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SORT IT: MALARIA ELIMINATION SUPPLEMENT

Changing distribution and abundance of the malaria vector *Anopheles merus* in Mpumalanga Province, South Africa

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Background: The malaria vector *Anopheles merus* occurs in the Mpumalanga Province of South Africa. As its contribution to malaria transmission in South Africa has yet to be ascertained, an intensification of surveillance is necessary to provide baseline information on this species. The aim of this study was therefore to map *An. merus* breeding sites in the Ehlanzeni District of Mpumalanga Province and to assess qualitative trends in the distribution and relative abundance of this species over a 9-year period.

Methods: The study was carried out during the period 2005–2014 in the four high-risk municipalities of Ehlanzeni District. Fifty-two breeding sites were chosen from all water bodies that produced anopheline mosquitoes. The study data were extracted from historical entomological records that are captured monthly.

Results: Of the 15 058 *Anopheles* mosquitoes collected, 64% were *An. merus*. The abundance and distribution of *An. merus* increased throughout the four municipalities in Ehlanzeni District during the study period.

Conclusion: The expanded distribution and increased abundance of *An. merus* in the Ehlanzeni District may contribute significantly to locally acquired malaria in Mpumalanga Province, likely necessitating the incorporation of additional vector control methods specifically directed against populations of this species.

Previously considered as only a minor, or even unimportant malaria vector, potentially unable to sustain transmission alone,[1] *Anopheles merus* has now been identified as an 'unexpectedly' important vector species along the Tanzanian coast and in Mozambique.[2,3] It has also been implicated in malaria transmission in Kenya and Madagascar.[4–6] Originally referred to as a 'salt water *An. gambiae*' variant, it is now characterised as a member of the *An. gambiae* species complex.[7] Other members in this complex include *An. gambiae* sensu stricto, *An. coluzzii* and *An. arabiensis* which, together with *An. funestus*, are recognised as the major African malaria vector species.[8]

As opposed to the fresh-water variants of the *An. gambiae* complex, *An. merus* mostly breeds along the eastern coastal salt-water areas of Africa. However, it has also been isolated further inland in both saline and fresh-water larval habitats in Mozambique, Zambia, Zimbabwe, Swaziland and South Africa.[9–14]

In the Ehlanzeni District of Mpumalanga Province in South Africa, an inland district with mostly fresh-water bodies, *An. arabiensis* is likely the major vector responsible for malaria transmission.[7,8,15] However, *An. merus* also occurs in this district, and it has been shown that, in sufficient numbers, *An. merus* can contribute significantly to the transmission of malaria.[2,3] Its increased breeding in habitats previously dominated by other mosquito species therefore has the potential to affect localised malaria epidemiology, and may also affect vector control strategies that are aligned to vector population localities, relative abundance, vulnerability and receptivity patterns of the vectors. Vector control in this region is based on indoor residual insecticide (IRS) spraying coupled with larval source management in selected areas.

It has been noted anecdotally that since its first identification in 1997 in the Ehlanzeni district,[12] *An. merus* has been increasing in terms of geographical range and relative abundance. This is thought to be a possible contributory factor to the persistence of malaria cases still reported in the district.[16]

Sporozoite infectivity rates and blood feeding index are markers used to incriminate *Anopheles* populations in malaria transmission. In Tanzania, *An. merus* was shown to have high sporozoite infectivity rates of up to 11.6%.[2] Two Mozambican studies also demonstrated a sharp increase in *An. merus* sporozoite infectivity rates, from 0.067% to 4.2%, between 2007 and 2009.[3,17] This species has also been incriminated as a vector of lymphatic filariasis in coastal East Africa.[18]

Although vector control primarily based on IRS, when coverage is sufficiently high, has been demonstrated to interrupt the malaria transmission cycle, successful implementation of vector control interventions is also somewhat dependent on precise knowledge of the ecology and behaviour of target species. With South Africa aiming to eliminate malaria by 2020, targeted vector control strategies need to be enhanced. Detailed knowledge of the breeding sites and abundance of *An. merus* populations will help in these interventions, given the encroachment of this species into new, fresh-water areas and its potential malaria infectivity. Recording the changes in the distribution over time of this species in inland districts of South Africa will help to elucidate the bionomics of this species. The aim of the present study was therefore to

†It is with great sadness that we acknowledge the passing of Mr Frans Mbokazi. This article is a tribute to him and his contribution to malaria vector research and control in South Africa.

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map *An. merus* breeding sites in the Ehlanzeni District and to assess qualitative trends in the distribution and relative abundance of this species over a 9-year period.

METHODS

Study design

This was a qualitative, descriptive cross-sectional study using routinely collected entomological data over a 9-year period, from July 2005 to June 2014, in Ehlanzeni District, Mpumalanga Province, South Africa.

Setting

General setting

Mpumalanga Province is one of the malaria-endemic provinces of South Africa. It is bordered by Gauteng and Limpopo Provinces in the west and north, respectively. It is estimated that over 1.7 million people, 43% of Mpumalanga Province's population, live in the low-lying areas and are at risk of contracting malaria. The disease is endemic in Ehlanzeni District, which is further subdivided into five municipalities, four of which are considered malaria-endemic.

Specific site

This study was carried out in the four high-risk municipalities of Ehlanzeni District: Bushbuckridge, Mbombela, Nkomazi and Umjindi. In these municipalities malaria transmission is unstable, seasonal and greatly influenced by climatic factors such as rainfall, temperature and relative humidity. In Mpumalanga Province, the malaria season typically starts after the first rains in October, peaks in December and January, and wanes in April–May.

Fifty-two breeding sites were chosen from water bodies that have produced members of both the *An. gambiae* complex and *An. funestus* group during the period under review.

Laboratory techniques and procedures

Larval sampling

Field collection teams visited each breeding site once a month. The anopheline larvae collected were transferred into labelled plastic cups and transported to the Driekoppies insectary (Mpumalanga Province), where they were reared to adults.

Morphological identification

The adult mosquitoes were killed by freezing and separated according to sex. Female mosquitoes were identified and then separated into *An. gambiae* complex and *An. funestus* groups using the morphological identification key of Gillies and Coetzee.[8] The mosquitoes were then individually placed in tubes containing silica gel desiccant and stored for molecular studies. The prepared specimens were assigned unique identifier numbers. Data on each specimen were recorded electronically in a separate MS Excel spreadsheet (Microsoft Corp, Redmond, WA, USA).

Molecular identification

Specimens belonging to the *An. gambiae* complex were identified to species level using the polymerase chain reaction (PCR) method of Scott et al.[19] Those belonging to the *An. funestus* group were identified using the PCR method of Koekemoer et al.[20] These procedures were conducted at the Vector Control Reference Laboratory of the National Institute for Communicable Diseases (NICD; Johannesburg, South Africa).

Data collection

Historical entomological data were extracted from the entomology database by two data assistants using standardised data abstraction forms. Data variables included a unique species identification per record, date of PCR identification, latitude and longitude coordinates, district, municipality, sector and locality where the collections were conducted, and total number of specimens collected by species. The extracted data were captured on the developed EpiData entry file (v3.1, EpiData Association, Odense, Denmark) during August and November 2015.

The Driekoppies entomology team verified the accuracy of the recorded global positioning system (GPS) coordinates for each *An. merus* breeding site identified.

Data analysis

The GPS coordinates were converted into degrees. Descriptive analyses were conducted and results presented as proportions. The recorded data files were converted to database files and then imported to ArcView software (Esri, Redlands, CA, USA) and visualised using an Ehlanzeni District shape file. Through the verified GPS coordinates for *An. merus* breeding sites, three sets of maps were produced.

Ethics approval

Ethical approval for the study was obtained from the Provincial Health and Research Ethics Committee of Mpumalanga Province and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (Paris, France), and Médecins Sans Frontières (Geneva, Switzerland).

RESULTS

The numbers of *Anopheles* mosquito breeding sites in each municipality are shown in Table 1. Nkomazi municipality had the most *Anopheles* breeding sites. The numbers and relative abundance of anopheline species collected in each of the municipalities over the 9-year period are shown in Table 2. *An. merus* predominated in all municipalities except Bushbuckridge.

TABLE 1 Number of water bodies identified as *Anopheles* mosquito breeding sites in each municipality, Ehlanzeni District, Mpumalanga Province, South Africa, 2005/2006–2014

Municipality	*n* (%)
Nkomazi	26 (50)
Mbombela	12 (23)
Bushbuckridge	9 (17)
Umjindi	5 (10)
Total	52

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Conflicts of interest: none declared.

TABLE 2 Numbers and relative abundance (percentages) of *Anopheles* mosquitoes collected by species by municipality, Ehlanzeni District, Mpumalanga Province, South Africa, 2005/2006–2014

Municipality	*An. merus* *n* (%)	*An. arabiensis* *n* (%)	*An. parensis* *n* (%)	*An. rivulorum* *n* (%)	*An. vaneedeni* *n* (%)	*An. quadrianulatus* *n* (%)	*An. leesoni* *n* (%)	Total
Nkomazi	7898 (63)	539 (4)	79 (<1)	937 (8)	124 (1)	2780 (22)	175 (1)	12532
Mbombela	1320 (82)	0	0	0	0	294 (18)	0	1614
Umjindi	360 (68)	0	0	0	0	171 (32)	0	531
Bushbuckridge	56 (15)	1 (<1)	0	0	0	324 (85)	0	381
Total	9634 (64)	540 (4)	79 (<1)	937 (6)	124 (<1)	3569 (24)	175 (1)	15058

The distribution trends over time showed that *An. merus* and *An. quadriannulatus* continuously increased in relative abundance (based on absolute numbers and proportions of specimens collected per season/year) from 2010/2011 to 2013/2014 (Figure 1). This trend was most pronounced in *An. merus*. The numbers collected and the relative abundance of the other species remained comparatively stable during the review period. Figures 2A–2C show the progressively increasing geographical distribution and relative densities of *An. merus* by municipality from 2005/06 to 2014.

During the period 2005/2006–2008, *An. merus* was confined to Nkomazi municipality, which had the highest mosquito density compared to the other three municipalities (Figure 2A). In the following years, this species spread to Mbombela and Nkomazi Districts, and finally to Bushbuckridge and Umjindi (Figures 2B and 2C).

DISCUSSION

This entomological survey spans a 9-year period and specifically focuses on the abundance and spatial distribution of *An. merus* in Ehlanzeni District. Of the 52 breeding sites identified for *An. merus*, half were in one municipality, Nkomazi. During the period under review, the spatial distribution of this species increased to include all the municipalities in the study. In addition, the preponderance of *An. merus* also increased with time in most of the municipalities.

The increasing abundance and distribution of *An. merus* in this region, for reasons unknown, is important because *An. merus* may be partially responsible for ongoing malaria transmission, which threatens South Africa's elimination programme.[16] Although this species has never been directly implicated in malaria transmission within South Africa, it has been implicated in neighbouring southern Mozambique.[3]

A strength of this study is that it extended over a long period—9 years—allowing the gathering of sufficient entomological data to map the spread of *An. merus*. Standardised mosquito collection and identification methods were used in all municipalities during this period. Nevertheless, some limitations are recognised. These data cannot be adequately correlated with clinical and epidemiological indicators because the mosquito abundances obtained by the larval sampling method may differ from those of adult sampling methods, and the actual contribution of *An. merus* to malaria transmission in South Africa has not been established.[21]

The increasing abundance and distribution of *An. merus* populations may hamper efforts to eliminate malaria in South Africa, especially if the reservoir of *Plasmodium* parasites increases via immigration of infected persons from other endemic areas. Significant population migration between the Ehlanzeni District and neighbouring malaria-endemic regions, including Mozambique, Swaziland and Zimbabwe, was recently noted.[22] The increasing distribution and abundance of *An. merus*, coupled with a possible increase in the *Plasmodium* parasite reservoir, may lead to an increase in the incidence of locally acquired malaria in Mpumalanga Province, necessitating the exploration of new vector control strategies specifically directed against populations of this species.

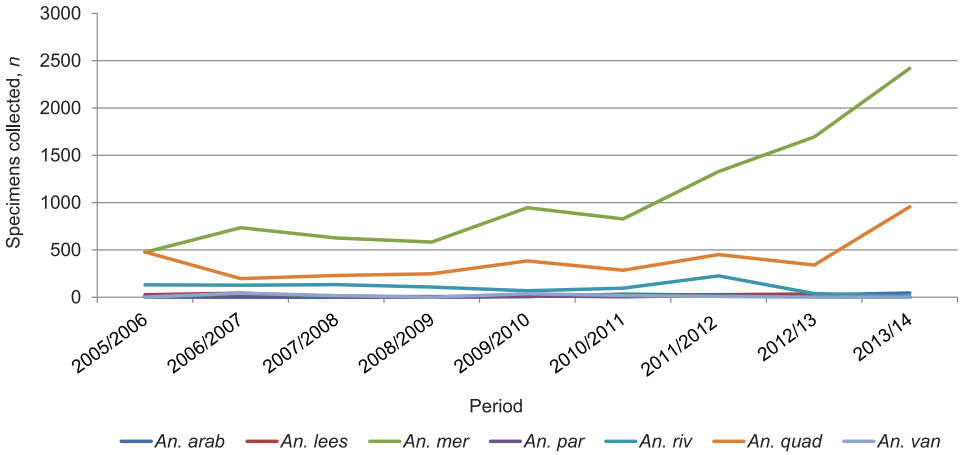

FIGURE 1 Trends in numbers of *Anopheles* mosquitoes collected by species over time in Ehlanzeni District, Mpumalanga Province, South Africa, 2005/2006–2014. *An. arab* = *An. arabiensis*; *An. lees* = *An. leesoni*; *An. mer* = *An. merus*; *An. par* = *An. parensis*; *An. riv* = *An. rivulorum*; *An. quad* = *An. quadriannulatus*; *An. van* = *An. vaneedeni*.

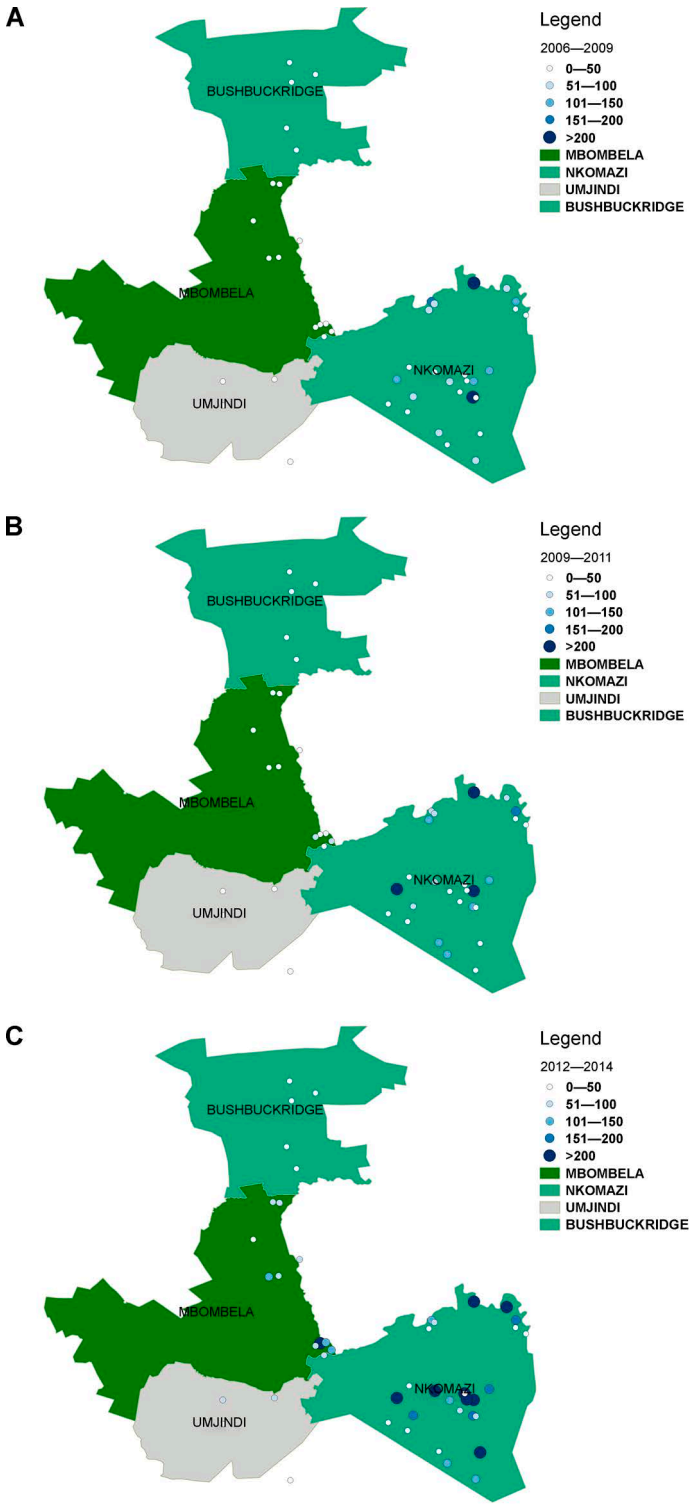

FIGURE 2 Distribution and relative abundance of identified *Anopheles merus* breeding sites by municipality in Ehlanzeni District, Mpumalanga Province, South Africa. **A)** During the period 2005/2006–2008. **B)** During the period 2009–2011. **C)** During the period 2012–2014.

Ongoing entomological surveillance is necessary to establish the role of *An. merus* and other *Anopheles* species in malaria transmission. These surveillance activities should also include insecticide susceptibility monitoring of the incriminated vector populations. Furthermore, information concerning the feeding, resting and breeding habits of incriminated species will enable the design of secondary control measures—such as targeted winter larviciding, screening of houses and direct focal spraying—that can be used to enhance the effectiveness of the IRS programme.

CONCLUSION

The distribution and abundance of *An. merus* has evidently increased in the Ehlanzeni District, most notably in the Nkomazi municipality. This trend may lead to an increase in the incidence of locally acquired malaria in Mpumalanga Province, likely necessitating the incorporation of additional vector control methods specifically directed against populations of this species.

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Contexte : Le vecteur du paludisme, *Anopheles merus*, sévit dans la province de Mpumalanga en Afrique du Sud. Comme sa contribution à la transmission du paludisme en Afrique du Sud reste à vérifier, une intensification de la surveillance est nécessaire afin de fournir des informations de départ sur cette espèce. Le but de cette étude a donc été de cartographier les sites de reproduction de *An. merus* dans le district d'Ehlanzeni de la province de Mpumalanga et d'évaluer les tendances qualitatives de la distribution et de l'abondance relative de cette espèce sur une période de 9 ans.
Méthodes : Cette étude a été réalisée pendant la période de 2005 à 2014 dans les quatre municipalités à risque élevé du district d'Ehlanzeni. Cinquante-deux sites de reproduction ont été choisis dans tous les plans d'eau qui ont produit des moustiques de l'espèce anophèle. Les données de l'étude ont été extraites de registres entomologiques historiques qui sont saisis chaque mois.
Résultats : Sur les 15 058 moustiques *Anopheles* recueillis, 64% ont été *An. merus*. L'abondance et la distribution d'*An. merus* ont augmenté dans les quatre municipalités du district d'Ehlanzeni pendant la période d'étude.
Conclusion : La distribution en expansion et l'abondance accrue d'*An. merus* dans le district d'Ehlanzeni peut contribuer significativement au paludisme acquis localement dans la province de Mpumalanga et nécessite l'incorporation de méthodes de lutte vectorielle supplémentaires spécifiquement dirigées contre les populations de cette espèce.

Marco de referencia: *Anopheles merus*, vector del paludismo, está presente en la provincia de Mpumalanga de Suráfrica. Puesto que no se ha determinado su contribución a la transmisión del paludismo en el país, es necesario intensificar la vigilancia, con el fin de aportar información de referencia sobre esta especie. El objetivo del estudio fue cartografiar los criaderos de *An. merus* en el distrito de Ehlanzeni de la provincia de Mpumalanga y evaluar la evolución cuantitativa de la distribución y la abundancia relativa de esta especie durante un período de 9 años.
Métodos: El estudio se llevó a cabo del 2005 al 2014 en cuatro municipios de alto riesgo de transmisión del distrito de Ehlanzeni. Se escogieron 52 criaderos de todas las masas de agua productoras de mosquitos anófeles. Los datos del estudio se extrajeron de los registros entomológicos históricos que se captan cada mes.
Resultados: De los 15 058 mosquitos anófeles recogidos, el 64% correspondía a *An. merus*; su abundancia y distribución aumentó en los cuatro municipios del distrito de Ehlanzeni durante el período del estudio.
Conclusión: La ampliación de la distribución y el aumento de la presencia de *An. merus* en el distrito de Ehlanzeni pueden contribuir de manera significativa a los casos de paludismo adquiridos localmente en la provincia de Mpumalanga, y es probable que sera necesario incorporar otros métodos de control de vectores dirigidos específicamente contra las poblaciones de esta especie.

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