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Yellow fever and dengue fever viruses' serosurvey in non-human primates of the Kedougou forest galleries in Southeastern Senegal

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The potential risk of non-human primates in Senegal to be natural hosts for arboviruses of importance for human has been assessed. A total of 58 wild monkeys, including 14 Erythrocebus patas and 44 Chlorocebus sabaeus, were trapped at three sites within forest galleries and the nearby village of Ngari, in the Kedougou area, Southeastern Senegal. Blood samples were taken and sera analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of Yellow Fever (YF) and/or Dengue 2 (DEN-2) reacting antibodies. An overall yellow fever seroprevalence of 22.4% was found, including 5.2% and 17.2% YF IgG positive E. patas (3/58) and C. sabaeus (10/58) respectively. Three of the positive C. sabaeus were trapped near Ngari village, and the others in forest galleries. All of the primates tested positive including 5.2% of E. patas and 6.9% of C. sabaeus, all of them were from the forest galleries. Ultimately Cercopithecidae act as potential amplificatory reservoir hosts for YF virus and, seroconversion observed within wild C. sabaeus and E. patas demonstrates also an active DENV-2 virus circulation within non-human primates in Senegal. The present study addresses and discusses new insight of both viruses’ natural enzootic cycles.

Key words: Yellow fever, Dengue, monkeys, Senegal.

INTRODUCTION

Yellow fever virus (YFV) and Dengue viruses (DENV) belong to the same Flavivirus genus of the Flaviviridae family. There are four DENV serotypes also distinguishable by

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their genome (DENV-1, DENV-2, DENV-3 and DENV-4), all of which can cause dengue fever (DF), and dengue hemorrhagic (DHF) (Gubler, 1997; Bhatt et al., 2013). YFV and DENV belong to the same clade within Flaviviridae. Despite the excellent protection afforded by the worldwide available 17D vaccine, YFV still causes, in unprotected persons, severe and often deadly illness (Nathan et al., 2001). Indeed, outbreaks occur annually in West Africa, and cases are typically underreported. The World Health Organization estimates that 200,000 cases of yellow fever occur worldwide each year, from which there are 30,000 deaths, most of which occurring in West Africa (Mutebi and Barrett, 2002). It still remains an important health risk in sub-Saharan Africa and tropical South America (Vainio and Cutts, 1998; Tomori, 2004). Vaccine coverage is often unreliable, particularly in remote regions, and the risk for outbreaks increases whenever routine vaccination breaks down (Nathan et al., 2001). In Senegal, outbreaks have been recorded and the epidemic risk remains (Thonnon et al., 1998a, b).

Dengue fever is now one of the most important arthropod-borne viral diseases in humans, accounting for the largest portion of global mosquito-borne disease morbidity and mortality. There is no licensed vaccine for DENV and control of this disease primarily relies on vector control and community. This disease sickens 50 to 100 million people every year, from which 200,000 to 500,000 cases of potential life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) are reported (Noi sakran and Chuen, 2008). Dengue infection can cause a spectrum of illness ranging from mild, undifferentiated fever illness to severe fatal hemorrhagic syndrome. The first phase of the illness can last for up to seven days with high fever, severe headache, retro-orbital pain, arthralgia and rash. In 3 to 5% of DENV infections, severe syndrome occurs, including DHF with hemorrhagic tendencies, thrombocytopenia and plasma leakage, and DSS with all the above criteria plus circulatory failure. DHF and DSS are potentially deadly however, patients with early diagnosis and appropriate therapy can recover without sequela (Guha-Sapir and Schimmer, 2005). Several investigations have been undertaken in West Africa concerning the natural cycle of DENV- wild mosquitoes - non-human primates but failed to prove a dengue sylvatic cycle. However in South East Asia, limited observations favoring a potential DENV sylvatic cycle have been documented: in the Philippines, Simmons et al. (1931) conducted some experiments in Manila and suspected a dengue sylvatic cycle; in Malaysia, extensive field and laboratory investigations conducted on the ecology of the dengue viruses hypothesized a sylvatic transmission cycle (Rudnick, 1986) and in some other countries of South East Asia, Yuwono et al. (1984) suggested the occurrence of a zoonotic reservoir of infection existing in all the primary tropical forests of Malaysia, Thailand, Vietnam, Cambodia and Indonesia.

In Senegal, serosurveillance programs led within wild monkeys in forested areas of the emergence zone also brought little information about the sylvatic cycle of dengue viruses (Cornet et al., 1984; Saluzzo et al., 1986; Diallo et al., 2003).

From June 2002 to November 2006, we performed a study in order to determine the role of feral monkeys in the sylvatic cycle of DENV. Seroepidemiological survey was carried out in Southeastern Senegal in order to assess if the most abundant non human primates of the region could potentially act as efficient DENV reservoirs or amplification hosts and play an important role in the virus natural perpetuation in forest galleries where mosquitoes have been found infected with DENV-2. Simultaneously, a YFV serosurvey was conducted.

MATERIALS AND METHODS

The present research complied with legal requirements of the Senegalese authorities and adhered to the principles for the ethical treatment of non-human primates. An authorization to conduct monkey trapping and blood sampling was granted by the Direction of wildlife Services, Ministry of Environment and Nature Protection, Senegal (Approval # 001270 DEF/DFG 2002, Direction des Eaux et Forêts, Chasses et de la Conservation des Sols), and ratified by the Research Institute for Development (IRD, Marseille, France).

Study sites

Ngari village (12° 38’ 0.57” N, 12° 14’ 59.77” W) is located 11 km north of Kedougou in a hilly region of the savanna-forest gallery mosaic of the Sudan–Guinean phytogeographic domain. The rainy season begins in May and ends in October. Ngari, as well as all others surrounding villages, is of traditional agricultural type, consisting of extended family compounds of 3 to 6 houses interspaced between fields of corn, millet and peanuts. Most houses are mud-walled with thatch roofs. Plantations of mango trees (Mangifera indica), baobab (Adansonia digitata) and Cola nitida’s fruits around the village supply a food source for monkeys according to the season. The Pont-Plateau site (12° 36’ 0.09” N, 12° 14’ 0.25” W) is located 2 km south of Ngari in the forest gallery named “PK10” (i.e.: 10 km away from Kedougou), bordered by a cool dense forest gallery erected in a depression where mostly baboons and green monkeys sleep. The “Two Rivières” site (12° 38’ 0.20” N, 12° 14’ 0.15” W), located 1 km North of Ngari, represents a temporary running water source bordered by a forest gallery, with high flow during all the rainy season (Figure 1). From May to December 2002, visual surveys were performed in the forest galleries around Kedougou, in order to identify simian species present in the area and to know their vital domains and daily activities. These preliminary studies allowed: 1) to establish the specific richness of monkey population; 2) assess male/female, sub-adult/juvenile ratio for each species. Based on these data, the trapping sites were selected, while also DENV-2 and YFV have been known for circulating in these targeted areas (Cornet et al., 1978; 1979; 1984; Diallo et al., 2003; Traore-Lamizana et al., 1994).

Monkeys trapping and blood collection

Before setting traps, peanut heaps were sparsely placed into rows around the trap places in order to attract monkeys and habituate
them feeding around the sites. An operator hiding place was set in a small shelter hut under dense vegetation, 150 m distant from each trap to look out for monkey arrivals. A soft green fishing net was designed for the African green monkey, Chlorocebus sabaeus (Gray, 1821) and the Patas monkey, Erythrocebus patas (Schreber, 1775) species. It was adapted as a tent trap of 6 m length, 4 m width and 2 m height, maintained vertically by six PVC tubes set at the four corners and two in the middle. Another trap for Guinea baboon, Papio papio (Erxleben, 1777) species was made and consisted of a metallic cage of 4 m length and 3 m wide, toughly fixed in the soil by four tubes. Entrance was designed as a sliding door attached to a rope, turning around a pulley, and linked to a tiny rope that ran into the hut for shutter release.

At 06:00 am all material was set ready for capture and blood collection. Trapped specimens were anaesthetized using insulin syringes with a dose of 10 mg/kg of ketamine (Imagen 1000®). While anaesthetized monkeys were taken out of the trap, 5 to 10 ml of blood were drawn from the femoral vein depending to the size of the animal using 10 ml disposable syringes and transferred from the syringe to 10 ml blood sterile collection tubes (VENOJECT® PLAIN SILICON-COATED Z). Samples were stored in a cooler at +4°C to be transported to the research station and processed for sera extraction and preservation. Sera aliquots were kept in Nunc® cryotubes and stored in a nitrogen tank until transferred to a -80°C freezer for later use. Morphometric data were recorded, each individual was weighed and an identification number allocated under his armpit using a dermography stylus.

**ELISA test for antibodies detection**

YFV and DENV-2 antibody detection were performed on 1/100 sera dilution: IgM were detected by MAC-ELISA following the protocol of Lhuillier and Sarthou (1983) and IgG were detected using the technique of indirect ELISA as previously described (Innis et al., 1989). Serum samples were tested with a positive and negative control. Briefly, specific antibodies bind to soluble antigens attached to the microwells (Tittertek, Flow Laboratories, McLean, VA). After a first wash, enzyme conjugate is added to the well that binds antibodies captured by the antigen. After a second wash, a substrate is added that turns blue in the presence of the enzyme complex. A stop solution turns the mixture yellow, and is then read with a spectrophotometer. Results are reported as optical density values (OD).

**RESULTS**

From June 2002 to December 2006, 58 serum samples were obtained from 51 and seven recaptured, specimens including: 14 E. patas and 44 C. sabaeus (Table 1).

Among the seven recaptured specimens, three were C. sabaeus juvenile males trapped for the first time from Ngari site in December 21st, 2002 (N1, N4 and N6). At their second trapping, on June 3, 2003, their sera were respectively identified as Re1N1, Re1N4 and Re1N6 (Re1N1 meaning 1st Recapture of monkey number N1). While recapturing these individuals at the “Pont” site for a second time, a sub-adult male E. patas was captured for the first time and marked as P8 (P for “Pont” site) that same June 3, 2003. Three other juvenile C. sabaeus were caught and marked as L1 (female juvenile, L for “Plateau” site), L4 and L10 (both male juvenile) during August, 2006. At that time, the P8 E. patas was recaptured (Re1P8, in August 2006). During our last trapping on December 2006, the three C. sabaeus previously marked on August 2006 were resampled as Re1L1, Re1L4 and Re1L10.

At the end of the 2002 rainy season, seven sera over 19 of C. sabaeus tested positive for YFV IgG, without any YFV IgM detection. Positive individuals were two adult male (D2 and P3), two adult female (D3 and P2) and three juveniles (D4, P5 and N3) (Table 2). Follow up
Table 1. Seroprevalence of YFV and DENV-2 antibodies from trapped monkeys.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorocebus sabaeus</td>
<td>7/19</td>
<td>NT</td>
<td>3/9</td>
<td>0/9</td>
<td>NT</td>
<td>4/16</td>
<td>10/28</td>
<td>4/25</td>
</tr>
<tr>
<td></td>
<td>(36.8)*</td>
<td></td>
<td>(33.3)</td>
<td>(0.0)</td>
<td>(25.0)</td>
<td>(3.6)</td>
<td>(16.0)</td>
<td></td>
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<tr>
<td>Total C. sabaeus</td>
<td>19</td>
<td>9</td>
<td>0/10</td>
<td>3/10</td>
<td>NT</td>
<td>3/4</td>
<td>3/10</td>
<td>3/14</td>
</tr>
<tr>
<td>Erythrocebus patas</td>
<td>0</td>
<td>0</td>
<td>0/10</td>
<td>0/10</td>
<td>NT</td>
<td>75.0</td>
<td>30.0</td>
<td>21.4</td>
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<tr>
<td>Total E. patas</td>
<td>0</td>
<td>10</td>
<td>4</td>
<td>14</td>
<td></td>
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</tbody>
</table>

*Number positive / total tested (Percentage); NT, not tested;

Table 2. Seroprevalence of anti-YFV and anti-DENV-2 antibodies in wild Chlorocebus sabaeus and Erythrocebus patas captured in Deux rivières (D), Pont (P)-Plateau (L) of PK10, and in Ngari (N) during our study. * (nt = not tested).

<table>
<thead>
<tr>
<th>Code</th>
<th>Species</th>
<th>Site</th>
<th>Sex</th>
<th>Age</th>
<th>2002 YF IgM</th>
<th>2002 IgG</th>
<th>2003 YF IgM</th>
<th>2003 IgG</th>
<th>2006 YF IgM</th>
<th>2006 IgG</th>
</tr>
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<tbody>
<tr>
<td>D2</td>
<td>C. sabaeus</td>
<td>2Rivieres</td>
<td>M</td>
<td>Adult</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>D3</td>
<td>C. sabaeus</td>
<td>2Rivieres</td>
<td>F</td>
<td>Adult</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>D4</td>
<td>C. sabaeus</td>
<td>2Rivieres</td>
<td>F</td>
<td>Juvenile</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>P2</td>
<td>C. sabaeus</td>
<td>Pont</td>
<td>F</td>
<td>Adult</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>P3</td>
<td>C. sabaeus</td>
<td>Pont</td>
<td>M</td>
<td>Adult</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
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<tr>
<td>P5</td>
<td>C. sabaeus</td>
<td>Pont</td>
<td>M</td>
<td>Juvenile</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>N3</td>
<td>C. sabaeus</td>
<td>Ngari</td>
<td>M</td>
<td>Juvenile</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>P7</td>
<td>E. patas</td>
<td>Pont</td>
<td>M</td>
<td>Subadult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P8</td>
<td>E. patas</td>
<td>Pont</td>
<td>M</td>
<td>Subadult</td>
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<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P9</td>
<td>C. sabaeus</td>
<td>Pont</td>
<td>M</td>
<td>Subadult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N7</td>
<td>E. patas</td>
<td>Ngari</td>
<td>M</td>
<td>Adult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N13</td>
<td>C. sabaeus</td>
<td>Ngari</td>
<td>M</td>
<td>Adult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N15</td>
<td>C. sabaeus</td>
<td>Ngari</td>
<td>F</td>
<td>Juvenile</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>L2</td>
<td>C. sabaeus</td>
<td>Plateau</td>
<td>F</td>
<td>Adult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>L6</td>
<td>C. sabaeus</td>
<td>Plateau</td>
<td>M</td>
<td>Adult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
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</tr>
<tr>
<td>L11</td>
<td>C. sabaeus</td>
<td>Plateau</td>
<td>M</td>
<td>Adult</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
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<tr>
<td>L14</td>
<td>E. patas</td>
<td>Plateau</td>
<td>F</td>
<td>Juvenile</td>
<td>nt</td>
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<td>C. sabaeus</td>
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<td>nt</td>
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At the end of the 2002 rainy season, all 19 samples were negative for both DENV-2 IgG and IgM (Tables 1 and 2). During the rainy season in 2006, over 20 sera collected from captured monkeys, seven [four C. sabaeus (L2, L6, L11 and L16) and three E. patas (Re1P8, L14 and L15)] tested positive for DENV-2 IgG without DENV-2 IgM. Among these, six newly captured individuals in 2006 tested positive for DENV-2 IgG (Table 2), including two juveniles less than 1 year old [one E. patas (L14) and one C. sabaeus (L16)], attesting that DENV-2 recently circulated within the monkeys of the forest gallery of PK10.
DISCUSSION

YFV IgG positive samples referred to two adult male, two adult female and three juvenile C. sabaues (Table 2). Morphometric and morphologic traits recorded on juveniles allowed for age estimation of approximately two to three years old. Then, one can estimate that these C. sabaues got an YFV infection earlier at the beginning of their life in 1999 and seroconverted that might explain YFV IgG circulation detected in 2002. Another scenario is that, they could have contracted the virus more recently (six months before they were caught and sampled, since YFV IgG disappear within 2 to 5 months). In all cases, YFV reacting antibodies among juvenile not older than 3 years old, in absence of any YF human case reported, attest about a YFV amplification and circulation within monkeys in a silent cycle in the PK10 forest gallery. Yellow fever (YF) occurs only in sub-Saharan Africa and the tropical regions of South America, where it is endemic and sporadically epidemic. In Africa, the YF sylvan cycle involves the non-human primate reservoir species ( Chlorocebus spp., Erythrocebus spp.) and the forest mosquitoes [ Aedes aegypti aegypti, Aedes aegypti formosus, Aedes (Stegomyia) africanus, Aedes (Stegomyia) bromeliae, Aedes (Diceromyia) furcifer, Aedes (Stegomyia) luteocephalus, Aedes (Stegomyia) metallicus, Aedes (Stegomyia) opok, Aedes (Stegomyia) simpsoni complex, Aedes (Diceromyia) taylori, Aedes (Aedimorphus ) vittatus] that bite and infect humans who enter the forest (Cordellier, 1991). The forest savannah mosaic of southeastern Senegal represents the YFV “zone of emergence” where transmission to humans occurs when the fundamental of emergence, including several sylvan and domestic infected mosquito vector species, a preexisting primate-mosquito sylvan YFV cycle and a non immune human population, are combined. The human intrusion in the sylvatic cycle fosters an intermediate YFV cycle that bridges the sylvan enzootic and urban endemic cycles. Ultimately, it is from this scenario that YFV transmission goes from human to human, causing outbreaks and even epizootics affecting several villages and towns in the urban cycle (Germain, 1986).

Moreover, our findings suggest that DENV-2 has been circulating in the PK10 forest gallery of southern Senegal within the local monkey population including E. patas as well as C. sabaues. DENV-2 isolation in Senegal was first obtained from blood of a young girl in Bandia (14 35”N, 17 01”W; Mbour Department, Thies Region), in the sahelosudanian area, in 1970 (Robin et al., 1980). Further entomological investigations conducted in the forest galleries of southeastern Senegal (zone of emergence) led to isolate DENV-2 from Aedes (Stegomyia) luteocephalus mosquitoes in 1974 (Robin et al., 1980). A retrospective non human-primates serosurvey in this area detected also epizootics of DENV-2 infection among monkeys, suggesting that primates might be efficient amplifying hosts for the virus (Saluzzo et al., 1986), and therefore involved in a sylvatic cycle of DENV-2.

Routine entomological surveillance and sero-survey programs set up and carried out by Pasteur Institute and ORSTOM (IRD) of Dakar reported recurrent DENV-2 amplifications in those forest gallery areas of Senegal: 1980-1982, a DENV-2 epizootic occurred with virus isolations from mosquitoes ( Aedes furcifer, Ae. taylori and Ae.luteocephalus) and from the red monkey, E. patas ( Cornet et al., 1984); 1989-1990, with virus isolation from the same mosquito species as previously found (Traore-Lamizana et al., 1994); 1999, when Aedes (Stegomyia) aegypti and Aedes (Aedimorphus) vittatus were, for the first time, found infected with DENV-2, while the known potential vectors ( Aedes furcifer, Ae. taylori and Ae luteocephalus), were again found infected with DENV-2 and, ultimately DENV-2 IgG were also detected in African green monkeys, C. sabaues (Diallo et al., 2003) captured from January 31 to February 6, 2000 in the same forest galleries (Diallo et al., 2003), as for the present study. Our findings appeared during August of the rainy season of 2006 that is six years after the last DENV-2 amplification of 2000 reported by Diallo et al. (2003), corroborative to the periodicity of occurrence with silent intervals of 5 to 8 years so far observed (Alhouse et al., 2012). Moreover, the seroconversion that we have detected from wild C. sabaues and E. patas living in forest galleries of southeastern Senegal support the role played by monkeys in the circulation and maintenance of sylvatic DENV-2. After an inter epizootic period, DENV-2 virus reemerged in this area, sharing the same Cercopithecidae vertebrate hosts with YF virus.

Stegomyia mosquitoes (Aedes aegypti formosus and Aedes luteocephalus) and Diceromyia (Aedes furcifer and Aedes taylori), which are specific to the forest gallery, have been found infected with DENV-2, as well as Aedes vittatus (Diallo et al., 2003). They play a major role in the mosquito-monkey maintenance wild cycle regarding their preferences to blood feed on monkeys when they return to the forest gallery at dusk to rest. Also Aedes furcifer and Ae. luteocephalus were highly susceptible to both sylvatic and urban DENV-2 strains and represent potential vectors of the virus (Diallo et al., 2005). Ultimately, entomological and sero-epidemiological surveillance of arboviruses circulation in Southeastern Senegal (Monlun et al., 1993; Diallo et al., 2003) revealed an amplification of DENV-2 within Aedes mosquitoes from the forest galleries, concomitant to DENV-2 infection in humans in the nearby villages (Zeller et al., 1992; Traore-Lamizana et al., 1994).

In other parts of West Africa, Fagbami et al. (1977) detected DENV-2 antibodies in non-human primates inhabiting both gallery and lowland forests in Nigeria; over 100 strains of DENV-2 were also isolated from forest Aedes taylori, Aedes furcifer, Aedes opok, Aedes luteocephalus and Aedes africanus in Guinea, Côte d’Ivoire, and Burkina Faso (Cordellier et al., 1983; Roche et al., 1983; Hervy et al.,
In West Africa, there has been no evidence of dengue epidemic from an enzootic transmission that bridge to a rural or urban cycle, affecting human population. Moreover, Rico-Hesse (1990) attributed the epidemic that arose in Burkina Faso in 1982 to a DENV-2 strain that originated from the Seychelles Islands.

In South East Asia, Simmons et al. (1931) conducted some experiments in Manila (Philippines) and prove for the first time that dengue virus can be transmitted by *Aedes* mosquitoes to monkeys species *Macacus fuscatus* and *Macacus philippinensis* and retransferred to other monkeys or to men through mosquito bites. In Penang, Malaya, Smith (1956) demonstrated that forest tree-dwelling mammal species were more exposed to dengue infection than ground-dwelling animals and suggested then, an implication of a canopy-dwelling forest vector. He postulated also that *Ae. albopictus* may be the bridge vector between monkeys in the forest and man in rural areas (Smith, 1958).

Rudnick (1965) demonstrated the presence of widespread DENV-neutralizing antibodies in wild monkeys (*Macaca nemestrina, M. fascicularis, Presbytis cristata* and *P. melaphos*).

Rudnick et al. (1986) isolated several strains of DENV-1, 2 and 4 from 27 sentinel monkeys [*Presbytis obscura* and *Macaca fascicularis* (=*virus*)] placed in the forest canopy while no isolation was obtained from 19 sentinel monkeys placed at ground level. Although DENV-3 has not been isolated, seroconversion in sentinel monkeys suggested their circulation (Rudnick, 1986). They also isolated DENV-2 from *Ae. albopictus*, a potential vector found at ground level in the study areas, and DENV-4 from an *Aedes* species of the *niveus* group. Furthermore, a serum survey of 300 forest-dwelling Orang Asli aborigines detected neutralizing dengue antibodies in the vast majority, although no clinical dengue was reported among this group (Rudnick, 1986). Based on those findings, they hypothesized that dengue serotypes were circulating in the forest canopy, between *Aedes* mosquitoes of the *niveus* group and monkey species of the genus *Macaca* and *Presbytis* and that the man was occasionally infected by intrusion in this cycle (Rudnick, 1965; Rudnick et al., 1967). Moreover, Yuwono et al. (1984) postulated that this enzootic cycle could occur in all primary forests of tropical Asia where the zoonotic reservoir exists.

This arboviral disease increases its range of occurrence, gaining the tropical and intertropical world because substantial vector control efforts have not stopped its rapid emergence and global spread (Bhatt et al., 2013). DENV epidemics occurred earlier in Zanzibar (Christie, 1881) and in Cairo, Egypt (Hirsch, 1883). Later, it emerged sporadically in Burkina-Faso, in 1925 (Legendre, 1926), in Senegal (Bideau, 1925) and in South Africa (Edington, 1927). After Nigeria epidemic in 1964 diagnosed by a retrospective serosurvey (Carey et al., 1971), the virus spread silently throughout Africa. Kading et al. (2013) recently reported prevalence of antibodies to DENV-2 in non human primates in the greater Congo basin. So far considered as benign without severe syndrome (no dengue hemorrhagic fever) (Gratz and Knudsen, 1996), dengue sporadically emerged in the non immune human population causing hemorrhagic fever and sometimes fatal cases. In fact, an imported DHF case caused by a West African sylvatic strain of DENV-2 in a healthy man returning to Madrid from Guinea Bissau through Senegal has been recently described (Franco et al., 2011). Moreover, an urban epidemic of DEN attributed to serotype 3 occurred in Senegal in 2009, affecting 196 persons with five cases of dengue hemorrhagic fever and one fatal case of dengue shock Syndrome (Faye et al., 2014). A DENV-3 epidemic has also been previously reported in Mozambique (Gubler et al., 1986).

DENV-2 isolates from the above mentioned studies, and isolates from mosquitoes in other parts of West Africa, are phylogenetically distinct from contemporaneous DENV-2 strains circulating in Asia and the Americas, and are therefore likely to constitute a distinct “African” sylvatic cycle (Vasilakis et al., 2012). Recently, a phylogenetic study from Vasilakis et al. (2008) demonstrated that the first dengue virus infection in Nigeria documented by Carey et al. (1971) was an African strain of sylvatic origin. Two distinct transmission cycles have been described for dengue virus: 1) the endemic and epidemic cycles involving human host and viruses are transmitted by main vectors as *Ae. aegypti, Aedes albopictus* and other mosquitoes as secondary vectors (Wang et al., 2000), and 2) the sylvatic natural transmission cycle involving monkeys and several *Aedes spp.* mosquitoes mostly identified in Asia and West Africa (Holmes and Twiddy, 2003).

For a better understanding of the DENV evolution and dissemination throughout Africa, a long term serosurveillance program including non-human primates, and eventually other mammals living in the forested areas, must be undertaken, particularly in West Africa. Moreover, as postulated by Vasilakis et al. (2012), it is possible that sylvatic dengue may be present but yet unrecognized in other regions of Africa.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

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