Pending Questions from the

MESA FORUM: **Responding to the threat of *Anopheles stephensi* invasion in Africa**

Questions for Fitsum G. Tadesse

1. **Which ecological form of *An. stephensi* is spreading in African countries.**

   The evidence we have so far from the region is limited to morphological and molecular tests that are not essentially capable of differentiating the three forms of *An. stephensi.* Egg ridge counts are suggested in literature but these are overlapping (egg ridge counts for the three forms) and make the identification difficult. We will need improved tools.

2. **What is the transmission capacity of the *An. stephensi* in Africa.**

   A proxy study (direct membrane feeding assays) using fresh patient's blood fed to adult *An. stephensi* raised from field caught immature stages highlighted that *An. stephensi* strongly supports sporogony of local parasite isolates even higher than native *An. arabiensis,* as proved at the oocyst and sporozoite stage, in Ethiopia. Further evidence from wild caught adult mosquitoes from a couple of studies detected *Plasmodium falciparum* and*Plasmodium vivax* infections in *An. stephensi*.

Questions for Seth Irish

1. **What means of transport support the invasion of this species? Is there any record of *An. stephensi* in boats, ships?**

   We don't yet know. As of yet we don't have any findings of *An. stephensi* in ships or other means of transport. However, many of the initial findings of *An. stephensi* were in port cities (Bosaso, Port Sudan, Djibouti) which may indicate that shipping is involved in its spread, but we still need more information.

2. **What should I do as a country in preparation for mounting surveillance for *An. stephensi* in highly endemic and pre-elimination Countries?**

   Several of us recently published a paper providing some guidance (https://www.sciencedirect.com/science/article/abs/pii/S0001706X22003631?via%3Dihub). There is also guidance for surveillance in the recently updated vector alert (https://www.who.int/publications/i/item/9789240067714).

3. **What are the structural and molecular distinguishing features of both adult and larva forms of *An. stephensi* from other malaria vectors. How do hidden morphological and genomic traits between *An. stephensi* and *An. gambiae* differ? Particularly overlapping morphological traits.**

   The key structural differences that identify *An. stephensi* are shown in the most recent identification key for African Anopheles (https://malariajournal.biomedcentral.com/articles/10.1186/s12936-020-3144-9), and are shown in the original version of the vector alert (https://www.who.int/publications/i/item/WHO-HTM-GMP-2019.09). There is not yet a good identification key for separating *An. stephensi* larvae from other African species of Anopheles. Sequencing ITS2/CO1 can allow for identification of the species (from larval or adult specimens).

4. **Are there other species we need to track?**

   It is important to identify mosquitoes collected during routine entomological surveys as much as possible. Additional sequencing (routine or of unamplified specimens) can assist in this.

5. **Is there a silver bullet against the *An. stephensi*? Is there any change in the vector control intervention? What are the strategies to control the spread of *An. stephensi*, and reduce vector population in sites with *An. stephensi*?**

   We do not yet have a silver bullet and don't understand the optimal vector control tools to control *An. stephensi*. In India and elsewhere, larviciding, larval source management, and larvivorous fish have been used. Further studies are needed to properly evaluate these and other methods.

6. **Present/future partnerships to contain spread and the collateral damage with the parasites especially *P. vivax.***

   Collaboration will be key to contain the spread.

7. **What are the pros and cons of IRS, ITNs, space spraying & larvicides.**

   There are limited high quality studies on these tools for the control of *An. stephensi*, so further work is needed.

Questions for Noble Surendran

1. **As *An. stephens*i is zoophilic, does it pose a greater risk than native zoophilic vectors such as arabiensis or gambiae.**

   It is difficult to say without any substantial data on feeding behavior and vectorial capacity of local vectors including invasive *An. stephensi* of a particular locality. We have seen the emergence of minor vectors as major malaria vectors under certain environmental changes. Considering the fact that *An. stephensi* can transmit malaria, presence of *An. stephensi* can pose a risk of malaria transmission in addition to the presence of other local vectors.

2. **What are the potential entomological sampling tools that could be used for trapping *An. stephensi* adults?**

   Adults can generally be collected through light-trap or pyrethrum spray collections or using animal baits such as cattle baited traps.

Questions for Ashwani Kumar

1. **What is the relative importance of the rural form of *An. stephensi* vs. the urban form.**

   The rural form of *An. stephensi* is known as *An. stephensi* mysorensis and is known to be primarily zoophagic and is a non vector so far in India.

2. **Is *An. stephensi* biting behaviour nocturnal as most Anopheles mosquitoes, or is it a mosquito species that can bite during the day as well?**

   It is a nocturnal species and its peak biting time is around midnight followed by a smaller peak around 4AM, although the active biting period of the species ranges from 6 PM - 6AM in India.

3. **Why not apply wide area suppression of vectors by employing aircraft to eliminate malaria.**

   Since *An. stephensi* breeds in man-made habitats e.g. overhead tanks, sump tanks, wells, tyres, curing waters in the construction sites, masonry tanks, barrels, etc. etc.

which can be either indoors or very close to human inhabitations, therefore, the use of Aircraft for larviciding may not be suitable in suppressing the vector populations.

4. **What lessons learned from India on urban planning, building regulations, and other environmental management can be applied?**

   Building by-laws, legislative measures in India include, mosquito-proof lid design of overhead tanks and sump tanks, fine imposed when breeding checkers/Field workers detect vector breeding in construction sites, wells, barrels etc. In metropolitan cities where high rise buildings have very large overhead water tanks, multiple lids are recommended and also an iron ladder is recommended for alighting on the roof of the tank and checking for mosquito breeding. Construction workers' registration with local health authorities and their routine blood testing for malaria. They may be issued a malaria card. The best examples are Bombay Municipal Act and Goa Public Health Act in India.

5. **What preventive tools are most efficient against this vector?**

   Mosquito-proofing of Overhead tanks, Sump tanks, introduction of larvivorous fishes in the wells and ornamental fountains (@5 fish/sq met surface area) in the gardens and parks, and also freshwater drains when present; application of larvicides/ bio-larvicides on curing waters (fortnightly), use of LLINs in migrant populations/workers engaged in the construction activities to prevent man-mosquito contact, are the main preventive measures suitable against *An. stephensi* and the transmission of malaria by this species.

6. **Is *An. stephens*i able to survive in a natural stream and in sewage as compared to other species of Anopheles?**

   This species usually prefers fresh/cleaner waters in man-made habitats and does not breed in natural streams and in the sewage water.

Question for Gudissa Assefa

1. **Is there support for health promotion officers to reach the community with information on *An. stephensi*, through stakeholders meetings, radio?**

   Yes, there is support for health promotion officers to deliver key messages of *An. stephensi* to reach the community. Advocacy and Communication/SBCC is a cross-cutting intervention boldly indicated in our (Ethiopia’s) actionable plan for the integrated surveillance and control of *An. stephensi* and *Aedes aegypti*. A stakeholders meeting was also held on a scientific forum that took place in 2022 along with the world malaria day celebration. Advocacy and launching of larval source management was done in the presence of Dire Dawa city mayor and top leadership representatives. In addition, *An. stephensi* SBC guide was also developed to support and guide health promotion officers including health extension workers on how to address the target audience especially where this vector is detected and deliver key messages. Radio and other channels were not used at this moment.

Questions for Sarah Zohdy

1. **What could be the climate factors which would enhance the spread of *An. stephensi* in sub-Saharan African countries?**

   Anopheles stephensi has the ability to thrive in both artificial and natural habitats and has the ability to transmit human *Plasmodium* parasites across a wider thermal range than endemic African vector, *An. gambiae* (Villena et al. 2022). By using containers as larval habitats, *An. stephensi* populations can persist throughout dry periods and due to its thermal tolerance can also thrive in more arid environments than most typical African malaria vectors.
   A recent modeling study revealed that seasonal malaria interventions, such as IRS and SMC may not be ideal for *An. stephensi*, because of its lack of population association with rainfall patterns like other malaria vectors.
   Climate shifts towards longer dry seasons may be conducive environments for *An. stephensi* to thrive where other malaria vectors do not.
   Extreme weather events and shifts in rainfall driven by climate change may also create an increase in artificial and natural larval habitats for *An. stephensi,* allowing populations to grow.

2. **What are future molecular diagnostic approaches?**

   Currently confirmation of *An. stephensi* requires Sanger sequencing which can be limiting for labs without sequencing capacity. However, recently a publication describing a conventional PCR assay as a first molecular line of detection for *An. stephensi* was published. This method can be used to initially see if a specimen is *An. stephensi* although sequencing will still be required for full confirmation and reporting to WHO.
   Morphological identification can be conducted according to Coetzee et al. 2020, ideally with reference specimens to compare to although again, this requires sequencing confirmation.
   With new detections being reported in new locations, there may be a shift in molecular entomology towards not only sequence confirmation of the species but population genomics approaches to determine where populations may have originated from, how long they have been there, and where they may be moving to. As more genetics data become available a more complete picture of the spread of *An. stephensi* can be established.

3. **Present/future partnerships to contain spread and the collateral damage with the parasites especially *P. vivax.***

   This can take shape at various levels. Higher level global and donor coordination, national level multisectoral collaboration, and in-country coordination is needed to ensure that epidemiologists and entomologists review data and plan response strategies together. As the vector spreads these partnerships are also essential to ensure there are no missed learning opportunities that can be shared. Coordination of local government/community level engagement is also critical in sustaining efforts to mitigate the impacts on disease. The elephant in the room is that there are not sufficient resources to tackle this new threat alone. National and local government and community engagement, to leverage efforts will be critical to this response.

General Questions

1. **What are available trainings in morphological identification and molecular characterisation of *An. stephensi*?**

   Seth: There are occasional trainings available for morphological identification that are offered by different partners (WHO, PMI VectorLink, etc). There may also be training on molecular work, although this would not be specific to *An. stephensi*. For additional information, inquiries can be sent to vectorsurveillance@who.int

   Noble: There are standard morphology-based identification keys available. Medical Entomology Laboratories in many countries where *An. stephensi* is present may offer training on morphology-based identification. However molecular tools are more reliable than morphology-based identification as one may lose certain morphological traits during handling adults. Following articles are useful for early detection of *An. stephensi* (https://wwwnc.cdc.gov/eid/article/29/1/22-0786_article)

   Ashwani: There are morphological keys available for the identification of the species (Christophers 1933, Fauna of British India: Diptera Family Culicidae Tribe Anophelini, vol. IV. London: Taylor & Francis). There are publications available on molecular identification of *An. stephensi* (Singh et al, Molecular tools for early detection of invasive malaria vector *Anopheles stephensi* mosquitoes. EID Vol. 29, Number 1-January 2023).

   Sarah: Trainings for morphological identification of *An. stephensi* for public health entomologists and National Malaria Programs have been held by the Pan African Mosquito Control Association (PAMCA), West African Aedes Surveillance Network (WAASuN), Horn of Africa Network for Monitoring Antimalarial Treatment (HANMAT), WHO EMRO, and PMI VectorLink Ethiopia with support from the US Centers for Disease Control and Prevention (CDC) and US President's Malaria Initiative (PMI).
   Pinned reference specimens may be made available for trainings upon request from CDC and other institutions. PMI VectorLink Ethiopia (soon to be PMI Evolve Ethiopia) will be holding a training in June 2023 which is open to interested participants from malaria programs. While travel costs will not be covered, the training will be no-cost and materials made available for participants. While space will be limited, interested participants can email for more information.
   As noted above, Coetzee et al. 2020 can be used as a morphological key, this image developed by, and pinned specimens can be used for morphological identifications.
   Molecular characterization should include sequencing confirmation but these papers (Singh et al. 2023; Carter et al. 2019 include relevant methods that laboratory teams can use at this point in time.

2. **How can the common man join in this sensitisation?**

Fitsum: The fight against the vector requires multisectoral engagement. Included in this would be major contributions from the community. This requires strategic community engagement.

Seth: Larval source management is a first step that can be taken, to ensure that there is no stagnant water in the household. Other community-based programs may come into play, such as community based larviciding.

Noble: Public awareness and participation in vector control is essential. Locals should be educated in lay terms highlighting their role in vector control by eliminating possible development sites of immature forms. Public should be part of any vector control programme to sustain it.

Ashwani: The common man can join hands with the local health authorities in dissemination of information in the neighbourhood and in the educational institutions on the threat posed by this invasive species to human health, bio-ecology, prevention measures to be taken at home and in the surroundings and also extending cooperation during the vector and disease surveillance when undertaken by the health authorities.

Sarah: Community engagement can be a strong pillar in the fight against *An. stephensi* and there are numerous forms this can take.
A social and behaviour change guidance document for households and communities was recently released here and could be used as a starting point. Additionally, community engagement is critical in launching and sustaining larval source management (LSM) activities so there are opportunities for community members to participate in and lead efforts to source-reduce larval habitats in their communities. Information sharing with communities is also a way to create vigilance and awareness around *An. stephensi* to support its mitigation and stop its spread.
Some researchers have encouraged citizen scientist efforts as a potential complementary way to identify new *An. stephensi* locations or as a starting point to guide conventional larval surveillance approaches. For example, the NASA Globe Observer mobile phone app contains a Mosquito Habitat Mapper application. This app, developed for *Aedes* vector surveillance, allows citizen scientists to map larval habitats by capturing GPS coordinates and document the habitats through photos. It also walks citizen scientists through a simple process in which they can report whether the habitat was source reduced or not. One interesting feature is also the ability for citizen scientists to use small clip-on phone lenses to capture photos of larvae. All of this information is then made openly available and public health entomologists can quickly sift through a map of larval habitats to see what kinds of habitats exist in a certain location and whether or not *Anopheles* (or other vectors) are being found in them. Other citizen science phone apps also exist to track mosquitoes and scientists from the University of South Florida recently put all of the mosquito detection data from those citizen science apps (iNaturalist, Globe Observer, Mosquito Alert, etc.) into a global dashboard for citizen science mosquito observations.