Anopheles stephensi and the risk of increased urban malaria in South Africa

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DATES:
Received: 05 Apr. 2024
Revised: 17 Mar. 2025
Accepted: 26 Mar. 2025
Published: 29 May 2025

HOW TO CITE:
Kaiser ML, Dahan-Moss Y, Koekemoer LL, Shandukani MB, Yewhalaw D, Brooke BD. *Anopheles stephensi* and the risk of increased urban malaria in South Africa. S Afr J Sci. 2025;121(5/6), Art. #18359. https://doi.org/10.17159/sajs.2025/18359

ARTICLE INCLUDES:
☒ Peer review
☐ Supplementary material

DATA AVAILABILITY:
☐ Open data set
☐ All data included
☐ On request from author(s)
☐ Not available
☒ Not applicable

EDITORS:
Pascal Bessong
Shane Redelinghuys

KEYWORDS:
urban malaria, malaria risk, *Anopheles stephensi*

FUNDING:
None

Owing to the scarcity of malaria vector mosquito populations in urban settings, urban malaria is rare in South Africa. *Anopheles stephensi*, an efficient urban malaria vector in South Asia and parts of the Arabian Peninsula (excluding southwest Saudi Arabia and Yemen), has recently expanded its range into African countries and territories where it has not previously occurred. The central hypothesis to explain the recent dispersal of *An. stephensi* out of its endemic range and into sub-Saharan Africa is via shipping, making seaports especially vulnerable to introductions of this species, although land crossings and general population movements are likely important as well. Based on an analysis of global shipping networks, South Africa is at risk of invasion by this species, although it has not been recorded in southern Africa to date. The World Health Organization has issued an alert for the spread of *An. stephensi,* including guidelines for surveillance in non-invaded areas, territories or countries that could be at risk. The aim of this review was to assess the risk of *An. stephensi* to South Africa and provide recommendations for vigilance and vector surveillance. We conclude that the range expansion of *An. stephensi* poses a significant threat to malaria control and elimination in southern Africa, and recommend regularised surveillance in at-risk areas, further engagement with existing multisectoral and cross-border initiatives, the mitigation of malaria in urban planning, and community awareness concerning water storage practices.

Significance:

The recent and continuing range expansion of the South Asian malaria vector mosquito *Anopheles stephensi* poses a significant threat to malaria control and elimination in sub-Saharan Africa. Fortunately, there are no records of this species in the southern African region to date, but this scenario will likely change and could lead to an increase in the incidence of urban malaria in South Africa and neighbouring countries.

Introduction – urban malaria in South Africa

Malaria in South Africa is primarily rural, and local transmission is restricted to the low-altitude, northeastern border regions of KwaZulu-Natal, Mpumalanga and Limpopo provinces. This restriction may, however, shift as urbanisation increases. Urban malaria is rare in South Africa due to the scarcity of malaria vector species in urban settings. However, there is increasing evidence that populations of some of these species can proliferate in such settings if conditions are favourable.[1] Favourable conditions may occur when urban planning fails to provide for proper drainage or where densely populated informal areas provide adequate breeding sites for mosquitoes, even in heavily polluted sites. Recent evidence shows that one of South Africa's principal malaria vectors, *Anopheles arabiensis*, is able to breed in water polluted with heavy metals, fertilisers and herbicides.[2-4] Added to the risk of adaptation of local vector populations to urban environments is the possible introduction of the major malaria vector *An. stephensi* into South Africa.

Background to *Anopheles stephensi*

Anopheles stephensi, an efficient urban and, to a lesser extent, rural malaria vector in South Asia and parts of the Arabian Peninsula (excluding southwest Saudi Arabia and Yemen), has recently expanded its range into countries and territories where it has not previously occurred.[5] The first detection of *An. stephensi* in Africa was from Djibouti in 2012, where it was implicated in a malaria resurgence.[6] Since then, it has been detected in Ethiopia and Sudan in 2016[7,8], in Somalia in 2019[9] and in Ghana, the Republic of Somaliland and Nigeria in 2020[10-12]. A year later, this invasive species was reported in Yemen[13], followed by reports in 2022 in Kenya and Eritrea[14,15].

Anopheles stephensi poses a threat to countries declared malaria-free. For example, Sri Lanka was declared malaria-free in 2016[16] and the detection of this species in December 2016 highlighted the threat it poses to maintaining Sri Lanka's malaria-free status[17]. This range expansion prompted the World Health Organization (WHO) to issue an alert for *An. stephensi*, acknowledging that there is potential for this species to spread throughout Africa, posing a serious threat to malaria control and elimination in affected territories.[9]

Anopheles stephensi general bionomics

Anopheles stephensi shares some morphological similarities with certain African malaria vector species, specifically those of the *An. gambiae* species complex, possibly leading to misidentifications. In cases of uncertainty, confirmation of species identity is by polymerase chain reaction (PCR) assay.[18] *Anopheles stephensi* has three forms (type, intermediate and variety (var.) *mysorensis* (hereafter *mysorensis*)), that can be separated by egg morphology.[19] Geographical distribution and ecotype vary by form, with the type form occupying urban areas, the intermediate form semi-urban areas and *mysorensis* rural areas.[19,20] All forms transmit malaria.[5,9]

Anopheles stephensi, described as anthropozoophilic (human and animal feeding), tends more toward zoophily, especially in rural areas. Female adults are commonly found in cattle sheds, although they will also enter houses, especially those that are more openly constructed and roofed with thatch, to feed on human occupants.[20,21] Feeding occurs mainly outdoors at dusk.[5] However, there have been reports of endophily (indoor-resting) and endophagy (indoor-feeding)[22], highlighting the plasticity of this species' behaviours that are not unlike those of *An. arabiensis*[23]. For example, in southeast Iran, the majority of the *mysorensis* form fed on bovine blood, while 11.8% fed on humans.[24] In Rajasthan, India, Nagpal et al.[25] reported outdoor-feeding by this species at dusk, followed by indoor-resting in houses. Their resting behaviour was somewhat affected by the use of DDT, causing blood-fed mosquitoes to rest on unsprayed surfaces (such as hanging clothing, utensils and furniture), and it was noted that ambient temperatures exceeding 35 °C reduced house entry of blood-fed female mosquitoes.

Adult collection methods for *An. stephensi* include aspiration from houses or shelters, pyrethrum spray catches, human landing catches, CDC light traps, black resting boxes and cattle-baited tents.[19,20,26,27] Black resting boxes only appear effective near horse stables.[27] Of the methods used by Balkew et al.[27] for collecting adult mosquitoes, the most effective were aspiration, black resting boxes and animal-baited traps. CDC light traps, human landing catches and pyrethrum spray catches were less effective.[27]

Typical *An. stephensi* larval sites are small water bodies, including artificial water containers such as cisterns, fountains and freshwater wells.[9,26,27] These are similar to sites typically occupied by certain important *Aedes* mosquito species, of which *Aedes aegypti* and *Ae. albopictus* are vectors of several arboviruses including dengue, Zika and chikungunya. *Anopheles stephensi* larval sites can also include pools, streambeds, river margins and marshy areas with slow-flowing water[24], similar to the preferred breeding sites of the African malaria vectors *An. funestus* and *An. arabiensis*[28].

Of the forms, 'type' and 'intermediate' are efficient vectors of *Plasmodium falciparum* and *P. vivax* malaria.[5,9] By comparison, a laboratory study showed that *An. stephensi* was more susceptible to *Plasmodium* infections than *An. arabiensis.*[29] This finding should, however, be interpreted with caution as field situations may differ. For example, of the *An. stephensi* specimens screened for *Plasmodium* parasites in Ethiopia, none were found to be infected with *P. falciparum*[26,27], and approximately 0.4% were infected with *P. vivax*[27]. Note that in this study the ELISA boiling step was not used – an omission that increases the likelihood of false positives from mosquitoes that fed on non-human animal blood.[30] Additionally, the *An. stephensi* human blood index was low.[27] Owing to its zoophilic nature, the *mysorensis* form is not a major malaria vector, although it has been implicated in malaria transmission in rural areas of Afghanistan and Iran.[5,9]

Interestingly, and unsurprisingly given the type of larval habitats *An. stephensi* utilises, seasonal rainfall is not the most accurate predictor of increases in population abundance. Temperature and land-use patterns, as opposed to rainfall, more accurately predict population densities of this species.[31]

Control methods

Primary control methods for *An. stephensi* in South Asia and the Arabian Peninsula are larval source management in urban areas (covering of potable water containers, drainage of potential breeding sites, larviciding using temephos and *Bacillus thuringiensis* var. *israelensis* (Bti) in aquatic breeding sites), indoor residual spraying of specially formulated insecticides[20,32] and the mass deployment of insecticide-treated nets. Larval control using larvivorous fish occurs in some urban areas of India.[20] Owing to the primarily exophilic nature of *An. stephensi*, indoor residual spraying and the distribution of insecticide-treated nets may not be particularly effective control options for this species in some regions. Nevertheless, these methods are used in certain rural localities where blood-fed female mosquitoes are found indoors[20], and in areas where these methods are effective against other vector species.

Of particular concern is resistance to multiple insecticides in many *An. stephensi* populations.[27,33,34] In Ethiopia, *An. stephensi* is resistant to pyrethroid, carbamate, organophosphate and organochlorine insecticides.[27,35,36]

Risk to South Africa

The central hypothesis to explain the recent dispersal of *An. stephensi* out of its endemic range is via shipping, making seaports especially vulnerable to introductions of this species, although land crossings, the importation of goods via containers transported overland, and general population movements are likely important as well.[5,6,8] Based on an analysis of global shipping networks, South Africa is at risk of invasion[37] because it has seaports in the malaria-endemic province of KwaZulu-Natal. In addition, there are several suitable urban habitats for *An. stephensi* in all endemic provinces where *Plasmodium* parasites occur (Figure 1). Specifically, Durban and Richard's Bay are higher-risk ports of entry because they are major international harbours situated in malaria-receptive eco-zones permissive to the proliferation of *An. stephensi.*[5] Importantly, Maputo harbour in Mozambique also represents an importation risk for South Africa owing to the transit of goods from Maputo into South Africa or via South Africa to neighbouring countries. This may also be true of seaports in Namibia. The risk of malaria transmission is substantially lower in major seaports of non-endemic provinces, like Gqeberha in the Eastern Cape Province and Cape Town in the Western Cape Province. While *An. stephensi* can be introduced at these ports, the absence of locally occurring *Plasmodium* parasites in these non-endemic regions mitigates the risk of transmission, although there is a low risk of inadvertent transport of imported mosquitoes from these ports to other parts of the country via modes of land transport.

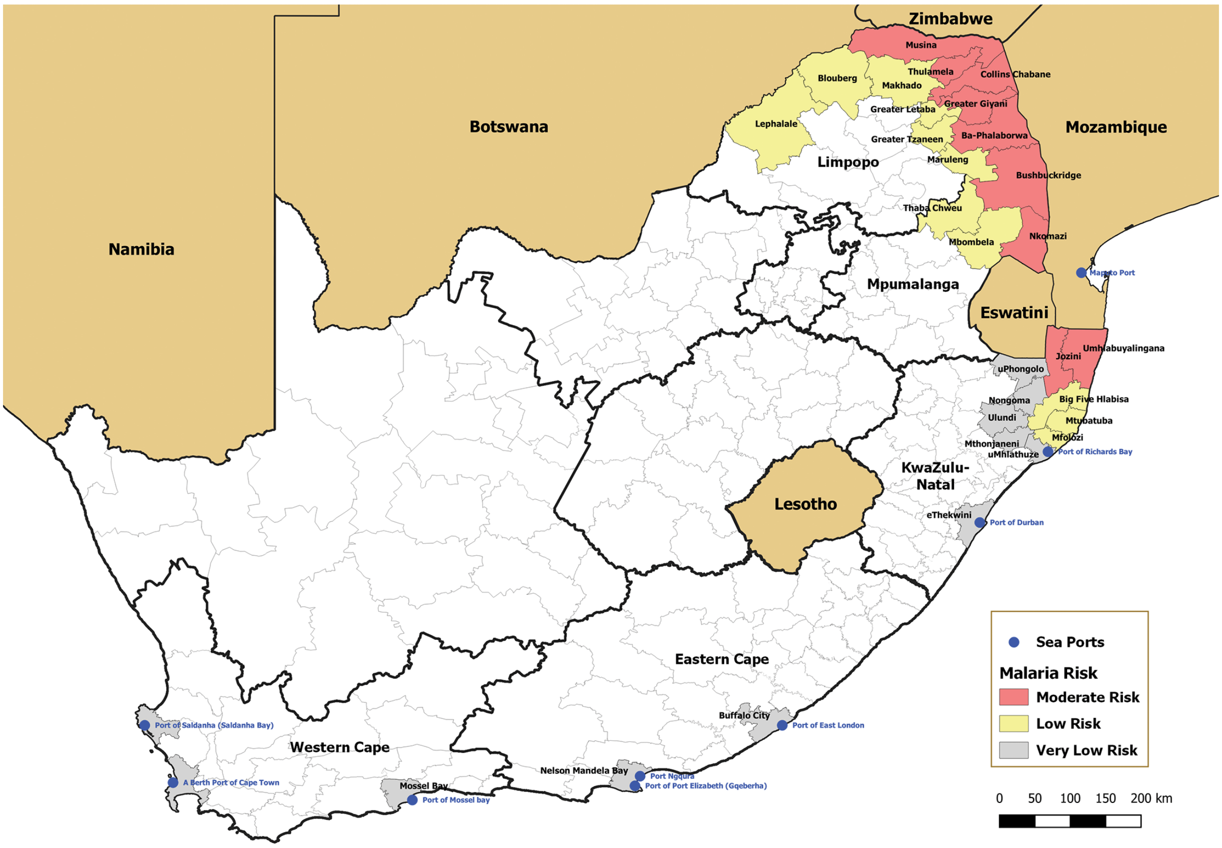
An introduction of *An. stephensi* into South Africa's malarious regions, including at-risk urban areas, is likely to increase the incidence and geographical spread of locally acquired malaria. This is because South Africa's annual vector control measures – indoor residual spraying and larval source management – are primarily implemented in rural and specific peri-urban settings where local malaria transmission regularly occurs. Owing to the current scarcity of urban malaria in South Africa, there is no proactive implementation of vector control in urban settings. Instead, a locally acquired case triggers a reactive response that may include localised indoor residual spraying, larval source management and vector surveillance, and an outbreak triggers a wider response using the same measures. If the incidence of locally acquired urban malaria substantially increases in South Africa, possibly caused by the localised establishment of *An. stephensi* populations, then proactive vector control measures will be necessary[38], especially in terms of achieving malaria-free status. As South Africa's national and provincial malaria control programmes aim to eliminate malaria within the country's borders by 2028, an introduction of *An. stephensi*, and its subsequent proliferation in urban settings, could pose a significant risk to this objective.

Vigilance, vector surveillance and response

The WHO's alert[9] includes guidelines for *An. stephensi* surveillance in non-invaded areas, territories or countries that could be at risk. Owing to the breeding characteristics of this species and the reduced likelihood of collecting good samples of adult specimens using many of the conventional adult collection methods, the recommended approach is sampling of aquatic stage mosquitoes from potential breeding sites, especially in urban and peri-urban areas.[9] Adult collection methods can nevertheless be used in addition to larval sampling, especially from animal shelters and those houses constructed in an open fashion and with thatched roofs.[9,21,27] Passive resting sites may also be useful for adult collections in and around animal shelters.[27]

Identification of larval specimens necessitates rearing them to adulthood. Adult mosquitoes are identified by external morphology using dichotomous keys[39] followed by PCR and sequencing for confirmation if necessary[14,19]. Larval habitats from where samples were collected should be thoroughly characterised and field surveillance officers are encouraged to report any newly detected *An. stephensi* localities through the WHO's reporting and tracking system.[40] Should sample sizes allow, determining insecticide susceptibilities and other key indicators (feeding, resting and breeding habits) in newly detected populations of this species is especially important, as these data inform control operations.

Anopheles stephensi surveillance is best implemented within an operational research framework by including information on collection site geolocation,



Map created using the QGIS Geographic Information System (Open Source Geospatial Foundation Project, http://qgis.org) and the malaria risk map courtesy of the National Department of Health https://www.health.gov.za/outbreaks-malaria/

Figure 1: Malaria risk map for South Africa showing major seaports through which the urban malaria vector *Anopheles stephensi* may be imported.

collection method, date and time of collection, climate parameters, land cover, land use, breeding site characteristics and other *Anopheles* species that occur in sympatry. These are important parameters for predictive modelling of malaria transmission dynamics against a backdrop of changing land-use patterns, increasing urbanisation and climate change.

Control of *An. stephensi*, where detected, ought to form part of an integrated vector management strategy[41], noting that indoor residual spraying and the mass deployment of insecticide-treated nets have proved effective in reducing malaria prevalence in *An. stephensi* populations in India, Pakistan, Iran and Afghanistan. Personal protection measures, including the use of repellent products containing Diethyl-3-methylbenzamide (DEET), are also indicated.[36,42-44] The Global Vector Control Response to invasive *An. stephensi*[45] has identified three key aspects to curb the spread of this species, i.e. enhanced surveillance, deployment of additional vector control approaches for malaria vectors and the consideration of the roles of different partners and funding sources in tackling and mitigating in-country risk posed by this species.

Conclusion and recommendations

The range expansion of *An. stephensi* poses a significant threat to malaria control and elimination in sub-Saharan Africa. This is especially pertinent in light of recent information suggesting that this species may be partly responsible for the introduction of antimalarial-drug- and diagnostic-resistant *P. falciparum* into the Horn of Africa.[46] Fortunately, there are no records of this species in the southern African region to date, but this scenario will likely change.

We recommend that South Africa remains vigilant through annual surveillance and additional spot checks at seaports, airports and land points of entry in those endemic districts where ongoing malaria transmission occurs. Incorporation of the required *An. stephensi* surveillance into the broader integrated vector management strategy for South Africa is necessary as soon as possible, as is the upskilling of provincial surveillance personnel for detection and control of this species, which has already been initiated. Continued monitoring at a national level of the molecular markers of antimalarial drug resistance in *P. falciparum* is especially important.[47] If, or when, necessary, South Africa and neighbouring countries should approach the implementation of vector control interventions for *An. stephensi* through multisectoral and cross-border initiatives including those of Elimination 8 and MOSASWA (Mozambique, South Africa and Eswatini).[48]

Other important recommendations, as suggested by the WHO Global framework for the response to malaria in urban areas[38], include: urban leadership, whereby city/municipal leaders ensure that urban malaria forms part of urban planning and policymaking; community engagement concerning water storage practices; and a multisectoral response to urban malaria, including quality clinical care.

Acknowledgements

Managers of South Africa's national and provincial malaria control programmes are thanked for supporting the training of surveillance personnel in the identification of malaria vector species.

Data availability

There are no data pertaining to this study/article.

Declarations

We have no competing interests to declare. We have no AI or LLM use to declare.

Authors' contributions

M.L.K.: Conceptualisation, writing – initial draft and revisions. Y.D.M.: Conceptualisation, writing – initial draft and revisions. L.L.K.: Writing –

revisions. M.B.S.: Writing – revisions. D.Y.: Writing – initial draft and revisions. B.D.B.: Conceptualisation, writing – initial and final drafts, project lead. All authors read and approved the final manuscript.

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