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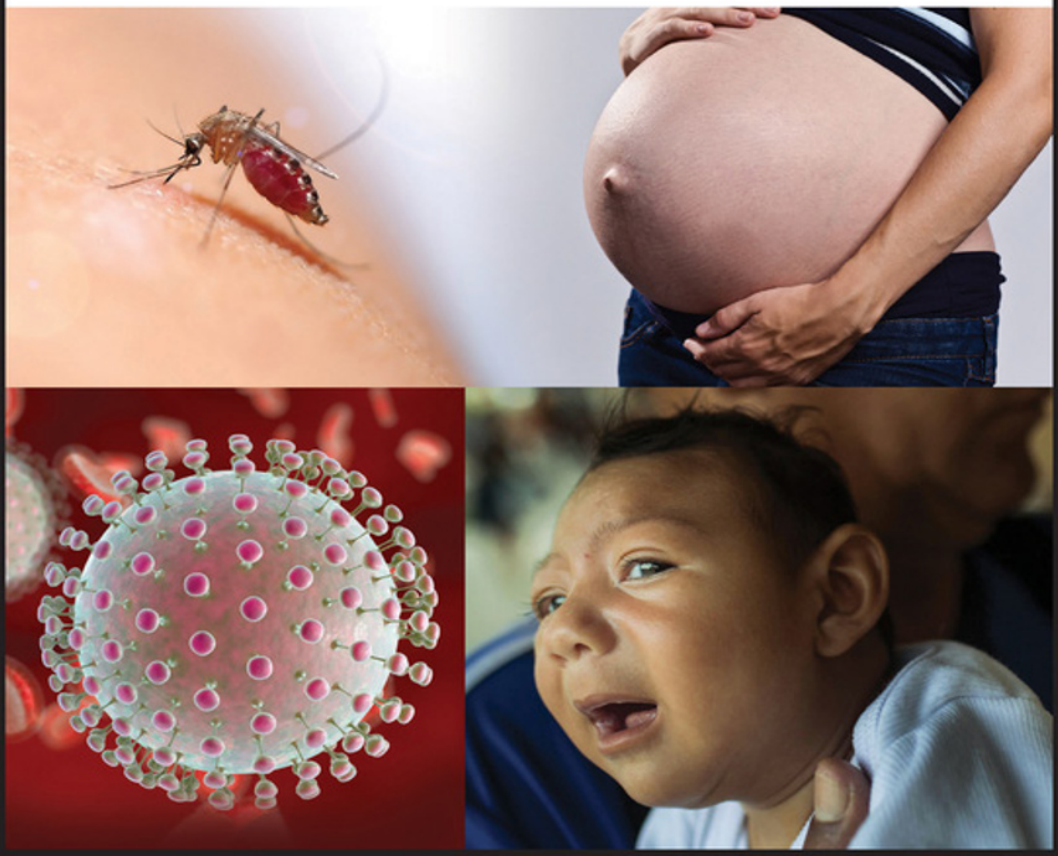
From Molecular Biology to Epidemiology



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Preface

Zika virus (ZIKV), a mosquito-borne flavivirus, is an emerging infectious agent associated with numerous neurological diseases. Discovered in 1947, the virus was silent for almost 60 years until the recent outbreaks in 2003 and 2013 in the Pacific Islands, and in 2015 in South and Central America. While the virus detected in Africa at the time of discovery was only associated with mild fever, rash, and pain, recent ZIKV outbreaks were associated with neurological disorders such as Guillain-Barré Syndrome in adults and microcephaly in newborns. The dynamic changes in ZIKV-associated pathology over the years has prompted extensive studies aimed at understanding the differences among the virus lineages (African vs. Asian/American) isolated from different regions with the goal of developing specific therapeutic drugs.

This book will describe the ZIKV story since its discovery in 1947 up to the most updated studies in 2017. We will cover more than 70 years of ZIKV history with details in the discovery, outbreaks, transmission, associated diseases, animal models that have been developed, ZIKV and cell/host interactions, the differences among ZIKV strains, and drugs that have been tested against ZIKV. This book should provide valuable information for both the general public and scientists.

List of Abbreviations

Abbreviations	Full name
2′-CMA	2′-C-methyladenosine
2′-CMC	2′-C-methylcytidine
2′-CMG	2′-C-methylguanosine
2′-CMU	2′-C-methyluridine
2′-O-Me	2′-O ribose methylation
3-MA	3-methyladenine
7-deaza-2′-CMA	7-deaza-2′-C-methyladenosine
7DMA	7-deaza-2′-C-methyladenosine
aa	amino acid
AEN	apoptosis enhancing nuclease
AIM	absent in melanoma
ALKBH	Alkylation repair homologs
AMPK	5′ adenosine monophosphate-activated protein kinase
Atg	autophagy-related protein
ATP	adenosine triphosphate
BPTI	bovine pancreatic trypsin inhibitor
BUNV	Bunyamvera virus
C	capsid protein
CCL	Chemokine (C-C motif) ligand
CCN	cyclin
CD	cluster of differentiation
CDK	cyclin-dependent kinase
CDKi	CDK inhibitor
CDKN	cyclin dependent kinase inhibitor
CFS	cerebrospinal fluid

CHIKV	chikungunya virus
CM	conditioned medium
C_{max}	maximum plasma concentrations
CPAP	centrosomal P4.1-associated protein
CPE	cytopathic effect
CRISPR	Clustered regularly interspaced short palindromic repeats
CXCL	Chemokine (C-X-C motif) ligand
CXCR	C-X-C motif chemokine receptor
D.P.C.	days post-coitus
D.P.I.	days post-infection
DC	dendritic cell
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DENV	dengue virus
DISC	death-inducing signaling complex
DNA	deoxyribonucleic acid
dsRNA	double-stranded RNA
E	envelope protein
EBV	Epstein-Barr virus
ELISA	enzyme-linked immunosorbent assay
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
FDA	Food and Drug Administration
FFU	focus forming units
FLE	fusion loop epitope
FLUAV	influenza A virus
FLUBV	influenza B virus
fNPC	fetal neural progenitor cell
fNSC	fetal NSC
FTO	fat mass and obesity-associated protein
GEF	guanine nucleotide exchange factor
GM	genetically modified
GO	gene ontology
GTP	guanosine-5′-triphosphate
H2AX	H2A histone family, member X
HAEC	human amnion epithelial cell
HC	Hofbauer cell
HCV	hepatitis C virus

hESC	human embryonic stem cell
HI	hemagglutination-inhibition
hiPSC	human inducible pluripotent stem cell
HIV	human immunodeficiency virus
hNPC	human neural progenitor cell
hnRNP	heterogeneous nuclear ribonucleoprotein
hpi	hour(s) post infection
HSV	herpes simplex virus
I.C.	intracerebrally
I.P.	intraperitoneally
IFIT	IFN-induced proteins with tetratricopeptide repeats
IFITM	IFN-inducible transmembrane protein
IFN	interferon
IKK	IκB kinase
IL	interleukin
IP-10	Interferon gamma-induced protein 10
IPS	interferon-promoter stimulator
iPSC	induced pluripotent stem cells
IRF	IFN regulatory factor
ISG	IFN-stimulated gene
ISRE	IFN-stimulated responsive element
IUGR	intrauterine growth restriction
JEV	Japanese encephalitis virus
KUNV	Kunjin virus
LC3	microtubule-associated protein 1A/1B-light chain 3
LDH	lactate dehydrogenace
LGP2	laboratory of genetics and physiology 2
LGTV	Langat virus
M	membrane protein
m^6A	N^6 methylation of adenosine
MAVS	Mitochondrial antiviral-signaling protein
MAYV	Mayaro virus
MBFV	mosquito-borne flaviviruses virus
MCM	Mauritian cynomolgus macaque
MCP1	monocyte chemoattractant protein-1
MDA 5	melanoma-differentiation-associated gene 5
MDE	mean day of euthanasia
MEF	mouse embryonic fibroblast
METTL	methyltransferase-like

MHC	major histocompatibility complex
MLD	mucin-like domain
moDC	monocyte-derived DC
MOI	multiplicity of infection
MORF	MOZ-related factor
MOZ	monocytic leukemic zinc-finger protein
MPA	mycophenolic acid
MR	monkey rhesus
MRI	magnetic resonance imaging
mRNA	messanger RNA
MTase	methyltransferase
mTORC	mammalian target of rapamycin complex
MyD88	myeloid differentiation primary response gene 88
NCX-NES cells	neocortical NES cells
NES cells	neuroepithelial stem cells
NF-κB	nuclear factor-κB
NKV	no known vector
NMR	nuclear magnetic resonance
NPC	neural progenitor cell
NPC	neural progenitor cells
NS	nonstructural protein
NSC	neural stem cell
NTPase	nucleoside triphosphatase
OAS	2′-5′-oligoadenylate synthetase
ORF	open reading frame
p.i.	post-infection
PAMP	pathogen-associated molecular pattern
PARP	poly-ADP ribose polymerase
PAS	pre-autophagosomal structure
PCD	programmed cell death
PCNT	Pericentrin
PE	phosphatidylethanolamine
PFU	plaque-forming units
PG	phosphatidylglycerol
PHT	primary human trophoblast
PI3K	phosphatidylinositol-3-kinase
PKR	protein kinase R
PKA	protein kinase A
PKI	PKA inhibitor
pNGB	p-nitrophenyl-p-guanidino benzoate

PQS	potential quadruplex sequence
prM	the precursor of membrane protein
PRNT	plaque reduction neutralization tests
$PRNT_{50}$	50% plaque reduction neutralizing titer
PRR	pattern recognition receptor
PTEN	phosphatase and tensin homolog
PTK	protein tyrosine kinase
qPCR	quantitative polymerase chain reaction
RANTES	regulated on activation, normal T cell expressed and secreted
RdRp	RNA-dependent RNA polymerase
RF	replicative form
RGC	radial glia cell
RGP	radial glial progenitor
RI	replicative intermediate
RIG-I	retinoid-inducible gene I
RLR	RIG-I-like receptors
RNA	ribonucleic acid
ROS	reactive oxygen species
RPE cells	retinal pigment epithelial cells
RSAD	radical S-adenosyl methionine domain containing
RSP	recombinant subviral particle
RTPase	RNA triphosphatase
RT-PCR	reverse-transcriptase polymerase chain reaction
RT-qPCR	quantitative reverse transcription PCR
S.C.	subcutaneously
SAM	S-adenosyl-methionine
SARS	severe acute respiratory syndrome
SD	standard deviation
sfRNA	subgenomic flaviviral RNA
SINV	Sindbis virus
SPOV	Spondweni virus
ssRNA	single stranded RNA
STAT	signal transducer and activator of transcription
STING	stimulator of interferon gene
TAM	Tyro3-Axl-Mer
TANK	TRAF family member-associated NF-κB activator
TBFV	tick-borne flavivirus
TBK	TANK-binding kinase
TEM	transmission electron microscopy

TIM	T-cell immunoglobin and mucin domain
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
t_{max}	duration to achieve C_{max}
TMD	transmembrane domain
TMEV	Theiler's mouse encephalomyelitis virus
TNF	tumor necrosis factor
TRAF	tumor necrosis factor receptor-associated factor
TRAIL	TNF-related apoptosis inducing ligand
TRIF	TIR-domain-containing adapter-inducing interferon-β
TSC	tuberous sclerosis
ULK1	Unc-51-like kinase
UTR	untranslated region
VEEV	Venezuelan equine encephalitis virus
VP	vesicle packet
WDR	WD40 repeat
WNV	West Nile virus
WS	Webster Swiss
xrRNA	Xrn1-resistent RNA
YF	yellow fever
YFV	yellow fever virus
YTH	YT521-B homology
YTHDF	YTH N6-methyladenosine RNA binding protein
ZFYVE	zinc finger FYVE-type
ZIKV	Zika virus
$ZIKV^{BR}$	ZIKV strain isolated in Brazil
γH2AX	phosphorylated histone H2AX

1

The History of ZIKV Discovery

1.1 ZIKV Isolation from Monkeys and Mosquitos

Zika virus (ZIKV) was first isolated in April 1947 in a forest named "Ziika" near Lake Victoria in Uganda (1, 2). It is interesting to note that the term *Ziika* means "overgrowth" in Luganda (the Bantu language of the Baganda people, commonly used in Uganda). The virus was isolated by researchers from The National Institute for Medical Research in London, United Kingdom (G. W. A. Dick), and The Rockefeller Foundation in New York, United States (S. F. Kitchen and A. J. Haddow), as part of collaborative studies with the Yellow Fever Research Institute in Entebbe, Uganda (Figure 1.1) (1, 2).

To monitor emerging infections, the investigators commenced studying the sentinel rhesus monkeys in Bwamba, Uganda, in 1946 (Figure 1.2) (1). Zika Forest was chosen because it was well-known that monkeys in that area had a high immunity to yellow fever virus (YFV) (3–6). Most of the forest was parallel to the Entebbe-Kampala Road, and the monkeys were kept in cages in the canopy of the trees (1, 7–9).

At that time, six monkey rhesus (MR) were monitored daily for any variation in their body temperature. One of the monkeys, named MR766, presented an increase in temperature on April 18; hence, a blood sample was collected on April 20. MR766 was monitored for more 30 days but no other symptom was detected. The blood sample collected from MR766 was injected subcutaneously (S.C.) into another monkey named MR771, and into Swiss albino mice, intracerebrally (I.C.) and intraperitoneally (I.P.), for further studies. No sign of

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Figure 1.1 Alexander J. Haddow in the Zika Forest. The base of the platform used to capture mosquitoes and keep the monkeys can be observed. Obtained from the University of Glasgow (AJ Haddow and University of Glasgow Archives & Special Collections, Papers of AJ Haddow, GB248 DC 068/80/63).

infection was observed in either MR771 or the mice injected by I.P. for up to 30 days after inoculation. However, the mice injected by I.C. became sick 10 days post-infection (d.p.i.), and the first ZIKV isolation was obtained from these animals. Since this virus was neutralized by serum taken from monkeys MR766 (on May 20) and MR771 (at 35 d.p.i.) but not by sera from these same monkeys before their exposure to ZIKV, the researchers proved that the virus isolated from the mice was originated from monkey MR766. For this reason, the first ZIKV strain isolated was named MR766. A neutralizing antibody is the antibody that can protect the cells from an infection by neutralizing its biological effect (in this case, infection). In this study, it was used in an assay to determine if the virus detected in one animal was the same as the one isolated from the previous animal (1).

In addition to analyzing and collecting samples from the monkeys, the researches were collecting mosquitos for the YF studies (Figure 1.3). *Aedes africanus* were among the captured ones in 1948. This mosquito was suspected to be involved in the YFV cycle at that time. From January 5 to January 20, nine lots of mosquitos were

Figure 1.2 Details of the steel tower used as a platform to collect mosquitos, and to keep the caged monkeys in the Zika Forest. The platforms can reach the canopy of the trees. Obtained from the University of Glasgow (AJ Haddow and University of Glasgow Archives & Special Collections, Papers of AJ Haddow, GB248 DC 068/80/62).

acquired, and their samples were processed and injected into mice by I.C. with both unfiltered supernatants and Seitz E.K. filtrates. The second isolation of ZIKV (strain E/1), which was also the first from mosquitos, was from lot E/1/48, captured on January 11–12, with 86 mosquitos (1). All six mice inoculated with the unfiltered sample were inactive at 7 d.p.i. For animals that received the filtrated sample, one died at 6 d.p.i. while other was sick at 14 d.p.i.

Figure 1.3 Details on the stairs used to access and recover the mosquitoes caught. A boy can be observed in the picture, since they were used to help the researchers to collect the samples in the high height. Obtained from the University of Glasgow (AJ Haddow and University of Glasgow Archives & Special Collections, Papers of AJ Haddow, GB248 DC 068/80/49).

Those inoculates were also injected S.C. into MR758, which showed no sign of sickness. Based on the results observed with the sick mice, blood samples from MR758 were collected on the 8^{th}, 9^{th}, and 10^{th} d.p.i., which were I.C. injected into six mice. From the first injection, one mouse died at 10 d.p.i. and another two became sick at 19 and 20 d.p.i. From the second group of injection, one died at 13 d.p.i., one was sick at 12 d.p.i., and another one developed paralysis, which was identified

as Theiler's encephalomyelitis (10, 11). Mice injected with samples from the third collection had no symptom. Neutralization tests with serum from MR758 proved that these animals had developed neutralization antibodies to ZIKV strains E/1 and MR766. It was concluded that ZIKV was identical to neither YFV, Dengue virus (DENV), nor Theiler's mouse encephalomyelitis virus (TMEV) (1).

Dick (1952) observed that the virus isolated from MR766 and mosquitos was well adapted after 90 passages in the mouse brain. Data from studies analyzing adaption and pathogenesis became more reproducible. Among the three ZIKV strains tested (MR766, MR758, and E/1), the virus from MR758 caused more cases of mice that presented with paralysis in early passage than the virus from MR766. With all the strains evaluated, the first sign of infection was roughness of the coat. Infection by I.P. injection in mice older than 2 weeks of age was not as efficient in those of 7 days old. Using a late-passage virus, no significant difference in the infection was observed between unweaned and 5- to 6-week-old mice (2).

The virus tropism was examined by analyzing infection in different organs, including brain, kidney, lung, liver, and spleen. The results of the mice inoculated by I.C. indicated that the brain was the main target of ZIKV. While other animals including cotton-rat and guinea pigs could also be infected with ZIKV, no symptom was observed. On the other hand, rabbits could produce antibodies by 21 d.p.i. Other species of monkeys—including rhesus (6 animals), grivet (13 animals), and redtails (2 animals)—were also infected and analyzed. Circulating antibodies were detected in Grivet 733 and Redtail 1044 after ZIKV infection. Interestingly, Grivet 1019 was naturally infected by ZIKV, but this monkey was captured in Sese Island, which was not in the Zika region. In 1950, among the monkey rhesus used for the YFV research, animal MR801 was naturally positive for ZIKV but the only symptom was minor pyrexia. MR801 was kept in the same platform (number 3) where the strain E/1 was isolated from the captured mosquitoes. Platform number 3 was 0.2 miles from platform number 5 where MR766 was infected (2). Antibodies against ZIKV were not detected in small mammals that were trapped in the forest, indicating that the infection was restricted to monkeys, mosquitos, and human beings at that time (12, 13).

Other ZIKV strains were isolated in 1958 from two different catches of *Aedes africanus*, consisting of 206 (strain Lunyo V) and 127 (strain Lunyo VI) mosquitoes. The Lunyo V strain caused viral encephalitis,

skeletal myositis, and myocarditis in adults and infant mice. The virus was passed through the brain and the heart into infant mice via I.C. or I.P. injections. Some of the mice injected with Lunyo VI were paralyzed. The strains were injected into monkeys MR1059 and MR1063, respectively, and no symptoms of infection were observed (14). ZIKV was further isolated from *Aedes luteocephalus* in Nigeria (15).

1.2 ZIKV Infection in Humans

The timing of the first ZIKV infection in humans is controversial (16). A paper published by MacNamara in 1954 described its isolation and exploited the possible association between ZIKV infection and jaundice (17). Another study, by Bearcroft in 1956, was on a volunteer that self-injected with the virus, who precisely described the symptoms following the infection (18). The only problem is that the virus isolated in the first study and used in the second one was not ZIKV but a Spondweni virus (SPOV) (16). MacNamara's study evaluated an epidemic of jaundice in Nigeria (Afikpo Division, Eastern Nigeria). From a study of three patients, the virus was isolated from a 10-year-old female patient who was not jaundiced but had symptoms of fever and headache, and her serological response to ZIKV was low (17).

Bearcroft's study was done to verify whether there was any association between ZIKV and the development of jaundice. A 34-year-old European male volunteer was exposed to the virus isolated by MacNamara (1956). Eighty-two hours post-infection (h.p.i.), the only symptom was a headache, followed by malaise and pyrexia in the 2nd and 3rd d.p.i. In the 5th d.p.i., there was a peak in the corporal temperature, accompanied by nausea and vertigo, which was diagnosed as histamine reaction. After 7 days, the volunteer had no sign of infection or jaundice. Mice infected with virus collected from the volunteer, in different periods, developed encephalitis after receiving sera collected at 4 and 6 d.p.i., which were around the peak of pyrexia. Meanwhile, the volunteer was exposed to *Aedes aegypti*, but the mosquitos were not able to transmit the infection to infant white mice (18).

The first clue that both studies were using SPOV was revealed in a study by Simpson (1964), which was also the first one to describe a natural infection of ZIKV in humans (19). In this paper he mentioned that previous isolations of ZIKV were made in Nigeria (West Africa), and Dr. Delphine Clarke had found out that those viruses were closely related to SPOV, which was named CHUKU strain. Another study in

1968 also pointed out that SPOV virus was isolated in Nigeria, and was wrongly identified as ZIKV (20). Simpson was actually the person who contracted the infection while working together with his team in Uganda. A detailed description of his symptoms following the natural infection was recorded. At the 1 d.p.i., he presented a headache, and by 2 d.p.i., he developed a red rash diffused throughout his face, neck, chest, and arms, without itching, and slight pain in the back and thighs. The rash covered all the limbs, including palms and soles. The fever started at 2 d.p.i., followed by malaise. At 3 d.p.i., the patient had no fever and did not feel sick, and at 5 d.p.i., there was no more rash (19). Actually, this was the first study that documented the presence of a rash on humans infected by ZIKV, one of the most common symptoms of ZIKV infection in today's patients (21).

The first isolation of ZIKV in Nigeria was reported in 1975 by Moore (1975) in a study describing the isolation of 15 arboviruses between 1964 and 1970 (22). Isolation of ZIKV in Oyo State, Nigeria, was described in 1979. The virus was isolated from two patients, a 2½-year-old boy with a mild fever in 1971, and a 10-year-old male in 1975, who presented with fever, headache, and pain in the body. This study suggested that ZIKV might be widespread, even if it had been isolated at a low rate. One important point mentioned in this study was that ZIKV infection numbers might be underestimated because of the mild symptoms or misdiagnosis with other arthropod-borne viral infections (23).

1.3 ZIKV Infection Spread to Other Hosts and Regions

Different serological studies were performed around the 1950s and 1960s and showed that the ZIKV infection had reached other areas in Africa and Asia (24, 25). Serological analysis, based on hemagglutination-inhibition (HI) tests of other animals, were described in 1977 with samples from 2,428 small mammals and 1,202 birds captured over a five-year period in Kano Plain, Kenya, close to Lake Victoria. The results revealed the prevalence of ZIKV antibodies as follows:

- In small mammals:
  - 4.0% (58/1,446) in *Arvicanthus niloticus*
  - 34.0% in (85/250) *Arvicanthis niloticus*
  - 3.1% (2/63) in *Crocidura occidentalis*

- In reptiles:
  - 40.0% (4/10) in *Boaedon fuliginosus*
  - 12.5% (1/8) in *Varanus niloticus*
- in birds:
  - 4.0% (2/49) in *Threskiornis aethiopicus*
  - 2.7% (1/37) in *Bubulcus ibis* and 50.0% (1/2) in *Philomachus pugnax*
- In other mammals:
  - 0.1% (1/655) in goats
  - 0.7% (2/283) in sheep
  - 0.6% (8/1361) in cattle living close to irrigated areas
  - 0.7% (7/963) in cattle from nonirrigated places (26)

Serological studies with human serum collected for the YFV research indicated that humans from some areas were exposed to ZIKV. There was no detection of ZIKV antibodies in the Zika and Kampala regions, while Bwamba had detection rates of ZIKV antibodies at 28.5% (2/7) in adults and 15.4% (2/13) in children, which were higher than the 9.5% (2/21) detection rate of West Nile antibodies in adults in this region (2). Dick (1952) was careful in his study and suggested that just because there was no evidence of an acute disease in humans caused by ZIKV infection, this did not indicate that ZIKV was not important or might not cause any problem in humans (2).

The detection of antibodies against ZIKV in South-East Asia was published in 1963, revealing that 75.0% (from 100 samples) of the population living in the Federation of Malayan (currently known as Peninsula of Malaya) was positive, while the presence of neutralization antibodies in the north region such as North Vietnam and Thailand (Bangkok and Chiang Mai) was rare (27). In 1965, ZIKV was detected in different regions of the Angola trough with 31.0% (40/129) and 1.5% (2/129) rates in children in the northwestern region by HI and neutralization tests, respectively, and with 57.7% (71/123) and 21.1% (26/123) rates in adults, respectively, by the same methods. In the southwestern region, 32.8% (20/61) and 21.3% (13/61) of the adults were positive by HI and neutralization tests, respectively, and for the eastern region, 3.5% (2/56) and 1.8% (1/56) of the adults were positive, also using HI and neutralization tests, respectively (28). Results from Kano Plain, Kenya, showed that ZIKV was endemic in 1973, but it was considered at a low level. By analyzing sera from children (ages 4–15+ years old) from schools distributed close to Lake Victoria,

ZIKV was detected by HI test in 7.2% (40/559) of the children grouped as 12 years old. Since this was considered a low rate, the other ages were not evaluated (29).

In 1974, a serological study to detect different arboviruses analyzed 1,649 human sera from Portugal and identified four (0.25%) individuals that reacted against ZIKV by the HI test, indicating the silent spread of the virus across the continents (30). In 1979, a serological study analyzed 235 samples from Hong Kong and detected 4.6% (11/235) ZIKV-positive individuals, which also cross-reacted with other flaviviruses. Among those who had gender and age information, 12.9% (4/31) females and 8.3% (1/12) males between 21 and 40 years old were positive, while 7.1% (1/14) males older than 41 years old were positive (31).

Interesting results were found at Kainji Lake Basin, Nigeria, in 1980, when ZIKV was detected by HI test, with cases concentrated in young adults and adults. Infection rate was correlated with increased age. Specifically, 9.3% (7/75) of 5- to 9-year-olds, 22.2% (8/36) of 10- to 14-year-olds, 46.1% (6/13) of 15- to 19-year-olds, 71.8% (61/85) of 20- to 39-year-olds, and 77.3% (68/88) of adults 40 years old and older (32) were positive. The continuous ZIKV detection by the HI test throughout Uganda villages in 1984 indicated that the incidence of ZIKV was not common in the region, with infection rates at 3.7% (1/27) in Tokora, 15.4% (2/13) in Nadip, 3.5% (2/58) in Namalu, and 20.0% (3/15) in other regions (33).

1.4 Cross-Paths between ZIKV and Other Flaviviruses

Analysis of sera collected from two different towns, Ilaro and Ilobi, in southwest Nigeria in 1951 and 1955 showed high detection rates of ZIKV antibodies in these populations. The distribution of ZIKV infection by age was as follows: 10.0% (3/29) among 5- to 9-year-olds, 22.0% (7/32) among 10- to 14-year-olds, 52.0% (13/25), among 15- to 19-year-olds, 76.0% (19/25) among 20- to 29-year-olds, 52.0% (16/31) among 30- to 39-year-olds, and 93.0% (28/30) for those adults 40 years old and older in Ilobi. In Ilaro, only children samples (4 to 16 years old) were collected, which showed a 44.0% positive rate for ZIKV antibodies. Besides ZIKV, high infection rates were also detected for DENV, YFV,

Uganda S virus (UGSV), and Bwamba fever virus (BWAV). There was association of antibodies against ZIKV, DENV, YFV, and UGSV, suggesting an overlapping protection. However, infection by one virus did not decrease the chance of being infected by another flavivirus, albeit it might reduce the pathogenesis. The most common combinations of infections were ZIKV or UGSV with YFV. Hence, a pre-infection with either ZIKV or UGSV might produce neutralization antibodies to YFV. ZIKV had a strong association with UGSV, YFV, and DENV. The DENV infection rate reached close to 100% in this region. It was suggested that ZIKV and UGSV might suppress YFV in the Forest Belt compared to other regions with high incidences of YFV and lower infection rates of ZIKV and UGSV (34).

The cyclic periodicity between ZIKV and chikungunya virus (CHIKV) was suggested by McCrae (1971) because there was no evidence that both viruses were maintained in the Entebbe region, but there were epizootic outbreaks with intervals of 5 to 8 and up to 10 years. The intervals were similar but not the same, with ZIKV following CHIKV outbreak after 1 to 2 years, which might be the result of the dynamic interactions of the viruses within the forest (35). In 1982, a study was published to address the possible interaction and interference in transmission between ZIKV and YFV. Mosquito's catches resulted in the isolation of 15 ZIKV strains from *Aedes africanus* and *Aedes apicoargenteus*. Dozens of monkeys were shot (after unsuccessful attempts of collecting blood) and captured to provide evidence of immunity change among the monkeys in the forest. Twenty-two Redtail monkeys were captured in Kisubi Forest while 68 monkeys (including Redtail, Mona, Colobus, and Mangabeys) were caught in Bwamba. After serological analysis, CHIKV was detected at high rates among the monkeys followed by ZIKV and YFV with similar rates, indicating that ZIKV infection did not prevent the circulation of YFV (36).

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