Dispatch

Occurrence of the Asian malaria-vector *Anopheles stephensi* in Eastern Sudan

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Abstract:

Anopheles stephensi is an important Asian malaria vector which has recently become established in Djibouti and Ethiopia, and represents a significant threat to malaria control in East Africa. Here, we report occurence of *An. stephensi* in two non-contiguous states of Eastern Sudan, with phylogenetic analysis suggesting the possibility of distinct invasions. Genomic analysis revealed xxxxxxxxxxxx Our findings highlight the utility of enhanced genomic surveillance for

Malaria is a major global public health threat, particularly in the Sub-Saharan countries whereas malaria control programs rely heavily on the vector control tools to reduce malaria burden, vector control strategy has contributed significantly in the global reduction of the relevant mortality and morbidity (1). Despite this global reduction, 228 million malaria cases were reported in 2018 and 93% from Africa (2).

In Sudan, malaria is a serious public health risk with about 2 million malaria cases and 5,000 deaths reported in 2018, and the total country population lives at risk of malaria (2). Additionally, the report showed that malaria vectors composition in Sudan includes *Anopheles arabiensis, An. funestus, An. gambiae, An. nili,* and *An. pharoensis,* with *An. arabiensis* as the major vector (2,3).

Invasive disease vectors are posing a serious risk that challenges the vector control programs globally (4). Therefore, early detection and control is the best strategy to avoid the establishment of a new competent vector locally (4), otherwise, the risk of disease emergence or re-emergence is highly potential (5). *Anopheles stephensi*, also known as the Asian malaria vector, is increasingly expanding its geographical distribution (6), particularly in Africa, where it was recently reported from Ethiopia (7) and Djibouti (8). In this communication, we report the first identification of *An. stephensi* in Sudan and discuss the potential complication of this emergence on malaria transmission.

The Study

Between September 2016 and February 2018 we collected mosquito larvae (*Anopheles sp*) from twelve sites around Sudan. Sites were selected to have a full representation of urban and peri-urban sites throughout the country. Larvae were collected and reared to adults, then, morphologically identified to species level and a well-established molecular PCR protocol was

used to confirm the species identification. However, 149 mosquito samples failed to amplify using several modifications of the protocols for *gambiae* and *funestus* complexes. Then the mitochondrial cytochrome c oxidase 1 (CO1) primers designed by Folmer et al. were used to amplify these samples and sequencing them (9). To identify our samples, we blasted the obtained sequences against reference sequences of *Anopheles* vectors in the database (VectorBase).

We have sequenced samples from six study sites distributed across four states (Red Sea, El-Gadarif, Sennar, and River Nile states). Our sequence analysis showed that out of the total 149 sequenced *Anopheles* mosquito samples, 67% (86/130) of the samples from the Red Sea sites, 100% (36/36) from Port Sudan, 54% (50/94) from Tokar samples were *An. stephensi*. Among the samples from El-Gadarif state sites, a single sample out of three from Abu Alnaja and a single sample out of five from Daim Bakur was *An. stephensi* too (Fig. 1). No *An. stephensi* was detected from both Sennar and River Nile states.

Phylogenetic analysis of our sequences showed that samples from Gadarif are clustering with Djibouti, Indian, and the Ethiopian 2019 H3 sequences of *An. stephensi*, while samples from the Red Sea state were clustering with Ethiopian populations of *An. stephensi* (7,8).

The emergence of *An. stephensi* in Sudan is of serious epidemiological importance to malaria control and elimination in Sudan and alarming to the neighbouring countries in East and North Africa particularly areas potential for urban malaria transmission (10). This risk grows greater considering that local surveillance systems in Africa, as well as the currently available knowledge and expertise, are mainly focused on the vectors member of the predominant *gambiae* and *funestus* complexes (10). Particularly that members of these complexes are rural species in contrast to the *An. stephensi* which is mainly urban vector, this might require a major change in the control measures and/or delivering activities to target the invasive species as well as the endemic vectors (10,3).

An. stephensi might have contributed to the current resurgence of malaria in the area because it is unknown when this vector was firstly introduced in the region (10). The ability of *An. stephensi* mosquitoes to efficiently transmit both *Plasmodium falciparum* and *P. vivax,* the rapid expansion of the geographical distribution, and adaptability to getting established in new environments is alarming for malaria control and eradication programs worldwide (5,8,7). Previous studies warned from the risk of malaria epidemics in densely populated African cities like Khartoum in Sudan because of *An. stephensi* invasion (10).

Conclusions:

Here we report the emergence of *An. stephensi* in Sudan. This finding is of great public health concern for Sudan, because of the risk of malaria pandemic if this competent Asian malaria vector spread throughout the country. Further systematic investigations are needed to detect the current distribution of this vector, determine its role in the local malaria transmission, and monitoring its susceptibility to the locally available control measures.

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Disclaimers:

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

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