Brazil	Primates	Callitrichidae	Marmoset	*Callithrix jacchus*	RT-PCR	[20]

Legend: ELISA - Enzyme-linked Immunosorbent Assay; PCR - Polymerase Chain Reaction; RT-PCR - Real Time PCR; HIA - Hemagglutination Inhibiting Antibodies; IFAT - Immunofluorescence Antibody Test; SN - Serum Neutralization; CTF - Complement fixation test

Acknowledgments

We would like to thank the Fundação Oswaldo Cruz (Fiocruz) for the support and we also thank Dr. Fernando Dias de Avila Pires for constructive comments.

References

1. International comittee on taxonomy of viruses. Virus taxonomy. 2014. Release 2015. Available: http://www.ictvonline.org/virustaxonomy.asp.

2. Pierson TC, Diamond MS. Flaviviruses. In: Knipe DM, Howley PM, editors, Fields Virol, Lippincott Williams & Wilkins, Philadelphia; 2013. pp. 747-794.

3. Russell PK. The Zika Pandemic - A Perfect Storm? PLoS Negl Trop Dis. 2016; 10: e0004589. doi: 10.1371/journal.pntd.0004589 PMID: 26991663.

4. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952; 46: 509–520. doi:10.1016/0035-9203(52)90042-4 PMID: 12995440.

5. Grard G, Caron M, Mombo IM, Nkoghe D, Ondo SM, Jiolle D, et al. Zika Virus in Gabon (Central Africa) – 2007: A New Threat from Aedes albopictus? PLoS Negl Trop Dis. 2014; 8: e2681. doi:10.1371/journal.pntd.0002681.

6. McCrae AW, Kirya BG, Yellow fever and Zika virus epizootics and enzootics in Uganda. Trans R Soc Trop Med Hyg. 1982;76(4):552-62. doi: 10.1016/0035-9203(82)90161-4 PMID: 6304948.

7. Faye O, Freire CCM, Iamarino A, Oliveira JVC, Faye O, Oliveira JVC, et al. Molecular

Evolution of Zika Virus during Its Emergence in the 20thCentury. PLoS Negl Trop Dis; 2014; 8: e2636. doi: 10.1371/journal.pntd.0002636 PMID: 24421913.

8. Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952; 46: 521–534. doi: 10.1016/0035-9203(52)90043-6 PMID: 12995441.

9. Hayes EB. Zika virus outside Africa. Emerg. Infect. Dis. 2009; 15: 1347-1350. doi: 10.3201/eid1509.090442 PMID: 19788800.

10. Zanluca C, Santos CND. Zika virus - an overview. Microbes Infect. 2016; 18: 295-301. doi:10.1016/j.micinf.2016.03.003 PMID: 26993028.

11. Althouse BM, Hanley KA, Diallo M, Sall AA, Ba Y, Faye O, et al. Impact of climate and mosquito vector abundance on sylvatic arbovirus circulation dynamics in Senegal. Am J Trop Med Hyg. 2015; 92: 88-97. doi: 10.4269/ajtmh.13-0617 PMID: 25404071.

12. Ioos S, Mallet H-P, Leparc Goffart I, Gauthier V, Cardoso T, Herida M. Current Zika virus epidemiology and recent epidemics. Médecine Mal. Infect. 2014; 44: 302–307. doi: 10.1016/j.medmal.2014.04.008 PMID: 25001879.

13. World Health Organization. Epidemiological Update Zika virus infection. 2015. Available:http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32021&lang=en.

14. Albuquerque, C. Fiocruz identifica mosquitos Aedes aegypti naturalmente infectados pelo vírus Zika. Portal Fiocruz. 25 May 2016. Available: http://portal.fiocruz.br/pt-br/content/fiocruz-identifica-mosquitos-aedes-aegypti-naturalmente-infectados-pelo-virus-zika. Accessed 27 May 2016.

15. Neiderud, CJ. How urbanization affects the epidemiology of emerging infectious diseases. Infection Ecology and Epidemiology. 2015; 5: 27060. doi: 10.3402/iee.v5.27060 PMID: 26112265.

16. Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver S, Hanley KA. Potential for Zika virus to establish a sylvatic transmission cycle in the Americas. [In press] BioRxiv. http://dx.doi.org/10.1101/047175. Accessed 5 Apr. 2016.

17. Faria NR, Azevedo RSS, Kraemer MUG, Souza R, Cunha MS, Hill SC et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science. 2016; 351: 1371-1494. doi: 10.1126/science.aaf5036 PMID: 27013429.

18. Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol. 2016;161: 665-668. doi: 10.1007/s00705-015-2695-5 PMID: 26611910.

19. 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. doi: 10.1590/0074-02760150192 PMID: 26061233.

20. Favoretto S, Araújo D, Oliveira D, Duarte N, Mesquita F, Zanotto P, et al. First detection of Zika virus in neotropical primates in Brazil: a possible new reservoir. [In press]. BioRxiv. doi: http://dx.doi.org/10.1101/049395. Accessed 20 April 2016.

21. Darwish MA, Hoogstraal H, Roberts TJ, Ahmed IP, Omar F. A sero-epidemiological survey for certain arboviruses (Togaviridae) in Pakistan. Trans R Soc Trop Med Hyg. 1983; 77: 442-445. doi:10.1016/0035-9203(83)90106-2 PMID: 6314612.

22. Henderson BE, Hewitt LE, Lule M. Serology of wild mammals. In: Virus Research Institute 409 Annual Report. East African Printer, Nairobi, Kenya. 1968, pp. 48–51. http://dx.doi.org/10.2471/BLT.16.171082

23. Cornet M, Robin Y, Chateau R, Hème G, Adam C, Valade M, et al. Isolements d'arbovirus au Sénégal Oriental h partir de moustiques (1972-1977) et notes sur l'épidémiologie des virus transmis par les *Aedes*, en particulier du virus amaril. sér. Ent. méd. et Parasitol. 1979; XVII: 149-163.

24. Monath TP, Kemp GE. Importance of non-human primate in yellow fever epidemiology in Nigeria. Trop Geogr Med. 1973; 25:28-38. PMID: 4632785.

25. Musso D, Cao-Lormeau VM, Gubler DJ. Zika virus: following the path of dengue and chikungunya? Lancet. 2015; 386: 243–244. doi: 10.1016/S0140-6736(15)61273-9 PMID: 26194519.

26. Johnson BK, Chanas AC, Shockle P, Squires EJ, Gardner P, Wallace C, et al. Arbovirus isolations from, and serological studies on, wild and domestic vertebrates from Kano Plain, Kenya. Trans. R. Soc. Trop. Med. Hyg. 1977; 71: 512-517. doi: 10.1016/0035-9203(77)90146-8 .

27. Gennaro AD, Lorusso A, Casaccia C, Conte A, Monaco F, Savini G. Serum Neutralization Assay Can Efficiently Replace Plaque Reduction Neutralization Test for Detection and Quantitation of West Nile Virus Antibodies in Human and Animal Serum Samples. Clin Vaccine Immunol. 2014; 1: 1460 -1462. doi:10.1128/CVI.00426-14.

28. Mansfield KL, Horton DL, Johnson N, Li L, Barrett AD, Smith DJ et al. Flavivirus-induced antibody cross-reactivity. J Gen Virol. 2011; 92: 2821-2829. doi: 10.1099/vir.0.031641-0 PMCID: PMC3352572.

29. Kuno G, Chang GJJ. Biological Transmission of Arboviruses: Reexamination of and New Insights into Components, Mechanisms, and Unique Traits as Well as Their Evolutionary Trends. CMR. 2005; 18: 608-637. doi:10.1128/CMR.18.4.608–637.2005 PMID: 16223950.

30. Haviland WA, Prins HEL, Walrath D, McBride B. Anthropology: The Human Challenge 14st ed. Belmont: Wadsworth, Cengange Learning. In: Living primates. Wadsworth/Cengage Learning; 2014. 61pp. ISBN-13: 9781133941323.

31. Unwin S, Ancrenaz M, Bailey W. Handling, anesthesia, health evaluation and biological sampling. In: Setchell JM, Curtis DJ, editors. Field and laboratory methods in primatology: A Practical Guide. 2nd ed. Cambridge, UK: Cambridge University Press; 2011. pp. 147-168.

32. Reagan RL, Rumbaugh H, Nelson H, Brueckner AL. Effect of Zika virus and Bwanba virus in the cave bat (Myotus lucifugus). Trans Am Microsc Soc. 1955; 74: 77-79. doi: 10.2307/3223847.

33. Barr KL, Anderson BD, Long MT. Working with Zika and Usutu Viruses in Vitro. [In press] BioRxiv. http://dx.doi.org/10.1101/040139. Accessed 18 Feb. 2016.

34. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008; 451: 990-993. doi:10.1038/nature06536 PMID: 18288193.

35. Wardeh M, Risley C, McIntyre MK, Setzkorn C, Baylis M. Database of host-pathogen and related species interactions, and their global distribution. Sci data. 2015. doi:10.1038/sdata.2015.49 PMID: 26401317 .

36. Romanelli C, Cooper D, Maiero M, Campbell-Lendrum D, Villalobos E, Sommerfeld J et

al. Biodiversity and human health linkages: concepts, determinants, drivers of change and approaches to integration. In: WHO. Connecting Global Priorities: Biodiversity and Human Health. A State of Knowledge Review, 2015, pp.28.

37. Pongsiri MJ, Roman J, Ezenwa VO, Goldberg TL, Koren HS, Newbold SC, et al. Biodiversity Loss Affects Global Disease Ecology. BioScience. 2009; 59: 945-954. doi:10.1525/bio.2009.59.11.6.

38. Karesh WB, Formenty P. Infectious diseases. In: WHO. Connecting Global Priorities: Biodiversity and Human Health. A State of Knowledge Review, 2015, pp.28.

39. Osofsky SA, Kock RA, Kock MD, Kalema-Zikusoka G, Grahn R, Leyland T, et al. 2005. Building support for protected areas using a 'one health' perspective. In Friends for life: New partners in support of protected areas, edited by J. A. McNeely. Gland, Switzerland and Cambridge, United Kingdom: IUCN. pp. 65-79.

40. Wolfe ND, Escalante AA, Karesh WB, Kilbourn A, Spielman A, Lal AA: Wild primate populations in emerging infectious disease research: the missing link? Emerg Infect Dis 1998; 4: 149–158. doi: 10.3201/eid0402.980202 PMID: 9621185.

41. Gillespie TR, Chapman CA, Forest fragmentation, the decline of an endangered primate, and changes in host–parasite interactions relative to an unfragmented forest. 2008; 70: 222–230. doi: 10.1002/ajp.20475.

42. Mittermeier R. Primate Diversity and the Tropical Forest. In: Wilson EO, Peter FM, editors. Biodiversity. Harvard University; National Academy of Sciences/Smithsonian Institution; 1999. pp. 521. ISBN/ASIN: 0309037395 ISBN-13: 9780309037396.

43. Bohrer CBA. Mata Atlântica e formações associadas. In: Serra MV, Serra, MTF, editors.

Guia de História Natural do Rio de Janeiro. Rio de Janeiro: Ed. Cidade Viva; 2012. pp. 138-157.

44. Marsh, LK. Primates in Fragments. Ecology and Conservation. New York: Kluwer Academic/ Plenum Publishers. 2003. ISBN 978-1-4757-3770-7.

45. Bicca-Marques, JC. Outbreak of yellow fever affects howler monkeys in southern Brazil. Oryx. 2009; 43:169-175. doi: http://dx.doi.org/10.1017/S0030605309432046.

46. Kraemer MUG, Sinka ME, Duda KA, Mylne A, Shearer FM, Brady OJ et al. The global compendium of Aedes aegypti and Aedes albopictus occurrence. Sci Data. 2015; 2:150035. doi: 10.1038/sdata.2015.35 PMID: 26175912.

47. Giovanini D. Rede Nacional de Combate ao Tráfico de Animais Silvestres. Relatório nacional sobre o tráfico de fauna silvestre. 2002. Accessed: http://www.renctas.org.br/wp-content/uploads/2014/02/REL_RENCTAS_pt_final.pdf

48. Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287:443.

49. Kilbourn AM, Karesh WB, Wolfe ND, Bosi EJ, Cook RA, Andau M. Health evaluation of free-ranging and semi-captive orangutans (Pongo pygmaeus pygmaeus) in sabah, malaysia. JWD. 2003; 39: 73-83, doi: http://dx.doi.org/10.7589/0090-3558-39.1.73 PMID: 12685070.

50. Kindhauser MK, Allen T, Frank V, Santhana RS, Dye C. Zika: the origin and spread of a mosquito-borne virus [In press]. Bull World Health Organ. 2016; 171082 doi: http://dx.doi.org/10.2471/BLT.16.171082.

51. Leung GH, Baird RW, Druce J, Anstey NM. Zika virus infection in Australia following a monkey bite in Indonesia. Seameo Tropmed. 2015; 46: 460-464. PMID: 26521519.

52. Olson JG, Ksiazek TG, Gubler DJ, Lubis SI, Simanjuntak G, Lee VH, Nalim S, et al. A survey for arboviral antibodies in sera of humans and animals in Lombok, Republic of Indonesia. Ann Trop Med Parasitol. 1983;77:131-137. PMID: 6309104.

53. Wolfe N, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp B et al. 2001. Sylvatic transmission of arboviruses among Bornean Orangutans. Am J Trop Med Hyg. 64: 310–316. PMID:11463123.

54. World Health Organization. 2016. Zika virus situation reports. Available: http://www.who.int/entity/emergencies/zika-virus/situation-report/9-june 2016/en/index.html.

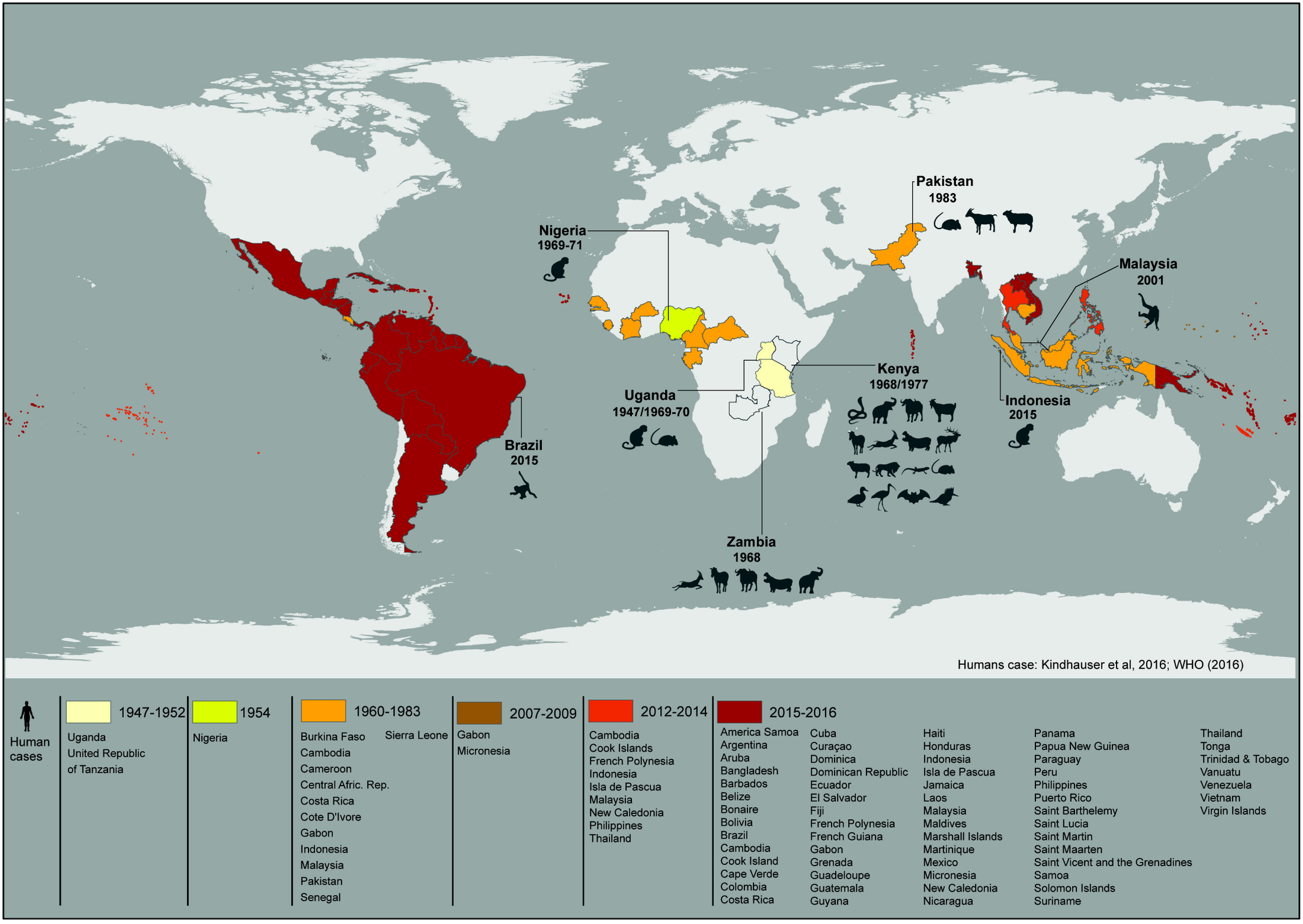

Fig 1. Historical time-line of Zika virus spread in humans and animals in the world. Colored countries have reported autochthonous vector-borne human cases, and those listed and with the years highlighted have reported diagnosed cases of ZIKV in naturally infected animals. The list of animal species is described in Table 1.