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Arbovirus Discovery in Central African Republic (1973-1993): Zika, Bozo, Bouboui, and More

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Abstract

The progressive research on yellow fever and the subsequent emergence of the field of arbovirology in the 1950s gave rise to the continued development of a global arbovirus surveillance network with a specific focus on human pathogenic arboviruses of the tropical zone. Though unknown at the time, some of the arboviruses studies would emerge within the temperate zone decades later (e.g.: West Nile, Zika, Chikungunya). However, initial research by the surveillance network was heavily focused on the discovery, isolation, and characterization of numerous arbovirus species. Global arboviral surveillance has revealed a cryptic circulation of several arboviruses, mainly in wild cycles of the tropical forest. Although there are more than 500 registered arbovirus species, a mere one third has proved to be pathogenic to humans (CDC, 2015). Indeed, most known arboviruses did not initially demonstrate a pathogenicity to humans or other vertebrates, and were considered "orphans" (i.e. without known of vertebrate hosts). As a part of this global surveillance network, the Institut Pasteur International Network has endeavored to understand the role played by arboviruses in the etiology of febrile syndromes of unknown origin as one of its research missions. Here, we report how The Pasteur Institute of Bangui (Institut Pasteur Bangui, or IPB) in the Central African Republic (CAR) actively participated in this mission and conducted an arbovirus survey from 1973 to 1993 that led to the isolation of 297 virus strains from 409,877 mosquitoes belonging to 78 different species. Ultimately, 24 new virus species were identified among these isolated strains, including two new orphan arboviruses Bozo, Bouboui, and two other unidentified arboviruses (ArB 11266 as a flavivirus-like virus and ArD28542 as a bunyavirus-like virus).The findings of this important, unpublished data from this survey are discussed here and give historical context to the recent global emergence and spread of Zika virus out of Africa. During its long journey from Africa emergence, into Asia, Oceania, and most recently to the Americas, the Zika virus has shown some significant genetic changes to its potential for pathogenicity. The original data presented here suggests that a significant number of other viruses, circulating in a hidden or discrete wild cycle with vertebrate hosts yet to be identified, represent a constant and undetermined risk of emergence among a non-immune human population with the possibility for similar natural genetic changes.

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Introduction

Many viruses that are isolated from vertebrate hosts and/or vectors are ignored if they are not pathogenic to humans or animals, and therefore maintained in a wild, cryptic, natural cycle. Viruses transmitted by arthropod vectors, or "arboviruses", are examples of those that circulate in this kind of natural cycle. These cycles are complicated and poorly understood, often occurring in the heart of the rainforest within the inter-tropical zone. As of 1992, 535 species of arboviruses had been registered in the International Catalogue of Arboviruses, currently hosted by the CDC (https://wwwn.cdc.gov/arbocat/). The majority of these viruses cause only subclinical infections which are retrospectively evidenced by the presence of circulating antibodies. Such infections do not provoke an evident effect on community health, but they indicate that a virus is circulating and that the viremic subjects may become reservoirs for insects or tick vectors [1]. When an arbovirus has a clinical effect, it usually presents as a mild febrile infection that often goes unnoticed. In rare instances, some of these infections may be responsible for serious conditions that manifest only among a reduced number

of infected individuals. Non-human pathogenic viruses, mainly arboviruses, are considered to be orphaned or insect-specific viruses (i.e. without a permissive vertebrate host) [2]. Ultimately, those arboviruses usually pass unnoticed until the moment they emerge in a non-immune population, which reveals hitherto unknown and subsequently unexpected clinical and epidemic patterns.

Arbovirus diseases are animal diseases (some are zoonotic), with a sylvatic cycle involving mosquitoes and wild animals [3]. The circulation of these viruses in their sylvatic cycle depends on the frequency of renewal of non-immune monkey populations. Humans are contaminated by penetrating these niches, which are shared with apes. Infections are rare and mainly affect children living in low population densities. The paucity of roads and the subsequent limits on travel help the virus remain confined to an endemic area more or less circumscribed. Once introduced into a non-immune human population, however, as was the case for the chikungunya virus on the island of la Réunion, the spread can be explosive [4].

Among the virus families to which arboviruses belong, it is within the Flaviviridae family (63 virus species) that most of viral species with human pathogenic potential are found. They are often responsible for a broad spectrum of clinical presentations, ranging from sub-clinical infection to deadly hemorrhagic syndrome. Most commonly, infected patients present a benign flu-like infection. These ignored arboviruses with mostly asymptomatic or mildly symptomatic infections can emerge unexpectedly, with improved methods of dispersion. These emergences are complex and difficult to anticipate, as exemplified by recent and original events: West Nile virus in North America; the establishment of a worldwide circulation of dengue viruses in less than a century; the re-emergence of yellow fever in Africa and South America; the recent emergence of the Alkhuma variant of Kyasanur Forest disease virus in Saudi Arabia; and Japanese encephalitis that emerged in Australia when wind-blown infected mosquitoes traveled from New Guinea to Australia, potentially introducing the virus to the continent in 1995 [5]. The Sindbis virus, an arbovirus transmitted between birds by ornithophilic mosquitoes, was isolated in Egypt and has been subsequently isolated in southern and central Europe and in several areas of Africa and in Asia and in Australia. Sindbis virus infection in humans causes a benign disease with arthralgia, rash, and occasional fever [6]. Chikungunya virus adapted quickly to a new environment and vectors among the non-immune populations of Indian Ocean island nations and presented unprecedented clinical severity after 50 years of silent circulation in Africa and Asia [7]. Usutu virus, after 40 years of a quasi-silent transmission in Africa among birds and mosquitoes, emerged in Europe, first appearing in Austria (2001) as a deadly virus for the Eurasian blackbird, but later showing a consistent prevalence among humans while few cases of men in go-encephalitis in immune-compromised patients were reported in Italy [8]. Tembusu flavivirus, originating in China and later considered as a potential zoonosis [9], emerged in Southeast Asia, devastating layer and broiler duck farms in Thailand [10]. Recently and most notably is the global emergence of Zika virus. The first significant outbreak of Zika virus occurred in 2007, outside of the African continent on the tiny Micronesian island of Yap. This outbreak was accompanied by what remain the most severe clinical forms ever described for this virus [11]. A second major outbreak of Zika virus was declared in 2013-2014 across Oceania, in French Polynesia, New Caledonia, the Cook Islands, and Easter Island. Zika virus has since been making its mark in this millennium by settling in the Americas. As 2015 saw Ebola Virus Disease recede after devastating West Africa, the Zika virus made its way to the Western Hemisphere, and took its place at the forefront of the media scene [12]. The WHO is currently supporting national governments and communities in order to prevent and manage Zika and its complications. The WHO Zika strategic response will cost approximately $122 million, and will implement elements of detection, prevention, care and support, and research across 60 countries. [13]. Although long known to cause limited or mild infection until it arose in Yap state in 2007, Zika virus did not attract the attention of health authorities or arouse great interest in the fight against epidemics since its discovery in monkeys in 1947, or its subsequent identification in humans in 1952 [14]. This singular and unexpected intrusion, and spread, of Zika virus in the landscape outside of its traditional bounds is reminiscent of the role played by arboviruses in the history of infectious diseases [15].

Background

During the 1950's, the number of isolations of previously unrecognized viruses from arthropods and vertebrates increased substantially around the world. In widely separated laboratories, investigators had begun to search for possible arboviruses among all likely sources in nature. These studies were aided by a global increase in staffing of investigators in laboratories primarily interested in arthropod-borne viruses. “The extension of studies into previously unexplored regions, and the development of new laboratory and field techniques for the isolation and serological identification of viruses contributed greatly to the outpouring of new information” [16]. In particular, the Rockefeller Foundation played a key role in the tracking and study of arboviruses in laboratories scattered throughout the tropics by creating a collaborative international network for the study of arboviruses, and bring "arbovirologists" together and thus promote progress within an innovative scientific frame work and ultimately by producing the first newsletter on American Arthropod-borne Virus Research in April 1960. The 1960’s saw both changes to existing arbovirus institutions, as well as growth to the overall scientific and surveillance network. In 1964, the Rockefeller Foundation transferred its research and reference activities from the Laboratory of Virology and Infectious Disease (The Rockefeller University) to Yale University’s Department of Epidemiology and Public Health. The same year these activities moved to a new building and became the Yale Arbovirus Research Unit (YARU), WHO recognized YARU as the International Reference Centre for Arboviruses [17]. The WHO International World Reference Centre for the study of arboviruses, an international network of WHO regional reference centers, was created in 1967 to include laboratory facilities in Atlanta, Bratislava, Brisbane, Dakar, Entebbe, Moscow, Paris, Tokyo, and Poona. This international collaboration between field laboratories and WHO reference centers was achieved through the isolation and identification of new viruses registered in the International Catalogue of Arboviruses created in 1967. The Institut Pasteur International Network would also play an important role in this collaboration on arbovirus discovery, especially in those parts of the inter-tropical zone where their laboratories were placed (https://hal-riip.archives-ouvertes.fr). The Pasteur Institute, through its international network, actively participated in the research presented here. Laboratories were developed within existing Pasteur Institutes around the world, including: Abidjan, Bangui, Cayenne, Nouméa, Papeete, Antananarivo, and Yaoundé. An international partnership had already been established in 1964 to connect the above-listed field laboratories to the reference laboratories for Arbovirus of WHO and YARU. We report here, within this historical framework of arbovirus discovery, the work performed at the Pasteur Institute

Table 1: Arboviruses isolated form arthropod in Central Africa (Central African Republic, 1973-1983).

Mosquito Genus Subgenus species	Registered arbovirus species*																			New arbovirus species				
	CHIKV	BAGV	BOUV	BWAV	ILEV	KAMV	MIDV	MOSV	MPOV	NDOV	ORUV	PATV	PGAV	SIMV	SINV	TATV	WSLV	WNV	YFV	ZIKV	BOZV	KEDV	ArD28542	Arb11266	TOTAL
A. St. africanus	43		22								7						5	3	25	46	40				191
A. St. opok	10		5								2						2		3	13	9				44
A. Aed. abnormalis													1				5								6
A. Aed. fowleri													1												1
A. Aed. tarsalis												1	1				7					4			13
A. Aed. vittatus							14							4			1								19
A. Neom palpalis														1											1
C. Lutziatigripes								1																	1
C. Cx. duttoni																								1	1
C. Cx. decens						1			1																2
C. Cx. perfuscus		1							3								1	1							6
C. Cx. pruina						1															1				2
C. Cx. Sp.															1										1
A. Celliagambia					2		1				1												1		5
A. Cellia funestus				1						1						1	1								4
Total Strain	53	1	27	1	2	2	15	1	4	1	10	1	3	5	1	1	22	4	28	59	50	4	1	1	297

Caption: *V: Virus; CHIK: Chikugunya; BAG: Bagaza; BOU: Bouboui; BWA: Bwamba; ILE: Ilesha; KAM: Kamese; MIDV: Middleburg; MOS: Mossuril ; MPO: M'Poko; NDO: Nyando; ORU: Orungo; PAT: Pata.; PGA: Pongola; SIM: Simbu; SIN: Sinbis; TAT: Tataguine; WSL: Wesselsbron; WN: West Nile; YF: Yellow Fever; ZIKV: Zika; BOZV: Bozo; KED: Kedougou; A.: Aedes; St.: Stegomya; Aed.: Aedimorphus; Neom.: Neomelaniconium; Cx.: Culex; An.: Anopheles. ArD28542: unidentified Bunyavirus* related; *Arb11266 unidentified Flavivirus* related to WNV and Usutu virus.

of Bangui (IPB) from 1973 to 1983, performed with the initial aim of studying the epidemiology of yellow fever in Central Africa.

Objective

Beginning in October 1973, the IPB and the Medical Entomology Laboratory of the Institute of Research for Development (commonly referred to as the Office de la Recherche Scientifique Technique Outre-Mer, or ORSTOM), both based in Bangui, Central African Republic, cooperatively conducted a continuous virological survey of mosquitoes near the village of Bozo (Ombella-M'Poko district; 5°10 N, 18°30 E). The Bozo field station was located in a savanna with a tropical mix of grasslands and scrublands, and luxurious, dense forest galleries; altogether these ecosystems are characteristic of the sub-Sudanese savannah ecosystem. The climate is tropical and rainy, save for the five month long dry season; the annual rainfall is 1,600 mm and the mean temperature is 25°C [18]. The joint project (IPB-ORSTOM) was specifically designed to understand the sylvatic cycle of yellow fever virus in West and Central Africa as a comparative study to what was already known from previously conducted studies in East Africa. Another similar project was conducted, simultaneously, at the Dakar Pasteur Institute of Senegal with its own ORSTOM, and carried out at the Kedougou research station, located in the broadleaf evergreen forest of southeastern Senegal.

Material and Methods

Priority was given to the collection of anthropophilic and primatophilic arthropod species from the forest gallery and the nearby village. The most numerous species were *Aedes (Stegomyia) africanus* and *Aedes (St.) opok* inside the forest gallery, and *Aedes (Aedimorphus) vittatus* in the neighboring savanna. Mosquito trapping was also performed inside the walls of human dwellings in the Bozo village, which allowed for the collection of anthropophilic *Anopheles* mosquitoes (*A. gambiae* and *A. funestus*). Trapping was performed using the human landing catch method [19], a method at the time considered the gold standard for the effective collection of *Aedes* mosquito species [20]. Mono-specific pools of females (not blood-fed) and/or males were constituted and frozen in situ (nitrogen) for further analysis. After thawing, each mosquito pool (max 100 mosquitoes by pool) was ground in Hanks balanced salt (3 ml) medium containing antibiotics, and bovine serum albumin. A total of 14,591 mosquito pools, sorted by species and sex, were prepared and tested for virus isolation. Virus isolation was performed by intracerebral (0.02 ml) and intraperitoneal (0.02 ml) inoculation to suckling mice. The mice were observed for two weeks for any clinical sign of virus infection (i.e. paralysis). Virus identification was performed using the hemagglutination inhibition (HI), complement fixation (CF), and neutralizing tests. Strain identification was confirmed at the WHO Collaboration Center for Arbovirus Reference and Research Center at Pasteur Institute, Dakar, Senegal. Ultimately, several epidemiological enquiries were carried out as a serosurvey process and/or seroprevalence study [21].

Ethics

The Pasteur Institute animal facilities received accreditation from both the national health authorities of CAR and the French Ministry of

Table 2: Arbovirus isolated during a ten years' field study at Bozo scientific station of Central African Republic (1973-1983).

Virus species* (isolate number)	Region** (Year) of origin	Geographic extension	Human pathogenic
Alphavirus Genus			
Chikungunya (53)	E Af. (1955)	Asia, Indian Ocean, Americas	+
Middelburg (15)	S Af. (1957)	South Africa, C Af.	unknown
Sindbis (1)	Af. (1952)	Israel, Philippines, Australia	++
Flavivirus Genus			
Bagaza, (1)	W Af. (1966)	India, Israel	+
Bouboui (27)	Af. (CAR, 1971)	C Af.	unknown
Kedougou (4)	W Af. (1977)	C & W Af.	mild
West Nile (4)	E Af. (1937)	WW	+++
Wesselsbron (22)	S Af. (1956)	C,W Af. ; Madagascar Thailand	++
Yellow fever (28)	Af. (1927)	Af., Oceania, Americas	VHF
Zika (59)	E Af. (1947)	Af., Asia, Oceania, Americas	++
Bunyavirus Genus			
Bozo (50)	C Af. (1883)	C Af.	unknown
Bwamba (1)	C Af. (1937)	C Af.	+++
Ilesha (2)	W Af. (1957)	Madagascar	VHF
M'Poko (4)	W Af. (1966)	W Af., E Af.	unknown
Pongola (3)	S Af. (1955)	E Af., W Af.	++
Simbu (5)	Af. (1957)	Af., Asia?	+
Orbivirus Genus			
Pata (1)	C Af. (1968)	Af.	unknown
Orungo (10)	W Af. (1976)	Af.	++
Tataguine (1)	W Af. (1962)	Af.	+
Rhabdovirus Genus			
Mossuril (1)	E Af. (1962)	Africa	Orphan
Kamese (2)	E Af. (1969)	E Af., C Af.	Orphan
Unclassified			
Nyando (1)	E Af. (1959)	C Af., W Af.	+

Caption: *: Two new arboviruses were isolated and not yet totally identified: 1/ArB 11266 (Flavivirus, West Nile virus /Usutu virus like); 2/ArD28542 (Bunyavirus); ** Af.: Africa, C: Central, W: West, E: East, S: South

Agriculture to perform diagnostic tests on live animals, in compliance with the French and European regulations following national and institutional guidelines for the care and use of animals. This study was approved by the Institutional Animal Care and Use Committee at Pasteur Institute. Epidemiological studies and serological surveys were conducted under the Pasteur Institute Scientific Counsel review and the CAR Ministry of Health agreement for diagnostic and public health epidemic surveillance, in accordance with the ethical institutional and national standards of medical research committee.

Results

Any viruses that showed potential for being arboviruses were isolated, locally identified, and referred for confirmation and further study to the WHO Reference Center for Africa (CRORA), located at the Pasteur Institute in Dakar. When a virus was suspected to be novel with our tests (i.e. sero-neutralization), it was flown to YARU for confirmation and entry into the International Catalogue of Arboviruses, a catalog that included, by the late 1980s, more than 500 registered viruses [15]. Between 1973 to 1982, field collection encompassed 409,877 female and 23,619 male mosquitoes identified as belonging to 78 and 39 mosquito species, respectively (unpublished), including only 15 species that yield virus isolates (Table 1). The three main vectors of YFV – *A. africanus, A. opok,* and *A. vittatus* – represented 75% of the specimens collected. Three hundred and twenty-one strains of 24 different virus species were recorded in all (Table 2).

Discussion

During the study period, 72 cases of arbovirus infections were reported, leading to the isolation of 17 arbovirus species from human cases in Central African Republic: Chikungunya, Igbo-Ora, o'nyong'nyong, Sindbis, Bouboui, yellow fever, Wesselsbron, West Nile, Zika, Ilesha, Bwamba, Crimean-Congo hemorrhagic fever, Dugbe, Tataguine, Nyando, Bangui, and Rift Valley fever viruses [22]. Altogether, six arboviruses were found living in the forest gallery, including Bouboui, Bozo, chikungunya, Orungo, yellow fever, and Zika viruses. These six showed common characteristics of having a similar wild cycle involving *A. africanus* as a vector of the virus, and had locally available primates as hosts for feeding and virus amplification [18]. We were able to clearly define epizootic manifestations on the basis of virus isolation and a concurrent serological survey of monkeys. Virus activity was observed to be highly variable from year to year. No

Table 3: Arbovirus strain isolated by mosquitos' species during a ten years' field study at Bozo scientific station of Central African Republic (1973-1983).

Family	Genus	Species	TOTAL (%)
Aedes			275 (92.6)
	Stegomya	*africanus*	168 ( )
	Stegomya	*opok*	44
	Stegomya	*gr. Africanus*	23
	aedimorphus	*gr. abnormalis*	6
	aedimorphus	*fowleri*	1
	aedimorphus	*tarsalis*	13
	aedimorphus	*vittatus*	19
	neomelaniconion	*palpalis*	1
Culex			13 (4.0)
	Lutzia	*tigripes*	1
	Culex	*duttoni*	1
	Culex	*decens*	2
	Culex	*perfuscus*	6
	Culex	*pruina*	2
	Culex	*sp*	1
Anopheles			9 (3.0)
	Cellia	*gambia*	5
	Cellia	*funestus*	4

seasonal periodicity was identified, but in some cases, an increase of virus activity was seen while the virus was successive isolated from mosquitoes during consecutive years (YFV in 1977 and 1978, Zika virus in 1979 and 1980, and Bozo virus in 1979-1980) suggesting a continuation of virus natural cycle through the mechanism of mosquito transovarial transmission, which allows the sustainment of the virus in nature. Even the host amplifiers (i.e. primates) have been observed to have had high herd immunity from the previous year or when migrating out of the forest gallery [18]. During these epidemiological studies, a serological survey by HAI was conducted among residents of the village of Bozo (4°55 N, 18°31 E). The results showed the activity of four main arboviruses circulating in the area: Bozo, Chikungunya, yellow fever, and Zika (Table 1 and 3). Other testing for viruses showed that 12% of the inhabitants of the region had neutralizing antibodies to Bozo virus and 33% had complement fixing antibodies against Orungo virus (Unpublished data).

At the time of this research, Bozo (ArB 7343) was a new arbovirus isolated from a pool of 100 *A. opok* collected during the current field study. The prevalence of Bozo virus neutralizing antibodies in CAR ranged from 3% in the north (above 5°N) to 25% in tropical forest (unpublished data). Bouboui virus was another new arbovirus that had been isolated in CAR prior to the commencement of this arbovirus survey. It closely reacts with yellow fever (using the CF test), and antibody seroconversion was observed [23]. During this study, the chikungunya virus, in addition to its epizootic circulation, reached the village of Bozo and caused a major epidemic, mainly among young children [24]. High prevalence of chikungunya virus is related to an outbreak in 1978 in the village affecting mainly young children. The isolation of seven different viruses, including Ilesha, Middelburg, Nyando, Tataguine, Bwamba, Orungo, and West Nile (Table 1 and 3) from two anthropophilic *Anopheles* species collected from inside human dwellings, suggested the possibility of a human-to-human transmission cycle; these viruses were previously known only to infect *Anopheles* spp. [25]. Moreover, Bwamba, Ilesha, Nyando, Orungo, and Tataguine viruses had previously been associated with exanthema febrile syndrome in humans [18]. It is worth mentioning that the minimum infectious rate percent (i.e., MIR% for one mosquito species and one virus species=(number of positive pools/(number of pools tested × pool size)) × 100)) [26] of the eight different virus species associated with the two species of *Anopheles* mosquitoes was very low (MIR from 0.043 to 0.089) as compared to chikungunya (0.216), Zika (0.222) and yellow fever viruses (0.103) isolated from *A. africanus* (Table 1) (Unpublished, JF Saluzzo and Max Germain, personal communication).

Among the arboviruses that have a natural cycle identical to yellow fever virus, two of them, chikungunya and Zika, have spread worldwide after more than 60 years of uneventful, geographically limited, and cryptic transmission [27,28]. Although yellow fever was under control in most of Africa and South America by means of an intensive vaccination campaign, Orungo virus has shown an intense amount of activity in human populations of Central and West Africa [29]. Can we expect that Bouboui and Bozo viruses will someday dominate international headlines? Explaining the role of arboviruses in human pathology has been a primary objective of the Pasteur Institute of Bangui, especially with regards to the elucidation of the etiology of "Congolese red fever" which had long been assumed as solely rickettsialin origin. Four viruses dominated the etiology of this so-called "Congolese red fever": Chikungunya, Ilesha, Bwamba, and Tataguine. Out of all, 60% of cases associated with these viruses presented a continuing fever, diffuse pains, and rash. The evolution was favorable and short; recovery showed a marked significant weakness for 8 to 10 days, and no severe clinical presentation was observed. These results again show the extreme difficulty of any differential diagnosis in the absence of laboratory support for virological diagnostics.

When diagnosing cases of febrile illness, malaria was almost always assumed, preliminarily, and treated for [30]. In such circumstances, it was and still remains nearly impossible to attribute such a non-specific clinical picture to any arbovirus when these viruses emerge in new are as among non-immune populations. In endemic areas, arbovirus infections mainly affect children, as adults are protected by successive contacts with several arboviruses resulting in a strong homologous (same virus) or heterologous (cross-species) immunity [31]. The emergences and pandemics of arboviruses teach us incrementally of the different mechanisms based on these viruses and their hosts, but mainly about the environmental, human, and physical changes that shape these occasions and promote the duration and severity of these outbreaks. Apart from the historical pandemics of yellow fever and dengue fever, with their multiple and successive emergences and re-emergences in several continents, only West Nile and chikungunya viruses have spread suddenly and globally. All these arboviruses seemed inconsequential at the time of their discovery over fifty years ago in their East African cradles. However, despite a few initial scattered and failed attempts to go beyond the African continent, most have spread far and wide in a short amount of time and among large and previously non-immune populations and areas, to the astonishment of health authorities and the global community [27,28]. The emergence of Zika in the western hemisphere has (until recently) appeared to have been along a somewhat narrow path, beginning in the forests of Uganda, over to Yap and Oceania, and then to Brazil and onwards. Our results, along with other recent

studies, reveal that Zika has been proliferating and circulating throughout West [32] and Central Africa for the last forty years. No arbovirus can settle permanently in an ecosystem in the absence of a natural cycle. This was the situation during the summer of 2007 in the area of Ravenna, Italy, where after a large chikungunya outbreak (249 cases reported) introduced to the area by an infected traveler returning from India, the virus disappeared permanently because a sylvatic cycle could not be established. Will this be true for Zika virus? The spread of Zika virus from Africa to Asia was astonishing, crossing the ocean from island to island, from one non-immune population to another and ultimately reaching South America [33,34]. To this end, there are some unique parallels worth noting between the spread of chikungunya virus and that of Zika. Like chikungunya, the Zika virus caused an explosive outbreak among a non-immune human population living on an isolated island, prior to its significant global emergence. It is possible, if not likely, that both arboviruses were under environmental pressure and were altered significantly evolve in a new phenotype during their time in these tropical, secluded, and biologically novel locations. This possibility is supported not only by epidemiological indicators suggesting a potential increase in virulence and ease of transmission among both virus species, but also by research suggesting genetic variation among Zika virus strains in particular when comparing those samples isolated in Africa versus those isolated in Asia and Oceania [35]. A few characteristics of this study make it remarkable. First, this study was performed in a remote, very limited, and relatively isolated location with, therefore, a limited spread that did not reach into the general health system. Second, the sensitivity of the methods for viral isolation that were used were subjectively based on the observation of neurotropic and viscera topic clinical manifestation of suckling mice that were experimentally infected. This study demonstrated as previously observed that even though arboviruses show a wide range of circulation, they usually are present at a low prevalence in forest-savanna area of the inter-tropical zone. Ultimately and beyond expectation, 90 species of arboviruses were isolated and characterized at the Pasteur Institute of Bangui because of this study, 30 of which were new virus species [36]. The presence of Zika in this study, with respect to the current level of interest in understanding the virus's spread, provides new information on its historical endemic zone. These findings teach us the necessity of conducting targeted epidemiological surveillance within areas where viruses with the potential for regional or global emergence are circulating. There is a need to maintain constant bio surveillance to detect unsung viruses that hide in wild cycles and can be potentially pathogenic to humans. Finally, given the complexity of arbovirus natural cycles and the variety of hosts and vectors, these studies must be considered as a part of the One Health approach. Beyond the consideration of arthropods and natural hosts, special attention must be paid to human and physical environments that play a central role in disease emergence. Vector dispersal and human mobility are essential factors leading arbovirus emergence toward new territories, and certainly have contributed to the expansion of Zika virus among non-immune populations [37].

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