Entomological surveillance for malaria control at a gold mine in Sadiola District, western Mali

Sue-Ellen Wragge

#1938168

A dissertation submitted to the faculty of Health Sciences,

University of Witwatersrand, Johannesburg.

In fulfilment of the requirements for the degree of

Master of Science in Medicine

Supervisors: Profs M Coetzee and L.L. Koekemoer

WRIM

Declaration

I, Sue-Ellen Wragge, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in Medicine at the University of Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at any other university.

SWragge

Signature

8th day of February 2021

In loving memory of my Father

John Wragge

1942 – 2009

Publications

Wragge S., Venter N., Touré D., Hunt RH., Coetzee M. (2020). New distribution record of *Anopheles rivulorum*-like in Sadiola, Mali, with notes on malaria vector insecticide resistance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* https://doi.org/10.1093/trstmh/traa113
(Accepted for publication on 5 October 2020. See Appendix 3. Published online, 22 October 2020).

Authors' contributions

1. Wragge, S-E. – part of the MSc research project
2. Venter, N. – assisted with insecticide resistance bioassays and processing mosquitoes at the NICD in Johannesburg.
3. Toure, D. – assisted with fieldwork.
4. Hunt, R.H. – responsible for previous surveys and gave advice on programmatic issues.
5. Coetzee, M. – supervisor.

Abstract

The aim of this research was to determine if vector control methods used in the SEMOS gold mine vector control programme (Kayes district, south-western Mali), were effective in reducing malaria transmission. Historical entomological surveys to manage insecticide resistance in the main *Anopheles* mosquito vectors were compared with current surveys.

Mosquitoes were collected from potential breeding habitats within and adjacent to the control zone. Adult *Anopheles* mosquitoes were exposed to insecticides using standard insecticide susceptibility bioassays and subsequently identified using morphological keys and molecular methods for members of the *Anopheles gambiae* complex and *An. funestus* group. The Walter Reed Biosystematics Unit's West African adult keys were used for identifying *Culex* and *Aedes* mosquitoes. Insecticide resistance data and species identification from 2018 were compared with previous surveys in 2006, 2011, 2014 and 2016. In all years, *An. gambiae* and *An. arabiensis* were present and most abundant. *Anopheles coluzzii* was present in all five years in small numbers. *Anopheles funestus* was collected in four of the five years, *An. leesoni* in 2016, *An. rivulorum* in 2006 and *An. rivulorum*-like in 2018. The last species is recorded for the first time in Mali.

Rotation of insecticide classes using a mosaic indoor spray pattern, has resulted in maintaining *An. arabiensis* susceptibility to five of the insecticides in three classes tested in 2018, with only resistance to DDT (86.9% mortality) still present. Including the treatment of pit toilets into the SEMOS vector control programme has reduced nuisance and other vector mosquito species in the area.

Keywords: Vector Control, entomological surveys, insecticide resistance, rotation, indoor residual spray, mosaic spray pattern.

Acknowledgements

I would like to thank friends at SEMOS gold mine who assisted during the study: Mr Francois Taljaard, Mr Geoff Gushee and Mrs Ellen van Schalkwyk for their assistance with analysing the data obtained during the research; Prof Richard Hunt, Mr Nelius Venter, Mr Craig Davis and the malaria spray team for their assistance with mosquito collections; the SEMOS goldmine management for their support; and AngloGold Ashanti for allowing me to carry out the research.

The National Institute for Communicable Diseases is thanked for the laboratory processing of mosquitoes for molecular species identification.

Profs Maureen Coetzee and Lizette L Koekemoer are thanked for giving me the opportunity to do this research and their direction and assistance is greatly appreciated. Thank you.

Last, but not least, to my husband Mr Morne Smuts who endured many proof reading sessions and listening to my mosquito stories, thank you!

Table of Contents

List of Figures

List of Tables

List of Nomenclature / Abbreviations

BPM	Bendiocarb in conjunction with pirimiphos-methyl
Bti	*Bacillus thuringiensis* var *israelensis*
BVIP	Blair Ventilation Improvement Pit
CHIKV	Chikungunya virus
CS	Capsular Suspension
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue virus
EC	Emulsifiable Concentrate
FNV	French Neurotropic Vaccine
GDP	Gross Domestic Product
IMF	International Monetary Fund
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
LF	Lymphatic Filariasis
LIDAR	Light Imaging and Detection and Ranging
LLIN	Long-Lasting Insecticide Net
NGO	Non-Governmental Organization
NICD	National Institute for Communicable Diseases
NMCP	National Malaria Control Programme
PBO	Piperonyl butoxide
PMI	The President’s Malaria Initiative
RDT	Rapid Diagnostic Test

RH%	Relative Humidity percentage
SEMOS	Société des Mines d’Or du Mali
UNICEF	United Nations Children’s Fund
WG	Wettable Granules
WHO	World Health Organization

Chapter 1.

Introduction

1.1 Malaria – the Global African Burden

The World Health Organization (WHO) stated in 2014 that vector-borne diseases account for about 17% of the global burden of all infectious diseases. Mosquitoes are some of the best disease-transmitting insect vectors globally, and malaria is one of the most devastating diseases with regards to morbidity and mortality (Ferguson, 2018). The WHO estimated that 228 million people contracted the parasite globally, resulting in 405,000 deaths in 2018, with 93% (213 million) of these positive malaria cases and 85% of the deaths occurring in Africa (WHO, 2019). Many of the worst affected countries fall into the low to low-middle economic bracket and are therefore often unable to raise the financial resources required to combat the malaria burden (Hay *et al.*, 2004). Lack of political will, corruption and conflict in the region impacts on the logistics for vector control programmes and medication needed for the treatment of these cases (Bates *et al*., 2004; Gayer *et al.*, 2007).

The global economic burden of malaria amounted to US$ 2.7 billion for 2018 of which 47% was allocated to low-income countries (WHO, 2019). UNICEF estimates that countries in Africa lose up to US$12 billion each year in Gross Domestic Product (GDP) as a direct cost of malaria (UNICEF Global Databases, 2019). Malaria places a heavy burden on individuals, households, communities and governments, with the disease being linked to the reduction of economic growth in some African countries by as much as 1.3% over a year (Hailu *et al.*, 2017). Alleviation of poverty in a country has a knock-on effect of reducing malaria cases and improvements in treatment. Poorer households are less likely to seek medical assistance due to costs and rather seek traditional healers or home remedies for treatment, increasing the severity of malaria or death (Worrall *et al.*, 2005). This is worse at the beginning of the rainy season in rural areas, when money is at its lowest, as income obtained for the previous years' crop harvest has been spent (Heggenhougen *et al.*, 2003).

1.2 Malaria in Mali

Mali is as one of the poorest countries in the world (Bleck & Michelitch, 2015) with approximately 65% of the population living in poverty. The African Economic Outlook for 2019 and the International Monetary Fund (IMF) show that Mali still faces a debt distress level described as “moderate risk”, meaning that any abrupt changes to the country’s macroeconomic policies will influence the country’s ability to repay its debt. Mali’s population estimate is 18.5 million (2015) of which 100% of the population are at risk of contracting malaria. Mali’s health care system is a decentralized model with community participation to extend health care coverage. The President’s Malaria Initiative (PMI) Mali 2018 reports that the state operated 1,204 functional health care centres, however the Ministry of Health is experiencing a medical staffing shortage with less than 1 doctor per 24,000 patients in rural areas. The WHO recommends a ratio of one doctor per 10,000 patients (PMI Mali, 2018).

Mali is divided into 10 regions namely: Gao, Kayes, Kidal, Koulikoro, Menaka Mopti, Sikasso, Ségou, Taoudeni, Tombouctou and the capital Bamako. Malaria is endemic in the central and southern regions where 90% of the population live. Malaria transmission varies in the five geo-climatic zones, occurring all year round in the Sudano-Guinean zone in the south of the country, with peak transmission during the main rainy season (June to November). The northern Sahelian zone’s transmission is during the months July/August to October. Malaria is endemic in the Niger delta area, around dams and rice fields. Transmission is regarded as low in the urban areas of Bamako and Mopti (PMI Mali, 2018).

Malaria is still the major cause of mortality and morbidity in Mali, with a malaria incidence per 1,000 population of 448.6. Children under the age of five years old are still the most vulnerable, with this age group making up 36% of all cases treated. Mortality rates from 2013 for children under the age of five was 98/1,000 live births (PMI Mali, 2018). Malaria in pregnancy is a large contributing cause of premature births, stillbirths, anaemia and maternal deaths (Berry *et al.*, 2018), this, despite a Malian Health Ministry national policy in line with the WHO prescribed standards of at least three doses of sulfadoxine-pyrimethamine at the beginning of the second trimester. Only 35% of pregnant women receive their first dose and less than 20% receive the second and third dose. The cost of this treatment is still a barrier as many are unable to pay and thus unable to access health services in their area (Klein *et al.*, 2016).

Since 2010 the number of malaria cases treated based on positive malaria tests, has increased from 18% in 2010 to 93% in 2016. Results obtained (PMI Mali, 2018) show that *Plasmodium falciparum* accounts for 95% of the malaria cases, with *P. malariae*, *P. vivax* and *P. ovale* making up the remaining 5%. Mali follows the WHO treatment guidelines of using combination therapy of artemether-lumefantrine as first line treatment for uncomplicated malaria or artesunate-amodiaquine as a second line therapy. Artesunate injections (intravenous or intramuscular) are recommended as the first line treatment for severe malaria, including pregnant women and children. Oral quinine is still the drug of choice in treating uncomplicated malaria in pregnancy. In remote areas of Mali the reliance on traditional healers' treatment before seeking modern medical treatments still exists due to travelling distance and road conditions, especially during the rainy season (Diallo *et al.*, 2006).

1.2.1 *Anopheles* species

There are four main malaria vector species in Mali: *Anopheles gambiae s.s., An. coluzzii, An. arabiensis* and *An. funestus* (Kyalo *et al.*, 2017; Wragge *et al.*, 2015). The first three are members of the *Anopheles gambiae* complex, with preferred larval breeding in temporary pools and rice fields. *Anopheles gambiae* and *An. coluzzii* are closely associated with humans and human habitats, feeding on humans and resting indoors. *Anopheles arabiensis* will feed on cattle and rest outdoors, thus reducing its contact with humans (Massebo *et al.*, 2013), but is still a recognised vector of malaria throughout Africa (Kyalo *et al.,* 2017). In Mali, the practice of placing cattle in the centre of the village for protection increases the chances of *An. arabiensis* feeding on humans (Balcha *et al.*, 2002). *Anopheles funestus* is known to favour permanent and semi-permanent water bodies for breeding. It is highly anthropophilic (preferring to bite humans) and endophilic (living indoors) (Gillies & De Meillon, 1968; Braack *et al.*, 2015) with feeding taking place mainly during the second half of the night. Irish *et al.* (2020) list 22 other species of *Anopheles* found in Mali.

1.2.2 Insecticide resistance in the malaria vectors

Coleman & Hemingway (2007) noted that the potential for insecticide resistance developing in *Aedes* and *Anopheles* mosquitoes existed during the 1950's, but was poorly documented. Currently, there are five insecticide classes approved by WHO for use in vector control: pyrethroids, carbamates, organophosphates, organochlorines and most recently, neonicotinoids. Resistance to compounds in the first four classes has been recorded in all African malaria vector mosquitoes (Wiebe *et al*., 2017). Management of insecticide resistance is key to effective control of adult malaria vectors. Fanello *et al.* (2003) commented on the development of pyrethroid resistance in Bamako in 1987. The Malian National Malaria Control Programme (NMCP) mainly used pyrethroid and carbamate insecticides for indoor residual spraying (IRS) from 2008 - 2013. An entomological survey carried out by the NMCP showed insecticide resistance to both classes, resulting in a change to organophosphate insecticides for IRS in 2014 (PMI Mali, 2018; Sovi *et al.*, 2020). WHO insecticide susceptibility assays using 10x the deltamethrin discriminating dose, gave a mortality rate of between 53-91% in *An. gambiae s.l.* (PMI Mali, 2018), confirming intense pyrethroid resistance. Insecticide resistance in *An. gambiae s.l.* also includes DDT and lambda-cyhalothrin, with evidence of developing resistance to bendiocarb and fenitrothion. The use of three IRS classes of insecticides in general pest control and agriculture in Mali, added to the use of pyrethroids on long-lasting insecticide nets (LLINs), has placed strain on insecticide resistance management (Cisse *et al.*, 2015; Kudom *et al.*, 2018). Wragge *et al.* (2015) showed that in Sadiola (Kayes region, southwest Mali) susceptibility to pirimiphos-methyl (0.25%) was still high, with *An. arabiensis* having 98.8% mortality, and *An. gambiae* and *An. coluzzii* 100% mortality 24 hours post-exposure on WHO test papers.

1.2.3 Malian Vector Control Strategy

The PMI allocated US$22 million to the NMCP for the year 2018. The NMCP, in line with the PMI 2018 strategy, used the funds for:

1. Entomological and insecticide resistance surveys.
2. The distribution of insecticide treated nets (ITN's) with 1.4 million nets provided to pregnant women and children.

3. Indoor residual spraying (IRS) covering 242,684 structures and protecting 778,884 people (2017) in the regions identified as “high risk” areas.
4. Enhanced case management with aims to increase testing.
5. Seasonal chemoprevention campaigns aimed at children under 5 years old.
6. Strengthening the prevention of malaria in pregnancy.
7. Strengthening health systems and capacity building.
8. Education regarding social and behaviour changes.
9. Enhancing surveillance and evaluation by improving data collection and analysis.

1.2.4 Sadiola District

Sadiola falls into the Kayes region in southwest Mali, within the Sudano-Guinean climatic zone. It is approximately 70km south of Kayes and 693km from the capital of Bamako. Large scale commercial gold mining started in the area in 1996 (Masurel *et al.*, 2017). Prior to the relocation of Sadiola village in 1999 due to the expansion of Société des Mines d’Or du Mali (SEMOS) gold mine, Sadiola (Fig. 1.1) consisted of 39 governmental related structures, with 512 people living in 129 houses, utilizing 57 pit toilets (MacKenzie *et al.*, 2003).

Figure 1.1 – Sadiola village prior to the relocation in 1999. (SEMOS photo archive)

With the influx of jobseekers into the area, Sadiola village has grown into the largest of the villages located in the district, consisting of 14,442 people living in 1,584 houses, with over 1,200 pit toilets (SEMOS Malaria Vector Control unpublished data, 2018). The expansion of Sadiola and other surrounding villages can be seen from Light Imaging and Detection and Ranging (LIDAR) mapping, satellite and drone images of the area (Figs 1.2 and 1.3). The mayor of Sadiola is in charge of the administrative functions of the area. There is one government primary health care facility in Sadiola. The main medical conditions seen at the clinic are malaria, diarrheal disease and maternity-related illnesses. All complicated medical cases are referred to the government hospital in Kayes for further evaluation and management. Roads in the area are laterite in nature and are often washed away during the rainy season making road transportation problematic.

There are no non-governmental organizations (NGO) or Mali government-run vector control programmes in the area.

Figure 1.2. Aerial photo of Sadiola Village from 2000. (From MacKenzie *et al.*, 2003).

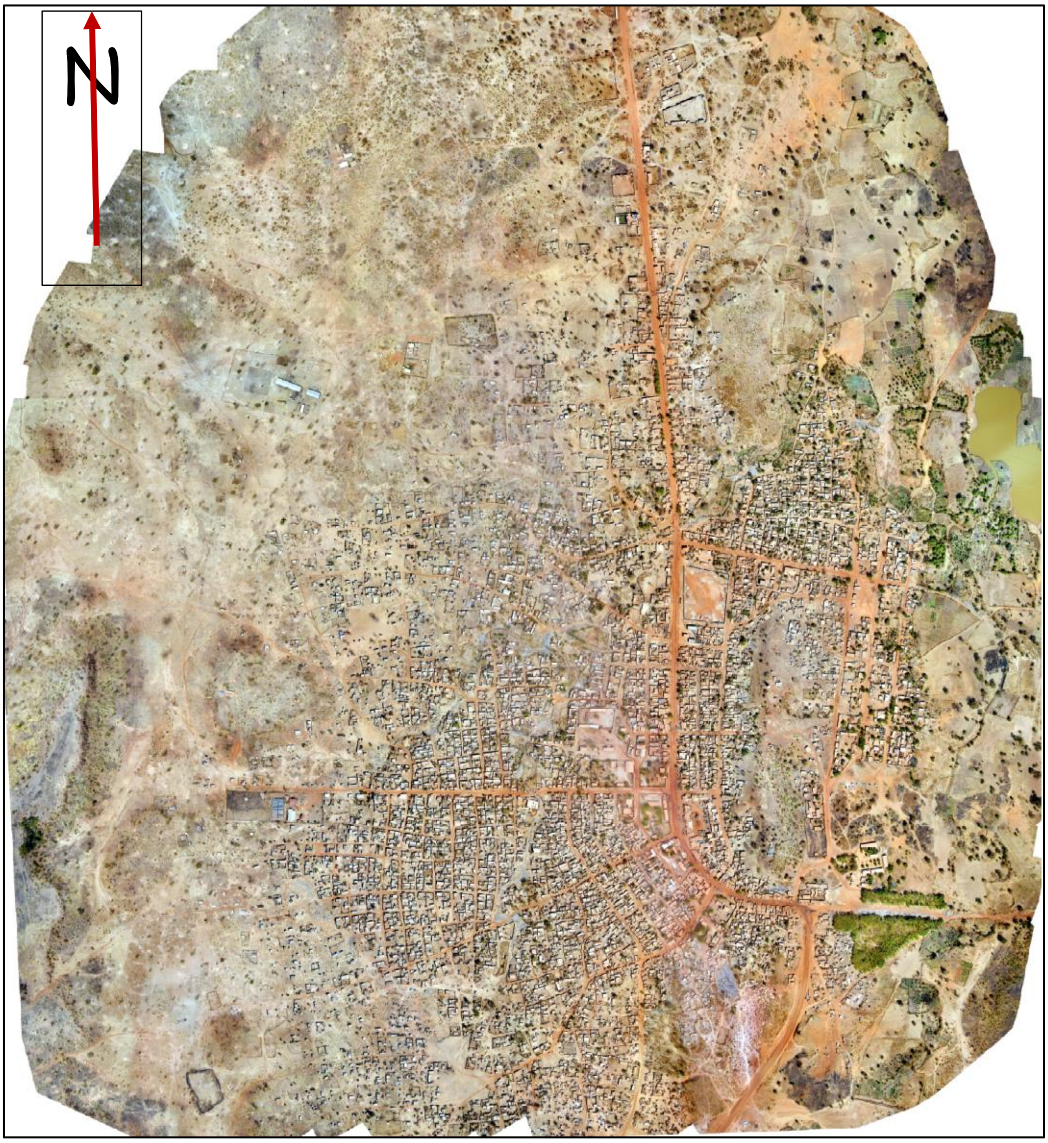


Figure 1.3. Sadiola Village February 2019, drone survey. Scale 1:3,000. SEMOS Technical Services Department.

1.2.5. Malian sanitation system

Mali has no public sanitation systems, with 85% of the population using pit toilets and the remaining population utilizing a soak pit sewage system. In the Sadiola District, the pit toilets are a ‘dry’ system. Namely, a hole dug approximately 2-6m deep and 1mx1m to 2mx2m wide, covered with a concrete slab (Martínez-Santos *et al.*, 2017). There is no water used for “flushing” (Fig. 1.4). These pit toilets are favoured over the “kassatan” toilets (meaning – “no smell”), a modified Blair Ventilation Improvement Pit (BVIP) toilet that was developed in Zimbabwe (MacKenzie *et al.*, 2003). Not much care is taken in the location of the toilet in relation to run-off and the water table. The financial situation of the family determines if the toilet is built at all, enclosed with bricks or woven reeds, and if the toilet will have an open or closed roof. Being often open roofed, the toilet fills up with water and become a breeding site for mosquitoes. A pit toilet will last a family approximately 10 years depending on the number of family members. Once the toilet has become full, a new one is constructed, often leaving the old toilet uncovered (Nakagiri *et al.*, 2016).

Figure 1.4. Examples of pit toilet construction in Madine village

1.3 Research Rationale

1.3.1 Malaria – SEMOS Gold Mine - Sadiola District

Prior to 2009, malaria cases seen at the SEMOS gold mine clinic were treated either based on a positive Rapid Diagnostic Test (RDT) or a patient presenting with signs or symptoms of malaria. The treating of malaria cases without a positive test result poses a problem concerning misdiagnosis of other pyrexia-causing diseases that can be found in the area. For example, dengue fever, yellow fever, typhoid, meningitis and Ebola (early stages), can all present with very similar symptoms to that of malaria (Tambo *et al.*, 2016).

Since 2009, only patients with confirmed positive tests are treated for malaria. The SEMOS clinic uses the BIO-RAD OptiMAL-IT® malaria RDT, due to its high sensitivity of detecting peripheral parasitaemia levels of 0.001-0.002% (50-100 parasites per µL of blood) (Moody & Chiodini, 2002). The test is able to confirm *P. falciparum* and identify possible infections of *P. vivax, P. ovale* and *P. malariae*. The test only shows a positive result when live parasites are present, thus making it ideal for malaria treatment monitoring in the remote environment (Mawili-Mboumba *et al.*, 2010).

1.3.2. *Anopheles* mosquito larval adaptation to breeding in sewage

A literature search showed that adaptation of *Anopheles* to breeding in sewage water has been reported by several groups. Young & Johnson (1949), carrying out a survey in Monrovia, Liberia, highlighted that wells constructed using oil drums became places of heavy breeding of *An. gambiae* even if these wells were contaminated with faecal matter. Sattler *et al.* (2005) in Tanzania, showed anopheline species could adapt to breed in nearly any kind of water, thus all water bodies should be considered as potential breeding sites and form part of an integrated vector control programme. A worrying trend was highlighted by De Silva *et al.* (2012) of *An. gambiae* becoming adapted to breeding in polluted water, some of which contained sewage in Côte d'Ivoire and Cameroon. Kamdem *et al.* (2012) commented that growth of urban populations was at the heart of promoting adaptive ecological divergence as seen in southern Cameroon. Fossog *et al.* (2013) had similar findings in two large urban areas in Cameroon where the *An. gambiae* M. form (now *An. coluzzii*) had adapted to survive in polluted breeding sites. They further commented that this evolution has likely followed phenomena such as deforestation and increases in urbanization, that are known to be driving forces in adaptation and composition of mosquitoes in the tropics. This adaptation has also been noted in Lagos, Nigeria where *An. gambiae s.s.* was found breeding in ponds containing human sewage (Awolola *et al.,* 2007). Studies in Ghana show a similar pattern (Klinkenberg *et al.,* 2008; King *et al.,* 2017; Dzorgbe Mattah *et al.,* 2017). Research articles by Keating *et al.* (2003), Walker *et al.* (2007) and Imponivil *et al.* (2008) all highlighted the ability of *An. gambiae* to quickly adapt to new breeding sites such as trash-filled pools, with some containing sewage in urbanized areas of Kisumu and Malindi in Kenya. They added that any water body, regardless of longevity, had the potential to be a breeding site periodically. This re-reinforces the suggestion by Sattler *et al.* (2005) that all water bodies should be considered when undertaking vector control programmes. For the first time in rural Muheza, Tanzania, Emidi *et al.* (2017) showed adaptation to breeding in polluted water. As recently as 2018 in Sudan, Azrag & Mohammed (2018) documented that a small population of *An. arabiensis* had adapted to breeding in polluted habitats.

1.3.3 Vector Control at SEMOS Gold Mine

SEMOS Gold Mine has been undertaking malaria vector control in Sadiola District as part of its community responsibility out-reach programme since 2005. The mine first used net dipping and indoor residual spraying with deltamethrin as the main tool for vector control. In 2008, *Bacillus thuringiensis* var *israelensis* (*Bti*) wettable granules (WG) formulation was added to control mosquito larvae and enhance water body management, and has become an integral component of the programme. Since 2011, house-based education was incorporated into the programme, with information being conveyed verbally in both French and Bambara (local dialect) by the IRS teams, with "talks" being directed at the family matriarch regarding open standing water and good housekeeping, to reduce breeding sites in and around the home (De Silva *et al.*, 2012). Entomological studies carried out by SEMOS clinic during 2011 showed a marked increase in insecticide resistance to deltamethrin (Wragge *et al.*, 2015) that prompted the change to pirimiphos-methyl (Actellic EC50). However, this was only introduced in 2013, because of political instability caused by a military coup in 2012 (Solomon, 2014) that interfered with the logistics needed to import the insecticide. This resulted in no IRS spraying during 2012, and a decision was made to start treating the pit toilets (n = 674) in the surrounding villages with 2-4mm pre-expanded polystyrene beads. This was carried out for two reasons: (1) maintaining a presence in the villages, and (2) as part of a community initiative to help reduce other nuisance mosquito species that prefer breeding in sewage, such as species of *Culex* and *Aedes* (Lam & Dharmaraj, 1982). An added benefit of inserting the polystyrene beads is its odour reducing effect, according to the local villagers, which helped make it a successful addition to the vector control toolbox. The remaining polystyrene beads from 2012 were rolled over into 2013, resulting in a further 683 pit toilets being treated until the supply on site ran out. The treating of pit toilets was implemented as an additional part of the control in 2014 (n = 800 toilets) and 2016 (n = 1,210 toilets). During those years, a noticeable decrease in malaria positive cases occurred.

Since then, the IRS insecticide was upgraded in 2015 from Actellic EC50 to Actellic 300CS, due to firstly, its authorization for use in IRS in Mali and secondly, for the longer lasting effects of the capsular-based active ingredients on interior mud walls (Rowland *et al.*, 2013) and used up to 2017. In 2018, Ficam VC (bendiocarb) was used in conjunction with the Actellic 300CS, resulting in a further drop in malaria cases for 2018 (n=271), achieving the lowest positive case numbers since 2006 (n=289).

1.4 Aims and Objectives

The SEMOS vector control programme was summarized by Wragge *et al.* (2015). This present study examines the entomological surveillance carried out since 2015, correlated with the vector control interventions and compares this with the previous surveys published in 2015. Insecticide resistance in the major vector species was analysed, and the impact of resistance management strategies assessed.

In addition, the reduction of positive malaria cases in the years of active pit toilet treatment prompted the question of whether a local adaptation had occurred in the *An. gambiae* complex mosquitoes, and whether the treating of toilets was reducing vector breeding and in turn reducing positive case numbers. Extensive mosquito collections in and around pit toilets were carried out to determine whether members of the *An. gambiae* complex could be found breeding in polluted water.

Chapter 2.

Research Methodology

2.1 Study Area

This research was carried out at the SEMOS Gold Mine (GPS co-ordinates: N13 53 23.9: W11 42 07.1; elevation 130m above sea level) in the vector control programme area and nearby villages (Fig. 2.1). The area covers 396Km², consisting of 17 local villages, a mine village and the mine working area. The average population within the spray zone is 20,750 (2014-2018, SEMOS malaria vector control spray data). The economic activity in the spray zone consists of subsistence-based farming practices, employment with the mine or one of the sub-contractors, or local commerce directly or indirectly linked to the mine. The area experiences two distinct seasons, a hot dry period from November to April/May with average dry season temperatures of 40°C and a humid wet season (average temperature of 36ºC) with annual rainfall between May to October, with a small amount of rain (Mango rains) in January. The 15-year (2004-2018) average rainfall obtained from the SEMOS mine Environmental Department is 826.3mm. As a result of the distinct dry and wet seasons, the SEMOS vector control spray programme runs from May to early November.

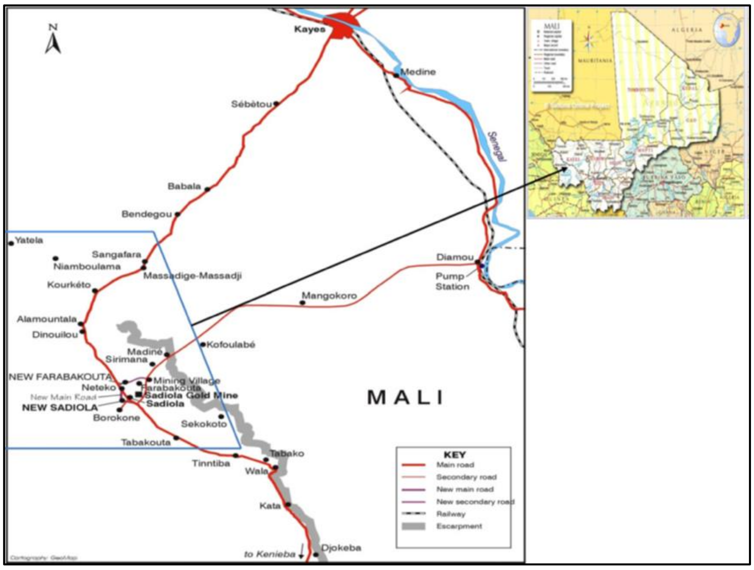


Figure 2.1. Sadiola District, showing the SEMOS spray zone (blue area). (Wragge *et al.*, 2015)

SEMOS mine clinic has been recording malaria positive case statistics since 2004 (Fig. 2.2). From 2010, only confirmed malaria positive cases received treatment. In 2012, data regarding malaria positive cases in children under five years old were included in the statistics. Data used for this research only include age, type of malaria infection, village and travel history, with no personal information given. Permission to use the data was given by the senior health manager of Anglo Gold Ashanti (see Appendix 1).

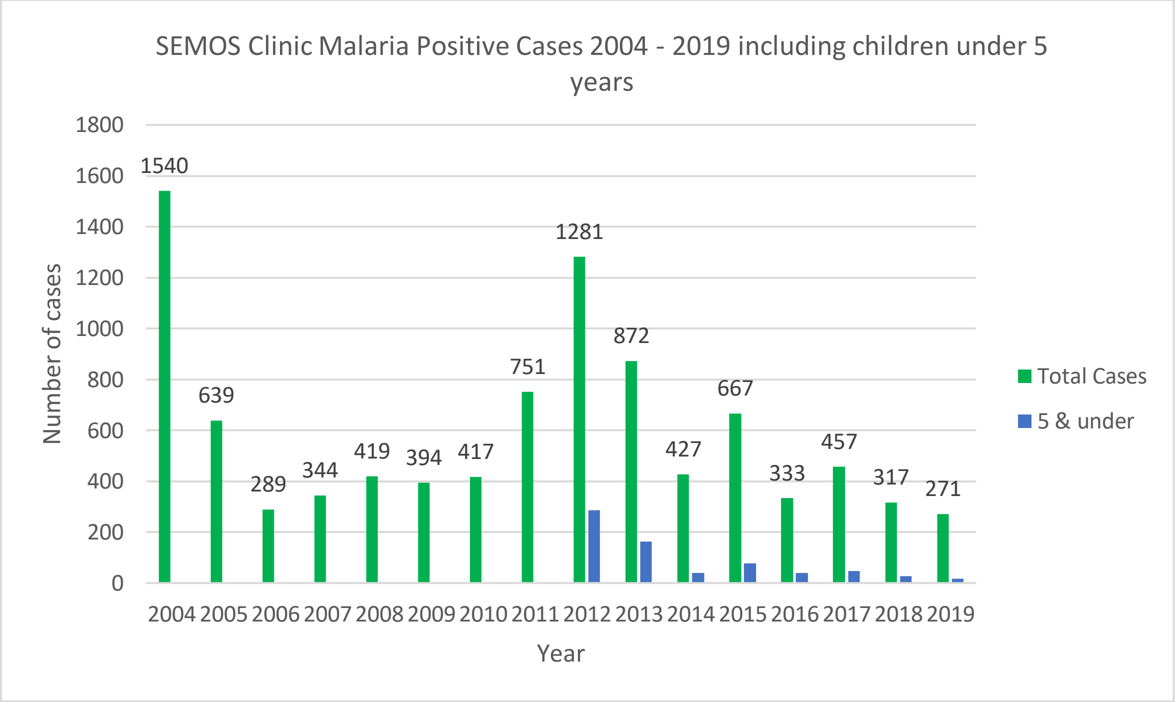


Figure 2.2. SEMOS clinic positive malaria cases including children under 5 years.

2.2 Mosquito Sampling Methods

Sampling of mosquito larvae and adults emerging from larval habitats was carried out over 3 months, from August to the end of October in 2018. This was done during the peak rainy season in the area.

2.2.1 Live larval collections

Live larvae were collected from three locations: a disused splash pool in the mine village, one site in Sadiola main village and a water puddle on one of the dirt roads in the mine village. Larvae were collected using a 120ml dipper in accordance with WHO guidelines (WHO, 2013). Twelve larval samples were taken over a 12-week period. The larvae were placed in collection bottles in separate cages according to the location they were obtained from and allowed to develop into adults. In order to kill the adults quickly to avoid anatomical damage and in the absence of an entomological killing jar with ethyl-acetate, each cage was placed next to an interior house wall that had been treated with Actellic 300CS. The fumes from the sprayed wall were sufficient to kill the mosquitoes within eight hours. Dead mosquitoes were removed, identified according to genera, and placed in micro centrifuge tubes for further possible molecular analysis.

2.2.2 Emerging adult collections

Five Hamer floating emergence traps (Hamer *et al.*, 2011) where used to capture adults emerging from natural breeding habitats at three localities (Fig. 2.3). One was placed in a sewage tank in the mine village sewage plant, one in the disused splash pool in the mine village and a third on a pit toilet (opened roof, untreated) located outside the mine village and used by the local bakery for their ablutions (Fig. 2.4). These three traps were placed four times per week between the hours of 17h00 and 07h00. A fourth Hamer trap was circulated amongst the IRS spray team members and placed on a pit toilet for a one-week period, in Sadiola main village, Madine village and Sirimana village. This was done to establish if the emergence pattern and numbers emerging was similar to that from the bakery pit toilet. A fifth trap was added during the 2018 entomological study to survey pit toilets outside of the spray zone. Mosquitoes caught in each Hamer trap's 500ml plastic collection jar were emptied into separate cages according to their capture location and placed near an IRS treated wall (Actellic 300CS) (Fig 2.5). Within 8 hours all caught mosquitoes had died. Dead mosquitoes were then identified to the main genera and placed in micro centrifuge tubes for further processing.

Climate data were obtained during the course of the research. This included rainfall and relative humidity (RH %).

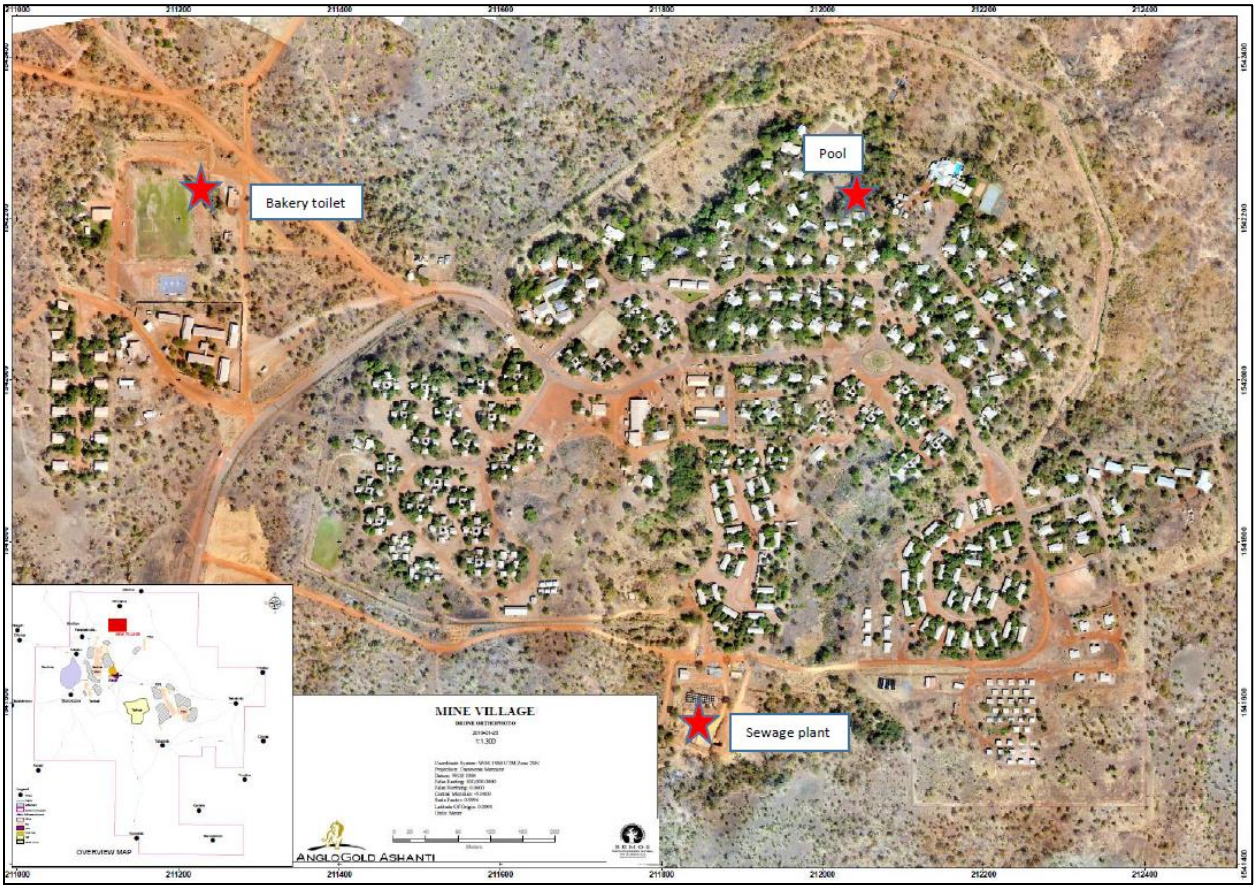


Figure 2.3. Placement of the Hamer Emergence traps in the mine village indicated with red stars.

Figure 2.4. Hamer Emergence trap with 500ml collection jar, placed over a pit toilet.

Figure 2.5. Captured mosquitoes from the pit toilet.

2.3 Insecticide resistance surveys

In collaboration with Professor R.H. Hunt, entomological surveys were carried out for SEMOS mine in October 2018. These results were compared with previous surveys done in September/October 2006, July/August 2011, August 2014 and August 2016. Mosquitoes were collected resting inside houses from villages that had not been sprayed during the routine SEMOS vector control implementation. Both male and female mosquitoes were exposed to the various insecticide classes approved by WHO for vector control, using the WHO (2016) insecticide susceptibility test methods. With an aspirator, 20-25 wild-caught *Anopheles* female mosquitoes where introduced into WHO holding tubes (Fig. 2.6). They were then gently transferred through into the tubes containing WHO insecticide treated papers (obtained from the National Institute for Communicable Diseases (NICD), Johannesburg). After one-hour exposure, the female mosquitoes were transferred back into the holding tubes and provided with cottonwool pads soaked with a 10% sugar solution. The results of the tests were recorded after 24 hours.

All four classes of WHO-approved insecticides were tested – pyrethroids (0.05% deltamethrin), organochlorines (4% DDT, 4% dieldrin), organophosphates (1% fenitrothion, 5% malathion, 0.25% pirimiphos-methyl) and carbamates (0.1% bendiocarb, 0.1% propoxur). In addition, the WHO (2016) guidelines recommend that where resistance is confirmed, the intensity of the resistance should be tested. This was done by exposing mosquitoes to 5 times the standard discriminating dose for 1 hour with 24 hours recovery time. As there were no survivors, the recommended 10 times test was not done.

Figure 2.6. WHO insecticide susceptibility test tubes

2.4 Mosquito Identification

The anopheline samples from the larval collections and emergence traps were identified using the morphological keys of Gillies & De Meillon (1968) and Gillies & Coetzee (1987). The identification of *Culex* mosquitoes relied on the Walter Reed Biosystematics Unit (http://wrbu.si.edu/index.html) - Culex (Cux.) Mosquitoes, West Africa (AFRICOM) adult keys. *Aedes* mosquitoes were identified using the Walter Reed Biosystematics Unit - *Aedes* Mosquitoes, Afrotropical (AFRICOM) adult keys in conjunction with Rueda (2004) pictorial keys for the identification of mosquitoes associated with dengue virus transmission.

A sample of 100 mosquitoes per day (or less depending on the catch) were preserved in micro centrifuge tubes with blue indicator silica gel and bagged according to date, location, sex and species in accordance with the WHO (2003) sampling and preserving of mosquito guidelines.

All mosquitoes used in the insecticide exposures were subsequently identified morphologically using the keys of Gillies & Coetzee (1987) and then underwent further molecular processing for identification of members of the *Anopheles gambiae* complex (Scott *et al.*, 1993, Fanello *et al.*, 2002) and the *An. funestus* group (Koekemoer *et al.*, 2002). The molecular identifications were carried out by the Vector Control Reference Laboratory at the National Institute for Communicable Diseases in Johannesburg, South Africa.

2.5 Data analysis

Quantitative descriptive statistics were used to analyse the data. Primary data collected included location, total number of mosquitoes emerging, sex of the mosquitoes and species. The data were captured in Microsoft Excel spreadsheets and compared graphically to determine if patterns or trends existed.

Chapter 3.

Results

3.1 Malaria transmission

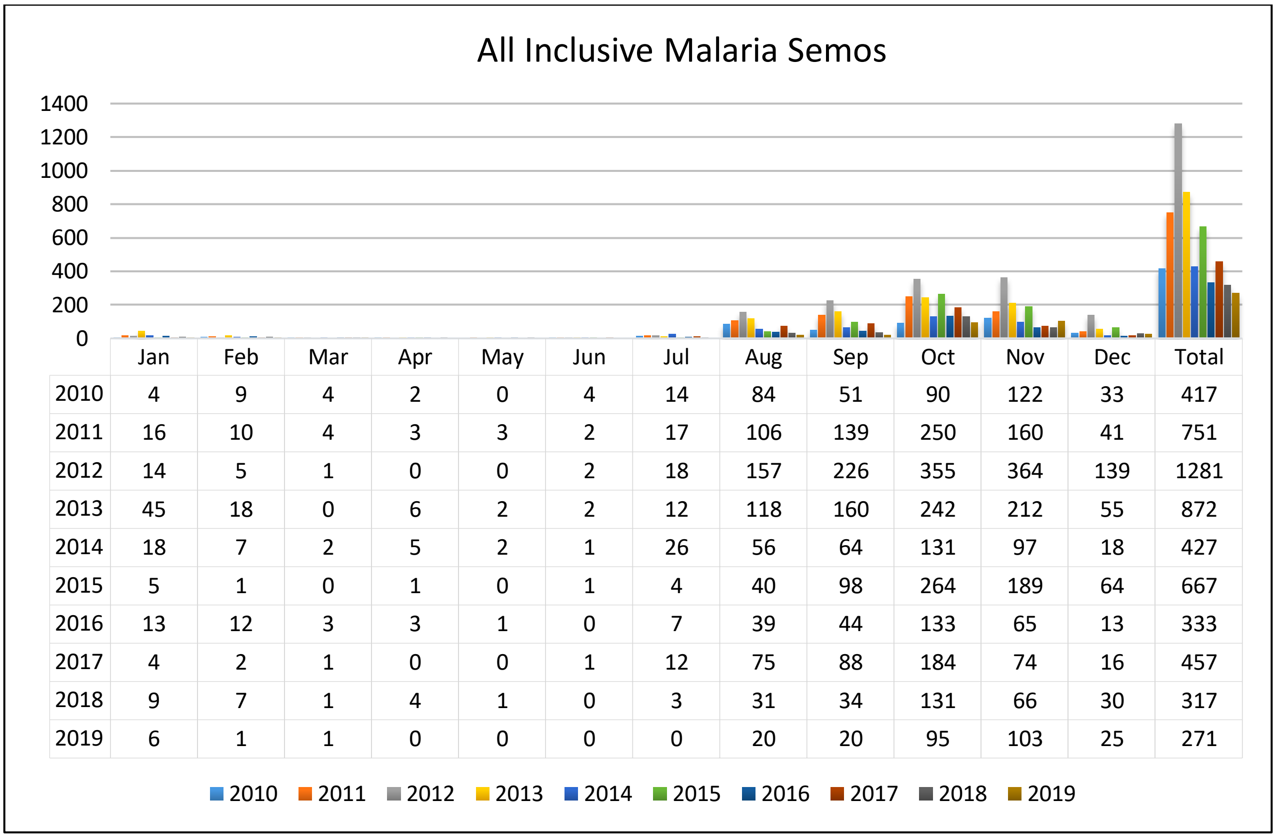
The highest all-inclusive number of cases was 1,540 in 2004, with the lowest number being 289 cases in 2006 (Fig. 2.2). The number of cases of malaria in children 5 years and younger has been decreasing, with only 27 (8.5%) cases (total n = 317) in 2018 being children in this age group. The highest malaria transmission in the Sadiola district occurs at the end of the rainy season between October and November (Fig. 3.1).

The rainfall figures for SEMOS mine from 2004 to 2018 are given in Table 3.1. Rainfall measurements was recorded were taken over a 24-hour period and at 06h00 each day.

Table 3.1. Rainfall at SEMOS mine from 2004 to 2019.

	2004	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**16-years Average (2004-2019)**
Jan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0	0.0	**0.3**
Feb	0.0	14.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.0	0.0	0.0	0.0	0.0	**3.1**
Mar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	0.0	**0.3**
Apr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	6.5	0.0	0.0	12.0	3.0	0.0	0.0	0.0	**1.4**
May	19.2	24.5	14.0	57.5	22.0	12.5	0.0	7.5	14.0	24.0	80.5	1.0	1.0	11.5	0.0	0.0	**18.1**
Jun	69.0	217.5	102.0	74.0	135.0	124.0	115.0	56.5	169.6	173.0	83.5	29.0	123.5	121.5	88.0	110.0	**111.9**
Jul	232.0	210.5	171.0	232.0	152.5	95.5	161.5	138.0	330.0	139.0	125.0	231.5	216.0	212.5	161.0	199.0	**187.9**
Aug	250.0	243.0	216.0	336.0	224.5	357.5	264.0	284.0	342.5	301.5	258.0	400.5	276.5	168.0	316.0	270.5	**281.8**
Sep	85.5	156.0	118.5	154.5	159.0	238.5	207.5	189.0	220.5	183.0	131.0	178.0	96.5	95.5	244.5	170.5	**164.3**
Oct	43.5	62.0	69.0	34.5	97.0	49.0	104.0	57.5	117.0	23.5	7.5	77.5	17.5	12.5	50.5	59.5	**55.1**
Nov	0.0	0.0	0.0	0.0	0.0	2.0	5.5	0.0	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**1.0**
Dec	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**
Total	699.2	927.5	690.5	889.5	790.0	879.0	857.5	733.0	1208.6	844.0	685.5	970.0	738.5	621.5	860.0	809.5	**825.2**

The number of malaria cases from 2004 to 2019, associated with total annual rainfall and insecticide used for IRS, is shown in Fig. 3.2.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2010	4	9	4	2	0	4	14	84	51	90	122	33	417
2011	16	10	4	3	3	2	17	106	139	250	160	41	751
2012	14	5	1	0	0	2	18	157	226	355	364	139	1281
2013	45	18	0	6	2	2	12	118	160	242	212	55	872
2014	18	7	2	5	2	1	26	56	64	131	97	18	427
2015	5	1	0	1	0	1	4	40	98	264	189	64	667
2016	13	12	3	3	1	0	7	39	44	133	65	13	333
2017	4	2	1	0	0	1	12	75	88	184	74	16	457
2018	9	7	1	4	1	0	3	31	34	131	66	30	317
2019	6	1	1	0	0	0	0	20	20	95	103	25	271

Figure 3.1. Malaria transmission per month from 2010 – 2019

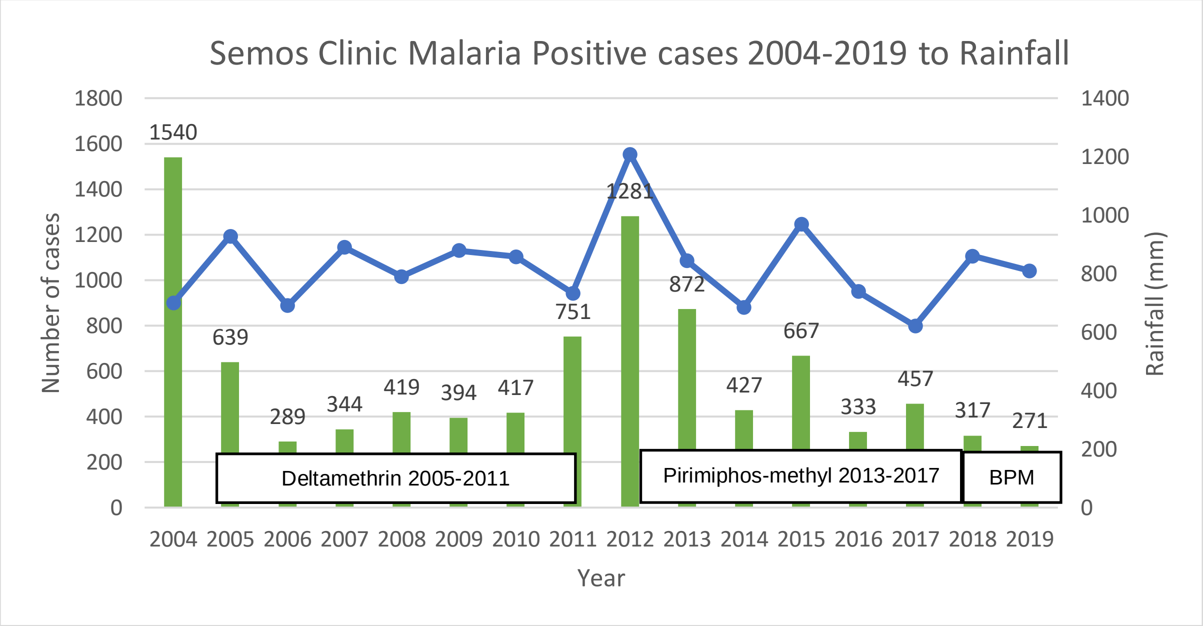


Figure 3.2. Malaria transmission in relation to rainfall and insecticide used (2004-2019). (BPM – bendiocarb in conjunction with pirimiphos-methyl; 2018 - 2019)

It can be seen in figure 3.2, from 2006 until 2011 there was an increase in malaria cases while using deltamethrin during those years. 2012 had the highest number of malaria cases with no IRS taking place. From 2013 until 2017 there is a fluctuation in case numbers. In 2018, case numbers drop to 317, with a further drop to 271 cases in 2019, while using a combination of bendiocarb and pirimiphos-methyl in a mosaic IRS application pattern.

3.2 Mosquito collections

3.2.1 Larval collections

A total of 94 larvae were collected from 12 dips over the 12 weeks of sampling. Only 12 anopheline larvae were found (12.8%) in a single collection from a pool at the side of the road in the mine village. The collection data are shown in Table 3.4.

The Anopheles specimens were identified morphologically as belonging to the An. gambiae complex, but were not further identified using molecular methods due to low numbers collected.

Table 3.2. Mosquitoes collected by larval dipping from three localities in and around the mine area.

	Mine village			Splash pool			Road puddle		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
Culex	2	2	4	0	0	0	13	24	37
Aedes	17	15	32	2	7	9	0	0	0
Anopheles	0	0	0	0	0	0	6	6	12

3.2.2 *Emergence traps*

A total of 3 Hamer emergence traps, placed as shown in Figure 2.4 over a 12-week period, produced 13,585 mosquitoes belonging to three genera. Table 3.2 gives the number of mosquitoes collected per locality. Only a single *Anopheles* male was collected, this from the sewage plant on the 1st August 2018.

Table 3.3. Hamer emergence traps placed at three localities in the SEMOS gold mine area from 1st August 2018 to 24 October 2018.

	Sewage plant			Splash pool			Bakery pit toilet		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
Culex	992	774	1,766	8	5	13	7,574	4,185	11,759
Aedes	25	4	29	7	3	10	7	0	7
Anopheles	0	1	1	0	0	0	0	0	0

The Hamer trap placed in the surrounding villages within the spray zone resulted in nine (2 males, 7 females) *Culex* mosquitoes from a pit toilet in Medine village that had been treated with expanded polystyrene balls in 2016. Both the traps over pit toilets in Sadiola and Sirimana villages produced zero mosquitoes. The trap placed over an untreated pit toilet at Babala village, which is outside of the vector control area, produced 883 *Culex* mosquitoes (470 males, 413 females) in one night (12 October 2018).

A sample of mosquitoes caught and preserved were analysed (Table 3.3) resulting in the following species being identified from the mosquitoes caught in the emergence traps: *Culex quinquefasciatus, Cx. univittatus, Aedes aegypti* and *Ae. vittatus.*

Table 3.4. Species identified from Hamer trap collections

Date	**Sample location**	***Ae. aegypti***	***Ae. vittatus***	***Cx. quinquefasciatus***	***Cx. univittatus***
6/8/2018	pit toilet	0	0	19	3
9/8/2018	sewage plant	0	0	2	0
	pit toilet	1	0	65	34
13/8/2018	pool	0	0	0	4
	pit toilet	0	0	25	38
14/8/2018	sewage plant	0	2	1	0
15/8/2018	sewage plant	0	3	0	0
3/9/2020	sewage plant	0	0	0	4
10/9/2018	pool	0	2	0	0
	sewage plant	0	0	3	20
	pit toilet	0	0	8	49
3/10/2018	sewage plant	1	0	9	20
10/10/2018	pit toilet	0	0	22	6
	sewage plant	0	0	15	45
15/10/2018	pit toilet	1	0	36	59
	Total	**3**	**7**	**205**	**282**

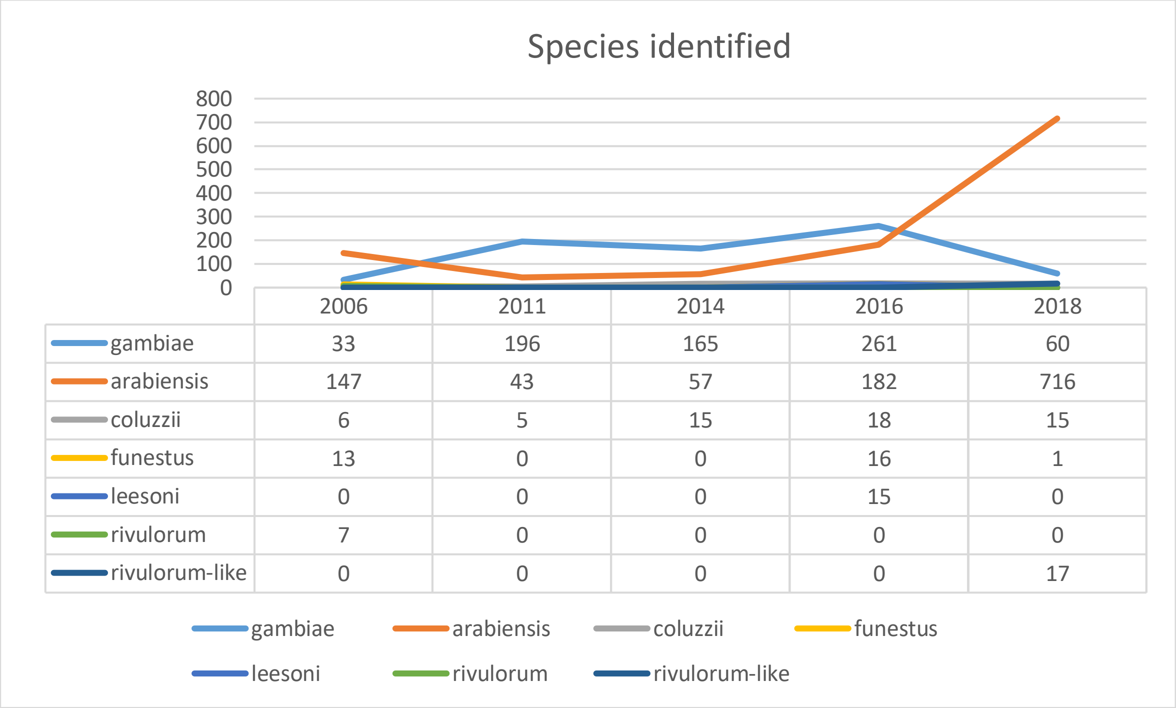
3.3 Entomological surveys

Five entomological surveys were carried out over 12 years to provide information on vectors species present (Table 3.5) and their susceptibility to insecticides approved for public health use by WHO (WHO, 2016). The data from 2006, 2011 and 2014 were published by Wragge *et al.* (2015).

Table 3.5. Indoor resting *Anopheles* species in Sadiola identified using PCR methods (2006, 2011 and 2014 data from Wragge *et al*., 2015).

Year	Species Identified	Number collected	% of Total/Year
2006 September- October	*An. gambiae* *An. coluzzii* *An. arabiensis* *An. funestus* *An. rivulorum*	6 6 147 11 7 Total=177	3.4 3.4 83.1 6.2 3.9
2011 July-August	*An. gambiae* *An. coluzzii* *An. arabiensis* *An. funestus*	196 5 43 10 Total=254	77.2 2.0 16.9 3.9
2014 August	*An. gambiae* *An. coluzzii* *An. arabiensis*	165 15 57 Total=237	69.6 6.3 24.1
2016 August	*An. gambiae* *An. coluzzii* *An. arabiensis* *An. funestus* *An. leesoni*	261 18 182 16 15 Total=492	53.0 3.6 37.0 3.3 3.1
2018 September - October	*An. gambiae* *An. coluzzii* *An. arabiensis* *An. funestus* *An. rivulorum*-like	60 15 716 1 17 Total=809	7.4 1.9 88.5 0.1 2.1

Three members of the *An. gambiae* complex and four of the *An. funestus* group were identified (Fig. 3.3). The species composition and abundance changed over the years, with *An. rivulorum* being identified only in 2006, *An. leesoni* only in 2016 and *An. rivulorum*-like only in 2018.



	2006	2011	2014	2016	2018
gambiae	33	196	165	261	60
arabiensis	147	43	57	182	716
coluzzii	6	5	15	18	15
funestus	13	0	0	16	1
leesoni	0	0	0	15	0
rivulorum	7	0	0	0	0
rivulorum-like	0	0	0	0	17

Figure 3.3. Species identified during entomological surveys carried out at SEMOS gold mine.

3.4 Insecticide Resistance

Insecticide susceptibility tests were only conducted on the *An. gambiae* complex samples because of the numbers collected.

As can be seen in Table 3.6, the largest sample size was obtained in 2018, with 1,231 mosquitoes being exposed to WHO insecticide susceptibility tests (WHO, 2016). 2018 also had the highest average mortality percentage of 85.4%, compared to 74% in 2006, 56.4% in 2011, 48.8% in 2014 and 58.4% in 2016. 133 mosquitoes were exposed to 5x deltamethrin for the first time in 2018, resulting in a 100% mortality. This 100% mortality to 5x deltamethrin can also be seen in Table 3.7, whereby all *An. arabiensis* and *An. gambiae* samples exposed had 100% mortality at 24 hours.

Table 3.6. Insecticide resistance in *An. gambiae* complex (pooled).

Insecticide Class	**Insecticide used**	**% Mortality at 24 hours**									
		2006		**2011**		**2014**		**2016**		**2018**	
		Sample size	**% Mortality**	**Sample size**	**% Mortality**	**Sample size**	**% Mortality**	**Sample size**	**% Mortality**	**Sample size**	**% Mortality**
Pyrethroids	5x Deltamethrin									133	100
	Deltamethrin	133	99	125	68	86	52	76	36	124	95
Organophosphates	Fenitrothion			83	99						
	Malathion	94	100	96	96			102	91	128	100
	Pirimiphos-methyl					213	100	121	89	123	100
Carbamates	Bendiocarb	81	74	59	12	214	88	71	94	118	96
	Propoxur			48	21			35	78	125	98
Organochlorines	DDT	104	94	47	38	48	2	49	21	130	80
	Dieldrin	123	75	17	82					114	100
Control		48	2	63	0	50	2	67	0	236	0

Table 3.7. Insecticide resistance results of *An. arabiensis* and *An. gambiae.*

Insecticide Class	**Insecticide used**	***An.arabiensis* compared to *An. gambiae* : % Mortality at 24 hours**											
		2014				2016				2018			
		An. arabiensis		*An. gambiae*		*An. arabiensis*		*An. gambiae*		*An. arabiensis*		*An. gambiae*	
		Sample size	% Mortality	Sample size	% Mortality	Sample size	% Mortality	Sample size	% Mortality	Sample size	% Mortality	Sample size	% Mortality
Pyrethroid	5x Deltamethrin									99	100	13	100
	Deltamethrin	28	85.7	58	19	29	86.21	42	11.9	91	98.9	6	66.67
Organophosphates	Malathion					43	100	41	87.8	78	100	8	100
	Pirimiphos-methyl	85	98.8	100	100	61	96.72	71	87.32	98	100	7	100
Carbamates	Bendiocarb	105	83.8	109	93.6	39	100	51	86.27	80	95	11	100
	Propoxur					2	100	28	85.71	88	97.73	8	100
Organochlorines	DDT			48	2.1	8	37.5	26	7.14	99	86.87	4	0
	Dieldrin									83	100	3	100

The change in pyrethroid resistance status can be seen in Tables 3.6 and 3.7. In 2006 deltamethrin had a pooled mortality of 99% dropping to a low of 36% in 2016. While the pooled resistance improved to 95% in 2018, with 98.9% mortality in *An. arabiensis* and 66.67% in *An. gambiae*, the very small sample size of *An. gambiae* (n=6) requires additional testing. DDT resistance in *An. arabiensis* in 2018 was 86.87% compared to 37.5% in 2016. Both *An. arabiensis* and *An. gambiae* had a 100% mortality at 24 hours to dieldrin, malathion, pirimiphos-methyl and propoxur in 2018.

The pooled mortality of bendiocarb in 2016 and 2018 has shown marked improvement since 2011 (Table 3.6), although *An. gambiae* gave only 86% mortality in 2016 (Table 3.7)

Chapter 4.

Discussion

4.1. Malaria Transmission

The rainfall season in Sadiola occurs from June to October, with malaria cases starting to increase in August and peaking in October/November. Other than following the seasonal trend, the malaria case data recorded since the beginning of the indoor spray programme in 2005 does not follow the trend of rainfall received (Fig. 3.2). The very high rainfall in 2012 (1,208.6mm) and the highest number of cases (n=1,281) post-2004 (Fig. 3.2) also coincided with the year that the IRS programme was not carried out due to political unrest and lack of availability of insecticides. This is a very clear indication of what happens in the area when there is no vector control.

There was a reduction in case numbers during the years of active toilet treatment in 2014 and 2016. However, the fact that no *Anopheles* mosquitoes emerged from the pit toilet collections, indicates that the reduction of cases during those years was not attributed to the treatment of the toilets but rather some other factor. Annual rainfall in 2014 (685.5mm) was one of the lowest over 16 years with 2016 (738.5mm) being only marginally higher (Table 3.1). It is doubtful that rainfall could have been the cause of the decreasing case numbers during those years. One possible reason for the steady decrease in cases over the years is the acceptance of the programme by the local population in the area.

Data gathered from the IRS programme (2010-2019) showed that on average, 30% of households in the area use pyrethroid treated LLINs and ITNs. In 2018, seven years after having stopped the use of pyrethroids for IRS in 2011, mosquito screening for insecticide resistance in the *An. gambiae* complex showed 95% mortality on the WHO standard assay and 100% mortality on 5 x deltamethrin intensity tests. The reduction in resistance to deltamethrin and subsequent restoration of insecticide treated bed nets effectiveness, together with the introduction of a mosaic spray pattern using a carbamate plus organophosphate in 2018, may explain the reduction in malaria transmission for the past two years.

Further entomological surveys will need to be carried out in the coming years to determine if this is indeed the case.

4.2. Insecticide Resistance

Currently there are five classes of insecticides approved by the WHO for use in malaria vector control, namely: organochlorines, pyrethroids, carbamates, organophosphates and most recently, neonicotinoids. The use of this fifth class of insecticides is not yet widespread, and, as with any new insecticide, cost often outweighs its initial use (Tangena *et al.*, 2020). Reports of developing resistance to the neonicotinoids Acetamiprid and Imidacloprid in *An. coluzzii* in Côte d'Ivoire, is concerning, as both insecticides have been used extensively in agriculture (Mouhamadou *et al.*, 2019). Currently only clothianidin has been approved for use in IRS in Mali (Institut du Sahel, 2019). Tests carried out in Bandiagora and the Mopti districts showed clothianidin to be effective (Oxborough *et al.*, 2019) and can therefore be considered for future SEMOS vector control programmes.

The first entomological survey carried out for the SEMOS vector control programme was in 2006. This and subsequent surveys have shown fluctuations in insecticide resistance to eight products from four out of the five classes (Wragge *et al.*, 2015) (Table 3.6).

4.2.1 Pyrethroids

Pyrethroid resistance in West Africa is well known (Cisse *et al.*, 2015; Wragge *et al.*, 2015; Sovi *et al.*, 2020) and was first documented in 1993 in Coté d' Ivoire (Elissa *et al.*, 1993). Entomological surveys carried out in Sadiola in 2006 showed a 99% mortality at 24 hours post-exposure to the pyrethroid deltamethrin. With the continual use of this insecticide in IRS from 2005 until 2011 (Fig. 3.2), the mortality fell to 68% in 2011 (Table 3.6; Wragge *et al.*, 2015). Only pyrethroid insecticides are used on bed nets due to their safety profiles (Cisse *et al.*, 2015). As a result of this increasing resistance to pyrethroid insecticides, a decision was made to stop all distribution of ITN, LLIN and net dipping as part of the SEMOS malaria vector control programme. The use of pyrethroids extensively in IRS, on LLIN and ITN, coupled with this insecticide being widely available for agricultural use, is the driving force behind the worsening resistance in the area (Klinkenberg *et al.*, 2008; Institut du Sahel, 2019, Zouré *et al.*, 2020). Interestingly, the mortality for pooled *An. gambiae* complex fell to as low as 36% at 24 hours in 2016, despite the use of this class of insecticide being stopped five years earlier (Table 3.6).

Anopheles gambiae had an improved mortality of 66.67% in 2018 compared to 11.9% in 2016 but the 2018 sample size was very small (n=6) so no conclusions can be drawn for this species. Further insecticide resistance surveys were carried out in 13 districts in Mali during 2019, revealing a high resistance to pyrethroid insecticides, with the Kayes district having mortality of 67% at 24 hours (PMI Mali, 2020). Tests carried out on *An. gambiae* in Mali between 2013–2016 in four districts in Mopti, two districts in Koulikoro and one district in Segou, showed 5 x deltamethrin only had an average mortality at 24 hours of 64.5% (PMI Africa IRS, 2017), indicating a high level of resistance. This resistance to 5 x deltamethrin was echoed in Accra, Ghana with mortality at 24 hours being 24% (Pwalia *et al.*, 2019). *Anopheles arabiensis* showed similar improvements when comparing 2016 (86.21%) and 2018 (98.9%) for mortality at 24 hours (Table 3.7). This coupled with 100% mortality at 24 hours when exposed to 5 x deltamethrin (Tables 3.6 & 3.7) is very promising as it could indicate that the effectiveness of LLIN and ITN nets in the area has been restored.

Further entomological surveys will need to be conducted to see if this resistance continues to improve. No pyrethroid/PBO (piperonyl butoxide) combination LLINs have been distributed in the area as yet, but PBO nets should be considered as an addition to SEMOS malaria vector control programmes, due to their higher efficacy than LLIN and ITNs' treated only with pyrethroids (Menze *et al.*, 2020).

4.2.2 Organophosphates

Resistance to pirimiphos-methyl was reported in *An. gambiae* for the first time in 2017 in three towns in Tanzania (Arumeru 87%, Geita 82.5% and Muleba 86.3%) (Kisinza *et al.*, 2017). In SEMOS in 2013, the insecticide used for IRS was changed from deltamethrin to pirimiphos-methyl and this chemical continued to be used until the end of the malaria season in 2017 (Fig. 3.2) The 2016 entomological survey showed 96.7% mortality for *An. arabiensis* but only 87.3% mortality for *An. gambiae* (Table 3.7), indicating a developing resistance. Exposures of *An. gambiae* to another organophosphate, malathion, also showed resistance with 87.8% mortality at 24 hours. In 2018 and 2019, the carbamate bendiocarb and pirimiphos-methyl were used for IRS in a mosaic spray pattern in an attempt to mitigate the insecticide resistance. Surveys carried out in 2018 showed a return to 100% mortality at 24 hours for both *An. gambiae* and *An. arabiensis*, to both pirimiphos-methyl and malathion (Table 3.7). The return to 100% mortality is encouraging as pirimiphos-methyl is often used as the alternative insecticide when resistance to pyrethroids is detected (Abong'o *et al.*, 2020)

4.2.3. Carbamates

Cisse *et al.* (2015) reported bendiocarb resistance in four towns in Mali, namely: Kita, Bla, Bounouni and Kadiolo. Three members of the *An. gambiae* complex (*An. gambiae, An. coluzzii* and *An. arabiensis*) were identified from their study sites but unfortunately they did not do species identifications on the mosquitoes that were exposed to the insecticides. Bendiocarb resistance was further identified in 2017 (PMI Africa IRS, 2017) with an additional four sites showing resistance (Bamako 90%, Selingue 86%, Bougouni 76% and Baroueli 89%). Again, the resistance results were not correlated with species identification.

Resistance to bendiocarb was first seen in entomological surveys carried out at SEMOS in 2006 when pooled *An. gambiae* complex had a mortality at 24 hours of 74%, indicating that there was a strong resistance to this class of insecticide (Table 3.6). Alarmingly this resistance worsened during 2011 when mortality fell to 12% for pooled *An. gambiae.* From 2014 resistance to bendiocarb began to improve with 88% in 2014, 94% in 2016 and 96% in 2018 (Table 3.6). Both *An. gambiae* and *An. arabiensis* showed evidence for resistance (Table 3.7). The first time bendiocarb was used in the SEMOS IRS programme was in conjunction with pirimiphos-methyl in 2018, as part of a mosaic spray pattern (Fig. 3.2). This was introduced to determine if the mosaic spray pattern would help mitigate the resistance developing to pirimiphos-methyl. Annual testing of the local mosquito populations is needed to ensure the vector control programme stays on track.

Propoxur, the second carbamate tested, showed similar trends. In 2011 (Table 3.6) mortality at 24 hours was 21%, this subsequently improved in 2016 to 78% and 98% in 2018. The effect of propoxur on *An. arabiensis* and *An. gambiae* was similar to bendiocarb in 2016, with *An. arabiensis* having a 100% mortality at 24 hours compared to *An. gambiae* with 85.7% mortality. In 2018 mortality at 24 hours in *An. gambiae* was 100% compared to 97.7% in *An. arabiensis* (Table 3.7). The future potential use of propoxur in the IRS programme utilizing a mosaic spray pattern is hindered, as currently, propoxur is not on the approved list of insecticides allowed for use in Mali (Institut du Sahel, 2018).

4.2.4. Organochlorines

The use of organochlorines in monitoring insecticide resistance is to determine if a mutation has occurred on the sodium channel receptors that the insecticide is targeted to attack, known as “knockdown resistance” (*kdr*). Both DDT and pyrethroids share the same mode of action (WHO, 2018). This *kdr* is used to determine cross-resistance between DDT and pyrethroid insecticides (Brown, 1986). Cisse *et al.* (2015) highlighted that cross-resistance between pyrethroids and DDT exists in *An. arabiensis*, *An. coluzzii* and *An. gambiae* in Mali.

This cross-resistance can be seen in Table 3.6 where the resistance to both organochlorines and pyrethroid decreases in a similar pattern. *Anopheles arabiensis* (Table 3.7) in 2016 showed a different resistance pattern, indicating there may be additional mechanisms coupled with the *kdr* mutation that is adding to DDT (37.5%) resistance, when comparing it to pyrethroid (86.2%) resistance.

Tests carried out in previous surveys showed resistance to dieldrin for pooled *An. gambiae* was 75% in 2006, 82% in 2011, and 100% mortality at 24 hours in 2018 (Table 3.6). Dieldrin resistance was documented in Nigeria in 1956 with both *An. gambiae* and *An. arabiensis* known to be resistant (Coetzee *et al.*, 1999).

The usage of dieldrin peaked in the mid-1960s, with as much as 50% of all production in the United States of America being used in pest control in 1964. Dieldrin was banned by the Stockholm Convention in 2010 (www.chm.pops.int) due to its high lipid solubility, with concentrations of the insecticide being found in human liver, fat, breast milk and semen (Jorgenson, 2001).

DDT is still permitted for use in malaria vector control, however its use is limited to IRS only (WHO, 2011). The effectiveness of DDT for IRS was highlighted by Maharaj *et al.* (2005). In South Africa, DDT for IRS was stopped in 1996 and swapped for a pyrethroid insecticide, resulting in a resurgence of malaria cases in 1999/2000 as a result of pyrethroid resistance in the main vector species *An. funestus* (Hargreaves *et al.*, 2000). Fourteen countries in sub-Saharan Africa have utilized DDT in their respective IRS programmes since 2001 (Van den Berg *et al.*, 2017). No organochlorines have been used for IRS in the SEMOS malaria vector control programme since its start in 2005 (Wragge *et al.*, 2015) and currently the use of this class of insecticide is not permitted in Mali (Institut du Sahel, 2019).

4.3 Vector Control

The SEMOS vector control programme aims to control all breeding mosquitoes, regardless of whether they are nuisance or vector mosquitoes.

4.3.1. Water management

The SEMOS programme aims to treat or remove all standing water, irrespective of its status as a known *Anopheles* breeding site or not. The programme achieves this by larviciding all large bodies of standing water, treating of pit toilets, removing refuse and community education.

Larviciding

The programme has been using *Bacillus thuringiensis* var. *israelensis* (Bti) since 2008 as part of its water body management (Wragge *et al.*, 2015). Bti is ideal for larviciding as its primary action is on Diptera species by attacking the mid-gut of mosquitoes, but it has a low toxicity to non-target insects, animals and humans. It is considered an environmentally friendly product (Brűhl *et al.*, 2020). Currently, larviciding is not part of Mali's national malaria control plan (PMI 2019), which is a common trend in sub-Saharan Africa, whereby countries rely predominately on the distribution of LLINs and ITNs, and limited IRS (Derua *et al.*, 2019).

During the dry season, no water body management is needed in the area. Once the first heavy rains have occurred, known water accumulating areas are identified, GPS coordinates taken and monitored. Larviciding is started in July/August as by this stage the seasonal rains are well established (Table 3.1). Larviciding is done on a rotational basis and after heavy rains, as previous applications of Bti are assumed to have been flushed out of the water body (Dambach *et al.*, 2014).

In 2012 the SEMOS malaria control programme relied only on water body management as all IRS was stopped due to political uncertainty. It can be clearly seen that relying on just larviciding as a control method saw the highest number of malaria cases and cases involving children under 5 years old. These high numbers were also compounded by heavy rainfall experienced during that year, the highest over a 16 year period (Figs 2.2 & 3.2). The WHO guidelines for malaria vector control advise against using only larviciding for vector control (WHO, 2019).

Pit toilet treatment

Treating of pit toilets using polystyrene beads started in 2012, as mentioned earlier in Chapter One, as a means to maintain a presence in the community since IRS spraying was not done in 2012 due the military coup and the resulting logistical issues. After engaging with the Sadiola village mayor and village elders, the go-ahead was given to treat 674 toilets in the village. A further 63 toilets were treated in smaller villages such as Madine. GPS data were taken at each treated toilet and were plotted using Google Earth (Fig. 4.1).

In 2013, the treating of pit toilets continued (n = 683) because of leftover polystyrene beads and the positive response from the surrounding villages. Treating of pit toilets was done again in 2014 (n = 800) and 2016 (n = 1, 210).

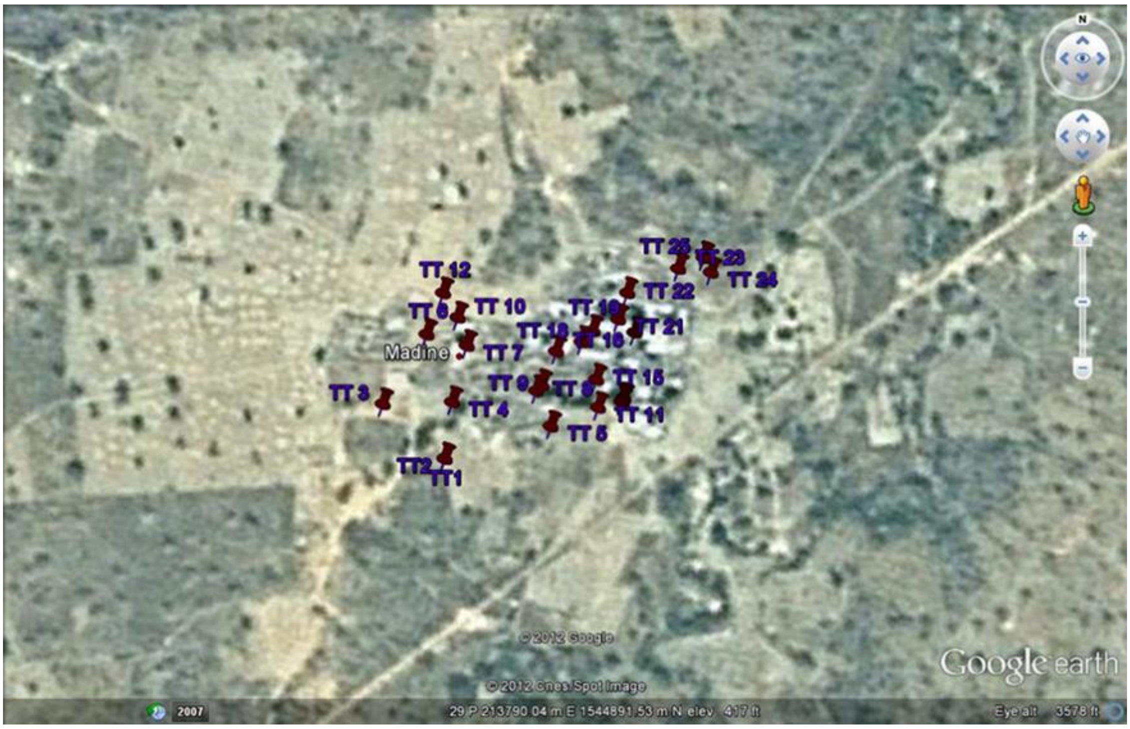


Figure 4.1. Treated toilets in Madine 2012.

The effectiveness of the polystyrene beads (Fig. 4.2) can been seen in the negligible emergence numbers of mosquitoes from these treated toilets (n = 7). When comparing adult emergence between treated and non-treated toilets, it was evident that the polystyrene beads drastically reduced emergence numbers. The non-treated bakery pit toilet produced 11,766 culicine mosquitoes (Table 3.2) over the project period with an average emergence of 324/day and 883 *Culex* mosquitoes caught during one night from a non-treated pit toilet in Babala village outside of the IRS spray zone. Treated toilets tested in Sadiola, Madine and Sirimana village produced 7 *Culex* mosquitoes. Sivagnaname *et al.* (2005) in a review article reported similar reductions in emerging *Cx. quinquefasciatus* between treated and non-treated pit toilets in India and Tanzania.

Figure 4.2. Treating a pit toilet with 2kg of 2-4mm polystyrene beads.

Refuse removal

The SEMOS vector control team assists households with regards to the correct way to dispose of refuse that might provide mosquito larval habitats. In some incidences, old items such as tin cans and disused tyres are removed, with the homeowner’s permission, and disposed of correctly in the mine’s landfill site (Shenton *et al.*, 2019). By doing this, it strengthens community/spray team relationships and has had a positive impact on helping to increase acceptability of IRS and larviciding in each respective village, as the communities feel that they are “getting something back”.

4.3.2. Indoor Residual Spraying (IRS)

In the 1930s, research on the malaria vectors in South Africa demonstrated that the female mosquitoes spent a large portion of time indoors after taking a blood meal, and that they could be killed by weekly spraying of pyrethrum inside houses (Coetzee *et al*., 2013b). From the 1940's, spraying the inside walls of houses became popular with the invention of long-lasting insecticides such as DDT and was used extensively in southern Africa and elsewhere as a means of vector control. From 2000 IRS was rolled out in many African countries, mainly through the President's Malaria Initiative (PMI). However, as of 2017, only 7% of the population in affected sub-Saharan Africa are covered by IRS compared to 50% of the population that are covered by ITN's and LLIN's (Wilson *et al.*, 2020).

The SEMOS malaria vector control programme's aim is to reduce malaria morbidity and mortality, while trying to prevent insecticide resistance from worsening in the area. The IRS component of the programme started in 2005 using deltamethrin (Wragge *et al.*, 2015). As mentioned earlier, IRS insecticides have been rotated to try to reduce insecticide resistance and incidences of malaria, using insecticides that are approved for use in IRS in Mali (Institut du Sahel, 2018). The programme tries to achieve an 80% or higher spray coverage in the area (WHO, 2019).

In the earlier days of the programme local politics in the surrounding villages resulted in members of the communities being reluctant to allow IRS teams into their houses and only wanting to rely on net dipping to protect themselves. A similar lack of political will by community leaders was seen in Mozambique regarding their involvement in either preventing or allowing the implementation of an IRS programme (Munguambe *et al.*, 2014). The village chiefs also felt that they should have representation on the team in that one of the IRS team members must come from their respective villages. From 2011, access to houses for IRS started to improve with the incorporation of the respective village chiefs' request.

Prior to 2011, the IRS teams consisted of illiterate members from the surrounding villages. From 2011, all team members had to pass a basic French reading and writing test, thus improving the quality of understanding regarding malaria, and in turn could pass this information onto their respective families and village communities. Having IRS team members that are literate has ensured that data regarding number of houses sprayed, LLIN used and number of people per house is more reliable.

Physical fitness assessments are done on all spray team members prior to employment. Year on year improvements in the IRS team physical fitness has ensured that proper IRS techniques are maintained when spraying the insecticide onto the wall surfaces (WHO, 2015; Desalegn *et al.*, 2018).

In 2013, pirimiphos-methyl (Actellic EC50) was introduced as the insecticide of choice for IRS (Wragge *et al.*, 2015). The emulsifiable concentrate (EC) version of the insecticide was used until 2015, when in 2016 it was changed to a capsule suspension (CS) that is more suited to mud covered interior walls than EC solutions (Rowland *et al.*, 2013). This could have further reduced the malaria case numbers over the years. Pirimiphos-methyl CS was continued in 2017.

In response to the increased malaria case numbers in 2017 (Fig. 3.2) and insecticide susceptibility data showing 89% mortality on pirimiphos-methyl (Table 3.6), a mosaic spray pattern was introduced in 2018 using the organophosphate (pirimiphos-methyl CS) and a carbamate (bendiocarb) (WHO, 2019). This was achieved by spraying each of the smaller villages within the spray zone with a different class of insecticide, and according to neighbourhood “blocks” in the larger Sadiola village (Corbel & N’Guessan, 2013; WHO, 2015). The mosaic pattern was repeated in 2019, further reducing malaria transmission in the area (Fig. 3.2), and helping reduce insecticide resistance. Since resistance to the different classes of insecticides is caused by different genetic mechanisms in the mosquito vectors, the IRS mosaic pattern aims to manage the resistance in *An. gambiae* and *An. arabiensis* so that if one insecticide does not kill an individual the other insecticide will.

4.3.3. Education

Education related to the cause of malaria and how to reduce mosquito breeding is re-enforced during each IRS spray round. The re-enforcing of household-based education each year, regarding good housekeeping practices involving the removal of refuse and keeping the areas around houses clear of possible breeding sites could have helped reduce malaria transmission further.

Additional educational drives regarding standing water, encouraged local building-block makers in the respective villages to insert a small hole at the bottom of each block to allow the water to drain out while the block is drying in the sun. This was done to prevent the accumulation of water and help reduce other mosquito populations, such as *Cx. quinquefasciatus*, *Cx. univittatus*, *Ae. aegypti* and *Ae. vittatus* that prefer breeding in these stagnant water bodies.

As women are involved in the daily collection of water and the subsequent storing of water in their respective homes, education was directed at local women's groups within each village to ensure that the storage pots were covered at all times. The women were also encouraged to ensure that all refuse that could hold water, was removed and disposed of correctly.

The SEMOS-run local TV station (Draman TV), which is broadcast to the surrounding villages, is used to highlight the effects of malaria and to encourage seeking early treatment. SEMOS uses an animated awareness programme called Buzz & Bite (French version) during the Malian school holidays (August – October) (http://www.chocmoose.com/portfolio-item/buzz-and-bite/), which has helped reinforce the IRS teams household education (Owusu-Addo E & Owusu-Addo SB, 2014).

As a result of the SARS-CoV-2 pandemic in 2020, Covid-19 education was incorporated into the IRS spray teams training. The aim of this was firstly for them to be aware of the virus and how it is spread, so to better protect themselves when undertaking IRS. Secondly, to assist with disseminating the correct information regarding Covid-19 to the surrounding communities such as hand washing techniques, the importance of wearing face masks and to seek medical attention soon if they are feeling unwell (Chanda-Kapata *et al.*, 2020; Rahi *et al.*, 2020).

4.4. *Anopheles* species diversity and abundance

The entomological surveys carried out at SEMOS goldmine revealed a number of effective malaria transmitting species (Fig. 3.3).

4.4.1. The *Anopheles gambiae* complex

It is well-known that the *An. gambiae* complex contains the most effective and efficient malaria vectors in the world and that they are widespread across sub-Saharan Africa (Sinka *et al.*, 2010). They are found in wide ecological zones, from the hotter arid areas of Mopti to the more humid forested areas of Mali, in both urban and rural settings.

Within the SEMOS vector control zone, three members of the *An. gambiae* complex have been identified: namely *An. gambiae s.s.*, *An. arabiensis* and *An. coluzzii*.

4.4.1.1 *Anopheles gambiae s.s.*

Anopheles gambiae is a relatively long-lived species with a short larval stage. It is highly anthropophilic – preferring to bite humans. The species is a late night feeder, preferring to bite and rest indoors to digest its blood meal. However, it is an adaptive, opportunistic species and can be found biting and resting outdoors (Sinka *et al.*, 2010). The species aestivates during the drier months and this ability is the main mechanism that allows malaria to persist in the Sahel region during the dry season (Yaro *et al.*, 2012; Gillies & De Meillon, 1968). As seen in Fig. 3.3, *An. gambiae* has been the main malaria vector in the area since 2011, with numbers peaking in 2016. In the 2018 survey carried out, there was a drastic drop in their numbers. This cannot be attributed to rainfall, as 2018 had only 121.5mm more rain (n= 860.0mm) compared to 2016 (n=738.5mm).

The 2018 surveys showed that both *An. gambiae* and *An. arabiensis* had 100% mortality when exposed to 5 x deltamethrin. However, *An. gambiae*'s preference for biting and resting indoors could be the reason for the sudden drop in species numbers with the rotational IRS and re-activation of the LLIN and ITN nets in the area. Kitau *et al.* (2012) commented on similar findings in a LLIN/ITN research project in Moshi and Muheza, Tanzania, whereby proportionally less *An. arabiensis* died due to LLIN and ITN than *An. gambiae* showing that *An. arabiensis* were avoiding the nets.

4.4.1.2. *Anopheles arabiensis*

The change from *An. gambiae* being the predominant malaria vector to that of *An. arabiensis*, is especially apparent in the 2006 and 2018 surveys (Fig. 3.3). A number of possible reasons could be the cause of this change. The time of the year in which the surveys were carried out, for example, was different in 2006 and 2018 (September/October) compared with the other surveys being done in August (Table 3.5). October is the end of the rainy season where relative humidity and rainfall is decreasing. Carrying out the survey at the end of the rainy season may have resulted in more favourable conditions for *An. arabiensis* (Sogoba *et al.*, 2007), thus resulting in the higher numbers seen in these two years.

Another possible reason for the 2018 increase is deforestation due to the influx of people into the area since 2016 following local “gold rushes”, producing breeding sites more favourable to *An. arabiensis* than *An. gambiae*. Small-scale artisanal mining involves clearing of vegetation, increase in foot and vehicle traffic, excavating holes and bringing in water for gold panning, creating a “moon like” environment (Fig. 4.3) (Aryee *et al.*, 2003). With this influx of people seeking their fortunes, an increase in agricultural activities around the small-scale mining area developed to supply food to the growing population, thus increasing possible larval sites (Yasuoka & Levins, 2007). The reduction in the natural vegetation may result in a higher water body temperature that is more favourable for *An. arabiensis* (Kirby & Lindsay, 2009). *Anopheles arabiensis* prefers a drier environment with sparse woodlands (Sinka *et al.*, 2010), thus small-scale mining may be adapting the environment more in their favour than for *An. gambiae*.

The decreased number of malaria cases in the two years when *An. arabiensis* was dominant (2006 – 289 cases, 2018 – 317 cases, Fig. 3.2) is interesting. Specimens from 2018 were processed by ELISA for circumsporozoite antigen at the NICD and 716 *An. arabiensis* tested negative while 1 *An. gambiae* (n=60) was positive (1.7% infectivity rate). Unfortunately, none of the 2006 samples were processed for parasite infectivity. The difference in sporozoite rates between *An. arabiensis* and *An. gambiae* has been recorded as far back as the 1970s with, for the most part, *An. arabiensis* being a much poorer vector (Gillies & Coetzee, 1987).

The outdoor tendencies of *An. arabiensis* also allows this species to avoid treated surfaces, making it difficult to control. A similar shift in vector species was seen in Kenya and in Senegal whereby the predominant species shifted from *An. gambiae* to *An. arabiensis* with the large-scale roll out of mosquito nets (Sougoufara *et al.*, 2017).

Figure 4.3. Effects of small-scale mining in Sadiola, on the environment. (May 2019, SEMOS Security department)

4.4.1.3. *Anopheles coluzzii*

The early work of Touré *et al.* (1998) on the banding patterns of the polytene chromosomes of the *An. gambiae* complex had demonstrated that at least two species under the name "*gambiae*" existed in Mali. Coetzee *et al.* (2013a) described and named one of these species as *An. coluzzii*, a new member of the *An. gambiae* complex, with the identification of this species relying on molecular methods. *Anopheles coluzzii* is an effective malaria vector, playing a role in malaria transmission in both central and West Africa (Main *et al.*, 2015). The species is rare in the SEMOS spray zone (Wragge *et al.*, 2015), with numbers only starting to increase from 2014, the highest number being caught during the 2016 (n=18) survey (Fig. 3.3). As Main *et al.* (2015) pointed out, *An. coluzzii* shows resistance to both permethrin and deltamethrin. Like *An. gambiae, An. coluzzii* is a prolific anthropophilic, endophilic and endophagic mosquito (Sougoufara *et al.*, 2016), thus the current IRS programme that SEMOS uses is able to target this species using the pirimiphos-methyl/bendiocarb mosaic IRS.

4.4.2. The *Anopheles funestus* sub-group

The *An. funestus* group in Africa consists of 11 species, of which *An. funestus s.s.* is considered to be the only major vector of malaria parasites (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). Minor vectors include *An. rivulorum* and *An. leesoni* in Tanzania (Wilkes *et al.*, 1996; Temu *et al.*, 2007) and more recently, *An. parensis* and *An. vaneedeni* in South Africa (Burke *et al.*, 2017, 2019). The latter two species do not occur in Mali (Irish *et al.,* 2020).

Anopheles funestus was identified in low numbers in numerous surveys carried out by the mine, with 16 identified in 2016 and 1 during the 2018 survey (Fig. 3.3). This species is often associated with rice cultivation with its preference for using large, permanent and semi-permanent fresh water bodies for egg laying (Gillies & De Meillon, 1968). *Anopheles funestus* is a late night biter with biting peaking after 22h00. It is generally anthropophilic and endophilic, but is an adaptable species allowing for a large distribution involving different habitat types and climatic conditions (Sinka *et al.*, 2010). Guelbeogo *et al.* (2014) highlighted this adaptation ability, whereby after mass distribution of ITNs, the species changed its biting times to peak during the dawn and early morning when the population was awake, and therefore no longer protected by the bed nets in rural villages of Koubri and Kuiti, Burkina Faso. The re-activation of the LLINs and ITNs in 2018 could be the reason for the drop in numbers of *An. funestus* caught during the survey. Further studies would need to be undertaken to establish if this avoidance adaptation, as mentioned by Guelbeogo *et al.* (2014), towards LLINs and ITNs, is occurring. Like *An. gambiae*, *An. funestus* is an effective transmitter of *Wuchereria bancrofti,* which is responsible for Lymphatic Filariasis in both Mali and elsewhere in West Africa (Coulibaly *et al.*, 2013).

The 2018 entomological survey identified *Anopheles rivulorum*-like, a member of the *An. funestus* group, for the first time in the area (Fig. 3.3) and in Mali. The furthest west this species has been recorded previously is from north-east Cote d'Ivoire (Adja *et al.*, 2006). A manuscript describing this has been accepted for publication by the *Transactions of the Royal Society of Tropical Medicine & Hygiene* (Appendix 3).

4.4.3 Adaptation of *Anopheles*

The samples obtained during the course of this research, shows that *Anopheles* mosquitoes in the Sadiola district have not currently adapted to breeding in polluted water. Of all the emergence traps placed throughout the area, only one male *Anopheles* mosquito was caught at the SEMOS mine village sewage plant on the first day of data collection.

4.5. Neglected Tropical Disease and Culicine Mosquitoes

4.5.1. Medical Significance of *Culex* mosquitoes.

Tandina *et al.* (2018) identified twenty-eight *Culex* species, belonging to four subgenera in Mali, with *Cx.* (*Culex*) *poicilipes*, *Cx. antennatus*, *Cx*. *quinquefasciatus* and *Cx. neavei* being possible *Flavivirus* (family: Flaviviridae) and lymphatic filariasis vectors.

West Nile Virus is wide spread in Africa, with *Culex* mosquitoes being one of the main vectors, and domestic and wild birds being the main hosts. In favourable conditions, the virus can be transmitted to humans and horses. Normally the virus in humans presents with mild febrile signs or is asymptomatic, however, in severe cases, the virus can result in meningitis and encephalitis, with a mortality of 26-43% (Chevalier *et al.*, 2004). Sule *et al.* (2018) reported that serological evidence of West Nile virus in humans is present in Mali.

Culex quinquefasciatus is one of the known vectors of *Wuchereria bancrofti,* mosquito-borne filarial nematodes that causes Lymphatic Filariasis (LF) in East Africa (Derua *et al.*, 2017). In West Africa the main vectors for LF are members of the *An. gambiae* complex and *An. funestus* group (Coulibaly *et al.*, 2013). As Harrus & Baneth (2005) pointed out, the large increase in travel, especially between the developing and the developed world, is allowing for the invasion of other known vector species through road, shipping and air travel. This movement can be one of the drivers for a possible emergence of LF in *Cx. quinquefasciatus* in West Africa.

Two studies carried out, one in Brazil (Guedes *et al.*, 2017) and another in China (Guo *et al.*, 2016), highlighted that *Cx. quinquefasciatus* has the potential to carry the Zika virus. If this is confirmed in further studies, it could have a significant impact on the control of this vector species.

Large numbers of *Cx. quinquefasciatus* and *Cx. univittatus* where caught in the emergence traps, especially the trap placed over the untreated pit toilet. The vast numbers emerging from this pit toilet was alarming and a similar pattern was seen in the trap placed in Babala village, which is outside of the vector control programme area. The lack of emerging mosquitoes from the treated pit toilets in the surrounding villages is promising as it shows that even after a number of years, the polystyrene beads are still effective in stopping mosquitoes from breeding in these pit toilets.

4.5.2. Medical significance of *Aedes* mosquitoes

Most of the *Aedes* mosquitoes were caught in the sewage plant (63%) or standing water (22%), with very low numbers emerging from the bakery pit toilet (15%). *Aedes aegypti* and *Ae. vittatus* (Fig. 4.4) were identified, both of which are known vector species for the transmission of dengue, yellow fever, Zika and chikungunya viruses (Sudeep & Shil, 2017).

Aedes mosquitoes can breed in a wide range of "water bodies", from tree-holes, artificial containers, in some cases sewage systems and cesspits. Rapid urbanisation has helped *Ae. aegypti* flourish by creating man-made breeding sites in and around households (Amarasinghe *et al.*, 2011). It is therefore important to include the non-*Anopheles* breeding sites in malaria vector control programmes where *Ae. aegypti* is present, to prevent the transmission of the diseases they can carry (Banerjee *et al.*, 2015).

Aedes mosquitoes are responsible for the spread of arboviruses, which is described as a threat to global health. The control of these mosquitoes has been left to the countries concerned, with very little financial and logistical support from international donor agencies. This is true regarding insecticide resistance surveys whereby there is little understanding of resistance in *Aedes* species responsible for the spread of arboviruses. The WHO now regards insecticide resistance in *Aedes* species as a major threat to the emergence/re-emergence and spread of arboviruses globally (Corbel *et al.*, 2016).

Figure 4.4. *Aedes vittatus* caught from the sewage plant.

Aedes aegypti is the main transmitter of Dengue virus (DENV) (family Flaviviridae; genus Flavivirus), in not only Africa, but also affecting the Asia-Pacific and Americas-Caribbean regions. It is estimated that between 30-54.7% of the global population are at risk of contracting DENV (Brady *et al.*, 2012). It is endemic in 34 countries in Africa, including Mali. In 2008 the Sadiola area reported 109 suspected cases of Dengue fever with 2 suspected deaths (Ministere De La Sante et des Affaires Socilales – Mali, 2019).

Yellow fever virus is caused by the same virus family as DENV, that is Flaviviridae, and transmitted by *Ae. aegypti*. The development of the French Neurotropic Vaccine (FNV) in the 1930's resulted in a drastic drop in yellow fever cases globally. In recent years however, the re-emergence of yellow fever in Africa is being driven by collapsing or poor health care facilities, inappropriate vector control methods and surveillance, rapid urbanisation coupled with poverty, influx of the population from rural to urbanised areas and deforestation (Oyewale, 2002).

The Zika virus was first reported in humans in Uganda in 1964. Testing carried out in Mali between 1964-1967 isolated Zika virus antibodies in human blood samples using the hemagglutination inhibitor test (Kindhauser *et al.*, 2016). *Aedes aegypti* is regarded as the main transmitter of the virus, however, it has now been isolated in a number of other *Aedes* species including *Ae. vittatus* in a study carried out in Cote d'Ivoire in 1999 (Diange *et al.*, 2015). Research by Herrera *et al.*, (2017) showed that Zika was endemic in Senegal and Nigeria, stating that data on Zika virus, especially in West Africa, was lacking. The Zika epidemic in South American countries in 2015 – 2016, causing severe neurological conditions, has increased global awareness of the virus. In under resourced rural African villages, pyrexia of unknown origin is commonly recorded as malaria and not their "true" diagnosis (Weetman *et al.*, 2018).

The Chikungunya virus (CHIKV) was first isolated in Tanzania in 1953 and is transmitted by *Ae. aegypti* and *Ae. albopictus*. Research carried out in Kenya highlighted that *Ae. vittatus* is also an effective transmitter of CHIKV (Mulwa *et al.*, 2018). Chikungunya has been identified in West African countries such as Guinea, Senegal, Sierra Leone, Nigeria and Benin (Simo *et al.*, 2019). Chikungunya is a neglected tropical disease that needs special attention due to its rapid spread to new areas and lack of specific treatment (Wahid *et al.*, 2017). Travel is promoting the spread of this virus (Mulwa *et al.*, 2018).

The similarities in clinical features between malaria and the above arboviral diseases of fever, joint pains and rash, results in a majority of the viral cases not being recorded correctly, as they are assumed to be malaria due to either no proper medical examination or no laboratory facilities to properly diagnose the virus. As such, malaria vector control programmes should consider integrating *Aedes* breeding sites and insecticide resistance monitoring into the existing programmes.

4.6. Methodology Limitations

4.6.1 Larval collections

Very few live mosquitoes were collected from the sites within the spray zone. The lack of mosquitoes developing in water bodies could be due to the puddles drying up fairly quickly and/or the fact that sampling was taking place in the vector control spray programme area, thus having a negative impact on the numbers obtained. However, a positive outcome was the fact that so few larvae were caught, showing that the current water body management in the vector control programme is effective.

4.6.2 Emergence trap collection

On three occasion’s ants and beetles got into the traps, resulting in either zero or low numbers caught. A skink lizard became trapped in one of the traps on one occasion, resulting in all the mosquitoes caught having to be released to ensure the safe releasing of the lizard. For a two-week period, a female donkey and her foal chased and harassed the researcher when collecting the trap from the bakery pit toilet, much to the delight of the bakery staff and school children on their way to school in the mornings.

The large numbers of mosquitoes caught in the bakery pit toilet emergence trap damaged the adult mosquitoes, with many of the mosquito scales being rubbed off, making identification difficult.

Chapter 5.

Conclusion

All malaria vector control programmes should rely on regular entomological surveys to ensure complete understanding of the biology of the vector mosquitoes in the area and their insecticide resistance status. This information is crucial for developing policies and strategies for vector control. The surveys carried out for the SEMOS control programme has guided choice of insecticide and has seen a decrease in malaria transmission over the past seven years. The entomological surveys should ideally not be limited to only the control of *Anopheles* species, but should take a holistic approach for targeting all disease-carrying mosquitoes. Including the biology and insecticide resistance status of *Culex* and *Aedes* vectors in future surveys is encouraged.

The use of a mosaic spray pattern, in conjunction with regular rotation of the different classes of insecticides, has helped improve the overall insecticide resistance seen in surveys carried out within the SEMOS malaria vector control programme. Mosaic spray patterns should be considered by other vector control programmes as an effective way of ensuring a sustained decrease in malaria transmission while limiting the development of insecticide resistance.

The limited entomological survey reported here produced no evidence for *Anopheles* mosquito adaptation to breeding in sewage-contaminated water within the SEMOS spray zone. However, a literature search has shown that such adaptations are occurring elsewhere in Africa and the possibility of it happening here is likely and should be monitored. It is therefore suggested that malaria vector control programmes incorporate those water bodies considered unfavourable for *Anopheles* breeding, into their water body management programmes.

The use of pre-expanded 2-4mm polystyrene beads offer a cheap, effective and long lasting method of controlling mosquitoes breeding in pit toilets and should be considered as an addition to the vector control “tool box”. The prevention of nuisance mosquitoes breeding in pit toilets has helped improve community / vector control programme partnerships, with the community noticing an overall reduction in mosquitoes in their villages, thus being more open to allowing other interventions, such as IRS, to be carried out within their community. The treating of these unfavourable water bodies can also assist in controlling the spread of other vector-borne diseases carried by *Culex* and *Aedes* species, which are known to favour polluted water bodies.

An important outcome of the research carried out here is the documenting of *Anopheles rivulorum*-like for the first time in Mali. Previously, the furthest west that this species had been found was northern Cameroun, so this is a new distribution record. Very little is known about the biology of this species or its role in malaria transmission and these studies need to be carried out as soon as possible.

References

Abong'o B., Gimnig JE., Torr SJ., Longman B., Omoke D., Muchoki M., ter Kuile F., Ochomo E., Munga S., Samuels AM., Njagi K., Maas J., Perry RT., Fornadel C., Donnelly MJ., Oxborough RM. (2020). Impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS) on entomological indicators of transmission and malaria case burden in Migori county, western Kenya. *Nature* 10: 4518.

http://doi.org/10.1038/s41598-020-61350-2

Adja AM., N'Goran KE., Kengne P., Koudou GB., Toure M., Koffi AA., Tia E., Fontenille D., Chandre F. (2006). Vectorial transmission of malaria in shrubby Savannah area at Ganse, Ivory Coast. *Médecine Tropicale* 66: 449-455.

African Development Bank Group. (2019). African Economic Outlook 2019. ISBN 978-9938-882-87-2.

Agossa FR., Gnanguenon V., Anagonou R., Azondekon R., Aïzoun N., Sovi A., Oké-Agbo F., Sèzonlin M., Akogbéto MC. (2015). Impact of insecticide resistance on the effectiveness of pyrethroid-based malaria vectors control tools in Benin: Decreased toxicity and repellent effect. *PLoS One* 10: e0145207.

http://doi.org/10.1371/journal.pone.0145207

Aiglo DY., Larbi RT., Kushitor MK., Biney A., Asante PY., Dodoo ND., Dodoo FN. (2018). Sense of community and willingness to support malaria intervention programme in urban poor Accra, Ghana. *Malaria Journal* 17: 289.

http://doi.org/10.1186/s12936-018-2424-0

Amarasinghe A., Kuristsky JN., Letson GW., Margolis HS. (2011). Dengue virus infection in Africa. *Emerging Infectious Diseases* 17: 1349-1354.

http://doi.org/10.3201.eid1708.101515

Aryee BNA., Ntibery BK., Atorkui E. (2003). Trends in the small-scale mining of precious minerals in Ghana: a perspective on its environmental impact. *Journal of Cleaner Production* 11: 131-140.

Awolola TS., Oduola AO., Obansa JB., Chukwurar NJ., Unyimadu JP. (2007). *Anopheles gambiae s.s.* breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *Journal of Vector Borne Disease* 44: 241-244.

Azrag RS., Mohammed BH. (2018). *Anopheles arabiensis* in Sudan: a noticeable tolerance to urban polluted larval habitats associated with resistance to Temephos. *Malaria Journal* 17: 204. http://doi.org/10.1186/s12936-018-2350-1

Banerjee SB., Mohan S., Saha N., Mohanty SP., Saha GK., Aditya G. (2015). Pupal productivity and nutrient reserves of *Aedes* mosquitoes breeding in sewage drains and other habitats of Kolkata, India: implications for habitat expansion and vector management. *Indian Journal of Medical Research* 142: 87-94.

http://doi.org/10.4103/0971-5916.176632

Bates I., Fenton C., Gruber J., Lalloo D., Lara AM., Squire SB., Theobald S., Thomson R, Tolhurst R. (2004). Vulnerability to malaria, tuberculosis, and HIV/AIDS infection and disease. Part II: determinants operating at environmental and institutional level. *The Lancet Infectious Diseases* 4: 368-374. http://infection.thelancet.com

Berry I., Walker P., Tagbor H., Bonjang K., Coulibaly SO., Kayentao K., Williams J., Oduro A., Milligan P., Chandramohan D., Greenwood B., Cairns M. (2018). Seasonal dynamics of malaria in pregnancy in West Africa: Evidence for carriage of infections acquired before pregnancy until first contact with antenatal care. *American Journal of Tropical Medicine and Hygiene* 98: 534-542. http://doi.org/10.4269/ajtmh.17-0620

Bleck J., Michelitch K. (2015). The 2012 crisis in Mali: Ongoing empirical state failure. *African Affairs* 114 (457): 598-623. https://doi.org/10.1093/afraf/adv038

Braack L., Hunt R., Koekemoer LL., Gericke A., Munhenga G., Haddow AD., Becker P., Okia M., Kimera I., Coetzee M. (2015). Biting behaviour of African malaria vectors: 1. Where do the main vector species bite on the human body? *Parasites & Vectors* 8: 76. http://doi.org/10.1186/s13071-015-0677-9

Brady OJ., Gething PW., Bhatt S., Messina JP., Brownstein JS., Hoen AG., Moyes CL., Farlow AW., Scott TW., Hay SI. (2012). Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Neglected Tropical Diseases* 6: e1760. http://doi.org/10.1371/journal.pntd.0001760

Brown AWA. (1986). Insecticide resistance in mosquitoes: A pragmatic review. *Journal of the American Mosquito Control Association* 2: 123-140.

Brűhl CA., Després L., Frör O., Patil CD., Poulin B., Tetreau G., Allgeier S. (2020). Environmental and socioeconomic effects of mosquito control in Europe using biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti). *Science of the Total Environment* 724: 137800. http://doi.org/10.1016/j.sciotenv2020.137800

Chanda-Kapata P., Kapata N., Zumla A. (2020). Covid-19 and malaria: A symptom screening challenge for malaria endemic countries. *International Journal of Infectious Diseases* 94: 151-153. http://doi.org/10.1016/j.ijid.2020.04.007

Chevalier V., de la Tocque S., Baldet T., Vial L., Roger F. (2004). Epidemiology processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese encephalitis and Crimean-Congo haemorrhagic fever. *Scientific and Technical Review of the Office International des Epizooties* (Paris). 23: 535-555.

Cisse MBM., Keita C., Dicko A., Dengela D., Coleman J., Lucas B., Mihigo J.., Sadou A, Belemvire A., George K., Fornadel C. (2015). Characterizing the insecticide resistance of *Anopheles gambiae* in Mali. *Malaria Journal* 14: 327.

http://doi.org/10.1186/s12936-015-0847-4

Coetzee M., Horne DWK., Brooke BD., Hunt RH. (1999). DDT, dieldrin and pyrethroid insecticide resistance in African malaria vector mosquitoes: an historical review and implications for future malaria control in South Africa. *South African Journal of Science* 95: 215-218.

Coetzee M., Hunt R., Wilkerson R., Della Torre A., Coulibaly MB., Besansky NJ. (2013a). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* 3619: 246-274.

http://doi.org/10.11646/zootaxa.3619.3.2

Coetzee,M., Kruger P., Hunt RH., Durrheim DN., Urbach J., Hansford CF. (2013b). Malaria in South Africa: 110 years of learning to control the disease. *South African Medical Journal* 103: 770-778.

Coleman M., Hemingway J. (2007). Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. *Journal of Pesticide Science* 32: 69-76 .

http://doi.org/10.1584/jpestics.R07-01

Corbel V., Achee NL., Chandre F., Coulibaly MB., Dusfour I., Fonseca DM., Grieco J., Juntarajumnong W., Lenhart A., Martins AJ., Moyes C., Ng LC., Pinto J., Raghavendra K., Vatandoost H., Vontas J., Weetman D., Fouque F., Velayudhan R., David J. (2016). Tracking insecticide resistance in mosquito vectors of arboviruses: The worldwide insecticide resistance network (WIN). *PLOS Neglected Tropical Diseases* 10: e0005054. https://doi.org/10.1371/journal.pntd.0005054

Corbel V., N'Guessan R. (2013). Distribution, mechanism, impact and management of insecticide resistance in malaria vectors: A pragmatic review. Anopheles *Mosquito: New insights into malaria vectors* 19: 579-633. ISBN: 9535111884, 9789535111887. http://dx.doi.org/10.5772/56117

Coulibaly YI., Dembele B., Diallo AA., Kristensen S., Konate S., Dolo H., Dicko I., Sangare MB., Keita F., Boatin BA., Traore AK., Nutman TB., Klion AD., Touré YT., Traore SF. (2013). *Wuchereria bancrofti* transmission pattern in southern Mali prior to and following the institution of mass drug administration. *Parasites & Vectors* 6: 247. http://www.parasitesandvectors.com/content/6/1/247

Coulibaly B., Kone R., Barry MS., Emerson B., Coulibaly MB., Niare O., Beavogui AH., Traore SF., Vernick K., Riehle MM. (2016). Malaria vector populations across ecological zones in Guinea Conakry and Mali, West Africa. *Malaria Journal* 15: 191. http://doi.org/10.1186/s12936-016-1242-5

Dambach P., Louis VR., Kaiser A., Ouedraogo S., Sié A., Sauerborn R., Becker N. (2014). Efficacy of *Bacillus thuringiensis* var. *israelensis* against malaria mosquitoes in northwestern Burkina Faso. *Parasite & Vectors* 7: 371.

http://doi.org/10.1186/1756-3305-7-371

Derua YA., Kweka EJ., Kisinza WN., Githeko AK., Mosha FW. (2019). Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. *Parasite & Vectors* 12: 426.

Http://doi.org/10.1186/s13071-019-3683-5

Derua YA., Rumisha SF., Batengana BM., Max DA., Stanley G., Kisinza WN., Mboera LEG. (2017). Lymphatic filariasis transmission on Mafia Islands, Tanzania: Evidence from xenomonitoring in mosquito vectors. *PLOS Neglected Tropical Diseases* 11:10. e0005938. http://doi.org/10.1371/journal.pntd.0005938

Desalegn Z., Wegayehu T., Massebo F. (2018). Wall-type and indoor residual spraying application quality affect the residual efficacy of indoor residual spray against wild malaria vector in southwest Ethiopia. *Malaria Journal* 17: 300.

http://doi.org/10.1186/s12936-018-2458-3

De Silva PM., Marshall JM. (2012). Factors contributing to urban malaria transmission in sub-Saharan Africa: a systematic review. *Journal of Tropical Medicine* 2012: 819563. http://doi.org/10.1155/2012/819563

Diagne CT., Diallo D., Faye O., Ba Y., Faye O., Gaye A., Dia I., Faye O., Weaver SC., Sall AA., Diallo M. (2015). Potential of selected Senegalese *Aedes* ssp. mosquitoes (Diptera: Culicidae) to transmit Zika virus. *BMC Infectious Diseases* 15: 492.

http://doi.org/10.1186/s12879-015-1231-2

Diallo D., Graz B., Falquet J., Traore AK., Giani S., Munkoro PP., Berthé A., Sacko M., Diakité C. (2006). Malaria treatment in remote areas of Mali: use of modern and traditional medicines, patient outcome. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100. 515-520. http://doi.org/10.1016/j.trstmh.2005.08.003

Directeur Generale de la Sante et De L’Hygiene Publique. (2019). Ministere de la Sante et des Affaire Sociales. No. 2822/MSAS/DGSHP. Republique Du Mali.

Elissa N., Mouchet J., Riviere F., Meunier JY., Yao K. (1993). Resistance of *Anopheles gambiae* s.s to pyrethroids in Cote D’Ivoire. *Annales de la Societe Belge de Medecine Tropicale* 73: 291-294.

Emidi B., Kisinza WN., Mmbando BP., Malima R., Mosha FW. (2017). Effect of physiochemical parameters on *Anopheles* and *Culex* mosquito larvae abundance in different breeding sites in a rural setting of Muhze, Tanzania. *Parasite & Vectors* 10: 304. http://doi.org/10.1186/s13071-017-2238-x

Fanello C., Petrarca V., della Torre A., Santolamazza F., Dolo G., Coulibaly M., Alloueche A., Curtis CF., Touré YT., Coluzzi M. (2003). The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *An. gambiae* s.s. *Insect Molecular Biology* 12: 241–245. http://doi.org/10.1046/j.1365-2583.2003.00407.x

Fanello C., Santolamazza F., della Torre A. (2002). Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology*, 16: 461–464. http://doi.org/10.1046/j.1365-2915.2002.00393.x

Ferguson NM. (2018). Challenges and opportunities in controlling mosquito-borne infections. *Nature* 559: 490-497. http://doi.org/10.1038/s41586-018-0318-5

Fossog BT., Antonio-Nkondjio C., Kengne P., Njokou F., Besansky NJ., Costantini C. (2013). Physiological correlates of ecological divergence along an urbanization gradient: differential tolerance to ammonia among molecular forms of the malaria mosquito *Anopheles gambiae*. *BMC Ecology* 13: 1.

http://wwwbiomedcentral.com/1472-6785/13/1

Gayer M., Legros D., Formety P., Connolly MA. (2007). Conflict and emerging infectious diseases. *Emerging Infectious Diseases* 13: 1625-1631. www.cdc.gov/eid

Gillies MT., Coetzee M. (1987). A Supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). Publications of the South African Institute of Medical Research, Johannesburg. No. 55. pp146.

Gillies MT., De Meillon B. (1968). The Anophelinae of Africa south of the Sahara (Afrotropical Region). Publications of the South African Institute for Medical Research, Johannesburg. No. 54. pp343 .

Guedes DRD., Paiva MHS., Donate MMA., Barbosa PP., Krokovsky L., Rocha SWS., Saraiva KLA., Crespo MM., Rezende TMT., Wallau GL., Barbos RMR., Oliveira CMF., Melo-Santos MAV., Pena L., Cordeiro MT., Franca RF., Oliveira ALS., Peixoto A., Leal WS., Ayres DFJ. (2017). Zika virus replication in the mosquito *Culex quinquefasciatus* in Brazil. *Emerging Microbes and Infections* 6: e69. http://doi.org/10.1038/emi.2017.59

Guelbeogo WM., Safnon N., Liu F., Besansky NJ., Costantini C. (2014). Behavioural divergence of sympatric *Anopheles funestus* populations in Burkina Faso. *Malaria Journal* 13: 65. http://doi.org/10.1186/1475-2875-13-65

Guo X., Li C., Deng Y., Xing D., Liu Q., Wu Q., Sun A., Dong Y., Cao W., Qin WC., Qin C, Zhao T. (2016). *Culex pipiens quinquefasciatus*: a potential vector to transmit Zika virus. *Emerging Microbes and Infections* 5: e102. http://doi.org/10.1038/emi.2016.102

Hailu A., Lindtjørn .B, Deressa W., Gari T., Loha E., Robberstad B. (2017). Economic burden of malaria and predictors of cost variability to rural households in south central Ethiopia. *PLoS One* e0185315. http://doi.org/10.10.1371/journal.pone.0185315

Hamer GI., Kelly PH., Focks DA., Goldberg TL. (2011). Evaluation of novel emergence traps to study *Culex* mosquitoes in urban catch basins. *Journal of the American Mosquito Control Association* 27: 142-147.

Hargreaves K., Koekemoer LL., Brooke BD., Hunt RH., Mthembu J., Coetzee M. (2000). *Anopheles funestus* is resistant to pyrethroid insecticides in South Africa. *Medical & Veterinary Entomology* 14: 181-189.

Harrus S., Baneth G. (2005). Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *International Journal for Parasitology* 35: 1309-1318. http://doi.org/10.1016/j.ijpara.2005.06.005

Hay SI., Guerra CA., Tatem AJ., Noor AM., Snow RW. (2004). The global distribution and population at risk of malaria: past, present, and future. *The Lancet Infectious Diseases* 4: 327-338. http://infection.thelancet.com

Heggenhougen HK., Hackethal V., Vivek P. (2003). The behavioural and social aspects of malaria and its control. An introduction and annotated bibliography. UNDP/World Bank/WHO. Special Programme for Research & Training in Tropical Diseases (TDR). www.who.int/tdr/publications/documents/seb_malaria.pdf

Herrera BB., Chang CA., Hamel DJ., Mboup S., Ndiaye D., Imade G., Okpokwu J., Agbaji O., Bei AK., Kanki PJ. (2015). Continued transmission of Zika Virus in humans in West Africa, 1992-2016. *The Journal of Infectious Diseases* 215: 1546-1550.

http://doi.org/10.1093/infdis/jix182

Impoinvil DE., Keating J., Mbogo CM., Potts MD., Chowdhury RR., Beier J. (2008). Abundance of immature *Anopheles* and culicines (Diptera: Culicidae) in different water body types in the urban environment of Malindi, Kenya. *Journal of Vector Ecology* 33: 107-116.

Institut du Sahel (2019). Liste globale des pesticides authorises par le Comité Sahélien des pesticides.

International Monetary Fund. List of LIC DSA's for PRGT – Eligible countries. 1 January 2019. https://www.imf.org/external/Pubs/ft/dsa/DSAlist.pdf

Irish SR., Kyalo D., Snow RW., Coetzee M. (2020). Updated list of *Anopheles* species (Diptera: Culicidae) by country in the Afrotropical Region and associated islands *Zootaxa* 4747: 401-449.

Jayaraj R., Megha P., Sreedev P. (2016). Organochlorines pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology* 9: 90-100. http://doi.org/10.1515/intox-2016-0012

Jorgenson JL. (2001). Aldrin and dieldrin: a review of research on their production, environmental deposition and fate, bioaccumulation, toxicity and epidemiology in the United States. *Environmental Health Perspectives* 109: 113-139.

http://doi.org/10.1289/ehp.01109s1113

Kamdem C., Fossog BT., Simard F., Etouna J., Ndo C., Kengne P., Bousse's P., Etoa F., Awono-Ambene P., Fontenille D., Antonio-Nkandjio C., Besansky NJ., Costantini C. (2012). Anthropogenic habitat disturbance and ecological divergence between incipient species of malaria mosquito *Anopheles gambiae*. *PLoS One* 7: e39453.

http://doi.org/10.1371/journal.pone.0039453

Keating J., MacIntrye K., Mbogo C., Githeko AW., Regens JI., Swalm C., Ndenga B., Steinberg LJ., Kobe L., Githure JI., Beier JC. (2003). A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa. *American Journal of Tropical Medicine and Hygiene* 63: 357-365.

Kindhauser MK., Allen T., Frank V., Santhana RS., Dye C. (2016). Zika: the origin and spread of a mosquito-borne virus. *Bulletin of the World Health Organization* 94: 675-686. http://doi.org/10.2471/BLT.16.171082

King SA., Onayifeke B., Akorli J., Sibomana JC., Manful-Gwira T., Dadzie S., Suzuki T., Wilson MD., Boakye DA., de Souza DK. (2017). The role of detoxification enzymes in the adaptation of the major malaria vector *Anopheles gambiae* Giles (Diptera: Culicidae) to polluted water. *Journal of Medical Entomology* 54: 1674-1683.

http://doi.org/10.1093/jme/tjx164

Kirby MJ., Lindsay SW. (2009). Effect of temperature and inter-specific competition on the development and survival of *Anopheles gambiae sensu stricto* and *An. arabiensis* larvae. *Acta Tropica* 109: 118-123. http://doi.org/10.1016/j.actatropica.2008.09.025

Kisinza WN., Nkya TE., Overgaard HJ., Massue DJ., Mageni Z., Greer G., Kaspar N., Mohamed M., Reithinger R., Moore S., Lorenz LM., Magesa S. (2017). Multiple insecticide resistance in *Anopheles gambiae* from Tanzania: a major concern for malaria vector control. *Malarial Journal* 16: 439. http://doi.org/10.1186/s12936-017-2087-2

Kitau J., Oxborough RM., Tungu PK., Matowo J., Malima RC., Magesa SM., Bruce J., Mosha FW., Rowland MW. (2012). Species shifts in the *Anopheles gambiae* complex: Do LLINs successfully control *Anopheles arabiensis*? *PLoS One* 7: e31481.

http://doi.org/10.1371/journal.pone.0031481

Klein MC., Harvey SA., Diarra H., Hurley EA., Rao N.., Diop S, Doumbia S. (2016). “There is no free here, you have to pay”: actual and perceived costs as barriers to intermittent preventive treatment of malaria in pregnancy in Mali. *Malaria Journal* 15: 158. http://doi.org/10.1186/s12936-016-1210-0

Klinkenberg E., McCall PJ., Wislon MD., Amerasinghe FP, Donnelly MJ. (2008). Impact of urban agriculture on malaria vectors in Accra, Ghana. *Malaria Journal* 7: 151. http://doi.org/10.1186/1475-2875-7-151

Koekemoer LL., Kamau L., Hunt RH., Coetzee M. (2002). A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene* 66: 804-811.

Kudom AA., Anane LN., Afoakwah R., Adokoh CK. (2018). Relating high insecticide residues in larval breeding habitats in urban residential areas to the selection of pyrethroid resistance in *Anopheles gambiae s.l.* (Diptera: Culicidae) in Akim Oda, Ghana. *Journal of Medical Entomology* 55: 490-495. http://doi.org/10.1093/jme/tjx223

Kyalo D., Amratia P., Mundia CW., Mobogo CM., Coetzee M., Snow RW. (2017). A geo-coded inventory of anophelines in the Afrotropical Region south of the Sahara: 1898 – 2016. *Wellcome Open Research* 2: 57.

http://doi.org/10.12688/wellcomeopenres.12187.1

Lam WK.., Dharmaraj D. (1982). A survey on mosquitoes breeding in septic tanks in several residential areas around Ipoh Municipality. *Medical Journal Malaysia* 37: 114-123 .

MacKenzie AG., Pooley J., Loots C., Samake B., Sangare S., Sidibe I. (2003). Four years after the move: Village relocation at Sadiola and Yatela Gold mines. Mali, West Africa. ResearchGate.

https://www.researchgate.net/publication/237621063_FOUR_YEARS_AFTER_THE_MOVE_VILLAGE_RELOCATION_AT_SADIOLA_AND_YATELA_GOLD_MINES_MALI_WEST_AFRICA

Maharaj R., Mthembu DJ., Sharp BL. (2005). Impact of DDT re-introduction on malaria transmission in KwaZulu Natal. *South African Medical Journal* 95: 871-874.

Main BJ., Lee Y., Collier TC., Norris LC., Brisco K., Fofana A., Cornel AJ., Lanzaro GC. (2015). Complex genome evolution in *Anopheles coluzzii* associated with increased insecticide usage in Mali. *Molecular Ecology* 24: 5145-5157.

http://doi.org/10.1111/mec.13382

Martens P., Hall L. (2000). Malaria on the Move: Human population movement and malaria transmission. *Emerging Infectious Diseases* 6: 103-109.

Martínez-Santos P., Martín-Loeches M., García-Castro N., Solera D., Díaz-Alcaide S., Montero E., García-Rincón J. (2017). A survey of domestic wells and pit latrines in rural settlements of Mali: Implications of on-site sanitation on the quality of water supplies. *International Journal of Hygiene and Environmental Health* 220:

1179–1189. http://doi.org/10.1016/j.ijheh.2017.08.001

Massebo F., Balkew M., Gebre-Michael T., Londtjorn B. (2013). Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in South-West Ethiopia. *Parasites & Vectors* 6: 44.

http://www.parasitesandvectors.com/content/6/1/44

Masurel Q., Thébaud N., Miller J., Ulrich S., Hein KAA., Cameron G., Béziat D., Bruguier O., Davis JA. (2017). Sadiola Hill: A world-class carbonate-hosted gold deposit in Mali, West Africa. *Society of Economic Geologists* 112: 23-47.

https://doi.org/10.2113/econgeo.112.1.23

Mazigo HD., Obasy E., Mauka W., Manyiri P., Zinga M., Kweka EJ., Mnyone LL., Heukelbach J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural Northwest Tanzania. *Malaria Research and Treatment* 794261. https://doi.org/10.4061/2010/794261

Mboumba DP., Akotet MKB., Ngoungou EB., Kombila M. (2010). Evaluation of rapid diagnostic tests for malaria case management in Gabon. *Diagnostic Microbiology and Infectious Disease* 66: 162-168. http://doi.org/10.1016/j.diagmicrobio.2009.09.011

Menze BD., Kouamo MF., Wondji MJ., Tchapga W., Tchoupo M, Kusimo MO., Mouhamadou CS., Riveron JM., Wondji CS. (2020). An experimental hut evaluation of PBO-based and pyrethroid-only nets against the malaria vector *Anopheles funestus* reveals a loss of bed nets efficacy associated with *GSTe*2 metabolic resistance. *Genes* 11: 143. http://doi.org/10.3390/genes11020143

Moody AH., Chiodini RL. (2002). Non-microscopic method for malaria diagnosis using OptiMal IT, a second-generation dipstick for malaria pLDH antigen detection. *British Journal of Biomedical Science* 59: 228-231.

http://doi.org/10.1080/09674845.2002.11783665

Mouhamadou CS., de Souza SS., Fodjo BK., Zoh MG., Bli NK., Koudou BG. (2019). Evidence of insecticide resistance selection in wild *Anopheles coluzzii* mosquitoes due to agricultural pesticide use. *Infectious Diseases of Poverty* 8: 64.

http://doi.org/10.1186/s40249-019-0572-2

Mulwa .F, Lutomiah J., Chepkorir E., Okello S., Eyase F., Tigoi C., Kanato M., Sang R. (2018). Vector competence of *Aedes bromeliae* and *Aedes vitattus* mosquito populations from Kenya for chikungunya. *PLoS Neglected Tropical Diseases* 12: e0006746. https://doi.org/10.1371/journal.pntd.0006746

Munguambe K., Pool R., Montgomery C., Bavo C., Nhacolo A., Fiosse L., Sacoor C., Nhalungo d., Mabunda S., Macete E., Alsono P. (2014). What drives community adherence to indoor residual spraying (IRS) against malaria in Manhiça district, rural Mozambique: a qualitative study. *Malaria Journal* 10: 344.

http://doi.org/10.1186/1475-2878-344

Nakagiri A., Niwagaba CB., Nyenje PM., Kulabako RN., Tumuhairwe JB., Kansiime F. (2016). Are pit latrines in urban areas of sub-Saharan African performing? A review of usage, filling insects and odour nuisances. *BMC Public Health* 16: 120.

http://doi.org/10.1186/s12889-016-2772-z

Owusu-Addo E., Owusu-Addo SB. (2014). Effectiveness of health education in community-based malaria prevention and control in sub-Saharan Africa: A systematic review. *Journal of Biology, Agriculture and Healthcare* 4: 22-34. ISSN 2225.093X (online).

Oxborough RM., Seyoum A., Yihdego Y., dabire R., Gnanguenon V., Wat'senga F., Agossa FR., Yohannes G., Coleman S., Musa Samdi L., Diop A., Faye O., Magesa S., Manjurano A., Okia M., Alyko E., Masendu H., Baber I., Sovi A., Rakotoson J., Varela K., Abong'o B., Lucas B., Fornadel C., Dengela D. (2019). Susceptibility testing of *Anopheles* malaria vectors with the neonicotinoid insecticide clothianidin; results from 16 African countries, in preparation for indoor residual spraying with new insecticide formulations. *Malaria Journal* 18: 264. http://doi.org/10.1186/s12936-019-2888-6

Oyewale T. (2002). Yellow fever in Africa: public health impact and prospects for control in the 21st century. *Biomédica* 22: 178-193.

http://www.redalyc.org/articulo.oa?id=84322213

PMI Africa IRS (AIRS). (2017). Project Indoor Residual Spraying (IRS2). Task order six. AIRS Mali entomological monitoring progress report. Abt. Associates, Inc.

Presidents' Malaria Initiative Mali. Malaria Operational Plan FY (2018). https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy-2018/fy-2018-mali-malaria-operational-plan.pdf?sfvrsn=5.

Presidents' Malaria Initiative Mali. Malaria Operational Plan FY (2020). https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy20/fy-2020-mali-malaria-operational-plan.pdf?sfvrsn=6

Pwalia R., Joannides J., Iddrisu A., Addae C., Acquah-Baidoo D., Obuobi D., Amlalo G., Akporh S., Gbagba S., Dadzie SK., Athinya DK., Hadi MP., Pates Jamet H., Chabi J. (2019). High insecticide resistance intensity of *Anopheles gambiae* (s.l.) and low efficacy of pyrethroid LLINs in Accra, Ghana. *Parasites & Vectors* 12: 299.

http://doi.org/10.1186/s13071-019-3556-y

Rahi M., Das P., Sharma A. (2020). Novel coronavirus disease (Covid-19) mitigation steps provide a blueprint for malaria control and elimination. *American Journal of Tropical Medicine and Hygiene* 103: 28-30. http://doi.org/10.4269/ajtmh.20-0394

Rowland M., Boko P., Odjo A., Asidi A., Akogbeto M., N'Guessan RN. (2013). A new long-lasting indoor residual formulation of the Organophosphate insecticide Pirimiphos Methyl for prolonged control of Pyrethroid resistant mosquitoes: An experimental hut trial in Benin. *PLoS One* 8: e69516 .

Rueda LM. (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. *Zootaxa* 589: 1-60. ISBN: 1-877354-47-3 (online edition).

Sattler MA., Mtasiwa D., Kiama M., Premji Z., Tanner M., Kileen GF., Lengeler C. (2005). Habitat characterization and spatial distribution of *Anopheles s.p.* mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malaria Journal* 4: 4.

Scott JA., Brogdon WG.., Collins FH. (1993). Identification of single specimens of *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 49: 520-529.

Seyoum A., Balcha F., Balkew M., Ali A., Gebre-Michael T. (2002). Impact of cattle keeping on human biting rate of anopheline mosquitoes and malaria transmission around Ziway, Ethiopia. *East African Medical Journal* 79: 485-490.

https://www.ajol.info/index.php/eamj/article/viewFile/9121/1865

Shenton FC., Addissie A., Alabaster G., Baziwe D., Tenezaca MC., chinula D., Jatta E., Jawara M., Jones R., Knudsen J., Krystosik AR., McCann R., Murima N., Mutuku F., Nguela RL., Sanchez CN., Nix E., Okumu F., Ruel-Bergeron S., Spitzen J., Tusting LS., Wilson AL., Wood H., Zahouli JZB., Davies M., Lindsay SW. (2019). Research agenda for preventing mosquito-transmitted diseases through improving the built environment in sub-Saharan Africa. *Cities & Health* (on-line)

http://doi.org/10.1080/23748834.2019.1684771

Simo FBN., Bigna JJ., Well EA., Sado FBY., Weaver SC., Moundipa PF., Demanou M. (2019). Chikungunya virus infection prevalence in Africa: a contemporaneous systematic review and meta-analysis. *Public Health* 166: 79-88.

http://doi.org/10.1016/j.puhe.2018.09.027

Simon-Oke IA., Olofintoye LK. (2015). The effects of climatic factors on the distribution and abundance of mosquito vectors in Ekiti State. *Journal of Biology, Agriculture and Healthcare* 5: 142-146. ISSN 2225-093X

Sinka ME., Bangs MJ., Mangiun S., Coetzee M., Mbogo CM., Hemingway J., Patil AP., Temperley WH., Gething PW., Kabaria CW., Okara RM., Van Boeckel T., Godfray HCJ., Harbach RE, Hay SI. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasite & Vectors* 3: 117.

http://www.parasitesandvectors.com/content/3/1/117

Sivagnaname N., Amalraj DD,. Mariappan T. (2005). Utility of expanded polystyrene (EPS) beads in the control of vector-borne diseases. *The Indian Journal of Medicine Research* 122: 291-296.

Sogoba N., Vounatsou P., Bagayoko MM., Doumbia S., Dolo G., Gosoniu L., Traore SF., Traore YT., Smith T. (2007). The spatial distribution of *Anopheles gambiae sensu stricto* and *An. arabiensis* (Diptera: Culicidae) in Mali. *Geospatial Health* 2: 213-222.

Soko W., Chimbari MJ., Mukaratirwa S. (2015). Insecticide resistance in malaria-transmitting mosquitoes in Zimbabwe: a review. *Infectious Diseases of Poverty* 4: 46. http://doi.org/10.1186/s40249-015-0076-7

Solomon H. (2014). Mali: West Africa's Afghanistan. *The RUSI Journal* 158: 12-19. http://doi.org/10.1080/3071847.2013.774635

Sougoufara S., Doucouré, Sembéne PMB., Harry M., Sokhna C. (2017). Challenges for malaria vector control in sub-Saharan Africa: Resistance and behavioural adaptions in *Anopheles* populations. *Journal of Vector Borne Diseases* 54: 4-15.

Sougoufara S., Harry M., Doucouré S., Sembène PM., Sokhna C. (2016). Shift in species composition in the Anopheles gambiae complex after implementation of long-lasting insecticidal nets in Dielmo, Senegal. *Medical and Veterinary Entomology* 30: 365-369. http://doi.org/10.111/mve.12171

Sovi A., Keita C.,Sinaba Y., Dicko A., Cisse MBM., Koita O., Dengela D., Flately C., Bankineza E., Mihigo J., Belemvire A., Carlson J., Fornadel C., Oxborough RM. (2020). *Anopheles gambiae* (*s.l.*) exhibit high intensity pyrethroid resistance throughout Southern and Central Mali (2016-2018): PBO or next generation LLINs may provide greater control. *Parasite & Vectors* 13: 239.

http://doi.org/10.1186/s13071-020-04100-7

Sudeep AB., Shil P. (2017). *Aedes vittatus* (Bigot) mosquito: An emerging threat to public health. *Journal of Vector Borne Diseases* 54: 295-300.

Sule WF., Oluwayelu DO., Hernández-Triana LM., Fooks AR., Venter M., Johnson N. (2018). Epidemiology and ecology of West Nile virus in sub-Saharan Africa. *Parasites & Vectors* 11: 414. http://doi.org/10.1186/s13071-018-2998-y

Tambo E., Yah CS., Ugwu CE., Olalubi OA., Wurie I., Jonhson JK., Ngogang JY. (2016). Fostering prevention and care delivery services capability on HIV pandemic and Ebola outbreak symbiosis in Africa. *The Journal of Infection in Developing Countries* 10: 001-012. http://doi.org/10.3855/jidc.6875

Tandina F., Doumbo O., Yaro AS., Traoré SF., Parola P., Robert V. (2018). Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. *Parasites & Vectors* 11: 467. http://doi.org/10.1186/s13071-018-3045-8

Tangena JA., Hendriks CMJ., Devine M, Tammaro M., Trett AE., Williams I., DePina AJ., Sisay A., Herizo R., Kafy HT., Chizema E., Were A., Rozier J., Coleman M., Moyes CL. (2020). Indoor residual spraying for malaria control in sub-Saharan Africa 1997-2017: an adjusted retrospective analysis. *Malaria Journal* 19: 150.

http://doi.org/10.1186/s12936-020-03216-6

Temu EA., Minjas JN., Tuno N., Kawada H., Takagi M. (2007). Identification of four members of the *Anopheles funestus* (Diptera: Culicidae) group and their role in *Plasmodium falciparum* transmission in Bagamoyo, coastal Tanzania. *Acta Tropica* 102: 119-125.

Touré YT, Petrarca V, Traoré SF, Coulibaly A, Maiga HM, Sankaré O, Sow M, Di Deco MA, Coluzzi M. (1998). The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia*, 40: 477-511.

Tustin AW., Lamson GE., Jacklitsch BL., Thomas RJ., Arbury SB., Cannon DL., Gonzales RG., Hodgson MJ. (2018) Evaluation of occupational exposure limits for heat stress in outdoor workers – United States, 2011-2016. *Morbidity and Mortality Weekly Report* 67: 733-737. Centers for Disease Control and Prevention.

Van den Berg H., Manuweera G., Kondradsen F. (2017). Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. *Malaria Journal* 16: 401. http://doi.org/10.1186/s12936-017-2050-2

Victor OA., Adekunle AJ., Tahiru IK., David OO. (2017). Influence of meteorological variables on diversity and abundance of mosquito vectors in two livestock farms in Ibadan, Nigeria: Public health implications. *Journal of Mosquito Research* 7: 70-78. http://doi.org/10.5376/jmr.2017.07.0009

Wahid B., Ali A., Rafique S., Idrees M. (2017). Global expansion of chikungunya virus: mapping the 64-year history. *International Journal of Infectious Diseases* 58: 69-76. http://doi.org/10.1016/j.ijid.2017.03.006

Weetman D., Kamgang B., Badolo A., Moyes CL., Shearer FM., Coulibaly M., Pinto J., Lambrechts L., McCall PJ. (2018). *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: current and future threats. *International Journal of Environmental Research and Public Health* 15: 220. http://doi.org/10.3390/ijerph15020220

Wiebe A., Longbottom J., Gleave K., Shearer FM., Sinka ME., Massey NC., Cameron E., Bhatt S., Gething PW., Hemingway J., Smith DL., Coleman M., Moyes CL. (2017). Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal* 16: 85.

http://doi.org/10.1186/s12936-017-1734-y

Wilkes TJ., Matola YG., Charlwood JD.. (1996) *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical & Veterinary Entomology* 10: 108-110.

Wilson AL., Courtency)., Kelly-Hope LA., Scott TW., Takken W., Torr SJ., Lindsay SW. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Neglected Tropical Diseases* 14: e0007831.

http://doi.org/10.1371/journal.pntd.0007831

World Health Organization (2003). Malaria entomology and vector control. Leaner's Guide. WHO/CDS/CPE/SMT/2002.18 Rev.1.

World Health Organization (2011). The use of DDT in malaria vector control. WHO/HTM/GMP/2011.

World Health Organization (2013). Malaria entomology and vector control. Guide for participants. ISBN 978 92 4 150581 9.

World Health Organization (2014). Vector-borne diseases 2014. Report of an informal expert consultation SEARO, New Delhi. 7-8 April 2014.

World Health Organization (2015). Indoor residual spraying. An operational manual for indoor residual spraying (IRS) for malaria transmission control and elimination. 2nd Edition. ISBN 978-92-4-150894-0.

World Health Organization (2016). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd Edition. ISBN 978 92 4 151157 5.

World Health Organization (2018). World Malaria Report 2018. ISBN 978-92-4-156565-3.

World Health Organization (2019). Guidelines for malaria vector control. ISBN 978-92-4-155049-9.

World Health Organization (2019). World Malaria Report 2019. ISBN 978-92-4-156572-1

Worrall E.., Basu S., Hanson K. (2005). Is malaria a disease of poverty? A review of the literature. *Tropical Medicine and International Health* 10: 1047-1059.

http://doi.org/10.1111/j.1365-3156.2005.01476.x

Wragge S., Toure D., Coetzee Marelize., Gilbert A., Christian R., Segoea G., Hunt RH., Coetzee Maureen. (2015). Malaria control at a gold mine in Sadiola District, Mali, and impact on transmission over 10 years. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 109: 755-762. http://doi.org/10.1093/trstmh/trv089

Yaro AS., Traoré AI., Huestis DL., Adamoi A., Timbiné S., Kassogué Y., Diallo M., Dao A., Traoré SF., Lehmann T. (2012). Dry season reproductive depression of *Anopheles gambiae* in the Sahel. *Journal of Insect Physiology* 58: 1050-1059.

http://doi.org/10.1016/j.jinsphys.2012.04.002

Yasuoka J., Levins R. (2007). Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *American Journal of Tropical Medicine and Hygiene* 76: 450-460. https://doi.org/10.4269/ajtmh.2007.76.450

Young MD., Johnson TH. (1949). A malaria survey of Liberia. *Journal of the National Malaria Society* 8: 4.

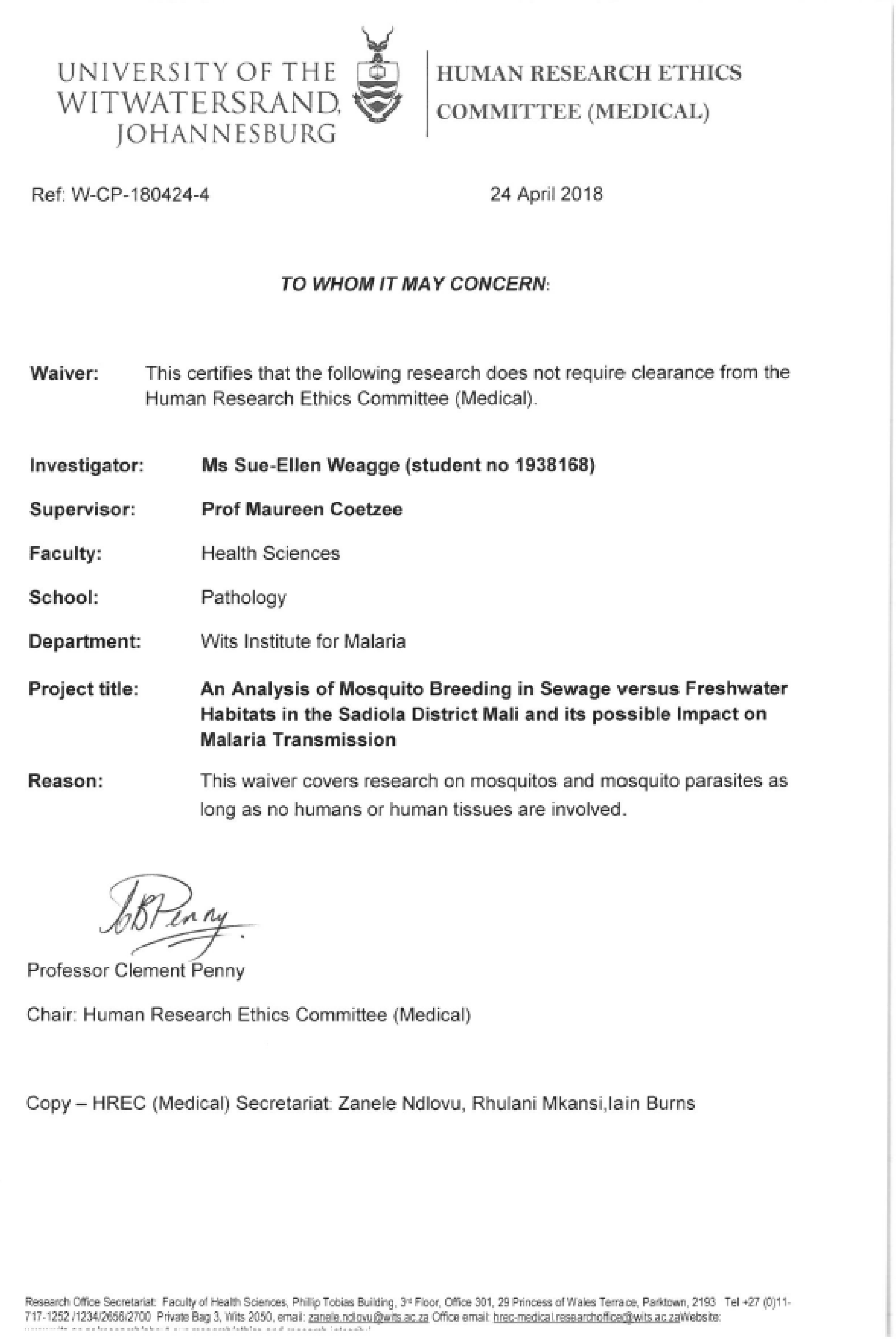
Zouré AA., Badolo A., Francis F. (2020). Resistance to insecticides in *Anopheles gambiae* complex in West Africa: A review of the current situation and the perspectives for malaria control. *International Journal of Tropical Insect Science*

http://doi.org/10.1007/s42690-020-00173-0

Appendices.

Appendix 1.

Ethics Approval



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

**HUMAN RESEARCH ETHICS
COMMITTEE (MEDICAL)**

Ref: W-CP-180424-4 24 April 2018

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator:	**Ms Sue-Ellen Weagge (student no 1938168)**
Supervisor:	**Prof Maureen Coetzee**
Faculty:	Health Sciences
School:	Pathology
Department:	Wits Institute for Malaria
Project title:	**An Analysis of Mosquito Breeding in Sewage versus Freshwater Habitats in the Sadiola District Mali and its possible Impact on Malaria Transmission**
Reason:	This waiver covers research on mosquitos and mosquito parasites as long as no humans or human tissues are involved.

CB Penny

Professor Clement Penny

Chair: Human Research Ethics Committee (Medical)

Copy – HREC (Medical) Secretariat: Zanele Ndlovu, Rhulani Mkansi,Iain Burns

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 301, 29 Princess of Wales Terrace, Parktown, 2193 Tel +27 (0)11-717-1252 /1234/2656/2700 Private Bag 3, Wits 2050, email: zanele.ndlovu@wits.ac.za Office email: hrec-medical.researchoffice@wits.ac.zaWebsite:

Appendix 2

SEMOS Gold Mine Research Approval

International Cyanide Management Code

ISO 14001 Certification

OHSAS 18001

SEMOS
Société d'Exploitation des Mines d'Or de Sadiola S.A.
Immatriculation Numéro 6587

Société d'Exploitation des Mines d'Or de Sadiola S.A.

R.C.M : Ma Bko-2005 M-5967 *N.I.F : 087800209E* *I.N.P.S : 18152/1*

Adresse postale:
Hamdallaye ACI 2000, Immeuble Bassekou GAMBY
1er Etage, Rue 311 – Porte 669, B.P. E-1194
Bamako, MALI
www.anglogoldashanti.com

Bamako Tél: (223) 20 21 46 06
Tél : (223) 20 21 47 28/67
Sadiola Tél : (223) 44 98 04 00
Tél : (223) 21 53 40 00/01/02
Fax : (223) 44 98 04 18

10 August 2018

Ms Sue-Ellen Wragge
Vector Control Manager
SEMOS S.A.

Dear Madam,

USE OF MALARIA CASE DATA FOR RESEARCH INTO ADAPTION OF MOSQUITO BREEDING HABITS

We refer to your official request dated 1 August 2018 and have the pleasure to inform you that formal approval was received from Dr. Bafedile Evah Chauke-Moagi, Senior Manager Health, Group Sustainable Development, Anglogold Ashanti, for you to use SEMOS positive malaria case data as part of your research.

This approval is granted on the condition that confidentiality is maintained at all times and that patient personal information or identifiers are not disclosed in any way.

We wish to take this opportunity to wish you every success with your research and related studies.

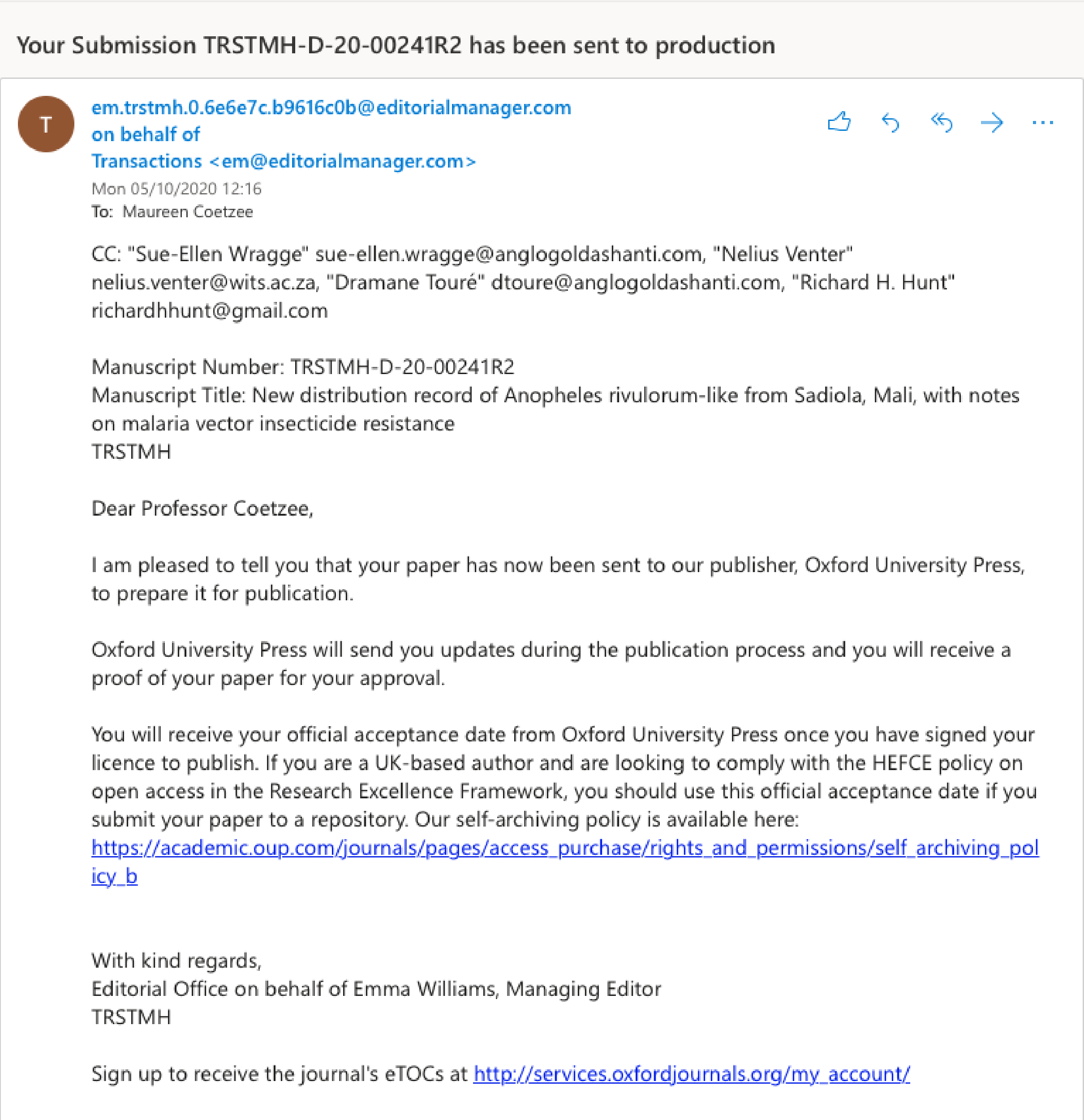
Yours faithfully

SEMOS S.A
Unit Manager - HR
Auth. Level 7

AJ BEHRENS
UNIT MANAGER HR & ADMINISTRATION

Appendix 3.

Article submission



Your Submission TRSTMH-D-20-00241R2 has been sent to production

em.trstmh.0.6e6e7c.b9616c0b@editorialmanager.com on behalf of Transactions <em@editorialmanager.com>
Mon 05/10/2020 12:16
To: Maureen Coetzee

CC: "Sue-Ellen Wragge" sue-ellen.wragge@anglogoldashanti.com, "Nelius Venter" nelius.venter@wits.ac.za, "Dramane Touré" dtoure@anglogoldashanti.com, "Richard H. Hunt" richardhhunt@gmail.com

Manuscript Number: TRSTMH-D-20-00241R2
Manuscript Title: New distribution record of Anopheles rivulorum-like from Sadiola, Mali, with notes on malaria vector insecticide resistance
TRSTMH

Dear Professor Coetzee,

I am pleased to tell you that your paper has now been sent to our publisher, Oxford University Press, to prepare it for publication.

Oxford University Press will send you updates during the publication process and you will receive a proof of your paper for your approval.

You will receive your official acceptance date from Oxford University Press once you have signed your licence to publish. If you are a UK-based author and are looking to comply with the HEFCE policy on open access in the Research Excellence Framework, you should use this official acceptance date if you submit your paper to a repository. Our self-archiving policy is available here: https://academic.oup.com/journals/pages/access_purchase/rights_and_permissions/self_archiving_policy_b

With kind regards,
Editorial Office on behalf of Emma Williams, Managing Editor
TRSTMH

Sign up to receive the journal's eTOCs at http://services.oxfordjournals.org/my_account/

Appendix 4

Turn It In Report

1938168:S_Wragge_thesis_final.pdf

ORIGINALITY REPORT

11%	8%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	hdl.handle.net Internet Source	1%
2	malariajournal.biomedcentral.com Internet Source	1%
3	www.bmmda.nt.ca Internet Source	1%
4	Wragge, Sue-Ellen, Dramane Toure, Marelize Coetzee, Allison Gilbert, Riann Christian, Godira Segoea, Richard H. Hunt, and Maureen Coetzee. "Malaria control at a gold mine in Sadiola District, Mali, and impact on transmission over 10 years", Transactions of the Royal Society of Tropical Medicine and Hygiene, 2015. Publication	1%
5	livrepository.liverpool.ac.uk Internet Source	<1%
6	edoc.unibas.ch Internet Source	<1%