WHO-EM/MAL/391/E

Summary report on the

Regional workshop to strengthen the capacity for morphological identification of primary malaria and dengue vectors in high-risk countries

Muscat, Oman
16–20 July 2023

World Health Organization
Eastern Mediterranean Region

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Contents

1. Introduction.....1

2. Summary of discussions 3

3. Evaluation and outcomes 9

4. Conclusions and recommendations.....9

1. Introduction

Vector-borne diseases, including malaria and dengue, contribute to substantial morbidity and mortality in the World Health Organization (WHO) Eastern Mediterranean Region. The spread of invasive vectors (*Anopheles* and *Aedes* mosquitoes) outside their geographical range in the Region constitutes an additional challenge that increases the risk of outbreaks of malaria and *Aedes*-borne diseases, particularly dengue, in the high-risk countries of the Region. The prevention and control of dengue depends on vector control of the Aedes mosquitoes that transmit the virus, while vector control of *Anopheles* mosquitoes that transmit malaria is an essential preventive intervention to reduce and eliminate the malaria burden in endemic countries and prevent the re-establishment of local transmission in malaria-free countries.

Entomological surveillance allows evidence-based decisions on the type and timing of vector control interventions for effective control of these diseases. An essential part of this surveillance is the identification of mosquito species, because vector control should target the mosquito species that are transmitting disease. While molecular methods for the identification of species are improving, morphological identification with a microscope remains the main method for identification. However, countries have reported that one of the main challenges is the lack of capacity to identify *Aedes* and *Anopheles* mosquitoes.

Accurate identification of the mosquito vectors allows describing the composition of the mosquito fauna, the species that feed most frequently on humans and those that contribute to the transmission of pathogens in an endemic area, both spatially and temporally. During vector surveillance, correct identification of vectors is the first step when processing field samples and allows evidence-based implementation of selective and timely effective vector control interventions. Capacity-building in morphological identification will

support national vector control programmes to conduct regular vector surveillance for rapid and appropriate vector control response.

In response to requests from high-priority countries, the WHO Regional Office for the Eastern Mediterranean held a training workshop in Muscat, Oman, on 16–20 July 2023 to strengthen their capacity for the morphological identification of primary malaria and dengue vectors. This training was organized with the support of the US Centers for Disease Control (CDC), the Ministry of Health of Oman and the WHO Global Malaria Programme.

The objectives of the workshop were to:

- illustrate the mosquito fauna, with a focus on the *Anopheles* and Aedes species in the Region;
- apply identification keys to identify primary *Anopheles* malaria and Aedes dengue vectors;
- distinguish morphologically the major *Anopheles* malaria vectors;
- distinguish morphologically the *Aedes* vectors, including *Aedes aegypti* and *Aedes albopictus*;
- prepare mosquitoes as voucher specimens for reference;
- preserve mosquito specimens for further molecular (ID PCR and others) and immunological (sporozoite and bloodmeal ELISA) analysis; and
- support national vector control programmes to prepare a plan for a similar workshop for national and subnational training.

The participants of the workshop included 30 entomologists and vector control officers from eight countries of the Eastern Mediterranean Region (Afghanistan, Bahrain, Egypt, Oman, Saudi Arabia, Somalia, Islamic Republic of Iran and Yemen) and two countries of the WHO African Region (Eritrea and South Sudan).

The workshop was conducted by a team of experts from WHO and the countries of the Eastern Mediterranean Region. It comprised theoretical and practical sessions, a field visit to demonstrate integrated vector surveillance and group work discussion to plan similar training in the participating countries. The training was delivered using an interactive teaching and learning approach, with presentations, for both theoretical and practical sessions. Pinned mosquito specimens were provided to each participant during the training for identification using the morphological keys.

2. Summary of discussions

Overview of microscopes and their use

For training on morphological identification of mosquitoes, microscopes are essential. The aim of this session was to provide an overview on the evolution of the microscopes over the years and those currently used in laboratory diagnosis, namely the compound and the dissecting microscope. The session also enabled participants to know the microscopes they would be using during the training as there are a variety of brands of microscope that may differ from country to country.

Phylum: Arthropoda

This session outlined the classification and general characteristics of the phylum Arthropoda, which include all insects that participants should be aware of. There are three classes of medical importance under the phylum Arthropoda. Among these classes, the Insecta, also known as Hexapoda (six legs) is the most important class that includes the major disease vectors, namely mosquitoes. Flies, fleas, bugs and lice are also included in the class Insecta.

Introduction to mosquitoes

While most participants had experience in monitoring and the identification of mosquitoes, a presentation reviewed the mosquito life cycle, systematics and anatomy. Particular attention was paid to the genera and species that participants are likely to encounter in the Eastern Mediterranean Region. The morphological features of the mosquito anatomy were presented, and these were used in subsequent practical sessions to demonstrate the identification of mosquitoes.

Major mosquito vectors and medical importance

Participants were provided an overview of the mosquito vectors responsible for transmitting malaria, dengue and other vector-borne diseases. The overlapping distribution of reported vector-borne diseases in the Eastern Mediterranean Region calls for effective vector surveillance and control interventions that can be achieved by generating local evidence, knowing the vectors and their ecology, monitoring insecticide susceptibility and building capacity. To combat vector-borne diseases, timely response, vigilance and information sharing are essential.

Insectary standard operating procedures

Participants were given a short presentation on the best practices to follow when building and maintaining insectaries. The end use determines the scope, facilities and security of any insectary. Some insectaries will only be used for the rearing of field collected larvae prior to insecticide susceptibility tests, whereas others will maintain insecticide-susceptible or insecticide-resistant strains of mosquitoes for other activities, such as monitoring the bio-efficacy of insecticide-treated nets. In all cases, cleanliness, temperature, humidity, lighting,

security, furniture and equipment must be considered. Furthermore, planning the work ahead of time will ensure larval and adult survival.

Introduction to the WHO initiative to stop the spread of Anopheles stephensi in Africa

In September 2022, the WHO launched an initiative against the spread of *An. stephensi* in Africa. Participants were introduced to the basic bionomics of *An. stephensi*, including its flexibility in larval site choice, host preference for cattle or goats, insecticide resistance and ability to transmit *Plasmodium* parasites. The spread of *An. stephensi* was shown using the **Malaria Threats Map** website, where it was noted that negative findings (where entomological surveillance has been conducted but *An. stephensi* has not been found) are also included on the map. The epidemiological impact of *An. stephensi* is not fully understood, although there appear to have been outbreaks due to *An. stephensi* in Djibouti and Dire Dawa, Ethiopia. The models that have been developed appear to show substantial increases in malaria risk if nothing is done. The WHO initiative has the following five aims: (1) exchanging information; (2) increasing collaboration; (3) strengthening surveillance; (4) prioritizing research; and (5) developing guidance. These aims target an appropriate surveillance and vector control response to the spread of *An. stephensi* in Africa.

Introduction to curation

Participants were introduced to two methods for pinning mosquitoes: card pointing and micropinning. Card pointing of mosquitoes involves gluing a mosquito on its thorax to a card point, which is more stable and protects the wings. Micropinning involves inserting a micro pin from a foam stage into the thorax between the legs, which allows complete visibility of both sides of the thorax but can be less stable. The

importance of recording specimen data on labels and maintaining these labels with the specimens was emphasized, and participants were then guided in a practical session to pin field-collected mosquitoes.

Use of Lucid matrix keys

Lucid matrix identification keys are a tool that are an important alternative to dichotomous identification keys. These identification keys are a computer-based tool, that provides users with four windows: (1) features available; (2) entities remaining; (3) features chosen; and (4) entities discarded. The "features available" window displays the morphological features that allows users to choose which features are visible (i.e. head. maxillary palpus (number of white bands): none, one, two, three, or four). Users can choose which features are clearly visible, without needing to choose them in order. The "entities remaining" window displays the genera or species that correspond to the features that have been chosen, while the "features chosen" records the features which have been chosen. Finally, the "entities discarded" window displays those that do not correspond to those features. The major advantages of Lucid matrix keys are that they do not require all parts of a mosquito to be visible to permit identification, and that they can easily be updated. However, not all the Lucid identification keys available (primarily on the **Walter Reed Biosystematics Unit** webpage) are complete (which is also true of dichotomous keys). Participants were given the opportunity to use these keys to identify mosquito specimens.

Morphology of Anopheles and identification keys

The external morphology of Anopheles mosquitoes used for the identification of species was explained by providing pinned mosquitoes. Trainers visualized the different parts of the mosquito body under the dissecting microscope and identified males from females by looking at the

antennae, and culicines from anophelines by observing the size and shape of the maxillary palps. For Anopheles species identification, pinned An. stephensi specimens were provided for each participant and the trainer walked them through the parts of the body such as the maxillary palps, legs, wings and abdomen which are used for species identification. The trainees were provided with mosquito specimens and two morphological identification keys prepared by Maureen Coetzee (2020) and Jayson I Glick (1992) and practised species identification with the assistance of the trainers.

Aedes identification

As there have been recent reports of Aedes aegypti and Aedes albopictus in areas where they had not been found previously, entomological monitoring of these dengue vectors is needed. A brief introduction to the identification of Aedes was provided (with an example from the Afrotropical identification keys) and participants were then provided with specimens for identification.

Field visit

The field visit day for integrated vector surveillance involved group visits to different sites accompanied by facilitators. The sites included houses with farms, animal shelters, irrigation tanks and outdoor areas. Various methods of collection were demonstrated, such as mechanical aspirators, light traps, ovitraps, larva collection and night catchers. Additionally, two Early Warning, Alert and Response Network (EWARN) sentinel sites were visited in populated areas with a history of dengue cases and positive breeding sites. During the visits, the vector control unit in the Ministry of Health of Oman demonstrated to participants the recording of entomological data on the mobile app Epicollect5, a free and easy-to-use mobile data-gathering platform. The second stop following the sentinel sites was the insectary and entomology laboratory at the Ministry of Health.

Feedback from participants about the field visit activity

After the field visit, participants provided feedback through a group discussion. They reported that, while use of CDC light traps was common in countries, they had observed some types of traps for the first time during the field visit, such as the ovitraps, Gravitraps and the night catcher. They expressed their interest in obtaining and using these traps, particularly the night catcher. Participants also found the data collection application Epicollect5 very useful and felt that it could be used in their countries. Regarding the visit to the insectary, participants found the digitalization of data and pictures to be a practical way for surveillance and very useful during training. Overall, the field visit provided valuable insights for enhancing entomological surveillance.

Group work for countries to plan similar training at a national level

On the final day of the training, participants were divided into groups to discuss developing a proposal for planning the implementation of cascade training on morphological identification of primary malaria and dengue. The following main components guided the discussion for development of the proposal to justify the need for this training:

- targeted trainees and criteria for selection
- number of trainees
- timeline and duration of training
- site of training
- estimated realistic budget and source of funding
- expected benefits
- challenges.

3. Evaluation and outcomes

Pre- and post-tests for evaluation of the training were conducted using an online form, composed of multiple-choice questions, images and specimens placed under microscopes for morphological identification. Analysis of the pre- and post-tests showed an increase in the number of participants with a score of 80% or above: from 21 to 30 participants out of a total of 30 participants.

The evaluation and feedback of the workshop were collected from participants through the Slido platform. The feedback provided was positive, with the workshop well-received by participants who reported high satisfaction rates with their learning experience. Participants also reported that a longer duration of the workshop would have benefitted them more and suggested adding sessions on surveillance and more mosquito species in future training. They noted that having refresher training would be helpful to sustain the information and skills gained.

4. Conclusions and recommendations

The training workshop achieved its set objectives. The participants acquired the knowledge and skills essential to strengthen vector surveillance and control through accurate morphological identification of mosquito vectors. Thirty participants from 10 high-risk countries in the Eastern Mediterranean Region and African Region became potential trainers able to plan and implement similar capacity-building workshops on morphological identification of primary malaria and dengue vectors. Given the various levels of knowledge among the participants, continuous engagement in vector surveillance activities would be beneficial in supporting countries to sustain and further improve the knowledge and skills of country focal points. WHO will review the proposals submitted by countries for the implementation of

cascade training adapted from the regional workshop curriculum and teaching materials, with support from WHO technical staff.

Recommendations were made at the end of the workshop. These will be used to guide future activities in the morphological identification of primary malaria and dengue vectors.

To Member States

1. Develop national plans for capacity-building in entomological surveillance that cover mosquito field sampling and accurate morphological identification of mosquito vectors.

To WHO

2. Continue supporting the strengthening of integrated vector surveillance through capacity-building on disease vectors and further entomological investigation.
3. Support the coordination of cross-border capacity-building activities and the sharing of information and skills.
4. Expand and strengthen the regional network of experts in the morphological identification of mosquito vectors and other areas to ensure sustainable support to capacity-building activities for countries.

World Health Organization
Regional Office for the Eastern Mediterranean
Monazamet El Seha El Alamia Street,
Extension of Abdel Razak El Sanhouri Street
P.O. Box 7608, Nasr City
Cairo 11371, Egypt
www.emro.who.int