Anopheles stephensi Deep Dive synthesis report

1. Introduction

The *Anopheles stephens*i is a mosquito of the genus Anopheles whose female is capable of transmitting the malaria parasites *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) to humans. It has been an efficient vector of malaria in parts of Asia and the Arabian Peninsula (ref). Within the past decade, *An. stephensi* has greatly expanded its geographical range. Its invasion of the Horn of Africa (HOA) was initially detected in Djibouti in 2012 following an epidemic of malaria (ref). Djibouti which was in its malaria pre-elimination phase had not recorded any local transmission since 1999 until the unusual outbreak in an urban area. Subsequently, annual outbreaks of *P. falciparum* and *P. vivax* malaria are recorded with documented increase in intensity (ref). *An. stephensi* was identified in Ethiopia in 2016 and is now widely distributed in the Eastern areas (ref). According to a 2022 study, the annual *P. falciparum* malaria cases in Ethiopia are estimated to rise by 50% if measures are not put in place to fight the invasive species (ref). In 2019, the serendipitous detection of the vector was made in Sudan during a survey for other anopheline species. Subsequent surveys report an extensive spread which include regions bordering six countries assumed not to harbor the mosquito (ref). Amidst these discoveries in Africa, Sri Lanka, a World Health Organization (WHO) certified malaria free country located in Asia, detected the unexpected presence of *An. stephensi* in 2017 (ref). This happened during a series of entomological investigations and highlighted the threat of re-establishment of transmission within the country. With concerns on the further spread of the vector, the WHO issued a vector alert on *An. stephensi* in 2019, warning Sri Lanka and African countries in and around the HOA to upgrade their vector surveillance (ref). Since then, sampling activities carried out in 2020 have detected *An. stephensi* in Somalia (HOA) and much further away in Nigeria, West Africa (ref). More recent entomological surveys carried out in Yemen in 2021 detected *An. stephensi* in the country for the first time (ref). Then in Dec 2022, Kenya announced the detection of *An. stephensi* following molecular surveillance (ref, ref). In a more recent discovery, Ghana reported the presence of *An. stephensi* (ref) after DNA sequencing on samples collected in 2022. This is the furthest West it has been detected so far on the African continent. Additionally, Eritrea has also recently identified *An. stephensi* larvae and adults through sampling conducted in 2022 (ref). The WHO, concerned by the spread, introduced an initiative in September 2022 to halt the further dispersion of *An. stephensi* in Africa (ref). This strategy emphasized a quintuple approach: augmenting collaboration, fortifying surveillance measures, enhancing information dissemination, formulating guidelines, and emphasizing research (ref). In January 2023, the WHO updated its 2019 vector alert, incorporating specific countermeasures against the *An. stephensi* spread in Africa and offered insights on surveillance, analysis, and reporting (ref).

Relevance

An. stephensi sets itself apart from other malaria vectors due to its ability to efficiently transmit malaria (*P. vivax* and *P. falciparum*) in urban areas. Furthermore, it is becoming increasingly evident that *An. stephensi* may be contributing to malaria transmission in Africa as well as playing a role in the spread of drug resistance and *pfhrp2/3* gene deletions (ref, ref). Thus, the invasion and establishment of *An. stephensi* poses a substantial threat to malaria control efforts, particularly in the rapidly urbanising Sub-Saharan African region. There is limited experience on surveillance, intervention and other aspects of controlling *An. stephensi* in

recently invaded areas. The rapidly evolving landscape of ongoing research, emerging hypotheses, and knowledge gaps suggests the imperative need to map *An. stephensi* research and investments to show activities being undertaken in order to facilitate the identification of best practices, research gaps and priority areas for funding to address knowledge gaps and encourage collaboration towards a coordinated effort to inform control.

We therefore carried out a Deep Dive (DD) exercise (landscaping review) to track *An. stephensi* research and investments. This DD is done in collaboration with the Roll Back Malaria Vector Control Working Group (RBM VCWG).

Objectives

1. Describe the geographic scale and scope of ongoing *An. stephensi* research and other projects.
2. Overview of the distribution of active *An. stephensi* surveillance or monitoring programmes.
3. Describe the funding sources for projects.
4. Document the list of questions under evaluation.
5. Identify knowledge gaps.

2. Methodology

The steps followed in the creation of a database of research on *An. stephensi* are outlined below.

Systematic data collection

- Systematic project searches were conducted in July 2022 and updated in July 2023. Information on *An. stephensi* projects were compiled in the following ways:

    - From *call for projects* targeting researchers and program managers with ongoing or recently completed projects or programmatic activities on *An. stephensi* to contribute to this active DD, by sharing their projects to be added to the MESA Track database of malaria projects.
    - From the WHO threat map by sourcing projects from the referenced publications of sites where *An. stephensi* has been detected.
    - Through a search of various research grant databases for projects and investments relating to *An. stephensi*.

- For each project, the information sought was the Project title; Project objective(s); Abstract and rationale; Start and end date; Project site; Principal investigator (PI); Principal institution; Funding institution; Partner institution; and Funding amount.

Eligibility criteria

The criteria used were:

- Projects/research related to *An. stephensi* in malaria
- Projects/research began/active ≥ 2012
- Projects in/translated to English.

Information verification

Once a project was identified and included in the MESA Track, the principal investigator was contacted via email. (S)He was given a link to the published project information in the MESA Track database of malaria projects and asked for additional information, if indicated, and verification. Projects for which we did not get a response to our verification request were still included in the DD.

Categorisation of projects

The projects identified as relevant for addition in the Deep Dive were categorised into different research areas in order to facilitate their analysis. The areas were chosen based on the research objectives the projects had in common.

3. Results

A total of 268 projects were identified. Of these, 68 were included for assessment in the DD because they fulfilled the eligibility criteria. This is an active DD and hence this number is liable to change over time as new projects on *An. stephensi* and related activities are included in the MESA Track database of malaria projects.

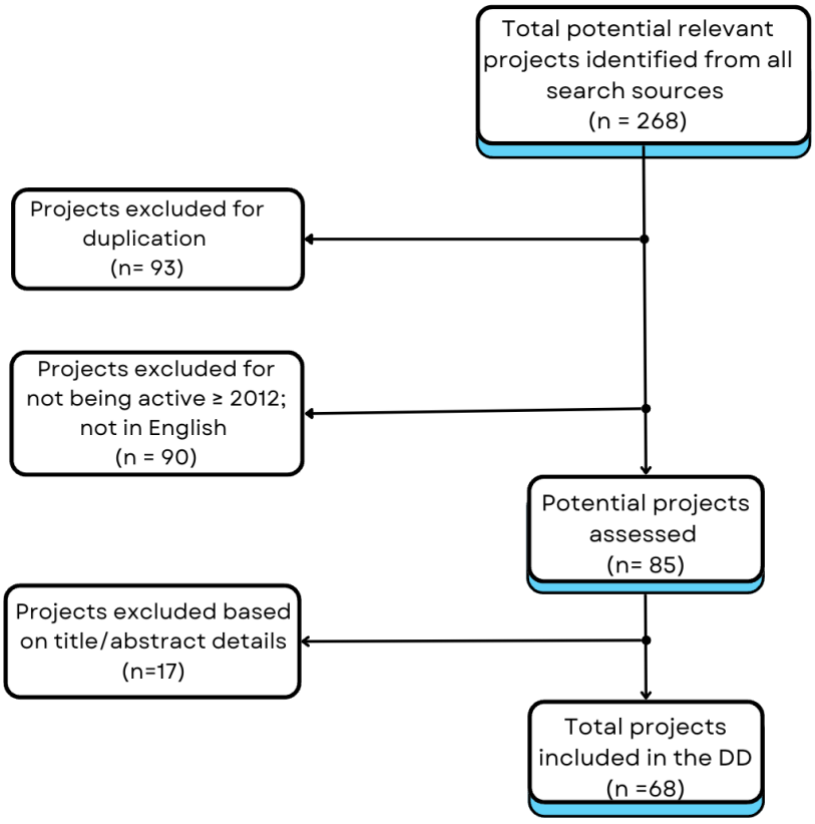

***Figure 1**: Project search flowchart*

Projects overview

The 68 projects identified as relevant are led by institutions in the USA, UK, Ethiopia, Japan, India, Australia, China, Djibouti, Italy, Kenya, Sri Lanka, Tanzania and Yemen.

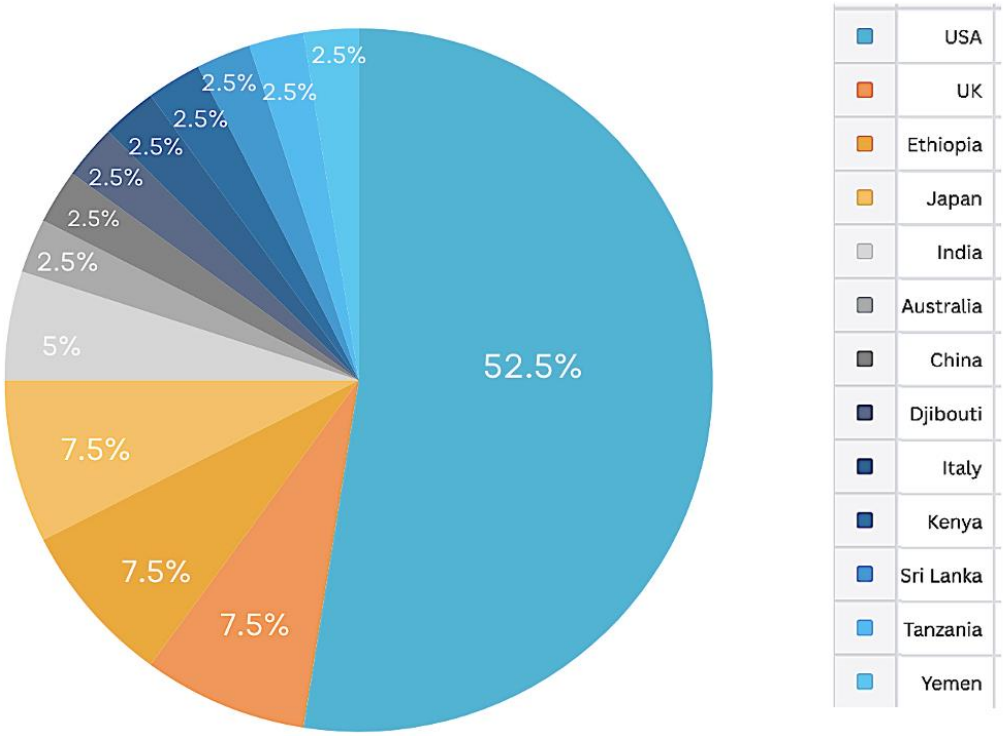

Figure 2: *Proportion: Lead principal institutions per country*

Of the above countries, Ethiopia, Kenya and Yemen have *An. stephensi* as an invasive malaria vector, while India is the only country which has *An. stephensi* as a native malaria vector.

Over half (52%) of the partners are research or academic institutions. The DD captured the involvement of the national malaria program in four countries (National Malaria Elimination Programme (NMEP) in Ethiopia, Division of National Malaria Programme (DNMP) in Kenya, Djibouti National Malaria Control Programme (PNLP) and National Malaria Control Programme (NMCP) in Yemen).

Active projects

There are 15 active and 53 completed projects. The active *An. stephensi* projects are ongoing in eight countries namely Djibouti, Ethiopia, Sudan, Tanzania, India, Sri Lanka, Mauritius, and the USA.

Three of these projects examine aspects of *An. stephensi* such as the role of cuticular hydrocarbons in aiding colonisation of new areas, the temperature-malaria transmission relationship, and the effectiveness of larvicide preparations.

Three others relate to the manufacture of a malaria vaccine.

Another three have aspects of genetic modification and aim to control *An. stephensi* population via the males which pass on a self-limiting gene that, when passed on, prevents their offspring from surviving to adulthood, to develop powerful gene drive tools that can be

used for the fast and reliable engineering of wild Anopheles populations, to limit parasite survival in the mosquito.

The remaining six projects have surveillance as part of their aim and are being executed in Ethiopia, Sudan, Tanzania, Mauritius, and Sri Lanka. These projects seek to:

- identify the route of invasion, current and potential distribution of *An. Stephensi*, estimate its importance for malaria transmission and evaluate multi-sectoral vector control strategies to combat its spread.
- improve entomological monitoring
- utilise artificial intelligence (AI) for automated species identification and spatiotemporal modelling.
- promote the development and use of entomological tools
- characterise the vector by analysis of the biotype, genotype, and entomological features.
- examine the arrival, establishment, and spread of *An. stephensi* in northern Sri Lanka and analyze this in relation to anthropogenic factors that favor its range expansion.

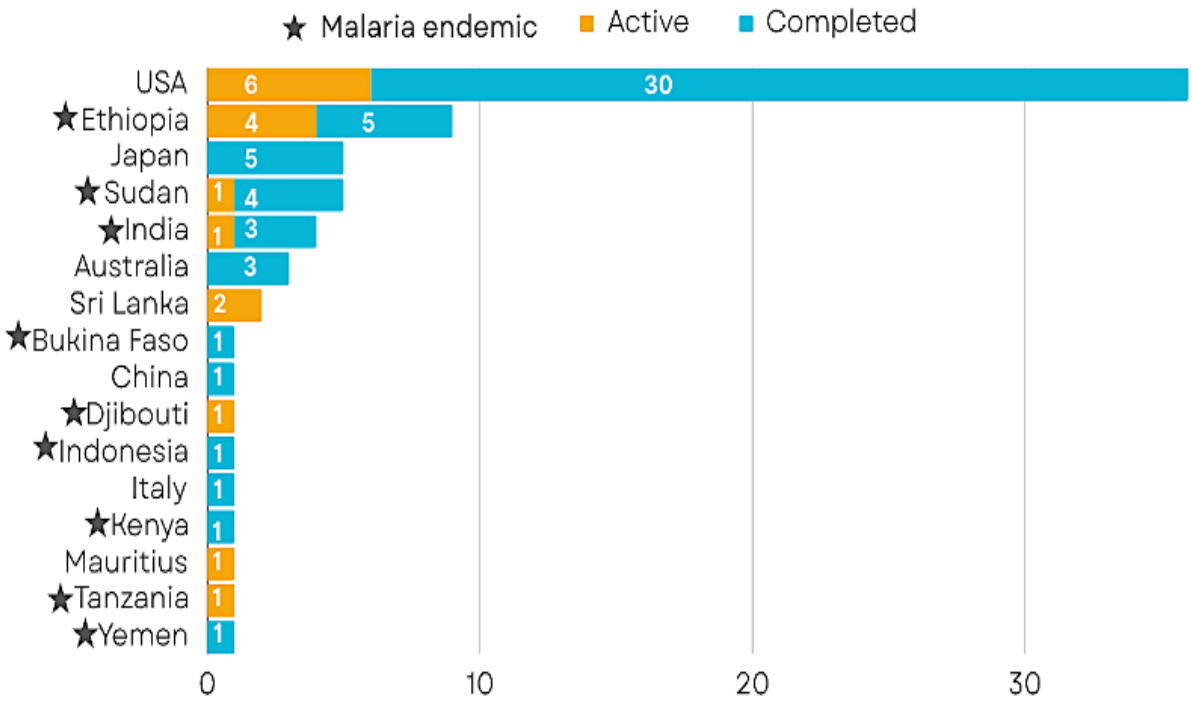

Figure 3: *Number of projects by country*

Category overview

The included projects were classified into eight different categories (liable to expand as more projects are added to the DD).

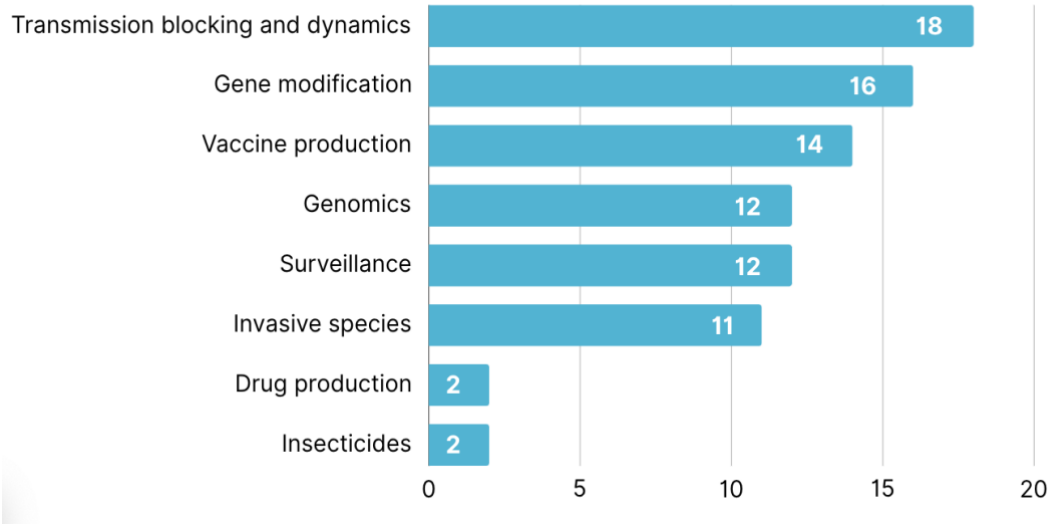

Figure 4: *Number of projects by category*

I. Transmission blocking and dynamics

Eighteen projects try to elucidate how certain environmental, mosquito and parasite factors such as temperature, bacterial infection, rare sugars ingestion, salivary gland secretion, midgut insulin signaling cascades (ISC), pantothenate kinase and parasite gametocyte infectivity affect *An. stephensi's* ability to transmit malaria parasites. The main outcome being to alter transmission intensity, internal physiological environment, fitness, immunity, and longevity of the vector.

II. Gene modification

Sixteen projects addressed modification of specific genes. Specifically, they examined and edited them to create highly fit transgenic mosquitoes. This included the modification of *Guy 1* for dosage compensation and/or sex-determination; leucine-rich repeat immune molecule for more intense mosquito Pf infections; single-chain antibodies (scFv) to disable midgut Pf; and midgut ISC to induce changes in mosquito Pf infection resistance, lifespan and reproduction. To achieve some of the modifications, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) and Maternal effect dominant embryonic arrest (Medea) gene drive technologies were employed. The ultimate goal of a majority of these projects was to create highly fit transgenic *Plasmodium* resistant mosquitoes with a few targeting mosquito population reductions.

III. Vaccine production

Fourteen projects deal with various research aspects in support of malaria vaccine production such as cost reduction of manufacture, and testing for safety and efficacy. These studies explore the use of attenuated-metabolically active *Plasmodium falciparum* (Pf) sporozoites (SPZ) (PFSPZ), genetically attenuated p52-/p36-/sap1-*Plasmodium falciparum* parasites (GAP3KO), radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS), nonattenuated sporozoites, and Anopheline anti-platelet protein (AAPP) for vaccine production. *An. stephensi* mosquitoes were used to either grow aseptic sporozoites, immunise volunteers, or infect volunteers with Pf during controlled human malaria infection trials.

IV. Genomics

Twelve projects were captured in this category. They revolve around basic biology, evolution, and genetic resources of *An. stephensi*. Some projects examine the structure and function of salivary glands and secretions, and their role in mosquito feeding. Others investigate signaling pathways both endogenous and interspecies (mosquito, parasite, and mammalian host) to understand physiological infection pathways. For the purpose of comprehending the impact on evolution, some studies characterized sex genes, proteomes and transcriptomes, while others map the genotype and biotype of novel and invasive *An. stephensi.*

V. Surveillance

The twelve projects in this category focus on identification, characterization, monitoring and control of *An. stephensi.* Two studies utilise information and communications technology to develop entomological surveillance planning tools for countywide control strategies, while another is based on automated species identification and spatiotemporal modelling using citizen observations. Some projects analysed the vector's genotype and biotype in order to establish presence, genetic diversity and assess its potential to transmit malaria.

VI. Invasive species

Eleven projects document the discovery of *An. stephensi* in new geographical areas, map its spread and attempt to elucidate factors responsible. Some factors examined include genotypes, biotypes, feeding preferences, sex communication, climatic adaptability, and habitat characteristics. In addition, the studies evaluate vector surveillance and multisectoral vector control strategies in the areas of spread.

VII. Drug production

Two studies relate to the development of oral antimalarials. One of these aims to develop a therapy using "bumped" kinase inhibitors to be taken in combination with artemetherlumefantrine and dihydroartemisinin-piperaquine. The other utilises the *Anopheles stephensi-Plasmodium yoelii* model to study the binding of recognition molecules to *Plasmodium* spp.

VIII. Insecticides

Two projects focus on *An. stephensi* larval control. One seeks to explore the efficacy and residual activity of three larvicide formulations. The other looks at the larvicide potential of solvent extracts of some plants.

Funding

The total amount of funding related to the investments captured in this DD is $89.0 M. The funding available for the active projects is $37.1 M. Projects that did not have a funding amount documented were 17 (25%). This is liable to change as we receive further information from the PIs. A review of the sources of funding and the distribution of these funds per project site and category is presented below.

● Funding sources

A total of 25 sources provided the $89.0 M documented in this DD. Fifteen (62%) of these funding sources were government institutions while nine were private institutions and one was a self-funded source.

The highest amount given by a single funding source was $53.0M. This was from the National Institute of Allergy and Infectious Diseases (NIAID), NIH to fund 31 projects and accounts for 60% of the total funding captured by this DD. The lowest was $3 K, however this project is self-funded by an individual. In addition, 10 funding sources did not provide the amount of money granted.

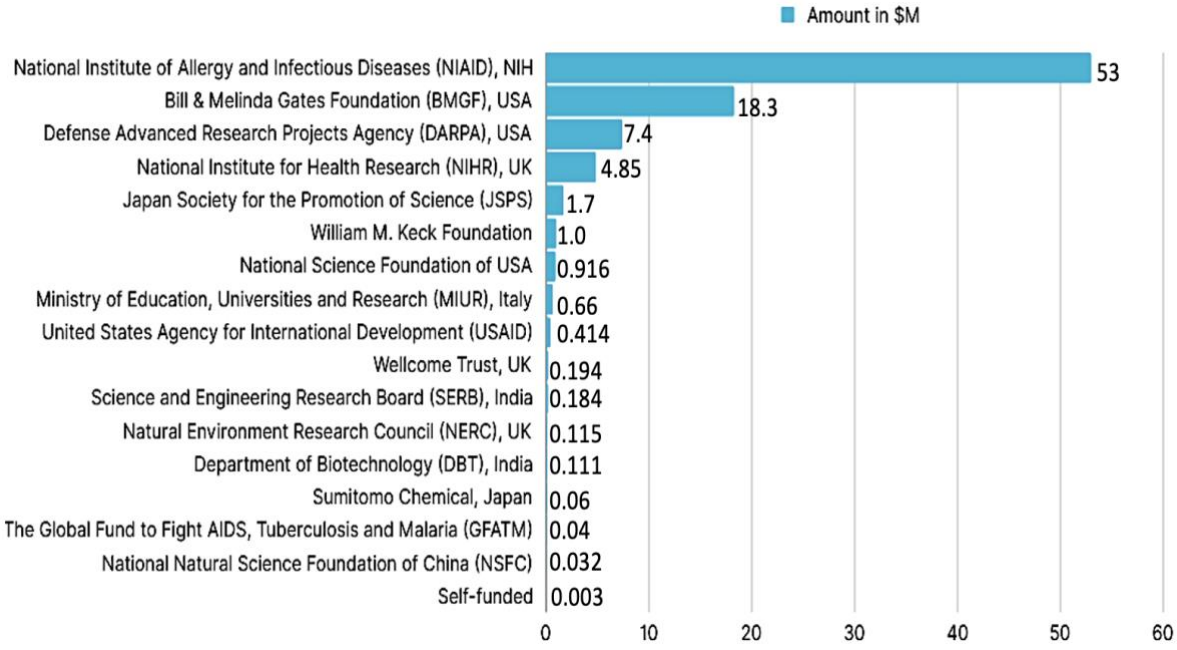

Figure 4: *Amount contributed per funding source (Organisations for which we did not have the project funding amount are not depicted)*

● Project Sites

The investments captured in this DD indicate that the USA is the project site which received the most funding, with $57.6 M for 36 investments (8 with undocumented funding amounts). This is followed by Djibouti with $18.3 M for one project. Sudan is next with $6.51 M for five projects. Closely following with nine projects is Ethiopia that received $6.36 M (3 projects with undocumented funding amounts). Burkina Faso follows with $2.77 M for its sole project and not far behind is Japan with $1.54M for five projects. The following sites received less than a million dollars for *An. stephensi* funding. Mauritius received $916K, India - $903K, Italy – 660K, Tanzania – 414K, Indonesia – 161K and China – 32K. Each of these countries had a single project, except India which had four. The remaining sites in Australia, Sri Lanka and Yemen did not state online the funding amounts in their three, two and one project respectively.

● Project Category

The amount of funds invested per category varies from $50 M for ‘Gene modification’ to 63K for ‘Insecticides’ depending largely on the total number of projects included

(See Table 1). However, sometimes a single project can receive a large grant as is the case of one project from the 'Gene modification' category which received $18.3 M.

Table 1: *Funding by category*

Category	Total Funding ($)	Total Projects
Gene modification	50.0 M	16
Vaccine production	22.8 M	14
Transmission blocking and dynamics	11.2 M	18
Genomics	7.35 M	12
Invasive species	7.03 M	11
Surveillance	6.75 M	12
Drug production	522 K	2
Insecticides	63.0 K	2

Urban Malaria

There is an overlap of six projects between the *An. stephensi* and Urban malaria Deep Dive. Three of these projects examine *An. stephensi* and urban malaria in Ethiopia. They focus on the epidemiologic and entomologic characteristics of malaria, *An. stephensi's* current and potential distribution, and ways to control the urban vector. Two projects carried out in India aim to determine the population genomics of *An. stephensi* and determine the thermal suitability for urban malaria transmission. The last project examines the anthropogenic factors responsible for *An. stephensi's* range of expansion into urban areas of Sri Lanka.

4. Discussion

The projects included in this DD cover a wide range of research areas and take place in both malaria endemic and non-endemic countries globally. Most of the research currently captured seeks to increase knowledge in the area of malaria transmission. There is a diverse array of funding sources, with government institutions and private organizations contributing significantly. However, the distribution of funds across different research categories varies. While "Gene modification" receives substantial funding, other areas such as "Insecticides" receive considerably less.

In the scarcity of funds, it is imperative to identify high priority knowledge gaps for a comprehensive response to the *An. stephensi* threat.

Potential gaps in knowledge and areas for further exploration include:

- **Surveillance and Monitoring:** There is a recognized need for intensified entomological surveillance, especially employing molecular techniques, in regions vulnerable to *An. stephensi* invasion. Such surveillance is critical for accurately

determining the presence, distribution, and expansion of this mosquito species as well as understanding how its presence is impacting malaria transmission.

- **Role in Drug- and Diagnosis-Resistant Malaria:** Evidence suggests *An. stephensi* plays a role in the spread of drug- and diagnosis-resistant malaria strains in Africa. Research on understanding the interactions or the co-occurrence of the threats is in its early stages.
- **Urban Malaria Dynamics:** Rapid urbanisation and associated construction activities are key factors in the establishment and proliferation of *An. stephensi* in urban settings. Such urban environmental changes are fostering habitats conducive to this mosquito species, thereby amplifying the risk of malaria in previously less-affected urban populations. This trend calls for a renewed focus on understanding and mitigating urban malaria dynamics, adapting existing control strategies to urban environments, and incorporating urban planning considerations into public health policies to combat the spread of *An. stephensi* and the associated increase in malaria transmission.
- **Climate Change Impact:** Predictive models are actively being developed to evaluate the environmental suitability of *An. stephensi* in varying climate scenarios. More of this research is crucial for anticipating the potential impact of climate change on the geographical distribution of this malaria vector and the associated risks of malaria transmission. By understanding these dynamics, strategies can be formulated to mitigate the potential escalation of malaria cases due to shifts in the vector's habitat driven by global climate change.
- **Control strategies:** Control strategies for *An. stephensi* are increasingly focused on the development and evaluation of targeted interventions. Gene modification and larvicides, being key strategies under exploration, require rigorous assessment to determine their effectiveness specifically against *An. stephensi*. These evaluations need to encompass not only immediate outcomes but also the long-term impact, scalability, and sustainability, ensuring that these methods are adaptable and effective across various settings. Additionally, research into Larval Source Management is crucial, targeting the reduction of larval populations. Exploring vector control opportunities, such as the cobreeding of *An. stephensi* with Aedes arbovirus vectors, offers potential for innovative and integrated control methods.
- **Social and behaviour change (SBC):** Research on social and behaviour change (SBC) related to *An. stephensi* is still in its nascent stages, particularly in the context of its recent invasion in Africa. Current studies contain limited exploration of the social and behavioural dimensions of *An. stephensi*. This gap highlights the need for more evidence-based guidance on individual, household, and community-level behaviours that could support the mitigation of *An. stephensi* and its impact on malaria transmission. Understanding how local populations perceive and react to the presence of *An. stephensi*, their knowledge about the vector, and their willingness to adopt preventive measures are crucial for developing effective SBC strategies. Such research is essential for tailoring interventions that can enhance community engagement and cooperation in controlling this emerging malaria threat.
- **Policy:** The current state of policy in *An. stephensi* research is characterized by several gaps. Firstly, there is a notable disconnect between research findings and the development of malaria policies and public health approaches, impeding the practical application of scientific knowledge to formulate effective strategies and policies. Secondly, policies are lacking in terms of adapting malaria control strategies to address the effects of climate change on vector distribution and behavior, which is

critical in the context of *An. stephensi's* spread and its impact on malaria transmission. Additionally, there is a need for policies that specifically address the dynamics of urban malaria, integrating urban planning with public health initiatives to effectively manage the challenges posed by *An. stephensi* in urban settings.

Addressing these knowledge gaps is crucial for developing and implementing effective strategies to combat *An. stephensi* and safeguard against malaria, particularly in urbanising regions and under changing climate conditions.

Main highlights

- There are 68 projects with $89.0 M total funding included in the DD.
- Up to 60% of total funding was contributed by the NIH.
- There are 15 active projects with $37.1M.
- It is necessary to track ongoing projects to improve exchange of information, know when new results will be obtained, boost partnership, aid research prioritisation, and work collectively across sectors in an integrated manner.
- The Ethiopian NMEP, Kenyan DNMP, Djibouti PNPL and NMCP Yemen were captured in this DD. It is important to involve local stakeholders in affected communities in order to tailor research and response to the local context. Funding to endemic country research institutions was low (34% of the total funding allocated to *An. stephensi* research and related activities as per this DD).
- Gaps in research were identified for surveillance and monitoring, *An. stephensi's* role in the spread of resistance and gene deletions, urban malaria dynamics, climate change impact, control strategies, SBC as well as policy for an integrated control.

5. Conclusion

Experts' opinion (name: **Dr. Corine Ngufor**, November 2022)

The invasion of Anopheles stephensi poses a major threat to malaria control in Africa. There are however major gaps in understanding how the vector spreads, transmits malaria, and how it can be effectively controlled. To better prioritise research efforts and limited funding and resources available, it is important to understand the current global research landscape around this vector species. MESA performed a landscaping review to track *An. stephensi* research and investments as part of an initiative by the Vector Control Working Group of the Roll Back Malaria Partnership to increase awareness and develop consensus towards addressing the invasion of *An. stephensi* on the African continent. The ensuing report highlights key areas of research that are currently covered, investments that have been made, what research is ongoing and in what areas of the world. The greater focus is on gene modification and vaccine production with less investments in surveillance and insecticide control. There is a need for more local research into more practical and community-focused strategies for controlling the vector. Funding in affected countries in the Horn of Africa is still limited and needs to be improved to provide a better understanding of the association between invasion of this vector and increase in malaria cases. Investments into vector surveillance in other African malaria affected countries that are prone to invasion by *An. stephensi* should be prioritised. The involvement of national malaria control and elimination programmes in research prioritisation efforts and activities is advisable to increase uptake.

If you would like to comment on the synthesis report, or are currently involved or planning research / programmatic activities on Anopheles stephensi please contact MESA (mesa@isglobal.org)