Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control on the Bijagós Archipelago, Guinea-Bissau.

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Preface

This thesis is presented as a 'Research Paper Style Thesis' in accordance with submission guidance provided by the London School of Hygiene and Tropical Medicine. Four of the seven chapters in this thesis have been published, submitted for publication or are in preparation for submission to particular peer-reviewed journals. Publication details and acknowledgement of co-author contributions are included on the individual cover sheets for each paper. The remainder of the thesis is comprised of 'linking material', and includes an introduction to the study, a detailed methods section on field and laboratory work conducted and a summary of the research findings.

All material within this thesis was written by Elizabeth Pretorius.

Abstract

Malaria continues to be a major threat to life for billions of people throughout the world. Regardless of widespread bednet distributions and high reported usage on the Bijagós Archipelago, Guinea-Bissau, malaria persists, reaching a qPCR prevalence of 17.5% during peak transmission. Should more control tools not be developed to target residual transmission in this setting, disease elimination is unlikely.

The Bijagós Archipelago is geographically isolated, with limited population movement between islands. It provides an ideal location to trial interventions. The primary aims of this PhD were to characterise the malaria vector population on the Bijagós and evaluate the impact of ivermectin in addition to dihydroartemisinin piperaquine mass drug administration (MDA) for malaria control on vectors using a cluster-randomised placebo-controlled trial design (alias: MATAMAL). In addition, this PhD described the built environment throughout the Archipelago and identified risk factors associated with mosquito house entry.

A baseline survey was performed in 2019 and showed that mosquitoes within the *Anopheles gambiae* complex were present in both indoor and outdoor CDC miniature light traps throughout the Archipelago. Results from the baseline survey were used to randomise clusters into either the ivermectin or ivermectin-placebo arm.

Monthly rounds of adjunctive ivermectin MDA at a dose of 300 µg/kg/day was distributed for three consecutive days in the months of July, August and September in 2021 and 2022. Cross-sectional surveys following completion of MDA and during a peak-transmission survey in November of 2021 and 2022 were conducted. From post-MDA collections, vector age structure was assessed using a newly validated technique of dry-preserving and rehydrating specimens prior to parity assessment. From all mosquito collections, vector density, species identification, sporozoite rate and entomological inoculation rate was investigated. The impact of ivermectin on the entomological outcomes was assessed using unadjusted and adjusted t-tests on cluster-level summaries. Adjustments were made for clustering and environmental variables. No significant impact from the intervention was seen in any entomological outcome at any time point, suggesting that ivermectin MDA does not significantly affect vector populations in this setting. Further investigation is needed into human and vector behaviour to better contextualise the results from the trial.

Households selected for entomological surveillance were asked to take part in a survey collecting variables on household demographics, bednet practices and the built environment. After adjusting for clustering, univariable and multivariable analyses were performed using a negative binomial regression to identify any risk factors associated with high *Anopheles* house entry. These results may

feed into future studies in order to design houses that are more resilient to mosquito entry, thereby building disease out of the Bijagós Archipelago.

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I would like to dedicate this thesis to the memory of my dad, Alan Westwood.

Abbreviations

Acronym	Description
ACT	Artemisinin-based Combination Therapy
CDC	Centre for Disease Control
CHW	Community Health Workers
CSP	Circumsporozoite Protein
DNA	Deoxyribonucleic Acid
DP	Dihydroartemisinin piperaquine
EIP	Extrinsic Incubation Period
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GTS	Global Technical Strategy for malaria 2016-2030
IPTi	Intermittent Preventative Treatment for infants
IPTp	Intermittent Preventative Treatment for pregnant women
IRR	Inter-Rater Reliability
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Nets
IVM	Ivermectin
LC50	Lethal Concentration 50
LF	Lymphatic Filariasis
LSHTM	London School of Hygiene & Tropical Medicine
LT	CDC miniature Light Traps
MDA	Mass Drug Administation
MIRS	Mid-Infrared Spectroscopy
MRC	Medical Research Council
NIRS	Near-Infrared Spectroscopy
NTD	Neglected Tropical Diseases
PBO	Piperonyl-Butoxide
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PTS	Peak Transmission Survey
qPCR	quantitative PCR
RDT	Rapid Diagnostic Test
RFLP	Restriction Fragment Length Polymorphism
SES	Socioeconomic status
SMC	Seasonal Malaria Chemoprevention
SP	Sulfadoxine pyrimethamine
SR	Sporozoite Rate
STH	Soil-Transmitted Helminths
WHO	World Health Organization

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Chapter 1. Introduction

The epidemiology and control of malaria

While great progress has been made in the control of malaria, there are still approximately 250 million cases and 600,000 deaths per year, most of which occur in children aged under 5 years old [1]. Malaria is caused by protozoan parasites of the genus *Plasmodium* and is transmitted through an infective bite from an *Anopheles* mosquito. Infection may produce a wide variety of symptoms, from absent or mild to acute anaemia and cognitive problems [2]. In 2022, around 94% of malaria cases were in sub-Saharan Africa (Figure 1) [1]. *Plasmodium falciparum*, the most prevalent malaria parasite, accounts for 99.7% of the region's malaria cases.

By 2030, the World Health Organization (WHO) Global Technical Strategy for malaria 2016-2030 (GTS) aims to reduce the global malaria mortality rate and case incidence by at least 90% and eliminate malaria in at least 35 of the more than 80 malaria-endemic countries [3]. Through the use of effective control interventions, an estimated 2 billion malaria cases and 11.7 million deaths were prevented globally between 2000-2021, the vast majority of which were in sub-Saharan Africa [1]. However, progress has stalled in recent years [4]. In 2019, only nine of the malaria-endemic countries in the region were on track to achieve the 40% reduction in case incidence by 2020, set out by the GTS [5].

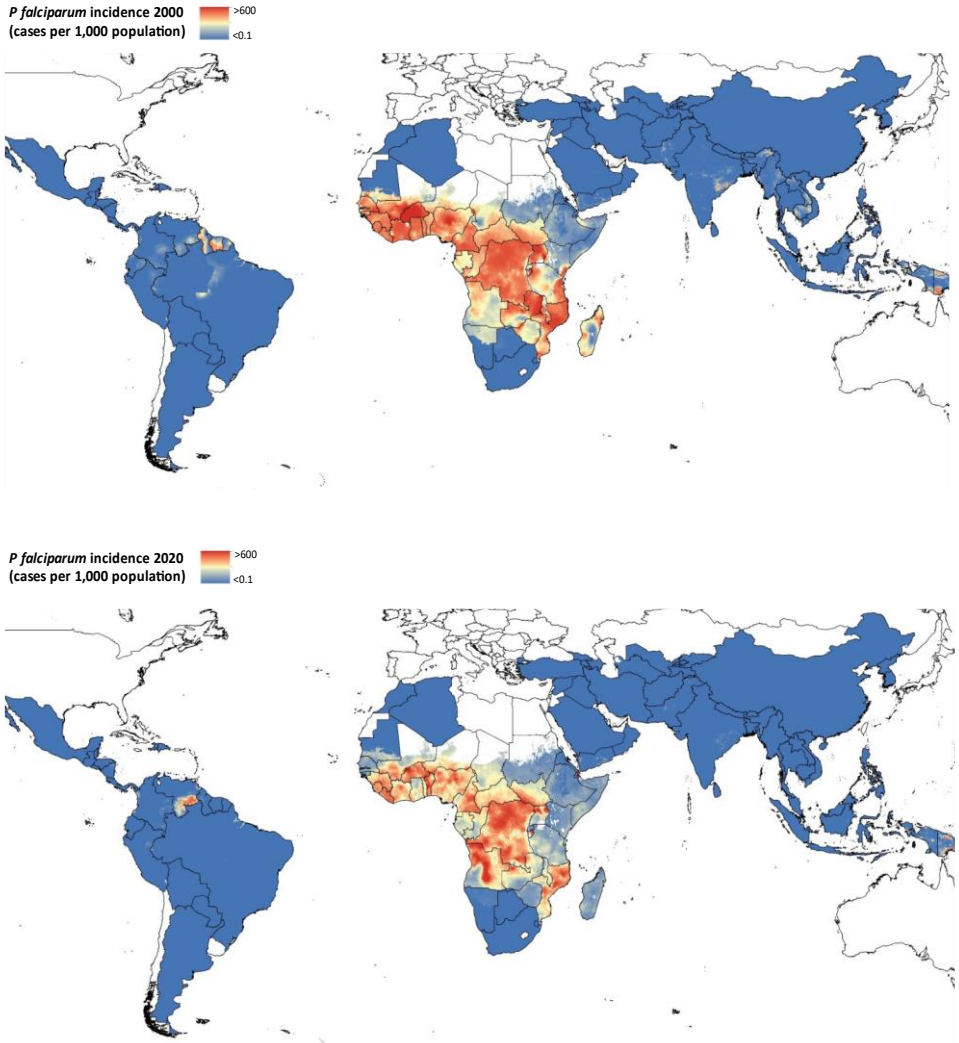

Figure 2. Spatial distribution of all-age *Plasmodium falciparum* incidence in 2000 (top) and 2020 (bottom) per 1,000 population from the Malaria Atlas Project 2000 and 2020 dataset. Areas without endemic *P. falciparum* are shown in white [6, 7].

Malaria control and its global challenges

From 1980 to the present day, malaria control has been dominated by insecticide-treated nets (ITNs). Between the early and late 1980s, ITNs were trialled and tested, and found to be effective at reducing malaria-related deaths and illness, reducing overall childhood mortality by 17% [8-10]. By creating a physical barrier, bednets prevent contact between the human and mosquito [11]. ITNs have the added advantage of reducing mosquito populations by using insecticides, whilst still maintaining a physical barrier. There are two classes of insecticides approved for use in ITNs, pyrroles and pyrethroids; pyrethroids are the backbone of insecticide-based interventions [12]. Permethrin, deltamethrin and α-cypermethrin are the most commonly used insecticides on ITNs. To reduce the primary vector population, ITNs and indoor residual spraying (IRS) are deployed at a community level in order to

continuously expose endophagic (indoor-feeding) and endophilic (indoor-resting) mosquitoes, largely responsible for malaria transmission, to insecticide. While IRS is regularly used, the reduction in malaria prevalence in the last few decades can be largely attributed to the wide-spread distribution of ITNs in areas with high malaria transmission (Figure 2) [4]. Since 2002, malaria-endemic countries have been scaling up their bednet distribution [12]. In 2021, 47% of the global population at risk of malaria were sleeping under nets; while this is an improvement from 29% coverage in 2010, there has been little progress since 2015 [1, 11, 13] .

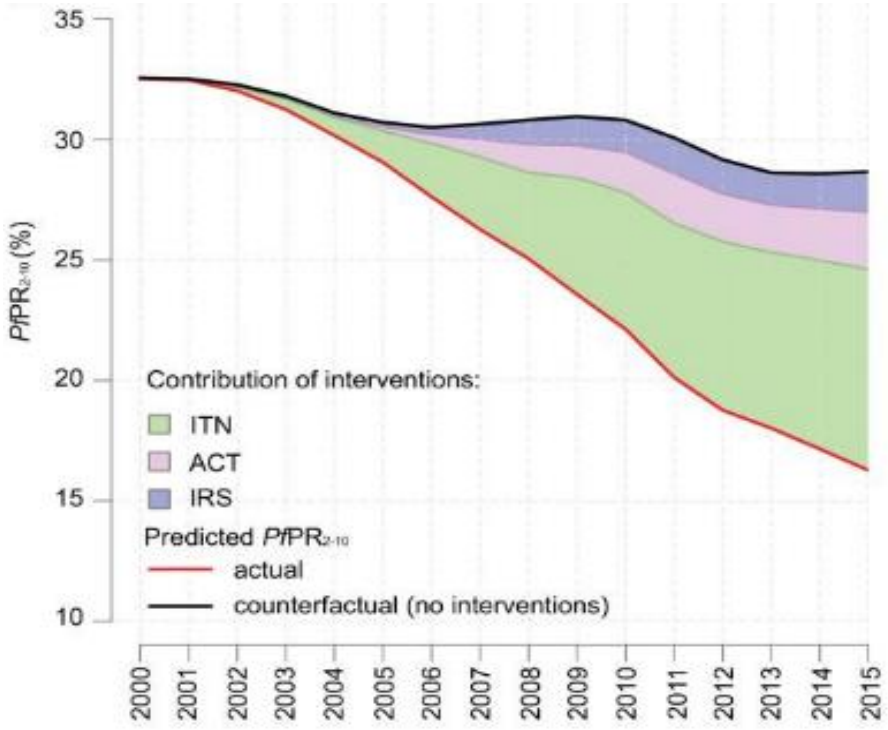

Figure 2. The relative contribution of malaria control interventions. Predicted time series of population-weighted mean *P. falciparum* prevalence ($PfPR_{2-10}$) across endemic Africa. The red line depicts the actual *P. falciparum* prevalence prediction when interventions are implemented. The black line represents a prediction in a scenario with no distribution of ITNs, artemisinin-based combination therapy or IRS. The coloured regions (green, pink and purple) indicate the relative contribution of each individual intervention in reduction of *P. falciparum* prevalence from 2000-2015 [4].

By 2030, the WHO GTS aims to reduce global malaria incidence by investing in three strategic pillars: (1) ensure universal access to malaria prevention, diagnosis and treatment, (2) accelerate efforts towards elimination and attainment of malaria-free status, and (3) transform malaria surveillance into a core intervention [3]. As well as vector control methods, the control measures outlined within the first strategic pillar include expanding chemoprevention programmes to the most vulnerable groups, such as administration of intermittent preventative treatment of malaria in pregnancy (IPTp) and

infants (IPTi) using sulfadoxine-pyrimethamine (SP), and seasonal malaria chemoprevention (SMC) for children under 5 years using amodiaquine plus SP [14, 15]. Ensuring universal diagnostic testing and provision of quality-assured treatment of fixed-dose drug formulations, such as artemisinin-based combination therapy (ACTs) is also a key component of the framework [3]. The contribution of effective ACT use to the reduction in malaria incidence is illustrated in Figure 4 [4]. To achieve the ambitious targets, efforts to control malaria must be integrated and multi-disciplinary, with vector control at its core.

Malaria control faces many challenges which need to be addressed in order to meet the targets outlined in the GTS. Seasonal and regional heterogeneity demands evidence-based decision-making on a country-by-country, or even within-country, basis. Up-to-date disease monitoring and evaluating must take precedence in order to adjust country interventions. A significant challenge is the funding gap between what is needed for malaria control and what is available. International investment has slowed in past years; in 2020 an estimated US$6.8 billion was required to meet the GTS targets, however only US$3.3 billion was spent [1]. The funding gap has been widening over recent years, increasing from US$2.8 billion in 2019 to US$3.8 billion in 2021. The COVID-19 pandemic, conflict and a global recession may further exacerbate this, leading to further reductions in funding. Meeting the GTS goals is likely to become more challenging if funding for malaria control is not increased.

In 2020, the COVID-19 pandemic caused disruptions to malaria services and bednet distributions. This is evident by the increase in cases from 227 million in 2019 to 241 million in 2020 [16]. Even though the disruptions caused by the pandemic to malaria services seems to have eased since 2020 in Sub-Saharan Africa, the long-term effects will need to be monitored [17].

Other challenges facing malaria control are biological. ACT efficacy has begun to wain due to artemisinin resistance being identified, particularly in countries in the Greater Mekong subregion [18]. Malaria control has become heavily dependent on the continued effectiveness of insecticides to maintain the status quo and ensure that there is no regression in disease incidence. Initially, in the 1990s, pyrethroid resistance in mosquitoes was thought to result from exposure to pyrethroids used in agricultural crops [19, 20]. However, as vector control programmes have increased throughout Africa, so have the reports of pyrethroid-resistance in *Anopheles* mosquitoes [21, 22]. The WHO has reported that 88% of malaria-endemic countries monitoring resistance have confirmed its presence in at least one vector to a least one pyrethroid [1, 23]. The negative impact of pyrethroid resistance has been difficult to quantify, with many studies generating inconclusive results [9, 24-27]. However, it is crucial that efforts are made to understand resistance to ensure the sustained efficacy of control measures.

Current control measures target those malaria vectors that are endophilic and/or endophagic. However, even with effective vector control interventions, malaria transmission continues. Residual malaria transmission refers to transmission that persists regardless of full universal coverage of ITNs and/or IRS which contain active ingredients which are effective against fully-susceptible local vector populations [28] . This is most commonly a result of behavioural differences in vectors which allows them to circumvent contact with ITNs or IRS [28, 29]. These behaviours include: (1) early exiting of houses and avoidance of contact with insecticide-treated surfaces within households, thereby minimizing exposure to hazards; (2) feeding upon animals, therefore, not entering houses and avoiding insecticides; (3) resting outdoors, away from insecticide-treated surfaces and (4) feeding on people whilst they are active outdoors. Currently, residual transmission remains sufficiently high across much of the tropics to discredit the notion of disease eradication [28]. Novel control interventions must be identified and tested to reduce residual malaria transmission in these areas. These interventions must address the vector behaviour patterns that are overlooked by current measures.

Socioeconomic development and housing for malaria control

Malaria is a disease of poverty and the environment; therefore, socioeconomic development is becoming increasingly important in the fight against the disease. Improvements in sectors outside of health, such as education, water and sanitation, agriculture and housing, could help in reducing the malaria burden [30]. This would not only have effects on malaria transmission, but also impact other diseases, for instance those caused by the waterborne-pathogens *Escherichia coli* and *Cholera vibrio* [31, 32].

There are currently major inequalities in the availability of safe and adequate housing [33]. Rapid urbanisation and ineffective planning has led to the formation of slums, with limited affordable options. With approximately 230 million people living in slums, sub-Saharan Africa has the highest population percentage of any other region. Tusting *et al.* quantified changes in housing throughout the region. 'Unimproved' housing was classified as such if it had at least one of four characteristics: (1) unimproved water supply; (2) unimproved sanitation; (3) more than three people per bedroom; and (4) built with natural or unfinished materials [34]. Finished materials were classified as those that were 'manufactured', such as cement, brick or corrugated iron. Houses that had none of the four characteristics were classified as 'improved'. They found that the prevalence of people living in improved houses doubled from 11% (95% CI 10-12%) in 2000 to 23% (95% CI 21-25%) in 2015, but unimproved housing persists throughout the region (Figure 3) [35, 36].

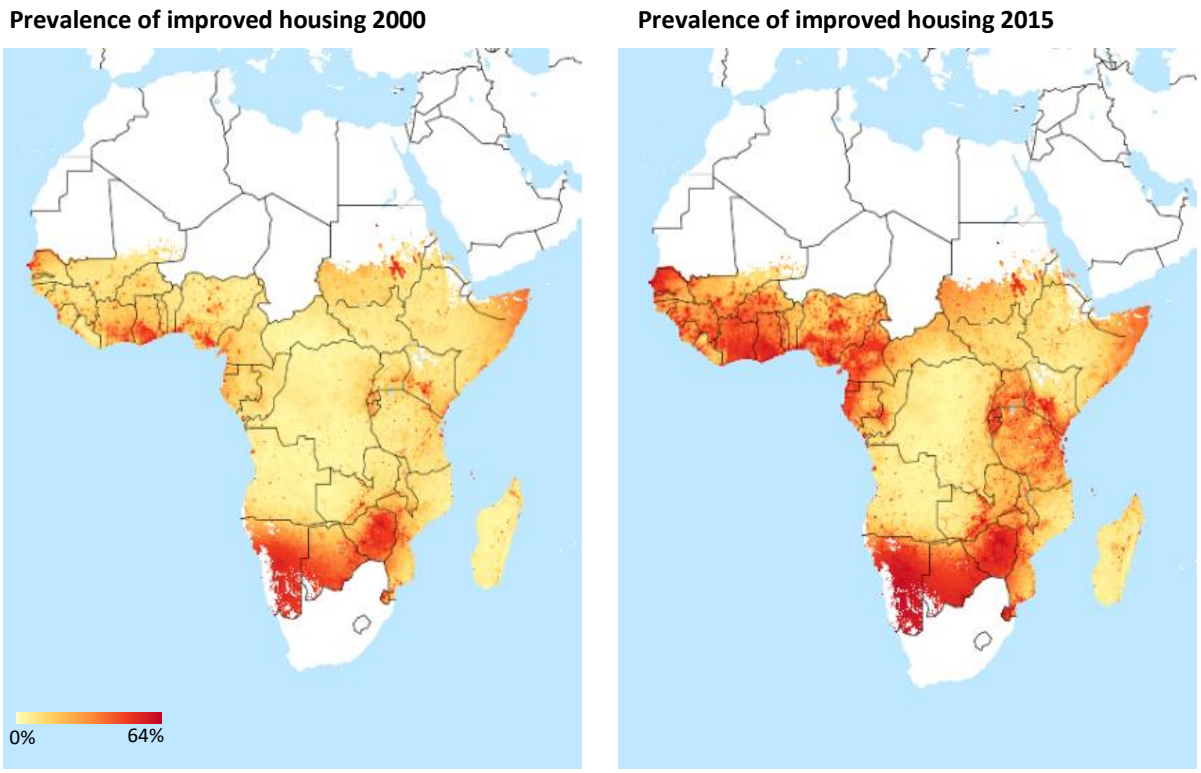

Figure 3 Percentage of improved housing in sub-Saharan Africa in 2000 and 2015. Maps taken from Malaria Atlas Project [35, 36].

As stated previously, malaria vectors usually show endophilic and endophagic behaviours. Entomological surveys from six different sites across sub-Saharan Africa estimated that 80-100% of malaria transmission occurs indoors at night [37]. This highlights the need to focus development on improving housing, to 'build' malaria out of the home. In sub-Saharan Africa, housing features such as open/unscreened eaves have been associated with an increase in clinical malaria incidence and higher mosquito numbers within the house [38-41]. Metal roofs, fewer windows, screened windows, brick walls and the presence of a ceiling have all been associated with a reduction in mosquito numbers within the house [30, 38, 40]. Localised knowledge of the vector population and the risk factors associated with mosquito entry will be necessary to ensure the correct housing improvements are applied to help prevent malaria transmission in the future. For instance, in areas where the main vectors are within the *An. gambiae* or *An. funestus* complexes, screened eaves or the presence of a ceiling will likely reduce mosquito house entry. However, in areas of southeast Asia where the main vector is *An. philippiensis*, house design seems to be less important, with the materials used for construction and the location used for cooking seeming to have the greatest impact on mosquito density within the house [42].

The global population is projected to increase to 9.8 billion by 2050, with high rates of growth in Africa. Nigeria's population, currently the seventh largest in the world, is projected to increase to the third largest by 2050 [43]. In 2021, 27% of global malaria cases and 31% of global malaria deaths were seen in Nigeria [1]. As populations increase, so will the demand for housing, and while Nigeria may be the most extreme example, many countries throughout sub-Saharan Africa follow similar population trends [43]. Using local knowledge of malaria transmission and the risk factors associated with mosquito house entry, housing may be better designed to help prevent disease transmission. This would be a sustainable approach to malaria control, relieving pressure on existing control measures.

Ivermectin for malaria control

As well as improving housing to reduce mosquito entry, novel approaches to vector control must also be explored. As our knowledge of different vector behaviours and preferences grows, so does our understanding of the complexity of transmission dynamics. One vector control measure is unlikely to be a 'silver bullet', therefore, novel interventions must work alongside existing ones to yield the best possible results, and hopefully progress towards large-scale malaria elimination in much of the affected region.

Endectocides are drugs that have endo- and ectoparasitocidal activity, and, for decades, have been used for medical and veterinary purposes. Ivermectin (IVM), a drug within the endectocide class of avermectins, has broad spectrum antiparasitic properties, with an excellent safety profile. IVM is one of the most successful drugs ever developed, with activity against parasitic nematodes and ectoparasites [44]. In 2015, William C. Campbell and Satoshi Ōmura were awarded a Nobel Prize in Physiology or Medicine "for their discoveries concerning a novel therapy against roundworm infections" [45]. IVM is now widely used in mass drug administration (MDA) for the control and prevention of lymphatic filariasis (LF) and onchocerciasis, both neglected tropical diseases (NTD). An MDA campaign is the distribution of a certain treatment to an entire eligible population to help control a disease. IVM MDA for NTD control is one example of a successful control strategy; MDA using albendazole or mebendazole has been given to preschool or school-aged children to kill certain soil-transmitted helminths (STHs) and azithromycin has been given to help prevent trachoma and the skin NTD yaws [46].

The effects of IVM on malaria vectors

IVM paralyses and ultimately kills invertebrates by blocking synaptic transmission in nerve and muscle cells by binding to glutamate-gated chlorine (GluCl) channels and hyperpolarizing the membrane potential. GluCl channels are part of the Cys-loop receptors and are thought to be the main mechanism of action of IVM, however, IVM has been shown to affect gamma-aminobutyric acid (GABA), histamine and pH-sensitive channels [47-49]. The effects of IVM on mosquitoes was first explored in 1985. Pampiglione and co-workers showed that IVM was an effective larvicide, and increased the mortality of adult females fed on IVM [50]. More recently, IVM *in vitro*, *in vivo* and direct feeding assays have been performed using a variety of *Anopheles* species (Table 1). In 2015, Meyers and co-workers characterised the GluCl channels within *An. gambiae*, and found that they were predominantly found in the motor and sensory system, in particular the antenna, Johnston's organ, supraesophageal ganglion and thoracic ganglia [48].

The common metric for IVM lethality is the lethal concentration 50 (LC_{50}), defined as the concentration of IVM blood needed to kill 50% of mosquitoes during a specific period of observation. Using different methodologies, multiple studies have investigated the IVM LC_{50} of malaria vectors (Table 1). Results vary greatly between *Anopheles* species and even strains of the same species. Interestingly, there is a marked difference between the IVM LC_{50} determined for malaria vectors *in vitro* and *in vivo* [51], suggesting that other factors play a role in the mosquitocidal effect when IVM is administered to humans. This is further supported by the finding that IVM, which has an estimated half-life of 1-3 days [52], continues to reduce mosquito survival up to 28 days post-administration [53]. Metabolites, produced by human liver microsomes, that break down IVM have since been identified and found to have a mosquitocidal effect, even in the absence of IVM [54-56].

When taken up in a bloodmeal, IVM has also been seen to reduce mosquito fecundity and fertility [57-59]. It has been suggested that this may be due to IVM changing the digestive responses to a blood meal in the mosquito's stomach, leading to a reduction in the proportion of blood proteins converted into nutrients for egg development [57]. This effect can be seen on mosquitoes exposed to sub-lethal doses of IVM.

Table 3. Key malaria vector susceptibility to ivermectin.

WHO geographic region	Reference	Species	Method	Susceptibility
African Region	Ouedraogo et al. [60]	*An. gambiae* (colony)	Membrane: blood from treated humans in combination with artemether-lumefantrine	7-day-LC_{50}: 8.6ng/ml
	Smit et al. [53]	*An. gambiae* (colony)	Membrane: blood from treated humans in combination with dihydroartemisinin-piperaquine	7-day-LC_{50}: 3.4 ng/ml
	Kobylinski et al. [56]	*An. gambiae* (G3 strain; colony)	Membrane: in vitro mixture (human blood + ivermectin)	5-day-LC_{50}: 22.4 ng/ml
	Kobylinski et al. [61]	*An. gambiae* (G3 strain; colony)		7-day-LC_{50}: 15.9 ng/ml
	Nicolas et al. [62]	*An. gambiae* (Kilifi strain; colony)	Membrane: in vitro mixture (human blood + ivermectin)	10-day-LC_{50}: 8 ng/ml
	Fritz et al. [63]	*An. gambiae* s.l. (colony)	Membrane: in vitro mixture (cattle blood + ivermectin)	9-day-LC_{50}: 19.8 ng/ml
	Chaccour et al. [64]	*An. arabiensis* (colony)	Feeding on treated cattle	10-day-LC_{50}: 3.7 ng/ml
	Fritz et al. [65]	*An. arabiensis* (Dongola strain; colony)	Membrane: in vitro mixture (cattle blood + ivermectin)	9-day-LC_{50}: 7.9 ng/ml
South-East Asia, Eastern Mediterranean and African Region	Dreyer et al. [66]	*An. stephensi* (colony)	Membrane: in vitro mixture (cattle blood + ivermectin)	4-day-LC_{50}: 7 ng/ml
South-East Asia and Western Pacific Region	Kobylinski et al. [51]	*An. minimus* (colony)	Membrane: blood from treated humans	10-day-LC50: 0.4 ng/ml
	Kobylinski et al. [67]	*An. minimus* (colony)	Membrane: in vitro mixture (human blood + ivermectin) Membrane: blood from treated humans	7-day-LC_{50}: 16.3 ng/ml
South-East Asia Region	Kobylinski et al. [51]	*An. dirus* (colony)	Membrane: blood from treated humans	10-day-LC_{50}: 2.9 ng/ml
	Kobylinski et al. [67]	*An. dirius* (colony)	Membrane: in vitro mixture (human blood + ivermectin) Membrane: blood from treated humans	7-day-LC_{50}:55.6 ng/ml
		An. campertris (colony)		7-day-LC_{50}: 26.4 ng/ml
		An. sawadwongporni (colony)		7-day LC_{50}: 27.1 ng/ml
Region of the Americas	Sampaio et al. [68]	*An. aquasalis* (colony)	Membrane: in vitro mixture (human blood + ivermectin)	5-day-LC_{50}: 47.05 ng/ml
	Pinilla et al. [69]	*An. darlingi* (colony)	Membrane: in vitro mixture (blood + ivermectin)	7-day-LC_{50}: 43.2 ng/ml
	Gardner et al. [70]	*An. quadrimaculatus* (colony)	Feeding on treated dogs	24-hour-LC_{50}: 6-12 ng/ml
	Dreyer et al. [66]	*An. albimanus* (colony)	Membrane: in vitro mixture (cattle blood + ivermectin)	4-day-LC_{50}: 1468 ng/ml
Western Pacific	Pasay et al. [71]	*An. farauti* (colony	Feeding on treated pigs	12-day-LC_{99}: 2.4 ng/ml*

In vitro and *in vivo* data for humans and/or animals presented. Note the variability in LC_{50} values when using the ivermectin spiked blood or blood from treated vertebrates. In all cases, *in vivo* data produce a stronger lethal effect when calculating LC_{50}s. Results by Dreyer et al. showing an in vitro LC_{50} of 1,468 ng/ml for *An. albimanus* have been published, so it is included here for completeness but there is data published by the same team showing that wild-type *An. albimanus* are susceptible to ivermectin-treated blood at concentrations normally found in treated cattle (i.e. 30-46 ng/ml) [72]. * only LC_{99} available

The desired outcome of IVM is to reduce the vectorial capacity of malaria vectors by targeting mosquitoes of blood-feeding age. The vectorial capacity describes the total number of potentially infectious bites that would arise from all the mosquitoes biting a single perfectly infectious human on a single day (Equation 1). The lifespan of a mosquito affects both the extrinsic incubation period (EIP) and the ability of the mosquito to complete gonotrophic cycles. Therefore, an intervention that i) shortens the lifespan of the mosquito so that the parasite is unable to mature to the infectious sporozoite stage, and ii) reduces fecundity so there are fewer mosquitoes in the next generation, is expected to reduce the vectorial capacity of the primary malaria vectors.

Equation 1. Vectorial capacity equation

$$V = \frac{ma^2 p^n}{-\ln(p)}$$

The classic vectorial capacity (*V*) comprises of four parameters: the parasite's EIP in *(n)* days; the ratio of mosquitoes to human (*m*); mosquito survival through one day (*p*); and the human biting rate (*a*)

A key metric to study the impact of an intervention on vectors is to examine the age structure of the population. Should an intervention successfully target mosquitoes of blood-feeding age, it would be expected that the proportion of mosquitoes that have completed at least one gonotrophic cycle (parous) within the population would decrease, leading to a higher proportion of nulliparous mosquitoes (those that have not completed a gonotrophic cycle). There are several ways to measure the age structure of vector populations, with the most commonly used method yielding a binary outcome [73]. The Detinova technique assesses the ovaries for the presence or absense of skeins, tightly-wound tracheoles which loosen with every gonotrophic cycle. The method is relatively simple to carry out, however does suffer from several limitations. Firstly, due to the binary nature of the outcome, it does not enable exact age measurements, thereby losing granularity; parous mosquitoes may be 3-5 days or two weeks old, however would simply be classified as parous. Secondly, mosquitoes which require multiple blood meals to produce eggs, or have ovarioles which develop unevenly during gonotrophic cycles so that one ovary may have skeins of mixed morphology, will be difficult to classify in an unbiased fashion [74-77].

Alternative methods have been developed to establish the exact age of mosquitoes and remove potential bias from age grading. The Polovodova method, another morphological technique, enables researchers to count the number of gonotrophic cycles completed [78]. Other, more modern techniques, such as near-infrared spectroscopy, mid-infrared spectroscopy, transcriptomic profiling, chromatographic analysis of cuticular hydrocarbons and quantifying wing wear, have all been developed [79-84]. Whilst these techniques show promise, they are often expensive and logistically challenging to use in remote resource-poor settings, needing extensive validation with local vector populations. Further work is required to enable large scale deployment of more modern techniques for mosquito age grading.

The potential use of IVM for malaria control

As with onchocerciasis and LF programmes, IVM delivery for malaria control would require an MDA strategy. Different approaches have been suggested which target different transmission settings [85] (Table 2). The most critical function of IVM MDA would be as a complementary tool to existing control interventions, targeting mosquitoes that are not killed through insecticide-based measures. IVM

administered to humans would target the primary malaria vectors. By combining IVM MDA with antimalarial drugs, human infections would be cleared simultaneously, thereby increasing the intervention's impact. Another alternative, in areas of seasonal transmission, is to co-administer IVM MDA with SMC. SMC would provide a platform from which IVM MDA could be distributed, therefore improving the logistical feasibility. In areas endemic with the microfilarial disease *Loa loa*, IVM is not recommended as there is an increased risk of fatal encephalopathy after treatment [86].

IVM is effective against a range of endo- and ectoparasites, so when administered to humans may result in a reduction in scabies and soil-transmitted helminths, as well as LF and onchocerciasis [87-92]. IVM MDA has been successful in reducing the prevalence of scabies and other ectoparasites, including bedbugs and lice, in several settings [93]. Results from cross-sectional surveys carried out following completion of the second year of MDA of a large cluster-randomized clinical trial in The Gambia, investigating IVM for malaria control, found a reduction in the prevalence of the parasitic roundworm *Strongyloides stercoralis* in intervention clusters [94]. The effect, however, did not last, with subsequent surveys finding the prevalence of *S. stercoralis* to be similar between the trial arms two years after MDA. Scabies and the other STHs investigated showed no significant reduction.

Table 4 The potential uses for ivermectin when delivered to target populations or co-administered with anti-malarial drugs. Taken and adapted from The Ivermectin Roadmappers [85].

Transmission setting	Rationale for ivermectin use	Target Blood source	Additional co-delivery[†]	Rationale for co-delivery
Higher	Reduce disease burden	Human	SMC	Using SMC as a platform for ivermectin delivery
Higher	Accelerate to elimination	Human	ACT MDA	IVM provides additional transmission reduction by targeting outdoor and early biting vectors
Higher	Reduce vectorial capacity	Livestock	Behaviour change interventions to boost ITN use and treatment of cases. Delivery of housing improvements to guard against vector house entry.	Protect household and drive vectors to zoophagy; this strategy allows the use of long-lasting veterinary formulations
Higher	Reduce vectorial capacity	Human + Livestock	With or without ACT MDA	Covering different blood sources could increase impact on local vector populations
Higher	Reduce vectorial capacity	Human	IRS timed after IVM MDA	Improve IRS efficacy by precipitating a sharp reduction in vectors right before the IRS campaign
Any	Reduce disease burden and reduce vectorial capacity	Human	NTD interventions such as azithromycin or IDA for lymphatic filariasis	As part of joint efforts with NTD programs
Any	Insecticide resistance management	Human ± Livestock	PBO and next-generation nets, other insecticide delivery vehicles, i.e. attractive toxic baits	As part of insecticide resistance management strategy
Any	Prevent or manage outbreaks	Human ± Livestock	MDA with ACT + IVM +other vector control tools	As a way to quickly reduce vectorial capacity
Lower	As part of reactive interventions	Human ± Livestock	As part of focal MDA with ACT ± other vector control tools	Prevention of secondary cases at low transmission levels

[†] National policy for malaria prevention will always be present. Interventions such as ITN distribution, IRS, case management and IPTp

ACT = artemisinin-based combination therapy; IDA = triple therapy with ivermectin, diethylcarbamazine and albendazole; IPTp = intermittent preventative treatment in pregnancy; IRS = indoor residual spraying; ITN = insecticide-treated nets; MDA = mass drug administration; NTD = neglected tropical disease; SMC = seasonal malaria chemoprevention; PBO = piperonyl butoxide.

The opportunity for the amalgamation or collaboration with existing successful NTD MDA programmes would be a great benefit to the use of IVM for malaria control. Whilst the IVM dose used in NTD MDA programmes is usually 150 µg/kg of body weight, less than that required to have a long-term impact on malaria vectors, previous studies have shown mosquito populations to be affected [95, 96]. In Ogun state, Nigeria, the parity rate (the proportion of mosquitoes within the population that have previously laid eggs, and used as a proxy for mosquito survival) of mosquitoes caught in indoor traps following IVM MDA for onchocerciasis was significantly reduced 13-14 days post-MDA [95]. Alout and co-workers also found that, following IVM MDA for LF and onchocerciasis using a dosage of 150 µg/kg, the parity rate of *An. gambiae* populations was significantly reduced for two weeks following MDA [96]. Sporozoite rates were also reduced significantly for two weeks; vector populations then recovered and plateaued in the third week after MDA.

Administering IVM to cattle and livestock could be performed in isolation or in conjunction with IVM MDA to humans [59]. Simultaneous IVM MDA administered to both humans and livestock would ensure that all vectors were targeted regardless of respective feeding preferences. The BOHEMIA trial, a large-scale clinical trial being conducted in communities in Mozambique and Kenya, is incorporating IVM-treated livestock into the study design [97, 98]. It would also improve the health of the livestock, reducing the burden of both endo- and ectoparasites. As there may be a considerable cost implication for incorporating livestock, further research is needed to establish the additional impact of a joint intervention. Local mosquito composition should also be considered when planning, to better understand the costs and benefits of livestock IVM MDA.

There are currently a number of large-scale clinical trials investigating the use of IVM MDA for malaria control [97-100]. They are investigating the two recommended regimens: a single dose of IVM at 400 µg/kg per month (“1 x 400”); and 300 µg/kg per day for three consecutive days per month (“3 x 300”) (Figure 4). The 1 x 400 option is expected to benefit from higher uptake, is simpler and shares similarities with existing NTD programmes, however it has a shorter effect time on the vector population, increasingly the likelihood of mosquito population bouncing back before the next MDA round is given. It also has the added benefit of already being approved by the European Medicines Agency [101]. The 3x300 regimen would have a longer effect time; however, coverage is expected to be lower and the dose has not been approved by regulators, therefore the regulatory pathway will be longer.

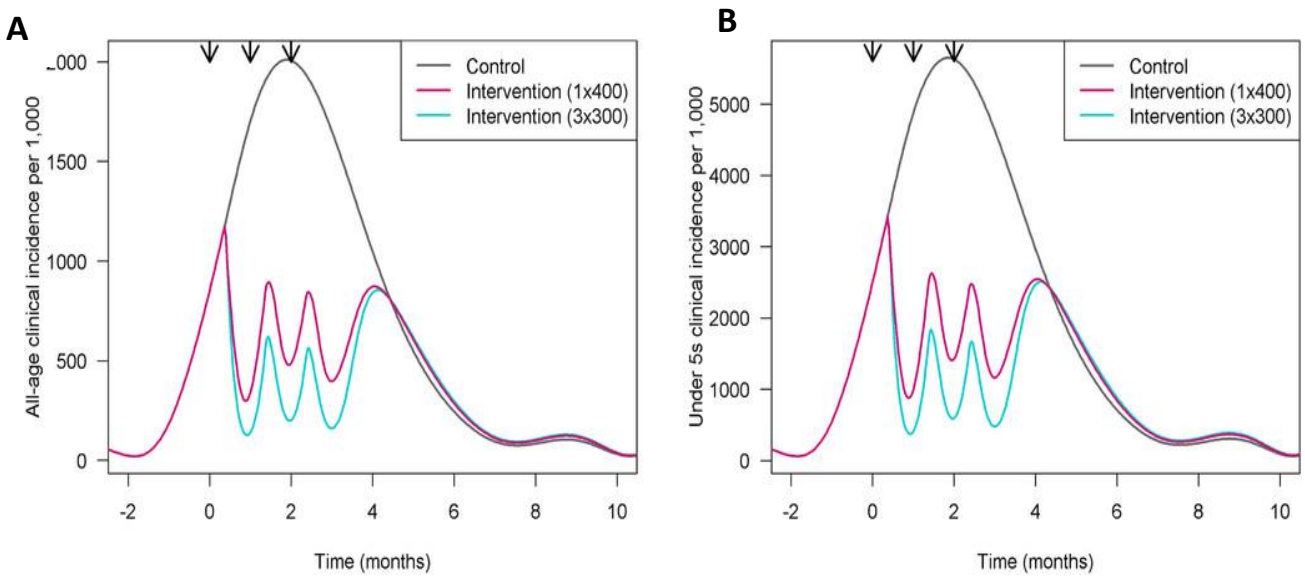

Figure 4. Model by Hannah Slater showing all-age clinical incidence per 1,000 (A) and under 5's clinical in incidence per 1,000 (B). The black arrows at the top of each graph indicate each MDA round. The black line indicates the clinical incidence without any intervention; the pink line indicates the clinical incidence in participants administered with a single dose of 400 µg/Kg; the blue line indicated the clinical incidence in participants administered with 300 µg/Kg for three consecutive days.

Combining IVM MDA with an MDA programme of an antimalarial, such as dihydroartemisinin-piperquine (DP), is also being trialled, as it would not only target vector survivorship, but also treat human hosts. Whilst no mosquitocidal effect has been shown when administering DP alone to patients, when co-administered with IVM, an increase in IVM concentrations in the blood has been seen, leading to a greater effect on mosquito mortality [51]

The MASSIV study, a cluster-randomised controlled trial in the Upper River region in eastern Gambia, trialled the use of co-administering IVM at the 3 x 300 regimen with DP MDA for malaria control in 2018 and 2019 [102]. Intervention clusters received IVM and DP MDA, while control clusters received standard control interventions. Coverage of both IVM and DP was low in the first year but improved in the second. In 2019, there was a significant reduction in malaria prevalence in the intervention cluster [103]. However, because of the study design, it is not possible to differentiate between the effect of the IVM and DP on malaria prevalence. The entomological outcomes, therefore, are the best indicator of the effect of the IVM MDA on malaria transmission. In 2019, when IVM MDA coverage was better, between 60.3% and 67.6% over the three MDA month, *Anopheles* density and the entomological inoculation rate (EIR; an estimate for the number of infectious bites per person per time unit) was significantly reduced in intervention villages [104]. There was no difference in parity rates between trial arms, indicating that IVM MDA did not have an effect on the vector survival.

The impact of IVM MDA on malaria prevalence will become clearer as more large clinical trials are completed. Administering both IVM MDA and DP MDA has cost implications, therefore, it is important for these trials to analyse the cost effectiveness and logistical feasibility of the intervention to inform future MDA practices.

This study is nested within a large clinical trial examining the impact of IVM MDA on malaria transmission (alias: MATAMAL). Below is an overview of the trial, for full details of the clinical trial design, please see published trial protocol (Appendix I) [105].

Overview of study site and MATAMAL trial

The Bijagós Archipelago, Guinea-Bissau

The Republic of Guinea-Bissau is a small West African nation bordering Senegal and Guinea (Figure 5). The country lies within the tropical zone, between the equator and the Tropic of Cancer and is dominated by two distinct seasons: the hot, dry season from November to April and the hot, rainy season from May to November. Currently ranked as the fourth poorest country in the world, Guinea-Bissau has an estimated population of just over 2 million people, an estimated 69.3% of which live below the poverty line [106]. Information on the malaria case incidence in Guinea-Bissau is limited, however, the majority of the cases are expected to be caused by *P. falciparum* as in neighbouring countries, and the overall *P. falciparum* incidence has decreased since 2000 (Figure 5). In 2016, an estimated 132,600 cases and 600 deaths occurred due to malaria [107]. Since then, Guinea-Bissau has reported an increase in malaria case incidence and the mortality rate, although exact figures are difficult to obtain [1]. Malaria control in the country is reliant on the distribution of ITNs every three years, IPTp and diagnosis and case management with ACT, with no IRS programme. An SMC programme started in 2016 and coverage has continued to grow yearly [1].

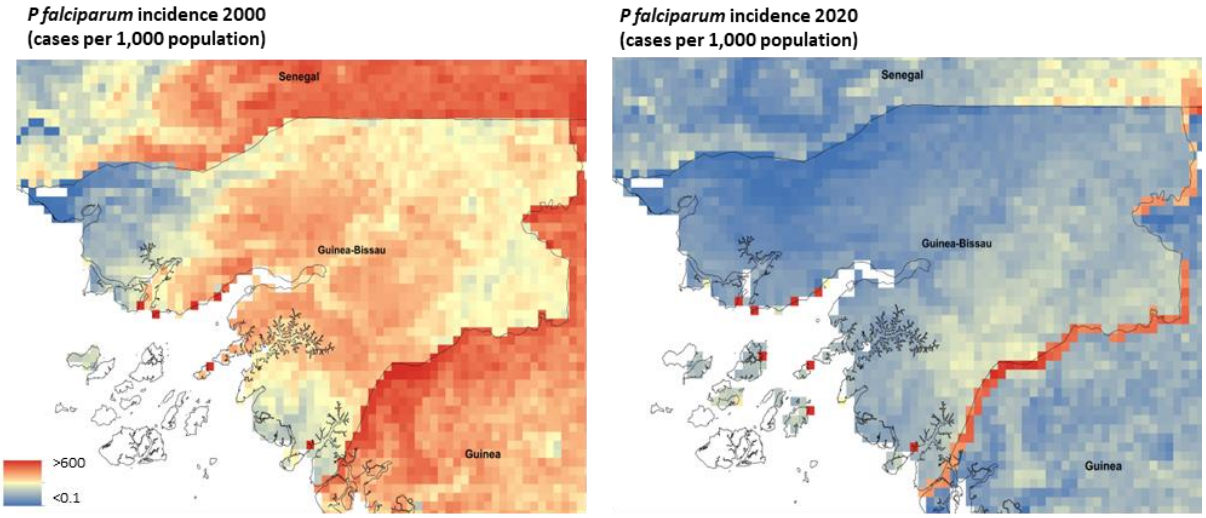

Figure 5. Map of *P. falciparum* case incidence in Guinea-Bissau in 2000 (left) and 2020 (right) from the Malaria Atlas Project dataset [6, 7].

The Bijagós Archipelago, a UNESCO biosphere reserve, is situated off the coast of mainland Guinea-Bissau [108]. It consists of 88 islands and islets, covering a total surface area of approximately 900 km². It is home to a great diversity of mammals, reptiles, birds and fish, and is the most important site in Africa for nesting green sea turtles, with 10,000 adults coming ashore each year [108]. Only around 20 of the islands are permanently inhabited by humans, with the remaining islands being seasonally inhabited for agriculture or periodically used for traditional ceremonies. The total human population of the Archipelago is approximately 25,000, with the population largely reliant on fishing and annual cashew harvest [109].

The Bijagós Archipelago is endemic to numerous infectious diseases, including many vector-borne diseases. Malaria in Guinea-Bissau continues to be a significant public health issue, however, whilst malaria prevalence has been well-characterised in the capital, less is known about malaria-transmission throughout the rest of the country [110, 111]. Knowledge of malaria incidence on the Bijagós Archipelago was limited. However, results from multiple surveys carried out by the London School of Hygiene & Tropical Medicine (LSHTM) project over the last five years show malaria transmission to be highly seasonal and stable. Baseline population-based *P. falciparum* prevalence (by PCR) was estimated to be 22.2% (95% CI 18.7-26.0%). ITN coverage is high at an estimated 92% (95% CI 86-96%), with bednet use also reported to be high (86%). On Bubaque island, the most populated island of the Archipelago, 97% (95% CI 91.5-99%) of people reported sleeping under bednets to prevent malaria [112].

Malaria-vectors within the *An. gambiae* complex have been identified on the Bijagós Archipelago [113, 114]. *Anopheles gambiae* s.s. was the most abundant *Anopheles* species during the wet season,

accounting for 50% of the *Anopheles* species caught during a survey on Bubaque in June/July 2017, and the species also had the highest sporozoite rate with 13.9%. The reported sporozoite rate is much higher than in surrounding regions, suggesting that more data is needed to get an accurate assessment of infection on the islands [96]. Full susceptibility to permethrin and partial resistance to alpha-cypermethrin has been observed using CDC bottle bioassays on the islands [114]. Testing for presence of the 'West African' 1014F *kdr* allele was conducted and found to be present in 36% of *An. gambiae s.s.,* 35% in *An. coluzzii*, and 42% in *An. gambiae/An. coluzzii* hybrids. The presence of the *kdr-w* allele in the vector population can most likely be attributed to the strong selective pressure imposed by the distribution of ITNs throughout Guinea-Bissau since 2011. Full susceptibility was seen when the insecticide synergist piperonyl butoxide (PBO) was added, suggesting a metabolic resistance mechanism through mixed-function oxidases [114, 115].

The MATAMAL trial

The MATAMAL trial is a cluster-randomised placebo-controlled trial investigating the impact of adjunctive IVM MDA in addition to DP MDA on the prevalence of *P. falciparum* parasitaemia in 2021 and 2022 in communities on the Bijagós Archipelago, Guinea-Bissau. Below is an introduction and overview of the MATAMAL trial, the protocol has been published and is included as an appendix to the thesis (Appendix I).

Trial Design

MATAMAL trialled the use of the IVM MDA regimen of 300 µg/kg per day for three days (Laboratorio Elea Phoenix, Argentina) co-administered with a full dose of the antimalarial DP MDA (Alfasigma, Italy). The trial was conducted on 24 clusters on the Bijagós Archipelago. Clusters were randomised into either an intervention or control arm. The intervention arm of the trial received three rounds of MDA of DP and IVM; the control arm received three rounds of MDA of DP and IVM placebo. Clusters were randomised into trial arms at a 1:1 ratio (Figure 6).

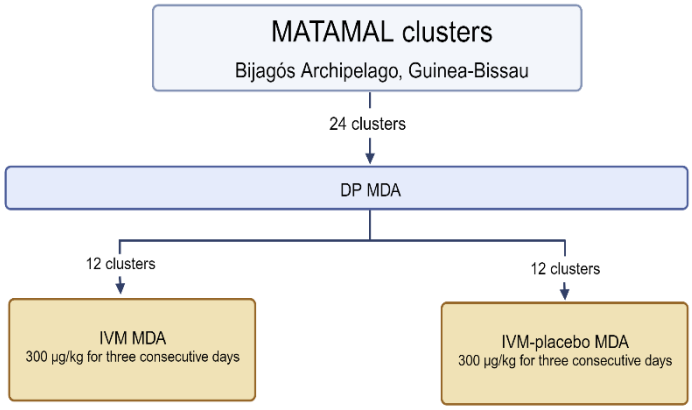

Figure 6. Diagram summarizing MATAMAL trial design. Twenty-four clusters randomised to either the control (DP + IVM-placebo) or intervention (DP + IVM) arm at a 1:1 ratio.

A model by Hannah Slater illustrating the impact of DP (control) versus DP+IVM (intervention) MDA at the 3 x 300 regimen on PCR prevalence on the Bijagós Archipelago can be seen in Figure 7 [105] . The model assumed a 70% coverage of the eligible population, as well as the baseline peak qPCR prevalence of 21.2%, a fixed lag time of 12.5 days from human infection to onward infectiousness, *An. gambiae* s.l. being the dominant vector and ideal conditions such as no contamination of participants and vectors across trial arms and no population movement. The model predicted that if the MDA be distributed each month for three consecutive months over the transmission season, there would be a difference in qPCR prevalence of 9.2% and 3.9% in the control and intervention arms respectively after year 1, and 3.9% and 0.8% respectively after year 3.

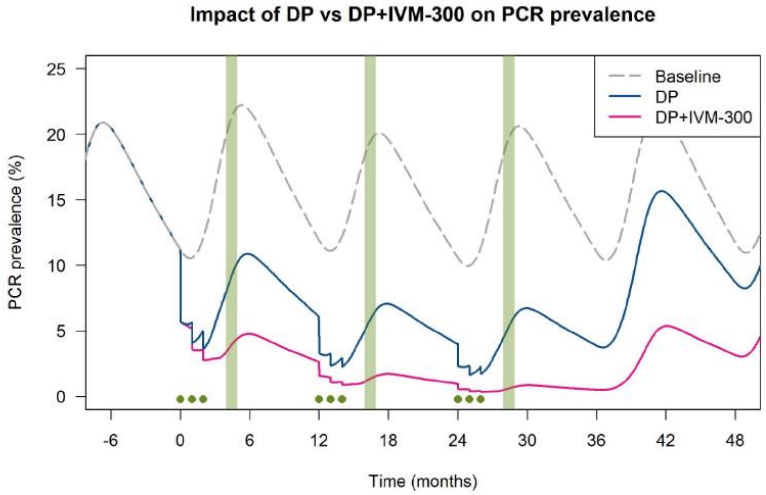

Figure 7. Model showing predicted qPCR prevalence of malaria over time, assuming 70% coverage and 75% DP efficacy on the Bijagós Archipelago. Dashed grey line: malaria prevalence in the absence of any MDA; Blue line: malaria prevalence with MDA of DP only; Pink line: malaria prevalence with MDA of DP and IVM at the 3 x 300 regimen. Green dots represent MDA rounds, and green bars show survey periods [105].

Trial arm randomisation was done using restricted randomisation (Figure 8) [116]. Restriction variables included baseline population, *P. falciparum* prevalence (qPCR and RDT), vector density, SMC coverage and presence of a health centre. The trial was quadruple-blinded, meaning that participants, intervention providers, investigators and analyst were unaware of which cluster was assigned to which arm.

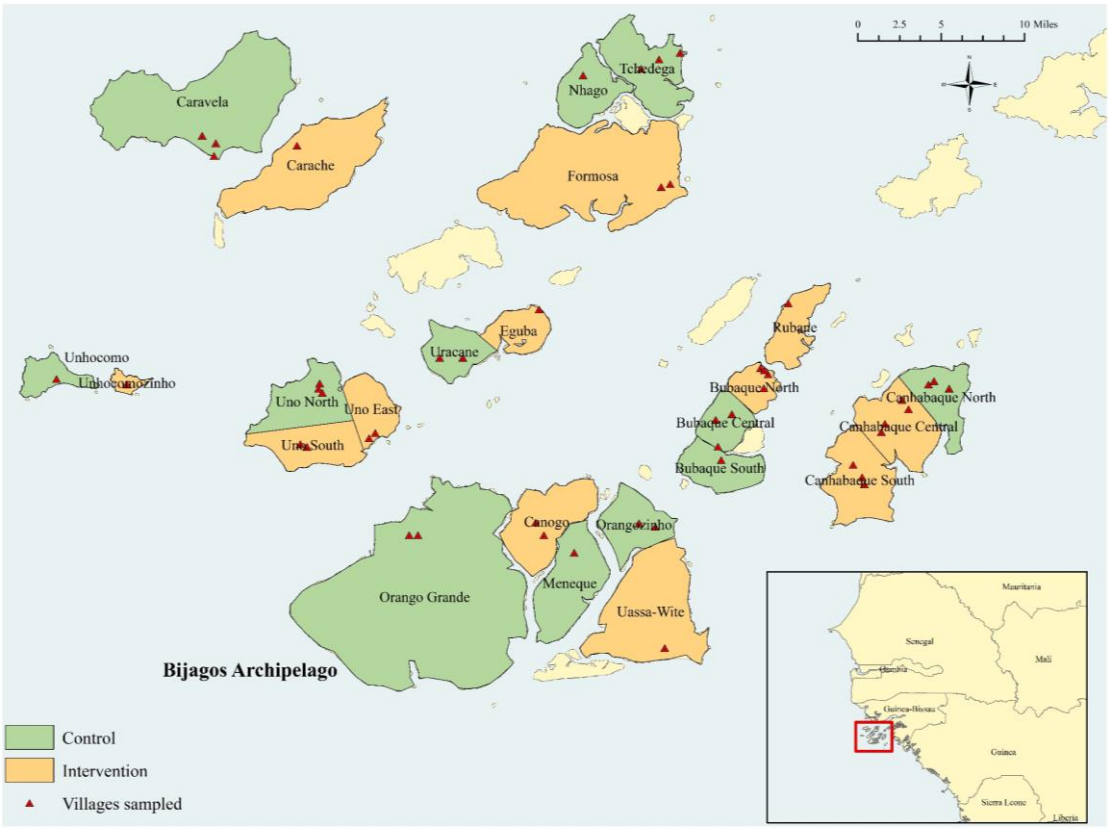

Figure 8. Map of clusters in the MATAMAL trial. Intervention clusters are seen in orange; control cluster in green. Villages sampled are depicted with red triangle. Inset map shows location of Guinea-Bissau in West Africa.

Nineteen of the permanently inhabited islands within the Bijagós Archipelago were included in the trial. The three most heavily populated islands, Bubaque, Canhabaque and Uno, were split into three clusters. All other clusters were entire islands. Samples for both clinical and entomological outcomes were collected using a fried-egg design. To avoid contamination between clusters, only villages within the cluster 'yolk' were sampled (Figure 9). There was at least a 2.2 km buffer zone between cluster 'yolks' [117].

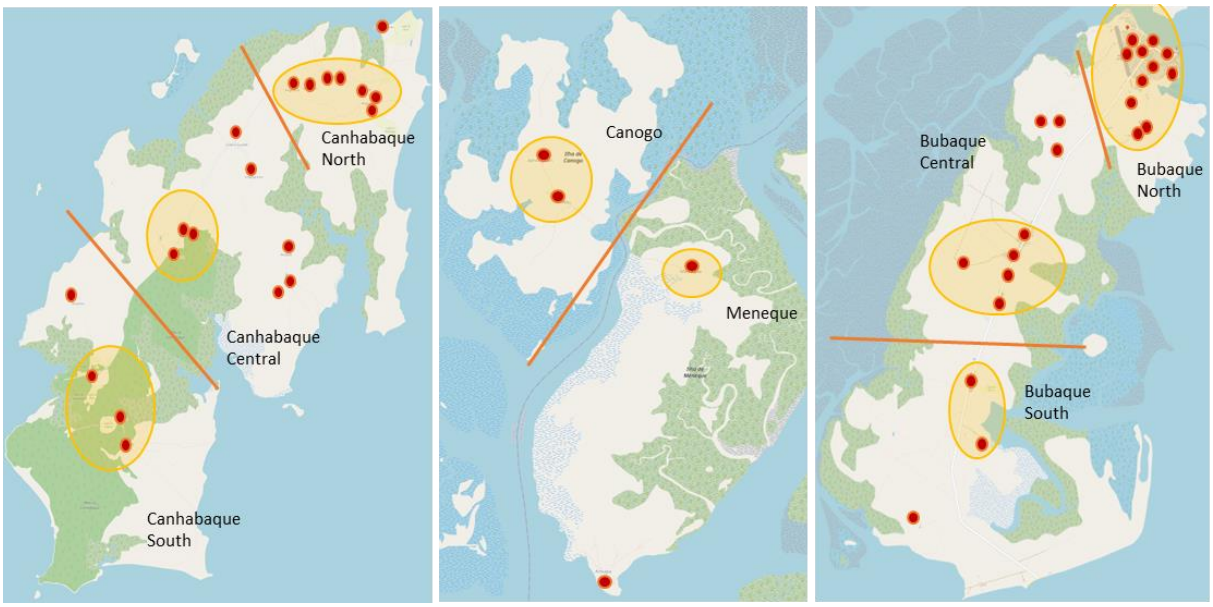

Figure 9. Examples of fried-egg design of MATAMAL clusters. Villages are represented with red dots. Cluster 'yolks' are shown in yellow circles.

All inhabitants of the Archipelago who resided within a cluster were invited to participate in the trial unless they met any of the following exclusion criteria:

1. Severe illness.
2. Age under 6 months (DP).
3. Height under 90 cm or weight under 15 kg (IVM/placebo).
4. Pregnancy (any trimester) or breast feeding (IVM/placebo). Pregnancy (first trimester) (DP).
5. Known hypersensitivity to either medication.
6. Concomitant use of drugs affecting cardiac function or the corrected QT interval (DP).
7. Travel to a country endemic for *Loa loa* (IVM/placebo).

The transmission season in the Bijagós Archipelago coincides with the rainy season from June to December, with peak-transmission in October/November [110]. MDA rounds were delivered in July, August and September, with the intention of reducing transmission throughout the season.

Trial outcomes

Primary outcome

The primary outcome for MATAMAL was the population-based qPCR *P. falciparum* prevalence in all age groups during the peak transmission season after two years of MDA. Data for this outcome was collected during a survey conducted in October/November 2022 (peak-transmission survey, PTS). qPCR prevalence was obtained from dried blood spots from 200 participants per cluster from all clusters.

Secondary outcomes (taken from Hutchins et al. [105])

1. Population-based prevalence of *P. falciparum* parasitaemia in all ages, detected by qPCR, during the peak transmission season after the first year of MDA.
2. Incidence of clinical malaria confirmed by *Plasmodium spp.* lactate dehydrogenase/histidine-rich protein 2 (pLDH/HRP2) rapid diagnostic test (RDT), determined through passive surveillance of all malaria cases presenting to health facilities throughout the trial.
3. Incidence of clinical malaria identified by RDT (CareStart Malaria PAN pLDH) during active surveillance of a cohort of children aged 5–14 years, during the intervention and peak transmission season.
4. Incidence of malaria infection identified by qPCR and serological analysis during the same period in this cohort of children.
5. Age-adjusted prevalence of serological markers indicating recent exposure to *P. falciparum*.
6. Prevalence of serological markers of recent *Anopheles* exposure.
7. Parity, as a measure of *An. gambiae* sensu lato survival, measured in mosquitoes caught using indoor CDC light traps 7–14 days after the final MDA round in year 1 and year 2.
8. Mosquito species composition, population density and SR in mosquitoes caught using indoor CDC light traps.
9. Prevalence of resistance to pyrethroids in anopheline mosquitoes using bioassay methodologies.
10. Prevalence of resistance to artemisinin and partner drugs in humans using molecular markers of resistance.
11. Safety of intervention through monitoring of adverse events.
12. Impact on IVM-susceptible neglected tropical diseases (NTDs; scabies, strongyloidiasis, other STHs and LF), headlice and bedbug infestation using clinical and serological parameters.
13. Cluster-level intervention coverage estimates.
14. MDA acceptability, feasibility and access.
15. Cost effectiveness of adjunctive IVM in this setting.

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Chapter 2. Study Rationale, Hypotheses, Aims and Objectives

Study Rationale

Malaria transmission persists throughout most endemic countries regardless of intervention programmes. It is therefore important to trial novel control measures that can work alongside mainstay interventions. On the Bijagós Archipelago of Guinea-Bissau, malaria transmission continues regardless of high bednet coverage and adherence. The islands are situated ~50 km off the mainland and movement between islands is limited. It was therefore an ideal setting to trial a new intervention using a cluster-randomised placebo-controlled design. Ivermectin (IVM) mass drug administration (MDA) used in conjunction with the antimalarial dihydroartemisinin-piperaquine (DP) may be a useful addition to the malaria control arsenal, targeting the parasite through the DP distribution and the vector population through the distribution of IVM.

The aim of this PhD was to evaluate the impact of IVM MDA on the mosquito population on the Bijagós. Mosquitoes collected from post-MDA and peak transmission surveys were identified. Further morphological and molecular analysis was performed on *Anopheles* mosquitoes to investigate the impact of IVM MDA on vector parity rate, density, species composition and infectivity rate (sporozoite rate). Comparisons between trial arms were done for each entomological outcome, adjusting for clustering.

Some preliminary surveys investigating the vector population had previously been conducted on the Archipelago, however they were small and limited to a few islands. The trial provided an opportunity to better understand the vector ecology and behaviour throughout all permanently inhabited islands. Surveys were conducted in houses selected for entomological sampling to characterise household variables, and a multi-variate analysis was performed to investigate individual risk-factors associated with mosquito house entry.

Hypotheses

1. The distribution of IVM MDA will result in a significant decrease in malaria vector parity rate and density.
2. When exposed to IVM MDA, the proportion of *An. gambiae* s.s. within the population will significantly decrease.
3. Vector populations exposed to DP + IVM MDA will have a significantly reduced sporozoite rate.
4. Poor quality houses will be associated with higher mosquito house entry.

Aims

The aim of this PhD was to characterise the mosquito population on the Bijagós Archipelago and evaluate the impact of IVM MDA for malaria control on vectors. The PhD also aimed to describe the built environment on the Archipelago for the first time and identify any risk factors associated with higher mosquito house entry.

Specific Objectives

1. To conduct a baseline survey on the Bijagós Archipelago, focusing on the *Anopheles* mosquito population.
2. To establish an appropriate method for storing *Anopheles* mosquitoes prior to dissection for parity assessment.
3. To evaluate the impact of IVM MDA on vector density, population age structure, species composition, sporozoite rate and entomological inoculation rate.
4. To evaluate the built environment of the Bijagós Archipelago and investigate how the quality of housing affects malaria vector house entry.

Chapter 3. Methods

Baseline survey

Design Overview

The malaria transmission season on the Bijagós Archipelago runs from June to December, with peak transmission occurring in October/November. The baseline survey was carried out on 16 of the permanently inhabited islands on the Bijagós Archipelago between October and December of 2019 (Figure 1). Islands were sampled sequentially, and sampling took place over six weeks between the 27th October and the 5th December 2019. Villages were selected using probability proportional to size sampling for a concurrent malaria prevalence survey. All villages selected for the prevalence survey were assigned a code, and one to two villages per island were selected at random for entomology sampling using a random number generator. For thirteen of the sixteen islands, one village was sampled for two consecutive nights. However, due to transport constraints, the entomology collection was heavily dependent on the prevalence survey schedule, therefore three islands had two villages sampled, each of which were trapped for one night.

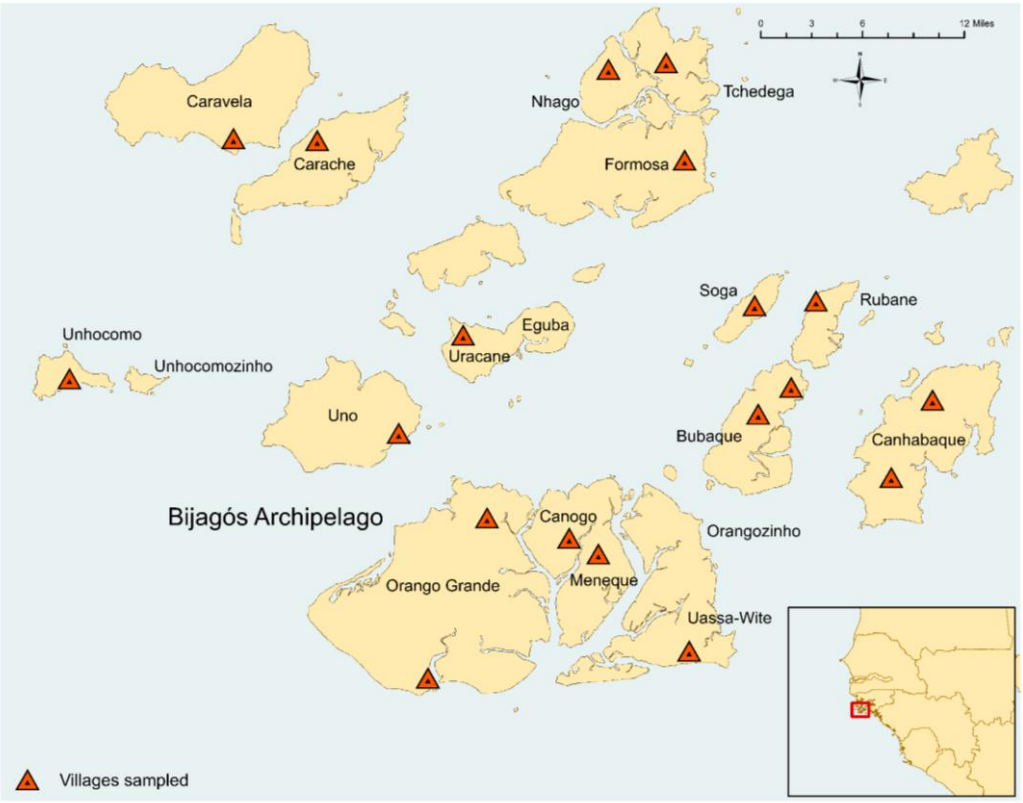

Figure 1. Map of the Bijagós Archipelago showing villages sampled in 2019. Location of the Archipelago in West Africa shown in inset.

Mosquito collection

Adult mosquitoes were caught using both indoor and outdoor Center for Disease Control (CDC) miniature light traps (LTs; John W. Hock, Gainesville, Florida, USA) from eight houses on each island sampled. Prior to trap set-up, verbal consent from the head of the household was taken. This involved explaining the methodology and rationale behind the work, ensuring that the participant understood the study correctly and giving the opportunity to answer any questions that the participant may have.

Following verbal consent being given, one room within the house was selected by the head of household. Bednets were required to be hung above beds in the trapping room; after ensuring this, one indoor LT was hung 50 cm from the base of an occupied ITN-protected bed, with the light of the LT at a height of 100 cm [1].

Outdoor feeding behaviour had not been previously investigated on the Bijagós Archipelago. Therefore as a pilot study, outdoor LTs were odour-baited using the MB5-lure blend [2]. The MB-5 lure comprises of five-compounds and simulates the smell of a human foot. The bait consists of ammonia, l-(+)lactic acid, tetradecanoic acid, 3-methyl-1-butanol and butan-1-amine at various concentrations (Table 1). The lures were pre-prepared at London School of Hygiene & Tropical Medicine (LSHTM) prior to travel to the Archipelago. Nylon strips cut from pantyhose (90% polyamide, 10% spandex; Marie Claire SA, Borriol, Spain) measuring 26.5 x 1.0 cm were used, they were soaked in 70% ethanol for 2 hrs prior to compound impregnation to ensure no unwanted compounds from manufacturer were still on the fabric. Each strip was impregnated by a single compound by submersing them in microcentrifuge tubes containing 1 ml of solution. They were then left to soak for 3-5 hours. Thereafter, the strips were hung under a fume hood for 30 min to allow excess fluid to drip off them. Finally, they were wrapped in aluminium foil according to the compound (i.e. all ammonia strips were stored together, etc) and stored in a refrigerator at 4°C until taken to the field.

Table 1. Compounds required to create MB-5 lure at specific concentrations.

Compound	Concentrations
Ammonia	25% solution
l-(+)lactic acid	88-92% solution
Tetradecanoic acid	16% (w/v) solution in ethanol
3-methyl-1-butanol	0.01% in paraffin oil
Butan-1-amine	0.001% in paraffin oil

Prior to outdoor trap set-up, the lure was made by taking one strip from each compound and securing them together with an elastic band (Figure 2). New strips were used for every island. The lure was then secured to the bracket of the LT using wire.

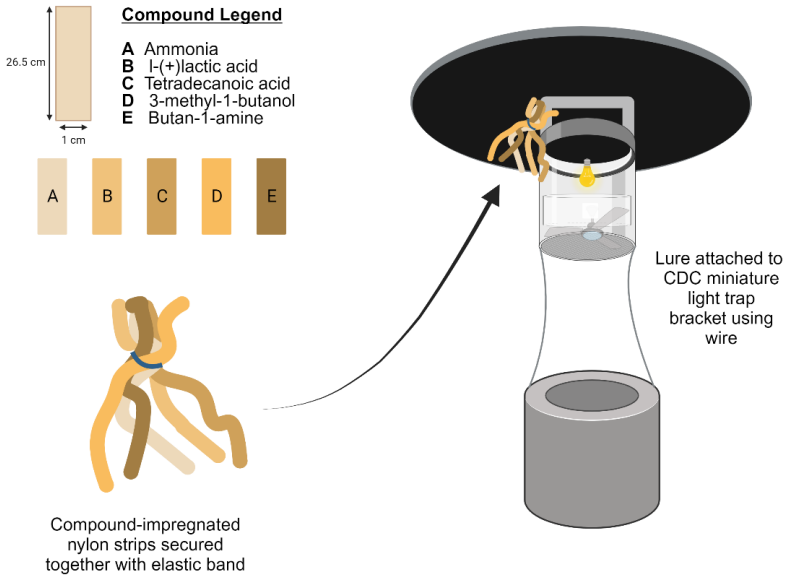

Figure 2. MB5-lure represented by multiple coloured strips (A) Ammonia, (B) l-(+)lactic acid, (C) tetradecanoic acid, (D) 3-methyl-1-butanol and (E) Butan-1-amine. Compounds were fastened together using elastic band and attached to LT using wire.

Outdoor LTs were set-up 5-10 m from the house in a clear area at a height of 100 cm. As well as the MB5-lure, outdoor LTs were also baited with CO2. To produce the CO2, 17.5 g of dried active yeast was added to 250 g of sugar in 2.5 L of water [2]. The CO2-generating solution was placed in a jug on the floor below the LT.

Both indoor and outdoor LTs ran from 19h00 to 07h00 for two consecutive nights. On islands where two villages were sampled, only one trapping night per village was possible.

Following nightly collections, mosquitoes were killed using acetone. Mosquitoes were morphologically identified using a hand-held magnifying glass using identification key [3]. Following identification, they were preserved in self-indicating silica gel and cotton wool in individual 1.5 ml microcentrifuge tubes.

A subsample of *An. gambiae* s.l. was sent to the Medical Research Council (MRC) The Gambia at London School of Hygiene & Tropical Medicine for molecular species identification using PCR and detection of the infective sporozoite stage using enzyme-linked immunosorbent assays (ELISA).

Larval surveys

A larval survey was conducted for every village sampled by searching the surrounding area for suitable larval habitats. When a larval habitat was found, it was characterised, and environmental variables were recorded. Variables included the size of the larval site perimeter, presence/absence of direct

sunlight, the presence/absence of vegetation and vegetation type, and habitat type (grouped into natural or man-made). Natural habitats included rain pools, drainage channels, salt marsh pans or potholes and erosion pits; man-made habitats included drainage pools from wells, cut-out palm tree hollows, salt pits and borrow pits [4]. Perimeter of larval habitats were grouped into four size categories: 0.01-1 m, 1.01-10 m, 10.01-100 m and >100 m [5]. The size of the larval habitat dictated the number of dips per site; 3, 5, 15 or 50 dips respectively. Dipping techniques varied depending on larval habitat, such as partial submersion of larval dipper around emergent vegetation, logs and tree stumps (Table 2) [6]. Larvae were collected and identified to be within the anopheline or culicine subfamily. Unfortunately, due to logistical difficulties, rearing the larvae to adulthood and identifying species was not possible. However, larval density and proximity to selected villages was recorded.

Table 2. Techniques for sampling mosquito larvae and pupae habitats using a standard pint dipper. Taken from O'Malley, 1989 [6].

Technique	Description	Habitats	Genera targeted
The Shallow Skim	A shallow, skimming stroke along the surface of the water, with one side of the dipper pressed just below the surface. End the stroke just before the dipper is filled to prevent overflowing.	Water with aquatic vegetation or floating debris.	*Anopheles*
Partial submersion	Larvae are drawn into the dipper by submerging one edge so that the water slows rapidly into the dipper.	Around emergent vegetation, logs and tree stumps.	*Anopheles*
Complete submersion	A quick plunge of the dipper below the surface of the water, bringing the dipper back up carefully through the submerged larvae.	Used in most habitats to sample larvae that have dived below surface of water when it is disturbed	*Aedes*
Dipper as a background	Submerge the dipper completely within the woodland pool, going down into the bottom litter if necessary. Come up underneath the larvae carefully with the dipper.	Woodland pools	*Early season species*
Flow-in method	Push the dipper down into the material on the bottom and letting the shadow surface water and mosquito larvae flow into the dipper.	Shallow water, with mud, leaf litter or other debris on the surface	*Aedes and Culex*
Scraping	Dip from the water in, toward the vegetation and end by using the dipper to scrape up against the base of the vegetation to dislodge any larvae present	Permanent or semi-permanent habitats, containing clumps of vegetation.	*Not specific*
Simple scoop	Simply scooping a dipperful of water out of a habitat	A variety of habitats	*Culex*
Salt marsh	In the case of salt marsh potholes, dip in a number of spots around the edge of the pothole, dipping in toward the edge. Sample in the middle of the pothole, using either a skimming or scooping stroke. Alternate between the two techniques..	Salt marshes	*Anopheles*

Molecular analysis

Species identification using PCR restriction fragment length polymorphism (PCR-RFLP)

Species within the *An. gambiae* complex are morphologically identical, therefore species genotyping is needed. PCR-RFLP was first described by Fanello et al in 2002, and is based on species-specific fixed differences in the rDNA region, including 28S coding region and intergenic spacer region [7]. DNA was extracted using the QIAcube Extractor robot (QIAgen, Hilden, Germany), following manufacturer guidelines. Firstly, a part of the sample, usually a mosquito leg or wing depending on sample condition,

was ground in a mixture of proteinase K and ATL buffer and lysed by incubating at 56°C overnight. It was then loaded into the extraction robot where DNA was extracted. A volume of 80 µl of DNA was eluted in AE buffer and stored at -20°C.

When performing the PCR-RFLP, 2 µl of sample DNA was added to 23 µl the PCR Master Mix, which included PCR water, deoxynucleotude triphosphates (dNTPs), buffer, primers and Taq polymerase. The primers used were (UN) (F) [GTG TGC CCC TTC CTC GAT GT], AR (R) [AAG TGT CCT TCT CCA TCC TA], GA (R) [CTG GTTTGG TCG GCA CGT TT], ME (R) [TGA CCA ACC CACTCC CTT GA]. PCR was carried out using the following amplification conditions: (1) 94°/5 mins followed by (2) (94° C / 30secs; 50°C / 30secs; 72 °C / 30secs) X 35 times followed by (3) 72° C/ 7mins. Following the PCR, to distinguish between *An. gambiae* s.s. and *An. coluzzii*, the PCR product was digested using the restriction enzyme Hha1 (10 µl of PCR product added to 5 µl of Master mix containing Hha1 enzyme). It was loaded into the PCR thermocycler for an incubation period of 18 hours at 37°C. Samples were then read using a QIAXcel Advanced Instrument (QIAgen, Hilden, Germany), analysed and scored. The expected product sizes of the various *Anopheles* species present can be seen in Table 3.

Table 3. Expected PCR product size of species found on the Bijagós Archipelago.

Anopheles species	Expected product size (10% tolerance)
An. arabiensis	292 bp (± 10%)
An. gambiae s.s	257 and 110 bp (± 10%)
An. coluzzii	367 bp (± 10%)
An. melas	435 bp (± 10%)

bp Base pairs
An. gambiae s.s./ *An. coluzzii* hybrids would have both expected products size base pairing for *An. gambiae* s.s. and *An. coluzzii*

Circumsporozoite protein (CSP) detection using ELISA

The ELISA detects the presence of the *Plasmodium* protein from the infective sporozoite stage in the thoracic salivary glands or mature oocysts in the mosquito midgut [8, 9]. To do this the head and thorax of *Anopheles* mosquitoes were dissected and ground in individual 1.5 ml microcentrifuge tubes. They are then stored at 4°C for 24 hours.

Monoclonal antibodies (Mab) solution was then pipetted into each of the plate wells and incubated at room temperature for 30 minutes or overnight (Figure 3). The Mab solution was then aspirated out and filled with a blocking buffer, again being incubated at room temperature for 1 hour. Following this period, the buffer was aspirated out and the mosquito triturate and controls were added and allowed to incubate for a further 2 hours. Mosquito triturate was removed, and plate wells were washed with PBS-Tween. A Mab-peroxidase conjugate was added to each well to be used as a marker and allowed to incubate in the dark for 1 hour; again this was aspirated, and wells were washed with PBS-Tween.

To visualise the results, a substrate solution was added and incubated for 30 minutes in the dark. Plates were then read at a wavelength measurement of 405 nm using a Vmax® or EmaxTM Kinetic ELISA absorbance microplate reader (Molecular Devices Corporation, Sunnyvale, California, USA).

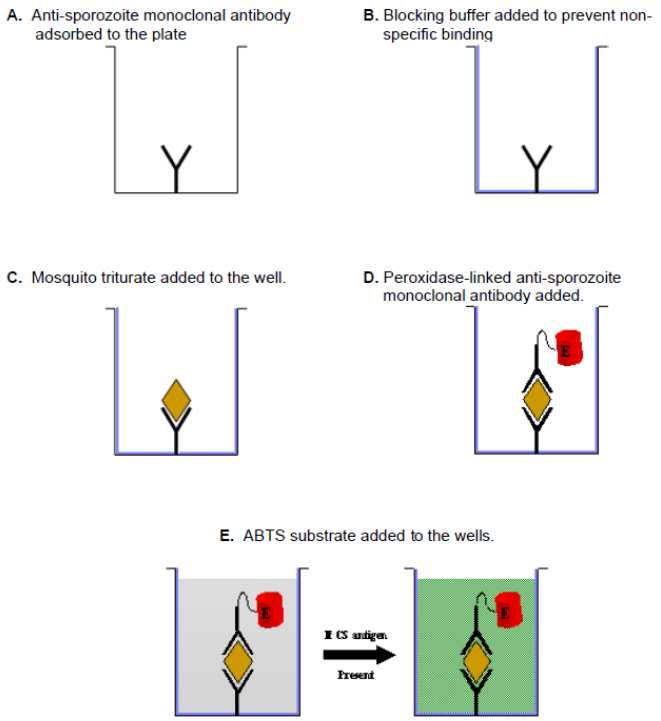

Figure 3. Step-by-step CSP-ELISA procedure. Taken from MRC Unit The Gambia at LSHTM ASSAY-MDEE-027 SOP describing methodology for CSP-ELISA.

To read the plate and calculate positive results, the mean negative control absorbance was used. Samples were considered positive if they had double the mean absorbance value of the negative controls.

Principal entomological data collection

As stated previously, this PhD project was nested within the MATAMAL trial. For full details on the MATAMAL protocol, please see Appendix I [10].

Design overview

The transmission season in the Bijagós Archipelago coincides with the rainy season from June to December, with peak-transmission in October/November [11]. The MATAMAL trial MDA rounds were

delivered in July, August and September, with the intention of reducing transmission throughout the season (Figure 4).

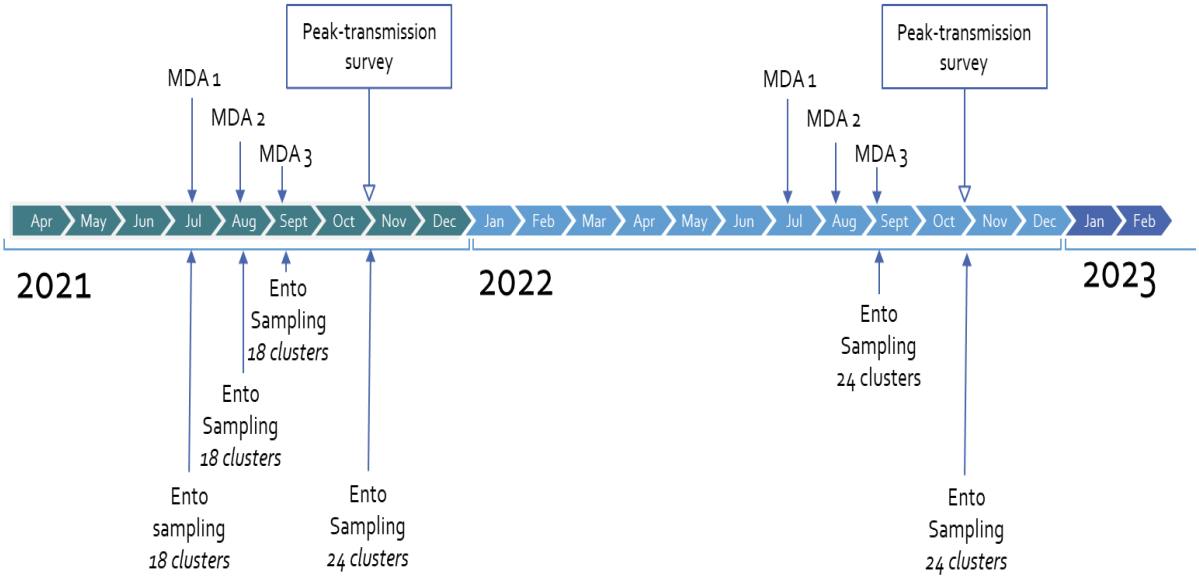

Figure 4. The MATAMAL trial timeline. MDA was delivered in July, August and September of 2021 and 2022. A peak-transmission survey (PTS) followed in October/November, one month after completion of final round of MDA. Entomological sampling (Ento sampling) following each MDA round and PTS in 2021, and the final MDA round and PTS in 2022 is shown.

The study required collection of adult *Anopheles* mosquitoes to determine whether there were any changes in population size, age or infectivity rate. To achieve this, mosquitoes were collected from within selected houses across study clusters from post-MDA collections and a peak-transmission survey (PTS) throughout the two years. In 2021, mosquito data collection occurred 7-14 days following completion of each round of MDA in 18 clusters (9 intervention and 9 control clusters). The same 18 clusters were repeatedly sampled throughout all three MDA months. Further mosquito collections were conducted in all clusters during the PTS. Following 2021, preliminary assessment of parity and density data indicated a high level of variability between clusters. In consultation with the trial principal investigator and statistician, it was decided that the entomology sampling should be increased to all 24 clusters in 2022. Due to the logistical challenges involved in increasing the number of clusters sampled, it was decided that sampling would only take place after the final round of MDA. Should there be a cumulative effect from the multiple rounds of MDA, then a difference between trial arms was most likely to be seen at this time point. Coverage in 2021 had also increased throughout the MDA rounds as the teams became more experienced, therefore it was felt that, should there be an effect from the intervention, this time point would provide the best opportunity to see it. In 2022, mosquitoes were also collected during the PTS in all clusters.

One field team was assigned to either one, two or three clusters depending on the population and geography of each cluster. Field teams were responsible for supervising the MDA delivery, continued cohort surveillance and entomological collection. Should a field team be in charge of two or three clusters, each cluster was sampled sequentially for three consecutive nights. The schedule of the entomological sampling was determined by the duration of the MDA on each cluster (i.e. whichever cluster finished first, was sampled first).

Village and household selection

At each time point (2021: MDA 1, 2, 3 and PTS; 2022: MDA 3 and PTS), one village was selected at random from within the yolk of each sampled cluster (Figure 5). In 2021 and 2022, ten and fifteen households were sampled from each selected village, respectively. The average size of households in the Bijagós is estimated at eight people [12]. Therefore, in 2021, to ensure there were enough houses in villages selected, only villages with a population greater than 80 were included in the randomisation (10 houses needed for sampling x average household size of eight people). In 2022, when 15 households per village were sampled, only villages with a population size greater than 120 were included in the randomisation (15 houses needed for sampling x average household size of eight people). Eligible villages within the cluster 'yolk' were assigned a number and one number was selected using the random number generator Random# (Random#, 2013 Nicholas Dean, iOS 12.0).

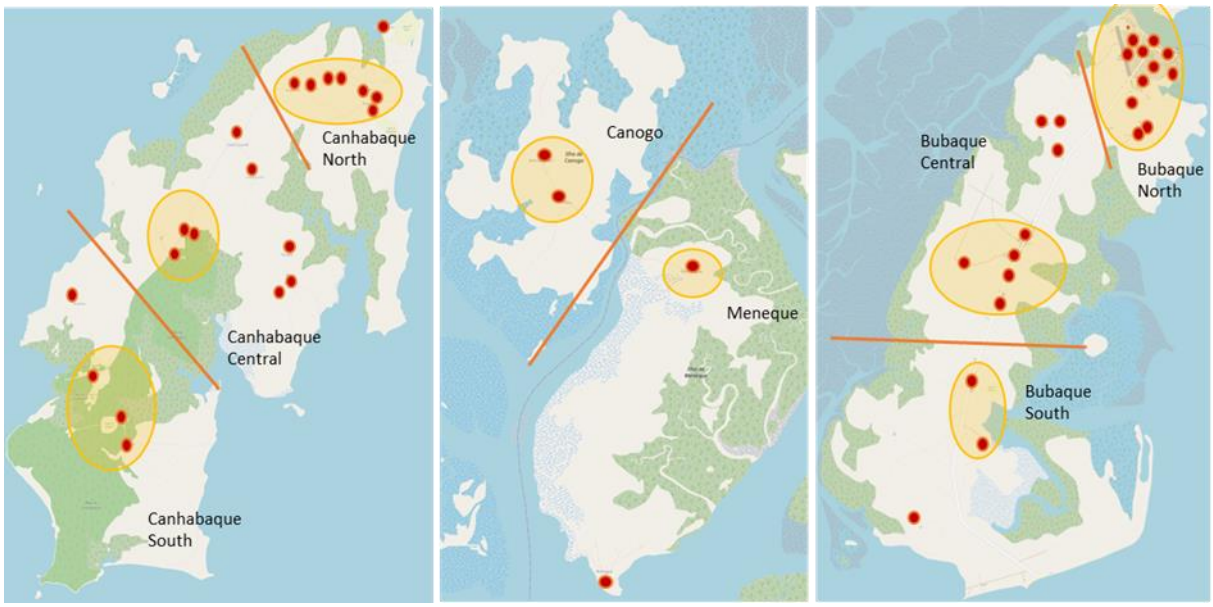

Figure 5. Examples of fried-egg design of MATAMAL clusters. Villages are represented with red dots. Cluster 'yolks' are shown in yellow circles.

As stated previously, in 2021 and 2022, ten and fifteen houses were sampled from each selected village, respectively. The number of households selected for sampling was determined by the number of female *Anopheles* mosquitoes needed per cluster for parity assessments (please see below for full details on parity assessment and sample size calculation). Using data from 2021, the number of households selected in 2022 for sampling was increased to ensure parity assessment sample sizes were met. Community health workers (CHW) operating across the islands were asked to create a household head list for each selected village. This was used to determine which houses within the selected village would be sampled for mosquito trapping. Using the Rand() function in Excel (Excel Version 2310, Microsoft, Redmond, USA), a random order was created for the household head list. This procedure was repeated for every timepoint sampled, resulting in a new random sample of houses for each entomological collection (Figure 6).

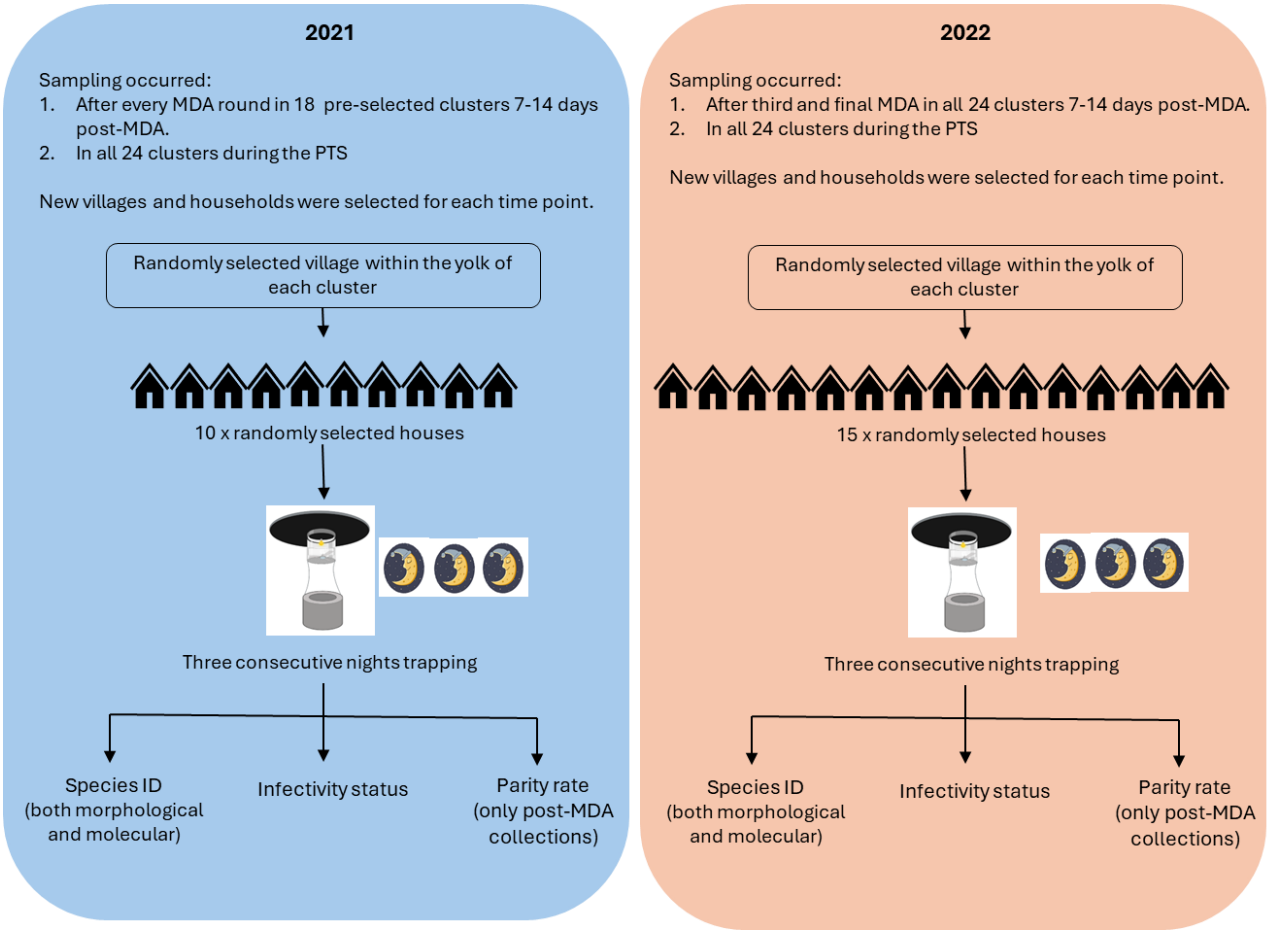

Figure 6. Sampling framework for entomological collections in 2021 and 2022. In 2021, ten households from 18 pre-selected clusters were sampled 7-14 days post each mass drug administration (MDA) round, followed by a peak-transmission survey (PTS) in which all clusters were sampled. In 2022, all 24 clusters were sampled 7-14 days post the third MDA round and during the PTS. New village and households were selected at each timepoint.

Households were identified and approached for mosquito collection. If the head of household was literate, they were offered a participant information sheet (Appendix II). If they were not literate, then the participant information was read to them in the presence of a witness (usually the head of the village or CHW). Verbal consent was then taken prior to trap set-up. If the head of household could not be found, the next house on the list was visited, until the required number of houses had been recruited.

Indoor trapping

One indoor LT was used to collect mosquitoes from each house. Trapping took place from 19h00 to 07h00 for three consecutive nights. LTs were hung at a height of 1 m from the floor at the foot of the bed in one room of each house. All beds in the trapping room were required to have an ITN. On the rare occasion that a household did not have an ITN for each bed, neighbours and village elders were consulted to see if a spare ITN could be found. Should a household be unable to find an ITN, then trapping was not conducted in that household, and a new household was selected.

Following each nights' trapping, all mosquitoes collected were killed using acetone and placed into a 15 ml universal tube containing self-indicating silica gel beads covered by cotton wool. During the MDA mosquito collection, the tubes were transported to the centralised laboratory on Bubaque for morphological identification and further analysis [3]. During PTS mosquito collection, morphological identification was done whilst on the islands.

At the time of sample collection, air temperature (°C) and relative humidity (%) were recorded at each house using a handheld hygrometer (Digital Thermo Hygrometer, Pro Signal, Bedforshire, UK). In 2022, a weather station (WTH600-E-KIT Wireless Weather Station Kit, Extech Instruments, Industrial Electronics Inc., Knoxville, Tennessee, USA) was installed at the project compound on Bubaque to monitor wind (m/s) and rainfall (mm).

Parity Analysis

The aim of current vector control strategies is to reduce the number of potentially infectious bites by targeting mosquitoes of blood-feeding age. Only female vectors that have taken blood and laid eggs are able to transmit malaria, therefore knowing the proportion of females that have laid eggs (parous mosquitoes) and have not laid eggs (nulliparous mosquitoes) within a population gives insight into whether a control intervention is having an effect. Should IVM MDA have an effect on the vector population, the proportion of parous mosquitoes in the intervention arm would be significantly lower than in the control arm.

Following morphological identification, a sub-sample of 200 female *Anopheles* mosquitoes per cluster were selected for parity assessment from post-MDA collections. In 2021, when ten households/cluster were sampled, 6.7 mosquitoes per trapping night were assessed, and in 2022, when 15 households/cluster were sampled, this decreased to 4.4 mosquitoes per trapping night. When there were not sufficient mosquitoes caught in a trap to reach the sample size (for instance, only three *Anopheles* females were caught), then all mosquitoes caught in that trap were used for parity assessment. Following selection and assessment of mosquitoes from all traps, the totals were added up, if the sample size was not reached, traps remaining with mosquitoes were allocated a number and selected at random using the random number generator Random#. One mosquito per trap was used and selection continued until the sample size was met. A sample size calculation determined that this number of specimens would provide >80% power to detect a 30% reduction in the proportion of parous mosquitoes in the intervention arm compared to the control arm (alpha = 0.05, assuming a coefficient of variation of 0.3).

Parity was assessed using a dry-preservation and rehydration method [13]. The method was validated with insectary-reared mosquitoes at LSHTM, and with wild-caught mosquitoes from the Bijagós Archipelago (full details can be found in Chapter 5) [14]. First, individual mosquitoes were placed in a 1.5 ml microcentrifuge tube containing 1 ml of 20% liquid soap solution for 20 minutes (Figure 7). They were then transferred to another tube containing 1 ml of distilled water for a further 20 minutes. The mosquitoes' abdomen was removed from the head and thorax. The mosquitoes' head and thorax were then put in individual 1.5 ml centrifuge tubes containing 70% ethanol solution, so that a subsample could be used for further molecular analysis (please see below for full details). The abdomen was dissected, ovaries were isolated and assessed using the ovarian tracheation method [15].

In 2021, ovary assessment was performed by one individual; photographs of the ovaries were taken of every 10th mosquito successfully identified using a microscope camera (Brunel Eyecam Plus; Brunel Microscopes Ltd; Chippenham, Wiltshire; UK). Photographs were then assessed by a second assessor blinded to the first assessor's results. In 2022, 68% of ovaries were assessed by two assessors at the laboratory in Bubaque. To determine the level of agreement between assessors, the inter-rater reliability (IRR) for each time point using Cohen's Kappa statistic was calculated [16]. For strength of agreement for the kappa coefficient, Landis and Koch proposed the following as standards: ≤0=poor, .01-.20=slight, .21-.04=fair, .41-.60=moderate, .61-.80=substantial, and .81-1=almost perfect [17].

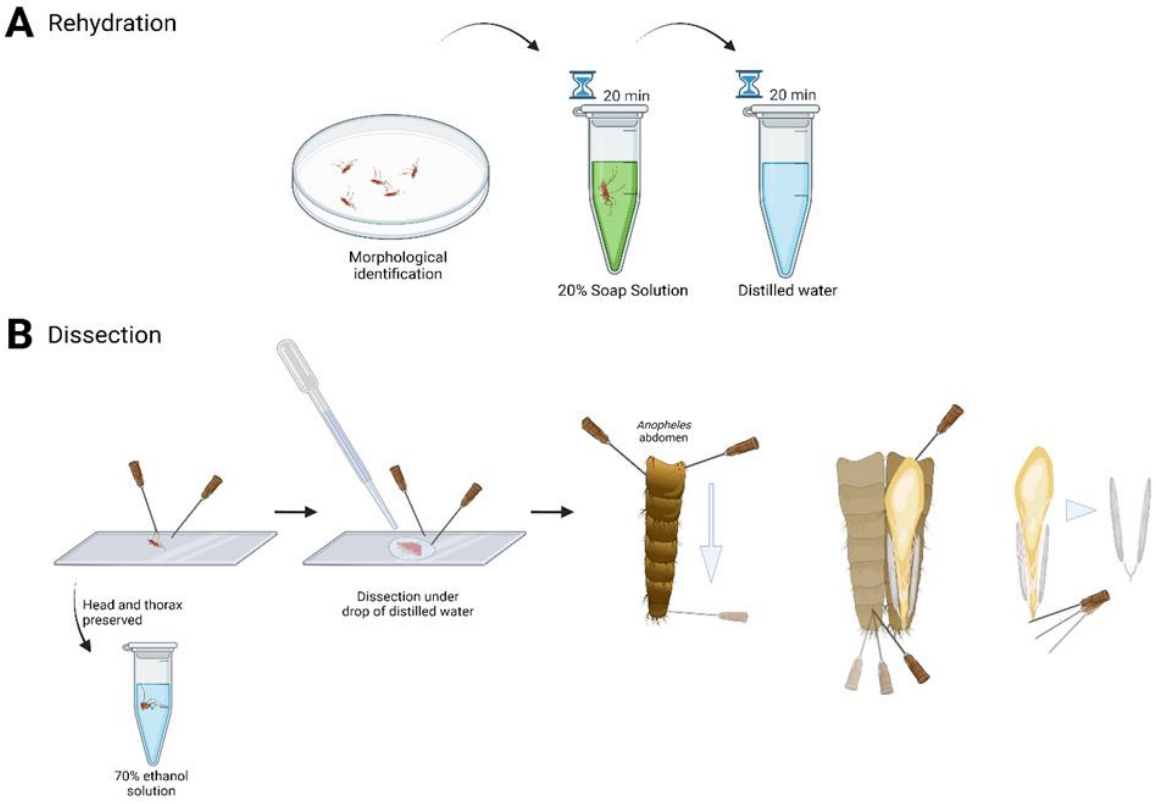

Figure 7. (A) Rehydration and (B) dissection of dry-preserved *Anopheles gambiae* s.l. for parity assessment.

Molecular methods

Species within the *An. gambiae* complex are morphologically identical, but molecularly distinct. To identify *An. gambiae* s.l. species present in the Bijagós Archipelago, a sub-sample of mosquitoes was sent to the MRC Unit The Gambia at LSHTM for molecular analysis. From post-MDA mosquito collections, 30 mosquitoes per cluster were sent for species identification. From PTS collections, 200 mosquitoes per cluster were sent for the same analysis. The primary outcome for the MATAMAL trial was the P. *falciparum* prevalence during the PTS in 2022, therefore, to support this with entomological data, a greater number of mosquitoes was analysed with PCR at these time points. Species identification was done using the same methodology as described above [7].

Two hundred *An. gambiae* s.l. from each cluster were tested for presence of CSP using ELISA from post-MDA 3 sample collections and PTS conducted in 2021 and 2022 (Figure 8). Full test details are described above. A sample size calculation determined that this number of specimens would achieve 96% power to detect a reduction from 5% CSP positivity rate in mosquitoes in the control arm to 2% in the intervention arm (alpha = 0.05, assuming a coefficient of variation of 0.3).

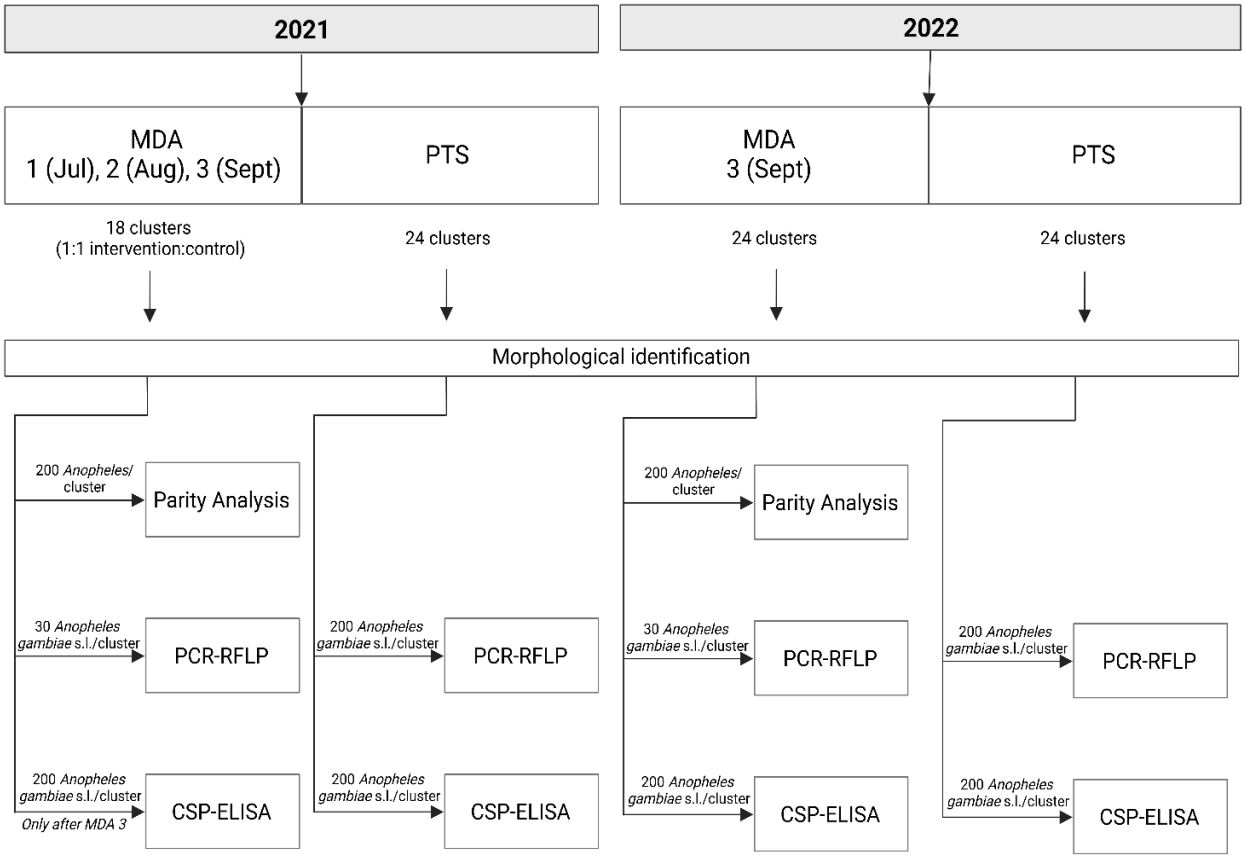

Figure 8. Workflow for sample testing and quantity of samples analysed at each time point for each test.

Household survey

A household survey was conducted on every house sampled. As with mosquito trapping, prior to the survey, a participant information sheet was offered to the head of household, if they were illiterate, the survey was explained (Appendix II). Written consent was taken from the head of household in the presence of a witness, usually a village chief or CHW (Appendix III). Questions to determine socioeconomic status were asked, as well as details on malaria prevention measures used and house built environment. Data was entered using Open Data Kit (Get ODK Inc., San Diego, USA). The full household survey is provided in Appendix IV.

Due to the concerns of workload on the field team in 2021, the surveys of houses recruited for entomological sample collection at each timepoint (MDA 1, 2, 3 and PTS) were conducted during the PTS, so that they did not need to be completed alongside trapping. However, in 2022, all surveys were conducted at the time of entomological sample collection (MDA 3 and PTS).

Statistical Analysis

Baseline survey

Descriptive statistics were used to illustrate the density, species composition and infectivity rate of adult mosquitoes caught in both indoor and outdoor LTs across the Archipelago. *Anopheles* density was calculated as total *Anopheles* females caught divided by the total trapping nights (total number of houses trapped multiplied by the number of nights). Larval sites were also fully described, and data was presented on *Anopheles* positive sites.

Principal Entomological outcomes

To compare anopheline parity rates between trial arms, a t-test on cluster-level parity rates was used. Comparisons were made for all sample collection time points.

Mosquito density was compared between arms using a t-test on *Anopheles* cluster-level densities. The distribution of densities was markedly skewed, so a log transformation was applied.

The proportion of each species identified was determined by cluster and by arm. *Anopheles gambiae* s.s. had previously been identified as the primary local vector, therefore, the proportion of *An. gambiae* s.s. was used to assess the impact of IVM on species composition [5]. A t-test was performed on mean cluster-level *An. gambiae* s.s. proportions.

Cluster-level sporozoite rates were compared between arms using t-test. The cluster-level entomological inoculation rate (EIR; an estimate for the number of infectious bites per person per time unit) was calculated using the formula 1.605 x (number of CSP-positive *Anopheles*/number of *Anopheles* tested) x (number of *Anopheles* collected from LTs/ number of trapping nights) x 180 (30 days per month multiplied by 6 months per season per year) [18]. The monthly cluster-level EIR summaries were then compared using a t-test.

For all analyses described above, adjustments, as described by Hayes and Moulton, were made for collection time point, temperature and humidity [19]. In 2022, rainfall was also adjusted for. Differences, 95% confidence intervals and P-values were presented for all tests.

Household survey

Household socioeconomic status was determined using Principal Component Analysis with variables on household education, income, goods and land owned. Households were then split into five socioeconomic groups: poorest, poor, middle, rich and richest.

Analysis was conducted in two stages. Univariable analysis using a negative binomial regression adjusting for cluster was first performed looking at the impact of timepoint, socioeconomic status, household demographics, bednet use, built environment and climatic variables on indoor mosquito

density (Table 4). Following this, a multivariable negative binomial regression was performed using all variables, irrespective of whether the univariable analysis indicated that the variables significantly impacted mosquito house entry.

Table 4. Variables used in univariable analysis looking at household risk factors associated with mosquito house entry.

	Variables	Categories or units
Timepoint		
1	Timepoint	-21MDA1 -21MDA2 -21MDA3 -21PTS -22MDA3 -22PTS
Socioeconomic status		
2	Socioeconomic status	-Poorest -Poor -Middle -Rich -Richest
Household particulars		
3	Number of occupants in household	-≤4 -5-8 -≥9
4	ITN use	-Yes -No
5	Number of household occupants per ITN	-≤1.5 -1.6–2.5 -≥1.6
6	ITN use last night	-Yes -No
7	Personal vector protection	-None -Smoking herbs -Repellent coil -Insecticide spray -Mosquito repellent -Combination of measures
Built environment		
8	House roofing material	-Thatch -Zinc -Grass matting or plastic sheeting
9	House flooring material	-Cement -Mud
10	House walling material	-Wooden poles -Blocks and cement -Palm fronds -Mud -Combination of materials
11	Eaves	-Open -Closed
12	Windows in household	-0 -1 -≥ 2
13	External door in trapping room	-Yes -No
14	Light in trapping room	-Yes -No
15	Fan in trapping room	-Yes -No

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Chapter 4. A survey of indoor and outdoor biting behaviour, species composition and circumsporozoite rate of malaria vectors in the Bijagós Archipelago, Guinea-Bissau.

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SECTION A – Student Details

Student ID Number	1903438	**Title**	Mrs
First Name(s)	Elizabeth Anne		
Surname/Family Name	Pretorius		
Thesis Title	Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control in the Bijagós archipelago, Guinea-Bissau.		
Primary Supervisor	Dr Anna Last		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where is the work intended to be published?	Malaria Journal
Please list the paper's authors in the intended authorship order:	Elizabeth Pretorius, Robert T. Jones, Harry Hutchins, Eunice Teixeira Da Silva, Sainey Ceesay, Mamadou Ousmane Ndiath, Umberto d'Alessandro, Amabelia Rodrigues, James G. Logan, Anna Last
Stage of publication	Choose an item. Yet to be submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	With the input from co-authors, I designed the study and conducted the fieldwork with the team in the Bijagos. SC performed the molecular analysis at the MRC. I cleaned and analysed the data, interpreted the findings and wrote the paper.

SECTION E

Student Signature	[redacted]
Date	11-03-2024

Supervisor Signature	Anna Last [redacted]
Date	18-03-2024

A survey of indoor and outdoor biting behaviour, species composition and circumsporozoite rate of malaria vectors in the Bijagós Archipelago, Guinea-Bissau.

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Abstract

Background: The malaria-endemic Bijagós Archipelago is situated 50 km off the coast of mainland Guinea-Bissau. It is a seasonal malaria transmission setting, with insecticide-treated bednets as its primary control strategy. Little is known about the vector diversity and behaviour across the Archipelago.

Methods: In 2019, a survey took place on 16 of the inhabited islands across the Archipelago. Adult mosquitoes were collected using odour-baited outdoor light traps and indoor light traps at houses selected at random. Larval surveys were conducted for each village sampled. *Anopheles* adults caught were morphologically identified and a sub-sample was analysed to identify species within the *Anopheles gambiae* complex using RFLP-PCR. Sporozoite positivity was detected within a sub-sample by CSP-ELISA.

Results: *Anopheles gambiae* sensu lato was present on all islands sampled. *Anopheles* density varied between islands, with densities ranging from 0.0 – 98.7 from indoor traps and 0.1 – 165.2 from outdoor traps. *Anopheles melas* was the most commonly observed species, accounting for 85.2% of all *Anopheles* caught from both indoor and outdoor light traps. A high level of hybridisation between *An. gambiae* s.s. and *An. coluzzii* was seen on some islands across the Archipelago. The overall sporozoite rate was 0.86% (0.2% for indoor traps; 1.4% for outdoor traps).

Conclusions: Species within *An. gambiae* s.l. are the primary vectors on the Bijagós. *Anopheles melas* may contribute to transmission throughout the year in the Bijagós. It is therefore important to better understand the vector species to tailor effective malaria control.

Keywords

Malaria, Bijagós Archipelago, Guinea-Bissau, vector survey, *Anopheles gambiae*

Background

While great strides have been made in malaria control over the last several decades through the mass distribution of insecticide-based interventions, progress has stalled since 2015 [1, 2]. This is multifactorial, including the observed increase in resistance in both the parasite to artemisinin-combination therapy and vector to insecticide, and residual transmission. Residual transmission refers to transmission that persists regardless of full universal coverage of insecticide-treated nets (ITNs) and/or indoor residual spraying (IRS) which contain active ingredients that are effective against fully-susceptible local vector populations [3].

Current mainstay vector control measures target endophilic (resting indoors) and night biting mosquitoes. This enables mosquitoes that display different behaviours to evade contact with insecticide-treated surfaces. These behaviours include resting and/or feeding outdoors, feeding earlier in the evening or later in the morning when human hosts are not protected by bednets, or not preferentially feeding on humans [3]. Between- and within- species variation in feeding behaviours has been well documented [4]. The diversity of vectors and their behaviours in each setting will impact the ability of interventions to effectively control malaria. It is, therefore, important to thoroughly understand the vector population prior to control measures being implemented.

The Bijagós Archipelago consists of 88 islands and islets which lie approximately 50 km off the coast of mainland Guinea-Bissau. Eighteen of the islands are permanently inhabited and home to approximately 25,000 people [5]. The population mainly consists of subsistence farmers and fishermen [6]. The islands' populations are relatively isolated, with travel between island usually being made to seek health care, attend cultural festivities or family events, or for income-generating and subsistence activities [6]. This isolation and the geographic topography of the islands makes it an ideal location for investigating vector control strategies [7].

Malaria on the Bijagós Archipelago is seasonal, primarily occurring during the rainy season in June to December, with peak prevalence in November [8]. Malaria vector control is almost exclusively reliant on the distribution of ITNs. In 2017, surveys were conducted on the island of Bubaque, the most heavily populated island in the Bijagós [8]. The survey identified *An. gambiae* sensu stricto (s.s) as the

primary vector on Bubaque during the transmission season, and *An. melas* was thought to be responsible for the low level of transmission that occurs during the dry season. However, larger surveys investigating the vector diversity on other islands, have yet to be published. Here, we present data from across the Archipelago to better understand the malaria vector population to inform future control programmes on the Bijagós.

Methods

Study site

The malaria transmission season on the Bijagós Archipelago runs from June to December, with peak transmission occurring in October/November. The survey took place on 16 of the inhabited islands on the Bijagós Archipelago over a six-week period between October and December 2019. Each island was sampled sequentially, with the team completing entomological sampling on one island before continuing onto the next (Figure 1). Villages were selected using probability proportional to size sampling for a concurrent malaria prevalence survey. All villages selected for the prevalence survey were assigned a code, and one to two villages per island were selected at random for entomology sampling using a random number generator. Due to transport constraints, the entomological sampling was highly dependent on the prevalence survey, therefore on three of the 16 islands, two villages were sampled. On those islands, each village was sampled for one night before moving to the second village (islands: Bubaque, Canhabaque and Orango Grande). On the remaining 13 islands, one village was sampled for two consecutive nights trapping. Eight households within each village were selected at random using Head of household lists generated by community health workers.

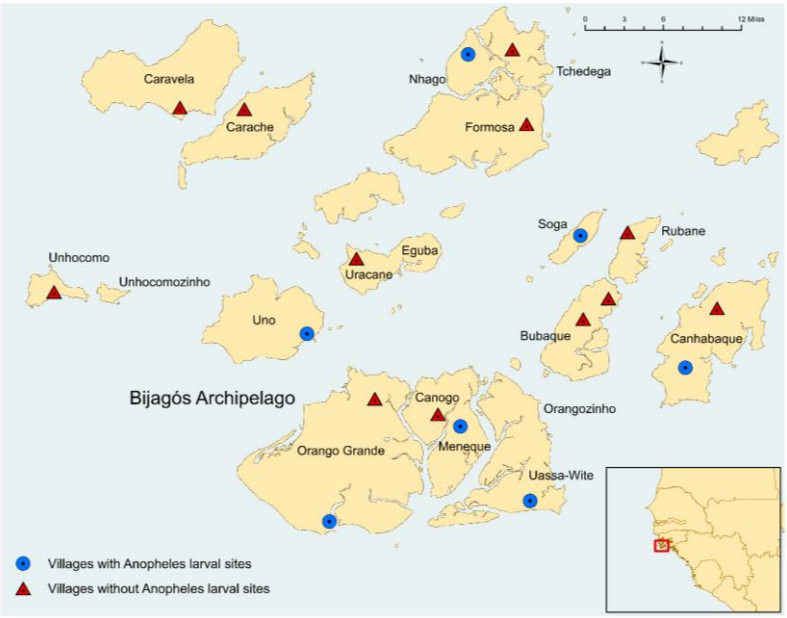

Figure 1. Map of the Bijagós Archipelago showing villages and *Anopheles* larval sites sampled in 2019. Location of the Archipelago in West Africa shown in inset.

Adult trapping and molecular analysis

Adult mosquitoes were caught using one indoor and one outdoor CDC Light trap (LTs; John W. Hock, Gainesville, Florida, USA) in each selected house on islands sampled. Verbal consent was given prior to LT set-up.

One indoor LT per house was placed 50 cm from the base of the bed at a height of 100 cm [9]. Outdoor LTs were placed 5-10 m from the house in a clear area at a height of 100 cm. Outdoor LTs were baited using the MB5-lure blend; these lures were hung from the side bracket of the trap using wire [10]. Carbon dioxide was also produced for the outdoor LTs using a 17.5 g dried active yeast with 250 g sugar in 2.5 L water [11]. Both indoor and outdoor LTs ran from 19h00 to 07h00 for two consecutive nights. Following each nights' trapping, mosquitoes were killed using acetone and morphologically identified [12]. Mosquitoes were then individually dry-preserved in self-indicating silica gel in 1.5 ml microcentrifuge tubes. Mosquito density was calculated as the number of mosquitoes caught divided by the number of trapping nights (number of houses multiplied by the number of nights trapped) for each island.

A subsample of 100 *An. gambiae* sensu lato (s.l.) from indoor and outdoor LTs was sent to the Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine for further molecular analysis.

To identify species within *An. gambiae* s.l., DNA was extracted using the automated QIA cube Extractor robot (Qiagen, Hilden, Germany) following manufacturer instructions. PCR was then performed using restriction fragment length polymorphism (RFLP) [13]. To test for the presence of the circumsporozoite protein (CSP), the head and thorax of *An. gambiae* s.l. were ground in 1.5 ml microcentrifuge tubes using BB NP40 solution. An enzyme-linked immunosorbent assay (ELISA) was performed on mosquito triturate [14].

Larval survey

A larval survey was conducted for every village sampled by searching the surrounding area for suitable mosquito larval habitats. Larval habitats were characterised, and environmental variables were recorded. Variables included size of larval site perimeter, presence/absence of direct sunlight, the presence/absence of vegetation and vegetation type, and habitat type (grouped into natural or man-made). Natural habitats included rain pools, drainage channels and erosion pits; artificial habitats included drainage pools from wells, cut-out palm tree hollows, salt pits and borrow pits [15]. Larval habitats were grouped into four size categories: 0.01-1 m, 1.01-10 m, 10.01-100 m and >100 m [8]. The size of the larval habitat dictated the number of dips per site; 3, 5, 15 or 50 dips respectively. Dipping techniques varied depending on larvae habitat, such as partial submersion of larval dipper

around emergent vegetation, logs and tree stumps. Full sampling techniques have been previously described [16]. Larvae were collected and identified to be within the anopheline or culicine subfamily. Larval density and proximity to selected villages was recorded.

Results

Mosquito density and species composition

A total of 8,625 female mosquitoes were caught; 6,229 (72%) were *Anopheles*, 2,391 (28%) were *Culex* genus and five (0.06%) were in the *Aedes* genus. Of the 6,229 *Anopheles* caught, 2,760 were from indoor LTs and 3,469 from outdoor LTs. All *Anopheles* females were morphologically identified as being within the *An. gambiae* complex.

The number of *Anopheles* females caught varied considerably between islands from both indoor and outdoor trapping (Figure 2). For indoor trapping, the density of *Anopheles* females caught ranged from none on Unhocomo island to 98.7 on Nhago island. For outdoor trapping, density ranged from 0.1 on a few islands to 165.2 on Nhago (Table 1). Except for the islands of Carache, Caravela and Unhocomo, *Anopheles* females dominated trap catches from both indoor and outdoor LTs.

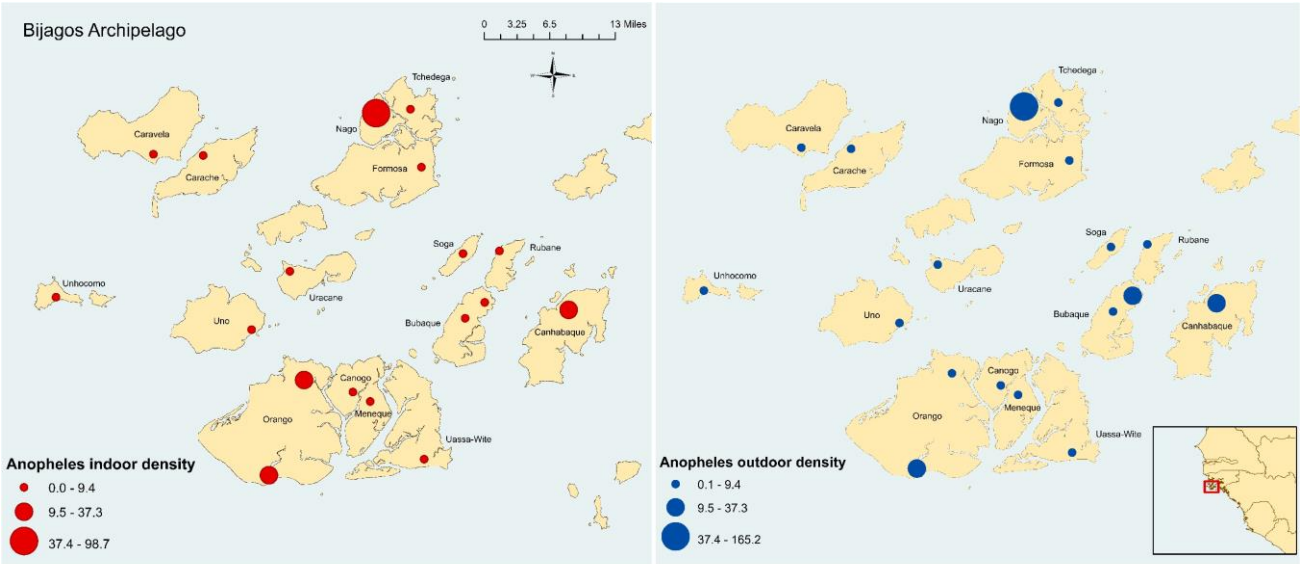

Figure 2. *Anopheles* density from indoor (red) and outdoor (blue) LTs across the Bijagós Archipelago.

Table 1. Total number of nights trapped, total female *Anopheles* caught, density and percentage of *Anopheles* females in trap catch for each island sampled using indoor and outdoor LTs.

Island	Num of households	Indoor trapping			Outdoor trapping		
		Total nights trapped	Total *Anopheles* females (% of trap catch[a])	*Anopheles* density	Total nights trapped	Total *Anopheles* females (% of trap catch[a])	*Anopheles* density
Bubaque	8	16	104 (50.2)	6.5	13	110 (83.3)	8.5
Canhabaque	7	13	278 (71.1)	21.4	14	391 (76.8)	27.9
Canogo	8	16	77 (62.6)	4.8	16	44 (50.0)	2.9
Carache	8	16	5 (2.7)	0.3	16	1 (1.5)	0.1
Caravela	8	15	11 (4.1)	0.7	16	4 (2.4)	0.2
Formosa	8	15	19 (19.6)	1.3	16	2 (6.1)	0.1
Meneque	8	7	25 (46.3)	3.6	8	39 (73.6)	4.9
Nhago	8	17	1678 (95.2)	98.7	15	2478 (96.7)	165.2
Orango Grande	8	15	374(88.6)	24.9	17	201 (84.4)	11.8
Rubane	8	15	46 (65.7)	3.1	15	56 (68.3)	3.7
Soga	8	15	19 (13.3)	1.3	16	31 (40.8)	1.9
Tchedega	8	15	69 (51.9)	4.6	15	5 (14.7)	0.3
Uassa-Wite	7	13	36 (48.0)	2.8	14	81 (71.0)	5.8
Unhocomo	8	15	0 (0.0)	0.0	16	2 (3.2)	0.1
Uno	8	16	12 (11.3)	0.7	16	12 (22.6)	0.7
Uracane	8	16	7 (7.1)	0.4	16	12 (11.8)	0.7

[a] Percentage of mosquitoes caught that were *Anopheles* females

A total of 902 *An. gambiae* s.l. specimens (409 from indoor LTs, 431 from outdoor LTs) were successfully amplified and their species identified. *Anopheles melas* dominated, accounting for 85.2% of all *An. gambiae* s.l. caught, followed by *An. gambiae* s.s./*An. coluzzii* hybrids (7.0%), *An gambiae* s.s. (6.1%) and *An. coluzzii* (1.7%). This was consistent throughout most islands sampled (Table 2). Formosa, Uassa-Wite and Uno were the only islands where the trap catch was below 50% *An. melas* from indoor LTs. From outdoor LTs, only Uassa-Wite carried on this trend. A high level of hybridisation between *An. gambiae* s.s. and *An. coluzzii* was seen on many islands, in particular from indoor LTs. Both *An. gambiae* s.s. and *An. coluzzii* were caught in small numbers throughout the islands.

Table 2. *Anopheles gambiae* s.l. species composition from indoor and outdoor LTs across the Bijagós Archipelago.

Island	Indoor					Outdoor				
	An. gambiae s.s. (%)	*An. coluzzii (%)*	*An. gambiae/ An. coluzzii* hybrids (%)	*An. melas (%)*	Total	*An. gambiae* s.s. (%)	*An. coluzzii (%)*	*An. gambiae/ An. coluzzii* hybrids (%)	*An. melas (%)*	Total
Bubaque	2 (5.9)	0 (0.0)	7 (20.6)	25 (73.5)	34	2 (3.3)	2 (3.3)	11 (18.3)	45 (75.0)	60
Canhabaque	0 (0.0)	0 (0.0)	2 (7.4)	25 (92.6)	27	0 (0.0)	0 (0.0)	1 (1.6)	60 (98.4)	61
Canogo	1 (1.7)	3 (5.1)	2 (3.4)	53 (89.8)	59	2 (5.9)	1 (2.9)	2 (5.9)	29 (85.3)	34
Carache	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	4	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1
Caravela	0 (0.0)	0 (0.0)	2 (18.2)	9 (81.8)	11	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	3
Formosa	6 (42.9)	1 (7.2)	4 (28.6)	3 (21.4)	14	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	2
Meneque	0 (0.0)	1 (4.2)	0 (0.0)	23 (95.8)	24	0 (0.0)	0 (0.0)	1 (3.3)	29 (96.7)	30
Nhago	0 (0.0)	0 (0.0)	0 (0.0)	19 (100.0)	19	2 (2.8)	0 (0.0)	0 (0.0)	70 (97.2)	72
Orango Grande	0 (0.0)	0 (0.0)	0 (0.0)	50 (100.0)	50	0 (0.0)	0 (0.0)	0 (0.0)	37 (100.0)	37
Rubane	2 (2.4)	0 (0.0)	3 (3.6)	79 (94.0)	84	6 (13.0)	1 (2.2)	0 (0.0)	39 (84.8)	46
Soga	3 (15.8)	0 (0.0)	2 (10.5)	14 (73.7)	19	0 (0.0)	0 (0.0)	3 (9.7)	28 (90.3)	31
Tchedega	3 (4.4)	0 (0.0)	3 (4.4)	62 (91.2)	68	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	5
Uassa-Wite	5 (31.2)	1 (6.2)	6 (37.5)	4 (25.0)	16	10 (43.5)	5 (21.7)	5 (21.7)	3 (13.0)	23
Unhocomo	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	2
Uno	5 (31.2)	1 (8.3)	4 (33.3)	2 (16.7)	12	0 (0.0)	0 (0.0)	0 (0.0)	12 (100.0)	12
Uracane	0 (0.0)	0 (0.0)	3 (50.0)	3 (50.0)	6	1 (8.3)	0 (0.0)	1 (8.3)	10 (83.3)	12

CSP-ELISA was conducted on a sample of 934 *An. gambiae* s.l. (434 from indoor LTs, 500 from outdoor LTs). Eight (SR 0.9%) specimens were found to be positive for CSP, seven of them were from outdoor LTs (SR 1.4%) with the remaining one caught indoors (SR 0.2%). All CSP-positive specimens were *An. melas* (Table 3). Seven positive *An. melas* were caught on Canogo, Nhago and Orango Grande from outdoor LTs; one was caught on Meneque from an indoor LT.

Table 3. Results from CSP-ELISA performed on *Anopheles gambiae* s.l. from across the Bijagós Archipelago.

	Total positive	Total negative	Infection rate (%)
An. gambiae s.s	0	55	0.0
An. coluzzii	0	15	0.0
An. gambiae/An. coluzzii hybrid	0	63	0.0
An. melas	8	761	1.0

Larval collections

In total 33 larval sites were sampled throughout the Bijagós, of which 13 (39.4%) contained *Anopheles* larvae (Figure 1). In total, 2113 larvae were collected, of those, 727 were anopheline larvae and 1386 were culicine larvae. *Anopheles* larvae were most commonly found in natural larval sites with a perimeter of 1.01-10 m (Table 4). They were more regularly found in sites with direct sunlight and no vegetation. When vegetation was present, both grasses and trees (either fallen or standing) were common.

Table 4. Characteristics of *Anopheles*-positive larval sites.

Variable	% of larval sites	% *Anopheles* larvae caught
Larval body type		
Natural	61.5	58.0
Artificial	38.5	37.6
Habitat perimeter		
0.01-1 m	38.5	17.4
1.01-10 m	61.5	68.2
10.01-100m	0.0	0.0
>100 m	0.0	0.0
Direct sunlight		
Yes	66.7	59.7
No	33.3	18.9
Vegetation present		
Yes	38.5	89.9
No	61.5	37.2
Vegetation type[a]		
Grasses, Reeds or Sedges	100.0	100.0
Trees	40.0	97.8

[a] Vegetation only found in five of the 12 larval sites

Discussion

Our survey provides, for the first time, descriptive data on the malaria vector population of 16 of the 18 permanently-inhabited islands of the Bijagós Archipelago. It reveals that vector species within *An. gambiae* s.l. are present throughout the islands. Thirteen of the 16 islands recorded a female *Anopheles* density of below ten from both indoor and outdoor LTs. The islands with higher densities were all sampled in the latter stages of the survey, towards the end of November and beginning of December. Environmental variables have been documented to impact the density and species diversity of *An. gambiae* s.l., with rain being a major driver in mosquito numbers, providing water bodies for oviposition and larval development [17, 18]. There was a late-season rain event in mid-November, which may have resulted in a late surge in *Anopheles* on the islands of Nhago, Canhabaque and Orango Grande, all of which were sampled in late November or early December.

As well as environmental changes, ecological differences likely contributed to the variation in density between islands. The Bijagós is a UNESCO biosphere reserve, and has a variety of different landscapes, including palm groves, lakes, wetlands, rivers, savannahs, mangroves and gallery forests [19]. Areas with more sandy, well-drained soil may be unable to provide suitable bodies of water to sustain large immature vector populations [20]. Other landscapes have been shown to be closely associated with specific species, such as the presence of *An. melas* larvae in mangroves [21-24]. Studies using remote-sensed data to model the vector population on the Bijagós would be useful to explain some of the variations in island densities.

The island of Nhago was an outlier with densities of 98.7 and 165.2 from indoor and outdoor LTs respectively, with PCR identifying the majority of the subsample analysed as *An. melas*. One of the largest and most productive larval bodies found during the survey was on Nhago, approximately 500 m outside of the village (larval site can be seen in Figure 1). *Anopheles melas* are able to sustain a population throughout the dry season by ovipositing and developing as larvae in brackish water with a higher salinity than most other species within the *An. gambiae* complex [4].

Anopheles melas had previously been identified as being important in transmission in November/December on the island of Bubaque [8]. Relatively little contemporary information on the behaviour of *An. melas* is available, and different feeding behaviours have been described. In Liberia and Senegal, *An. melas* has been documented as being highly anthropophilic, whereas in Nigeria it is reported to be more opportunistic and less anthropophilic than other *An. gambiae* s.l. species [23, 25, 26]. The presence of *An. melas* within households from this survey indicates potential human host-seeking. All CSP-positive *An. gambiae* s.l. analysed were *An. melas*. The presence of *An. melas* outdoors and proportion of outdoor CSP-positive *An. melas* indicate that the species may contribute

to residual transmission on the Archipelago, but future work is needed to better understand the *An. melas* population and its feeding-preferences. Malaria continues to be a problem across the islands, so understanding the roles of different vectors and how they might be targeted through interventions is important [27].

Species within *An. gambiae* s.l. have been described on mainland Guinea-Bissau, with studies identifying *An. gambiae* s.s. and *An. coluzzii* as the dominant malaria vectors [28-31]. A high level of hybridisation between *An. gambiae* s.s. and *An. coluzzii* has also been described on mainland Guinea-Bissau [32, 33]. Vicente et al. showed that there was introgressive hybridisation between the two species in coastal areas in mainland Guinea-Bissau [32]. Introgressive hybridisation is when hybrid speciation arises through the accumulation of genetic material from parental lineages in an admixed population [34, 35]. This results in a population with high genetic diversity, enabling a distinct, ecologically divergent population to establish which may be able to adapt to new or marginal niches. In Guinea-Bissau, a stable population of hybrids has been established, with hybridisation rates >20% being recorded for almost 20 years [8, 32, 33, 36, 37]. Overall, hybrids accounted for 7.0% of *An. gambiae* s.l. caught throughout our survey (ranging from 0-50% from indoor LTs and 0-21.7% from outdoor LTs), accounting for more of the *Anopheles* caught than either of the parent species, following the same trend as seen in coastal regions of the mainland [32, 33, 37]. A survey looking at a panel of genetic markers in *Anopheles* mosquitoes caught on the islands and mainland to assess for evidence of genetic isolation between the two populations was conducted in 2012 [36]. Despite the distance between the mainland and the islands being greater than known *An. gambiae* s.l. dispersal capabilities (>7 km with wind), it found no evidence of population isolation suggesting there is considerable gene flow [36, 38-40]. It is not surprising therefore, that vector populations on the islands are comparable to those seen in similar ecosystems on the mainland. From the specimens sampled, we saw no CSP-positive hybrids, therefore their contribution to malaria transmission from October to December is unknown.

Outdoor biting is a key driver of residual transmission [3]. ITN use is high in the Bijagós Archipelago, with 97% of participants surveyed indicating they slept under a net [41]. However, transmission persists regardless, indicating that the human population remains exposed to infective vectors. Our survey results, which found similar densities of host-seeking female *Anopheles* outdoors and female *Anopheles* trapped indoors, supports the hypothesis that outdoor biting plays a role in malaria transmission throughout the Archipelago. This is further reinforced by seven of the eight CSP-positive *Anopheles* mosquitoes being caught in outdoor LTs.

There are several limitations to the survey. Firstly, the use of different trapping techniques for indoor and outdoor LTs makes it difficult to draw conclusions about vector feeding behaviour. It is advised that the MB5 lure used for outdoor LTs is refrigerated prior to use [10]. Unfortunately, this was logistically infeasible in the Bijagós, therefore, lures were not refrigerated, which may have led to degradation in the lure quality over time. The MB5 lure also suffers from inherent limitations in this setting. Firstly, when deployed outdoors, it must compete with livestock, which routinely sleep amongst houses in Bijagós villages. Secondly, the blend of semiochemicals within the lure are standardised, which will likely result in specific mosquito species being more attracted to the lure than others, as different species, and even individual mosquitoes, prefer different odour blends [42]. The outdoor traps using the MB5 lure are therefore unlikely to catch a representative sample of the outdoor vector population. It is therefore recommended that in future surveys on the Bijagós Archipelago, a standardised methodology should be used for both indoor and outdoor trapping.

The terrain on the Bijagós made it difficult to have a systematic approach to larvae surveillance, and this was especially the case in thickly-forested areas. We were reliant on paths through the forest and local knowledge of potential larval sites. Some larval sites will have been missed, and it is, therefore, important to state that ours was not a comprehensive larval survey. More work needs to be done to better locate and characterize larval sites on the Bijagós. We also did not have the capacity to rear larvae to adulthood and identify specimens to species level, therefore, it is unknown whether we were locating larval sites associated with one species more frequently.

Conclusions

Despite ITN use being high in the Bijagós Archipelago, malaria transmission persists, indicating that the human population remains vulnerable to infective bites. It is, therefore, important to thoroughly understand the vector population on the islands to better inform future control strategies. Here, we present for the first time details of vector density, species composition and infectivity rate from across the islands. *An. gambiae* s.l. was found throughout the Archipelago. Density and species composition varied between islands, with *An. melas* identified as playing a key role in transmission. This is highlighted by its occurrence at high percentages in both indoor and outdoor LTs on most of the islands and also the presence of the infective sporozoite stage in eight of the specimens analysed. Although the trapping technique was different between indoor and outdoor LTs, the outdoor density relative to that of indoor LTs suggests that outdoor biting is likely to play a role in residual transmission of malaria on the Archipelago.

Abbreviations

ITN: insecticide-treated nets; LT: CDC Light trap; RFLP: restriction-fragment length polymorphism; CSP: circumsporozoite protein; ELISA: enzyme-linked immunosorbent assay; SR: sporozoite rate.

Declarations

Ethics approval and consent to participate.

The work presented was discussed and approved by ethics committees at the London School of Hygiene and Tropical Medicine (Ref No. 14431) and locally in Guinea-Bissau (Comité de Éticas na Saúde) Ref No. 076/CNES/INASA/2017.

Consent for publication

Not applicable.

Availability of data and materials

The dataset collected and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

AL and JGL conceived the study. EP, RTJ, HH, AR, JL and AL designed the study. EP, HH and ETS performed the field work. SC, MON and UA coordinated and performed the molecular analysis. EP analysed the data. EP wrote the manuscript. All authors provided comments and feedback on the final draft. All authors read and approved the final manuscript.

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Chapter 5. Validation of a method for dry preservation and rehydration of *An. gambiae* sensu lato for parity analysis to assess impact of vector control measures in the field.

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Student ID Number	1903438	**Title**	Mrs
First Name(s)	Elizabeth Anne		
Surname/Family Name	Pretorius		
Thesis Title	Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control in the Bijagós archipelago, Guinea-Bissau.		
Primary Supervisor	Dr Anna Last		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where was the work published?	Parasite & Vectors		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	With the input of co-authors, I designed the study, reared the mosquitoes, performed dissections and assessed all ovaries alongside RTJ. I conducted fieldwork and field dissections alongside team in the Bijagos. I analysed the data and interpreted it with guidance from JB. I wrote the manuscript and responded to reviewers feedback

SECTION E

Student Signature	[redacted]
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Validation of a method for dry preservation and rehydration of *An. gambiae* sensu lato for parity analysis to assess impact of vector control measures in the field.

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Abstract

Background: As the control of malaria remains heavily dependent on vector management, it is important to understand the impact of these interventions on mosquito populations. Age grading is a valuable tool for this, however logistical challenges in remote resource-poor areas make current methodologies difficult to incorporate into clinical trials and routine surveillance. Our aim was to validate a methodology that would be easily implemented in such settings. Using dried specimens instead of freshly-killed ones, we validated the commonly used ovarian tracheation technique for assessing population age structure.

Methods: Laboratory-reared *Anopheles coluzzii* mosquitoes with known parity-status were dry-preserved in silica gel for up to 12 weeks and rehydrated prior to parity assessment. Results were compared to parity results from freshly-killed mosquitoes from the same colony. Preserved, field-caught *An. gambiae* sensu lato. from Guinea-Bissau were assessed by three different assessors blinded to each other's scores. An overall index of agreement was calculated using all assessor-pairings inter-rater reliability (IRR). The impact of time preserved was investigated using a one-way ANOVA to look for differences in assessor agreement over three time periods.

Results: When dry-preserved and rehydrated, the parity status of 90% of insectary-reared *An. coluzzii* were correctly identified compared to 98% in freshly-killed mosquitoes. IRR of freshly-killed *An.*

coluzzii was highest (0.94). Results at all time points showed excellent strength of agreement between assessors. For field-caught *An. gambiae* s.l., the overall index of agreement between all three assessors was 0.86 (95% CIs 0.78-0.93), indicating an almost perfect agreement. There was no significant difference between assessor agreement between timeframes.

Conclusions: Dry preserving and rehydrating *Anopheles* mosquitoes to assess the efficacy of a control intervention provides an alternative to using freshly-killed mosquitoes in remote settings where it is logistically difficult to dissect fresh specimens. It also provides the flexibility required for parity assessment to be done on a larger scale over a greater area.

Keywords

Age grading, malaria, parity, vector control, mosquito, *Anopheles*, Guinea-Bissau.

Background

Even after half a century of successful campaigns and control interventions during the 'elimination era', there are still approximately 250 million malaria cases and 600 000 deaths annually, most of which are seen in children aged under 5 years old [1]. Control efforts remained largely dependent on vector control strategies, mainly through the widespread distribution of insecticide-treated nets (ITNs) and indoor residual spraying (IRS). However, whilst great strides have been made, progress has stalled in recent years [2-4]. As well as continued efficacy monitoring of existing measures, novel interventions to control mosquito populations are required to safeguard and continue the progress made thus far.

The overall aim of current vector control measures is to reduce the number of potentially infectious bites by targeting mosquitoes of blood-feeding age. This decreases both the likelihood of the extrinsic incubation period (EIP; the period between the parasite being taken up in the blood meal and developing to its infective sporozoite stage) being completed and the mosquito's ability to complete gonotrophic cycles, leading to fewer mosquitoes in the next generation [5]. The parity rate of a mosquito population is a key indicator in studies assessing the entomological impact of interventions [6]. It represents the average age of the mosquito population, assuming the population is stable i.e. recruitment and loss are similar [7]. Mosquitoes that have taken blood and laid eggs are parous mosquitoes, whereas those that have not laid eggs are nulliparous mosquitoes. If an intervention was successful in killing a proportion of mosquitoes of blood-feeding age, then the proportion of parous mosquitoes within that population would decrease [6]. Small reductions in parity can represent large drops in malaria transmission [8].

There are various techniques used to investigate the age of mosquitoes. Techniques such as chromatographic analysis of cuticular hydrocarbons, transcriptomic profiling, and mid-infrared (MIRS) and near-infrared (NIRS) spectroscopy, show promise. However, they are often expensive and logistically challenging to use in remote resource-poor settings [6, 9-12]. Morphological assessment of the ovaries has been most frequently used in vector control studies. There are multiple techniques to morphologically classify the parity of a mosquito. The Polovodova ovarian separation technique and ovarian oil injection can estimate the number of gonotrophic cycles completed by a mosquito [10, 11]. These techniques are technically difficult and require a high level of skill and expertise. The technique most frequently used in studies was first described in 1962, and is called the ovarian tracheation technique [13]. It is the most technically and logistically simple of the morphological methods, generating a binary parous/nulliparous outcome. It only requires a stereomicroscope, a compound microscope and dissection tools.

Whilst the ovarian tracheation technique is relatively simple to perform, there are challenges to its use in certain settings. The technique requires mosquitoes to be freshly-killed prior to dissection. Mosquitoes that have died one or more days before dissection, are either too brittle to dissect, or too decomposed to assess. The implications of this are that all specimens trapped overnight in field studies need to be dissected that same day. If a study involves trapping from multiple and distant sites at the same time, as might be required for an intervention trial with multiple clusters, this could only be achieved with multiple parity assessment teams. This has cost implications requiring trained personnel and equipment, and decentralises oversight, and requires high levels of quality control. By dry-preserving mosquito samples soon after they have been collected and rehydrating them later in a central laboratory, a study can achieve greater oversight and quality control of the procedure, whilst also reducing transport and equipment costs.

Preservation methods have been explored using laboratory-reared mosquitoes prior to dissection [14]. These include dissection of mosquitoes dried in silica gel, preserved in fixatives including formalin, ethanol, Bouin's and Carnoy's solutions, and frozen. Preserved mosquitoes were rehydrated prior to dissection, whilst frozen specimens were dissected after thawing without rehydration. All three techniques were feasible, but little detail was given on the effect of the length of preservation time on the accuracy of the parity scoring, and no detail was provided on the accuracy in the context of field-caught mosquitoes. In this study we use insectary-reared mosquitoes, in addition to field-caught mosquitoes collected in the Bijagós Archipelago, Guinea-Bissau, to validate the dry-preserving and rehydration method for parity analysis. We also investigate whether the length of time preserved affects the accuracy of parity assessment.

Methods

Validation of the desiccation and rehydration method for parity assessment was carried out in two stages: (1) with female *Anopheles coluzzii* reared at the London School of Hygiene & Tropical Medicine (LSHTM), and (2) with field-caught female *An. gambiae* sensu lato collected on the Bijagós. The *An. coluzzii* N'gousso strain is a laboratory strain colonized from field mosquitoes collected around Yaoundé, Cameroon in 2006 [15]. Confirmatory PCR was done at LSHTM to verify species [16].

Validation using laboratory-reared An. coluzzii mosquitoes

Mosquitoes

Anopheles coluzzii were maintained in a 12 h light:12 h dark photocycle at 27 ± 2°C with a relative humidity of 70 ± 10% at the insectaries at LSHTM. Mosquitoes were provided with a constant supply of 10% glucose.

Validation design

To evaluate the methodology, mosquitoes with known parity-status were prepared. Cages of parous mosquitos were generated by blood-feeding female mosquitoes aged 3-5 days. The mosquitoes were provided a blood meal on two consecutive days, then allowed to lay eggs once. Any females that did not take a blood meal were removed from the cages. Nulliparous cages contained female mosquitoes of the same age, but were not provided a blood meal. When mosquitoes were approximately 8-10 days old (after egg-laying in the parous cages), they were killed using ethyl acetate and dry-preserved in 15ml universal tubes containing silica gel beads and cotton wool.

To investigate whether the preservation period affects the accuracy of the parity assessment, mosquitoes were dry-preserved for 1, 2, 6, 9 and 12 weeks. The temperature at the time of preservation was 30 °C with a relative humidity of 62%. A subset of all cages was kept, killed using ethyl acetate, and immediately dissected, using the ovarian tracheation technique for comparison with those that were dry-preserved and rehydrated [13]. Over 100 mosquitoes were used at a 1:1 ratio for freshly-killed and dry-preserved mosquitoes at each time-point.

Rehydration and dissection of dry-preserved mosquitoes

Prior to rehydration, mosquitoes were randomly assigned to individual 1.5 ml microcentrifuge tubes by a third-party. Assessors were blind to parity status until after assessment was complete. Mosquitoes were rehydrated by soaking in 1ml of 20% liquid detergent solution for 20 min (Multipurpose detergent; Teepol Products, Orpington, Kent, UK). They were then transferred to distilled water for a further 20 min. Rehydration was performed in batches of 40. To dissect the mosquitoes, a specimen was placed onto a microscope slide and a drop of distilled water applied. Firstly, using two 28G needles, the head and thorax were removed. A lateral incision was then made

along the length of the abdomen (Figure 1). The abdomen was opened to expose the internal organs. The ovaries were identified and carefully isolated. The ovaries were moved to a clean drop of distilled water and allowed to dry in the same way as used in the ovarian tracheation method performed on freshly-killed mosquitoes. Once dry, ovaries were examined using a compound microscope at 40x to identify the presence or absence of skeins (Figure 2).

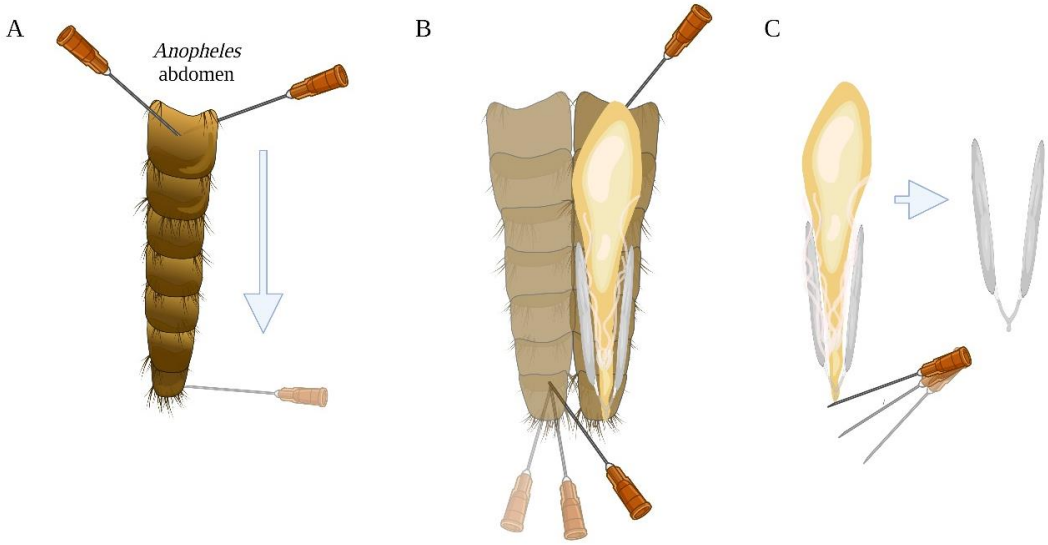

Figure 1. Technique used to dissect ovaries from dried and rehydrated mosquitoes using 28G needles. (A) lateral abdominal incision; (B) peeling cuticle back to reveal midgut, Malpighian tubules and reproductive organs; (C) identification and isolation of ovaries.

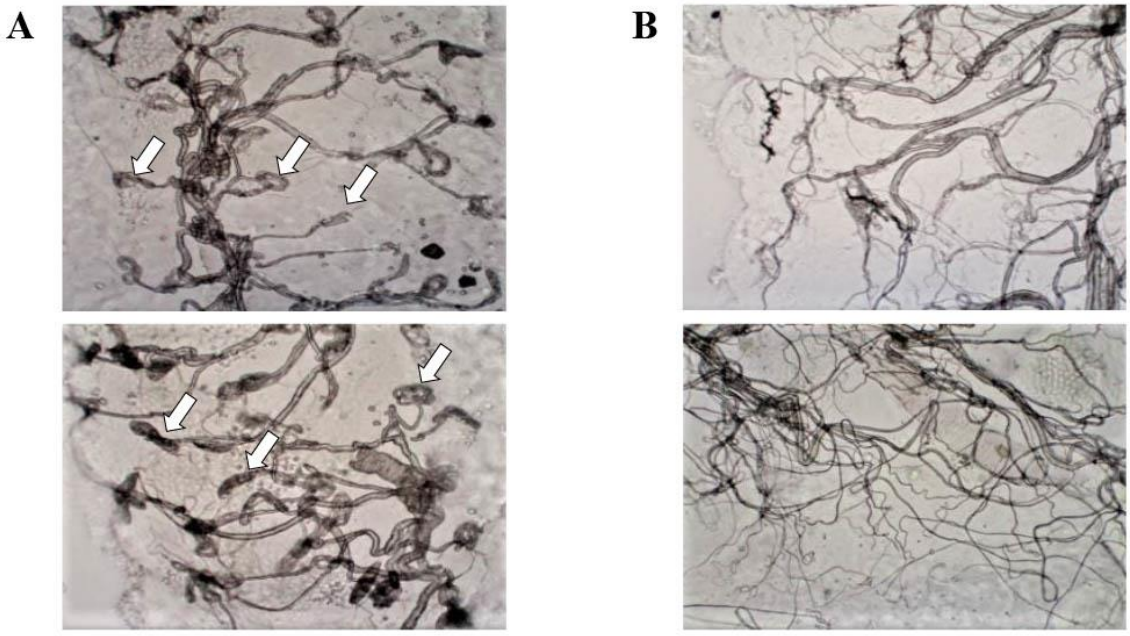

Figure 2. Dried and rehydrated ovaries from *An. coluzzii* showing tracheation. (A) Ovary tracheation from nulliparous ovary showing skeins (arrows); (B) Tracheation from a parous ovary showing unravelled tracheoles (photographs taken by EP).

All dissections were performed by one individual. The parity status of each pair of ovaries was then evaluated by two assessors. Once both assessors had determined parity status of all mosquitoes at each time point, true parity status was revealed. Those mosquitoes that could not be dissected due to them being too brittle, or an error in the technique of the individual performing the dissection, was classified as a 'loss to dissection'. Mosquitoes that had begun to decompose and were unable to be dissected were classified as a 'loss to decomposition'.

Validation using field-caught An. gambiae s.l. mosquitoes

Anopheles mosquitoes were caught in the Bijagós as part of ongoing studies by our group [17, 18]. Catches were made using indoor CDC miniature light traps (LTs; Model 512; John W. Hock Company, Gainesville, Florida, USA) using previously described methodology [19]. All *An. gambiae* s.l. caught were identified using previously described morphological keys [20], killed using acetone, and stored dry using the same method described above. The temperature at time of preservation was 29 ± 3°C with a relative humidity of 80 ± 11%. Mosquitoes were then transported to a central laboratory where 350 randomly selected mosquitoes from across the Archipelago were rehydrated and dissected. Ovaries were then scored by the first assessor. Photographs of the ovaries were taken using a microscope camera (Brunel Eyecam Plus; Brunel Microscopes Ltd; Chippenham, Wiltshire; UK), and then sent to two additional assessors for independent scoring by each. As the true parity status of field-caught mosquitoes was unknown, three assessors were used to validate the technique to give greater certainty in the methodology. All assessors were blind to each others' scores.

Confirmatory PCR, using restriction fragment length polymorphism (RFLP), was performed on a sub-set of 45 samples at the Medical Research Council Unit The Gambia at LSHTM [16]. DNA was extracted with the QIAcube extraction robot (QIAcube; QIAgen, Venlo, Netherlands) using the manufacturer protocol.

Statistical analysis

For insectary-reared mosquitoes, the results for each assessor from freshly-killed mosquitoes were compared to dry-preserved and rehydrated mosquitoes at each time point using a continuity adjusted Chi-square test. The inter-rater reliability (IRR), which indicates the extent to which both assessors agree, was calculated using Cohen's kappa statistic [21]. For strength of agreement for the kappa coefficient, Landis and Koch proposed the following as standards: ≤0=poor, .01-.20=slight, .21-.04=fair, .41-.60=moderate, .61-.80=substantial, and .81-1=almost perfect [22].

For field-caught mosquitoes from Guinea-Bissau, kappa-statistics was calculated for all assessor pairs. An overall index of agreement was then calculated using the arithmetic mean of all-pair kappa statistics. To assess the impact of time spent desiccated (i.e. the dry-preservation period between

mosquitoes being sacrificed and rehydrated) on assessor agreement, results were split into three timeframes; (1) 16-70 days; (2) 71-90 days; and (3) 91-110 days. An overall index of agreement was calculated using the method described above for each timeframe. A one-way ANOVA was then carried out to investigate the difference between the index of agreement at each time. All statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Validation using insectary-reared An. coluzzii mosquitoes

The process of desiccating and rehydrating mosquitoes can result in some damage or decomposition that prevents them from being scored for parity status. The highest loss to dissection or decomposition was after one week of preservation, prior to rehydration, with a 17% loss (Table 1). By comparison only 2% of freshly-killed mosquitoes could not be scored.

Table 1. Loss to dissection or decomposition for lab-reared mosquitoes assessed at LSHTM.

	Time preserved (weeks)	Total randomised	Total successfully dissected and assessed	Loss to dissection (%)	Loss to decomposition (%)
Freshly-killed	-	138	136	2 (1.4)	0 (0.0)
Dry-preserved and rehydrated	1	132	110	5 (4.5)	17 (12.9)
	2	133	128	2 (1.5)	3 (2.3)
	6	110	107	3 (2.7)	0 (0.0)
	9	116	115	1 (0.9)	0 (0.0)
	12	113	101	10 (8.8)	2 (1.8)

Following unblinding of the parity status of the insectary-reared mosquitoes, the IRR was calculated to assess the level of agreement between assessors' scoring. Comparisons between the accuracy of scoring of mosquitoes that were freshly-killed, and those that had been dry-preserved for all time points up to 12 weeks was also estimated. Overall, the proportion of freshly-killed mosquitoes that were correctly scored was 0.98 (0.98 parous and 0.97 nulliparous) when averaged over the two assessors. The proportion that were correctly scored after dry-preservation and rehydration was lower, at 0.90 (0.90 parous and 0.90 nulliparous).

Whilst the ability to accurately determine parity status was reduced in the desiccated samples, there was no clear trend of reduced accuracy as time of preservation increased. For assessor 1, there were significant differences in the accuracy of parity scoring between mosquitoes that were freshly-killed, and those that were dry-preserved and rehydrated at three time points: 1-week, 2-weeks and 12-weeks (Table 2). For assessor 2, there was one significant difference at the 6-week time point. The IRR was the highest, at 0.94, in freshly-killed mosquitoes, meaning that when the mosquitoes were freshly-killed it was more likely that both assessors scored the same way.

Table 2. Results from validation of methodology carried out on insectary-reared *Anopheles coluzzii* mosquitoes at LSHTM.

	Time preserved (weeks)	Assessor 1						Assessor 2						IRR[b]
		Correctly identified as parous		Continuity Ad. Chi-square for proportion correct parous[a]	Correctly identified as nulliparous		Continuity Ad. Chi-square for proportion correct nulliparous[a]	Correctly identified as parous		Continuity Ad. Chi-square for proportion correct parous[a]	Correctly identified as nulliparous		Continuity Ad. Chi-square for proportion correct nulliparous[a]	
		n/N (Prop)	(95% CI)		n/N (Prop)	(95% CI)		n/N (Prop)	(95% CI)		n/N (Prop)	(95% CI)		
Freshly-killed	-	65/66 (0.98)	(0.95-1.00)	-	68/70 (0.97)	(0.93-1.00)	-	65/66 (0.98)	(0.92-1.00)	-	68/70 (0.97)	(0.93-1.00)	-	0.94
Dry-preserved and rehydrated	1	43/54 (0.80)	(0.69-0.90)	$\chi2$=9.7, P=0.002*	44/45 (0.98)	(0.93-1.00)	$\chi2$=0.0, P=1.000	60/65 (0.92)	(0.86-0.99)	$\chi2$=1.6, P=0.203	43/45 (0.96)	(0.89-1.00)	$\chi2$=0.0, P=1.000	0.83
	2	48/58 (0.83)	(0.73-0.92)	$\chi2$=7.6, P=0.006*	48/51 (0.94)	(0.88-1.00)	$\chi2$=0.1, P=0.716	70/76 (0.92)	(0.86-0.98)	$\chi2$=1.8, P=0.173	45/52 (0.87)	(0.77-0.96)	$\chi2$=3.5, P=0.062	0.76
	6	53/59 (0.90)	(0.82-0.97)	$\chi2$=2.9, P=0.087	42/49 (0.86)	(0.76-0.95)	$\chi2$=3.6, P=0.058	55/58 (0.95)	(0.89-1.00)	$\chi2$=0.4, P=0.522	40/49 (0.82)	(0.71-0.92)	$\chi2$=6.5, P=0.011*	0.79
	9	60/67 (0.90)	(0.82-0.97)	$\chi2$=3.2, P=0.072	46/48 (0.96)	(0.90-1.00)	$\chi2$=0.9, P=0.362	62/66 (0.94)	(0.88-1.00)	$\chi2$=0.8, P=0.362	41/46 (0.89)	(0.80-0.98)	$\chi2$=3.2, P=0.072	0.87
	12	49/53 (0.92)	(0.82-0.98)	$\chi2$=0.4, P=0.242	38/48 (0.79)	(0.65-0.89)	$\chi2$=8.2, P=0.004*	50/52 (0.96)	(0.91-1.00)	$\chi2$=0.04, P=0.833	45/48 (0.94)	(0.87-1.00)	$\chi2$=1.2, P=0.665	0.77

[a] Degree of freedom=1 in all continuity adjusted chi-square tests

[b] IRR calculated using Cohen's Kappa statistic [21].

*Proportion of parity status correctly identified at time point significantly different from freshly-killed mosquitoes.

Validation using field-caught An. gambiae s.l. mosquitoes

Field-caught mosquitoes were scored for parity by three assessors. Out of 357 mosquitoes analysed, 324 were scored by all three assessors. This has been broken down into the three timeframes in Table 3. Since it is not possible to know the true parity status of the field-caught specimens, the ability to score them correctly was determined using their degree of agreement. The index of agreement between all three assessors was 0.83 (95% CIs 0.74-0.92), indicating an almost perfect strength of agreement between the three assessors, who were blind to each other's scores. There was no significant difference between the index of agreement between the three timeframes ($F(2,6) = 0.15$, $p=0.866$).

Table 3. Total number of mosquitoes dissected and identified by all three assessors. Index of agreement calculated by arithmetic mean of all-pair kappa statistics

Time desiccated (days)	Total dissected	Total assessed by all three assessors	Total unable to be identified by one or more assessor (%)	Index of agreement (95% CIs)
16-70	69	63	6 (9.5)	0.82 (0.78-0.87)
71-90	141	127	14 (9.9)	0.84 (0.79-0.89)
91-110	140	134	6 (4.3)	0.82 (0.55-1.00)

Anopheles gambiae sensu stricto (s.s.) (4.4%), *An. gambiae* s.s./*An. coluzzii* hybrids (11.1%) and *An. melas* (84.4%) were present within the sub-set of 45 samples identified to species level using PCR-RFLP (Table 4).

Table 4. Number of species within the *Anopheles gambiae* complex identified using RFLP-PCR.

***Anopheles gambiae* s.l. species**	**N (%)**
An. gambiae s.s.	2 (4.4)
An. gambiae s.s./*An. coluzzii* hybrid	5 (11.1)
An. melas	38 (84.4)

Discussion

This study demonstrates that the ovarian tracheation method can be performed on dry-preserved and rehydrated mosquitoes, and used in a remote setting with little infrastructure [6]. It builds on the work validating the methodology in both a laboratory and field setting [14]. Laboratory-reared *An. coluzzii*

mosquitoes reared and dry-preserved were able to be successfully rehydrated and scored after being preserved for up to 12 weeks. Similarly, field-caught *An. gambiae* s.l. from Guinea-Bissau were dry-preserved in the field for up to 16 weeks, then successfully rehydrated and scored. This technique would be beneficial in remote resource-poor settings, like the Bijagós, where transport is challenging. By centralising the parity analysis, greater oversight and quality control can be achieved in clinical trials or routine surveillance.

The 20% soap solution used during rehydration disrupts the phospholipid layer of cells, enabling water to permeate the cells [23]. Once this has taken place, specimens may be vulnerable to decomposition or disintegration [14]. This decomposition is most evident in the mosquito digestive tract, with the ovaries seeming more resistant. The high loss to decomposition seen in laboratory-reared mosquitoes rehydrated at the one week time point (12.9%) may be due to either poor mosquito handling during randomisation, or slow dissection speed. Mosquitoes were rehydrated in batches of 40. Therefore, at the one week time point, mosquitoes may have been in distilled water for a number of hours prior to dissection. This effect was reduced as the skill of the individual dissecting improve. As freshly-killed mosquitoes are not rehydrated, the risk of decomposition is low. It is challenging to estimate the maximum time mosquitoes can be rehydrating prior to decomposition, as the conditions at the time of preservation and rehydration will play a role. When the temperature and humidity is higher, faster decomposition may be expected, therefore rehydrating in smaller batches may be necessary. It is also crucial to ensure that the silica gel continues to properly preserve specimens, therefore using self-indicating silica gel is recommended. Self-indicating silica gel will also help in safe-guarding against decomposition should there be a handling error while preserving; for instance, a sample tube not being properly capped or there being too much moisture in the tube before samples are added.

Compared to freshly-killed mosquitoes, rehydrated specimens lack elasticity in their abdominal tissue making it harder to dissect. Results from the laboratory-reared mosquitoes showed a 0.9-8.8% loss to dissection in rehydrated mosquitoes compared to a 1.4% loss in freshly-killed mosquitoes. As time progressed, the dissection technique improved. This was evident by the decrease in the loss to dissection at the 2, 6 and 9 week time points. Loss to dissection at the 12 week time point may be attributed to specimens becoming more brittle after being preserved for longer. Results from field-caught mosquitoes showed a relatively stable loss to dissection at the 16-70 day and 71-90 day timeframe (9.5% and 9.9% respectively), however, that was halved to 4.28% in the 91–110 day timeframe. This may be due to the improved skill of the individual dissecting or conditions being more favourable when preserving or rehydrating. If using this methodology, a greater loss to dissection should be taken into consideration while planning. When planning a study, the experience of the

individual performing the dissection should be taken into consideration; if the individual is highly skilled, a 10-20% loss to dissection should be factored in.

With regards to the mosquitoes reared in the insectary, those that were incorrectly scored by Assessor 1 did not always correspond to those that were incorrectly scored by Assessor 2. For instance, when compared to freshly-killed mosquitoes, at the 2 week time point, Assessor 1 correctly assessed significantly fewer mosquitoes. This difference was not seen in Assessor 2's results at the same point. The divergence in the IRR at this time-point also illustrates this, indicating that while one assessor may incorrectly identify the parity status of a mosquito, the second assessor is likely to correctly identify the parity status of that same mosquito. In field-caught mosquitoes, the index of agreement was relatively stable, indicating an 'almost perfect' result throughout. However, the wider 95% CIs at the 91-110 day timeframe indicates a greater level of uncertainty in agreement between assessors. When designing a study and planning to dry-preserve specimens, a cut-off for the length of time specimens are allowed to be preserved prior to rehydration should be made. The conditions in which specimens are dry-preserved and rehydrated will vary depending on the setting, so trialling the methodology prior to large-scale implementation is important.

It is also important to trial the methodology, as, although damage to mosquitoes caught in LTs on the Bijagós was low, this will not be the case everywhere. Conducting pilot studies to investigate the impact of LTs on the local vector population, as well as the effect of climatic conditions on rehydrated specimens will help to ensure robust data is collected. As stated previously, rehydrated mosquitoes lack the elasticity of freshly-killed ones; specimens are more fragile, making dissections more challenging. The ovaries can often stick to the cuticle, making them difficult to see. To ensure an individual is able to successfully identify the ovaries, and gently remove them, thorough training and practice is necessary. It is recommended to have multiple trained assessors to ensure the best possible quality of results, and, wherever feasible, a third-party assessor should arbitrate in scoring specimens with differing parity assessments.

The technique of dry-preserving and rehydrating mosquitoes could be used for other established techniques. Ungureanu [14] successfully removed the salivary glands, identifying sporozoites in infective mosquitoes, and performed more complex morphological assessments of mosquito ovaries, such as Polodova's ovariole separation technique. However, little detail of these results was provided, except to point out that the dissections were mainly performed on recently dried specimens. The ovarian tracheation method gives a binary outcome, i.e. parous or nulliparous, and does not give information on the quantity of gonotrophic cycles completed by the mosquito. Validation of the ovariole separation technique on rehydrated mosquitoes is required. Future work is also needed to

investigate whether it is possible to perform other complex morphological identification, such as the ovarian oil injection, on dry-preserved and rehydrated specimens [10, 11].

Unlike the laboratory-reared mosquitoes, we are not certain of the actual parity status of the field-caught *An. gambiae* s.l., which leaves us heavily reliant on assessor IRR. Ideally, assays using mosquitoes with known-parity status at the study site would precede validation using field-caught mosquitoes. However, in the Bijagós it was not feasible to do any assessments on laboratory-reared mosquitoes due to lack of an available colony. This study also focuses on mosquitoes within *An. gambiae* s.l., and, therefore, further validation using other vector species is needed. A further limitation of the study is that all dissections were made by one individual, raising concerns of generalisability. However, since completing the validation work, multiple individuals have been successfully trained, and are able to remove the ovaries using this methodology.

Conclusions

Anopheles gambiae s.l. mosquitoes can be dry-preserved for up to 110 days and successfully rehydrated to allow parity assessment. Wherever possible, freshly-killed mosquitoes should still be used as a first option. However, this method may be used in remote settings where parity assessment of freshly-killed mosquitoes is not feasible. It may also provide a good alternative for large-scale concurrent surveillance. In such circumstances, this technique enables greater quality control and oversight over data collection.

Abbreviations

EIP: Extrinsic incubation period; IRR: Inter-rater reliability; IRS: Indoor residual spraying; ITN: insecticide-treated bednet; LSHTM: London School of Hygiene and Tropical Medicine; LT: CDC miniature light trap; MIRS: mid-infrared spectroscopy; NIRS: near-infrared spectroscopy.

Declarations

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Availability of data and materials

All data presented in this manuscript is available from the corresponding author upon reasonable request.

Authors' contributions

EP, RTJ, MK, AL and JGL conceived the study. AL acquired funding. EP, RTJ and JL designed the study. EP, ETS, HH and AR supported work in Guinea-Bissau and collection of mosquitoes in the Bijagós. EP, FB, AC and RTJ performed mosquito dissections. EP, RTJ and MK assessed mosquito ovaries. SC and MN coordinated and carried out the molecular analysis at the MRC Unit, The Gambia at LSHTM. EP and JB analysed the data. EP wrote the manuscript. All authors provided feedback on the final draft. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study is nested within a larger cluster-randomised placebo-controlled trial investigating the use of adjunctive ivermectin mass drug administration for malaria control on the Bijagós Archipelago of Guinea-Bissau (Alias: MATAMAL). Ethics approval has been obtained from LSHTM Research Ethics Committee (UK) (19156) and Comité Nacional de Ética em Pesquisa na Saúde (CNES; Guinea-Bissau) (084/CNES/INASA/2020).

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Chapter 6. Impact of adjunctive ivermectin mass drug administration for malaria control on vectors on the Bijagós Archipelago, Guinea-Bissau, a region with high bednet coverage: a cluster-randomised placebo-controlled trial

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SECTION A – Student Details

Student ID Number	1903438	**Title**	**Mrs**
First Name(s)	Elizabeth Anne		
Surname/Family Name	Pretorius		
Thesis Title	Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control in the Bijagós archipelago, Guinea-Bissau.		
Primary Supervisor	Dr Anna Last		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Nature Scientific Reports
Please list the paper's authors in the intended authorship order:	Elizabeth Pretorius, Robert T. Jones, Harry Hutchins, Eunice Teixeira Da Silva, Sainey Ceesay, Fatusha Barri, Bubacarr Darboe, Laura Reis Oliveira, Hristina Vasileva, Harouna M. Soumare, Mamadou Ousmane Ndiath, Umberto d'Alessandro, Amabelia Rodrigues, John Bradley, James G. Logan, Anna Last
Stage of publication	Choose an item. Yet to be submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	With the input from co-authors, I designed the study, conducted the training for the fieldwork and supervised the team in the Bijagos during data collections. I dissected and assessed mosquito ovaries for parity assessment. With guidance from JB, I cleaned and analysed the data. I wrote the manuscript.

SECTION E

Student Signature	[redacted]
Date	11-03-2024

Supervisor Signature	Anna Last [redacted]
Date	18-03-2024

Impact of adjunctive ivermectin mass drug administration for malaria control on vectors on the Bijagós Archipelago, Guinea-Bissau, a region with high bednet coverage: a cluster-randomised placebo-controlled trial

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Abstract

Ivermectin (IVM) mass drug administration (MDA) is being assessed as a complementary malaria control tool in seasonal settings of low transmission. In 2021 and 2022, a cluster-randomised placebo-controlled trial investigating IVM in combination with the antimalarial dihydroartemisinin piperaquine (DP) MDA was conducted in 24 clusters (12 intervention; 12 control) on the Bijagós Archipelago, Guinea-Bissau. Intervention clusters received DP at the standard dose and 300 μg/kg regimen of IVM each day for three consecutive days. Control cluster received DP and IVM-placebo. MDA was given each month for three consecutive months throughout the transmission season. *Anopheles* density, parity rate, species composition, sporozoite rate and entomological inoculation rate were investigated from mosquito collections using indoor CDC light traps conducted 7-14 days after completion of MDA and during a peak-transmission survey. There was no difference in parity rates between trial arms at any time point (primary entomological endpoint; 2022 post-MDA 3: unadjusted difference: 4.55%, 95%CI: -5.43-14.53, p=0.35). Inter-rater reliability between parity assessors was almost perfect at all

time points. Nor was there any difference in vector density between trial arms at any time point (2022 post-MDA 3: unadjusted difference: 0.33, 95%CI: 0.38-15.00, p=0.33). There was no significant difference in the proportion of *An. gambiae* sensu lato, sporozoite rate or entomological inoculation rate between trial arms at any time point. In this setting, IVM had no impact on the vector population, thereby suggesting that it is not an effective malaria control tool in this setting.

Background

Malaria continues to pose a major risk to life for billions of people, killing over 600,000 people a year [1]. Whilst great strides have been made in controlling malaria, progress has stalled since 2015 [1, 2]. Insecticide-based mosquito control programmes have been the backbone of malaria control thus far. Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have been successful in reducing case incidence and mortality throughout affected regions; however, transmission persists. This is due to a variety of different factors, including the funding gap between what is needed and what is required resulting in inadequate coverage of interventions, an increase in resistance in both the parasite, to artemisinin-combination therapy (ACT), and vector resistance, to insecticide, and residual transmission [1, 3-7]. Residual malaria transmission refers to transmission that persists regardless of full universal coverage of ITNs and/or IRS using active ingredients that are effective against fully-susceptible local vector populations [8]. It is attributed to the presence of vector species which shown more exophilic or zoophilic tendencies, or are less specific about blood-meal sources [3, 9-12]. Even in areas with high coverage of mainstay vector control measures, these behavioural adaptations allows vectors to evade insecticide-treated surfaces, resulting in sustained malaria transmission. To address these challenges, novel interventions are required.

Ivermectin (IVM) is a broad-spectrum anti-parasitic agent, within the avermectin class, that blocks the glutamate-gated chlorine channels between the nerve and muscle cells within invertebrates, leading to paralysis and death [13]. IVM has been used to control lymphatic filariasis (LF) and onchocerciasis, through mass drug administrations (MDAs) for decades [13]. IVM has since been repurposed as an endectocide as it has been shown to cause mortality in malaria vectors that have fed on the blood of humans or livestock treated with a high enough dose of IVM [14-18]. These studies suggest that IVM MDA could be used as a complementary control tool to help reduce residual transmission by targeting vectors that exhibit behaviours which enable transmission to persist [19]. In several sites in West Africa, a reduction in *Anopheles gambiae* sensu lato (s.l.) density, parity and sporozoite rate (SR) was observed after completion of IVM MDA using a 150 µg/kg dose, during programmatic IVM MDA for LF and onchocerciasis control [20]. The impact of IVM MDA on vector density, parity and SR lasted for two weeks before returning to pre-MDA conditions. The IVERMAL study showed that a higher dose of 300 µg/kg of IVM administered each day for three consecutive days (3 x 300 µg/kg regimen) in

combination with dihydroartemisinin-piperaquine (DP) to individuals presenting with uncomplicated clinical malaria was safe, well-tolerated and had a mosquitocidal effect on *Anopheles gambiae* sensu stricto (s.s.) fed on blood taken from participants 28 days post-treatment [14, 15]. It is important to note that there was no difference between the 3 x 300 µg/kg regimen and a 600 µg/kg dose regimen for three consecutive days. IVM has a half-life of 1-3 days, with metabolites, shown to increase the mosquitocidal effects of IVM, persisting for up to three days [13, 21].

To better control residual malaria transmission, IVM MDA may be administered with an artemisinin-combination therapy (ACT) antimalarial MDA, targeting both vector and parasite. Studies assessing the safety, efficacy and appropriate dosing regimen for an ACT-IVM combination MDA have been conducted and indicate that it is a safe and effective tool to control malaria [14-16]. There have since been two published clinical trials assessing adjunctive IVM MDA on malaria control [22, 23]. The RIMDAMAL trial, in Burkina Faso, investigated the impact of a single dose of IVM at 150-200 µg/kg in addition to 400 mg of the anti-parasitic agent, albendazole on clinical malaria episodes in children. Following discussion surrounding statistical methods for adjusting for clustering, results from this trial are contested [24, 25]. The MASSIV trial in The Gambia assessed the impact of two years of MDA with the antimalarial dihydroartemisinin-piperaquine (DP) in combination with IVM at the 3 x 300 µg/kg regimen against standard programmatic malaria control measures, including seasonal malaria chemoprevention (SMC), deployment of ITN and IRS, intermittent preventative treatment during pregnancy (IPTp) and case diagnosis and treatment with the ACT artemether-lumefantrine in their control arm [23, 26]. Though MASSIV demonstrated a reduction in malaria prevalence in the intervention arm in the second year of the trial, due to the design of the trial, it was not possible to establish whether the impact was due to DP MDA alone or the DP-IVM combination MDA. Again, following the second year of the intervention, a significant reduction in vector density were observed in the intervention arm, however there was no difference between arms in the primary outcome measure of vector parity [27]. No difference was seen following year one.

A quadruple-blind (participant, intervention provider, investigator and analyst) cluster-randomised placebo-controlled trial (MATAMAL) was conducted on the Bijagós Archipelago, 50 km off the coast of Guinea-Bissau. The MATAMAL trial protocol and primary outcomes are reported elsewhere (Hutchins et al. 2024, unpublished, [28]). Here, we assess the impact of adjunctive IVM MDA on the malaria vectors of the Bijagós and present the complete entomological outcomes from the MATAMAL trial.

Methods

Study site and trial procedures

The Archipelago consists of 19 permanently inhabited islands with a total population of around 25,000 [29]. Malaria is endemic and transmission is highly seasonal on the Bijagós. Programmatic malaria control measures consist of an ITN distribution every three years, IPTp, symptomatic case management with ACT and deployment of SMC in four regions on mainland Guinea-Bissau. There is no IRS programme in Guinea-Bissau [30]. ITN coverage (92%) and reported use (86%) is high [30, 31]. Despite maximal deployment of key interventions, malaria transmission is sustained. *Anopheles gambiae* s.s. has been suggested to be the primary malaria vector during the transmission season [32]. While logistically challenging, the Archipelago's geographic isolation and relative lack of between-island movement by the human and mosquito populations makes it a suitable setting for a cluster-randomised trial testing novel vector control tools [33].

The MATAMAL trial consisted of 24 clusters across 18 permanently inhabited islands on the Bijagós. All clusters received monthly rounds of MDA with a full dose of DP (Alfasigma, Bologna, Italy) following manufacturer's guidelines. Intervention clusters received a dose of IVM at the 3 x 300 µg/kg regimen. Control clusters received an IVM-placebo using the same methods as in the intervention clusters [28]. Clusters were sampled using a fried-egg design, will all villages within clusters receiving interventions [34]. To prevent cross-contamination, yolks were at least 2km apart and only villages within yolks were sampled [35]. Clusters were randomised into one of two trial arms (intervention and control) in a 1:1 ratio (Figure 1).

Monthly rounds of MDA were completed in July, August and September (MDA 1, 2 and 3 respectively) in 2021 and 2022 with the intention of reducing the burden of malaria throughout the entire transmission season. A peak-transmission survey (PTS) was conducted one month after the completion of the third MDA round in October/November in 2021 (PTS21) and 2022 (PTS22).

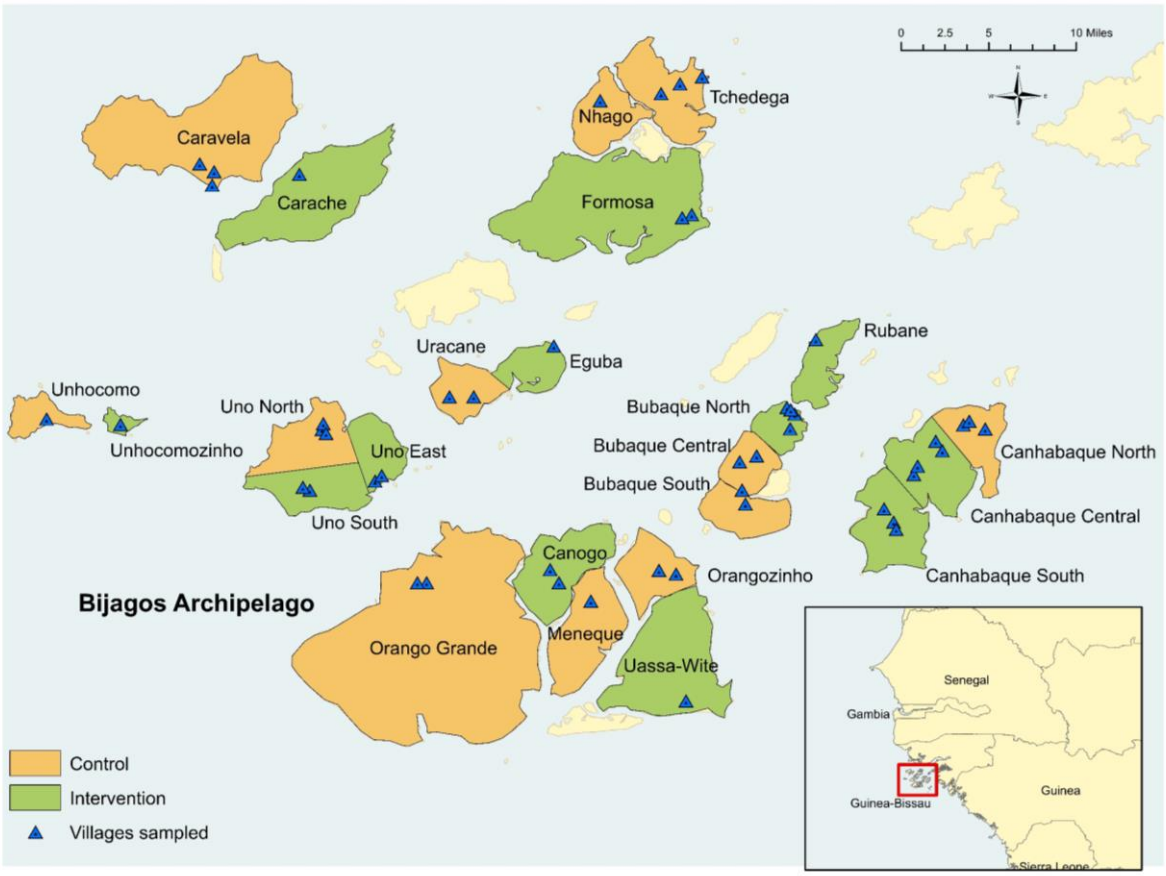

Figure 1. Map of the Bijagós Archipelago, Guinea-Bissau. MATAMAL intervention clusters (green); control clusters (orange); villages sampled are represented with blue triangles.

Mosquito collections

To collect mosquitoes, indoor CDC light traps (LTs; John W. Hock, Gainesville, Florida, USA) were used in one village selected at random from each cluster. LTs were deployed from 19h00 to 07h00, following previously described methodology, for three consecutive nights in each randomly selected house [36]. In 2021, ten houses from selected villages in 18 clusters (nine intervention and nine control clusters) were sampled 7-14 days after completion of every MDA round. Trapping also occurred in ten households from each of the 24 clusters during the PTS 2021 (PTS21). Following completion of entomological sampling in 2021, an assessment was made on the quantity of *An. gambiae* s.l. caught per cluster. As a result of this assessment, trapping was increased to 15 households per cluster in 2022 to ensure the sample size required to detect a difference in parity rate between trial arms was met (please see below for further details). Following preliminary assessment of the variance of vector density and parity between clusters in 2021, trapping was increased to include all 24 clusters in 2022. Due to the logistical implications of increasing the number of clusters sampled, trapping only occurred 7-14 days after completion of the third MDA round and during the PTS of 2022 (PTS22). Village and household selection was performed afresh for each MDA round and PTS sampled.

Following each nights' collection, mosquitoes we killed using acetone and dry-preserved in self-indicating silica gel. During the MDA sample collection, all mosquitoes from each household were placed into one tube and transported to the project laboratory on Bubaque island, the most heavily populated island in the Bijagós. Here they were morphologically identified; mosquitoes within the *Anopheles* genus were separated into new tubes for further analysis (described below) [37]. During PTS sample collection, morphological identification was completed whilst on the islands.

Entomological parameters and statistical analysis

Using collections from the LTs, *Anopheles* density, parity rate, species composition and SR was determined. The entomological inoculation rate (EIR), which estimates the number of infectious bites per person per time unit, was calculated using the formula 1.605 x (number of CSP-positive *Anopheles*/number of *Anopheles* tested) x (number of *Anopheles* collected from LTs/ number of trapping nights) x 180 (30 days per month multiplied by 6 months per season per year) [38].

The parity rate was estimated using 200 mosquitoes per cluster from collections following all MDA rounds in 18 clusters in 2021 and the final MDA round in 24 clusters in 2022. No parity assessment was conducted on mosquitoes collected during PTS21 or PTS22. This sample size estimate assumed that with the addition of IVM MDA in the intervention arm, there would be a difference in mosquito parity rate of 80% in the control arm and 50% in the intervention arm, giving 83% power to detect a difference (alpha=0.05, assuming a coefficient of variation of 0.3).

To carry out the parity assessments, dried mosquitoes were rehydrated prior to dissection using previously described methodology [39]. Mosquitoes were then dissected and assessed as parous (had laid eggs) or nulliparous (had not previously laid eggs) [40]. The parity rate was then calculated by dividing the number of parous mosquitoes by the total number of mosquitoes assessed. In 2021, the parity status of every one in ten mosquitoes was validated by an independent scorer. In 2022, 68% of mosquitoes assessed were scored by two scorers and were completed within two months of collection. The inter-rater reliability (IRR) was calculated for each time point using Cohen's Kappa statistic [41]. For strength of agreement for the kappa coefficient, Landis and Koch proposed the following as standards: ≤0=poor, .01-.20=slight, .21-.04=fair, .41-.60=moderate, .61-.80=substantial, and .81-1=almost perfect [42].

The mosquito density was calculated as the total number of *Anopheles* mosquitoes collected divided by the number of trapping nights (number of houses multiplied by number of nights trapped). A subsample of mosquitoes morphologically identified as being within the *An. gambiae* complex were sent to the Medical Research Council (MRC) Unit The Gambia at London School of Hygiene & Tropical Medicine (LSHTM) for molecular analysis. As *An. gambiae* s.s. is thought to be the primary malaria

vector, analysis focused on the difference in proportion of *An. gambiae* s.s. between arms [32]. From post-MDA collections, a subsample of 30 mosquitoes per cluster was identified by PCR using restriction fragment length polymorphism (RFLP), generating 89% power in 2021 (18 clusters) and 96% power in 2022 (24 clusters) to detect a difference of 50% *An. gambiae* s.s. in the control arm to 20% in the intervention arm [43]. The primary outcome for the trial was population-based qPCR prevalence of *P. falciparum* parasitaemia in all age groups, measured during the PTS22 [28]. To complement this endpoint, 200 mosquitoes per cluster were analysed from the PTS21 and PTS22, generating 99% power to detect the same difference (all species identification sample size calculations were made assuming coefficient of variation of 0.4).

To determine the SR, circumsporozoite (CSP) enzyme-linked immunosorbent assay (ELISA) was conducted on 200 mosquitoes/cluster after the last round of MDA and during the PTS each year [44]. This would generate 96% power if 2% of mosquitoes in the intervention arm and 5% of mosquitoes in the control arms were found to be CSP positive (alpha=0.05, assuming a coefficient of variation of 0.3).

The impact of IVM MDA on *Anopheles* density, parity rate, species composition, SR and EIR was assessed using unadjusted and adjusted t-tests on cluster-level summaries [34]. A T-test on cluster-level summaries is a robust and efficient method, that accounts for within-cluster variation and minimises the risk of overestimation of standard errors, which results in deceptively narrow confidence intervals and p-values that are too small. T-tests also enable adjustments to be made for contributing covariates, such as environmental variables, which, in this case, are associated with vector development. Adjustments were made for average temperature and relative humidity in 2021. In 2022, data on rainfall was collected, therefore adjustments included average rainfall.

Results

Trial eligibility, MDA coverage and the primary outcome are reported elsewhere (Hutchins et al. 2024, unpublished, [28]). The coverage of the eligible population (89.9% of total population) to receive a monthly single-dose of IVM/placebo ranged from 71.8-84.4%. The coverage of the eligible population to receive all three-doses monthly of IVM/placebo ranged from 58.0-77.1%. Importantly, in 2022, coverage exceeded the required 70% in accordance with models predicting intervention impact [28]. The distribution speed for all MDA rounds ranged from 20-26 days.

During the trial, mosquito sampling took place over 4,415 trapping nights in 1,506 households. Of the 158,617 mosquitoes caught, 71% were female mosquitoes in the *Anopheles* genus, 25% were female mosquitoes in the *Culex* genus and the remaining 4% was made up females in the *Aedes* genus and males from all three genera. All 113,069 *Anopheles* females caught were morphologically identified as being within the *An. gambiae* sensu lato (s.l.) [37].

Parity assessment was successfully performed on a total of 14,725 female *An. gambiae* s.l. from post-MDA collections from all three MDA rounds in 2021 and the final MDA round in 2022. This represented 17% (14,725/84,391) of *An. gambiae* s.l. females caught from post-MDA collections, with a similar proportion of mosquitoes being assessed in the control (20%; 7,565/38,138) and intervention (16%; 7,258/46,253) arms. An overall loss of 17% (2,979/17,802; 18% in control arm: 16% in intervention arm) to parity dissection was observed, with a greater loss in mosquitoes assessed from 2021 collections (18%; 2,240/12,174) compared to the 2022 collection (13%; 739/5,628). There was no significant difference observed in parity rates between study arms at any time point (Table 1). The IRR was almost perfect at all time points.

Table 1. The parity rate of *Anopheles gambiae* s.l. from post-MDA collections in the intervention and control arms in 2021 and 2022.

	Mean[a] parity rate, % (n/N)	Unadjusted t-test		Adjusted t-test		IRR[b]
		Diff (95%CI)	P-value	Diff (95%CI)	P-value	
2021						
MDA1						
Control	67.4 (1058/1551)					
Intervention	69.4 (1070/1621)	-0.93 (-12.80 – 10.95)	0.87	1.69 (-22.74 – 26.11)	0.88	0.83
MDA2						
Control	78.8 (1451/1846)					
Intervention	76.6 (1341/1763)	-2.19 (-13.05 – 8.68)	0.67	-2.68 (-32.68 – 27.31)	0.85	0.84
MDA3						
Control	74.1 (1265/1693)					
Intervention	62.4 (948/1469)	-11.77 (-23.85 – 0.30)	0.05	-14.26 (-40.39 – 11.87)	0.26	0.94
2022						
MDA3						
Control	67.8 (1679/2475)					
Intervention	72.3 (1740/2414)	4.55 (-5.43 – 14.53)	0.35	-1.32 (-14.77 – 12.12)	0.84	0.91

MDA mass drug administration; *PTS* peak-transmission survey; *Diff* difference; *CI* confidence interval; *IRR* inter-rater reliability

[a]Mean calculated using cluster-level summaries

[b]IRR calculated using Cohen's kappa statistic [41]

There was no difference in the density of *An. gambiae* s.l. between study arms at any time point (Table 2). In 2021, when all timepoints were sampled, the density of *An. gambiae* s.l. appears to peak in both trial arms after MDA 2 in August (Appendix V), consistent with seasonal expansion of vectors (Figure 2).

Table 2. *Anopheles gambiae* s.l. species composition of the two trial arms in 2021 and 2022.

	***An. gambiae* s.l. density**					***An. gambiae* s.l. species composition**								
	Mean[a] *An. gambiae* s.l. density (n/N)	Unadjusted t-test		Adjusted t-test		Total PCR-RFLP	*An. melas* n (%)	*An. coluzzii* n (%)	Hybrid[b] n (%)	*An. gambiae s.s.*				
		RR (95% CIs)	P-value	RR (95% CIs)	P-value					n (%)	Unadjusted t-test Diff (95%CI)	P-value	Adjusted t-test Diff (95%CI)	P-value
2021														
MDA1														
Control	11. 9 (3219/270)					246	193 (78.4)	1 (0.4)	32 (13.0)	20 (8.1)				
Intervention	21.5 (5069/267)	1.49 (0.63 – 3.52)	0.34	1.73 (0.69 – 4.32)	0.22	256	178 (69.5)	13 (5.1)	37 (14.4)	28 (10.9)	3.29 (-8.89 – 15.45)	0.57	1.96 (-7.33 – 11.25)	0.66
MDA2														
Control	48.2 (12468/262)					460	251 (54.6)	26 (5.6)	127 (27.6)	56 (12.2)				
Intervention	49.6 (13028/266)	0.95 (0.39 – 2.31)	0.91	0.90 (0.42 – 1.95)	0.79	456	225 (49.3)	46 (10.1)	112 (24.6)	73 (16.0)	5.32 (-5.87 – 16.51)	0.33	3.45 (-6.80 – 13.71)	0.49
MDA3														
Control	28.6 (7701/267)					340	171 (50.3)	35 (10.3)	39 (11.5)	95 (27.9)				
Intervention	15.4 (4171/270)	0.42 (0.11 – 1.63)	0.19	0.31 (0.07 – 1.38)	0.12	316	175 (55.4)	37 (11.7)	24 (7.6)	80 (25.3)	-2.58 (-20.78 – 15.62)	0.77	-8.34 (-25.97 – 9.28)	0.33
PTS														
Control	21.6 (7120/330)					1628	974 (59.8)	52 (3.2)	304 (18.7)	297 (18.2)				
Intervention	15.8 (5680/360)	0.79 (0.35 – 2.69)	0.96	0.89 (0.40 – 2.00)	0.77	1946	1202 (61.8)	44 (2.3)	413 (21.2)	286 (14.7)	0.88 (-12.53 – 14.30)	0.89	-0.14 (-13.02 – 12.74)	0.98
2022														
MDA3														
Control	28.1 (14749/525)					622	296 (47.6)	62 (10.0)	186 (29.9)	78 (12.5)				
Intervention	44.8 (23445/516)	1.46 (0.66 – 3.24)	0.33	1.29 (0.57 – 2.90)	0.53	874	375 (42.9)	96 (11.0)	251 (28.7)	152 (17.4)	2.18 (-2.93 – 7.29)	0.39	-0.66 (-7.08 – 5.76)	0.83
PTS														
Control	15.5 (8530/543)					1835	927 (50.5)	300 (16.3)	384 (20.9)	224 (13.3)				
Intervention	13.5 (7263/539)	0.87 (0.27 – 2.75)	0.81	0.46 (0.31 – 2.81)	0.89	2100	1121 (53.4)	380 (18.1)	381 (18.1)	218 (10.4)	-1.52 (-12.35 – 9.31)	0.77	-2.49 (-12.08 – 7.10)	0.59

MDA mass drug administration; *PTS* peak-transmission survey; *RR* rate ratio; *Diff* Difference; *CI* confidence intervals; *n* number of mosquitoes caught (density) or number of mosquitoes of species identified; *N* number of trapping nights.

[a]Mean density calculated using cluster-level summaries.

[b]*An. gambiae* s.s./ *An. coluzzii* hybrids.

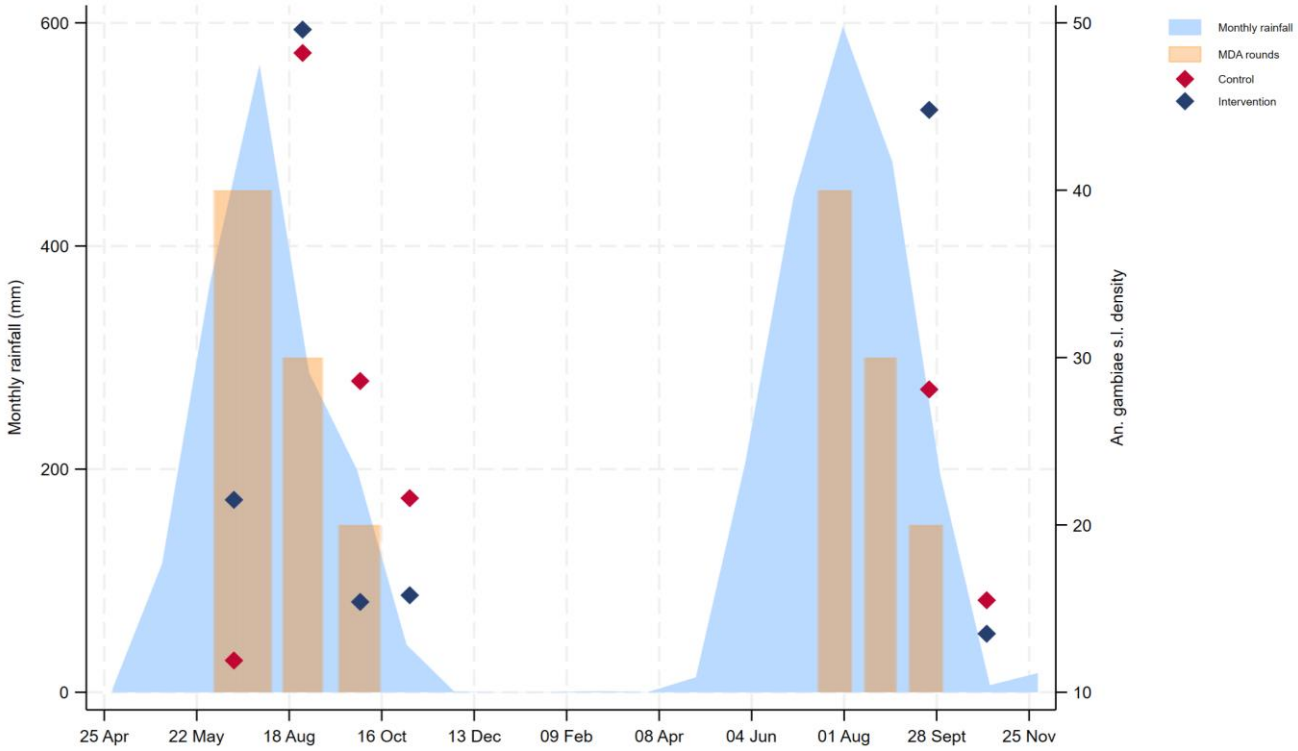

Figure 2. Monthly rainfall (mm), duration of MDA rounds and *An. gambiae* s.l. density for both 2021 and 2022. The width of bars (orange) represents the duration of each MDA round in both years. *An. gambiae* s.l. density for both the control (red) and intervention (blue) arm of each MDA round and PTS (2021: MDA 1, 2, 3 and PTS; 2022: MDA 3 and PTS) is denoted on the date mosquito trapping began.

Identification of species within the *An. gambiae* complex using PCR-RFLP was conducted on a total of 11,079 specimens (7% of all trapped *Anopheles*). There was no difference in the proportion of *An. gambiae* s.s. between trial arms at any time point (Table 2). *Anopheles melas* was the most common species in the study area. A high proportion of *An. gambiae* s.s./*An. coluzzii* hybrids was observed at all collection points, accounting for more than 10% of all identified species in all but one time point.

A total of 16,493 *An. gambiae* s.l. from post-MDA 3 and PTS were tested for the presence of CSP using ELISA over 2021 and 2022. There was no significant difference in SR between trial arms at any time point (Table 3). Of the 93 CSP-positive mosquitoes, species identification was performed on 54; 34 (63%) were *An. melas* (SR: 0.64%, 95%CI: 0.34 – 0.96), 8 (15%) were *An. gambiae*/*An. coluzzii* hybrids (SR: 0.16%, 95%CI: 0.02-0.29), 6 (11%) *An. gambiae* s.s. (SR: 0.54%, 95%CI: 0.01-1.06)) and 6 (11%) *An. coluzzii* (SR: 1.18%, 95%CI: 0.02-2.33)). There was no significant difference seen in EIR at any time point.

Table 3. *Anopheles gambiae* s.l density, SR and EIR from post-MDA3 and PTS collections in 2021 and 2022.

	Density	SR					EIR[a]		
	Mean[b]	Mean[b] SR (n/N)	Unadjusted t-test		Adjusted t-test		Mean[b] EIR (95% CIs)	Unadjusted t-test	
			Diff (95% CIs)	P-value	Diff (95% CIs)	P-value		Diff (95%CI)	P-value
2021									
MDA3									
Control	28.6	0.7 (14/1937)					87.7 (-44.9 – 220.3)		
Intervention	15.4	0.9 (19/1678)	0.23 (-0.58 – 1.03)	0.56	-0.25 (-2.12 – 1.62)	0.78	62.9 (6.7 – 119.1)	-24.75 (-157.17 – 107.66)	0.70
PTS									
Control	21.6	1.00 (18/1784)					36.8 (10.6 – 62.9)		
Intervention	15.8	0.5 (13/2064)	-0.47 (-0.99 – 0.05)	0.08	-0.47 (-1.49 – 0.55)	0.35	17.6 (3.1 – 32.0)	-19.19 (-8.14 – 46.52)	0.16
2022									
MDA3									
Control	28.1	0.2 (4/2458)					5.1 (-1.2 – 11.4)		
Intervention	44.8	>0.0 (1/2317)	-0.13 (-0.33 – 0.07)	0.20	-1.83 (-2.86 – 6.53)	0.43	6.5 (-7.9 – 21.0)	1.45 (-13.38 – 16.29)	0.84
PTS									
Control	15.5	1.8 (14/2014)					21.3 (0.5 – 42.1)		
Intervention	13.5	0.4 (10/2241)	-1.46 (-4.44 – 1.51)	0.32	-1.45 (-4.15 – 1.26)	0.28	32.4 (-16.9 – 81.6)	11.05 (-39.36 – 61.45)	0.65

MDA mass drug administration; *PTS* peak-transmission survey*; Diff* difference; *CI* confidence intervals; *SR* sporozoite rate; *EIR* entomological inoculation rate; *n* number of CSP positive specimens; *N* total number of specimens assessed by CSP-ELISA.

[a]EIR: calculated using the formula 1.605 x (number of CSP-positive *Anopheles*/number of *Anopheles* tested) x (number of *Anopheles* collected from LTs/ number of trapping nights) x 180 (30 days per month multiplied by 6 months per season per year) [38].

[b]Mean density, SR and EIR calculated from cluster-level summaries.

Discussion

The MATAMAL trial aimed to determine whether IVM in conjunction with DP MDA reduced the prevalence of *Plasmodium falciparum* parasitaemia in the Bijagós population when compared to DP MDA alone in an area with high coverage of ITNs. The intervention targeted both the parasite, through distribution of DP, and the vector, through the distribution of IVM. Here, we report there was no difference between trial arms in any of the entomological outcomes at any time point.

The lack of a difference in entomological outcomes between trial arms is likely due to a number of different factors. Firstly, while the coverage of the eligible population that received all three-doses monthly of IVM/placebo was high, there was still a proportion of the population that did not receive IVM. This, coupled with the speed of distribution and the ineligible residents, would likely result in a lower proportion of the population with sufficiently high levels of IVM in their blood to have an effect on the vector population. For the MDA to work, IVM treated individuals must also come into contact with vectors. On the Bijagós, the behaviours of both the human and vector population that drive residual transmission are still unknown. More research is needed on the outdoor biting and host-preferences of vectors to better contextualise our results.

As expected, seasonal variation in parity rates was observed, with a peak in the percentage of parous *Anopheles* females in August. There are natural fluctuations in vector parity rates throughout the season [46]. At the beginning of the season, as the rains increase, parity rates tend to be lower, driven by an influx of new emergence. As the transmission season progresses and rainfall continues, parity rates increase and then stabilise. This seasonal trend was seen in mosquitoes collected in both trial arms, with no difference seen in the intervention arm.

Alout et al. recorded a 20% and 22% reduction in parity rates in intervention villages in the first and second week respectively post-treatment with a single round of IVM at the standard 150 µg/kg dose in Burkina Faso, Liberia and Senegal [20]. Parity rates then reverted to baseline in the third week post-MDA. In Nigeria, following distribution of IVM MDA for LF and onchocerciasis control, parity rates from indoor mosquito collections in intervention villages was seen to reduce 2-3 days following MDA. Rates remained reduced at 13-14 days post-MDA, however, there was a visual rebound in parity rates between the two time points [47]. The RIMDAMAL trial, conducted parity analysis on just over 430 *An. gambiae* s.l. collected from indoor light traps (LTs) from the four control and four intervention villages, and found no difference between arms [22].

The MASSIV trial, a large Phase III cluster-randomised trial, designed to test the impact of IVM on malaria transmission, consisted of 32 clusters randomised at a 1:1 ratio into the control and intervention arms [23]. As stated previously, the trial tested the use of IVM MDA at 3 x 300 µg/kg dose

in addition to DP MDA in the intervention arm against standard control measures in the control arm. To collect mosquitoes, indoor trapping was conducted seven to 14 days following each MDA round in all intervention and eight control villages, and human landing catches (HLCs) were conducted in four intervention and four control villages [26, 27]. A subsample of *An. gambiae* s.l. caught from both indoor LTs and HLCs was assessed for parity status. No reduction in parity rates in the intervention arm was seen from either sampling method over the two years. The MASSIV and MATAMAL parity results align, indicating that when IVM is tested using a robust powerful study design, it has no impact on parity rates in these settings.

A potential explanation for the reduction in parity rates seen in the smaller studies described above may be the sampling period following completion of MDA [20, 47]. These studies sampled 2-3 days following MDA completion using a 150-200 µg/kg single dose of IVM. It would be expected that a 3 x 300 µg/kg IVM regimen would have a longer effect time on the vector population [14, 15]. Many in vitro and in vivo studies have shown a mosquitocidal effect in vectors fed on IVM-treated blood seven days post-treatment [48]. The killing effect is even more pronounced in mosquitoes fed on blood meals taken from treated individuals, with active metabolites shown to lengthen IVM's mosquito-lethal effect [18, 21, 49]. Smit et al. showed a killing effect up to 28-days post-treatment with a 3 x 300 µg/kg regimen on laboratory-reared *An. gambiae* s.s., however, since then, there has been a lack of pharmacokinetic and pharmacodynamic (PK/PD) data on IVM's mosquitocidal effect over more prolonged periods [14]. It is also worth noting that populations of wild mosquitoes may contain multiple species, be more genetically diverse and have varying levels of insecticide resistance, all of which may alter the level of success IVM MDA has to control vector populations.

The MASSIV trial conducted direct-membrane feeding assays to study the mosquitocidal effect following MDA [27]. They found that mortality among laboratory-reared *An. coluzzii* remained high when they had fed on blood taken from participants up to 21 days after MDA. While this showed that in principle IVM should continue to work up to 21 days following MDA, it is dependent on the proportion of the population with sufficient IVM concentrations in their blood at the time. The mosquitocidal effect on wild and laboratory-reared mosquitoes may also differ, accounting for the results presented here.

A reduction in *An. gambiae* s.l. density was found in intervention clusters within the MASSIV trial following the second year of intervention [27]. It is worth highlighting that this was seen in only one of the two years. MATAMAL sampled from more clusters and analysed each time point independently and found no reduction in *An. gambiae* s.l. density throughout the trial. It is unclear why MASSIV found a significant reduction in density when MATAMAL did not, however complex and heterogenous vector

feeding and human behaviour may explain some of the variability in entomological results presented here.

Anopheles melas and *An. gambiae* s.s./*An. coluzzii* hybrids were common throughout the transmission season in both years on the Archipelago. The rate of hybridisation on the islands is similar to vector populations studied in coastal regions on mainland Guinea-Bissau [11, 50, 51]. Little is known about the feeding behaviours and preferences of both *An. melas* and hybrids on the Bijagós. In some settings *An. melas* feeds opportunistically on humans, whereas in others it appears to be highly anthropophilic [52-54]. Should the *An. melas* population on the Bijagós preferentially feed on livestock or other non-human hosts, a large proportion of the vector population could evade the intervention. This may also be the case in future studies in areas where the major vectors, such as *An. arabiensis*, exhibit more zoophilic, exophagic and exophilic tendencies. Future studies are required to better understand the feeding behaviours of *An. melas* and hybrids on the islands. Furthermore, no previous IVM susceptibility testing has been performed on *An. melas* or hybrids, and although there is no physiological reason to suggest that either species would not be susceptible to IVM, future work is needed to confirm this.

ITN use is high throughout the Bijagós [30, 31]. Vector feeding behaviours, in particular the time at which vectors are host-seeking, is important in determining the impact of IVM MDA. Should host-seeking largely be conducted late at night, when the human population are protected by ITNs, vectors may not come into contact with IVM-treated hosts. Currently little is known about the vector biting behaviour on the Bijagós, it is therefore important to conduct further studies to better characterise the drivers of residual transmission and contextualise the results from the trial.

MATAMAL found no significant difference in the SR or EIR between trial arms at any time point. Interestingly, even though no difference was seen in the SR and human biting rate (HBR) between arms, the MASSIV trial reported a significant reduction in the EIR from human landing catches (HLC) performed on four intervention and four control clusters [27]. The difference between trial outcomes here is likely due to human-vector interactions differing between sites, and variation in vector biting behaviour.

There are a number of limitations of this study. Parity assessments from these collections took 11 months to complete. The methodology published suggests that dissections should be done within three months of preservation in order to reduce the possibility of incorrectly scoring parity [39]. As the trial was quadruple-blinded, if a dissection bias occurred, for instance parous mosquitoes being more easily dissected than nulliparous mosquitoes, then one would expect the bias to be observed in both trial arms. Due to the logistic constraints, we were unable to perform membrane-feeding assays

during our trial. These assays may have given clarity on the impact of IVM on the local vector species, in particular *An. melas*, and should be an avenue for future research.

The 3 x 300 µg/kg IVM regimen did not show any impact on the vector population in this setting. These results are consistent with the primary and other secondary outcomes from the trial (Hutchins et al. 2024, unpublished). It is important to note that the coverage sustained throughout the MATAMAL trial is unlikely to be met in a programmatic setting, therefore, as no impact on the vector population was evident, IVM MDA would not be appropriate to incorporate into a national programme to control malaria in this setting. Further understanding of the reasons why IVM did not have an impact on the vector population will be important for the future consideration of ivermectin to reduce residual transmission following maximal deployment of control measures of proven benefit (ITN, ACT).

Declarations

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Author contributions

EP, RTJ, HH, HMS, SL, JB, JGL and AL designed the study. AL acquired funding. EP, RTJ, HH, ETS, AR and AL supported the work in Guinea-Bissau, in particular the delivery of MDA. EP, RTJ, FB, LRO performed mosquito dissections. SC, BD, HV and MON coordinated and carried out the molecular analysis at the MRC Unit The Gambia at LSHTM. EP and JB analysed the data. EP wrote the manuscript. All authors provided feedback on the final draft. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data availability statement

All data presented in this manuscript is available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethics approval for the MATAMAL trial was obtained from LSHTM Research Ethics Committee (UK) (19156) and the Comité Nacional de Ética em Pesquisa na Saúde (CNES; Guinea-Bissau) (084/CNES/INASA/2020).

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Chapter 7. Risk factors associated with house entry of malaria vectors in the Bijagós Archipelago, Guinea-Bissau

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Student ID Number	1903438	**Title**	Mrs
First Name(s)	Elizabeth Anne		
Surname/Family Name	Pretorius		
Thesis Title	Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control in the Bijagós archipelago, Guinea-Bissau.		
Primary Supervisor	Dr Anna Last		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, trained the team in the Bijagos and supervised data collection. With guidance from JB and LT, I cleaned and analysed the data. I wrote the manuscript

SECTION E

Student Signature	[redacted]
Date	11-03-2024

Supervisor Signature	Anna Last
Date	18-0302024

Risk factors associated with house entry of malaria vectors in the Bijagós Archipelago, Guinea-Bissau.

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Abstract

Improved housing has been associated with lower mosquito house entry, and could represent a sustainable approach to malaria control, working independently or adjunctively to insecticide-based interventions. In the present study, we characterised the built environment of the Bijagós Archipelago and identified risk factors associated with higher indoor vector density. Surveys were carried out across 18 of the inhabited islands during the malaria transmission seasons of 2021 and 2022. Mosquitoes were collected using indoor CDC light traps from 1,506 houses for three consecutive nights. Each household was asked to participate in a survey which collected data on education level, income, goods or land owned, number of occupants, bednet use, materials used for house construction, open or closed eaves, number of windows and presence of fan and/or light in trapping room. A sub-sample of *Anopheles gambiae* s.l. were identified to species level by PCR-RFLP. The study was nested within a large cluster-randomised placebo-controlled trial, and mosquito counts were compared using a negative binomial regression, adjusting for cluster. Risk factors associated with higher proportions of the various *An. gambiae* s.l. species was investigated using a logistic regression. A total of 158,617 mosquitoes were caught, 113,069 (71.3%) of which were *An. gambiae* s.l. Higher numbers of *An. gambiae* s.l. were found in houses with lower socioeconomic status, which were typically made of more traditional building materials and had open eaves (IRR: 1.35, 95% CI 1.08-1.68). Risk factors associated with higher proportions of *An. gambiae* s.l. varied between species. The study

demonstrated that open eaves were associated with a higher rate of *An. gambiae* s.l. house entry in the Bijagós Archipelago, therefore indicating that eave closure or eave-specific technologies could be an appropriate house improvement to help reduce the burden of malaria on the islands.

Introduction

Although there have been huge advances in malaria control over the past several decades, the disease continues to pose a major risk to life for billions of people globally [1]. Reductions in malaria can largely be attributed to insecticide-based interventions to control the mosquito vector population, most notably the large-scale deployment of insecticide-treated bednets (ITNs) and indoor residual spraying (IRS) across affected regions [1, 2]. However, since 2015, little progress has been made towards malaria elimination [1].

The current dependence on insecticide-based interventions to control malaria may potentially expose affected populations to future risk should interventions become less effective [3, 4]. Insecticide resistant phenotypes have been documented in vectors throughout much of sub-Saharan Africa, with alarming increases in the prevalence of pyrethroid and DDT resistance between 2005 and 2017 [5, 6]. Improved housing poses an opportunity to prevent disease transmission without the use of insecticides, relieving the selection pressure on vectors. In addition to the direct health benefits, improved housing gives the opportunity to work with other sectors outside of health, including water and sanitation, education, city planning and agriculture, to better meet long-term sustainable development goals [7, 8].

An estimated 79% of bites by major malaria vectors occur indoors when residents are in bed [9]. Mosquito density indoors is affected by multiple contributing factors, including proximity and abundance of larval breeding sites [10-12], indoor temperature and humidity [13], house design [10, 13-17], use of personal vector protection against mosquitoes [11], human cooking behaviour [11, 18], number of residents [19], and individuals' attractiveness to mosquitoes [20]. Factors affecting indoor vector density are geographically heterogenous and setting specific. Characterising the built environment and vector feeding behaviour is important to identify appropriate improvements to better build out vector-borne diseases.

The malaria-endemic Bijagós Archipelago is situated ~50 km off the coast of mainland Guinea-Bissau. It consists of 88 islands and islets, with a population of 25,000 on 19 permanently inhabited islands [21]. Malaria control in Guinea-Bissau is largely dependent on the widespread distribution of ITNs triennially, with high bednet coverage and adherence. A survey conducted on the main island of Bubaque found that 97% of participants reported sleeping under bednets, however, even with high bednet adherence, malaria persists [22]. In 2017 on Bubaque island, an overall malaria prevalence was

estimated at 5.8% by rapid diagnostic test and 16.9% by qPCR, indicating continued malaria transmission despite maximal reported use of interventions [23]. Moderate insecticide resistance to α-cypermethrin and deltamethrin has been reported in *Anopheles* vectors on Bubaque island [Moss et al., 2024, unpublished, 24]. Data is currently lacking from other islands, however, the presence of insecticide resistance on Bubaque and on the mainland indicates that future monitoring is required [25].

The ongoing transmission of malaria and the presence of potential insecticide resistance on the Bijagós highlights the need for supplementary interventions that are not reliant on current insecticides. A household survey was conducted in conjunction with mosquito collections to test the hypothesis that house structure and socioeconomic position may be associated with vector house entry. The findings from this study will inform future research and implementation of potential housing improvements that could be promoted in this setting to prevent and reduce malaria transmission.

Methods

The study was nested within a cluster-randomised placebo-controlled trial investigating the impact of adjunctive ivermectin mass drug administration in conjunction with distribution of the anti-malarial dihydroartemisinin-piperaquine for malaria control (alias: MATAMAL). The trial consisted of 24 clusters, randomised 1:1 to intervention and control arms [26].

Mosquito collections

Mosquito collections were conducted over the transmission seasons of 2021 and 2022 on the Bijagós Archipelago using a fried-egg sampling design [27]. Sampling villages was selected at random from within the 'yolk' of a cluster. Selection was carried out afresh for each time point. In 2021, collections took place in July, August and September in 18 villages across 18 clusters (one village per cluster) to evaluate the vector population throughout the season. An additional survey was conducted in November 2021, where all 24 clusters were sampled (again, one village per cluster). In 2022, for logistical purposes, the number of clusters sampled increased to one village in each of the 24 clusters across the Archipelago in the months of September and November.

To select villages for sample collection, villages within cluster 'yolks' were assigned a number and one was selected using the random number generator Random# (Random#, 2013 Nicholas Dean, iOS 12.0) (Figure 1). In 2021, ten households per village were sampled; in 2022, this was increased to 15 households per village to ensure that 200 *Anopheles* females were collected to satisfy the sample size required for the MATAMAL trial parity outcome. Villages were sampled multiple times in clusters with fewer villages. To select households, a village heads of households list was generated prior to

sampling. Using the Rand() function in Excel (Excel Version 2310, Microsoft, Redmond, USA), a random order was created; following that order households were approached, informed consent was taken and traps were set-up.

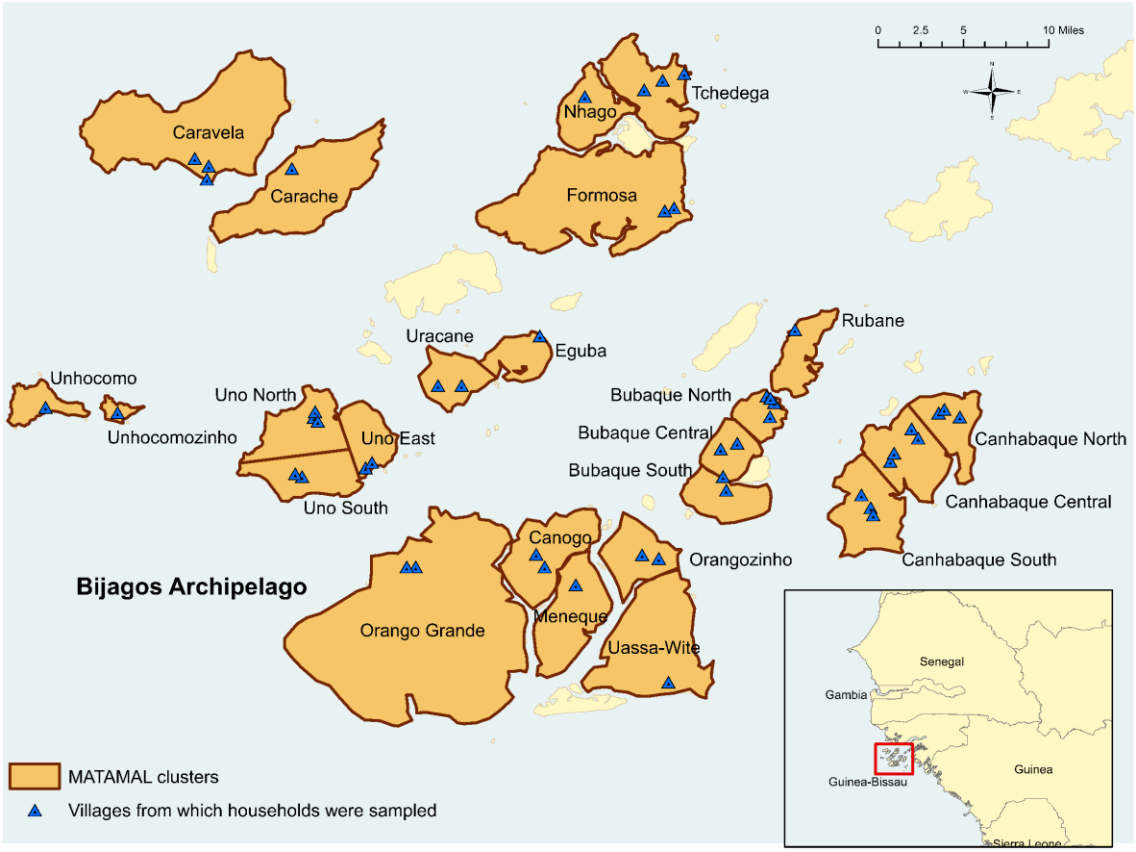

Figure 1. Map of the Bijagós Archipelago, illustrating different clusters (labelled and outlined with thick brown line) and villages from which households were selected (blue triangles).

Indoor US Center for Disease Control and Prevention (CDC) miniature light traps (LTs; CDC, Atlanta, GA, USA) were used for mosquito collections. LTs were hung 50 cm from the foot of an occupied bed, with the participant sleeping under a bednet, the light of the trap at a height of 1 m from the ground. Trapping was conducted in each household from 19h00 to 07h00 on three consecutive nights.

Following each night's collection, mosquitoes we killed using acetone and dry-preserved in self-indicating silica gel. Mosquitoes were morphologically identified to species complex level [28]. A subsample of *Anopheles gambiae* sensu lato (s.l.) were sent to the Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine for molecular species identification by PCR using restriction fragment length polymorphism (RFLP) [29].

Household Survey

A household survey was conducted on all households sampled, with heads of household being asked to answer survey questions. Variables to assess socioeconomic status (SES) of household were collected, these included: household head education level, marital status, spouse's education level, income, and goods, animals and land owned. Household demographic and behaviour variables collected were: number of occupants, age of occupants, ITN use, number of individuals per ITN within household and use of anti-mosquito measures. Built environment risk factors collected were: roof, floor and walling material, eave closure, quantity of windows in house, external door in trapping room and the presence of light and/or fans in trapping room (Figure 2). The full questionnaire is provided in Appendix IV.

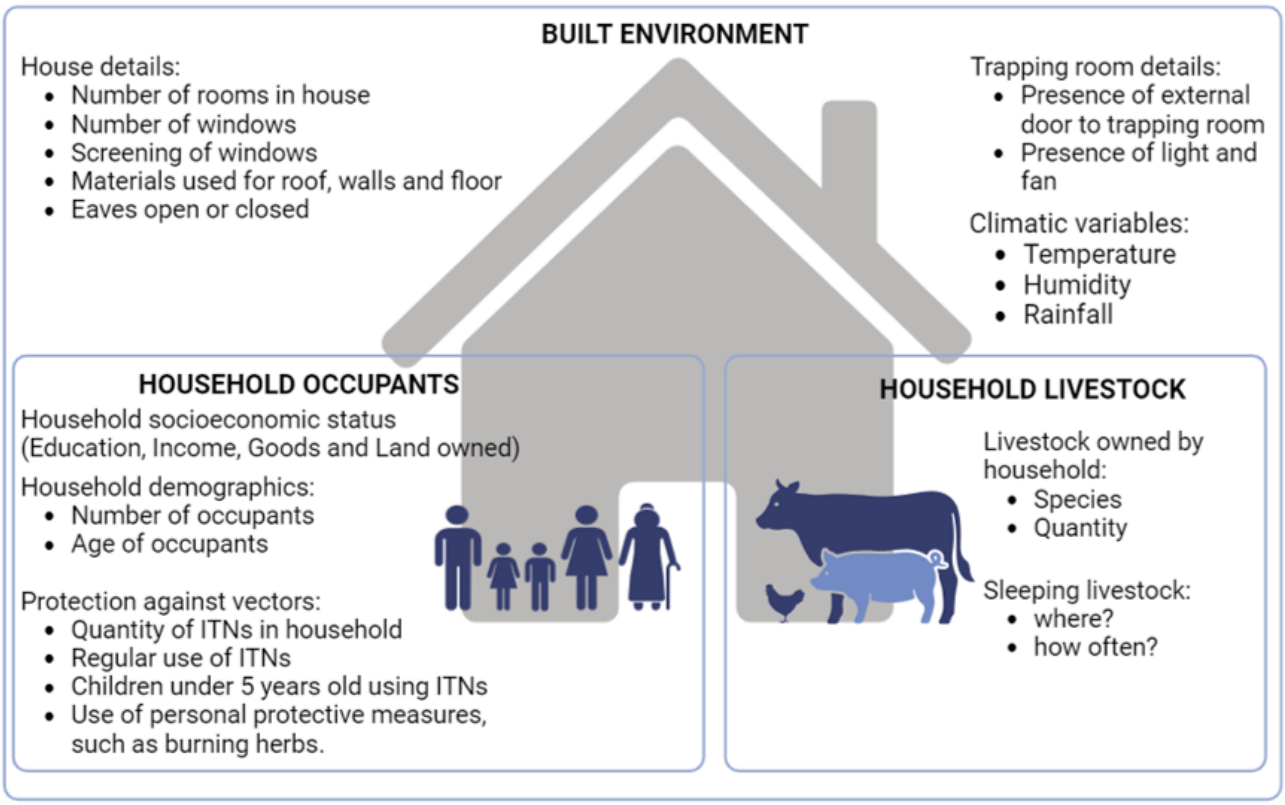

Figure 2. Variables collected during household survey thought to affect the abundance of indoor malaria vectors included: (1) Household demographic variables to determine socioeconomic status, the number and age of occupants, and measures taken by household to protect against vectors, including quantity and use of ITNs by household and personal protective measures, such as burning herbs; (2) Variables associated with household livestock including quantity of different species and sleeping location and; (3) Built environment variables of both the entire house and trapping room. Climatic variables measured were average temperature (°C), relative humidity (%) and average rainfall (mm).

Statistical Methods

Prior to analysis, data was cleaned, and the quality of individual variables was assessed. Any variable perceived as unreliable was excluded from the analysis.

To determine SES, a principal component analysis (PCA) was performed to generate a factor score for each household. The PCA was performed using data on household education level, income, and goods, animals and land owned. SES factor scores were then ranked, and households were divided into five wealth quintiles: poorest, poor, middle, rich and richest. To test for association between household SES and built environment, a chi-squared test for association was performed with a Bonferroni correction for multiple comparisons.

Due to the cluster-randomised design of the MATAMAL trial, adjustments for cluster were made to all analyses. As mosquito counts were overdispersed, a negative binomial regression was used to perform univariable analyses for each risk factor. Univariable analysis was also performed for time point sampled (2021: July, August, September and November 2022: September and November). Following univariable analyses, all risk factors were included in a multivariable model. The incidence rate ratio (IRR) and 95% confidence intervals (CIs) were estimated for each risk factor for both the univariable and multivariable analyses.

To identify any risk factors associated with a specific species, univariable and multivariable analyses were performed on the proportion of each species identified from the sub-sampled analysed by PCR-RFLP. Following adjustments for clustering, a logistic regression was used for each risk factor. Again, all risk factors were included in the multivariable model. The odds ratio (OR) and 95% CIs were estimated for each risk factor. All analysis was conducted using STATA 18.0 (StatCorp LLC, Texas, USA).

Results

Mosquitoes were collected over 4,415 nights from a total of 1,506 households. A total of 158,617 mosquitoes were caught, 113,069 (71%) were *An. gambiae* s.l. females [Chapter 6]. Of the 11,079 *An. gambiae* s.l. identified to species level, *An. melas* accounted for the majority of species identified, (Table 1) [Chapter 6].

Bednet use across the Archipelago was very high, with 99.6% of participants reportedly using bednets (Table 2). The average number of occupants in each household was 6.5 (95%CI: 6.3- 6.7), with children under 5 years old on average representing 12.1% (95%CI: 11.3-12.8) of inhabitants.

Table 1. Numbers of *Anopheles gambiae* s.l. identified using PCR-RFLP from indoor CDC miniature light traps during cross-sectional surveys conducted in ten households per cluster in 2021 (July, August, September and November) and 15 households per cluster in 2022 (September and November) on the Bijagós Archipelago, Guinea-Bissau.

	An. gambiae s.s. n (%)	*An. coluzzii* n (%)	Hybrid[a] n (%)	*An. melas* n (%)	Total PCR-RFLP
2021					
July	48 (9.5)	14 (2.7)	69 (13.7)	371 (73.9)	502
August	129 (14.1)	72 (7.9)	239 (26.1)	476 (50.3)	916
September	175 (26.7)	72 (11.0)	63 (9.6)	346 (52.7)	656
November	583 (16.3)	96 (2.7)	717 (20.1)	2176 (60.9)	3574
2022					
September	230 (15.4)	158 (10.6)	437 (29.2)	671 (44.8)	1496
November	442 (11.2)	680 (17.3)	765 (19.4)	2048 (52.0)	3935
All timepoints combined	1607 (14.5)	1092 (9.9)	2290 (20.7)	6088 (54.9)	11079[b]

[a]*An. gambiae* s.s./ *An. coluzzii* hybrids
[b]Two *An. arabiensis* females were identified during the November 2021 survey

There was limited diversity in the built environment characteristics (Table 2). Most houses had mud flooring and walling (91.2% and 90.0% respectively), open eaves (94.8%), and no light (88.8%) or fan (99%) in the trapping room. Household SES was significantly associated with all built environment characteristics, except eaves open/closed or the presence of an external door in the trapping room (Table 3). Wealthier households were more likely to have zinc roofs, cement flooring, cement block walls, more windows, a fan and light in the trapping room. Open eaves were common throughout all SES quintiles.

Table 2. Percentage of households, total number of trapping nights and mean female *Anopheles gambiae* s.l. density for each potential risk factor.

Variable	Number of households (%)	Number of trapping nights	Mean female *Anopheles* density per trap night[a] (95% CI)
Time point (N=1506)			
2021 July	182 (12.2)	537	17.7 (14.7 – 20.7)
2021 August	180 (12.1)	528	52.9 (44.6 – 61.1)
2021 September	180 (12.1)	537	22.0 (17.8 – 26.2)
2021 November	230 (15.4)	690	19.1 (13.4 – 24.8)
2022 September	360 (24.1)	1041	36.5 (32.2 – 40.9)
2022 November	361 (24.2)	1082	14.6 (11.8 – 17.3)
Socioeconomic status of household head (N=1386)			
Poorest	249 (18.0)	731	30.2 (24.6 – 35.9)
Poor	335 (24.2)	986	26.1 (21.6 – 30.6)
Middle	331 (23.8)	984	25.7 (21.8 – 29.6)
Rich	198 (14.2)	583	23.6 (19.4 – 27.7)
Richest	273 (19.7)	800	24.2 (19.6 – 28.9)
Number of occupants in household (N=1397)			
≤ 4	446 (31.8)	1314	21.5 (18.3 – 24.7)
5-8	626 (44.9)	1840	27.6 (24.3 – 30.9)
≥ 9	325 (23.3)	960	28.7 (24.5 – 32.8)
Use of ITN (N=1397)			
No	6 (0.4)	18	17.1 (5.2 – 29.0)
Yes	1391 (99.6)	4096	25.9 (23.9 – 28.0)
Use of ITN last night (N=1397)			
No	7 (0.5)	21	15.1 (4.4 – 25.9)
Yes	1390 (99.5)	4093	26.0 (23.9 – 28.0)
Number of occupants per ITN within household (N=1397)			
≤ 1.5	360 (25.7)	1055	24.3 (20.5 – 28.1)
1.6-2.5	622 (44.6)	1824	26.7 (23.4 – 29.8)
≥ 2.6	415 (29.7)	1235	26.2 (22.4 – 29.9)
Personal vector protection (N =1395)			
None	870 (62.1)	2529	26.5 (24.0 – 29.0)
Smoking herbs	390 (28.0)	1159	28.3 (23.9 – 32.7)
Repellent coil	82 (5.9)	240	14.4 (8.2 – 20.6)
Insecticide spray	45 (3.3)	138	15.8 (4.1 – 27.5)
Mosquito repellent	3 (0.2)	9	25.9 (-39.3 – 91.1)
Combination of measures	5 (0.4)	12	8.3 (-11.1 – 27.8)
Household roofing material (N=1397)			
Thatch	975 (69.7)	2863	27.9 (25.3 – 30.5)
Zinc	418 (30.0)	1239	21.5 (18.4 – 24.7)
Grass matting or plastic sheeting	4 (0.3)	12	4.9 (0.58 – 9.25)
Household flooring material (N=1392)			
Cement	123 (8.8)	366	31.0 (23.2 – 38.9)
Mud	1269 (91.2)	3733	25.5 (23.4 – 27.6)
Household walling material (N=1397)			
Wooden poles	28 (2.0)	76	2.7 (1.5 – 3.9)
Brick and cement	44 (3.2)	132	20.5 (13.9 – 27.0)
Palm fronds	25 (1.8)	71	32.7 (16.3 – 49.0)
Mud	1258 (90.0)	3709	27.0 (24.6 – 29.2)
Grass matting/Zinc/ Plastic sheeting	42 (3.0)	126	11.6 (7.0 – 16.2)
Eaves (N=1397)			
Closed	73 (5.2)	205	23.0 (12.0 – 34.0)
Open	1324 (94.8)	3909	26.1 (24.0 – 28.1)
Windows in household (N=1501)			
0	681 (45.3)	1990	24.7 (21.9 – 27.5)
1	535 (35.7)	1584	27.4 (23.7 – 31.0)
≥ 2	285 (19.0)	841	28.6 (24.2 – 33.1)
External door in trapping room (N=1397)			
No	720 (48.4)	2000	27.0 (24.1 – 29.6)
Yes	677 (51.6)	2114	24.9 (22.0 – 27.8)
Light in trapping room (N=1397)			
No	1241 (88.8)	3653	25.5 (23.3 – 27.7)
Yes	156 (11.2)	461	28.8 (22.1 – 35.5)
Fan in trapping room (N=1397)			
No	1384 (99.0)	4075	25.8 (23.7 – 27.9)
Yes	13 (1.0)	39	30.6 (8.6 – 52.6)

[a]Mean *An. gambiae* s.l. density calculated from household-level densities

Table 3. Built environment characteristics by household socioeconomic status (SES). Chi-squared test for association between built environment and SES.

	Socioeconomic status of household					χ^2 test for association
	Poorest (N=250) n (%)	Poor (N=336) n (%)	Middle (N=331) n (%)	Rich (N=198) n (%)	Richest (N=274) n (%)	
Roof material						
Thatch	216 (86.4)	257 (76.5)	233 (70.4)	134 (67.7)	125 (45.6)	χ^2 = 124.1
Zinc	34 (13.6)	76 (22.6)	97 (29.3)	64 (32.3)	149 (52.8)	p <0.001*
Floor material						
Mud	245 (98.4)	319 (95.2)	304 (92.1)	175 (88.8)	216 (80.0)	χ^2 = 66.5
Cement	4 (1.6)	16 (4.8)	26 (7.9)	22 (11.2)	54 (20.0)	p <0.001*
Wall material						
Mud	239 (95.6)	311 (92.6)	306 (92.4)	167 (84.3)	226 (82.5)	χ^2 = 57.7
Wooden poling	1 (0.4)	8 (2.4)	3 (0.9)	5 (2.5)	11 (5.5)	p <0.001*
Cement blocks	2 (0.8)	4 (1.0)	6 (1.8)	11 (5.6)	22 (8.0)	
Palm fronds	5 (2.0)	6 (1.8)	6 (1.8)	6 (3.0)	2 (0.7)	
Grass matting/ Zinc/ Plastic sheeting	3 (1.2)	7 (2.1)	10 (3.0)	9 (4.5)	13 (4.7)	
Eaves						
Closed	16 (6.4)	24 (7.1)	16 (4.8)	6 (3.0)	11 (4.0)	χ^2 = 6.0
Open	234 (93.9)	312 (92.9)	315 (95.2)	192 (97.0)	263 (96.0)	p= 0.197
Windows in household						
0	135 (54.2)	166 (49.6)	130 (39.3)	61 (30.8)	84 (30.7)	χ^2 = 56.1
1	79 (31.7)	105 (31.3)	141 (42.6)	79 (39.9)	125 (45.8)	p <0.001*
≥ 2	35 (14.1)	64 (19.1)	60 (18.1)	58 (29.3)	64 (23.4)	
External door in trapping room						
No	129 (51.8)	171 (51.0)	173 (52.3)	95 (48.0)	143 (52.4)	χ^2 = 1.2
Yes	120 (48.2)	164 (49.0)	158 (47.7)	103 (52.0)	130 (47.6)	p= 0.885
Light in trapping room						
No	236 (94.8)	311 (92.8)	313 (94.6)	181 (91.4)	190 (69.6)	χ^2 = 128.2
Yes	13 (5.2)	24 (7.2)	18 (5.4)	17 (8.6)	83 (30.4)	p <0.001*
Fan in trapping room						
No	249 (100.0)	333 (99.4)	330 (99.7)	198 (100.0)	263 (96.3)	χ^2 = 27.9
Yes	0 (0.0)	2 (0.6)	1 (0.3)	0 (0.0)	10 (3.7)	p <0.001*

*Statistically significant (p<0.05)

Univariable analysis revealed five risk factors associated with increased *An. gambiae* s.l. house entry (Table 4): Time point of collection, open eaves, presence of a fan in the trapping room, and average temperature and humidity. Compared to July, *Anopheles* house entry increased in August (2021 IRR= 2.19, 95%CI: 1.88-2.56) and then reduced in September (2021 IRR= 1.06, 95%CI: 0.89-1.26) and November (2021 IRR: 0.86, 95%CI: 0.73-1.01), following a seasonal pattern. Interestingly, the presence of a fan in the trapping room increased the risk of *Anopheles* entry (IRR: 1.55, 95%CI: 1.02-2.33), however the presence of a fan was only reported in a small number of houses (1.0%). From the univariable analysis, higher indoor mosquito numbers were seen between 28-32°C (IRR: 0.94, 95%CI: 0.92 – 0.97) and 70-90% relative humidity (IRR: 1.04, 95%CI: 1.03 – 1.04).

Table 4. Risk factors for *Anopheles gambiae* s.l. caught in houses. Incidence rate ratio (IRR) and 95% confidence intervals (CIs) are presented.

Variable	Univariable Analysis		Multivariable Analysis	
	IRR (95% CI)	P-value	IRR (95% CI)	P-value
Time point				
2021 July	1		1	
2021 August	2.19 (1.88 – 2.56)	<0.001*	2.26 (1.89 - 2.72)	<0.001*
2021 September	1.06 (0.89 – 1.26)		1.15 (0.94 – 1.41)	
2021 November	0.86 (0.73 – 1.01)		0.92 (0.76 – 1.11)	
2022 September	1.69 (1.46 – 1.95)		1.90 (1.60 – 2.27)	
2022 November	0.73 (0.63 – 0.85)		0.74 (0.62 – 0.89)	
Socioeconomic status of household head				
Poorest	1		1	
Poor	0.90 (0.78 – 1.04)	0.599	0.92 (0.80 – 1.05)	<0.021*
Middle	0.99 (0.86 – 1.14)		1.12 (0.98 – 1.29)	
Rich	0.94 (0.80 – 1.11)		1.03 (0.88 – 1.22)	
Richest	0.95 (0.82 – 1.11)		0.95 (0.83 – 1.12)	
Number of occupants in household				
≤ 4	1		1	
5-8	1.10 (0.99 – 1.22)	0.099	1.11 (1.00 – 1.24)	0.091
≥ 9	1.13 (1.00 – 1.28)		1.14 (1.00 – 1.31)	
Regular use of ITN				
No	1		1	
Yes	0.93 (0.47 – 1.83)	0.831	0.30 (0.05 – 1.91)	0.201
Use of ITN last night				
No	1		1	
Yes	1.02 (0.53 – 1.94)	0.961	2.45 (0.43 – 13.84)	0.311
Number of occupants per ITN within household				
≤ 1.5	1		1	
1.6-2.5	1.08 (0.96 – 1.21)	0.295	1.05 (0.93 – 1.18)	0.705
≥ 2.6	1.09 (0.97 – 1.23)		1.05 (0.92 – 1.20)	
Personal vector protection				
No	1		1	
Yes[a]	0.91 (0.82 – 1.01)	0.052	1.03 (0.92 – 1.14)	0.630
Household roofing material				
Thatch	1		1	
Zinc	1.06 (0.95 – 1.18)	0.537	1.14 (1.01 – 1.28)	0.093
Grass matting or plastic matting	1.17 (0.52 – 2.61)		1.14 (0.53 – 2.46)	
Household flooring material				
Cement	1		1	
Mud	1.00 (0.85 – 1.18)	0.987	1.01 (0.84 – 1.21)	0.911
Household walling material				
Mud	1		1	
Bricks and cement	0.95 (0.73 – 1.24)	0.838	1.00 (0.72 – 1.37)	0.985
Other[b]	1.05 (0.84 – 1.31)		1.02 (0.81 – 1.28)	
Eaves				
Closed	1		1	
Open	1.31 (1.05 – 1.64)	0.016*	1.35 (1.08 – 1.68)	0.007*
Windows in household				
0	1		1	
1	1.00 (0.90 – 1.11)	0.148	1.04 (0.94 – 1.16)	0.726
≥ 2	1.12 (0.99 – 1.26)		1.02 (0.90 – 1.17)	
External door in trapping room				
No	1		1	
Yes	1.07 (0.97 – 1.18)	0.152	1.11 (1.01 – 1.23)	0.034
Light in trapping room				
No	1		1	
Yes	1.11 (0.96 – 1.28)	0.146	1.05 (0.89 – 1.22)	0.578
Fan in trapping room				
No	1		1	
Yes	1.55 (1.02 – 2.33)	0.038*	0.98 (0.64 – 1.52)	0.940
Average temperature (°C)	0.94 (0.92 – 0.97)	<0.001*	0.96 (0.93 – 0.98)	<0.001*
Average relative humidity (%)	1.04 (1.03 – 1.04)	<0.001*	1.01 (1.00 – 1.01)	0.147

[a]Households smoked herbs, used repellent coils, insecticide spray, mosquito repellent or a combination of measures

[b]Other walling material includes grass matting, mangrove leaves, zinc sheeting, palm fronds and plastic sheeting

In the final multivariable model, as with the univariable results, the month of mosquito collection was associated with fluctuations in *Anopheles* house entry. Households with lower SES and open eaves increased the risk of higher mosquito numbers (IRR: 1.30, 95%CI: 0.95-1.33) within the house (Figure 3). From the multivariable results, only temperature was shown to have a significant impact on vector numbers.

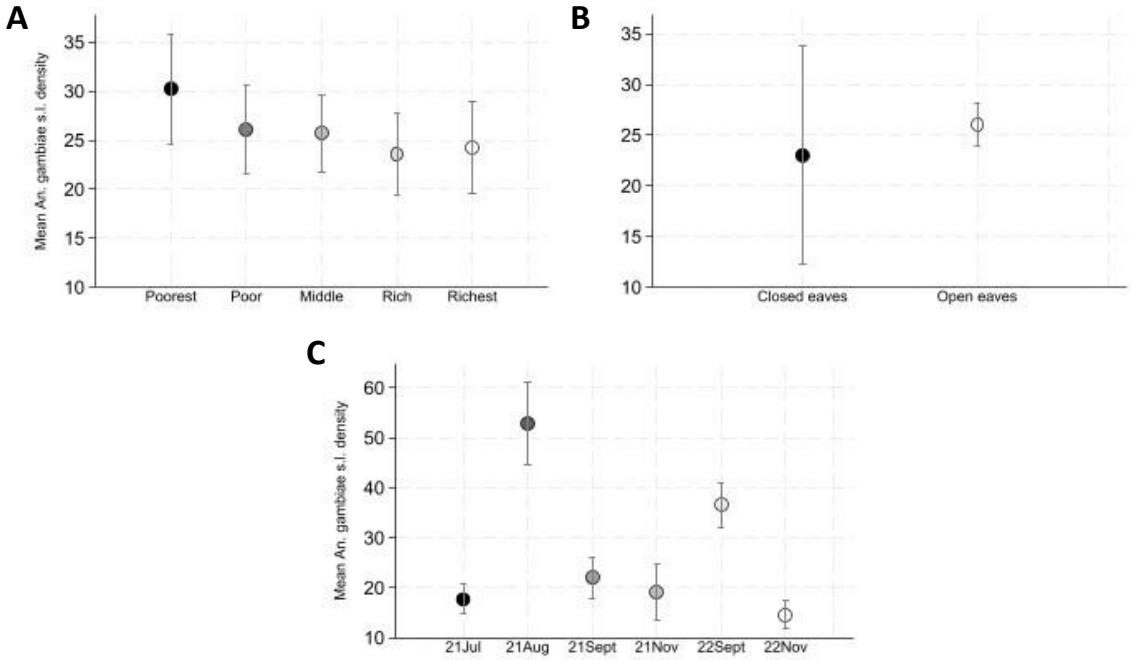

Figure 3. Mean *Anopheles gambiae* s.l. density by SES (A) eave closure (B) and time point (C). Densities were calculated using household-level densities.

It was not possible to detect an association between species proportion and bednet use because the number of households not using a bednet was too small (0.04%; 60/1506 houses). Factors associated with higher proportions of *An. melas* in the trap catch were: the month of collection, lower SES, personal vector protection taken by the household, and having an external door in the trapping room (Table 5). *Anopheles gambiae* s.s. was found at significantly higher proportions in houses with: lower SES, fewer residents, used personal vector protection, traditional mud flooring, more windows and no external door to trapping room. The proportion of *An. coluzzii* was higher in houses with: lower SES, fewer residents, thatched roofing, more windows and no external door in trapping room. A higher proportion of hybrids was found in houses with: personal vector protection, cement floors, more windows and no external door in trapping room. Often the numbers of species caught for each characteristic was small, which was particularly the case with *An. gambiae* s.s. and *An. coluzzii*, therefore the data have large confidence intervals.

Table 5. Risk factors associated with the proportion of *An. gambiae* s.s., *An. coluzzii, An. gambiae* s.s./*An. coluzzii* hybrids and *An. melas* within houses.

Variable	***An. gambiae* s.s.**			***An. coluzzii***			**Hybrid[a]**			***An. melas***		
		Multivariable Analysis			Multivariable Analysis			Multivariable Analysis			Multivariable Analysis	
	n/N[b] (%)	OR (95% CI)	P-value	n/N[b] (%)	OR (95% CI)	P-value	n/N[b] (%)	OR (95% CI)	P-value	n/N[b] (%)	OR (95% CI)	P-value
Time point												
2021 July	48/502 (9.6)	1		14/502 (2.8)	1		69/502 (13.7)	1		371/502 (73.9)	1	
2021 August	129/916 (14.1)	1.89 (0.83 – 4.29)	<0.01*	72/916 (7.9)	11.03 (2.65 – 45.83)	<0.01*	239/916 (26.1)	8.64 (3.70 – 20.14)	<0.01*	476/916 (52.0)	0.13 (0.05 - 0.32)	<0.01*
2021 September	175/656 (26.7)	13.3 (5.96 – 25.79)		72/656 (11.0)	78.54 (25.4 – 426.0)		63/656 (9.6)	0.48 (0.22 – 1.06)		346/656 (52.7)	0.06 (0.03 – 0.15)	
2021 November	583/3574 (16.3)	0.00 (0.00 – 0.00)		96/3574 (2.7)	0.00 (0.00 – 0.00)		717/3574 (20.1)	0.00 (0.00 – 0.00)		2176/3574 (60.9)	0.01 (0.00 – 0.03)	
2022 September	230/1496 (15.4)	1.24 (0.58 – 2.69)		158/1496 (10.6)	32.04 (7.93 – 129.52)		437/1495 (29.2)	10.65 (5.17 – 21.92)		670/1495 (44.8)	0.36 (0.16 – 0.80)	
2022 November	442/3935 (11.2)	0.00 (0.00 – 0.01)		680/3935 (17.3)	0.69 (0.16 – 2.85)		765/3935 (19.4)	0.01 (0.00 – 0.02)		2048/3935 (52.0)	0.03 (0.01 – 0.08)	
Socioeconomic status of household head												
Poorest	292/1839 (15.9)	1		224/1839 (12.2)	1		451/1839 (24.5)	1		872/1839 (47.4)	1	
Poor	346/2251 (15.4)	1.89 (0.52 – 1.79)	<0.01*	231/2251 (10.3)	1.77 (0.92 – 3.41)	<0.01*	488/2251 (21.7)	1.20 (0.65 – 2.22)	0.20	1186/2251 (52.7)	0.66 (0.31 – 1.37)	0.05*
Middle	397/2969 (13.4)	0.42 (0.22 – 0.81)		299/2969 (10.1)	0.53 (0.27 – 1.06)		631/2969 (21.2)	0.78 (0.40 – 1.54)		1641/2969 (55.3)	0.46 (0.20 – 1.04)	
Rich	200/1437 (13.9)	0.19 (0.09 – 0.40)		104/1437 (7.2)	0.71 (0.32 – 1.58)		253/1437 (17.6)	0.52 (0.24 – 1.14)		880/1437 (61.2)	1.46 (0.58 – 3.65)	
Richest	252/1937 (13.0)	0.34 (0.17 – 0.71)		194/1937 (10.0)	0.91 (0.41 – 1.99)		322/1937 (16.6)	0.72 (0.35 – 1.50)		1169/1937 (60.3)	0.89 (0.38 – 2.10)	
Number of occupants in household												
≤ 4	425/2882 (14.7)	1		320/2882 (11.1)	1		480/2882 (16.6)	1		1657/2882 (57.5)	1	
5-8	696/4862 (14.3)	0.56 (0.35 – 0.90)	0.01*	465/4862 (9.6)	0.47 (0.28 – 0.78)	<0.01*	1025/4862 (21.1)	1.04 (0.63 – 1.71)	0.41	2675/4862 (55.0)	0.87 (0.49 – 1.54)	0.24
≥ 9	371/2727 (13.6)	0.41 (0.23 – 0.73)		267/2727 (9.8)	0.33 (0.18 – 0.61)		355/2727 (24.0)	0.74 (0.41 – 1.35)		1434/2727 (52.6)	0.56 (0.27 – 1.13)	
Number of occupants per ITN within household												
≤ 1.5	394/2604 (15.1)	1		237/2604 (9.1)	1		480/2604 (18.4)	1		1493/2604 (57.3)	1	
1.6-2.5	652/4794 (13.6)	0.69 (0.41 – 1.15)	0.32	492/4794 (10.3)	1.55 (0.88 – 2.73)	0.06	941/4794 (19.6)	0.72 (0.42 – 1.25)	0.42	2708/4794 (56.5)	1.35 (0.72 – 2.54)	0.22
≥ 2.6	446/3073 (14.5)	0.67 (0.38 – 1.19)		323/3073 (10.5)	0.90 (0.48 – 1.71)		739/3073 (24.0)	0.90 (0.48 – 1.66)		1565/3073 (50.9)	0.84 (0.42 – 1.68)	
Personal vector protection												
No	818/6428 (12.7)	1		757/6428 (11.8)	1		1336/6428 (20.8)	1		3517/6428 (54.7)	1	
Yes[c]	674/4043 (16.7)	5.10 (3.16 – 8.24)	<0.01*	295/4043 (7.3)	0.71 (0.42 – 1.21)	0.21	824/4043 (20.4)	3.72 (2.27 – 6.09)	<0.01*	2249/4043 (55.6)	3.29 (1.71 – 6.34)	<0.01*
Household roofing material												
Thatch	1074/7365 (14.6)	1		756/7365 (10.3)	1		1598/7365 (21.7)	1		3937/7365 (53.4)	1	
Zinc	414/3074 (13.5)	0.57 (0.33 – 0.99)	0.14	292/3074 (9.5)	0.34 (0.19 – 0.61)	<0.01*	559/3074 (18.2)	0.56 (0.32 – 0.98)	0.12	1808/3074 (58.8)	1.52 (0.77 – 3.01)	0.48
Grass matting or plastic matting	4/32 (12.5)	0.51 (0.02 – 13.25)		4/32 (12.5)	0.78 (0.05 – 13.15)		3/32 (9.4)	0.58 (0.00– 232.20)		21/32 (65.6)	0.99 (0.01 – 72.75)	
Household flooring material												
Cement	106/1012 (10.5)	1		95/1012 (9.4)	1		160/1012 (15.8)	1		651/1012 (64.3)	1	
Mud	1385/9445 (14.7)	2.75 (1.18 – 6.40)	0.02*	956/9445 (10.1)	0.97 (0.43 – 2.18)	0.94	1995/9445 (21.1)	0.36 (0.15 – 0.88)	0.02*	5108/9445 (54.1)	1.48 (0.50 – 4.41)	0.48
Household walling material												
Mud	1369/9493 (14.4)	1		983/9493 (10.3)	1		1992/9493 (21.0)	1		5148/9493 (54.2)	1	
Brick and cement	43/352 (12.2)	4.97 (1.01 – 24.29)	0.13	26/352 (7.4)	0.60 (0.13 – 2.85)	0.75	62/352 (17.6)	0.51 (0.11 – 2.37)	0.53	221/352 (62.8)	1.22 (0.15 – 9.89)	0.85
Other[d]	80/626 (12.8)	0.85 (0.27 – 2.62)		43/626 (6.9)	0.76 (0.23 – 2.50)		106/626 (16.9)	1.47 (0.46 – 4.71)		397/626 (63.4)	1.44 (0.37 – 5.56)	
Eaves												
Closed	43/369 (11.6)	1		50/369 (13.5)	1		68/369 (18.4)	1		207/369 (56.1)	1	
Open	1449/10102 (14.3)	2.20 (0.86 – 5.68)	0.10	1001/10102 (9.9)	1.49 (0.58 – 3.85)	0.41	2092/10102 (20.7)	0.59 (0.24 – 1.44)	0.24	5559/10102 (55.0)	2.23 (0.79 – 6.31)	0.13
Windows in household												
0	677/4625 (14.6)	1		507/4625 (11.0)	1		1092/4625 (23.6)	1		2348/4625 (50.8)	1	
1	615/4572 (13.4)	0.80 (0.49 – 1.29)	<0.01*	368/4572 (8.0)	0.66 (0.38 – 1.12)	<0.01*	852/4572 (18.6)	0.48 (0.28 – 0.79)	<0.01*	2736/4572 (59.8)	1.26 (0.70 – 2.28)	0.72
≥ 2	315/1873 (16.8)	3.59 (1.99 – 6.48)		217/1873 (11.6)	4.89 (2.59 – 9.22)		346/1873 (18.5)	1.39 (0.74 – 2.61)		995/1873 (53.1)	1.23 (0.60 – 2.52)	
External door in trapping room												
No	829/4943 (16.8)	1		523/4943 (10.6)	1		1101/4943 (22.3)	1		2490/4943 (50.4)	1	
Yes	663/5528 (12.0)	0.38 (0.24 – 0.59)	<0.01*	529/5528 (9.6)	0.44 (0.26 – 0.74)	<0.01*	1059/5528 (19.2)	0.18 (0.11 – 0.30)	<0.01*	3276/5528 (59.3)	1.95 (1.07 – 3.54)	0.03*
Light in trapping room												
No	1348/9587 (14.1)	1		953/9587 (9.9)	1		1974/9587 (20.6)	1		5311/9587 (55.4)	1	
Yes	144/884 (16.3)	1.50 (0.77 – 2.93)	0.24	99/884 (11.2)	1.83 (0.88 – 3.81)	0.10	186/884 (21.0)	1.26 (0.64 – 2.47)	0.49	455/884 (51.5)	1.09 (0.45 – 2.65)	0.84
Fan in trapping room												
No	1482/10390 (14.3)	1		1045/10390 (10.1)	1		2142/10390 (20.6)	1		5720/10390 (55.0)	1	
Yes	10/81 (12.3)	3.33 (0.40 – 27.34)	0.26	7/81 (8.6)	1.42 (0.15 – 13.59)	0.76	18/81 (22.2)	5.15 (0.67 – 39.37)	0.11	46/81 (56.8)	0.90 (0.06 – 14.01)	0.94
Average temperature (°C)		1.14 (1.01 – 1.28)	0.03*		1.00 (0.89 – 1.14)	0.94		1.08 (0.96 – 1.22)	0.19		1.04 (0.89 – 1.22)	0.61
Average relative humidity (%)		1.00 (0.97 – 1.04)	0.74		0.99 (0.95 – 1.03)	0.62		1.01 (0.97 – 1.05)	0.65		0.99 (0.94 – 1.03)	0.54

[a]*An. gambiae* s.s./ *An. coluzzii* hybrids

[b]*n* is number of species identified; *N* total number of specimens identified using PCR-RFLP

[a]Households smoked herbs, used repellent coils, insecticide spray, mosquito repellent or a combination of measures

[d]Other walling refers to walling made from wooden poles, palm fronds, grass matting, mangrove leaves, metal or plastic sheeting

Discussion

Improved housing has been viewed favourably by many communities, who have perceived it as a means to reduce mosquitoes inside the house but expressed concerns about costs and potential increased temperatures [30-33]. In the fight against malaria, improved housing is not only expected to reduce mosquito entry but also remove the burden of responsibility on the end user by removing the daily task of maintaining and assembling an ITN. It also provides equal protection to all residents within the household and could potentially remove the dependence on insecticides. Where housing improvements prevent the entry of mosquitoes, they could simultaneously prevent contact with a range of vectors. This includes *Culex* mosquitoes responsible for the transmission of lymphatic filariasis and *Aedes* mosquitoes, which transmit the arboviruses dengue, yellow fever, Zika and chikungunya, which are most commonly transmitted in and around buildings [34].

This study is the first to characterise and investigate risk factors associated with vector density within houses across the Bijagós Archipelago. As reported previously, and seen in results here, ITN use is high throughout the islands [22, 23], however, in spite of this, malaria transmission persists. The built environment across the Archipelago is homogenous, with the majority of houses being constructed using traditional materials with thatched roofs, mud walls and floors, and open eaves. Wealthier households were found to use modern materials, such as zinc roofs and cement walls and floors, more frequently. Interestingly, contrary to other studies, closed eaves were not seen to be associated with higher SES [35]. On the Bijagós, there were only a few houses with closed eaves in each SES group, therefore comparing groups was challenging. However, the lack of closed eaves throughout all SES groups is interesting. Wealthier households are more likely to have zinc roofs, which may make closing eaves more challenging, or households may perceive the eave gap to be too small to be a risk factor for mosquito house entry. Increased indoor temperatures may also be a barrier to eave closure. Houses with metal roofs and closed eaves have been shown to have higher indoor temperatures compared to those with thatched roofs and closed eaves [13]. Future qualitative studies on the Bijagós, including focus-group discussions and key informant interviews, will be needed to better understand community perceptions of closed eaves and identify any potential barriers to uptake of eave-base interventions.

Studies throughout sub-Saharan Africa have found a range of different risk factors associated with mosquito entry, with the majority identifying higher numbers of mosquitoes in houses with open eaves [10, 13, 14, 19, 36-38]. Open eaves have been found to be responsible for higher numbers of the major sub-Saharan African vectors, *An. gambiae* s.l. and *An. funestus* s.l., within houses [19]. Although 94.8% of houses on the Bijagós had open eaves, those that did not saw a significant reduction in *An. gambiae* s.l. density. On the basis of our findings, we would recommend an eave-based intervention as a potential housing improvement to reduce mosquito house entry. An experimental hut trial was conducted in The Gambia investigating the relationship between eave closure, roofing material and door screening [13]. Using various combinations of features in houses identical in size, shape and walls, the investigators found that simply closing the eaves in a thatched house, even with a poorly fitted door resulted in a 94% reduction in indoor *An. gambiae* s.l. density. Eave closure was also found to reduce the number of other mosquito species, primarily within the *Culex* and *Mansonia* genera, found indoors by 43%. Similar reductions were seen when doors were well-fitted and screened. However, interestingly, this effect was not seen in houses with metal roofs, where mosquito entry increased relative to thatched houses when eaves were closed.

In Malawi, the impact on mosquito house entry of differing levels of eave closure has been trialled using an experimental hut design [14]. Three different levels of partial eave closure were compared alongside fully open and closed eaves. Although the numbers of female *Anopheles* caught was low, once again the indoor *Anopheles* density was reduced significantly in houses with fully closed eaves. In houses with four small openings in the eaves, anopheline numbers were seen to increase significantly. This is likely due to variations in airflow, leading to higher concentrations of carbon dioxide and host odours at eave openings, allowing vectors to locate small points of entry to the house [14, 34]. This study found no impact on culicine mosquitoes, which might reduce acceptability within communities should an intervention not be seen to affect mosquito numbers within houses.

Researchers have aimed to capitalise on the impact of partial closure of eaves on anopheline mosquitoes through the development of eave tubes as a novel way to deliver insecticides to vectors [39-41]. These are tubes with insecticide-treated inserts that expose mosquitoes to insecticide when entering the house. They have been shown to reduce mosquito entry by 60% even when windows were left open and lower vector blood-feeding indoors by 64% as

mosquito mortality increases following exposure to insecticide [40]. A large cluster-randomised trial investigating the use of eave tubes in addition to mosquito-proofing the houses with window screening and closing any gaps when mosquitoes could enter [41, 42]. In clusters where coverage was above 70%, the risk of a malaria case in recruited children was 47% lower than in control clusters [41].

Other ways to prevent mosquitoes using eaves to enter houses have been explored, insecticide-treated ribbons are a low-cost intervention that can be fitted onto multiple housing types [43-45]. Ribbons treated with the spatial repellent transfluthrin are currently estimated to cost $7.00 per house per year (unsubsidized) and provide 79% protection from indoor vector biting and 60% protection from outdoor biting [44]. They may provide an alternative to full eave closure as the costs associated with installation and maintenance may be a potential barrier to uptake. Jatta *et al.* estimated the cost of filling the eaves with mud, cement and broken bricks at $26 per house [13]. Should further alterations need to be made to the house, for instance metal-screened doors and windows, costs would increase. In Guinea-Bissau, where, in 2017, 22% of the population was living on less than $2.15 per day, this would be a heavy financial burden for the average household to bear [46]. Future work is needed to establish the long-term efficacy of eave closure, and the potential need for replacement or maintenance to accurately assess its cost-effectiveness.

Another possible barrier to community uptake of eave closure is the potential increase in temperature within households. Increased temperatures have been reported by communities across sub-Saharan Africa as a reason to not use ITNs [47, 48]. Temperatures in thatched houses with closed eaves have been reported to be more comfortable by residents than equivalent houses with metal roofs. Indeed, temperatures have been shown to be greater in metal-roofed houses, even reaching temperatures which increase mosquito mortality [49]. Temperature and community perception of any built environment intervention should be monitored alongside implementation [13].

With the exception of *An. melas*, the most commonly identified species, all species were seen at higher proportions in thatched houses. The proportion of *An. gambiae* s.s., thought to be the primary vector on the Bijagós, was greater in houses with thatched roofs, mud walls, more than two windows and no external door to the trapping room [24]. *Anopheles gambiae* s.s./*An. coluzzii* hybrids, were common on the islands and seen at higher proportions in similar

houses, indicating a potential similarity to *An. gambiae* s.s. in host-seeking behaviours. Metal roofs have been associated with lower mosquito house entry [19, 35, 37], with high indoor temperatures reducing vector survival [49]. While higher indoor temperatures may help in reducing vector numbers within houses, it may make living conditions for residents uncomfortable, potentially leading to more time spent outdoors. This may in turn expose communities to potentially infective bites from vectors exhibiting behaviours associated with residual transmission [9, 50]. In The Gambia, improved ventilation in metal-roof houses has been found to reduce temperatures enough to be comparable to those seen in thatched houses [13]. Further studies in the Bijagós are needed to examine the interaction between different housing characteristics to determine the best possible combination of interventions to reduce mosquito numbers.

Vector populations are complex and heterogenous, therefore understanding how different combinations of housing characteristics work in the local context is important. Seasonal shifts in species composition was seen throughout the study; the proportion of *An. melas* decreased while the other two species and hybrid form increased throughout the transmission season (in August and September). Different housing characteristics were found to be associated with higher proportions of the various species, highlighting the need to thoroughly investigate the host-seeking and feeding behaviours of the local vector population. With the exception of *An. gambiae* s.s./*An. coluzzii* hybrids, higher proportions of *Anopheles* mosquitoes were seen in households with lower SES. However, these results are challenging to interpret as significant differences in the proportion of one species may be a result of a behaviour or preference of another species. For instance, an external door to the trapping room, a common feature in the Bijagós, is associated with an increase in the proportion of *An. melas* and a decrease in the proportion of the other species and hybrid form. External doors may either be a risk factor associated with *An. melas,* or it may increase air flow and protect against the other three species. Results presented here generate further questions and highlight the complexity of species specific behaviour.

Improved housing has increased throughout sub-Saharan Africa, with an increase from 11% in 2000 to 23% in 2015, however a large proportion of the population are still living in unimproved housing [51]. The health sector alone is unable to deliver improved housing, it requires collaboration across multiple sectors, with political leadership and engagement, as

well as sufficient funding to drive the change. Community participation and education are also needed to sustain progress. This can be done through reviewing school curriculum and incorporating teachings on diseases associated with poverty and unimproved housing. Ways to better prevent transmission through environmental management and good bednet use could also be incorporated in educational programmes [34]. It is worth noting, that most home improvements will be done gradually by households, therefore engaging with communities to help further their understanding of potential benefits from different house designs will lead to more sustainable change.

The study would have benefitted from several additional components. Firstly, concurrent larval surveys would have given information about the changing water bodies throughout the season and given the opportunity to investigate how proximity to larval bodies affected mosquito house entry. However, this would have been a considerable undertaking given the number of houses sampled in our study and due to the logistical constraints, particularly the geographical difficulties of working in the Bijagós Archipelago, we were unable to incorporate this. Secondly, qualitative surveys, including focus group discussions and key informant interviews would have given information on community perceptions about improved housing and identified potential barriers to uptake.

On the Bijagós Archipelago, higher vector densities were seen in households with open eaves. This identifies a possible avenue for future control measures using eave-based interventions. Eave ribbons impregnated with the spatial repellent transfluthrin provide an opportunity to reduce mosquito house entry without closing eaves, allowing air to continue to circulate. They have also been shown to reduce the risk of both indoor and outdoor biting around the house [44]. Eave ribbons could target residual transmission driven by outdoor biting around houses, whilst providing an opportunity for an integrated vector management approach, reducing both *Anopheles*- and *Aedes*-borne diseases [44]. Understanding how eave ribbons interact with different built environment features, for instance metal vs thatched roofs, will be needed prior to implementation. The Bijagós Archipelago would benefit from future experimental hut trials to examine different combinations of eave-based interventions and built environment characteristics to prevent mosquito house entry, with the ultimate aim being to build-out malaria.

Abbreviations

IRR Incidence rate ratio; *IRS* Indoor residual spraying; *ITN* Insecticide-treated bednet; *LT* CDC miniature light trap; *PCA* Principal component analysis; *PCR-RFLP* polymerase chain reaction using restriction fragment length polymorphism; *OR* Odds ratio; *SES* socioeconomic status.

Declarations

Author contributions

EP, JB, SL, JGL, LST, RTJ and AL designed the study. AL acquired funding. EP, ETS, HH, AM and AL supported the data collection in Guinea-Bissau. SC and MN coordinated and performed the molecular analysis at the MRC Unit The Gambia at LSHTM. EP, JB and LST analysed the data. EP wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data presented in this manuscript is available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethics approval for the MATAMAL trial was obtained from LSHTM Research Ethics Committee (UK) (19156) and the Comité Nacional de Ética em Pesquisa na Saúde (CNES; Guinea-Bissau) (084/CNES/INASA/2020).

Competing interests

The authors declare no competing interests.

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Chapter 8. Discussion and conclusions

The persistence of malaria in most endemic countries, even in the presence of large-scale control programmes, highlights a need for novel interventions that may work alongside existing ones [1]. New vector interventions may specifically target vectors that are not currently being controlled by mainstay measures, such as bednets and indoor residual spraying. It is therefore important to understand the context in which these measures are deployed.

Previous vector surveys on the Bijagós Archipelago, in Guinea-Bissau, had only been on a few islands and had not been comprehensive. The aims of this thesis were to better understand the vector population and evaluate the impact of a mass drug administration (MDA) of ivermectin (IVM) in addition to the antimalarial dihydroartemisinin-piperaquine (DP) on vector age structure, density, species composition, infectivity rate and entomological inoculation rate (EIR) through a cluster-randomised placebo-controlled trial design across the Bijagós. It also aimed to characterise the built environment and identify any risk factors associated with mosquito entry to the house, with the hope to better inform future building practices.

Summary of research findings

This section provides a brief overview of the findings of this thesis in relation to its objectives. The study limitations and conclusions are also included in this section. For full details, please see chapters which are highlighted below for every objective.

Objective 1: To conduct a baseline survey on the Bijagós Archipelago, focusing on the *Anopheles* mosquito population.

(Research Paper – Chapter 4)

An entomological baseline survey was conducted on 16 of the 19 permanently inhabited islands across the Bijagós Archipelago from October to December in 2019. Mosquitoes were caught using both indoor and outdoor CDC miniature light traps (LTs). This was the largest entomological survey that had been conducted on the Archipelago, with many islands being sampled for the first time. *An. gambiae* sensu lato (s.l.) were found throughout the Archipelago in both indoor and outdoor LTs, with densities varying considerably between sampling sites. *An. melas* was common in both indoor and outdoor LTs, accounting for 85.2% of all *An. gambiae* s.l. caught. High levels of hybridisation were also seen between *An. gambiae* sensu stricto (s.s.) and *An. coluzzii*, with hybrid numbers regularly seen above both parent species. High frequencies of both *An. melas* and hybrids have been found on mainland Guinea-Bissau [2, 3].

The trapping techniques used for indoor and outdoor traps were different, therefore it was difficult to compare the two. However, the presence of *An. gambiae* s.l. in outdoor LTs on all islands sampled, and, in particular, the occurrence of outdoor CSP-positive *An. melas* on some islands, indicates that the population may be at risk of exposure to transmitting vectors when outdoors.

Larval surveys were conducted to identify any site characteristics which were more favoured by *Anopheles* larvae. Site identification proved extremely challenging, with the team being unable to reach and survey many areas. However, the sites that were found, identified natural larval sites with a perimeter of 1.01-10 m as being more prone to harbouring *Anopheles* larvae. *Anopheles* were also found more frequently in water bodies that had direct sunlight and an absence of vegetation. This larval survey highlighted the need to explore other ways to better identify and sample larval sites, in particular in heavily forested areas.

Objective 2: To establish an appropriate method for storing *Anopheles* mosquitoes from trial clusters for parity assessment.

(Research paper – Chapter 5)

Current control strategies target mosquitoes of blood-feeding age, therefore it is important to monitor the age structure of vector populations [4]. To do this, mosquitoes are killed and then dissected, ovaries are isolated and assessed to see whether mosquitoes have laid eggs (parous) or not (nulliparous) [5]. Parity assessment using freshly-killed mosquitoes is often difficult to implement in trial or programmatic settings, and while more modern techniques are promising, they are often costly and require certain infrastructure, such as an established laboratory, which is not always available [6-9].

The validation of the dry-preservation and rehydration technique described previously by Ungureanu in 1972, enabled large scale deployment of the technique throughout the trial [10]. The validation was conducted in two stages. The first was performed with laboratory-reared *An. coluzzii* at LSHTM and showed that mosquitoes may be preserved for up to 12-weeks prior to rehydration and dissection [11]. Parity status of dry-preserved and rehydrated mosquitoes was correctly identified in 90% of assessments, in comparison to 98% of freshly-killed mosquitoes. Excellent strength of agreement between assessors was seen. The second stage was conducted on the Bijagós with field-caught *An. gambiae* s.l. from indoor LTs. The overall index of agreement was 0.86 (95% CIs: 0.78-0.93), indicating almost perfect agreement.

The technique provides flexibility to study logistics and could be deployed on a large scale to be used in programmatic settings. While the technique is more challenging to master, once learnt, it could be

a valuable tool for assessing vector age structure. The technique may also be modified and used for other assessments, such as sporozoite detection in the salivary glands or sperm detection in the spermatheca. These techniques would need further validation, however, may be useful to future programmes and studies.

Objective 3: To evaluate the impact of IVM MDA on vector density, population age structure, species composition, sporozoite rate and entomological inoculation rate.

(Research paper – Chapter 6)

Mosquito collections were done following completion of all MDA rounds and during the PTS in 2021 using indoor LTs. Data was assessed after 2021 trapping completion and concerns were raised about the variability in *Anopheles* parity rates and density between trial clusters, therefore sampling increased in 2022. In 2021, 18 clusters were sampled during post-MDA collections, this was increased to all 24 clusters in 2022. However, due to logistical constraints, in 2022, sampling only occurred following completion of the third MDA round. In total, 1,506 households were sampled over 4,415 trapping nights, collecting over 113,000 *Anopheles* females.

All *Anopheles* mosquitoes were morphologically identified and found to be within the *An. gambiae* s.l. complex. These findings are consistent with past identifications done on the islands [12, 13]. Surveys on mainland Guinea-Bissau have also found that species within the *An. gambiae* s.l. complex dominate [14-16]. *Anopheles gambiae* s.l. are the primary vector for the surrounding region, however, other *Anopheles* species occur, most notably mosquitoes in the *An. funestus* complex and *An. pharoensis* [17-19]. Relatively few entomological surveys have occurred throughout Guinea-Bissau, therefore continued monitoring throughout the mainland and the Bijagós is necessary to monitor species composition and how the changing climate and environmental conditions are affecting populations.

The identification of *Anopheles* mosquitoes was used to calculate the vector density across the Archipelago. No difference in *Anopheles* density was seen at any time point between trial arms. This indicates that IVM MDA did not have an impact on *Anopheles* density in this setting. The MASSIV trial in The Gambia, trialled the same IVM MDA regimen, and found a significant decrease in *Anopheles* density caught 7-14 days following completion of MDA in indoor LTs from intervention villages in their second year of the study [20, 21]. This decrease was not seen in the first year or from human landing catch (HLC) collections conducted over the same time periods. Density was also seen to be affected by IVM MDA in two smaller studies [22, 23]. These studies both used a single IVM dose of 150 µg/kg used in lymphatic filariasis and onchocerciasis control programmes and sampled 2-3 days following completion of MDA.

The design outlined in this PhD followed data collection on IVM PKPD and mosquitocidal studies conducted on high-dose IVM MDA [24, 25]. When combined with DP, IVM MDA has been seen to kill *Anopheles* mosquitoes up to 28 days post-treatment [25]. Those studies however, were relatively small. Should those results not translate to large-scale IVM and DP distributions, we may have been sampling mosquitoes too late to see an effect. If so, this poses questions about the viability of IVM MDA in a programmatic setting. Should the effect time be shorter than originally thought, IVM MDA would need to be given more frequently, leading to costly implementation and logistical challenges.

Another potential explanation for the variation between the MATAMAL and MASSIV results could be due to the heterogeneity in host-seeking and feeding behaviours of the vector populations. *Anopheles melas* and *An. gambiae* s.s./*An. coluzzii* hybrids were common throughout the transmission season across the Archipelago. Little is known about the feeding behaviours of these populations; therefore further studies are needed to assess the interaction between the malaria vectors and human host on the Bijagós.

Parity assessment was successfully performed on over 14,000 *Anopheles* mosquitoes from post-MDA collections in 2021 and 2022 using the dry-preservation and rehydration method. Following statistical analysis, IVM MDA was seen to have no impact on parity rates between trial arms at any time point. The inter-rater reliability was almost perfect at all time points [26, 27]. This was the case even when trapping was increased to all trial clusters in 2022. As with density, two small studies looking at the impact on vector populations of IVM MDA using a dose of 150 µg/kg saw a significant reduction in parity rates from mosquitoes collected 2-3 days following MDA. However, when assessed on a larger scale, using a more robust cluster-randomised design, IVM MDA trials for malaria control showed no significant difference in parity rates when exposed to IVM MDA [20, 21, 28].

Over 11,000 *An. gambiae* s.l. were identified to species level using PCR-RFLP over six time points (2021: MDA 1, 2, 3 and PTS; 2022: MDA 3 and PTS). As *An. gambiae* s.s. had previously been reported as the primary vector on the Bijagós, comparisons of the percentage of *An. gambiae* s.s. present in the population were made between trial arms. No difference between arms was seen at any time point.

The species composition across the islands show a high percentage of *An. melas* and *An. gambiae* s.s./*An. coluzzii* hybrids throughout the Archipelago, mirroring the species compositions seen in coastal regions on mainland Guinea-Bissau [2, 3]. Relatively little behaviour data is available for *An. melas*. In some settings, it has been described as opportunistically feeding on humans, while in others, it has been documented to be highly anthropophilic, leading to questions about its role in transmission throughout the season on the Bijagós [29-31].

The high level of hybridisation found on the Bijagós and throughout Guinea-Bissau is unusual. Although *An. gambiae* s.s. and *An. coluzzii* often fill similar ecological niches, hybrid rates are usually below 1% [32]. On the mainland, hybrids have been seen to be more common in coastal areas [3]. Very little is known about the feeding preferences of hybrids, however a survey that occurred in Senegal found that hybrids fed on humans and livestock [33]. Further studies are required to understand the feeding-preferences and behaviours of hybrids on the Bijagós and their contribution to malaria transmission.

Following completion of the third MDA round and the PTS of 2021 and 2022, 200 *Anopheles* from all sampled clusters were analysed using CSP-ELISA to detect presence of sporozoites. In total, 16,493 samples were analysed, 93 of those were CSP-positive. The sporozoite rate varied between 0.0 and 1.8 % across trial arms and time points. When statistical analyses were performed, no significant difference was found between trial arms at any time point, indicating that the sporozoite rate was not significantly affected by IVM+DP MDA. These results are consistent with other large clinical trials published that investigated the use of IVM for malaria control [20, 21, 28]. The MASSIV trial in The Gambia found no difference between trial arms from mosquitoes caught using LTs or HLC. A smaller study in villages in Senegal, Liberia and Burkina Faso did find significant reductions when vector populations were exposed to 150 µg/kg of IVM [22]. These villages were sampled consistently for 20 days following MDA completion; by day 15, sporozoite rates in intervention villages had bounced back. A lower dose was used for this MDA, therefore we would expect a longer effect time at 300 µg/kg for three consecutive days.

Species identification using RFLP-PCR was done on 54 of the 93 CSP-positive *Anopheles* mosquitoes. *Anopheles melas* accounted for 63% of those analysed (overall sporozoite rate (SR): 0.64%), 15% were *An. gambiae* s.s./*An. coluzzii* hybrids (SR: 0.16%), and *An. gambiae* s.s. (SR: 0.54%) and *An. coluzzii* (SR:1.18%) each accounted for 11%. The presence of CSP-positive *An. melas* and hybrids indicates that they may play a role in malaria transmission on the Bijagós.

There are likely multiple factors contributing to the lack of effect of IVM MDA on malaria vectors on the Bijagós. Firstly, IVM's short half-life means that the coverage and speed of the distribution are important. While the coverage of the eligible population who received three-doses of IVM monthly during the MATAMAL trial was high, ranging from 58.1-77.1%, the proportion of the population at any given point whose blood contained IVM at a lethal dose to vectors was likely to be lower due to the speed of distribution. Secondly, to work, IVM-treated participants must come into contact with vectors. A large proportion of the vector population on the Archipelago consist of *An. melas* and An. gambiae s.s./*An. coluzzii* hybrids. Little is currently known about the feeding preferences and

behaviours of this species and hybrid form. Therefore, to better contextualise our results, more research is needed on the transmission dynamics of the Bijagós and the drivers of residual transmission.

Objective 4: To conduct household surveys on houses selected for entomological surveillance and perform a multivariable analysis to identify any risk factors associated with vector density.

(Research paper – Chapter 7)

The household survey shadowed the entomological sampling. In total, the household survey was conducted in 1,506 houses across the Archipelago. This was linked to 4,415 trapping nights to better understand the risk factors associated with mosquito entry. This was the first large scale survey on the Archipelago linking mosquito density data with household demographics and the built environment. As previously reported and seen in this survey, the use of insecticide-treated bednets (ITNs) was high, with 99.6% of respondents reporting sleeping under an ITN [34, 35]. Household socioeconomic status was calculated using a principal component analysis and found to be significantly associated with all built environment characteristics, with the exception of presence of open eaves or an external door in the trapping room. There was some variation in the roofing material used between houses, however little variation was seen for flooring and walling, where mud was used in the majority of houses (88% and 90% respectively). Eaves were open in 94.8% of houses.

Collection month, socioeconomic status and open eaves were associated with higher numbers of *An. gambiae* s.l. within houses. Open eaves have been identified as a risk factor for high mosquito house entry across multiple studies in sub-Saharan Africa [36-41], with one study demonstrating that by simply closing the eaves in a thatched house with a poorly-fitted door, numbers of indoor *An. gambiae* s.l. could be reduced by 94% [39]. Eave closure has been seen to have different effects when used in combination with different roofing material [39]. The impact of different combinations of built environment interventions on vector house entry needs to be evaluated in context to ensure the best possible results. The presence of *An. gambiae* s.l., in particular *An. melas*, in outdoor traps deployed during the survey in 2019, indicates that the population on the Bijagós may be at risk of outdoor biting. Further work is needed to confirm this; however, future interventions should consider the risk of both indoor and outdoor biting. Eave ribbons have been shown to reduce both indoor and outdoor biting [42]. In experimental hut trials conducted in Tanzania, eave ribbons were seen to reduce indoor catches by 77% and outdoor catches by 56% [42]. They also provide an opportunity for an integrated vector management approach, controlling both *Anopheles*- and *Aedes*-borne diseases.

Study limitations and lessons learnt

Each sections' limitations of the thesis have been discussed in their respective chapters. This section reviews the major limitations.

Many of the results reported in this thesis are from data collected during cross-sectional surveys. This study design has inherent strengths and weaknesses. Entomological studies often use this design, however it only provides data at a specific moment in time, and as vector populations are ever changing, it can often be challenging to draw conclusions from the data. By collecting samples at multiple time points and investigating multiple outcomes, this study tried to better understand the impact of IVM MDA on the vector population. Environmental variables, which have an impact on vector populations, were also collected throughout sampling to explore the study outcomes more accurately, adjusting for climatic variables during analysis [43].

The survey conducted in 2019 included both indoor and outdoor trapping, however, the same methodology was not used for the two trap positions. Comparing indoor and outdoor mosquito behaviour was therefore not possible. There are also inherent limitations with using the MB5 lure. Firstly, if deployed outdoors, it must compete with other host odours. In the case of a village setting in the Bijagós, were livestock sleep amongst houses, an outdoor synthetic lure is unlikely to attract a representative sample of mosquitoes. Secondly, difficulties also arise due to a lack of variation of odour blends in the lure. Different mosquito species, and even individual mosquitoes, have varying levels of attraction to different blends of semiochemicals released from vertebrate host bodies and breath [44, 45]. The MB5 lure is standardised, which will attract certain mosquitoes, however the trap catch is unlikely to be representative of the vector population as a whole. In future surveys on the Bijagós looking at both indoor and outdoor vector feeding-behaviour, it is recommended that a consistent methodology should be used for all trapping positions to better understand the risk of outdoor biting and its role in residual transmission. LTs hung at the base of occupied beds, protected by bednets, in both an indoor and outdoor setting would be a suitable alternative to better understand vector feeding behaviours across the Archipelago.

Variability in entomological outcomes between clusters was seen throughout the study. In 2021, following post-MDA collections from 18 trial clusters, the data was assessed and high levels of variability were seen in density and parity rates between clusters. The number of clusters sampled was therefore increased to help combat this (see appendices V-IX from cluster-level results and exploratory graphs). Increasing the number of clusters sampled created logistical challenges, therefore we were unable to sample after every MDA round in 2022. While it was thought that collections following completion of the last MDA round gave us the best possible chance of assessing

the impact of IVM MDA on the vector population, it meant that any trends throughout the MDA rounds in 2022 were unable to be assessed.

The assessment of the impact of IVM MDA on vector age structure was conducted following completion of MDA rounds. Our analysis focused on differences between trial arms, however it would have been beneficial to conduct parity assessments on the populations prior to MDA implementation. A baseline parity survey was attempted during the 2019 survey described in Chapter 3. This survey attempted to dissect freshly-killed mosquitoes in situ on each island and was plagued with logistical challenges, including a lack of suitable location (protected, well-lit and furnished) and electricity. The attempt to do this highlighted the need to find an appropriate technique that would enable samples to be analysed in a central laboratory, where there could be greater control over data quality. Vector population age structure is an important entomological parameter to assess the impact of an intervention, it would have therefore been interesting to have known the parity rate prior to MDA to better understand how the age structure changes throughout the transmission season.

A further limitation of the project was the inability to assess the bioefficacy of IVM on the vector population on the Bijagós Archipelago. This was mainly due to logistical constraints. Membrane-feeding rates in wild-caught mosquitoes are notoriously low, therefore it would have required a vast amount of rearing to provide sufficient mosquitoes, which the trial site does not have the capacity to accommodate. However, the recent development of a field bioassay to assess ivermectin bio-efficacy using a combination of glucose solution and blood spiked with IVM, may provide an alternative to standard membrane-feeding assays [46]. Future studies on ivermectin susceptibility in the vector population on the Bijagós are required to better contextualise the results of the trial.

The household survey conducted was targeted at better understanding the risk factors associated with house mosquito entry. However, it would have been beneficial to link the malaria prevalence surveys to a subsample of the households surveyed in the PTS to better understand how housing may impact malaria prevalence.

Future studies

From this PhD, there are several areas of research that should be explored to better contextualise the results, and further the knowledge base of malaria transmission dynamics on the Bijagós Archipelago.

Firstly, the use of the dry-preservation and rehydration method presented in Chapter 5 was validated for parity assessment using the ovarian tracheation method. However, further validation could be done using other dissection techniques. These may include more complex parity assessments, such as the Polovodova technique, a challenging dissection method that allows researchers to assess how

many gonotrophic cycles a female mosquito has gone through, thereby giving more granularity to age assessment. The dry-preservation and rehydration method may also be beneficial for dissections of the salivary glands to detect sporozoites. Ungureanu described that salivary glands could be dissected from dry-preserved and rehydrated specimens, however, did not give details about the length of time a specimen could be preserved for prior to rehydration. Where appropriate, these techniques could be used on a large-scale in a programmatic setting, enabling more data to be collected and ultimately contribute to more evidence-based decisions on vector control.

Residual transmission persists on the Bijagós Archipelago, regardless of wide-spread bednet use. Ivermectin (IVM) mass drug administration (MDA) showed no additional gains in controlling malaria or the vector population. Further work is needed to better understand the transmission dynamics, in particular regarding vector feeding-preferences and human-vector interactions. To do this, a study investigating human behaviour, in particular nighttime routines through observational studies, and a bednet survey examining knowledge, attitudes and practices, would be beneficial. Combining this data with a more accurate human biting rate would help develop a clearer understanding of who is most vulnerable to infective bites and when they are most at risk of exposure. As little is known about *An. melas* and *An. gambiae* s.s./*An. coluzzii* hybrid feeding-preferences on the Bijagós, outdoor biting could be incorporated, to better understand species specific behaviours. All the data collected from these surveys could be fed into models to better contextualise the trial results, and better predict how IVM MDA may perform in a different setting.

In addition to further investigations into residual transmission on the Bijagós, IVM susceptibility testing of *Anopheles* species present would be beneficial. Direct human-feeding assays on treated individuals with *Anopheles* mosquitoes reared in the laboratory on Bubaque island would give reassurance that the vector population is fully susceptible to IVM. No previous testing has been done on *An. melas* or hybrids due to a lack of an established colony, therefore these assays would be particularly informative.

Improved housing provides an opportunity for a sustainable, long-term approach to disease control. The household survey and risk factor analysis performed during this PhD could inform further studies on appropriate house improvements to reduce vector house entry on the Bijagós. As with many studies throughout sub-Saharan Africa, open eaves were associated with higher *Anopheles* density within houses across the Archipelago. Experimental hut trials could test potential housing improvements, with a focus on eave-based interventions. Eave closure, eave tubes and eave ribbons could all be testing alongside different house characteristic (for instance, roofing material) to help establish the best combination of interventions. Community perceptions and acceptability would also

need to be monitored to ensure long-term uptake. Following on from this, a cluster-randomized controlled trial could be performed looking at various disease outcomes associated with poor housing in the Bijagós.

As populations grow and resources become more finite, the development of more sustainable approaches to disease control are needed. Interventions that have an impact on multiple disease are required to help national programmes meet targets and progress towards disease elimination. Vector surveillance on the Bijagós must continue with a focus on multiple vector-borne diseases. Little is known about the prevalence of arboviruses across the Archipelago, therefore integrated vector surveillance, combined with serological assays to investigate past infection history could guide future management practices. As the majority of malaria and arbovirus transmission occurs in and around houses and buildings, this data, combined with results from the proposed future studies described above on the built environment, could help establish appropriate interventions to impact multiple vector-borne diseases.

Study Conclusions

The vector population of the Bijagós Archipelago was largely unexplored, in particular on the more remote islands. This was the first study to assess the vector population across all inhabited islands on the Archipelago, establishing that *An. gambiae* s.l. was present on all islands sampled.

The primary aims of this study were to assess the impact of IVM in conjunction with DP MDA on malaria vector age structure, density, species composition, sporozoite rate and EIR across the Bijagós Archipelago. From the baseline survey, it became clear that a method was needed to preserve and analyse samples at a centralised laboratory. A method was therefore validated to dry-preserve and rehydrate samples prior to dissection for parity assessment. Validation was successfully conducted with laboratory-reared *An. coluzzii* at LSHTM and with wild-caught *An. gambiae* s.l. at the laboratory on Bubaque island in the Bijagós. The dissection method is more challenging, however, once trained, provides practitioners the ability to assess greater numbers of samples and may be deployed on large scale programmes. Currently, the method has been validated to assess mosquito ovaries using the ovarian tracheation method, however, it could be used for other dissection techniques, such as sporozoite detection in mosquito salivary glands or sperm detection in the spermatheca.

Regardless of good coverage of ITNs, malaria transmission on the Bijagós Archipelago persists. The distribution of IVM to target vector populations continues to be trialled in multiple sites across the world, with the intent to target vectors that evade mainstay control measures and contribute to residual transmission. The malaria transmission season in the Bijagós runs from June to December. The three MDA rounds occurred in July, August and September, with the hope of reducing

transmission throughout the season. In 2021, cross-sectional surveys were conducted following completion of each MDA round in 18 of the 24 clusters and in all clusters in the PTS. In 2022, all 24 clusters were sampled following completion of the last round of MDA and during the PTS. Results from all time points in both 2021 and 2022 indicate that there was no impact of IVM MDA on the vector population density, age structure, species composition, sporozoite rate or EIR across the Archipelago.

Multiple factors may contribute to the lack of effect of IVM MDA on the vector populations. IVM has a short half-life, and although a mosquitocidal effect has been seen in laboratory-reared *An. gambiae* s.s. fed on blood taken from participants up to 28 days post treatment, the effect on wild vector populations may not be as long-lasting [25]. A high percentage of *An. melas* and *An. gambiae* s.s./ *An. coluzzii* hybrids is seen throughout the season on the Bijagós. IVM susceptibility testing has yet to be done on these species, and whilst there is no known physiological reason for them not to be affected by IVM, it is still important to investigate and ensure that they are fully susceptible to IVM. It is also important to better understand both the human and vector behaviour that may be contributing to the ongoing transmission. Should the human population go to bed earlier and be protected by bednets, or the vector population is host-seeking later in the evening when humans are already protected, then IVM may not have an opportunity to impact the vector population. The drivers of transmission need further investigation to better understand why IVM MDA does not have an effect on the mosquito vectors of the Bijagós.

The findings of this study indicate that IVM MDA for malaria control is not an appropriate intervention to be used in this setting. Should the effect on the vector population not be as long-lasting as previously thought, the intervention would need to be administered more regularly. This raises questions about the feasibility of the intervention to be deployed by national control programmes, which, as is the case in Guinea-Bissau, are already under considerable strain. Long-lasting formulations of IVM would mitigate this problem, however, currently it would not be appropriate to distribute these formulations to women of child-bearing age, raising further questions about coverage.

There are clinical trials investigating IVM MDA for malaria control that have yet to publish their findings. These trials will help us to better understand the impact of IVM MDA in different settings. However, from the results presented here and from trials that have already been published, there is little evidence to recommend IVM MDA as a future malaria control tool.

As part of this study, the first large household survey on inhabited islands was conducted and a multivariable analysis was performed looking at the risk factors associated with mosquito entry. It was also the first time the built environment of the Bijagós was characterised and potential risk factors identified. Unimproved housing is common throughout the Bijagós, with open eaves, thatch roofing

and mud walling and flooring accounting for the majority of houses. *Anopheles* house entry increased significantly when eaves were open, therefore eave closure may be a useful tool to help prevent vector house entry and ultimately malaria in the future. Improving housing worldwide is a sustainable approach to disease prevention, building out malaria and other diseases associated with poverty, such as lymphatic filariasis and chagas disease. Characterising the built environment of each setting is key to determining the measures that would be most effective for disease control.

In summary, this study characterised the vector population of the Bijagós Archipelago and assessed the impact of IVM MDA on mosquito density, age structure, species composition, sporozoite rate and EIR. There was no effect on any entomological outcome from IVM, which raises questions about the future suitability of the intervention in this setting. Future studies must be done to better contextualise the trial results and predict how the intervention would affect malaria transmission in another setting. The built environment was surveyed, and risk factors associated with higher mosquito entry were identified. This provides guidance to future house building practices and can be built upon to further reduce malaria transmission in the Bijagós.

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Appendix I. Protocol for a cluster randomised placebo-controlled trial of adjunctive ivermectin mass drug administration for malaria control on the Bijagós Archipelago of Guinea-Bissau: the MATAMAL trial.

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Student ID Number	1903438	**Title**	**Mrs**
First Name(s)	Elizabeth Anne		
Surname/Family Name	Pretorius		
Thesis Title	Evaluating the entomological effects of adjunctive ivermectin mass drug administration for malaria control in the Bijagós archipelago, Guinea-Bissau.		
Primary Supervisor	Dr Anna Last		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
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SECTION E

Student Signature	[redacted]
Date	11-03-2024

Supervisor Signature	Anna Last
Date	18-03-2024

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Protocol for a cluster randomised placebo-controlled trial of adjunctive ivermectin mass drug administration for malaria control on the Bijagós Archipelago of Guinea-Bissau: the MATAMAL trial

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ABSTRACT

Introduction As malaria declines, innovative tools are required to further reduce transmission and achieve elimination. Mass drug administration (MDA) of artemisinin-based combination therapy (ACT) is capable of reducing malaria transmission where coverage of control interventions is already high, though the impact is short-lived. Combining ACT with ivermectin, an oral endectocide shown to reduce vector survival, may increase its impact, while also treating ivermectin-sensitive co-endemic diseases and minimising the potential impact of ACT resistance in this context.

Methods and analysis MATAMAL is a cluster-randomised placebo-controlled trial. The trial is being conducted in 24 clusters on the Bijagós Archipelago, Guinea-Bissau, where the peak prevalence of *Plasmodium falciparum* (*Pf*) parasitaemia is approximately 15%. Clusters have been randomly allocated to receive MDA with dihydroartemisinin–piperaquine and either ivermectin or placebo. The primary objective is to determine whether the addition of ivermectin MDA is more effective than dihydroartemisinin–piperaquine MDA alone in reducing the prevalence of *P. falciparum* parasitaemia, measured during peak transmission season after 2 years of seasonal MDA. Secondary objectives include assessing prevalence after 1 year of MDA; malaria incidence monitored through active and passive surveillance; age-adjusted prevalence of serological markers indicating exposure to *P. falciparum* and anopheline mosquitoes; vector parous rates, species composition, population density and sporozoite rates; prevalence of vector pyrethroid resistance; prevalence of artemisinin resistance in *P. falciparum* using genomic markers; ivermectin's impact on co-endemic diseases; coverage estimates; and the safety of combined MDA.

Ethics and dissemination The trial has been approved by the London School of Hygiene and Tropical Medicine's Ethics Committee (UK) (19156) and the Comite Nacional de Eticas de Saude (Guinea-Bissau) (084/CNES/INASA/2020). Results will be disseminated in peer-reviewed publications and in discussion with the Bissau-Guinean Ministry of Public Health and participating communities.

Trial registration number NCT04844905.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The use of a placebo control arm allows identification of ivermectin's effect on malaria and neglected tropical disease transmission, above that of dihydroartemisinin–piperaquine.
⇒ Blinding of participants, distributors, investigators and outcome-assessors greatly reduces the risk of bias.
⇒ The geographical separation of island clusters minimises effects of contamination and spillover between clusters.
⇒ Sample size of 200 over 24 clusters achieves power of 80% for the primary outcome measure.
⇒ The unique setting means results may not be directly generalisable elsewhere, although malaria endemicity and transmission dynamics appear similar to those elsewhere in the region.

INTRODUCTION

Malaria in sub-Saharan Africa has declined dramatically since 2000, with much of the decrease due to vector control methods such as insecticide-treated nets and indoor residual spraying.[1] However, these methods are threatened by increasing insecticide resistance in vectors[2] and their limited efficacy against outdoor-biting or outdoor-resting mosquitoes.[3] There is a clear need for additional

vector-control methodologies, including novel tools or novel uses of existing tools.

Mass drug administration (MDA) with artemisinin-based combination therapy (ACT) could reduce transmission where coverage of vector-control interventions is high by impacting the human parasite reservoir,[4] reaching even asymptomatic cases, which help perpetuate transmis- sion in such settings.[5] Although MDA temporarily reduces malaria prevalence, there is little evidence of prolonged effect[6] and modelling predicts that without additional vector-control measures, efficacy is short-lived.[7]

Dihydroartemisinin–piperaquine (DP) is an efficacious and safe antimalarial.[8–10] Piperaquine's long half-life makes it attractive for ACT MDA.[11–14] A cluster-randomised controlled trial in Zambia showed reduced community-level parasite prevalence after two rounds of DP MDA to households with at least one case, particularly in low-transmission areas.[15] DP MDA in The Gambia also resulted in a significant reduction in infection incidence; however, these gains were short-lived in higher transmission areas.[16] There is currently little evidence of DP resistance in Africa,[17] although surveillance data following MDA are lacking.[18]

Ivermectin (IVM) has been widely used in MDA campaigns against onchocerciasis and lymphatic filariasis (LF) in Africa for decades.[19–23] It is an effective endectocide and could be used in MDA to complement vector control strategies, particularly where existing measures have been maximised.[19 24] In West Africa, IVM MDA using 150 µg/kg decreased *Anopheles gambiae* survival and sporozoite rates (SR).[25] Modelling predicts that 3 consecutive days at this dosage would reduce infectious vector populations by 68% for 60 days.[26] Three daily doses of 300 µg/kg/day or 600 µg/kg/day were mosquitocidal at 28 days post treatment[27 28] and while there was a slight increase in minor adverse events (AE) at the higher dose, significantly higher doses have been safely used to treat head- lice and onchocerciasis.[20–23 29]

Several clinical trials have demonstrated that combined IVM/ACT MDA is safe, and that it remains an effective antimalarial treatment and endectocide.[27 28 30–32] IVM/ACT MDA has been shown to be more lethal to vectors than IVM MDA alone[33] and population-level transmission modelling predicts adjunctive IVM would boost the efficacy of DP MDA in reducing malaria prevalence in both high and low prevalence settings.[26 34] Clinical trial data are needed to confirm these findings.

The RIMDAMAL trial in Burkina Faso reported that communities receiving IVM-only MDA (150–200 µg/kg), given 5 times at 3 weekly intervals after a single dose of IVM and albendazole, saw reduced incidence of clinical malaria in young children compared with communities receiving the single dose alone.[31] However, independent statistical analysis has questioned these findings.[35] The MASSIV trial in The Gambia compared 2 years of IVM (300–400 µg/kg) and DP MDA on 3 consecutive days in 3 consecutive months, against no intervention.[32] It found significantly lower malaria prevalence in the intervention arm; however, it is impossible to separate the effect of IVM from DP due to the absence of MDA in the control arm. Further trials are, therefore, required not only to confirm optimal dosage and regimen for IVM/ACT MDA, but also its impact on malaria transmission.

The Bijagós Archipelago lies 50 km off the Atlantic coast of Guinea-Bissau. Eighteen of the 88 tropical islands are inhabited, supporting a population of approximately 25 000 fishermen, hunter-gatherers and subsistence farmers. There is a long dry season alternating with a rainy season (June–October). The mean temperature is 27.3 °C with little monthly variation.[36] Qualitative surveys and daily activity mapping have shown that population movement, commonly for farming or ceremonies, is more limited than in continental Guinea-Bissau, likely due to the islands' remoteness and lack of transport.[37]

Malaria transmission is highly seasonal, peaking at the end of the rains. Serial cross-sectional surveys, powered to estimate prevalence with ±3% precision, 80% power and 95% confidence, were conducted across the Archi- pelago prior to this trial and showed that, in 2018, qPCR prevalence of *Plasmodium falciparum* infection was 8.5% in January (95% CI 6.2 to 10.8, n=578), 12.3% in July (95% CI 9.4 to 15.3, n=486) and 17.5% in November (95% CI 16.0 to 19.1, n=2305) (manuscript in preparation). Infection is highest in the 5–14 years age group. Bed net coverage is estimated at 92% (95% CI 86% to 96%) with reportedly high usage (86%); however, intermittent preventative therapy in pregnancy (IPTp) coverage is lower and, in some areas, non-existent.[38] IRS is not used. One pilot round of seasonal malaria chemoprevention (SMC) was conducted on the islands during the 2020 rainy season. Indoor CDC light trapping indicates that *An. gambiae* sensu stricto is the predominant mosquito species during peak malaria transmission, and SR, determined by Circumsporozoite (CSP) ELISA, suggest it is the primary malaria vector.[39]

IVM-sensitive neglected tropical diseases (NTDs) such as LF, soil-transmitted helminths (STH) and scabies are co-endemic, the qPCR prevalence of any STH infection being 47.3%, for instance.[40] NTD studies and control measures have demonstrated that MDA is feasible, accept- able and effective in this setting.[41]

Malaria remains a significant public health problem on the Bijagós Archipelago despite high coverage with control measures. The discrete geographical nature of the islands and the associated limitation of population and vector movement further serve to highlight the Bijagós as an ideal study site.[42]

MATAMAL will fill an important knowledge gap as no trial has successfully assessed the effect on malaria of IVM in addition to DP MDA.[43] It will provide valuable data on the effects on co-endemic NTDs, with a view to future integrated strategies. It will complement previous studies in evaluating acceptability, feasibility and cost effec- tiveness, adding to the evidence-base examining IVM's role in malaria control. Data will be used to inform and

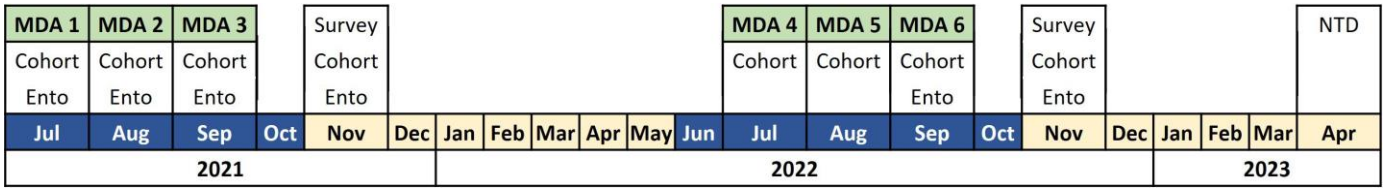

Figure 1 Timeline of the MATAMAL clinical trial showing months of MDA, cohort surveys, Ento, Survey and NTD survey. Rainy season months indicated in dark colour. Ento, entomological surveys; MDA, mass drug administration; NTD, neglected tropical disease; Survey, major cross-sectional surveys.

update existing models of the impact of MDA on malaria transmission.

METHODS AND ANALYSIS

Study AIM

To determine whether adjunctive IVM MDA co-administered with DP MDA significantly reduces the population-based prevalence of *P. falciparum* parasitaemia during peak malaria transmission season compared with DP MDA alone.

Study design

This is a quadruple-blind (participant, intervention provider, investigator and analyst) cluster-randomised placebo-controlled trial.

Twenty-four clusters have been assigned in a 1:1 ratio to one of two trial arms using restricted randomisation[44]:

1. Intervention (DP and IVM MDA).
2. Control (DP and IVM-placebo MDA).

Restriction variables included population, baseline *P. falciparum* prevalence (qPCR and RDT), vector density, SMC coverage and presence of a health centre. Of 100 000 randomisations, the final randomisation was selected from the approximately 10% satisfying the criteria.

This protocol was developed using SPIRIT reporting guidelines.[45 46] A timeline is shown in figure 1.

Population and setting

The trial is being conducted on the Bijagós Archipelago, Guinea-Bissau (figure 2). Government projections using the last formal census (2009) estimate a population of 25 589.[47] Individual islands will constitute 15 clusters. Three larger islands will each be subdivided into 3 clusters providing 24 clusters in total (figure 3). There will be buffer zones of at least 2.2 km between settlements in different clusters using a modified fried-egg principle.[44] Only one populated island, Soga, will be excluded as its very high baseline malaria prevalence is an outlier.

All residents of the islands, defined as anyone sleeping on the island for the majority of a given month, will be invited to participate unless they meet any of the following exclusion criteria:

1. Severe illness.
2. Age under 6 months (DP).
3. Height under 90 cm or weight under 15 kg (IVM/placebo).
4. Pregnancy (any trimester) or breast feeding (IVM/placebo). Pregnancy (first trimester) (DP).
5. Known hypersensitivity to either medication.
6. Concomitant use of drugs affecting cardiac function or the corrected QT interval (DP).
7. Travel to a country endemic for *Loa loa* (IVM/placebo).

Residents excluded from MDA will still be eligible for participation in surveys.

An extensive sensitisation campaign will take place ahead of the intervention, led by community health workers (Agentes de Saude Communitaria, ASCs). Informed written consent will be obtained after providing written/spoken information (according to literacy) in participants' own language. An independent witness will sign consent forms of illiterate participants. Parents/guardians will be asked to consent on behalf of all children under 18 years. Children aged 12–17 years will be invited to sign informed assent forms. Forms and participant information sheets for MDA are included as online supplemental materials 1–4. We anticipate very low rates of refusal based on experience with previous interventions, including MDA (for trachoma), in these communities and the perceived importance of malaria.[38]

Intervention and control

The intervention will be entire community MDA using:

1. DP (Alfasigma, Italy).
2. IVM (Laboratorio Elea Phoenix, Argentina).
3. IVM placebo (Laboratorio Elea Phoenix, Argentina).

Standard National Malaria Control Programme interventions (triennial distribution of bed nets, IPTp, case detection and treatment with artemether–lumefantrine) will continue in both arms. The intervention arm will receive DP and IVM; the control arm will receive DP and placebo (figure 4). A full course of MDA involves three sequential daily doses of both medications, given monthly in July, August and September of 2021 (year 1) and 2022 (year 2). Monthly MDA will begin in all clusters simultaneously, with at least 28 days between each round. Distribution will be conducted by ASCs in their own villages, supervised by experienced cluster-level field assistants.

Participant age, sex, weight, pregnancy and eligibility will be recorded by household. Example case record forms, in English, are included in online supplemental material 5.

DP is a rapidly acting artemisinin-based schizontocidal drug used to treat uncomplicated malaria. It is safe, well tolerated, efficacious in clearing *P. falciparum* infection

Figure 2 Map of Guinea-Bissau showing the Bijagós Archipelago off the south-west coast.[74]]

and exhibits prophylactic activity for approximately 4 weeks.[48]

IVM is an avermectin, active against a range of human parasitic infections and infestations. It is active against *Anopheles* spp at concentrations present in human blood post ingestion.[49]

All treatment will be directly observed. Doses will be tablet formulations taken orally with water, with no food for 3 hours before or after. IVM and IVM–placebo will be visually identical 6 mg tablets dosed at 300 µg/kg to the nearest tablet. DP will be available at doses of 20/160 mg and 40/320 mg per tablet, administered according to bodyweight. DP can be crushed and mixed with water, and will be readministered in case of vomiting within 30 min (half-dose if between 30 min and 60 min).

Before delivery to the field, an independent pharmacy team will relabel all IVM and placebo bottles with identical 'IVM or placebo' labels bearing pre-printed cluster codes and recoded lot numbers to prevent unblinding. Only the pharmacist and the independent statistician who conducted the randomisation and anonymised the labels will be unblinded. All laboratory samples will bear only alphanumeric codes. Statistical analysis will be conducted by a blinded statistician. Unblinding will only occur after completion of the primary analysis.

Outcome measures

Primary outcome

The population-based qPCR prevalence of *P. falciparum* parasitaemia in all age groups during the peak malaria transmission season after 2 years of intervention.

This was selected as the most appropriate method for quantifying malaria transmission due to its sensitivity, especially in submicroscopic infections, the reproducibility of methods and results for this well-established assay and comparability to other trials. qPCR prevalence can also be reliably obtained from dried blood spots (DBS), a cheap, simple and robust tool with good partic- ipant acceptability.

Secondary outcomes

1. Population-based prevalence of *P. falciparum* parasitaemia in all ages, detected by qPCR, during the peak transmission season after the first year of MDA.

Figure 3 Map of the Bijagós Archipelago. MATAMAL clusters marked by stars and separated by lines where sharing a landmass.

2. Incidence of clinical malaria confirmed by *Plasmodium spp.* lactate dehydrogenase/histidine-rich protein 2 (pLDH/HRP2) rapid diagnostic test (RDT), determined through passive surveillance of all malaria cases presenting to health facilities throughout the trial.
3. Incidence of clinical malaria identified by RDT (CareStart Malaria PAN pLDH) during active surveillance of a cohort of children aged 5–14 years, during the intervention and peak transmission season.

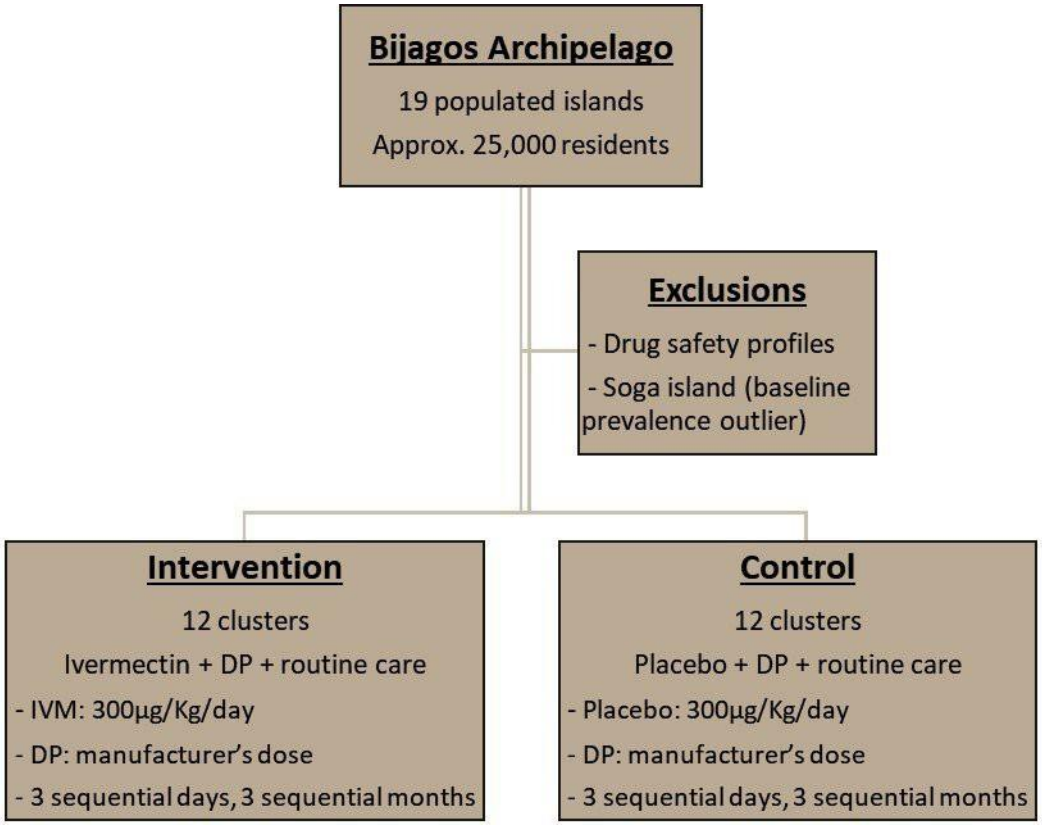

Figure 4 Design elements of the MATAMAL clinical trial. DP, dihydroartemisinin–piperaquine; IVM, ivermectin.

4. Incidence of malaria infection identified by qPCR and serological analysis during the same period in this cohort of children.
5. Age-adjusted prevalence of serological markers indicating recent exposure to *P. falciparum*.
6. Prevalence of serological markers of recent *Anopheles* exposure.
7. Parity, as a measure of *An. gambiae* sensu latu survival, measured in mosquitoes caught using indoor CDC light traps 7–14 days after the final MDA round in year 1 and year 2.
8. Mosquito species composition, population density and SR in mosquitoes caught using indoor CDC light traps.
9. Prevalence of resistance to pyrethroids in anopheline mosquitoes using bioassay methodologies.
10. Prevalence of resistance to artemisinin and partner drugs in humans using molecular markers of resistance.
11. Safety of intervention through monitoring of AE.
12. Impact on IVM-susceptible NTDs (scabies, strongyloidiasis, other STHs and LF), headlice and bedbug infestation using clinical and serological parameters.
13. Cluster-level intervention coverage estimates.
14. MDA acceptability, feasibility and access.
15. Cost effectiveness of adjunctive IVM in this setting.

Assessment of outcomes

Primary

A cross-sectional survey will be conducted across all clusters beginning 4 weeks after the completion of the second year of MDA, during peak transmission. Two hundred participants will be selected by a two-step randomisation (household and individual) from within a 'yolk' of villages in each cluster, purposively defined to capture sufficiently populated villages far from other clusters but logistically feasible to reach. Socio-demographic and GPS data will be collected alongside DBS for varATS qPCR analysis, a technique capable of detecting 0.03–0.15 parasites/ µL blood, 10 times the sensitivity of standard 18S rRNA qPCR.[50] Standard operating procedures are included as online supplemental materials 6–8. Participants will also be asked if they were resident in other clusters during MDA.

In November 2019, mean malaria 18S qPCR prevalence across the islands was 14.8% (95% CI 14.5% to 14.9%) (Last *et al*, mapping NTDs and Malaria on the Bijagós Archipelago of Guinea-Bissau, unpublished). These results informed a conservative estimate of the coefficient of variation of 0.46. Two hundred participants/cluster in 12 clusters/arm provides >80% power to detect a difference between arms if the primary outcome prevalence is 10% in the control arm and 5% in the intervention arm.

A mathematical model was generated to inform sample size calculations and cluster numbers. It was parameterised to simulate prevalence, seasonality, vector control and coverage. Assumptions included coverage (70% of eligible persons), DP efficacy (75% of recipients clearing *P. falciparum* parasites), baseline peak qPCR prevalence (21.2%), dominant vector species (*An. gambiae*), fixed time lag to onward infectiousness (12.5 days from infection to gametocyte presence) and ideal conditions such as no population movement and no contamination of participants or vectors across trial arms. A validated pharmacokinetic model was used for estimating participant IVM levels over time, and mosquito mortality was assumed to increase relative to this when feeding on a given day after receiving IVM. This model predicts control arm qPCR prevalence of 9.2% after 1 year and 6.5% at 3 years, compared with 3.9% and 0.8% in the intervention arm, an effect size of 87.8% over control (figure 5).

Secondary

1. A survey, identical to the primary outcome survey, will be conducted after 1 year of MDA.
2. On the last day of every month, data on RDT-confirmed incident malaria cases will be recorded from each of the 10 health centres on included islands: age, sex, cluster, fever, RDT performed, RDT result and treatment provided.
3. Cohorts of 50 children aged 5–14 years will be followed in 18 clusters. Households will be randomly selected from household-head lists of yolk villages. One child will be randomly selected to participate from each household, more if there are fewer than 50 eligible households. A new cohort will be selected in year 2. Participants will be visited 7–14 days after each round of MDA, as well as during the peak transmission surveys for a total of 8 timepoints. Age, sex, village, GPS and temperature will be recorded. Febrile children will be offered an RDT and treated if positive. All children will be asked about intercurrent clinical malaria episodes, trial adherence and bed net usage. A DBS will be taken for molecular and serological analysis.
4. Assessed as for outcomes 3 and 5.
5. Serological analysis will be performed on DBS taken during the cross-sectional and cohort surveys described above using a multiplex bead assay on the Luminex MAGPIX platform. The included antigens have antibodies, which are associated with recent or long-term exposure to, and/or protection from, *P. falciparum*: MSP1.19, AMA1, GLURP.R2, EBA175.RIII.V, EBA181.RIII.V, Etramp5.Ag1, Etramp4.Ag2, HSP40.Ag1, CSP, SBP1, Hyp2, GEXP18, Rh2.2030, Rh4.1 and Rh5.2.[51–53]
6. Assessed as for outcome 5.
7. One yolk village will be randomly selected per cluster. Households will be randomly selected in these villages using household-head lists (10 households/ cluster in year 1; 15/cluster in year 2). A CDC light trap will be placed inside each household overnight for 3 consecutive nights, 7 days after each round of MDA in year 1 (18 clusters) and 7 days after the 3rd round of MDA in year 2 (24 clusters). There will be three further nights of trapping during the peak

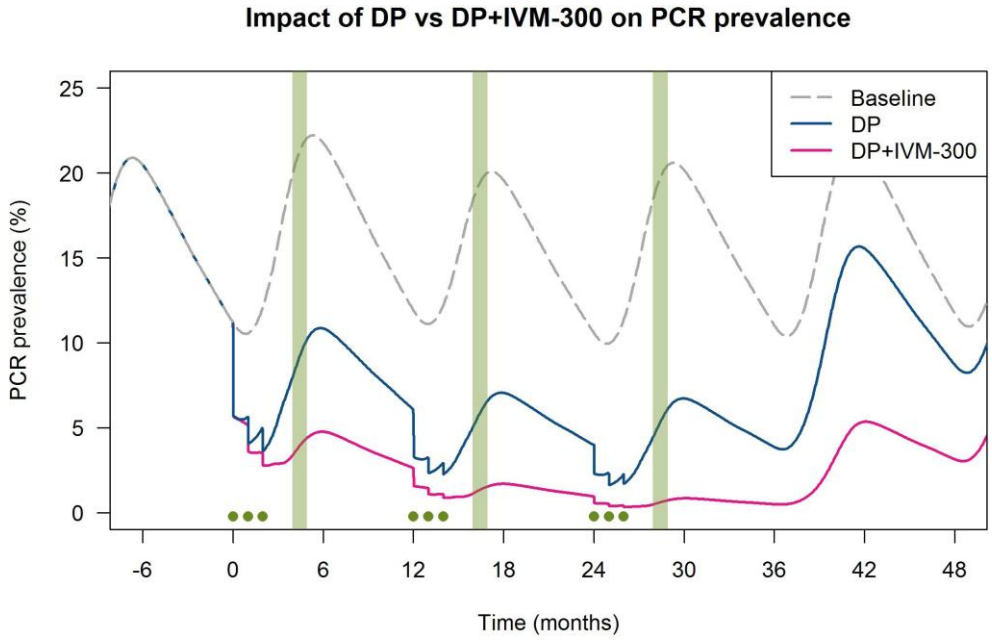

Figure 5 Graph showing modelled qPCR prevalence of malaria over time, assuming 70% coverage and 75% DP efficacy. Green dots: MDA rounds. Green bars: survey periods. Dashed line: no intervention. Blue line: MDA with DP only. Pink line: MDA with DP and IVM at 300 µg/Kg. DP, dihydroartemisinin–piperaquine; IVM, ivermectin; MDA, mass drug administration.

transmission survey. *An. gambiae* s.l. will be identified morphologically.[54] Parity, estimating mosquito survival, will be done using the Detinova ovarian tracheation method.[55]

8. These mosquitoes will also be used to estimate cluster-level and trial arm-level species composition (proportion of species present within *An. gambiae* complex), population density (number of mosquitoes/trap/night and a proxy for human biting rate) and sporozoite detection using CSP ELISA.[56] The entomological inoculation rate (EIR), a proxy for human exposure to infectious mosquitoes and a key indicator of local transmission, will be estimated using these data.[57]
9. Anopheline mosquitoes will be reared from locally caught larvae during the year 2 rainy season. Batches of 100 adult females will be tested for resistance to alpha-cypermethrin, permethrin and deltamethrin, with and without the synergist piperonyl butoxide, using CDC bottle bioassays and WHO test tubes at once, two and five times the discriminating dose.[58 59] Two hundred mosquitoes, including all resistant individuals, will undergo PCR species identification.[60]
10. DBS samples positive for *P. falciparum* DNA will undergo further extraction, amplification by PCR[61] and sequencing using Illumina-based technology to identify genetic mutations previously associated with antimalarial drug resistance, including polymorphisms in *pfkelch*, *pfmdr1*, *pfcrt* and *pfexo* genes, before being analysed using bioinformatic tools.[62 63] Selective whole genome amplification steps will be conducted on the initial DNA extract to increase the quantity of *P. falciparum* DNA. A cohort of RDT-positive malaria patients will also be recruited from select health centres during the rainy season to provide serial DBS throughout treatment. These will then undergo qPCR analysis for evidence of treatment success/failure and identification of resistance markers as described above.
11. AE data will be collected actively by distributors during MDA and for 48 hours after, and passively by sensitising participants and health centre nurses to report AEs. ASCs will monitor births, deaths and miscarriages. Supervisors will send daily reports to the trial manager, who will generate annual reports for the Data Safety Monitoring Board (DSMB). Cross-sectional and cohort surveys include post hoc questions on AEs. All doses will be directly observed. *Loa loa* is not endemic to this region, greatly reducing the risk of IVM-associated encephalitis. Appropriate health-seeking advice will provided by distributors to all participants reporting AEs.
12. A population-based cross-sectional survey will be performed in all clusters during the 2023 dry season, that is, after all MDA has been delivered: 100 households will be randomly selected from household-head lists in each cluster yolk, and 1 child aged 2–10 years invited to participate from each household. Multiple children will be invited if the sample size is not met. Skin will be systematically examined to identify scabies, and impetigo in positive cases. Headlice examination and fine-tooth combing above a white cloth will occur over 10 min. Bedframes, mattresses, nets and adjacent walls will be inspected with torchlight for signs of bedbugs, or eggs, over 10 min. A stool sample (5 g) will be collected and stored in ethanol to undergo multiplex parasite PCR (STH, Strongyloides).[64–66] DBS will be collected for serological testing for LF, strongyloidiasis and STH.[67–69]

13. MDA distributors enter administration data into forms listing every householder, including absent members, and capture data on daily refusals, absences and exclusions. These forms generate a de facto census and can be used calculate monthly cluster-level coverage, absence, refusal and exclusion statistics.
14. Qualitative and quantitative analysis of MDA acceptability, feasibility and access, including population movement, will be outlined in a separate protocol.
15. Cost-effectiveness analysis will be detailed in a separate protocol.

Data analysis plan

Data will be entered by trained MDA delivery teams under the supervision of highly trained and experienced local supervisors. Senior trial management will regularly review data entry and offer retraining as needed. Sample ID numbers will be electronically captured or double entered. A communication network will be established with remote villages to facilitate monitoring and correction of errors found on review.

All analysis will be intention-to-treat. Analysis will be performed using STATA software (StataCorp) and R.

Cluster-level, arm-level and overall demographic characteristics will be presented using descriptive statistics, including age, sex, bed net use, household size and village/cluster populations.

Primary outcome

Trial arms will be compared using a t-test on cluster-level malaria qPCR prevalences. A risk difference, 95% CI and p value will be presented. Analyses adjusting for age groups (<5 years, 5–14 years, 15 years and above), bed net use and the presence of a health centre will be presented for this and all secondary outcomes.

Secondary outcomes

1. As for the primary outcome, using data from the year 1 survey.
2. Cluster-level incidence rates will be compared using a t-test. If the distribution is markedly skewed, then the natural logarithm of each cluster-level summary will be taken. If some clusters have zero events, then 0.5 events will be added to each cluster. Rate differences, 95% CI and p value will be presented.
3. As for outcome 2, using RDT-confirmed incidence rates from the two annual cohorts of children.
4. As for outcome 3, using qPCR-confirmed incidence rates. Serological analysis described in outcome 5.
5. Cluster-level serological responses to malaria antigens will be presented as both continuous median fluorescent intensity (MFI) data, a proxy for anti- body titres, and binary seropositivity prevalence, with a seropositivity threshold of three standard deviations above the mean malaria-naïve MFI re- sponses. MFI will be compared between arms us- ing mixed effect linear regression, with a random effect for cluster. Seropositivity will be compared in the same way as the primary outcome, including analysis by age group. Cluster-level antigen-specific seroconversion rates (SCR) will be generated using serocatalytic models relying on seroprevalence, and age-seroprevalence plots will be generated by fitting data to a reversible catalytic conversion model using maximum likelihood methods. Trial arms' SCR will be compared using a t-test.[53]
6. As for outcome 5, using the gSG6 antigen and not employing SCR.
7. Cluster-level anopheline parity will be compared between arms using a t-test. Adjustment will be made for species, MDA round, time relative to MDA date, temperature, humidity and rainfall. Parity difference, 95% CI and p value will be presented.
8. The proportion of each species identified, and mosquito densities, will be presented by cluster and arm, and compared using χ^2 and t-tests. Adjustment will be made for study month and year. Differences, 95% CIs and p values will be presented. If the distribution of densities is markedly skewed, a log transformation will be applied. SR will be compared between arms using a t-test on cluster-level SR with adjustments and covariates as described for parity analysis above. Risk difference, 95% CI and p value will be present- ed. EIR will be compared with a t-test on cluster level summaries.
9. Species composition and level of resistance will be presented using descriptive statistics. Dose–response curves will be generated for each insecticide and probit regression analysis will be performed on these data using maximum likelihood or least squares methods.
10. The number of alleles associated with antimalarial resistance will be compared over time, before and after MDA. However, appropriate statistical tests will need to be selected depending on the allele sample size before and after MDA.
11. Descriptive statistics will be presented on the number, nature, severity and relatedness of all reported AE, with additional details for serious AE. Cluster-level and age-group data will be presented. Rates will be compared between arms.
12. As for the primary outcome and outcome 5.
13. Cluster-level, arm-level and overall coverage will be presented for each month and year of intervention, and for the trial overall. The denominator for these proportions will be the total number of people recorded on MDA administration records by distributors in each cluster, whether receiving MDA or not. Refusals, exclusions and absences will be presented similarly.
14. Qualitative and quantitative analysis of MDA acceptability, feasibility and access will be outlined in a separate protocol.
15. Cost-effectiveness analysis will be detailed in a separate protocol.

Patient and public involvement

Qualitative studies report that this population almost unanimously consider malaria to be a significant problem in their homes and the region, that additional malaria control measures would be welcome, and that MDA is acceptable,[38 70] all of which informed MATAMAL's design. All field assistants and the deputy trial manager are local residents and MDA will be delivered by ASCs within their own communities. Methods were finalised in discussion with these stakeholders, the Regional Health Directorate and MINSAP. There will be monthly feedback sessions with ASCs and staff during MDA and surveys. Trial outcomes, social science, public engagement and qualitative work will be presented to stakeholders and communities using plans developed with the contributors themselves.

Trial status

All necessary approvals are in place. A baseline malaria prevalence survey was completed in November 2019. Two years of MDA has successfully been delivered across all clusters, alongside two annual cohort surveys and entomological sample collection. Two annual peak transmission surveys and a dry season NTD survey have been completed. Qualitative and social science work has taken place throughout. Laboratory testing is ongoing ahead of statistical analysis.

ETHICS AND DISSEMINATION

MATAMAL will be reported according to CONSORT guidance.[71] It is a collaboration between LSHTM, Medical Research Council The Gambia and MINSAP. Ethical approval has been obtained from LSHTM Research Ethics Committee (UK) (19156) and CNES (Guinea-Bissau) (084/CNES/INASA/2020), with additional regulatory approvals from The Gambia. Any changes to the protocol will be agreed by investigators and submitted to these same bodies for approval. The independent DSMB will oversee trial safety through annual meetings, advising the Trial Steering Committee on ongoing trial conduct. Members of these committees are listed in online supplemental material 9. Independent audit may be carried out by LSHTM, our funders or MRC The Gambia. This work abides by the Declaration of Helsinki[72] and Good Clinical Practice.[73]

Electronic forms are encrypted on submission to a LSHTM HERA-compliant secure server. Paper forms will be kept in limited-access locked storage at the site office. Data will be de-identified, except for consent and MDA record forms. All data will be held for a minimum of 7 years, including in LSHTM electronic repositories, whence access to de-identified data can be requested. All investigators will have access to cleaned databases.

Results will be published in peer-review journals, presented at conferences and to local collaborators. Field staff, nurses and ASCs will disseminate findings to participating communities in appropriate and accessible formats. Authorship will be decided on paper-by-paper basis, to recognise significant contributions to design, conduct, analysis and reporting. Writing will not be out-sourced.

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Contributors ARL, the principal investigator, conceived the initial design of the study, secured funding and contributed significantly to writing this protocol. HH, the trial manager, runs all field operations, consulted on field methods and generated this manuscript. EP, RTJ and JGL drafted the entomology methods. JB provided statistical expertise. HS modelled the trial's anticipated impact. CD and HV provided serological expertise. MON and HdMS provided laboratory supervision and support. ETdS, CM, AR and EJN, our local collaborators, provided extensive information on local context and logistics. UD and DM supervised the writing of this protocol and provided expertise on trial conduct.

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Competing interests JGL declares he is founder/CEO of Arctech Innovation, a company which aims to design mosquito lures and malaria diagnostics.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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Appendix II. Participant information sheet

Participant Information Sheet

Title of Project: Adjunctive Ivermectin Mass Drug Administration for Malaria Elimination: A cluster randomized placebo-controlled trial

Introduction

We would like to invite you to take part in a research study. Joining the study is entirely up to you. Before you decide, you need to understand why the research is being done and what it would involve. One of our team will go through this information sheet with you, and answer any questions you may have. Ask questions if anything you read is not clear or you would like more information. Please feel free to talk to others about the study if you wish. Take time to decide whether or not to take part.

What is the purpose of the study?

The London School of Hygiene and Tropical Medicine (LSHTM) are conducting research into whether the combination of drugs (the antimalarial Dihyrdoartemisinin-piperaquine (DP) and the anti-parasitic drug Ivermectin) can be used to control malaria when given to the whole population. DP works directly to help fight the malaria parasite within humans, whilst Ivermectin helps by killing malaria-transmitting mosquitoes that bite humans that have taken the drug. We need new ways to continue to help control malaria, and it is important to test whether the intervention works.

Why have I been asked to take part?

You have been invited to join the study because we need to look at the mosquito populations within people's houses. If mosquito populations change then we can see whether the drug is working to help reduce infective mosquito numbers. We would also like to know if there are any features of your house that may let mosquitoes into your house more easily. This can help us to find better ways to build houses in the future to reduce malaria. All the households within the study were randomly selected using a head of household list generated by your community health worker and a random number generator

Do I have to take part?

No. It is up to you to decide to take part or not. If you don't want to take part, that's ok. Your doctor will still care for you should you present with any adverse events resulting from the DP and ivermectin/placebo distribution and your decision will not affect the quality of care you receive.

We will discuss the study together and give you a copy of this information sheet. If you agree to take part, we will then ask you to sign a consent form.

What will happen to me if I take part?

We will come to your house at 19h00 in the evening and set up one mosquito trap. The trap will be inside your house, and we will hang it 100cm off the ground at the foot of your bed, just outside your mosquito net. We will turn it on and it will stay on throughout the night. We will collect it in the morning at 07h00. The trap will emit a small amount of light to attract the mosquitoes. This will happen for three consecutive nights. The team may also ask you if they can put a mosquito trap outside your house, this is to see what types of mosquitoes are biting outdoors.

You will also be asked to take part in a household survey, this will involve answering questions about your family and house and will be conducted by one of our team members. This will take about half

an hour to complete. They will need to go into your house to take measurements of the room where the mosquito trap is being set up.

What will I have to do?
You will need to let us into your house at 19h00 and 07h00 so that we can set-up and collect the traps. We will also need half an hour of your time to answer the any questions for the survey.

What are the possible risks and disadvantages?
There is no increased risk or disadvantage from taking part in this study.

What are the possible benefits?
We cannot promise the study will help you but the information we get from the study will help our knowledge and understanding of malaria control.

What if something goes wrong?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions <969 171 828 >. If you remain unhappy and wish to complain formally, you can do this by contacting Patricia Henley at rgio@lshtm.ac.uk or +44 (0) 20 7927 2626.

The London School of Hygiene and Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation.

Can I change my mind about taking part?
Yes. You can withdraw from the study at any time. If you withdraw from the study we will need to use the data collected on you up to your withdrawal.

What will happen to information collected about me?
All information collected about you will be kept private. Only the study staff and authorities who check that the study is being carried out properly will be allowed to look at information about you. Data may be sent to other study staff in London or Bubaque, Guinea-Bissau, but this will be anonymised. This means that any information about you will have your name and address removed so that you cannot be recognised.

Your personal details will be stored securely on Bubaque.

At the end of the project, the study data will be archived and maintained at LSHTM in London. The data will be made available in a sharing repository accessible to other researchers worldwide for research and to improve medical knowledge and patient care. Your personal information will not be included and there is no way that you can be identified. Please also be advised that you can consent or refuse to have you data added to any data sharing repository.

Paper records (consent forms) will be managed and stored in the secure study site office in locked cabinets. After study completion, all the relevant study documentation will be retained in accordance with the local legislation, for a minimum period of ten years after completion of the study.

What will happen to the results of this study?
The study is part of a PhD project conducted by a student at the London School of Hygiene and Tropical Medicine. Results from the study will be published in a medical journal so that others can learn from them. The results will also be published in the student's PhD thesis at the end of the

project. Your personal information will not be included in the study report and there is no way that you can be identified from it.

Who is organising and funding this study?
London School of Hygiene & Tropical Medicine is the sponsor for the research and they have full responsibility for the project including the collection, storage and analysis of your data.

Who has checked this study?
All research involving human participants is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by The London School of Hygiene and Tropical Medicine Research Ethics Committee. The Comité Nacional de Ética na Saúde has also reviewed the study and have agreed that it is okay for us to ask people to take part.

Further information and contact details
Thank you for taking time to read this information leaflet. If you think you will take part in the study please read and sign the consent form. If you would like any further information, please contact Elizabeth Pretorius who can answer any questions you may have about the study. Contact details:

Liz Pretorius (969 171 828)
Harry Hutchins (956 580 914)
Eunice da Silva (955 386 560)

Appendix III. Consent form for participant and representative

CONSENT FORM FOR PARTICIPANT AND REPRESENTATIVE

Title of Project: Adjunctive Ivermectin Mass Drug Administration for Malaria Control on the Bijagós Archipelago of Guinea Bissau: A cluster randomised placebo-controlled trial

Name of PI/Researcher responsible for project: Anna Last

Statement	**Please initial or thumbprint* each box**
I confirm that I have read and understood the information sheet dated 02/06/2021 (version 1.0) for the above named study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
I understand that my consent is voluntary and that I am free to withdraw this consent at any time without giving any reason and without my/the participant's medical care or legal rights being affected.	
I understand that relevant sections of my/the participant's data collected during the study may be looked at by authorised individuals from London School of Hygiene and Tropical Medicine, where it is relevant to my/the participant's taking part in this research. I give permission for these individuals to have access to these records.	
I give consent to data about/from me/the participant being shared via a public data repository or by sharing directly with other researchers, and that I will not be identifiable from this information	
I give permission for a copy of this consent form, which contains my/the participant's personal information, to be made available to the Trial Coordinating Centre for monitoring purposes only.	
I agree to me/the participant taking part in the above named study.	

Printed name of participant/Representative (or thumbprint/mark if unable to sign)	Signature of participant/Representative	Date
Printed name of person obtaining consent	Signature of person obtaining consent	Date

The participant/representative is unable to sign. As a witness, I confirm that all the information about the trial was given and the participant/representative consented to taking part *(*only required if the participant/representative is unable to read or write)*

Printed name of impartial witness*	Signature of impartial witness*	Date

A copy of this informed consent document has been provided to the participant.

Participant Identification Number:

[Informed Consent for Participant and Representative for adults_14.09.2021_v1.1]

Appendix IV. Household Survey

MATAMAL Entomology
Household questionnaire Survey (English) v2.0 20/06/2022

Date (dd/mm/yy)	__ __/ __ __/ __ __
Interviewer Initials	|_____|
Identification	
Cluster Name	
Village Name	
Head of Household	

TAKE GPS COORDINATES

Resident Information					
No	**Question description**	**Options**	**Answer**	**If**	**Goto**
1	How many people live in your house?	Number (Don't Know = 98)	_ _		
2	How many adults live in your house?				
3	How many people under 18 live in your house?	Number	_ _		
4	How many children under 5 years old live in your house?				

Socioeconomic Status					
No.	**Question description**	**Options**	**Answer**	**If**	**Goto**
5	Has the head of the household ever attended school?	Yes	1		
		No	0	**0**	**7**
		Don't Know	98	**98**	**7**
6	What is the highest level of school the head of the household attended:	Primary	1		
		Secondary/technic	2		
		Higher	3		
		Don't Know	98		
7	Is the head of the household married?	Yes	1		
		No	0	**0**	**8**
		Don't Know	98	**98**	**8**
8	Has the head of the household WIFE ever attended school?	Yes	1		
		No	0	**0**	**10**
		Don't Know	98	**98**	**10**

9	What is the highest level of school the head of the household WIFE attended:	Primary	1		
		Secondary/technic	2		
		Higher	3		
		Don't Know	98		
10	How many rooms are there in this household? >>***Include all structures (huts etc)***	Number (Don't Know = 98)	_ _		
11	How many sleeping places are there in this household (beds, mattresses or mats)? ***>>Ask for both inside the hut and outside***	Number	_ _		
12	How many bednets does the household have?	Number (Don't Know =98)	_ _		
13	Where does the household's main income come from?	Fishing/Farming/ Selling crops	1	**1**	**15**
		Business/Shop	2	**2**	**15**
		Medical/ Teacher/ Government	3	**3**	**15**
		Other	98		
14	If Other kind of income, please specify	Free text			
15	Does the household (any member) have any of the following?	**Check box**			
		Electricity	|__|		
		Radio	|__|		
		mobile phone	|__|		
		Bicycle	|__|		
		Motorbike	|__|		
		Car or truck	|__|		
		Canoe or boat with motor	|__|		
		Sewing machine	|__|		
		Livestock	|__|		
		Television	|__|		
		Canoe or Boat without motor	|__|		
16	Does the household own land used for farming?	Yes	1		
		No	0		
		Don't Know	98		

If Livestock is selected in Q15, complete Livestock Section
If Livestock is NOT selected in Q15, then move to Protection Against Section Section

Livestock Section					
No.	**Question description**	**Options**	**Answer**	**If**	**Goto**
17	What animals does the household own?	Poultry/birds	|__|		
		Goats and sheep	|__|		
		Pigs	|__|		
		Cows/Donkeys	|__|		
		Other	|__|		
18	If Other kind of animal, please specify	Free text			
19	How many animals does the household own? ***>> Write number of animals owned***	Poultry/birds	_ _		
		Goats and sheep	_ _		
		Pigs	_ _		
		Cows/Donkeys	_ _		
		Other	_ _		
20	Are the goat/sheep or cows staying inside the house at night?	Yes	1		
		No	0	**0**	**22**
		Don't Know	98	**98**	**22**
21	If Yes, do they sleep there every night?	Yes	1		
		No	0	**0**	
		Don't Know	98	**98**	

Protection against vectors					
No.	**Question description**	**Options**	**Answer**	**If**	**Goto**
22	Has the household ever used aerosol can/coil/repellent/ herbs or plants to protect themselves against mosquitoes?	Yes	1		
		No	0	**0**	**24**
		Don't Know	98	**98**	**24**
23	If so, what have they used?	Free text			
24	Summarize how many mosquito nets were used last night (based on the each net section)	Number nets used	_ _		
25	How many children under the age of 5 slept under a mosquito net last night?	Number nets used	_ _		

26	What is the main reason the net was used?	Prevent malaria	1	**1**	**28**
		Privacy	2	**2**	
		Warmth	3	**3**	
		Protection against mosquitoes	4	**4**	
		Prevent Malaria & Protection against mosquitoes	5	**5**	
		Protection against other insects	6	**6**	
		I was advised to use it by the CHW	7	**7**	
		Don't Know	98	**98**	
		Other	8	**8**	**27**
27	If Other reason for net used specify	Free text			

Housing materials and build					
No.	**Question description**	**Options**	**Answer**	**If**	**Goto**
28	What is the main material of the roof? (observed)	Grass/Papyrus/leaves	1		**29**
		Metal sheets	2		
		Other	4	4	**28b**
28b	If Other type of roof specify	Free text			
29	What is the main material of the floor?	Mud/Sand	1	1	**30**
		Cement	2	2	
		Tiles	3	3	
		Mud/sand and cement	4	4	
		Other	5	5	**29b**
29b	If Other type of floor specify	Free text			
30	What is the main material of the walls?	Grass/Leaves	1	1	**18**
		Mud	2	2	
		Wood	3	3	
		Cement	4	4	
		Plastic Sheeting	5	5	
		Woven bamboo	6	6	
		Other	7	7	**30b**

30b	If Other type of wall specify	Free text			
31	Are eaves open? (Is there is a gap between the top of the wall and the roof?)	Yes	1	1	
		No	0	0	
32	Does the trapping room have windows?	Yes	1		
		No	0	**0**	**34**
33	How many windows does the room have?	Number	_ _		
34	Does the room have any external doors?	Yes	1	1	**34b**
		No	0	0	**35**
34b	If yes, is the external door screened?	Yes	1	1	
		No	0	0	
35	Is there light inside the bedroom?	Yes	1	1	
		No	0	0	
36	Is there fan inside the bedroom?	Yes	1	1	
		No	0	0	

If Household has windows in Q32, Windows Section will be repeated for amount of windows entered in Q33

Windows Section					
No.	**Question description**	**Options**	**Answer**	**If**	**Goto**
37	What is the width of window #?	Number (cm)	_ _		
38	What is the length of window #?	Number (cm)	_ _		
39	Is window # screened?	Yes	1		
		No	0		
40	Is window # shuttered?	Yes	1	1	**40d**
		No	0	0	
40b	If yes, if the shutter on window # closed at night?	Yes	1	1	
		No	0	0	

FORM COMPLETE

Appendix V. Exploratory analysis and cluster-level summaries of female *Anopheles* density following IVM MDA

Anopheles density was calculated the total number of *Anopheles* mosquitoes collected divided by the number of trapping nights (number of houses multiplied by number of nights trapped) for all time points in 2021 and 2022. This was done by dividing the total number of *Anopheles* females caught by the total number of trapping nights. The baseline survey conducted in October to December of 2019 sampled 16 of the 24 clusters (eight control clusters; eight intervention clusters). In the Novembers of 2021 and 2022, the PTS was conducted in all 24 clusters.

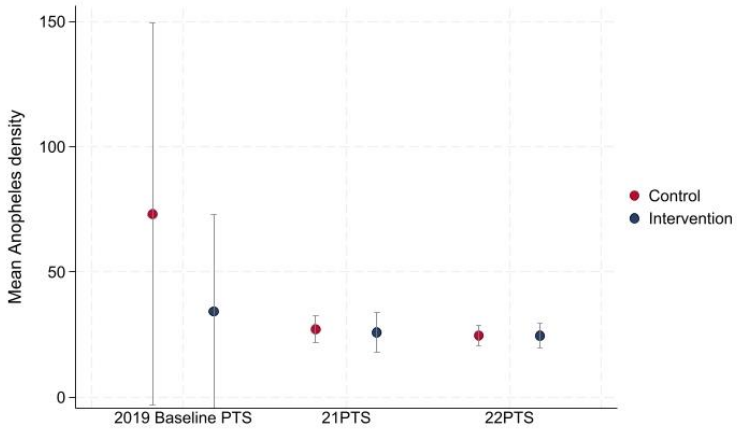

Figure 1. Mean female *Anopheles* density from control (red) and intervention (blue) arms from peak-transmission surveys conducted in 2019 (2019 Baseline PTS), 2021 (21PTS) and 2022 (22PTS). Trail arm densities estimated using cluster-level summaries. *PTS* peak-transmission survey.

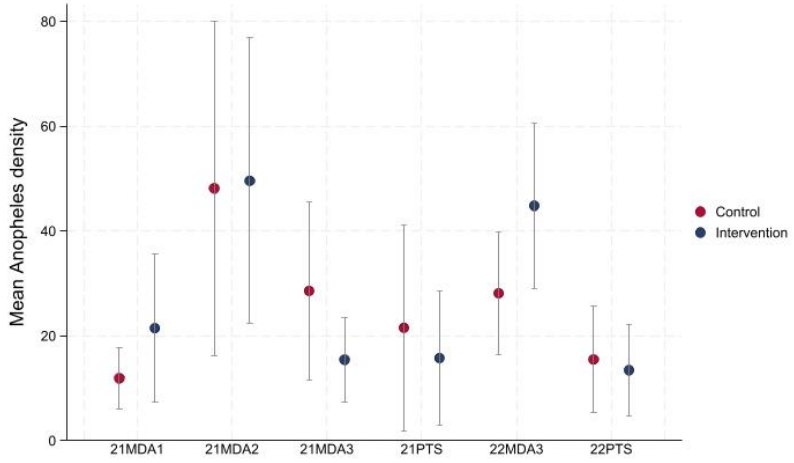

Figure 2. Mean female *Anopheles* density from control (red) and intervention (blue) arms across all time points in 2021 (21MDA1; 21MDA2; 21MDA3; 21PTS) and 2022 (22MDA3; 22PTS). Trail arm densities estimated using cluster-level summaries. *MDA* mass drug administration; *PTS* peak-transmission survey.

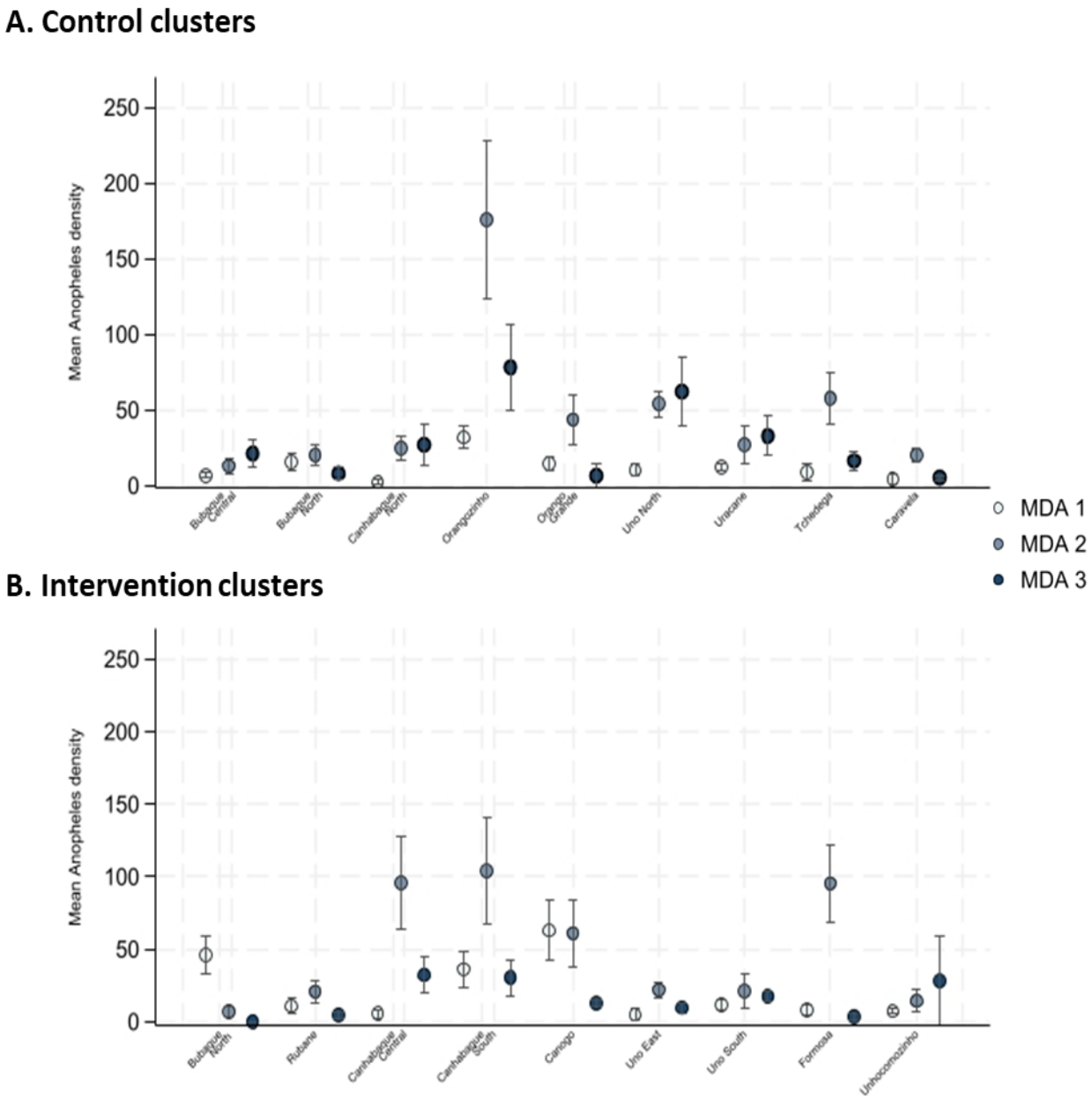

Figure 2. Mean *Anopheles* density for each cluster sampled in the (A) control arm and (B) intervention arm following all MDA rounds in 2021. *MDA* mass drug administration; *PTS* peak-transmission survey.

Table 1. Cluster-level female *Anopheles* densities at all time points sampled in 2021.

	2021 MDA 1			2021 MDA 2			2021 MDA 3			2021 PTS		
	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[b]	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[b]	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[b]	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[b]
Control												
Bubaque Central	206	30	6.9 (4.8 – 8.9)	385	30	12.8 (7.0 – 18.7)	628	30	20.9 (10.7 – 31.1)	381	30	12.7 (7.4 – 18.0)
Bubaque South	467	30	15.6 (9.0 – 22.1)	549	27	20.1 (12.3 – 27.9)	244	30	8.1 (3.8 – 12.4)	31	30	1.0 (0.4 – 1.7)
Canhabaque North	74	30	2.5 (0.7 – 4.2)	745	30	24.8 (16.1 – 33.6)	808	30	26.9 (11.9 – 42.0)	664	30	22.1 (9.6 – 34.6)
Caravela	126	30	4.2 (0.9 – 7.5)	610	30	20.3 (15.5 – 25.1)	152	30	5.1 (3.0 – 7.2)	164	30	5.5 (2.7 – 8.2)
Meneque[a]										919	30	30.6 (17.3 – 43.9)
Orango Grande	429	30	14.3 (9.3 – 19.3)	1251	27	43.6 (24.3 – 62.9)	186	27	6.4 (-2.3 – 15.3)	58	30	1.9 (0.25 – 3.6)
Orangozinho	962	30	32.1 (23.6 – 40.5)	4758	28	175.9 (115.6 – 236.2)	2350	30	78.3 (45.7 – 110.9)	3513	30	117.1 (27.8 – 206.4)
Tchedega	270	30	9.0 (2.4 – 15.6)	1740	30	58.0 (38.5 – 77.5)	487	30	16.2 (8.8 – 23.7)	423	30	14.1 (6.2 – 22.0)
Unhocomo[a]										65	30	2.2 (0.7 – 3.6)
Uno North	310	30	10.3 (5.9 – 14.8)	1615	30	53.8 (44.1 – 63.5)	1858	30	61.9 (35.6 – 88.3)	535	30	17.8 (9.7 – 25.9)
Uracane	375	30	12.5 (9.8 – 15.2)	816	30	27.2 (12.7 – 41.7)	988	30	32.9 (18.1 – 47.8)	367	30	12.2 (6.7 – 17.7)
Intervention												
Bubaque North	1374	30	45.8 (30.4 – 61.2)	213	30	7.1 (2.8 – 11.4)	5	30	0.2 (0.0 – 0.4)	186	30	6.2 (0.9 – 11.5)
Canhabaque Central	161	30	5.4 (1.9 – 8.9)	2836	27	95.7 (58.1 – 133.3)	971	30	32.4 (17.6 – 47.1)	481	30	16.0 (4.0 – 28.1)
Canhabaque South	1079	30	36.0 (22.0 – 49.9)	3114	30	103.8 (61.5 – 146.1)	909	30	30.3 (15.8 – 44.8)	2525	30	84.2 (6.2 – 162.1)
Canogo	1704	27	63.1 (39.1 – 87.1)	1672	30	60.8 (34.0 – 87.7)	378	30	12.6 (9.5 – 15.6)	155	30	5.2 (3.4 – 6.9)
Carache[a]										800	30	26.7 (9.5 – 43.9)
Eguba[a]										150	30	5.0 (2.6 – 7.4)
Formosa	240	30	8.0 (3.6 – 12.4)	2857	30	95.2 (65.0 – 125.5)	112	30	3.7 (0.8 – 6.7)	80	30	2.7 (1.1 – 4.3)
Rubane	327	30	10.9 (4.5 – 17.3)	619	30	20.6 (12.1 – 29.1)	141	30	4.7 (1.8 – 7.6)	225	30	7.5 (3.9 – 11.1)
Uassa-wite[a]										316	30	10.5 (4.0 – 17.1)
Unhocomozinho	223	30	7.4 (5.0 – 9.8)	434	30	14.5 (5.2 – 23.7)	840	30	28.0 (-7.6 – 63.6)	425	30	14.2 (5.8 – 22.5)
Uno East	152	30	5.1 (0.6 – 9.5)	652	30	21.7 (15.3 – 28.2)	291	30	9.7 (5.4 – 14.0)	179	30	6.0 (3.1 – 8.8)
Uno South	349	30	11.6 (8.1 – 15.1)	631	30	21.0 (7.7 – 34.1)	524	30	17.5 (13.3 – 21.6)	158	30	5.3 (3.3 – 7.3)

[a] Clusters only sampled during peak-transmission survey.

[b] Mean *An. gambiae* s.l. density calculated using household-level densities.

Table 2. Cluster-level female *Anopheles* densities at all time points sampled in 2022.

	2022 MDA 3			2022 PTS		
	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[a]	Total *Anopheles* caught	Total trap nights	Mean *An. gambiae* s.l. density (95% CI)[a]
Control						
Bubaque Central	319	42	7.6 (4.7 – 10.5)	203	45	4.5 (3.2 – 5.8)
Bubaque South	379	45	8.4 (2.9 – 13.9	342	45	7.6 (5.4 – 9.8)
Canhabaque North	354	45	7.9 (5.3 – 10.4)	146	45	3.2 (1.9 – 4.6)
Caravela	470	43	10.6 (7.0 – 14.2)	79	45	1.8 (1.1 – 2.4)
Meneque	1449	44	32.8 (28.5 – 37.1)	1404	45	31.2 (13.5 – 48.9)
Nhago	3101	45	68.9 (40.4 – 97.4)	428	45	9.5 (5.9 – 13.1)
Orango Grande	1276	45	28.3 (18.0 – 38.7)	438	45	9. 7 (3.1 – 16.3)
Orangozinho	1702	45	58.7 (37.0 – 80.4)	834	45	18.5 (0.8 – 36.3)
Tchedega	918	45	20.4 (11.8 – 29.0)	27	45	0.6 (0.0 – 1.2)
Unhocomo	862	45	19.2 (9.0 – 29.3)	127	45	2.8 (2.2 – 3.4)
Uno North	1278	43	29.2 (22.5 – 35.9)	2618	48	54.5 (40.1 – 69.0)
Uracane	1702	38	41.7 (5.6 – 77.7)	1884	45	41.9 (31.4 – 52.3)
Intervention						
Bubaque North	134	134	3.1 (2.2 – 3.9)	67	45	1.5 (1.0 – 1.9)
Canhabaque Central	3110	3110	69.1 (31.2 – 107.0)	261	45	6.0 (3.2 – 8.8)
Canhabaque South	4291	4291	95.4 (69.2 – 121.5)	1174	45	26. 1 (12.4 – 39.8)
Canogo	1042	1042	23.2 (13.2 – 33.1)	290	45	6.4 (2.8 – 10.1)
Carache	813	813	18.1 (3.2 – 32.9)	147	45	3.3 (2.7 – 3.8)
Eguba	2001	2001	47.6 (25.0 – 70.3)	195	45	4.3 (4.1 – 4.6)
Formosa	2206	2206	64.6 (29.7 – 98.8)	717	45	15.9 (9.7 – 22.1)
Rubane	181	181	5.3 (3.4 – 7.1)	43	45	1.0 (0.5 – 1.4)
Uassa-wite	3032	3032	67.4 (38.9 – 95.8)	46	45	1.0 (0.5 – 1.5)
Unhocomozinho	2197	2197	48.8 (26.4 – 71.2)	2369	45	52.6 (6.9 – 98.4)
Uno East	2077	2077	47.5 (35.9 – 59.1)	996	45	22.1 (11.5 – 32.8)
Uno South	2361	2361	53.5 (31.8 - 75.3)	958	45	21.3 (11.5 – 31.0)

[a] Mean *An. gambiae* s.l. density calculated using household-level densities.

Appendix VI. Exploratory analysis and cluster-level summaries of female *Anopheles* parity following IVM MDA

Exploratory graphs and tables were made during analysis of the female *Anopheles* parity following IVM MDA in 2021 and 2022. Parity rate was determined as the percentage of parous mosquitoes in the sub-sample assessed. Results from statistical analysis can be seen in Chapter 6.

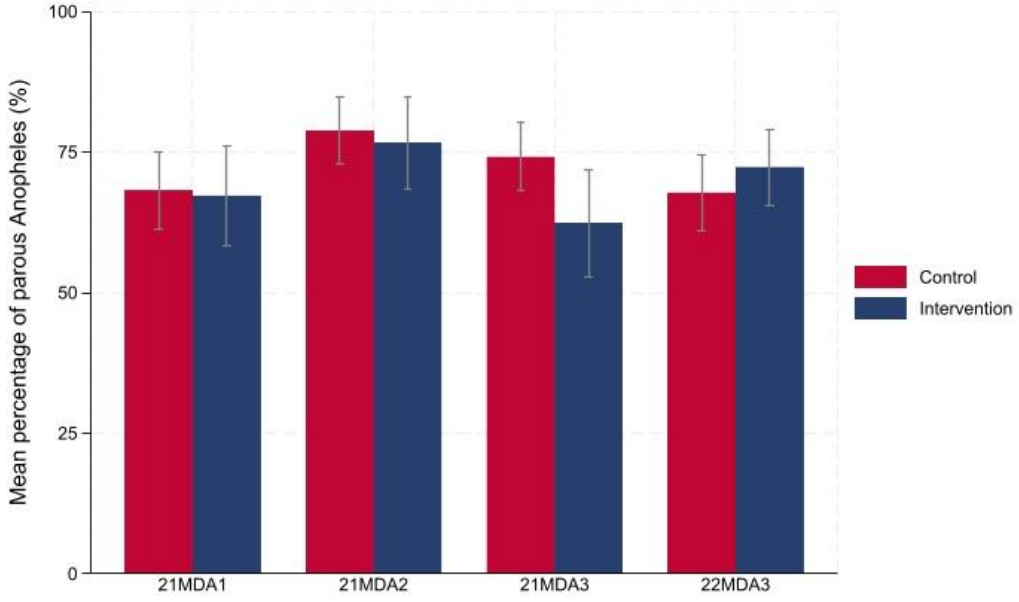

Figure 1. Mean *Anopheles* parity rate for both study arms calculated using cluster-level parity rates from post-MDA collections from the intervention and control trial arms. *MDA* Mass drug administration.

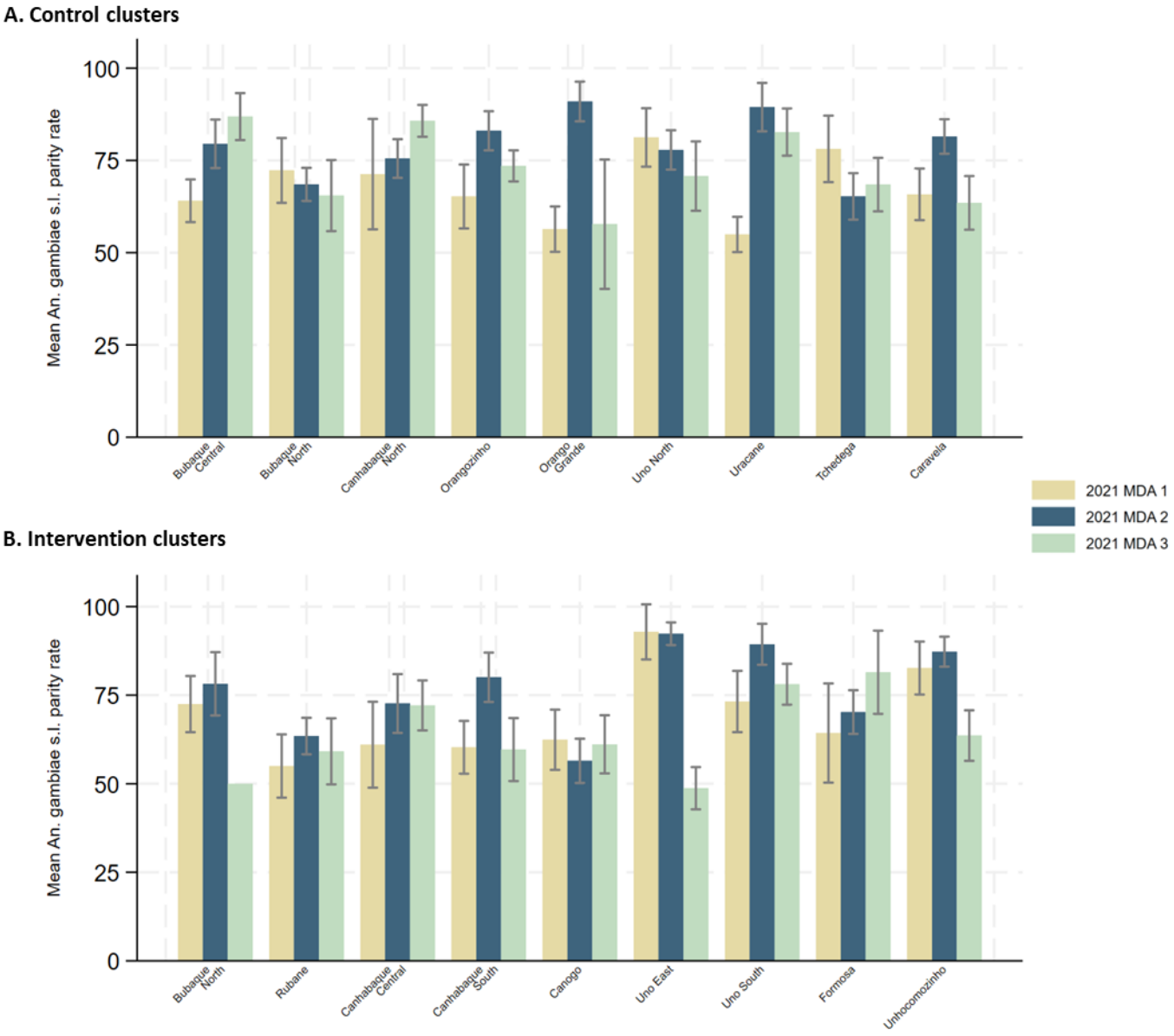

Figure 2. Mean *Anopheles* parity rates for each cluster sampled in the (A) control arm and (B) intervention arm following completion of each MDA round in 2021. *MDA* mass drug administration.

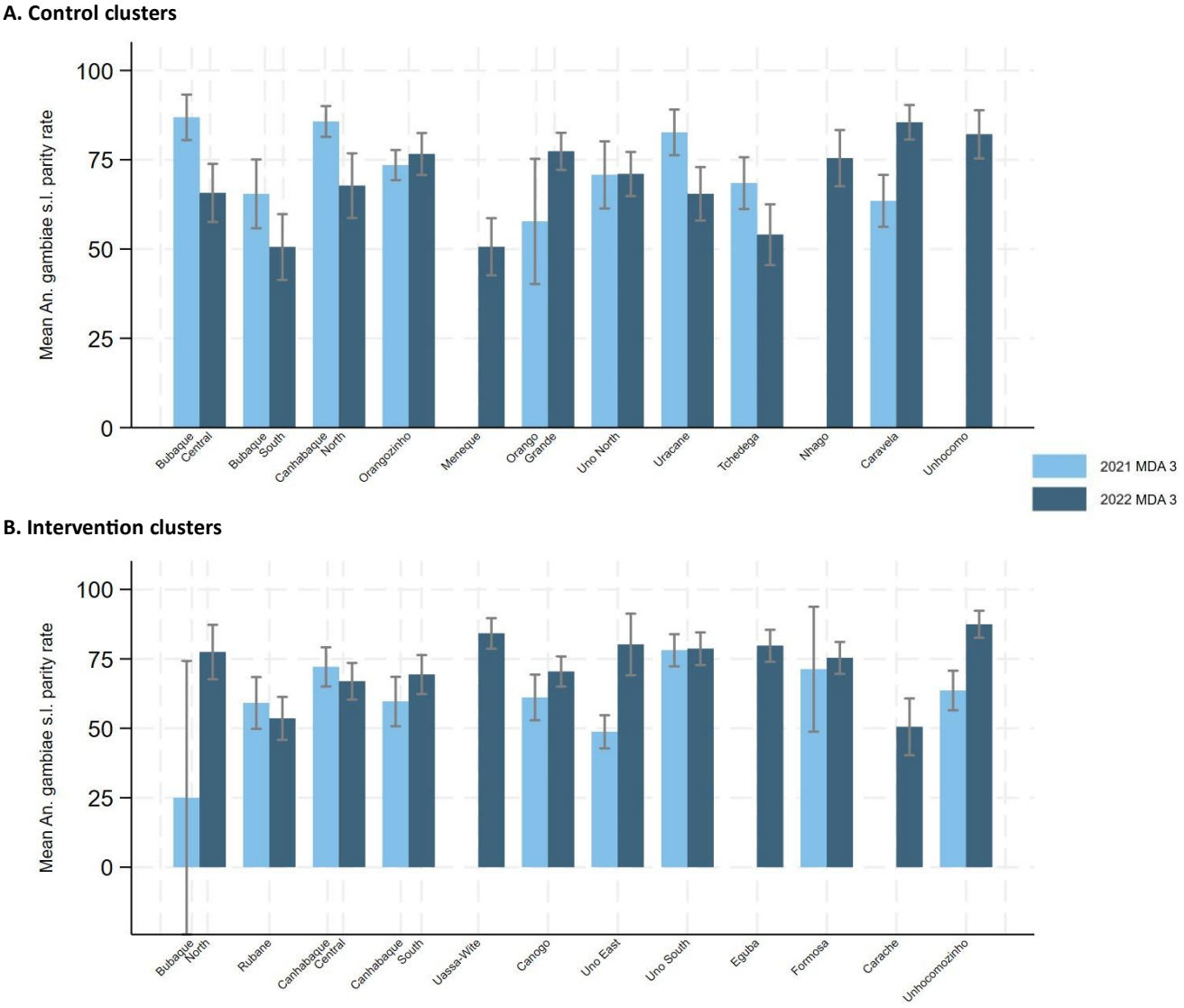

Figure 3. Mean *Anopheles* parity rates for each cluster sampled in the control arm (A) and intervention arm (B) following completion of the last MDA round in 2021 (light blue) and 2022 (dark blue). *MDA* mass drug administration

Table 1. Cluster-level *Anopheles* parity rates for control and intervention arms from post-MDA rounds in 2021 and 2022

	2021 MDA 1			2021 MDA 2			2021 MDA 3			2022 MDA 3		
	Total parous	Total assessed	Mean *Anopheles gambiae* s.l. parity rate (95% CI)[b]	Total parous	Total assessed	Mean *Anopheles gambiae* s.l. parity rate (95% CI)[b]	Total parous	Total assessed	Mean *Anopheles gambiae* s.l. parity rate (95% CI)[b]	Total parous	Total assessed	Mean *Anopheles gambiae* s.l. parity rate (95% CI)[b]
Control												
Bubaque Central	122	187	64.1 (57.4 – 70.7)	156	196	79.5 (71.9 – 87.1)	173	202	86.9 (79.5 – 94.2)	129	205	65.7 (56.7 – 74.6)
Bubaque South	141	200	72.3 (62.2 – 82.4)	137	202	68.5 (63.3 – 73.6)	116	190	65.4 (54.4 – 76.5)	113	208	54.2 (47.2 – 61.1)
Canhabaque North	41	61	71.3 (53.3 – 89.2)	162	215	75.5 (69.5 – 81.0)	179	205	85.7 (80.8 – 90.7)	131	205	67.7 (57.9 – 77.6)
Caravela	76	112	65.8 (57.4 – 74.2)	160	199	81.4 (76.1 – 86.8)	87	130	63.5 (55.1 – 71.8)	180	210	85.5 (80.2 – 90.7)
Meneque[a]										102	203	50.6 (41.9 – 59.3)
Nhago[a]										165	222	75.4 (66.9 – 84.0)
Orango Grande	110	200	56.4 (49.3 – 63.4)	204	223	91.0 (84.8 – 97.1)	108	158	66.0 (56.4 – 75.5)	159	204	77.3 (71.7 – 83.0)
Orangozinho	131	201	65.2 (55.3 – 75.2)	164	200	83.0 (76.9 – 89.1)	147	200	73.5 (68.7 – 78.3)	156	205	76.6 (70.2 – 83.0)
Tchedega	166	200	78.1 (67.3 – 88.9)	144	224	65.2 (57.5 – 69.4)	145	204	68.4 (60.1 – 76.8)	107	206	54.0 (44.7 – 63.3)
Unhocomo[a]										166	203	82.1 (74.8 – 89.5)
Uno North	161	191	81.2 (72.1 – 90.3)	159	204	77.8 (71.7 – 83.9)	142	202	70.7 (60.0 – 81.5)	142	201	71.0 (64.3 – 77.7)
Uracane	110	199	54.9 (49.4 – 60.4)	165	183	89.4 (81.9 – 97.0)	168	202	82.7 (75.3 – 90.0)	129	203	65.5 (57.3 – 73.6)
Intervention												
Bubaque North	144	200	72.5 (63.4 – 81.6)	64	82	78.2 (67.9 – 88.4)	1	3	33.3 ()	75	103	77.4 (66.8 – 88.1)
Canhabaque Central	87	152	61.0 (46.8 – 75.2)	157	218	72.6 (63.1 – 82.1)	147	206	72.1 (64.0 – 80.2)	158	235	66.9 (59.7 – 74.1)
Canhabaque South	122	204	60.3 (51.7 – 68.9)	166	205	80.0 (72.1 – 88.0)	120	198	59.6 (49.4 – 69.9)	151	218	69.3 (61.7 – 77.0)
Canogo	117	200	62.4 (52.8 – 72.0)	117	205	56.5 (49.3 – 63.6)	124	204	61.1 (51.7 – 70.5)	171	243	70.4 (64.5 – 76.3)
Carache[a]										112	232	50.5 (39.4 – 61.6)
Eguba[a]										161	204	79.7 (73.3 – 86.0)
Formosa	114	191	64.3 (48.2 – 80.4)	124	179	70.2 (63.1 – 77.3)	78	96	81.4 (66.9 – 96.0)	154	204	75.3 (69.1 – 81.6)
Rubane	103	200	55.0 (44.7 – 65.3)	162	262	63.5 (57.5 – 69.4)	84	133	59.1 (48.4 0 69.8)	93	174	53.6 (45.1 – 62.0)
Uassa-wite[a]										159	190	84.2 (78.2 – 90.1)
Unhocomozinho	130	162	82.6 (74.0 – 91.2)	178	200	87.2 (82.4 – 92.1)	132	211	63.6 (55.4 – 71.8)	176	201	87.4 (82.2 – 92.7)
Uno East	114	123	92.9 (83.9 – 101.8)	185	201	92.3 (88.6 – 96.0)	104	206	48.7 (41.9 – 55.6)	171	210	80.2 (68.1 – 92.3)
Uno South	139	189	73.2 (63.3 – 83.1)	188	211	89.4 (81.9 – 96.9)	158	203	78.1 (71.4 – 84.7)	159	200	78.6 (72.2 – 85.0)

[a] Clusters only sampled for parity in 2022

[b] Mean *Ano. gambiae* s.l. parity rates calculated using household-level parity rates

Appendix VII. Exploratory analysis and cluster-level summaries of female *An. gambiae* s.l. species composition from post-MDA and PTS collections in 2021 and 2022.

Exploratory graphs and tables were made during analysis of the female *An. gambiae* s.l. species composition following IVM MDA and PTS in 2021 and 2022. *Anopheles gambiae* sensu stricto (s.s.) is thought to be the primary vector of the Bijagós and is therefore the focus of our analysis. Following mosquito collection, all *Anopheles* mosquitoes caught were morphologically identified, all appeared to come from the *Anopheles gambiae* complex. Molecular identification using PCR with restriction fragment length polymorphism to identify species within the complex and differentiate between *An. gambiae* s.s. and *An. coluzzii* was performed at the MRC Unit The Gambia. From post-MDA mosquito collections, 30 *An. gambiae* sensu lato (s.l.) per cluster sampled were sent for species id. A further 200 *An. gambiae* s.l. per cluster were sent from the peak-transmission survey (PTS). Results from statistical analysis can be seen in Chapter 6.

The baseline survey conducted in October to December of 2019 sampled 16 of the 24 clusters (eight control clusters; eight intervention clusters). In the Novembers of 2021 and 2022, the PTS was conducted in all 24 clusters.

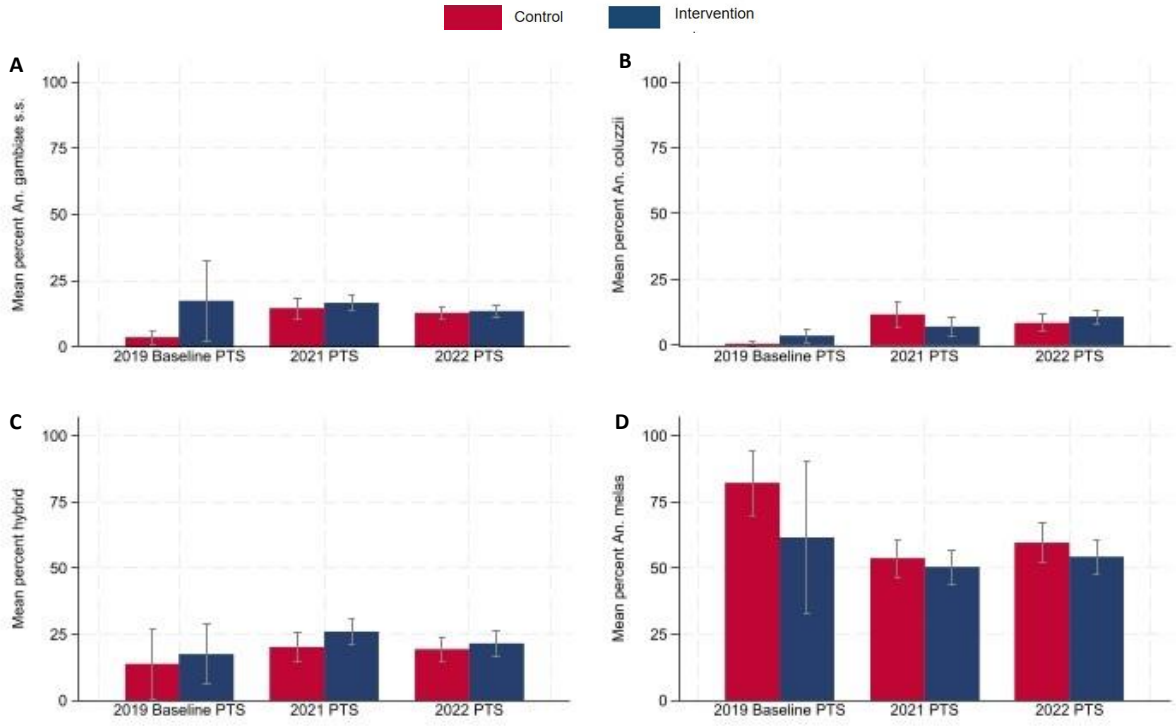

Figure 1. Mean percentage of (A) *An. gambiae* s.s., (B) *An. coluzzii*, (C) *An. gambiae* s.s./ *An. coluzzii* hybrids and (D) *An. melas* calculated using cluster-level percentages for peak-transmission surveys of 2019 (baseline), 2021 and 2022 from the intervention and control trial arms. *PTS* peak-transmission survey.

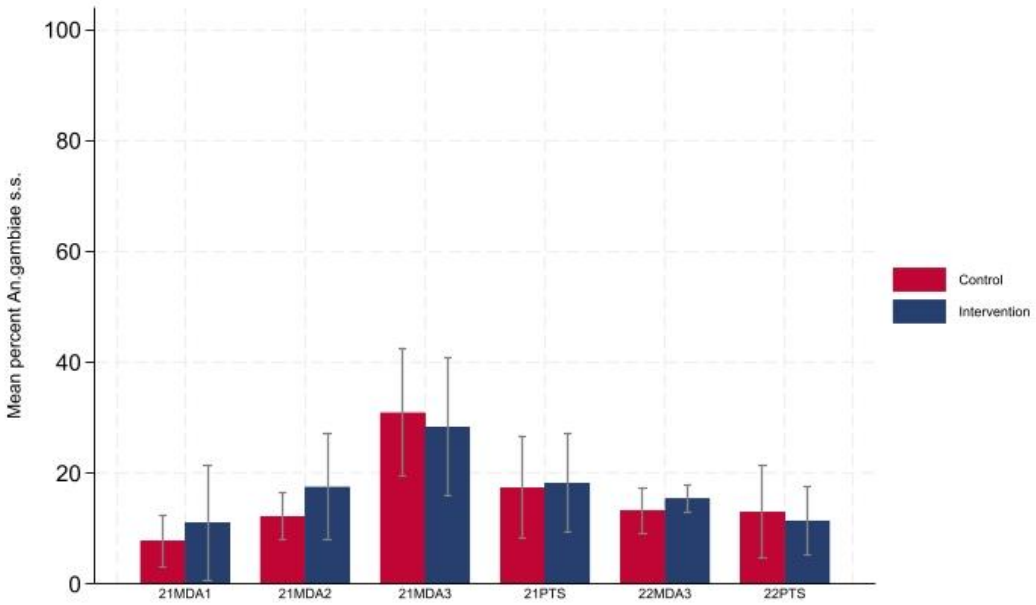

Figure 2. Mean percentage of *An. gambiae* s.s. calculated using cluster-level percentages for all timepoints sampled from the intervention and control trial arms. *MDA* Mass drug administration; *PTS* Peak-transmission survey.

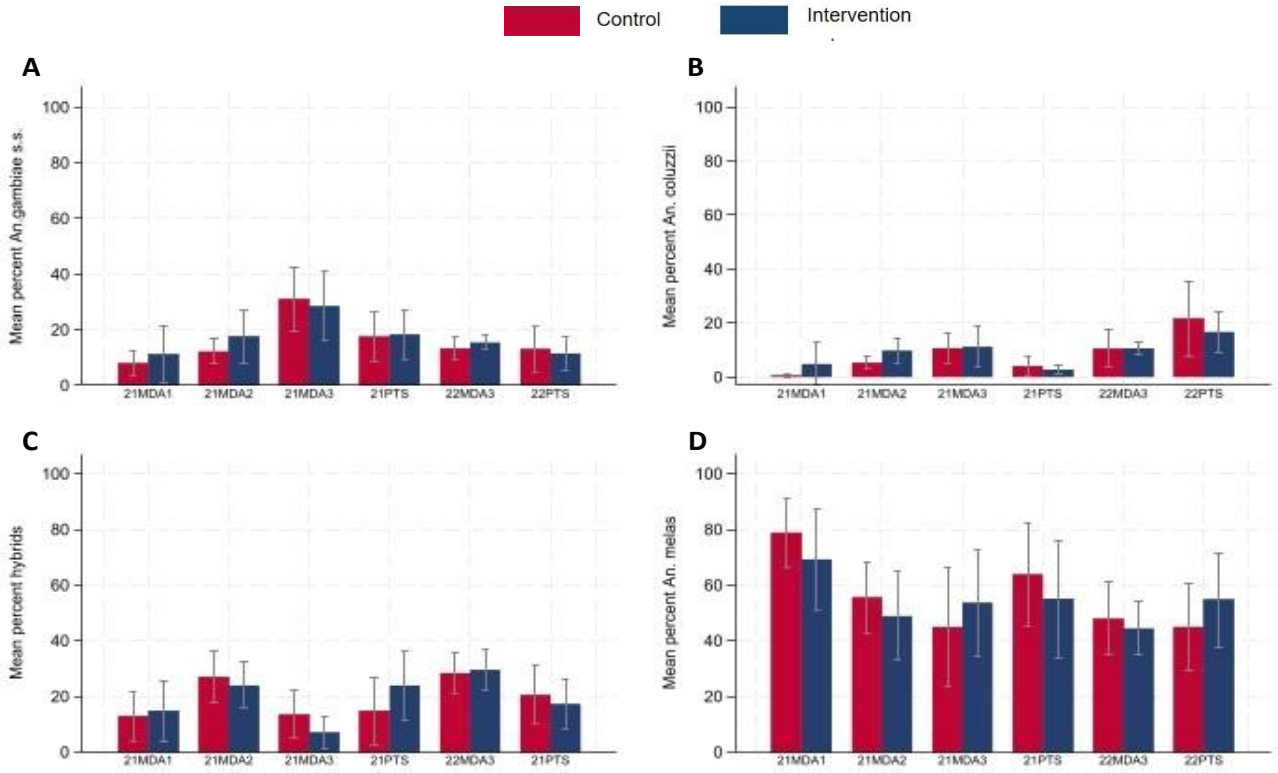

Figure 3. Mean percentage of (A) *An. gambiae* s.s., (B) *An. coluzzii*, (C) *An. gambiae* s.s./ *An. coluzzii* hybrids and (D) *An. melas* calculated using cluster-level percentages for all timepoints sampled from the intervention and control trial arms. *MDA* mass drug administration; *PTS* Peak-transmission survey.

Table 1. Totals and percentages of all *An. gambiae* s.l. caught from all time points sampled in 2021

	2021 MDA 1				2021 MDA 2				2021 MDA3				21PTS[b]			
	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)
Control																
Bubaque Central	3 (10.0)	0 (0)	1 (3.4)	25 (86.2)	6 (10.2)	4 (6.8)	21 (35.6)	28 (47.4)	24 (21.6)	12 (10.8)	8 (7.1)	67 (60.4)	8 (4.8)	0 (0)	4 (2.4)	155 (92.8)
Bubaque South	0 (0)	0 (0)	2 (9.1)	20 (90.9)	6 (10.5)	2 (3.5)	10 (17.5)	39 (68.4)	2 (6.4)	0 (0)	0 (0)	29 (93.5)	0 (0)	0 (0)	2 (6.4)	29 (93.5)
Canhabaque North	7 (23.3)	0 (0)	10 (33.3)	13 (43.3)	3 (7.0)	0 (0)	15 (34.8)	25 (58.1)	6 (20.7)	0 (0)	0 (0)	23 (79.3)	52 (28.0)	2 (1.1)	20 (10.7)	111 (59.7)
Caravela	2 (7.8)	1 (3.8)	5 (19.2)	19 (69.2)	2 (6.7)	1 (3.3)	2 (6.7)	25 (83.3)	4 (13.8)	2 (6.9)	9 (31.0)	14 (48.3)	6 (4.5)	4 (3.0)	2 (1.5)	122 (91.0)
Meneque[a]													11 (5.4)	2 (1.0)	12 (5.9)	177 (87.6)
Orango Grande	3 (11.1)	0 (0)	5 (18.5)	19 (70.4)	10 (25.6)	1 (2.6)	13 (33.3)	15 (38.5)	9 (26.5)	3 (8.8)	1 (2.9)	21 (61.8)	2 (2.3)	0 (0)	0 (0)	83 (97.6)
Orangozinho	1 (3.8)	0 (0)	0 (0)	25 (96.1)	10 (17.2)	3 (5.2)	16 (27.6)	29 (50.0)	14 (56.0)	3 (12.0)	5 (20.0)	3 (12.0)	75 (40.5)	6 (3.2)	43 (23.2)	61 (33.0)
Tchedega	2 (6.9)	0 (0)	0 (0)	27 (93.1)	5 (9.3)	4 (7.4)	10 (18.5)	35 (64.8)	9 (45.0)	5 (25.0)	4 (20.0)	2 (10.0)	46 (23.0)	12 (6.0)	31 (15.5)	111 (55.5)
Unhocomo[a]													12 (41.4)	6 (20.7)	1 (3.4)	10 (34.5)
Uno North	2 (7.1)	0 (0)	9 (32.1)	17 (60.7)	10 (17.5)	7 (12.3)	30 (52.6)	10 (17.5)	16 (53.3)	4 (13.3)	10 (33.3)	0 (0)	30 (15.0)	12 (6.0)	142 (71.0)	16 (8.0)
Uracane	0 (0)	0 (0)	0 (0)	10 (100.0)	4 (6.3)	4 (6.3)	10 (15.9)	45 (71.4)	11 (35.5)	6 (19.3)	2 (6.4)	12 (38.7)	55 (26.3)	8 (3.8)	47 (22.5)	99 (47.4)
Intervention																
Bubaque North	9 (31.0)	1 (3.4)	13 (44.8)	6 (20.7)	15 (48.4)	3 (9.7)	8 (25.8)	5 (16.1)	2 (50.0)	0 (0)	0 (0)	2 (50.0)	20 (32.8)	4 (6.6)	19 (31.1)	18 (29.5)
Canhabaque Central	5 (21.7)	0 (0)	5 (21.7)	13 (56.5)	2 (6.7)	0 (0)	2 (6.7)	26 (86.7)	4 (14.3)	0 (0)	0 (0)	24 (85.7)	15 (8.0)	1 (0.5)	10 (5.3)	161 (86.1)
Canhabaque South	0 (0)	0 (0)	1 (3.6)	27 (96.4)	14 (23.3)	7 (11.7)	8 (13.3)	31 (51.7)	3 (12.5)	5 (20.8)	1 (4.2)	15 (62.5)	12 (5.6)	0 (0)	10 (4.7)	191 (89.7)
Canogo	0 (0)	0 (0)	0 (0)	31 (100.0)	4 (6.4)	2 (3.2)	17 (27.4)	39 (62.9)	3 (10.0)	0 (0)	1 (3.3)	26 (86.7)	4 (2.9)	0 (0)	10 (7.1)	126 (90.0)
Carache[a]													1 (0.5)	0 (0)	2 (0.9)	209 (98.1)
Eguba[a]													40 (33.9)	4 (3.4)	46 (39.0)	28 (23.7)
Formosa	12 (40)	0 (0)	5 (16.7)	13 (43.3)	13 (28.3)	5 (10.9)	13 (18.3)	15 (32.6)	16 (53.3)	3 (10.0)	5 (16.7)	6 (20.0)	27 (45.8)	3 (5.1)	20 (33.9)	9 (15.2)
Rubane	0 (0)	1 (3.6)	2 (7.1)	25 (89.3)	4 (6.4)	2 (3.4)	4 (6.7)	49 (83.0)	4 (14.3)	2 (7.1)	0 (0)	22 (78.6)	13 (6.3)	11 (5.3)	18 (8.7)	164 (79.6)
Uassa-wite[a]													71 (36.0)	11 (5.6)	98 (49.7)	17 (8.6)
Unhocomozinho	0 (0)	11 (36.7)	0 (0)	19 (63.3)	5 (9.8	10 (19.6)	21 (41.2)	15 (29.4)	25 (21.9)	12 (6.0)	8 (7.0)	69 (60.5)	11 (5.4)	0 (0)	5 (2.4)	189 (92.2)
Uno East	0 (0)	0 (0)	1 (3.4)	28 (96.5)	4 (6.3)	13 (20.6)	24 (38.1)	22 (34.9)	7 (24.1)	5 (17.2)	7 (24.1)	10 (34.5)	37 (18.9)	5 (2.5)	79 (40.3)	75 (38.3)
Uno South	2 (7.1)	0 (0)	10 (35.7)	16 (57.1)	12 (2.22)	4 (7.4)	15 (27.8)	23 (79.3)	16 (53.3)	10 (34.4)	2 (6.9)	1 (3.4)	35 (23.2)	5 (3.3)	96 (63.6)	15 (9.9)

[a] Clusters only sampled during PTS

[b]Two *An. arabiensis* were caught during the PTS 2021. One was caught on Canhabaque North and the other was caught on Carache

Table 2. Totals and percentages of all *An. gambiae* s.l. caught from all time points sampled in 2022

	2022 MDA 3				2022 PTS			
	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)	Total *An. gambiae s.s* (%)	Total *An. coluzzii* (%)	Total hybrid (%)	Total *An. melas* (%)
Control								
Bubaque Central	6 (20.0)	1 (3.3)	8 (26.7)	15 (50.0)	62 (47.3)	7 (5.3)	21 (16.0)	41 (31.3)
Bubaque South	7 (21.9)	0 (0)	5 (15.6)	20 (62.5)	29 (22.7)	10 (7.8)	45 (35.2)	44 (34.4)
Canhabaque North	4 (8.3)	2 (4.2)	20 (41.7)	22 (45.8)	21 (15.0)	40 (28.6)	13 (9.3)	66 (47.1)
Caravela	5 (8.9)	4 (7.1)	12 (21.4)	35 (62.5)	2 (3.0)	35 (53.0)	17 (25.7)	12 (18.2)
Meneque	5 (10.0)	2 (4.0)	7 (14.0)	36 (72.0)	4 (2.2)	2 (1.1)	4 (2.2)	171 (94.5)
Nhago	2 (4.2)	0 (0)	3 (6.4)	42 (89.4)	12 (5.8)	18 (8.7)	14 (6.8)	162 (78.6)
Orango Grande	17 (15.9)	14 (13.1)	40 (37.4)	36 (33.6)	5 (2.3)	3 (1.4)	15 (7.0)	192 (89.3)
Orangozinho	6 (9.2)	4 (6.1)	23 (35.4)	32 (49.3)	3 (1.4)	125 (58.9)	0 (0)	84 (39.6)
Tchedega	12 (21.4)	6 (10.7)	16 (28.6)	22 (39.3)	0 (0)	15 (65.2)	2 (8.7)	6 (26.1)
Unhocomo	7 (25.9)	12 (44.4)	8 (29.6)	0 (0)	36 (32.1)	20 (17.8)	33 (29.5)	23 (20.5)
Uno North	3 (6.0)	10 (20.0)	16 (32.0)	21 (42.0)	28 (14.1)	8 (4.0)	101 (51.0)	61 (30.8)
Uracane	4 (7.4)	7 (13.0)	28 (51.8)	15 (27.8)	22 (9.9)	17 (7.6)	119 (53.4)	65 (29.1)
Intervention								
Bubaque North	3 (9.4)	2 (6.2)	7 (21.9)	20 (62.5)	21 (37.5)	7 (12.5)	13 (23.2)	15 (26.8)
Canhabaque Central	45 (23.2)	23 (11.9)	55 (28.3)	71 (36.6)	30 (13.8)	29 (13.3)	78 (35.8)	81 (37.2)
Canhabaque South	9 (13.8)	5 (7.7)	11 (16.9)	40 (61.5)	11 (5.1)	20 (9.2)	7 (3.2)	178 (82.4)
Canogo	6 (15.8)	3 (7.1)	7 (18.4)	22 (57.8)	12 (6.2)	50 (25.8)	9 (4.6)	123 (63.4)
Carache	9 (16.1)	4 (7.1)	14 (25.0)	29 (51.8)	1 (0.7)	0 (0)	0 (0)	142 (99.3)
Eguba	2 (6.9)	4 (13.8)	4 (13.8)	19 (65.5)	43 (20.5)	59 (28.1)	15 (7.1)	93 (44.3)
Formosa	7 (15.2)	7 (15.2)	24 (52.2)	8 (17.4)	36 (21.3)	25 (14.8)	59 (34.9)	49 (29.0)
Rubane	15 (16.1)	8 (8.6)	31 (33.3)	39 (41.9)	1 (2.4)	3 (7.1)	1 (3.1)	37 (88.1)
Uassa-wite	12 (21.0)	4 (7.0)	24 (42.1)	17 (29.8)	22 (10.3)	82 (38.5)	83 (39.0)	26 (12.2)
Unhocomozinho	9 (15.2)	6 (10.2)	15 (25.4)	29 (49.1)	6 (2.4)	2 (0.9)	7 (3.1)	206 (93.3)
Uno East	30 (17.4)	24 (13.9)	42 (24.4)	76 (44.2)	9 (4.1)	28 (12.9)	82 (37.8)	98 (45.2)
Uno South	5 (15.1)	6 (18.2)	17 (51.5)	5 (15.1)	26 (13.1)	75 (37.9)	27 (13.6)	70 (35.3)

Appendix VIII. Exploratory analysis and cluster-level summaries of female *Anopheles* sporozoite rate from post-MDA 3 and PTS collections in 2021 and 2022.

Exploratory graphs and tables were made during analysis of the female *Anopheles* sporozoite rate following IVM MDA and PTS in 2021 and 2022. To investigate the impact of ivermectin MDA on *Anopheles* sporozoite rate, CSP- ELISAs were performed on 200 mosquitoes/cluster at four different time points at the MRC Unit The Gambia. Mosquitoes from post-MDA 3 collection and the peak-transmission survey (PTS) in both years were used. Results from statistical analysis can be seen in Chapter 6.

The baseline survey conducted in October to December of 2019 sampled 16 of the 24 clusters (eight control clusters; eight intervention clusters). In the Novembers of 2021 and 2022, the PTS was conducted in all 24 clusters.

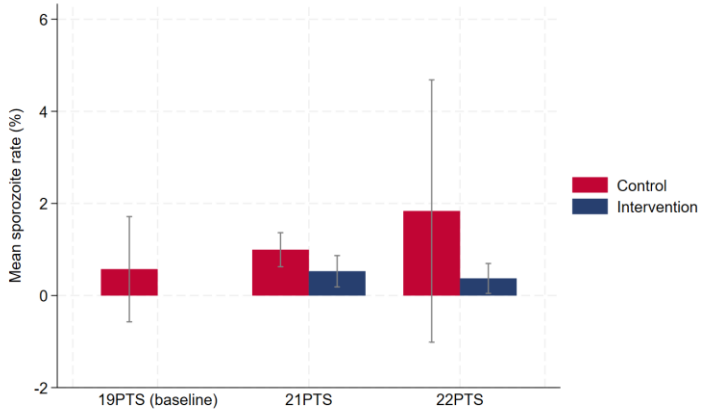

Figure 1. Mean *Anopheles* sporozoite rate calculated using cluster-level percentages for the peak-transmission survey in 2019 (baseline), 2021 and 2022 from the intervention and control trial arms. *PTS* peak-transmission survey.

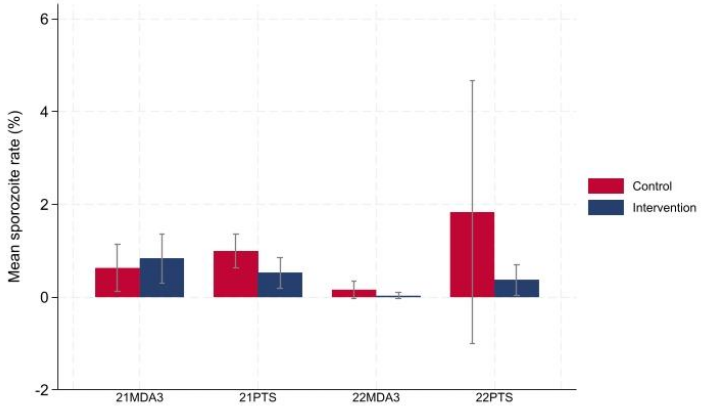

Figure 2. Mean *Anopheles* sporozoite rate calculated using cluster-level percentages from the intervention and control trial arms. *MDA* Mass drug administration; *PTS* Peak-transmission survey.

Table 1. Total *Anopheles* CSP positive, assessed and sporozoite rate for each cluster sampled from post-MDA 3 and PTS collections in 2021 and 2022.

	2021 MDA 3			2021 PTS			2022 MDA 3			2022 PTS		
	Total CSP +ve	Total assessed	Mean sporozoite rate (95% CI)[b]	Total CSP +ve	Total assessed	Mean sporozoite rate (95% CI)[b]	Total CSP +ve	Total assessed	Mean sporozoite rate (95% CI)[b]	Total CSP +ve	Total assessed	Mean sporozoite rate (95% CI)[b]
Control												
Bubaque Central	2	212	0.8 (-0.4 – 2.0)	3	163	1.6 (-1.0 – 4.2)	2	205	0.6 (- 0.7 – 2.0)	0	155	0 (0)
Bubaque South	1	206	0.3 (-0.4 – 1.1)	0	32	0 (0)	0	199	0 (0)	3	204	1.3 (-0.7 – 3.4)
Canhabaque North	0	258	0 (0)	1	210	0.4 (-0.6 – 1.5)	1	204	0.5 (-0.6 – 1.7)	0	145	0 (0)
Caravela	0	143	0 (0)	2	157	1.6 (-1.2 – 4.5)	0	210	0 (0)	0	77	0 (0)
Meneque[a]				3	210	1.4 (-0.2 – 3.1))	0	192	0 (0)	0	178	0 (0)
Nhago[a]							0	213	0 (0)	3	224	1.3 (-0.2 – 2.7)
Orango Grande	0	178	0 (0)	1	90	1.1 (-1.4 - 3.6)	0	215	0 (0)	0	223	0 (0)
Orangozinho	6	251	2.5 (0.9 – 4.1)	0	211	0 (0)	0	211	0 (0)	2	218	1.1 (-0.5 – 2.6)
Tchedega	3	241	1.2 (-0.2 – 2.6)	2	210	1.2 (-1.5 – 3.8)	1	206	0.4 (-0.4 – 1.2)	4	23	18.2 (-16.5 – 52.8)
Unhocomo[a]				1	65	2.8 (-3.6 – 9.2)	0	203	0 (0)	0	118	0 (0)
Uno North	1	231	0.4 (-0.5 – 1.2)	3	208	1.4 (-0.2 – 3.1)	0	197	0 (0)	0	224	0 (0)
Uracane	1	217	0.6 (0.8 – 2.0)	2	228	1.0 (-5.2 – 2.5)	0	203	0 (0)	2	225	0.9 (-0.4 – 2.2)
Intervention												
Bubaque North	0	4	0 (0)	0	85	0 (0)	0	92	0 (0)	0	67	0 (0)
Canhabaque Central	5	226	2.0 (-0.8 – 4.9)	1	207	0.5 (-0.6 – 1.5)	1	254	0.4 (-0.5 – 1.4)	0	222	0 (0)
Canhabaque South	3	246	1.0 (-0.7 – 2.7)	0	209	0 (0)	0	218	0 (0)	2	228	0.9 (-0.4 – 2.2)
Canogo	1	228	0.4 (-0.6 – 1.5)	0	143	0 (0)	0	200	0 (0)	0	200	0 (0)
Carache[a]				1	218	0.5 (-0.6 – 1.5)	0	203	0 (0)	0	148	0 (0)
Eguba[a]				0	122	0 (0)	0	200	0 (0)	0	213	0 (0)
Formosa	0	108	0 (0)	0	79	0 (0)	0	202	0 (0)	1	223	0.4 (-0.5 – 1.4)
Rubane	0	139	0 (0)	2	210	0.9 (-0.5 – 2.3)	0	180	0 (0)	0	41	0 (0)
Uassa-wite[a]				2	209	1.0 (-0.5 – 2.4)	0	167	0 (0)	2	224	0.8 (-0.6 – 2.2)
Unhocomozinho	5	260	1.4 (-0.4 – 3.3)	4	211	1.9 (0.1 – 3.6)	0	202	0 (0)	4	225	1.8 (0.1 – 3.5)
Uno East	2	240	0.6 (-0.4 – 1.7)	2	209	0.9 (-0.5 – 2.4)	0	227	0 (0)	1	223	0.4 (-0.5 – 1.4)
Uno South	4	227	1.9 (0.1 – 3.6)	1	162	0.5 (-0.7 – 1.8)	0	220	0 (0)	0	227	0 (0)

[a] Clusters not sampled during 2021 MDA 3. In the case of Nhago, error in archipelago mapping meant it was not sampled during the 2021 PTS.

[b] Mean sporozoite rate calculated using household-level rates.

Appendix IX. Exploratory analysis and cluster-level summaries of female *Anopheles* entomological inoculation rate from post-MDA 3 and PTS collections in 2021 and 2022.

Exploratory graphs and tables were made during analysis of the female *Anopheles* entomological inoculation rate (EIR) following IVM MDA and PTS in 2021 and 2022. To calculate the EIR, we used the formula 1.605 x (number of CSP-positive *Anopheles*/number of *Anopheles* tested) x (number of *Anopheles* collected from LTs/ number of trapping nights) [1]. To detect the infective sporozoites, CSP-ELISA was performed on post-MDA 3 and peak transmission survey (PTS) collections in 2021 and 2022, therefore the EIR has been calculated for these timepoints.

The baseline survey conducted in October to December of 2019 sampled 16 of the 24 clusters (eight control clusters; eight intervention clusters). In the Novembers of 2021 and 2022, the PTS was conducted in all 24 clusters.

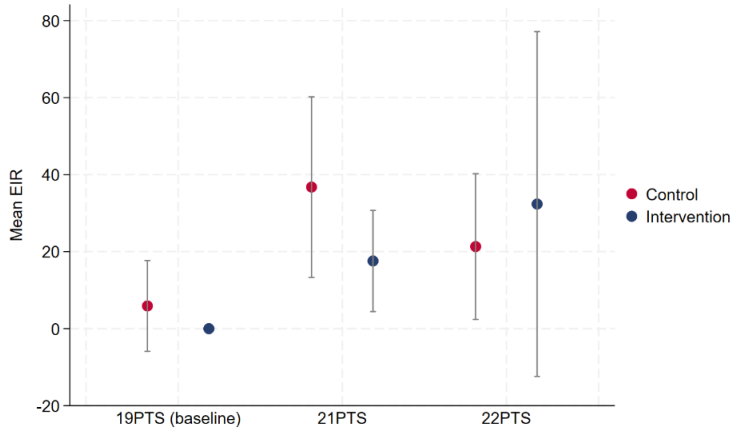

Figure 1. Mean *Anopheles* EIR calculated using cluster-level percentages for the PTS in 2019 (baseline), 2021 and 2022 from the intervention and control trial arms.

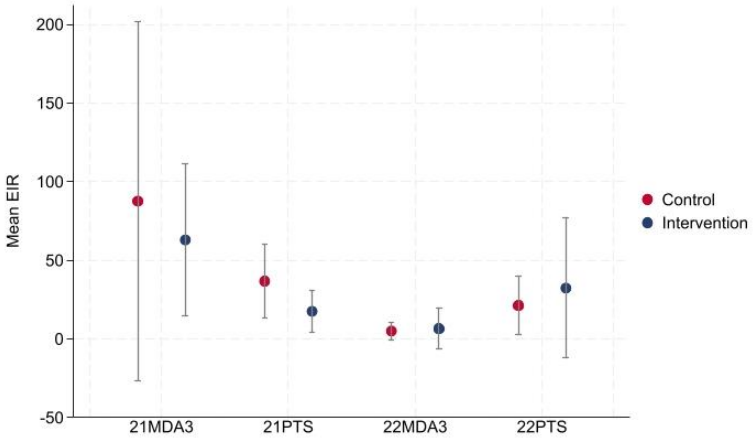

Figure 2 Mean *Anopheles* EIR calculated using cluster-level rates. Mass drug administration (MDA); Peak-transmission survey (PTS) from the intervention and control trial arms.

Table 1. *Anopheles* density (proxy for HBR), sporozoite rate and EIR for each cluster sampled from post-MDA 3 and PTS collections in 2021 and 2022.

	2021 MDA 3			2021 PTS			2022 MDA 3			2022 PTS		
	Anopheles density	SR (%)	Mean EIR (95% CI)[b]	*Anopheles* density	SR (%)	Mean EIR (95% CI)[b]	*Anopheles* density	SR (%)	Mean EIR (95% CI)[b]	*Anopheles* density	SR (%)	Mean EIR (95% CI)[b]
Control												
Bubaque Central	20.9	0.8	55.9 (-31.3 – 143.0)	12.7	1.6	44.1 (-23.8 – 112.0)	7.7	0.6	21.3 (-24.7 – 67.2)	4.5	0	0 (0)
Bubaque South	8.1	0.3	8.0 (-10.1 – 26.2)	1.0	0	0 (0)	8.4	0	0 (0)	7.6	1.3	39.8 (-32.1 – 111.7)
Canhabaque North	26.9	0	0 (0)	22.1	0.4	10.9 (-13.8 – 35.7)	7.9	0.5	11.8 (-13.5 – 37.0)	3.2	0	0 (0)
Caravela	5.1	0	0 (0)	5.5	1.6	12.2 (-6.2 – 30.6)	10.6	0	0 (0)	1.8	0	0 (0)
Meneque[a]				30.6	1.4	59.9 (-21.9 – 141.8)	32.8	0	0 (0)	31.2	0	0 (0)
Nhago[a]							68.9	0	0 (0)	9.5	1.3	45.0 (-7.9 – 97.9)
Orango Grande	6.4	0	0 (0)	6.0	5.0	16.1 (-20.3 – 52.3)	29.0	0	0 (0)	9.7	0	0 (0)
Orangozinho	78.3	2.5	521.5 (139.9 – 903.0)	117.1	0	0 (0)	58.7	0	0 (0)	18.5	1.1	15.3 (-7.4 – 38.0)
Tchedega	16.2	1.2	45.6 (-7.6 – 98.9)	14.1	1.2	10.7 (-13.5 – 34.9)	20.4	0.4	15.2 (-17.4 – 47.8)	0.6	18.2	21.0 (-24.0 – 66.1)
Unhocomo[a]				2.2	2.8	5.3 (-6.7 – 17.4)	19.2	0	0 (0)	2.8	0	0 (0)
Uno North	61.9	0.4	77.4 (-97.7 – 252.5)	17.8	1.4	47.8 (-23.0 – 118.5)	29.2	0	0 (0)	55.2	0	0 (0)
Uracane	32.9	0.6	0.3 (-0.3 – 0.9)	12.2	1.0	19.3 (-9.8 – 48.4)	40.9	0	0 (0)	41.9	0.9	128.0 (-60.4 – 316.3)
Intervention												
Bubaque North	0.2	0	0 (0)	6.2	0	0 (0)	3.1	0	0 (0)	1.5	0	0 (0)
Canhabaque Central	32.4	2.0	226.8 (-115.0 – 568.7)	16.0	0.5	9.2 (-11.6 – 29.9)	69.1	0.4	128.0 (-147.5 – 402.4)	5.8	0	0 (0)
Canhabaque South	30.3	1.0	123.3 (-66.9 – 1.7)	84.2	0	0 (0)	95.4	0	0 (0)	26.1	0.9	55.2 (-41.4 – 151.8)
Canogo	12.6	0.4	19.7 (-27.9 – 64.3)	5.2	0	0 (0)	23.2	0	0 (0)	6.4	0	0 (0)
Carache[a]				26.7	0.5	7.6 (-9.6 – 24.9)	18.1	0	0 (0)	3.3	0	0 (0)
Eguba[a]				5.0	0	0 (0)	48.0	0	0 (0)	4.5	0	0 (0)
Formosa	3.7	0	0 (0)	2.7	0	0 (0)	64.4	0	0 (0)	15.9	0.4	20.1 (-23.0 – 63.3)
Rubane	4.7	0	0 (0)	7.5	0.9	11.3 (-9.6 – 32.2)	5.3	0	0 (0)	1.0	0	0 (0)
Uassa-wite[a]				10.5	1.0	20.1 (-13.4 – 53.7)	67.4	0	0 (0)	1.0	0.8	4.1 (-4.7 – 12.8)
Unhocomozinho	28.0	1.4	147.5 (-109.8 – 404.8)	14.2	1.9	31.0 (-7.4 – 69.5)	48.8	0	0 (0)	52.6	1.8	117.7(-20.6 – 256.0)
Uno East	9.7	0.6	27.6 (-14.5 – 69.7)	6.0	0.9	10.7 (-5.8 – 27.2)	47.5	0	0 (0)	22.1	0.4	24.8 (-28.4 – 78.1)
Uno South	17.5	1.9	96.8 (3.0 – 190.7)	5.3	0.5	5.3 (-6.7 – 17.4)	53.5	0	0 (0)	21.3	0	0 (0)

SR sporozoite rate; *EIR* entomological inoculation rate.

[a] Clusters not sampled during 2021 MDA 3. In the case of Nhago, error in archipelago mapping meant it was not sampled during the 2021 PTS.

[b] Mean EIR calculated using household-level rates using the formula 1.605 x (number of CSP-positive *Anopheles*/number of *Anopheles* tested) x (number of *Anopheles* collected from LTs/ number of trapping nights) x 180 [1].

References

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