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# Gene Flow, Subspecies Composition, and Dengue Virus-2 Susceptibility among *Aedes aegypti* Collections in Senegal

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## Abstract

*Background: Aedes aegypti*, the ''yellow fever mosquito'', is the primary vector to humans of the four serotypes of dengue viruses (DENV1-4) and yellow fever virus (YFV) and is a known vector of Chikungunya virus. There are two recognized subspecies of *Ae. aegypti sensu latu* (*s.l.*): the presumed ancestral form, *Ae. aegypti formosus (Aaf)*, a primarily sylvan mosquito in sub-Saharan Africa, and *Ae. aegypti aegypti (Aaa)*, found globally in tropical and subtropical regions typically in association with humans. The designation of *Ae. aegypti s.l.* subspecies arose from observations made in East Africa in the late 1950s that the frequency of pale ''forms'' of *Ae. aegypti* was higher in populations in and around human dwellings than in those of the nearby bush. But few studies have been made of *Ae. aegypti s.l.* in West Africa. To address this deficiency we have been studying the population genetics, subspecies composition and vector competence for DENV-2 of *Ae. aegypti s.l.* in Senegal.

*Methods and Findings:* A population genetic analysis of gene flow was conducted among 1,040 *Aedes aegypti s.l.* from 19 collections distributed across the five phytogeographic regions of Senegal. Adults lacking pale scales on their first abdominal tergite were classified as *Aedes aegypti formosus (Aaf)* following the original description of the subspecies and the remainder were classified as *Aedes aegypti aegypti (Aaa)*. There was a clear northwest-southeast cline in the abundance of *Aaa* and *Aaf*. Collections from the northern Sahelian region contained only *Aaa* while southern Forest gallery collections contained only *Aaf*. The two subspecies occurred in sympatry in four collections north of the Gambia in the central Savannah region and *Aaa* was a minor component of two collections from the Forest gallery area. Mosquitoes from 11 collections were orally challenged with DENV-2 virus. In agreement with the early literature, *Aaf* had significantly lower vector competence than *Aaa*. Among pure *Aaa* collections, the disseminated infection rate (DIR) was 73.9% with a midgut infection barrier (MIB) rate of 6.8%, and a midgut escape barrier (MEB) rate of 19.3%, while among pure *Aaf* collections, DIR = 34.2%, MIB rate = 7.4%, and MEB rate = 58.4%. Allele and genotype frequencies were analyzed at 11 nuclear single nucleotide polymorphism (SNP) loci using allele specific PCR and melting curve analysis. In agreement with a published isozyme gene flow study in Senegal, only a small and statistically insignificant percentage of the variance in allele frequencies was associated with subspecies.

*Conclusions:* These results add to our understanding of the global phylogeny of *Aedes aegypti s.l.*, suggesting that West African *Aaa* and *Aaf* are monophyletic and that *Aaa* evolved in West Africa from an *Aaf* ancestor.

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## Introduction

*Aedes aegypti*, the ''yellow fever mosquito'', is the primary vector to humans of the four serotypes of dengue viruses (DENV1-4), yellow fever virus (YFV) and is a known vector of Chikungunya virus. Dengue is a major public health problem in tropical regions of the world, causing millions of dengue fever and hundreds of thousands of dengue hemorrhagic fever cases annually [1]. In endemic areas the annual number of cases has risen steeply since the 1950s [2]. With multiple serotypes circulating in endemic areas, 100 million infections of dengue fever (DF) occur annually, including up to 500,000 cases of the more severe form of disease called dengue hemorrhagic fever (DHF) with a case fatality rate of up to 5% [3]. Despite the development of a safe, effective YFV vaccine, yellow fever remains an important health risk in sub-Saharan Africa and tropical South America [4,5]. The WHO estimates there are 200,000 cases and 30,000 deaths attributable to YFV infection each year, most of which occur in Africa [6].

There are two recognized subspecies of *Ae. aegypti s.l.*: the presumed ancestral form, *Ae. aegypti formosus (Aaf)*, a primarily

## Author Summary

We conducted a population genetic study with 1,040 *Aedes aegypti sensu latu* (*s.l.*) collected from 19 sites distributed across the five phytogeographic regions of Senegal. Adult mosquitoes without pale scales on their first abdominal tergite were classified as *Aedes aegypti formosus* (*Aaf*) and those having pale scales as *Aedes aegypti aegypti* (*Aaa*). We found the two forms distributed along a northwest-southeast cline. Northern Sahelian collections contained only *Aaa* while the southern Forest gallery collections consisted of only *Aaf*. The two subspecies were sympatric in four collections north of The Gambia. *Aaa* was a minor component of two collections from the Forest gallery area. Eleven of these collections were fed a dengue-2 virus-infected bloodmeal. Consistent with the early literature, *Aaf* had lower vector competence than *Aaa*. In agreement with a recently published isozyme gene flow study in Senegal, analyzes of allele frequencies indicated only a small, nonsignificant percentage of the variance associated with subspecies. These results improve our understanding of the global phylogeny of *Aedes aegypti s.l.*, suggesting that West African *Aaa* and *Aaf* are monophyletic and that *Aaf*, the black ''sylvan'' species, is the ancestor of *Aaa*, the lighter ''domestic'' species in West Africa.


sylvan mosquito in sub-Saharan Africa, and *Ae. aegypti aegypti (Aaa)*, found globally in tropical and subtropical regions typically in association with humans. The designation of *Ae. aegypti s.l.* subspecies arose from observations made in East Africa in the late 1950s that the frequency of pale ''forms'' of *Ae. aegypti* was higher in populations in and around human dwellings than in those of the nearby bush [7]. The implied correlation between color and behavior prompted Mattingly [8] to revisit the biology and taxonomy of *Ae. aegypti*. He described *formosus* (Walker) as a subspecies of *Ae. aegypti* that was restricted to sub-Saharan Africa and in West Africa ''is the only form known to occur except in coastal districts and in one or two areas of limited island penetration.'' He also suggested that it most frequently breeds in natural containers such as tree holes and feeds on wild animals. Mattingly also stated that, in addition to the dark-scaled parts of the body being generally blacker, ''*ssp. formosus* never has any pale scales on the first abdominal tergite.'' The type form of *Ae. aegypti aegypti* was alternatively defined as ''either distinctly paler and browner (at least in the female) than *ssp. formosus* or with pale scaling on the first abdominal tergite or both.'' He also suggested that *Aaa* breeds in artificial containers provided by humans, will breed indoors, and has a preference for feeding on human blood [9]. McClelland [10] made a comprehensive study of differences in scale patterns in the abdominal dorsum in 74 *Ae. aegypti s.l.* collected from 69 different worldwide locations. He concluded that many of Mattingly's subspecies distinctions were not always clear cut in Africa, the only region in the world where both forms are found. In East Africa, pure *Aaa* or *Aaf* collections as defined by both color and behavior were found but there were also collections where the subspecies were mixed. In areas of sympatry, he found intermediate forms, with peridomestic habits and a wide range of pale scaling. Populations widely overlapped in the extent of pale scaling. McClelland [10] concluded that, with a large enough sample size, populations could be distinguished on the basis of body color, although peridomestic populations overlapped with the distributions of both *Aaa* and *Aaf* populations. Body color alone, however, was unreliable as a means to assign individuals to a particular subspecies and instead, he recommended using the number of pale scales on the first abdominal tergite.

Later, mark-release-recapture studies in Kenya [11] demonstrated that immature mosquitoes collected from sylvan, peridomestic, or domestic breeding containers showed an overwhelming preference for their respective habitat as adults. In contrast, in West Africa, mosquitoes morphologically consistent with *Aaf* were found breeding domestically indoors in Nigeria [12] and Gabon [13]. Therefore, the classic behavioral/habitat descriptions given by Mattingly [8] for these two subspecies were not valid throughout Africa. In eastern Kenya, genetic crosses between *Aaf* and *Aaa* showed that preferences for endophily had a strong genetic component [14]. These authors speculated that these sympatric populations remained behaviorally and morphologically distinct because of adaptations that limited genetic exchange. *Aaf* rarely entered houses, and the authors proposed that those that did would not be likely to oviposit in water jars but would instead seek natural breeding sites in the forest. They speculated that the offspring of those that oviposit in water jars would not be adapted to surviving in the low nutritional content of drinking water. Conversely, they argued that gravid *Aaa* rarely enter the forest, and were not therefore attracted to tree holes. If they oviposited there, the larvae would not be adapted to avoiding predators found in natural containers. Those larvae that survived to adults would be anthropophilic and unlikely to find a suitable host. It was further hypothesized that the subspecies evolved allopatrically, and that *Aaa* was reintroduced into East Africa after adaptation to human habitats. Therefore these layers of behavioral differences were fully developed when the subspecies came into contact again, greatly restricting gene flow between them. Laboratory experiments crossing *Aaa* and *Aaf* from Kenya showed no evidence of assortative mating [15]. Furthermore, there was no decrease in fecundity in hybrids, nor any morphological defects.

The monumental works of Tabachnick, Powell, Munstermann and Wallis [16–27] on the global population genetics and vector competence of *Ae. aegypti s.l.* showed that collections made throughout the species distribution fell into one of two clades (Figure 1). One clade contained *Aaa* from East Africa, South America, the Caribbean and Texas/Northeastern Mexico suggesting that these New World populations were derived from East Africa. The other clade contained Asian and Southeastern U.S. *Aaa* and a basal clade consisting of *Aaf* from East and West Africa. This tree topology suggested therefore independent New World and Asian introductions. Their parallel work with Beaty [17–19] on vector competence suggested that West African *Aaf* had lower competence for YFV than other global collections of *Aaf* and *Aaa*.

Despite the importance of these early groundbreaking studies they had, in retrospect, a number of deficiencies. They did not use the number of pale scales on the first thoracic tergite [9] to identify individual mosquitoes. Instead, whole *Ae. aegypti s.l.* collections were classified as either *Aaa* or *Aaf* based upon geographic origin, collection location (indoor *Aaa* vs. outdoor *Aaf*) and/or their general body coloration of ''light'' (*Aaa*) or ''dark'' (*Aaf*). Furthermore, they assumed that all West African *Ae. aegypti* were *Aaf*. Thus notice in Figure 1 that no *Aaa* were sampled from West Africa. This assumption was based upon Mattingly's [8] claim that in West Africa ''*formosus* is the only form known to occur except in coastal districts and in one or two areas of limited island penetration.'' But this statement was based largely upon collections from Ghana and Burkina Faso. Finally, all early vector competence work was based upon the Asibi strain of YFV. No work was done with DENV because dengue was not a prevalent disease at that time. In order to address these deficiencies, we have been studying the population genetics, subspecies composition and vector competence for DENV-2 of *Ae. aegypti s.l.* in Senegal. Here we report an analysis of 1,040

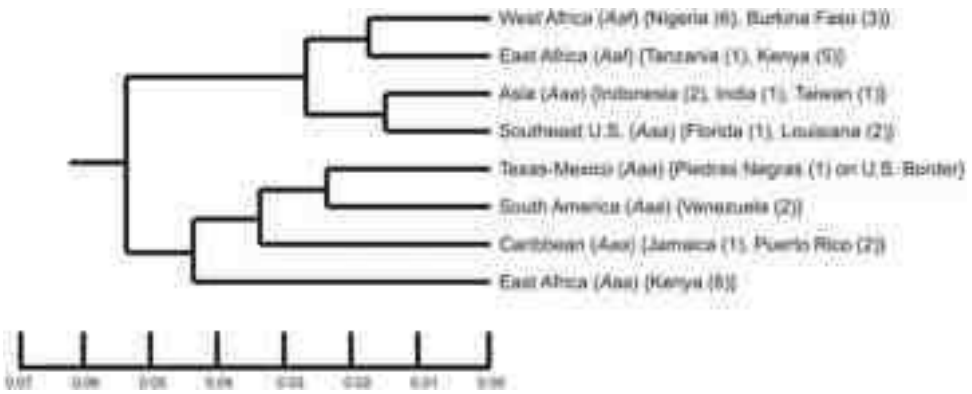


Figure 1. Genetic relationships among 34 worldwide collections of *Ae. aegypti s.l.* Each clade is labeled according to the original names followed by the country or location where the material was collected and, in parentheses, the number of collections. Modified from [25].
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*Aedes aegypti sensu latu* (*s.l.*) from 19 collections distributed across the 5 phytogeographic regions of Senegal.

## Materials and Methods

### *Aedes aegypti* collections and extraction of DNA

From January 8, 2005–July 20, 2007 we collected *Ae. aegypti s.l.* immature stages (larvae and pupae) and eggs from the 19 locations in Senegal listed in Table 1 and mapped in Figure 2. At each urban and rural site, we collected immature stages from at least 30 different breeding sites in each of three different, distant locations at least 100 m apart. Breeding sites consisted of water storage containers and discarded trash such as plastic pails, tires, and cans. In the forest gallery sites of PK10 and Deux Rivieres, immature stages were collected from treeholes and from the discarded husks of *Saba senegalensis* (Apocynacea) which collect water during the rainy season. Eggs collection were also made using ten ovitraps in both of these forest gallery sites.

Eggs and immature stages were returned to the laboratory where they were reared to adults and then identified to species [28]. *Aedes aegypti s.l* were further identified as *Aaa* or *Aaf* based upon the number of pale scales on the first abdominal tergite [10]. If the first abdominal tergite lacked pale scales (McClelland's F range [10]) it was scored as *Aaf* and was otherwise scored as *Aaa*.

Table 1. Name, date, phytogeographic region, location, habitat and sample sizes of collection sites in Senegal.

| City | Date collected | Phytogeographic region | Latitude (N) | Longitude (W) | Habitat | N[a] | N(SNP)[b] | N(VC)[c] |
|---|---|---|---|---|---|---|---|---|
| Saint-Louis | 7/1/2007 | Sahel | 16u 1932.440 | 16u30917.940 | Urban | 26 | 26 | 83 |
| D´igale | 7/2/2007 | Sahel | 16u10960.000 | 15u4590.000 | Rural Village | 58 | 63 | 18 |
| Louga | 7/1/2007 | Sahelo-sudan | 15u36955.030 | 16u13917.560 | Urban | 58 | 56 | - |
| Dakar | 1/8/2005 | Sahelo-sudan | 14u44959.970 | 17u27959.120 | Urban | 61 | 46 | 54 |
| N'goye | 6/29/2007 | Sudano-sahelian | 14u36951.250 | 16u24942.790 | Rural Village | 59 | 58 | 52 |
| Touba | 4/16/2007 | Sudano-sahelian | 14u51933.670 | 15u52943.800 | Urban | 73 | 67 | - |
| Mindin | 7/16/2006 | Sudanian | 14u 3958.550 | 15u17958.760 | Rural Village | 36 | 36 | - |
| Kaffrine | 7/16/2006 | Sudano-sahelian | 14u 6923.830 | 15u3397.250 | Urban | 43 | 37 | - |
| Koungheul | 7/16/2006 | Sudano-sahelian | 13u58933.490 | 14u48915.110 | Urban | 52 | 48 | - |
| Tambacounda | 7/16/2006 | Sudanian | 13u46923.130 | 13u40938.350 | Urban | 105 | 58 | 50 |
| Saraya | 7/18/2006 | Sudanian | 12u49960.000 | 11u4590.000 | Urban | 25 | 54 | - |
| Dienoudialla | 7/17/2006 | Sudanian | 13u12952.050 | 13u6943.150 | Rural Village | 26 | 57 | - |
| Goudiry | 7/8/2007 | Sudano-sahelian | 14u11913.020 | 12u42943.910 | Urban | 58 | 60 | 58 |
| Niemenike | 7/17/2006 | Sudanian | 13u0925.520 | 12u32948.140 | Rural Village | 69 | 59 | 49 |
| Ngari | 11/20/2006 | Sudanian | 12u3890.570 | 12u14959.770 | Rural Village | 57 | 49 | 51 |
| PK10 | 11/20/2006 | Sudanian | 12u3690.090 | 12u1490.250 | Forest Gallery | 40 | 59 | 35 |
| Deux rivières | 11/20/2006 | Sudanian | 12u3890.200 | 12u1490.150 | Forest Gallery | 83 | 51 | 38 |
| Simenti | 7/20/2007 | Sudanian | 13u 1959.720 | 13u17958.770 | Rural Village | 58 | 58 | - |
| Fongolimbi | 7/23/2006 | Sudano-Guinean | 12u24944.880 | 12u0941.760 | Rural Village | 53 | 56 | 26 |
| TOTAL | | | | | | 1040 | 998 | 514 |

[a]N = number of mosquitoes examined for number of white scales on the first abdominal tergite.
[b]N(SNP) = number of $F_1$ mosquitoes in the SNP genotype assays.
[c]N(VC) = number of $F_1$ mosquitoes in the vector competence assays.
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Figure 2. *Aedes aegypti s.l.* collection sites and associated sample sites in Senegal. Predominant vegetation zones are also shown.
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These adults were provided access to sugar, allowed to mate for three days; males were then aspirated, and stored at 280uC. Every third day, over a two-week period, sugar was removed from the cages 24 h prior to bloodfeeding on mice. Bloodfed females were then given constant access to wet paper towels as an oviposition substrate. After two weeks females were aspirated and stored at 280uC. DNA was obtained from individual adults by salt extraction [29], suspended in 300 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0), and stored at 280uC.

## Vector competence

Mosquito collections were characterized for vector competence using an immunofluorescence assay (IFA) at 14 days post-oral challenge. The DENV-2 strain used was dengue 2 JAM1409 which was isolated in 1983 in Jamaica [30] and belongs to the American Asian genotype [31]. This DENV-2 strain was used rather than one from West Africa because we wished to compare vector competence data in *Ae. aegypti* from Senegal with all of our other collections including our standard susceptible Dengue 2 Susceptible on 3 chromosomes (*D2S3*) strain and our resistant Dengue 2 Midgut Escape Barrier (*D2MEB*) strains [32]; all of which have been characterized with JAM1409. All procedures for growing virus in 14 day cell culture, quantifying the virus, and infecting mosquitoes with membrane feeders covered with sterile hog-gut are published [33]. *D2S3* [32] served as a positive control to test for consistency in the quality and quantity of DENV-2 preparation and infection. Undiluted virus titers ranged from 7.5–8.5 $\log_{10}$ infectious virus/mL.

After exposure to the infectious bloodmeal, fully engorged mosquitoes were removed from the feeding carton and held for 14 days at a constant 27uC and 80% relative humidity in an insectary with a 12-hour photoperiod. Heads and abdomens were assayed for infection by IFA using a mouse derived primary monoclonal antibody directed against a flavivirus E gene epitope [34,35]. Heads were checked first for DENV-2 infections. If the head was uninfected, the abdomen was checked for infection.

## SNP discovery

Table 2 lists the primers and annealing temperatures for the eight gene regions from which we identified SNPs. Figure 3 shows the locations of SNPs in the amplified regions. These gene regions were amplified in the 57 *Ae. aegypti* listed in Table 3. Amplified products were screened for polymorphisms with Single Stranded Conformation Polymorphism (SSCP) analysis [29]. All novel SSCP genotypes were then sequenced to screen for SNPs. These sequences were then assembled into a single dataset and translated to assess whether each SNP encoded a synonymous or replacement substitution. Once a SNP locus was selected it was assigned the name of the gene followed by a numeric label indicating its distance in nucleotides from the adenine in the ATG start site.

## SNP genotype identification

Genotypes at SNP loci were detected using allele specific PCR. Genotypes were determined in a single-tube PCR using two

Table 2. Sequences of primers used for PCR amplification of the eight gene regions in *Ae. aegypti s.l.* from Senegal.

| Gene Name (E.C. No.) | Vector Base # | Forward Primer | Reverse primer | Amplicon size (bp) |
|---|---|---|---|---|
| a-Amylase (3.2.1.1) | AAEL013421 | ATGACGTTGGAGTGCGAATC | ACCAGGTTGCCGTAGATGAA | 350 |
| a-Glycerophosphate dehydrogenase (1.1.1.8) | AAEL003873 | GCAGAGGATTCGTCGCAA | ATATCCAGCCCCAAAATG | 258 |
| Aminopeptidase N (3.4.11.2) | AAEL012783 | TCCATCACGGCAATCACA | AGATCCAGCCAGCATTCG | 203 |
| Fumarase (4.2.1.2) | AAEL008167 | CAGAAAGCAACAGCAAGT | GTGTCCATTAGGGAGTGAT | 282 |
| Glucose-6-phosphate Isomerase (5.3.1.9) | AAEL012994 | CGTGCCGAGTTGGAAAGT | CGAATCGTGCGAGGTAGT | 239 |
| Glutamate dehydrogenase (1.4.1.2) | AAEL010464 | GTCGGCTCTGATGACCTTC | CGTCCGTAAATACCACCCT | 312 |
| Phosphoglucomutase (5.4.2.2) | AAEL010037 | CCCAATCTCACTTACGCA | CATCAGGTTACCGAAATAC | 593 |
| Trypsin (early) (3.4.21.4) | AAEL007818 | CCCAAAGCCAACAACCT | TTTYGTCCAACTCCAGCA | 510-523 |

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different ''allele-specific'' primers, each of which contained a 39 nucleotide corresponding to one of the two alleles and an opposite primer that amplified both alleles. Allele specific primers were manufactured (Operon Inc., Huntsville, AL) with 59 tails [36,37] designed to allow discrimination between SNP alleles based on size or melting temperature. Primer sequences are provided in Table 4. An intentional transversion mismatch was introduced three bases in from the 39 end of allele specific primers to improve specificity and each allele specific primer differed by a transition at this site [38]. Melting curve PCR was performed as previously described [39].

Figure 3. The amplified region of each of the 7 nuclear genes. PCR primer sites are underlined, all SNP sites are underlined, and the selected SNP is placed in a box.
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Table 3. Geographic origin, sex, and sample sizes of *Aedes aegypti s.l.* used to screen for SNPs.

| Collection Location | Females | Males |
|---|---|---|
| *Ae. aegypti formosus* Deux Rivieres | 4 | 9 |
| *Ae. aegypti formosus* Ngari | 0 | 7 |
| *Ae. aegypti formosus* Pk10 strain | 8 | 7 |
| *Ae. aegypti aegypti* Dakar | 15 | 7 |
| Total | 27 | 30 |

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## Statistical analysis of haplotype and allele frequencies

Variation in allele frequencies among and within years, subspecies, phytogeographic regions, vegetation zones and habitats was determined by analysis of molecular variance (AMOVA) using the computer program Arlequin 3.01 [40]. This program also estimated pairwise $F_{ST}$ values and Slatkin's linearized $F_{ST}$ [$F_{ST}/(12F_{ST})$] [41] among collections and computed the significance of the variance components associated with each level of genetic structure by a nonparametric permutation test with 100,000 pseudoreplicates [40]. Pairwise linearized $F_{ST}$ values were used to construct a dendrogram among all collections by means of unweighted pair-group method with arithmetic averaging analysis [42] in the NEIGHBOR procedure in PHYLIP3.5C [43]. Wright's F-Statistics were calculated using Weir and Cockerham's method [44].

Table 4. Oligonucleotides used for allele specific PCR.

| Gene Name | SNP locus | Oligonucleotide sequences (59 end) | Oligonucleotide sequences (39 end) |
|---|---|---|---|
| a-Amylase | Amy2.447Gf | 59-GCGGGCAGGGCGGCGGGGGCGGGGCC | ACCGAACGACTTCAATGCG-39 |
| | Amy2.447Tf | 59-GCGGGC | ACCGAACGACTTCAATACT-39 |
| | Amy2.447r | 59-CCAGCAGTTACGCACCTGATAG-39 | |
| | Amy2.450f | 59-AACTTCCCTGCAGTCCCC-39 | |
| | Amy2.450Tr | 59-[long tail] | TAGTCGTAGATTTCAGAA-39 |
| | Amy2.450Gr | 59-[short tail] | TAGTCGTAGATTTCAAAC-39 |
| a-Glycerophosphate | aGPDH.55f | 59-GCAGAGGATTCGTCGCAA-39 | |
| dehydrogenase | aGPDH.55Gr | 59-[long tail] | GTGACTGGACCTGTTCCTAC-39 |
| | aGPDH.55Ar | 59-[short tail] | GTGACTGGACCTGTTCCCAT-39 |
| Aminopeptidase N | Apn.1938Gf | 59-[long tail] | TCACTCTAAAACTCATTGAG-39 |
| | Apn.1938Af | 59-[short tail] | TCACTCTAAAACTCATTAAA-39 |
| | Apn.1938r | 59-GAGCGATGCCCAAGGAAC-39 | |
| Fumarate hydratase | Fum.-294Gf | 59-[long tail] | GGAAAGTGGATTCTTCTTGTTAGCG-39 |
| | Fum.-294Af | 59-[short tail] | GGAAAGTGGATTCTTCTTGTTAACA-39 |
| | Fum.-294r | | |
| Glucose-6-phosphate | Gpi.1,500Gf | 59-[long tail] | GCTGATTGCCATGTACGAACACCAG-39 |
| Isomerase | Gpi.1,500Af | 59-[short tail] | GCTGATTGCCATGTACGAACACTAA-39 |
| | Gpi.1,500r | 59-CGTCCCAGATGACACCCT-39 | |
| Glutamate | GlutDH.507Gf | 59-[long tail] | GATGACCTTCAAGTGTGCCTGCTTG-39 |
| Dehydrogenase | GlutDH.507Af | 59-[short tail] | GATGACCTTCAAGTGTGCCTGCCTA-39 |
| | GlutDH.507r | 59-ATGYTCCGAATACTGCTTGGG-39 | |
| | GlutDH.567Gf | 59-[long tail] | CCCCAAGCAGTATTCGCAG-39 |
| | GlutDH.567Af | 59-[short tail] | CCCCAAGCAGTATTCGTAA-39 |
| | GlutDH.567r | 59-CGGTCCRATGAAGCCCTTTT-39 | |
| | GlutDH.627Cf | 59-[long tail] | TGTCCAAAAAGGGCTTCCTC-39 |
| | GlutDH.627Tf | 59-[short tail] | TGTCCAAAAAGGGCTTCTTT-39 |
| | GlutDH.627r | 59-CCCATATCGGGAGCKGGCA-39 | |
| Phosphoglucomutase | Pgm.954Cf | 59-[long tail] | GTCATTGCTCACTACGTC-39 |
| | Pgm.954Af | 59-[short tail] | GTCATTGCTCACTACGTA-39 |
| | Pgm.954r | 59-CTGTTGGCATACTTCTGGC-39 | |
| Trypsin (early) | TrypEarlIf | 59-[long tail] | GGCTACCGCATAACCCTGAACCACA-39 |
| | TrypEarlDf | 59-[short tail] | CTACCGCATAACCATGAACC-39 |
| | TrypEarlr | 59-TGGCTGAGTCCCAGAAGG-39 | |

The sequences of the short and long tails are provided in bold for the first gene only. The 39 allele specific nucleotide is bold and the mismatch at the third nucleotide from the 39 end is underlined.

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## Results

### Subspecies distribution

Figure 4 shows the proportion and distribution of mosquitoes classified as *Aaa* or *Aaf* in the 19 collection sites. This figure suggests a northwest-southeast cline in the abundance of the two subspecies. Six collections from the Sahelian region in northwest Senegal where the primary vegetation type is *Acacia*-Savannah contained only *Aaa*. Six collections from the southern Forest gallery area in southern Senegal where the primary vegetation type is deciduous forest and scrub consisted of only *Aaf* (Ngari, PK-10 and Deux Rivieres are placed under a single pie chart in Figure 4). Only *Aaf* was found in Goudiry in the central *Acacia*-Savannah region. The two subspecies were sympatric in four sites north of The Gambia in the central Savannah region containing predominantly tall grass savanna and scrub and in Dienoudialla and Saraya in the southern Forest gallery area. Letters in the pie charts in Figure 4 indicate the results of pairwise 2⊗2 heterogeneity $x^2$ tests. Four statistically homogeneous groups were detected. Group 'a' are the pure *Aaa* collections while group 'd' are the pure *Aaf*, and the Dienoudialla and Saraya collections, groups 'b' and 'c' overlap and contain all of the collections in which the two subspecies are sympatric.

### Vector competence

We incorporated our standard *D2S3* strain [32] as a positive control and standard refractory *D2MEB* [32] strain as a negative control. The Disseminated Infection Rate (DIR) was 92.3% in *D2S3* and 51.2% in *D2MEB* (sample sizes = 65 and 80 females, respectively). Figure 5 shows the proportion and distribution of mosquitoes with a disseminated infection (DIR), a midgut infection barrier (MIB) and a midgut escape barrier (MEB). There is a northwest-southeast cline in the susceptibility of *Ae. aegypti s.l.* populations. Northwestern *Aaa* collections have a high disseminated infection rate (DIR) while southeast *Aaf* collections have a low DIR associated with a MEB. Letters in the pie charts in Figure 5 indicate the results of pairwise 2⊗2 heterogeneity $x^2$ tests. Five statistically homogeneous groups were detected. N'goye (group 'a') had a higher DIR than the other 10 collections. Group 'b' contains the pure *Aaa* collections from the Sahel. Group 'e' contains the pure *Aaf* collections from the Forest Gallery. Groups 'c' and 'd' overlap and contain all of the other collections. There was a positive correlation between the proportion of *Aaf* among *Ae. aegypti s.l.* and the proportion of mosquitoes with a midgut escape barrier for the 11 sites (Spearman's rank correlation; $r_s = 0.797$, $P = 0.003$).

### SNP discovery

Using the primers in Table 2, the regions of the Aminopeptidase N (Apn) (3.4.11.2) AAEL012783, a-amylase 2 (Amy2) (3.2.1.1) AAEL013421, a-Glycerophosphate dehydrogenase (aGPDH) (1.1.1.8) AAEL003873, Glucose-6-phosphate isomerase (GPI)

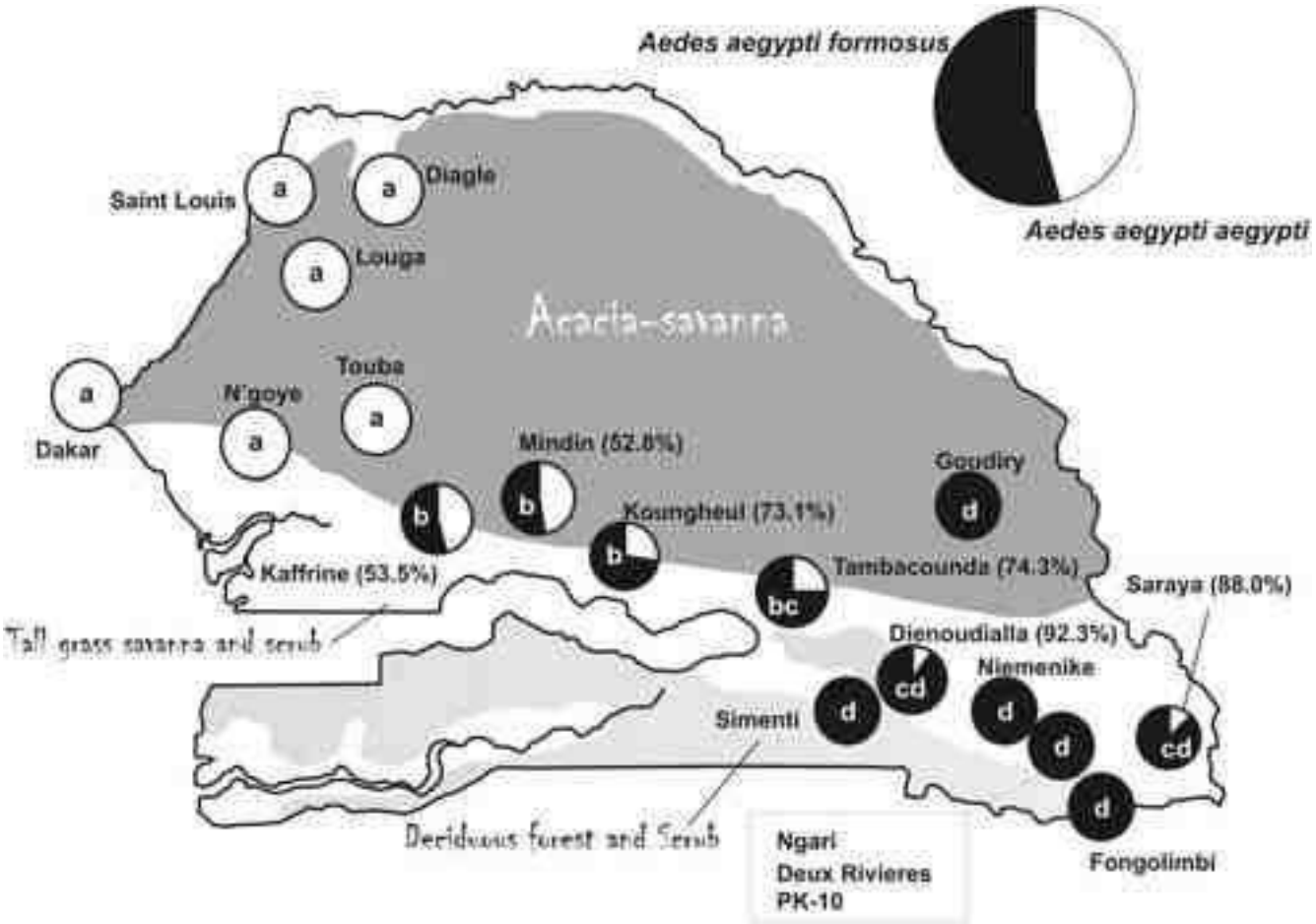


Figure 4. Distribution of *Aaa* or *Aaf* in Senegal. Pairwise Fisher's Exact Tests were performed on all collections. Strains with equivalent rates have the same labels and these were significantly different from one another.
doi:10.1371/journal.pntd.0000408.g004

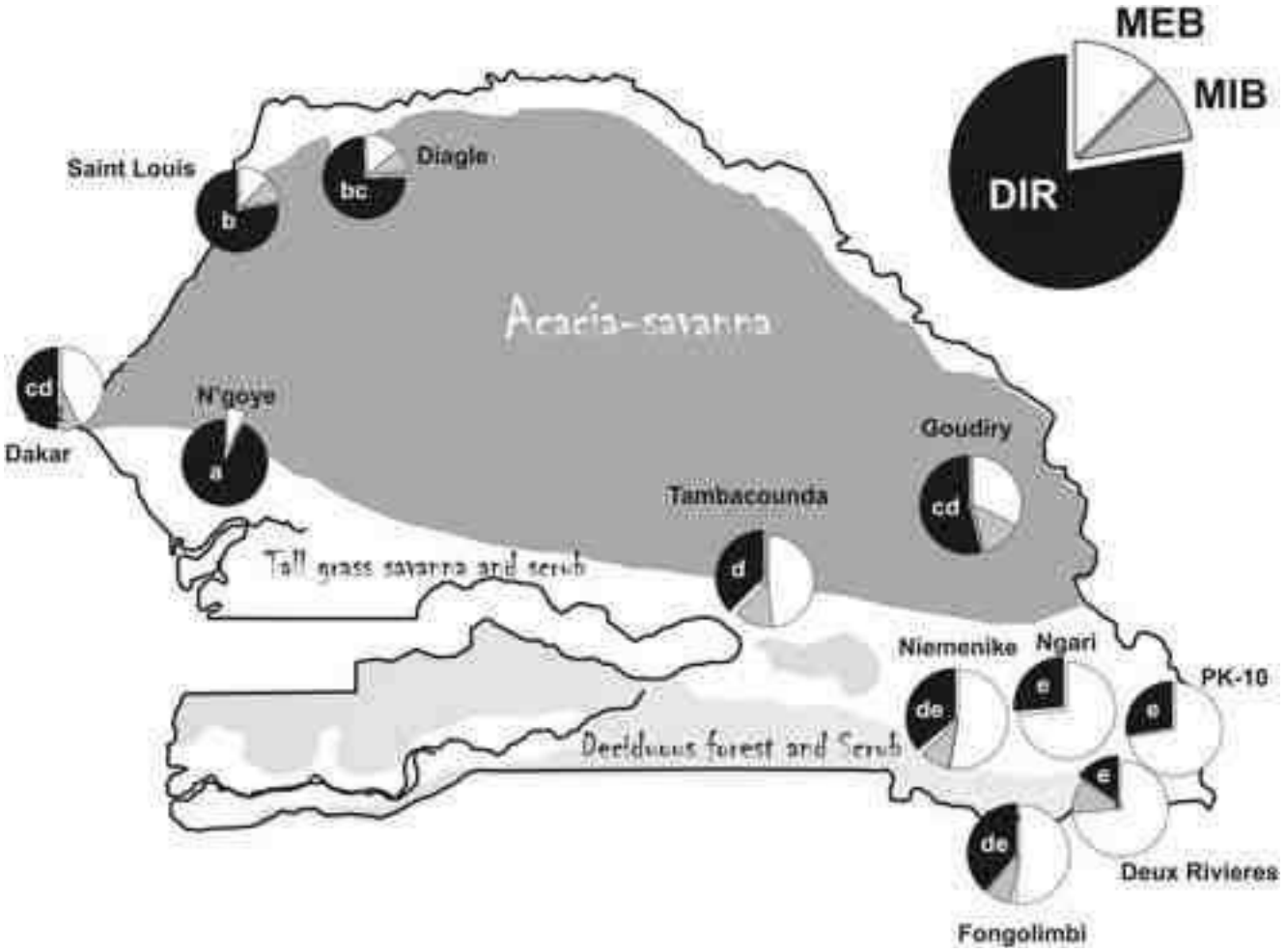


Figure 5. Vector competence of *Ae. aegypti s.l.* collections in Senegal. Disseminated infection rate (DIR) appears in black, midgut infection barrier rate (MIB) appears in grey, and midgut escape barrier rate (MEB) appears in white. Pairwise Fisher's Exact Tests were performed on all collections. Strains with equivalent rates have the same labels and these were significantly different from one another. Sample sizes = 50-65 females.
doi:10.1371/journal.pntd.0000408.g005

(5.3.1.9) AAEL012994, Glutamate dehydrogenase (GluDH) (1.4.1.2) AAEL010464, Fumarase (Fum) (4.2.1.2) AAEL008167, and Phosphoglucomutase (Pgm) (5.4.2.2) AAEL010037 genes shown in Figure 3 were amplified in the 57 mosquitoes listed in Table 3. These were then screened for sequence variation using SSCP. All of the primers and the associated analyses for the *Early Trypsin* gene are published [45].

Figure 3 shows the region that was amplified with the PCR primers underlined. All SNP sites are also underlined and the chosen SNP site is in a box. Our selection of SNPs was biased in many ways. We only used SNP loci that demonstrated two alternate nucleotides because more nucleotides would require additional, more expensive SNP detection. In addition only those SNPs were used in which the most common allele had a frequency #0.95 among the 57 initial mosquitoes. The remaining SNPs were then screened as candidates for allele specific PCR. Each SNP was analyzed using Primer Premier 5.0H (Premier Biosoft International, Palo Alto, CA) to identify primers that would amplify a product #70 bp because this was the maximum size for discrimination by melting curve PCR. Furthermore, primers were eliminated that had potential to form hairpins or might anneal to one another.

aGPDH.55 is a synonymous G«A transition in the third position of a Arg codon. Apn.1938 is a synonymous G«A transition in the third position of a Gln codon. Amy2.447 is a synonymous G«T transversion in the third position of a Pro codon, while Amy2.450 is a synonymous G«T transversion in the third position of the adjacent Pro codon (Figure 3). Fum.-294 resides 294 bp upstream from the ATG start in the Fumarate hydratase gene. GPI. 1,500 is a synonymous G«A transition in the third position of a Lys codon. GlutDH.507, 567, and 627 are all synonymous transitions in the third position of Val, Glu, and Iso codons, respectively. Pgm.954 is a synonymous A«C transversion the third position of a Leu codon. TrypEarl detects a 13 bp deletion immediately 59 to the ATG start in the Early Trypsin gene [45].

## SNP allele and genotype frequencies in collections

SNP allele frequencies were compared among and within years, subspecies, phytogeographic regions, vegetation zones and habitats by AMOVA [40]. We first tested whether alleles shifted in frequency among collection years (Table 5A) because this would have required partitioning by year any further analyses. Results indicate that 1% of the variation in allele frequencies arose among the three years and this was not significant in the permutation tests. All subsequent analyses, therefore, combined samples from different years.

Table 5. AMOVA of SNP allele frequencies among and within A) years, B) subspecies, C) regions, D) vegetational zones, E) phytogeographic regions, and F) habitats.

| Source of variation | d.f. | Sum of squares | Variance Component | F | % variation |
|---|---|---|---|---|---|
| A) Among collection years | | | | | |
| Among years | 2 | 16.6 | 0.0052 | 0.010 | 1.0 |
| Among collections in years | 16 | 91.3 | 0.0505 | 0.095 *** | 9.4 |
| Among mosquitoes in collections | 972 | 428.3 | 20.0408 | 20.085 | 27.6 |
| Within mosquitoes | 991 | 517.5 | 0.5222 | 0.028 | 97.2 |
| Total | 1981 | 1053.8 | 0.5371 | | |
| B) Among subspecies in sympatry | | | | | |
| Among six mixed collections | 5 | 20.4 | 0.0437 | 0.080 *** | 8.0 |
| Between subspecies in collections | 6 | 2.7 | 20.0013 | 20.003 | 20.2 |
| Among mosquitoes in collections | 244 | 119.7 | 20.0115 | 20.023 | 22.1 |
| Within mosquitoes | 256 | 131.5 | 0.5137 | 0.057 | 94.3 |
| Total | 511 | 274.4 | 0.5446 | | |
| Between subspecies | 1 | 4.2 | 20.0016 | 20.087 | 20.3 |
| Among collections in subspecies | 23 | 101.9 | 0.0531 | 0.100 *** | 10.1 |
| Among mosquitoes in collections | 939 | 407.4 | 20.0414 | 20.003 | 27.9 |
| Within mosquitoes | 964 | 498.0 | 0.5166 | 0.019 | 98.1 |
| Total | 1927 | 1011.5 | 0.5268 | | |
| C) Among Northern, Central and Eastern Regions | | | | | |
| Between regions | 2 | 20.7 | 0.0072 | 0.013 | 1.3 |
| Among collections in zones | 16 | 87.2 | 0.0484 | 0.091 *** | 9.0 |
| Among mosquitoes in collections | 972 | 428.3 | 20.0408 | 20.085 | 27.6 |
| Within mosquitoes | 991 | 517.5 | 0.5222 | 0.028 | 97.2 |
| Total | 1981 | 1053.8 | 0.5371 | | |
| D) Among three vegetational zones | | | | | |
| Among three vegetational zones | 2 | 15.9 | 0.0030 | 0.006 | 0.6 |
| Among collections in zones | 16 | 92.0 | 0.0510 | 0.096 *** | 9.6 |
| Among mosquitoes in collections | 972 | 428.3 | 20.0410 | 20.085 | 27.6 |
| Within mosquitoes | 991 | 517.5 | 0.5220 | 0.026 | 97.4 |
| Total | 1981 | 1053.8 | 0.5360 | | |
| E) Among five phytogeographic regions | | | | | |
| Among five phytogeographic regions | 4 | 42.8 | 0.0173 | 0.032 * | 3.2 |
| Among collections in regions | 14 | 65.1 | 0.0409 | 0.078 *** | 7.6 |
| Among mosquitoes in collections | 972 | 428.3 | 20.0408 | 20.085 | 27.6 |
| Within mosquitoes | 991 | 517.5 | 0.5222 | 0.032 | 96.8 |
| Total | 1981 | 1053.8 | 0.5397 | | |
| F) Among four habitats | | | | | |
| Among four habitats | 3 | 24.8 | 0.0050 | 0.009 | 0.9 |
| Among collections in habitats | 15 | 83.1 | 0.0499 | 0.094 *** | 9.3 |
| Among mosquitoes in collections | 972 | 428.3 | 20.0408 | 20.085 | 27.6 |
| Within mosquitoes | 991 | 517.5 | 0.5222 | 0.026 | 97.4 |
| Total | 1981 | 1053.8 | 0.5364 | | |

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Next, we tested for variation in allele frequencies between the subspecies. In the first AMOVA we analyzed only the six collections in which the two subspecies were sympatric to avoid confounding differences among sites with differences among subspecies. Table 5B indicates that no variation was found between the subspecies. We then compared all *Aaa* collections with all *Aaf* collections, and again no variation was found between the subspecies. All subsequent analyses combined the subspecies in the six sympatric collection sites.

We next analyzed for variation among northern, central and eastern collections and Table 5C indicates that 1.3% of the variation in allele frequencies arose among the three regions but

this was not significant in the permutation tests. All collections were next grouped into one of the three vegetation zones in Figure 2. Table 5D indicates that 0.6% of the variation in allele frequencies arose among these zones and that this was not significant. All collections were next grouped into the five phytogeographic regions (Table 1). Table 5E shows that 3.2% of the variation in allele frequencies arose among these regions and this was significant. Finally, all collections were grouped into the three habitat types (Table 1), and Table 5F indicates that 0.9% of the variation in allele frequencies arose among habitats and that this was not significant.

Table 6 lists Wright's F-statistics estimated using Weir and Cockerham's methods [44] for the entire study. $F_{ST}$ estimates at each locus were significantly (P#0.0001) greater than 0. The largest amount of variance was detected at the GlutDH.507 locus, the least occurred at the TrypEarl locus. Many $F_{IS}$ estimates at each locus were significantly (P#0.0001) greater or less than 0. Of 185 independent tests 56 were significant; far in excess of the nine expected with 5% Type 1 error rate. However, there was no general trend towards excess homozygotes ($F_{IS}$ - 0) or excess heterozygotes ($F_{IS}$ , 0). In half of the tests $F_{IS}$ - 0 and in the other half $F_{IS}$ , 0. The largest deviance in $F_{IS}$ was seen at GlutDH.627 ($F_{IS}$ = 20.276) with excess heterozygotes in six collections. The smallest deviance in $F_{IS}$ was seen at GlutDH.507 ($F_{IS}$ = 20.012) with a slight excess of heterozygotes in one collection.

Unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis [46] of pairwise $F_{ST}/(12F_{ST})$ among the Senegalese collections (Figure 6) indicates four clusters labeled A–D. The collection year was distributed independently among clades (Fisher's Exact Test (FET), p = 0.1397). Subspecies were distributed independently among clades (FET, p = 1.0000). The vegetative zone in which the collection was made was also independent among clades (FET, p = 0.0643). However, collections were clustered by phytogeographic region (FET, p = 0.0010) and habitats (FET, p = 0.0068) with a disproportionately large number of Urban and *Acacia* Savanna collections occurring in Clade A. Thus, aside from habitats, the cluster analysis largely confirms the AMOVA results.

A Mantel analysis of pairwise $F_{ST}/(12F_{ST})$ against geographic distances indicated a highly significant correlation between genetic and geographic distances among collections (Figure 7). While a significant correlation is usually interpreted as evidence of isolation by distance, the regression coefficients were small ($R^2$ = 0.03–0.05) and general inspection of the data points in the untransformed geographic distance graph suggests only a weak trend.

Table 6. Wright's F-statistics estimated by Weir and Cockerham's method [44] among the 19 Senegal collections.

| Locus | $F_{IS}$ ($F_{IS}$?0/no.tests: $F_{IS}$ - 0, $F_{IS}$ , 0) | $F_{ST}$ | $F_{IT}$ |
|---|---|---|---|
| aGPDH.55 | 20.023 (3/15: 2+, 12) | 0.100*** | 0.079 |
| Apn.1,938 | 0.098 (6/19: 5+, 12) | 0.110*** | 0.197 |
| Amy2.447 | 0.096 (6/18: 5+, 12) | 0.086*** | 0.174 |
| Amy2.450 | 20.166 (6/19: 1+, 52) | 0.116*** | 20.031 |
| Fum.-294 | 20.050 (7/17: 4+, 32) | 0.146*** | 0.104 |
| GPI.1,500 | 20.143 (3/15: 2+,12) | 0.090*** | 20.041 |
| GlutDH.507 | 20.012 (5/12: 4+, 12) | 0.209*** | 0.200 |
| GlutDH.567 | 20.183 (6/19: 1+, 52) | 0.090*** | 20.076 |
| GlutDH.627 | 20.276 (7/18: 1+, 62) | 0.081*** | 20.173 |
| Pgm.954 | 20.166 (6/18: 2+, 42) | 0.135*** | 20.009 |
| TrypEarl | 20.026 (1/15: 1+, 02) | 0.038*** | 0.013 |
| Mean | 20.083 (56/185: 28+, 282) | 0.110*** | 0.035 |
| JackKnife Mean | 20.084 | 0.109 | 0.035 |
| Std. Dev. | 0.047 | 0.01 | 0.045 |

***P#0.0001.

Under $F_{IS}$ are indicated the number of tests for goodness-of-fit to Hardy-Weinberg expectation in which $F_{IS}$?0 over the number of tests. This is followed by the number of tests in which $F_{IS}$ - 0 and the number in which $F_{IS}$ , 0.

doi:10.1371/journal.pntd.0000408.t006

## Discussion

We have demonstrated a northwest–southeast cline in the abundance of *Aaa* and *Aaf* in Senegal as determined by the number of pale scales on the first abdominal tergite of individual mosquitoes. The vector competence of mosquitoes in some of these collections was analyzed for susceptibility to DENV-2 susceptibility and was correlated with the distribution of the two subspecies. Population genetic analyses with SNPs revealed large and significant differences in allele frequencies among collections. However, none of this variation was attributable to the year of collection, subspecies, the vegetation zone, or the habitat in which the collections were made. Minor amounts of the variation in allele frequencies were attributable to the geographic distance among collection sites and to the phytogeographic region in which the collections were made.

Huber *et al.* [47] recently published an in-depth examination of gene flow among five cities in Senegal using variation at 10 isozyme markers. They collected five samples from Barkedji in the Sahel; Diourbel, Kaffrine and Koungheul from the Savannah region; and Kedougou from the Forest gallery for a total of 25 samples containing 1,086 mosquitoes. Their overall $F_{ST}$ value was 0.078. Most (74%) of $F_{ST}$ was accounted for by variation among the five collections within each city, while the remainder was accounted for by differences among the five cities. Our overall $F_{ST}$ value was slightly larger (0.109) but we did not compare multiple collections within cities; some of our sites had small sample sizes (which inflate $F_{ST}$ estimates [48]) and our study included 19 sites over a much larger geographic range. Huber *et al.* [47] also performed an AMOVA among collections in the same vegetation zones as in Figure 2 and, as with our study, more variation arose within (5.5%) rather than among (2.6%) zones. Huber *et al.* also performed an AMOVA on subspecies. As with our study, more of the variation arose among collections within a subspecies (5.7%) rather than among subspecies (3.6%). However, even though this was a small percentage, it was significant in their permutation tests. We only examined gene flow in the six collections where the subspecies are sympatric and found a non-significant 1.4% of frequency variation arose between subspecies. In contrast Huber *et al.* compared Kedougou (*Aaf*) with all other cities (*Aaa*). Thus their subspecies variance included, and was therefore inflated by, variation among cities. Huber *et al.* performed a cluster analysis of linear $F_{ST}$ values and, as in Figure 6, found no clusters corresponding to cities, subspecies or vegetation zones. They also tested for isolation by distance using the same analyses as presented here and found none. While our regression was significant, the linear regression model explained little of the overall variance.

There is a major discrepancy between our $F_{IS}$ results and those of Huber *et al.* The number of significant tests in their study was the number expected with a 5% Type 1 error rate but the number of significant tests in our study was far in excess of this expected rate. This initially suggested to us that our melting curve PCR

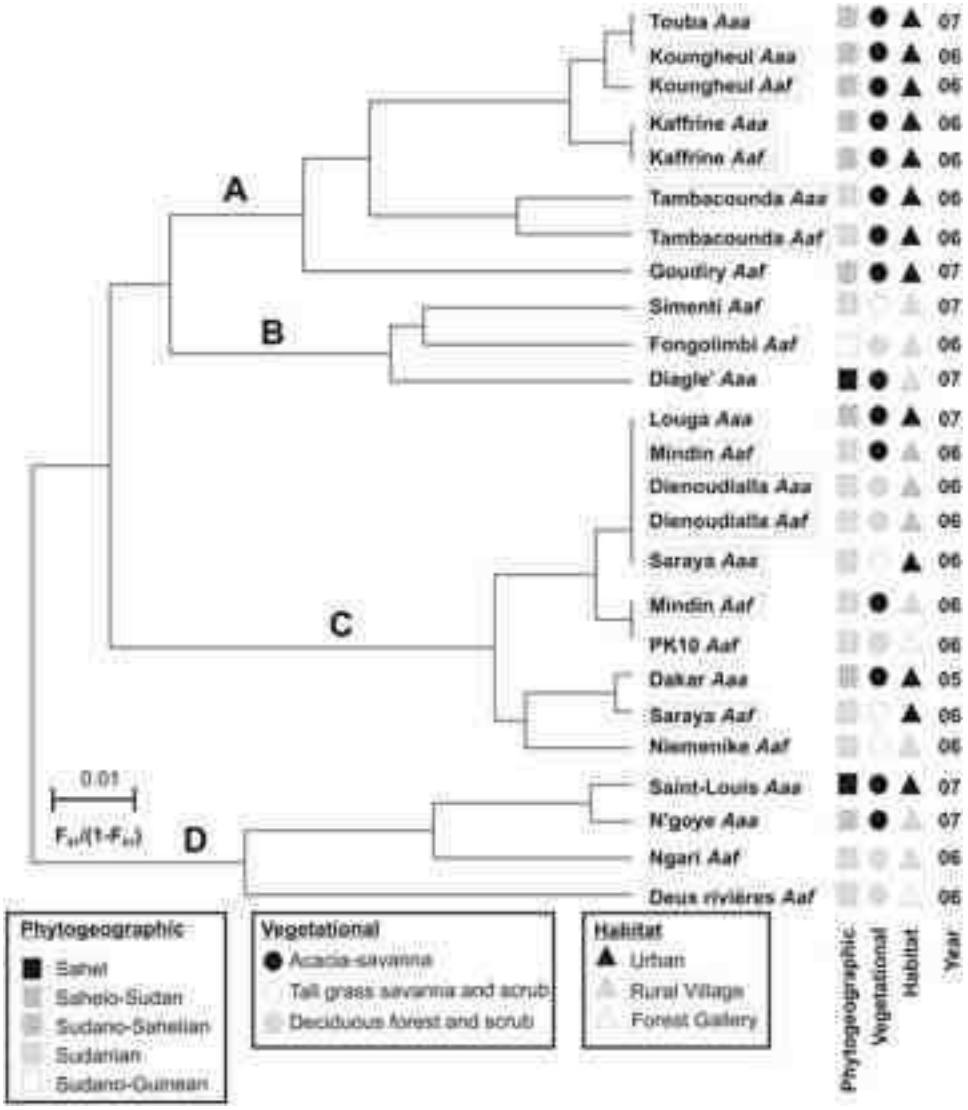


Figure 6. UPGMA cluster analysis of pairwise $F_{ST}/(12F_{ST})$ markers among the 25 collections.
doi:10.1371/journal.pntd.0000408.g006

assay was inaccurate. The assay might not be equally sensitive to both nucleotides at a locus and thus indicate an apparent homozygote for one allele in mosquitoes that are in reality heterozygotes, thus yielding $F_{IS} < 0$. The assay might also not be specific and thus indicate an apparent heterozygote in mosquitoes that are in reality homozygotes, thus yielding $F_{IS} > 0$. The problem with this interpretation is that $F_{IS} = 0$ for the majority of tests at each locus and $F_{IS}$ was not consistently greater or less than zero in any one collection or at any one locus. Nevertheless, we amplified and sequenced PCR products from 2–3 individuals in a collection and at a locus where $F_{IS} \neq 0$ and in every case confirmed the genotype reported by melting curve PCR assay. In addition, we reviewed our initial sequence results from some of the 57 mosquitoes listed in Table 3. These also did not conform to Hardy-Weinberg expectations. Sometimes there was an excess of homozygotes at a locus but for other loci there was an excess of heterozygotes. At this time, we have no explanation for this discrepancy.

Both studies agree that very little or no variation exists between the subspecies. This is in stark contrast to similar studies [25] done in East Africa where allozyme frequencies differed markedly between the subspecies. Our results were presaged by McClelland [10] who found intermediate forms in areas of sympatry. These forms exhibited a wide range of pale scaling and occurred in peridomestic habitats. More recently, mosquitoes morphologically consistent with *Aaf* were found breeding domestically indoors in Nigeria [12] and Gabon [13]. Huber *et al.* [47] readily identified both forms in Senegal. Therefore, the classic behavioral/habitat descriptions given by Mattingly [8] for these two subspecies are not valid throughout Africa.

This tautology between *Aaa* and *Aaf* in West Africa therefore suggests a revision to Figure 1 in which West African *Aaa* and *Aaf* are monophyletic within the upper clade (Figure 8). This revision suggests three fundamental conclusions. First, because *Aaf* is only found in Sub-Saharan Africa, and West African *Aaa* and *Aaf* are monophyletic, our results strongly support Mattingly's original suggestion [9] that *Aaa* arose from a sylvan *Aaf* population probably in West African forests. Second, Asian and Southeastern US *Aaa* populations originated from West Africa *Aaa* rather than *Aaf* as was previously suggested [27]. Third, West African *Aaa*

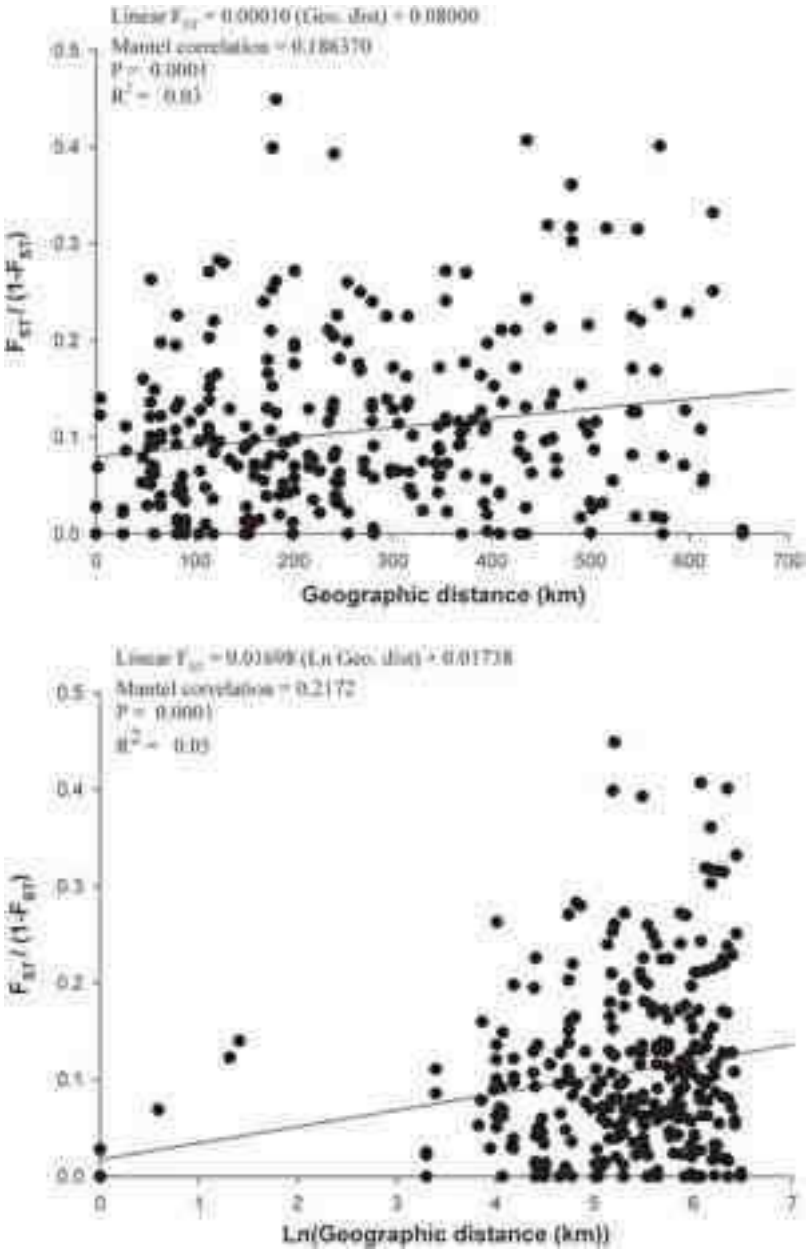


Figure 7. Regression analysis of pairwise $F_{ST}/(12F_{ST})$ for the SNP markers against geographic distances (km) (upper panel), pairwise $F_{ST}/(12F_{ST})$ for SNP markers against ln(geographic distances (km)) (lower panel).
doi:10.1371/journal.pntd.0000408.g007

subsequently spread into East Africa where they adapted to human habitats, and subsequently gave rise to the Texas/ Northeastern Mexico, Caribbean, and South American *Aaa*.

In agreement with the early literature [17–19], we also found that *Aaf* had significantly lower vector competence than *Aaa*. Among pure *Aaa* collections, the disseminated infection rate (DIR) was 73.9% with a midgut infection barrier (MIB) rate of 6.8%, and a midgut escape barrier (MEB) rate of 19.3% while among pure *Aaf* collections, DIR = 34.2%, MIB rate = 7.4%, and MEB rate = 58.4%. These patterns are consistent with those reported earlier for the two subspecies with YFV and DENV1-4 [17–19,49], but are inconsistent for specific locations. DENV-2 virus has been isolated from both western Senegal (*Bandia Village in Figure 2) [50] and extensively from the Ke´dougou area in eastern Senegal (near Ngari in Figure 2) [51,52]. However, a comprehensive serosurvey for DENV exposure has not been made and so we cannot test for a correlation between *Aaa* abundance and risk for DENV exposure.

When Tabachnick *et al.* [17] examined the susceptibility of ''West African Sylvan'' populations from Dakar and N'goye to YFV infection they found the DIR to be 11 and 7% respectively. This is odd in two respects. First we found no *Aaf* in our Dakar and N'goye collections, and secondly, the DIRs with DENV-2 were 50 and 90% respectively. It is possible that vector competence for the long passaged Asibi strain of YFV used by Tabachnick *et al.* [17] is low (their most competent population only had a 53% DIR). But it is also possible that the subspecies composition of these sites has changed.

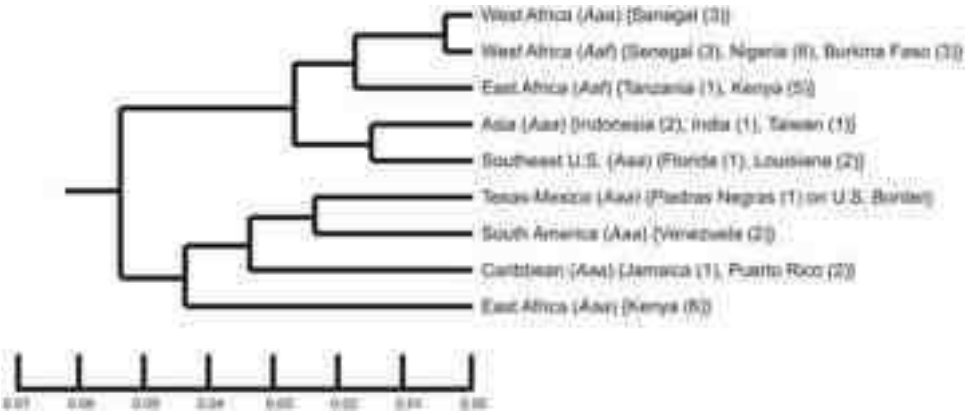


Figure 8. Addition of Senegal collections to Figure 1.
doi:10.1371/journal.pntd.0000408.g008

A group at Institut Pasteur de Dakar published a paper in 2008 [53] also measuring vector competence of *Ae. aegypti s.l.* populations from six locations in different bioclimatic zones and habitats of Senegal. They examined competence using a sylvatic (ArD 140875) and an epidemic (ArA 6894) DENV-2 isolate. They found that Senegalese *Ae. aegypti s.l.* populations had a high MIB rate (74–100%) and a highly variable DIR (10–100%). Both their study and ours examined vector competence in Dakar and N'goye and their findings are completely incongruent with ours. We believe three factors explain the discrepancies. First, they did not use standard susceptible and refractory strains as controls. Thus they have no baseline for comparison. Secondly, their MIB rates were very high resulting in DIR estimates based on #2–10 midgut-infected females. Third, their TCID50/ml titers were $10^{6.5–7.0}$ plaque forming units (pfu) while we used titers of $10^{7.5–8.5}$ pfu and Tabachnick *et al.* [17] used YFV TCID50/ml titers of $10^{7.8–8.8}$ pfu. Their low DIR was therefore probably due to low blood meal titers of both DENV-2 isolates.

Taken as a whole, our descriptions of subspecies distributions, vector competence and allele frequencies provide a very incomplete picture. In fact, they present a paradox. Why are the distributions of subspecies and vector competence rates distributed along a northwestern-southeastern cline while no such pattern is seen with either isozymes or SNPs? Why are SNP or isozyme phylogenies not distributed along the same cline? Our current knowledge of the distribution and vector competence of the two subspecies in West Africa in general and in Senegal in particular is still very incomplete. An additional deficiency in the current study is that no data were collected as to feeding, resting, or oviposition behaviors exhibited by mosquitoes at each sites. In addition, Figures 4 and 5 suggest a northwest-southeast cline in subspecies composition and vector competence but, in fact, the sampling locations were mostly distributed from northwest to southeast. Note that there are no collections from the northern or western marshes, the southern broadleaf evergreen forest, the western tall grass savanna and scrub, nor from the western deciduous forest and scrub south of The Gambia. A broader study of subspecies, vector competence and allele frequencies throughout West Africa may provide clues towards resolving this paradox.

## Author Contributions

Conceived and designed the experiments: MS CB MN WCB. Performed the experiments: MS CB LUM. Analyzed the data: CB LUM WCB. Contributed reagents/materials/analysis tools: WCB. Wrote the paper: CB LUM WCB.

## References


1. Monath TP (1994) Dengue - the Risk to Developed and Developing-Countries. Proc Natl Acad Sci U S A 91: 2395–2400.
2. Ooi EE, Goh KT, Gubler DJ (2006) Denque prevention and 35 years of vector control in Singapore. Emerging Infectious Diseases 12: 887–893.
3. Halstead SB, Suaya JA, Shepard DS (2007) The burden of dengue infection. Lancet 369: 1410–1411.
4. Tomori O (2004) Yellow fever: The recurring plague. Crit Rev Clin Lab Sci 41: 391–427.
5. Robertson SE, Hull BP, Tomori O, Bele O, LeDuc JW, et al. (1996) Yellow fever - A decade of reemergence. Jama-Journal of the American Medical Association 276: 1157–1162.
6. Vainio J, Cutts F (1998) Yellow fever. WHO/EPI/GEN/98.11. Geneva: World Health Organization.
7. McClelland GAH (1960) A preliminary study of the genetics of abdominal color variations in *Aedes aegypti* (L.) (Diptera: Culicidae). Ann Trop Med Parasitol 54: 305–320.
8. Mattingly PF (1957) Genetical aspects of the *Aedes aegypti* problem, I. Taxonomy and bionomics. Ann Trop Med Parasitol 51: 392–408.
9. Mattingly PF (1967) Taxonomy of *Aedes aegypti* and Related Species. Bull World Health Organ 36: 552–554.
10. McClelland GA (1974) Worldwide Survey of Variation in Scale Pattern of Abdominal Tergum of *Aedes-Aegypti* (L) (Diptera-Culicidae). Transactions of the Royal Entomological Society of London 126: 239–259.
11. Trpis M, Hausermann W (1975) Demonstration of Differential Domesticity of *Aedes-Aegypti* (L) (Diptera, Culicidae) in Africa by Mark Release Recapture. Bull Entomol Res 65: 199–208.
12. Nasidi A, Monath TP, Decock K, Tomori O, Cordellier R, et al. (1989) Urban Yellow-Fever Epidemic in Western Nigeria, 1987. Trans R Soc Trop Med Hyg 83: 401–406.
13. Vazeille-Falcoz M, Failloux AB, Mousson L, Elissa N, Rodhain F (1999) Oral receptivity of *Aedes aegypti formosus* from Franceville (Gabon, Central Africa) for dengue type 2 virus. Bull Soc Pathol Exot 92: 341–342.
14. Trpis M, Hausermann W (1978) Genetics of House-Entering Behavior in East-African Populations of *Aedes-Aegypti* (L) (Diptera-Culicidae) and Its Relevance to Speciation. Bull Entomol Res 68: 521–532.
15. Moore DF (1979) Hybridization and Mating-Behavior in *Aedes-Aegypti* (Diptera, Culicidae). J Med Entomol 16: 223–226.
16. Wallis GP, Tabachnick WJ (1990) Genetic-Analysis of Rock Hole and Domestic *Aedes-Aegypti* on the Caribbean Island of Anguilla. J Am Mosq Control Assoc 6: 625–630.
17. Tabachnick WJ, Wallis GP, Aitken THG, Miller BR, Amato GD, et al. (1985) Oral Infection of *Aedes-aegypti* with Yellow-Fever Virus - Geographic-Variation and Genetic Considerations. Am J Trop Med Hyg 34: 1219–1224.
18. Wallis GP, Aitken THG, Beaty BJ, Lorenz L, Amato GD, et al. (1985) Selection for Susceptibility and Refractoriness of *Aedes-Aegypti* to Oral Infection with Yellow-Fever Virus. Am J Trop Med Hyg 34: 1225–1231.
19. Lorenz L, Beaty BJ, Aitken THG, Wallis GP, Tabachnick WJ (1984) The Effect of Colonization Upon *Aedes-Aegypti* - Susceptibility to Oral Infection with Yellow-Fever Virus. Am J Trop Med Hyg 33: 690–694.
20. Wallis GP, Tabachnick WJ, Powell JR (1984) Genetic-Heterogeneity among Caribbean Populations of *Aedes-Aegypti*. Am J Trop Med Hyg 33: 492–498.

21. Wallis GP, Tabachnick WJ, Powell JR (1983) Macrogeographic Genetic-Variation in a Human Commensal - *Aedes-Aegypti*, the Yellow-Fever Mosquito. Genet Res 41: 241–258.
22. Tabachnick WJ (1982) Geographic and Temporal Patterns of Genetic-Variation of *Aedes-Aegypti* in New-Orleans. Am J Trop Med Hyg 31: 849–853.
23. Powell JR, Tabachnick WJ, Arnold J (1980) Genetics and the Origin of a Vector Population - *Aedes-Aegypti*, a Case-Study. Science 208: 1385–1387.
24. Tabachnick WJ, Powell JR (1979) World-Wide Survey of Genetic-Variation in the Yellow-Fever Mosquito, *Aedes-Aegypti*. Genet Res 34: 215–229.
25. Tabachnick WJ, Munstermann LE, Powell JR (1979) Genetic Distinctness of Sympatric Forms of *Aedes-Aegypti* in East-Africa. Evolution 33: 287–295.
26. Tabachnick WJ, Powell JR (1978) Genetic Structure of East-African Domestic Populations of *Aedes-Aegypti*. Nature 272: 535–537.
27. Tabachnick WJ (1991) Evolutionary genetics and arthropod born disease. The yellow fever mosquito. American Entomologist 37: 14–24.
28. Huang YM, Ward RA (1981) A pictoral key for the identification of the mosquitoes associated with yellow fever in Africa. Mosquito Systematics 13: 138–149.
29. Black WC, DuTeau NM (1997) RAPD-PCR and SSCP analysis for insect population genetic studies. In J Crampton, CB Beard, C Louis. The Molecular Biology of Insect Disease Vectors: A Methods Manual.
30. Deubel V, Kinney RM, Trent DW (1986) Nucleotide-Sequence and Deduced Amino-Acid-Sequence of the Structural Proteins of Dengue Type-2 Virus, Jamaica Genotype. Virology 155: 365–377.
31. Diaz FJ, Black WC, Farfan-Ale JA, Lorono-Pino MA, Olson KE, et al. (2006) Dengue virus circulation and evolution in Mexico: A phylogenetic perspective. Arch Med Res 37: 760–773.
32. Bennett KE, Beaty BJ, Black WC (2005) Selection of D2S3, an *Aedes aegypti* (Diptera:Culicidae) strain with high oral susceptibility to Dengue 2 virus and D2MEB, a strain with a midgut barrier to Dengue 2 escape. J Med Entomol 42: 110–119.
33. Bennett KE, Olson KE, Munoz MD, Fernandez-Salas I, Farfan-Ale JA, et al. (2002) Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. Am J Trop Med Hyg 67: 85–92.
34. Gould EA, Buckley A, Cammack N (1985) Use of the biotin-streptavidin interaction to improve flavivirus detection by immunofluorescence and ELISA tests. J Virol Methods 11: 41–48.
35. Gould EA, Buckley A, Cammack N, Barrett AD, Clegg JC, et al. (1985) Examination of the immunological relationships between flaviviruses using yellow fever virus monoclonal antibodies. J Gen Virol 66: 1369–1382.
36. Germer S, Higuchi R (1999) Single-tube genotyping without oligonucleotide probes. Genome Research 9: 72–78.
37. Wang J, Chuang K, Ahluwalia M, Patel S, Umblas N, et al. (2005) High-throughput SNP genotyping by single-tube PCR with Tm-shift primers. Biotechniques 39: 885–893.
38. Okimoto R, Dodgson JB (1996) Improved PCR amplification of multiple specific alleles (PAMSA) using internally mismatched primers. Biotechniques 21: 20–22, 24, 26.
39. Urdaneta-Marquez L, Bosio C, Herrera F, Rubio-Palis Y, Salasek M, et al. (2008) Genetic relationships among *Aedes aegypti* collections in Venezuela as determined by mitochondrial DNA variation and nuclear single nucleotide polymorphisms. Am J Trop Med Hyg 78: 479–491.
40. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. Genetics 131: 479–491.
41. Slatkin M (1993) Isolation by Distance in Equilibrium and Nonequilibrium Populations. Evolution 47: 264–279.
42. Sneath PH, Sokal RR (1962) Numerical Taxonomy. Nature 193: 855–860.
43. Lim A, Zhang LX (1999) WebPHYLIP: a web interface to PHYLIP. Bioinformatics 15: 1068–1069.
44. Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38: 1358–1370.
45. Gorrochotegui-Escalante N, Lozano-Fuentes S, Bennett KE, Molina-Cruz A, Beaty BJ, et al. (2005) Association mapping of segregating sites in the early trypsin gene and susceptibility to dengue-2 virus in the mosquito *Aedes aegypti*. Insect Biochem Mol Bio 35: 771–788.
46. Sneath PHA, Sokal RR (1973) Numerical Taxonomy. San Francisco: Freeman.
47. Huber K, Ba Y, Dia I, Mathiot C, Sall AA, et al. (2008) *Aedes aegypti* in Senegal: Genetic diversity and genetic structure of domestic and sylvatic populations. Am J Trop Med Hyg 79: 218–229.
48. Apostol BL, Black WC, Reiter P, Miller BR (1996) Population genetics with RAPD-PCR markers: The breeding structure of Aedes aegypti in Puerto Rico. Heredity 76: 325–334.
49. Miller BR, Mitchell CJ (1991) Genetic Selection of a Flavivirus-Refractory Strain of the Yellow-Fever Mosquito *Aedes-aegypti*. Am J Trop Med Hyg 45: 399–407.
50. Robin Y, Cornet M, Heme G, Legonidec G (1980) Dengue Virus Isolation in Senegal. Annales De Virologie 131: 149–154.
51. Cornet M, Saluzzo JF, Hervy JP, Digoutte JP, Germain M, et al. (1984) Dengue 2 au Se´ne´gal oriental: une pousse´e e´pizootique en milieu selvatique; isolements du virus à partir d'un singe et considérations épidémiologiques. Cahier ORSTOM Série Entomologie Médicale et Parasitologie 22: 313–323.
52. Diallo M, Ba Y, Sall AA, Diop OM, Ndione JA, et al. (2003) Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999–2000: Entomologic findings and epidemiologic considerations. Emerg Infect Dis 9: 362–367.
53. Diallo M, Ba Y, Faye O, Soumare ML, Dia I, et al. (2008) Vector competence of *Aedes aegypti* populations from Senegal for sylvatic and epidemic dengue 2 virus isolated in West Africa. Trans R Soc Trop Med Hyg 102: 493–498.

# *Aedes* species in treeholes and fruit husks between dry and wet seasons in southeastern Senegal

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ABSTRACT: During the dry season in February, 2010 and the wet season in September, 2011 we sampled mosquito larvae and eggs from treeholes of seven native hardwood species and the husks of *Saba senegalensis* in 18 sites in the PK-10 forest in southeastern Senegal. Larvae were reared to adults for species identification. In the dry season, we recovered 408 *Aedes* mosquitoes belonging to seven species. *Aedes aegypti* s.l. comprised 42.4% of the collection, followed by *Ae. unilineatus* (39%). In contrast to reports from East Africa, both *Ae. aegypti aegypti* and *Ae. aegypti formosus* were recovered, suggesting that both subspecies survive the dry season in natural larval habitats in West Africa. In the wet season, 455 mosquitoes were collected but 310 (68.1%) were the facultatively predaceous mosquito *Eretmapodites chrysogaster.* The remaining 145 mosquitoes consisted of ten *Aedes* species. *Aedes aegypti* s.l. comprised 55.1% of these, followed by *Ae. apicoargenteus* (15.2%) and *Ae. cozi* (11.7%). Similar to East Africa, most (90%) of *Ae. aegypti* s.l. in the wet season were subspecies *formosus.* ***Journal of Vector Ecology* 38 (2): 237-244. 2013.**

*Keyword Index: Aedes*, *Stegomyia, Eretmapodites,* natural larval habitats, treeholes.

## INTRODUCTION

The mosquito *Aedes aegypti* (Linneus) is the major vector of yellow fever (YF) and dengue fever (DENV1-4) flaviviruses (Gubler 2012), and chikungunya alphavirus (CHIK) (Dupont-Rouzeyrol et al. 2012) throughout most tropical and subtropical regions of the world. The species is taxonomically subdivided on the basis of scaling patterns on the abdominal tergites into three subspecies, two of which occur in Africa. The initial need for designation of *Ae. aegypti* s.l. subspecies in Africa arose from observations made in East Africa in the late 1950s that the frequency of pale "forms" of *Ae. aegypti* was higher in populations in and around human dwellings than in those of the nearby forest (McClelland 1960). The implied correlation between color and behavior prompted a revision of the biology and taxonomy of *Ae. aegypti* (Mattingly 1957). He described *formosus* (Walker) as a subspecies of *Ae. aegypti* that was restricted to sub-Saharan Africa and in West Africa "is the only form known to occur except in coastal districts and in one or two areas of limited island penetration" (Mattingly 1957, p. 395). He also suggested that it most frequently breeds in natural containers such as tree holes and feeds on wild animals. Mattingly also stated that in addition to the dark-scaled parts of the body being generally blacker, "ssp. *formosus* never has any scales on the first abdominal tergite (p. 395)." The type form of *Ae. aegypti aegypti* was alternatively defined as "either distinctly paler and browner (at least in the female) than ssp. *formosus* or with pale scaling on the first abdominal tergite or both (p. 395)." These two forms are hereafter referred to as *Aaa* and *Aaf*.

Mattingly (1957) suggested that *Aaa* breeds in artificial containers provided by humans, will breed indoors, and has a preference for feeding on human blood. A comprehensive study was subsequently made of differences in scale patterns on the abdominal dorsum in 74 *Ae. aegypti* s.l. collections from 69 different worldwide locations (McClelland 1974). A 15-point scale running from F (*formosus*-like = no scales on the first abdominal tergite) to Q (*queenslandensis*-like = entire abdominal dorsum covered with scales) was used to categorize *Ae. aegypti* s.l. from these many collections. McClelland (1974) concluded that many of Mattingly's distinctions between subspecies were not always clear-cut. For example, collections from North America (east coast of Mexico, United States, and various Caribbean Islands) contained *Ae. aegypti* lacking any scales on the first abdominal tergite (McClelland 1974). Both *Aaa* and *Aaf* were collected throughout Africa but forms with minimal scaling tended to occur in coastal regions.

In a recent study carried out in Senegal, Sylla et al. (2009) demonstrated that there was a northwest–southeast cline in the abundance of *Aaf,* with *Aaa* occurring exclusively in the northwest and *Aaf* exclusively in the southeast. The southern margins of the arid Acacia-Savannah habitat run from east to west through central Senegal. Along this margin, mixed *Aaa* and *Aaf* collections were observed. Paupy et al. (2010) examined one such mixed site in and around Niakhar, a town 115 km southeast of Dakar, Senegal. They examined the abundance of subspecies in adults raised from larvae collected in domestic larval habitats during the dry season and from domestic, peridomestic, and natural containers during the wet season. They noted a significant seasonal shift towards female mosquitoes with higher McClelland scores (greater scaling) during the wet season. Paupy et al. (2010) also performed

an analysis of genetic differentiation at eight microsatellite loci. Allele frequencies were then compared between sexes, among the two subspecies, and among collections in the dry vs rainy seasons. In agreement with earlier studies (Huber et al. 2008, Sylla et al. 2009), they found no differences between sexes nor among the two subspecies. However, a significant difference was detected between mosquitoes collected in the dry vs rainy seasons.

There are two deficiencies in the existing literature on seasonal shifts in subspecies abundance in West Africa. Sylla et al. (2009) only made collections during the wet season while Paupy et al. (2010) did not examine subspecies abundance in natural containers during the dry season. To partially address these deficiencies, we have tested for seasonal shifts in subspecies abundance in treeholes and fruit husks in a natural forested area. During the dry season in February, 2010 and in the wet season in September, 2011, we sampled mosquito larvae and eggs from treeholes of seven native hardwood species and the husks of *Saba senegalensis* in 18 collection sites in the PK-10 forest near the town of Kedougou in southeastern Senegal, 610 km southeast of Dakar. We also found many other *Aedes* species in abundance in treeholes and fruit husks in PK-10 and these are also reported here.

## MATERIALS AND METHODS

From February 15-19, in 2010, treeholes were excavated and flooded at 14 locations in the PK-10 forest gallery. The locations of all sites were recorded with a GPSMAP® 62s (Garmin Inc. Wichita KS) and coordinates are listed in Table 1 and mapped in Figure 1. All native trees were identified to species using Berhaut (1967).

All treeholes were completely dry during the February "dry season" collection. In treehole cavities that were large enough to reach into, we scooped all loose detritus into a plastic bag. A cold chisel was then used to scrape the inside of the cavity and this loosened material was collected into the same plastic bag. The tree hole was immediately filled to the brim with water and then, using a siphon, the majority of this water was recovered from the treehole into a collecting container. This was immediately followed by a second wash and siphoning. Treeholes that were too small to reach into were washed and siphoned twice. We intentionally made no collections during August-September, 2011 to allow PK10 treeholes and *S. senegalensis* husks to go through one wet and dry season following our February, 2010 collections.

The same procedures were followed and the same treeholes were sampled from September 7-30, 2011. All treeholes contained free standing water or moist detritus and mud during the September "wet season" collection. All free standing water was first removed into a plastic pail. The hole was subsequently immediately filled to the brim with water brought from the laboratory and stirred. All liberated material was scooped into the pail. Any remaining water was removed to the pail using a siphon. This was immediately followed by a second wash, stirring, and siphoning. Approximately 50 *Saba senegalensis* husks that had been split open (presumably by foraging monkeys) were collected into a plastic pail at sites 3, 11, 12, and 18 (Figure 1).

All 14 plastic bags and pails containing treehole water and

| Site | Tree species | Latitude | Longitude |
|---|---|---|---|
| PK10-01 | *Adansonia digitata* | 12°36'45.11"N | 12°14'51.24"W |
| PK10-02 | *Diospyros mespiliformis* | 12°36'45.11"N | 12°14'51.24"W |
| PK10-03 | *Saba senegalensis* husks | 12°36'45.11"N | 12°14'51.24"W |
| PK10-04 | *Anogeissus leiocarpus* | 12°36'41.66"N | 12°14'49.35"W |
| PK10-05 | *Cola nitida* | 12°36'41.24"N | 12°14'49.46"W |
| PK10-06 | *Combretum glutinosum* | 12°36'41.10"N | 12°14'50.15"W |
| PK10-07 | *A. leiocarpus* | 12°36'41.10"N | 12°14'50.15"W |
| PK10-08 | *A. leiocarpus* | 12°36'41.10"N | 12°14'50.15"W |
| PK10-09 | *A. leiocarpus* | 12°36'40.59"N | 12°14'49.84"W |
| PK10-10 | *A. leiocarpus* | 12°36'40.80"N | 12°14'49.54"W |
| PK10-11 | *S. senegalensis* husks | 12°36'40.80"N | 12°14'49.54"W |
| PK10-12 | *S. senegalensis* husks | 12°36'39.99"N | 12°14'49.57"W |
| PK10-13 | *D. mespiliformis* | 12°36'38.63"N | 12°14'50.44"W |
| PK10-14 | *A. leiocarpus* | 12°36'38.63"N | 12°14'50.44"W |
| PK10-15 | *Parkia biglobosa* | 12°36'36.09"N | 12°14'47.49"W |
| PK10-16 | *C. nitida* | 12°36'31.68"N | 12°14'45.17"W |
| PK10-17 | *A. leiocarpus* | 12°36'37.88"N | 12°14'45.16"W |
| PK10-18 | *S. senegalensis* husks | 12°36'41.65"N | 12°14'46.16"W |
| Maginot Tower | | 12°36'39.73"N | 12°14'50.39"W |
| Station Tower | | 12°36'43.41"N | 12°14'47.27"W |

Table 1. Location of the 18 collection sites in PK10 and the tree species from which mosquito larvae were collected. Approximately 50 husks were collected per site at PK10-03, 11, 12, and 18. The locations of collecting towers are indicated for reference with earlier literature.

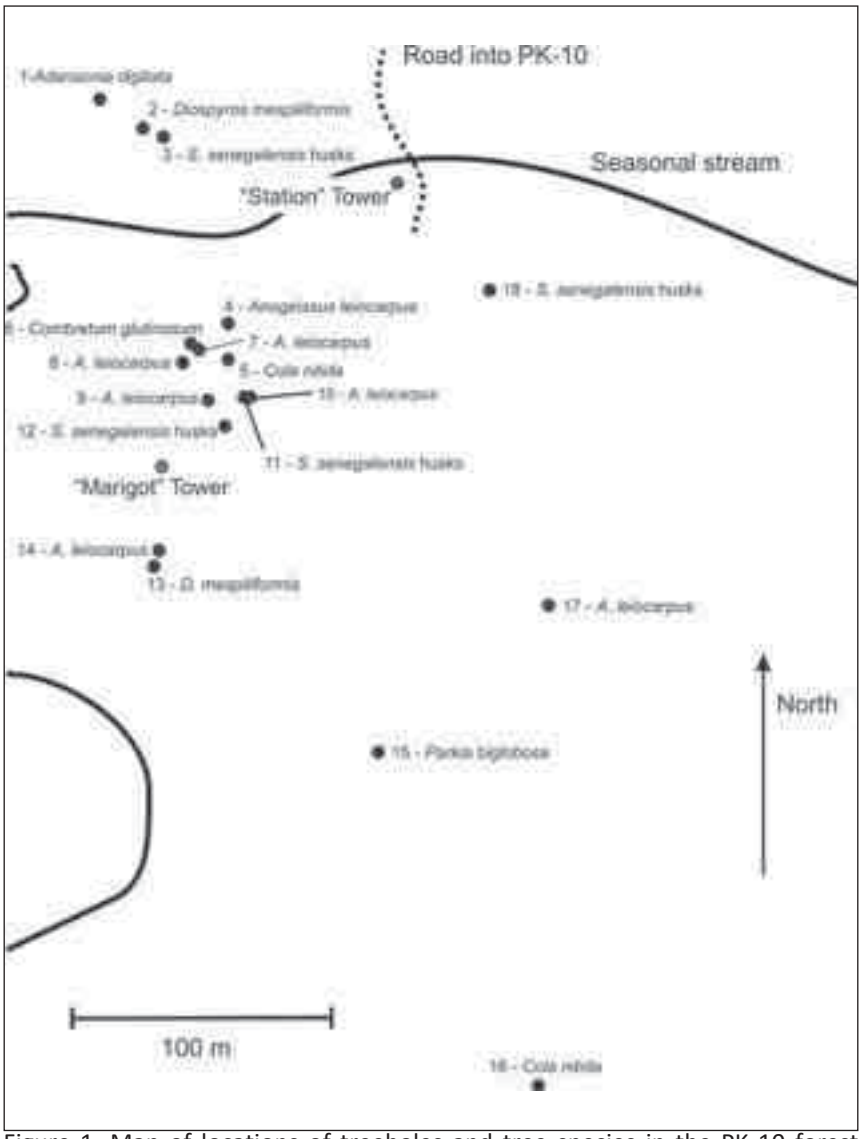


Figure 1. Map of locations of treeholes and tree species in the PK-10 forest sampled in the present study. Also shown are the locations of *Saba senegalensis.* In each of these four locations, only fruit husks were sampled. The locations of all sites were recorded with a GPSMAP® 62s (Garmin) and coordinates are listed in Table 1. Marigot and Station Towers are shown to provide reference points relative to earlier studies that included PK-10 (Raymond et al. 1976, Monlun et al. 1993, Cornet et al. 1975).

four pails containing *S. senegalensis* husks were returned to our local laboratory where husks and dry contents of treeholes were separately flooded. All containers were checked daily in the laboratory, larvae were collected and transferred into cups, supplemented with Brewer's yeast, and reared to adults in Bug-dorm® DP1000 cages (Bug-dorm Store, Taichung, Taiwan). Larval mortalities were not recorded. Adult mosquitoes which had eclosed within the previous 12-16 h period were aspirated and transferred into one-pint cartons that had been covered with mesh. These were then knocked down (in most cases killed) with FlyNap (Triethylamine) (Carolina Biological Supply Company, Burlington, NC). Adults were then individually removed with forceps to the stage of a dissecting microscope (Olympus) where they were identified to species. We progressively developed a regional key of adult *Aedes* for southeastern Senegal. Initially we used Huang and Ward (1981), followed by Huang (2004) for the subgenus *Stegomyia* and Huang (1990) for the *africanus* group. When these failed (e.g., *Ae. capensis* and *Ae. simulans*), we used Edwards (1941). *Aedes aegypti* s.l. were also scored on a scale from 1 ("F") to 15 ("Q") by separating the wings and examining the amount of scaling on the abdominal tergites and scoring these based upon the diagrams in McClelland (1974) to distinguish between *Aaa* and *Aaf*. All mosquitoes were then individually stored in Purell® Advanced Hand Sanitizer as voucher specimens and for eventual extraction of DNA.

Proportions of species were compared among larval

habitats, collection methods, and seasons by calculating Bayesian 95% Highest Density Intervals (95% HDI) using WinBUGS (Lunn et al. 2000) and the analysis of contingency tables script (Box 6.13 in McCarthy 2012). Species diversity was summarized with Shannon's diversity index H (Shannon 1948) where $H = -\sum p_i \ln(p_i)$ and $p_i$ is the proportion of species *i* in a collection. The 95% HDI around H was estimated to compare H among collections using WinBUGS and the script in Box 3.15 of McCarthy (2012). Proportions and diversity indices with non-overlapping 95% HDI were considered credible.

## RESULTS

### Seasonal shifts in abundance of *Aedes aegypti* s.l. subspecies

There was a credible lower abundance of *Aaf* during the dry season (60.7%) as compared with the wet season (90.0%), mainly due to an excess of McClelland G mosquitoes in the dry season (Table 2a). During the dry season, percentages of *Aaf* were the same regardless of whether larvae were collected in treeholes by excavation, flooding, or by submerging *S. senegalensis* husks (Table 2b). This was also true during the wet season (Table 2c).

Dry season treehole collections captured 355 mosquitoes of which 147 (41.4%) were *Ae. aegypti* s.l. (Table 3). A similar percentage was obtained from the same treeholes sampled during the wet season (43.1%, Table 4). Flooded treehole collections contained the same percentage of *Ae. aegypti* s.l. in the dry (34.4%, Table 3) and wet seasons (43.1%, Table 4). However, excavated contents of treeholes in the dry season produced a credibly higher percentage of *Ae. aegypti* s.l. (64.8%, Table 3) than flooded treehole collections. Half (49.1%, Table 3) of the mosquitoes from flooded *S. senegalensis* husks in the dry season were *A. aegypti* s.l. compared to 6.6% during the wet season (Table 4).

### Seasonal shifts in species abundance

Seven species were collected during the dry season and their abundances were similar in excavated treeholes, flooded treeholes, and *S. senegalensis* husks with two exceptions. Credibly more *Ae. aegypti* s.l. were obtained from excavated treeholes, while more *Ae. unilineatus* were obtained from flooded treeholes (Table 3). Shannon diversity indices were uniform among species collected in excavated and flooded treeholes, and *S. senegalensis* husks (Table 3).

Thirteen species were collected during the wet season and the Shannon diversity index (1.79, Table 4) was credibly greater than in the dry season (1.11-1.30, Table 3). However, in *S. senegalensis* husks an opposite trend was seen, wherein the diversity index was credibly greater in the dry season (1.30, Table 3) than in the wet season (0.32, Table 4). Only three species were collected during the wet season and two of these were represented by a single individual and the remainder was all *E. chrysogaster* s.l.

## DISCUSSION

There are five principal findings of this study. First, we detected a lower abundance of *Aaf* during the dry season as compared with the wet season. This contrasts with Paupy et al. (2010) who instead reported a greater abundance of *Aaf* in the dry season. However, they only sampled domestic containers in the dry season. Nevertheless, they reported that ~45-50% of female mosquitoes had high McClelland scores of 8-10 (letter scores of L-M) during the rainy season as compared to ~10% during the wet season. Paupy et al. (2010) observed a decrease in the relative abundance *Aaf* during the wet season, while we noted an increase. However, we only noted this trend in treeholes not in *S. senegalensis* husks. They also found a difference in McClelland scores between males and females while McClelland (1974) and the present study detected no such differences (analyses not shown). Furthermore, gender differences were not large in natural containers in the wet season (Paupy et al. 2010). The present study and Paupy et al. (2010) document shifts in the relative abundance *Aaf* between the dry and wet seasons.

Secondly, while dry and wet season flooded treeholes yielded similar percentages of *Ae. aegypti* s.l., its abundance differed greatly between *S. senegalensis* collections in the dry and wet seasons. It is most likely that the lower percentage in wet season husks was due to the abundance of facultatively predaceous *E. chrysogaster* s.l. (92.8%) larvae as compared to treeholes (10.9%, Table 4). *Eretmapodites* (Theobald) is a small genus containing 24 species. Haddow (1946) first commented on the predatory habits of *Eretmapodites* larvae in Africa noting that the mouthparts "have all been found to possess a group of thickened, comb-like hairs on the medio-ventral aspects of the mouth brushes (p. 58)." He further observed that "an *Eretmapodites* larva, after seizing its victim, holds it between the half-flexed head and ventral surface of the thorax. The prey is consumed rapidly - a large larva may be devoured in about 10 minutes - and larvae are attacked even in the presence of abundant other food material (p. 59)." We made similar observations. Haddow (1946) also made the first major taxonomic review of *Eretmapodites* species in Africa and identified four groups: *E. chrysogaster* s.l. (Graham) group (five species), *E. inornatus* (Newst.) group (two species), *E quinquevittatus* (Theo.) group (two species) and *E. oedipodius* (Edw.) (two species). Near Mombasa, Kenya, Lounibos (1978) showed that *E. subsimplicipes* (*E. chrysogaster* s.l. group) has a strong preference for fruit husks including the congeneric species *Saba florida,* while *E. quinquevittatus* has a preference for tap water. Lounibos (1981) reported a peak abundance of *E. subsimplicipes* in August and September from bamboo traps in forested collecting sites near Mombasa in Kenya. Raymond et al. (1976) sampled treeholes and fruit husks near Kedougou and also reported a large number of *E. chrysogaster* s.l. in husks collected from August-September, 1974. As in the present study, *E. chrysogaster* s.l. constituted 90-94% of mosquitoes collected in fruit husks and adults were easily identified based upon their large size, lack of ornamentation on the scutum, and the presence of large plumes on the hind tarsi of males. *Eretmapodites* males lacking these plumes or

Table 2. Abundance of each *Aedes aegypti* subspecies and McClelland's forms during the dry and wet seasons in the PK-10 forest of southeastern Senegal. Values defining the 95% HDI appear in parentheses. The first number in parentheses is the 2.5% HDI, the second underlined value is the mean percentage, while the third value is the 97.5% HDI. Rows in which mean percentages had overlapping 95% HDI are highlighted in grey. Values are not highlighted when credible differences exist between mean percentages.

| a) Between seasons | Dry season | | Wet season | |
|---|---|---|---|---|
| *Species* | Numbers collected | (95% HDI) | Numbers collected | (95% HDI) |
| *Ae. aegypti formosus* (F) | 105 | (53.3%, 60.7%, 67.9%) | 72 | (82.4%, 90.0%, 95.6%) |
| *Ae. aegypti aegypti* (G-) | 26 | (10.2%, 15.0%, 20.7%) | 4 | (1.4%, 5.0%, 10.6%) |
| *Ae. aegypti aegypti* (G) | 25 | (9.6%, 14.4%, 20.1%) | 2 | (0.4%, 2.5%, 6.8%) |
| *Ae. aegypti aegypti* (H) | 17 | (5.9%, 9.8%, 14.7%) | 2 | (0.4%, 2.5%, 6.8%) |
| | 173 | | 80 | |

b) Within dry season (Total = 173)

| *Species* | Excavated treeholes | (95% HDI) | Flooded treeholes | (95% HDI) | *Saba senegalensis* husks | (95% HDI) |
|---|---|---|---|---|---|---|
| *Ae. aegypti formosus* | 32 | (47.0%, 60.4%, 73.2%) | 57 | (50.5%, 60.6%, 70.3%) | 16 | (41.9%, 61.5%, 78.9%) |
| *Ae. aegypti aegypti* | 21 | | 37 | | 10 | |
| | 53 | | 94 | | 26 | |

c) Within wet season (Total = 80)

| | | | | | | |
|---|---|---|---|---|---|---|
| *Ae. aegypti formosus* | - | - | 55 | (85.3%, 93.2%, 97.6%) | 17 | (60.7%, 80.9%, 95.2%) |
| *Ae. aegypti aegypti* | - | - | 4 | | 4 | |
| | - | - | 59 | | 21 | |

Table 3. Dry season abundance of *Aedes* species in different sampling habitats in the PK10 forest of southeastern Senegal. Values defining the 95% HDI appear in parentheses. The first number in parentheses is the 2.5% HDI, the second underlined value is the mean percentage, while the third value is the 97.5% HDI. Rows in which mean percentages had overlapping 95% HDI are highlighted in grey. Values are not highlighted when credible differences exist across mean percentages.

| Species | Excavated treeholes | (95% HDI) | Flooded treeholes | (95% HDI) | *Saba senegalensis* husks | (95% HDI) |
|---|---|---|---|---|---|---|
| *Ae. aegypti* s.l. | 53 | (54.0%, 64.6%, 74.8%) | 94 | (28.9%, 34.4%, 40.2%) | 26 | (35.6%, 49.1%, 62.4%) |
| *Ae. apicoargenteus* | 0 | (0.0%, 0.0%, 1.6%) | 7 | (1.0%, 2.6%, 4.6%) | 1 | (0.0%, 1.9%, 7.0%) |
| *Ae. luteocephalus* | 7 | (3.2%, 8.5%, 15.6%) | 25 | (6.0%, 9.2%, 12.9%) | 7 | (5.4%, 13.2%, 23.3%) |
| *Ae. metallicus* | 3 | (0.4%, 3.7%, 8.5%) | 5 | (0.6%, 1.8%, 3.7%) | 5 | (2.5%, 9.4%, 18.5%) |
| *Ae. opok* | 0 | (0.0%, 0.0%, 1.4%) | 1 | (0.0%, 0.4%, 1.2%) | 0 | (0.0%, 0.0%, 2.9%) |
| *Ae. stokes* | 4 | (0.9%, 4.9%, 10.4%) | 11 | (2.0%, 4.0%, 6.6%) | 0 | (0.0%, 0.0%, 3.4%) |
| *Ae. unilineatus* | 15 | (10.7%, 18.3%, 27.5%) | 130 | (41.7%, 47.6%, 53.6%) | 14 | (15.5%, 26.4%, 39.3%) |
| TOTAL | 82 | | 273 | | 53 | |
| Shannon Diversity Index (95% HDI) | | (0.92, 1.11, 1.29) | | (1.19, 1.29, 1.39) | | (1.12, 1.30, 1.45) |

females with scutum ornamentation were never recovered in the present study.

Third, we detected large seasonal shifts in species abundance and diversity. Fewer species were collected during the dry season. This result is not surprising given that species richness is expected to be greater in wet vs dry treeholes. Furthermore, ephemeral fruit husks would not be expected to support the number of species as are found in stable treeholes. Large seasonal shifts in larval species composition have been previously documented by Haddow in East Africa (Haddow 1945), Teesdale (Teesdale 1959) and later Lounibos along the Kenya coast (Lounibos 1981), and Corbert in Uganda (Corbet 1964). The same was noted in West Africa in the Northern Guinea Savannah of Nigeria (Service 1965), in southern Nigeria (Dunn 1927, Kerr 1933, Mattingly 1949a,b), in Liberia (Rozeboom and Burgess 1962), in Ghana (Addy et al. 1996), and in Senegal (Raymond et al. 1976).

Fourth, sampling technique and location affected species composition during the dry season (Table 3). Specifically far more *Ae. unilineatus* were collected by flooding while the relative abundances of *Ae. aegypti* were greater in excavated material. The effect of location on species composition was even greater during the wet season in which the relative abundances of the 11 species were significantly different between flooded treeholes (ten species) and *S. senegalensis* husks (four species), and most (92.8%) of the mosquitoes collected from husks were *E. chrysogaster* s.l. Lounibos (1981) described different hatching patterns among treehole *Aedes* and demonstrated that *Stegomyia* spp. hatched first, followed by other subgenera. This may explain why wet season samples contained a higher proportion of non-*Stegomyia* aedines compared to flooded, dry-season tree holes. Also Sota and Mogi (1992) showed that eggs of forest-dwelling *Stegomyia* are less desiccation-resistant than non-forest counterparts, which may explain the differences between *Aaf* and *Aaa* in treeholes in the dry vs wet seasons. At present, we are uncertain of the reasons that treehole excavation and flooding would yield different numbers of species. More careful excavation of different regions of the treehole might provide answers. In addition, more careful sampling might have detected differences in species abundance among different sizes of treeholes and possibly differences among tree species.

Fifth, this study suggests that both *Aaa* and *Aaf* may survive the tropical dry season in natural larval habitats such as treeholes and husks in West Africa. Many reports from coastal Kenya (e.g., Trpis & Hausermann 1975, 1978) concluded that *Aaf* and *Aaa* may hybridize peridomestically in the rainy season. Sylla et al. (2009) reported *Aaf* domestic indoor larval habitats in Senegal. This is consistent with earlier reports from Nigeria and Gabon (Dunn 1927, Kerr 1933, Mattingly 1949a,b, Service 1963, 1965). Furthermore, in our collections from treeholes in PK-10 we recovered an abundance of *Aaa*. Both trends contrast with previous reports from East Africa (Mattingly 1957, Trpis and Hausermann 1975, 1978, 1986) of 1) strict endophily in *Aaa* and exophily in *Aaf*, and 2) household containers as the exclusive larval habitats for *Aaa* and treeholes as the predominant larval habitats for *Aaf*.

Table 4. Wet season abundance of each *Aedes* species in different sampling habitats in the PK10 forest of southeastern Senegal. Values defining the 95% HDI appear in parentheses. The first number in parentheses is the 2.5% HDI, the second underlined value is the mean percentage, while the third value is the 97.5% HDI. Mean percentages with overlapping 95% HDI are highlighted in grey. Values are not highlighted when credible differences exist between mean percentages.

| Species | Flooded treeholes | (95% HDI) | *Saba senegalensis* husks | (95% HDI) |
|---|---|---|---|---|
| *Ae. aegypti s.l.* | 59 | (34.8%, 43.1%, 51.6%) | 21 | (4.1%, 6.6%, 9.7%) |
| *Ae. apicoargenteus* | 22 | (10.5%, 16.1%, 22.5%) | 0 | (0.0%, 0.0%, 0.5%) |
| *Ae. capensis* | 4 | (0.9%, 2.9%, 6.1%) | 0 | (0.0%, 0.0%, 0.3%) |
| *Ae. cozi* | 17 | (7.4%, 12.4%, 18.3%) | 0 | (0.0%, 0.0%, 0.5%) |
| *Ae. furcifer-taylori* | 4 | (0.9%, 2.9%, 6.1%) | 0 | (0.0%, 0.0%, 0.3%) |
| *Ae. luteocephalus* | 0 | (0.0%, 0.1%, 1.0%) | 1 | (0.0%, 0.3%, 1.4%) |
| *Ae. metallicus* | 7 | (0.1%, 5.1%, 4.7%) | 0 | (0.0%, 0.0%, 0.0%) |
| *Ae. simulans* | 1 | (0.1%, 0.7%, 2.4%) | 0 | (0.0%, 0.0%, 0.2%) |
| *Ae. unilineatus* | 7 | (0.1%, 5.1%, 4.7%) | 0 | (0.0%, 0.0%, 0.0%) |
| *Ae. vittatus* | 1 | (0.1%, 0.7%, 2.7%) | 1 | (0.0%, 0.3%, 1.1%) |
| *E. chrysogaster* | 15 | (6.0%, 10.9%, 16.8%) | 295 | (89.6%, 92.9%, 95.3%) |
| *TOTAL* | 137 | | 318 | |
| *Shannon Diversity Index* | | (1.639, 1.79, 1.927) | | (0.234, 0.32, 0.414) |
| *E. chrysogaster excluded from diversity analysis* | | (1.46, 1.63, 1.779) | | (0.211, 0.50, 0.810) |

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REFERENCES CITED

Addy, P.A.K., R.K. Esena, and S.K.N. Atuahene. 1996. Possible contributing factors to the paucity of yellow fever epidemics in the Ashanti region of Ghana, West Africa. E. Afr. Med. J. 73: 3-9.

Berhaut, J. 1967. *Flore du Sénégal*. Dakar, Éditions Clairafrique.

Corbet, P.S. 1964. Observations on mosquitos ovipositing in small containers in Zika Forest, Uganda. J Anim Ecol 33: 141-164.

Cornet, M., H. Raymond, and P.Y. Dieng. 1975. Etudes sur les vecteurs sylvatiques du virus amaril. Essai de mise en évidence de la survie de femelles en saison sèche dans un foyer endémique au Sénégal Oriental. Cahiers O.R.S.T.O.M., Sér. Entomol. Méd. Parasitol. 13: 215-222.

Dunn, L.H. 1927. Tree-holes and mosquito breeding in West Africa. Bull. Entomol. Res. 18: 39-144.

Dupont-Rouzeyrol, M., V. Caro, L. Guillaumot, M. Vazeille, E. D'Ortenzio, J.M. Thiberge, N. Baroux, A.C. Gourinat, M. Grandadam, and A.B. Failloux. 2012. Chikungunya virus and the mosquito vector *Aedes aegypti* in New Caledonia (South Pacific Region). Vector Borne Zoonot. Dis. 12: 1036-1041.

Edwards, F.W. 1941. *Mosquitoes of the Ethiopian Region III.- Culicine Adults and Pupae*. Oxford University Press.

Gubler, D.J. 2012. The economic burden of dengue. Am. J. Trop. Med. Hyg. 86 : 743-744.

Haddow, A.J. 1946. The mosquitoes of Bwamba County, Uganda. 4. Studies on the Genus *Eretmapodites*, Theobald. Bull. Entomol. Res. 37: 57-82.

Haddow, A.J. 1945. On the mosquitoes of Bwamba County, Uganda. 1. Description of Bwamba, with special reference to mosquito ecology. Proc. Zool. Soc. Lond. 115: 1-13.

Huang, Y.M. 1990. The subgenus *Stegomyia* of *Aedes* in the Afrotropical region. I. The *Africanus* group of species (Diptera: Culicidae). Contrib. Am. Entomol. Inst. 26: 1-90.

Huang, Y.M. 2004. The subgenus *Stegomyia* of *Aedes* in the Afrotropical region with keys to the species (Diptera: Culicidae). Zootaxa : 700: 3-123.

Huang, Y.M. and R.A. Ward. 1981. A pictorial key for the identification of the mosquitoes associated with yellow fever in Africa. Mosq. Syst. 13: 138-149.

Huber, K., Y. Ba, I. Dia, C. Mathiot, A.A. Sall, and M. Diallo. 2008. *Aedes aegypti* in Senegal: Genetic diversity and genetic structure of domestic and sylvatic populations. Am. J. Trop. Med. Hyg. 79: 218-229.

Kerr, J.A. 1933. Studies on the abundance, distribution and

feeding habits of some West African Mosquitos. Bull. Entomol. Res. 24: 493-510.

Lounibos, L.P. 1978. Mosquito breeding and oviposition stimulant in fruit husks. Ecol. Entomol. 3: 299-304.

Lounibos, L.P. 1981. Habitat segregation among african treehole mosquitoes. Ecol. Entomol. 6: 129-154.

Lunn, D.J., A. Thomas, N. Best, and D. Spiegelhalter. 2000. WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. Stat. Comput. 10: 325-337.

Mattingly, P.F. 1949a. Studies on West African forest mosquitoes .1. The seasonal distribution, biting cycle and vertical distribution of 4 of the principal species. Bull. Entomol. Res. 40: 149-168.

Mattingly, P.F. 1949b. Studies on West African forest mosquitoes. 2. The less commonly occurring species. Bull. Entomol. Res. 40: 387-402.

Mattingly, P.F. 1957. Genetical aspects of the *Aedes aegypti* problem, I. Taxonomy and bionomics. Ann. Trop. Med. Parasitol. 51: 392-408.

McCarthy, M.A. 2012. *Bayesian Methods for Ecology*. New York, Cambridge University Press.

McClelland, G.A.H. 1960. A preliminary study of the genetics of abdominal color variations in *Aedes aegypti* (L.) (Diptera: Culicidae). Ann. Trop. Med. Parasit. 54: 305-320.

McClelland, G.A. 1974. Worldwide survey of variation in scale pattern of abdominal tergum of *Aedes aegypti* (L.) (Diptera Culicidae). Trans. R. Entomol. Soc. Lond. 126: 239-259.

Monlun, E., H. Zeller, B. Leguenno, M. Traorelamizana, J.P. Hervy, F. Adam, L. Ferrara, D. Fontenille, R. Sylla, M. Mondo, and J. P. Digoutte. 1993. Arbovirus affecting humans in southeastern Senegal - Surveillance in humans and mosquitoes (1988-1991). Bull. Soc. Pathol. Exot. 86: 21-28.

Paupy, C., C. Brengues, O. Ndiath, C. Toty, J.P. Herve, and F. Simard. 2010. Morphological and genetic variability within *Aedes aegypti* in Niakhar, Senegal. Infect. Genet. Evol. 10: 473-480.

Raymond, H.L., M. Cornet and P.Y. Dieng. 1976. Etudes sur les vecteurs sylvatiques du virus amaril : inventaire provisoire des habitats larvaires d'une forêt-galerie dans le foyer endémique du Sénégal oriental. Cah. ORSTOM Sér. Entomol. Méd. Parasitol. 14: 301-306.

Rozeboom, L.E. and R.W. Burgess. 1962. Dry-season survival of some plant-cavity breeding mosquitoes in Liberia. Ann. Entomol. Soc. Am. 55: 521-524.

Service, M.W. 1963. Checklist and distribution of the Nigerian Culicidae (Diptera). J. West Afr. Sci. Assoc. 8: 80–110.

Service, M.W. 1965. The ecology of the tree-hole breeding mosquitoes in the northern Guinea savanna of Nigeria. J. Appl. Ecol. 2: 1–16.

Shannon, C.E. 1948. A mathematical theory of communication. Bell System Tech. J. 27: 379-423.

Sota, T. and M. Mogi. 1992. Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. Oecologia 90: 353-358.

Sylla, M., C. Bosio, L. Urdaneta-Marquez, M. Ndiaye, and W. C. Black. 2009. Gene flow, subspecies composition, and dengue virus-2 susceptibility among *Aedes aegypti* collections in Senegal. PLoS Neglect. Trop. Dis 3: e408.

Teesdale, C. 1959. Observations on the mosquito fauna of Mombasa. Bull. Entomol. Res. 50: 191-208.

Trpis, M. and W. Hausermann. 1975. Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark release recapture. Bull. Entomol. Res. 65: 199-208.

Trpis, M. and W. Hausermann. 1978. Genetics of house-entering behavior in east-African populations of *Aedes aegypti* (L.) (Diptera-Culicidae) and its relevance to speciation. Bull. Entomol. Res. 68: 521-532.

Trpis, M. and W. Hausermann. 1986. Dispersal and other population parameters of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. Am. J. Trop. Med. Hyg. 35: 1263-1279.

Vazeille, M., A. Yebakima, R. Lourenco-de-Oliveira, B. Andriamahefazafy, A. Correira, J.M. Rodrigues, A. Veiga, A. Moreira, I. Leparc-Goffart, M. Grandadam, and A.B. Failloux. 2013. Oral receptivity of *Aedes aegypti* from Cape Verde for yellow fever, dengue, and chikungunya viruses. Vector Borne Zoonot. Dis. 13: 37-40.

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*Full Length Research Paper*

# 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. Also, 12.0% 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 (Noisakran 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 sequelae (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/DGF 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 Sudano-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

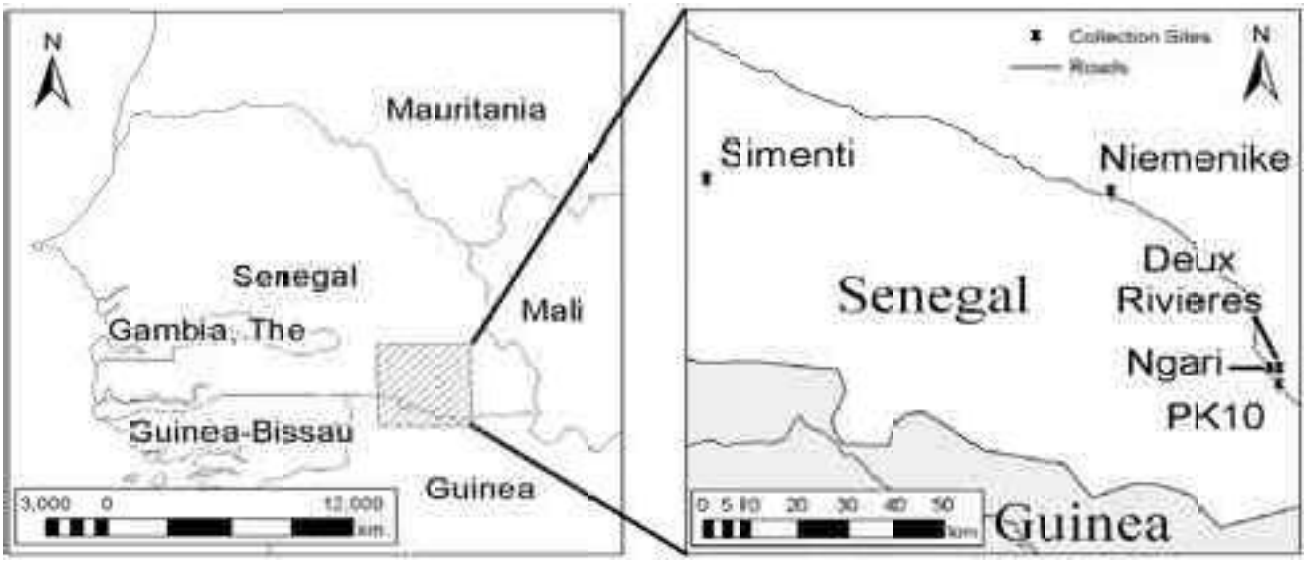


**Figure 1.** Study sites: Map of Senegal indicating locations of the three trapping sites in southeastern Senegal. Site (N) located about 100 m away from Ngari village; Site (P) for Pont and Site (L) for Plateau are located in the forest gallery of PK10; Site (D) located in Deux Rivieres.

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 lookout 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 (Imalgen 1000®). While anesthetized monkeys wer 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 (Titertek, 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.

| Parameter | 2002 | | 2003 | | 2006 | | Total | |
|---|---|---|---|---|---|---|---|---|
| | YF | DENV-2 | YF | DENV-2 | YF | DENV-2 | YF | DENV-2 |
| *Chlorocebus sabaeus* | 7/19 (36.8)* | NT | 3/9 (33.3) | 0/9 (0.0) | NT | 4/16 (25.0) | 10/28 (3.6) | 4/25 (16.0) |
| Total *C. sabaeus* | 19 | | 9 | | 16 | | | 44 |
| *Erythrocebus patas* | 0<br>0 | 0<br>0 | 3/10 (30.0) | 0/10 (0.0) | NT | 3/4 (75.0) | 3/10 (30.0) | 3/14 (21.4) |
| Total *E. patas* | 0 | | 10 | | 4 | | 14 | |
| Total monkeys | 19 | | 19 | | 20 | | 58 | |

*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).

| Code | Species | Site | Sex | Age | 2002 | | | | 2003 | | | | 2006 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | YFV | | DENV-2 | | YFV | | DENV-2 | | YFV | | DENV-2 | |
| | | | | | IgM | IgG | IgM | IgG | IgM | IgG | IgM | IgG | IgM | IgG | IgM | IgG |
| D2 | *C. sabaeus* | 2Rivieres | M | Adult | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| D3 | *C. sabaeus* | 2Rivieres | F | Adult | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| D4 | *C. sabaeus* | 2Rivieres | F | Juvenile | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| P2 | *C. sabaeus* | Pont | F | Adult | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| P3 | *C. sabaeus* | Pont | M | Adult | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| P5 | *C. sabaeus* | Pont | M | Juvenile | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| N3 | *C. sabaeus* | Ngari | M | Juvenile | - | + | - | - | nt | nt | nt | nt | nt | nt | nt | nt |
| P7 | *E. patas* | Pont | M | Subadult | nt | nt | nt | nt | - | + | - | - | nt | nt | nt | nt |
| P8 | *E. patas* | Pont | M | Subadult | nt | nt | nt | nt | - | + | - | - | nt | nt | - | + |
| P9 | *C. sabaeus* | Pont | M | Subadult | nt | nt | nt | nt | - | + | - | - | nt | nt | nt | nt |
| N7 | *E. patas* | Ngari | M | Adult | nt | nt | nt | nt | - | + | - | - | nt | nt | nt | nt |
| N13 | *C. sabaeus* | Ngari | M | Adult | nt | nt | nt | nt | - | + | - | - | nt | nt | nt | nt |
| N15 | *C. sabaeus* | Ngari | F | Juvenile | nt | nt | nt | nt | - | + | - | - | nt | nt | nt | nt |
| L2 | *C. sabaeus* | Plateau | F | Adult | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |
| L6 | *C. sabaeus* | Plateau | M | Adult | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |
| L11 | *C. sabaeus* | Plateau | M | Adult | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |
| L14 | *E. patas* | Plateau | F | Juvenile | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |
| L15 | *E. patas* | Plateau | F | Adult | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |
| L16 | *C. sabaeus* | Plateau | | Juvenile | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | - | + |

studies on the same monkey population during the subsequent 2003 rainy season allowed to test 19 sera from which six were positive for YFV IgG, including three *C. sabaeus* (P9, N13 and N15) and three *E. patas* (P7, P8 and N7). Among them were two adult males [one *C. sabaeus* (N13), one *E. patas* (N7)], three sub-adult males [two *E. patas* (P7 and P8), one *C. sabaeus* (P9)], and one juvenile *C. sabaeus* (N15). No YFV IgM, nor DENV2-IgG or DENV-2 IgM were detected (Table 2). Among the red monkeys (*E. patas*), one sub-adult male (P8) tested positive for YFV IgG on June 2003, and subsequently when recaptured in August 2006, it tested positive for DENV-2 IgG (Table 2).

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. sabaeus* (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. sabaeus* 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 IgM 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, Ae. aegypti formosus, Ae. (Stegomyia) africanus, Ae. (Stegomyia) bromeliae, Ae. (Diceromyia) furcifer, Ae. (Stegomyia) luteocephalus, Ae. (Stegomyia) metallicus, Ae. (Stegomyia) opok, Ae. (Stegomyia) simpsoni complex, Ae. (Diceromyia) taylori, Ae. (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 epidemics 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*. *sabaeus*. 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 sahelo-sudanian 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 (*Ae. furicifer*, *Ae. taylori* and *Ae.luteocaphalus*) 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 (*Ae. 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. sabaeus* (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 (Althouse et al., 2012). Moreover, the seroconversion that we have detected from wild *C. sabaeus* 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 (*Ae. aegypti formosus* and *Ae. luteocephalus*) and *Diceromyia* (*Ae. furcifer* and *Ae. taylori*), which are specific to the forest gallery, have been found infected with DENV-2, as well as *Ae. 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 *Ae. 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 *Ae. taylori, Ae. furcifer, Ae. opok, Ae. luteocephalus* and *Ae. africanus* in Guinea, Côte d’Ivoire, and Burkina Faso (Cordellier et al., 1983; Roche et al., 1983; Hervy et al.,

1984; Rodhain, 1991). 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* (=*irus*)] 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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**REFERENCES**

Althouse BM, Lessler J, Sall AA Diallo M, Hanley KA, Watts M, Weaver SC, Cummings DAT(2012). Synchrony of Sylvatic Dengue Isolations: A Multi-Host, Multi-Vector SIR Model of Dengue Virus Transmission in Senegal. PLOS Negl. Trop. Dis. 6(11):e1928.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Cameron P, Simmons CP, Scott TW, Farrar JJ, Hay SI (2013). The global distribution and burden of dengue. Nature 496:504-507.

Bideau J (1925). Une épidémie de dengue avec complications à bord de l'avion "Antares". Rev. de Méd.e nav. 107-136.

Carey DE, Causey OR, Reddy S, Cooke AR (1971). Dengue viruses from patients in Nigeria. Lancet 1:105.

Christie J (1881). On epidemics of dengue fever: their diffusion and etiology. Glasgow Med. J. 16:161-176.

Cordellier R (1991). L'épidémiologie de la fièvre jaune en Afrique de l'ouest. Bull. OMS. 69 (1):73-84.

Cordellier R, Bouchite B, Roche JC, Monteny N, Diaco B, Akoliba P (1983). Circulation selvatique du virus Dengue 2, en 1980, dans les savanes subsoudaniennes de Côte d'Ivoire. Cahier ORSTOM, Sér. Ent. Méd. et Parasitol. 21:165-179.

Cornet M, Chateau R, Valade M, Dieng PL, Raymond H, Lorand A (1978). Données bio-écologiques sur les vecteurs potentiels de virus amaril. Cahier ORSTOM, Sér. Ent. Méd. Parasitol. 16:315-341.

Cornet M, Robin Y, Chateau R, Heme G, Adam C, Valade M, Legonidec G, Jan C, Renaudet J, Dieng PL, Bangoura JF, Laurent A (1979). Isolement d'arbovirus au Sénégal oriental à partir de moustiques (1972-1977) et note sur l'épidémiologie des virus transmis par les *Aedes*, en particulier du virus amaril. Cahier ORSTOM, Sér. Ent. Méd. Parasitol. 17:149-163.

Cornet M, Saluzzo JF, Hervy JP, Digoutte JP, Germain M, Chauvancy MF, Eyraud M, Ferrara L, Heme G, Legros F (1984). Dengue 2 au Sénégal oriental: une poussée épizootique en milieu selvatique; isolements du virus à partir d'un singe et considérations épidémiologiques. Cahier ORSTOM, Sér. Ent. Méd. Parasitol. 22:313-323.

Diallo M, Bâ Y, Sall AA , Diop OM, Ndione JA, Mondo M, Girault L, Mathiot C (2003). Amplification of the sylvatic cycle of dengue virus type 2, Senegal. 1999-2000: Entomologic findings and epidemiologic considerations. Emerg. Infect. Dis. 9:362-367.

Diallo M, Sall AA, Moncayo AC, Ba Y, Fernandez Z, Ortiz D, Coffey LL, Mathiot C, Tesh RB, Weaver SC (2005). Potential role of sylvatic and domestic African mosquito species in dengue emergence. Am. J. Trop. Med. Hyg. 73:445-449.

Edington AD (1927). Dengue, as seen in the recent epidemic in Durban. J. Med. Assoc. South Afr. 1:446-448.

Fagbami AH, Monath TP, Fabiyi A (1977). Dengue virus infections in Nigeria: a survey for antibodies in monkeys and humans. Trans. R. Soc. Trop. Med. Hyg. 71(1):60-65.

Faye O, Ba Y, Faye O Talla C, Diallo D, Chen R, Mondo M, Ba R, Macondo E, Siby T, Weaver SC, Diallo M, Sall AA (2014). Urban Epidemic of Dengue Virus Serotype 3 Infection, Senegal, 2009. Emerg. Infect. Dis. 20 (3): 456-459.

Franco L, Palacios G, Martinez JA, Vazquez A, Savji N, De Ory F, Sanchez-Seco MP, Martın D, Lipkin WI, Antonio Tenorio A (2011) . First Report of Sylvatic DENV-2 Associated Dengue Hemorrhagic Fever in West Africa. PLOS Negl. Trop. Dis. 5(8):e1251.

Germain M (1986). La fièvre jaune en Afrique de l'Ouest : une dynamique spatiale. ORSTOM Actu. (14):9-12.

Gratz NG, Knudsen AB. (1996). The rise and spread of Dengue, Dengue Haemorrhagic Fever and its vectors. A historical review (up to 1995). Document of the WHO CTD/FIL (DEN) 96.7:197 pp.

Gubler DJ (1997). Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: D. J. Gubler and G. Kuno (ed.), Dengue and dengue hemorrhagic fever. CAB International, London, United Kingdom. pp. 1-22.

Gubler DJ, Sather GE, Kuno G, Cabral JR (1986). Dengue 3 virus transmission in Africa. Am. J. Trop. Med. Hyg. 35:1280-1284.

Guha-Sapir D, Schimmer B (2005). Dengue fever: new paradigms for a changing epidemiology. Emerg. Themes Epidemiol. 2 (1): 1-10.

Hervy JP, Legros F, Roche Monteny N, Diaco B (1984). Circulation du virus Dengue 2 dans plusieurs milieux boisés des savanes soudaniennes de la région de Bobo-Dioulasso (Burkina Faso). Considérations entomologiques et épidémiologiques. Cahier ORSTOM, Sér. Entomol. Méd. Parasitol. 22**:** 135-143.

Hirsch A (1883). Dengue, a comparatively new disease: its symptoms. Trans. C. Creighton. In. Handbook of Geographical and Historical Pathology, III vols. Syndenham Society. Vol. I, pp. 55-81.

Holmes EC, Twiddy SS (2003). The origin, emergence and evolutionary genetics of dengue virus. Infect. Genet. Evol. 3(1): 19-28.

Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Koke CH (1989). An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trop. Med. Hyg. 40(4):418-427.

Kading RC, Borland EM, Cranfield M, Powers AM (2013). Prevalence of antibodies to alphaviruses and flaviviruses in free-ranging game animals and nonhuman primates in the greater Congo basin. J. Wildl. Dis. 49(3):587-599.

Legendre J (1926). La dengue ouest-africaine. Presse méd. 34: 1012-1014.

Lhuillier M, Sarthou JL (1983). Intérêt des IgM antiamariles dans le diagnostic et la surveillance épidémiologique de la fièvre jaune. Ann. Virol. Institut Pasteur 134E: 349-359.

Monlun E, Zeller H, Le Guenno B, Traore-Lamizana M, Hervy JP, Adam F, Ferrara L, Fontenille D, Sylla R, Mondo M, Digoutte JP (1993). Surveillance de la circulation des arbovirus d'intérêt médical dans la région du Sénégal Oriental (1988-1991). Bull. Soc. Pathol. Exot. 86: 21-28.

Mutebi JP, Barrett ADT (2002). The epidemiology of yellow fever in Africa. Microbes Infect. 4:1458-1468.

Nathan N, Barry M, Van Herp M, Zeller H (2001). Shortage of vaccines during a yellow fever outbreak in Guinea. Lancet 358: 2129-2130.

Noisakran S, Chuen Perng G **(**2008). Alternate Hypothesis on the Pathogenesis of Dengue Hemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS) in Dengue Virus Infection. Exp. Biol. Med. 233 (4): 401-408.

Rico-Hesse R (1990). Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology 174: 479-493.

Robin Y, Cornet M, Heme G, LE Gonidec G (1980). Isolement du virus de la dengue au Sénégal. Ann. Virol. (Institut Pasteur) 131E:149-154.

Roche JC, Cordellier R, Hervy JP, Digoutte JP, Monteny N (1983). Isolement de 96 souches de virus Dengue 2 à partir de moustiques capturés en Cote d'Ivoire et en Haute Volta. Ann. Virol. (Institut Pasteur) 134E:233-244.

Rodhain F (1991). The role of monkeys in the biology of dengue and yellow fever. Comp. Immunol. Microbiol. Infect. Dis. 14: 9-19.

Rudnick A (1965). Studies of the ecology of dengue in Malaysia: a preliminary report. J. Med. Entomol. 2(2): 203-208.

Rudnick A (1986). Dengue virus ecology in Malaysia. Rudnick A, Lim TW. Editors. Dengue fever studies in Malaysia. Institute of Medical Research of Malaysia. Bull. 23: 51-153.

Rudnick A, Marchette NJ, Garcia R (1967). Possible jungle Dengue. Recent studies and hypotheses. Symposium on Arbovirus Diseases, Animal Vectors and Reservoirs, Tokyo. pp. 69-74.

Saluzzo JF, Cornet M, Adam C, Eyraud M, Digoutte JP (1986). Dengue 2 au Sénégal oriental: enquête sérologique dans les populations simiennes et humaines. Bull. Soc. Pathol. Exot. 79: 313-322.

Simmons JS, St John, JH, Reynolds FHK. (1931). Experimental studies of dengue. Philippine J. Science. 44: 1-252.

Smith CEG (1958). The distribution of antibodies to Japanese encephalitis, dengue, and yellow fever viruses in five rural communities in Malaysia. Trans. R. Soc. Trop. Med. Hyg. 52: 237-252.

Smith CEG (1956). The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. J. Trop. Med. Hyg. 59:243-251.

Thonnon J, Fontenille D, Tall A, Diallo M, Renaudineau Y, Baudez B, Raphenon G (1998a). Re-emergence of yellow fever in Senegal in 1995. Am. J. Trop. Med. Hyg. 59 (1):108-114.

Thonnon J, Spiegel A, Diallo M, Sylla R, Fall A, Mondo M, Fontenille D (1998b). Yellow fever outbreak in Kaffrine, Senegal 1996: epidemiological and entomological findings. Trop. Med. Int. Health 3:872-877.

Tomori O (2004). Yellow fever: the recurring plague. Crit. Rev. Clin. Lab. Sci. 41:391-427.

Traore-Lamizana M, Zeller H, Monlun E., Mondo M, Hervy JP, Adam F, Digoutte JP (1994). Dengue 2 outbreak in southeastern Senegal during 1990: virus isolations from mosquitoes (Diptera: Culicidae). J. Med. Entomol. 31(4):623-627.

Vainio J, Cutts F (1998). Yellow fever. World Health Organization /EPI/GEN/98.11.

Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC (2012). Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nat. Rev. Microbiol. 9(7):532-541.

Vasilakis N, Tesh RB, Weaver SC (2008). Sylvatic dengue virus type 2 activity in humans, Nigeria, 1966. Emerg. Infect. Dis. 14:502-504.

Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ, Weaver SC (2000). Evolutionary relationship of endemic/epidemic and sylvatic dengue viruses. J. Virol. 74 (7): 3227-3234.

Yuwono J, Suharyono W, Koiman I, Tsuchiya Y, Tagaya I (1984). Seroepidemiological survey on dengue and Japanese encephalitis virus infections in Asian monkeys. Southeast Asian J. Trop. Med. Public Health 15(2):194-200.

Zeller HG, Traore-Lamizana M, Monlun E, Hervy JP, Mondo M, Digoutte JP (1992). Dengue-2 virus isolation from humans during an epizootic in Southeastern Senegal in November, 1990. Res. Virol. 143(2):101-102.

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*Review*

# Chiropteran and Filoviruses in Africa: Unveiling an ancient history

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***Ebolavirus* and *Marburgvirus* belong to the Filovirus family and are responsible for hemorrhagic fevers in Africa. The first documented Filovirus outbreak in Africa occurred in Central Africa and was attributed to *Ebolavirus* species. In the last four decades, Filoviral hemorrhagic fevers (FHFs) outbreaks caused by Ebola and Marburg viruses have been on the increase in Africa. The 2013-2015 outbreak has been the largest outbreak in human and has had the most devastating human and economic impact. Epidemics usually originate from a primary single introduction of the virus into simian or human population followed by an interspecies spill over. Multiple, short and isolated transmissions to humans have been also observed. Since the 1976 Yambuko (Democratic Republic of Congo) and Nzara (Sudan) epidemics, several investigations of different animal species have been undertaken but failed to identify the natural reservoirs of *Ebolavirus*. Further studies identified bats as probable reservoirs of *Ebolavirus* in Gabon, and major natural reservoirs of *Marburgvirus* in Uganda, supposed central forested areas of Africa as the epicenter where these viruses originated from, before dissemination. Chimpanzees, gorillas and duikers have been identified as highly sensitive hosts of *Ebolavirus* within wildlife. However, the relative importance of potential vertebrate hosts in the FHFs emergence into human population remains unclear. Different transmission routes involving bats have been proposed. Filoviruses have a zoonotic origin; amplified and maintained in nature between potential reservoirs in a jungle cycle. *Ebolavirus* mostly escapes these natural foci, when other sensitive secondary simian are infected and transmit the virus to human population via hunting, bat's saliva infected wild fruit collection or land monitoring, while *Marburgvirus* emergence was linked to monkey's tissues handling or human entry into bat sheltering habitats. This review discusses the dissemination of filoviruses circulating within their possible chiropteran reservoir species. Vertebrate hosts suspected in the maintenance/transmission cycles are reviewed and their bioecological features discussed. Despite the importance of the findings about reservoirs' discovery, several other questions such as plurispecific associations, migration routes, breeding cycles need to be addressed and are pointed out in this review, in order to generate risk maps for filoviruses' (re)emergence in West Africa.**

**Key words:** *Ebolavirus*, *Marburgvirus*, Chiropteran, emergence, bioecology, West Africa.

## INTRODUCTION

Filoviral hemorrhagic fevers (FHFs) are endemic to Africa. Certainly confined in a jungle cycle for a long time, their etiological agents, namely Ebola and Marburg viruses circulated silently without any manifestation in human population until 1976, when *Ebolavirus* hemorrhagic fever was first simultaneously diagnosed from human communities in Yambuko (Democratic Republic of Congo, DRC) (Johnson, 1978) and Nzara and Maridi (Sudan) (Smith, 1978). Its closest relative, *Marburgvirus* was first recognized in Marburg, Germany and Belgrade, Serbia (formerly Yugoslavia) in 1967 causing an outbreak of severe viral hemorrhagic fever among laboratory workers. African green monkeys (*Chlorocebus aethiops*) imported from Uganda for research purpose were the source of the infection (Smith et al., 1967; Siegert et al., 1968). In Africa, it appeared first in Johannesburg, South Africa (Gear et al., 1975). Since those first recorded emergences, filoviruses increasingly manifest their pathogenic potential, sporadically emerging or re-emerging, enlarging their areas of incidence into Africa and threatening public health and animal biodiversity. There has been a mystery overlapping their natural emergence for decades. Nowadays, bats are much more known involved in their transmission cycle. The emergence of *Ebolavirus* in West Africa inspired several interrogations and request detailed research-action studies in order to understand the extent that the viral amplification, within the reservoir species, has reached. It is likely that the 2013 Guekedou emergence in Guinea was induced by a fruit bat, *Eidolon helvum* (Funk and Piot, 2014). If the virus circulates within the local West African fauna, it will then have the opportunity to set in new ecological niches, in a West African sylvatic cycle, and sporadic epidemics are predictable in West Africa. Surveillance study programs across West African countries, along a west-east prospection transect bordering the northern limit of the forested areas of Central Africa needs to be entirely undertaken. This will aim to detect virus circulation or specific antibodies in reservoir and incidental hosts using serology and RT-PCR for viral nucleic acid sequences detection from wild samples in order to infer the natural history of *Ebolavirus* circulation, and map the geographic range of the virus' amplification. This review discusses the filoviruses associated with bats, and proposes future directions for epidemiological and ecological studies that need to be undertaken, in order to better understand the involvement of chiropteran populations and the patterns of FHFs emergence.

We reviewed the literature on chiropteran found naturally infected with filoviruses in Africa. Other bat species or wild animals from which filovirus nucleic acid sequences or serological evidence of filovirus circulation has been detected are also listed. Considering the ecological and ethological features so far known about chiropteran (Rosevear, 1965; Walker, 1999), we speculate on the potential filoviruses' extension due to their migration, roosting and reproduction.

A literature analysis allowed us to discuss each potential reservoir species' implication in the epidemiology of Ebola and Marburg viruses. Future orientation studies are proposed to pinpoint the areas at risk for eventual filovirus' emergence in West Africa. Systematic terminology of chiropteran used in this paper follows Rosevear (1965) and Walker (1999), while classification of filoviruses follows the revised filovirus taxonomy of the 9th report of the International Committee on Taxonomy of Viruses (ICTV) (Kuhn et al., 2010; 2013). The distribution maps of bats are documented from the available bibliographic data and unpublished collection data from the IRD laboratory of medical zoology, in Dakar, Senegal. We hypothesize the potential amplifying mechanisms, and the ways from which human populations might become infected from sylvatic cycles. We also specify the eventual role of various potential bat reservoir species.

## BACKGROUND OF FILOVIRAL HEMORRHAGIC FEVER OUTBREAKS

### Filoviruses, the causative agents

The causative agents of FHF are non-segmented, enveloped negative-sense, single-stranded RNA viruses, that morphologically resemble rhabdoviruses and functionality paramyxoviruses, similar also in their genome organization, expression and replication (Feldmann et al., 1993; Beer and Kurth, 1999). RNA viruses have a high ability to rapidly evolve in response to changing host and environmental circumstances via multiple genetic mechanisms, what classify them among the most dangerous emerging and re-emerging pathogens (Morens and Fauci, 2013). The family *Filoviridae* (filo derived from: *filum*, Latin) comprises three genera: *Ebolavirus*, *Marburgvirus* and *Cuevavirus*. The two first ones are the most known because they were described during deadly filoviral hemorrhagic fever epidemics. A third genus, *Cuevavirus*, (species *Lloviu cuevavirus*) less known than the precedents, was only described after a filoviral outbreak [viral pneumonia due to Lloviu virus (LLOV)] which affected a population of the Schreiber's bats, *Miniopterus schreibersii* Kuhl, 1817 in Spain, Europe (Negredo et al., 2011). The genus *Ebolavirus* includes

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five genetic and antigenic subtypes: *Bundibugyo ebolavirus* (BEBOV), *Zaire ebolavirus* (ZEBOV), *Reston ebolavirus* (REBOV), *Sudan ebolavirus* (SEBOV) and *Taï Forest ebolavirus* (TAFEBOV) or *Ivory Coast ebolavirus* (ICEBOV). The genus *Marburgvirus* accounts for a single species, *Marburgvirus marburgvirus* (formerly *Lake Victoria marburgvirus*), which consists of two very divergent "viruses": Marburg virus and Ravn virus, approximately 20% divergent at a genetic level (Carroll et al., 2013; Kuhn et al., 2010, 2013; Towner et al., 2006, 2009). This is in contrast to the known diversity for Ebolavirus species, with *Zaire ebolavirus* having only a 2.7% nucleotide difference between sequences, *Sudan ebolavirus* 5.2%, and *Reston ebolavirus* 4.5% (Lauber and Gorbalenya, 2012; Carroll et al., 2013).

Despite increasing numbers of viruses being detected, some species are represented by single viral lineage (for example, *Taï Forest ebolavirus* by Forest virus and *Lloviu cuevavirus* by Lloviu virus). During the 1998 Marburg Viral Disease outbreak that occurred in northeastern DRC, nine genetic lineages of the virus were involved (Bausch et al., 2006). In 1976, when *Ebolavirus* described 9 years after *Marburgvirus* presented the same filament-like structure as *Marburgvirus*, both were included in the same family of *Filoviridae,* newly described (Kiley et al., 1982). With the growing awareness of the rising threats to humans and wildlife caused by filoviruses, the importance of bats as potential reservoirs of viruses are much more investigated and will probably provide more divergent lineages within *Filoviridae*, that will enrich these taxonomic classifications.

## Discovery of filoviruses

### *Ebolavirus*

The first emergences of *Ebolavirus* were documented from Yambuko (DRC), Nzara and Maridi (Sudan) in 1976 with very high case fatality rates of 88 and 53%, respectively, caused by two distinct species of *Ebolavirus*: *Z. ebolavirus* (ZEBOV) (Johnson, 1978), and *Sudan ebolavirus* (SEBOV) (Smith, 1978). The source of transmission remains unknown. The causative agent was then named *Ebolavirus* after the Ebola River running along the Yambuku village, in the North Equator province of the Democratic Republic of Congo (formerly Zaire), where it was first diagnosed in the human population in 1976, simultaneously as in Nzara, Sudan (Smith, 1978). The number of cases has risen steeply and *Ebolavirus* outbreaks re-emerged after a long silent period (1980-1993), with increased frequency and new species discovery: *Côte d'Ivoire ebolavirus* (CIEBOV) in 1994 in the Ivory Coast and, *Bundibugyo ebolavirus* (BEBOV) in 2007 in Uganda (Towner et al., 2008). While re-emerging in Gabon and Republic of the Congo, *Ebolavirus* incidence in human was concomitant with amarkedmortality amongst gorillas and chimpanzees infected with the ZEBOV strain. *Ebolavirus* epidemics occurred between latitudes 10°N and 10°S, on both sides of the equator (Peterson et al., 2004; Groseth et al., 2007), approximately corresponding to the Afrotropics, with exception of *S. ebolavirus* which emerged at the extreme Eastern. The disease spread from Central to West Africa. Four of the known *Ebolavirus* species have emerged in sub-Saharan Africa, causing deadly outbreaks: *S. ebolavirus* (SEBOV), *Ivory Coast ebolavirus* (CIEBOV), *Bundibugyo ebolavirus* (BEBOV), and *Zaire ebolavirus* (ZEBOV) recently incriminated in the biggest Ebola epidemic ever recorded touching Guinea, Sierra Leone, Liberia (Baize et al., 2014) and lastly Nigeria, Senegal and Mali. From the past, epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo and Uganda (Smith, 1978; Le Guenno et al., 1995, 1999).

### *Marburgvirus*

The other member of the *Filoviridae* family is *Marburgvirus*, the silent cousin of Ebola. The virus Marburg was named after Marburg in Germany, but originated from Uganda, in Central Africa. Vervet monkeys [*Chlorocebus aethiops* (Gray, 1821)] importation for research purpose in Marburg and Belgrade (formerly Yugoslavia) brought the virus to these countries in 1967 (Smith et al., 1967). The first manifestation of *Marburgvirus* in Africa was a sporadic and fatal case, documented in Johannesburg, South Africa, in February 1975 from an Australian who came from Zimbabwe. Marburg hemorrhagic fever epidemiology will be discussed below. Ebola and Marburg viruses occurred in Africa, and at a much lesser extent in a primatology research center, in Manilla, Phillipines where *Reston Ebolavirus* (REBOV) has been described from cynomologus monkeys (*Macaca fascicularis* Raffles, 1821) imported into America (Philadelphia, 1989; Alice, Pennsylvania, 1990, 1996) and Italy (1996) (Rollin et al., 1999; WHO, 1992).

## Epidemiology of Filoviral hemorrhagic fevers

### *Ebola hemorrhagic fever (EHF) or ebola virus disease (EVD)*

EHF (EVD, International Classification of Diseases, ICD-10) is of major public health concern in the rural areas of sub-Saharan Africa, where *Ebolavirus* reached human population, after escaping its sylvatic foci first, then spread into rural/urban areas where it caused deadly hemorrhagic manifestations in human population. Multiple *Ebolavirus* species are co-circulating in endemic areas and the emerging zoonosis remains one of the most important zoonotic viral diseases of human in sub-Saharan Africa, because there is no approved treatment

and no licensed vaccine. EVD outbreaks occurred sporadically in Africa, scattered, within 10° latitude of the equator (Peterson et al., 2004; Groseth et al., 2007). This area is of dense and humid rainforest, characterized by succession of two rainy seasons and two dry seasons, providing the ecological niches favorable for *Ebolavirus* spp. amplification, maintenance and circulation.

It is likely that the vertebrate animals involved in *Ebolavirus* circulation find the optimal conditions necessary for sheltering, feeding and breeding and that the factors modulating *Ebolavirus* emergence are associated with those ecosystems. Spatio-temporal distributions of human *Ebolavirus* spp. outbreaks in Africa have already been well documented and mapped (Peterson et al., 2004; Pourrut et al., 2005; Groseth et al., 2007; Changula et al., 2014; Rougeron et al., 2015). *Ebolavirus* epidemics arose generally at the same time of the year (end of the dry season-beginning of the rainy season), when reservoir species of the virus gather with other sensitive hosts because of scarcity of food source, modification of ecological habitats which imply encroachment of different vertebrate animals. Also, population dynamic over time (physiological status such as reproduction time, demographic explosion of sensitive naive species) and space (migration) might conduct to amplification and emergence of *Ebolavirus*.

### *Ebolavirus dissemination*

When the optimal conditions for *Ebolavirus* spp. circulation into those ecosystems are met, their probability to escape from these foci is enhanced. Peterson et al. (2004) used an ecologic niche modeling of outbreaks and sporadic cases of filovirus-associated hemorrhagic fever (HF) to provide a large-scale perspective on the geographic and ecologic distributions of Ebola and predicted that EVD would occur in the humid rain forests of central and western Africa. They observed that filovirus' transmission to humans is not common, and most occurrences can be traced to a single index case (WHO, 1978), followed by a spillower reaching the population. The following hypotheses can be considered for the introduction of the virus to nonhuman primate populations: 1) Non-human primates might have shared and eaten fruit rests containing virus in residual bat saliva and directly infected themselves. Gonzalez et al. (2007) theorized this pathway, stating that chronically *Ebolavirus* spp. infected bats might drop down partially eaten and masticated fruit spats or pulp picked from the canopy to the ground, promoting indirect transmission of the virus to some terrestrial dwelling mammals. Viral particles shed in bat saliva infected by the way, infect the rests of fruits secondly eaten by ground mammals. It has been shown that females chimpanzees mostly gave some collected fruit to their depending offspring and that adult male share meat with females and juveniles (de Wall, 1989); 2) Infected individuals can contaminate their group during care and social behavior, 3) Great apes also hunt and share other primates preys such as vervets, galagos and colobes and can be infected with contaminated meat. Assessing that infection of primates colonies begin with a single index case is then more difficult to support. Several individuals can contract the virus at the same time and contribute to disseminating it, because of their social behavior, 4) Natural secretions such as feces, urine, body fluid, placental rest and secretion might be shed in nature and represent a potential source of contamination to other small terrestrial mammals. Great apes and forest duikers fed on fruit rests become infected and might later represent the first link of a human transmission chain if rural communities enter into contact with those wild animals, via hunting. It is an epidemiological schema that might transpose the virus in a human population.

Olival and Hayman (2014) summarized, in their proposed transmission dynamic, that chiropteran are the potential reservoirs maintaining an intra-interspecies *Ebolavirus* circulation, and transmitting it to non-human primates and forest duikers; while direct transmission to human as well as rodents and pigs remain to be elucidated. Also, there is no yet evidence that wild animals, excepted non-human primates, can transmit directly the virus to human populations. The role of mosquitoes in their transmission model is questionable, interhuman transmission via natural secretions favors the virus spreading. Bausch et al. (2007) tested several body fluids as saliva, stool, semen, breast milk, tears, and nasal blood and concluded that EBOV is shed in a wide variety of bodily fluids during the acute period of illness but that the risk of transmission from vomits in an isolation ward and from convalescent patients is low. Humans can transmit the virus as soon as symptoms appear and continue to be infectious during the later stages of the disease as well as after death. Burial ceremonies in which mourners have direct contact with the body of the deceased person can also play a role in the transmission. *Ebolavirus* has been detected in semen for up to 82 days, and *Marburgvirus* for up to 13 weeks (Martini and Smith, 1968; Bausch et al., 2007), after the onset of illness, suggesting that these viruses could be eventually transmitted by sexual route (Bausch et al., 2007).

### *Analyzing the origin of contaminations*

After the first Ebola outbreaks that occurred between 1976 -1979 (DRC and Sudan), the second waves of *Ebolavirus* spp. epidemics occurred between 1994-1997, after a silent period of 15 years; a first case was linked to a chimpanzee autopsied by a Swiss ethnologist in Ivory Coast, West Africa, and was attributed to a new strain, CIEBOV. The Kikwit epidemic (DRC), Mekouka, Mayibout and Booue (Gabon) were due to ZEBOV reemergence (Amblard et al., 1997; Georges et al., 1999). The source was a deep forest gold-mining camp, suggesting that

workers of the mine entered the reservoir/vectors biota. Mayibout outbreak was related to Mekouka's epidemic. Booue epidemic also began by an infected hunter who accidentally entered the sylvatic cycle at this time, while a high viral sylvatic amplification was going on as suggested by died chimpanzees that tested positive for *Ebolavirus* infection. From 2000 to 2004, multiple epidemics were recorded and attributed to ZEBOV at the border of Gabon and the Republic of Congo and to SEBOV in Sudan and Uganda, affecting simultaneously large populations of gorillas and chimpanzees (Leroy et al., 2002, 2004b; Bermejo et al., 2006). The first findings that the Swiss ethnologist was infected by a chimpanzee and the fact that the Mayibout outbreak originated in deep forest and was related to a gold-mine, drew the schema of an implication of forest mammals, more specifically cave dwelling mammals. ZEBOV remerged in 2005 in the Republic of Congo, in 2007-2009 in Democratic Republic of Congo, twelve years after the 1995 Kikwit outbreak. Two successive epidemics arose in the Luebo region (Kasai Occidental Province, DRC) in 2007 and 2008 and were caused by *Zaire ebolavirus* (Grard et al., 2011). Phylogenetic analyses performed on the full-length genomes of the two Luebo strains revealed that they were nearly identical, but not related to the lineage including ZEBOV strains from the 1976-1996 outbreaks (DRC and Gabon), nor to the descendants of the lineage including animal-derived sequences since 2001 and the human strains from the Mbandza-Mbomo 2003 and Etoumbi 2005 outbreaks (Gabon-RDC), with which they do, however, share a common ancestor (Grard et al., 2011). The Luebo 2007 outbreak represented an independent viral emergence, favored by a viral spillover caused by a dispersed reservoir species. Like the 1994-1997 Gabonese epidemics, these cross-border outbreaks were concomitant to marked wildlife epizootics (Leroy et al., 2004b; Rouquet et al., 2005; Lahm et al., 2007).

Chimpanzees, gorillas and duikers were susceptible hosts responsible for viral introduction into human populations. SEBOV emergence was also recorded in Uganda from 2011-2012, as in the DRC in 2012 (http://www.cdc.gov/vhf/ebola/resources/outbreak-table.html). In their modeling of geographic distribution of filovirus disease across Africa, Peterson et al. (2004) predicted the eastern extreme as the predilection area of *S. ebolavirus*, but this species emerged in DRC, the viral spillover being probably favored by widely dispersed reservoirs. In the past decades, in particular, FHFs incidences have increased and have been seen in areas they were not reported previously. Before, FHFs have never been recorded in Guinea until December 2013 when the first cases arose in the Southeast (Baize et al., 2014). Ebola virus disease was spreading unrecognized, while typical hemorrhagic fever cases such as Lassa fever or yellow fever, endemic in the area, were suspected but not proven. The hemorrhagic disease has been spreading quietly until late March 2014 when the diagnosis was finally confirmed Ebola virus disease. Human to human transmission via contact of fluids favored a spillover and the disease reached the neighboring countries of Sierra Leone and Liberia bordering the original epicenter of the outbreak. Lastly, the outbreak reached unexpected proportion in two months (Baize et al., 2014; Gire et al., 2014; Pigott et al., 2014; Wauquier et al., 2015), overwhelming the fragile health system in those developing West African countries. The epidemic touched the cities of Conakry (Guinea), Freetown (Sierra Leone), Monrovia (Liberia), Lagos (Nigeria), Dakar (Senegal) and Kayes (Mali), reaching the specter of a regional, even international dissemination. In fact, imported cases have been noticed in the USA (Dallas, Texas; Chevalier et al., 2014), Spain (Madrid; Parra et al., 2014) and the United Kingdom (London; Kuhn et al., 2014). Also, contaminated healthcare workers have been transferred to Hamburg (Germany) and Lyon (France) for care. The disease spread from Central Africa to West Africa. Among the known *Ebolavirus* species, four have emerged in sub-Saharan Africa, causing deadly outbreaks: *S. ebolavirus* (SEBOV), *Ivory Coast ebolavirus* (ICEBOV), *Bundibugyo ebolavirus* and *Z. ebolavirus* (ZEBOV) recently incriminated in the biggest Ebola epidemic ever recorded. The forested area of Guinea has been the epicenter and the source of contamination is discussed subsequently. While the Guinean EVD outbreak was spreading in the neighboring countries of West Africa, Ebolavirus reemerged in July 26, 2014, for the seventh time, in Democratic Republic of Congo, in Inkanamongo village, in the vicinity of Boende town (Equateur province). A total of 69 cases were reported, including 8 cases among health care workers, with 49 deaths (Maganga et al., 2014). A coding-complete genome sequence of EBOV that was isolated during this outbreak showed 99.2% identity with the most closely related variant from the 1995 outbreak in Kikwit (DRC) and 96.8% identity to EBOV variants that are currently circulating in West Africa (Maganga et al., 2014). The two outbreaks were in fact caused by two novel EBOV variants, consensually named Makona (West Africa) and Lomela (Middle Africa), after the Makona River close to the border between Liberia, Guinea and Sierra Leone and the Lomela River that runs through DRC's Boende District, respectively (Kuhn et al., 2014). The genetic characterization of the virus, combined with the geographic location of the outbreak, demonstrate that the DRC outbreak is an independent event, without any epidemiologic or virologic connection with the continuing epidemic in West Africa (Kuhn et al., 2014; Maganga et al., 2014).

**Marburg hemorrhagic fever (MHF) or Marburg viral disease (MVD)**

*Marburgvirus* was described from the Behring laboratory,

in Marburg, Germany from Vervet monkeys (*Chlorocebus aethiops*) imported from Uganda (Smith et al., 1967). Infected monkeys presented typical hemorrhagic fever clinical tables (Jahrling et al., 1990; Peters et al., 1992). That first Marburg outbreak reported with severe viral hemorrhagic fever was related to the handling of organs and tissues from those green monkeys (Smith et al., 1967; Martini, 1969). Eight years later, the first manifestation of *Marburgvirus* in Africa happened, in Johannesburg, South Africa, in February 1975, sporadic and fatal. It concerned an Australian just returning from a trip to Zimbabwe where he slept frequently in the open and once in an abandoned house which loft was inhabited by numerous bats (Gear et al., 1975). The third recognized Marburg manifestation affected a French engineer in Kenya in 1980 that subsequently infected his doctor before dying. He visited the Kitum cave (Mont Elgon National Park) where large populations of bats were sheltering. Next, another Marburg case has been reported and concerned a Danish who died after visiting the Kitum cave in August 1987 (Kenyon et al., 1994). After a silent period of more than 30 years, Marburg virus, the long neglected Ebola virus relative, called for attention in its cradle of Central Africa, hitting twice recently, and in large proportion: 1) 1998-2000, a gold-mining community in Durba, in the northeastern region of the Democratic Republic of the Congo, was affected with a high mortality rate reaching 83% (Rec, 1999; Baush et al., 2006); 2) 2004 and 2005, a second and large Marburg outbreak followed in northern Angola (West Africa), in the province of Uige (Rep, 2005; Towner et al., 2006) with a mortality rate higher than that during the 1998-2000 outbreak of Durba above cited (Towner et al., 2006).

Surprisingly, an Ebola outbreak was expected because of the large area affected reaching a big community since a first single infected case working in a gold-mining company. In July and September 2007, miners working in Kitaka Cave, Uganda, were diagnosed with MHF (Towner et al., 2009). At the same time (June-September 2007), 4 miners from Ibanda District contracted MHF through exposure to bats secretions in a mine in Kamwenge District, Uganda (Adjemian et al., 2011). Genetically diverse viruses isolated from tissues of the Egyptian Fruit Bat as well as detection of RNA MARV from these bats supported that *Rousettus aegyptiacus* was responsible for the epidemic. In late 2007, an American tourist contracted MVD in the python cave and in July 2008, another tourist from Netherlands was also infected with MARV in the same cave, from which diverse genetically MARVs were also isolated from *R. aegyptiacus* (Amman et al., 2014). Confined in a jungle cycle as *Ebolavirus*, *Marburgvirus* emerged and expressed its pathogenic potential, such as that one for *Ebolavirus*, without any doubt. As for *Ebolavirus* epidemics, *Marburgvirus* outbreaks in Africa were also well mapped and documented (Bausch et al., 2006; Feldmann, 2006; Brauburger et al., 2012; Rougeron et al., 2015). Imported human cases of Marburg virus infection from Uganda have been also reported in the USA (Timen et al., 2009) and in Netherlands (Fujita et al., 2010). Practically, all MARV emergences have been related to bat shelters (caves, gold-minning areas) and contact with infected monkeys (Cercopithecidae). These events clearly traced back the source of contamination to chiropters and primates Cercopithecidae. Both filoviruses are afrotropical, originally infectious of fruit bats (Chiroptera, Pteropidae) that seem playing the major role in their epidemiology, namely their maintenance and circulation in nature that will be discussed in a comparative manner in this review. *Ebolavirus* emerged mostly than *Marburgvirus*, but in terms of epidemiology both filoviruses are very similar. They share bats as the same vertebrate hosts.

## Clinical manifestations and pathology of Ebola and Marburg viral diseases

At several times that a FHF arose in Africa, other endemics diseases such as Lassa fever, Yellow fever, malaria, cholera or typhoid fever were suspected. That has been the case for this ongoing Ebola epidemic in West Africa, where local Guinean healthcare workers attributed the first reported hemorrhagic cases to Lassa fever (Vogel, 2014). In 2007, the RDC ZEBOV emergence was also concomitant to an epidemic of typhoid and shigellosis. Then, the clinical table of filovirus-infected patients is non-specific and difficult to separate from other endemic diseases. The asymptomaticincubation period of filoviruses is 2-21 days. Symptoms usually manifest abruptly by a fever (greater than 38.6°C), severe headache, muscle pain and malaise. Secondly, severe diarrhea, nausea, vomiting, respiratory disorders, abdominal pain and weakness appear, accompanied with a lack of appetite. Hemorrhagic manifestations are observed in 30-50% of patients and vary in severity. Spontaneous abortion has been recorded within pregnant woman (Baize et al., 2014; Vogel, 2014). The pathogenesis of thesehemorrhagic fevers includes necrosis of many organs, particularly liver (Martines et al., 2014). It has been suggested that the hemorrhages and shock manifestations may be a consequence of endothelial cell infection, with consequent loss of endothelial integrity leading to rapid hypovolaemic shock, multiple effusions and bleeding (Fisher-Hock et al., 1985). Death ensues within few days but some infected people recover.

However, patients who die usually have not developed a significant immune response to Ebola infection. *Z. ebolavirus, S. ebolavirus, Bundibugyo ebolavirus* and *Forest ebolavirus* cause severe illness in humans, although Forest virus infections have rarely been documented. *Reston ebolavirus* does not seem to be pathogenic for humans, but people may seroconvert after exposure to infected nonhuman primates or pigs.

Infection with *Marburgvirus* develops an acute illness for up to three weeks at least, accompanied by the following signs and symptoms: fever, generalized body pain, nausea and vomiting, headache, anorexia, malaise, abdominal pain, diarrhea, dyspnea, dysphagia, hiccups, conjunctivitis, rash or petechiae and abnormal bleeding from the nose, mouth, gastrointestinal tract, or genitourinary tract (Bausch et al., 2006). Death arises within few days, but as for EVD, some MVD infected people recovered.

## The reservoir search

Several investigations targeting different vertebrate animals have been undertaken to identify the natural vertebrates that host and lurk Ebola virus in nature, after the first emergences. Arata and Johnson (1977) tested 100 specimens from 501 vertebrates collected in 1977 from Sudan; Germain (1978) screened more than 800 bedbugs and 147 mammals in DRC; Breman et al. (1999) collected 1664 animals of 117 species around the areas where the 1976 Ebola hemorrhagic fever occurred in the DRC and in Cameroon; Leirs et al. (1999) screened 3000 animals primarily from forest areas near the home of the index case after the Kikwit Ebola epidemic (DRC). Samples were representative of the different class of mammalia, reptilia and birds; even plants were suspected and tested. Globally, no evidence of *Ebolavirus* infection was found. Swanepoel et al. (1996) conducted experimental inoculation of thirty-three varieties of 24 species of plants with *Z. ebolavirus*, no evidence of infection was observed. Vertebrate animals inoculated included pigeons, young snakes, rodents, laboratory mice colonies, tortoises, lizards, frogs, toads and bats. Two microchiroptera of the family Molossidae, the Angola free-tailed bat, *Tadarida condylura* and the little free tailed bat, *Tadarida pumila* and one megachiroptera of the family Pteropidae, the Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* were able to asymptomatically replicate the ZEBOV with high viral titers, 4 weeks after inoculation, demonstrating for the first time that bats might be reservoirs hosts of *Ebolavirus* (Swanepoel et al., 1996). Invertebrates as cockroaches, leafhoppers, spiders, social ants, myrmicine ants, millipede and land snails were also inoculated but did not yield any proof of virus replication (Swanepoel et al., 1996). Turrell et al. (1996) negatively tested the ability of three mosquitoes *Aedes albopictus, Aedes taeniorhynchus* and *Culex pipiens* (Diptera, Culicidae), and one soft tick, *Ornithodoros sonrai* (Ixodida, Argasidae) for *Ebolavirus*. Arthropods have never been successfully infected following inoculation (Swanepoel et al., 1996, Turell et al., 1996), although several observations suggest they can transmit Ebola virus to humans, as demonstrated by Kunz et al. (1968) who showed that Marburg virus persist for more than 3 weeks in *Aedes* mosquitoes after experimental inoculation. Since their first emergences in 1976 (Ebolavirus in Yambuko, RDC and Nzara, Sudan), and in 1975 (*Marburgvirus* in Johannesburg, South Africa), natural reservoirs of filoviruses remained elusive for 3 decades and any investigation was not able to reveal where these viruses persist in nature, during inter-epidemic periods until 2005 when Leroy et al. (2005) provided the first evidence of bats as possible natural reservoirs.

The first documented primary infections of natural MVD outbreaks in Africa have been linked to human visiting caves inhabited by bats: gold mining in Kitaka Cave in the Kamwenge District, Uganda (Adjemian et al., 2011); visit of python Cave in Maramagambo Forest Uganda (Fujita et al., 2010; Timen et al., 2009). These findings provided the first clues that bats might play an important role in the transmission cycle of MVD (Monath, 1999; Peterson et al., 2004; Bausch et al., 2003), and evidence of MARV circulation in bats was only been documented when Towner et al. (2007) first detected MARV nucleic acids and antibodies from the common Egyptian fruit bat, *Rousettus aegyptiacus* in 2002 and 2005 in Gabon, without any virus islation. Swanepoel et al. (2007) also found MARV nucleic acid and antibody to the virus in the serum of insectivorous and fruit bats trapped in the Goroumbwa Mine, in northeastern DRC, but their attempts to isolate the virus were unsuccessful. Later, Towner et al. (2009) isolated MARV nine months apart from Egyptian fruit bats of the Kitaka cave in Uganda, demonstrating long-term virus circulation among the bat reservoir species. Genome sequences of MARV isolated from bats closely matched those isolated from miners during this epidemic, indicating that common Egyptian fruit bats represent major natural reservoir and source of Marburg virus with potential for spillover into humans. Despite the isolation of MARV from naturally infected Egyptian fruit bats captured in the Kitaka cave near Ibanda, in Western Uganda (Towner et al., 2009) and the python cave in the Queen Elisabeth National Park, Uganda (Amman et al., 2014), experimental inoculation of *R. aegyptiacus* with MARV were conducted and showed that the species is a natural reservoir host for MARV and demonstrated routes of viral shedding via rectal and oral routes capable of infecting humans and other animals (Amman et al., 2015). While the *Marburgviruses* exhibit high overall genetic diversity (up to 22%), only 6.8% nucleotide difference was found between the West African Angolan viruses and the majority of East African viruses, suggesting that the virus reservoir species in these regions are not substantially distinct. Remarkably, few nucleotide differences were found among the Angolan clinical specimens (0 to 0.07%), consistent with an outbreak scenario in which a single (or rare) introduction of virus from the reservoir species into the human population was followed by person-to-person transmission with little accumulation of mutations. This is in contrast to the 1998 to 2000 *Marburgvirus* outbreak, where evidence of several virus

genetic lineages (with up to 21% divergence) and multiple virus introductions into the human population was found (Towner et al., 2006).

### *Wild vertebrate hosts sensitive to Filoviruses*

With the exception of *Reston ebolavirus,* all African filoviruses cause severe illness in nonhuman primates and some other animals. While there is no formal evidence for a causative role in some species, *Ebolavirus* outbreaks have been linked to reports of massive die-off of gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations. An outbreak of Ebola decimated in November 1994, 25% of a wild chimpanzee community of 43 members in the Taï National Park, in Ivory Coast (Formenty et al., 1999), as did another in great apes of Minkebe Forest, north-eastern Gabon and in western equatorial Africa (Huijbregts et al., 2003; Walsh et al., 2003). Between 2001 and 2003, the epidemics that occurred in Gabon and Republic of Congo were also, for the first time, linked to concurrent animal mortality, mainly gorillas, chimpanzees and duikers (Leroy et al., 2004b; Bermejo et al., 2006). Detection of EBOV infected corpses in these three species strongly incriminated *Ebolavirus* as the causative agent.

Their population decreased and duikers were estimated to have fallen by 50% between 2002 and 2003 in the Lossi sanctuary, Republic of Congo, while chimpanzees lost 88% of their populations (Leroy et al., 2004b). *Ebolavirus* was also incriminated in a marked decline in gorilla and chimpanzee populations in the same areas, at the same point in time in Mekouka and Mayibout outbreaks. Small EBOV-specific genetic sequences were amplified from organs of six mice (*Mus setulosus* and *Praomys* sp., Rodentia, Muridae) and a shrew (*Sylvisorex ollula*, Insectivora, Erinaceidae), in Central African Republic and provided the first documented biological evidence of EBOV presence in healthy animals (Morvan et al., 1999), however this data was not sufficient enough, to attribute a reservoir status to these animals, being given lack of specific serologic responses, nucleotide specificities in the amplified viral sequences, failure of virus isolation, and the non-reproducible nature of the results. *Ebolavirus* infects a large variety of animal species, as attested by exploration of dead wild animal carcasses analyses. During the Gabon and RC epidemics (2001- 2004), the remains of animals were found in the surrounding forest (Rouquet et al., 2005). Thirty four samples taken from those carcasses (bones, muscles and skin) were analyzed using a panel of highly sensitive techniques, such as reverse-transcription polymerase chain reaction (RT–PCR), serology, histopathology and immunohisto-chemistry (IHC). Fourteen of them (10 gorillas, 3 chimpanzees and 1 duiker) tested positive for Ebola infection, indicating that these three animal species can be naturally infected by EBOV.

Most infected animals probably died rapidly, as suggested by the rapidly fatal nature of experimental EBOV infection in a variety of non-human primate species (Pourrut et al., 2005). Analyses of animal carcasses show that the great apes of the central African forests are particularly at risk for Ebola. This was confirmed by a serologic survey based on 790 samples taken from about 20 primate species in Cameroon, Gabon and Republic of Congo (Leroy et al., 2004a). Interestingly, some positive samples largely preceded the first human outbreaks in these regions, suggesting a viral sylvatic amplification chronologically happening before human contact with the virus. The results suggest that these animals are in regular contact with the EBOV reservoir, that some of them survive the infection, and that EBOV has probably been present for a very long time in the central African forest region. EBOV-specific antibodies were also found in other monkey species such as mandrills (*Mandrillus* sp.), vervets (*Cercopithecus* sp.), baboon, and drills suggesting that EBOV circulation between Cercopithecidae may be very complex, and some of their representative might be amplifying hosts because some great apes developed an Ebola viremia after eating their congeners Cercopithecidae. *Ebolavirus* epidemiology might involve other reservoir/amplifying hosts' species different to bats, and the passage of the virus to gorillas and chimpanzees might be more complex than a simple direct contact from the main reservoir. It is also possible that there are several reservoir species, and that many other animal species are susceptible to the virus and thereby participate in the natural EBOV life cycle (Figure 1). These include duikers (forest antelope, *Cephalophus dorsalis,* Onguligrades, Artiodactyla, Bovidae) and bush pigs (red river hog, *Potamochoerus porcus,* Onguligrades, Artiodactyla, Suidae). Overall, non-human primates of the family Cercopithecidae (colobus, baboons, mandrills, vervets and guenons) seem less sensitive to *Ebolavirus* infection as compared to non-human primates of the family Hominidae (chimpanzees and gorillas).

The Egyptian fruit bat is the potential reservoir of MARV. Marburg virus has been circulating in this species between the python cave and the Kitaka cave in Uganda as suggested by virus' isolation obtained by Towner et al. (2009) and Amman et al. (2014). The fact that Marburg and Belgrade epidemics were caused by *Chlorocebus aethiops* imported from Uganda support a typical reservoir role of this green monkey for the virus Marburg. In fact, the monkeys that carried the virus to Europe in 1967 were kept on Lake Victoria island, in a holding facility where large numbers of fruit bats were sheltering (Swanepoel et al., 2007). Uganda represents a "hotspot" for MARV circulation. It's actually known that transmission cycle can be schematized as presented in Figure 2.

### *Chiropteran as probable natural reservoirs of filoviruses*

Enquiries were carried out in Central Africa, aiming to

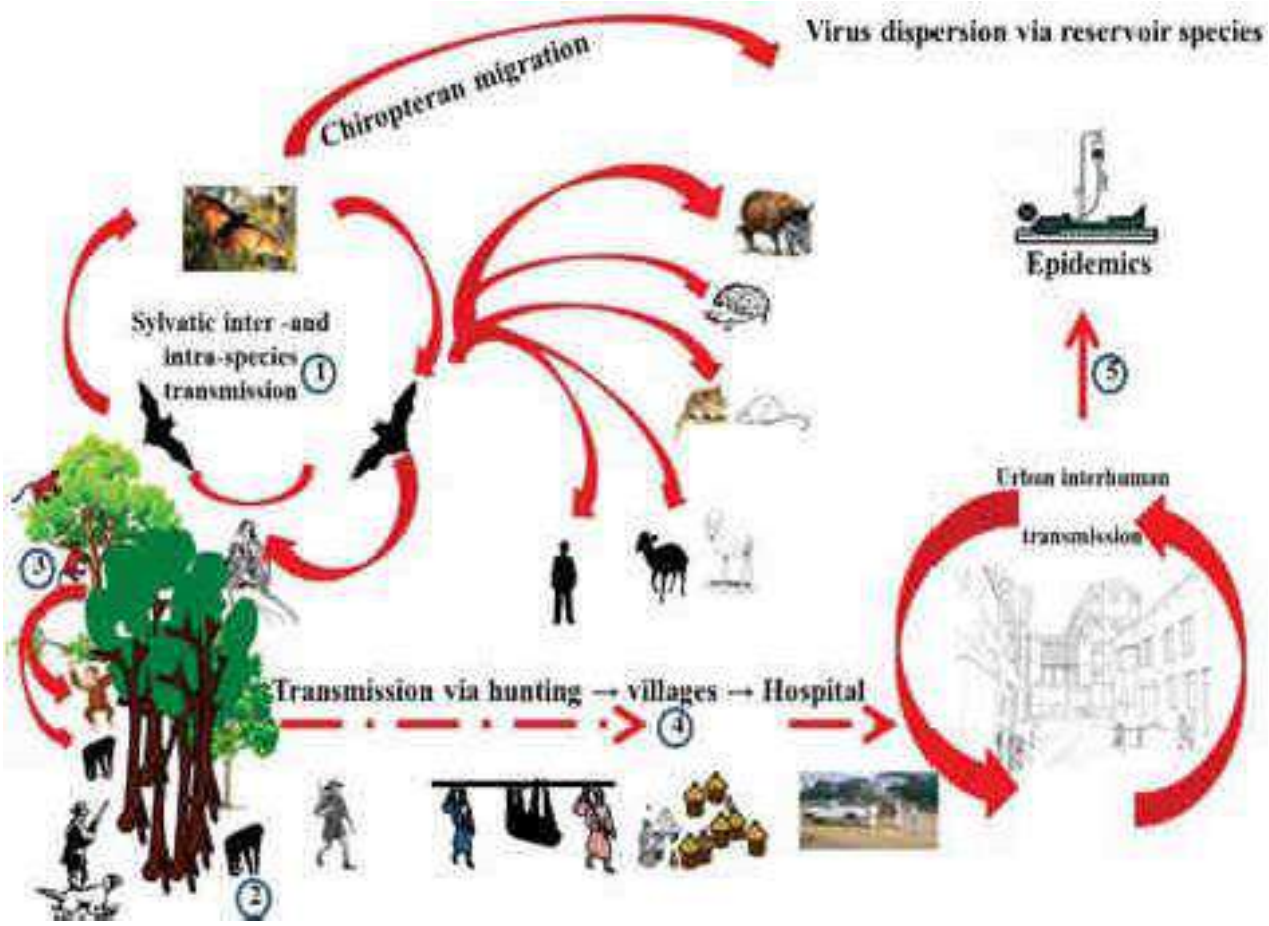


**Figure 1.** Ecoiagram of *Ebolavirus* transmission in nature. Fruit bats infected with Ebolavirus partially eat wild fruits in the forests (1). Partially chewed fruit contain virus particles enrobed in bat's saliva and dropped down from trees, contaminate other ground animals such as rodents, Insectivora, Onguligrades and non-human primates (2). Infected bats and Cercopithecidae are also eaten by great apes that are subsequently infected (3). Man can also be infected after intrusion in the canopy (caves and bat shelters) receiving directly bat's secretion infected with Ebolavirus. Mostly, hunting and handling of bushmeat (4) transposed ebolavirus from a sylvatic to an rural/urban transmission cycle causing deadly epidemics (5).

identify the natural reservoirs species of filoviruses (Leroy et al., 2005; Gonzalez et al., 2007; Pourrut et al., 2009). They found that bats belonging to the family Pteropidae were the major susceptible population, asymptomatically infected by the virus as attested by antibodies and viral nucleic acid detection. Serological studies conducted allowed to detect specific anti Ebola IgG from 16 bats: 4 Hammer-headed Fruit Bat, *Hypsignathus monstrosus* H. Allen, 1861, 8 Franquet's Epauletted bat, *Epomops franqueti* Tomes, 1860 and 4 Little Collared Fruit bat, *Myonycteris torquata* Dobson, 1878 (Chiroptera, Pteropidae) (Leroy et al., 2005; Gonzalez et al., 2007; Pourrut et al., 2009). Their studies also detected viral nucleic acid sequences in the tissues of 13 bats (3 *H. monstrosus,* 5 *E. franqueti* and 5 *M. torquata*) and provided the first evidence of bats' role as probable potential reservoirs of *Ebolavirus* in nature (Table 1). Swanepoel et al. (2007) investigated the reservoir hosts for Marburg virus (MARV) after the epidemic that hit the gold mining-community in Durba and detected MARV viral nucleic acid sequences from two insectivorous bats, the Greater Long-fingered Bat, *Miniopterus inflatus* Thomas, 1903 and the Eloquent horseshoe bat, *Rhinolophus eloquens* K. Anderson, 1905 (Microchiroptera, Rhinolophidae), and the Egyptian fruit bat, *Rousettus aegyptiacus* E. Geoffroy, 1810 (Megachiroptera, Pteropidae). Serological evidence of MARV circulation was detected by ELISA in *R. eloquens* and *R. aegyptiacus*. They concluded that these bats were implicated in *Marburgvirus* circulation around the Goroumbwa mine and its immediate surroundings. Towner et al. (2007) detected MARV-specific RNA, IgG antibody from *R. aegyptiacus* and isolated MARV for the first time from this species in Gabon, acting now as a typical reservoir of *Marburgvirus* (Towner et al., 2007). Pourrut et al. (2009) documented that both Ebola and

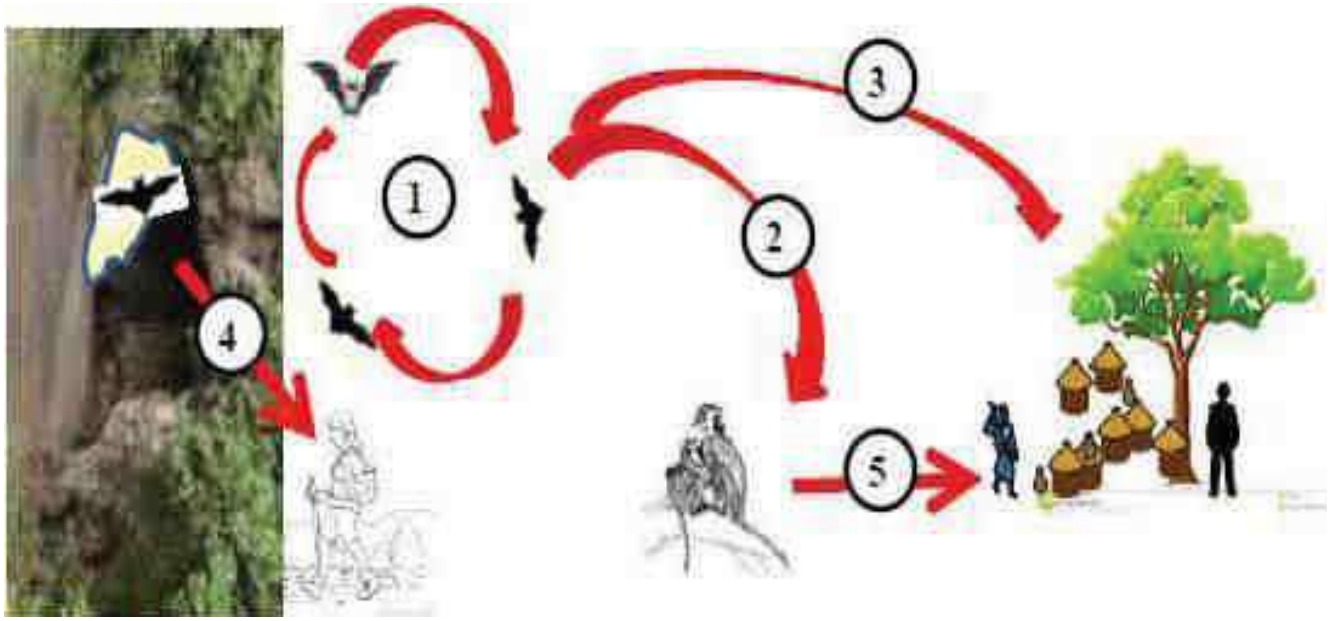


**Figure 2.** Ecodiagram of *Marburgvirus* transmission in nature. High intra-interspecific contact in roost facilitates rapid transmission of MARV between bats (1). Partially chewed fruit containing virus particles shed in bat's saliva and dropped down from trees, contaminate Cercopithecidae (2) and Hominidae (3). Man can also be infected after intrusion into the sylvatic (caves and bat shelters) receiving directly bat's secretion infected with MARV (4). Handling of monkeys tissues also directly infect human beings (5).

Marburg viruses co-circulated within the Egyptian Fruit Bat. Hayman et al. (2010) detected Zaire EBOV (ZEBOV) antibodies in a single Straw-colored Fruit Bat, *Eidolon helvum* Kerr, 1792 (Megachiroptera, Pteropidae) from a roost in Accra, Ghana; another fruit bat *Epomophorus gambianus* Ogilby, 1835 (Megachiroptera, Pteropidae) has been found infected with *Ebolavirus* by Hayman et al. (2012), as well as *E. franqueti* and *H. monstrosus*. Serological evidence of EBOV antibodies has been also detected in a serum sample of the Little flying Cow, *Nanonycteris veldkampii* Matschie, 1899 (Megachiroptera, Pteropidae) (Hayman et al., 2012). ZEBOV-IgG were detected again in *E. franqueti, H. monstrosus, R. aegyptiacus* and *M. torquata*; while the Lesser Epaulet bat, *Micropteropus pusillus* Peters, 1867 (Megachiroptera, Pteropidae) and *Mopscondylurus* Lesson (Microchiroptera, Molossidae) tested for the first time ZEBOV-IgG positive in nature (Pourrut et al., 2009). MARV-IgG were also found in *R. aegyptiacus* and *H. monstrosus* (Pourrut et al., 2009). Amman et al. (2012) investigated the Python Cave inhabited by the Egyptian Fruit Bat in Uganda and detected viral nucleic sequences of MARV; also seven of the bats yielded Marburg virus isolates (Table 1). Using an enzyme-linked immunosorbent assay based on the viral glycoprotein antigens, Ogawa et al. (2015) detected IgG ZEBOV, and MARV in serum samples collected from the fruit bats (*Eidolon helvum*) in Zambia during 2006-2013. Distinct specificity for *Reston ebolavirus*, so far known only from Philippines and China, in Asia (Barrette et al., 2009; Pan et al., 2014), has been shown also from *E. helvum* for the first time in Zambia (Ogawa et al., 2015). Serological evidence of antibodies directed against flaviviruses and detection of viral nucleic acid incriminate those chiropters as potential reservoirs of filoviruses in nature. The isolation of MARV in nature supports a typical status of *Marburgvirus* reservoir species for *R. aegyptiacus.* Overall, these findings suggest a closer follow-up of the other bats, particularly of the family Pteropidae that can play the major role. Researches on the role of bats as reservoirs of filoviruses, particularly *Ebolavirus* are still ongoing, several vertebrate animals as Great apes and duikers are naturally infected by this virus, probably directly from the reservoir, but the pathways of its emergence in human environment is not yet fully understood. However, the epidemiological scenario so far advanced, make bats the most probable reservoir candidates for filoviruses.

### *Domestic vertebrate animals sensitive to filoviruses*

Dogs and pigs are the only domestic animals so far identified as species that can be infected with EBOV. A survey conducted in Gabon on dogs eating dead animals showed over 30% seroprevalence for EBOV during the Ebola outbreak in 2001-2002 (Allela et al., 2005). Dogs asymptomatically incubate the virus; while pigs experimentally infected with EBOV can develop clinical disease, depending on the virus species. Pigs were experimentally able to transmit Zaire-Ebola virus to naive pigs and macaques; however, their role during Ebola

**Table 1.** *Marburgvirus* (MARV) and *Zaire ebolavirus* (ZEBOV), antibodies (IgG), and viral RNA sequences detected from bats in Africa.

| Date | Bat species | Vernacular name (Order, Family) | Filovirus isolated | | | | Filoviral event | |
|---|---|---|---|---|---|---|---|---|
| | | | Ebolavirus | | Marburgvirus | | | |
| | | | Antibodies detected | RNA sequences | Antibodies detected | RNA sequences | Locality | Reference |
| January 2008 | *Eidolon helvum* | Straw-colored Fruit Bat | IgG | PCR (-) | - | PCR (-) | Ghana<br>Zambia* | Hayman et al., 2010<br>Ogawa et al., 2015* |
| May-June 2007 | *Epomophorus gambianus* | Gambian Epauleted Bat (Megachiroptera, Pteropidae) | IgG | PCR (-) | - | PCR (-) | Ghana | Hayman et al., 2012 |
| June 2003-March 2008 | *Epomops franqueti* | Franquet's Epauletted Bat (Megachiroptera, Pteropidae) | IgG | PCR (+) | IgG | PCR (-) | Gabon, Ghana* | Pourrut et al. (2005, 2007; 2009)<br>Hayman et al., 2012* |
| May-June 2007 | *Hypsignathus monstrosus* | Hammer-headed Fruit Bat (Megachiroptera, Pteropidae) | IgG | PCR (+) | IgG* | PCR (-) | Gabon*, RC*, Ghana | Pourrut et al., 2009*<br>Hayman et al., 2012 |
| June 2003-March 2008 | *Micropteropus pusillus* | Lesser Epauleted Bat (Megachiroptera, Pteropidae) | IgG | PCR (-) | IgG | PCR (-) | Gabon | Pourrut et al., 2009 |
| June 2003-March 2008 | *Myonycteris torquata* | Little Collared Fruit Bat (Megachiroptera, Pteropidae) | IgG | PCR (+) | - | PCR (-) | Gabon, RC | Pourrut et al., 2009 |
| June 2003-March 2008 | *Hyposideros gigas* | Giant Leaf-nosed Bat (Microchiroptera, Hypossideridae) | - | PCR (-) | IgG | PCR (-) | Gabon, RC | Pourrut et al., 2009 |
| June 2003-March 2008 | *Mops condylurus* | Greater Mastiff Bat (Microchiroptera, Molossidae) | IgG | PCR (-) | IgG | PCR (-) | Gabon | Pourrut et al., 2005; 2007; 2009 |
| May-October 1999 | *Miniopterus inflatus* | Greater Long-fingered Bat (Microchiroptera, Vespertilionidae) | - | PCR (-) | - | PCR (+) | DRC | Swanepoel et al., 2007 |
| May-October 1999 | *Rhinolophus eloquens* | Eloquent Horseshoe Bat (Microchiroptera, Rhinolophidae) | - | PCR (-) | IgG | PCR (+) | DRC, Gabon* | Swanepoel et al., 2007<br>Pourrut et al., 2009* |
| June 2003-March 2008 | *Nanonycteris veldkampii* | Little flying Cow (Megachiroptera, Pteropidae) | - | PCR (-) | IgG | PCR (-) | Ghana | Hayman et al., 2012 |
| May-October 1999 | *Rousettus occidentalis* | Egyptian Fruit Bat (Megachiroptera, Pteropidae) | - | PCR (-) | IgG | PCR (+) | RDC | Swanepoel et al., 2007 |
| May-June 2007 | *Rousettus occidentalis* | Egyptian Fruit Bat | IgG | PCR (-) | IgG* | PCR (-) | Ghana, Gabon*, DRC* | Hayman et al., 2010<br>Pourrut et al., 2009* |
| June 2003-March 2008 | *Rousettus occidentalis* | Egyptian Fruit Bat | IgG | PCR (+) | IgG | PCR (-) | Gabon, Republic of Congo | Pourrut et al., 2009 |

**Table 1.** Contd

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2005-January 2006 | *Rousettus occidentalis* | Egyptian Fruit Bat | - | PCR (+) | Virus isolation | PCR (+) | Gabon* | Towner et al. (2007 ; 2009)* |
| Aug 2008-Nov 2009 | *Rousettus occidentalis* | Egyptian Fruit Bat | - | PCR (-) | Virus isolation | PCR (+) | Uganda | Amman et al., 2012 |
| June-July 2007 | *Rousettus occidentalis* | Egyptian Fruit Bat | - | PCR (-) | - | PCR (+) | Kenya | Kuzmin et al., 2010 |

When several documented filoviral events happened in different localities, the mark on the locality's name refer to the author with the same mark. Republic of Congo (RC), Democratic Republic of Congo (DRC).

outbreaks in Africa needs to be clarified (Weingartl et al., 2013). In 2009 Reston-EBOV was the first EBOV reported to infect swine with possible transmission to humans (Weingartl et al., 2013).

## ECOLOGY OF BATS AS POTENTIAL RESERVOIRS OF FILOVIRUSES

*Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata* approximately share the same vital domains, the two last species being sympatric (Pourrut, 2007). They are confined to the tropical Central Africa and extent their distribution range to the wetter part of West Africa (Figure 3). They are found natively along and on either side of the equator, between latitudes 10°N and 10°S. They have been also recorded eastwards to Uganda and southwards to Angola and Congo (Rosevear, 1965). *H. monstrosus* is the less gregarious species among these; living in companies of a maximum of 20 individuals hanging close together daily up in trees or low down in shrubs. The Hammer-headed Fruit Bat has a preference for the closed forest what affiliate it to the Guinean woodlands where it finds dense patched of forest, with a variety of fruits maturing successively over seasons. Rosevear (1965) postulated that a little is known about its mode of life. Dispatched records of *H. montrosus*' occurrence have been noted, but nobody gave information about its migration range north and south the equator according to the season. Other bioecological features related to mating, breeding, feeding and roosting are not well known. Sanderson (1940) recorded a little colony of the Hammer-headed Fruit Bat resting into rocks, what seems unusual in current scientific literature, the species might have switched to a tree sheltering bat, because of scarcity of cave-dwelling structures. The Franquet's Epauletted bat, *E. franqueti*, occurs in West Africa, from Ghana to Loanda in Angola, and across the continent to the great Lakes as far south as Tanganyika. As the Hammer-headed Fruit Bat, it is a closed forest species and does not appear to be gregarious too; only few specimens have been found roosting together, hanging freely from trees or low bushes (Rosevear, 1965). Its bioecological features are not also well known. The Little Collared Fruit bat, *M. torquata*, shares the same predilection areas as the previous two other Ebola probable reservoirs, but a little is known about its habits (Rosevear, 1965). *R. occidentalis*, a potential filovirus reservoir species, is common and widely distributed in Africa (Figure 4). Its migration range can lead to a large variety of epidemiological situations. Over the ten species of the genus *Rousettus* known worldwide, *Rousettus occidentalis* is the mostly represented in Africa, numbering several subspecies, *R. a. arabicus* of the Arabic Peninsula (Saudi Arabia, Yemen, Oman, Pakistan, Iran), *R. a. aegyptiacus* in Egypt, Turquia, Syria, *R. a. unicolor* in West Africa, *R. a. leachi* in East, *R. a. angolensis* (or *Lissonycteris angolensis*) from Guinea to Kenya and from South Angola to Zimbabwe and *R. a. princeps*, *R. a. tomasi, R. a. unicolor* on the islands of Guinea gulf. The genus *Rousettus* is widely distributed and colonizes a large range of areas including dry and humid ecosystems, within altitudes reaching 4000 m. It is the only megachiroptera actually found roistering into caves and treeholes, thousands of individuals can also shelter into roofs of non-occupied human habitations, bridges. Bats of the genus *Rousettus* leave their shelter at sun down and fly around 30 km for feeding. A little is known about their migratory behavior (1 individual has been caught 500 km far away from its previous shelter in South Africa few days after). Widely common in sub- Saharan Africa (Figure 5), *Eidolon helvum* live in large colonies reaching 1, 000, 000 individuals of both sex (Walker, 1999), hanging on trees, often in cities. This fruit bat is of interest in *Ebolavirus* epidemiology because of its wide range migration, reaching more than 2,500 km (Richter and Cumming, 2008). The typical predilection area of the Straw-colored Fruit Bat is the forested areas of Central Africa where it is

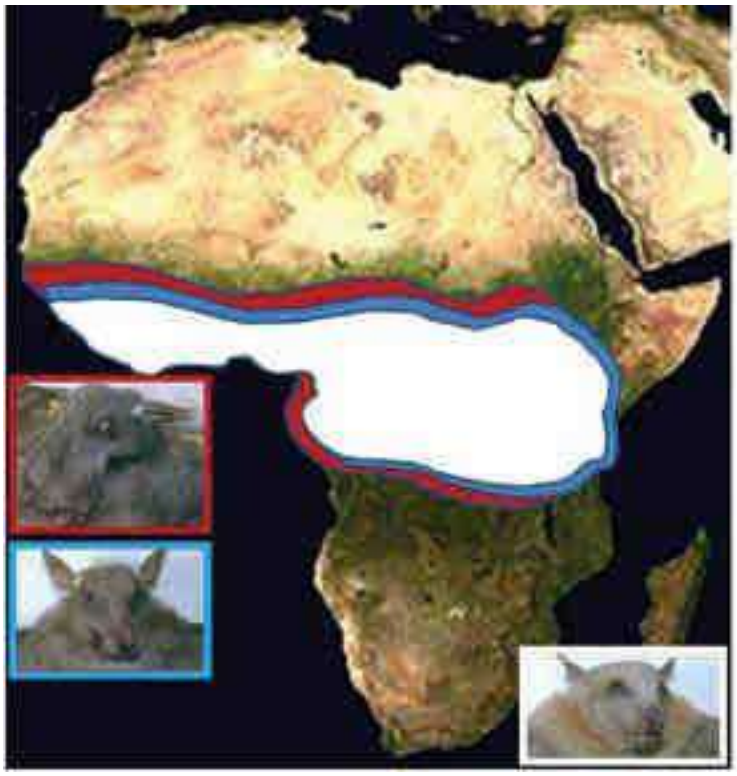

**Figure 3.** Distribution of *Hypsignsthus monstrosus* (red), *Epomops franqueti* (white) and *Myonycteris torquata* (blue) in Africa. The vital domains of the three species are overlapping.


**Figure 4.** Distribution of *Rousettus aegyptiacus occidentalis* in Africa.

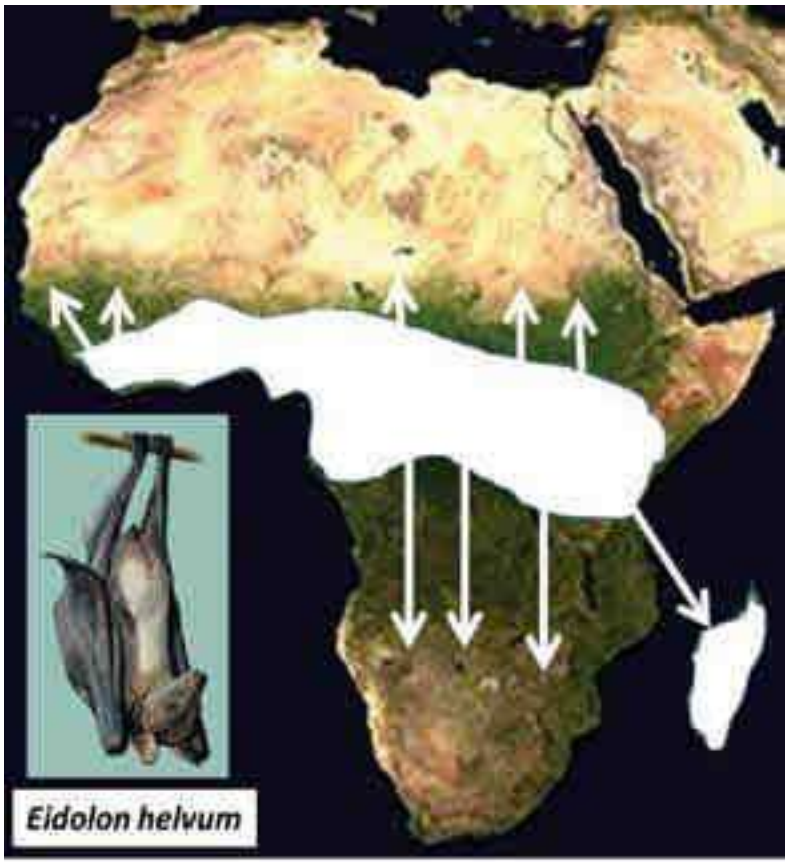


**Figure 5.** Distribution of *Eidolon helvum* in Africa. Arrows indicate their migration routes.

present year-round but its migration routes conduct numerous colonies of the fruit bat to North and South of Africa. Anderson (1907) reported its distribution from Somalia, Djibouti, southeastern Ethiopia and Sudan in the northeast; Senegal, Gambia and Mali in the northwest, to Malawi, South Africa and Zimbabwe in the south. The transition of filovirus species causing outbreaks in Central and West Africa during 2005-2014 seemed to be synchronized with the change of the serologically dominant virus species in the species *E. helvum* (Ogawa et al., 2015), but surveillance programs seem too limited over time and space to state that the serological status of these bats has changed. *Epomophorus gambianus*, contrarily to the other Pteropodids suspected to be reservoirs of *Ebolavirus*, is not associated with the forested areas of Central Africa. Indeed, the Gambian Epauleted bat prefers open grasslands, woodlands and savannah of Western Africa (Figure 6). It has been recorded in the forest edges, and occurs from Senegal to Southern Sudan and Ethiopia (Rosevear, 1965). The Sahel Acacia-wooded grassland and deciduous bush land form its northern limit of predilection. Its particular ecological features might involve it in a less manner in *Ebolavirus* ecology; in fact the species roosts singly or in groups of a maximum of 50 individuals (Rosevear, 1965), and does not compete with the other known Ebola potential reservoirs. *N. veldkampi* migrates northward from the forest of Ivory Coast and into the savannah during rainy season. They can fly 500 km and roost in small groups of well-spaced individuals (Reeder, 1999). Plurispecific associations have been noted between bats of the genus *Rousettus* and other microchiroptera such as the Giant Leaf-nosed Bat, *Hipposideros gigas* (Wagner), the Benito Leaf-nosed Bat, *Hippossideros beatus* K. Anderson, 1906 and the High-crowned Bat, *Miniopterus inflatus* (Thomas, 1903) in Gabon (Pourrut, 2007). Considering that ecological feature, an eventual role of microchiroptera as reservoir or amplificatory hosts of filoviruses needs to be investigated. In fact, Saez et al. (2015) recently suspected that *M. condylurus* might be involved in the zoonotic origin of the ongoing 2013-2015 West African EVD epidemic. The Eloquent horseshoe bat, *Rhinolophus eloquens* is found in Eastern Africa (Ethiopia, Kenya, Rwanda, Somalia, South Sudan, Tanzania and Uganda). This cave dwelling microchiroptera is associated with natural habitats of the subtropical or tropical moist lowland forests, dry savanna and moist savanna. The Greater Long-fingered Bat, *Miniopterus inflatus* is a


**Figure 6.** Distribution of *Epomophorus gambianus* in Africa.

species inhabiting high forested areas where they roast in colonies reaching 1000 of individuals in caves, crevices and rocks sometimes in association with other insectivorous bats as *Hypossideros caffer* or fruit bats as *Lyssonycteris angolensis*. It is common in Central Africa (Cameroon, Gabon, Central African Republic, Democratic Republic of the Congo, Equatorial Guinea, Uganda) and East Africa (Ethiopia, Rwanda, Tanzania and Kenya). It has been recorded in West Africa (Guinea, Liberia) and south to Africa (Mozambique, Namibia and Zimbabwe). Epidemiological scenari can be amplified by a response to environmental modifications, often resulting from human activities. *Ebolavirus* amplification in nature has been documented by Pourrut (2007) who found that it was correlated with reproduction time, changing from a country to another because of climatic specificities. *Hypposideros gigas*, *Mops condylurus*, *Miniopterus inflatus*, and *Rhinolophus eloquens* are the microchiroptera so far suspected as potential reservoirs of *Ebolavirus* spp. They proliferate in most of the African biota south to Sahara and in the island of Madagascar, of the Indian Ocean. Generally, microchiropters are not migratory bats. Their seasonal movements are not well studied but seem to be local. The four microchiropters so far found associated with *Ebolavirus* in nature are present between the latitudes 10°N and 10°S, on both sides of the equator. Occurrences areas of *H. gigas* and *R.s eloquens* almost overlap (Figures 7 and 8), covering the western central part of Africa; while some dispatched records are noted for *M. inflatus* which share the same ecosystems with the two precedents (Figure 9). This species has been recorded in Ethiopia, Uganda, Kenya and Tanzania in East Africa; and from Namibia, Zimbabwe and Mozambique in southern Africa. The predilection areas of *Mop condylurus* are much larger; this species is widely distributed over much of sub-Saharan Africa, ranging from Senegal, Gambia and Mali in the west, to the Sudan, Ethiopia and Somalia in the east (Figure 10). It has been also recorded southwards through much of eastern and southern Africa, and Swaziland. The species appears to be largely absent from the Congo Basin (Figure 10). As most of the microchiropters, they eat insects that abound in greater or less profusion all year long under the tropics (Rosevear, 1965). Involved in the filoviruses' epidemiological cycle, microchiropters will then maintain local enzootic cycles of


**Figure 7.** Distribution of *Hypposideros gigas* in Africa.


**Figure 8.** Distribution of *Rhinolopus eloquens* in Africa.


**Figure 9.** Distribution of *Miniopterus inflatus* in Africa.

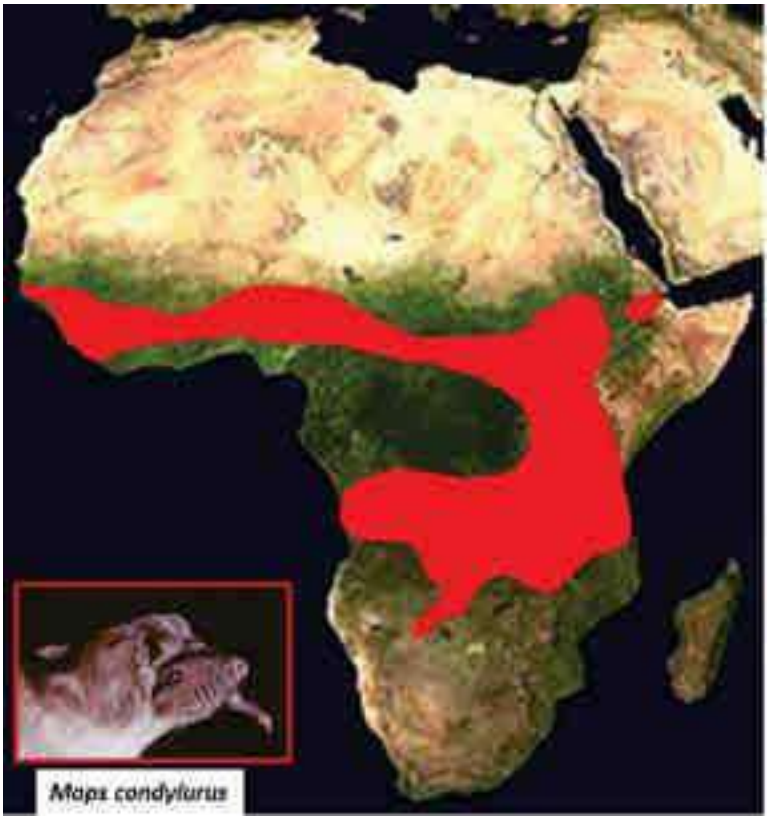


**Figure 10.** Distribution of *Mops condylurus* in Africa.

infection and play an important role in the perpetuation of filoviruses within ecosystems.

The microchiropters, at the opposite of megachiropters which include the single family of Pteropidae, account for fifteen different families known worldwide among which eight have an Afrotropical biogeographical distribution: Emballonuridae, Megadermatidae, Molossidae, Myzopodidae (Malagasian Subregion),Nycteridae, Rhinolophidae/Hipposideridae, Vespertilionidae (http://planet-mammiferes.org). Rosecvear (1965) noticed that they breed at most times of the year, though there are indications of preferences for the dry season.

## INVESTIGATION OF THE ZOONOTIC ORIGIN OF FILOVIRAL HEMORRHAGIC FEVERS

The natural source of the first Ebola outbreaks occurring from 1976 to 1979 has never been elucidated despite several research tentative targeting different vertebrate animals (Breman et al., 1999; Germain, 1978; Arata and Johnson, 1977; Leirs et al., 1999). Later, the Swiss ethnologist's infection with *Ebolavirus* was related to a chimpanzee she was autopsying (Le Guenno et al.,1995). Similarly, the 1996 Mayibout outbreak in Gabon originated from children who found and butchered a chimpanzee in the forest (Georges et al., 1999). Similar sources have been reported for Marburg virus which caused the 1967 outbreak in Marburg and Belgrade linked to the handling of organs and tissues of *C. aethiops* monkeys imported from Uganda (Smith et al., 1967; Martini, 1969). Practically all the sources of *Ebolavirus* outbreaks in Democratic Republic of Congo and Gabon were related to animal carcasses of gorillas, chimpanzees and duikers, hunted and handled since the forest (Olloba, 2001; Grand-Etoumbi, 2002; Entsiami 2002; Yembelengoye, 2002; Leroy et al., 2004b) as well as for the epidemics of Etakangaye 2001, Olloba 2002, Mendemba 2001, Ekata 2001 and Mvoula 2003. The presence of bats were recorded several times in the warehouses of the cotton factory, where the first people infected during the 1976 and 1979 outbreaks in Nzara, Sudan were working. No other likely source of infection was identified in either outbreak. It is also noteworthy that the Australian who was infected by Marburg virus (and subsequently infected two other people in Johannesburg in 1975) had just returned from a trip to Zimbabwe, during which he had slept frequently in the open and once in an abandoned house, the loft of which was inhabited by numerous bats. A few days before becoming ill, the French engineer who was infected by Marburg virus in Kenya in 1980 (and who subsequently infected his doctor) had visited caves containing large bat populations (Smith et al., 1982). However, when baboons and Vervet monkeys were placed in cages inside the same caves, none became infected (Johnson, 1996 personal communication), the experience might be set up into the caves out of the virus' amplification period in bats, or monkeys were resistant to infection and had developed an immunity following a previous contact with the virus. The fact that bats have already been implicated as source of infection in some previous filovirus outbreaks such as the Marburg hemorrhagic fever outbreak of Durba (Democratic Republic of Congo) inspired the IRD Research Unit 178 (Fundamentals and Domains of Disease Emergence) and opened the way to investigation of an eventual role of bats as reservoirs of those mysterious filoviruses. Swanepoel et al. (1996) experimentally proved that the Angola free-tailed bat, *Tadarida condylura* and the little free tailed bat, *Tadarida pumila* (Microchiroptera, Molossidae) and the Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* (Megachiroptera, Pteropidae), were able to asymptomatically replicate ZEBOV with high viral titers, 4 weeks after inoculation, but the first attempts to isolate the virus from bats in nature were not successful (Germain, 1978; Arata and Johnson, 1977; Breman et al., 1999; Leirs et al., 1999). The mystery was dissipated when an IRD (UR 178) team based at the CIRMF first discovered that bats of the family Pteropidae might be involved in replication, incubation and filoviruses (Ebola and Marburg) maintenance and transmission in nature (Pourrut et al., 2005; Leroy et al., 2005; Towner et al., 2007) and enhanced future directions for the research on reservoir species. Hypothetical transmission routes that seem plausible are proposed (Gonzalez et al., 2007; Olival and Heyman, 2014); however more investigations are needed to elucidate the ways that filoviruses borrow from the reservoir to nonhuman primates and to humans. While the struggle for containing the deadly EVD outbreak in West Africa was going on, few studies searched to figure out where it came from, and what was its zoonotic carrier. It is hypothesized that the ongoing EVD epidemic originated from a little 2 years old girl who might have been infected by *Eidolon helvum* in Guekedou (Funk and Piot, 2014). There has been no handling or consummation of bush meat in the village, the toddler might have collected a partially chewed fruit dropped from a tree by the straw-colored fruit bat and subsequently became infected with virus particles in residual bat saliva (1st hypothesis). Saez et al. (2015) investigated the zoonotic origin of the West African *Ebolavirus* outbreak around Meliandou where the toddler first contracted the ZEBOV strain, but did not find any evidence of virus circulation in wildlife. Particularly, bats belonging to the incriminated species (*E. helvum*) that were captured and tested did not allow any virus isolation or ZEBOV sequences detection. Also, their enquiries conducted on wildlife did not reveal any decline of sensitive wild animals, but observed that there was a tree with large hollow in the index home, inhabiting microchiroptera among which *M. condylurus* has been identified. This insectivorous bat already tested ZEBOV-IgG positive (Pourrut et al., 2009) and might be the source

of the infection, because kids usually caught and played with bats in this tree (2nd hypothesis). Free-tailed bats have been already incriminated in such infection as for the first Sudan Ebola virus outbreaks (World Health Organization/International Study Team, 1978). Cases of Marburg virus infection via exposure to bat colonies have been already documented with the Kitum cave in Mont Elgon National Park, Kenya, and in Zimbabwe. A total of 12 bats have been suspected to be potential hosts of Ebola and Marburgviruses in the Afrotropical biogeographic region (Table 1). They include 8 megachiropters of the family Pteropidae: *H. monstrosus, M. torquata* and *E. franqueti,* mostly associated with the forested areas as previously discussed. *E. gambianus, E. helvum* and *R. occidentalis* found positive for filoviruses have tested negative in June 2006, in Senegal supposed Ebola free and used as a control site (Pourrut, 2007), *M. pusillus* and *N. weldkampi*. 4 microchiropters are identified as probable reservoirs: *M. condylurus M. inflatus*, *H. gigas* [Pourrut et al. (2009) list it as IgG ZEBOV positif], and *R. eloquens*.

## PLACE OF CHIROPTERS IN THE EPIDEMIOLOGY OF EMERGING ZOONOTIC DISEASES

Bats harbor a potential role as reservoirs for zoonotic diseases. About 66 different viruses have been isolated from bats (Calisher et al., 2006) and serological evidence for infection of bats with many viruses has been found (Kuno, 2001; Messenger et al., 2003; Gonzalez et al., 2008). Studies of their bioecology, dynamic and natural behavior have been enhanced from the 1970s since they have been incriminated in zoonoses' emergence due to coronaviruses, filoviruses and paramyxoviruses. They considerably participate on diseases dispersal across a vast range of regions where they are involved in the increasing threat of emerging infectious diseases to human societies: the severe acute Middle East respiratory syndrome-like coronavirus (MERS-CoV) (Ithete et al., 2013; Memish et al., 2013), paramyxoviruses Nipah virus (NiV) in Malaysia and Bangladesh (Luby, 2013), Hendra (HeV) in Australia (Clayton et al., 2013), and lyssavirus disease in America, Europe and Australia (Warrell and Warrell, 2004; Van der Poel et al., 2006) plus the emerging filoviruses, Ebola and Marburg in Africa (Leroy et al., 2005; Calisher et al., 2006). It has been already established that rabies virus infections in France have been associated with the migratory routes of the Nathusius' pipistrelle, *Pipistrellus nathusii* Keyserling and Blasius, 1839 (Brosset, 1990). In Africa, the widely separated geographic locations of Ebola outbreaks have supported that the reservoir and the transmission cycle are probably closely associated with the rainforest ecosystem, assertion supported by antibodies distribution. The fact that outbreaks seldom occur suggests the presence of a rare or ecologically isolated reservoir species having few contact with human and non-human primate species (Gonzalez et al., 2005). In the Class Mammalia of the vertebrate animals, the order Chiroptera represents the second in terms of species diversity, behind the order of Rodentia, but is the most important because of its potential for harboring zoonotic pathogens. It includes the suborders of Microchiroptera and Megachiroptera; the last accounting for the unique family of Pteropidae which include the Old World fruit bats or flying foxes found in tropical and subtropical Africa and east to the Western Pacific. Most of the actually suspected filoviruses' reservoirs belong to that family. The Microchiroptera are found throughout most of the world and include small insectivorous bats, few bat species fruit and flower feeders, few carnivorous bats, and lastly vampire bats which have a Neotropical geographic distribution, found in tropical areas of the American continent, principally in Mexico, Chile, Argentina and Brazil. Rodents are terrestrial and commensally mammals, closely associated with human environment and carry significant diseases with a real public health concern (Mills, 2006). As examples, Hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome are due to hantaviruses pathogens hosted by rodents of the family Muridae (Schmaljohn and Hjelle, 1997). Lymphocytic choriomeningitis, Lassa fever, Argentina, Bolivian, Venezuelan and Brazilian hemorrhagic fevers are caused by rodent's arenaviruses. These small mammals are also incriminated in Congo Crimean Hemorrhagic Fever and Rift Valley Fever epidemiology (Camicas et al., 1990; Pretorius et al., 1997). They become less studied than bats which do not directly interact with human environment, because they are phytophilous (associated with forest vegetation) or lithophilous (associated with caves, rocks and similar sheltering structures) (Rosevear, 1965). Compared with rodents, bats are unique in their propensity to host zoonotic viruses, they are natural reservoirs of a number of high-impact viral zoonoses. In their quantitative analysis, Luis et al. (2013) demonstrated that bats indeed host more zoonotic viruses per species than rodents, because their sympatry with other species of the same taxonomic order promote interspecific transmission and zoonotic viral richness.

## THE PROBABLE ROLE OF ANIMALS INVOLVED IN FILOVIRAL HEMORRHAGIC FEVERS

In the light of reservoir species theory of Rodhain (1998), the following criteria can be considered: 1-Efficient vertebrate reservoirs (or good reservoirs) of filoviruses need to be receptive to these viruses, not just slightly sensitive. They must be able to asymptomatically replicate the virus, develop an efficient and sufficient viremia, and once infected, the animal must survive; ensuring maintenance and circulation of the virus in nature, and therefore the foci's continuity. 2- the reservoir species must be of an abundant and prolific population, able to replicate and disseminate the pathogen. Neonate

or naive individuals are non-immune, which allow their receptivity to the virus and infection, ensuring continuation. 3- The viremia must be of a high viral titer, last longer enough, the time to allow it to infect other receptive hosts of the same population for virus perpetuation.

In its natural foci, a filovirus circulates between several vertebrate hosts, playing different roles in its epidemiology. For Ebola and Marburg viruses, bats are the potential candidates for the reservoir status: 1) Filovirus RNA characterization associated with virus specific antibodies and virus isolation within some bats species provided clues that chiropters might be incriminated; 2) It is also likely that the reservoir species are ecologically isolated, associated with the rainforest ecosystem with an important potential of migration which might justify the scattered geographic occurrences of Ebola outbreaks. Bats satisfy this statement. Other vertebrates are just activating the foci for a while, acting as amplifying hosts: in this category, belong some monkeys of the family *Cercopithecidae* such as vervet, *Chlorocebus aethiops*, found infected with a filovirus in Marburg (Smith et al., 1967) and the red colobus, *Procolobus badius,* hunted and eaten by chimpanzees, who subsequently became infected by *Ebolavirus* (Boesch, 1994). The virus can also reach some other non-susceptible animals unable to replicate it or who just present a temporary short viremia with a low viral titer: the dead-end hosts. Birds that tested refractory to *Ebolavirus* (Swanepoel et al., 1996) must be listed in this category. Widely divergent orders or families of the avian fauna were unable to experimentally replicate *Ebolavirus*. Then, efforts on field reservoir search should focus more on other animals able to replicate the virus than birds. Migratory vertebrates will disseminate the virus: bats again fit in this case, spreading pathogens through migration; and other sensitive hosts will serve as sentinel hosts or biological markers, allowing the epidemiologists to detect the virus' activity. That's the case for great apes (chimpanzees and gorillas) which have a wide range of vital domain but do not move as far as migratory bats. Once in contact with the virus, they die, promoting about a probable emergence. Animals involved in a filovirus' activity are not necessarily all reservoirs. Incidental hosts are just accidentally involved in the cycle, like mosquitoes that might be infected after a viremic blood meal taken on a wild animal. In addition, filoviruses generally do not replicate in arthropods or arthropod cell lines (Peterson et al., 2004). Due to their dispersal, several index cases should be reported if anthropophilic mosquitoes were able to disseminate filoviruses. A filovirus can adopt several different reservoirs, in different environmental conditions; an animal species might be a good reservoir in a certain environment and a bad one in another. In the case of bats for example, food is found in some restricted areas, depending to the phenology of wild fruit trees, which varies from season to season (even month to month). If the availability of food is good, they stay around for several nights or even weeks, and chronically infected bats would increase the length of time during which they can infect other receptive species and are qualified as good reservoirs in such environmental conditions. If their survival conditions are not met, they must necessarily travel further afield and will not stay longer enough to perpetuate their carried pathogen in this specific ecosystem and are circumstantially qualified as bad reservoirs. Mostly wild vertebrates (birds and mammals) act as usual reservoirs for most of the pathogens. Domestic or commensal mammals, as well as human beings, are rarely involved as reservoirs. In the case of many arboviruses, arthropods are involved in their maintenance because of their longevity and their vectorial competence allowing them to replicate and transmit the virus through vertical transmission to the offspring. The bats might do the same for filoviruses, but will transmit the virus to the offspring through placental exchanges. In fact, Leroy et al. (2006) postulated that great apes might be contaminated while touching bat placental tissues and biological fluids, during parturition. Bat's ability for long distance flying provides an intensive selective force for coexistence with viral parasites through a daily cycle that elevates metabolism and body temperature analogous to the febrile response in other mammals (O'Shea et al., 2014). These factors imply a large diversity of epidemiological situations according to the virus, the bat reservoir species and the region. Understanding epidemiological situations need a comprehension of the evolution of these linked systems in correlation with the modification of ecosystems, often resulting from human induced activities on the environment. Repeated passages of filoviruses from a vertebrate host to another will, sooner or later, develop modifications of their viral genome in response to new environmental adaptation, by emergence of reassortants during coinfections. In such conditions two situations are predictable: 1- the virus might lose some virulence and this can lead to extinction of its foci, 2- after genome modification, the foci are activated after a short silent interval, increasing the ability of the virus to last longer. This last scenario happened in Sierra Leone and contributed to maintaining the virus' adaptation. In Fact, Gire et al. (2014) tracked *Ebolavirus*' evolution during this West African epidemic and found that it was changing as it spread. Their genetic analysis revealed that the outbreak in Sierra Leone was sparked by at least two distinct viruses, introduced from Guinea at about the same time. One of this disappearing from patients sampled later in the outbreak, while a third lineage appeared. Then, for several different reasons, it appears puzzling, to predict the ending of the outbreak because of those mutations, and to set efficient preventive measures axed at level of natural reservoirs.

## FACTS, THEORY AND HYPOTHESIS

Zoonoses are diseases that originate from wildlife and

strike living animals, threatening animal biodiversity and public health (Daszak et al., 2000; Leroy et al., 2004a; Woolhouse et al., 2005; Lahm et al., 2007; Jones et al., 2008). Filoviral hemorrhagic fever asymptomatically develops in the wild vertebrate host and cause fatal manifestations when it reaches human beings (anthropozoonose). Filoviruses are circulating in a sylvan cycle among reservoir species and other sensitive hosts. EVD is an anthropozoonose, benign within the reservoir species, fatal within sensitive human population where it is associated with a mortality rate ranging from 50 (SIEBOV) (Smith, 1978; Baron et al., 1983) to 80% (ZEBOV) (Bwaka et al., 1999; Nkoghé et al., 2004), depending on the virus species (Johnson, 1978). The duikers and great apes (gorilla and chimpanzee) are also sensitive to *Ebolavirus* infection and represent intermediate hosts that can bridge the virus to human population. Humans entering the forest can be infected while hunting bats or other apes, antelopes and sensitive hosts. It is in that occasion that the virus reaches rural population, spreading from human to human, causing outbreaks and even epidemics affecting several villages and towns. These outbreaks can provide a source for potentially devastating urban epidemics, which are the most dangerous, because of concentration of susceptible people; typically higher mortality rates associated with urban situation are recorded after prolonged human-to-human transmission. However, the role of bats with their spectrum of behavioral variation, in the forested areas of central Africa where the virus originated from is unclear.

## ECONOMIC AND SOCIAL IMPACT OF FILOVIRUSES EMERGENCE

The public health and economic burden imposed by FHFs on the developing world with limited medical coverage are enormous. The West African EVD outbreak caused global societal and economic impact due to the unexpected magnitude of the epidemic killing thousands of people; the socioeconomic impacts in Guinea, Sierra Leone and Liberia include job losses, smaller harvests and food insecurity. Travel, global business and other life activities were affected, taking a significant human toll as well as cause public fear, economic loss and other adverse outcomes. While the primary cost of this tragic outbreak is in human lives and suffering, the crisis will secondly worsen already entrenched poverty. The Bank Group estimates that Guinea, Sierra Leone and Liberia will lose at least US$1.6 billion in economic growth in 2015 (http://www.worldbank.org). As of April 2015, the World Bank Group's response to the Ebola crisis has mobilized US$1.62 billion to support the affected countries containing and preventing the spread of infections, providing treatment and care, and improving public health systems. They also mobilized funds for providing 10,500 tons of maize and rice to seed more than 200,000 farmers in Guinea, Liberia and Sierra Leone, averting hunger in Ebola-affected countries and reviving agriculture. In terms of morbidity and mortality, EVD accounts largely among the global disease burden of humankind. As of April 19, 2015, 23816 cases of EHF (14893 laboratory confirmed were reported, accounting for 10736 deaths in Guinea, Sierra Leone, Liberia and to a less degree, in Nigeria and Mali (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html) (Figure 11). The bulk of FHF mortality occurs in sub-Saharan Africa where it is seeded by the lethal emergence of the most deadly *Ebolavirus* species, *Z. ebolavirus* (ZEBOV) and the existence of a wide range of potential bat reservoirs. Despite the rarity and ecologically isolation of the reservoir species, the force of FHF transmission in some areas of sub-Saharan Africa is extremely high (25,907 cases suspected, probable and confirmed), intensively driven by interhuman transmission. FHF are socially devastating diseases of the developing world and the risk of epidemics remains. Since the last emergence of ZEBOV in Gueckedou and Macenta, Southeastern Guinea (Baize et al., 2014), on December 2013, the disease continues to sicken and kill thousands of people in the affected countries of sub-Saharan Africa. It is difficult to control because of repetitive health care workers, medical doctors and laboratory diagnosis personnel direct contamination. Nosocomial infections occurred in the hospital, during the Yambuko epidemic (1976), a Belgian nuns inadvertently started the epidemic by giving vitamin injections to pregnant women, through reuse of unsterilized syringes, needles or other medical equipment contaminated with body fluids (Piot, personal communication). Inadequate dispositions for contact with Ebola infected patients throughout herbalist care, burial preparation, including body washing and long intimate funeral ritual greatly increased the risk of the virus spillover, by fluid transmission. By September 14, 2014, a total of 318 cases, including 151 deaths, had been reported among health care workers (WHO Ebola Response Team, 2014).

It is the first West Africa Ebola outbreak and the largest ever recorded in history; morbidity and mortality recorded are higher than in all previously Ebola outbreaks combined in Africa. This EVD epidemic is very similar to the 1976 outbreak. Both were caused by *Z. ebolavirus,* hitting rural forest communities first, before spreading into urban areas, without any link to bush meat handling. Hemorrhagic cases were suspected due to malaria, typhoid, Lassa fever, yellow fever or influenza. From the past, epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo and Uganda (Smith, 1978; Le Guenno et al., 1995, 1999). Filoviruses and mammals co-evolved since the Paleocene. The existence of orthologous filoviruslike elements shared among mammalian genera whose divergence dates have been estimated suggesting that filoviruses are at least tens of millions of years old (Taylor et al., 2010). Phylogenetic and sequencing evidence from gene boundaries was consistent with integration of filoviruses in mammalian genomes.

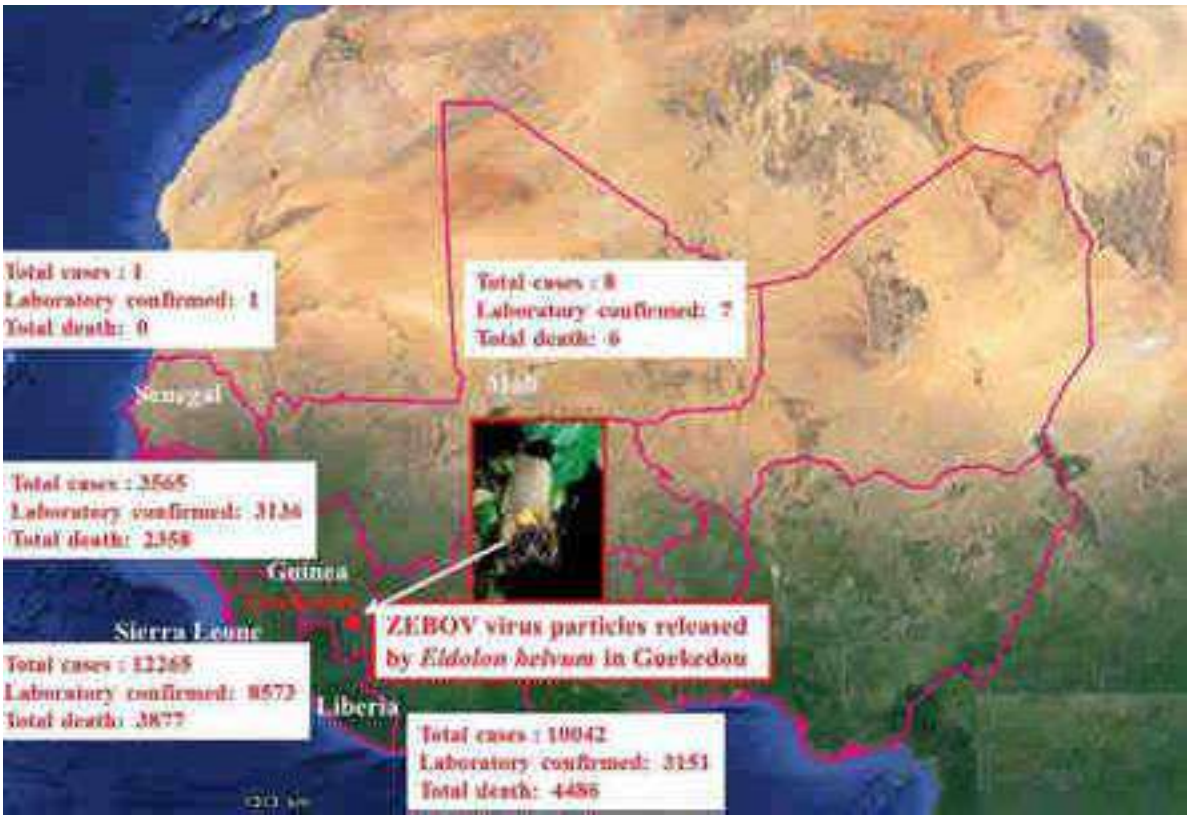


**Figure 11.** Incidence of ZEBOV activity in West Africa as at April 19, 2015.

## FUTURE STUDIES

Despite the importance of the studies achieved on the epidemiology of filoviruses, a number of deficiencies have been pointed out and need to be addressed. A fundamental aim needs to assess the ecology of reservoirs in the rural/sylvan interface, where EVD transmission spills over into human populations. Filoviruses might silently breed in some West African forested ecosystems, introduced since the emerging areas of central Africa by some potential reservoirs as *E. helvum*. They can extend their amplification areas and reach other sensitive secondary hosts. Peterson et al. (2004) suggested that a large-scale ecologic and geographic comparison is an unexplored approach to identifying the natural reservoir of filoviruses in order to detect patterns of co-occurrence and co-distribution of viruses with potential hosts.

### Studies extended to other Pteropidae sub families to see if any other potential reservoirs exist

Understanding the ecological features of the major suspected reservoirs of *Ebolavirus*, that is, *H. monstrosus*, *E. franqueti* and *M. torquata* is a major goal. Their principal known domains of occurrence is concern with the central forested areas of Africa, but some studies recorded *H. monstrosus* in Southern Senegal (Feiler, 1986; Koopman, 1975; Koopman et al., 1978), as well as *E. franqueti* and *M. torquata* (Pourrut, 2007). The roosting behavior of *R. aegyptiacus* needs to be investigated. Plurispecific associations have been observed among Pteropidae (Kunz, 1982; Kuzmin et al., 2010). Many bat species are gregarious, living in dense colonies: for example, *Eidolon helvum* aggregations can reach a population of 50,000 to 100,000 individuals per roost (Jones, personal communication; Rosevear, 1965). Roosting sites can also account for assemblages of multiple species where high intra and interspecific contact rates of bats from different origins and unknown pathologic and immune status directly promote rapid transmission of pathogens and their spread. The Egyptian Fruit Bat roosts daily in trees or caves, often with large groups of other bats. High-densities bat colonies have been observed, sometimes numbering in the thousands. They emerge from the roost to forage for food in the late evening, and return just before dawn. They hang upside down, with their wings folded closely around their bodies (http://en.wikipedia.org/wiki/Rousettus_aegyptiacus). We hypothesize that following those pluri-specific associations, competitionfor territory conquest or simply daily association into shelters might lead to infection of potential reservoirs

such as *R. aegyptiacus* which is known widespread in all the Afrotropical biogeographic region excepted the Saharan domain (Figure 4). A scenario such as this one might extend the known occurrence area of *Ebolavirus* since its natural foci of central forested African areas, *R. aegyptiacus* acting as the bridge vector. 1) Occurrence areas of the three known potential reservoirs (*H. monstrosus, E. franqueti* and *M. torquata*) need to be updated and mapped as well as for the other potential filoviruses reservoirs. In fact, several vector-borne, parasitic or zoonotic diseases have (re)-emerged and spread within Africa these recent years, because of global and local changes caused by either climate change, human-induced landscape changes like constant reduction in size of natural forests tending to make the original epidemiologic sylvatic cycle somewhat a relic one, switching to a rural cycle. This implies encroachment of people and livestock into wildlife habitats and in another direction increases wildlife migration from degraded areas into rural and peri-urban regions. Impacted landscape variation induced by environmental factors and human behaviors (hunting, irrigation; deforestation; cattle breeding...), added to climatic changes, directly impact human health. 2) Their dynamic over time (reproduction period) and space (migration) need to be completely understood for modeling the risk of *Ebolavirus* emergence. It has been already proven that most reservoirs are efficient *filovirus* vectors during sexual activity (reproduction time). In fact, Amman et al. (2012) observed that birthing seasons represent times of increased infection among juveniles and that most human MVD cases coincided with those periods. 3) Serologic studies undertaken along a West-East transect study across West Africa will assess to what extent the *Ebolavirus* amplification has been observed. Other Pteropidae close to the known reservoirs such as *Rousettus angolensis smithi*, *Eidolon* spp., *Micropteropus* spp., *Nanonycteris,* etc.., existing in Africa, need to be studied in order to discover other eventual filoviruses and bat reservoirs.

## Migration routes and distribution areas of the potential bat species reservoirs

To fully understand their migration circuits and areas of predilection, the above cited transect study needs to be entirely prospected. The actually known EBOV serotypes might have circulating in a primeval cycle, among certain bat species (*Hypsignathus, Myonycteris*, *Epomops*…) without any symptomatic infection in the forest of Central Africa in a silent cycle. Man entering the forest gallery for the purpose of hunting might be occasionally involved in this cycle. Such a zoonotic reservoir of infection could exist in all forested areas (primary forest galleries, isolated patches of forest, forest-savanna mosaics) of West Africa. Ecosystems modification and environmental conditions linked to global change can influence spatial and temporal distribution and dynamics of human pathogenic agents. A high viral amplification of *Ebolavirus* in the forest ecosystem probably favoured its escape from its naturally sylvatic cot increasing the probability for the virus to reach directly human population or via other sensitive hosts. As shown by the phylogenetic study from Baize et al. (2014), the bottom clade contains *Ebolavirus* (ZEBOV) described from Gabon, suggesting that the other top clades derived from it. In fact, the derived clades show that ZEBOV emerged in DRC in 1976, simultaneously ɛs SEBOV in Sudan, in 1976 before the Ivory Coast emergence of CIEBOV. Their ancestor, the Gabon strain (ZEBOV) emerged later in 1994, probably confined in a jungle cycle, before its emergence. All available data about the implication of bats in the epidemiology of EVD are limited to Central Africa, because the disease first emerged in this area. Little information is obtained from West Africa. Senegal is the extreme limit of the geographical range of the known Ebola reservoir species, that is, *H. monstrosus, E. franqueti,* and *M. torquata*. Ninety eight (98) bats belonging to the genus *Eidolon helvum, Epomoporus gambianus* and *Rousettus aegypticus occidentalis* were captured near Mbour (14°25' N, 16°57' W; MBour Dpt. [Thiès Reg.]), 80 km far away from Dakar in June 2006, and tested negative for EBOV (Pourrut et al., 2007). However, a serologic study of human and simian populations undertaken by Gonzalez et al. (2005) detected IgG from human population in Africa. The demonstration of neutralizing antibody to EBOV in the human sera suggested that there might be a sylvatic cycle of EBOV in West Africa. Marburg and Ebola viruses are endemic in Central African countries where outbreaks are unpredictable and just sporadically emerge.

## Bioecology of the microchiropters, potential species reservoirs

Four species belonging to three different microchiropters' families (*Molossidae, Vespertilionidae*, and *Rhinolophidae*) are suspected for now in filoviruses' epidemiology. Some detailed studies need to be undertaken in order to clarify the following points: 1) are members of different families breeding at the same time of the year? 2) Do they successively breed over time? Responding to those questions will assess if seasonal amplification of a filovirus is short over time because of reproduction at the same period, with a sexual pause during which neonate bat species do not exist, corresponding to the inter-epizootic period. In the other case, the amplification period can last long and promoted by the opportunity of continuous contact of naive offspring with infectious bats in the colonies during a certain time of the year. This will conduct logically to a seasonal pulse of filoviruses in the ecosystem characterized by amplification periods separated by silent intervals. This scheme of amplification/silencing makes

sense if microchiropters were only proliferating in the ecosystem. Plurispecific associations include micro-chiropters and megachiropters, the last accounting individuals with large migration range (*Hypposideros* species and *R. aegypticus occidentalis* have been recorded together in the Kitaka cave, Uganda). Do both incubate filoviruses at the same time in nature? Are there reproduction/amplification periods synchronic? One might be a relay while another is entering a silent period. A comprehensive approach will investigate the natural reservoir of filoviruses which is large-scale ecologic and geographic comparisons in order to elucidate the patterns of (co) occurrence of viruses within potential hosts. Dynamic of the bat reservoir species of these filoviruses as well as interactions between sensitive hosts and bats in the rural/sylvan interface are not fully understood. Breman et al. (1999) conducted several researches aiming to identify the wild animal species hosting the virus in nature but failed to find the reservoirs. Extensive field and laboratory studies of the wide range of filoviruses activity in Central and West Africa need to be undertaken. The main emphasis will be the bioecology of the chiropteran with regard to the specific filovirus they carry. Sensitive serological assays need to be processed on a wide range of bats captured from diverse ecological forested areas as well as from other sensitive apes and Cercopithecidae in order to figure out the extent of the filoviruses amplification and dissemination. The 2013-2015 outbreak of EVD shows a higher fatality rate attributed to the strain ZEBOV, Quantitative Trait Loci maps of genetic factors that condition virulence of the Ebola strains isolated during these concomitant epidemics might be elucidated from a locality to another, and the already known Ebola virus strains so far isolated and incriminatedduringpreviousepidemics. Understanding the immune responses to filoviruses that ensure apathogenic, persistent infections in the reservoirs, without any sign of disease is a major goal.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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### REFERENCES

Adjemian J, Farnon EC, Tschioko F, Wamala JF, Byaruhanga E, Bwire GS, Kansiime E, Kagirita A, Ahimbisibwe S, Katunguka F, Jeffs B, Lutwama JJ, Downing R, Tappero JW, Formenty P, Amman B, Manning C, Towner J, Nichol ST, Rollin PE (2011). Outbreak of Marburg hemorrhagic fever among miners in Kamwenge and Ibanda Districts, Uganda, 2007. J. Infect. Dis. 204(3):S796-799.

Allela L, Boury O, Pouillot R, Délicat A, Yaba P, Kumulungui B, Rouquet P, Gonzalez JP, Leroy EM (2005). Ebola virus antibody prevalence in dogs and human risk. Emerg. Infect. Dis. 11(3):385-390.

Amblard J, Obiang P, Edzang S, Prehaud C, Bouloy M, Le Guenno B (1997). Identification of the Ebola virus in Gabon in 1994. Lancet 349: 181-182.

Amman BR, Carroll SA, Reed ZD, Sealy TK, Balinandi S, Swanepoel R, Kemp A, Erickson BR, Comer JA, Campbell S, Cannon DL, Khristova ML, Atimnedi P, Paddock CD, Crockett RJK, Flietstra TD, Warfield KL, Unfer R, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Rollin PE, Ksiazek TG, Nichol ST, Towner JS (2012). Seasonal Pulses of Marburg Virus Circulation in Juvenile *Rousettus aegyptiacus* Bats Coincide with Periods of Increased Risk of Human Infection. PLoS Pathog. 8. doi:10.1371/journal.ppat.1002877.

Amman BR, Nyakarahuka L, McElroy AK, Dodd KA, Sealy TK, Schuh AJ, Shoemaker TR, Balinandi S, Atimnedi P, Kaboyo W, Nichol ST, Towner JS (2014). Marburgvirus resurgence in Kitaka Mine bat population after extermination attempts, Uganda. Emerg. Infect. Dis. 20(10):1761-4.

Amman BR, Jones ME, Sealy TK, Uebelhoer LS, Schuh AJ, Bird BH, Coleman-McCray JD, Martin BE, Nichol ST, Towner JS (2015). Oral shedding of Marburg virus in experimentally infected Egyptian fruit bats (*Rousettus aegyptiacus*). J. Wildl. Dis. 51(1):113-24.

Anderson K (1907). On *Pterocyon, Rousettus* and *Myonycteris*. Ann. Mag. Nat. Hist. 8 (1): 431-435.

Arata AA, Johnson B (1977). Approaches towards studies on potential reservoirs of viral haemorrhagic fever in southern Sudan (1977). In: Pattyn SR, Ebola virus haemorrhagic fever. Amsterdam: Elsevier/Netherland Biomedical. pp. 191-202.

Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, Soropogui B, Sow MS, Keïta S, De Clerck H, Tiffany A, Dominguez G, Loua M, Traoré A, Kolié M, Malano EM, Heleze E, Bocquin A, Mély S, Raoul H, Caro V, Cadar D, Gabriel M, Pahlmann M, Tappe D, Schmidt-Chanasit J, Impouma B, Diallo AK, Formenty P, Van Herp M, Günther S (2014). Emergence of Zaire Ebola Virus Disease in Guinea - Preliminary Report. N. Engl. J. Med. 371(15):1418-1425.

Baron RC, McCormick JB, Zubeir OA (1983). Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. Bull. WHO 61: 997-1003.

Barrette RW, Metwally SA, Rowland JM, Xu L, Zaki SR, Nichol ST, Rollin PE, Towner JS, Shieh WJ, Batten B, Sealy TK, Carrillo C, Moran KE, Bracht AJ, Mayr GA, Sirios-Cruz M, Catbagan DP, Lautner EA, Ksiazek TG, White WR, McIntosh MT (2009). Discovery of swine as a host for the *Reston ebolavirus*. Science 325(5937): 204-206. doi: 10.1126/science.1172705.

Bausch DG, Borchert M, Grein T, Roth C, Swanepoel R, Libande ML, Talarmin A, Bertherat E, Muyembe-Tamfum JJ, Tugume B, Colebunders R, Kondé KM, Pirad P, Olinda LL, Rodier GR, Campbell P, Tomori O, Ksiazek TG, Rollin PE. (2003). Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. Emerg. Infect. Dis. 9(12):1531-1537.

Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H, Campbell P, Tshioko FK, Roth C, Colebunders R, Pirard P, Mardel S, Olinda LA, Zeller H, Tshomba A, Kulidri A, Libande ML, Mulangu S, Formenty P, Grein T, Leirs H, Braack L, Ksiazek T, Zaki S, Bowen MD, Smit SB, Leman PA, Burt FJ, Kemp A, Swanepoel R (2006). Marburg hemorrhagic fever associated with multiple genetic lineages of virus. N. Engl. J. Med. 355: 909-919.

Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, Nichol ST, Ksiazek TG, Rollin PE (2007). Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J. Infect. Dis. 196 (2):S142-147.

Beer B, Kurth R (1999). Characteristics of Filoviridae: Marburg and Ebola Viruses. Naturwissenschaften 86: 8–17.

Bermejo M, Rodríguez-Teijeiro JD, Illera G, Barroso A, Vilà C, Walsh PD. (2006). Ebola outbreak killed 5000 gorillas. Science 314(5805):1564.

Boesch C (1994). Chimpanzees-red colobus monkeys: a predator-prey system. Anim. Behav. 47: 1135-1148.

Brauburger K, Hume AJ, Mühlberger E, Olejnik J (2012). Forty-Five Years of Marburg Virus Research. Viruses 4: 1878-1927.

Breman JG, Johnson KM, van der Groen G, Robbins CB, Szczeniowski MV, Ruti K, Webb PA, Meier F, Heymann DL (1999). A Search for

Ebola Virus in Animals in the Democratic Republic of the Congo and Cameroon: Ecologic, Virologic, and Serologic Surveys, 1979-1980. J. Infect. Dis. 179 (1): S139-S147.
Brosset A (1990). The migrations of *Pipistrellus nathusii* in France-possible implication on the spreading of rabies. Mammalia 54: 207-212. (In French).
Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, Katwiki KR, Kibadi K, Kipasa MA, Kuvula KJ, Mapanda BB, Massamba M, Mupapa KD, Muyembe-Tamfum JJ, Ndaberey E, Peters CJ, Rollin PE, Van den Enden E, Van den Enden E (1999). Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J. Infect. Dis. 179: S1-S7.
Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006). Bats: important reservoir hosts of emerging viruses. Clin. Microbiol. Rev. 19: 531-545.
Camicas JL, Wilson ML, Cornet JP, Digoutte JP, Calvo MA, Adam F, Gonzalez JP (1990). Ecology of Ticks as potential vectors of Crimean-Congo hemorrhagic fever virus in Senegal: epidemiological implications. Arch. Virol. (Suppl. 1): 303-322.
Carroll SA, Towner JS, Sealy TK, McMullan LK, Khristova ML, Burt FJ, Swanepoel R, Rollin PE, Nichol ST (2013). Molecular evolution of viruses of the family Filoviridae based on 97 whole-genome sequences. J. Virol. 87(5): 2608-2616.
Changula K, Kajihara M, Mweene AS, Takada A (2014). Ebola and Marburg virus diseases in Africa: Increased risk of outbreaks in previously unaffected areas? Microbiol. Immunol. 58: 483-491.
Chevalier MS, Chung W, Smith J, Weil LM, Hughes SM, Joyner SN, Hall E, Srinath D, Ritch J, Thathiah P, Threadgill H, Cervantes D, Lakey DL, Centers for Disease Control and Prevention-CDC (2014). Ebola virus disease cluster in the United States-Dallas County, Texas, 2014. MMWR Morb. Mortal. Wkly. Rep. 63(46):1087-1088. Erratum in: MMWR Morb. Mortal. Wkly. Rep. 63(48): 1139.
Clayton BA, Wang LF, Marsh GA (2013). Henipaviruses: An Updated Review Focusing on the Pteropid Reservoir and Features of Transmission. Zoonoses Public Health 60: 69-83.
Daszak P, Cunningham AA, Hyatt AD (2000). Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287(5452): 443-449.
Feldmann H (2006). Marburg Hemorrhagic Fever- The Forgotten Cousin Strikes. N. Engl. J. Med. 355 (9): 866-859.
Feldmann H, Klenk HD, Sanchez A (1993). Molecular biology and evolution of filovirures. Arch. Virol. Suppl. 7: 81-100.
Fisher-Hock SP, Platt GS, Neild GH, Southee T, Baskerville A, Raymond RT, Lloyd G, Simpson DI (1985). Pathophysiology of shock and haemorrhage in a fulminating viral infection (Ebola). J. Infect. Dis. 152 (5): 887-894.
Formenty P, Boesch C, Wyers M, Steiner C, Donati F, Dind F, Walker F, Le Guenno B (1999). Ebola Virus Outbreak among Wild Chimpanzees Living in a Rain Forest of Cote d'Ivoire. J. Infect. Dis.179 (1): S120–S126.
Fujita N, Miller A, Miller G, Gershman K, Gallagher N, Marano N, Hale C (2010). Imported Case of Marburg Hemorrhagic Fever-Colorado, 2008 (Reprinted from MMWR, Vol. 58, pp. 1377-1381, 2009). J. Am. Med. Assoc. 303: 413-415.
Funk S, Piot P (2014). Mapping Ebola in wild animals for better disease control. eLife 3: e04565.
Gear JS, Cassel GA, Gear AJ, Trappler B, Clausen L, Meyers AM, Kew MC, Bothwell TH, Sher R, Miller GB, Schneider J, Koornhof HJ, Gomperts ED, Isaäcson M, Gear JH (1975). Outbreak of Marburg virus disease in Johannesburg. Br. Med. J. 4: (5995): 489-493.
Georges AJ, Leroy EM, Renaut AA, Benissan CT, Nabias RJ, Ngoc MT, Obiang PI, Lepage JP, Bertherat EJ, Bénoni DD, Wickings EJ, Amblard JP, Lansoud-Soukate JM, Milleliri JM, Baize S, Georges-Courbot MC (1999). Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and Health control issues. J. Infect. Dis. 179: S65-S75.
Germain M (1978). Collection of mammals and arthropods during the epidemic of haemorrhagic fever in Zaire. In: Pattyn SR, ed. Ebola virus haemorrhagic fever. Amsterdam: Elsevier/Netherland Biomedical. pp.185-189.
Gire SK, Goba A, Andersen KG, Sealfon RSG, Park DJ, Kanneh L, Jalloh S, Momoh M, Fullah M, Dudas G, Wohl S, Moses LM, Yozwiak NL, Winnicki S, Matranga CB, Malboeuf CM, Qu J, Gladden AD, Schaffner SF, Yang X, Jiang PP, Nekoui M, Colubri A, Coomber MR, Fonnie M, Moigboi A, Gbakie M, Kamara FK, Tucker V, Konuwa E, Saffa S, Sellu J, Jalloh AA, Kovoma A, Koninga J, Mustapha I, Kargbo K, Foday M, Yillah M, Kanneh F, Robert W, Massally JL, Chapman SB, Bochicchio J, Murphy C, Nusbaum C, Young S, Birren BW, Grant DS, Scheiffelin JS, Lander ES, Happi C, Gevao SM, Gnirke A, Rambaut A, Garry RF, Khan SH, Sabeti PC (2014). Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345 (6202): 1369-1372.
Gonzalez JP, Gouilh MA, Reynes JM, Leroy E (2008). Bat-Borne Viral Diseases. Human health and forests. A Global Overview of Issues, Practices and Policy. Edited by Carol J. Pierce Colfer. pp. 161-200.
Gonzalez JP, Herbreteau V, Morvan J, Leroy EM (2005). Ebola virus circulation in Africa: a balance between clinical expression and epidemiological silence. Bull. Soc. Pathol. Exot. 98: 210-217.
Gonzalez JP, Pourrut X, Leroy E, 2007. *Ebolavirus* and other filoviruses. In Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and cross species transmission. JE Child, JS Mackenzie and JA Richt eds.pp. Spring verlag berlin Heidelberg. 315: 363-388.
Grard G, Biek R, Tamfum JJ, Fair J, Wolfe N, Formenty P, Paweska J, Leroy E (2011). Emergence of divergent Zaire ebola virus strains in Democratic Republic of the Congo in 2007 and 2008. J. Infect. Dis. 204 (3):S776-784.
Groseth A, Feldmann H, Strong JE. (2007). The ecology of Ebola virus. Trends Microbiol. 15 (9): 408-416.
Hayman DTS, Emmerich P, Yu M, Wang LF, Suu-Ire R, Fooks AR, Cunningham AA, Wood JLN (2010). Long term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. PLoS ONE 5:e11978.
Hayman DTS, Meng Y, Gary C, Lin-Fa W, Suu-Ire R, Wood JLN, Cunningham AA (2012). Ebola Virus Antibodies in Fruit Bats, Ghana, West Africa. Emerg. Infect. Dis.18 (7): 1207-1209.
Huijbregts B, De Wachter P, Obiang, LSN, Akou ME (2003). Ebola and the decline of gorilla Gorilla gorilla and chimpanzee *Pan troglodytes* populations in Minkebe Forest, north-eastern Gabon. Oryx 37:437-443.
Ithete, NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, Drosten C, Drexler JF, Preiser W (2013). Close Relative of Human Middle East Respiratory Syndrome Coronavirus in Bat, South Africa. Emerg. Infect. Dis. 19 (10): 1697-1699.
Jahrling PB, Geisbert TW, Dalgard DW, Johnson ED, Ksiazek TG, Hall WC, Peters CJ (1990). Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet 335 (8688): 502-505.
Johnson KM (1978). Ebola haemorrhagic fever in Zaire, 1976. Bull WHO 56: 271-293.
Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008). Global trends in emerging infectious diseases. Nature 451(7181): 990-993.
Kenyon RH, Niklasson B, Jahrling PB, Geisbert T, Svensson L, Frydén A, Bengtsson M, Foberg U, Peters CJ (1994). Virologic investigation of a case of suspected haemorrhagic fever. Res. Virol. 145(6): 397-406.
Kiley MP, Bowen ET, Eddy GA, Isaacson M, Johnson KM, McCormick JB, Murphy FA, Pattyn SR, Peters D, Prozesky OW, Regnery RL, Simpson DI, Slenczka W, Sureau P, van der Groen G, Webb PA, Wulff H (1982). Filoviridae: a taxonomic home for Marburg and Ebola viruses? Intervirology 18 (1-2): 24-32.
Kuhn JH, Andersen KG, Baize S, Bào Y, Bavari S, Berthet N, Blinkova O, Brister JR, Clawson AN, Fair J, Gabriel M, Garry RF, Gire SK, Goba A, Gonzalez JP, Günther S, Happi CT, Jahrling PB, Kapetshi J, Kobinger G, Kugelman JR, Leroy EM, Maganga GD, Mbala PK, Moses LM, Muyembe-Tamfum JJ, N'Faly M, Nichol ST, Omilabu SA, Palacios G, Park DJ, Paweska JT, Radoshitzky SR, Rossi CA, Sabeti PC, Schieffelin JS, Schoepp RJ, Sealfon R, Swanepoel R, Towner JS, Wada J, Wauquier N, Yozwiak NL, Formenty P (2014). Nomenclature-and database-compatible names for the two Ebola virus variants that emerged in Guinea and the Democratic Republic of the Congo in 2014. Viruses 6(11):4760-4799.
Kuhn JH, Bao Y, Bavari S, Becker S, Bradfute S, Brister JR, Bukreyev

AA, Caì Y, Chandran K, Davey RA, Dolnik O, Dye JM, Enterlein S, Gonzalez JP, Formenty P, Freiberg AN, Hensley LE, Honko AN, Ignatyev GM, Jahrling PB, Johnson KM, Klenk HD, Kobinger G, Lackemeyer MG, Leroy EM, Lever MS, Lofts LL, Mühlberger E, Netesov SV, Olinger GG, Palacios G, Patterson JL, Paweska JT, Pitt L, Radoshitzky SR, Ryabchikova EI, Saphire EO, Shestopalov AM, Smither SJ, Sullivan NJ, Swanepoel R, Takada A, Towner JS, van der Groen G, Volchkov VE, Wahl-Jensen V, Warren TK, Warfield KL, Weidmann M, Nichol ST (2013). Virus nomenclature below the species level: A standardized nomenclature for filovirus strains and variants rescued from cDNA. Arch. Virol. Virus nomenclature below the species level: a standardized nomenclature for filovirus strains and variants rescued from cDNA.159(5):1229-37.

Kuhn JH, Becker S, Ebihara H, Geisbert TW, Johnson KM, Kawaoka Y, Lipkin WI, Negredo AI, Netesov SV, Nichol ST, Palacios G, Peters CJ, Tenorio A, Volchkov VE, Jahrling PB (2010). Proposal for a revised taxonomy of the family Filoviridae: Classification, names of taxa and viruses, and virus abbreviations. Arch. Virol. 155 (12): 2083-2103.

Kuno G (2001). Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts. Rev. Med. Virol. 11: 165-190.

Kunz C, Hofmann H, Aspöck H (1968). Propagation of "Marburg-virus" (vervet monkey disease agent) in *Aedes aegypti*, Zentralbl. Bakteriol. Parasitol. Orig. 208: 347-349.

Kunz TH (1982). Ecology of bats. New York, NY: Plenum Press.

Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Markotter W, Breiman RF, Shieh WJ, Zaki SR, Rupprecht CE (2010). Marburg Virus in Fruit Bat, Kenya. Emerg. Infect. Dis. 16: 352–354.

Lahm SA, Kombila M, Swanepoel R, Barnes RF (2007). Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994–2003. Trans. R. Soc. Trop. Med. Hyg. 101(1): 64-78.

Lauber C, Gorbalenya AE (2012). Genetics-Based Classification of Filoviruses Calls for Expanded Sampling of Genomic Sequences. Viruses 4: 1425-1437.

Le Guenno B, Formenty P, Boesch C (1999). Ebola virus outbreaks in Ivory Coast and Liberia, 1994-1995, in: H.-D. Klenk (Ed.), Current Topics in Microbiol. and Immunol. Marburg and Ebola viruses. Springer-Verlag, Berlin. 235: 77-84.

Le Guenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C (1995). Isolation and partial characterization of a new strain of Ebola. Lancet 345: 1271-1274.

Leirs H, Mills JN, Krebs JW, Childs JE, Akaibe D, Woollen N, Ludwig G, Peters CJ, Ksiazek TG (1999). Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: Reflections on a vertebrate collection. J. Infect. Dis. 179 (Suppl. 1): S155-S163.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P (2005). Fruit bats as reservoirs of Ebola virus. Nature 438: 575-576.

Leroy EM, Pourrut X, Gonzalez JP (2006). Bats, reserves of the Ebola virus : The mystery is dissipated. Med. Sci. 22(1): 78-79.

Leroy EM, Rouquet P, Formenty P, Souquière S, Kilbourne A, Froment JM, Bermejo M, Smit S, Karesh W, Swanepoel R, Zaki SR, Rollin PE (2004b). Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science 303 (5658) : 387-390.

Leroy EM, Telfer P, Kumulungui B, Yaba P, Rouquet P, Roques P, Gonzalez JP, Ksiazek TG, Rollin PE, Nerrienet E (2004a). A serological survey of Ebola virus infection in central African nonhuman primates. J. Infect. Dis. 190 (11):1895-1899.

Luby SP (2013). The pandemic potential of Nipah virus. Antivir. Res. 100: 38-43.

Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JR, Mills JN, Timonin ME, Willis CK, Cunningham AA, Fooks AR, Rupprecht CE, Wood JL, Webb CT (2013). A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proc. Biol. Sci. B 280(1756): 20122753.

Maganga GD, Kapetshi J, Berthet N, Kebela Ilunga B, Kabange F, Mbala Kingebeni P, Mondonge V, Muyembe JJ, Bertherat E, Briand S, Cabore J, Epelboin A, Formenty P, Kobinger G, González-Angulo L, Labouba I, Manuguerra JC, Okwo-Bele JM, Dye C, Leroy EM. (2014). Ebola virus disease in the Democratic Republic of Congo. N. Engl. J. Med. 371(22):2083-2091.

Martines RB, Ng DL, Greer PW, Rollin PE, Zaki SR. (2015). Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. J. Pathol. 235(2):153-174.

Martini GA (1969). Marburg agent disease in man, Trans. R. Soc. Trop. Med. Hyg. 63: 295-302.

Martini GA, Schmidt HA (1968). Spermatogenic transmission of the 'Marburg virus' (causes of 'Marburg simian disease'). *Klin Wochenschr.* 46: 398-400.

Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Alhakeem R, Durosinloun A, Al Asmari M, Islam A, Kapoor A, Briese T, Daszak P, Al Rabeeah AA, Lipkin WI (2013). Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg. Infect. Dis. 19(11):1819-1823.

Messenger SL, Rupprecht CE, Smith JS (2003). Bats, emerging virus infections, and the rabies paradigm, In: T. H. Kunz and M. B. Fenton (ed.), Bat ecology. University of Chicago Press, Chicago, Ill: 622-679.

Mills JN (2006). Biodiversity loss and emerging infectious disease: an example from the rodent borne hemorrhagic fevers. Biodiversity 7: 9-17.

Monath TP (1999). Ecology of Marburg and Ebola viruses: Speculations and directions for future research. J. Infect. Dis. 179: S127–S138.

Morens DM, Fauci AS (2013). Emerging Infectious Diseases: Threats to Human Health and Global Stability. PLoS Pathog. 9(7): e1003467.

Morvan JM, Deubel V, Gounon P, Nakoune E, Barriere P, Murri S, Perpète O, Selekon B, Coudrier D, Gautier-Hion A, Colyn M, Volehkov V (1999). Identification of Ebola virus sequences present as RNA or DNA in organs of terrestrial small mammals of the Central African Republic. Microbes Infect. 1(14): 1193–201.

Negredo A, Palacios G, Vázquez-Morón S, González F, Dopazo H, Molero F, Juste J, Quetglas J, Savji N, de la Cruz Martínez M, Herrera JE, Pizarro M, Hutchison SK, Echevarría JE, Lipkin WI, Tenorio A (2011). Discovery of an Ebolavirus-Like Filovirus in Europe. PLoS Pathog. 7(10): e1002304.

Nkoghé D, Formenty P, Nnégué S, Mvé MT, Hypolite I, Léonard P, Leroy E (2004). Comité International de Coordination Technique et Scientifique. Practical guidelines for the management of Ebola infected patients in the field. Med. Trop. 64 (2): 199-204. [article in French].

O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DTS, Luis AD, Peel AJ, Plowright RK, Wood JLN (2014). Bat Flight and Zoonotic Viruses. Emerg. Infect. Dis. 20 (5): 741-745.

Ogawa H, Miyamoto H, Nakayama E, Yoshida R, Nakamura I, Sawa H, Ishii A, Thomas Y, Nakagawa E, Matsuno K, Kajihara M, Maruyama J, Nao N, Muramatsu M, Kuroda M, Simulundu E, Changula K, Hang'ombe B, Namangala B, Nambota A, Katampi J, Igarashi M, Ito K, Feldmann H, Sugimoto C, Moonga L, Mweene A, Takada A. (2015). Seroepidemiological Prevalence of Multiple Species of Filoviruses in Fruit Bats (*Eidolon helvum*) Migrating in Africa. J. Infect. Dis. pii: jiv063.

Olival KJ, Hayman DTS (2014). Filoviruses in Bats: Current Knowledge and Future Directions. Viruses 6: 1759-1788.

Pan Y, Zhang W, Cui L, Hua X, Wang M, Zeng Q (2014). Reston virus in domestic pigs in China. Arch. Virol. 159(5): 1129-1132.

Parra JM, Salmerón OJ, Velasco M (2014). The first case of Ebola virus disease acquired outside Africa. N. Engl. J. Med. 371 (25): 2439-2440.

Peters CJ, Johnson ED, Jahrling PB (1992). Filoviruses. In Emerging viruses. Edited by M. S. S. Oxford: Oxford University Press, pp. 159-175.

Peterson AT, Bower JT, Mills JN (2004). Ecological and Geographic Distribution of Filovirus Disease. Emerg. Infect. Dis. 10 (1): 40-47.

Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ, Brady OJ, Kraemer MU, Smith DL, Moyes CL, Bhatt S, Gething PW, Horby PW, Bogoch II, Brownstein JS, Mekaru SR, Tatem AJ, Khan K, Hay SI (2014). Mapping the zoonotic niche of Ebola virus disease in Africa. Elife 8(3):e04395.

Pourrut X (2007). Implication des chauves-souris dans les épidémies de filovirus en Afrique : modalités de contamination de l'homme et des grands singes. Thèse de Doctorat Université de la Méditerranée IX, Marseille II. 234 pp.

Pourrut X, Delicat A, Rollin PE, Ksiazek TG, Gonzalez JP, Leroy EM (2007). Spatial and Temporal Patterns of *Zaire ebolavirus* Antibody

Prevalence in the Possible Reservoir Bat Species. J. Infect. Dis. 196: S176-183.
Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Délicat A, Yaba P, Nkoghe D, Gonzalez JP, Leroy EM (2005). The natural history of Ebola virus in Africa. Microbes Infect. Inst. Pasteur. 7:1005-1014.
Pourrut X, Souris M, Towner JS, Rollin PE, Nichol ST, Gonzalez JP, Leroy EM (2009). Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus.* BMC Infect. Dis. 9:159.
Pretorius MJ, Oelofsen MS, Smith MS, Van Der Ryst E (1997). Rift Valley Fever virus: A seroepidemiologic study of small terrestrial vertebrates in South Africa. Am. J. Trop. Med. Hyg. 57 (6): 693-698.
Rec WE (1999). Marburg fever, Democratic Republic of the Congo. Wkly. Epidemiol. Rec. 74:145.
Rep MMW (2005). Outbreak of Marburg virus hemorrhagic fever-Angola, October 1,2004-March 29, 2005. Morb. Mortal. Wkly. Rep. 54:308-309.
Richter HV, Cumming GS (2008). First application of satellite telemetry to track African straw-coloured fruit bat migration. J. Zool. (Lond). 275: 172-176.
Rodhain F (1998). La notion de réservoir en arbovirologie. Bull. Soc. Pathol. Exot. 91 (4): 279-282.
Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, Sanchez A, Trappier SG, Peters RL, Greer PW, Zaki S, Demarcus T, Hendricks K, Kelley M, Simpson D, Geisbert TW, Jahrling PB, Peters CJ, Ksiazek TG (1999). Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. J. Infect. Dis.179: S108-S114.
Rosevear DR (1965). Bats of West Africa. Trustees of the British Museum (Natural History) London. 418 pp.
Rougeron V, Feldmann H, Grard G, Becker S, Leroy EM (2015). Ebola and Marburg haemorrhagic fever. J. Clin. Virol. 64:111-119.
Rouquet P, Froment JM, Bermejo M, Kilbourne A, Karesh W, Reed P, Kumulungui B, Yaba P, Délicat A, Rollin PE, Leroy EM (2005). Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001-2003. Emerg. Infect. Dis. 11(2): 283-290.
Saéz AM, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Düx A, Kuhl HS, Kaba M, Regnaut S, Merkel K, Sachse A, Thiesen U, Villányi L, Boesch C, Dabrowski PW, Radoni A, Nitsche A, Leendertz SAJ, Petterson S, Becker S, Krähling V, Couacy-Hymann E, Akoua-Koffi C, Weber N, Schaade L, Fahr J, Borchert M, Gogarten JF, Calvignac-Spencer S, Leendertz FH (2015). Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol. Med. 7(1):17-23.
Sanderson IT (1940). The mammals of the North Cameroons forest area. Being the results of the Percy Sladen expedition to the Mamfe Division of the British Cameroons. Trans. Zool. Soc. London 24: 623-725.
Schmaljohn C, Hjelle B (1997). Hantaviruses: a global disease problem. Emerg. Infect. Dis. 3:95-104.
Siegert R, Shu HL, Slenczka HL, Peters D, Müller G (1968).The aetiology of an unknown human infection transmitted by monkeys (preliminary communication). Ger. Med. Mon. 13(1): 1-2.
Smith CEG, Simpson DIH, Bowen ETW (1967). Fatal human disease from vervet monkeys. Lancet II: 1119-1121.
Smith DH, Isaacson M, Johnson KM, Bagshawe A, Siongok T, Keruga WK (1982). Marburgvirus disease in Kenya. Lancet 1(8276):816-820.
Smith DIH (1978). Ebola haemorrhagic fever in Sudan, 1976. Bull. WHO 56:247-270.
Swanepoel R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, Rollin PE, Zaki SR, Peters CJ (1996). Experimental inoculation of plants and animals with Ebola virus. Emerging Infectious Diseases. 2(4):321-325.
Taylor DJ, Leach RW, Bruenn J (2010). Filoviruses are ancient and integrated into mammalian genomes. BMC Evol. Biol. 10:193.
Timen A, Koopmans MPG, Vossen A, van Doornum GJJ, Gunther S, van den Berkmortel F, Verduin KM, Dittrich S, Emmerich P, Osterhaus AD, van Dissel JT, Coutinho RA (2009). Response to Imported Case of Marburg Hemorrhagic Fever, the Netherlands. Emerg. Infect. Dis. 15:1171-1175.
Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PB, Albarino CG, Miller DM, Reed ZD, Kayiwa JT, Mills JN, Cannon DL, Greer PW, Byaruhanga E, Farnon EC, Atimnedi P, Okware S, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Ksiazek TG, Nichol ST, Rollin PE **(**2009). Isolation of Genetically Diverse Marburg Viruses from Egyptian Fruit Bats. PLoS Pathog. 5(7):e1000536.
Towner JS, Khristova ML, Sealy TK, Vincent MJ, Erickson BR, Bawiec DA, Hartman AL, Comer JA, Zaki SR, Ströher U, Gomes da Silva F, del Castillo F, Rollin PE, Ksiazek TG, Nichol ST (2006). Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. J. Virol. 80 (13): 6497-6516.
Towner JS, Pourrut X, Albarino CG, Nkogue CN, Bird BH, Grard G, Ksiazek TG, Gonzalez JP, Nichol ST, Leroy EM (2007). Marburg virus infection detected in a common African bat. PLoS ONE 2(8): e764.
Towner JS, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, Quan PL, Lipkin WI, Downing R, Tappero JW, Okware S, Lutwama J, Bakamutumaho B, Kayiwa J, Comer JA, Rollin PE, Ksiazek TG, Nichol ST (2008). Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. PLoS Pathog. 4(11):e1000212.
Turell MJ, Bressler DS, Rossi CA (1996). Lack of virus replication in arthropods after intrathoracic inoculation of Ebola Reston virus. Am. J. Trop. Med. Hyg. 55: 89-90.
Van der Poel WH, Lina PH, Kramps JA (2006). Public health awareness of emerging zoonotic viruses of bats: a European perspective. Vector Borne Zoonotic Dis. 6 (4): 315-24.
Vogel G (2014). Infectious Disease. Genomes reveal start of Ebola outbreak. Science 345 (6200): 989-990.
Walker EP (1999). Mammals of the World. (2 volumes). (Sixth Edition). Volume I- Baltimore and London: The Johns Hopkins University Press, 836 pp.
Walsh PD, Abernethy KA, Bermejo M, Beyers R, De Wachter P, Akou ME, Huijbregts B, Mambounga DI, Toham AK, Kilbourn AM, Lahm SA, Latour S, Maisels F, Mbina C, Mihindou Y, Obiang SN, Effa EN, Starkey MP, Telfer P, Thibault M, Tutin CE, White LJ, Wilkie DS (2003). Catastrophic ape decline in western equatorial Africa. Nature 422 (6932): 611-614.
Warrell MJ, Warrell DA (2004). Rabies and other lyssavirus diseases. Lancet 363:959-969.
Wauquier N, Bangura J, Moses L, Khan SH, Coomber M, Lungay V, Gbakie M, Sesay MSK, Gassama IAK , Massally JLB, Gbakima A, Squire J, Lamin M, Kanneh L, Yillah M, Kargbo K, Roberts W, Vandi M, Kargbo D, Vincent T, Jambai A, Guttieri M, Fair J, Souris M, Gonzalez JP (2015). Understanding the Emergence of Ebola Virus Disease in Sierra Leone: Stalking the Virus in the Threatening Wake of Emergence. Plos Current Outbreaks. doi: 10.1371/currents.outbreaks.9a6530ab7bb9096b34143230ab01cdef.
Weingartl HM, Nfon C, Kobinger G (2013). Review of Ebola virus infections in domestic animals. Dev. Biol. (Basel).135:211-218.
WHO Ebola Response Team (2014). Ebola Virus Disease in West Africa-The First 9 Months of the Epidemic and Forward Projections. N. Engl. J. Med. 1-15.
Woolhouse ME, Haydon DT, Antia R (2005). Emerging pathogens: the epidemiology and evolution of species jumps. Trends Ecol. Evol. 20: 238-244.
World Health Organization (WHO) (1978). Ebola haemorrhagic fever in Zaire, 1976. Bull. World Health Organ. 56:271–293.
World Health Organization (WHO) (1992). Viral haemorrhagic fever in imported monkeys. Wkly. Epidemiol. Rec. 67:142-143.
World Health Organization/International Study Team (1978). Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. Bull World Health Organ. 56:247-270.


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# Ticks (Acari: Ixodida) of the genus *Haemaphysalis* Koch, 1844 in Senegal: a review of host associations, chorology, and identification

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## ABSTRACT

The *Haemaphysalis* genus (Acari, Ixodidae) in Senegal is reviewed. This embodies a summary of specimens collected from vertebrate hosts over three decades. 454 collections were performed over this period (408 from mammals and 46 from birds), representing a total of 5752 ticks in different developmental stages. Seven *Haemaphysalis* spp. were collected, identified, and inventoried including: *H.* (*Kaiseriana*) *rugosa*, *H.* (*Ornithophysalis*) *hoodi*, *H.* (*Rhipistoma*) *houyi*, as well as four other species belonging to the *leachi* group, namely *H.* (*Rhipistoma*) *leachi*, *H.* (*Rh.*) *moreli*, *H.* (*Rh.*) *muhsamae* and *H.* (*Rh.*) *spinulosa*. Vertebrate hosts of *Haemaphysalis* species were identified and listed in different ecological zones of Senegal. An identification key of the haemaphysalids of Senegal is proposed, which is also applicable to the haemaphysalid fauna of the Occidental sub-region of the Afrotropical zoogeographical region. The role of these species as potential vectors of zoonotic diseases in Senegal is also discussed.

**Keywords** *Haemaphysalis*; hosts; distribution; diagnosis; Ixodidae; vectors; West Africa
**Zoobank** http://zoobank.org/2C4DABA4-796F-42F6-8E0E-25712CC53E93

## Introduction

The genus *Haemaphysalis* Koch, 1844 belongs to the *Ixodidae* family (*Hyalomminae* sub-family). Haemaphysalid ticks are characterized by a scutum without ornamentation, a distinctive feature among ixodid ticks of the Metastriata group. These eyeless and inornate ticks lack adanal and subanal plates in males, while females have two short spurs of unequal length on coxa 1. Some other notable characteristics include: short palpi, usually conical with a large palpal segment 2 extending laterally beyond the basis capituli, at least two times longer than segment 1; short mouthparts; a large rear-facing spur on trochanter I; festoons are also present on the posterior margin. *Haemaphysalis* spp. have a three-host life cycle, are mostly associated with wild animals, but sometimes infest livestock (Morel, 2003). Few studies have been carried out on the *Haemaphysalis* genus so far, and those performed mostly focused on their economic importance to livestock in Eurasia, Africa, Australia, and New Zealand (Levin, 2016), with less emphasis on their diversity and role as potential vectors and reservoirs of human and animal pathogens. Indeed, some adaptive changes in haemaphysaline behavior such as a shift from wild deer and antelope to domestic cattle, sheep and goats have been noticed (Levin, 2016),

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implying a potential for the emergence of pathogens associated with wild populations into the peridomestic environment within livestock.

Research was performed on hard ticks (i.e. Ixodids) infesting domestic livestock and wild animals in Senegal in order to establish a list of all ixodid ticks found in Senegal (Sylla *et al*., 2007; 2008). The knowledge previously acquired on the *Haemaphysalis* genus includes: the description of immature stages of *Haemaphysalis rugosa* (Camicas, 1978); revisiting the taxonomy of the *H. leachi* group, with the description of *H. moreli* (Camicas *et al*., 1972); the delineation of the *aciculifer* group from the *rugosa* group (Hoogstraal and El Kammah, 1972; Camicas, 1978). More recently, Apanaskevich *et al*. (2007) screened many collections of *Haemaphysalis*, previously identified as *Haemaphysalis* (*Rhipistoma*) *leachi*, and compared them with typed *H*. (*Rh*.) *leachi* specimens from North Africa and the holotype specimen of *Haemaphysalis* (*Rhipistoma*) *elliptica*, concluding that many of the Southern and East African ticks previously identified as *H*. (*Rh*.) *leachi* were misdiagnosed and are actually *H*. (*Rh*.) *elliptica*.

The present study reviews *Haemaphysalis* species known to occur in different geographic areas of Senegal and describes features of their specific diagnosis, their host associations and their chorology.

## Materials and methods

Most of the data presented here originate from a collection currently held at the laboratory of Medical Zoology, Research Institute for Development (IRD, Mbour Centre, Senegal). This collection has been continuously enriched by different research projects including: research conducted on birds, wild and domestic mammals during the Crimean-Congo Hemorrhagic Fever (CCHF) program (1987-1993) (Camicas *et al*., 1990); a project on climatic change and health (Action Thematique Interdisciplinaire, 2003-2004) focusing on tick inventories and the impact of climatic change on their geographical distribution and associated risk of CCHF emergence (Wilson *et al*., 1990). For these studies, rodents were caught in different geographical areas of Senegal, and ticks were removed from the hosts and preserved in a 70% ethanol solution for further study. More recently, the EDEN project (European Commission Project on "Emerging Diseases in a Changing European Environment: http://www.eden-fp6project.net, 2004-2007) included an investigation of West Nile virus ecology leading to bird trapping and tick collection in the Djoudj National Park of Birds (16°25'N, 16°18'W, Saint Louis Department, Saint Louis Region) and Barkedji (15°17'N, 14°52'W, Linguere Department, Louga Region).

Study sites and tick collections have been presented in detail elsewhere (Sylla *et al*., 2004; 2007; 2008). Tick collections were mapped at the scale of the squared degree including the locality where each tick sample was collected. Maps were generated using SavGIS software (IRD, www.savgis.org). Geographical position of each locality was given by the gazetteer of Senegal (Board on Geographic Names, 1965, Dpt. of the Interior, Washington, D.C., 20240, 1965, IV + 194 pp.) or by using a GPSMAP® 62S (Garmin Inc. Wichita KS).

*Haemaphysalis* species diagnosis followed an in-house identification key adapted to the ixodid fauna of the Occidental sub-region of the Afrotropical Region (Matthysse and Colbo, 1987A; Elbl and Anastos, 1966) that follows the systematic terminology of Camicas and Morel (Camicas and Morel, 1977; Camicas *et al*., 1998; Morel, 2003). Features pointed out by Apanaskevich *et al*. (2007) for the diagnosis of the *H*. (*Rh*.) *leachi* group are considered in this study. Vertebrate host terminologies follow Walker (1999a and b) and Wilson and Reeder (1992) for mammals and Cabot *et al*. (1992A and B) for birds.

## Results

A total of 7 *Haemaphysalis* species, including 3 species of *Haemaphysalis* stricto sensu (*Haemaphysalis* (*Kaiseriana*) *rugosa* Santos Dias, 1956; *Haemaphysalis* (*Ornithophysalis*)

**Table 1** Association of *Haemaphysalis* ticks with their known class of vertebrate hosts in Senegal. The number of records per species is given in brackets. % = the percentage of individuals within each life stage for a given species and host class. Total immature = Larvae+Nymph, Total mature = Male+Female.

| | Class of Mammalia | | | | Class of Aves | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Tick species | Total Immature L+N | % | Total Mature M+F | % | Total Immature L+N | % | Total Mature M+F | % | Total collected per species |
| *H. (Kaiseriana) rugosa* (12) | 41 | 89.1 | 5 | 10.9 | 0 | 0 | 0 | 0 | 46 |
| *H. (Ornithophysalis) hoodi* (41) | 0 | 0 | 0 | 0 | 41 | 69.50 | 18 | 30.50 | 59 |
| *H. (Rhipistoma) houyi* (143) | 730 | 33.4 | 1457 | 66.6 | 0 | 0 | 0 | 0 | 2187 |
| *H. (Rhipistoma) leachi* (203) | 1012 | 33.3 | 2010 | 66.2 | 15 | 0.5 | 1 | 0.0 | 3038 |
| *H. (Rhipistoma) moreli* (52) | 3 | 0.7 | 415 | 99.3 | 0 | 0 | 0 | 0 | 418 |
| *H. (Rhipistoma) muhsamae* (1) | 0 | 0 | 1 | 100 | 0 | 0 | 0 | 0 | 1 |
| *H. (Rhipistoma) spinulosa* (3) | 3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Total ticks collected/life stage | 1789 | 31.5 | 3888 | 68.5 | 56 | 74.7 | 19 | 25.3 | |
| Total ticks collected/class (455) | | 5677 (98.7%) | | | | 75 (1.3%) | | | 5752 |

*hoodi* Warburton & Nuttall, 1909; *Haemaphysalis* (*Rhipistoma*) *houyi* Nuttall & Warburton, 1915) and four species of the *Haemaphysalis leachi* group (*Haemaphysalis* (*Rhipistoma*) *leachi* Audouin, 1826; *Haemaphysalis* (*Rhipistoma*) *moreli* Camicas, Hoogstraal & El Kammah, 1972; *Haemaphysalis* (*Rhipistoma*) *muhsamae* Santos Dias, 1954; *Haemaphysalis* (*Rhipistoma*) *spinulosa* Neumann, 1906), were collected from mammals and birds in Senegal (Table 1).

### *Haemaphysalis* (*Kaiseriana*) *rugosa* Santos Dias, 1956

The African antelope spurred-rugose haemaphysalid, was only recorded from mammals (Table 2). The first specimens were collected from wild bovid, mongoose and civet in the Niokolo Koba National Park, NKNP (13°N, 13°W, Tambacounda Reg.) in 1971 and recorded as *Haemaphysalis aciculifer* Warburton, 1913 by Morel (1956), re-identified later by Hoogstraal and El Kammah (1972) as *H.* (*Ka.*) *rugosa*. This species has also been recorded from Kedougou (12°33'N, 12°11'W, Kedougou Department, Kedougou Region), Kolda (12°53'N, 14°57'W, Kolda Dpt., Kolda Reg.), and Sangalkam (14°47'N, 17°13'W, Dakar Dpt., Dakar Reg.) (Figure 1).

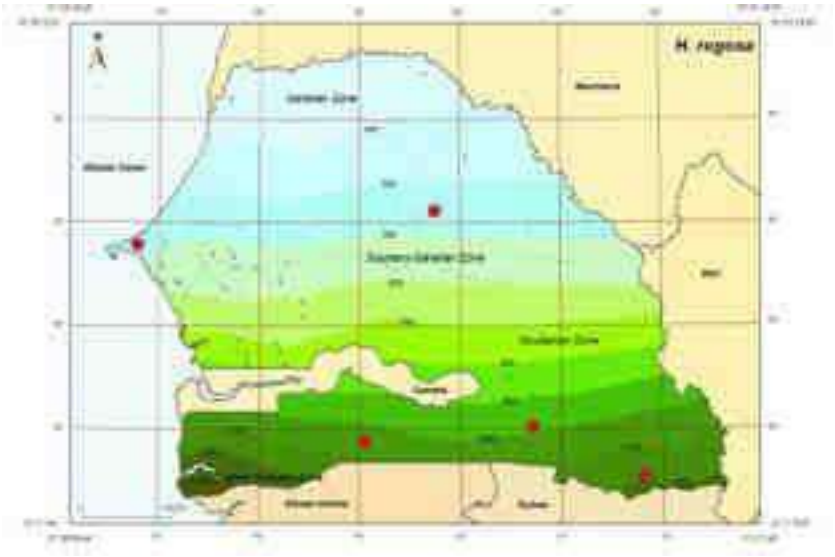

**Figure 1** Collecting sites of *Haemaphysalis* (*Kaiseriana*) *rugosa* indicated in red; blue dots show localization of all other collection sites.

### *Haemaphysalis* (*Ornithophysalis*) *hoodi* Warburton & Nuttall, 1909

*Haemaphysalis* (*Ornithophysalis*) *hoodi* infested birds only (Table 3). Several collection points were obtained for this species throughout Senegal: Gorom (14°49'N, 17°09'W, Dakar Reg.); Sangalkam (14°47'N, 17°13'W, Rufisque Dpt., Dakar Reg.); Saboya (13°39'N, 16°07'W, Nioro Dpt., Kaolack Reg.); Bandia (14°35'N, 17°01'W, Mbour Dpt., Thies Reg.); Kedougou (12°33'N, 12°11'W, Kedougou Dpt., Kedougou Reg); Kaffrine (14°05'N, 15°33'W, Kaffrine Dpt., Kaffrine Reg.); Missira (13°31'N, 13°31'N; Tambacounda Dpt., Tambacounda Reg.); Kolda (12°53'N, 14°57'W, Kolda Dpt., Kolda Reg.) (Figure 2).

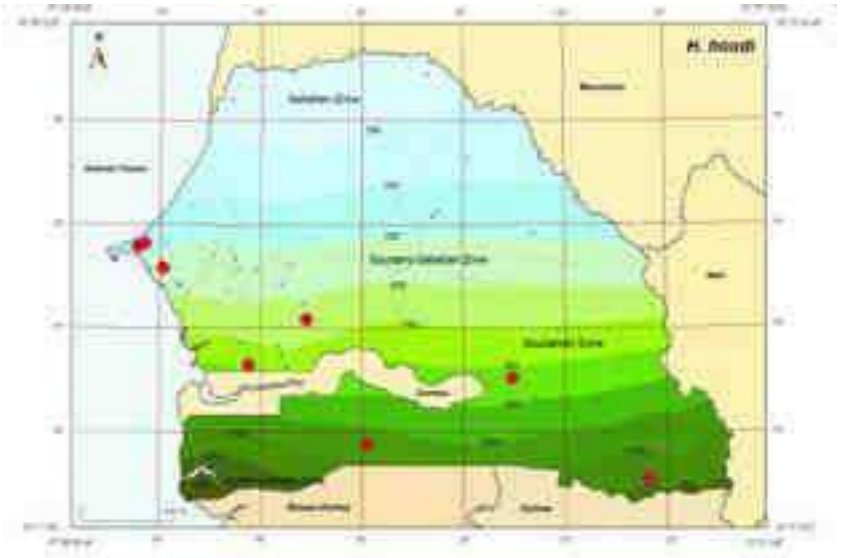

**Figure 2** Collecting sites of *Haemaphysalis* (*Ornithophysalis*) *hoodi* indicated in red; blue dots show localization of all other collection sites.

### *Haemaphysalis* (*Rhipistoma*) *houyi* Nuttall & Warburton, 1915

*Haemaphysalis* (*Rhipistoma*) *houyi* was exclusively collected from the African Ground Squirrel, *Xerus erythropus* (Desmarest, 1817) (Mammalia, Rodentia, Sciuridae) (Table 4). Records of this African tick in Senegal are from several locations: Bandia Forest reserve (14°35'N, 17°01'W, Mbour Dpt., Thies Reg.); Saboya Forest reserve (13°39'N, 16°07'W, Nioro-du-Rip Dpt., Kaolack Reg.); 10km North of Kedougou (12°33'N, 12°11'W, Kedougou Dpt., Kedougou Reg.); Santhiaba Mandjak (12°22'N, 16°33'W, Ziguinchor Dpt., Casamance Reg.); National Park of Basse Casamance, NPBC (12°N, 16°W, Oussouye Dpt., Casamance Reg.); Fadiga (12°33'N, 12°12'W, Kedougou Dpt., Kedougou Reg.); from the area between Sare Dioulde and Sinthian Koundara (13°27'N, 14°20'W, Velingara Dpt., Kolda Reg.) (Figure 3).

### *Haemaphysalis leachi* group Camicas, Hoogstraal & El Kammah, 1972

*Haemaphysalis* (*Rhipistoma*) *leachi* Audouin, 1826: 203 collections were obtained from wild animals including 5 from birds (Table 5) and 198 from mammals (Table 6). At the pre-imaginal stages, it is associated with Carnivores of the Herpestidae and Viverridae families. Immatures are also frequently collected from Insectivora species. In Senegal, the yellow dog-tick, *H. leachi*, has been recorded from: Bandia Forest reserve (14°35'N, 17°01'W, Mbour Dpt., Thies Reg.); Richard-Toll 16°28'N, 15°41'W, Saint-Louis Dpt., Saint Louis Reg.); Bode, Podor (16°27'N, 14°21'W, Podor Dpt., Saint Louis Reg.); Savoigne (16°12'N, 16°17'W, Saint-Louis Dpt., Saint Louis Reg.); Kedougou (12°33'N, 12°11'W, Kedougou Dpt., Kedougou Reg.).

**Table 2** Hosts of *Haemaphysalis* (*Kaiseriana*) *rugosa* in Senegal. The number of records per species is given in brackets. Total immature = Larvae+Nymph, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name / Common name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Artiodactyla (3) | Bovidae (3) | *Ourebia ourebi* (1) Oribi | 0 | 0 | 0 | 1 | 0 | 1 |
| | | *Redunca redunca* (1) Reedbuck | 0 | 0 | 0 | 1 | 0 | 1 |
| | | *Tragelaphus scriptus* (1) Bushbuck | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total per stage | | 0 | 0 | 0 | 3 | 0 | 3 |
| | Total (Im+M) | | | | | 3 | | |
| Total | Mammalia, Artiodactyla (3) | | | | | 3 | | |
| Carnivora (7) | Herpestidae (2) | *Herpestes ichneumon* (1) African mongoose | 0 | 0 | 0 | 1 | 0 | 1 |
| | | *Ichneumia albicauda* (1) White-tailed mongoose | 1 | 0 | 1 | 0 | 0 | 0 |
| | Total per stage | | 1 | 0 | 1 | 1 | 0 | 1 |
| | Total (Im+M) | | | | | 2 | | |
| | Viverridae (5) | *Civettictis civetta* (1) African civet | 0 | 0 | 0 | 1 | 0 | 1 |
| | | *Genetta pardina* (1) Forest genet | 0 | 1 | 1 | 0 | 0 | 0 |
| | | *Pseudogenetta villiersi* (3) Villiers' genet | 30 | 4 | 34 | 0 | 0 | 0 |
| | Total per stage | | 30 | 5 | 35 | 1 | 0 | 1 |
| | Total (Im+M) | | | | | 36 | | |
| Total | Mammalia, Carnivora (7) | | | | | 38 | | |
| Lagomorpha (1) | Leporidae (1) | *Lepus crawshayi* (1) Crawshay's hare | 0 | 1 | 1 | 0 | 0 | 0 |
| | Total per stage | | 0 | 1 | 1 | 0 | 0 | 0 |
| | Total (Im+M) | | | | | 1 | | |
| Total | Mammalia, Lagomorpha (1) | | | | | 1 | | |
| Rodentia (1) | Muridae (1) | *Gerbillus sp* . (1) Pygmy gerbil | 4 | 0 | 4 | 0 | | 0 |
| | Total per stage | | 4 | 0 | 4 | 0 | | 0 |
| | Total (Im+M) | | | | | 4 | | |
| Total | Mammalia, Rodentia (1) | | | | | 4 | | |
| General Total | Mammalia (12) | | | | | 46 | | |

**Table 3** Hosts of *Haemaphysalis (Ornithophysalis) hoodi* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name<br>Common name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Coraciadiforma (7) | Bucerotidae (6) | *Tockus erythrorhynchus* (6)<br>Red-billed hornbill | 7 | 0 | 7 | 7 | 1 | 8 |
| (=Coraciiforma) | Coraciidae (1) | *Coracias cyanogaster* (1)<br>Blue-bellied roller | 2 | 1 | 3 | 0 | 0 | 0 |
| | Total per stage | | 9 | 1 | 10 | 7 | 1 | 8 |
| | Total (Im+M) | | | | 18 | | | |
| Total | Aves, Coraciadiforma (7) | | | | 18 | | | |
| Cuculiforma (15) | Cuculidae (15) | *Centropus senegalensis* (15)<br>Senegal coucal | 6 | 8 | 14 | 0 | 0 | 0 |
| | Total per stage | | 6 | 8 | 14 | 0 | 0 | 0 |
| | Total (Im+M) | | | | 14 | | | |
| | Aves, Cuculiforma (15) | | | | 14 | | | |
| Galliforma (9) | Phasianidae (9) | *Francolinus bicalcaratus* (7)<br>Double spurred francolin | 6 | 5 | 11 | 1 | 0 | 1 |
| | | *Gallus gallus domesticus* (1)<br>Domestic chicken | 1 | 0 | 1 | 0 | 0 | 0 |
| | | *Numida meleagris galeata* (1)<br>Helmeted guineafowl | 2 | 0 | 2 | 0 | 0 | 0 |
| | Total per stage | | 9 | 5 | 14 | 1 | 0 | 1 |
| | Total (Im+M) | | | | 15 | | | |
| | Aves, Galliforma (9) | | | | 15 | | | |
| Passeriforma (10) | Corvidae (1) | *Ptilostomus afer* (1)<br>Piapiac | 1 | 1 | 2 | 0 | 0 | 0 |
| | Motacillidae (1) | *Macronyx croceus* (1)<br>Yellow-throated long claw | 0 | 0 | 0 | 0 | 1 | 1 |
| | Ploceidae (2) | *Bubalornis albirostris* (2)<br>White-billed buffalo-weaver | 0 | 0 | 0 | 1 | 2 | 3 |
| | Strunidae (6) | *Lamprotornis caudatus* (6)<br>Long-tailed glossy starling | 0 | 1 | 1 | 1 | 4 | 5 |
| | Total per stage | | 1 | 2 | 3 | 2 | 7 | 9 |
| Total | Total (Im+M) | | | | 12 | | | |
| | Aves, Passeriforma (10) | | | | 12 | | | |
| General Total | Aves (41) | | | | 59 | | | |

Ultimately, Villiers (1955) reported it in Dakar (14°40'N, 17°26'W, Dakar Dpt., Dakar Reg.) from the Golden jackal, *Canis aureus* (1♂, December 13, 1946, Institut Fondamental d'Afrique Noire, IFAN), the Serval, *Felis serval* (8♂♂ 3♀♀, October 1945, IFAN) and, the White-tailed mongoose, *Ichneumia albicauda* (2♂♂ 1♀, June 1947, IFAN). Moreover, the yellow dog-tick has been also collected and identified from an extended variety of hosts in Senegal including: the African hedgehog, *Atelerix albiventris* in Bel-Air, Dakar (2♂♂, March 1948, IFAN), in Thiaroye, Dakar (1♂, February, 1955, IFAN); the Serval, *Felis serval* (2♂♂, June 1946, IFAN) in Bignona (12°47'N, 16°14'W, Bignona Dpt., Casamance Reg); (10♂♂, 3♀♀, May 18, 1956) in Sandiara (14°25'N, 16°48'W, Mbour Dpt., Thies Reg.); the Domestic cat, *Felis catus* (2♀♀, January 4, 1956) in Hann, Dakar (Morel,1958; 1961); in Sangalkam (14°47'N, 17°13'W, Dakar Dpt., Dakar Reg.) (2♀♀, January 14, 1956); in Popenguine Forest reserve (14°34'N, 17°05'W, Mbour Dpt., Thies Reg) (1♀, January 2, 1956); the Common genet, *Genetta genetta senegalensis* (2♂♂, April 29, 1955) in Nioro-du-Rip (13°45'N, 15°48'W, Nioro Dpt., Kaolack

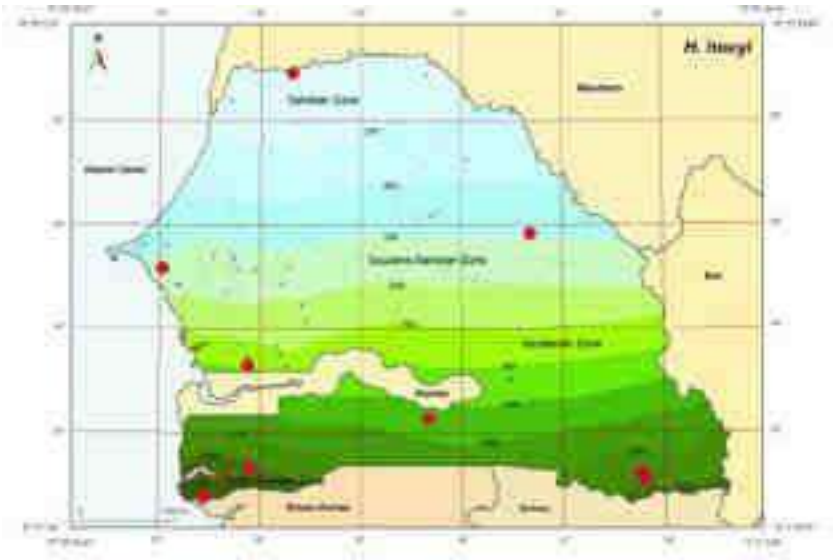

**Figure 3** Collecting sites of *Haemaphysalis* (*Rhipistoma*) *houyi* indicated in red; blue dots show localization of all other collection sites.

Reg); Missira (13°31'N, 13°31'N; Tambacounda Dpt., Tambacounda Reg.) (1♂, August 28, 1948, IFAN), Richard-Toll (16°28'N, 15°41'W, Saint-Louis Dpt., Saint Louis Reg.) (2♂♂ 1♀, September 24, 1948, IFAN); Kidira (14°27'N, 12°13'W; Kidira Dpt., Tambacounda Reg.); the African civet, *Civettictis civetta* (1♂, 1♀, August 30, 1954) in Tambacounda (13°46'N, 13°39'N; Tambacounda Dpt., Tambacounda Reg.); the domestic dog, *Canis familiaris* (1♀, October 1947, Dakar); the African giant rat, *Cricetomys gambianus* (7NN, 3LL in June 19, 1956, Dakar); Morel (1956) collected *H. leachi* in the NKNP from the Side-striped jackal, *Canis adustus* (2♂♂, September 1955; 1♂, December 6, 1955); African civet, *Civettictis civetta* (8♂♂, 5♀♀, September 1955, 3♂♂, February, 1956); African mongoose, *Herpestes ichneumon* (4♂♂, February 1956, 7♂♂, 3♀♀); White-tailed mongoose, *Ichneumia albicauda* (September 12, 1955); Bushbuck *Tragelaphus scriptus scriptus* (1♀, September 1955); Oribi, *Ourebia ourebi* (4♂♂, 1♀, September 1955). Morel (1961) recorded it again in the NKNP (13°2'N, 13°17'N; Tambacounda Dpt., Tambacounda Reg.) from *Panthera pardus* (1♂ 1♀, March 1955); African wild cat, *Felis libyca* (1♀, March 25, 1957); Villiers' genet, *Pseudogenetta*

**Table 4** Hosts of *Haemaphysalis houyi* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name / Common name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Rodentia (143) | Sciuridae (143) | *Xerus erythropus* (143) African ground squirrel | 195 | 535 | 730 | 1164 | 293 | 1457 |
| | Total per stage | | 195 | 535 | 730 | 1164 | 293 | 1457 |
| | Total (Im+M) | | | | | 2187 | | |
| | Mammalia, Rodentia | | | | | 2187 | | |
| General Total | Mammalia | | | | | 2187 | | |

**Table 5** Avian hosts of *Haemaphysalis (Rhipistoma) leachi* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name / Common name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Cuculiforma (4) | Cuculidae | *Centropus senegalensis* (4) Senegal coucal | 10 | 5 | 15 | 0 | 0 | 0 |
| | Total per stage | | 10 | 5 | 15 | 0 | 0 | 0 |
| | Total (Im+M) | | | | | 15 | | |
| Total | Aves, Cuculiforma (4) | | | | | 15 | | |
| Passeriforma (1) | Muscicapidae | *Myrmecocichla aethiops* (1) Northern anteater chat | 0 | 0 | 0 | 0 | 1 | 1 |
| | Total per stage | | 0 | 0 | 0 | 0 | 1 | 1 |
| | Total (Im+M) | | | | | 1 | | |
| Total | Aves, Strigiforma (1) | | | | | 1 | | |
| General Total | Aves (5) | | | | | 16 | | |

*villiersi* (1♂, March 25, 1955). It was also collected from a Domestic dog (1♀, June 7, 1959) in Fann, Dakar; Common genet, *Genetta g. senegalensis* (3♂♂, 2♀♀, September 28, 1959) in Thiaroye, Dakar; Mbaouane, Kayar (14°53'N, 17°07'W, Tivaouane Dpt., Thies Reg.); *Civettictis civetta* (3♂♂, March, 16 1963) in Kolda (12°53'N, 14°57'W, Kolda Dpt., Kolda Reg.), Bandafassi (12°32'N, 12°19'W, Kedougou Dpt., Kedougou Reg.) in August 2006, and *Pseudogenetta villiersi* (12♂♂, 8♀♀, January 6, 1964; 2♀♀ January, 10, 1964) (Figure 4). Ultimately, the species was recorded by flagging or hand picking *in natura* (1♀, June 23, 1962 in Diender near Tanma lake (15♂♂, 8♀♀, June 12, 1962), and also form different unidentified rodents spp. (2NN, March 2, 1962; 4LL, July 25, 1962; 32LL, 2NN, February 25, 1963).

### *Haemaphysalis* (*Rhipistoma*) *moreli* Camicas, Hoogstraal & El Kammah, 1972

*Haemaphysalis* (*Rhipistoma*) *moreli* has been only collected from mammals in Senegal (Table 7). Collection reference sites are as follow: Ngohé (14°34'N, 16°3'W; Diourbel Dpt., Diourbel Reg.); Bao Bolon River (13°38'N, 15°45'W, Nioro du Rip Dpt., Kaolack Reg.); Bandia Forest reserve; Saboya Forest reserve; Dialakoto (13°19'N, 13°18'W, Tambacounda Dpt., Tambacounda Reg.); Bandafassi (12°32'N, 12°19'W, Kedougou Dpt., Kedougou Reg.). It was collected from *Civettictis civetta* in August 2006; between Thies and Bambey on the road; in Kedougou (12°33'N, 12°11'W, Kedougou Dpt., Kedougou Reg.); Sandiara (14°25'N, 16°48'W, Mbour Dpt., Thies Reg.); Etiess (12°34'N, 12°26'W, Salemata Dpt., Kedougou Reg.); Dakar-Yoff (14°45'N, 17°28'W, Dakar Dpt., Dakar Reg.) (Figure 4).

### *Haemaphysalis* (*Rhipistoma*) *muhsamae* Santos Dias, 1954

*Haemaphysalis* (*Rhipistoma*) *muhsamae* is typically a parasite of carnivores of the Mustelidae and Viverridae families. It was confused for a long time with *H. leachi*, which shares the same hosts and the same geographical distribution. A specimen collected from the zorilla or Striped polecat, *Ictonyx striatus* (Carnivora, Mustelidae) in Hann, Dakar is deposited (1♂, August 5, 1939, IFAN) (Figure 4). Although not abundant in the collection, several records from Senegal are attributed to this species. Those are from Diourbel (14°40'N, 16°15'W, Diourbel Dpt., Diourbel Reg.); Mahekor Forest reserve; Bao Bolon (13°38'N, 15°45'W, Nioro du Rip Dpt., Kaolack Reg.); Kassas Forest reserve (14°14'N, 15°35'W, Kaffrine Dpt., Kaffrine Reg.); Saboya Forest reserve (13°39'N, 16°07'W, Nioro-du-Rip Dpt., Kaolack Reg.); Bandia Forest reserve (14°35'N, 17°01'W, Mbour Dpt., Thies Reg.); Ngoyé = Ngohé (14°34'N, 16°3'W; Diourbel Dpt., Diourbel Reg.); Dialakoto (13°19'N, 13°18'W, Tambacounda Dpt.,

**Table 6** Mammalian hosts of *Haemaphysalis (Rhipistoma) leachi* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| | | *Canis adustus* (1) | 0 | 2 | 2 | 126 | 87 | 213 |
| | | *Canis aureus* (9) | 0 | 0 | 0 | 34 | 17 | 51 |
| | Canidae (17) | *Canis lupus familiaris* (1) | 0 | 0 | 0 | 0 | 1 | 1 |
| | | *Vulpes pallida* (6) | 0 | 0 | 0 | 20 | 12 | 32 |
| | Total per stage | | 0 | 0 | 2 | 180 | 117 | 297 |
| | Total (Im+M) | | | | | 299 | | |
| | | *Felis catus* (5) | 0 | 1 | 1 | 9 | 0 | 9 |
| | | *Felis lybica* (10) | 0 | 4 | 4 | 133 | 57 | 190 |
| | Felidae (21) | *Felis serval* (2) | 0 | 0 | 0 | 1 | 0 | 1 |
| | | *Felis sylvestris* (2) | 0 | 0 | 0 | 2 | 4 | 6 |
| | | *Panthera leo* (1) | 8 | 0 | 8 | 0 | 0 | 0 |
| | | *Panthera pardus* (1) | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total per stage | | 8 | 5 | 13 | 146 | 61 | 207 |
| | Total (Im+M) | | | | | 220 | | |
| | | *Atilax paludinosus* (9) | 0 | 1 | 1 | 96 | 33 | 129 |
| Canivora (131) | | *Herpestes ichneumon* (3) | 0 | 4 | 4 | 22 | 1 | 23 |
| | | *Ichneumia albicauda* (25) | 358 | 50 | 408 | 194 | 78 | 272 |
| | Herpestidae (40) | *Mungos gambianus* (1) | 0 | 0 | 0 | 2 | 0 | 2 |
| | | *Mungos mungo* (1) | 0 | 0 | 0 | 10 | 2 | 12 |
| | | *Mongoose, ind* (1) | 0 | 0 | 0 | 0 | 1 | 1 |
| | Total per stage | | 358 | 55 | 413 | 324 | 115 | 439 |
| | Total (Im+M) | | | | | 1062 | | |
| | Mustelidae (1) | *Ictonyx striatus* (1) | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total per stage | | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total (Im+M) | | | | | 1 | | |
| | | *Civettictis civetta* (9) | 0 | 0 | 0 | 70 | 31 | 101 |
| | Viverridae (53) | *Genetta g. senegalensis* (14) | 0 | 0 | 0 | 63 | 33 | 96 |
| | | *Pseudogenetta villiersi* (30) | 91 | 2 | 93 | 147 | 68 | 215 |
| | Total per stage | | 91 | 2 | 93 | 280 | 132 | 412 |
| | Total (Im+M) | | | | | 505 | | |
| Total | Mammalia, Carnivora (130) | | | | | 2085 | | |
| | Suidae (1) | *Potamochoerus porcus* (1) | 0 | 1 | 1 | 0 | 0 | 0 |
| Artiodactyla (1) | Total per stage | | 0 | 1 | 1 | 0 | 0 | 0 |
| | Total (Im+M) | | | | | 1 | | |
| Total | Mammalia, Artiodactyla (1) | | | | | 1 | | |
| | Erinaceidae (52) | *Atelerix albiventris* (52) | 284 | 177 | 461 | 467 | 180 | 647 |
| Insectivora (52) | Total per stage | | 284 | 177 | 461 | 467 | 180 | 647 |
| | Total (Im+M) | | | | | 1108 | | |
| Total | Mammalia, Insectivora (53) | | | | | 1108 | | |
| | Leporidae (2) | *Lepus aegyptius* (1) | 0 | 0 | 0 | 0 | 1 | 1 |
| Lagomorpha (2) | | *Lepus crawshayi* (1) | 2 | 0 | 2 | 0 | 0 | 0 |
| | Total per stage | | 2 | 0 | 2 | 0 | 1 | 1 |
| | Total (Im+M) | | | | | 3 | | |
| Total | Mammalia, Lagomorpha (2) | | | | | 3 | | |
| | | *Arvicanthis niloticus* (2) | 1 | 1 | 2 | 0 | 0 | 0 |
| | | *Mastomys erythroleucus* (4) | 13 | 9 | 22 | 0 | 0 | 0 |
| | Muridae (11) | *Mastomys huberti* (1) | 1 | 0 | 1 | 0 | 0 | 0 |
| | | *Rattus rattus* (1) | 0 | 1 | 1 | 0 | 0 | 0 |
| | | *Tatera guinea* (1) | 1 | 0 | 1 | 0 | 0 | 0 |
| Rodentia (12) | | *Rodent's burrow* (2) | 2 | 0 | 0 | 2 | 3 | 5 |
| | Total per stage | | 17 | 11 | 27 | 2 | 3 | 5 |
| | Total (Im+M) | | | | | 32 | | |
| | Hystricidae (1) | *Hystrix cristata* (1) | 0 | 0 | 0 | 0 | 1 | 1 |
| | Total per stage | | 0 | 0 | 0 | 0 | 1 | 1 |
| | Total (Im+M) | | | | | 1 | | |
| Total | Mammalia, Rodentia (12) | | | | | 33 | | |
| General Total | Mammalia (198) | | | | | 3022 | | |

**Table 7** Hosts of *Haemaphysalis moreli* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Carnivora (47) | Canidae (1) | *Vulpes pallida* (1) Pale fox | 0 | 1 | 0 | 0 | 0 | 0 |
| | Total per stage | | 0 | 0 | 1 | 0 | 0 | 0 |
| | Total (Im+M) | | | | 1 | | | |
| | Felidae (4) | *Felis catus* (1) Domestic cat | 0 | 0 | 0 | 0 | 0 | 1 |
| | | *Felis libyca* (3) African wild cat | 0 | 0 | 0 | 3 | 4 | 7 |
| | Total per stage | | 0 | 0 | 0 | 3 | 4 | 7 |
| | Total (Im+M) | | | | 7 | | | |
| | Herpestidae (1) | *Atilax paludinosus* (1) Marsh mongoose | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total per stage | | 0 | 0 | 0 | 1 | 0 | 1 |
| | Total (Im+M) | | | | 1 | | | |
| | Viverridae (41) | *Civettictis civetta* (5) African civet | 0 | 0 | 0 | 8 | 5 | 13 |
| | | *Genetta g. senegalensis* (13) Common genet | 0 | 2 | 2 | 68 | 44 | 112 |
| | | *Genetta pardina* (1) Forest genet | 0 | 0 | 0 | 17 | 17 | 34 |
| | | *Genetta tigrina* (1) Large-spotted genet | 0 | 0 | 0 | 5 | 10 | 15 |
| | | *Pseudogenetta villiersi* (21) Villiers' genet | 0 | 0 | 0 | 164 | 57 | 221 |
| | Total per stage | | 0 | 2 | 2 | 262 | 133 | 395 |
| | Total (Im+M) | | | | 397 | | | |
| Total | Mammalia, Carnivora (47) | | | | | | | |
| Insectivora (5) | Erinaceidae (5) | *Atelerix albiventris* (5) African hedgehog | 0 | 0 | 0 | 11 | 1 | 12 |
| | Total per stage | | 0 | 0 | 0 | 11 | 1 | 12 |
| | Total (Im+M) | | | | 12 | | | |
| Total | Mammalia, Insectivora (5) | | | | 12 | | | |
| General Total | Mammalia (52) | | | | 418 | | | |

Tambacounda Reg.); Bambey, CNRA (14°N, 16°W, Thiès Dpt., Thies Reg.); Almadies, Dakar; on the road to Thies-Bambey; on the road Bambey-Fatick-Mbour (14°N, 16°W, Kaolack and Thies Regs.); on the road Bandia-Fatick; Sandiara-Khombole; Dakar; on the road to Fatick-Mbour; Sandiara-Mbour; Mbour; on the road to Bandia-Mbour; Kedougou; Sandiara (14°25'N, 16°48'W, Mbour Dpt., Thies Reg.); Thies; Louly; Baria Forest reserve (13°37'N, 16°13'W, Nioro du Rip Dpt., Kaolack Reg.); Sidioli (12°43'N, 12°16'W, Kedougou Dpt., Kedougou Reg.); on the road to Kedougou-Mako (12°N, 12°W, Kedougou Dpt., Tambacounda Reg.); and Bandafassi (12°32'N, 12°19'W, Kedougou Dpt., Kedougou Reg.).

### *Haemaphysalis* (*Rhipistoma*) *spinulosa* Neumann, 1906

*Haemaphysalis* (*Rhipistoma*) *spinulosa* typically from the Oriental subarea, is known in this subarea as *Haemaphysalis ethiopica* Santos Dias, 1958 (Hussein and Mustafa, 1983) (Table 8). Numerous collection references are attributed to *H. spinulosa* in Senegal: Bandia Forest

**Table 8** Hosts of *Haemaplysalis (Rhipistoma) spinulosa* in Senegal. The number of records per species is given in brackets. Total immature = L+N, Total mature = Male+Female.

| Host | | | Stage collected | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order | Family | Scientific name<br>Common name | Larva | Nymph | Total Immature (Im) | Male | Female | Total Mature (M) |
| Carnivora (2) | Herpestidae (1) | *Ichneumia albicauda* (1)<br>White-tailed mongoose | 1 | 0 | 1 | 0 | 0 | 0 |
| | Viverridae (1) | *Genetta g. senegalensis* (1)<br>Common genet | 0 | 1 | 1 | 0 | 0 | 0 |
| | Total (Im+M) | | 1 | 1 | 2 | 0 | 0 | 0 |
| | Mammalia, Carnivora (2) | | | | 2 | | | |
| Rodentia (1) | Cricetomyidae (1) | *Cricetomys gambianus* (1)<br>African giant pouched rat | 0 | 1 | 1 | 0 | 0 | 0 |
| | Total (Im+M) | | | | 1 | | | |
| | Mammalia, Rodentia (1) | | | | 1 | | | |
| General Total | Mammalia (3) | | | | 3 | | | |

reserve (14°35'N, 17°01'W, Mbour Dpt., Thies Reg.); Saboya Forest reserve; (13°39'N, 16°07'W, Nioro-du-Rip Dpt., Kaolack Reg.); on the road to Sandiara-MBour; Kedougou; Bandafassi (12°32'N, 12°19'W, Kedougou Dpt., Kedougou Reg.); Sidioli (Figure 4).

## Determination key of Western African *Haemaphysalis* spp. (Senegal, Mauritania, Mali, Gambia, Guinea, Bissau Guinea, Burkina Faso, Ivory Coast)

### Males

*1.* Coxa IV with a long internal lanceolate spur, longer than the width of the coxa itself, conscutum with dense to moderate punctuations, parasite of ruminants ..... *H. rugosa*
— Coxa IV with an internal spur shorter than the width of the coxa itself, or absent ..... 2

*2.* Palpal segment III with a strong retrograde dorsal spur, prominent; lateral grooves very short, almost reaching half of the conscutum; parasite of antelopes..... *H. parmata*
— Palpal segment III without retrograde dorsal spur..... 3

*3.* Basidorsal margin of palpi in a straight line, forming a right angle with the inner axis of the palps. Basis capituli rectangular. Scutum proximately large with marked punctuations; parasite of birds ..... *H. hoodi*
— Basidorsal margin of palps drawing a retrograde spur more or less developed, or forming an angle. Parasite of mammals ..... 4

*4.* Coxal spurs moderately long, prominent, each one reaching almost the next coxa. Trochanter I with a strong ventral spur, other trochanters with moderately short spurs, not strong nor pointed. Tarsi short and strong, bluntly pointed. Scutum broadly elongate with few scattered, shallow punctuations. Lateral grooves long reaching the level of intercoxal space II + III, Parasite of *Xerus erythropus* ..... *H. houyi*
— Trochanter I without ventral spur. Coxae with a short pointed spur on at least two pairs. Palpal profile laterally straight or very slightly concave; palpal segment 3 ventrally bearing a fine retrograde spur, pointed, relatively long. Common on carnivores, rare on other animals . . . . . 5

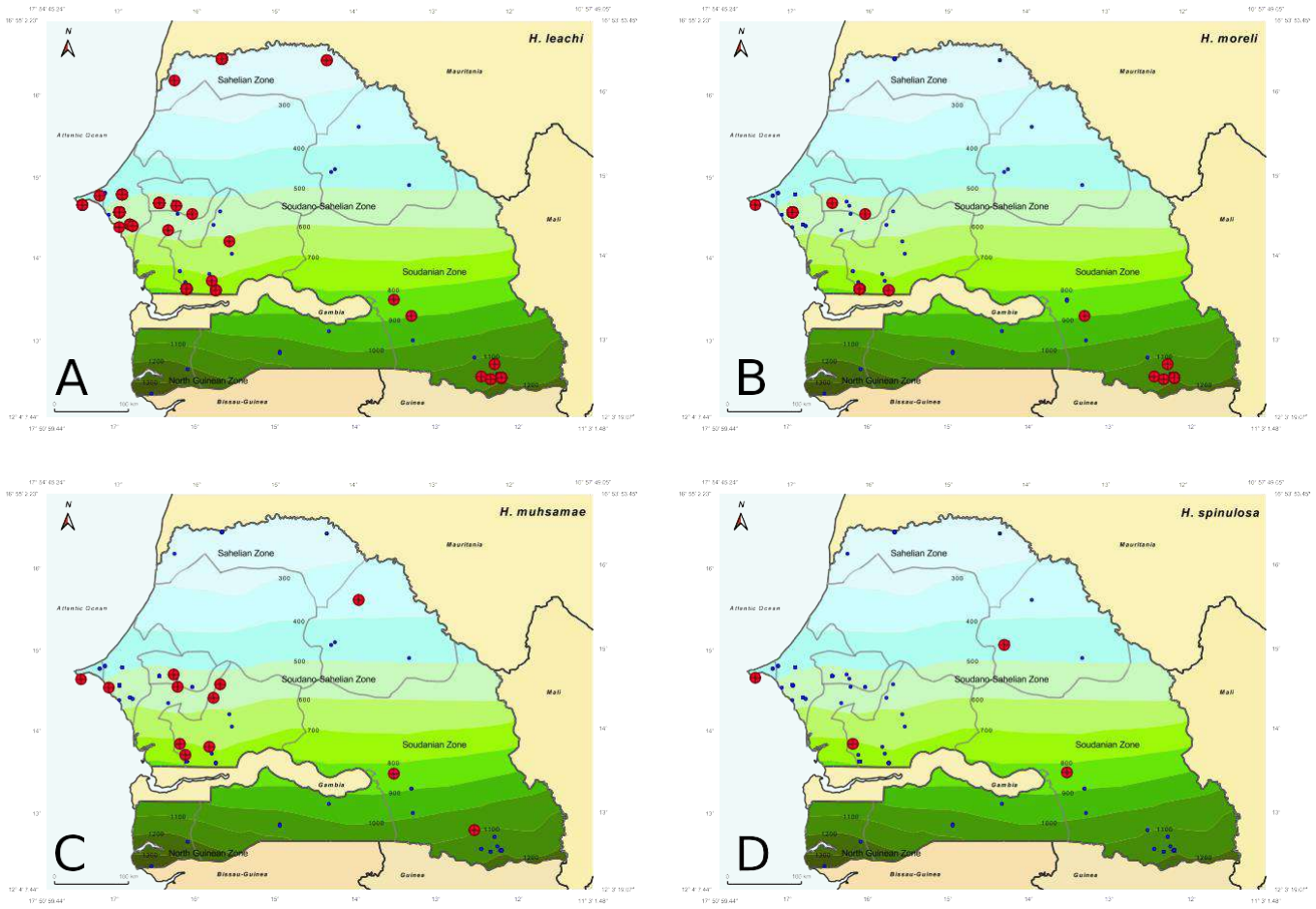


**Figure 4** Collecting sites of *Haemaphysalis* of the *leachi* group indicated in red; blue dots show localization of all other collection sites: A – *Haemaphysalis* (*Rhipistoma*) *leachi*;B – *Haemaphysalis* (*Rhipistoma*) *moreli*;C – *Haemaphysalis* (*Rhipistoma*) *muhsamae*;D – *Haemaphysalis* (*Rhipistoma*) *spinulosa*.

*5.* Conscutum broadly rounded posteriorly showing dense, deep separated punctuations, of moderate size, basis capituli external margin diverging, ventral spur of palpal segment 3 straight, more acutely pointed ..... *H. punctaleachi*
— Conscutum shagreened, showing numerous dense, deep contiguous punctuations, of small to moderate size ..... 6

*6.* Moderate-sized punctuations, lateral grooves enclosing only the first festoon, cornua triangular, palpal segment 2 interno-dorsal margin strongly bulging..... *H. moreli*
— Punctuations clearly separated, moderate to high in number, tiny to moderate in size. Hypostomal formula varying from 4/4 to 6/6 ..... 7

*7.* Hypostomal formula 5/5 or 6/6. Large species measuring 3.5-4.0 mm in length over 1.7-1.9 mm in width. Nine to eleven festoons. Lateral groove enclosing one festoon or sometimes partially two ..... *H. paraleachi*
— Hypostomal formula 4/4, smaller tick species, 1.3-3.8 over 0. 8-1.9 mm. Eleven festoons, lateral groove enclosing only one festoon ..... 8

8. Small ticks, 1.3-2.2 mm length over 0.8-1.2 mm in width. Eleven festoons. Large scutum: 1.6 times longer than width. Coxal spurs small; tarsi II and IV short, robust, bumped and

bluntly pointed. Lateral margins of basis capituli largely diverging forward, cornua short and wide. Lateral groove enclosing 1 festoon. (Common on small carnivores) ..... *H. spinulosa*
— Small to moderately thin species, 1.88-2.97 mm length for 0.82-1.29 mm width. Eleven festoons. Coxal spurs long, distinctly overlapping the next coxal margin, tarsi II-IV elongated, gradually. Conscutum narrow and slightly convex, 2 times longer than wide and smoothly rounded posteriorly. Cervical pits narrow, deep and converging, cervical grooves short, shallow, slightly visible and diverging. Lateral margins of basis capituli slightly diverging forward, cornua sharp, elongated and triangular. Lateral grooves conspicuous, distinct, extending ¼ scutal length and enclosing 2 to 3 festoons (Common on small and large carnivores) . . . . . *H. leachi*

## Females

1. Basidorsal margin of palpal segment 3 with a spur or markedly oblique anteriorly. Scutum approximately as long as wide ..... 2
— Basidorsal margin of palpal segment 3 horizontal, approximately perpendicular to the long axis of the palp, without angle nor spur..... 3

*2.* Palpal segment 3 with a postero-dorsal spur. Basis capituli with small cornua. Scutum wider than long. Coxa I with a small spur overlapping the external basal margin, but without a postero-internal spur..... *H. parmata*
— No postero-dorsal spur on palpal segment 3. Basis capituli with sharp cornua. Scutum rough or shagreened with dense and numerous punctuations of moderate size, as long as wide, or slightly longer than width. Short spur at the internal basal angle of coxa 1 ..... *H. rugosa*

3. Basidorsal margin of palps straight, without any marked angle or spur. Basis capituli almost straight laterally, vestigial cornua. Scutum almost as long as wide. Parasites of birds . . . . . *H. hoodi*
— Basidorsal margin of palps with an angle or bearing a spur. Lateral margins of basis capituli diverging. Cornua moderate to well-developed. Parasites of mammals ..... 4

*4.* Palps with a basiventral spur, fine and moderately long; dorsal margin distinctly pointed sublaterally; segment 3 with a ventral fine spur, elongated and pointed. Each coxa with a well-developed spur (spur-like ridge) not overlapping the trochanter's margin; tarsi bluntly pointed, short, well-developed and sometimes bumped. Claws as long as tarsi. Scutum slightly longer than wide, broadly rounded posteriorly; few widely scattered shallow punctuations (20-30), essentially located in the anterior part, posterior margin broad and regularly rounded. Basiventral margin of palpi with a strong median spur. Parasite of the Geoffroy's ground squirrel (*Xerus erythropus*). ..... *H. houyi*
— Combination of characters different. Palpi generally bearing a conspicuous basal spur, dorsal or ventral, palpal segment 3 with a retrograde ventral spur, elongated, pointed and narrow; lateral profile straight or slightly curved, rarely concave. Basis capituli with strong cornua (generally not parasite of the Geoffroy's ground squirrel, common on carnivores, but rare on other mammals)..... 5

5. Basiventral margin of palpal segment 2 with a strong, pointed spur. Few scutal punctuations . . . . . *H. spinulosa*
— Species of small to moderate size, 1.17-1.70 mm long and 0.66-0.96 mm wide. Lateral margins of scutum slightly diverging anteriorly, converging posteriorly giving a bluntly rounded aspect. Cervical grooves narrow arcs extending 2/3 of total scutal length. Numerous scutal punctuations. Palpi broadly salient (*leachi* type), basiventral margin of palpal segment 2 without a strong, pointed spur. Spur reduced to a short obtuse angle looking like a curve..... 6

*6.* Conspicuous spurs on all coxae. Long and pointed cornua ..... *H. leachi*
— Small coxal spurs. Short and rounded cornua ..... 7

*7.* Hypostomal formula 5/5 or 5.5/5.5. Large elongate *Haemaphysaline* (3.59-3.93 mm long and 1.77-1.88 mm wide for unengorged specimens). External margins of basis capituli diverging slightly anteriorly. Tarsi II to IV bluntly pointed..... *H. paraleachi*
— Hypostomal formula 4/4. Moderate size *Haemaphysalis*, from 2.1-3.0 mm long and 1.0-1.6 mm wide. Basis capituli diverging anteriorly. Tarsi II to IV curving proximally ..... 8

*8.* Basis capituli approximately 2.8 times longer than wide. Spurs on coxae II and III reduced to small protrusions. Basiventral margin of palpal segment 2 with a small, narrow indentation, nearly at mid-width of the posterodorsal segment..... *H. moreli*
— Basis capituli approximately 2.1 times wider than long. Conspicuous spurs on coxae II and III. Basiventral margin of palpal segment 2 with a large emargination, approximately near the mi-width of the segment, showing a sinuous aspect..... *H. punctaleachi*

## Discussion

*Haemaphysalis rugosa* infests antelopes and buffalo as principal hosts for adults, but the species can also be found on carnivores and livestock. In Senegal, immature stages were collected on Carnivores of the Herpestidae and Viverridae families, adult stages were recorded from wild Bovidae including bushbuck, reedbuck and oribi (Table 2). Immature ticks are known to feed on civets, genets, rodents, and hares (Camicas, 1978; Hoogstraal and Kim, 1985; Yeoman and Walker, 1967, Hoogstraal and El Kammah, 1972). It is a typical species of wet savannas. *H. rugosa* has been recorded from Senegal in the south and the West Coast of Dakar. It is known in Burkina Faso, Ivory Coast, Ghana, Benin, Uganda, Sudan, Central African Republic (north-eastern), and Democratic Republic of Congo (north-eastern).

*Haemaphysalis hoodi* preferentially parasitizes ground feeding birds at all stages. All stages were collected from different bird orders including: Cuculiforma, Galliforma and Passeriforma, in the sudanian and sudano-guinean domains of Senegal (Table 3). Hoogstraal (1958) also recorded it from domestic chicken, *Gallus domesticus*. *Haemaphysalis hoodi* is known from sub-Saharan Africa and Morocco (Walker, 1991).

*Haemaphysalis houyi* is characterized by strict host-parasite specificity, only known to infest the African Ground Squirrel, *X. erythropus.* It is recorded in all geographic domains of Senegal, due to the wide distribution of its hosts. This species is also reported to occur in Mali, Burkina Faso, Ivory Coast, Ghana, Chad, Central African Republic, Cameroon, Sudan, Ethiopia, Uganda, and Kenya (Hoogstraal and Kim, 1985; Ntiamoa-Baidu *et al*., 2004).

*Haemaphysalis spinulosa* adults appear to feed on various small and medium-sized carnivores, as well as hedgehogs. In Senegal, immature stages are found on Carnivora of the families Herpestidae and Viverridae; they can also feed on small mammals as rodents (Table 7). This species has been recorded in sub-Saharan Africa and Yemen (Hussein and Mustafa, 1983; 1985; Hoogstraal and Kim, 1985; Walker, 1991).

*Haemaphysalis parmata* was not recorded in Senegal, but is known from Ivory Coast, in the same biogeographic Occidental subarea, and is considered in the proposed key.

*Haemaphysalis* (*Rhipistoma*) *leachi* group (subgroup *leachi*) includes five species of which three are described from the Occidental subarea of the Afrotropical region: 1) *H.* (*Rh.*) *leachi* (Audouin, 1826) was originally described in Egypt from grass-rats, *Arvicanthis niloticus* (Desmarest, 1822), and the Long-eared hedgehog, *Hemiechinus auritus aegyptius* E. Geoffroy, 1803 and their resting habitats (Hoogstraal, 1958). Adults can also feed on jungle cat, *Felis chaus nilotica* de Winton, 1898; the common fox, *Vulpes vulpes aegyptica* (Sonnini, 1816) and the wolf jackal, *Canis aureus lupaster* Hemprich and Ehrenberg, 1833; 2) *H.* (*Rh.*) *moreli*

Camicas, Hoogstraal & El Kammah, 1972 was described from genets, civets and also recorded on Felid carnivores, including lions, leopards, servals, hyaena, jackals, foxes, domestic cats and dogs of Western and Eastern Africa; 3) *Haemaphysalis* (*Rhipistoma*) *paraleachi* Camicas, Hoogstraal & El Kammah, 1983 parasitizing small and medium-sized carnivores as civets in Central Africa; *Haemaphysalis.* (*Rhipistoma*) *punctaleachi* Camicas, Hoogstraal & El Kammah, 1973 is also found on civets, leopard, jackal and antelope of West African Guinean forests, and *H.* (*Rhipistoma*) *elliptica* (Koch, 1844) being known only from Austral and Oriental Africa. For comparative purposes, *H. punctaleachi* is included in the key.

The *Haemaphysalis* (*Rh*.) *spinulosa* subgroup incorporated four species, namely *H.* (*Rh.*) *muhsamae* Santos Dias, 1954, *H.* (*Rh*.) *spinulosa* Neumann, 1906; *Haemaphysalis* (*Rhipistoma*) *norvali* Hoogstraal & Wassef, 1983 and *Haemaphysalis* (*Rhipistoma*) *subterra* Hoogstraal, El Kammah & Camicas, 1992 also from Austral and Oriental Africa.

The taxonomic status of *H.* (*Rh.*) *elliptica* and *H.* (*Rh.*) *leachi* was updated by Apanaskevich *et al*. (2007); they also gave some collection references of *H.* (*Rh*.) *leachi* from the same carnivore hosts as in our study in Senegal [*Leptailurus serval* = *Felis* (*Leptailurus*) *serval*, *Felis silvestris lybica* and *Civettictis civetta*], but a taxonomic revision of the whole *H.* (*Rh*.) *leachi* group, including host associations and distribution ranges, is still needed. In West Africa, *H.* (*Rh*.) *leachi* infests a wide range of carnivore hosts including the domestic dog, domestic cat, lion, *Panthera leo* Linnaeus, 1758; and leopard, *Panthera pardus* Linnaeus, 1758 as adults, and immature stages are found on a variety of rodent species. Adults of both species have been recorded in several collections obtained from a single host.

Although the haemaphysalid tick species of Senegal described here are not known to carry or vector pathogens of medical importance, detailed studies are currently lacking. Several other species from the Amblyommid family (i.e. *Amblyomma, Rhipicephalus* and *Hyalomma spp.*) have been found infected with a variety of viruses in Senegal including: Bandia, Bhanja, Coxsackie B4, CCHF, Dugbe, Jos, Koutango, NDelle, Ndumu, Somone, Wad Medani or Ngoye (Anonyme, 1998; Grard *et al*., 2006). Moreover, other *Haemaphysalis* spp. are known worldwide as pathogen carriers: protozoan parasites *stricto sensu* [e.g.: *Babesia gibsoni, B. bigemina, B. ovata, Toxoplasma gondi, Hepatozoon canis* (Dubey and Beattie, 1988*;* Zhou *et al*., 2016); *Theileria orientalis, T. recondita*, *T. mutans* (Fujisaki *et al*., 1994, Alani and Herbert, 1998; Gao *et al*., 2008); bacteria as *Rickettsia heilongjiangensis, R. japonica, R. siberica, Coxiella burnetii* (Jongejan and Uilenberg, 2004, Liu *et al*., 2014); *Anaplasma phagocytophilum, Borrelia burgdorferi, Francisella tularensis ssp., Mycobacterium, Sphingomonas spp., Pseudomonas spp., Wolbachia spp., Brucella melitensis* (Nosek, 1971b; Raoult and Roux, 1997; Gyuranecz *et al*., 2011)]. A wide range of viruses (e.g.: Bhanja, Barur, Crimean-Congo hemorrhagic fever, Ganjam, Kaisodi, Kyasanur Forest Disease, New Minto, Powassan encephalitis, Louping-ill, Russian spring-summer encephalitis, Burana, Sawgrass, Silverwater or Tribec) are also carried by *Haemaphysalis* ticks (Work *et al*., 1957; Work and Trapido, 1957; Trapido *et al*., 1959; Anonyme, 1964; Bhatt *et al*., 1966; Dandawate and Shah., 1969; Dandawate *et al*., 1969; Boshell *et al*., 1970; Rajagopalan *et al*., 1970; Nosek *et al*., 1971a and b; Vesenjak-Hirjan *et al*., 1977; Pavlov *et al*., 1978; Pattnaik, 2006; Yadaw *et al*., 2011; Holbrook, 2012; Lasecka and Baron, 2013; Lvov *et al*., 2014; Mourya *et al*., 2014; Walker *et al*., 2015; Yang *et al*., 2016). Again, other newly identified emerging viruses have been isolated from *Haemaphysalids* including: the Tick-borne zoonosis with severe fever and thrombocytopenia syndrome virus (Liu *et al*., 2014; Fujisaki *et al*., 1994; Jongejan and Uilenberg, 2004); the Huaiyangshan virus (Yu *et al*., 2011; Zhang *et al*., 2011; Zhang *et al*., 2012; Kuhn *et al*., 2016), the Khasan virus from Maritime Territory China, as well as the Kwatta virus (Rhabdoviridae) and the Burana virus (Khoo *et al*., 2016).

Ultimately, the haemaphysalid tick fauna of Senegal requires more in depth investigations to complete our knowledge, including biological, morphological and taxonomic studies and an evaluation of the transmission risk of pathogens. We postulate, for example, that the entomological surveillance conducted on hemaphysalid ticks in Senegal is too limited to declare these species of no risk and thus merit further investigation. Indeed *H. leachi*, the

yellow or African dog tick, is found in tropical and southern Africa and can transmit canine and feline babesiosis, Mediterranean spotted fever, Q-fever, and Boutonneuse fever (Sonenshine, 1992b; Khoo *et al*., 2016). With respect to climate change, migration and commercial trade, understanding the health risks associated with tick-borne pathogens appears of public health importance in the geographic domains where haemaphysalid tick circulate. Indeed, their relative abundance, their seasonality, their geographical extension need to be assessed; the involvement of the *Haemaphysalis* genus in pathogen transmission may be underestimated.

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## References

Alani A. J., Herbert I. V. 1998. Morphology and transmission of *Theileria recondita* (Theileriidae: Sporozoa) isolated from *Haemaphysalis punctata* from North Wales. Vet. Parasitol., 28: 283-291. doi:10.1016/0304-4017(88)90075-1

Anonyme 1964. Indian Council of Medical Research. Kyasanur Forest Disease 1957-1964. Cambridge Print. Works Delhi, 1 volume, 30 pages.

Anonyme 1998. Rapport annuel, Centre collaborateur OMS de référence et de recherche sur les arbovirus. Institut Pasteur de Dakar, 141 pp.

Apanaskevich D. A., Horak I. G., Camicas J. L. 2007. Redescription of *Haemaphysalis* (*Rhipistoma*) *elliptica* (Koch, 1844), an old taxon of the *Haemaphysalis* (*Rhipistoma*) *leachi* group from East and southern Africa, and of *Haemaphysalis* (*Rhipistoma*) *leachi* (Audouin, 1826) (Ixodida, Ixodidae). Onderstepoort J. Vet. Res., 74:181-208. doi:10.4102/ojvr.v74i3.122

Bhatt P. N., Work T. H., Varma M. G. R., Trapido H., Murthy N. D. P., Rodrigues F. M. 1966. Isolation of Kyasanur forest disease from infected humans and monkeys of Shimoga District, Mysore State. Indian J. Med. Sci., 20: 316-320.

Board on Geographic Names. 1965. Senegal. Names for places and features in Senegal. U.S. Govt. Printing Office, Division of Geography, Department of the Interior, Washington, D. C., pp. IV + 194.

Boshell J., Desai P. K., Dandawate C. N., Goverdhan M. K. 1970. Isolation of Ganjam virus from ticks Haemaphysalis intermedia. Indian J. Med. Res., 58: 561-562.

Cabot J., Carboneras C., Elliott A., Folch A., del Hoyo J., de Juana E., Llimona F., Martínez I., Martínez-Vilalta A., Matheu E., Motis A., Orta J. 1992A. Handbook of the birds of the world. Volume 1 Ostrich to Ducks.. Lynx eds, Barcelona, Spain. 696 pp.

Cabot J., Carboneras C., Elliott A., Folch A., del Hoyo J., de Juana E., Llimona F., Martínez I., Martínez-Vilalta A., Matheu E., Motis A., Orta J. 1992B. Handbook of the birds of the world. Volume 2 New world Vultures to Guineafowls. Lynx eds. 638 pp.

Camicas J. L., Hoogstraal H., El Kammah K. M. 1972. Notes on African *Haemaphysalis* ticks. VIII. *H.* (*Rhipistoma*) *moreli* sp. n., a carnivore parasite of the *H.* (*R.*) *leachi* group (Ixodoidea: Ixodidae). J. Parasitol., 58: 1185-1196. doi:10.2307/3278163

Camicas J. L., Morel P. C. 1977. Position systématique et classification des tiques. (Acarida: Ixodida). Acarologia, 18 (3): 410-420.

Camicas J. L. 1978. Contribution à l'étude des tiques du Sénégal (Acarida : Ixodida) 1. Description des stases préimaginales d'*Haemaphysalis* (*Kaiseriana*) *rugosa* Santos Dias, 1956. Cah. ORSTOM, Ser. Ent. Med. Parasitol., XVI (1): 23-28.

Camicas J. L., Wilson M. L., Cornet J. P., Digoutte J. P., Calvo M-A., Adam F., Gonzalez J. P. 1990. Ecology of Ticks as potential vectors of Crimean-Congo hemorrhagic fever virus in Senegal: epidemiological implications. Arch. Virol., [Suppl. 1]: 303-322.

Camicas J. L., Hervy J. P., Adam F., Morel P. C. 1998. Les tiques du monde nomenclature, stades décrits, hôtes, répartition. ORSTOM eds. Paris, 240 pp.

Dandawate C. N., Shah K. V. 1969. Ganjam virus: A new arbovirus isolated from ticks *Haemaphysalis intermedia* Warburton and Nuttall, 1909 in Orissa, India. Indian J. Med. Res., 57: 799-804.

Dandawate C. N., Work T. H., Webb J. K., Shah K. V. 1969. Isolation of Ganjam virus from a human case of febrile illness: A report of a laboratory infection and serological survey of human sera from three different states of India. Indian J. Med. Res., 57:975-982.

Dubey J. P., Beattie C. P. 1988. Toxoplasmosis of Animals and Man. Boca Raton: CRC Press Inc.

Elbl A., Anastos G. 1966. Ixodid ticks (Acarina: Ixodoidea) of Central Africa. Ann. Mus. Roy. Afrique Centrale Tervuren. Volume IV. Genus *Aponomma* Neumann, 1899, *Boophilus* Curtis, 1891, *Dermacentor* Koch, 1844, *Haemaphysalis* Koch, 1844, *Hyalomma* Koch, 1844 and *Rhipicentor* Nuttall & Warburton, 1908 pp.

Fujisaki K., Kawazu S., Kamio T. 1994. The taxonomy of the bovine *Theileria* spp. Parasitol. Today, 10: 31-33. doi:10.1016/0169-4758(94)90355-7

Gao J., Luo J., Fan R., Fingerle V., Guan G., Liu Z., Li Y., Zhao H., Ma M., Liu J., Liu A., Ren Q., Dang Z., Sugimoto C., Yin H. 2008.. Cloning and characterization of a cDNA clone encoding

calreticulin from *Haemaphysalis qinghaiensis* (Acari: Ixodidae). Parasitol. Res., 102(4): 737-746. doi:10.1007/s00436-007-0826-y
Grard G., Lemasson J. J., Sylla M., Dubot A., Cook S., Molez J. F., Pourrut X., Charrel R., Gonzalez J. P., Munderloh U. Holmes E. C., De Lamballarie X. 2006.. Ngoye virus: a novel evolutionary lineage within the genus Flavivirus. J. Genet., 87(11): 3273-3277. doi:10.1099/vir.0.82071-0
Gyuranecz M., Rigo K., Dan A., Foldvari G., Makrai L., Denes B., Fodor L., Majoros G., Tirjak L., Erdelyi K. 2011.- Investigation of the Ecology of *Francisella tularensis* During an Inter-Epizootic Period.- Vector-Borne Zoon. Dis., 11 (8): 1031-1035. doi:10.1089/vbz.2010.0091
Holbrook M. R. 2012.. Kyasanur forest disease. -Antivir. Res., 96(3): 353-362. doi:10.1016/j.antiviral.2012.10.005
Hoogstraal H. 1958.. Notes on African Haemaphysalis ticks. IV. Description of Egyptian populations of the yellow dog-tick, *H. leachii leachii* (Audouin, 1827) (Ixodoidea, Ixodidae). J. Parasitol., 44: 548-558. doi:10.2307/3274429
Hoogstraal H., El Kammah K. M. 1972. Notes on African *Haemaphysalis* ticks. X. *H.* (*Kaiseriana*) *aciculifer* Warburton and *H.* (*Ka.*) *rugosa* Santos Dias, the African representatives of the *spinigera* subgroup (Ixodoidea: Ixodidae). J. Parasitol., 960-978. doi:10.2307/3286594
Hoogstraal H., Kim K. C. 1985.. Chapter 10. Tick and mammal coevolution with emphasis on *Haemaphysalis*. Pp. 505-568, in Coevolution of parasitic arthropods and mammals.. Pr. Ke Chung Kim eds. John Wiley and sons, New York.
Hussein H. S., Mustafa B. E. 1983. *Haemaphysalis* (*Rhipistoma*) *spinulosa* Neumann, 1906: Description of immature stages, adult structural variations, and notes on biology (Ixodoidea: Ixodidae). J. Parasitol., 69 (2): 405-412. doi:10.2307/3281245
Hussein H. S., Mustafa B. E. 1985. *Haemaphysalis spinulosa* and *Rhipicephalus simus* (Acari: Ixodidae): seasonal abundance of immature stages and host range in the Shambat area, Sudan. J. Med. Entomol. 22:72-77. doi:10.1093/jmedent/22.1.72
Jongejan F., Uilenberg G. 2004. The global importance of ticks. Parasitol. 129:S3-S14. doi:10.1017/S0031182004005967
Khoo J. J., Chen F., Kho K. L., Ahmad Shanizza A. I., Lim F. S., Tan K. K., Chang L. Y., AbuBakar S. 2016. Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. -Ticks and Tick-borne Dis., 7: 929-937. doi:10.1016/j.ttbdis.2016.04.013
Kuhn J. H., Wiley M. R., Rodriguez S. E., Bào Y., Prieto K., Travassos da Rosa A. P., Guzman H., Savji N., Ladner J. T., Tesh R. B., Wada J., Jahrling P. B., Bente D. A., Palacios G. 2016.. Genomic Characterization of the genus Nairovirus (Family Bunyaviridae). Viruses 8(6): 164. doi:10.3390/v8060164
Lasecka L., Baron M. D. 2013. The molecular biology of nairoviruses, an emerging group of tick-borne arboviruses. Arch. Virol., 159: 1249-1265. doi:10.1007/s00705-013-1940-z
Levin M. L. 2016. Ticks. The Merck Veterinary Manual, 11th Edition. S. E. Ajello and M. A. Moses edds. Kenilworth, NJ, Merck Publishing and Merial: 925-942.
Liu Q., He B., Huang S. Y., Wei F., Zhu X. Q. 2014. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. Lancet Infect. Dis., 14:763-772. doi:10.1016/S1473-3099(14)70718-2
Lvov D. K., Alkhovsky S. V., Shchelkanov M. Yu., Shchetinin A. M., Deryabin P.G., Gitelman A. K., Aristova V. A., Botikov A. G. 2014. Taxonomic status of the Burana virus (BURV) (Bunyaviridae, Nairovirus, Tamdy group) isolated from the ticks *Haemaphysalis punctata* Canestrini & Fanzago, 1877 and *Haem. concinna* Koch, 1844 (Ixodidae, Haemaphysalinae) in Kyrgyzstan [in Russian] . Vopr Virusol., 59:10-15.
Matthysse J. G., Colbo M. H. 1987A. The Ixodid ticks of Uganda, together with species pertinent to Uganda because of their present known distribution. Entomol. Soc. America, College Park, Maryland, XII + 426pp.
Morel P. C. 1956.. Le Parc National du Niokolo-Koba (Premier fascicule). XV – Tiques d'animaux sauvages. Mémoires de l'Institut Français d'Afrique Noire N48. IFAN-Dakar. 228-232.
Morel P. C. 2003. Les tiques d'Afrique et du Bassin méditerranéen, 1350 pp. + Contribution à la connaissance de la distribution des tiques (Acariens, Ixodidae et Amblyommidae) en Ethiopienne continentale. 388 pp. + Annexe cartographique de 62 cartes. CD-Rom, Cirad 2003. Alfort (France) et L.N.R.V. Dakar (Sénégal), manuscrit non publié, 1342 + x pp.
Mourya D. T., Yadav P. D., Patil D. Y. 2014. Highly infectious tick-borne viral diseases: Kyasanur forest disease and Crimean-Congo haemorrhagic fever in India. WHO South-East Asia J. Pub. Health., 3(1): 8-21.
Nosek J. Z. 1971a. The ecology, bionomics and behaviour of *Haemaphysalis* (*Haemaphysalis*) *concinna* Tick.. Parasitenk., 36: 233-241.
https://doi:10.1007/BF00348561
Nosek J. Z. F. 1971b. -The ecology, bionomics, and behaviour of *Haemaphysalis* (*Aboimisalis*) *punctata* tick in central Europe. Parasitenk., 37(3): 198-210.
https://doi:10.1007/BF0025949.
Ntiamoa-Baidu Y., Carr-Saunders C., Matthews B. E., Preston P.M., Walker A. R. 2004. An updated list of the ticks of Ghana and an assessment of the distribution of the ticks of Ghanaian wild mammals in different vegetation zones.. Bull. Ent. Res., 94(3): 245-260. doi:10.1079/BER2004302
Pattnaik P. 2006. Kyasanur forest disease: an epidemiological view in India. Rev. Med. Virol., 16 (3): 151-165. doi:10.1002/rmv.495
Pavlov P., Rosický B., Hubálek Z., Daniel M., Bárdos V., Minár J., Juricová Z. 1978. Isolation of Bhanja virus from ticks of the genus *Haemaphysalis* in southeast Bulgaria and presence of antibodies in pastured sheep. Folia Parasitol., 25(1): 67-73.
Rajagopalan P. K., Sreenivasan M. A., Paul S. D. 1970. Isolation of Ganjam virus from the bird tick *Haemaphysalis wellingtoni* Nuttall and Warburton 1907. Indian J. Med. Res., 58: 1195-1196.

Raoult D., Roux V. 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin. Microbiol. Rev., 10(4): 694-719. doi:10.1128/CMR.10.4.694
Sonenshine D. E. 1992b. Biology of ticks. Vol. 2. Oxford University Press, New York, 465 pp.
Sylla M., Pourrut X., Faye, N., Bâ K, Cornet, J. P., Camicas J. L. 2004. Argasidae (Acari: Ixodida) parasite of wild and domestic animals in Senegal: 1-Review and distribution. Acarologia, 44 (3-4): 137-150.
Sylla M., Molez J. F., Cornet J. P., Mondet B., Camicas J. L. 2007. Les tiques (Acari : Ixodida) du Sénégal : Fréquence des hôtes répertoriés, dynamique saisonnière et chorologie d'*Amblyomma* (*Xiphiasto*r) *variegatum* (Fabricius, 1794). Acarologia, 47 (1-2): 13-23.
Sylla M., Molez J. F., Cornet J. P., Camicas J. L. 2008. Impact du changement climatique sur la répartition des tiques au Sénégal et en Mauritanie. Acarologia, 48 (3-4) : 137-153.
Trapido H., Rajagopalan P. K., Work T. H., Varma M. G. 1959. Isolation of Kyasanur Forest disease virus from naturally infected ticks of the genus *Haemaphysalis*. Indian. J. Med. Res., 47:133-138.
Vesenjak-Hirjan J., Calisher C., Brudnjak Z., Tovornik D., Skrtic N., Lazvick J. S.1977. Isolation of Bhanja virus from ticks in Yugoslavia. Am. J. Trop. Med. Hyg., 26: 1003-1008. doi:10.4269/ajtmh.1977.26.1003
Villiers A. 1955.. Notes sur quelques Ixodidae et Argasidae parasites des vertébrés rencontrés en A.O.F. Bull. IFAN, 17 (A, 2): 444-454.
Walker J. B. 1991. A review of the ixodid ticks (Acari, Ixodidae) occurring in southern Africa. Onderstepoort J. Vet. Res., 58:81-105.
Walker E. P. 1999a. Mammals of the World. (2 volumes). (Sixth Edition). Volume I. Baltimore and London. The Johns Hopkins University Press, 836 pp.
Walker E. P. 1999b. Mammals of the World. (2 volumes). (Sixth Edition). Volume II. Baltimore and London. The Johns Hopkins University Press, 1085 pp.
Walker P. J., Firth C., Widen S. G., Blasdell K. R., Guzman H., Wood T. G., Paradkar P. N., Holmes E. C., Tesh R. B., Vasilakis N. 2015.. Evolution of Genome Size and Complexity in the Rhabdoviridae. PLOS Pathog., 11(2): e1004664. doi:10.1371/journal.ppat.1004664
Wilson M. L., Gonzalez J. P., LeGuenno B., Cornet J. P., Guillaud M., Calvo M. A., Digoutte J. P., Camicas J. L. 1990. Epidemiology of Crimean-Congo hemorrhagic fever in Senegal: temporal and spatial patterns. Volume 1 of the series Arch. Virol., (Suppl. 1): 323-340.
Wilson D. E., Reeder D. M. 1992. Mammal species of the world. A taxonomic and geographic reference. Second edition, Smithsonian Institution Press. Washington, 1206 pp.
Work T. H., Trapido H. 1957. Kyasanur forest disease: a new virus disease in India: summary of preliminary report of investigations of the VRC on the epidemic disease affecting forest villagers and wild monkeys of Shimoga district, Mysore State. Indian J. Med. Sci, 11:41-342.
Work T. H., Trapido H., Murthy D. P., Rao R. L., Bhatt P. N., Kulkarni K. G. 1957. "Kyasanur forest disease. III. A preliminary report on the nature of the infection and clinical manifestations in human beings. Indian J. Med. Sci., 11(8): 619-645.
Yadav P. D., Vincent M. J., Khristova M., Kale C., Nichol S. T., Mishra A. C., Mourya D. T. 2011. Genomic analysis reveals Nairobi sheep disease virus to be highly diverse and present in both Africa, and in India in the form of the Ganjam virus variant. Infect. Genet. Evol., 11: 1111-1120. doi:10.1016/j.meegid.2011.04.001
Yang J., Tian Z., Liu Z., Niu Q., Han R., Li Y., Guan G., Liu J., Liu G., Luo J., Yin H. 2016.. Novel spotted fever group rickettsiae in *Haemaphysalis qinghaiensis* ticks from Gansu, Northwest China.. Parasit Vectors 9:146.
https://doi:10.1186/s13071-016-1423-1427.
Yeoman G. H., Walker J. B. 1967. The ixodid ticks of Tanzania. A study of the zoogeography of the Ixodidae of an east African country. London and Reading: Commonwealth Institute of Entomology. xii + 215 pp.
Yu X. J., Liang M. F., Zhang S. Y., Liu Y., Li J. D., Sun Y. L., Zhang L., Zhang Q. F., Popov V.L., Li C., Qu J., Li. Q., Zhang Y.P., H ai R.,Wu W., Wang Q., Zhan F. X.,Wang X. J., Kan B.,Wang S. W., Wan K. L., Jing H. Q., Lu J. X.,Yin W. W., Zhou H., Guan X. H., Liu J. F., Bi Z. Q., Liu G. H., Ren J.,Wang H., Zhao Z., Song J. D., He J. R.,Wan T., Zhang J. S., Fu X. P., Sun L. N., Dong X. P., Feng Z. J., Yang W. Z., Hong T., Zhang Y.,Walker D. H., Wang Y., Li D. X. 2011. Fever with thrombocytopenia associated with a novel bunyavirus in China. N. Engl. J. Med., 364: 1523-1532. doi:10.1056/NEJMoa1010095
Zhang Y. Z., Zhou D. J., XiongY., Chen X. P., He Y. W., Sun Q.,Yu B., Li J., Dai Y. A., Tian J. H., Qin X. C., Jin D., Cui Z., Luo X. L., Li W., Lu S.,WangW., Peng J. S., Guo W. P., Li M. H., Li Z. J., Zhang S., Chen C., Wang Y., deJong M. D., Xu J. 2011.. Hemorrhagic fever caused by a novel tick-borne Bunyavirus in Huaiyangshan,China. Zhonghua Liu Xing Bing Xue Za Zhi, 32: 209-220.
Zhang Y. Z., Zhou D. J.,Qin X. C.,Tian J. H., Xiong Y.,Wang J. B.,Chen X. P., Gao D. Y.,He Y. W. Jin D., Sun Q., Guo W. P., Wang W., Yu B., Li J., Dai Y. A., Li W., Peng J. S., Zhang G. B., Zhang S., Chen X. M., Wang Y., Li M. H., Lu X., Ye C., de Jong M. D., Xu J. 2012. The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. J. Virol., 86: 2864-2868. doi:10.1128/JVI.06192-11
Zhou Y., Zhang H., Cao J., Gong H., Zhou J. 2016.. Epidemiology of toxoplasmosis: role of the tick *Haemaphysalis longicornis*. Infect. Dis. Poverty, 5: 14. doi:10.1186/s40249-016-0106-0