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Short Communication

Yellow fever virus susceptibility of two mosquito vectors from Kenya, East Africa

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ABSTRACT

Yellow fever is an unpredictable disease of increasing epidemic threat in East Africa. *Aedes (Stegomyia) aegypti* has never been implicated as a vector in this region and recent outbreaks have involved a newly emerging virus genotype (East African). To better understand the increasing epidemic risk of yellow fever in East Africa, this study is the first to investigate the vector competence for an emerging East African virus genotype in Kenyan *A. aegypti sensu latu (s.l.)* and *A. (Stegomyia) simpsoni s.l.* mosquito species. Using first filial generation mosquitoes and a low passage yellow fever virus, this study demonstrated that although *A. aegypti s.l.* is a competent vector, *A. simpsoni s.l.* is likely a more efficient vector.

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1. Introduction

In East Africa, yellow fever remains a disease of increasing epidemic risk. The most recent yellow fever outbreak in the region was reported by the WHO in late 2010 and included the first human cases reported in Uganda in almost 50 years.¹ Prior to this, outbreaks occurred in Sudan (2003 and 2005) and were the first reports of yellow fever from that country in approximately 50 years.¹ These events were preceded by the first outbreak ever reported in Kenya (1992–1993), which were the first reported human cases in East Africa for close to 25 years.¹

The most recent outbreaks in Kenya and Sudan involved the re-emerging East African genotype of yellow fever virus that had been undetected for over 40 years.¹ To date, the most important vector of epidemic yellow fever virus in East Africa is *Aedes simpsoni sensu latu (s.l.)* (likely *A. bromeliae*), which was the principal vector during the largest recorded epidemic of yellow fever worldwide, in Ethiopia, from 1960 to 1962.¹ Urban epidemics of yellow fever virus, vectored by *A. aegypti s.l.*, have been completely absent in East Africa despite the abundance of this species, strong anthropophilic behavior and laboratory evidence suggesting specimens from East Africa to be among the most yellow fever competent species worldwide.²

To better understand the increasing epidemic risk of yellow fever in East Africa, this study is the first to investigate the vector competence for an emerging East African yellow fever virus genotype in Kenyan *A. aegypti s.l.* and *A. simpsoni s.l.* mosquito species.

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Table 1
Yellow fever susceptibility in female mosquitoes according to species and location

Species	Location	Infected/total (%)	χ^2	df	Disseminated/total (%)	χ^2	df	Disseminated/infected (%)	χ^2	df
<i>Aedes (Stegomyia) aegypti sensu latu (s.l.)</i>	Nairobi	5/75 (7)	8.64 ^a	2	0/75 (0)	–	–	0/75 (0)	–	–
	Mariakani	31/75 (41)	0.93	2	14/75 (19)	0.6	2	14/31 (45)	0.87	2
	Kerio Valley	8/75 (11)	9.58 ^a	3	3/75 (4)	5.79	3	3/8 (38)	–	–
	Kakamega	19/75 (25)	3.52	2	8/75 (11)	4.48	2	8/19 (42)	1.42	2
Total	63/300 (21)	47.79 ^a	12	25/300 (8)	30.27 ^a	12	25/63 (40)	–	–	
<i>Aedes (Stegomyia) simpsoni s.l.</i>	Nairobi	10/21 (48)	3.23	1	6/21 (29)	0.58	1	6/10 (60)	0.15	1
	Mariakani	2/3 (67)	–	–	0/3 (0)	–	–	0/2 (0)	–	–
	Kerio Valley	51/85 (60)	10.40 ^a	3	46/85 (54)	17.00 ^a	3	46/51 (90)	4.05	3
	Kakamega	1/11 (9)	–	–	1/11 (9)	–	–	1/1 (100)	–	–
Total	64/120 (53)	23.92 ^a	7	52/120 (43)	30.94 ^a	7	52/64 (81)	6.44	7	

Infected: presence of virus in abdomen; disseminated: presence of virus in head tissue.

^a $p < 0.05$.

2. Materials and methods

Human-landing *A. aegypti s.l.* and *A. simpsoni s.l.* were collected from domestic/peridomestic biotopes at four locations in Kenya: Nairobi, Rabai, Kerio Valley and Kakamega (January to October 2004).³ An average of 20 human-landing female mosquitoes were collected and reared from each site, except in Kakamega where only three adult female *A. simpsoni s.l.* (likely *A. bromeliae*) mosquitoes were collected. For convention, and because of inconsistencies in morphological characteristics, the specimens studied here are referred to as *A. aegypti s.l.* and *A. simpsoni s.l.*^{3,4} For additional information regarding mosquito collections and identification refer to Ellis et al., 2007.³

Vector competence experiments were performed using first filial (F1) generation mosquitoes derived from the pooled eggs obtained from human-landing collections. Batches of 50–100 female mosquitoes, aged 5–7 days, were fed a 1:2 mixture of yellow fever virus (6.7–7.5 log₁₀PFU/ml) and defibrinated sheep blood. The yellow fever virus East African genotype was isolated in suckling mouse brain from human serum (Sudan 2003). The virus was passaged a second time in suckling mouse brain to obtain sufficient quantities. Fully engorged mosquitoes were held for 14 days at 27 °C, 80% relative humidity and 12-h photoperiod after which the head and abdomen were separated at the thorax and homogenized separately. Homogenates were inoculated into LLC-MK2 cell culture and following 10 days of incubation viral infection was assayed using an indirect fluorescent antibody test (IFA) as previously described.⁵ The χ^2 and contingency table statistical tests were performed to detail heterogeneity within/between locations and to calculate odds ratios (see Table 1).

3. Results and Discussion

This is the first study to revisit the competence of important yellow fever virus vectors in East Africa using locally derived mosquitoes and a geographically relevant yellow fever virus genotype of emerging epidemiological importance. Results indicate significant geographical variability in the yellow fever virus susceptibility of both mosquito

species (Table 1). Significant differences in yellow fever virus dissemination rates were observed between the four sites tested (χ^2 30.27) and within Nairobi (χ^2 9.58) for *A. aegypti s.l.* This observation concurs with a previous report using a long-passaged yellow fever virus Asibi strain and *A. aegypti* from East Africa *s.l.*²

We hypothesized that *A. simpsoni s.l.* would prove competent, because of the documented importance of this vector in the natural history of yellow fever in the region, and anticipated highlighting the threat of urban yellow fever emergence with comparable *A. aegypti* vector competence rates.¹ However, the overall yellow fever virus dissemination rate in *A. simpsoni s.l.* averaged five times greater (OR 5.2, 95% CI 3.1–8.8; $p < 0.001$) and the finding of a completely refractory *A. aegypti s.l.* population in Nairobi is noteworthy. Interestingly, the infection and dissemination rates of *A. aegypti s.l.* reported here (21% and 8%, respectively) are strikingly similar to a study of *A. aegypti* from West Africa that used a local yellow fever virus isolate (infection and dissemination rates were 26% and 7%, respectively), of which this relatively low vector competence was sufficient to support a large epidemic.⁶

Interpreting the epidemiological significance of these results is not without limitations. A variety of factors may be involved in transmission, including mosquito behavior, vector abundance and proximity to susceptible human populations.³ Extrapolating laboratory results to field populations also has limitations and the within-population genetic diversity or sub-species structure was not studied. Finally, the presence of the virus was only detected in the head tissue of mosquitoes and did not include quantification of virus shed in saliva.

One of the greatest public health concerns related to yellow fever is that it will become efficiently transmitted in urban environments. For this to occur in East Africa, it is likely that *A. aegypti s.l.* will have to be involved in urban epidemic transmission. This study demonstrates that *A. aegypti s.l.* is mostly a competent vector for the recently emerging yellow fever virus genotype in East Africa, although relatively poor in comparison to *A. simpsoni s.l.* A number of interesting questions remain about the natural history of yellow fever, *A. aegypti s.l.* in East Africa

and the potential for future epidemic transmission in the region.^{1,2,4} However, without renewed research the only certainty (without a vaccine-protected population) is that yellow fever will continue to emerge unpredictably and remain an important public health threat in East Africa.

Authors' contributions: BRE, RCS and DMW were involved in the study design; BRE and RCS were responsible for field work; BRE, KMH and SH were responsible for design and carrying out immunofluorescent assay protocols; BRE drafted the manuscript; RCS, DMW, KMH, and SH critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. BRE is guarantor of the paper.

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Competing interests: None declared.

Ethical approval: This study (SSC No. 803) was approved by the Kenya Medical Research Institute (KEMRI)/National Ethical Review committee.

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