Malaria vector control in South Africa

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Malaria vector control is primarily insecticide based and relies on indoor residual spraying (IRS) and the distribution of long-lasting insecticide-treated bednets (LLINs). These interventions have generally proved effective where appropriately implemented. However, the increasing incidence of insecticide resistance in target vector populations can, and in several cases already has, undermined the effectiveness of IRS and LLINs. Today there are very few localities on the African continent where populations of vector mosquitoes are still susceptible to the approved classes of insecticides. If elimination is to be achieved, SA needs to invest more resources into malaria control.

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Malaria vector control is primarily insecticide based. It relies on indoor residual spraying (IRS) of houses and the distribution of long-lasting insecticide-treated bednets (LLINs). Only twelve insecticides from four different chemical classes (organochlorines, organophosphates, pyrethroids, and carbamates) are currently recommended by the World Health Organization (WHO) for IRS.[1] The oldest classes of these insecticides are the organochlorines, of which dichlorodiphenyltrichloroethane (DDT) is the only approved insecticide, and the organophosphates, which are derived from phosphoric acid. Both have been used since the 1940s. All 12 insecticides collectively target two insect neurological sites. Currently, only pyrethroids are approved by the WHO for LLIN treatment.

These interventions have generally proved effective where appropriately implemented.[2] However, the increasing incidence of insecticide resistance in target vector populations can, and in several cases already has, undermined the effectiveness of IRS and LLINs.[3-6] Insecticide resistance in target vector populations, coupled with environmental and health concerns associated with insecticide use, has led to a burgeoning interest in the development of alternative forms of vector control,[7] as well as to the implementation of integrated vector-management strategies.[8, 9]

1. Malaria mosquitoes in South Africa

1.1 Species identification

The first essential step in a malaria vector control programme is the accurate identification of the mosquitoes involved in transmission of the disease so that scarce resources are not wasted on harmless insects. The first step in this process is the microscopic examination of the external morphology of the adults or larvae. This starts with recognition of characters that separate *Anopheles* mosquitoes from other culicine mosquitoes and then 'fine-tuning' the process to identify species within the genus *Anopheles* so as to group mosquitoes into possible vectors and non-vectors.[10,11] However, the process does not stop there as many species are now known to belong to 'species complexes' or group of species that look identical under the microscope. These species need to be identified using modern molecular methods. One such group is the *Anopheles gambiae* complex. It comprises eight members of which *An. gambiae*, *An. coluzzii* and *An. arabiensis* are major malaria vectors, *An. merus*, *An. melas* and *An. bwambae* are minor/localised vectors, and *An. quadriannulatus* and *An. amharicus* are not known to transmit malaria.[11-14]

Similarly, *An. funestus* is the nominal member of a group of nine African species and the only one considered to be of any importance in malaria transmission.[15] While other members of the group (*An. parensis*, *An. vaneedeni* and *An. rivulorum*) are either non-vectors or of very limited importance, the fact that they are easily confused with *An. funestus* using morphological characters means that accurate, molecular identification techniques developed in South Africa (SA)[16,17] are needed for this group, just as they are for the *An. gambiae* complex.

1.2 Vector incrimination

Mosquitoes are incriminated in malaria transmission based on the detection of *Plasmodium* sporozoites by direct dissections (Fig. 1) in the salivary glands of identified females or circumsporozoite protein (CSP) detection by enzyme-linked immunosorbent assays (ELISAs) and polymerase chain reaction (PCR). Infection rates for *An. funestus* in SA in the past have been recorded as high as 27% in the Letsitele Valley of Limpopo Province in the 1930s[18] while in the 1999/2000 epidemic the *An. funestus* infection rate was approximately 5% in northern KwaZulu-Natal (KZN) Province.[19] There are no recent records of *Plasmodium* sporozoite-positive *An. arabiensis* or *An. merus* specimens from SA; older reports are unreliable due to lack of definitive species identification. In general, very few studies have been carried out in the past 40 years on the infectivity of South African mosquito populations[20,21] (unpublished National Institute for Communicable Diseases (NICD) data, 2005 - 2012). By comparison, Table 1 gives sporozoite infection rates for the three species in the Maputo region of southern Mozambique, showing the clear

impact on transmission that was achieved by the Lubombo Spatial Development Initiative (LSDI).[22] In SA in general, *An. funestus* is usually associated with outbreaks and epidemics while *An. arabiensis* is considered largely responsible for comparatively low-level seasonal transmission in the malaria-affected regions.[23]

The study carried out by De Meillon *et al.*[20] in 1977 showed that *An. vaneedeni* was readily infected with *Plasmodium falciparum*, but this species has never been implicated in malaria transmission in nature.[13]

1.3 Bionomics and distribution of vector species

An. arabiensis occurs in all three of SA's malaria affected provinces. It tends to prefer dry, savannah environments and generally breeds in small, sunlit, temporary, fresh-water pools. However, breeding sites can also be found along the margins of dams in cattle hoof prints as well as in rice paddies.[11,13] Adult females will feed on both cattle and humans and can be found resting both inside human habitations and outdoors.[11,13] The outdoor resting components of *An. arabiensis* populations are largely unaffected by IRS and are almost certainly responsible for the low-level seasonal malaria transmission experienced in SA each year.[23] The saltwater breeding member of the *An. gambiae* complex, *An. merus*, is widespread both inland and on the coastal plains.[13]

An. funestus prefers to breed in permanent and semi-permanent fresh-water pools or slow-moving streams containing emergent vegetation.[11,13] Unlike *An. arabiensis*, this species is highly anthropophilic and endophilic making it especially amenable to control by IRS.

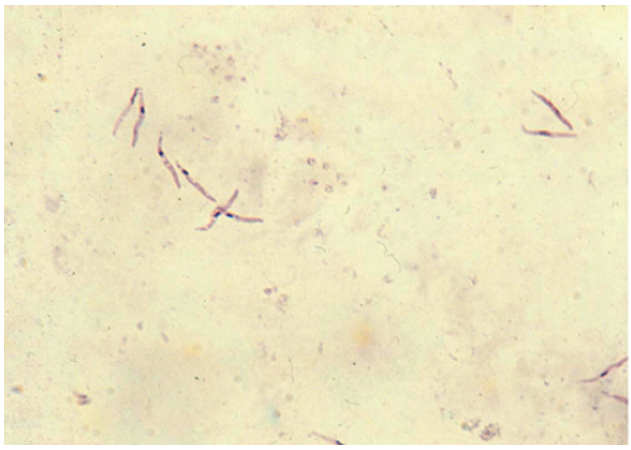

Fig. 1. Sporozoites from mosquito salivary glands.

2. Insecticide resistance

Insecticide resistance in malaria vector populations is widespread. The increasing use of insecticides for malaria vector control over the past decade coupled with ongoing agricultural pesticide use has led to selection for resistance genes in a great many vector populations.[24] Resistance to insecticides in South African malaria vector populations is a relatively recent occurrence. The first documented tests for insecticide susceptibility in local malaria vectors took place in 1978 (unpublished newsletters of the National Institute for Tropical Diseases, 1978 - 1980) with no resistance reported. No further tests were carried out until those described by Gericke *et al.*[25] Samples of *An. arabiensis* were collected in 1996 from Thomo and Malahlapanga in Limpopo Province and Mamfene in KZN. They were assayed against DDT, deltamethrin, fenitrothion and propoxur using the standard WHO bioassay technique for testing the susceptibility of adult anopheline mosquitoes.[26] In these tests, the Malahlapanga population was used as a control population from an insecticide-free area (Kruger National Park), against which the populations from Mamfene and Thomo were assessed, both of which are located in areas that, at that time, had experienced insecticide use either for agricultural and public health purposes (Thomo) or for public health only (Mamfene). All populations showed full susceptibility to DDT, deltamethrin and fenitrothion. The Malahlapanga and Mamfene populations also proved fully susceptible to propoxur whereas the Thomo population showed evidence of propoxur resistance.[25]

In response to the South African malaria epidemic that peaked in 2000, samples of anopheline mosquitoes were collected from the Ndumu region of northern KZN during 1999.[19] These samples were primarily collected in window exit traps or by pyrethrum spray catches. Male anophelines, morphologically identified as members

Table 1. Sporozoite infection rates (%) in three vector species in southern Mozambique

Period	*An. funestus*	*An. arabiensis*	*An. merus*	Reference
1994 - 1996	2.4	1.1	-	Mendis *et al.*, 2000[49]
1999	4.7	7.1	2.2	Sharp *et al.*, 2007[22]
2000	4.3	9.6	4.2	Cuamba and Mendis, 2009[50]
2005	2.2	0.8	0.7	Sharp *et al.*, 2007[22]

Table 2. Insecticide resistance reported in South Africa

Insecticide	Species	Year	24-hour post exposure mortality rate, % (insecticide)	Reference
DDT	*An. arabiensis*	2002	86.5	Hargreaves *et al.*, 2003[30]
	An. arabiensis	2005	78 (permethrin)	Mouatcho *et al.*, 2009[21]
Pyrethroid	*An. funestus*	1999	86 (permethrin)	Hargreaves *et al.*, 2000[19]
Carbamate	*An. funestus*	2000	56 (propoxur)	Brooke *et al.*, 2001[28]
Organophosphate	None reported			

DDT = dichlorodiphenyltrichloroethane.

of the *An. funestus* group, that had been collected live in the window traps, were assessed for their susceptibility to permethrin in the field using a bottle bioassay.[27] The progeny of wild-caught females, also identified as *An. funestus* group, were assayed under laboratory conditions for permethrin resistance using the bottle bioassay as well as the standard WHO method.[26] Species identification of the wild mosquitoes used the new molecular method of Koekemoer *et al.*[16] The insecticide bioassays revealed high levels of permethrin resistance in *An. funestus* but full susceptibility to DDT (Table 2). Samples of the closely related species *An. rivulorum* and *An. parensis* from the same collections gave no indication of resistance to permethrin.[19] Subsequently, more substantial collections of *An. funestus* were carried out in 2000 from the Beluluane region of southern Mozambique. Resistance to the pyrethroids deltamethrin and lambda-cyhalothrin was demonstrated. Furthermore, resistance to propoxur was detected while full susceptibility to DDT was confirmed.[28] It has since been established that southern African *An. funestus* are fully susceptible to the pyrrole insecticide chlorfenapyr regardless of their resistance status to pyrethroids.[29]

Samples of *An. arabiensis* and *An. merus* from the Beluluane region of southern Mozambique, collected in 2000, did not show any indication of resistance to pyrethroids (deltamethrin and lambda-cyhalothrin), DDT, carbamates (propoxur and bendiocarb) or the organophosphate fenitrothion.[28] However, collections of *An. arabiensis* in 2002 from northern KZN revealed resistance to DDT, but full susceptibility to deltamethrin.[30] Subsequent collections in Mamfene during 2005 revealed resistance to permethrin and suspected resistance to deltamethrin.[21] At the same time, resistance to deltamethrin was also recorded in the non-vector species *An. parensis* from Mamfene.[31]

2.1 Mechanisms of resistance

The insecticide susceptibility assays for adult mosquitoes are direct response-to-exposure tests that provide no insight on the underlying genetic mechanisms of resistance, when detected. This information can be obtained using various molecular and biochemical assays.

The elucidation of the underlying mechanisms of pyrethroid resistance in southern African *An. funestus* was made possible by the successful laboratory colonisation of this species using wild-caught material from southern Mozambique.[32] It has been demonstrated that pyrethroid resistance is primarily based on the upregulated detoxifying capabilities of at least two P450 monooxygenase genes.[15,28,33-36] The resistance phenotype is most likely inherited as a single, incompletely dominant factor and the expression of pyrethroid resistance does not affect developmental and reproductive fitness in resistant mosquitoes.[37,38] Furthermore, the expression of pyrethroid resistance is enhanced by blood-feeding in those females carrying the resistance genotype,[39] and pyrethroid-resistant mosquitoes are likely to have thicker cuticles than their insecticide susceptible counterparts.[40] The expression of pyrethroid resistance in southern African *An. funestus* generally decreases with age although this effect was mitigated by successive blood feeding and mating under laboratory conditions.[32,36]

Pyrethroid resistance in the wild population of *An. arabiensis* from Mamfene is also primarily based on monooxygenase detoxification.[21,41] Microarray analysis of a DDT and pyrethroid-resistant laboratory colony, which was established using wild-caught material from Mamfene, revealed over-transcription of 20 genes associated with the DDT/pyrethroid resistance phenotype.[42] Most of these genes are monooxygenases and glutathione s-transferases. It is interesting to note that although no knockdown resistance (*kdr*) mutations have ever been recorded in wild *An. arabiensis* from Mamfene,[21] including the most recent sample (n=100) collected in 2012 (unpublished data, NICD), the laboratory colony on which these studies are based and which has been intensively selected for resistance to DDT, is now fixed for the L1014F *kdr* mutation. Association analyses revealed that DDT and permethrin resistance in this laboratory colony is closely linked to the L1014F mutation while deltamethrin resistance is primarily based on enzyme detoxification.[42]

2.2 Insecticide resistance management (IRM)

The occurrence of insecticide resistance in malaria vector populations in SA necessitates the design of tailored strategies by region/province to manage resistance and thereby maintain vector control programme efficacy. As insecticide resistance has been detected in vector populations in nearly two-thirds of those countries experiencing ongoing malaria transmission, a Global Plan for Insecticide Resistance Management (GPIRM) has been developed by the WHO.[43] This plan lists several strategies for IRM including rotations of insecticides, mosaic insecticide spraying and combinations of interventions. These are designed to preserve the efficacy of those insecticides available for public health use either by circumventing the development of resistance in target populations or by slowing the spread of resistance where it already occurs. These strategies can easily be incorporated into those programmes, based on an integrated vector management approach.

Currently, malaria vector control in SA's three malaria-affected provinces is based on an IRS mosaic approach in which pyrethroids are used for cement-brick structures while DDT is used for traditional mud-walled structures. Carbamates are also used for IRS in some instances. Cross-border malaria control initiatives with neighbouring countries[22] also formed part of an integrated plan that was designed to achieve malaria elimination within SA's borders by 2015.[44,45]

3. IRS programme logistics

3.1 Programmatic organisation

While policy development on all malaria-control interventions takes place at national level, their implementation is the responsibility of the Provincial Departments of Health. Vertically-structured provincial malaria control programmes were established in KZN, Mpumalanga and Limpopo in 1995. Each province has its own malaria control structure, headed by a provincial Malaria Control Programme Manager.

IRS activities are carried out by malaria spray operators divided into malaria spray teams. These teams have bases in their respective operating areas from where IRS services are delivered to surrounding communities. The size of spray teams varies depending on the operational area, community sizes and access routes. Malaria spray teams consist of a number of spray operators (2 - 10), 1 - 2 foremen and a team leader. Teams are transported daily to their respective work areas in the communities from the malaria unit or base.

Due to the seasonal nature of malaria in SA, with IRS only taking place during the early summer, the majority of spray operators are employed as temporary workers. Malaria spray teams are supervised by qualified environmental health practitioners with reporting lines to district and provincial malaria units. Training of spray operators, in line with WHO guidelines, takes place annually. The training focuses on the correct application of insecticides, safe handling (Fig. 2) and disposal of waste.

DDT and WHO Pesticide Evaluation Scheme (WHOPES)-accredited pyrethroids (e.g. deltamethrin and alpha-cypermethrin) are currently used for spraying operations. Dwellings are sprayed

Fig. 2. Spray operators are required to wear protective clothing during indoor residual spraying operations. (Image: R. Hunt, NICD.)

before the main transmission season, commonly before the end of December. Follow-up or mop-up spraying, in areas where needed, then continues until March. At district and sub-district level there is integration and collaboration with other health programmes, e.g. Primary Health Care and Health Promotion. Approximately 1.77 million structures are covered with IRS every year in SA (National Department of Health, unpublished data).

Record-keeping systems are in place to record all IRS activities. These consist of 'hutcards' that are completed at each sprayed dwelling. Pertinent information including date, insecticide used and spray operator's details are recorded on each hutcard. This card remains with the householder for future monitoring and record purposes. The daily performance of spray operators is also recorded, with spray data entered into electronic information systems. The IRS programmes are fully funded out of each respective provincial Health Department's budget. The annual budget for malaria control at provincial level currently ranges from R35 million to R88 million depending on province and vector control accounts for about 70% of the provincial malaria budgets in any given year.

Although the IRS programmes have been highly successful in reducing malaria transmission to low levels, some operational challenges are experienced. Some of these are:

- Growth of communities in endemic areas exceeds the capacity of the malaria spray teams.
- Dwellings have become more sophisticated with extensive furnishings that inadvertently provide more vector mosquito resting sites. High IRS coverage is difficult to achieve in such dwellings. The deposits left on walls by DDT are also not always accepted by communities.

Opportunities to improve IRS through better stratification of spraying activities are currently being explored. These include the use of geographical information systems to monitor and record spray performance at household level, as well as linking IRS information to malaria case notifications within communities.

4. Use of DDT in the malaria control programme

The introduction of the highly effective public health insecticide, DDT, shortly after the end of World War II, allowed the malaria control programme to greatly expand its activities and dramatically reduce the burden of disease. Given that much of the cost of a malaria control spraying programme is for labour and logistics, the fact that DDT remains effective for the whole transmission season meant that households could be protected with just one spray round per year. As a result of the expansion of the spray programme, malaria, which historically occurred as far south as Port St Johns and as far inland as Pretoria, was pushed back to the north-eastern Lowveld areas bordering Mozambique and Zimbabwe.[46]

Since the 1960s, DDT has been receiving progressively more attention from various lobby groups with respect to its harmful effects on humans and the environment, often based on suspect data. In 2000, at the height of SA's malaria epidemic, the Stockholm Convention on Persistent Organic Pollutants (POPs) held its fifth and final negotiating committee meeting in Johannesburg. The South African government, along with the assistance of international scientists and an independent advocacy group, managed to secure an exemption for those countries wishing to use DDT for malaria vector control purposes. Currently, the SC acknowledges that there is an ongoing need for DDT and allows its use for vector control according to strict guidelines drawn up by the WHO.[47,48] Countries that wish to use DDT are required to register with the Convention Secretariat and report back on a regular basis as to quantities of DDT used and what research has been undertaken to investigate equally effective, safe and affordable alternatives. To this end, some countries are being supported by the Global Environment Facility to carry out integrated vector management with the aim of reducing reliance on DDT. In the meantime, DDT remains an important insecticide in the arsenal of public health insecticides for malaria vector control.

5. The role of entomologists in malaria vector control

Entomological surveillance is a fundamentally important activity in a malaria vector control programme. Without skilled entomological support, analysis and decision making for IRS, particularly in terms of IRM, is not possible. Therefore, there is a need for the national and provincial malaria control programs to develop capacity within this field.

Malaria entomologists provide guidance and support to malaria vector control programmes. They should participate in the planning, implementation, monitoring and evaluation of the programme. The skills required for adequate entomological surveillance include mosquito sampling in the field, species identification using morphological and molecular methods, vector incrimination using immunological methods, and insecticide susceptibility testing using standard biological (bioassay) techniques. Follow-up operational research activities and specialised diagnostics involve more detailed and sophisticated techniques that are usually undertaken by a specialist laboratory.

The WHO has recently recommended that routine insecticide susceptibility monitoring and evaluation should be supervised by a qualified entomologist with at least an MSc degree in entomology.[43] There is currently a shortage of trained entomologists on the African continent, including SA. It is important to note that this role cannot be adequately performed by field technologists or technicians as their skills lie in a different area and are not sufficiently specialised to include entomological surveillance.

6. Challenges for malaria vector control

6.1 Increasing spread of resistance

This is an increasing problem in all African malarious countries. Today there are very few localities on the continent where populations of vector mosquitoes are still susceptible to the approved classes of

insecticides. Pyrethroid resistance has exploded in concert with the scale-up of pyrethroid-treated bednets, causing concern for those countries that depend on LLINs for vector control. In SA, pyrethroid resistance in the Mozambican population of *An. funestus* remains a threat, particularly in the face of changes in housing construction in rural areas that is leading to less traditional-style housing. There are therefore fewer structures sprayed with DDT, opening the way for *An. funestus* to return to SA once again.

6.2 Lack of new chemicals

While this is being addressed by initiatives such as the Innovative Vector Control Consortium, the process is slow and expensive and it is likely that no new chemicals will be available for at least 5 years.

6.3 Diminishing resources

As other diseases are perceived to be more important for the health of South Africans, so resources are redirected away from malaria control. This has serious potential for us to see history repeat itself, with epidemics occurring on a scale last seen 13 years ago. If elimination is to be achieved, SA needs to invest more resources into malaria control, not less.

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