Monkey in the middle: monkeys serve as amplification hosts but not reservoir hosts of sylvatic chikungunya virus

Benjamin M. Althouse[1,2,3,4,*, †], Mathilde Guerbois[5,†], Derek A. T. Cummings[6], Ousmane M. Diop[7], Ousmane Faye[7], Abdourahmane Faye[7], Diawo Diallo[7], Bakary Djilocalisse Sadio[7], Abdourahmane Sow[7], Oumar Faye[7], Amadou A. Sall[7], Mawlouth Diallo[7], Brenda Benefit[4], Evan Simons[8], Douglas M. Watts[9], Scott C. Weaver[10], and Kathryn A. Hanley[4]

[1]Epidemiology, Institute for Disease Modeling, Bellevue, WA
[2]Information School, University of Washington, Seattle, WA
[3]Santa Fe Institute, Santa Fe, NM
[4]Department of Biology, New Mexico State University, Las Cruces, NM
[5]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX
[6]Emerging Pathogens Institute, University of Florida, Gainesville, FL
[7]Institut Pasteur de Dakar, Dakar, Senegal
[8]Department of Anthropology, New Mexico State University, Las Cruces, NM
[9]Office of Research and Sponsored Projects, University of Texas at El Paso, El Paso, TX and Center for Biodefense and Emerging Infectious Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX
[10]Departments of Pathology and Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, USA

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Abstract

Background Novel pathogens can emerge into humans via one-step transmission from a *reservoir host*, an animal species in which the pathogen is maintained, or a two-step process in which the pathogen is transmitted from the reservoir host into a different *amplification host* species and thence to humans. Here we use serosurveillance and mathematical modeling to discover whether monkeys serve as reservoir or amplification hosts for mosquito-borne chikungunya virus (CHIKV). CHIKV invaded the Americas in 2013, and our study provides key data for predicting whether and where CHIKV will establish *enzootic* transmission among animal hosts in the New World.

*To whom correspondence should be addressed. Email: balthouse@idmod.org
†These authors contributed equally to this work.

Results Over three years we captured 219 African green monkeys, 78 patas monkeys, and 440 Guinea baboons, the three monkey species near Kédougou, Senegal. Monkey age was determined by anthropometry and dentition, and exposure of each animal to CHIKV was determined via detection of neutralizing antibodies. Age and exposure were used to estimate age-specific CHIKV seroprevalence, force of infection (FoI), and basic reproductive number (R_0) in each species. CHIKV FoI were extremely high, ranging from 0.13 (95% CI, 0.07–0.22) in patas in 2012 to 1.12 (95% CI, 0.81–2.28) in African greens in 2011. R_0 ranged from 1.5 (95% CI, 1.3–1.9) in patas in 2012, to 6.6 (95% CI, 5.1–10.4) in baboons in 2011.

Conclusions These findings demonstrate that monkeys in this region are constantly exposed to CHIKV transmission, even when population seropositivity, and therefore immunity, was too high for monkeys themselves to support continuous CHIKV transmission. We therefore conclude that monkeys in this system serve as amplification rather than reservoir hosts of CHIKV.

Considering the potential for CHIKV to spill back in to monkeys in the Americas and elsewhere, improved understanding of its sylvatic cycle is essential to understanding and perhaps controlling the spread of this virus.

keywords: chikungunya virus — sylvatic arbovirus — non-human primate — age-stratified serosurvey

Introduction

Arthropod-borne viruses (arboviruses) can emerge from *enzootic* cycles, i.e. transmission cycles maintained in non-human *reservoir hosts* and arthropod vectors, into humans via vector transmission directly from the reservoir host to a human host [1]. Yellow fever virus, for example, can be transmitted directly from its non-human primate (NHP) reservoir hosts to humans in transitional habitats where the two interact. Alternatively, an arbovirus may first be transmitted from the reservoir host into an *amplification host* species that supports robust replication of the virus, and from the amplification host to humans. For example, Japanese encephalitis virus is maintained in avian reservoir hosts but usually must be amplified in pigs before human infections occur [2]. Identifying the suite of reservoir and amplification hosts that support transmission and enable emergence of specific arboviruses is key to predicting and, in some instances, preventing human infections.

Mosquito-borne chikungunya virus (CHIKV) circulates in two genetically-distinct, enzootic transmission cycles in the forests of West Africa and East/Central/South Africa (ECSA) [3]. Evidence to date has suggested that non-human primates (NHPs) serve as the reservoir hosts in these cycles [4], though Chevillon and colleagues [5] have questioned this claim. Sylvatic CHIKV periodically spills over from both the enzootic cycles into humans to cause individual cases and small outbreaks. Moreover explosive outbreaks and long-range geographic spread have occurred due to transmission by the anthrophilic mosquitoes *Aedes aegypti* and *Ae. albopictus*. CHIKV causes significant morbidity in humans, including debilitating arthralgia and myalgia that can become chronic [6]. CHIKV outbreaks in the Indian Ocean [7] and in India [6] have involved millions of cases; outbreaks on islands in the Indian Ocean, involving hundreds-of-thousands of cases and some fatalities, have included many tourists returning to Europe and the Americas [7, 6, 8, 9]. Recently, CHIKV established transmission in the Americas, resulting in thousands of chikungunya cases in the islands of the Caribbean as well as mainland South America [8]. There have been over 150 deaths from CHIKV in the Caribbean since the beginning of the outbreak in 2013 [10]. In July 2014, local transmission of CHIKV was detected in Florida, U.S.A. [11].

It is not clear whether the sporadic nature of CHIKV outbreaks in Asia over the last five decades [12, 13, 14] are due to sustained transmission in multiple enzootic foci in Asia or repetitive

geographic expansion and retreat from reservoir transmission cycles within Africa. Moreover, the potential for CHIKV to establish novel enzootic cycles in the Americas is not clear, because the hosts responsible for persistence of CHIKV in its endemic enzootic cycles in Africa have not been fully characterized [15, 5]. Certainly NHPs are abundant in the islands and mainland regions of tropical America in which CHIKV is currently circulating, including large populations of Old World NHPs [1]. A recent report of serological evidence of CHIKV infection in captive pig-tailed macaques (*Macaca nemestrina*) [16] as well as crab-eating macaques (*Macaca fascicularis*) and brown lemurs (*Eulemur fulvus*) on islands in the Indian Ocean [17] suggests that the virus has the potential to establish novel sylvatic transmission cycles, as yellow fever virus did when it was transported via the slave trade from Africa into the Americas [1].

In Senegal, primatophilic, sylvatic mosquitoes have been collected in the Department of Kédougou (Figure 1) via human landing capture and screened for arbovirus infection annually since the early 1970s by personnel from the Institut Pasteur. CHIKV has been isolated from mosquitoes, primarily in the genus *Aedes* (e.g. *Ae. furcifer, Ae. taylori, Ae. luteocephalus*, and *Ae. africanus*) [18], at 4.1 year intervals over this timespan [13]. Periods during which the virus can be detected in mosquitoes are termed amplifications. During amplifications, CHIKV has been isolated from all three monkey species resident in Kédougou; African green monkeys (*Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*), as well as from from a bushbaby (*Galago senegalensis*), *Scotophilus* bats and one palm squirrel (*Xerus erythropus*) [18, 5]. Additionally CHIKV has been isolated from guenons (*Cercopithecus aethiops*) in Senegal [18], although guenons are not resident in Kédougou [19]. Sera from mandrills (*Mandrillus sphinx*) in Gabon [20], red-tail monkeys (*Cercopithecus ascanius schmidti*) in Uganda [21], and African green monkeys (*Cercopithecus (Chlorocebus) aethiops sensu lato*) and Chacma baboon (*Papio ursinus*) in South Africa and Zimbabwe [22], have also tested positive for CHIKV antibodies. Together, these findings serve as the basis for the common assertion in the literature that NHPs serve as the reservoir hosts of CHIKV, i.e. hosts that are required for pathogen persistence, and the 4 year periodicity in observed CHIKV amplifications is characterized by depletion of susceptible NHP hosts during epizootics, local extinction of the virus, recruitment of susceptible hosts via births, and reintroduction of the virus from distant sites [3].

In the current study, we sought to test the dynamics of CHIKV transmission in monkeys in

Senegal and thereby discern their role in the West African sylvatic CHIKV cycle. Specifically, we hypothesized that monkeys serve as reservoir hosts for CHIKV, and that the four year periodicity observed in CHIKV amplifications is driven by depletion of susceptible NHP hosts during epizootics (epidemics in the reservoir hosts), local extinction of the virus, recruitment of susceptible hosts via births, and reintroduction of the virus from distant sites [3]. To test this hypothesis, we conducted a three-year study of the seroprevalence of CHIKV among individuals of known age from the three monkey species resident in the Department of Kédougou. These data were used to estimate key epidemiological parameters describing the transmission dynamics of CHIKV: age-specific seroprevalence, forces of infection (FoI), and basic reproductive numbers in each of these three species. Contra our hypothesis, we found that rates of CHIKV seropositivity in juvenile monkeys and CHIKV FoI were high in all three monkey species in the periods between amplifications in primatophilic mosquitoes. These findings suggest that vertebrate species other than monkeys serve as reservoir hosts in this area, while monkeys instead act as amplification hosts. To our knowledge this is the first quantitative characterization of CHIKV transmission dynamics in its sylvatic cycle, the only age-stratified serological survey of CHIKV in NHPs in Africa, and the first time that this approach has been used to distinguish whether a particular species or group of species serves as reservoir host, amplification host or both for a zoonotic pathogen. Moreover, our findings will inform future work integrating data and models to assess risk to humans living near African sylvatic hotspots [23] as well as surveillance of potential enzootic CHIKV hosts outside of Africa.

Methods

Ethics Statement

All animal research was approved by the Institutional Animal Care and Use Committee (IACUC) of University of Texas Medical Branch, Galveston, protocol number: 0809063 (principal investigator: SCW), and the entire protocol was approved on November 27, 2008 by the Consultative Committee for Ethics and Animal Experimentation of the Interstate School for Veterinary Sciences and Medicine, Dakar, Senegal (principal investigator: AAS). No other specific permits were necessary. This approval is necessary and sufficient to conduct wildlife research in Senegal. Animals were trapped in large, open air containers (see Figure 1, panel **c**) with access to water and food, sedated

and retained only long enough to take anthropomorphic measurements and draw a blood sample. Animals were released together as an intact troop upon recovery from ketamine anesthesia.

Study site

NHPs were trapped at sites around Kédougou, Senegal (12°33 N, 12°11 W) close to the borders of Mali and Guinea (Figure 1). The area is comprised of a mosaic of open savanna, woody savanna, outcrops of laterite (bowé), and relictual gallery forest, the latter concentrated along valleys and rivers. The Kédougou region is characterized by a tropical savanna climate, and receives an average of 1,300 mm of total annual rainfall, with one rainy season from approximately June through November. Mean temperatures fluctuate around 25–33°C throughout the year. Three monkey species reside in Kédougou: African green monkeys (AGM; *Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*). A relictual population of chimpanzees (*Pan troglodytes*) is present in the region [24], albeit at numbers too small to significantly affect CHIKV transmission. Senegal bushbabies (*Galago senegalensis*) are the only other NHP resident in Kédougou; populations sizes for this species in Senegal are not known [25]; because bushbabies are nocturnal and primarily consume arthropods it was not possible to collect them using the methods employed in this study. Humans in Kédougou have typically lived at low density ($4/km^2$) in small dispersed villages. In the last ten years, however, the region has experienced a "gold rush", and the expanding scope of mining operations is creating dramatic changes in population density, occupation and mobility [26].

The Kédougou area features a rich diversity of mosquito species including *Aedes aegypti* formosus, *Ae. africanus*, *Ae. centropunctatus*, *Ae. dalzieli*, *Ae. furcifer*, *Ae. hirsutus*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. neoafricanus*, *Ae. taylori*, *Ae. vittatus*, *Anopheles coustani*, *An. domicola*, *An. funestus*, *Culex poicilipes*, and *Mansonia uniformis*. *Ae. luteocephalus, Ae. taylori* and *Ae. africanus* show high rates of CHIKV infection but their distributions tend to be confined to forest canopies, thus they have been implicated in the maintenance of transmission of CHIKV among NHPs. *Ae. furcifer* has comparable CHIKV infection rates compared to the former three species, but a distribution that encompasses both the forest canopy and villages equally. We have therefore proposed that this species is the principal vector for spillover of sylvatic arboviruses into human communities around Kédougou [27].

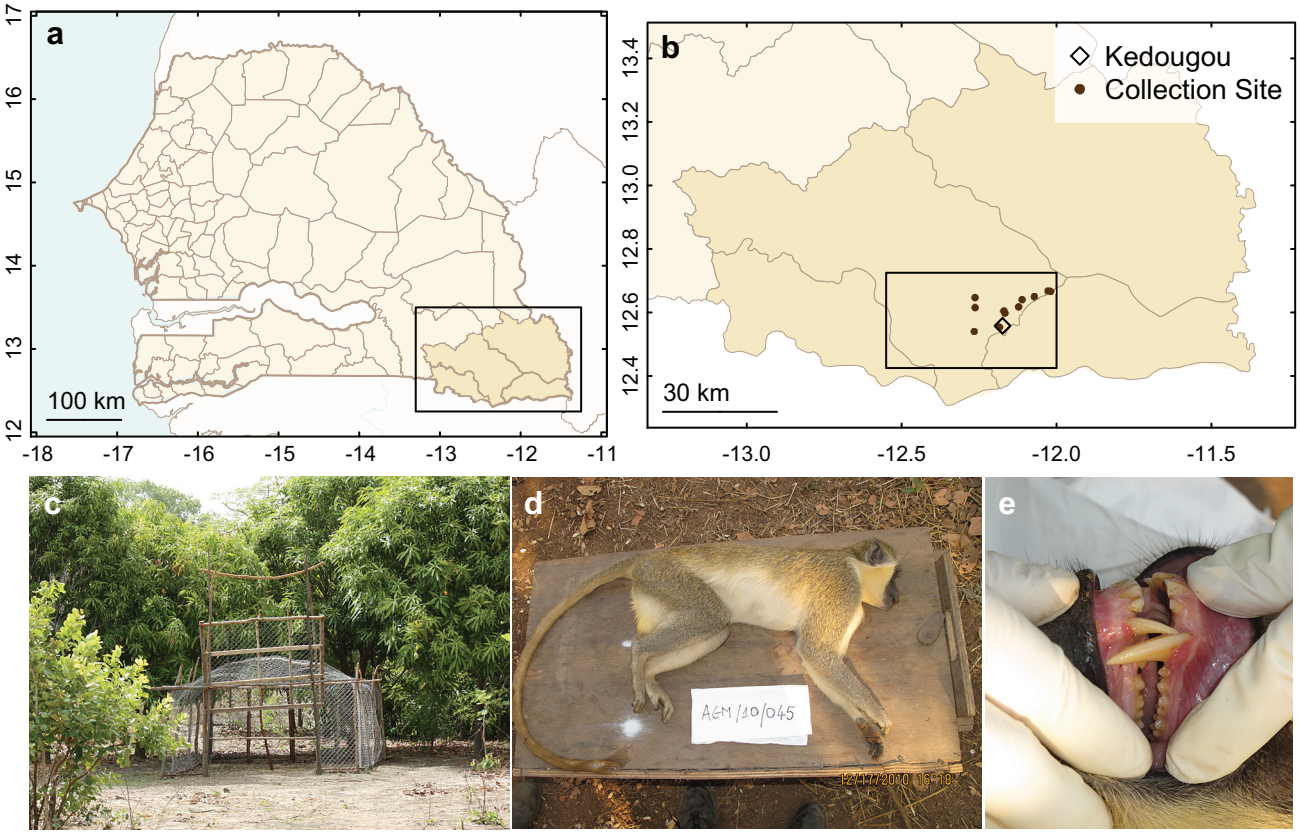

Figure 1: **NHP Collection Sites and Sample NHP** Panel **a** shows a map of Senegal with the Kédougou Department boxed. Panel **b** shows a map of the Kédougou region with the study region boxed and presented in detail in Figure 2. Panel **c** shows a typical trap, and panels **d** and **e** shows a male *C. sabaeus* estimated to be approximately 5 years (between 4-6 years) of age.

Monkey and Mosquito Collections

E. patas, *C. sabaeus*, and *P. papio* were trapped during the dry season (generally December to May) in 2010, 2011, 2012, from 15 sites in the Department of Kédougou (Figure 1). Monkeys were captured in ground traps (see Figure 1 and Supplemental Information) during the dry season, when other foods are scarce. Monkeys were sedated with 10 mg/kg of ketamine administered intramuscularly. Anthropological measurements were taken (weight, arm length, leg length, tail length, and body length), gender was determined, and nipple and scrotum conditions were noted. Dental casts and dental photographs were taken to assess which teeth were erupted (based on gingival emergence and complete eruption).

Monkey captures were conducted during the dry season, while mosquito collection was conducted during the rainy season (June–January) [27]. An amplification of CHIKV occurred in June

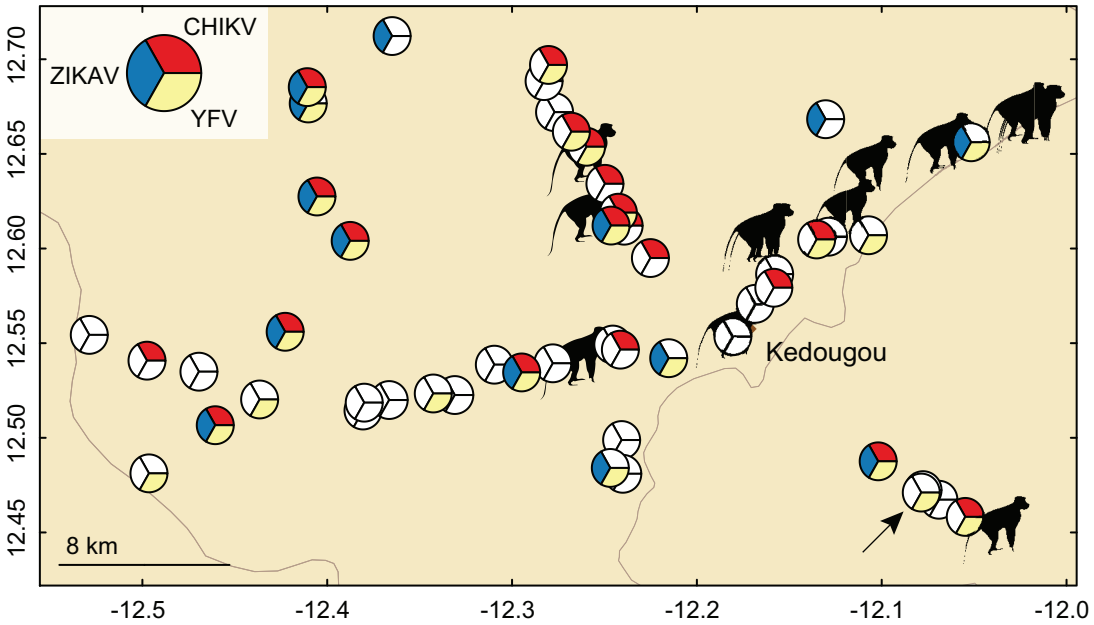

Figure 2: **Distribution of non-human primate (NHP) collection sites relative to CHIKV, YFV, and ZIKV Isolations from Mosquitoes, 2009–2011** Figure shows the spatial distribution of NHP collection sites (monkey symbols) and the mosquito collection sites (pie charts). Pie slices indicate mosquito collection moving clockwise from 2009 at the top. Red indicates CHIKV isolates in 2009, Yellow indicates YFV isolates in 2010, and Blue indicates ZIKAV isolates in 2011; unfilled (white) slices indicate that there was no virus isolation in that year. Diamond indicates Kédougou town. Arrow indicates a ZIKAV isolate that is obscured.

2009–January 2010, but CHIKV was not then detected in mosquitoes in 2010, 2011, or 2012. Yellow fever virus (YFV; [28]) and Zika virus (ZIKAV; unpublished data) were amplified in 2010 and 2011, respectively. Figure 2 shows the relative location of the NHP sites and mosquito sites where CHIKV, YFV, and ZIKAV were isolated.

Determination of Monkey Age

Chlorocebus sabaeus, *Erythrocebus patas*, and *Papio papio* were sorted into age classes based on the tooth eruption and degree of molar wear. The sequence of tooth eruption and molar occlusal wear was first determined separately for males and females of each species. Tooth presence, absence and gingival eruption information taken from casts and photographs was placed in order of tooth appearance to reveal the dental eruption sequence (see Supplemental Information). Published ages of dental eruption based on individuals of known age from captive and/or wild populations of the same species (*Chlorocebus aethiops* and *Erythrocebus patas*), or closely related species (*Papio*

cynocephalus and *Papio anubis*) were used to estimate the chronological age of infant through young adult individuals in the Senegal populations [29, 30, 31, 32, 33]. See Supplemental Information for more information including age classes for NHPs used in this study.

Serology

Monkeys were bled from the inguinal vein while sedated and serum was frozen for later testing. Sera ware tested for CHIKV, dengue virus, and YFV antibody by plaque reduction neutralization tests (PRNT) to determine the dilutions of sera that neutralized 50% and/or 80% of available virus [34]. $PRNT_{80}$ data are presented here. O'nyong nyong virus, an alphavirus with a close antigenic relationship to CHIKV, is present in Senegal. While antibodies raised against CHIKV will bind O'nyong nyong virus; antibodies raised against O'nyong nyong virus will not generally bind CHIKV [35]. This one-way antigenic cross-reactivity ensures the results presented here are likely true CHIKV antibody responses and not responses to O'nyong nyong [36].

Associations with CHIKV Seropositivity

To identify associations between NHP characteristics and CHIKV seropositivity, mixed-effects logistic and linear regressions were estimated. $PRNT_{80}$ IgG seropositivity and inverse $PRNT_{80}$ titers were the two outcomes of interest. Covariates of interest were NHP age, month of collection, and species, with NHP troop as a random effect to account for possible correlation of seropositivity at the troop level. As true NHP troops were not tracked, and indeed may not exist as consistent entities in some species, we considered those NHPs collected on the same day in the same site to belong to the same troop.

Force of CHIKV Infection

Increases in seropositivity with age reflect the rate at which hosts acquire infection as a function of time as well as their risk of acquiring infection at different ages. The force of infection gives an indication of the intensity of transmission in a given area; high forces of infection indicating high prevalence of the pathogen in a population. Catalytic models of infection were fit to age-stratified data to determine annual forces of infection (denoted throughout as $\lambda(t)$). Models fit here are based on Grenfell et al. [37], and have been employed for dengue virus in Brazil [38] and Thailand [39].

Briefly, the proportion of the population susceptible to CHIKV infection of age a at time t is given by

$$x(a,t) = \exp\left(-\int_0^a \lambda(t-\tau)d\tau\right). \tag{1}$$

The proportion of individuals of age a infected with CHIKV at time t is

$$z(a,t) = x(a,t)\left[\exp\left(-\int_0^a \lambda(t-\tau)d\tau\right) - 1\right]. \tag{2}$$

We can discretize the model by age and use maximum likelihood methods for estimating $\lambda(t)$. The binomial log-likelihood (seropositive for CHIKV or not) of $\lambda_k(t)$ for age class $k \in [1,m]$ is

$$\ell(\lambda_k(t)) \quad = \quad \sum_{k=1}^{m}\Big[n_{xk}\log[x(a_k,t_0)] + n_{yk}\log\left[z(a_k,t_0) + (1-z(a_k,t_0))\right]\Big], \tag{3}$$

where n_{xk} and n_{yk} are the numbers susceptible and seropositve for CHIKV infection in age class k, respectively. We can compare the maximum likelihood estimates, $\ell_{\max}$, to the saturated likelihood to estimate the goodness-of-fit of each model. The saturated likelihood, ℓ_{sat}, is given by

$$\ell_{\text{sat}} = \sum_{k=1}^{m}\left[n_{xk}\log\left[\frac{n_{xk}}{N_k}\right] + n_{yk}\log\left[\frac{n_{yk}}{N_k}\right]\right]. \tag{4}$$

The statistic $X^2 = 2\cdot(\ell_{\max} - \ell_{\text{sat}})$ is χ^2 distributed with $m-P$ degrees of freedom, where m is the number of age classes and P is the number of parameters being estimated. As per Ferguson et al. [40], smaller X^2 values are better and models with p-values greater than 0.05 are considered to fit the data well, as this indicates models that are statistically indistinguishable from saturated models. We calculated bootstrap confidence intervals to estimate uncertainty in estimates of $\lambda(t)$ by sampling NHPs with replacement and recalculating $\lambda(t)$. We estimate both constant and age-varying forces of infection.

Calculating of the Basic Reproductive Number of CHIKV

The basic reproductive number, R_0, gives important information about the infectiousness of a pathogen in a population, and the feasibility of its eradication or control in that population. Higher values of R_0 would indicate higher numbers of infections and that a larger fraction of the of population would need to be removed from the transmission pool (e.g. through vaccination or treatment) to stop transmission. R_0 can be calculated from $\lambda(t)$ if assumptions are made about the age structure of the population experiencing infection by using hazards to estimate the fraction of the population that remains susceptible and taking its reciprocal [40]. Let $f(a)$ be the fraction of the population aged a, and $w(a,t)$ be the fraction of the population aged a unexposed to CHIKV at time t, then

$$R_0 = \frac{1}{1 - \int_0^\infty f(a)w(a,t)da}. \tag{5}$$

We estimate $w(a,t)$ from $\lambda(t)$ as

$$w(a,t) = 1 - \exp\left(-\int_0^a \lambda(t-\tau)d\tau\right). \tag{6}$$

As the age structure of the NHP populations under study are not known, we assume three distributions of ages: Uniform(0, maximum observed age); Exponential(rate = 1/captive mean lifespan); and Exponential(rate = 1/mean observed age). We compared these to the observed age distributions of captured NHP. We use reported lifespans of NHP species in captive settings as an upper-bound on the lifespan.

Sensitivity Analyses

Substantial sensitivity analyses were conducted to assess the effects of biased sampling by age; these are presented in the Supplemental Information.

Results

Non-human Primates Collected

Across all years of the study, 737 NHPs were collected in the 15 sites (Table 1). This included 219 *C. sabaeus*, 78 *E. patas*, and 440 *P. papio*. Sites differed substantially in numbers of NHPs collected. *P. papio* were the most frequently collected NHP, but were only caught at 6 of the 15 sites (see Supplemental Information, Table S1). *E. patas* were collected at 7 of 15 sites, and *C. sabaeus* at 9 of the 15 sites. The mean ages of collected NHPs were relatively low, ranging from 3.5 years for *E. patas* to 6.7 years for *P. papio* (Figure 3). These ages are consistent with previous estimates of the lifespan of wild *P. papio* and *E. patas* [41, 42, 43]. Ages were approximately exponentially distributed. As might be expected in collections biased toward juvenile animals [44], more male *C. sabaeus* (N=147) and *P. papio* (N=260) were collected than females (N=70 *C. sabaeus* and N=180 *P. papio* females), though more female *E. patas* (N=64) were collected than males (N=14). The sex of two individuals was not recorded. Captured females of all species were older than captured males (*C. sabaeus* 5.4 vs. 3.4 years [1-sided t-test, $p = 0.0001$], *P. papio* 8.7 vs. 5.4 years [$p < 0.0001$], and *E. patas* 3.9 vs. 1.6 years [$p = 0.003$]).

	2010	2011	2012	Total
Chlorocebus sabaeus	52	78	89	219
Erythrocebus patas	34	4	40	78
Papio papio	103	200	137	440
Total	189	282	266	737

Table 1: **NHPs Collected by Year** Table shows the numbers of NHPs collected per year across all sites.

Seropositivity

Rates of CHIKV seropositivity in all three species were high. Among 667 NHPs tested (198 *C. sabaeus*, 399 *P. papio*, and 70 *E. patas*) 479 (72%) were seropositive for for CHIKV by PRNT. The remaining animals were not tested either because (i) adequate volumes of blood could not be drawn, (ii) identification data were not recorded, (iii) dental casts or photographs were inadequate, or (iv) samples were lost during shipment. As expected during the dry season, no animals were positive for IgM antibody. Moreover, agreement between $PRNT_{50}$ and $PRNT_{80}$ was excellent, only 14 of

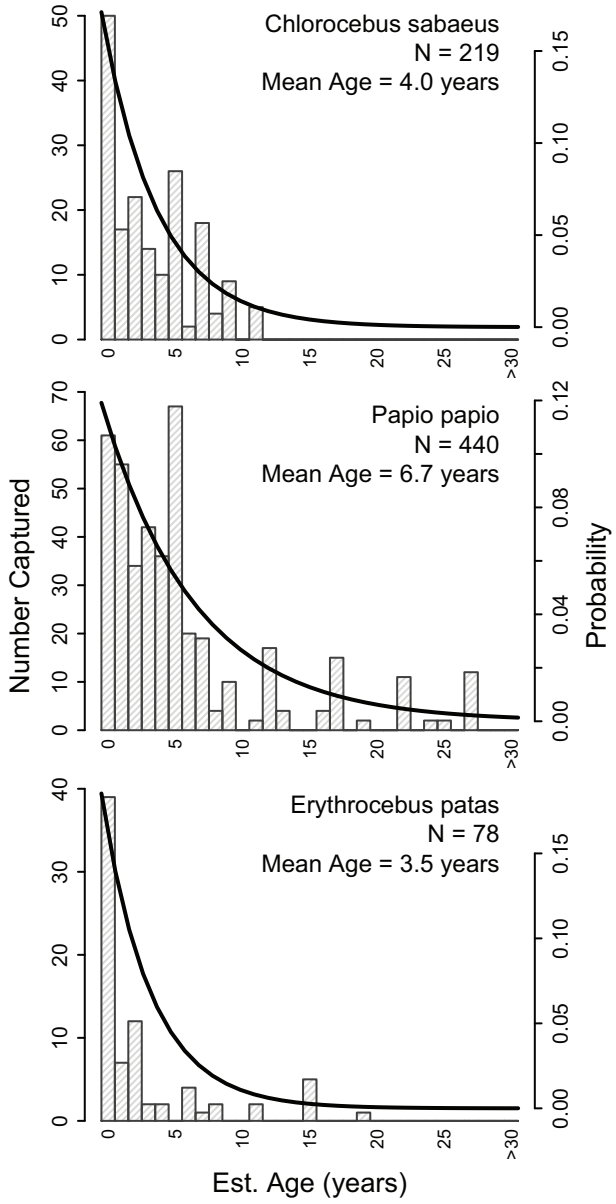

Figure 3: **Age Distributions of Collected NHPs** Panels show the observed age distributions of collected NHPs with exponential distributions (thick line) with rates equal to the mean age of collected individualls, for *Chlorocebus sabaeus*, *Papio papio*, and *Erythrocebus patas*, respectively.

493 were positive by $PRNT_{50}$ and not $PRNT_{80}$ (2% of all NHPs tested, Cohen's $\kappa = 0.95$ [95% CI 0.87–1]). Mixed effects regression models were preferred to fixed effects models by AIC (432.7 versus 446.0). Baseline seropositivity was high with the intercept and random effect indicating 95% of *C. sabaeus* primate infants (< 1 year old) collected in January to have $PRNT_{80}$ positivity rates between 0.060 and 0.52 (Table 2). Age of NHP was strongly positively associated with odds

Covariate	OR (95% CI)
Intercept (β_0)	0.26 (0.05, 1.24)
Age	2.14 (1.84, 2.50)
Erythrocebus patas	0.18 (0.05, 0.66)
Papio papio	1.18 (0.45, 3.13)
Feb. Collection	1.51 (0.19, 11.78)
Mar. Collection	0.84 (0.17, 4.23)
Apr. Collection	1.34 (0.23, 7.81)
May Collection	1.86 (0.25, 13.87)
Dec. Collection	2.32 (0.28, 19.35)
RE Troop (b_0)	0.72 (-0.69, 2.12)
ICC	0.135

Table 2: **Mixed Effects Logistic Regression** Table reports the estimates from a mixed effects logistic regression with CHIKV IgG seropositivity as the outcome and NHP age, species, month of collection as fixed effects and troop (same collection site and date) as a random effect. Intercept corresponds to 0.26 probability of IgG positivity in the first year of life in *Chlorocebus sabaeus* primates collected in January, with the random effect indicating 95% of *Chlorocebus sabaeus* primate infants ($<$ 1 year old) collected in January have PRNT_{80} positivity rates between 0.060 and 0.52 $(\exp(\beta_0 \pm 1.96 \cdot b_0)/[1+\exp(\beta_0 \pm 1.96 \cdot b_0)])$. ICC is the intraclass correlation for the random effect, and indicates about 13.5% of the total observed variance is due to variance within NHP troops.

of seropositivity (Odds Ratio [OR] = 2.14 [95% CI, 1.84–2.50] for each additional year of life), and *E. patas* had significantly smaller odds of seropositivity (OR 0.18 [95% CI, 0.05–0.66]) than the other two species. Intraclass correlation (ICC) calculated from the random intercept indicates about 13.5% of the total observed variance is due to variance within NHP troops.

Mixed effects linear regressions for the inverse PRNT_{80} titers were preferred to fixed effects models by AIC (1670.3 versus 1802.2) and are presented in the Supplemental Information. Age was significantly negatively associated with inverse titer, with each year of age corresponding to about a 4% decrease in titer ($\beta = 0.96$ [95% CI, 0.94–0.98]). This decrease is driven largely by *P. papio* in 2010 and 2011 (see Supplementary Figure 3). Large differences in antibody titers were seen across study years, with 2011 having 75% lower titers ($\beta = 0.25$ [95% CI, 0.13–0.49]). This is likely due to there being no inverse titers of 1280 observed in 2011 (Figure 4).

Force of Infection

In general, forces of infection (FoI) were high, ranging from 0.13 (95% CI, 0.07–0.22) in *E. patas* in 2012, to well over 1 in *C. sabaeus* in 2011 ($\lambda(t) = 1.12$, [95% CI, 0.81–2.28]). Only two of the constant FoI models provided a better fit than the saturated model ($p > 0.05$): *C. sabaeus* in 2010

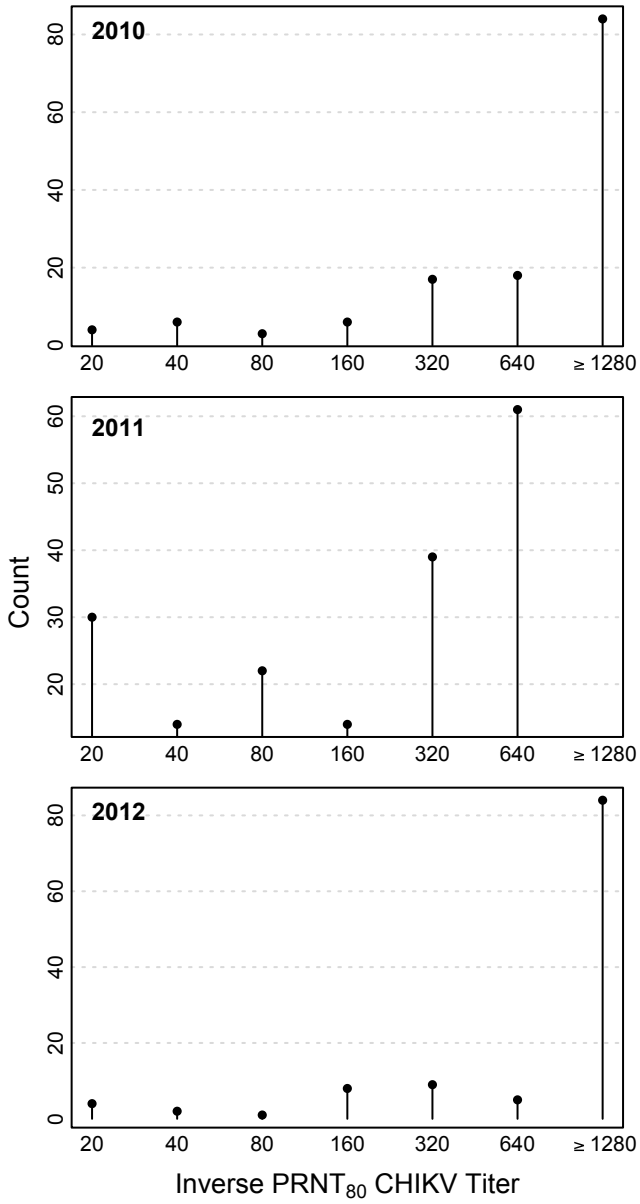

Figure 4: **Inverse $\mathbf{PRNT_{80}}$ CHIKV titers by Year** Figure shows number of animals (counts) per antibody titer by year for all three species of NHP.

and 2011. *P. papio* in 2010 was marginally better than the saturated model (p=0.05). As might be expected, the age-varying FoI was more flexible and provided a better fit (see Supplemental Information). FoI were high for younger NHPs, but there was a spike in FoI for NHPs aged about 8 years. Sensitivity analyses revealed the potential for over-estimation of $\lambda(t)$ when the sampling is very biased by age (see Supplemental Information). Note the presence of smoldering CHIKV

transmission: there is high CHIKV seropositivity observed in young NHPs of all species ($\leq$ 2 years old) in 2012 – four years after the most recent CHIKV amplification (Figure 5 and Supplementary Figure 4).

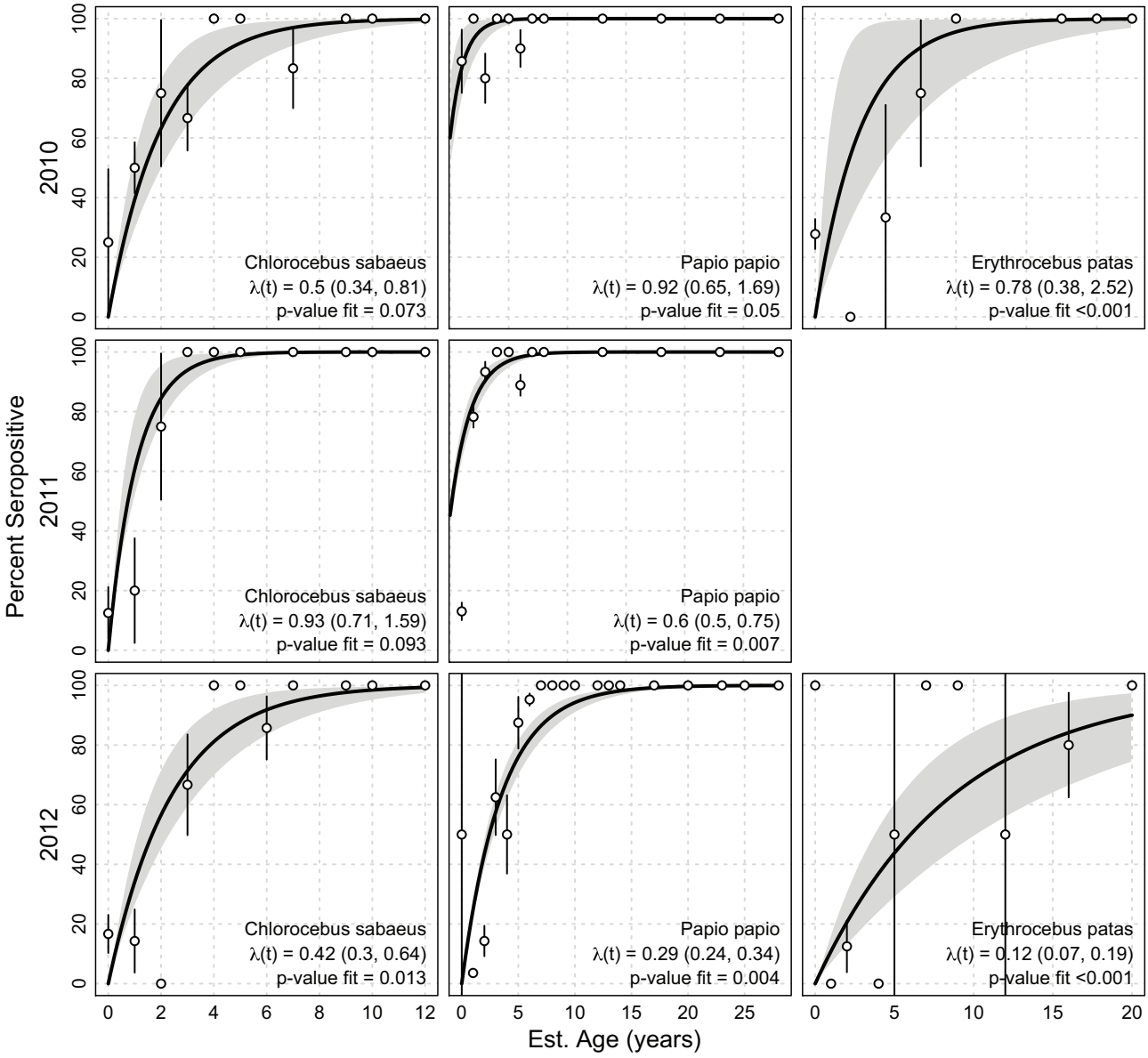

Figure 5: **Forces of Infection by NHP and Year** Panels show the forces infection ($\lambda(t)$) and p-values for the fit across years for *C. sabaeus*, *E. patas*, and *P. papio*, respectively. Too few *E. patas* were collected in 2011 to obtain estimates. We included all NHPs less than one year old in the 0 age category (we present seropositivity results for NHPs under 3 years of age in the Supplementary Information). Points are the proportion of seropositve NHPs per age year with confidence intervals. Thick black line is the fit of the force of infection, grey bands are bootstrap confidence intervals for the fit.

Year	Age Distribution	*Chlorocebus sabaeus* R_0 (95% CI)	*Papio papio* R_0 (95% CI)	*Erythrocebus patas* R_0 (95% CI)
2010	Flat	5.9 (4.1, 9.4)	22.9 (16.8, 38.0)	6.8 (3.5, 20.5)
	Literature Ages	4.1 (3.0, 6.1)	15.3 (11.5, 25.1)	5.8 (3.1, 16.8)
	Mean Ages	2.7 (2.2, 3.8)	6.6 (5.1, 10.4)	2.1 (1.6, 4.4)
2011	Flat	10.7 (8.3, 17.5)	15.7 (13.1, 19.0)	
	Literature Ages	6.9 (5.5, 10.8)	10.7 (9.1, 12.9)	
	Mean Ages	4.3 (3.5, 6.4)	4.0 (3.6, 4.7)	
2012	Flat	5.0 (3.6, 7.5)	7.8 (6.5, 9.3)	2.5 (1.8, 3.8)
	Literature Ages	3.5 (2.8, 5.0)	5.6 (4.8, 6.6)	2.2 (1.6, 3.0)
	Mean Ages	2.4 (2.0, 3.1)	2.5 (2.3, 2.8)	1.5 (1.3, 1.9)

Table 3: **Estimates of $\mathbf{R_0}$** Table reports the estimates of the basic reproduction number for the three species of NHP each year of the study period. Estimates are dependent on the assumed underlying population structure. "Flat" structure assumes a uniform population structure, "Literature Ages" and "Mean Ages" assume exponentially-distributed population structures with rates equal to the mean lifespan reported in the literature for captive NHPs, and the mean ages of the collected NHPs, respectively.

Basic Reproductive Number

Estimates of R_0 varied by NHP, year, and assumed population structure. Assuming an exponential population structure with rate equal to the observed mean ages, estimates of R_0 varied from 1.5 (95% CI, 1.3, 1.9) in *E. patas* in 2012, to 6.6 (95% CI, 5.1, 10.4) in *P. papio* in 2011. Generally, R_0 was highest in 2010 and in *P. papio*. *P. papio* consistently had the highest estimates of R_0 with estimates up to 4 times as high as either species in each year. See Table 3 and the Supplemental Information.

Discussion

In the Kédougou region, sylvatic CHIKV has been isolated from pools of primatophilic mosquitoes collected via human landing capture at roughly four-year intervals since the early 1970s [18, 13]. During these amplifications, outbreaks of CHIKV among humans occurred in Senegal in 1966, 1982, 1996, 2004, and in 2010 ([27] and unpublished data), and the virus was isolated from humans in 1975 and 1983. We have hypothesized that monkeys are the reservoir hosts of CHIKV, and that during CHIKV amplifications, most susceptible monkeys are infected and rendered immune, so that the interval between CHIKV amplifications reflects the time needed for a sufficient number of susceptible monkeys to be born (susceptible recruitment) [13]. However, to date no studies have

systematically examined the transmission dynamics of sylvatic CHIKV in NHP hosts.

As expected based on its 4 year amplification cycle, CHIKV was isolated from 42 of 4,211 mosquito pools collected across the Kédougou study region during the rainy season (June–January) of 2009. Infection rates among mosquito species differed temporally, with *Ae. furcifer, Ae. luteocephalus, Ae. taylori*, and *Ae. dalzieli* having significantly higher rates in December [27]. Despite similar mosquito collection efforts, and consistent with a 4.1 year periodicity in the CHIKV amplification cycle, the virus was not isolated from mosquitoes in the wet seasons of 2010, 2011, and 2012. To assess whether susceptible NHP hosts were indeed depleted during this amplification, leading to local CHIKV extinction and consequent cessation of NHP infection, we initiated a three-year age-stratified, serological survey of NHPs in Kédougou in 2010, immediately following the 2009 amplification. Over 700 NHPs were captured in the 2010, 2011, and 2012 dry seasons and we found high IgG seropositivity rates (72% by $PRNT_{80}$). Catalytic models found correspondingly high forces of infection, in some cases approaching 1, making infection in the first year of life a near certainty. Even in 2012, three years after the last detected amplification of CHIKV in mosquitoes, we detected relatively high rates of infection in NHP infants ($<$ 1 year old), with seropositivity rates approaching 50% in those under 3 months old (see Supplemental Information). One interpretation of this finding is that infants are seropositive due to transfer of maternal antibody. However, while there is evidence of maternal transfer of CHIKV antibody in humans, the rates are not 100% and antibody levels decay rapidly [45, 46]. Additionally maternal transfer would be unlikely to sustain infant seroprevalence over several years. Thus we conclude that the majority of seropositive infants in this study were infected with CHIKV in their first year of life, despite the failure to detect infected primatophilic mosquitoes during these years. This finding contradicts the hypothesis that monkeys serve exclusively as necessary and sufficient reservoir hosts with primarily primatophilic mosquitoes as vectors. It is likely that these monkey species act as CHIKV amplification hosts.

Our data suggest that an alternate cycle of CHIKV involving reservoir hosts other than monkeys and non-primatophilic vectors exists in Kédougou and is supporting CHIKV transmission. Although previous studies have suggested the existence of such cryptic reservoirs [5], our results provide the strongest evidence to date that the dynamics of CHIKV in monkeys preclude them from serving as dominant long-term reservoirs of the virus. We do note that we have only investigated three species of NHP in this study, however, they are the most common NHPs in Senegal and the

only three monkey species resident in the region. CHIKV has been isolated from several small mammals in Senegal, including *Scotophilus* bats, a palm squirrel (*Xerus erythropus*), and a bushbaby (*Galago senegalensis*) [18, 5]; moreover, bushbabies are important reservoir hosts of yellow fever virus in East Africa [1]. CHIKV may be maintained in cycles involving small mammals and non-primatophilic mosquitoes, which would not necessarily be detected by human landing capture methods. Alternatively, or in addition, it is possible that birds serve as a reservoir host for the virus. The source of bloodmeals from purportedly primatophilic mosquitoes that are known CHIKV vector species in Kédougou has been identified via PCR amplification of vertebrate cytochrome *b* [47]. This study found 60% (39 individual bloodmeals) of vector bloodmeals were taken from birds, with meals from Western Plantain-eater *Crinifer piscator* being the most common (26 bloodmeals, or 40% of the total). Primates accounted for 35% (23 bloodmeals) of the bloodmeals, and 5% (3 bloodmeals) of fed mosquitoes contained both human and Western Plantain-eater blood. Although previous studies discounted a possible role for birds as amplification hosts in India, where only the urban human-mosquito cycle is known to occur [48], further effort should be made to investigate the possible role of birds in the African enzootic cycle of CHIKV.

Making assumptions about the population structure of Senegalese NHPs, we determined the basic reproductive number of CHIKV in these populations to range from 1.6 to 6.6. Interestingly, we found large differences among species of NHP, with *P. papio* having estimates of R_0 up to three times that of the other NHPs. The forces of infection and reproductive numbers seen here indicate that all three of these species could initiate an explosive amplification of CHIKV. In geographic regions where sylvatic CHIKV transmission occurs, spillover into humans occurs frequently during CHIKV amplifications. Full emergence presumably is initiated when humans infected via spillover come into contact with the urban vectors *Ae. aegypti aegypti* and *Ae. albopictus* [49]. Thus, amplification hosts of CHIKV both directly and indirectly generate risk for human disease. In the last 60 years, CHIKV has emerged into sustained human transmission only from the reservoirs in the ECSA sylvatic cycle [50], but the West African cycle has the potential to launch new CHIKV strains into urban transmission. Maps of areas with high risk of spillover infection could be created if estimates of the range of movement and population numbers for the monkey species implicated as amplification hosts were known. Based on our estimates of force of infection, *P. papio* could be playing a larger role in the amplification of CHIKV than previously recognized, especially con-

sidering the substantial spatial heterogeneity mosquito density in the region [27]. In areas with low mosquito density, NHPs with higher forces of infection or values of R_0, may have a larger role in transmission. More importantly, future studies should focus on identifying levels of CHIKV seroconversion and isolating CHIKV in other, non-monkey species. Improved understanding of the enzootic, sylvatic cycle of CHIKV is essential to understanding and perhaps controlling sylvatic arbovirus transmission generally and thereby reducing human disease.

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References

[1] Kathryn A Hanley, Thomas P Monath, Scott C Weaver, Shannan L Rossi, Rebecca L Richman, and Nikos Vasilakis. Fever versus fever: The role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. *Infect Genet Evol*, Mar 2013.

[2] Leon Rosen. The natural history of japanese encephalitis virus. *Annual Reviews in Microbiology*, 40(1):395–414, 1986.

[3] Scott C Weaver, Richard Winegar, Ian D Manger, and Naomi L Forrester. Alphaviruses: population genetics and determinants of emergence. *Antiviral research*, 94(3):242–257, 2012.

[4] Peter Daszak, Andrew A Cunningham, and Alex D Hyatt. Emerging infectious diseases of wildlife–threats to biodiversity and human health. *science*, 287(5452):443–449, 2000.

[5] Christine Chevillon, Laurence Briant, François Renaud, and Christian Devaux. The chikungunya threat: an ecological and evolutionary perspective. *Trends in microbiology*, 16(2):80–88, 2008.

[6] Chandrakant Lahariya and S K Pradhan. Emergence of chikungunya virus in indian subcontinent after 32 years: A review. *J Vector Borne Dis*, 43(4):151–60, Dec 2006.

[7] Rémi N Charrel, Xavier de Lamballerie, and Didier Raoult. Chikungunya outbreaks–the globalization of vectorborne diseases. *N Engl J Med*, 356(8):769–71, Feb 2007.

[8] G Rezza, L Nicoletti, R Angelini, R Romi, A C Finarelli, M Panning, P Cordioli, C Fortuna, S Boros, F Magurano, G Silvi, P Angelini, M Dottori, M G Ciufolini, G C Majori, A Cassone, and CHIKV study group. Infection with chikungunya virus in italy: an outbreak in a temperate region. *Lancet*, 370(9602):1840–6, Dec 2007.

[9] Marc Grandadam, Valérie Caro, Sébastien Plumet, Jean-Michel Thiberge, Yvan Souarès, Anna-Bella Failloux, Hugues J Tolou, Michel Budelot, Didier Cosserat, Isabelle Leparc-Goffart, et al. Chikungunya virus, southeastern france. *Emerging infectious diseases*, 17(5):910, 2011.

[10] World Health Organization. Fact sheet 327: Chikungunya. Technical report, WHO, http://www.who.int/mediacentre/factsheets/fs327/en/, October 2014.

[11] Press release: Florida department of health confirms first locally acquired cases of chikungunya fever (http://newsroom.doh.state.fl.us/wp-content/uploads/newsroom/2014/05/071714-locally-aquired-chikungunya.pdf). 2014.

[12] PJ Mason and AJ Haddow. An epidemic of virus disease in southern province, tanganyika territory, in 1952–1953: An additional note on chikungunya virus isolations and serum antibodies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 51(3):238–240, 1957.

[13] Benjamin M Althouse, Justin Lessler, Amadou A Sall, Mawlouth Diallo, Kathryn A Hanley, Douglas M Watts, Scott C Weaver, and Derek A T Cummings. 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, Nov 2012.

[14] Benjamin M Althouse, Kathryn A Hanley, Mawlouth Diallo, Amadou A Sall, Yamar Ba, Ousmane Faye, Douglas M Watts, Scott C Weaver, and Derek A T Cummings. Impact of

climate and mosquito vector abundance on sylvatic arbovirus circulation dynamics in senegal. *American Journal of Tropical Hedicine and Hygiene*, in press, 2014.

[15] David M Morens and Anthony S Fauci. Chikungunya at the door—déjà vu all over again? *New England Journal of Medicine*, 2014.

[16] Khajornpong Nakgoi, Narong Nitatpattana, Worawidh Wajjwalku, Pornsawan Pongsopawijit, Supakarn Kaewchot, Sutee Yoksan, Voravit Siripolwat, Marc Souris, and Jean-Paul Gonzalez. Dengue, japanese encephalitis and chikungunya virus antibody prevalence among captive monkey (macaca nemestrina) colonies of northern thailand. *American journal of primatology*, 76(1):97–102, 2014.

[17] Gwenaël Vourc'h, Lénaïg Halos, Amélie Desvars, Franck Boué, Michel Pascal, Sylvie Lecollinet, Stéphan Zientara, Thomas Duval, Angella Nzonza, and Michel Brémont. Chikungunya antibodies detected in non-human primates and rats in three indian ocean islands after the 2006 chikv outbreak. *Veterinary research*, 45(1):1–5, 2014.

[18] M Diallo, J Thonnon, M Traore-Lamizana, and D Fontenille. Vectors of chikungunya virus in senegal: current data and transmission cycles. *Am J Trop Med Hyg*, 60(2):281–6, Feb 1999.

[19] Jill D Pruetz, A Socha, and D Kante. New range record for the lesser spot-nosed guenon (cercopithecus petaurista) in southeastern senegal. *African Primates*, 7(1):Pp–64, 2010.

[20] Rebekah C Kading, Erin M Borland, Mike Cranfield, and Ann M Powers. Prevalence of antibodies to alphaviruses and flaviviruses in free-ranging game animals and nonhuman primates in the greater congo basin. *Journal of wildlife diseases*, 49(3):587–599, 2013.

[21] AWR McCrae, BE Henderson, BG Kirya, and SDK Sempala. Chikungunya virus in the entebbe area of uganda: isolations and epidemiology. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 65(2):152–168, 1971.

[22] BM McIntosh. Antibody against chikungunya virus in wild primates in southern africa. *The South African journal of medical sciences*, 35(3):65, 1970.

[23] Shannon L LaDeau, Gregory E Glass, N Thompson Hobbs, Andrew Latimer, and Richard S Ostfeld. Data-model fusion to better understand emerging pathogens and improve infectious disease forecasting. *Ecological Applications*, 21(5):1443–1460, 2011.

[24] Wwf: Chimpanzees (http://wwf.panda.org/what_we_do/endangered_species/great_apes/chimpanzees/). 2014.

[25] Iucn red list of threatened species: Galago senegalensis (http://www.iucnredlist.org/details/8789/0). 2014.

[26] Lamine Daffé. Gold rush in kédougou, senegal: Protecting migrants and local communities. http://publications.iom.int/bookstore/free/ GlobalEyeissue11_29Feb2012.pdf, March 2012.

[27] Diawo Diallo, Amadou A Sall, Michaela Buenemann, Rubing Chen, Oumar Faye, Cheikh T Diagne, Ousmane Faye, Yamar Ba, Ibrahima Dia, Douglas Watts, Scott C Weaver, Kathryn A Hanley, and Mawlouth Diallo. Landscape ecology of sylvatic chikungunya virus and mosquito vectors in southeastern senegal. *PLoS Negl Trop Dis*, 6(6):e1649, 2012.

[28] Diawo Diallo, Amadou A Sall, Cheikh T Diagne, Oumar Faye, Kathryn A Hanley, Michaela Buenemann, Yamar Ba, Ousmane Faye, Scott C Weaver, and Mawlouth Diallo. Patterns of a sylvatic yellow fever virus amplification in southeastern senegal, 2010. *The American journal of tropical medicine and hygiene*, 90(6):1003–1013, 2014.

[29] Jane E Phillips-Conroy and Clifford J Jolly. Dental eruption schedules of wild and captive baboons. *American Journal of Primatology*, 15(1):17–29, 1988.

[30] P Kahumbu and RM Eley. Teeth emergence in wild olive baboons in kenya and formulation of a dental schedule for aging wild baboon populations. *American Journal of Primatology*, 23(1):1–9, 1991.

[31] T R Turner, F Anapol, and C J Jolly. Growth, development, and sexual dimorphism in vervet monkeys (cercopithecus aethiops) at four sites in kenya. *Am J Phys Anthropol*, 103(1):19–35, May 1997.

[32] Debra R Bolter and Adrienne L Zihlman. Morphometric analysis of growth and development in wild-collected vervet monkeys (cercopithecus aethiops), with implications for growth patterns in old world monkeys, apes and humans. *Journal of Zoology*, 260(1):99–110, 2003.

[33] Yu Okuda Jogahara and Masahito Natori. Dental eruption sequence and eruption times in erythrocebus patas. *Primates*, 53(2):193–204, 2012.

[34] BJ Beaty, CH Calisher, and RE Shope. *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, pages 189–212. American Public Health Association, 1995.

[35] NK Blackburn, TG Besselaar, and G Gibson. Antigenic relationship between chikungunya virus strains and o'nyong nyong virus using monoclonal antibodies. *Research in virology*, 146(1):69–73, 1995.

[36] Ann M Powers, Aaron C Brault, Robert B Tesh, and Scott C Weaver. Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *Journal of General Virology*, 81(2):471–479, 2000.

[37] BT Grenfell and RM Anderson. The estimation of age-related rates of infection from case notifications and serological data. *Journal of Hygiene*, 95(02):419–436, 1985.

[38] Isabel Rodriguez-Barraquer, Marli T Cordeiro, Cynthia Braga, Wayner V de Souza, Ernesto T Marques, and Derek A T Cummings. From re-emergence to hyperendemicity: the natural history of the dengue epidemic in brazil. *PLoS Negl Trop Dis*, 5(1):e935, 2011.

[39] Isabel Rodríguez-Barraquer, Rome Buathong, Sopon Iamsirithaworn, Ananda Nisalak, Justin Lessler, Richard G Jarman, Robert V Gibbons, and Derek A T Cummings. Revisiting rayong: Shifting seroprofiles of dengue in thailand and their implications for transmission and control. *Am J Epidemiol*, Nov 2013.

[40] N M Ferguson, C A Donnelly, and R M Anderson. Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. *Philos Trans R Soc Lond B Biol Sci*, 354(1384):757–68, Apr 1999.

[41] Jeanne Altmann, Stuart A Altmann, Glenn Hausfater, and Sue Ann McCuskey. Life history of yellow baboons: physical development, reproductive parameters, and infant mortality. *Primates*, 18(2):315–330, 1977.

[42] Naofumi Nakagawa, Hideyuki Ohsawa, and Yasuyuki Muroyama. Life-history parameters of a wild group of west african patas monkeys (erythrocebus patas patas). *Primates*, 44(3):281–90, Jul 2003.

[43] Lynne A Isbell, Truman P Young, Karin Enstam Jaffe, Anne A Carlson, and Rebecca L Chancellor. Demography and life histories of sympatric patas monkeys, erythrocebus patas, and vervets, cercopithecus aethiops, in laikipia, kenya. *Int J Primatol*, 30(1):103–124, Feb 2009.

[44] Anna J Jasinska, Christopher A Schmitt, Rita M Cantor, Ken Dewar, James D Jentsch, Jay R Kaplan, Trudy R Turner, Wesley C Warren, George M Weinstock, Roger P Woods, et al. Systems biology of the vervet monkey. *ILAR Journal*, 54(2):122–143, 2013.

[45] Duksha Ramful, Sylvain Sampériz, Xavier Fritel, Alain Michault, Marie-Christine Jaffar-Bandjee, Olivier Rollot, Brahim Boumahni, and Patrick Gérardin. Antibody kinetics in infants exposed to chikungunya virus infection during pregnancy reveals absence of congenital infection. *Journal of Infectious Diseases*, page jit814, 2013.

[46] Veerachai Watanaveeradej, Timothy P Endy, Sriluck Simasathien, Angkool Kerdpanich, Napuschon Polprasert, Chanchai Aree, David W Vaughn, and Ananda Nisalak. Transplacental chikungunya virus antibody kinetics, thailand. *Emerging infectious diseases*, 12(11), 2006.

[47] Diawo Diallo, Rubing Chen, Cheikh T Diagne, Yamar Ba, Ibrahima Dia, Amadou A Sall, Scott C Weaver, and Mawlouth Diallo. Bloodfeeding patterns of sylvatic arbovirus vectors in southeastern senegal. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 107(3):200–203, 2013.

[48] SD Bedekar and KM Pavri. Studies with chikungunya virus. i. susceptibility of birds and small mammals. *The Indian journal of medical research*, 57(7):1181–1192, 1969.

[49] Scott C Weaver and William K Reisen. Present and future arboviral threats. *Antiviral research*, 85(2):328–345, 2010.

[50] Konstantin A Tsetsarkin, Rubing Chen, Michael B Sherman, and Scott C Weaver. Chikungunya virus: evolution and genetic determinants of emergence. *Current opinion in virology*, 1(4):310–317, 2011.