# *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). 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 harbour the mosquito (ref). Amidst these discoveries in Africa, Sri Lanka, a 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). According to a 2022 study, the annual *P. falciparum* malaria cases in Ethiopia are expected to rise by 50% if measures are not put in place to fight the invasive species (ref). In September 2022, the WHO published a new initiative to stop the further spread of *An. stephensi* in Africa. To encourage an effective response, it recommends a five-pronged approach which includes: increasing collaboration; strengthening surveillance; improving information exchange; developing guidance; and prioritising research (ref).

**Relevance**

*An. stephensi* sets itself apart from other malaria vectors due to its ability to efficiently transmit malaria in urban areas. 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 no or limited experience on surveillance, intervention and other aspects of controlling *An. stephensi* in recently invaded areas. It is therefore imperative to map *An. stephensi* research and investments to show major activities being undertaken in this area. This will facilitate the identification of best practices, possible research gaps and priority areas for funding towards further research to drive evidence-based policy development.

**Collaboration**

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 or draw on any overlaps between the urban malaria Deep Dive and the *An. stephensi* Deep Dive.

## 2. Methodology

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

**Systematic data collection**

During the period of March 2022 – July 2022, information on *An. stephensi* projects were compiled. 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.

This data was acquired in four main ways:

- From researchers in the VCWG
- From a shoutout published in the February edition of the MESA newsletter, in the MalariaWorld newsletter and other MESA social media channels
- From the WHO threat map by sourcing backwards through the referenced publications
- Through a search of various research grant databases for projects and investments relating to *An. stephensi* using the keywords: *stephensi, stephensi* AND malaria and invasive species.

**Eligibility criteria**

The criteria used were:

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

**Information validation**

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 on the MESA Track and asked for additional information, if indicated, and verification. Using this link, a check for any errors in the project description was made by the PI and any required amendments made by the MESA team. Projects for which we did not get a response to our validation request were still included in the DD.

**Categorization 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

The database search yielded 260 projects. Of these, 200 were excluded due to either not fulfilling the eligibility criteria or duplication. A total of 60 projects were added to the MESA Track and subsequently included in the DD. This is an active DD and hence this number is liable to change over time as and when new projects are included in the MESA Track.

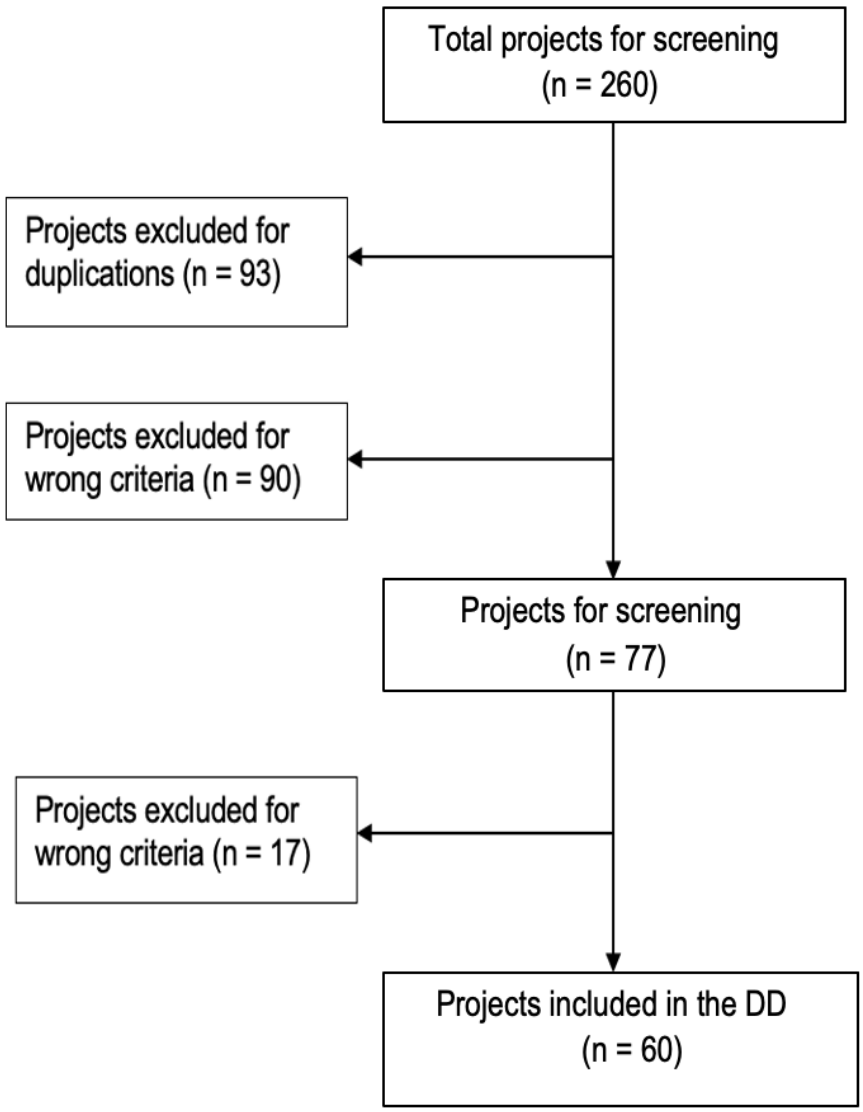


*Figure 1: Project search flowchart*

**Projects overview**

The 60 projects identified as relevant are led by institutions in the USA, Ethiopia, Japan, UK, India, Sri Lanka, Yemen, Australia, Italy and China.

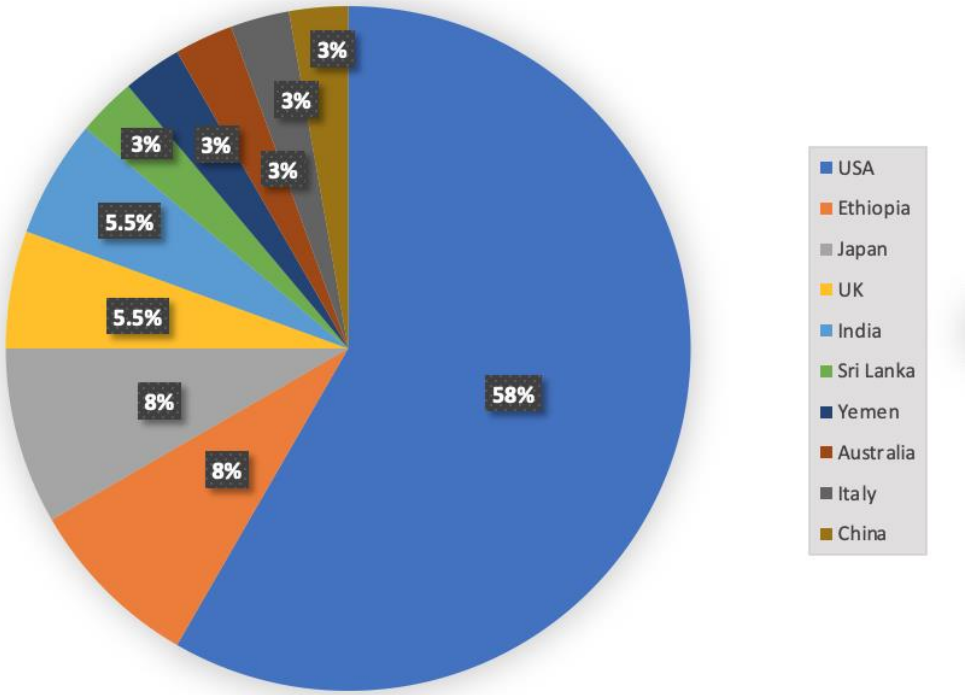


***Figure 2:*** *Proportion: Lead principal institutions per country*

India is the only country which has *An. stephensi* as a native malaria vector. There are 14 active projects and 46 completed.

Institutions that partner with these lead institutions are often located in either the same country as the funding source or the project site. Over half (52%) of the partners are research or academic institutions. The DD captured the involvement of the national malaria program in two countries (NMEP in Ethiopia and NMCP in Yemen).

**Active projects**

The 14 active *An. stephensi* projects are ongoing in 6 countries namely Ethiopia, Sudan, 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. Two others relate to the manufacture of a malaria vaccine while another looks at transgenics *via* the manipulation of *Guy 1* gene for the purpose of mosquito population control.

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

- track, observe, characterise, and predict the expansion of *An. stephensi*.
- promote the development and use of entomological tools.
- utilise artificial intelligence (AI) for automated species identification and spatiotemporal modelling.
- characterise the vector by analysis of the biotype, genotype, and entomological features.

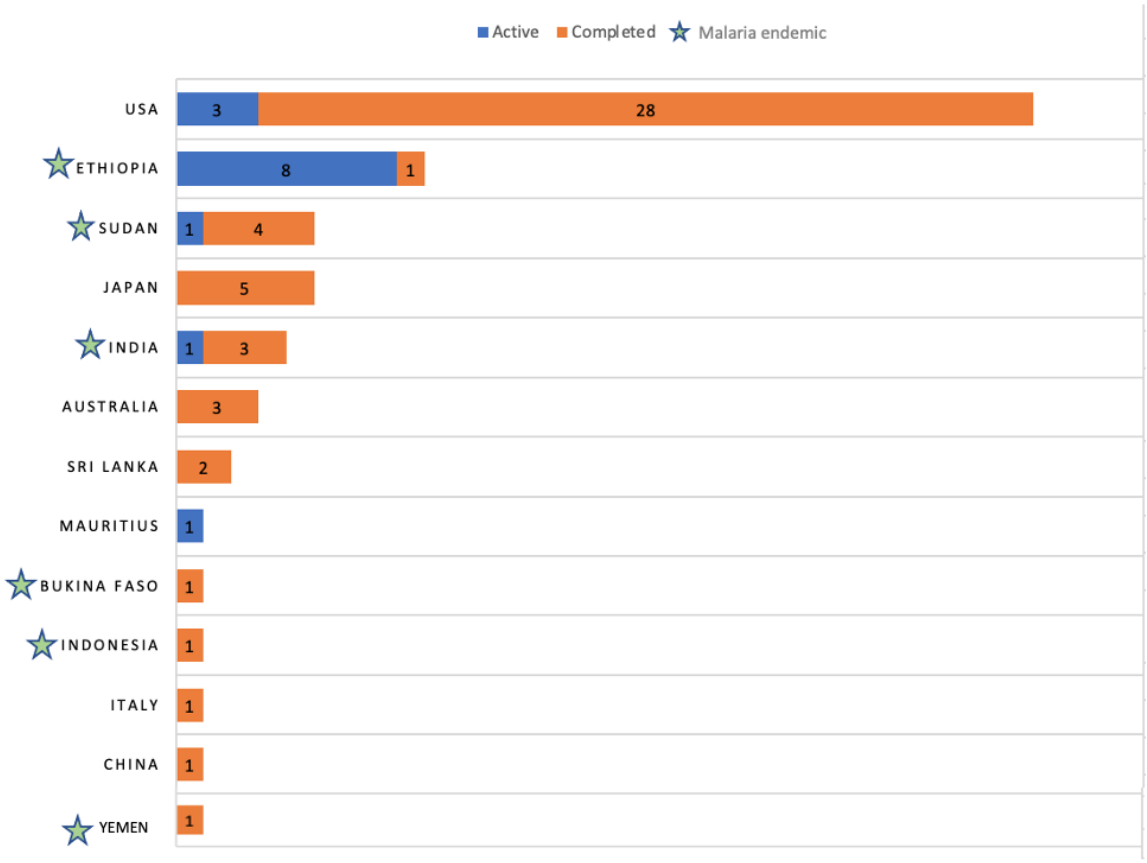


***Figure 3:*** *Number of projects by country*

**Category overview**

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

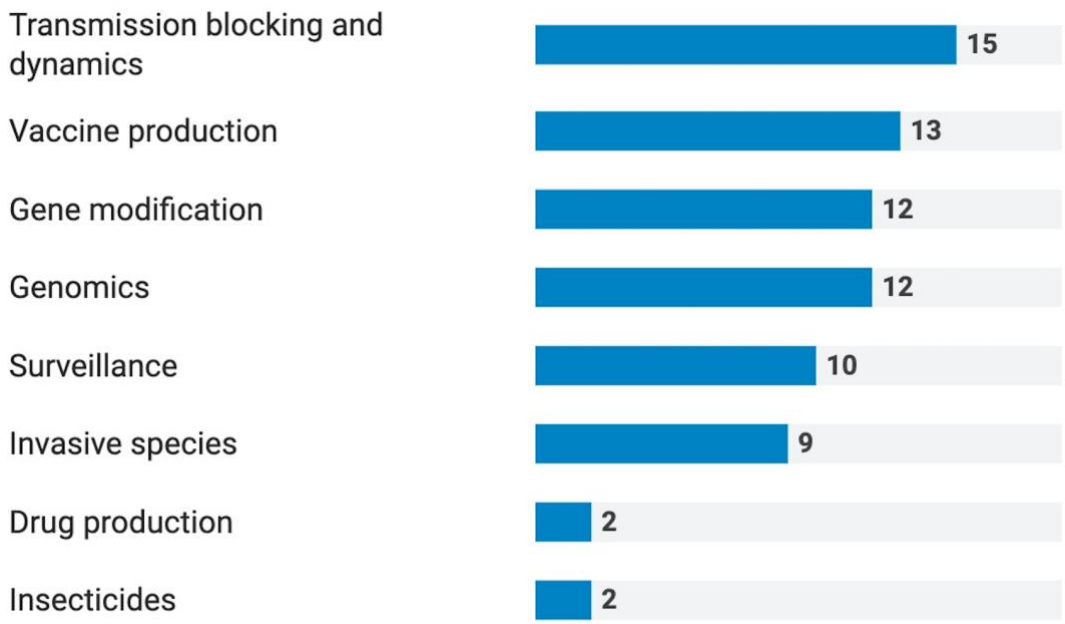


***Figure 4:*** *Number of projects by category*

### I. Transmission blocking and dynamics

Fifteen projects try to elucidate how certain environmental, mosquito and parasite factors such as temperature, bacterial infection, rare sugars ingestion, salivary gland secretion, midgut insulin signalling cascades (ISC), 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. Vaccine production

Thirteen 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), non-attenuated 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.

### III. Gene modification

Twelve 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.

### 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 ten 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

Nine 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 artemether-lumefantrine 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 $66.1 M. The funding available for the active projects is $17.6 M. Projects that did not have a funding amount documented were 17 (28%). 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 24 sources provided the $66.1 M documented in this DD. Fifteen (62%) of these funding sources were government institutions while eight were private institutions and one was a self-funded source.

  The highest amount given by a single funding source was $48.8M. This was from the National Institute of Allergy and Infectious Diseases (NIAID), NIH to fund 26 projects and accounts for 74% of the total funding captured by this DD. The lowest was $3 K for a project funded by an individual. In addition, 10 funding sources did not state online the amount of money granted.

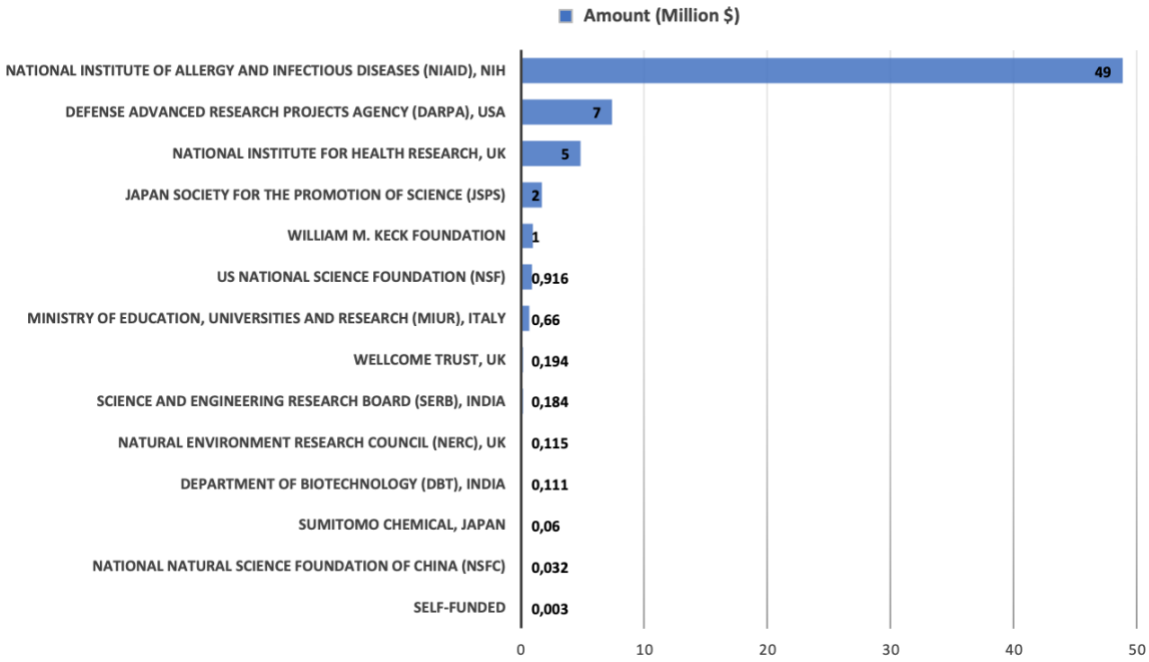


***Figure 4:*** *Amount contributed per funding source (Organisations for which we did not have the project funding amount are not depicted in the graph)*

- **Project Sites**
  The investments captured in this DD indicate that the USA is the project site which received the most funding, with $53.4 M to fund 31 projects (8 projects with undocumented funding amounts). This is followed by Sudan 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, 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 $29.4 M for 'Gene modification' to 63K for 'Insecticides' depending largely on the total number of projects included (See Table1). However, sometimes a single project can receive a large grant as is the case of two projects from the 'Gene modification' category which received approximately $7 M each.

Table 1: Funding by category

| Category | Total Funding ($) | Total Projects |
|---|---|---|
| Gene modification | 29.4 M | 12 |
| Vaccine production | 21.5 M | 13 |
| Transmission blocking and dynamics | 9.27 M | 15 |
| Genomics | 7.35 M | 12 |
| Invasive species | 6.58 M | 9 |
| Surveillance | 6.30 M | 10 |
| 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, while the bulk of funding is spent on genetic modification of *An. stephensi*. Though the invasive nature of this vector is under investigation, more needs to be done. Higher investments should be made towards understanding factors facilitating its spread, on surveillance studies and vector control strategies.

**Main highlights**

- There are 60 projects with $66.1 M total funding included in the DD.
- Up to 74% of total funding contributed by the NIH.
- There are 14 active projects with $17.6M.
- 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.
- 89% of the project leads are men and perhaps suggests an opportunity to identify strategies for accelerating involvement of women in sustained support for vector control interventions.
- Only the Ethiopian NMEP and NMCP Yemen were captured in this DD. It is important to encourage involvement of national malaria control and elimination programs in research activities ongoing in their countries. Funding to endemic country research institutions needs to be improved.

## 5. Conclusion

Experts' opinion (name: Dr. Corine Ngufor)

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)*