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Peer review history for:

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>

HOW TO CITE:

Anopheles stephensi and the risk of increased urban malaria in South Africa [peer review history]. *S Afr J Sci.* 2025;121(5/6), Art. #18359. <https://doi.org/10.17159/sajs.2025/18359/peerreview>

Reviewer 1: Round 1

Date completed: 08 September 2024

Recommendation: Accept / **Revisions required** / Resubmit for review / Resubmit elsewhere / Decline / See comments

Conflicts of interest: None

Does the review fall within the scope of SAJS?

Yes/No

Is the review written in a style suitable for a non-specialist and is it of wider than only specialist interest?

Yes/No

Do the Title and Abstract clearly and accurately reflect the content of the review?

Yes/No

Does the review provide a significantly novel perspective or significant recent advances in the field?

Yes/No

Is the objective of the review concisely stated?

Yes/No

Is appropriate and adequate reference made to other work in the field?

Yes/No

Do current debates and points of contention receive appropriate coverage?

Yes/No/Not applicable

Are gaps in the literature adequately identified?

Yes/No/Not applicable

Does the review provide direction for future research?*

Yes/No/Not applicable

Are the methodology and statistical treatment appropriate?

Not applicable/Yes/No/Partly/Not qualified to judge

Are the interpretations and recommendations aligned with the objective?

Yes/Partly/No

Please rate the manuscript on overall contribution to the field

Excellent/Good/Average/Below average/Poor

Please rate the manuscript on language, grammar and tone

Excellent/Good/Average/Below average/Poor

Is the manuscript concise and free of repetition and redundancies?

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If accepted, would you recommend that the article receives priority publication?

Yes/No

Are you willing to review a revision of this manuscript?

Yes/No

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Yes/No

Comments to the Author:

Very informative and well-written paper on the potential threat of the invasive urban malaria vector *Anopheles stephensi* to South Africa. I only have a few minor suggestions:

1. As I imagine that not all readers will be familiar with mosquitoes and mosquito terms, I suggest giving a bit more info on *Aedes* (you now only mention they inhabit similar habitats, but what is it and what does it transmit?) and explain what 'exophilic' means.
2. You mention that mosquitoes could be imported from Maputo, but how about other countries (e.g. Namibia) and other main routes of entry. Are containers being imported across borders by road from e.g. Botswana?
3. You also mention that ports in areas without local malaria transmission are not important, but mosquitoes could quickly spread to other (endemic) areas, correct? Especially if they 'hitchhike', see e.g.

<https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003648>

<https://www.nature.com/articles/s41598-017-12652-5>

Author response to Reviewer 1: Round 1

All comments have been addressed as suggested except that the correct abbreviation for '*Anopheles*' is '*An.*'. This is because there are other mosquito genera that also begin with 'A' such as *Aedes*, and so the nomenclature for this group of insects has been adjusted to avoid ambiguity. Hence '*An. stephensi*' is the correct abbreviation for this species. Additionally, changing land use patterns, increasing urbanization, water storage practices and climate change are discussed in the context of future operational research within the section on Vigilance, vector surveillance and response (page 6), and in the Recommendations (page 7). The risk of overland importation of *An. stephensi* has been addressed on page 4 and more context on *Aedes* mosquitoes has been given on page 3.

Reviewer 2: Round 1

Date completed: 26 November 2024

Recommendation: Accept / Revisions required / **Resubmit for review** / Resubmit elsewhere / Decline / See comments

Conflicts of interest: None

Does the review fall within the scope of SAJS?

Yes/No

Is the review written in a style suitable for a non-specialist and is it of wider than only specialist interest?

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Yes/No

Is appropriate and adequate reference made to other work in the field?

Yes/No

Do current debates and points of contention receive appropriate coverage?
Yes/ No /Not applicable
Are gaps in the literature adequately identified?
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Does the review provide direction for future research?*
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Are the interpretations and recommendations aligned with the objective?
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Is the manuscript concise and free of repetition and redundancies?
Yes/ No
Is the supplementary material relevant and separated appropriately from the main document?
Yes/No/ Not applicable
Please rate the manuscript on overall quality
Excellent/Good/ Average /Below average/Poor
If accepted, would you recommend that the article receives priority publication?
Yes /No
Are you willing to review a revision of this manuscript?
Yes /No
With regard to our policy on ‘ Publishing peer review reports ’, do you give us permission to publish your anonymised peer review report alongside the authors’ response, as a supplementary file to the published article? Publication is voluntary and only with permission from both yourself and the author.
Yes /No

Comments to the Author:

1. English grammar needs to be fixed all through the document.
2. Abstract: The abstract does not contain a specific hypothesis or aim of the review. It does not give a definite conclusion as well. Kindly fill in the gaps.
3. A section should be included that discuss the implications for Malaria Control in South Africa, this can highlight potential Vulnerability of South African cities, the challenges involved in controlling this Vector, highlighting the use of insecticide-treated nets and indoor spraying which isn't a common practice in South African urban settings. The author can also discuss the potential for outbreaks, and how stephensi could disrupt malaria elimination efforts in South Africa. This would give the manuscript more rigour and quality information.
4. Line 133: While there is a need for vector surveillance, identifying research gaps is a key factor in addressing environmental issues. Please add more information on the need for studies on stephensi's biology under South African conditions and Its interaction with existing malaria vectors. The discussion on predictive modeling would come in handy here as a method for surveillance and research. A paragraph stating the use of climate and urbanization data to map potential spread might be helpful in the manuscript.
5. Conclusion: Although the vector stephensi is not yet in South Africa, your conclusion might have specific information that relates to policies and urban specific interventions. e.g., larval source management in urban water storage systems,. Your recommendation states that the upskilling of provincial surveillance personnel for detection and control of this species is already in place, so I think you should delete that and include new recommendations, for example community engagement, there is a need to educate urban population about water storage practices and to incorporate vector management into urban planning.
6. At least, a table should be included in the review. This can center on reported distribution of

Anopheles stephensi in Africa, the countries with references and any other information that will be suitable for the manuscript.

[See Appendix 1 for Reviewer 2's comments made directly on the manuscript]

Author response to Reviewer 2: Round 1

English grammar needs to be fixed all through the document.

AUTHOR: We apologize for the grammatical errors. An expert editor has reviewed the manuscript.

Abstract: The abstract does not contain a specific hypothesis or aim of the review. It does not give a definite conclusion as well. Kindly fill in the gaps.

AUTHOR: Added: "The aim of this review was to assess the risk of An. stephensi to South Africa and provide recommendations for vigilance and vector surveillance. These include regularized surveillance in at-risk areas, multisectoral and cross-border initiatives, the mitigation of malaria in urban planning and community engagement concerning water storage practices."

A section should be included that discuss the implications for Malaria Control in South Africa, this can highlight potential Vulnerability of South African cities, the challenges involved in controlling this Vector, highlighting the use of insecticide-treated nets and indoor spraying which isn't a common practice in South African urban settings. The author can also discuss the potential for outbreaks, and how *A. stephensi* could disrupt malaria elimination efforts in South Africa. This would give the manuscript more rigour and quality information.

AUTHOR: Added: 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 - IRS and larval source management (LSM) - are primarily implemented in rural and specific peri-urban settings where local malaria transmission has recently occurred. 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 localized IRS, LSM 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 localized establishment of An. stephensi populations, then proactive vector control measures will be necessary (see the global framework for the response to malaria in urban areas. <https://www.who.int/publications/i/item/9789240061781>), 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.

Line 133: While there is a need for vector surveillance, identifying research gaps is a key factor in addressing environmental issues. Please add more information on the need for studies on *A. stephensi's* biology under South African conditions and its interaction with existing malaria vectors. The discussion on predictive modeling would come in handy here as a method for surveillance and research. A paragraph stating the use of climate and urbanization data to map potential spread might be helpful in the manuscript.

AUTHOR: Added: "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, 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 urbanization and climate change."

Conclusion: Although the vector *A. stephensi* is not yet in South Africa, your conclusion might have specific information that relates to policies and urban specific interventions. e.g., larval source management in urban water storage systems,. Your recommendation states that the upskilling of provincial surveillance personnel for detection and control of this species is already in place, so I think you should delete that and

include new recommendations, for example community engagement, there is a need to educate urban population about water storage practices and to incorporate vector management into urban planning.

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

At least, a table should be included in the review. This can center on reported distribution of *Anopheles stephensi* in Africa, the countries with references and any other information that will be suitable for the manuscript.

AUTHOR: In the section "Background to *Anopheles Stephensi*:" we list the African countries thus far affected by *An. stephensi*. We thus feel that a table with the same information is probably unnecessary, especially given that the spread of this species is quite fluid.

Appendix 1: Reviewer 2's comments on manuscript (Round 1)

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

2

3 **Abstract**

4 Urban malaria is rare in South Africa due to the scarcity of malaria vector species in urban settings.
5 *Anopheles stephensi*, an efficient urban malaria vector in South Asia and parts of the Arabian
6 Peninsula (excluding south-west Saudi Arabia and Yemen), has recently expanded its range into
7 African countries and territories where it has not previously occurred. The central hypothesis to
8 explain the recent dispersal of *An. stephensi* out of its endemic range and into sub-Saharan Africa
9 is via shipping, making seaports especially vulnerable to introductions of this species, although
10 land crossings and general population movements are likely important as well. Based on an
11 analysis of global shipping networks, South Africa is at risk of invasion by this species, although
12 it has not been recorded in southern Africa to date. The WHO has issued an alert for the spread of
13 *An. stephensi* including guidelines for surveillance in non-invaded areas, territories or countries
14 that could be at risk. Here we assess the risk of *An. stephensi* to South Africa and provide
15 recommendations for vigilance and response.

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

21

22 **Introduction - urban malaria in South Africa**

23 Malaria in South Africa is primarily rural and local transmission is restricted to the low-altitude,
24 north-eastern border regions of KwaZulu-Natal, Mpumalanga and Limpopo provinces. This
25 restriction may however shift as urbanisation increases. Urban malaria is rare in South Africa due
26 to the scarcity of malaria vector species in urban settings. However, there is increasing evidence
27 that populations of some of these species can proliferate in such settings if conditions are
28 favourable¹. Favourable conditions can occur when urban planning fails to provide for proper
29 drainage or where densely populated informal areas provide adequate breeding sites for
30 mosquitoes, even in heavily polluted sites. Recent evidence shows that one of South Africa's
31 principal malaria vectors, *Anopheles arabiensis*, is able to breed in water polluted with heavy
32 metals, fertilisers and herbicides^{2,3,4}. Added to the risk of adaptation of local vector populations to
33 urban environments is the possible introduction of the major malaria vector *An. stephensi* into
34 South Africa.

35 **Background to *Anopheles stephensi***

36 *Anopheles stephensi*, an efficient urban and, to a lesser extent, rural malaria vector in South Asia
37 and parts of the Arabian Peninsula (excluding south-west Saudi Arabia and Yemen), has recently
38 expanded its range into countries and territories where it has not previously occurred⁵. The first
39 detection of *An. stephensi* in Africa was from Djibouti in 2012, where it was tentatively implicated
40 in a malaria resurgence⁶. Since then, it has been detected in Ethiopia and Sudan in 2016^{7,8}, in
41 Somalia in 2019⁹ and in Ghana, the Republic of Somaliland and Nigeria in 2020^{10,11,12}. A year

Commented [A1]: This should be abbreviated as *A. stephensi*

Commented [A2]: For proper understanding, it's better to use the word "confirmed" to replace this.

42 later, this invasive species was reported in Yemen¹³, followed by reports in 2022 in Kenya and
43 Eritrea^{14, 15}.

44 *Anopheles stephensi* poses a threat to countries declared malaria-free. For example, Sri Lanka was
45 declared malaria-free in 2016¹⁶ and the detection of this species in December 2016 highlighted the
46 threat that this species could pose to maintaining Sri Lanka's malaria-free status¹⁷. This range
47 expansion prompted the World Health Organization (WHO) to issue an alert for *An. stephensi*
48 because there is potential for this vector to spread throughout Africa, posing a serious threat to
49 malaria control and elimination in affected territories⁹.

50 ***Anopheles stephensi* general bionomics**

51 *Anopheles stephensi* shares some morphological similarities with certain African malaria vector
52 species, specifically those of the *An. gambiae* species complex, and can therefore be difficult to
53 distinguish using external morphological characteristics alone. Confirmation of species identity is
54 by subsequent polymerase chain reaction (PCR) assay¹⁸. *Anopheles stephensi* comprises three
55 forms (type, intermediate and variety (*var.*) *mysorensis* (hereafter *mysorensis*)), that can be
56 separated based on egg morphology¹⁹. Geographical distribution and ecotype vary by form, with
57 the type form occupying urban areas, the intermediate form semi-urban areas and *mysorensis* rural
58 areas^[19,20]. All forms transmit malaria^{5,9}.

59 *Anopheles stephensi*, described as anthropozoophilic (human and animal feeding), tends more
60 toward zoophily, especially in rural areas. Adult females are commonly found in cattle sheds,
61 although they will also enter houses, especially those that are more openly constructed and roofed
62 with thatch, to feed on human occupants^{20,21}. Feeding occurs mainly outdoors at dusk⁵. There are
63 however reports of endophily (indoor-resting) and endophagy (indoor-feeding)²², highlighting the
64 plasticity of this species' behaviours that are not unlike those of *An. arabiensis*²³. For example, in
65 southeast Iran, the majority of the *mysorensis* form fed on bovine blood while 11.8% fed on
66 humans²⁴. Nagpal et al.²⁵ reported that adults were found to bite outdoors at dusk and then to enter
67 and rest in houses in Rajasthan, India. The resting behavior changed from resting indoors on walls
68 at all heights before the use of DDT, to resting indoors but mostly on unsprayed surfaces following
69 the use of DDT. Untreated surfaces included hanging clothing, utensils, furniture and wall surfaces
70 missed during spraying²⁵. There were no significant differences in the resting surfaces chosen by
71 mosquitoes in the sprayed and unsprayed sites once DDT was in use. There also appeared to be an
72 association with increasing outdoor temperature (increasing to 35°C and above) and *An. stephensi*
73 house entry²⁵.

74 Adult collection methods for *An. stephensi* include aspiration from houses or shelters, pyrethrum
75 spray catches, human landing catches, CDC light traps, black resting boxes and cattle baited tents
76^{19,20,26,27}. Black resting boxes only appear effective near horse stables²⁷. Of the methods used by
77 Balkew et al.²⁷ for collecting adult mosquitoes, the three best methods were aspiration, black boxes
78 and animal baited traps. The more routinely used surveillance methods of CDC light traps, human
79 landing catches and pyrethrum spray catches were much less effective²⁷.

80 Typical larval sites of *An. stephensi* are small water bodies, including artificial water containers
81 such as cisterns, fountains, sewage water and fresh-water wells^{9,25,26}, which are similar to sites
82 typically occupied by *Aedes* species. Larval sites can also include pools, streambeds, river margins
83 and marshy areas with slow-flowing water²⁴, which resemble the larval sites of the African malaria
84 vectors *An. funestus* and *An. arabiensis*²⁸.

Commented [A3]: delete

85 Of the forms, ‘type’ and ‘intermediate’ are considered efficient vectors of *Plasmodium falciparum*
86 and *P. vivax* malaria^{5,9}. In fact, *An. stephensi* were more susceptible to malaria parasite infections
87 than *An. arabiensis* in a laboratory study²⁹. This should however be interpreted with caution as the
88 situation in the field may differ. For example, of *An. stephensi* specimens screened for *Plasmodium*
89 parasites in Ethiopia, none were found to be infected with *P. falciparum* parasites, and
90 approximately 0.4% were infected with *P. vivax*^{26,27}. Note that in this study the boiling step was
91 not used, an omission that can increase the chances of false positives from mosquitoes that fed on
92 animal blood³⁰. Additionally, the human blood index of *An. stephensi* was low²⁶. Due to its
93 zoophilic nature, the *mysorensis* form is not a major malaria vector. It has, however, been
94 implicated in malaria transmission in rural areas of Afghanistan and Iran^{5,9}.

95 Control methods

96 Primary control methods for *An. stephensi* in South Asia and the Arabian Peninsula are larval
97 source management in urban areas (covering of potable water containers, drainage of potential
98 breeding sites, larviciding using temephos and *Bacillus thuringiensis* var. *israelensis* (Bti) in
99 aquatic breeding sites) and indoor residual spraying (IRS) with specially formulated insecticides
100 in rural areas²⁰. Larval control using larvivorous fish occurs in urban areas of India²⁰. Owing to
101 the primarily exophilic nature of *An. stephensi*, IRS and insecticide treated bed net distribution
102 may not be particularly effective control options for this species.

103 Nevertheless, these methods are used in some rural localities when the vectors have been found
104 indoors²⁰ and in areas where these tools are effective against other vector species. Of particular
105 concern is resistance to multiple insecticides in many *An. stephensi* populations^{27,31,32}. In Ethiopia,
106 *An. stephensi* is resistant to pyrethroid, carbamate, organophosphate and organochlorine
107 insecticides^{27,33,34}. Interestingly, and unsurprisingly given the type of larval habitats this species
108 utilises, seasonal rainfall is not the most accurate predictor of increases in abundance of *An.*
109 *stephensi*. Temperature and land-use patterns, as opposed to rainfall, appear to more accurately
110 predict population densities of this species³⁵.

111

112 Risk to South Africa

113 The central hypothesis to explain the recent dispersal of *An. stephensi* out of its endemic range is
114 via shipping, making seaports especially vulnerable to introductions of this species, although land
115 crossings and general population movements are likely important as well^{5,6,8}. Based on an analysis
116 of global shipping networks, South Africa is at risk of invasion³⁶ because it has seaports in the
117 malaria-endemic province of KwaZulu-Natal. In addition, there are several suitable urban habitats
118 for *An. stephensi* in all endemic provinces where *Plasmodium* parasites occur (Figure 1).
119 Specifically, Durban and Richard’s Bay are higher-risk ports of entry because they are major
120 international harbours situated in malaria-receptive eco-zones permissive to the proliferation of
121 *An. stephensi*⁵. Importantly, Maputo harbour also represents an importation risk for South Africa
122 owing to the transit of goods from Maputo into South Africa or via South Africa to neighbouring
123 countries. The risk of malaria transmission is low in major seaports of non-endemic provinces, like
124 Gqeberha in the Eastern Cape Province and Cape Town in the Western Cape Province. While *An.*
125 *stephensi* can be introduced at these ports, the absence of locally occurring *Plasmodium* parasites
126 in these non-endemic regions mitigates the risk of transmission.

Commented [A4]: Aside from the risk of invasion through sea ports, other risks should be included e.g. climate, urbanization and water storage practices in South Africa.

148 of this species is especially important, as these data inform control operations. Surveillance for *An.*
149 *stephensi* can form part of routine vector surveillance operations for other native *Anopheles*
150 malaria vectors as well as arbovirus vectors such as *Aedes* species. Control of *An. stephensi*, where
151 detected, should form part of an integrated vector management strategy (IVM)³⁹.

152 The Global Vector Control Response to invasive *An. stephensi*⁴⁰ has identified three key aspects
153 to curb the spread of this species i.e. enhanced surveillance, deployment of additional vector
154 control approaches for malaria vectors and the consideration of the roles of different partners and
155 funding sources in tackling and mitigating in-country risk posed by this species.

156 **Conclusion & recommendations**

157 The range expansion of *An. stephensi* poses a significant threat to malaria control and elimination
158 in sub-Saharan Africa. This is especially pertinent in light of recent information suggesting that
159 this species may be partly responsible for introductions of antimalarial drug and diagnostic
160 resistant *P. falciparum* into the Horn of Africa⁴¹. Fortunately, there are no records of this species
161 in the southern African region to date, but this scenario will likely change.

162 We recommend that South Africa remain vigilant through annual surveillance and additional spot
163 checks at seaports, airports and land points of entry in those endemic districts where ongoing
164 malaria transmission occurs. Incorporation of the required *An. stephensi* surveillance into the
165 broader IVM strategy for South Africa is necessary as soon as possible, as is the upskilling of
166 provincial surveillance personnel for detection and control of this species, which has already been
167 initiated. Continued monitoring at national level for the molecular markers of antimalarial drug
168 resistance in *P. falciparum* is especially important⁴². If or when necessary, South Africa and
169 neighboring countries should approach the implementation of vector control interventions for *An.*
170 *stephensi* through multisectoral and cross-border initiatives including those of the Elimination 8
171 and MOSASWA (Mozambique, South Africa and ESwatini)⁴³.

172

173 **Acknowledgements**

174 Anonymised

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176 **Conflicts of interest**

177 The authors declare no conflicts of interest

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