Title: Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently.

Author: Rengina Vorou

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Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread worldwide: what we know and what we should investigate urgently.

REVIEW

Dr Rengina Vorou, MD, DTM&H, MPH, Ph.D.

Medical Microbiologist,

Hellenic Center for Diseases Control and Prevention, Athens, Greece.

Hellenic Center for Diseases Control and Prevention,

3-5 Agrafon str, Marousi, Athens,PC 15 123

Greece.

e-mail: vorou@keelpno.gr

Abstract

Objectives

The widespread epidemic of Zika virus infection in South and Central America and the Caribbean in 2015 along with increased incidence of microcephaly in fetuses born to mothers infected with Zika virus and a potential for a worldwide spread indicate the need to review the current literature regarding vectors, reservoirs and amplification hosts.

Vectors

The virus has been isolated in Africa in mosquitoes of the genera *Aedes, Anopheles and Mansonia*, as well as in *Aedes* in Southeastern Asia and the Pacific area. *Aedes albopictus* has invaded several countries in Central Africa and all Mediterranean countries and continues to spread throughout Central and Northern Europe. The wide distribution of the virus in animal hosts and vectors favors the emergence of its recombinants.

Animal hosts

The virus has been isolated in monkeys, and antibodies have been detected in domestic sheep, goats, horses, cows, ducks, rodents, bats, orangutans, and carabaos.

Conclusions

It is a public health imperative to define the domestic or wild animal reservoirs, amplification hosts and vectorial capacity of the genera *Aedes*, *Anophelines*, and *Mansonia.* These variables will define the geographic distribution of Zika virus along with the indicated timing and scale of the environmental public health interventions worldwide.

Key words: Zika virus infection; vector; animal; *Aedes; Anophelines;* migrants

Highlights

- The aim of this review is to present the wide distribution of Zika virus in animal hosts and vectors and to stress the lack of evidence about the animals capable of being reservoirs or amplification hosts and their vectorial capacity.
- It is highlighted that the RNA structure of the virus facilitates the emergence of recombinants and, additionally, its circulation in a wide range of animals and vectors in West Africa has also been correlated with such emergence, a fact that raises concerns regarding the potential of a novel clinical presentation.
- It is highlighted that in Africa, Zika virus has been isolated in mosquitoes of the genera *Aedes, Anopheles,* and *Mansonia*.
- It is stressed that while in South America, southeastern Asia and the Pacific, *Aedes aegypti* is the principal vector, *A. albopictus* is being established as a competent vector as well, having invaded Central Africa, the Mediterranean and parts of Central and Northern Europe.
- It is highlighted that antibodies against the virus have been detected in domestic sheep and goats, horses, cows, ducks, rodents, bats, orangutans, and carabaos in southeastern Asia, separate from the strains of the virus isolated from monkeys in Africa.
- It is discussed that it is a public health emergency to define the domestic or wild animal reservoirs and amplification hosts, as well as the vectorial capacity of the genera *Aedes*, *Anophelines*, and *Mansonia* as they will define

the geographic distribution of Zika virus and the indicated public health interventions worldwide.

Introduction

A widespread epidemic of Zika virus infection was reported in 2015 in Central and South America and the Caribbean and was associated with increased incidence of microcephaly in fetuses born to mothers infected with Zika virus (ZIKV), with Brazil being the most affected country by both epidemics[1-4]. This rare, devastating, and untreatable complication of the fetuses was declared to be a Public Health Emergency of International Concern (PHEIC)[1-4]. The early recognized neurotropism of the virus [5] has been in concordance with the recent rise in microcephaly incidence in Brazil[3, 4, 6]. The virus has been found in the amniotic fluid of two pregnant women with fetuses suffering a reduction in the circumference of the head [6]. The virus has also been found in the central nervous systems (CNS) of the fetuses[3, 4], while negative tests excluded other congenital infections [6].

Thirty-two additional countries and territories of the Americas are affected by ZIKV, including Rio de Janeiro, Brazil, where the Olympics and Paralympics are to be held in August 2016, thus posing a threat for international travelers and local host-country residents[2]. Local transmission has not been observed in Europe yet, and there is a global alert about travelers returning from endemic countries with symptoms consistent with Zika virus infection.

The immediate public health measures feasible at present, in the absence of a vaccine, are interventions relevant to the vector and a better understanding of the wide and unknown range of possible amplification hosts. Two main concerns prompted this exhaustive review of the literature: the historical paradigm of the introduction of yellow fever virus from Africa to the Americas, where it adapted to local sylvatic vectors and primates[7], and the fact that we do not yet know which could

be the competent vectors or amplifying hosts of Zika virus in the temperate climate, thus hampering any future surveillance and intervention control programs. The range of vectors and animals in which the virus has been detected worldwide were reviewed in order to assess the likelihood of an established circulation of the virus in novel areas.

Epidemiology

Zika virus is an emerging vector-borne pathogen that was first isolated in 1947 in a sentinel rhesus macaque monkey and again in 1948 from a pool specimen of *Aedes africanus* mosquitoes of the Zika Forest in Uganda[5]. From 1947 through 2007, only serological data, entomological data and the diagnosis of 14 human cases with viral isolation or serology had been reported in Asia and Africa[8-13]**.** It has been speculated that among those human cases, a few may have been the tip of an unrecognized outbreak. One unrecognized outbreak took place in 1977-1978 in Indonesia [14, 15]. In 2007, on Yap Island in the Federated States of Micronesia, the first outbreak outside of Africa and Asia occurred, with 49 confirmed cases and 73% of residents three years or older infected according to the IgM seropositivity[16, 17]. There are limitations in the seroprevalence studies resulting from the cross-reactive nature of ZIKV with dengue and other flaviviruses. However, IgM antibodies against dengue virus persist up to 12 weeks, and the specimens for the IgM measurement were collected during a 12-week period from April 1 through July 31, 2007. During this time, the specimens had been collected within 10 days from symptom onset. They tested positive only for Zika virus RNA and not for dengue or other flavivirus RNA[17].

From 2007 through 2013, no new instances of human seropositivity or disease had been reported. In 2013, the virus reemerged in French Polynesia,and from 2013 through 2014, it disseminated to the Cook Islands, New Caledonia, Easter

Island and throughout the Pacific[18]. French Polynesia reported 396 epidemiologically confirmed cases and 29,000 suspect cases[19]. The virus has been either isolated or had its nucleic acid extracted by PCR and then sequenced from samples collected in Southeast Asia (e.g., Thailand, Indonesia, Cambodia[20-22]). All strains collected in Asia and the Pacific belong to the Asian lineage and are closely related, indicating that the virus was present in the area for several years but remained undetected as the clinical manifestations resemble those of other known endemic arboviruses such as dengue and chikungunya virus[20]. However, we cannot exclude the possibility that the virus has spread to the Pacific and SE Asia successively, causing a wave of clinical disease and subsequent detection of the virus[20].

In March 2015, Brazil notified the World Health Organization of an illness compatible with but not suspected to be a ZIKV infection. Soon after, in May 2015,it documented the first confirmed Zika virus transmission in mainland South America, along with the assumption that the virus was introduced to the country via the Va'a World Sprint Championship canoe race held in Rio de Janeiro during August 2014[23]. Four Pacific countries (French Polynesia, New Caledonia, the Cook Islands, and Easter Island) participated; subsequently, the virus was transferred from the major cities across the country via the infected Brazilian participants and spectators who returned to their home towns[24] . The strain found in Brazil was phylogenetically closer to the strain in the French Polynesia outbreak of 2013–2014, with both belonging to the Asian lineage.It is estimated that 440,000 to 1,3 million cases have been reported as of December 2015 [23].

Virus

Zika virus is an approximately 11-kb single-stranded, positive-sense ribonucleic acid (RNA) virus from the Flaviviridae family. It is related to Dengue, West Nile and Yellow fever viruses and is a member of the Spondweni serocomplex whose transmission cycle consists mainly of vectors from the *Aedes* genus (*A. furcifer, A. taylori, A.luteocephalus* and *A. africanus*) and monkeys [25, 26]. Phylogenetic analyses have revealed three lineages: two African lineages ie the MR 766 cluster and the Nigerian cluster, and one Asian lineage (Table 1) [16, 27, 28].

All lineages share a common origin in Uganda early in the 20th century, from where it dispersed west to West Africa via two introductions and east to Southeast Asia and then to the Pacific as follows: a) A relative of the MR 766 prototype strain was introduced from Uganda to Côte d'Ivoire in 1940 and from there to Senegal in 1985 resulting in the MR 766 lineage, b) a relative of the Nigerian strain was introduced from Uganda to the Central African Republic and Nigeria around 1935, and from Nigeria to Senegal and Côte d'Ivoire around 1960 forming the Nigerian lineage, and c) a ZIKV cluster was probably spread from Uganda to Malaysia in 1945, making its way to Micronesia sometime around 1960, where it formed the Asian lineage [25]. However, it is unknown whether to attribute this migration only to human and vector movements, or also to birds carrying the virus along migratory routes.

Regarding the African lineages, the phylogenetic analysis of ZIKV strains collected from 1968 through 2002 in Senegal, Côte d'Ivoire, Burkina Faso and the Central African Republic indicated that there are more recombinants than in other flaviviruses; however, they all clustered in the two African lineages, the MR 766 cluster and the Nigerian cluster.

Transmission

Transmission has been demonstrated to occur mostly via infected female mosquito vectors of the *Aedes* genus, Culicidae family. Transmission is mainly urban and sylvatic, with humans serving as primary amplification hosts in areas where there are no non-human primates [17]. The latter constitute the amplification host in a sylvatic cycle[17]. Mosquitoes, as hematophagous arthropods, acquire the virus via a blood meal, and they host it throughout their life span without being affected. They transmit it to the next amplification host, i.e., their target during the next blood meal[29]. Other routes of transmission are sexual intercourse[30-33], perinatal transmission[34] from mother to fetus, and blood transfusion[35].Breast feeding has not been reported as a mode of transmission.

Clinical manifestations

The majority of infections are subclinical, estimated to reach 81% of infected individuals. The clinical manifestations mimic those of other arboviral infections,e.g., dengue and the chikungynya endemic in tropical areas (West Africa, SE Asia, Pacific area, South America)[2, 17, 22, 36]. Macular or papular rash (90%), fever (65%), arthritis or arthralgia (65%), non purulent conjunctivitis (55%), myalgia (48%), headache (45%), and retro-orbital pain (39%) were the most commonly reported symptoms[20], followed by anorexia, vomiting, diarrhea, stomach aches, dizziness, leg pain, lymphadenopathy and hypotension. No deaths, hospitalizations, or hemorrhagic manifestations were documented[17, 20]. In the Indonesian outbreak among humans in 1977-1978, no rash was reported[14].

In Eastern Nigeria, two patients appeared with jaundice [37]. There is early evidence for neurotropism of the virus [5], which spares all body tissue except for nervous tissue [38]. Similarly, intracerebral inoculation of infected human blood in

suckling albino Swiss mice was followed by proliferation of the virus in their nervous tissue [8].

Zika virus infection has been associated with Guillain-Barré syndrome (GBS) in Martinique and French Polynesia[39, 40]. During the Zika virus outbreak of French Polynesia in 2013-2014, the incidence of GBS multiplied by 20 [39],and a case control study of a large series of patients confirmed the link between Zika virus infection and Guillain-Barré syndrome. Further, the same study did not find any evidence that past or concurrent dengue infection was a causative or predisposing factor to GBS[41]. Additionally, microcephaly and various ophthalmological findings and neurological symptoms have been observed in fetuses and infants born to infected mothers[1-4]. The autopsy findings in the central nervous systemof fetuses were a very small brain, a complete absence of cerebral gyri, severe dilation of both cerebral lateral ventricles, dystrophic calcifications throughout the cerebral cortex, and hypoplasia of the brain stem and spinal cord. Particles consistent with Zika virus were detected on electron microscopy of brain tissue but not in other tissues[3,4].

Diagnosis

It is challenging to diagnose pregnant women or symptomatic individuals living in or returning from areas endemic for flaviviruses. Flaviviruses trigger the production of cross-reactive antibodies in humans, and they cause dengue, Japanese encephalitis, Saint Louis encephalitis, West Nile fever, yellow fever and Zika infection, diseases which share partly similar symptoms[42-44]. Emphasis for diagnostics should be on molecular testing such as reverse-transcription polymerase chain reaction (RT-PCR) during the first seven days after symptom onset. After the seventh day, viraemia decreases gradually; consequently, a negative RT-PCR does not exclude flavivirus infection, and serological testing should be performed[20]. IgM

antibodies persist for about two to twelve weeks and can be detected with enzyme-linked immunosorbent assay (ELISA). If this assay is positive, neutralizing antibody detection assays, e.g., plaque reduction neutralization tests (PRNT), may enable us to determine the virus causing infection[43, 44].

The confirmation of the diagnosis of Zika virus infection relies on the detection of Zika virus RNA (RNA extraction) in blood through RT-PCR or pan-flavivirus PCR amplification followed by sequencing, or viral isolation. Alternatively, a confirmatory diagnosis may be achieved with the co-detection of anti-Zika IgM antibodies (ELISA) and a Zika virus $PRNT_{90}$(or $PRNT_{80}$) titer of at least 20 and, if West Nile virus (WNV) or dengue virus need to be ruled out, a ratio of Zika to either dengue virus or WNV PRNT titers of at least four. In contrast, a probable case of Zika virus infection tests negative by RT-PCR but positive for IgM antibody (ELISA), and has a Zika virus PRNT titer of at least 20, and a ratio of Zika to dengue virus or to WNV PRNT titers less than four[17, 20, 43, 45].

The detection of the virus in pool specimens of mosquitoes is performed with quantitative real-time PCR [46].

Vectors

The vectors of ZIKV in Africa are distinct from those in South America, Southeast Asia and the Pacific area. Outside Africa, *Aedes aegypti* is the principal vector, while *A. albopictus* is being established as a competent vector as well (Table 2).

In Africa, Zika virus has been first isolated from mosquitoes, *Aedes africanus,* collected in Zika Forest, Bwama county, Uganda, in 1948[5],1958 [47] and 1964 [10]. In 1969, ZIKV was isolated from *Aedes africanus* and *Aedes apicoergenteus* collected

in Zika Forest, Uganda[12]. In 2014, a retrospective study investigated *Aedes africanus* and *Aedes opoc* collected in Central African Republic, Western Africa, from 1976 through 1980. The phylogenetic analysis of this research revealed that the detected ZIKV strains clustered together in the African lineages of ZIKV[48] . Viral isolates from 1968 to 2002 in West Africa revealed that ZIKV detected in *Aedes dalzieli, Aedes africanus, Aedes aegypti* and *Aedes furcifer* exhibited many recombination events which could be attributed principally to the zoophilic mosquitoes *Aedes dalzieli,* which take blood meals from distinct animal species harboring different ZIKV strains at the same time[25]. Another retrospective study in Senegal, Western Africa, investigated samples collected from 1962 through 2008 and detected the Zika virus in *Ae.aegypti, Ae. dalzieli, Ae. furcifer (*known as *A. taylori), Anopheles africanus coustani, Anopheles gambiae s.l.,* and *Mansonia uniformis*[49]. In 2011, in southeastern Senegal, ZIKV was isolated from *Mansonia uniformis*, *Culex perfuscus*, and *Anopheles coustani*, but without adequate clarification about their vectorial competency [50].

However, in 2007, the first human infection with Zika was recognized in Gabon, Central Africa, as a result of the presence of *Aedes albopictus*, a species that has invaded the urban areas of the country[36]. Until then, the Zika virus epidemic had gone unrecognized as a result of co-existence with Dengue and Chikungunya viruses[36]. *A. albopictus* has invaded Cameroon in Central Africa and Mozambique in Southeast Africa, gradually replacing the indigenous *A. aegypti*[51], and depicts a dynamic expansion in temperate climates across the globe[52].

In the Pacific, vectors of Zika virus are mosquitoes of the genus *Aedes*, principally the prevalent *Aedes aegypti*, followed by the invading *Aedes albopictus*[53], which are known to transmit Chikungunya virus, Dengue virus and Zika Virus[42]. They are considered to be competent vectors of the ZIKV Asian lineage and have enhanced their prevalence from 2011 to 2014[42]. In 1969 in Malaysia, Zika virus was

isolated from *Aedes aegypti*[11]. In Indonesia in 1978, a human epidemic with Zika virus was presumably propagated by *Aedes aegypti* during the rainy season, but no study has been conducted to establish the presence of the virus in mosquitoes [14]. In Singapore, the experimental inoculation of local *A. aegypti* with the Uganda strain of ZIKV was followed by transmission of the virus[54].

However, on Yap island, in the Federated States of Micronesia, the most prevalent among the 12 mosquito species belonging to four genera collected was *Aedes henselli*, and pool specimens tested negative for Zika virus in two studies; consequently, we are not certain about the vector [17, 55]. The laboratory inoculation of Zika virus into this mosquito was conducted on Yap Island, with 80% (when high inoculum was provided) being colonized, among which 23% developed infection[55]. Similarly, in French Polynesia, where an extended epidemic occurred in October 2013, the prevalence of *Aedes henselli* is higher than any other species, supposedly rendering it to be a vector for Zika virus; however, there has been no study to detect the virus in pool specimens [24] and at the peak of the epidemic the entomological study pointed at *Aedes aegypti* and *Aedes polynesiensis* [19].

In Brazil, transmission has been attributed to *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti* can be found in rural and urban areas transmitting Chikungunya virus and four Dengue serotypes, but *Aedes albopictus* is prevalent in the country [2, 31]. Human travel among several commercial urban areas allow for the rapid movement of vectors (in cars, trucks, planes) along with the infected humans.

Aedes albopictus has colonized almost every Mediterranean country and continues to spreadthrough Central and Northern Europe. *Aedes japonicus* has spread widely in Central Europe; *Aedes atropalpus* in Northern Italy and the Netherlands; *Aedes Koreicus*in Swiss-Italian border, Belgium [56] and Germany; and

Aedes aegypti, has established itself on Madeira and around the Black Sea coast (Russia, Abkhazia, Georgia) [29].

Similar to all arboviruses, Zika vectors are influenced by animal population density. Thus, Zika virus could reveal surprising vector, reservoir and amplifying host range should it be introduced to novel tropical or temperate natural ecosystems, including Europe[25, 57]. Additionally, vectors such as *Aedes aegypti* and *A. dalzieli*, which feed on several animal species and humans, enhance the transmission rate as well as the concurrent infections and the recombination and reassortment of the genetic material of Zika virus strains [24,57].

As *Aedes aegypti* and *Aedes albopictus* thrive in stagnant water collections like those in peridomestic water supplies used in the absence of piped water provision, proliferation may be encouraged by human population growth or the migratory waves from areas with civil upheaval and the possible subsequent uncontrolled slum formation. Therefore, consistent public awareness about the significance of eliminating any peridomestic stagnant water is of critical public health importance[56, 58-60].

Animal hosts

The virus reservoir was not identified in a 1947 study in Zika Forest, Uganda, where the Zika virus was first isolated from rhesus monkeys [5]. The monkeys displayed mild or absent clinical presentation, while 5 days after experimental infection, they developed neutralizing antibodies [38]. Additionally, anti-Zika antibodies have been detected in wild mammals in Senegal in 1967-1968[61]. In 1969, in Zika Forest, ZIKV was isolated in samples taken from monkeys [12]. In Lombok, Indonesia, in 1978, anti-Zika antibodies were detected in ducks, goats, cows, horses, bats, and carabaos (water buffalo), but not in chickens, rats or wild birds, indicating the widespread

circulation of the virus in domestic animals[15]. The question of whether birds transfer the virus over long distances remains unanswered [15].

In 1982 in Gabon, antibodies against the virus were again detected in monkeys [13]. In 1983, antibodies against Zika virus were detected in Pakistan among rodents, domestic sheep and goats, as well as in human sera [9]. Samples collected in 1996-1997 from wild and semi-captive orangutans in Borneo, Malaysia, tested positive for anti-ZIKA antibodies[62]. Samples collected from monkeys in West Africa from 1968 through 2002 were examined and the virus detected with RT-PCR [25], and samples collected between 1962 and 2008 from monkeys in West Africa tested positive for specific ZIKA antigens with serology assays (Table 3)[49].

The antibody detection assays run the risk of cross-reaction with other flaviviruses co-circulating with the Zika virus, thus challenging the safe interpretation of published data. Furthermore, early laboratory methods for the detection of antibodies were of uncertain specificity and sensitivity whereas antigen and molecular assays were not developed.

Conclusions

There are few entomologic and vectorial capacity studies and limited literature regarding the investigation of the prevalence of the virus in wild and domestic animals in temperate and tropical climates. This is probably because the virus has caused mild or no clinical symptoms in humans and animals until recently.

The dissemination of the virus throughout the Pacific and South America after the French Polynesia outbreak with Zika virus in 2013 is undoubted. There is no information about the possibility of transovarian transmission of the virus in mosquitoes. The virus has been identified in the genera *Aedes, Anophelines*, and

Mansonia only in Africa, and there is no such pool specimen examination of those genera in the Asia, the Pacific, or the Americas. In the Pacific and SE Asia, however, experimental inoculation of *Aedes aegypti* and *Aedes henselli* was successful.

Aedes albopictus has expanded worldwide and can adapt to distinct ecosystems and trigger arboviral outbreaks. *Aedes aegypti,* which is prevalent in densely populated areas of South America, is recognized as difficult to eradicate and control[31].

There are concerns about the vast majority of asymptomatic while contagious infections, the recent neurologic complications and sequelae in adults and fetuses/newborns[25] and the potential genetic evolution of this RNA virus, which pose a threat for subsequent novel neurological or other manifestations. It is a vector-borne infection that can be transmitted via sexual intercourse.

In sum, we do not have sufficient information regarding the animal reservoirs and amplification hosts, including domestic animals, and the vectors of Zika virus as well as the vectorial capacity of the genus *Aedes* and genus *Anophelines*. It is a public health emergency to shed light our understanding of these factors because these will define the transmission dynamics and geographic distribution of Zika virus as well as indicate the timing and scale of environmental public health interventions.

Conflict of interest statement

None declared.

Ethical Approval

Approval was not required.

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Table 1: Lineages of Zika virus.

Lineages of Zika virus	
1. African lineages (two clusters):	**2. Asian lineage.**
a. MR 766 cluster	One Asian genotype
b. Nigerian cluster	

Table 2: Mosquitoes in which Zika virus was detected

Years of sampling	Location	Mosquito genus species	Study/Assay	Ref
AFRICA				
1948	Zika forest, Uganda	*Aedes africanus*	Mosquito catches in Zika forest and first isolation of Zika virus from *Aedes africanus* pool specimens	[5, 38]
1958	Zika forest, Uganda	*Aedes africanus*	Virus isolation	[47]
1964	Zika Forest, Uganda	*Aedes africanus*	Virus isolation	[10]
1969	Uganda, Bwama county, Zika forest	*Aedes africanus, Aedes apicoergenteus*	Virus isolation from pool specimens of mosquitoes trapped in Zika forest.	[12]
1976-1980	Central African Republic	*Aedes africanus,* aedes opok	Retrospective entomologic study with RT-PCR and sequencing	[48]
1968-2002	West Africa: Côte d'Ivoire And Senegal Burkina Faso, Central Africa Republic	*Aedes dalzieli, Aedes africanus, Aedes aegypti, Aedes furcifer, Aedes grahamii Ae. luteocephalus Aedes vittatus A. opock*	Retrospective study. Phylogenetic analysis Reverse transcription-PCR, nucleotide sequencing. Numerous recombination events were detected.	[25]
1962-2008	Senegal	*Aedes aegypti, Ae. custani, Ae. dalzieli, Ae. fowleri, Ae. furcifer (known as A. taylori), Aedes luteocephalus, Ae. vittatus,Ae. neoafricanus, Ae. metallicus, Ae. minutus, Anopheles africanus coustani, Anopheles gambiae s.l., and Mansonia uniformis* The higher number of ZIKV isolation events was detected in *Ae. furcifer (known as A. taylori), Aedes luteocephalus,* and *Aedes dalzieli.*	Virus isolation in the mosquito cell line AP61 (Ae. pseudoscutellaris) Identification of isolates with immunofluorescence with virus-specific immune ascitic fluid. This was confirmed by complement fixation or neutralization tests.	[49]
2011	southeastern Senegal	*Ae. africanus, Ae. hirsutus, Ae. metallicus, Ae. unilineatus and Cx. perfuscus* had the highest infection rates compared to *Aedes (Diceronyia) furcifer, Aedes (Fredwardsius) vittatus, Aedes taylori, Aedes luteocephalus, Aedes dalzieli, Aedes aegypti, Mansonia uniformis and Annopheles coustani, with the lower infection rates*	Virus isolation, RT-PCR	[50]
2007	Gabon	*Aedes albopictus*	Retrospective seroepidemiologic and entomologic study in 2014. RT-PCR and sequencing of pool specimens	[36]
ASIA				
1969	Malaysia	*Aedes aegypti*	Virus isolation	[11]
Non applicable. Experiment in 2012	Singapore	(local in Singapore) *Aedes aegypti*	Inoculation of Ugandan ZIKV in (local in Singapore) *Aedes aegypti* and subsequent mosquito-borne transmission of the virus	[54]
Non Applicable Experiment in 2014	Yap island, Federated States of Micronesia. *Human outbreak in 2007*	Field collected *Aedes henselli* and *Culex quinquefasciatus* tested negative for Zika virus. *Aedes henselli* laboratory infection .	Experiment. Laboratory infection of *Aedes henselli*	[55]

Table 3: Animals in which Zika virus was detected.

Years of sampling	Location	Animal	Assay virus/antibody	Number of animals tested	Positive animals (%)	Reference
1947	Zika forest, Uganda	Rhesus monkeys,	Intracerebral inoculation into mice and subsequent virus isolation	One Rhesus diagnosed with the virus, Two others underwent successful experimental inoculation		5 38
1967-1968 1962 and 1964	Senegal 1967-1968 Ethiopia 1962 and 1964	Wild mammals monkeys	Antibody detection with hemagglutination inhibition (HI)		wild mammals 2.4% monkeys 25%	61
1969	Uganda, Kisubi, Bwama county, zika forest	Bwamba county monkeys[a]	Antibody detection with hemagglutination Inhibition test	Kisubi Redtail 21 Bwama Redtail 52 Bwama others 16	4/21 23/52 7/16	12
1978	Lombok, Indonesia. *Human outbreak in 1977.*	Ducks, goats, cows, horses, bats, rats, carabaos (water buffalo)	Hemagglutination inhibition antibodies (HIA), Detection of antibodies (DA)	HIA[c] 15 horses 41 cows 13 Carabao 35goats 78 chicken 52 ducks 71 bats 25 rats 17 wild birds DA[d] 6 horses 8 cows 1 carabao 9 goats 1 chicken 2 ducks 1 bat	HIA[c] 3/15 (20%) 4/41 (10%) 1/13 (8%) 7/35 (20%) 0/78 (0) 2/52 (4%) 6/71 (8%) 0/25 (0) 0/17 (0) DA[d] All tested negative	15
1982	Gabon	monkeys	Antibody detection with hemagglutination inhibition (HI) /Complement fixation test			13
1983	Pakistan	Rodents, domestic sheep and goats (at slaughter) humans	Antibody detection	157 rodents, 45 Cows 33 Buffaloes 46 Sheep 48 Goats	6/157(3.8%) 0 0 1(2.57%) 1(2.083%)	9
1996-1997	Malaysia, Borneo	Wild orangutans, Semi-captive orangutans	antibodies	40 Wild orangutans, 31 Semi-captive orangutans	5/40 (13%)wild, 1/31 (3%) semi-captive	62
1968-2002	West Africa: Côte d'Ivoire,Senegal Burkina Faso, Central Africa Republic Retrospective study of viral isolates	monkeys	RT-PCR, nucleotide sequencing, recombination detection.	N/A[b]		25
1962-2008	Senegal Retrospective study of viral isolates	Monkey (Erythrocebus patas), Chlorocebus aethiops,(also named Grivet and African Green Monkey)	Virus isolation in the mosquito cell line AP61 (Ae. pseudoscutellaris) identification of isolates with immunofluorescence with virus-specific immune ascitic fluid and confirmed by complement fixation or neutralization tests.	N/A[b]		49

[a] Redtail monkey, Black mangabey, Lowland Colombus were positive for Zika virus. [b]Non applicable

[c]Hemaglutination inhibition antibodies [d] Detection of antibodies.