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Bloodfeeding patterns of sylvatic arbovirus vectors in southeastern Senegal

Diawo Diallo^a, Rubing Chen^b, Cheikh T. Diagne^a, Yamar Ba^a, Ibrahima Dia^a, Amadou A. Sall^c, Scott C. Weaver^{b,*}, and Mawlouth Diallo^a

^aUnité d'Entomologie Médicale, Institut Pasteur de Dakar, 36 Avenue Pasteur, BP 220, Dakar, Senegal

^bInstitute for Human Infections and Immunity, Center for Tropical Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0610, USA

^cUnité des Arbovirus et Virus de Fievres Hemorragiques, Institut Pasteur de Dakar, 36 Avenue Pasteur, BP 220, Dakar, Senegal

Abstract

Background—Dengue (DENV), yellow fever (YFV) and chikungunya (CHIKV) viruses circulate in sylvatic, enzootic transmission cycles in southeastern Senegal, but understanding of the vector–host interactions involved is limited.

Methods—The vertebrate hosts of several potential mosquito vectors of the three viruses were identified by PCR amplification and sequencing portions of the cytochrome *b* gene from bloodmeals of mosquitoes collected in Kedougou, Senegal, June 2010–January 2011.

Results—We identified the sources of 65 bloodmeals of 82 engorged mosquitoes. *Aedes taylori* was the only species that fed on monkeys (*Chlorocebus sabaeus* and *Papio papio*). The majority of the avian-derived blood-meals were from the Western Plantain-eater (*Crinifer piscator*).

Conclusion—These findings corroborate the importance of *Ae. taylori* and African monkeys in the sylvatic cycles of YFV, DENV and CHIKV and suggest the possible involvement of other vertebrates.

Keywords

Arboviruses; Vertebrate hosts; Mosquitoes; Disease vectors; Bloodmeal; Senegal

Introduction

In eastern Senegal, dengue (DENV), yellow fever (YFV) and chikungunya (CHIKV) viruses have shown recurrent amplifications at 5–8 year intervals since 1972. Their main mosquito vectors appear to be *Aedes taylori*, *Ae. furcifer* and *Ae. luteocephalus*, although they have

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*Corresponding author: Tel: +1 409 266 6500; Fax: +1 409 266 6810; sweaver@utmb.edu.

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also been isolated from several other species. Indeed, these species are repeatedly associated with these viruses in nature. *Aedes furcifer* is highly susceptible to YFV and DENV-2 and *Ae. luteocephalus* to DENV-2. The major vertebrate hosts identified in their sylvatic, enzootic cycles are believed to be non-human primates (NHP) including *Chlorocebus sabaeus*, *Erythrocebus patas* and *Papio papio*.^{1–3}

The maintenance of DENV, YFV and CHIKV over time may be ensured by their vertical transmission in mosquito vectors and/ or acute infections of NHP reservoirs. However, vertical transmission frequency appears to be too low to serve as the only mechanism of virus maintenance in nature, and an exclusive role for NHPs as the principal amplifying and/ or reservoir hosts is still questioned due to, among other factors, their short durations of viraemia.⁴ Therefore, secondary cycles involving other vectors/vertebrates should be investigated.

To identify potential vertebrate hosts and reservoirs of DENV, YFV and CHIKV, and to seek evidence of secondary enzootic transmission cycles not involving NHPs that might be suggested by the feeding of enzootic vectors on other hosts, we characterized the host-feeding patterns of their mosquito vectors through analysis of bloodmeals of wild-caught mosquitoes by sequencing of the vertebrate cytochrome *b* gene. Here, we report preliminary data suggesting the need for further research on these potential secondary cycles.

Materials and methods

Mosquitoes were collected from natural resting sites (tree holes, under rocks and low vegetation in forest-galleries, savannahs and villages) by aspiration using the Modified CDC Backpack Aspirator model 1412 (John W. Hock Co., Gainesville, FL, USA) in Kédougou, located in southeastern Senegal (12°33'N, 12°11'W), from June 2010 to January 2011. Kédougou is characterized by an average annual rainfall of 1200 mm, mean temperatures of 33–39.5°C, a low human population density and a diverse fauna.³ Mosquitoes were identified on a chill table using morphological keys and a dissecting microscope,^{5,6} and blood-engorged specimens identified visually were transferred individually into 2-ml microfuge tubes and stored at –20°C.

DNA was isolated from the abdominal contents using DNA-zol BD (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's recommendation, with some modifications.⁷ The PCR reactions were performed using two pairs of primers based on cytochrome *b* sequences of vertebrate species available in the GenBank library. Vertebrate-specific primer pair 1 had the sequences 5'-CCvTCIAACATCTCHGCHTGATGRAA-3' (forward) and 5'-CHCCTCARAADGATATTTGDCCTCA-3' (reverse), with a predicted amplicon size of 358 bp. Vertebrate-specific primer pair 2 had the sequences 5'-CTGHGAYAAAATCCCRTTCCA-3' (forward) and 5'-GGYCTTCARTCTTTGGYTTACAAGAC-3' (reverse), with a predicted amplicon size of 552 bp. PCR cycling conditions were those described by Molaei et al.⁷ A 25-μL reaction volume was prepared from a master mix with 1.5 μL of template DNA, 2.2 μL of each primer (10 nmol/μL), 2.75 μL of 10× QIAGEN PCR Buffer (QIAGEN, Valencia, CA, USA), 0.508 μL dNTP mix (10 mmol/L each), 0.1375 μL of *Taq* DNA Polymerase (1.25 U/ reaction) and 16.92 μL of water. PCR amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN) and sequenced using the Applied Biosystems BigDye Terminator version 3.1 Cycle Sequencing Kit and ABI 3500 Genetic Analyzer following the manufacturer's protocols (Applied Biosystems, Foster City, CA, USA). DNA sequences were annotated using ChromasPro version 1.5 (Technelysium Pty Ltd, Tewantin, Australia) and identified by BLAST searches of the GenBank library.

Results

We identified the sources of 65 bloodmeals (79%) of 82 engorged mosquitoes, which comprised 12 species in four genera (Table 1). Identification failures occurred because detectable PCR amplicons were not produced or because the sequences did not match a host with a geographic distribution which includes Senegal. All anophelines and *Ae. taylori* had fed mainly (57–100%) upon mammals. The other vectors, including *Ae. aegypti* (67%), *Ae. furcifer* (75%), *Ae. luteocephalus* (80%) and *Ae. vittatus* (100%), had fed principally (67–100%) on avian hosts. Three mixed bloodmeals were identified, all of which were taken from humans (*Homo sapiens*) and Western Plantain-eaters (*Crinifer piscator*). All eight bloodmeals identified from *Ae. taylori* were taken from NHPs: seven from the African green monkey and one from the Guinea baboon. The majority (10/15) of the *Anopheles* mosquitoes had fed on the domestic cow (*Bos taurus*). Among the seven avian species that served as hosts for the mosquitoes tested (Table 1), the Western Plantain-eater was most frequent.

Discussion

The collection of wild engorged females of sylvatic arbovirus vectors is technically and logistically challenging, explaining the small sample sizes in this study and the relative lack of prior data on the feeding activity of these vectors in Africa. Another limitation of our study is the lack of information about the relative abundance of vertebrate host species within the area.

Despite these limitations, the avian-derived bloodmeals, especially from *Crinifer piscator*, detected in *Ae. furcifer* and *Ae. taylori* were unexpected because these species have been described as primarily primatophilic.⁸ *Crinifer piscator*, the Grey Plantain-eater, is a large arboreal bird restricted to sub-Saharan Africa.⁹ To our knowledge, this bird has never been identified as a host for any mosquito species nor associated with any arbovirus. Our findings support further investigation of a potential role of birds in the transmission of DENV, YFV and CHIKV. This possibility is further suggested by early studies that associated several wild animals, including the arboreal strata birds (hornbills, parrots and touracos), with CHIKV, YFV and Zika virus in Cameroon and Senegal by seroprevalence.^{4,10}

Historically, *Ae. taylori* has the highest infection rate of these three arboviruses among the sylvatic vectors in southeastern Senegal. The two NHP species (African green monkey and Guinea baboon) fed on by this species in our study generally exhibit the highest arbovirus (YFV, DENV and CHIKV) seropositivity in Africa.⁴ These data confirm the importance of *Ae. taylori* and NHPs in the sylvatic cycles of these arboviruses. However, the feeding by *Ae. taylori* on other hosts, including birds, suggests the possibility of secondary enzootic transmission cycles.

The tendency of other important vectors like *Ae. luteocephalus*, *Ae. vittatus*, *Ae. aegypti* and *Ae. dalzieli* to feed on avian hosts was not expected. This suggests the possibility of secondary enzootic transmission cycles and points to the need for more research into avian reservoirs for these sylvatic arboviruses. The possible avian reservoirs should be further investigated by studying seroprevalence and performing experimental infections on species with high rates of seropositivity.

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Number and percentage of avian and mammalian bloodmeals identified from mosquitoes, naturally associated with dengue, yellow fever and/or chikungunya viruses,³ collected in Kedougou, Senegal, June 2010–January 2011

Table 1

Common name	Scientific name	Order	<i>Aedes aegypti</i> ^a	<i>Aedes dalziel</i>	<i>Aedes argenteo-punctatus</i>	<i>Aedes furcifer</i>	<i>Aedes taylori</i>	<i>Aedes lateocephalus</i>	<i>Aedes vitatus</i>	<i>Anopheles junestus</i>	<i>Anopheles domicola</i>	<i>Anopheles rufipes</i>	<i>Culex decens</i>	<i>Eretmapodites chrysogaster</i> ^b	Total
Avian hosts															
Scaly Francolin	<i>Pternistis squamatus</i>	Galliformes	1 (33)	0	0	2 (50)	0	0	0	0	0	0	0	0	3
Western Plantain-eater	<i>Cinifer piscator</i>	Musophagiformes	1 (33)	4 (80)	1 (50)	0	4 (29)	4 (80)	9 (90)	1 (12)	1 (20)	0	0	1 (50)	26
Red-backed Shrike	<i>Lanius collurio</i>	Passeriformes	0	0	1 (50)	0	0	0	0	0	0	0	0	0	1
Red-tailed Ant-thrush	<i>Neocossyphus rufus</i>	Passeriformes	0	0	0	0	0	0	0	0	0	0	1 (20)	0	1
Southern Pied-babbler	<i>Turdoides bicolor</i>	Passeriformes	0	0	0	0	1 (7)	0	1 (10)	0	0	0	1 (20)	0	3
African Thrush	<i>Turdus pelius</i>	Passeriformes	0	0	0	1 (25)	1 (7)	0	0	0	0	0	2 (40)	0	4
Grey Heron	<i>Ardea cinerea</i>	Pelecaniformes	0	0	0	0	0	0	0	0	0	0	1 (20)	0	1
Total			2 (67)	4 (80)	2 (100)	3 (75)	6 (43)	4 (80)	10 (100)	1 (12)	1 (20)	0	5 (100)	1 (50)	39
Mammalian hosts															
Green monkey	<i>Chlorocebus sabaeus</i>	Primates	0	0	0	0	7 (50)	0	0	0	0	0	0	0	7
Guinea baboon	<i>Papio papio</i>	Primates	0	0	0	0	1 (7)	0	0	0	0	0	0	0	1
Black-fronted duiker	<i>Cephalophus nigrifrons</i>	Artiodactyles	0	0	0	1 (25)	0	0	0	0	0	0	0	1 (50)	2
Bushbuck	<i>Tragelaphus scriptus</i>	Artiodactyles	0	1 (20)	0	0	0	0	0	0	0	0	0	0	1
Domestic cow	<i>Bos taurus</i>	Artiodactyles	0	0	0	0	0	0	0	4 (50)	4 (80)	2 (100)	0	0	10
Donkey	<i>Equus asinus</i>	Perissodactyles	0	0	0	0	0	0	0	2 (25)	0	0	0	0	2
Total			0	1 (20)	0	1 (25)	8 (57)	0	0	6 (75)	4 (80)	2 (100)	0	1 (50)	23
Mixed hosts															
Humans and Western Plantain-eater	<i>Homo sapiens</i> and <i>Crinifer piscator</i>		1 (33)	0	0	0	0	1 (20)	0	1 (12)	0	0	0	0	3
Total identified			3	5	2	4	14	5	10	8	5	2	5	2	65
Total tested			5	10	2	4	14	6	16	8	6	3	6	2	82

^a *Aedes aegypti formosus* is the only subspecies of *Ae. aegypti* that exists in southeastern Senegal.

^b *Eretmapodites chrysogaster* is a species complex, requiring male genitalia for species determination.