Investigating the Effects of Ivermectin plus Dihydroartemisinin-Piperaquine MDA on Malaria Transmission by Measuring Serological Markers of Exposure and its Effects on the Prevalence of Ectoparasites and Soil-Transmitted Helminths

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Declaration

I declare that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated within the thesis.

Signature Date:

10.11.2023

Preface

According to the submission guidelines provided by the London School of Hygiene and Tropical Medicine, this thesis is presented as a "Research Paper Style Thesis". Four of the chapters in the thesis contain a total of two papers that have been submitted for publication and published, and two papers that are currently being prepared for submission to specific peer-reviewed journals. Due to the varying requirements of the journals, there may be some repetition of material and differences in formatting within these chapters. The publication details and acknowledgments for co-author contributions are provided on the cover sheets for each individual paper. The rest of the thesis consists of additional material which includes an introduction and methods section to the research project as a whole.

All material within this thesis was written by Christian Kositz.

Abstract

The goal of eliminating malaria is constantly facing challenges by adaptations of both the vector and parasite. Ivermectin, a broad-acting endectocide against several non-vertebral species, is a promising additional tool for malaria control. However, large-scale studies on the effects of ivermectin for this purpose were still missing, and the MASSIV trial in Eastern Gambia was the first to evaluate its value along with mass drug administration (MDA) with dihydroartemisinin-piperaquine.

My project was nested within the MASSIV trial, and I was investigating several on and off target effects of ivermectin and dihydroartemisinin-piperaquine MDA. Firstly, I evaluated the ability of the Luminex MagPix© platform to detect changes in malaria antibodies. Furthermore, I evaluated this system as a first step to using it as a tool for the surveillance of malaria transmission using serology and to establish it in The Gambia as an ongoing surveillance method for future projects in The Gambia and West Africa.

Secondly, I was investigating the effects of ivermectin on non-malaria targets, specifically ectoparasites, including scabies, headlice, bedbugs, and soil-transmitted helminths. For these, prevalence data in the study area and The Gambia were lacking. Therefore, I conducted a survey before and after MDA and two years after MDA to examine its effect between the study arms. Ectoparasites were examined with clinical/physical examinations. For soil-transmitted helminths, qualitative PCR was performed on a single stool sample to detect soil-transmitted helminths (*Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Trichuris trichiura and Strongyloides stercoralis*).

Lastly, I used the collected data on MDA coverage from the MASSIV trial to look at potential reasons for reduced coverage, such as systematic non-compliance and what factors can be attributed to non-participation or not receiving the MDA.

The overall objective was to demonstrate that, in addition to its effect on malaria, ivermectin would decrease the burden of several other parasites, therefore improving health and quality of life on a broader scale.

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Table of abbreviations

ABCB1	ATP-binding Cassette Subfamily B Member 1
ABER	Annual Blood Examination Rate
ACT	Artemisinin-based Combination Therapies
AQ	Amodiaquine
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis* subsp. *Israelensis*
COVID-19	Coronavirus Disease 2019
DBS	Dried Blood Spot
DHP	Dihydroartemisinin-piperaquine
EIR	Entomological Inoculation Rate
Fcy	fragment crystallisable y
GluCls	Glutamate-Gated Chloride Channels
GMIS17	Gambia Malaria Indicator Survey 2017
GR	Gametocyte Rate
HTLV-1	Human T-Lymphotropic Virus
IgG	Immunoglobulin G
IPTi	Intermittent Preventive Treatment in Infants
IPTp	Intermittent Preventive Treatment in Pregnancy
IRS	Indoor Residual Spraying
ITL	Insecticide-Treated Livestock
IVERMAL	Efficacy and Safety of High-Dose Ivermectin for Reducing Malaria Transmission
IVM	Ivermectin
LLIN	Long Lasting Insecticidal Net
LSHTM	London School of Hygiene and Tropical Medicine
malERA	Malaria Eradication Research Agenda
MASSIV	Mass Drug Administration of Ivermectin and Dihydroartemisinin-piperaquine as an Additional Intervention for Malaria Elimination
MATAMAL	Adjunctive Ivermectin Mass Drug Administration for Malaria Control: A Cluster Randomised Placebo-controlled Trial
mcg/kg	Micrograms per Kilogram
MDA	Mass Drug Administration
MDR1	Multidrug Resistance Protein 1
MFI	Mean Fluorescence Intensity
MRCG	Medical Research Center The Gambia
MRP	Multidrug resistance-associated protein

MSD	Merck Sharpe & Dome
NAAT	Nucleic Acid Amplification Test
NIBSC	National Institute for Biological Standards and Control
NMCP	National Malaria Control Programme
NTD	Neglected Tropical Disease
ODK	Open Data Kit
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PF*Pf*	Proportion of Fevers Parasitaemic
P.f.	*Plasmodium falciparum*
PR	Parasite Rate
RDT	Rapid Detection Test
R-PE	R-Phycoerythrin
SCC	Scientific Coordinating Committee
SCH	Survey of Schistosomiasis
SMC	Seasonal Malaria Chemoprophylaxis
SOP	Standard Operating Procedure
SP	Sulfadoxine-Pyrimethamine
SPR	Slide Positivity Rate
STH	Soil-Transmitted Helminths
UNICEF	United Nations International Children's Emergency Fund
URR	Upper River Region
WHO	World Health Organization

Chapter 1 Introduction

Malaria is estimated to have caused up to 255 million cases and approximately 405,000 deaths in 2018, with the majority of cases and deaths in sub-Saharan Africa (1). Globally, malaria is one of the most important human diseases and has left its imprint on human genetics (2,3), art (4), empires (5) and even outcomes in human conflict (6–8).

Since the first introduction of treatments against the malaria parasite and insecticides for vector control, both the vectors and Plasmodium species have shown an incredible ability to develop resistance (1,9,10), including vector behavioural changes to avoid the physical and chemical barrier that of modern long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) (11–14). The development of resistance against interventions is a continuous challenge for malaria control and elimination efforts and warrants the ongoing development and use of new drugs, methods, and strategies.

1.1 Current tools for Malaria control

1.1.1 Vector control tools

At present the toolkit for malaria control includes a broad variety of interventions that either attack the vector directly or prevent vector/host contact, its breeding sites or the parasite itself.

Most malaria vectors, especially *An. gambiae*, are known for their late night, indoor biting, and resting behaviour, making them an excellent target for long-lasting insecticidal nets (LLINs) and indoor IRS. Therefore preventing the mosquito from biting the host (LLINs) or from surviving their resting phase on walls inside houses (IRS) have been key control strategies (15). However, insecticide resistance is on the rise reducing the effectiveness of these interventions (16). Moreover some species have an early evening outdoor biting and outdoor resting mode which are more challenging to control with these methods (11,17). Additionally, it appears that other *Anopheles* spp. can change their original biting behaviour (9,28), again offsetting these otherwise successful control methods (8). Finally, on the host side, a person's night-time behaviour could significantly negate the effects of LLINs or IRS(18).

Vector breeding control usually involves components that attack the vector's larvae. These range from chemical compounds such as Temephos, specific bacterial species such as

Bacillus thuringiensis subsp. *Israelensis* (Bti) and *Bacillus sphaericus* (Bs) or bacterial toxins (Spinoyns), insect growth regulators and biological control using larvivorous fish (19).
Not yet included in the official WHO recommendations but potentially future methods include the use of toxic sugar baited traps which showed some success in a recent trial with another trial being underway (20,21) Additionally, the use genetically modified mosquitoes to reduce transmission of malaria directly or the fertility of female mosquitoes by using a gene drive has shown promise in small proof of principle trials (22–24). Another less commonly applied but additional form of vector control is the use of livestock to divert the more zoophilic *Anopheles* species away from humans and/or using these as traps by feeding them ivermectin or insecticide-treated livestock(ITL) (25–28).

1.1.2 Parasite Control Tools

Improved treatment of malaria with the discovery and development of artemisinin-based combination therapies (ACTs) helped to markedly reduce the number of deaths. Nonetheless this success is threatened by emerging resistance to different compounds (29). Specific ACTs such as dihydroartemisinin-piperaquine have the advantage of not only treating malaria but elicit a prophylactic effect of 14 to 28 days after treatment due to the prolonged half-life of piperaquine (30). This pharmacological characteristic gives DHP a significant advantage for MDA and several MDA trials in a variety of settings have shown its benefits for malaria control (31,32) and modelling suggesting even the possibility of interruption (33). However, it has been shown that as soon as the effect of piperaquine abates, malaria rates rise again, even within the same season (32,34).
Seasonal malaria prophylaxis (SMC), intermittent preventive treatment in pregnancy (IPTp) and Intermittent preventive treatment in infants (IPTi) are different variants of anti-malaria drug applications to target specifically vulnerable groups such as young children and pregnant women, with SMC being specifically aimed at countries with highly seasonal malaria such as The Gambia (35,36).
More recently, the first malaria vaccine has been approved recently and shown to be non-inferior to SMC and in combination with SMC resulted in a markedly reduced incidence in uncomplicated and severe malaria, and deaths (37).

1.2 Ivermectin (Mectizan@)

1.2.1 Background

Ivermectin (IVM) is an *endectocide* (i.e., a drug active against endo- and ectoparasites) of the avermectin group. It was discovered in 1975 by a private/public partnership between the Kitasato Institute in Japan and Merck Sharpe & Dome (MSD), with the Nobel Prize for its discovery being awarded in 2015 (38). It is one of the most used drugs in veterinary medicine, treating a wide variety of infestations (39,40). In human medicine, it became well known through its use to treat, control and eliminate onchocerciasis through the Mectizan Donation Programme (41), and subsequently its use for filariasis (42), thus reducing the burden of these diseases for millions of people. Furthermore, it is an effective intervention for a wide variety of human parasites, such as helminths and ectoparasites, specifically *Sarcoptes scabiei* and *Strongyloides stercoralis* (43–46).

1.2.2 Mechanism of Action

Although its mechanism of action is not fully understood, one of ivermectin's main functions is its action on glutamate-gated chloride channels (GluCls), which are found only in invertebrates, affecting mobility, feeding, and reproduction (47). However, IVM interacts with a wider range of ligand-gated channels in vertebrates and invertebrates, and fatalities, especially in certain dog breeds have been reported (48), these are caused by the lack of P-glycoprotein 1, also known as multidrug resistance protein 1 (MDR1), ATP-binding cassette subfamily B member 1 (ABCB1), Multidrug resistance-associated proteins (MRPs) and others for which ivermectin itself also acts as an inhibitor (49). A lack of this protein, which protects against the effects of ivermectin (50,51) has been reported only once so far in a human and use of ivermectin showed clearly the potential toxicity in humans (52). Additionally, the unlicensed use of IVM for COVID-19 lead to several recorded cases of overdosing with signs of neurotoxicity and even death (53).

However, IVM has been shown to have very few side effects, even in comparatively high doses or repeated dosing (54–56). Restriction of its use has been based on the fact that in vertebrates, it must penetrate the blood-brain barrier which was postulated to be incomplete in new-borns or younger children. However, though possibly the case in some rats, in humans this hypothesis lacks serious scientific evidence (57–59).

Due to a lack of safety, its use in humans under a certain age, pregnancy, and breastfeeding is off-label and not recommended. However, newer data indicate that ivermectin may be safe at a standard dose of 200 mcg/kg body weight in children < 15 kg or < 90 cm, broadening its applicability (60).

1.3 Importance of Ivermectin in Vector Control in Malaria

IVM has become a topic of interest in the malaria community due to its mosquitocidal effects to address some of the challenges that especially LLINs and IRS are facing. Some of these challenges are related to mosquito behaviour, as not all Anopheles species and even within the same species are indoor resting or late evening biters, therefore LLINs and IRS do lose efficacy as their main effect is based on people resting or sleeping under a bed net or if the mosquito rests inside to die from the insecticide on the walls (61–64). This difference in behaviours gives ivermectin an advantage as it directly turns the human participant into a deadly trap, as after ingestion of a standard dose, the blood of a participant becomes highly toxic for a wide variety of *Anopheles* species involved in malaria transmission (65–67).

However, ivermectin has a distinct pharmacological downside as its plasma half-life is only 18 hours and, with a standard dose (200 mcg/kg once), this mosquitocidal effect lasts on average only 1.9 days, reducing the drug's usefulness for mass drug administration (68,69). For this reason the IVERMAL trial investigated a different schedule in which participants took three days of ivermectin at a dose of 300 µg/kg for three consecutive days, showing an extension of the effects on *Anopheles gambiae* up to 28 days , solving this problem (54,70). Nonetheless, the application of such an extended schedule may cause logistical problems, as many of the areas hit hardest with malaria are often not easily accessible.

Furthermore, this extended schedule makes the drug a valid option for mosquito control, regardless of feeding and resting behaviour.

Another important downside of ivermectin the main downside is the fact that an infected mosquito can still potentially transmit malaria at least once before dying of ivermectin poisoning from its blood feed. Therefore, the addition of a drug to treat / prevent malaria such as DHP makes sense as part of this intervention but also due to the ethical implications of potentially exposing people to malaria. Pharmacologically, DHP is the partner drug of

choice, as it provides a full course of treatment and piperaquine on its own prevents malaria for up to a month after such a full course.

This is an important point to consider, as ivermectin in comparison to SMC, post discharge malaria chemotherapy (PDMC) or perennial malaria chemotherapy (PMC) is neither a treatment for malaria nor does it suppress the parasite.

Additional effects of ivermectin on mosquitoes include a knockdown effect that reduces the ability to fly and, therefore, evasion of predators and other natural causes similar to other insecticides used, and interestingly reduced fertility, further broadening the use and impact on mosquito mortality of ivermectin in the field (71–73).

1.3.1 Modelling the Effect of Ivermectin on Malaria

Modelling from 2014 showed adding IVM to an ACT MDA could significantly reduce malaria transmission, primarily by a sustained impact on reduction in vector density and longevity and reduced parasite prevalence (74). The most recent modelling data used the IVM results from IVERMAL incorporating the 300mcg/kg for three consecutive days and 1x400 mcg/kg once based pharmacokinetic–pharmacodynamic model (70). The results of this work showed that IVM on its own and in combination with an ACT MDA leads to a reduction in incidence and prevalence in a variety of settings, with the 300mcg/kg schedule showing a greater effect than the 1x400 mcg/kg that was more pronounced in a seasonal malaria setting (75).

Both models show that ivermectin would be expected to lead to a change in age distribution of the female mosquitos, with a reduction in the proportion of older female mosquitoes (i.e. those who can transmit malaria as the parasite development in the mosquito takes at least 10 days (76)), thus leading to an additional lag time after MDA with a prolonged reduction in vector capacity (74,75). Specifically, the assumed reduction in clinical incidence for a DHP-MDA plus ivermectin combination such as the one in the MASSIV trial was calculated to be at 78 - 90% for seasonal and highly seasonal malaria(75). However, one the assumptions was that the intervention group was a constant population with no movement of infected humans or vectors in or out of the study area (75). On top of that the coverage needed was expected to be at least 70% of everyone above 5 years of age without large population movements of parasite carrying people and mosquitoes, putting emphasis on the logistics of conducting a trial.

Additionally, it is important to note that all the data available at present on the potential impact of ivermectin is from models, though several studies are either finishing or conducted at present. These will answer the question where ivermectin is positioned within the toolset of options for malaria control, specifically, vector control and in extension interruption of malaria transmission. For example, the Cochrane review for LLITNs and ITNs shows a reduction in malaria incidence of 56% and 41% compared to no nets(77), and in case of SMC which depending on the country showed a protective effect in reducing incidence of 88% (78.7 – 93.3 %). Assuming the modelling assumptions for ivermectin hold true, with 78 – 90% reduction in incidence of clinical malaria it would be comparable to SMC(35,78). However, it should be pointed out, that the DHP-IVM combination is very similar to a SMC in its reduction of incidence, and therefore a trial comparing SMC vs. DHP-IVM is needed to clarify that the effect seen is not just based on the DHP part of the intervention.

1.4 Potential NTD targets of the MASSIV IVM MDA

Malaria is often co-endemic with a large group of ectoparasites such as scabies, head lice, bed bugs, and soil-transmitted helminths that fall into the category of neglected tropical diseases (NTDs). They are rarely fatal, but exert a large morbidity burden and are a major public health problem in many settings (79,80).

1.4.1 Scabies (*Sarcoptes scabiei*)

Scabies is a parasitic skin infestation common in low- and middle-income countries with a prevalence of up to 50% in some areas in under 18-year olds (81,82). Data from suburban areas of The Gambia show a prevalence of about 15% (83), with no data available from rural areas.

Typical of ectoparasites, the scabies mite causes an intense itching sensation leading to scratching and possible bacterial skin disease. These secondary bacterial infections increase morbidity, and can lead to mortality through bacterial sepsis, deep skin infections, kidney disease, and rheumatic heart disease (83–86).

Several topical treatments such as permethrin or benzylbenzoate cream are available for scabies. However, they are less suited for population-level control because they require a prolonged duration of application, can cause skin irritation, and depending on the product, can be expensive (85,87). Oral medications such as ivermectin offer a more straightforward

solution, ingestion can be observed, and asymptomatic individuals, as well as entire populations, can be treated and have been shown to be highly effective (88). The MASSIV trial will use the IVERMAL dosing schedule, i.e., higher doses, and therefore an effect on scabies is expected.

1.4.2 Headlice (*Pediculus humanus capitis*)

Headlice are a common infestation of the human scalp, especially in crowded areas such as schools and refugee camps (89,90). Lice infestation is associated with severe itching, occasionally enlarged cervical lymph nodes, bacterial skin infections, and social stigma (91,92). Several bacteria have been found in headlice (93).
However, the importance of its role, compared to body lice (*Pediculus humanus corporis*), as a cause of secondary diseases is not yet evident (93,94). One study from The Gambia showed a prevalence of 28% in children (95). Data were available from the URR.
Ivermectin is an effective treatment for head lice (46,96). However, resistance to ivermectin has been observed and the mechanism has recently been quantified as a mutation of a glutamate-gated chloride channel in Senegal (97).

1.4.3 Bedbugs (*Cimex* spp.)

Bedbugs are a global pest and a growing public health concern. They are divided into two species: the tropical bedbug *C. hemipterus* and the common bedbug *C. lectularius*. Their role in the transmission of infectious diseases has not been proven so far (98). More common in low-income areas (99,100), they can be imported into any social strata and cause outbreaks. Resistance to insecticides is a common feature, and ivermectin has the potential to be a new solution in hard-to-treat infestations (101). Data in The Gambia were scarce but showed significant infestation, with a prevalence of 37.5% in the beds investigated. Data from the URR were non-existent. A caveat in the study area is the high environmental temperature of up to the 45°C, which can lead to inhibition and death of bedbugs (102). Ivermectin is capable of killing bedbugs in human feeding and animal experiments (103,104).

1.4.4 Soil-Transmitted Helminths

Globally, soil-transmitted helminths infest an estimated 1.5 billion people, causing chronic anaemia, malabsorption in general, and clinical presentations such as intestinal

obstruction in severe infestation of *Ascaris lumbricoides* and rectal prolapse in *Trichuris trichiura*, responsible for a large amount of morbidity, delayed development, low birth weight, reduced school performance, and social stigma (105,106). Furthermore, they are strongly associated with poverty and the stage of a country's development (107,108). In the case of The Gambia, there is a relative lack of prevalence data on helminths, the most recent data being from 1995 and the earliest from 1952 (109,110), both from the same geographical area. There are no data for the Upper River Region in eastern Gambia.

To reduce the burden of disease for soil-transmitted helminths, the WHO recommends MDA with benzimidazoles such as albendazole and mebendazole, depending on local prevalence (111,112), with ivermectin being used less frequently due to its reduced effect on hookworms (113,114). However, data on multiple dose schedules of ivermectin for hookworm and the possibility of a stacking effect are lacking. Furthermore, ivermectin has a greater impact than albendazole on *Strongyloides stercoralis* (115).

The group of soil-transmitted helminths includes several species, including hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*), to a lesser extent, *Strongyloides stercoralis* and whipworm (*Trichuris trichiura*) (79,108). Of these, the first three cause a larva migrans visceralis syndrome, which means that the larval stages must travel through the human body, explicitly reaching the lungs as an intermediate stage to complete their development before ending up in the intestines where they produce eggs or larvae (116–119). This process can cause eosinophilia and Löffler syndrome, which presents with asthma-like symptoms such as wheezing and coughing (120).

With regard to their way of transmission and lifecycle, there are both similarities and some distinct differences between species. Hookworms and *S. stercoralis* larvae penetrate human skin directly when skin is in direct contact with contaminated soil, while *A. lumbricoides* and *T. trichiura* are transmitted by ingestion of eggs in contaminated food or hands (116–119). This also means that exposure could be reduced to the former by wearing shoe wear and the latter by handwashing (121,122).

Life expectancy can be years or decades for most of these helminths, and without reinfection seems to be self-limiting (123,124), with the exception of *S. stercoralis*, which has the ability to autoinfect and could potentially maintain infection for decades, perhaps for life, even after the person has left the endemic area (125). Furthermore, under immunosuppressed conditions, most commonly corticosteroids and human T-lymphotropic

virus (HTLV-1) infection, which can cause hyperinfection syndrome that, if not treated, carries a high mortality rate (126).

1.5 Tools for Monitoring Malaria Transmission

Malaria transmission surveillance depends on the sensitivity of the diagnostic method used, and as more progress is achieved in malaria elimination many areas become low transmission settings. This in turn is correlated with a higher proportion of infections being sub-microscopic (127).

A summary of tools used for measuring malaria transmission and their problems in low transmission settings is given in Table 1.

Metric	Definition(128)	Measure of Transmission	Method	Discriminatory Power
Annual blood examination rate (ABER)	The number of people receiving a parasitological test for malaria per unit population per year	Level of diagnostic monitoring activity	Microscopy or RDT	•Dependent on health-system provision
Case, confirmed	Malaria case (or infection) in which the parasite has been detected in a diagnostic test	Current transmission or incidence if data collection is repeated or routine	Microscopy or RDT positive	•Insensitive at low transmission; saturates at high transmission •Underestimates due to system inadequacies and poor health seeking behaviour
Case, fever	The occurrence of fever (current or recent) in a person	Current transmission or incidence if data collection is repeated or routine	Reported or observed fever	•Depends on diagnostic sensitivity •Insensitive at low transmission
Proportion of fevers parasitaemic (PFPf) *	Proportion of fever cases found to be positive for Plasmodium	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	•Depends on ABER •Insensitive at low transmission
Slide positivity rate (SPR)	Proportion of blood smears found to be positive for Plasmodium among all blood smears examined	Current transmission or incidence if data collection is repeated or routine	Microscopy	•Depends on RDT sensitivity •Insensitive at low transmission
Parasite rate (PR)	Proportion of positive results among all RDTs performed	Current transmission or incidence if data collection is repeated or routine	RDT	•Depends on RDT sensitivity •Insensitive at low transmission
Parasite rate (PR)	Proportion of the population found to carry asexual blood-stage parasites	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	•Depends on diagnostic sensitivity •Insensitive at low transmission
Gametocyte rate (GR)	Percentage of individuals in a defined population in whom sexual forms of malaria parasites have been detected	Potentially infectious human population	Microscopy; NAAT	•Depends on diagnostic sensitivity •Insensitive at low transmission

*No WHO definition is available for this term. Abbreviations: ABER, annual blood examination rate; GR, gametocyte rate; NAAT, nucleic acid amplification test; PF*Pf*, proportion of fevers parasitaemic; PR, parasite rate; RDT, rapid diagnostic test; RDT-PR, RDT positivity rate; SPR, slide positivity rate.

Table 1: An overview of malaria transmission metrics and the methods used to detect them and their discriminatory power by malERA(129)

1.5.1 Use of Serology as a Tool for Malaria Surveillance

Techniques such as the entomological inoculation rate (EIR), gametocyte rate (GR), parasite rate (PR), rapid diagnostic test positive rate (RDT-PR), or slide positive rate (SPR) become insensitive as the prevalence of malaria declines (130). Therefore, new methods are needed, especially in the end stage of malaria elimination.

As with most infections, the malaria parasite elicits an immune response that leads to the production of antibodies, thus providing a library of past infections (131–133). Although

malaria antibodies do decline when tested after years of successful control efforts (134), a successful surveillance method would need to test long-term and short-term antibody markers to receive an overall profile of local transmission or the effect of a control programme. Helb et al. (90) have described a selection of these required antibodies and their use in conjunction with the Luminex MagPix© platform, a multiplex assay system that has been standardised by Wu et al. (135). The use of dry blood spots has been shown to provide adequate antibody concentrations (136) which can be collected through cross-sectional surveys. These samples can be stored and transported quite easily and are used with the Luminex system.

The combination of these methods would provide easy-to-perform and standardised methods for transmission surveillance, and the MASSIV trial provides a potential testing ground for these approaches.

A list of the potential antibodies used and their position within the malaria lifecycle and how a DHP-IVM MDA would affect them is shown in Table 4. As an example, gSG6 and CSP-antigen occur only in the mosquito and on sporozoites respectively, therefore it is not expected that DHP would have a direct impact on them, however IVM which kills the mosquito might by reducing the EIR(137,138). On the other hand, GLURP R2 or MSP-1.19 are either expressed throughout from the hepatic stage onwards or during the erythrocyte stage, which DHP impacts by killing the parasite (139,140).

Furthermore, the expected duration of antibody responses is dependent on the age of person and the repeated duration of exposure. Also, antibodies considered to be short term are categorized by the fact, that in children without exposure to the respective antigen, their seroprevalence will drop. However, repeated exposure will lead to a more solid immune response and increase of seroprevalence over time. Long term antibodies on the other hand, do not necessarily require repeated exposure and are more indicative of previous lifetime infection with malaria. It is therefore expected that an intervention would show the largest impact on antibody levels and seroprevalence in young children and only minor changes in older adolescents or adults. These assumptions are based on available data regarding the immunogenicity of the different antigens (141–154). Unfortunately, precise data for short term decline is limited to 6-month post intervention(155). A hypothetical graphical display of the expected increase or decrease of antibodies over time is presented in Figure 1.

Antigen	Infection Cycle Stage	Life cycle stage expression	Likely to be directly affected by IVM	Likely to be affected by DHP	Timeframe	Reference
gSG6	Pre-Skin	mosquitoe saliva antigen	yes	no	Mosquito exposure	Koffi et al(156);
PfMSP-1-19	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Long-term	Kerkhof et al.(141);Pizarro et a.(157), Moss et al.(140) Helb et al.(142); van den Hoogen et al.(158); Wu et al.(135,159); Koffi et al.(156);Richards et al.(160)
PfAMA-1	Pre-hepatic; 1st generation hepatic merozoites; blood stage;	sporozoite/merozoite	yes?	yes	Long-term	Silvie et al. (161); Helb et al.(142); van den Hoogen et al.(162); Wu et al.(135,159); Richards et al.(160)
GLURP R2	Hepatic; 1st generation hepatic merozoites; blood stage;	hepatic/merozoite/schizont	no	yes	Long-term	Borre et al.(139); Helb et al.(142); van den Hoogen et al.(162); Wu et al.(135,159); Richards et al.(160)
HSP40	Hepatic; 1st generation hepatic merozoites; blood stage;	hepatic/schizont/trophozoite	no	yes	Short-term	Botha et al.(163); Mathews et al(164); Helb et al.(142); van den Hoogen et al.(162); Wu et al.(135,159);
ETRAMP5.Ag1	blood stage	parasitophorous vacuole membrane / ring stage	no	yes	Short-term	Helb et al.(142); van den Hoogen et al.(162); Wu et al.(135,159); Achan et al.(144); Spielmann et al.(165)

PfSEA-1	Hepatic stage, blood stage	schizont	no	yes	Short-term	Peng et al.(166); Helb et al.(142); van den Hoogen et al.(162)
CSP	Pre-hepatic	sporozoite	yes	no	Short-term	Kerkhof et al.(141);Helb et al.(142); van den Hoogen et al.(162);
Hyp2	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Short-term	Wu et al.(135,159);
MSP2.Dd2	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Short-term	Richards et al.(160);
MSP2.CH150	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Short-term	Richards et al.(160);
Rh4.2	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Short-term	Reiling et al.(167); Ord et al.(168);Richards et al.(160)
SBP1	erythrocytes	Maurer's clefts	no	yes	Short-term	Cooke et al.(169);
Etramp4.Ag2	blood stage	parasitophorous vacuole membrane / ring stage	no	yes	Short-term	Wu et al.(135,159); Helb et al.(142);Spielmann et al.(165)
EBA175.RIII.V	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Variable marker	Cowman et al.(170); Wu et al.(135,159); Richards et al.(160,171);

EBA181.RIII.V	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Variable marker	Cowman et al.(170); Richards et al.(160,171);
EBA140.RIII.V	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Variable marker	Cowman et al.(170); Richards et al.(160,171);
Rh2.2030	1st generation hepatic merozoites; blood stage;	merozoite	no	ye	Variable marker	Achan et al.(144); Ord et al.(168);Richards et al.(160)
Rh5.1	1st generation hepatic merozoites; blood stage;	merozoite	no	yes	Variable marker	Ord et al.(168); Minassian et al.(172); Richards et al. (160)

Table 2: Selected antibodies for the planned study and their position within the parasite life cycle as well whether they may be influenced by IVM or DHP interventions; Of note the position of within the life cycle could still change with future research.

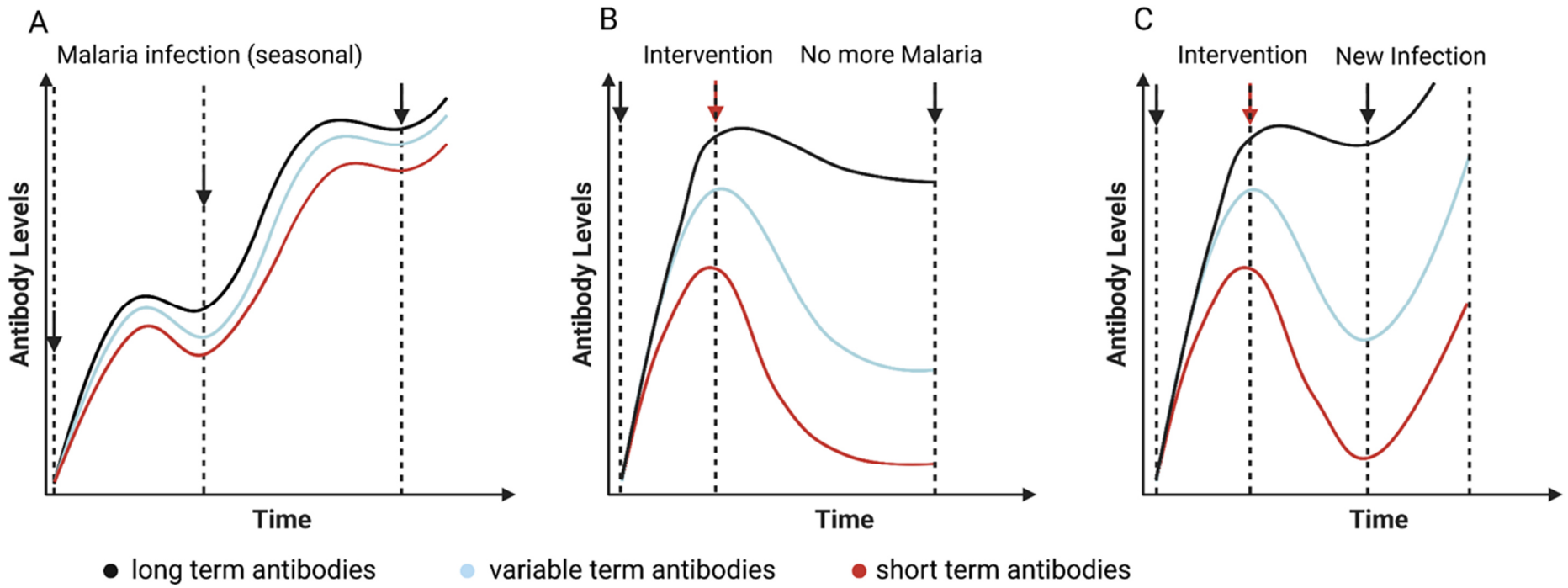


Figure 1: Expected changes in antibody levels in a seasonal malaria region for long term, variable term and short-term antibody levels based on the available literature. Unfortunately, precise data on very short-term changes < 6 months is not yet available.

1.6 Multiplex PCR for Soil-Transmitted Helminths

The detection of soil-transmitted helminth infection strongly depends on the method and the local prevalence. For example, Kato Katz was initially developed for *Schistosoma mansoni* detection (173) and is now one of the most commonly used methods for STH but has problems in detecting hookworm if the stool sample is not immediately prepared (174,175), and is insensitive to detect *S. stercoralis* infection altogether, due to its lack of egg production, leading to its original exclusion as an STH-neglected tropical disease by the WHO (176). A single specimen Kato Katz shows good sensitivity for infections with moderate to high intensities and loses sensitivity at lower intensity infections, while qPCR keeps its level of sensitivity regardless of intensity of infection (177) and potentially has a very low limit of detecting a helminth DNA going down to a handful of eggs per sample (178,179). This was an additional benefit for the Gambian setting, where prevalence data were out of date and non-existing for the trial area.

Molecular methods do have their drawbacks, leading to possible false negatives if species are indistinguishable by microscopy, specifically in the case of different hookworm species, but not detected by very species-specific primers (99,100).

1.7 The MASSIV Trial Background

The MASSIV trial (NCT03576313) was carried out in the Upper River Region (URR) of The Gambia. Despite continuous efforts to control malaria, URR experiences moderate to high rates of malaria transmission, and the vector *Anopheles gambiae* is long-lived (180,181).

The rationale of the MASSIV trial was to administer dihydroartemisinin-piperaquine (DHP) and IVM for three months during the main malaria season to evaluate if such a combined MDA could reduce or even interrupt transmission. The DHP part would treat malaria and protect from disease and IVM would add its vector control potential to reduce transmission (182).

1.7.1 Methods:

The MASSIV trial was set up as a cluster randomised controlled trial with two arms, intervention and control arm, and each arm includes 16 clusters (villages). DHP and IVM were implemented in the intervention arm in addition to the already established national malaria control methods, including IRS and LLINs, which were therefore in routine use in both the intervention and control arm and distributed by the national malaria control programme (NMCP) independently of the MASSIV trial and unfortunately no data on coverage could be obtained from the NMCP. Ivermectin was administered at a dose of 300 mcg/kg for three consecutive days once a month for three consecutive months at the beginning of the transmission season and dihydroartemisinin-piperaquine according to the manufacturer's protocol for a standard treatment course of three days. This dosing and administration schedule was based on earlier modelling data by Slater et.al (74,75). The original timeframe consisted of two years of MDA, 2018 and 2019.

1.7.2 Results:

The intervention led to significant reduction in prevalence in all ages in the intervention arm with 5.1% compared to the control arm with 12.8% (OR 0.3, 95%CI 0.16 – 0.59; $p < 0.0001$) and in incidence of clinical malaria, with an incidence rate of 1.1 in the control arm and 0.24 in the intervention arm (IRR 0.21, 95% CI: 0.1 – 0.43; $p < 0.0001$). No effect was seen on primary entomological end point of parous rate (OR: 0.9; 95%CI: 0.66 –

1.25, p = 0.537). However, the secondary endpoints of vector density and entomological inoculation rate (EIR) were significantly reduced in the intervention arm in 2019 with an OR of 0.36 (95%CI: 0.21 – 0.64, p < 0.0001) and 0.26 EIR ratio (95%CI 0.13 – 0.51) respectively.

1.8 Relationship of my PhD within the MASSIV Trial

This PhD was nested within the MASSIV trial. My PhD questions were included within the main trial protocol. My contribution to the everyday trial was my time for fieldwork, laboratory work, and data analysis, and the funds for my fieldwork, the laboratory work and data analysis.

I was responsible for the planning, organisation and supervision of the elements of the trial related to my PhD; specifically, the ectoparasite field surveys, including the stool samples collected in the November 2019 and 2021 surveys. Furthermore, I analysed the dried blood spots (DBS) collected during the trial for the malaria serology part using the Luminex® platform and a qPCR platform for STH at LSHTM for the collected stool samples and I conducted the analysis on the factors associated with non-participation with MDA.

1.9 Scientific Rationale and Knowledge Gaps

1.9.1 Malaria Questions:

Question 1:

Does mass drug administration (MDA) with ivermectin (IVM) and dihydroartemisinin-piperaquine (DHP) cause a change in the antibody titre levels of selected antibodies for short-term, variable-term and long-term parasite exposure as well as vector between the control and intervention arms?

Question 2:

Does the serology of the chosen correlate with measures such as PCR prevalence of malaria within arms?

1.9.2 NTD Questions

Question 1:

Does the use of ivermectin as a Malaria MDA using the IVERMAL dosing schedule lead to a reduction in prevalence of soil-transmitted helminths, specifically a composite of *A. duodenale, N. americanus, A. lumbricoides, T. trichiura*, and *S. stercoralis*?

Question 2:

Does the use of ivermectin as a Malaria MDA using the IVERMAL dosing schedule lead to a reduction in prevalence of ectoparasites, specifically a composite of *S. scabiei, Cimex* spp. and *P. humanus capitis*?

1.9.3 MDA-Compliance Question:

Are there potential causes for systematic non-participation in the MDA and are they related to each other?

Chapter 2 Study Aim and Objectives

2.1 Aim

1. To evaluate the impact of ivermectin and dihydroartemisinin-piperaquine MDA on serological markers for malaria parasite and vector exposure (transmission);

2. To assess the effects of ivermectin and dihydroartemisinin-piperaquine MDA of the MASSIV trial on non-malaria targets (SHT and ectoparasites).

2.2 Objectives

1. To use Luminex® MagPix platform multiplex serological assays to assess differences in exposure to malaria parasites and vectors between control and intervention arms of the MASSIV study and evaluate its use for measuring malaria transmission.
    a. To determine the correlation of the malaria serology results and the malaria PCR results

2. To determine the prevalence of soil-transmitted helminths using a STH qPCR.

3. To determine the prevalence of ectoparasites (scabies, headlice and bedbugs) using clinical examination

4. To identify risk factors for systematic non-participation in the MDA using the coverage data collected by the MASSIV trial

Chapter 3 Methodology

3.1 Study Site and Population

The study is set in the Upper River Region of The Gambia in West Africa an area of the country that still experiences highly seasonal malaria with peak transmission between September and November during the short rainy season from July to November followed by a longer dry season (November to June). The population consists of members of the Fula, Mandinka, Sarahule, and to lesser extent Woloff people and is primarily of Islamic faith.
A census conducted by the MASSIV trial in 2018 estimated 11518 people within the 32 villages. Each of these villages represented a single trial cluster.

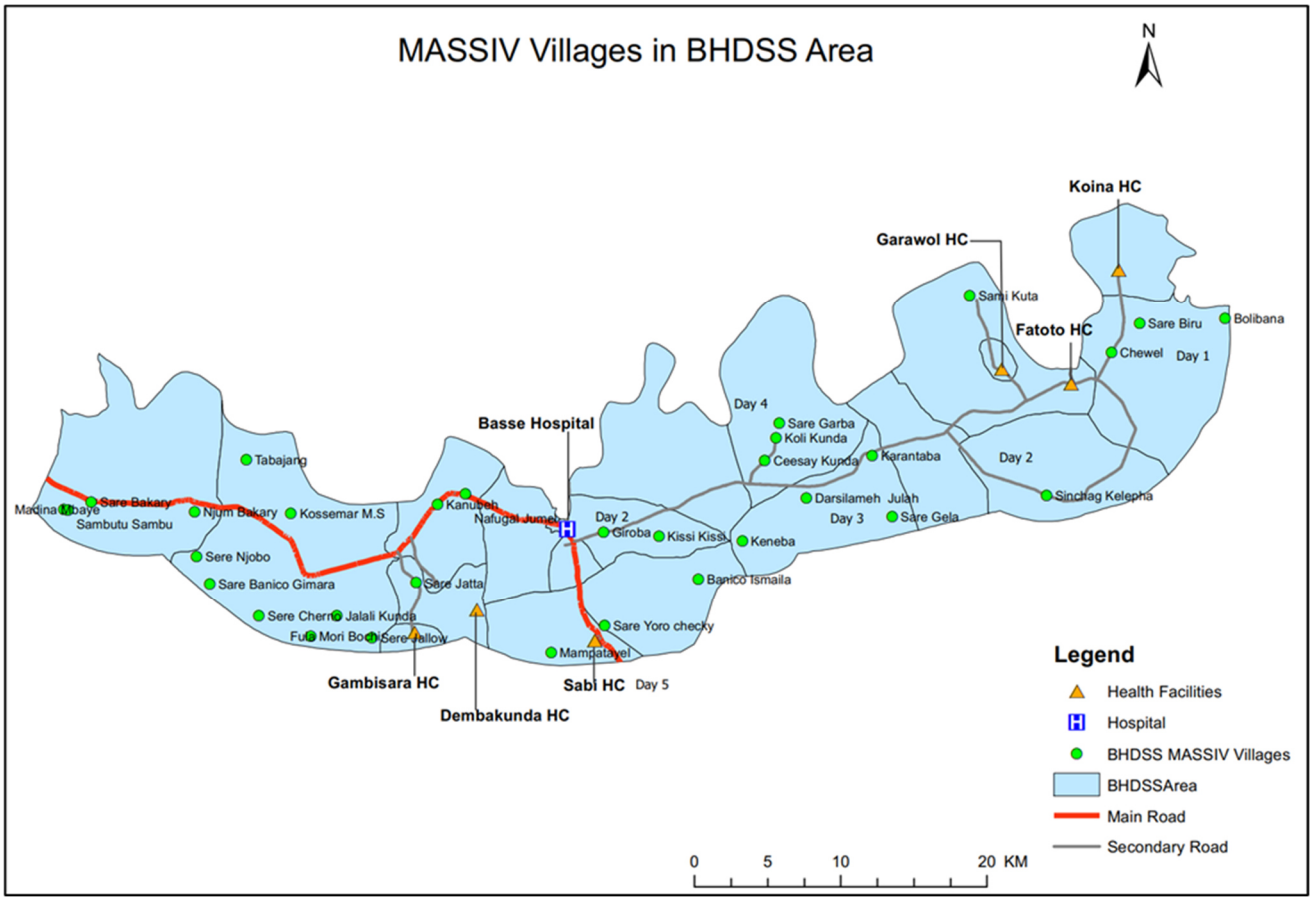


Figure 1: Map of the MASSIV trial study area showing the villages, health centres and main roads, generously provided by the MASSIV trial data team.

3.1.1 Prevalence of Malaria in the Upper River Region (URR) and the National Malaria Control Programme (NMCP) of The Gambia:

The prevalence of malaria in the URR has been surveyed with both molecular and serological methods shown in Table 1. The two studies used different survey units. Mwesiga et al. conducted a cross-sectional survey using PCR surveying five villages spread over the URR with a prevalence ranging from 21 – 49% (181). The seroprevalence used by Okebe et al. was conducted in schools throughout the Gambia, including three schools located within the URR. The age ranged from 4 to 20 years. In this survey, antibodies against MSP-1-19 were measured and showed a prevalence ranging from 34 to 35% (183).

The national malaria control programme in the Gambia uses IRS, LLIN, IPTp, using SP/Fansidar combination, and since 2014 SMC (seasonal malaria chemotherapy) was introduced with the support of UNICEF. The precise details are described in the GMIS 2017 (Gambia Malaria Indicator Survey 2017) (184). In summary, for SMC, no data were given on the drug used, although UNICEF can be expected to use WHO recommendations for those years, hence amodiaquine (AQ) and sulfadoxine-pyrimethamine (SP) for 3 – 59 months of age every year. The URR has been part of the SMC implementation, but no data on the villages and coverages were provided in the GMIS17 (184).

Paper	Year	Type (area)	Age group	Method	Area	Prevalence (% per survey unit)
Mwesiga et al.	2015	Village survey (MASSIV area)	All age groups	PCR	URR (MASSIV area)	21.17/26.16/35.21/35.86/49.13
Okebe et al.	2014	School survey (MASSIV area)	4 – 20 years	MSP-1 (serology)	URR (MASSIV area)	34.4/34.7/35.7

Table 3:PCR prevalence and seroprevalence for malaria in the URR The Gambia

3.1.2 Available Data on Prevalence of STH in The Gambia:

In May 2015, a national mapping survey of schistosomiasis (SCH) and soil-transmitted helminthiases (STH) was conducted in The Gambia (185).

The MASSIV study area was found to have a low to moderate prevalence, with roughly a third of the MASSIV study area having no survey data. The only MDA conducted was the use of praziquantel for schistosomiasis, and no mass drug administration for STH was carried out in this area.

The STH investigated included *A. lumbricoides, T. trichiura,* and hookworms. However, *S. stercoralis* was not included. Furthermore, the data showed the highest prevalence of STH in the country's capital Banjul, which was rather unexpected, considering the level of urbanisation, e.g., tarmac roads and use of concrete. However, the data did correlate with the climate data for the Gambia regarding the land surface temperature requirements for STH (186,187). Regarding *S. stercoralis*, available data were sparse and ranged from 3 to 16%, and the three studies having a rather low number of participant (188–190). A summary of the available data is given in Table 2.

Study	Year	Location	Nr of Participants	Age Range	*A. lumbricoides* (%)	*N. americanus* (%)	*A. duodenale* (%)	*T. trichiura* (%)	*S. stercoralis* (%)	Total STH** prevalence (%)
McGregor et al.	1952	Keneba (LRR)	90 (12/23/20/23)	2-5/ 6-10/ 11-16/ 17+	NA/ 4,3/ 10/ 4,3	25/ 91.3/ 75/ 69.5	N/A	N/A	N/A	N/A
Palmer et al.	1995	Marakissa	216	1-30+	25	30	N/A	2.4		N/A
Knight et al.	1981	Marakissa	168	1-4, 5+	N/A	80 -87; 93 - 94;	N/A	N/A	N/A	N/A
Knight et al.	1981	Mandinari	174	1-4, 5+	N/A	39 - 56; 80 - 92	N/A	N/A	N/A	N/A
Marsden et al.	1964	Sukuta	93	18 months	9	12	N/A	N/A	16,0	N/A
McGregor et al.	1970	Keneba (LRR)	215	< 6 / 6-23 / 24 - 60 months	N/A	N/A	N/A	N/A	N/A	0/1-2/5-8
Nyan et al.	2001	Banjul / Farafenni	448	< 35 >= /< 35 >=	N/A	N/A	N/A	N/A	N/A	17/15; 4.7/8.2
Stettler et al.	1998	Keneba (LRR)	42	11-13	12	10	N/A	3	3	79.0
Bradbury et al.	2015	West Kiang (Keneba)	128	adults (18+)	N/A	10	N/A	N/A	7,8	13.0
						Hookworm				
Camara et al.*	2021	National (The Gambia)	10434	school children (7 – 14)	1.8	0.6	N/A	0.1	N/A	N/A
		Central River Region	1915		0.6	0.5	N/A	0	N/A	N/A
		Lower River Region	1100		3.1	0.5	N/A	0	N/A	N/A
		North Bank East Region	1157		0.6	0.2	N/A	0	N/A	N/A
		North Bank West Region	607		0.2	0	N/A	0	N/A	N/A
		Upper River Region	1451		1.5	0.2	N/A	0.1	N/A	N/A
		Western Region 1	2551		4	0.4	N/A	0.5	N/A	N/A
		Western Region 2	1653		60	2.0	N/A	0	N/A	N/A

Table 4: Prevalence data from published sources for The Gambia; *National survey in schools conducted in 2015 with microscopy, **some studies only presented the total % of all STH combined, others did not report this measure;

3.2 Study Components

The study consisted of four components to address the two aims and four objectives of this PhD.

1. Evaluation of the IVM and DHP MDA on malaria serological markers (Aim 1 and Objective 1)
    a. Evaluating the correlation between malaria serological markers and malaria PCR positivity (Objective 1a)
2. Impact of the MDA on the prevalence of ectoparasites and soil-transmitted helminths (Objectives 2 and 3)
3. Evaluation of causes for systematic non-participation in the MDA (Objective 4)

An overview of each component of the PhD is given below. Detailed introductions and methodologies for each component are reported in the respective Research Papers addressing each objective.

3.2.1 Evaluation of the IVM and DHP MDA on Malaria Serological Markers

(Detailed methodology and results reported in Research Paper 1– Chapter 5)

The use of malaria serology to measure the effect of an intervention is still a recent idea and has not yet been widely adopted.

This part of my PhD aimed to evaluate a panel of 19 antigens using the Luminex Magpix© system for their potential use as tools for surveillance post-MDA and potentially later on in low-endemic settings.

The fieldwork part of this component included several cross-sectional surveys to collect DBS; this was in November 2018 after the first MDA from August to October 2018, in June 2019 before the second MDA in 2019 and November 2019 after the second MDA. These surveys were conducted by the MASSIV trial staff and included all age groups in the participating villages.

The lab work component included the analysis of the 19 antigens using the Luminex Magpix© system, which was carried out in part by myself and as part of the local transfer of skills and knowledge by appointed staff of the MRC The Gambia as well as a LSHTM Luminex specialist for training and oversight. A detailed outline of the lab work methodology is reported in Research Paper Chapter 1.

The outcome of the Luminex analysis was measured in mean fluorescence intensity (MFI). Linear regression with random effects for clusters was used to assess associations at the village level, with the difference in MFI between arms as the outcome variable, with fixed variables for the different age groups and sex. The data are presented in unadjusted and adjusted geometric means.

Logistic regression with random effects was used to assess the correlation between PCR positivity and MFI village level, with fixed variables for the different age groups and sex. The data are presented in adjusted and unadjusted odds ratios.

3.2.2 Impact of the MDA on the Epidemiology of Ectoparasites and Soil-Transmitted Helminths

(Detailed methodology and results reported in Research Paper 2– Chapter 6)

Ivermectin has been shown to be effective against a variety of ectoparasites and endoparasites (citation). Furthermore, data on the prevalence of ectoparasites and endoparasites in the Upper River Region was sparse to non-existent. This element of my PhD aimed to establish the prevalence of these parasites and to assess the effect of the ivermectin part of the MASSIV MDA on the prevalence of these ecto- and endoparasites within the trial community. The fieldwork component of this part consisted of three cross-sectional surveys. Survey number one was conducted in June/July 2019 before the 2nd MASSIV MDA to establish the prevalence of scabies, headlice, and bedbugs in the community. During this survey no signs of living headlice or bedbugs were discovered, and therefore these two parasites were dropped from later surveys. Survey number two was conducted in November 2019 after the 2nd MASSIV MDA to assess the impact on scabies and soil-transmitted helminths. The third survey was originally planned for 2020 but due to the COVID-19 pandemic, delayed to November 2021 as a long-term follow-up of the effects of the MDA. Standardised protocols for physical examination for scabies, headlice, and bed bugs were used (95,191). Stool samples were collected from each study participant and stored in 98% ethanol for further transport and processing. An outline of the fieldwork methodology for the study is given below (Section 3.3 Fieldwork Protocol).

Logistic regression adjusted for clusters and random effects was used to assess the impact of the MDA with fixed variables for the different age groups, sex. The data are presented in adjusted and unadjusted odds ratios.

3.2.3 Evaluation of Causes for Systematic Non-Participation in the MDA

(Detailed methodology and results reported in Research Paper 3– Chapter 7)

One of the most important components of successful MDAs is the cooperation and participation of the targeted communities. If a non-participating area in an MDA overlap with hotspots of the targeted disease, these would act as reservoirs of the disease and continue to maintain transmission in the community (192,193).

During the MASSIV trial, the MDA consisted of two drugs with each drug consisting of nine doses in total with three continuous days a month for three months, each day a dose. Importantly, for ivermectin, only completion of the MDA is considered to create a significant impact on malaria transmission (74,75).

To look for quantitative factors involved in non-participation, I used the MASSIV trial coverage database. The data included only the participants in the intervention arm and every participant was given a score for taking a single dose of MDA from 0 to 1 and with the sum of all MDA doses taken ranging from 0 for no dose at all, to 9 for all doses. Additionally, this allowed to calculate the number of completed months with each completed monthly MDA consisting of three doses respectively.

These scores were then used to create an UpSetR plot to display the distribution of how many participants received what number of doses for the most common combinations of MDA intake.

Additionally, logistic regression was used to assess the association between taking at least one dose and in a second analysis association between having complete MDA and no or incomplete MDA respectively. These two models were then adjusted for whether the household head took the MDA, household size, age group, and sex. A detailed outline of the methodology is reported in Research Paper Chapter 3.

3.3 Fieldwork Protocol

3.3.1 Participant Inclusion and Exclusion Criteria

Inclusion Criteria:

2019 June/July survey

Children 3 – 14 years of age were selected for whom informed consent was obtained from either their parents or guardian.

2019 November survey

Children 3 – 14 years of age were selected for whom informed consent was obtained from either their parents or guardian.

2021 November follow-up survey

Children 3 – 14 years of age were selected for whom informed consent was obtained from either their parents or guardian.

Exclusion Criteria: all surveys

Any participant who declined to participate or was unable to consent or participate in the sample collection due to

- Illness
- Incapacity
- Inability to communicate
- Repeated absence

3.3.2 Informed Consent

The MASSIV field trial team obtained informed consent from any participant in the study. This consent was the basis for the surveys in 2019. For the survey in 2021 consent had to be taken anew from children aged 3 – 14 years of age. For each participating child, the parent or guardian provided the necessary consent.

3.3.3 Data and Sample Collection

Baseline demographic data was collected during the MASSIV census survey in 2018, which included sex, age, household number, compound number, head of household, and parents. During the MDAs the status of how many and which drugs had been taken.

3.3.4 Scabies Examination

The examination of scabies was performed clinically without the use of a dermatoscope or ink test as the established guidelines for clinical signs and symptoms are highly sensitive and specific (194).

3.3.4.1 The Clinical Definition of Scabies

- Visible lesions involving typical sites for scabies (i.e., interdigital spaces of hands, wrists, axillae, elbows, knees, buttocks, and genitalia in men, breast areolae in women, palms and soles in children
- Presence of itching involving at least two sites of the body
- Presence of others in the household with an itch

3.3.4.2 The Clinical Findings for Scabies

- Pruritic inflammatory papules/nodules/blisters/pustules with a typical distribution of lesions (Fig.1)
- Webs of fingers, hands, wrist, elbows, knees, trunk (back, groin, buttock) and ankles
- Possible infection with pus-filled sores and crusted sores with the collection of scabies lesions

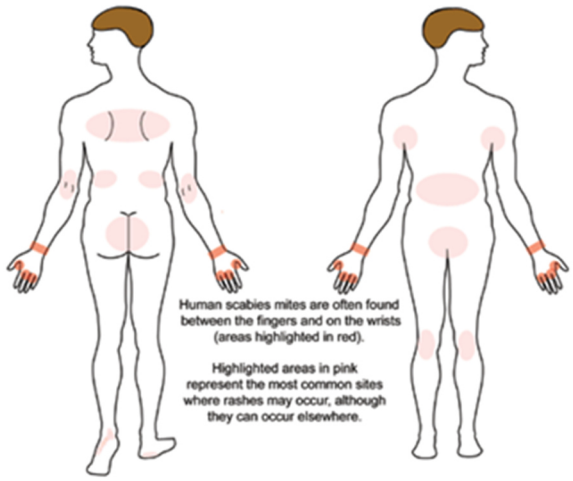


Figure 2: Typical Scabies distribution. *(195)*

3.3.4.3 Algorithm

- Examination of the skin is conducted in the community of visible skin and skin the participant is comfortable to expose
- Examine specifically finger and toe webs
- Examine the person even if they say they have no skin problems

- Make notes whether there are any other skin problems

3.3.5 Head Lice Examination

3.3.5.1 The Clinical Definition of Head Lice

- Live adult or nymph louse with or without eggs (nits)
- If only lice eggs (nits) are found, then:
    - < 0.25 inch (0.63mm) from the hair shaft/scalp => suggestive of active infestation
    - > 0.25 inch (0.63mm) from the hair shaft/scalp => possible old or no active infestation

3.3.5.2 Clinical Findings for Head Lice

- Itch on the scalp, neck and around ears (+/- enlarged nuchal lymph nodes)
- Life lice crawling through hair and on the scalp
- Lice eggs (nits) on the hair shaft. The nits stick to the hair

3.3.5.3 Definition of Hair Length

- Hair can hold a comb is defined as long or braided
- Hair that cannot hold a comb is defined as short
- If most of the scalp is without hair, it is defined as shaved

3.3.5.4 Algorithm

- The examination is carried out in a standardised way by using metal combs with 0.1 mm teeth size.
- The examination is started by direct visual inspection of sites of predilection for head lice: the back of the ears, temples, and neck.
- Hair is then parted into four sections (first down the sagittal plane and then ear to ear). Each section was examined using the nit comb, starting very close to the scalp.
- If a live head louse is found, it is caught and placed on a piece paper for confirmation. The participant is considered positive.
- If a head louse is caught in the comb, but when transferred to paper, no movement is detected, a diagnosis of dead adult lice was made.
- If only head lice eggs/nits were seen, the full examination was carried out in search of a live louse.

- Eggs and lice were deliberately not removed from the hair during the examination to avoid biasing results regarding the effectiveness of ivermectin by physical delousing.
- Following examination, nit combs must be carefully cleaned using toothbrushes to avoid cross-contamination and risk of infesting the next participant.

3.3.6 Bedbug Examination

To assess the prevalence of bedbugs in the communities of both arms, 30 sleeping quarters in each cluster will be screened.

3.3.6.1 Clinical Signs and Symptoms of Bedbugs

- Itching around the site of bite marks
- Bite marks are usually located on exposed skin (any body part)
- Bite marks can be random or in a straight line
- Bite marks may appear up to 14 days after being bitten

3.3.6.2 Findings for Bedbugs Infestation

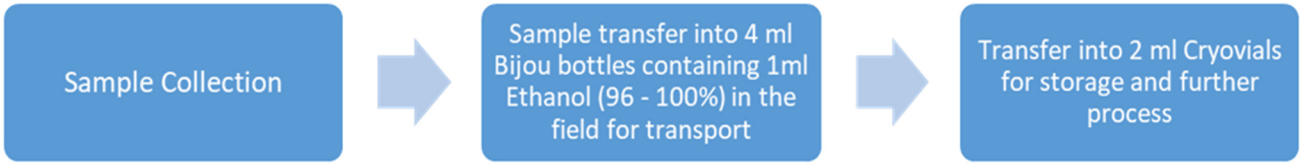
- Bed bugs' exoskeletons after moulting
- Bed bugs in the fold of mattresses and sheets
- Rusty–coloured blood spots due to their blood-filled faecal material that they excrete on the mattress or nearby furniture
- A sweet, musty odour

3.3.6.3 Algorithm

- Inspection of Sleeping quarters
    - Look for moulted exoskeletons of small blood stains on the mattress/bedsheets
    - Look for living bedbugs in the fissures and folds of the mattress, the bedframe and the wall the bed is attached to
- Grading of infestation
    - Active bedbug infestation
        - Live bedbugs detected in the living quarters
    - Suspected bedbug infestation
        - dead bed bugs, shed skins, eggs, faeces, blood smears from crushed bed bugs, and units of residents with complaints about bed bug bites but no live bugs

3.3.7 Stool Sample Collection and Processing for STH

Stool samples were collected during the surveys in November 2019 and November 2021.



After collection in the field the samples were stored in a -20°C freezer at the Biobank at the MRC Unit The Gambia and later for further processing transported to LSHTM.
Detailed methodologies of the fieldwork are provided in the Research Paper (Chapter 6) which report on the findings of these studies.

3.4 Data Analysis Software

Statistical analysis for the epidemiological part of the studies was performed using STATA 14 and STATA 17 software specifically for metobit regression (Stata Corporation, College Station, Texas USA) and R software with R studio version 4.0.2 (2020-06-22). Details of the specific analysis plans for each component of the project are provided in the individual component chapters below.

3.5 Ethics

All elements of the study were conducted in accordance with the Declaration of Helsinki(196).
Ethical approval for this project has been granted by LSHTM (LSHTM ethics ref: 17123 and 17002), the MRCG Scientific Coordinating Committee (SCC) (LSHTM ref: 17123), and the Joint MRCG and Gambian Ethics Committee (LSHTM ethics ref: 17123).
Ethical approval for the MASSIV trial has been granted by LSHTM (Ref: 15823) and the SCC/ Joint MRCG and Gambian Ethics Committee (Ref: SCC 1593v1.1).

3.6 Data Management

All participants in the MASSIV trial received a unique identifier ID which was used to link clinical, demographical, geographical, and laboratory data collected by the trial and me. All personal identifiers were removed from the data sets prior to analysis. During the active time of the MASSIV trial, REDCap® software was used to collect data in the field and link to

link it with the MASSIV database using unique identifiers. After the trial ended, for the last survey of my PhD, Opendatakit (ODK) was used using the same unique identifiers as before to link the collected data with the already existing databases (197). This system performs real-time data validity checks, ensures that all data are automatically entered into the database in a predetermined coded format, and minimises missing data. Data from this system are uploaded directly to a secure, password-protected, cloud-based server. All data were verified, and inconsistencies resolved prior to analysis.

Data collected during the fieldwork to determine the whereabouts of participants was collected on the fieldwork paper sheets used to find participants in the villages. These data were later incorporated into the fieldwork database using unique identifiers.

Data will be kept for a minimum of seven years after publication (or ten years after specimen collection if no publication results from the work).

3.6.1 Quality Assurance

Quality control measures have been addressed in the relevant sections of the manuscript. These include the following:

1. Use of REDCap® or ODK data entry forms on Android smartphones
    a. Avoids missing data
    b. Validation checks on data at the time of entry
    c. Ensures data coded appropriately
2. Standardisation of recording of scabies lesions
3. Labelling of all stool samples with a unique identification number to link samples to Individuals and consent forms
4. Laboratory work conducted by operators blinded to the MASSIV trial data using standardised SOPs

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Chapter 4 Literature Review

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REVIEW ARTICLE

Broadening the range of use cases for ivermectin – a review of the evidence

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Ivermectin is a broad-spectrum antiparasitic agent that interferes with glutamate-gated chloride channels found in invertebrates but not in vertebrate species. Mass drug administration (MDA) with ivermectin-based regimes has been a mainstay of elimination efforts targeting onchocerciasis and lymphatic filariasis for more than 3 decades. More recently, interest in the use of ivermectin to control other neglected tropical diseases (NTDs) such as soil-transmitted helminths and scabies has grown. Interest has been further stimulated by the fact that ivermectin displays endectocidal efficacy against various *Anopheles* species capable of transmitting malaria. Therefore there is growing interest in using ivermectin MDA as a tool that might aid in the control of both malaria and several NTDs. In this review we outline the evidence base to date on these emerging indications for ivermectin MDA with reference to clinical and public health data and discuss the rationale for evaluating the range of impacts of a malaria ivermectin MDA on other NTDs.

Keywords: ivermectin, neglected tropical diseases, malaria, mass drug administration, soil-transmitted helminths

Introduction

Ivermectin is a macrocyclic lactone compound and part of the avermectin family. Avermectins were discovered by Satoshi Omura and William C. Campbell in Japan in the 1970s, during analysis of *Streptomyces avermitilis* compounds, and they subsequently discovered ivermectin. In 2015, both scientists received the Nobel Prize in Physiology or Medicine for their discovery.[1] Since its introduction, the drug's utility has seen its use extended in veterinary medicine and animal husbandry to treat endo- and ectoparasites.[2–4]

Ivermectin is a mainstay in the success of the control and elimination of *Onchocerca volvulus*, the causative agent of river blindness. It has been extensively used by the African Programme for Onchocerciasis Control, the Expanded Special Project for the Elimination of Neglected Tropical Diseases in Africa and the Onchocerciasis Elimination Program of the Americas. Ivermectin is also known to affect a variety of invertebrate species.[5–7] Due to its broad application, it is considered an endectocide, a drug affecting several ecto- and endoparasites, and its use has steadily expanded in the years since its discovery. In recent years ivermectin has been successfully applied on a larger scale against several pathogens/parasites, including scabies mites (*Sarcoptes scabiei*), lice (*Pediculus humanus* sp.) and helminths such as *Strongyloides stercoralis*[8–11] and there is growing interest in its use as a mosquitocidal agent as part of malaria control.

We aimed to summarise data on the use of oral ivermectin in non-immuncompromised patients across a range of emerging indications. We highlight key data on the rationale, dosage considerations and existing evidence supporting the use of ivermectin for each new indication. The pharmacology and mode of action of ivermectin have been extensively reviewed elsewhere[12–16] and we therefore primarily limit this literature review to factors of direct relevance to its extended use. However, a short summary of the mode of action and pharmacology will be given for completeness. Finally, this literature review is restricted to multicellular parasites, excluding suggested but unproven applications in oncology[17] or virology,[18,19] including severe acute respiratory syndrome coronavirus 2.

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Mode of action

In invertebrates, ivermectin interferes with glutamate-gated chloride channels (GluCls), which are not expressed in vertebrates. GluCls play a role in several processes in invertebrates and their inhibition affects motility, feeding and reproduction.[15,20] These effects are shown at nanomolar concentrations. At higher concentrations, ivermectin interacts with a variety of receptors such as γ-aminobutyric acid, glycine, histamine and nicotinic acetylcholine receptors, which are expressed in both invertebrates and vertebrates.[20]

Vertebrates, including humans, express P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), in their blood–brain barrier, which functions as a transport efflux pump of ivermectin out of the central nervous system.[16,21] The combination of its receptor specificity and the existence of P-gp is thought to be the major factor behind the safety and side-effect profile of ivermectin. Notably, some species, such as certain dog or horse breeds, do not possess the gene encoding P-gp, and recently a human case was found.[22] Therefore, in specific animal species, the use of ivermectin, especially at high dosages, can lead to drowsiness, coma and death,[23,24] clearly demonstrating the protective role of P-gp in humans.[16]

Safety considerations

Ivermectin has an extremely well-established safety profile, with billions of doses being administered since the inception of the Mectizan Donation Programme by Merck in 1987 for onchocerciasis and filariasis control.[25] Pharmacokinetic dosing studies have suggested that doses of ivermectin up to six times the recommended dose as well as repeated daily or monthly doses[26–32] are well tolerated. There is a well-established risk associated with the use of ivermectin in *Loa loa* (a filariform parasite) endemic areas. In this setting, ivermectin can lead to a rapid die-off of large numbers of *Loa loa* microfilaria in the central nervous system, leading to a potentially fatal encephalopathy.[33,34]

Currently, due to a lack of safety data, ivermectin should not be given to pregnant women[35], however, inadvertent use in control programmes has occurred regularly.[36] The majority of data currently are based on observed teratogenicity from animal models using P-gp-deficient mice[37] or very high doses in rats and rabbits with 10–50 and 7–30 times the human equivalent, respectively.[38–40] The relevance of these animal data to humans is therefore questionable and better data are needed. Currently children whose weight or height is <15 kg or <90 cm are also not recommended to receive ivermectin. The basis for these restrictions is the unproven concept of an immature 'leaky' blood–brain barrier, for which there is no scientific support.[41–43] In contrast to theoretical concerns, there is an increasing accumulation of real-world data showing safety among young children.[44–50]

Malaria

Malaria control measures over the past 2 decades have resulted in a significant reduction in morbidity and mortality, driven by a combination of long-lasting insecticidal nets, indoor residual spraying, artemisinin-based combination therapy and rapid diagnostic tests.[51] However, the emergence of drug and insecticide resistance, and changes in vector behaviour, such as increased outdoor biting and resting behaviour, is threatening this progress.[52–54] Over the past decade, interest has emerged in the use of ivermectin as an additional tool for the control of malaria.[55,56]

Anopheles mosquitoes predominantly express GluCls in organs and tissues responsible for their sensory and motor function.[14] The same channels exist in the culicine nervous system; however, ivermectin appears to be unable to penetrate into the haemocoel and only exerts an effect at levels 10 times greater than shown for *Anopheles* sp. Its effect on culicine species such as *Aedes* and *Culex* is therefore greatly reduced[57,58] unless the drug is injected directly into the haemocoel.[59]

Several historical studies have explored the use of ivermectin and its impact on mosquito control,[60–62] but significant interest for malaria vector control has re-emerged recently.[63] These studies use different methods to assess ivermectin's effect. Specifically, membrane feeding assays (MFAs) involve feeding mosquitos on donated blood, either from donors who have taken oral ivermectin or on blood spiked with ivermectin. Direct feeding assays (DFAs) involve feeding mosquitos on volunteers treated with ivermectin. Different *Anopheles* species, such as *Anopheles gambiae* (MFA, DFA), *Anopheles arabiensis* (MFA), *Anopheles aquasalis* (MFA, DFA), *Anopheles minimus* (DFA), *Anopheles campestris* (DFA), *Anopheles sawadwongporni* (DFA), *Anopheles dirus* (MFA), *Anopheles darlingi* (MFA), *Anopheles farauti* (DFA), and *Anopheles stephensi* (human MFA, mouse DFA), have all shown high mortality after ingesting blood containing ivermectin levels comparable to those reached in humans after an oral dose of 200, 400 and 600 μg/kg body weight.[58,64–69] The IVERMAL trial found no difference in ivermectin mosquitocidal toxicity between MFAs and DFAs against *A. gambiae* using placebo (n=23), 300 μg/kg/d (n=24) or 600 μg/kg/d (n=22).[70] Although DFAs showed higher mosquitocidal toxicity than MFAs in a trial by Sampaio et al.,[64] the number of participants was small (n=6).

Pharmacokinetic considerations limit the effectiveness of a single standard dose of ivermectin of 200 μg/kg for malaria control programmes. The half-life of 18 h means that these dosing regimens only generate a mosquitocidal effect lasting for about 5–6 d,[71] which is inadequate for malaria control. Furthermore, vectors from outside the treated areas, especially in open systems on larger landmasses, will quickly repopulate these losses. To improve the pharmacokinetic profile, and hence the duration of its endectocidal effect, alternative dosages have been suggested: a single dose of 400 μg/kg or three consecutive daily doses of 300 μg/kg.[72] The latter regime was investigated in the IVERMAL trial conducted in Kenya and was given once a month for three consecutive months in human volunteers. The treatment had a good safety profile and the mosquitocidal effect lasted for up to 28 d.[73]

In the Repeat Ivermectin Mass Drug Administrations for Control of Malaria: a Pilot Safety and Efficacy Study (RIMDAMAL) conducted in Burkina Faso, villages were randomly assigned to ivermectin (150–200 μg/kg) and albendazole (400 mg) at baseline in both arms followed by the same single doses of ivermectin every 3 weeks over 18 weeks in the intervention arm or no treatment in the control arm. The study aimed to evaluate the effect on

the cumulative incidence of uncomplicated malaria. The results showed evidence of a reduction in incidence in children <5 y of age,[74] although the statistical methods for analysis have been disputed.[75,76]

The results of these relatively small trials have led to the planning of larger trials. The 300 μg/kg/d for 3 d treatment schedule is now being evaluated in ongoing or planned cluster randomized trials: the MASSIV trial (NCT03576313) in Gambia,[77] the Adjunctive Ivermectin Mass Drug Administration for Malaria Control (MATAMAL) trial in the Bijagos Islands, in Guinea Bissau (NCT04844905) and RIMDAMAL II in Burkina Faso (NCT03967054). The BOHEMIA trial is currently planned to be conducted in Tanzania and Mozambique, in which ivermectin will be administered to both livestock and humans. Another trial is planned in Thailand using ivermectin in rubber plantation workers, but it has not yet started.

Potential veterinary application of ivermectin as part of malaria MDA

Several *Anopheles* species, such as *A. arabiensis* and *A. farauti*, exhibit both anthropophagy and zoophagy, particularly for peridomestic animals such as cattle and pigs.[78,79] These alternative feeding sources can therefore sustain the mosquito population and complicate control efforts.[80] Treating livestock therefore offers a possible addition for vector control for malaria transmission and has been shown to be feasible in field studies in Belize, Burkina Faso and Tanzania.[81–83] Veterinary applications of ivermectin allow for higher and repeated dosing than are possible in humans, as well as application of potential long-lasting formulations.[84–86]

Similarly, *Glossina palpalis* and *Glossina morsitans*, the vectors for *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, West and East African sleeping sickness, respectively, take their blood meal from humans, wild animals and livestock alike. Field studies have shown these species exhibit similar susceptibility to ivermectin as *Anopheles* mosquitos. This included dose-dependent reduced lifespan and fecundity.[87–89] Similar data from animal models exist for some triatomine bugs (*Triatoma infestans* and *Rhodnius neglectus*), vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease.[90]

This 'One Health' approach could offer additional advantages by treating animals for endoparasites and ectoparasites, improving the health and economic value of domestic animals,[91] while also providing vector control for malaria and other diseases. The use of ivermectin in animals is restricted by public health policies, such as the withdrawal times for slaughter or milking,[92] which could make this strategy technically challenging.[93] Another important aspect is the effect of ivermectin in livestock on dung degradation and non-target fauna, which could cause environmental concerns[94–98] and needs to be addressed.

Soil-transmitted helminths (STHs)

STHs are among the most prevalent parasitic infections in humans both in tropical and subtropical regions of the globe[99,100] and are associated with broad health impacts including anaemia, stunting and delays in cognitive development.[101]

MDA with benzimidazol derivatives (albendazole and mebendazole) is recommended to reduce the STH burden in a community,[102] because these drugs have a significantly greater efficacy compared with ivermectin in most STH species.[103,104] Data on the effect of ivermectin on hookworms show a variable reduction of 0–33%,[105,106] with the most successful application being two doses of 200 μg/kg 10 d apart reported from Brazil.[8] In comparison, both *Ascaris lumbricoides* and *Strongyloides stercoralis* respond well to a single standard ivermectin dose of 200 μg/kg each, with field studies finding cure rates of 98–100% and 83–96%,[107,108] respectively. Reports on *Trichuris trichiura* are mixed, ranging from 11% in Tanzania to 84% in Peru.[8,103,105,109,110] The reasons for these geographical differences in susceptibility are not yet well understood but could be due to different species.[111] Other nematodes such as *Ancylostoma braziliense*, *Ancylostoma caninum* and *Uncinaria stenocephala* are primarily zoonotic diseases that cause cutaneous larva migrans (CLM) syndrome in humans. Depending on the clinical presentation, one to two standard doses of ivermectin have been used and have been shown to resolve the lesions in 81–100% of cases.[112,113]

Currently there are no published data evaluating the impact of higher-dose multiple treatment regimes, as utilised for malaria control, on STHs. Ongoing malaria MDA provides an additional opportunity to investigate these potential synergistic impacts.

Filarial worms

Filarial infections were the first human disease targeted for control using ivermectin. Widespread roll-out of ivermectin MDA has produced a significant impact on filarial disease–related morbidity, including blindness and severe pruritus caused by *O. volvulus* and lymphatic obstruction and secondary bacterial skin disease caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*.[114–116]

Ivermectin as a single dose administered annually at 150–200 μg/kg for onchocerciasis will reduce the microfilarial load by 99% after 1–2 months and administered over 16–18 y interrupts transmission and leads to elimination.[117,118] Recent data have shown that a sterilizing effect on adult onchocercal filaria can be achieved with administration every 3 months over 3 y.[119]

In lymphatic filariasis (LF), caused by *W. bancrofti*, *B. malayi* and *B. timori*, ivermectin (200 μg/kg) lacks activity against the adult filaria responsible for the pathology and it is therefore used in combination with either albendazole or diethylcarbamazine citrate (DEC) or as a triple combination of all three outside onchocerciasis areas.[120–122] The latter combination of ivermectin, DEC and albendazole has shown superior efficacy compared with the dual combination[120,122–124] and is now recommended by the World Health Organization for use in many LF-endemic regions.

Ivermectin is used with caution in *Loa loa*-endemic areas with a surveillance system for early detection and management of post-treatment severe adverse events, as it results in rapid killing of microfilaria (mf),[125] which can cause acute encephalitis, leading to disability and even death.[33,34,126] For other common filarial parasites such as *Mansonella streptocerca* and *Mansonella ozzardi*, ivermectin treatment with 150 μg/kg and

150–200 μg/kg, respectively, leads to a reduction of microfilaria and possibly some impact on macrofilaria.[127–130] *Mansonella perstans* was shown not to be affected by a standard single dose of ivermectin,[131–134] with reports of repeated doses being potentially more successful.[32,135] Importantly, ivermectin does not appear to affect the vector of these filaria, *Culicoides* sp.[136,137]

Food-borne nematodes

For food-borne nematodes such as *Gnathostoma* sp., the recommended daily dosage is 200 μg/kg for 2–3 d.[138,139] Caution is advised in infections of the central nervous system, as treatment could cause deleterious inflammation. For trichinellosis, ivermectin was effective in rat and mouse models against the free-living stage in the gut but was ineffective against the encysted stage of the parasite.[140,141]

Other nematodes

Enterobius vermicularis, colloquially known as pinworm/threadworm, is a common cosmopolitan parasite primarily causing anal pruritus and in rare cases appendicitis. It has been successfully treated with a single dose of ivermectin (200 μg/kg), with a study from Peru reporting cure rates of 89% 3 d after treatment and 78% after 30 d,[109] but a study from China showed a lower cure rate of 52.9%.[105]

Ectoparasites

Scabies is a globally occurring skin disease caused by the scabies mite (*Sarcoptes scabiei* var. *hominis*) that is especially common in poor and crowded communities in tropical and subtropical areas[142] and causes both significant morbidity and mortality through its downstream sequelae.[143,144]

There is limited pharmacodynamic data available on the use of ivermectin for scabies, although an animal model in pigs is available.[145] Doses ≤150 μg/kg have lower efficacy,[146] and even at standard doses of 200 μg/kg, increased survival times have been found *in vitro* over the last decade.[147] The use of a higher dose and repeated administration may improve the cure rate.[143]

Several large-scale trials have demonstrated significant reductions in the prevalence of scabies following MDA with ivermectin. The Skin Health Intervention Fiji Trial was a three-arm randomised trial in which communities were randomized to standard of care, MDA with topical permethrin or MDA with ivermectin. MDA was superior to other treatment options, with a relative reduction in prevalence of 94% for ivermectin, 62% for permethrin and 49% for standard of care.[9] The Azithromycin Ivermectin Mass Drug Administration trial on the Solomon Islands, a prospective single-arm, before and after community intervention trial using ivermectin and azithromycin in combination and permethrin 5% for pregnant and breastfeeding women and children weighing <12.5 kg, showed an 88% relative reduction of baseline scabies prevalence after 12 months.[148] Similar results have been reported from studies in Australia using ivermectin MDA for scabies control in remote aboriginal communities[10] and Brazil using ivermectin as a community intervention for several susceptible parasites.[8,149]

Success of ivermectin-based MDA for scabies control is dependent on treating individuals with a contraindication to ivermectin. Currently this is through topical permethrin treatment, but increasing safety data on ivermectin in these populations, especially for children <5 y of age, may increase the proportion of the population who can be treated with ivermectin.

Humans are host to three species of closely related lice: *Pediculus humanus capitis*, *Pediculus humanus corporis* and *Phtirus pubis*. Of these, only the body louse *P. humanus corporis* commonly acts as a vector of potentially life-threatening infectious diseases. However, recent data have shown the potential for head lice to also transmit similar pathogens,[150] are a cause of bacterial pyoderma of the scalp[151] and even cause iron deficiency in heavy infestations.[152] All three of these species cause pruritus and hence morbidity.[153,154]

In a cluster randomized trial including centres in the UK, Ireland, France and Israel, a dose of 400 μg/kg/d 1 week apart resulted in a 97.1% reduction of head lice on day 15.[155] Another randomized household-level trial in Brazil using 200 μg/kg/d twice 10 d apart led to 16% in the intervention arm being louse free compared with 4% in the control arm at 60 d post-intervention.[156] Several non-randomized studies from Egypt and Mexico using 200 μg/kg/d showed cure rates of 92.5–97% after a second dose 8 d later if the first dose failed.[157–159] A study in the Solomon Islands using MDA with a dose of 200 μg/kg/d on days 0 and 7 resulted in a 89% reduction of head lice at day 14 post-MDA[160] and a study in Thailand using the same schedule showed a 95% reduction at 14 d post-MDA.[161]

A study from Senegal using 400 μg/kg/d resulted in a 77.4% reduction in the ivermectin arm compared with 32.3% in the d-phenothrin shampoo arm at day 15. However, 7.4% of the children showed treatment failure to ivermectin[162] and there was some evidence of potential ivermectin resistance in head lice. Additional molecular analysis confirmed a genetic mutation of the GluCl receptor, the primary target of ivermectin in arthropods.[163]

Data on ivermectin for the treatment of body lice and pubic lice are scarce and mainly from smaller case series or cohort studies. These data appear to show a significant reduction in prevalence.[164,165] In this context, a potential ivermectin resistance pathway has been described outside of the GluCl receptor, called complexin, a synaptic exocytosis and neurotransmitter release regulator protein.[166] Aside from resistance, reintroduction and re-infestation is a common problem in all three species of lice even after successful MDA.[160,164,167]

Data from Brazil on the treatment of *Tunga penetrans* with a standard dose of ivermectin did not show efficacy, although it may be dependent on seasonality and the timing of the application.[149,168] In myiasis, which is common in tropical communities and can cause significant morbidity, ivermectin has been successfully used to facilitate extraction of larvae.[169,170]

There are only experimental blood feeding data from human studies using ivermectin to treat *Cimex lectularius and Cimex hemipterus*, the cause of bed bugs, a global nuisance. These data show some impact, but real-world data are unavailable.[171–173] Ivermectin has also been used with variable success for the treatment of *Demodex* mites, which are associated with a variety of

Table 1. Ivermectin use for endoparasites

Endoparasites	Potential impact of ivermectin MDA	Ivermectin dose (individual treatment)	Ivermectin MDA schedule for control	Reduction at recommended dose (%)[a]	References
Ascaris lumbricoides	Yes	200 μg/kg, once		98–100%	8, 103, 106
Necator americanus	Unclear	Not recommended, two doses of 200 μg/kg 10 d apart		0–33% single dose of 20 μg/kg, 68% two doses of 200 μg/kg 10 d apart	8, 103, 105, 106
Ancylostoma duodenale	Unclear	Not recommended[b]		[b]	[b]
Strongyloides stercoralis[c]	Yes	200 μg/kg once or multiple several days apart (day 1, 2, 15 and 16)		83–96%	8, 103, 106, 107, 108, 109
Trichuris trichiura[d]	Yes	200 μg/kg for 3 d[e], 200 μg/kg twice 10 days apart		11–88%[c]; 81.7–84% 200 μg/kg twice 10 d apart	8, 103, 105, 109, 110
Enterobius vermicularis	Yes	200 μg/kg once, plus repeat after 14 d		52.6–89%	105, 109
Onchocerca volvulus	Yes		150–200 μg/kg biannually or annually	99% reduction in microfilaria after 1–2 months; transmission interruption and elimination after 16–18 y	117–119
Loa loa	Yes	Not recommended			125
Wuchereria bancrofti	Yes	Ivermectin monotherapy not recommended	200 μg/kg annually in combination with a second drug or as triple therapy	94% reduction in microfilaria using IDA	120–124
Brugia malayi	Yes		see *W. bancrofti*		
Brugia timori	Yes		see *W. bancrofti*		
Mansonella perstans	Unclear	200–600 μg/kg once, not recommended	400 μg/kg once then 800 μg/kg annually for 3 y or 400 μg/kg twice then 800 μg/kg every 3 months for 3 y[20]	No effect short term; MDA 85–97% reduction	131–135
Mansonella streptocerca	Yes	150 μg/kg once		55–60% reduction in microfilaria[f]	127, 128
Mansonella ozzardi	Yes	150–200 μg/kg once		94–100% reduction in microfilaria	128–130
Gnathostoma sp.	Yes	200 μg/kg for 2 d		76–100%	138, 139
Trichinella spiralis	Mixed	200 μg/kg once, not recommended		No effect on encysted form; 80–90% in free living forms[g]	140, 141
Ancylostoma braziliense, *Ancylostoma canium*, *Uncinaria stenocephala*[h]	Yes	200 μg/kg, 1–2 doses depending on the clinical picture		81–100%	112, 113

[a]Cure rate if not otherwise indicated.
[b]Possibly a similar situation as *N. americanus*; no speciation conducted.
[c]In immunocompetent patients.
[d]*T. trichiura* may consist of several species explaining the geographically different rates in reduction after treatment.
[e]Unknown.
[f]Potential effect on macrofiliaria similar to *O. volvulus*.
[g]Only animal model data available.
[h]All responsible for CLM.

Table 2. Use of ivermectin for ectoparasites

Ectoparasites (excluding *Anopheles*)	Potential impact of ivermectin MDA	Ivermectin dose (individual treatment)	Ivermectin MDA schedule for control	Reduction at recommended dose (%)[a]	Parasite mortality (%) after n days	References
Sarcoptes scabiei var. *hominis* (scabies)	Yes	200 μg/kg/day, 2 weeks apart or a single dose	200 μg/kg/d 1–2 weeks apart	83–100% at 12 months[b]		8–10, 146–149
Pediculus humanus capitis (head louse)	Yes	200–400 μg/kg/d 1 week apart		77.4–97.1% for 400 μg/kg/d, 89.1–95% for 200 μg/kg/d		154–162
Pediculus humanus corporis (body louse)	Yes	200 μg/kg on day 0, 7 and 14		78%		164
Phtirus pubis (pubic louse)	Yes	200 μg/kg/d 1–2 weeks apart		100%		165
Cimex lectularius (common bedbug)	Yes	200 μg/kg once			67% after 20 d; blood meal 3 h after oral ivermectin: moulting reduced to 0% at 20 d in the same group[c]	171–173
Cimex hemipterus (tropical bedbug)	Unclear[d]	Unclear		Unclear	Unclear[e]	[d]
Demodex sp.	Likely	200 μg/kg		Unclear		174–176
Tunga penetrans	No	200 μg/kg				149, 168
Myiasis (botfly larva)	Unclear[f]	200 μg/kg		Unclear		169, 170[d]

[a]Cure rate if not otherwise indicated.
[b]Topical treatment for children <15 kg.
[c]Without molting sexual maturity does not occur.
[d]Circumstantial observation.
[e]Expected to be similar to *C. lectularius*.
[f]Recommended only in conjunction with surgery.

inflammatory skin diseases, including acne, rosacea, blepharitis and peri-oral dermatitis,[174–176] but larger randomized studies are needed to show specific efficacy of ivermectin.

Conclusions

Ivermectin has been the mainstay of onchocerciasis and LF control programmes worldwide. Within the last decade, ivermectin has shown considerable promise for use in a broader range of diseases, in particular for malaria, scabies and as an adjunct for STH control. These diseases have highly overlapping distributions, suggesting that in some circumstances MDA for malaria may also result in additional health and economic benefits through 'off-target' effects.

Ongoing and planned malaria control trials utilising ivermectin MDA provide opportunities to explore these potential synergies (Box 1). Incorporating STH and scabies endpoints into these trials should be strongly considered to more fully capture the potential health impacts of these programmes. On the other hand, current onchocerciasis, LF, STH and scabies dosing schedules are unlikely to have significant impacts on mosquito populations or malaria transmission. A key question is whether the platforms can be coordinated alongside newer malaria control efforts to accelerate progress. The expansion of ivermectin use requires careful consideration of the development of resistance in both on- and off-target organisms. Potential environmental problems could also arise from its use in animals for malaria vector control or its impact on non-target insect species.[94,96]

In summary, as we enter the decade of the Sustainable Development Goals, it appears the role of ivermectin may be expanding not contracting. Data emerging from recently completed, ongoing and future well-designed clinical trials using ivermectin MDA

for malaria control in varied settings, as mentioned in the malaria section, will answer key programmatic questions about its future role in disease control programmes worldwide.

Box 1.

After >30 y as the mainstay for control and elimination programmes for onchocerciasis and LF there is increasing evidence for a range of expanded indications including scabies and malaria control.

Extended use of ivermectin MDA for malaria vector control has the potential to impact several co-endemic parasites by reducing their burden of disease.

There is a need for exploration of reliable affordable generic supply of ivermectin to support expanded applications for which donations are currently unlikely.

Safety data on use in, at present, excluded populations such as pregnant or breastfeeding women and younger children (<5 y of age) is needed.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	386243	**Title**	Dr.
First Name(s)	Christian Hermann		
Surname/Family Name	Kositz		
Thesis Title	Investigating the Effects of Ivermectin plus Dihydroartemisinin-Piperaquine MDA on the Transmission of Malaria by Measuring Serological Markers of Exposure and its Effects on the Prevalence of Ectoparasites and Soil-Transmitted-Helminths		
Primary Supervisor	Michael Marks		

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?	Transactions of the Royal Society of Tropical Medicine and Hygiene		
When was the work published?	2021		
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Please list the paper's authors in the intended authorship order:	Christian Kositz, John Bradley, Harry Hutchins, Anna Last, Umberto D'Alessandro, Michael Marks
Stage of publication	**In press**

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)	Drafted the manuscript, conducted the literature review, prepared the final version; responded to peer review comments

SECTION E

Student Signature	[redacted]
Date	11.10.2022

Supervisor Signature	[redacted]
Date	11.10.2022

Chapter 5 Research Paper 1

Impact of Mass Drug Administration with Ivermectin and Dihydroartemisinin on serological Markers of Malaria Transmission

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Key Words:
Malaria, Luminex, MDA

Abstract:

Malaria serology is an established approach to detect historical patterns of malaria endemicity. Recent advances in serological technologies combined with standardised methods for epidemiological surveillance have allowed these approaches to be extended as new tools for malaria surveillance, in particular in low transmission areas, where other methods such as rapid diagnostic test or entomological inoculation rates may not be sufficiently sensitive.

Methods:

Cross-sectional surveys collecting dried blood spots (DBS) were conducted in June and November 2019 as part of a cluster randomized trial using MDA with ivermectin and dihydroartemisinin-piperaquine, conducted in The Gambia. MDA was implemented in three rounds during August-October 2018 and July-September 2019, corresponding to the main transmission season. In each of 32 clusters 200 participants were randomly selected for DBS collection and of these a random subset of at least 70 up to 100 DBS per cluster were selected for analysis. The Luminex MAGPIX© system was used to measure the difference in median fluorescence intensity (MFI) of a range of antibodies reflecting long, intermediate, and short-term exposure to malaria.

Findings:

No impact of a combined MDA of dihydroartemisinin-piperaquine and ivermectin on serological markers of malaria exposure was found. The mean fluorescent intensity against serological markers was strongly correlated with PCR positivity for malaria, and seasonal correlation for the vector exposure marker (gSG6) with an aOR of 1.12 (95% CI: 0.88 - 1.41, p = 0.359) in the dry season and an aOR of 1.17 (95% CI: 1.04 - 1.33, p = 0.007) in the rainy season and for sporozoite markers an aOR of 1.04 (95% CI: 0.92 - 1.18, p = 0.45) and 1.52 (95% CI: 1.34 - 1.71 p < 0.001).

Conclusion:

Although the serological data did not show any evidence of an impact of MDA on markers of exposure to malaria or to the vector in the month following MDA, this is the first study to investigate this question one month after the MDA, therefore setting time point from when

to further explore the use of serological markers in malaria. The clear correlation of mosquito and sporozoite exposure markers to season is encouraging and could be a first step to show the feasibility of this method for surveillance purposes.

Background

Despite substantial progress over the last 20 years, malaria still results in a significant health burden (1). Many endemic countries have highly heterogeneous transmission, driven by focal hot spots (2). In these settings it is important to detect small changes in malaria incidence to prevent flare up epidemics. However, this becomes increasingly difficult in low transmission areas as many of the available diagnostic tools, such as rapid diagnostic tests, microscopy and even PCR, and vector surveillance approaches, such as entomological inoculation rate (EIR), do not have an adequate sensitivity at extremely low prevalence of infection (3).

Serological methods to detect malaria antibodies are a highly sensitive way of assessing past exposure and could potentially differentiate between exposures within the last month and the last year, particularly in younger age groups (4,5). Selected combinations of antigens detecting specific antibodies may therefore be a suitable surveillance tool for malaria exposure in a low transmission setting (6). In addition, an immunogenic *Anopheles gambiae* spp. salivary protein, *An. Gambiae* salivary gland antigen 6 (gSG6), has potential as a marker to detect recent vector exposure and to act as a potential proxy marker for the effects of vector control methods (7).

High throughput serological methods, such as the Luminex MAGPIX© system, combined with the development of standardized recombinant malaria antigens, has made large scale sero-epidemiological methodology logistically feasible (8–10). However, large scale field studies evaluating this methodology are still needed.

We utilised a *Plasmodium falciparum* (*P.f.)* multiplex serological panel within the MASSIV trial, which investigated Mass Drug Administration (MDA) of dihydroartemisinin-piperaquine and ivermectin for malaria control in The Gambia (11). Here, we report the findings of the impact of MDA on the serological markers for long- and short-term exposure to both *P.f.* and to the *An. gambiae* vector.

Methods:

MASSIV Trial:

The MASSIV trial (NCT03576313) was a cluster randomized trial that included 32 clusters (villages) randomized 1:1 to either the intervention or control arm (12). Its primary aim was to evaluate the effect of mass drug administration of dihydroartemisinin-piperaquine and ivermectin on malaria transmission (11). Residents on the intervention villages received dihydroartemisinin-piperaquine (DHP) at 320/40mg and 160/20mg depending on bodyweight and with ivermectin at a dose of 300 – 400 mcg/kg bodyweight on three consecutive days at monthly intervals starting in July for three months with the beginning of the malaria season (July – November). Individuals whose weight was below 15 kg, pregnant or breastfeeding women did not receive ivermectin. Individuals under 6 months of age and who took QTc prolonging medication did not receive DHP. The intervention was carried out in 2018 and 2019. The intervention led to a significantly lower prevalence of infection) and incidence of clinical malaria in the intervention arm. Although vector parity, a proxy of vector survival, was not significantly reduced between arms, vector density and entomological inoculation rate were significantly lower in the intervention arm, however, a seasonality effect on these parameters are not excluded.

In this study, we determined whether antibody responses to a panel of antigenic markers for short-term, variable-term, and long-term exposure alongside markers of exposure to vector saliva could reflect the observed decrease in malaria prevalence and incidence, and in vector density.

Study Design:

The MASSIV study population included all members of the 32 village communities in the Upper River Region (URR) fulfilling the entry criteria and having provided a written informed consent. This study utilises samples collected during two community-based cross-sectional surveys carried out in all study villages. in June 2019, before the 2nd MDA, and nine months after the 1st and in November 2019 one month after the 2nd MDA. For the cross-sectional surveys, up to 200 participants per village of all ages out of the original MASSIV trial census of 11518 participants were randomly selected and a blood sample was collected from a finger prick for dried blood spot (DBS) testing. For the current study, 100 DBS per village were randomly selected for the Luminex assay.

Originally, two years of MDA were planned, but due to logistical and staffing problems the 2018 MDA had less coverage than needed, as well as not reaching the calculated sample size needed for an analysis and was therefore excluded(11).

Sample size calculation:

A sample size of 70 participants of all ages per cluster gives a power of 90% to detect a difference between 40% sero-prevalence in the control clusters compared to 20% in the intervention clusters at the end of the transmission season assuming a coefficient of variation of 0.5 (13).

Recombinant Antigen selection:

A panel of *P.f.* antigens were selected according to their potential capacity to differentiate between a long- or short-term exposure to infection, according to the respective antibodies. (Table 3)

Sample processing and data generation:

Collected DBS were immediately stored at 4°C, then transferred to -20^{0}C until further processing. A 6mm diameter punch (ca. 4µL serum) of each DBS was eluted in 400µL PBS with sodium azide (NaN3) on a shaking platform overnight, resulting in a serum dilution of 1/400, assuming 50% haematocrit. The chemical coupling of *P.f.* recombinant antigens to Luminex MagPlex© COOH-microspheres or 'beads' (Luminex Corp., Austin TX) was optimised and tested against 50µl of dry blood spot eluent per sample as described previously. (10) Briefly, 50µl of antigen-coupled beads were co-incubated with 50µl of DBS eluent followed by 50µl of a fluorescent secondary antibody (goat anti-human Fcy-fragment specific IgG conjugated to R-Phycoerythrin (R-PE), Jackson ImmunoResearch©, 109-116-098) diluted 1/200 to detect antibody binding. Each Luminex plate included a pool of hyperimmune Tanzanian serum samples (CP3) in a six point, five-fold serial dilution at 1/10, 1/50, 1/250, 1/1250, 1/6250, 1/31250 to serve as a standard curve. The 1st WHO international reference standard for *P.f.* (NIBSC, 10/198) diluted 1/400 and 1/4000 was used as a second positive control, two malaria-naive European blood donors (Public Health England, 2016) diluted 1/400 and 1/4000 were used as negative controls, and two wells of elution buffer only served as background controls. The Median Fluorescence Intensity (MFI), a proxy measure of

antigen-specific antibody titres present in the sample, was acquired for each sample using a MagPix© Bioanalyser.
The Luminex assay was conducted by trained personnel at the MRC Unit The Gambia under supervision of LSHTM.

Statistical Analysis

MFI data was background adjusted and the quality of the output was checked by comparing control standard curves variation between plates and looking at Levey-Jennings plots of mean MFI data to fall in the acceptable variation of 2 standard deviations. This was done using an in-house R script generated by Wu et al (14).

The primary outcome was the difference in MFI detected between the intervention and control arm at the village level. For adjusted analyses, age was split into three groups, <5 years, 5 – 14 years and 14 > years. MFI was considered a continuous variable. The log MFI was used for the regression analysis and results were then transformed into the geometric mean. To compare study arms, a metobit regression model with fixed effects for age, gender, and random effects for study clusters was fitted.
For the antibody seroprevalence estimates, MFI cut-offs were calculated using the mean MFI responses of malaria-naive European blood donors HE) +3SD and the resulting MFI data dichotomised into a categorical variable with seropositives above the cut-off value and seronegatives below. These variables were then used to calculate the seroprevalence for each antibody by comparing the difference between the arms for each survey and age-groups split into 0 – 5, >5 – 10, >10 – 15, > 20 – 30, > 30 – 40, >40 – 50, 50+.
The same DBS used for the serological analysis were also used for malaria qPCR to measure the PCR positive rate in each survey. The qPCR positive results were then paired with the respective logMFI data from the same DBS surveys and analysed using a logistic regression model with fixed effects for age, gender and arm, and with random effects for study cluster was fitted.
Figures containing statistical data were created using R version 4.1.2 (2021-11-01). Data analysis was conducted using Stata 14.1.

Ethics Statement:

Ethical clearance was given by the ethics board of the London School of Hygiene and Tropical Medicine, the Scientific Coordinating Committee at the Medical Research Centre The Gambia @LSHTM, and the Gambian government/MRCG Joint Ethics Committee (LSHTM Ethics Ref. Nr. 17123, and Ref. Nr. 15823; SCC Ref Nr. 1593v1.1).

As part of the MASSIV trial, communities and local authorities were informed about the content of this survey and only consented participants were included. Parents consented for their children and additional written consent was obtained for children ≥ 12 years of age.

Results:

A total of 5251 DBS samples were collected from participants from both arms, with 2722 in the intervention and 2529 in the control arm. Samples of the June and November survey were paired and randomly selected. In case no pairing could be found, additional samples were selected. This resulted in 3,103 DBS randomly selected samples for the June 2019 survey with the final analysis included 3010 samples (1540 in the control and 1470 in the intervention arm) as 93 did not generate results (6) or the age of the participants was not available (87).

For the November 2019 survey, this resulted in 3076 DBS being selected with the final analysis included 2975 samples (1496 in the control and 1479 in the intervention arm) as 101 did not generate results (16) or the age of the participants was not available (85) (Figure 1). Baseline demographic details for each specific survey are described in Table 1.

MFI results for exposure marker antibodies:

There was no evidence for an effect of the MDA on the MFI for any of the antigens tested in the June 2019 survey**.** However, despite lacking significance there was a consistent trend for higher MFI levels in the intervention arm.

For the November 2019 survey there was also no evidence for an effect of the MDA between arms detected on the MFI for any of the marker antigens tested. The results for both surveys are shown in Table 3. Only the November 2019 data was available for additional adjusting for village level malaria PCR positive prevalence. No evidence was found for and effect of the MDA between arms and the results are shown in Table 3a.

Graphical representations of both surveys are presented in Figures 2 – 5.
The respective prevalence for the MFI of each antibody marker is presented in Figure 6 - 8 for each of the surveys. When we categorised each antibody result as either positive or negative, we did not find any difference in the seroprevalence of any marker, irrespective of short, long or variable term status, between study arms at either timepoint.

Relation between PCR positive samples and MFI results

Distribution of PCR positive malaria samples correlated with the MFI from the MASSIV trial are shown in Table 2.
The mosquito exposure marker antigen gSG6 showed no evidence of a correlation with PCR positivity for June 2019 with an aOR was 1.12 (95%CI 0.96 – 1.41, p-value = 0.359), however, evidence was shown in the results post-MDA in November 2019 with an aOR was 1.17 (95%CI 1.04 – 1.33, p-value = 0.007). Also, the sporozoite marker CSP which is pre-hepatic showed no evidence of PCR positivity for June 2019 with an aOR was 1.04 (95%CI 0.92 – 1.18, p-value = 0.45), but evidence was shown in the results post-MDA in November 2019 with an aOR was 1.52 (95%CI 1.34 – 1.71, p-value = 0.001). Interestingly, Rh5.1, a variable term marker, and a molecule for RBC invasion, showed no correlation with PCR positivity in June 2019 with an aOR of 1.02 (95%CI (0.82 – 1.27), p-value = 0.82) nor in November 2019 with an aOR of 1.1 (95%CI 0.95 – 1.29, p-value = 0.179).
All other markers independent of their short, long, or variable term status, were markers of either of the parasite's blood stage, showed clear correlation with PCR positivity.
The results for both surveys can be seen in Table 4.
Table 4a and Table 4b present the data by splitting it into the respective arms for June and November, with June reflecting the overall results for June in Table 4, but no evidence for a correlation between MFI and PCR positivity found in November for gSG6 with an aOR of 1,12 (95%CI 0.93 – 1.36, p-value = 0.218), and PfAMA1 with an aOR 1.08 (95%CI 0.98 – 1.19, p-value = 0.104).

Discussion:

In this study we did not find any clear evidence of an impact of MDA combining dihydroartemisinin-piperaquine with ivermectin MDA on serological markers of exposure to either the plasmodium parasite or the malaria vector. This result stands in contrast to the main result of the MASSIV trial itself which showed a significant reduction of malaria prevalence between intervention and control arm of 12.8% vs 5.1%, measured using PCR. We found a strong correlation between a positive PCR result for malaria during the MASSIV trial and the respective MFI for most of the exposure markers both before and after the malaria season, with the main exception being RH5.1, a potential vaccine candidate (15), gSG6, a mosquito exposure marker, and CSP, a sporozoite antigen. Interestingly, the mosquito exposure marker gSG6 was not correlated with PCR positivity pre-malaria season, when no Anopheles vectors are present in the area, but at the end of the malaria season when they are plentiful. However, splitting the data further into the respective arms for each June and November, found that the June data as well as the results from the control arm in November showed very similar results, though no correlation was found in the intervention arm in November for gSG6, which could point to reduction in mosquito exposure, and no correlation for PfAMA1. Though this is harder to interpret and could be either due to a reduction in sporozoite exposure as PfAMA1 is expressed on sporozoites, which seems to be unlikely as CSP correlated with PCR, or a lack of additional merozoite generations due to the MDA. Another hypothesis could be, that PfAMA1 is just a bit less immunogenic than the other two long term markers.

To our knowledge this was the first study to use a large variety of serological markers against malaria in conjunction with a large MDA including ivermectin. Samples were collected about a month after the intervention was conducted. Plausibly this may have been too early to show any significant difference in MFI reduction of the selected marker antibodies. It may be therefore advisable for other studies investigating malaria serology to select different a wider range of time points as some of the more recent studies measured the effect of MDA between 3 – 6 months(9,16) post MDA or even in annual surveys (17).

In MASSIV the MDA combined dihydro-artemisinin (DHP) with ivermectin. DHP is active against asexual stages and early gametocytes but not against merozoites or pre-hepatic stages. Therefore, it is possible even in the intervention arm, an infection, although treated, still provided sufficient antigen load from the pre-erythrocytic stages for the immune system

to react to. These antigens present during specific lifestyle stages including sporozoites and any merozoites directly exiting the liver and which are shared to some extent with asexual stages of the erythrocytic part of the parasite's lifecycle. The CSP results for PCR positivity seem to indirectly reflect this with no correlation seen before the malaria season starts and evidence of a correlation with PCR positivity at the end of the season.

This study had several limitations. The MFI thresholds were calculated using Public Health England (PHE) samples adults for negative data and this could have slightly biased the antibody responses towards European negative controls. Ideally negative exposure serum samples from the local population would be used as controls but there is considerable difficulty identifying individuals with no malaria exposure at all in this setting. Secondly, we utilised DBS not serum samples. DBS show a lower absolute MFI level in negative samples compared to negative samples from fresh serum (Greenhouse B, 2022; personal correspondence), but not for positive samples. This could bias the cut-off values for positive samples at the lower end and vice versa for negative ones at the upper end of the MFI values. Thirdly, the CP3 positive controls are designed towards PfMSP-1-19, PfAMA-1 and GLURP.R2 and could therefore influence results for the other antigens. A fourth point being the less-than-optimal conducted 1st year of the trial which led to the exclusion of that particular dataset. Finally, SMC was delivered as part of the national control programme in the control arm and in those not eligible in the intervention arm and potentially all eligible children in post MDA. It can therefore not be excluded that the SMC may have influenced the MFI levels in the respective age group (18).

Overall, the results in this study were mixed with no effect shown for serological markers, but clear correlation between PCR positive results and MFI with some markers such as CSP and gSG6 showing an expected seasonal variability due to lack of mosquito exposure during the dry season. Interestingly, despite no significant difference of MFIs between arms, the appears to be a consistent trend towards higher antibody MFI levels in the intervention arm. As of now, there is no good explanation for this effect with chance being a possibility. The study delivers important data for future research as there is now data available for a broad antigen panel showing a potential first cut-off at one month post MDA in this studies' setting. This opens up the possibility for studies to investigate the potential decline of antibody MFIs at time points more removed from such a MDA to find the best possible time point for measuring changes in antibody levels and therefore using this serological method

as a way to measure mosquito and parasite exposure post MDA and potential low level re-exposure for epidemiological surveillance.

Author Contributions:

Conceptualization: CK, JB, KT, CD, MM, JH; Review & Editing: CK, JB, HH, FC, SS, CD, KT, UD, MM; Original Draft Preparation: CK; Analysis and interpretation of data: CK, JB, HV, CD, KT, UD, MM; Read and approved final version: CK, MN, FC, SS, JA, HV, ED, CD, KT, JB, UD, MM;

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Conflicts of interest: None declared.

Data Sharing:

After publication, trial data will be made available on reasonable request to the corresponding author. A proposal with a detailed description of the study objectives and a statistical analysis plan is needed for the evaluation of the requests. Additional materials might also be required during the process. Deidentified participant data will be provided after approval by the sponsor and the trial management group.

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Tables:

Demography	June 2019		November 2019	
	Control	Intervention	Control	Intervention
Age Group (years)	N (%)		N (%)	
0 - 5	295 (19.1)	254 (17.3)	266 (17.8)	243 (16.4)
<5 - 14	540 (35.1)	513 (34.9)	559 (18.8)	524 (35.4)
>14	705 (45.8)	703 (47.8)	677 (45.4)	712 (48.2)
Sex				
Female	887 (57.6)	833 (56.7)	859 (57.4)	844 (57.1)
Male	653 (42.4)	637 (43.3)	637 (42.6)	635 (42.9)
Arm	1540 (51.2)	1470 (48.8)	1496 (50.3)	1479 (49.7)
Total	3010		2975	

Table 1: Baseline characteristics of the study participants for malaria DBS surveys

PCR positive	June 2019		November 2019	
	Control	Intervention	Control	Intervention
Age Group (years)	N (%)		N (%)	
0- <5	8/295 (2.7)	12/254 (4.7)	33/266 (12.4)	12/243 (4.9)
5 - 14	35/540 (6.4)	36/513 (7.1)	58/559 (10.4)	22/524 (4.2)
≥14	58/705 (8.2)	60/703 (8.5)	100/677 (14.8)	48/712 (6.7)
Sex				
Female	61/887 (6.9)	54/833 (6.5)	101/859 (11.8)	45/844 (5.3)
Male	40/653 (6.1)	54/637 (8.5)	90/637 (14.1)	37/635 (5.8)
Arm	101/1540 (6.5)	108/1470 (7.3)	191/1496 (12.7)	82/1479 (5.5)
Total DBS samples	3010 (1540/1470)		2975 (1496/1479)	

Table 2: Baseline distribution of PCR positive malaria cases per arm and survey in relation to the DBS samples used for PCR and serological analysis

Survey	Jun-19				Nov-2019			
Antibody	unadjusted geom Mean (95% CI)	p-value	adjusted geom Mean (95% CI)	p-value	unadjusted geom Mean (95% CI)	p-value	adjusted geom Mean (95% CI)	p-value
Marker of Mosquito Exposure								
gSG6	1.09 (0.89 - 1.33)	0.38	1.08 (0.89 - 1.33)	0.39	0.92 (0.65 - 1.32)	0.67	0.94 (0.65 - 1.32)	0.7
Long Term Markers of Exposure								
PfMSP-1-19	1.13 (0.65 - 2.01)	0.63	1.1 (0.63 - 1.93)	0.72	0.5 (0.22 - 1.15)	0.1	0.48 (0.2 - 1.12)	0.09
PfAMA-1	1.13 (0.46 - 2.8)	0.76	1.07 (0.44 - 2.61)	0.86	0.67 (0.31 - 1.44)	0.31	0.64 (0.3 - 1.37)	0.25
GLURP R2	1.73 (0.74 - 4.09)	0.2	1.61 (0.7 - 3.78	0.25	0.64 (0.26 - 1.56)	0.33	0.6 (0.24 - 1.49)	0.27
Short Term Markers of Exposure								
HSP40	1.11 (0.88 - 1.4)	0.35	1.09 (0.86 - 1.37)	0.43	0.86 (0.65 - 1.15)	0.32	0.85 (0.63 - 1.12)	0.26
ETRAMP5.Ag1	1.15 (0.96 - 1.4)	0.13	1.13 (0.94 - 1.38)	0.18	0.71 (0.45 - 1.1)	0.13	0.69 (0.44 - 1.09	0.11
PfSEA-1	1.18 (0.92 - 1.55)	0.19	1.17 (0.9 - 1.52)	0.22	0.86 (0.68 - 1.08)	0.21	0.85 (0.67 - 1.07)	0.17
CSP	1.32 (0.86 - 2.03)	0.19	1.25 (0.83 - 1.93)	0.27	0.93 (0.57 - 1.5)	0.77	0.87 (0.53 - 1.41)	0.58
Hyp2	1.17 (0.96 - 1.44)	0.13	1.16 (0.94 - 1.43)	0.16	0.95 (0.82 - 1.09)	0.51	0.95 (0.81 - 1.09)	0.44
MSP2.Dd2	1.27 (0.91 - 1.78)	0.15	1.24 (0.89 - 1.75)	0.2	0.94 (0.62 - 1.4)	0.74	0.91 (0.6 - 1.36)	0.64
MSP2.CH150	1.13 (0.46 - 2.82)	0.77	1.07 (0.43 - 2.66)	0.86	0.54 (0.22 - 1.33)	0.18	0.51 (0.2 - 1.26)	0.14
Rh4.2	1.27 (0.94 - 1.73)	0.11	1.24 (0.92 - 1.69)	0.15	0.84 (0.51 - 1.37)	0.49	0.82 (0.5 - 1.33)	0.43
SBP1	1.15 (0.96 - 1.39)	0.13	1.12 (0.95 - 1.36)	0.18	0.91 (0.71 - 1.17)	0.5	0.89 (0.69 - 1.15)	0.39
Etramp4.Ag2	1.12 (0.81 - 1.25)	0.91	0.99 (0.79 - 1.23)	0.96	0.87 (0.54 - 1.37)	0.562	0.85 (0.53 - 1.34)	0.49
Variable Markers of Exposure								
EBA175.RIII.V	0.96 (0.35 - 2.58)	0.94	0.92 (0.33 - 2.48)	0.86	0.43 (0.13 - 1.4)	0.16	0.41 (0.12 - 1.39)	0.14
EBA181.RIII.V	1.09 (0.84 - 1.43)	0.68	1.07 (0.82 - 1.4)	0.58	0.91 (0.71 - 1.16)	0.48	1.11 (0.69 - 1.14)	0.38
EBA140.RIII.V	0.8 (0.36 - 1.75)	0.58	0.77 (0.35 - 1.71)	0.53	0.48 (0.16 - 1.47)	0.2	0.46 (0.14 -1.43)	0.18
Rh2.2030	1.02 (0.76 - 1.39)	0.86	0.99 (0.73 - 1.35)	0.96	0.63 (0.26 - 1.5)	0.3	0.6 (0.25 - 1.4)	0.24
Rh5.1	0.99 (0.83 - 1.15)	0.82	0.99 (0.84 - 1.16)	0.91	0.93 (0.76 - 1.15)	0.55	0.95 (0.77 - 1.61)	0.6

Table 3: Results for the difference in geometric means between trial arms of the MFI for markers to exposure; *adjusted for age group, sex, arm and clustering at the village level

Nov-19			
unadjusted geom Mean (95% CI)	p-value	adjusted geom Mean (95% CI)	p-value
Marker of Mosquito Exposure			
0.92 (0.65 - 1.32)	0.67	0.95 (0.64 - 1.4)	0.81
Long Term Markers of Exposure			
0.5 (0.22 - 1.15)	0.1	0.55 (0.22 - 1.39)	0.42
0.67 (0.31 - 1.44)	0.31	0.71 (0.31 - 1.63)	0.24
0.64 (0.26 - 1.56)	0.33	0.73 (0.27 - 1.95)	0.54
Short Term Markers of Exposure			
0.86 (0.65 - 1.15)	0.32	0.88 (0.74 - 1.31)	0.93
0.71 (0.45 - 1.1)	0.13	0.85 (0.52 - 1.36)	0.49
0.86 (0.68 - 1.08)	0.21	0.95 (0.74 - 1.21)	0.68
0.93 (0.57 - 1.5)	0.77	1.06 (0.63 - 1.76)	0.82
0.95 (0.82 - 1.09)	0.51	1.03 (0.89 - 1.19)	0.64
0.94 (0.62 - 1.4)	0.74	0.96 (0.61 - 1.52)	0.88
0.54 (0.22 - 1.33)	0.18	0.63 (0.23 - 1.68)	0.36
0.84 (0.51 - 1.37)	0.49	0.86 (0.51 - 1.49)	0.61
0.91 (0.71 - 1.17)	0.5	0.97 (0.74 -1.27)	0.84
0.87 (0.54 - 1.37)	0.562	0.96 (0.57 - 1.58)	0.86
Variable Markers of Exposure			
0.43 (0.13 - 1.4)	0.16	0.44 (0.12 - 1.6)	0.23
0.91 (0.71 - 1.16)	0.48	0.97 (0.75 - 1.27)	0.87
0.48 (0.16 - 1.47)	0.2	0.44 (0.12 - 1.52)	0.19
0.63 (0.26 - 1.5)	0.3	0.65 (0.25 - 1.68)	0.38
0.93 (0.76 - 1.15)	0.55	0.95 (0.75 - 1.19)	0.63

Table 3a: Results for the difference in geometric means between trial arms of the MFI for markers to exposure;
*adjusted for age group, sex, arm, PCR positive village prevalence and clustering at the village level; PCR village prevalence was only available for November.

Survey	Jun-19				Nov-19			
Antibody	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value
Marker of Mosquito Exposure								
gSG6	1.13 (0.9 - 1.14)	0.261	1.12 (0.88 - 1.41)	0.359	1.14 (1.01 - 1.28)	0.025	1.17 (1.04 - 1.33)	0.007
Long Term Markers of Exposure								
PfMSP-1-19	1.46 (1.25 - 1.72)	<0.001	1.37 (1.16 - 1.62)	<0.001	1.23 (1.15 - 1.32)	<0.001	1.24 (1.15 - 1.33)	<0.001
PfAMA-1	1.27 (1.15 - 1.39)	<0.001	1.24 (1.11 - 1.38)	<0.001	1.19 (1.12 - 1.26)	<0.001	1.22 (1.14 - 1.3)	<0.001
GLURP R2	1.43 (1.29 - 1.59)	<0.001	1.53 (1.34 - 1.75)	<0.001	1.24 (1.17 - 1.32)	<0.001	1.29 (1.21 - 1.38)	<0.001
Short Term Markers of Exposure								
HSP40.Ag1	2.71 (2.21 - 3.33)	<0.001	2.76 (2.19 - 3.46)	<0.001	2.31 (1.98 - 2.69)	<0.001	2.49 (2.12 - 2.93)	<0.001
ETRAMP5.Ag1	2.5 (2.09 - 2.99)	<0.001	2.46 (2.03 - 2.99)	<0.001	2.11 (1.86 - 2.39)	<0.001	2.14 (1.88 - 2.44)	<0.001
PfSEA-1	2.31 (1.85 - 2.88)	<0.001	2.16 (1.72 - 2.73)	<0.001	1.87 (1.61 - 2.17)	<0.001	1.9 (1.63 - 2.23)	<0.001
CSP	1.14 (1.03 - 1.26)	0.007	1.04 (0.92 - 1.18)	0.45	1.37 (1.25 - 1.5)	<0.001	1.52 (1.34 - 1.71)	<0.001
Hyp2	2.9 (2.1 - 4.03)	<0.001	2.61 (1.84 - 3.69)	<0.001	3.28 (2.56 - 4.21)	<0.001	3.5 (2.74 - 4.65)	<0.001
MSP2.Dd2	1.32 (1.12 - 1.57)	0.001	1.2 (1.01 - 1.42)	0.034	1.66 (1.43 - 1.93)	<0.001	1.84 (1.53 - 2.21)	<0.001
MSP2.CH150	1.43 (1.29 - 1.59)	<0.001	1.48 (1.3 - 1.68)	<0.001	1.3 (1.22 - 1.4)	<0.001	1.38 (1.27 - 1.51)	<0.001
Rh4.2	1.57 (1.31 - 1.89)	<0.001	1.44 (1.19 - 1.75)	<0.001	1.58 (1.37 - 1.82)	<0.001	1.61 (1.39 - 1.88)	<0.001
SBP1	2.22 (1.72 - 2.88)	<0.001	2.06 (1.53 - 2.76)	<0.001	2.24 (1.87 - 2.68)	<0.001	2.59 (2.11 - 3.17)	<0.001
Etramp4.Ag2	1.85 (1.55 - 2.21)	<0.001	1.75 (1.45 - 2.13)	<0.001	1.93 (1.66 - 2.23)	<0.001	2 (1.71 - 2.33)	<0.001
Variable Markers of Exposure								
EBA175.RIII.V	1.31 (1.2 - 1.43)	<0.001	1.31 (1.19 - 1.45)	<0.001	1.19 (1.12 - 1.25)	<0.001	1.19 (1.12 - 1.26)	<0.001
EBA181.RIII.V	1.7 (1.44 - 1.99)	<0.001	1.64 (1.36 - 1.97)	<0.001	1.91 (1.67 - 2.17)	<0.001	2.13 (1.82 - 2.48)	<0.001
EBA140.RIII.V	1.3 (1.17 - 1.44)	<0.001	1.25 (1.13 - 1.4)	<0.001	1.22 (1.15 - 1.3)	<0.001	1.22 (1.14 - 1.3)	<0.001
Rh2.2030	1.89 (1.62 - 2.19)	<0.001	2.03 (1.69 - 2.44)	<0.001	1.33 (1.22 - 1.44)	<0.001	1.42 (1.28 - 1.58)	<0.001
Rh5.1	0.93 (0.75 - 1.15)	0.543	1.02 (0.82 - 1.27)	0.82	1.04 (0.9 - 1.19)	0.587	1.1 (0.95 - 1.29)	0.179

Table 4: Results for the ORs for PCR positivity and MFI level for markers to exposure; *adjusted for age group, sex, arm and clustering at the village level

Survey	Jun-19 Control				Jun-19 Intervention			
Antibody	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value
Marker of Mosquito Exposure								
gSG6	1.11 (0.81 - 1.51)	0.518	1.08 (0.78 - 1.51)	0.635	1.17 (0.85 - 1.61)	0.331	1.16 (0.83 - 1.61)	0.384
Long Term Markers of Exposure								
PfMSP-1-19	1.29 (1.7 - 1.56)	0.007	1.18 (0.99 - 1.42)	0.62	1.75 (1.36 - 2.26)	< 0.001	1.75 (1.33 - 2.29)	< 0.001
PfAMA-1	1.33 (1.15 - 1.55)	< 0.001	1.29 (1.09 - 1.53)	0.02	1.22 (1.07 - 1.38)	0.002	1.2 (1.04 - 1.38)	0.01
GLURP R2	1.47 (1.27 - 1.71)	< 0.001	1.55 (1.28 - 1.86)	< 0.001	1.41 (1.21 - 1.63)	< 0.001	1.52 (1.25 - 1.85)	< 0.001
Short Term Markers of Exposure								
HSP40.Ag1	2.79 (2.1 - 3.7)	< 0.001	2.77 (2.01 - 3.81)	< 0.001	2.71 (2.01 - 3.65)	< 0.001	2.84 (2.04 - 3.95)	< 0.001
ETRAMP5.Ag1	2.6 (2.02 - 3.35)	< 0.001	2.49 (1.9 - 3.27)	< 0.001	2.47 (1.91 - 3.19)	< 0.001	2.52 (1.91 - 3.33)	< 0.001
PfSEA-1	2.48 (1.82 - 3.39)	< 0.001	2.29 (1.65 - 3.19)	< 0.001	2.24 (1.64 - 3.06)	< 0.001	2.15 (1.55 - 2.97)	< 0.001
CSP	1.15 (1.01 - 1.31)	0.041	1.04 (0.89 - 1.21)	0.62	1.14 (0.98 - 1.32)	0.083	1.06 (0.87 - 1.3)	0.542
Hyp2	2.74 (1.79 - 4.18)	< 0.001	2.35 (1.5 - 3.68)	< 0.001	3.26 (1.96 - 5.42)	< 0.001	3.12 (1.81 - 5.38)	< 0.001
MSP2.Dd2	1.1 (0.93 - 1.31)	0.247	1.01 (0.85 - 1.19)	0.947	1.93 (1.46 - 2.56)	< 0.001	2.05 (1.47 - 2.88)	< 0.001
MSP2.CH150	1.58 (1.35 - 1.86)	< 0.001	1.67 (1.38 - 2.02)	< 0.001	1.32 (1.15 - 1.52)	< 0.001	1.35 (1.14 - 1.6)	0.001
Rh4.2	1.33 (1.06 - 1.68)	0.016	1.18 (0.94 -1.49)	0.152	1.95 (1.49 - 2.55)	< 0.001	1.91 (1.43 - 2.56)	< 0.001
SBP1	2.45 (1.71 - 3.5)	< 0.001	2.17 (1.45 - 3.25)	< 0.001	2.05 (1.41 - 2.97)	< 0.001	1.98 (1.3 - 3.02)	0.001
Etramp4.Ag2	1.97 (1.53 - 2.53)	< 0.001	1.84 (1.39 - 2.44)	< 0.001	1.75 (1.37 - 2.26)	< 0.001	1.73 (1.32 - 2.26)	< 0.001
Variable Markers of Exposure								
EBA175.RIII.V	1.47 (1.28 - 1.68)	< 0.001	1.48 (1.26 - 1.74)	< 0.001	1.22 (1.09 - 1.36)	< 0.001	1.22 (1.08 - 1.38)	0.002
EBA181.RIII.V	1.85 (1.47 - 2.34)	< 0.001	1.76 (1.35 - 2.29)	< 0.001	1.57 (1.27 - 1.97)	< 0.001	1.56 (1.21 - 2.02)	0.001
EBA140.RIII.V	1.38 (1.18 - 1.61)	< 0.001	1.31 (1.11 - 1.54)	< 0.001	1.25 (1.09 - 1.43)	0.001	1.23 (1.08 - 1.42)	0.003
Rh2.2030	2.01 (1.61 - 2.5)	< 0.001	2.12 (1.63 - 2.78)	< 0.001	1.8 (1.47 - 2.21)	< 0.001	1.98 (1.54 - 2.54)	< 0.001
Rh5.1	0.81 (0.59 - 1.1)	0.181	0.88 (0.64 - 1.21)	0.448	1.07 (0.79 - 1.42)	0.661	1.17 (0.87 - 1.58)	0.306

Table 4a: Results for the ORs for PCR positivity and MFI level for markers to exposure for the June survey split by study arms; *adjusted for age group, sex, and clustering at the village level

Survey	Nov-19 Control				Nov-19 Intervention			
Antibody	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value	unadjusted OR (95% CI)	p-value	adjusted OR* (95% CI)	p-value
Marker of Mosquito Exposure								
gSG6	1.17 (1.01 - 1.36)	0.035	1.21 (1.04 - 1.42)	0.013	1.09 (0.91 - 1.31)	0.342	1.12 (0.93 - 1.36)	0.218
Long Term Markers of Exposure								
PfMSP-1-19	1.26 (1.16 - 1.37)	< 0.001	1.29 (1.17 - 1.41)	< 0.001	1.17 (1.05 - 1.31)	0.005	1.15 (1.03 - 1.3)	0.012
PfAMA-1	1.24 (1.15 - 1.33)	< 0.001	1.32 (1.21 - 1.44)	< 0.001	1.11 (1.01 - 1.21)	0.026	1.08 (0.98 - 1.19)	0.104
GLURP R2	1.25 (1.17 - 1.34)	< 0.001	1.32 (1.21 - 1.43)	< 0.001	1.22 (1.1 - 1.36)	< 0.001	1.24 (1.1 - 1.41)	0.001
Short Term Markers of Exposure								
HSP40.Ag1	2.44 (2.03 - 2.94)	< 0.001	2.72 (2.23 - 3.32)	< 0.001	2.02 (1.55 - 2.65)	< 0.001	2.11 (1.56 - 2.83)	< 0.001
ETRAMP5.Ag1	2.14 (1.84 - 2.5)	< 0.001	2.21 (1.88 - 2.57)	< 0.001	2 (1.58 - 2.52)	< 0.001	2.02 (1.58 - 2.58)	< 0.001
PfSEA-1	1.81 (1.51 - 2.17)	< 0.001	1.86 (1.54 - 2.25)	< 0.001	1.96 (1.51 - 2.55)	< 0.001	1.99 (1.51 - 2.62)	< 0.001
CSP	1.41 (1.26 - 1.59)	< 0.001	1.6 (1.38 - 1.86)	< 0.001	1.3 (1.11 - 1.52)	0.001	1.35 (1.09 - 1.67)	0.005
Hyp2	3.1 (2.29 - 4.19)	< 0.001	3.45 (2.5 - 4.77)	< 0.001	3.68 (2.39 - 5.65)	< 0.001	3.82 (2.42 - 6.04)	< 0.001
MSP2.Dd2	1.69 (1.41 - 2.03)	< 0.001	1.95 (1.55 - 2.45)	< 0.001	1.61 (1.24 - 2.09)	< 0.001	1.655 (1.21 - 2.27)	0.002
MSP2.CH150	1.29 (1.19 - 1.41)	< 0.001	1.38 (1.25 - 1.53)	< 0.001	1.34 (1.16 - 1.55)	< 0.001	1.41 (1.18 - 1.67)	< 0.001
Rh4.2	1.67 (- 1.41 - 2)	< 0.001	1.74 (1.44 - 2.09)	< 0.001	1.42 (1.12 - 1.79)	0.004	1.38 (1.07 - 1.78)	0.011
SBP1	2.35 (1.89 - 2.93)	< 0.001	2.88 (2.24 - 3.71)	< 0.001	2 (1.46 - 2.73)	< 0.001	2.1 (1.47 - 3)	< 0.001
Etramp4.Ag2	1.91 (1.59 - 2.28)	< 0.001	1.99 (1.65 - 2.41)	< 0.001	1.98 (1.52 - 2.58)	< 0.001	2.01 (1.52 - 2.66)	< 0.001
Variable Markers of Exposure								
EBA175.RIII.V	1.18 (1.12 - 126)	< 0.001	1.19 (1.12 - 1.28)	< 0.001	1.19 (1.08 - 1.31)	< 0.001	1.18 (1.065 - 1.31)	0.002
EBA181.RIII.V	1.95 (1.67 - 2.3)	< 0.001	2.21 (1.82 - 2.67)	< 0.001	1.83 (1.47 - 2.29)	< 0.001	1.99 (1.52 - 2.61)	< 0.001
EBA140.RIII.V	1.23 (1.14 - 1.34)	< 0.001	1.25 (1.15 - 1.35)	< 0.001	1.19 (1.06 - 1.33)	0.003	1.17 (1.03 - 1.32)	0.01
Rh2.2030	1.34 (1.21 - 1.49)	< 0.001	1.47 (1.29 - 1.67)	< 0.001	1.31 (1.19 - 1.52)	0.001	1.32 (1.11 - 1.59)	0.002
Rh5.1	1.04 (0.87 - 1.4)	0.634	1.12 (0.93 - 1.34)	0.229	1.03 (0.799 - 1.32)	0.822	1.08 (0.83 - 1.41)	0.565

Table 4b: Results for the ORs for PCR positivity and MFI level for markers to exposure for the November survey split by study arms; *adjusted for age group, sex, and clustering at the village level

Figures:

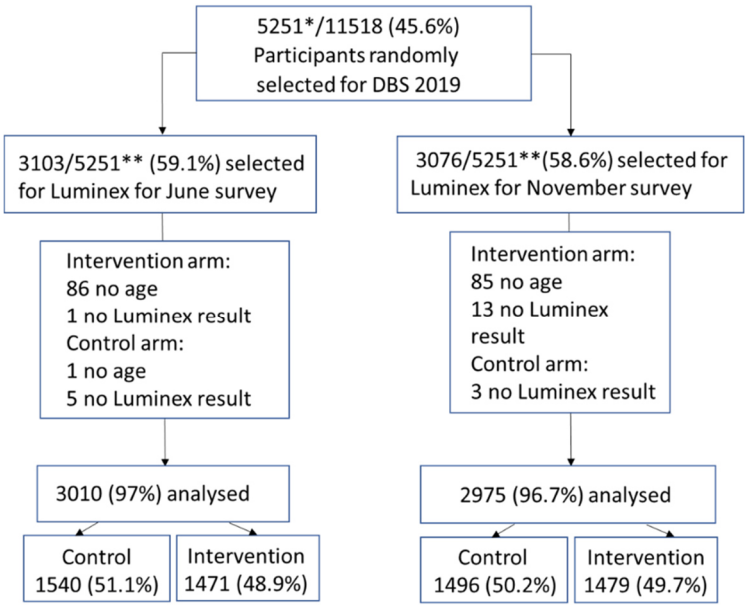

Figure 1 Participants selected for DBS collection. *Number of DBS per village may be lower than 200 as some villages did not reach 200 inhabitants; ** Number of DBS participants between surveys differs slightly due to absent participants, or missing sample.

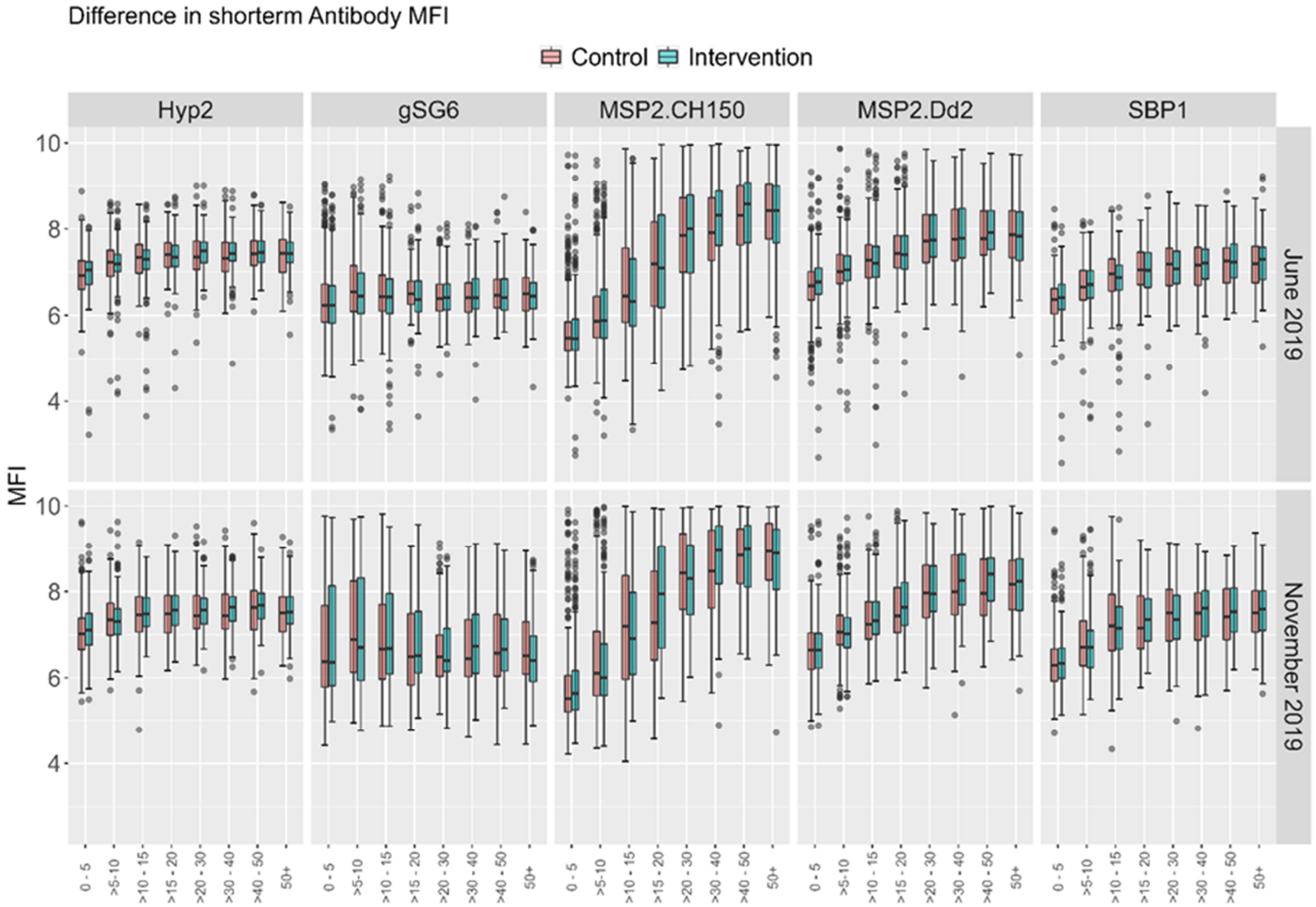

Figure 2: Boxplots presenting the difference between arms in short term marker antibody MFIs for both surveys for the respective age groups. gSG6 represents exposure to *Anopheles gambiae* spp saliva

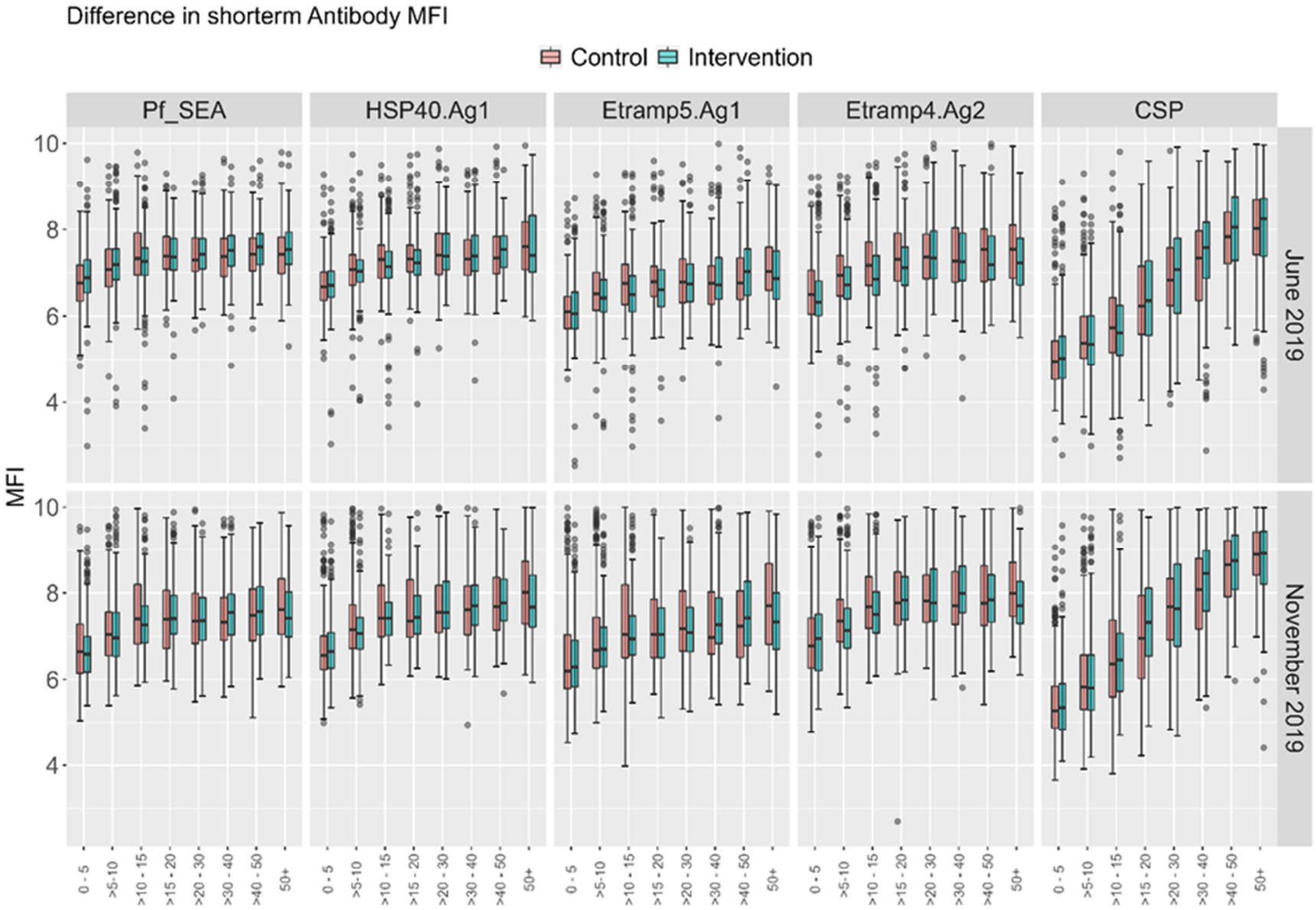

Figure 3: Boxplots presenting the difference between arms in short term marker antibody MFIs for both surveys for the respective age groups

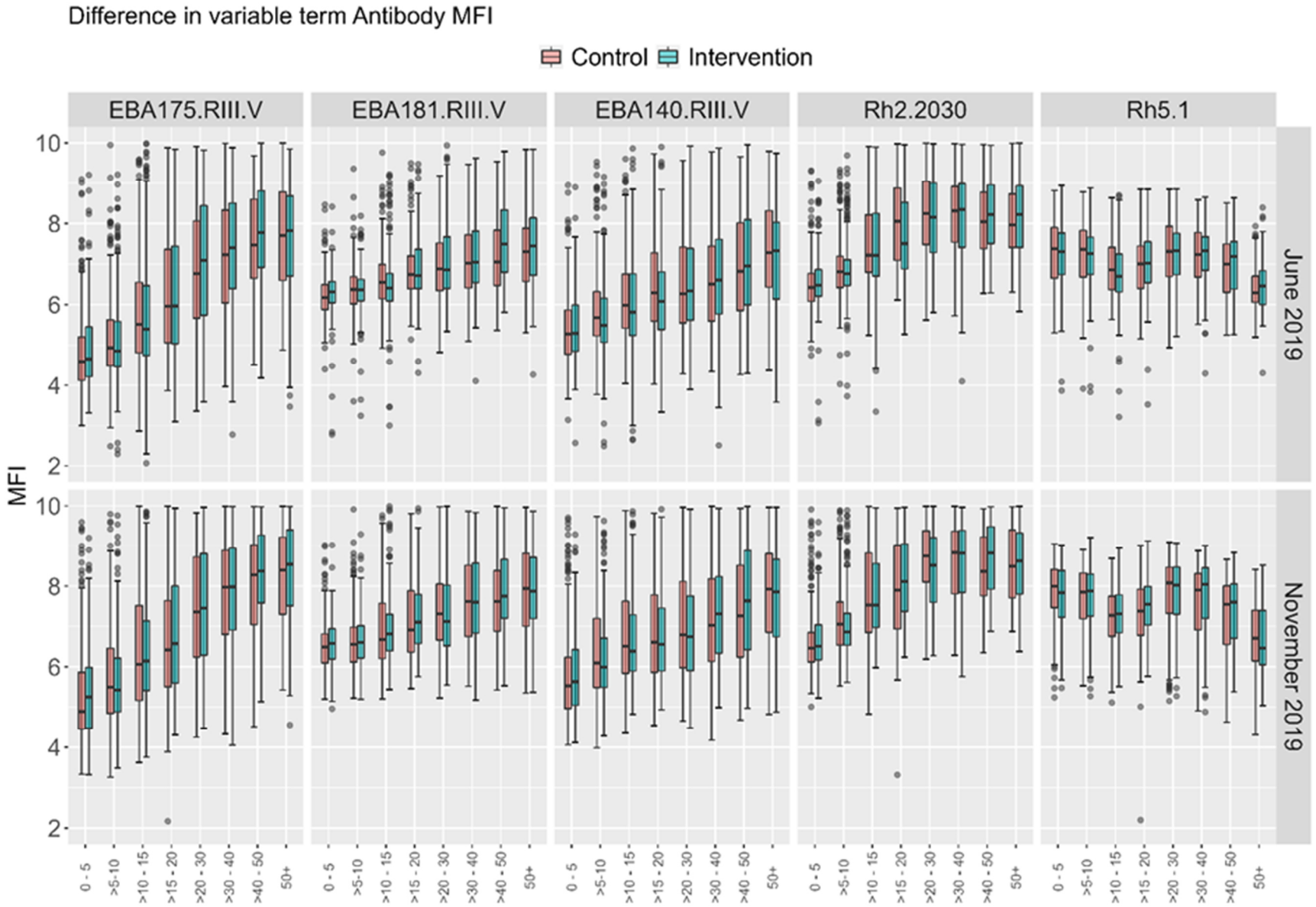

Figure 4: Boxplots presenting the difference between arms in variable markers of exposure antibody MFIs for both surveys for the respective age groups

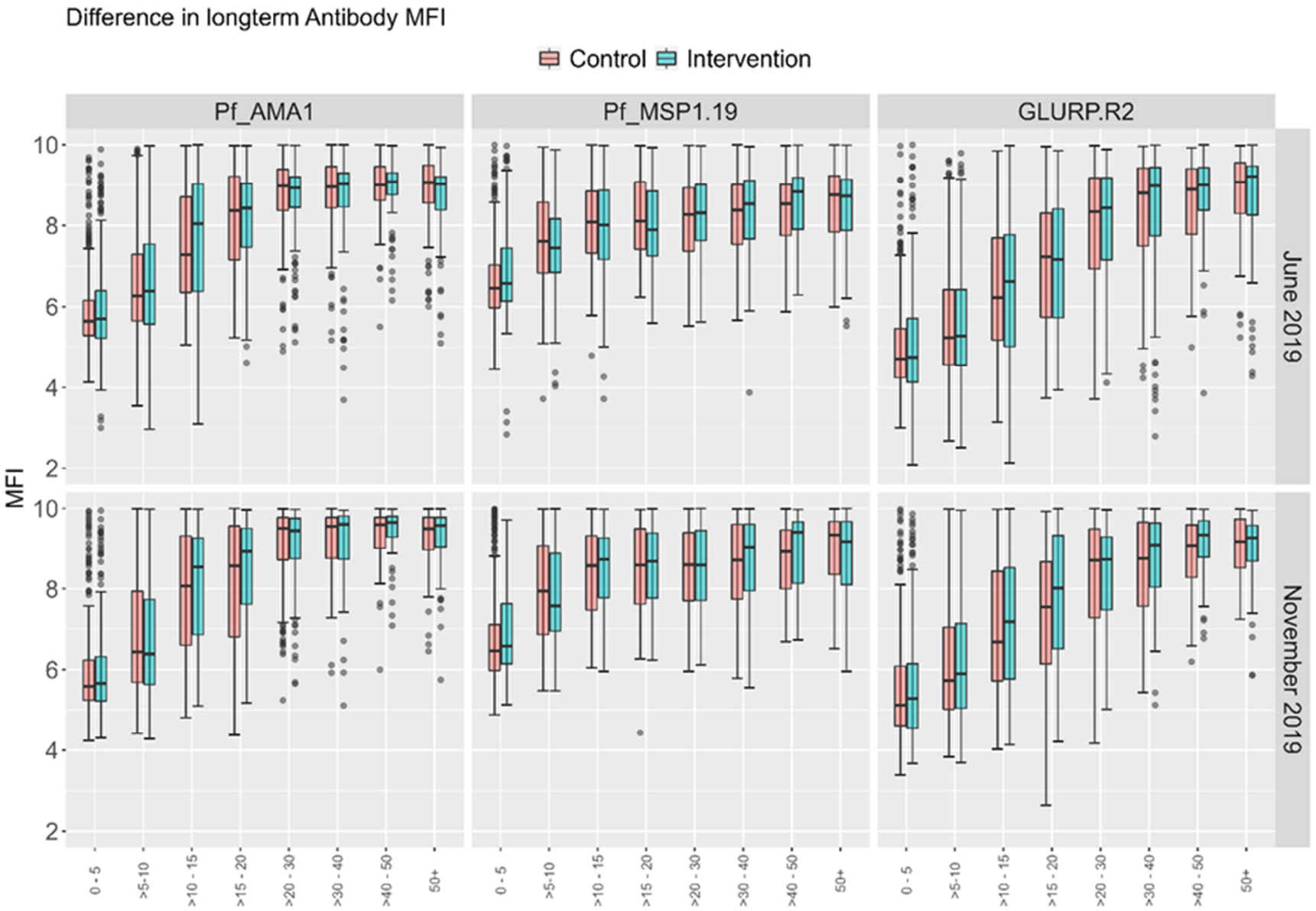

Figure 5: Boxplots presenting the difference between arms in long term markers of exposure antibody MFIs for both surveys for the respective age groups

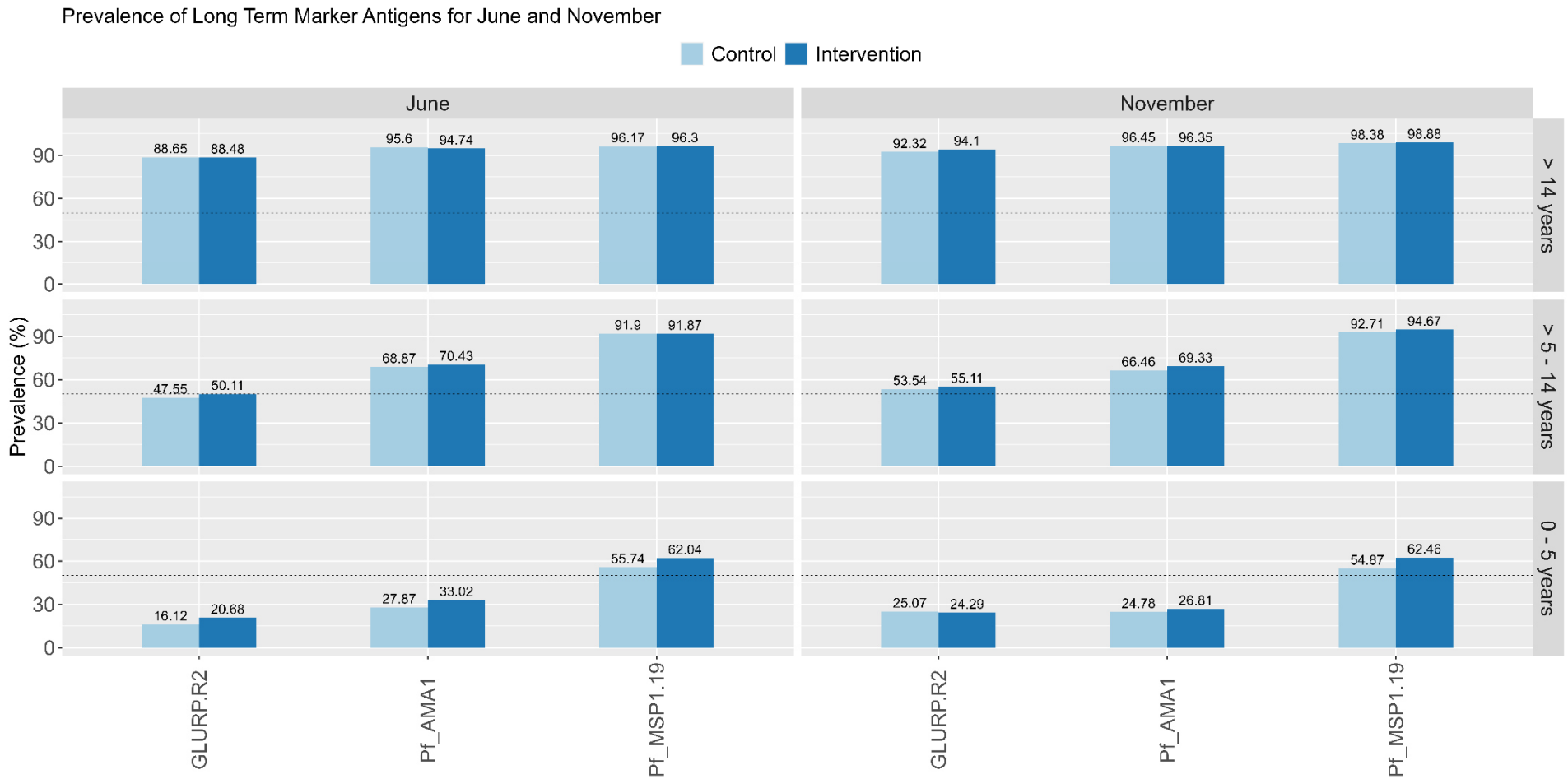

Figure 6: Barplot showing the prevalence of long-term marker antigens for June and November surveys in 2019 for the respective age groups

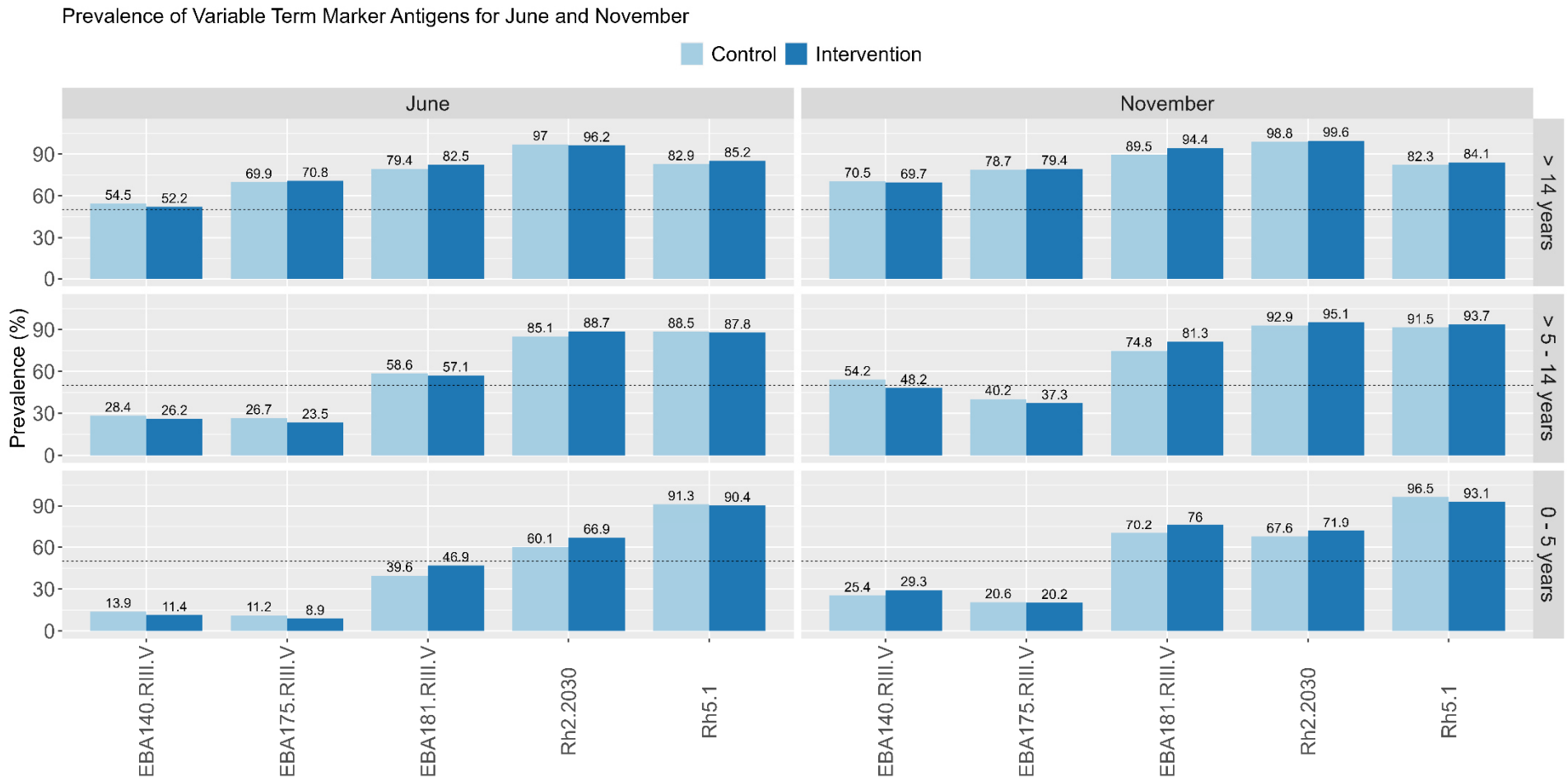

Figure 7: Barplot showing the prevalence of variable term marker antigens for June and November surveys in 2019 for the respective age groups

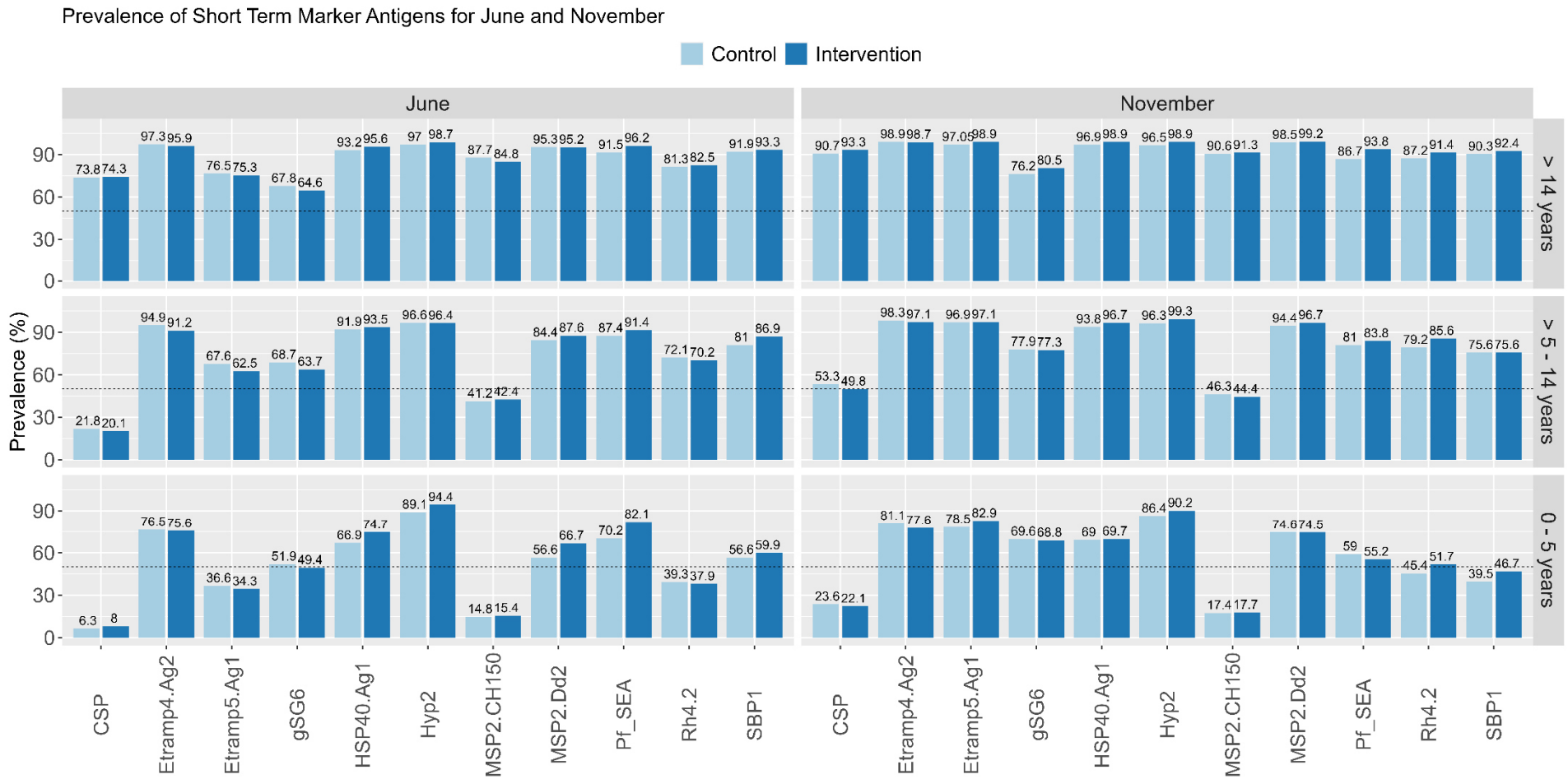

Figure 8: Barplot showing the prevalence of short-term marker antigens for June and November surveys in 2019 for the respective age groups

5.1 Research Paper 1 Supplementary

Antigen	Protein Name	Exposure to	Stage of Expression	Timeframe	Reference
Tetanus toxoid	Tetanus toxoid	Tetanus toxoid		assay control	Wu et al.(14,19);
GST	Glutathione S-transferase	N/A	GST expression tag	NA	Harper et al.(20);
gSG6	*Anophelese gambiae* salivary gland protein 6	vector	mosquitoe saliva antigen	Marker of mosquito exposure	Koffi et al(21);
PfMSP-1-19	merozoite surface protein 1-19	parasite	merozoite	Long-term marker of exposure	Helb et al.(4); van den Hoogen et al.(22); Wu et al.(14,19); Koffi et al.(21);
PfAMA-1	apical membrane antigen 1	parasite	sporozoite/merozoite	Long-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23); Wu et al.(14,19);
GLURP R2	glutamate-rich protein region 2	parasite	merozoite/schizont	Long-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23); Wu et al.(14,19);
HSP40	heat shock protein 40, type II ag 1	parasite	schizont/trophozoite	Short-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23); Wu et al.(14,19);
ETRAMP5.Ag1	early transcribed membrane protein 5	parasite	parasitophorous vacuole membrane / ring stage	Short-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23); Wu et al.(14,19); Achan et al.(24);
PfSEA-1	schizont egress antigen 1	parasite	schizont	Short-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23)
CSP	circumsporozoite protein	parasite	sporozoite	Short-term marker of exposure	Helb et al.(4); van den Hoogen et al.(23);
Hyp2	hypothetical protein 2	parasite	infected red blood cell/parasitophorous vacuole membrane	Short-term marker of exposure	Wu et al.(14,19);
MSP2.Dd2	merozoite surface protein 2, Dd2 allele	parasite	merozoite	Short-term marker of exposure	Richards et al.(25);
MSP2.CH150	merozoite surface protein 2, CH150/9 allele	parasite	merozoite	Short-term marker of exposure	Richards et al.(25);

Rh4.2	Reticulocyte binding protein homologue 4	parasite	merozoite	Short-term marker of exposure	Reiling et al.(26); Ord et al.(27);
SBP1	skeleton-binding protein 1	parasite	Maurer's clefts	Short-term marker of exposure	Cooke et al.(28);
Etramp4.Ag2	early transcribed membrane protein 4	parasite	infected red blood cell/parasitophorous vacuole membrane / ring stage	Short-term marker of exposure	Wu et al.(14,19); Helb et al.(4);
EBA175.RIII.V	erythrocyte binding protein 175, region III-V	parasite	merozoite	Variable marker of exposure (mid time predictor)	Wu et al.(14,19); Richards et al.(25,29);
EBA181.RIII.V	erythrocyte binding protein 181, region III-V	parasite	merozoite	Variable marker of exposure (mid time predictor)	Richards et al.(25,29);
EBA140.RIII.V	erythrocyte binding protein 140, region III-V	parasite	merozoite	Variable marker of exposure (mid time predictor)	Richards et al.(25,29);
Rh2.2030	Reticulocyte binding protein homologue 2	parasite	merozoite	Variable marker of exposure (mid time predictor)	Achan et al.(24); Ord et al.(27);
Rh5.1	reticulocyte binding protein homologue 5	parasite	merozoite	Variable marker of exposure (mid time predictor)	Ord et al.(27); Minassian et al.(15);

Table S1: List of antigen markers for the respective time frame; all proteins except for tetanus toxoid (*Clostridium tetani*) and GST (*Schistosoma japonicum*) are derived from *P. falciparum*.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	386243	**Title**	Dr.
First Name(s)	Christian Hermann		
Surname/Family Name	Kositz		
Thesis Title	Investigating the Effects of Ivermectin plus Dihydroartemisinin-Piperaquine MDA on the Transmission of Malaria by Measuring Serological Markers of Exposure and its Effects on the Prevalence of Ectoparasites and Soil-Transmitted-Helminths		
Primary Supervisor	Michael Marks		

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

Where is the work intended to be published?	Malaria Journal

Please list the paper's authors in the intended authorship order:	Christian Kositz, Hristina Vasileva, Fatima Ceesay, Sarata Sambou, Catriona Patterson, Mamadou O Ndiath, Jane Achan, Annette Erhart, Edgard Dabira, John Bradley, Michael Marks, Umberto D'Alessandro, Chris Drakeley, Kevin K A Tetteh
Stage of publication	**Not yet 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)	Drafted the manuscript, part of the laboratory work; statistical analysis, graphics, will prepare the final version and respond to reviewer comments

SECTION E

Student Signature	[redacted]
Date	03.01.2023

Supervisor Signature	[redacted]
Date	03.01.2023

Chapter 6 Research Paper 2

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Effects of ivermectin mass drug administration for malaria vector control on ectoparasites and soil-transmitted helminths: a cluster randomized trial

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ABSTRACT

Objectives: Ivermectin, used to control several neglected tropical diseases, may also reduce malaria transmission. Mass drug administration (MDA) for malaria control therefore might have off-target impacts on neglected tropical diseases.

Methods: In The Gambia, nested in a trial of ivermectin MDA, cross-sectional surveys measuring ectoparasites and soil-transmitted helminths in children aged 3 to 14 years took place in June and November 2019 and in November 2021.

Results: After MDA, scabies prevalence was 41.2% (237/576) in the control and 38.2% (182/476) in the intervention arm (odds ratio [OR] 0.89 (95% confidence interval [CI] 0 67-1.2), *P*-value = 0.471) but by 2021, had rebounded to 38.8% (180/464) in the control and 53.2% (245/458) in the intervention arm. After MDA, prevalence of *Strongyloides stercoralis* was 16.8% (87/518) in the control and 9.1% (40/440) in the intervention arm (OR 0.4 (95% CI 0.16-0.94), *P*-value = 0.039). In 2021, it was 9.2% (38/413) in the control and 11.3% (45/399) in the intervention arm (OR 1.31 (95% CI 0.74-2.28), *P*-value = 0.35).

Conclusion: Scabies prevalence was similar between the two study arms. *S. stercoralis* prevalence was reduced. However, this effect did not last long: the prevalence 2 years after MDA was similar between study arms.

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Background

Despite substantial progress over the last 20 years, malaria remains an important cause of morbidity and mortality in many endemic countries (World Health Organization. World malaria report, 2021). Ivermectin has been identified as an additional tool for vector control due to its mosquitocidal properties against *Anopheles* spp. (The Ivermectin Roadmappers *et al.*, 2020). Mathematical modeling suggests that mass drug administration (MDA) of ivermectin at a dose of 300 mcg/kg for 3 days or 400 mcg for 1 day and with 70% coverage of those aged over 5 years would reduce malaria transmission (Slater *et al.*, 2020). These dosing regimens have been shown to be both safe and mosquitocidal (Smit *et al.*, 2018).

In addition to its potential for vector control, ivermectin is already established as an effective drug against several neglected tropical diseases, particularly ectoparasites and soil-transmitted helminths (STH), which are a major cause of morbidity in lower- and middle-income countries. Ectoparasites occur at low levels in almost all countries but can be highly prevalent in hyperendemic foci in vulnerable or neglected populations (Feldmeier and Heukelbach, 2009; Gbakima *et al.*, 2002; Heukelbach *et al.*, 2003). STH, including *Strongyloides stercoralis, Ascaris lumbricoides, Trichuris*

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the container and the amount needed. Instructions included storing the sample in a cold, dark place, and preferably taking a morning stool sample to reduce the time between sample production and collection. Samples produced at the time of a study visit were collected the same day. Collected samples were aliquoted into a 7-ml Bijou container containing 98% ethanol to preserve DNA and prevent bacterial overgrowth at a 1 : 2 to 1 : 4 ratio, avoiding larger objects. Samples were then transported into the research facility and aliquoted into 1.8-ml cryotubes for further cold storage at -20C.

STH detection by quantitative polymerase chain reaction (qPCR)

Stool samples in 98% ethanol were thawed at ambient temperature and processed as previously described (Farrant *et al.*, 2020). Due to the potentially low prevalence and therefore, lack of sensitivity, no microscopy was done (Farrant *et al.*, 2020). qPCR was performed on a Rotorgene 3000 platform, using previously evaluated STH qPCR protocols (Farrant *et al.*, 2020). Briefly, *S. stercoralis* was analyzed using a singleplex qPCR (Verweij *et al.*, 2009), whereas *A. lumbricoides, Necator americanus, Ancylostoma duodenale,* and *T. trichiura* were analyzed together using a multiplex qPCR (Basuni *et al.*, 2011; Mejia *et al.*, 2013). For both qPCRs described, 3 µl of target DNA template was used in a total volume of 25 µl reaction. Table S2 in supplementary materials shows primer and probe sequences.

Statistical analysis

The co-primary outcomes were the prevalence of each ectoparasite and a composite end point of overall STH prevalence. For adjusted analyses, age was stratified into three groups, 3-6 years, 7-10 years, and 11-14 years, roughly corresponding to preschool, primary school, and secondary school children, respectively. To compare between arms, a logistic regression model with fixed effects for age and sex, and with random effects for the study cluster was fitted.

The statistical analysis was conducted using STATA 14. Figures containing statistical data were made using R software with R studio version 4.0.2 (June 22, 2020).

Role of the funding source

The Wellcome Trust did not have any involvement in data collection, analysis, or interpretation; trial design; patient recruitment; or any aspect pertinent to the study.

Results

Using the MASSIV trial census, 1342 children were randomly selected in July 2019, 1321 in November 2019, and 1378 in November 2021; 81.7 % (1096/1342) of these children were identified in July 2019, 79.6 % (1052/1321) in November 2019, and 66.9 % (922/1378) in November 2021 (Figs. 1 and 2). Stool samples were provided by 72.5% (957/1321) children in November 2019, and 58.9% (812/1378) in November 2020. Baseline demographic details by the survey are described in Tables 1 and 2.

Head lice and bedbugs

During the June 2019 survey, no bedbugs were found. Only eight (0.7%) individuals, four in each study arm, had nits with no living head lice detected. Therefore, no further search for these ectoparasites was carried out in the following surveys.

Scabies

In June 2019, the overall prevalence of scabies was 43.7% (478/1096); 38.8 % (205/529) in the intervention, and 48.2% (273/567) in the control arm (Table S3). After adjustment for clustering, scabies prevalence was significantly lower in intervention villages (odds ratio [OR]: 0.64; 95% confidence interval [CI]: 0.43-0.95, *P*-value = 0.027).

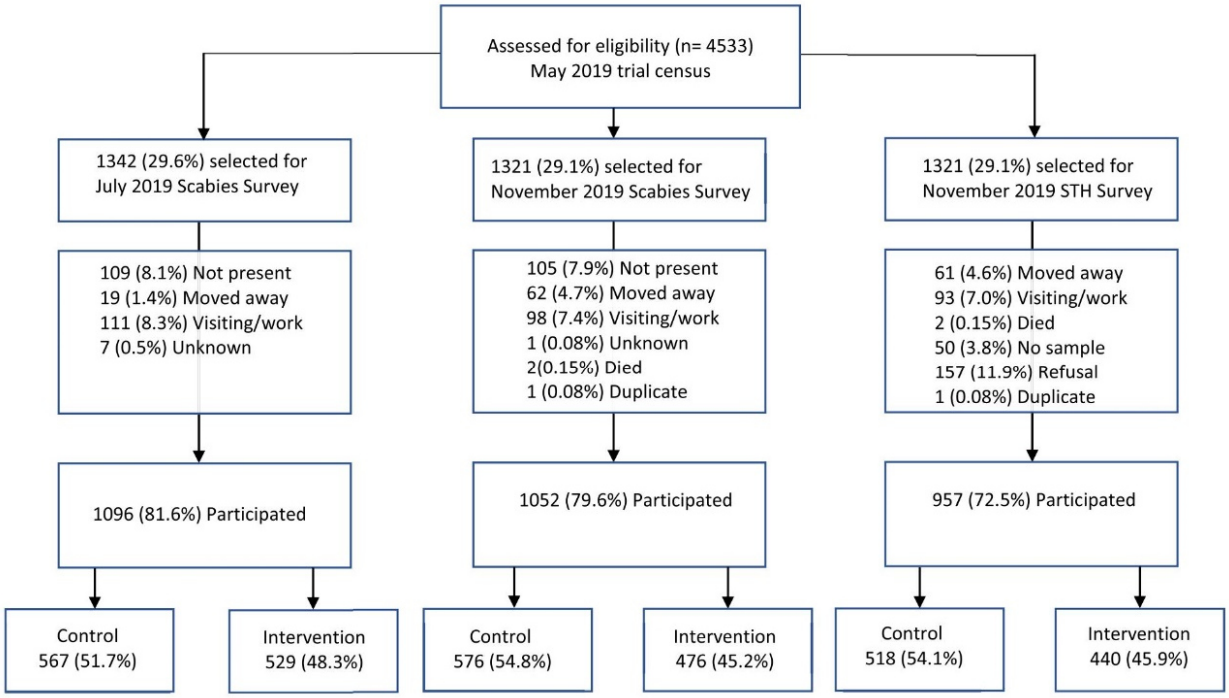

Fig. 1. Participants and their whereabouts in 2019 STH, soil-transmitted helminths.

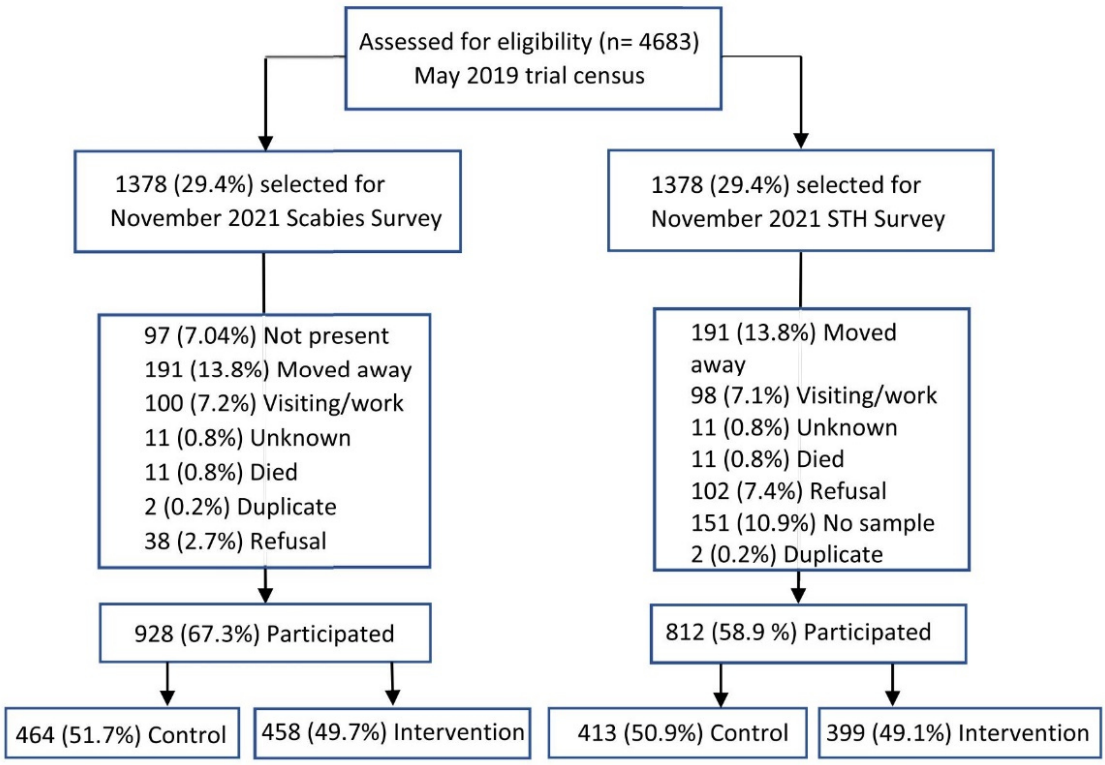

Fig. 2. Participants and their whereabouts in 2021
STH, soil-transmitted helminths.

Table 1
Baseline characteristics of the study participants for scabies surveys.

Demography (scabies)	July-19		November-19		November-21	
	Control	Intervention	Control	Intervention	Control	Intervention
Age group	N (%)		N (%)		N (%)	
3-6 years	244 (22.3)	217 (19.7)	215 (20.4)	172 (16.4)	184 (19.9)	175 (18.9)
7-10 years	210 (19.2)	183 (16.7)	215 (20.4)	172 (16.4)	176 (19.1)	180 (19.5)
11-14 years	113 (10.3)	129 (11.8)	146 (13.9)	132 (12.5)	104 (11.3)	103 (11.2)
Sex						
Female	280 (25.5)	272 (24.8)	278 (26.4)	257 (24.4)	250 (27.1)	206 (19.6)
Male	287 (26.2)	257 (23.5)	298 (28.3)	219 (20.8)	214 (20.3)	252 (23.9)
Arm	567 (51.7)	529 (48.3)	576 (54.8)	476 (45.2)	464 (50.3)	458 (49.7)
Total	1096		1052		922	

Table 2
Baseline characteristics of the study participants for soil-transmitted helminths surveys.

Demography (soil-transmitted helminths)	November-19		November-21	
	Control	Intervention	Control	Intervention
Age group	N (%)		N (%)	
3-6 years	195 (20.4)	161 (16.8)	162 (19.9)	154 (18.9)
7-10 years	207 (21.6)	165 (17.2)	161 (19.8)	162 (19.9)
11-14 years	116 (12.1)	114 (11.9)	90 (11.2)	83 (10.3)
Sex				
Female	254 (26.5)	226 (23.6)	228 (28.1)	166 (20.4)
Male	264 (25.6)	214 (22.3)	185 (22.8)	233 (28.7)
Arm	518 (54.1)	440 (45.9)	413 (50.8)	399 (49.1)
Total	958	812		

In the second survey in November 2019, the overall prevalence was 39.9% (419/1052); 41.2 % (237/576) in the intervention and 38.2 % (182/476) in the control arm (OR: 0.88; 95% CI: 0.66-1 8, *P*-value = 0.406).

In November 2021, 2 years after MDA, the overall prevalence of scabies was 46.5% (425/922); 53.3% (245/458) in the intervention, and 38.7% (180/464) in the control arm (OR: 1.94; 95% CI: 1.1-3.43, *P*-value = 0.022) (Table 3).

Table 3
OR and *P*-values for all three scabies surveys; adjusted for sex and age.

Scabies					
Survey	Prevalence	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
July-19					
Control	273/567 (48.2%)	1		1	
Intervention	205/529 (38.8%)	0.64 (0.43-0.95)	0.027	0.65 (0.44-0.97)	0.037
November-19					
Control	237/576 (41.2%)	1		1	
Intervention	182/476 (38.2%)	0.88 (0.66-1.8)	0.406	0.89 (0.67-1.2)	0.471
November-21					
Control	180/464 (38.8%)	1		1	
Intervention	245/458 (53.2%)	1.94 (1.1-3.43)	0.022	1.89 (1.07-3.37)	0.029

OR, odds ratio.

Table 4
OR and *P*-values for both STH surveys and *S. stercoralis* surveys; adjusted for sex and age.

Soil-transmitted helminths					
Survey	Prevalence	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
November-19					
Control	121 (23.4%)	1		1	
Intervention	72 (16.4%)	0.63 (0.33-1.19)	0.158	0.63 (0.34-1.21)	0.169
November-21					
Control	57 (13.8%)	1		1	
Intervention	75 (18.7%)	1.47 (0.86-2.52)	0.157	1.51 (0.86-2.63)	0.149
Strongyloides					
Survey	Prevalence	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
November-19					
Control	87 (16.8%)	1		1	
Intervention	40 (9.1%)	0.40 (0.16-0.95)	0.037	0.4 (0.17-0.96)	0.039
November-21					
Control	38 (9.2%)	1		1	
Intervention	45 (11.3%)	1.26 (0.72-2.17)	0.41	1.31 (0.74-2.28)	0.35

OR, odds ratio.

STH 2019 and 2021

In November 2019, the overall prevalence of STH was 20.2% (193/958). *S. stercoralis* was the most prevalent STH (13.3%; 127/958), followed by *N. americanus* (4.8%; 46/958), and *A. lumbricoides* (4.1%; 39/958). No *T. trichiura* or *A. duodenale* were detected (Table S4, supplementary material).

No evidence of an effect of the intervention on the overall STH prevalence was detected (adjusted OR: 0.63; 95% CI 0.34-1.21, *P*-value = 0.169). There was evidence of an effect of the intervention on *S. stercoralis*, with 16.9% in the control and 9.1% in the intervention arm, with an adjusted OR: 0.4 (95% CI 0.17-0.96, *P*-value = 0.039) (Table 4).

In November 2021, the overall STH prevalence was 16.3% (132/812). In 2019, *S. stercoralis* was the most prevalent STH with 10.2% (83/812), followed by *N. americanus* 4.8% (40/812) and *A. lumbricoides* 1.2% (10/812). The prevalence for *A. duodenale* and *T. trichiura* were 0.24 and 0.12%, respectively (Table S4).

No evidence of an effect of the intervention on overall STH prevalence was detected with an unadjusted OR of 1.47 (95% CI 0.86-2.52, *P*-value = 0.15) and an adjusted OR of 0.63 (95% CI 0.34-1.21; *P*-value = 0.169). In the case of *S. stercoralis*, no evidence for a long-term effect was seen, with an OR of 1.26 (95% CI 0.72-2.17, *P*-value = 0.41) and an adjusted OR of 1.31 (95% CI 0.74-2.28; *P*-value =0.35) (Table 4).

Discussion

In this study, we did not show any clear evidence of the impact of ivermectin MDA conducted in the context for malaria control on either scabies or STH prevalence, except for *S. stercoralis*. The prevalence of scabies was relatively high in all three surveys, whereas the STH prevalence varied around 15-20%, most of the infections caused by *S. stercoralis*. The prevalence of other STH was low and comparable to that of a survey carried out in 2015 among Gambian school children; however, this survey did not include *S. stercoralis* (Camara *et al.*, 2021).

The study results initially suggested a change in scabies prevalence in July 2019. However, this effect was not seen again after the second MDA in 2019, and the effect appeared to be reversed in the following survey in 2021. This is probably explained by the lack of substantial impact of the MDA on scabies combined with local fluctuations in prevalence (see Fig. S1) because the survey, a few months after the MDA, showed no change in prevalence. Our results differ from most of the previous studies. A lack of impact on scabies has been shown in a study in an area with high population movement (Kearns *et al.*, 2015; Lake *et al.*, 2022). One of the strongest contributors to our results is probably the presence of a substantial untreated reservoir of study participants, including individuals with >15 kg body weight and pregnant and breastfeeding women. The MDA conducted for MASSIV differed importantly from most of the previous studies, where MDA was conducted for scabies. In studies conducted specifically for scabies, individuals who remained untreated due to ineligibility for ivermectin would have been offered treatment with permethrin. In addition, there is a potential of children who were treated being reinfected in schools or while visiting other villages outside the study area or due to contamination between clusters. Using larger clusters might have avoided this risk but is not as statistically robust, and cluster randomized trials have been successfully used for scabies control programs elsewhere. Furthermore, it may be that the coverage reported by the MASSIV trial is enough to impact malaria, but not enough to impact scabies.

The lack of detection for head lice is probably caused by the local habit of shaving the boys' heads, as well as braiding of girl's hair, thus denying lice the necessary living space. In the case of bedbugs, it is possible that, based on temperature data, the climate is too hot in the URR to establish a perennial presence (How and Lee, 2010).

The intervention did not have any effect on the overall STH prevalence. In the 2019 survey, we did detect an impact on *S. stercoralis*, which represented more than half of all STH infections. Of note, only three of 16 intervention villages had two-thirds of all individuals infected with *Strongyloides* (see Fig. S2). Whether this clustered higher burden was due to differences in coverage in these villages is unknown. Despite this initial impact, the effect of ivermectin was not detectable 2 years after MDA. This absence of effect may be due to reinfection from individuals who were untreated within MASSIV or from environmental sources.

This study faced several limitations. The STH survey in 2021 had less than the expected number of participants. Secondly, the prevalence of *A. lumbricoides, N. americanus, A. duodenale,* and *T. trichiura* were much lower than anticipated, resulting in reduced statistical power to detect differences between arms. Unfortunately, this negated the opportunity to shine more light on the potential effect of this repeated ivermectin MDA on *T. trichiura* and hookworm species. Also, not every selected individual provided a stool sample, which may have introduced a bias into the data. In addition, we observed a high level of population movement, either by people moving away to different areas for better job opportunities or seasonal workers migration, which may have impacted the observed efficacy of the intervention (see Figs. 1 and 2).

Finally, although study clusters are all from within the same region of The Gambia and we are not aware of any other factors outside the trial itself that could have influenced scabies prevalence in the control clusters but not the intervention clusters, we cannot completely exclude the possibility that an unidentified external factor influenced the prevalence of scabies in the control villages. Because routine socioeconomic and health interventions are not typically delivered to some villages, we believe that external secular changes are unlikely to account for the lack of effect seen.

Overall, the results of the study were mixed with no effect shown for scabies and only *S. stercoralis* being significantly impacted. Further work is required to investigate the effect of MDA with ivermectin when implemented for malaria control on ectoparasites and STH because this may depend on both the intensity of the MDA coverage and local parasite prevalence.

Funding

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Ethical approval

This study was nested within the MASSIV trial, and all participants were consented for the main study. The communities and local authorities were informed about these surveys and only consented participants were included. Parents consented for their children and additional written assent was obtained for children aged ≥12 years. Ethical clearance was given by the ethics board of the London School of Hygiene and Tropical Medicine and the Gambian government/Medical Research Council Unit Gambia Joint Ethics Committee (Ethics Ref. Nr. 17123).

Author contributions

Conceptualization: CK, JB, MM, JH; review & editing: JB, HV, AL, UD, MM; methodology: HV, JH, JA; original draft: CK; analysis and interpretation of data: CK, JB, HV, JH, JA, UD, MM; read and approved final version: CK, MD, JB, HV, JH, JA, UD, MM.

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Data sharing

After publication, trial data will be made available on reasonable request to the corresponding author. A proposal with a detailed description of study objectives and a statistical analysis plan is needed for assessment of requests. Additional materials might also be required during the process. Deidentified participant data will be provided after approval by the sponsor and trial management group.

Declaration of competing interest

The authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.10.043.

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6.1 Research Paper 2 Supplementary

Tables:

	July 2019	November 2019	November 2021
Scabies	480	420	429
B1	99	128	77
B2	-	-	-
B3	340	217	307
C1	39	74	41
C2	2	1	4
Total	478	419	425

Table S1: Clinical cases defined by the international consensus criteria for scabies

Species	Target	Primer	Sequence 5'-3'	Final Concentration (nM)	Reference
Ascaris lumbricoides	ITS1	Forward	GTAATAGCAGTCGGCGGTTTCTT	60	Basuni et al., 2011
		Reverse	GCCCAACATGCCACCTATTC	60	
		Probe	[FAM]TTGGCGGACAATTGCATGCGAT[BHQ1]	100	
Ancylostoma duodenale	ITS2	Forward	GAATGACAGCAAACTCGTTGTTG	200	Basuni et al., 2011
		Reverse	ATACTAGCCACTGCCGAAACGT	200	
		Probe	[Cy5]ATC[+G]TTTA[+C][+C][+G]A[+C]TTTAG[BHQ2]	200	
Necator americanus	ITS2	Forward	CTGTTTGTCGAACGGTACTTGC	200	Basuni et al., 2011
		Reverse	ATAACAGCGTGCACATGTTGC	200	
		Probe	[JOE]CT[+G]TA[+C]TA[+C][+G][+C][+A]TT[+G]TATAC[BHQ1]	100	
Trichuris trichiura	ITS1	Forward	TCCGAACGGCGGATCA	60	Mejia et al., 2013
		Reverse	CTCGAGTGTCACGTCGTCCTT	60	
		Probe	[ROX]TTGGCTCGTAGGTCGTT[BHQ2]	100	
Strongyloides stercoralis	18SRNA	Forward	GAATTCCAAGTAAACGTAAGTCATTAGC	100	Verweij et al., 2009
		Reverse	TGCCTCTGGATATTGCTCAGTTC	100	
		Probe	[FAM]ACACACCGGCCGTCGCTGC[BHQ1]	100	

Table S2: Primer and probe sequences used in qPCR detection of STH in stool DNA. Bases in brackets and indicated by a + are locked nucleic acids.

Scabies	Prevalence		
Survey	**July 2019**	**November 2019**	**November 2021**
Age Group	N = 1096 (%)	N = 1052 (%)	N = 922 (%)
3 - 6	226/461 (49%)	175/387 (45.2%)	179/359 (49.8%)
7 – 10	160/393 (40.7%)	152/387 (39%)	168/356 (47.1%)
11 – 14	92/242 (38%)	92/278 (33.1%)	78/207 (37.6%)
Sex			
Female	217/552 (39.3%)	205/535 (38.3%)	175/456 (38.3)
Male	261/544 (47.9%)	214/517 (41.4%)	250/466 (53.6)
Arm			
Control	273/567 (48.2%)	237/576 (41.2%)	180/464 (38.8%)
Intervention	205/529 (38.8%)	182/476 (38.2%)	245/458 (53.2%)
Total	43.6%	39.8%	46.1%

Table S3: Prevalence results by demography for all three scabies surveys

Nov-19	Prevalence STH N (%)				
Age Group	**Participants**	**Strongyloides**	**Ascaris**	**Necator**	**STH total**
3 – 6	356 (37.2%)	50 (14.04%)	12 (3.4%)	17 (4.8%)	73 (20.5%)
7 – 10	372 (38.8%)	57 (15.3%)	20 (5.4%)	16 (4.3%)	84 (22.6%)
11 – 14	230 (24%)	20 (8.7%)	7 (3%)	13 (5.7%)	36 (15.7%)
Sex					
Female	480 (50.1%)	63 (13.1%)	18 (3.8%)	20 (4.2%)	93 (19%)
Male	478 (49.9%)	64 (13.3%)	21 (4.4%)	26 (5.4%)	100 (20.9%)
Arm					
Control	518 (54.1%)	87 (16.9%)	22 (4.4%)	26 (5%)	121 (23.4%)
Intervention	440 (45.9%)	40 (9.1%)	17 (4%)	20 (4.6%)	72 (16.4%)
Total	958	127 (13.3%)	39 (4.1%)	46 (4.8%)	193 (20.2%)
Nov-21	**Prevalence STH N (%)**				
Age Group	**Participants**	**Strongyloides**	**Ascaris**	**Necator**	**STH total**
3 – 6	316 (38.9%)	32 (10.1%)	4 (1.3%)	15 (4.7%)	48 (15.1%)
7 – 10	323 (39.8%)	29 (8.9%)	3 (0.9%)	16 (4.9%)	51 (15.7%)
11 – 14	173 (21.3%)	22 (12.7%)	3 (1.7%)	9 (5.2%)	31 (17.9%)
Sex					
Female	394 (48.52%)	43 (10.9%)	5 (1.2%)	15 (3.8%)	64 (16.2%)
Male	418 (51.48%)	40 (9.6%)	5 (1.1%)	25 (5.9%)	68 (16.3%)
Arm					
Control	413 (50.86%)	38 (9.2%)	4 (0.9%)	15 (3.6%)	57 (13.8%)
Intervention	399 (49.14%)	45 (11.3%)	6 (1.5%)	25 (6.6%)	75 (18.7%)
Total	812	83 (10.2%)	10 (1.2%)	40 (4.8%)	132 (16.2%)

Table S4: Prevalence results by demography for both STH surveys, *T. trichiura* und *A. duodenale* are omitted from the 2021 survey with only 0.12% and 0.24% respectively

Figures:

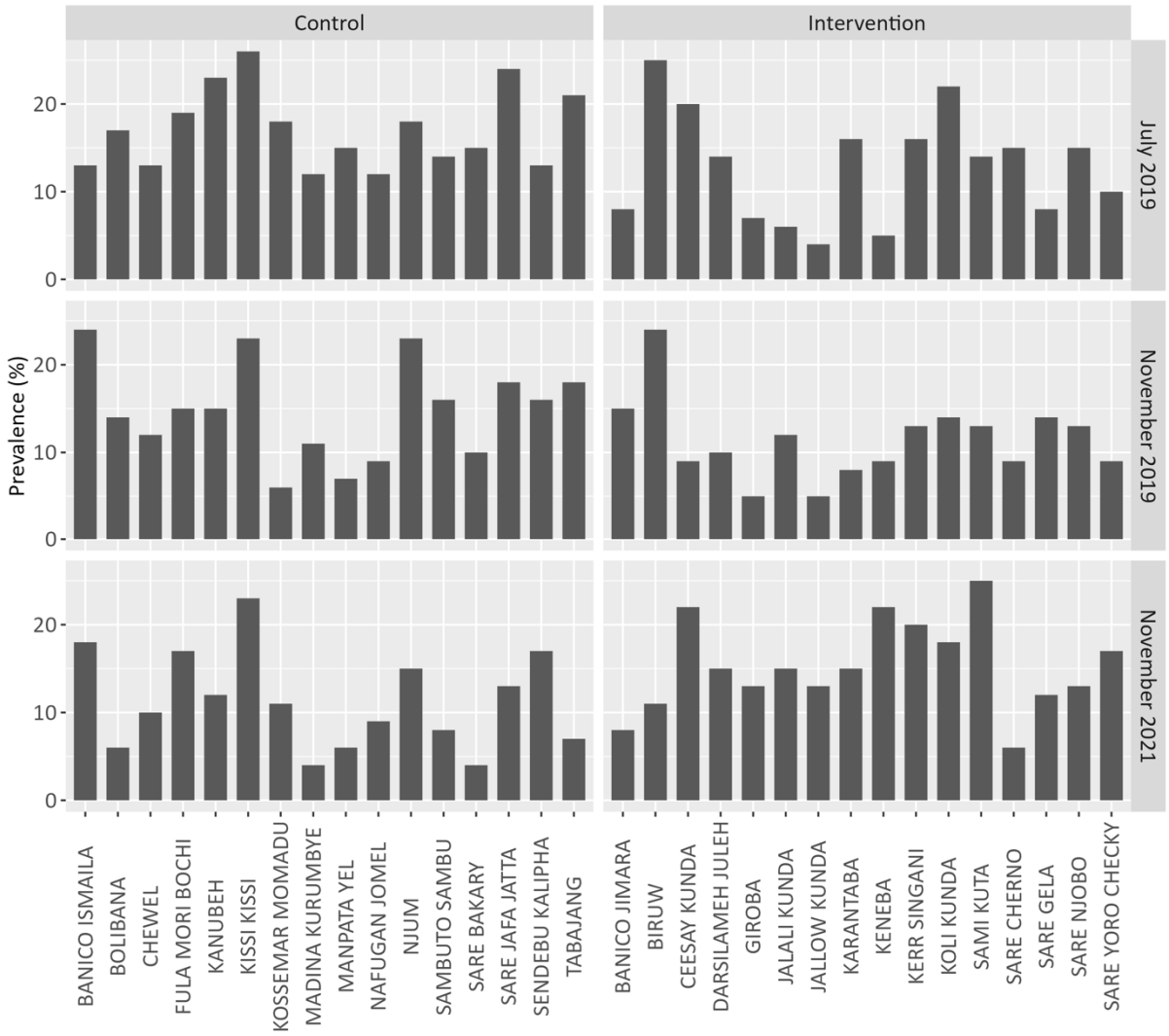

Figure S1: Prevalence of Scabies per Village and Arm, 2019 and 2021 showing the local variability

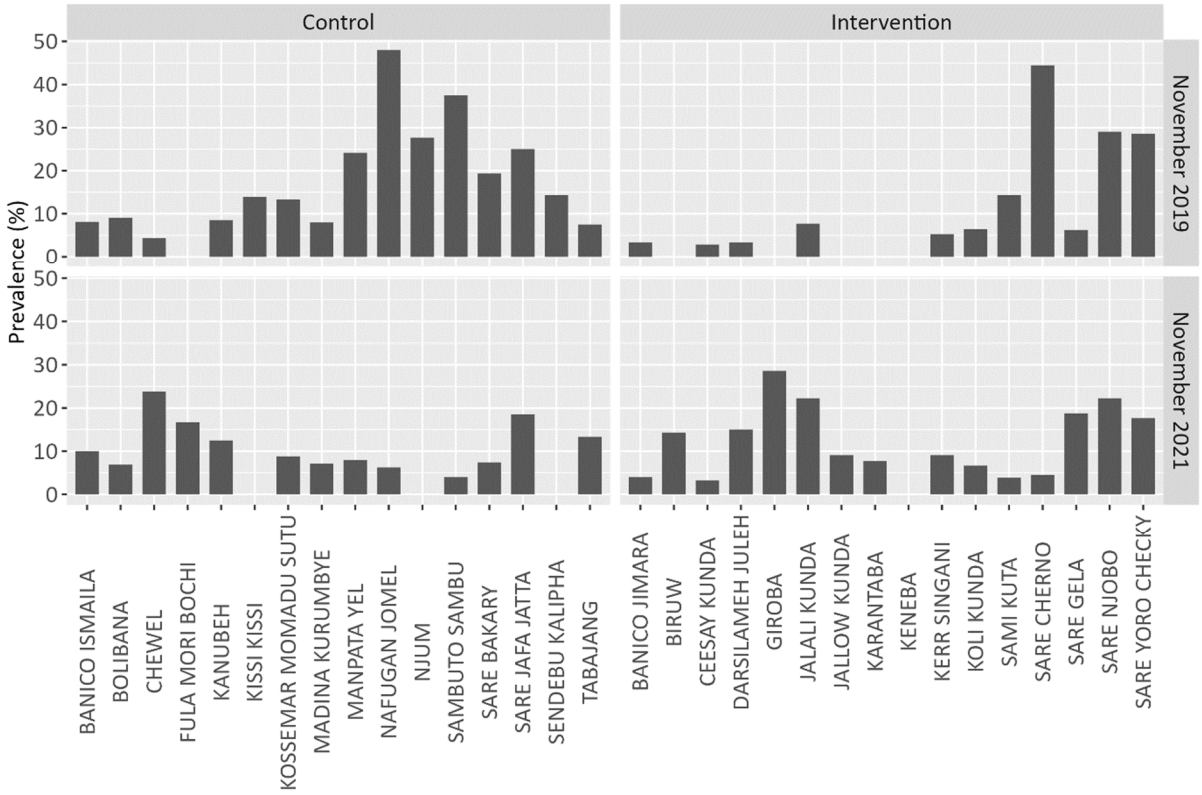

Figure S2: Prevalence of *S. stercoralis* per Village and Arm, 2019 and 2021 showing the local variability

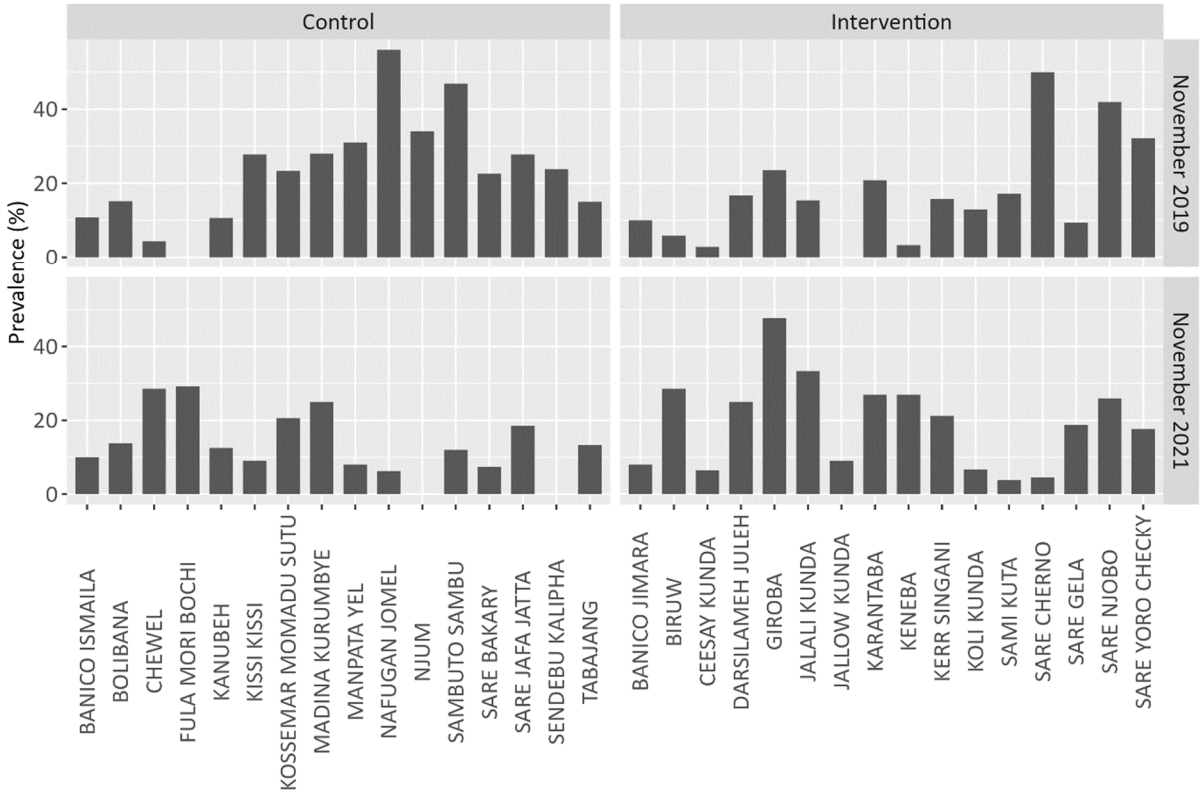

Figure S3: Prevalence of STH per Village and Arm, 2019 and 2021 showing the local variability

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	386243	**Title**	Dr.
First Name(s)	Christian Hermann		
Surname/Family Name	Kositz		
Thesis Title	Investigating the Effects of Ivermectin plus Dihydroartemisinin-Piperaquine MDA on the Transmission of Malaria by Measuring Serological Markers of Exposure and its Effects on the Prevalence of Ectoparasites and Soil-Transmitted-Helminths		
Primary Supervisor	Michael Marks		

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?	International Journal of Infectious Diseases		
When was the work published?	2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	

Please list the paper's authors in the intended authorship order:	Christian Kositz, MD Mariama Drammeh, MD Hristina Vasileva, MSc Joanna Houghton, PhD James Ashall, MSc Prof Umberto D'Alessandro, MD Michael Marks, MD John Bradley, PhD
Stage of publication	**In press**

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)	Drafted the manuscript, conducted the fieldwork, specimen collection, laboratory work and statistical analysis; prepared the final version; responded to peer review comments

SECTION E

Student Signature	[redacted]
Date	11.11.2022

Supervisor Signature	[redacted]
Date	11.11.2022

Chapter 7 Research Paper 3

Risk Factors for Non-Participation in Ivermectin and Dihydroartemisinin-Piperaquine Mass Drug Administration for Malaria Control in the MASSIV Trial

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Key Words:
MDA, non-compliance, ivermectin

Abstract:

Background:

Mass Drug Administration (MDA) has become a mainstay for the control of several diseases over the last two decades. Successful implementation of MDA programs requires community participation and can be threatened by systematic non-participation. Such concerns are particularly pertinent for MDA programmes against malaria, as they require multi-day treatment over several consecutive months. Factors associated with non-participation to the MDA campaign with ivermectin and dihydroartemisinin-piperaquine (DHP) implemented within the MASSIV cluster randomized trial were determined.

Methods:

Coverage data was extracted from the MASSIV trial study database. We classified a complete month of MDA as receiving all three daily doses of treatment. For both ivermectin and DHP, we used ordinal logistic regression to identify individual and household level variables associated with non-participation.

Findings:

For ivermectin, 51.5% of eligible participants received all three months of treatment while 30.7% received either one or two complete months. For DHP, 56.7% of eligible participants received all three months of treatment and 30.5% received either one or two complete months. Children aged 5-15 years and adults aged more than 50 years were more likely to receive at least one complete month of MDA than working age adults, both for ivermectin (aOR 4.3 – 95% CI 3.51-5.28 and aOR 2.26 95% CI 1.75-2.95) and DHP (aOR 2.47 – 95%CI 2.02 – 3.02 and aOR 1.33 – 95%CI 1.01 – 1.35).

Households whose head received a complete month of MDA were more likely to have had a complete month of MDA, both for ivermectin (aOR 1.71 – 95%CI 1.35 – 2.14) and for DHP (aOR 1.64 - 95%CI 1.33 – 2.04).

Interpretation:

Personal and household-level variables were associated with participation to the MDA programme for malaria control. Specific strategies to increase participation amongst some groups may be important to ensure maximum impact of MDA strategies in achieving malaria elimination.

Background

Mass drug administration (MDA) has become established as a mainstay in the control of various diseases over the past 15-20 years (1,2). Successful MDA programmes require cooperation with local communities to ensure high coverage of the treated population. For many MDA-based interventions, the degree to which individuals participate in MDA programmes over a number of years is an important consideration. The impact of MDA may be markedly reduced if there is systematic non-compliance of participants or households, or if specific segments of the population are frequently missed (3–7).

Previous studies have suggested that non-participation in MDA programmes does not occur at random. Instead, systematic non-participation is often observed and may be linked to both individual and household-level factors such as the participation of the household head (8,9). The impact of systematic non-participation likely varies depending on the extent to which MDA is providing direct treatment of individuals or indirect effects on transmission of a pathogen, or a mix of both features. When the primary effect is on transmission, high levels of coverage can still result in a significant impact on disease, even if a section of the population is systematically excluded.

Ivermectin is among the most commonly used drugs for MDA programmes and is currently in programmatic use for the control of several neglected tropical diseases (NTDs) (10). More recently, the drug has been identified to have the potential to become a malaria vector control tool, due to its mosquitocidal effects on *Anopheles* spp when taken with a blood meal (11). In contrast to NTD programmes that administer a single annual dose of ivermectin, to achieve significant mosquitocidal effects, the ivermectin needs to be taken for three consecutive days in a row. In this context, ivermectin generates a long-lasting effect on mosquito populations up to 28 days. The safety of such dosing regimens has been shown in the IVERMAL trial in Kenya (12). However, repeated ivermectin dosing, requiring both multiple consecutive days and multiple months of treatment may cause challenges in maintaining high population coverage which could ultimately impact the efficacy of this intervention.

The MASSIV trial conducted in the Upper River Region in the eastern part of The Gambia is the first large scale trial to investigate the effects of MDA with ivermectin and dihydroartemisinin-piperaquine, an antimalarial treatment, in an integrated MDA, with both drugs being given on three consecutive days for three consecutive months. Given the

pharmacokinetic characteristics of ivermectin, the success of these MDA programmes is likely to be highly dependent on the coverage of the intervention. The trial therefore provides the ideal opportunity to investigate the frequency and determinants of systematic non-compliance participation in more complex MDA programmes.
In this study, we use data from the MASSIV trial to assess both individual and household-level factors that influence uptake and coverage of the trial intervention.

Methods:

Study Design:

The MASSIV (NCT03576313) trial design, baseline findings, and outcomes have been described previously (13,14). In summary, 32 clusters (villages) were randomized (1:1) to either the intervention or the control arm. The intervention clusters received MDA orally with ivermectin (IVM) at a dose of 300 – 400 mcg/kg bodyweight plus dihydroartemisinin-piperaquine (DHP) at 320/40mg and 160/20mg depending on body weight on three consecutive days at monthly intervals for three months during the start of the malaria season (July – October), which coincides with the geographical wet season. The MDA schedule is based on modelling data that suggests ivermectin would exert its mosquitocidal effect for up to 28 days at this dose when taken on three consecutive days (12).
Exclusion criteria for (IVM) were chronic illnesses, bodyweight under 15 kg measured at distribution, pregnancy, excluded with point of care pregnancy tests, hypersensitivity to the drug and for DHP < 6 months of age, 1st trimester pregnancy, hypersensitivity to the drug and QT prolonging medication. The intervention was carried out in 2018 and 2019. The primary outcome was malaria prevalence after MDA and the results of this have been previously reported (14). A tertiary outcome was STH and prevalence of ectoparasites, which were thought to be specifically affected by ivermectin (rather than DHP) and has been reported elsewhere (15).

Study Population:

The trial was implemented in Upper River Region (URR), eastern Gambia. The population includes several ethnic groups, specifically, Fula, Mandinka, Sarahule, and Wollof. Malaria transmission occurs during the rainy season (June-November) and the following 2 months. The database for this study included every registered participant in the 16 villages in URR included in the intervention arm. Study participants were visited

each day of treatment, which was directly observed (three consecutive days on each of three consecutive months). At baseline data was collected on demographics including age and gender, the household size and the head of the household. On each study day eligible participants were offered ivermectin and DHP (as outlined above). For each study day the team recorded if either, both, one or no drugs were taken by each participant.

Statistical Analysis:

We analysed patterns of compliance in the intervention arm of the 2nd year of the MASSIV trial. The main outcome was either a completed monthly course of MDA defined as having received the three daily doses of treatment, ranging between one to three completed monthly treatments, or incomplete MDA if no MDA was received or no month was completed. In addition, as the dosing required for an impact on NTDs differs from that for malaria, we looked at receiving no MDA or at least one dose of MDA.

The proportion of individuals who received 0-3 completed monthly courses of ivermectin and DHP was calculated separately for each drug in-line with their specific inclusion and exclusion criteria. Frequencies of non-treatment within and across rounds were displayed with histograms generated with the UpSetR package in R software with R studio version 4.0.2 (2020-06-22) (16).

For the primary outcome of a complete monthly course of MDA, an ordered logistic regression model that included fixed effects for age group, sex, household size, ethnicity, and treatment status of the household head, and random effects for study clusters (villages) was used. Age was stratified into five groups, <5, 5 – 15, >15 – 25, >25 – 50 and over >50 years of age with the 25-50 years age group used as reference. The median household size of 25 members per household was used to stratify the study population into five groups, < 6, 6 – 12, >12 – 24, 25 – 50 and > 50 members per household. Data on ethnicity were self-reported and included members of Fula, Mandinka, Sarahule, Wollof, and not specified. Participation of household heads was categorised in complete, partial or no MDA received. For the analysis of receiving of at least one dose of ivermectin a logistic regression model was fitted using a similar approach.

In a post hoc analysis, we assessed interactions between the treatment status for household heads. The interaction variable was age group (<5, 5 – 15 and >15 years of age) and sex, and the outcome variable was a complete monthly course of MDA.

Statistical analysis was conducted using STATA 17.

Ethics Statement:

Ethical clearance for the MASSIV study was provided by the ethics board of the London School of Hygiene and Tropical Medicine, and the Gambian government/MRCG Joint Ethics Committee (Ethics Ref. Nr. 15823).

Results:

The MASSIV database contained 5036 participants in the intervention arm of which 3311 had complete data. Within this population, 2730 were eligible for ivermectin MDA and 3291 for DHP-MDA (Figure 1). Most participants (2065 ,62.4%) were Fula, followed by Mandinka (638, 19.3%), Sarahule (134, 4.1%), Wollof (3, 0.1%); 471 (14.2%) did not specify their ethnicity. There were more females than males (Table 1). Data on the MDA coverage of individuals with missing demographic data are reported in Supplementary Table 1.

Ivermectin MDA

Excluding non-eligible participants, 1407/2730 (51.5%) participants received all three-monthly courses of ivermectin MDA, 327 (11.9%) received at least one complete monthly course, 512 (18.8%) received at least two complete monthly courses and 484 (12.8%) did not receive any MDA or completed a single monthly round of MDA (Table 2, Figure 2, Supplementary Figure 1 & 2).

Eligible children under 5 (aOR 1.43 - 95%CI 0.97 – 2.1), children aged 5-15 (aOR 4.3 - 95%CI 3.51 – 5.28) and older adults (aOR 2.26 - 95%CI 1.75 – 2.95) were all more likely to receive MDA than working age adults. Males were also more likely than women to receive at least one complete month of MDA (aOR 1.54 – 95%CI 1.31 – 1.81). Neither household size nor ethnicity were associated with receiving a complete month of ivermectin MDA, except individuals who did not specify their ethnicity, who had an aOR 0.05 (95%CI 0.04 – 0.06) for receiving one complete month of MDA (Table 3). Individuals living in a house where the household head received a complete course of MDA were more likely to receive a complete month of MDA (aOR 1.71 - 95%CI 1.35 – 2.14) than those living in households whose head did not receive a complete course of MDA (Table 3).

There was no evidence of an interaction between an individual's age and whether the household heads received MDA with an interaction p-value of 0.171 (Supplementary Table 2). In contrast, there was strong evidence of an interaction with the gender of adult participants, with adult males much more likely to receive MDA compared to women if the household head had received MDA with an interaction p-value of 0.0001 (Supplementary Table 3).

For the secondary analysis of receiving at least a single dose of ivermectin, eligible children aged under 5 (aOR 4.61 95%CI 1.52 – 13.97), children aged 5-15 (8.81 95%CI 5.51 – 14.07)

and older adults (aOR 3.43 - 95%CI 2.13 – 5.48) were more likely to receive treatment. Men were also more likely than women to receive at least one dose of IVM (aOR 2.71 95%CI 1.98 – 3.71). Neither household size nor ethnicity appeared to be associated with receiving at least one dose of IVM (Supplementary Table 4).

DHP MDA

Excluding non-eligible participants, 1865/3290 (56.7%) participants received all three months of DHP MDA, 623/3290 (18.9%) received at least two complete months of MDA. 380/3290 (11.6%) received at least one complete month of MDA and 422 (12.8%) did not receive any complete months of DHP MDA (Table 4, Figure 3, and Supplementary Figure 1 & 2).

Similar to our findings for IVM MDA, eligible children under 5 (aOR 2.01 – 95%CI 1.59 – 2.54), children aged 5-15 (2.47 – 95%CI 2.02 – 3.02) and older adults (aOR 1.33 – 95%CI 1.01 – 1.35) were all more likely to receive at least one complete month of DHP MDA than working age adults. Males were also more likely than women to receive at least one complete month of MDA (aOR 1.17 - 95%CI: 1.01. – 1.35). Similar to IVM MDA, neither household size nor ethnicity were associated with receiving a complete month of DHP MDA. Individuals living in a house where the household head received a complete month of MDA were more likely to receive a complete month of MDA themselves (aOR 1.64 - 95%CI 1.33 – 2.04) than those living in households whose head did not receive any complete months of treatment (Table 4).

There was evidence of a week interaction between age groups and the impact of whether a household head had received MDA (interaction p-value 0.039). Similar to our findings for IVM MDA, strong evidence of an interaction between the gender of adults and the MDA status of the household head, with males much more likely to receive MDA if the household head had also been treated (interaction p-value < 0.0005) (Supplementary Table 2 & 3).

Discussion:

Within the MASSIV trials, some individuals and households did not participate across multiple monthly rounds of an integrated ivermectin and DHP MDA programme delivered for malaria control. Systemic non-compliance has been previously recognized as a threat to the successful implementation of MDA interventions (4,9,17). This may be particularly true for ivermectin when used for malaria vector control in addition with or without an additional drug, as the MDA schedule requires several doses of MDA per month over three months in a relatively short timeframe and a relatively high coverage to result in a measurable impact (18,19). The MASSIV trial provided a unique opportunity to explore the factors associated to non-participation in more detail and use the data to inform future ivermectin-DHP MDA rollouts in other settings. Similar factors were associated with MDA participation for ivermectin and DHP, suggesting that individuals either participated in all or no parts of the intervention.

In keeping with previous studies, we found that there was a strong relationship between participation of the household head and the other members of the family. Similar associations with household head participation haves been reported from the Gambia in the context of MDA with azithromycin for trachoma elimination. These data highlight the key role family structures have on the participation in some community-based health interventions such as MDA. We also noted that the lowest rate of participation was seen in adolescents, young adults and working aged individuals who are often absent during public health interventions. Previous studies on MDA for soil-transmitted helminths and onchocerciasis (3) have also found high rates of absenteeism in these groups (20). Collectively, data across these studies highlight the fact that specific measures such as adjusting the timing of MDA delivery may be required to increase uptake of MDA amongst these population segments.

In line with other studies, we also found a strong association with gender and receiving MDA, with men being more likely than women to receive both ivermectin and DHP. It might be anticipated that exclusion criteria related to pregnancy or breastfeeding would affect the uptake of ivermectin a similar phenomenon would not be expected for DHP. Previous studies have highlighted that not receiving ivermectin during previous pregnancies can encourage women not to participate to the MDA even if not pregnant (20,21). Men have also been reported to be more likely to receive MDA for soil transmitted helminths in Kenya

but no difference between sexes was seen amongst children in a previous study examining participation in trachoma MDA in the Gambia or in adults for soil-transmitted helminths in Uganda (3,6,22). Continuous monitoring is required to facilitate equal distribution of treatment during MDA and ensure women are not more likely to be excluded from such interventions.

The major limitation of our study was that we had to exclude of a fifth of the participations from the analysis due to a lack of complete demographic data. However, accounting for the fact that a proportion of excluded participants likely had an age < 5 the overall distribution of MDA participation was similar to the individuals whose data was available (see Table S1). In addition, there was no information on socioeconomic factors such as household income, education levels and road access, some of which have previously been reported to be associated with the likelihood of participating in MDAs (7,23). Finally, we could only use data from the second year of the MASSIV trial due to difficulties with the It would be important to assess if similar evidence of systematic non-participation was observed over a multi-year MDA cycle.

Our study highlights several key areas that must be addressed to optimise the use of ivermectin as a potential tool for malaria vector control. Engagement with household heads must be a central pillar of such strategies as their participation influences the entire household. In particular, enhanced strategies to improve coverage amongst adolescents and working age adults should be considered such as amending or adapting the MDA timing, considering evening drug distribution and potentially improved engagement with the community in the implementation of the intervention. Our data shows that MDA implementation must be adapted to the participating community, its cultural background, infrastructural realities on the ground and agricultural seasons in particular. Addressing these findings will be key to achieve the maximum benefit of ivermectin MDA for malaria control.

Contributions:

Conceptualisation: CK, JB, MM; Review & Editing: JB, UD, MM; Methodology: JB, CK; Original Draft: CK; Analysis and interpretation of data: CK, JB, MM; Read and approved final version: CK, JB, MM, HV, UD;

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Conflicts of interest:

None declared.

Declaration of interest

We declare no competing interests.

Data Sharing

After publication, trial data will be made available on request to the corresponding author. A proposal with a detailed description of the study objectives and a statistical analysis plan is needed for the evaluation of the requests. Additional materials might also be required during the process.

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Tables:

Table 1: Baseline demographics of the intervention group of the MASSIV database

Number of Participants Selected for Analysis from the Intervention Arm (%)	
Total	3311
Age	
< 5 years	585 (17.7)
5 - 15 years	1065 (32.2)
> 15 - 25 years	543 (16.4)
> 25 - 50	780 (23.6)
> 50 years	338 (10.2)
Sex	
Female	1780 (53.8)
Male	1531 (46.2)
Ethnicity	
Fula	2065 (62.4)
Mandinka	638 (19.3)
Sarahule	134 (4.1)
Wollof	3 (0.1)
Not specified	471 (14.2)
Household Size	
< 6	51 (1.5)
6 - 11	391 (11.8)
12 - 24	1373 (41.5)
25 - 50	1141 (34.5)
> 50	355 (10.7)
MDA Eligible	
DHP	3291 (99.4)
Ivermectin	2730 (82.5)

Table 2: Ivermectin treatment by demographic factors.

	Total Population (N = 3311)				
	Eligible Population/Total Population	Eligible Population for Ivermectin (N = 2370)			
		No MDA complete (%)	1 month of MDA complete (%)	2 months of MDA complete (%)	3 months of MDA complete (%)
Total	2730/3311 (82.5)	484 (17.73)	327 (11.98)	512 (18.8)	1407 (51.5)
Houshold Head MDA					
Complete	1445/1754 (82.4)	222 (15.3)	140 (9.7)	242 (16.8)	841 (58.2)
Incomplete	816/1006 (81.1)	149 (18.2)	131 (16.1)	186 (22.8)	350 (42.9)
None	469/551 (85.1)	113 (24.1)	56 (11.9)	84 (17.9)	216 (46.1)
Agegroup					
< 5 years	116/585 (19.8)	10 (8.6)	16 (13.8)	31 (26.7)	59 (50.9)
5 - 15 years	1010/1065 (94.8)	35 (3.5)	70 (6.9)	177 (17.5)	728 (72.1)
> 15 - 25 years	524/543 (96.5)	157 (30)	84 (16)	110 (21)	173 (33)
> 25 - 50	742/780 (95.1)	233 (31.4)	110 (14.8)	129 (17.4)	270 (36.4)
> 50 years	338/338 (100)	49 (14.5)	47 (13.9)	65 (19.2)	177 (52.4)
Sex					
Female	1457/1780 (81.9)	309 (21.2)	183 (12.6)	253 (17.4)	712 (48.9)
Male	1273/1531 (83.1)	175 (13.8)	144 (11.31)	259 (20.4)	695 (54.6)
Ethnicity					
Fula	1716/2065 (83.1)	166 (9.7)	206 (12)	353 (20.6)	991 (57.8)
Mandinka	526/638 (82.4)	54 (10.3)	44 (8.4)	89 (16.9)	339 (64.5)
Sarahule	115/134 (85.8)	8 (6.9)	9 (7.8)	23 (20)	75 (65.2)
Wollof	3/3 (100)	0 (0)	0 (0)	1 (33.3)	2 (66.7)
Not specified	370/471 (78.6)	256 (69.2)	68 (18.4)	46 (12.4)	0 (0)
Household Size					
< 6	46/51 (90.2)	5 (10.9)	5 (10.9)	11 (23.9)	25 (54.4)
6 - 11	322/391 (82.4)	57 (17.7)	55 (17.1)	61 (18.9)	149 (46.3)
12 - 24	1137/1373 (82.8)	195 (17.2)	119 (10.5)	195 (17.2)	628 (55.2)
25 - 50	937/1141 (82.1)	168 (17.9)	104 (11.1)	177 (18.9)	488 (52.1)
> 50	288/355 (81.1)	59 (20.5)	44 (15.3)	68 (23.6)	117 (40.6)

Table 3: Odds ratios for participants eligible for ivermectin receiving a completed month of MDA.

Variables associated with receiving ivermectin amongst the eligible population (by completed Nr of monthly MDAs)				
	aOR	95% CI	p - value for specific variables	Likelihood Ratio - p - value (aOR)
Houshold head receiving MDA				
None	1			< 0.001
Complete	1.71	1.35 - 2.14	< 0.001	
Incomplete	1.14	0.89 - 1.47	0.287	
Houshold Size				
<6	1			0.0457
>6 - 12	0.64	0.33 - 1.25	0.198	
>12 - 25	0.75	0.39 - 1.42	0.379	
>25 - 50	0.59	0.31 1.14	0.119	
> 50	0.53	0.26 - 1.05	0.069	
Ethnicity				
Fula	1			< 0.001
Mandinka	1.04	0.74 - 1.45	0.8	
Sarahule	1.16	0.64 - 2.11	0.61	
Wollof	1.14	0.11 - 11.5	0.911	
Not specified	0.05	0.04 - 0.06	< 0.001	
Age Group				
<5	1.43	(0.97 – 2.1)	0.065	< 0.001
>5 - 15	4.3	(3.51 – 5.28)	< 0.001	
>15 - 25	0.92	(0.73 – 1.14)	0.45	
>25 - 50	1			
>50	2.27	(1.75 – 2.95)	< 0.001	
Sex				
Female	1			<0.001
Male	1.54	1.31 - 1.81	< 0.001	

Table 4: DHP treatment by demographic factors.

	Total Population (N = 3311)				
	Eligible Population/Total Population	Eligible Population for DHP (N = 3290)			
		No MDA complete (%)	1 month of MDA complete (%)	2 months of MDA complete (%)	3 months of MDA complete (%)
Total	3290/3311 (99.4)	422 (12.8)	380 (11.6)	623 (18.9)	1865 (56.7)
Houshold Head MDA					
Complete	1742/1754 (99.3)	191 (10.9)	162 (9.3)	281 (16.1)	1108 (63.6)
Incomplete	999/1006 (99.3)	128 (12.8)	155 (15.5)	240 (24)	476 (47.7)
None	549/551 (99.6)	103 (18.8)	63 (11.4)	102 (18.6)	281 (51.2)
Agegroup					
< 5 years	564/585 (96.4)	47 (8.3)	51 (9)	104 (18.4)	362 (64.2)
5 - 15 years	1065/1065 (100)	65 (6.1)	69 (6.5)	167 (15.7)	764 (71.7)
> 15 - 25 years	543/543 (100)	117 (21.6)	92 (16.9)	127 (23.4)	207 (38.1)
> 25 - 50	780/780 (100)	145 (18.6)	121 (15.5)	159 (20.4)	355 (45.5)
> 50 years	338/338 (100)	48 (14.2)	47 (13.9)	66 (19.5)	177 (52.4)
Sex					
Female	1768/1780 (99.3)	230 (13)	225 (12.7)	332 (18.8)	981 (55.5)
Male	1522/1531 (99.4)	192 (12.6)	155 (10.2)	291 (19.1)	884 (58.1)
Ethnicity					
Fula	2062/2065 (99.9)	88 (4.3)	228 (11.1)	425 (20.6)	1321 (64.1)
Mandinka	637/638 (99.8)	30 (4.7)	49 (7.7)	108 (16.9)	450 (70.7)
Sarahule	134/134 (100)	6 (4.5)	12 (8.9)	25 (18.7)	91 (67.9)
Wollof	3/3 (100)	0 (0)	0 (0)	0 (0)	3 (100)
Not Specified	454/471 (96.4)	298 (65.6)	91 (20)	65 (14.3)	0 (0)
Household Size					
< 6	51/51 (100)	6 (11.8)	5 (9.8)	11 (21.6)	29 (56.8)
6 - 11	390/391 (99.7)	48 (12.3)	60 (15.4)	75 (19.2)	207 (53)
12 - 24	1365/1373 (99.4)	163 (11.9)	144 (10.6)	236 (17.3)	822 (60.2)
25 - 50	1136/1141 (99.5)	149 (13.1)	121 (10.7)	219 (19.3)	647 (56.9)
> 50	348/355 (98)	56 (16.1)	50 (14.4)	82 (23.6)	160 (45.9)

Table 5: Odds ratios for participants eligible for DHP receiving a completed month of MDA.

Variables associated with receiving DHP amongst the eligible population (by completed Nr of monthly MDAs)				
	OR	95% CI	p - value for specific variables	Likelihood Ratio p – value
Houshold head receiving MDA				
None	1			< 0.001
Complete	1.64	1.33 – 2.04	< 0.001	
Incomplete	1.07	0.85 – 1.34	0.564	
Houshold Size				
<6	1			0.06
>6 – 12	0.85	0.45 – 1.62	0.628	
>12 – 25	0.94	0.51 – 1.75	0.858	
>25 – 50	0.77	0.41 – 1.43	0.41	
> 50	0.66	0.34 – 1.28	0.219	
Ethnicity				
Fula	1			< 0.001
Mandinka	1.05	0.76 – 1.46	0.74	
Sarahule	0.78	0.44 – 1.39	0.399	
Wollof	6.21E+07	(0 - .)	0.998	
Not specified	0.03	0.02 – 0.03	0.021	
Age Group				
<5	2.01	(1.59 – 2.54)	< 0.001	< 0.001
>5 – 15	2.47	(2.02 – 3.02)	<0.001	
>15 – 25	0.76	(0.61 – 0.94)	0.014	
>25 – 50	1			
>50	1.33	(1.01 – 1.35)	0.036	
Sex				
Female	1			0.036
Male	1.17	1.01 – 1.35	0.036	

Figures:

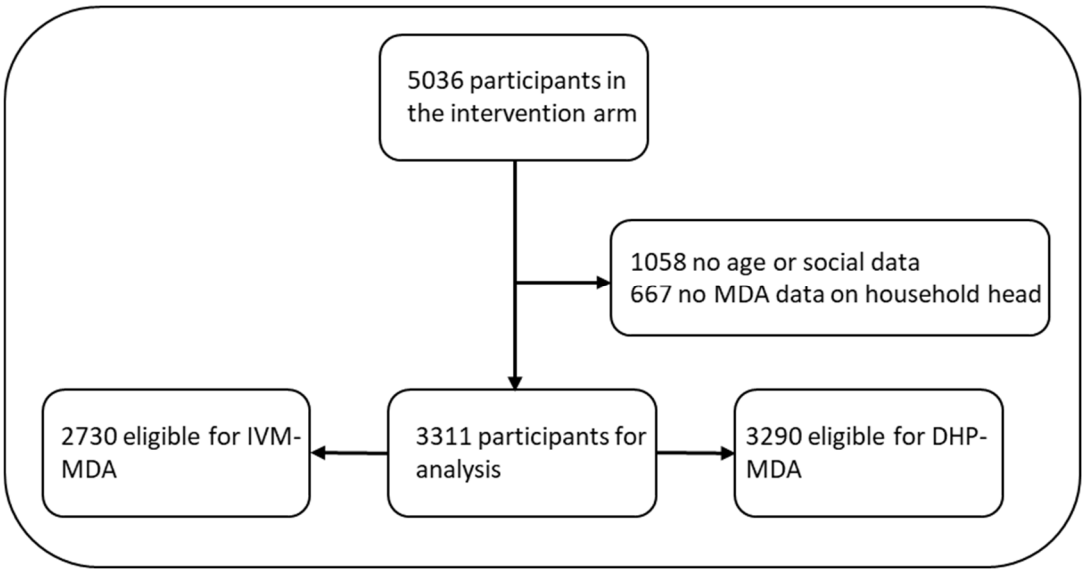


Figure 1: Number of participants in the intervention arm and their exclusion criteria for this study. IVM = ivermectin, DHP = dihydroartemisinin-piperaquine.

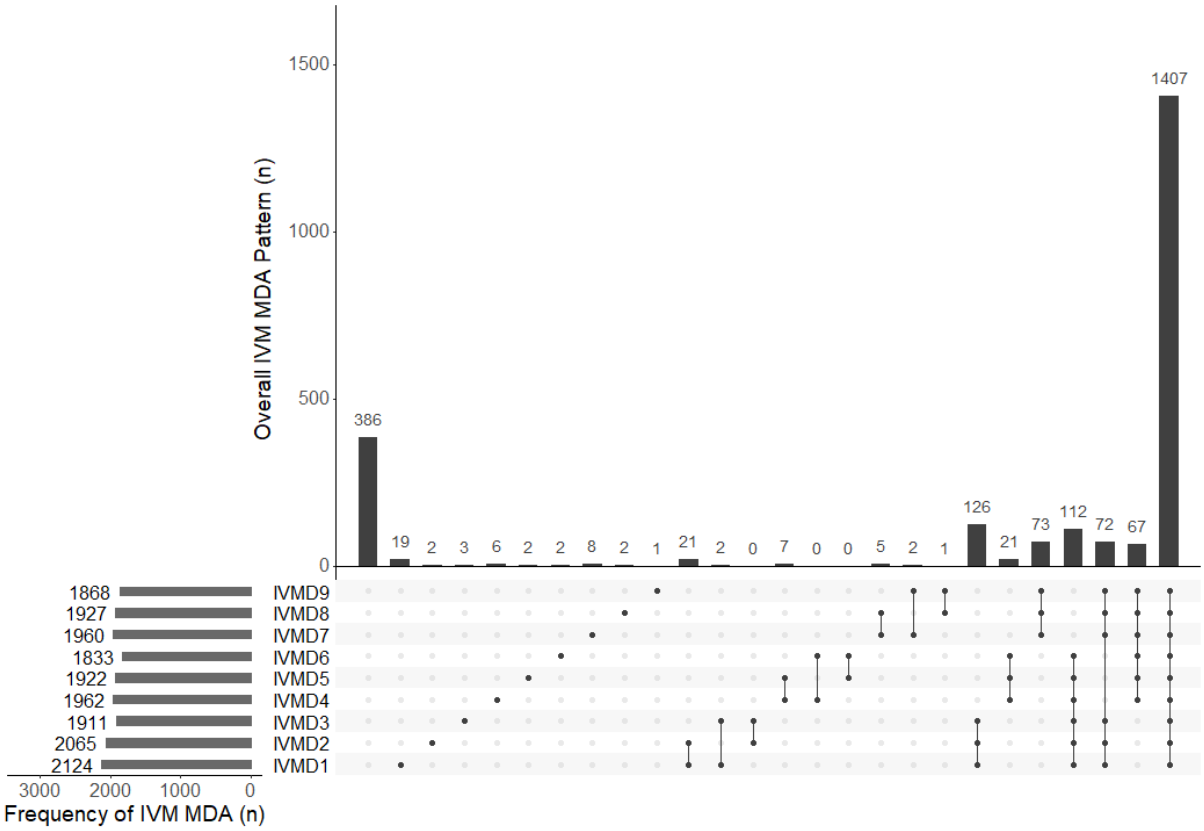


Figure 2: Overall treatment frequency and pattern for the ivermectin MDA excluding non-eligible participants. Full points indicate MDA received. IVMD# denotes the number of ivermectin MDA received, and the pattern such as a single dose or all nine MDA rounds. Certain patterns, such as received 1st and 9th MDA but none in between are excluded for convenience, therefore data for only 2347/2730 participants are show are shown.

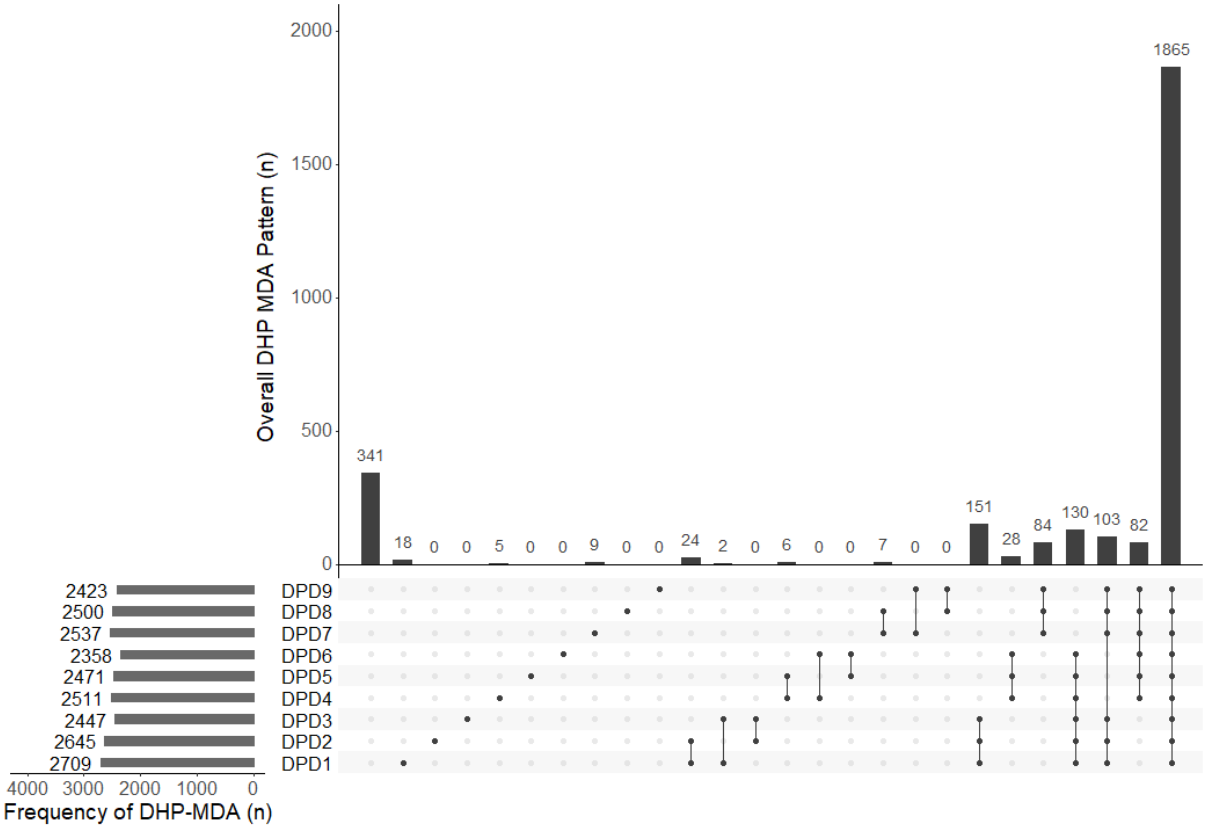


Figure 3: Overall treatment frequency and pattern for the dihydroartemisinin-piperaquine (DHP) MDA excluding non-eligible participants. Full points indicate MDA received. DPD# denotes the number of DHP MDA received, and the pattern such as a single dose or all nine MDA rounds. Certain patterns, such as received 1st and 9th MDA but none in between are excluded for convenience, therefore data for only 2855/3290 participants are shown.

7.1 Research Paper 3 Supplementary

Supplementary Appendix

Supplementary Table 1: MDA coverage of individuals missing demographic data.

Supplementary Table 2: Interaction between age-groups and MDA status of household heads.

Supplementary Table 3: Interaction between sex and MDA status of household heads.

Supplementary Table 4: Factors associated with receiving at least one dose of ivermectin.

Figure S1: Overall number of doses received for both dihydro-artemisinin piperaquine and ivermectin MDA for eligible participants.

Figure S2: Overall number of doses received for both dihydro-artemisinin piperaquine and ivermectin MDA for eligible participants including individuals with missing demographic data.

Supplementary Table 1: MDA coverage of individuals missing demographic data.

Characteristic		No Month	1 Month MDA	2 Months MDA	3 Months MDA
Population Missing Data (DHP MDA)	1,726 (100.0)	242 (14.0)	311 (18.0)	377 (21.8)	796 (46.1)
Population Missing Data (IVM)	1,726 (100.0)	543 (31.5)*	265 (15.4)	303 (17.6)	615 (35.6)

Table shows the distribution of the excluded participants who either lacked age, social data or household head data; *lack of age data might lead to inclusion of <5-year-olds who are not eligible for IVM MDA

Supplementary Table 2: Interaction between age groups and MDA status of household heads.

		Ivermectin		DHP	
Age	Head of house hold	OR (95%CI)	p – value	OR	p - value
< 5	None	1	0.75	1	0.72
	Complete	0.74 (0.08 - 6.77)		1.05 (0.41 - 2.7)	
	Incomplete	0.48 (0.05 - 4.70)		0.81 (0.31 - 2.13)	
5 - 15	None	1	0.82	1	0.96
	Complete	0.86 (0.33 - 2.20)		0.99 (0.5 - 1.96)	
	Incomplete	1.09 (0.376 - 3.18)		0.93 (0.44 - 1.94)	
> 15	None	1	< 0.001	1	< 0.001
	Complete	2.23 (1.62 - 3.04)		2.38 (1.7 - 3.35)	
	Incomplete	1.74 (1.24 - 2.45)		1.99 (1.37 - 2.89)	
Interaction p-value		0.171		0.039	

Supplementary Table 3: Interaction between sex and MDA status of household heads.

Age > 15		Ivermectin		DHP	
Sex	Head of Household	OR	P-value	OR	p - value
Female	None	1	0.3	1	0.357
	Complete	1.35 (0.9 - 2.02)		1.37 (0.85 - 2.2)	
	Incomplete	1.15 (0.73 - 1.81)		1.44 (0.84 - 2.45)	
Male	None	1	< 0.001	1	< 0.001
	Complete	4.75 (2.86 - 7.91)		4.75 (2.86 - 7.91)	
	Incomplete	2.85 (1.67 - 4.85)		2.93 (1.72 - 5.00)	
Interaction p-value		0.0001		0.0005	

Supplementary Table 4: Factors associated with receiving at least one dose of ivermectin.

Variables associated with receiving ivermectin amongst the eligible population (IVM No/Yes)					
		aOR	95% CI	p - value for specific variables	Likelikood Ratio p - value
Household Size					
<6	43/46 (93.5)	1			0.175
>6 - 12	272/322 (84.5)	0.23	0.05 - 1.09	0.065	
>12 - 25	986/1137 (86.7)	0.24	0.056 - 1.08	0.064	
>25 - 50	800/937 (85.4)	0.2	0.044 - 0.89	0.035	
> 50	243/288 (84.4)	0.25	0.05 - 1.18	0.082	
Ethnicity					
Fula	1607/1716 (93.7)	1			0.84
Mandinka	495/526 (94.1)	1.37	0.74 - 2.53	0.304	
Sarahule	108/115 (93.9)	1.48	0.51 - 4.38	0.47	
Wollof	3/3 (100)	1	-	-	
Not specified	131/370	0.032	0.022 - 0.04	< 0.001	< 0.001
Age Group					
<5	112/116 (96.6)	4.61	1.52 - 13.97	0.007	< 0.001
>5 - 15	983/1010 (97.3)	8.81	5.51 - 14.07	< 0.001	
>15 - 25	397/524 (75.8)	1.15	0.83 - 1.61	0.83	
>25 - 50	552/742 (74.4)	1			
>50	300/338 (88.8)	3.43	2.13 - 5.48	< 0.001	
Sex					
Female	1209/1457 (82.9)	1			< 0.001
Male	1135/1273 (89.2)	2.71	1.98 - 3.71	< 0.001	

Figure S1: Overall distribution of the MDA dose uptake by number of MDA doses received for IVM or DHP respectively for all eligible participants with complete data for analysis out of the total population in the intervention arm (IVM N = 2730/5036, DHP N = 3290/5036).

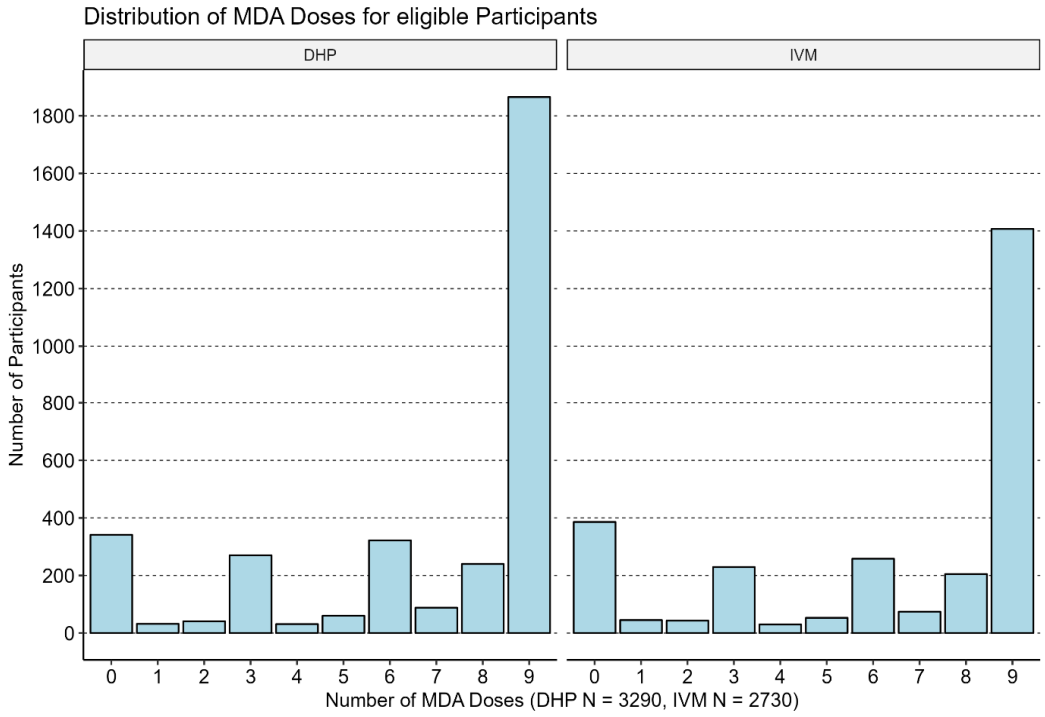
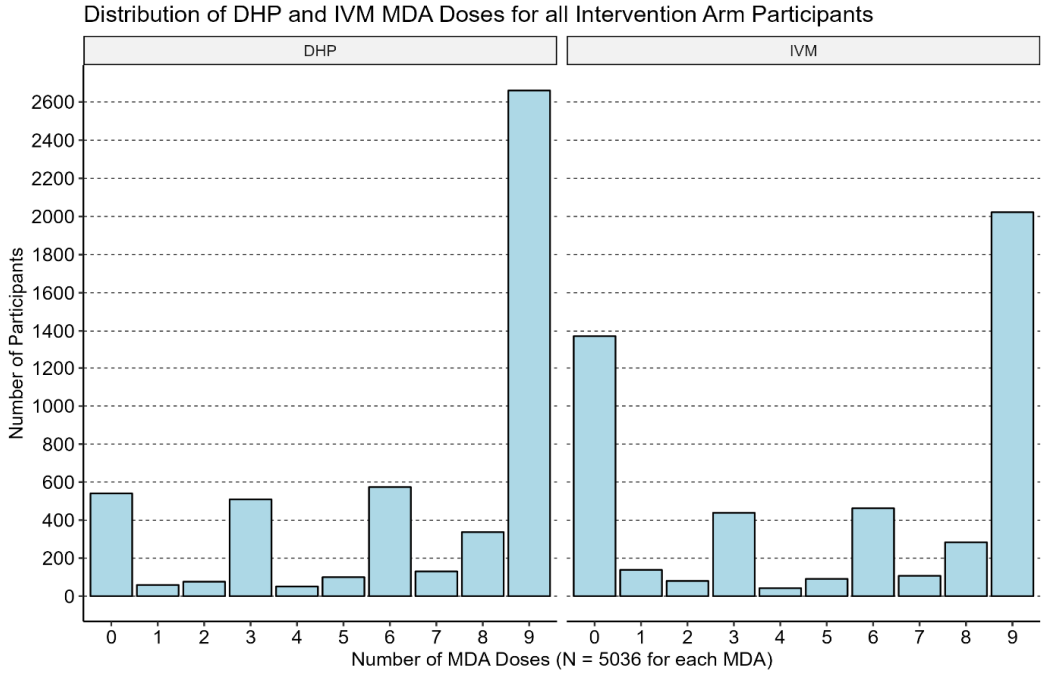


Figure S2: Overall number of doses received for both dihydroartemisinin piperaquine and ivermectin MDA for eligible participants including individuals with missing demographic data (N = 5036).



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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	386243	**Title**	Dr.
First Name(s)	Christian Hermann		
Surname/Family Name	Kositz		
Thesis Title	Investigating the Effects of Ivermectin plus Dihydroartemisinin-Piperaquine MDA on the Transmission of Malaria by Measuring Serological Markers of Exposure and its Effects on the Prevalence of Ectoparasites and Soil-Transmitted-Helminths		
Primary Supervisor	Michael Marks		

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?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Malaria Journal

Please list the paper's authors in the intended authorship order:	Christian Kositz, Hristina Vasileva, Nuredin Mohammed, Jane Achan, Edgard Dabira, John Bradley, Umberto D'Alessandro, Michael Marks
Stage of publication	**Not yet 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)	Drafted the manuscript; data cleaning, statistical analysis, graphics, manuscript draft, will prepare the final version and respond to reviewer comments

SECTION E

Student Signature	[redacted]
Date	10.04.2023

Supervisor Signature	[redacted]
Date	10.04.2023

Chapter 8 Summary of research findings

In this section, I provide a brief overview over the results of my studies in relation to the objectives and aims of this thesis. For each of the objectives and aims the respective research paper, in which a detailed description of the findings is reported, is highlighted.

8.1 Research Paper 1 - Chapter 5

- Aim 1: To evaluate the impact of ivermectin and dihydroartemisinin-piperaquine MDA on serological markers for malaria parasite and vector exposure (transmission)
- Objective 1 and 1a: To use Luminex® MagPix platform multiplex serological assays to assess differences in exposure to malaria parasites and vectors between control and intervention arms of the MASSIV study and evaluate its use for measuring malaria transmission.

The first DBS collection was conducted in June before the 2019 MDA (July to September) and nine months after the 2018 MDA and the second DBS collection in November 2019, a month after the 2nd MDA. In both surveys, antigens for detecting 19 malaria associated antibodies used with the Luminex® MagPix platform measuring mean fluorescence intensity. No evidence for a difference between the arms in any of these tested antigen/antibody combinations was found. This is in contrast to other studies that have shown a variety of interventions may lead to a difference in antibody MFI levels. In many of these studies there has been a period of more than 3 months (1,2) between intervention and sample collection (3,4) and the shorter time frame in MASSIV may explain the absence of effect seen. Although no effect was seen our data may still inform a potential cut off for time frame in which there is value in serology as a monitoring tool in a highly seasonal malaria area. Future similar studies should therefore collect samples at different timepoints for example at monthly intervals post intervention.

Malaria PCR was conducted from the same DBS samples as the Luminex serology results. When correlating the serology with malaria positivity gSG6 at the individual level, an *A. gambiae* spp. saliva antigen showed no correlation with infection in the June survey, however there was correlation in the November 2019 survey post MDA. Similarly, CSP, the sporozoite antigen, showed no correlation in the adjusted model in the June 2019 survey but did in the adjusted model for the November 2019 survey post MDA. This is likely unrelated to the MDA and more likely related to no exposure to mosquitoes and sporozoites during the dry season (January to June) and could be seen a confirmation that the serological results are correlated to season and therefore biologically sound.

From the other antibodies, only Rh5.1 was not correlated with PCR positivity in either survey. What the explanation or consequence of this is, is not clear as this antigen is considered a vaccine candidate(5).

8.2 Research Paper 2 - Chapter 6

Aim 2: To assess the effects of ivermectin and dihydroartemisinin-piperaquine MDA of the MASSIV trial on non-malaria targets (SHT and ectoparasites).
Objective 2: To determine the prevalence of soil-transmitted helminths using a STH qPCR.

Both cross-sectional surveys determining the effect of the MDA on the prevalence of STH in 3 – 14-year-olds found a low prevalence of *A. lumbricoides* with 4.4% vs 4% in the control vs intervention arm in 2019 and 0.9% vs 1.2% in 2021, respectively, and 5% vs 4.6% in 2019 and 3.6% vs 6.6% for *N. americanus* in 2021, respectively. Numbers for *T. trichiura* and A. *duodenale* were negligibly low with an overall prevalence of 0.12% and 0.24% respectively. The low prevalence limited the study's power to detect an effect of ivermectin. Nonetheless, these numbers are broadly in line with the findings of a mapping survey conducted in the Gambia in 2015(6), conducted via microscopy, which is the most likely reason why *S. stercoralis* was missed. A reason for these low numbers for STH that transmit via egg contamination could be the rigorous habit of handwashing before meals.

In the first survey in 2019 *S. stercoralis* showed a lower prevalence in the intervention arm with 9.1% vs 16.9% ($p = 0.039$) in the control arm, suggesting an initial effect of the ivermectin intervention. In the survey conducted in 2021, *S. stercoralis* prevalence in the intervention was 11.3%, compared to 9.2% in the control arm, but this difference was not statistically significant. This prevalence was comparable to previous studies in the Western part of The Gambia and it could be that *S. stercoralis* is the predominant STH in the country but also specifically the URR. This would fit well with the rather short 3 – 4 months of rain season and much longer dry season of the Upper River Region (7) favouring a parasite that does not necessarily require a maturation process outside the human body and potential lifelong persistence in the host compared with other STH (8,9).

8.3 Research Paper 2 - Chapter 6

Objective 3: To determine the prevalence of ectoparasites (scabies, headlice and bedbugs) using clinical examination.

During the first survey in June 2019 before the MDA, no physical evidence for bedbugs and no clear signs for headlice were found in either study arm and these measures were therefore dropped from the subsequent surveys. This result was unexpected as a survey from western URR found 37.5% of all children's beds infested with bedbugs and 28.8% of children under 10 had head lice (10). In case of head lice it was suspected that the cultural habit of clean shaving the boys' heads and regular braiding of the girls' hair may prevent headlice infestation, in addition to head lice having lower survival rates in high temperature low humidity environments (11). Similarly, for bed bugs the climate of the URR itself is too hot for perennial bed bug infestation.

The scabies survey conducted at the same time in June 2019 showed almost half of the children aged 3 – 14 years had clinical signs of scabies, with 48.2% in the control and 38.8% in the intervention arm respectively. This was almost 8 months after the 2018 MDA conducted by the MASSIV trial and the effect was statistically significant. However, in the survey of November 2019 set one and a half months after the 2019 MDA, no effect of ivermectin was found anymore, primarily due to a reduction in scabies prevalence in the control arm to 41.2% whereas in the intervention arm prevalence was almost the same at 38.2%. This trend reversed in the last survey in November 2021, which was delayed by the COVID-19 pandemic by one year. Prevalence changed to 38.8% in the control arm and increased to 53.2% in the intervention arm, not showing any sign of a long-term effect of the intervention. It is possible that the ivermectin MDA coverage of the eligible population may not have been sufficient to impact scabies. Additionally, the presence of several reservoirs of non-IVM-eligible study participants such as the < 5 year olds, roughly 17.4% of the population on average in The Gambia (12), as well as potential contamination of the study area due to population movement and from other village might have contributed to this result. Although the outcome itself is disappointing, it is important data to show that scabies may not be as easily controlled or even eliminated in an open system with a mobile population such as West Africa compared to pacific islands (13).

8.4 Research Paper - Chapter 7

Objective 4: To identify risk factors for systematic non-participation in the MDA using the coverage data collected by the MASSIV trial.

Risk factors for non-participation in the MDA was assessed using the coverage data from the second round of MDA in the MASSIV trial in 2019 and combining it with the existing MASSIV census data. The results showed that for both ivermectin and DHP whether the household head participated in the MDA was a major factor associated with participation of other household members.

In keeping with other studies, I found participation was associated with both age and gender but did not find any clear evidence of an impact of household size or ethnicity on participation. The lower uptake amongst women has been seen in previous studies and is thought to be related to women not having participated in previous rounds of MDA when they have been pregnant previously (12,13). The observed age specific differences may be related to these age groups including adolescents, secondary grade students and the main demographic of the working population and suggest interventions to improve coverage in these groups may be needed.

Chapter 9 Study Limitations:

The work conducted in my PhD has a number of limitations. The work took place during the COVID-19 pandemic, and this inevitably interfered with the timings of some study activities. Specific challenges of each study component are discussed below.

9.1 Evaluation of the IVM and DHP MDA on malaria serological markers

The MASSIV trial's use of two drugs (DHP and IVM) in the intervention arm and no drug in the control arm inherently restricted the serological analysis, as it is not possible to clearly differentiate the effect of each drug apart. This limitation is likely going to be solved by the MATAMAL trial carried out in the Bijagos Islands which contains two arms, DHP alone + Placebo versus DHP+ ivermectin.

The data on the kinetics of each of the tested malaria antibodies, especially the short term to medium term antibodies is limited and no clear pharmacokinetic curve starting from exposure to several months for each of the tested antibodies exist. As of date, the available data is at least three months post intervention and more commonly six months or older (1,2,4), therefore it is possible that the results may have been influenced by the short post MDA timeframe, which was 1 – 1.5 months between MDA and DBS collection, leading no difference in MFI levels between the arms.

9.2 Impact of the MDA on the prevalence of ectoparasites and soil-transmitted helminths

The study's findings primarily relied on cross-sectional studies, which have inherent limitations. This design provides a mere snapshot of the current epidemiology of the disease of interest at a specific time point. This is especially notable in the changes of scabies prevalence in each survey (48.2 vs 38.8; 41.2 vs 38.2; 38.8 vs 53.2, control vs intervention respectively), which appeared to be independent of the intervention.

Because of the very limited pre-existing data, sample sizes were estimated. In case of scabies, this was not a problem, but the complete lack of bed bugs and head lice did reduce the information gained on the impact the intervention could have had. For soil-transmitted helminths, I noted more complex societal issues regarding the collection of stool samples, which was sometimes seen with suspicion in adults or embarrassment in adolescents. This led to a considerable decline in participation on several villages and consequently underpowering of the STH cross sectional survey in 2021.

9.3 Evaluation of potential causes associated with systematic non-participation in the MDA

The biggest limitation was the missing data for age and households, which resulted in a loss of 20% of participants who could be included. These individuals did not differ in their participation in MDA. However, the additional data would have strengthened the associations seen. Additionally, only the data from 2019 was available due to a less then optimal execution and data collection of the trial in 2018.

9.4 Conclusions

The aim of the first part of my PhD was to evaluate the use of malaria serology on the Luminex platform for its potential as serological tool for measuring the impact of a DHP/IVM MDA and potentially for surveillance. Cross-sectional DBS surveys were conducted before and after mass treatment to establish accurate estimates of malaria antibody MFIs and the difference MFI between arms as well as the prevalence of each antibody.

Although no difference in MFI was found after the intervention, the data show that the Luminex essay for malaria serology worked as expected, with an increase in MFI levels and prevalence of the antibodies against malaria with age in both arms. Additionally, we found a seasonal association between malaria PCR positivity and vector and sporozoite exposure markers, which biologically fits with the lack of exposure to mosquitoes and sporozoites during the dry season.

It is possible that the DBS collection happened to early after exposure and alternatively that the overall coverage of the intervention was not high enough. The latter seems less likely as PCR positivity results showed a clear impact of the MDA (14) . The first hypothesis requires further studies to allow it to be proven or disproven. Such work will be undertaken in the ongoing MATAMAL trial, in which the timeframe between MDA and DBS collection is similar. Hence, if confirmed this would establish a cut-off for the timing of serological assays for malaria and would be a valuable advance for future studies.

The second part of my PhD was to evaluate specifically the impact of the ivermectin MDA on off-targets of the original trial including ectoparasites and soil-transmitted helminths. To obtain accurate estimates on the prevalence of scabies and soil-transmitted helminths and assess the impact of the intervention on these parasites between arms, cross-sectional

surveys were conducted before and after mass treatment. As no bedbugs and head lice were found the ectoparasite part focused on scabies for which no evidence of an impact of the repeated application of ivermectin was found. Although not necessarily the result that was expected, it does show that the control of scabies in a setting where movement of people is not restricted by geographical boundaries, as is the case in the pacific (13), proved to be more difficult. This adds important data for future studies for scabies control as data in such open geographical settings is missing. Also, the lack of additional treatment of participants that were excluded from taking ivermectin likely acted as an ongoing source for reinfection as described in previous study in the pacific (15).

Unfortunately, most STH species had such low prevalence that the loss of power would not justify making any claim on the impact of ivermectin. The exception was *Strongyloides stercoralis*, which turned out to be the most prevalent species and up to this date no prevalence data on it existed in this area as the previous national survey used microscopy (6), which is unsuitable for detecting *S. stercoralis* (16,17). For *S. stercoralis* the intervention showed an impact right after the MDA, however this difference in prevalence was not found again 2 years later in the follow survey.

In the third part of my PhD, I investigated the potential causes for non-participation in the MDA. This was of particular interest as the MASSIV trial MDA is substantial with two drugs given for three consecutive days once a month for three month adding up to nine tablets per single round (5 tablets of ivermectin and 4 tablets of DHP) and studies on systematic non-compliance and associated variables are still few (18–22). In this case the participation of household heads was an important factor that increased compliance, whereas being of within the age group of 15 – 25 and 25 – 50 reduced the odds of receiving MDA. Interestingly, men had a higher odd of receiving MDA for both drugs. However, this analysis only pointed to certain factors influencing the MDA uptake and more investigation is needed support or dismiss potential hypothesis generated with each of these variables.

In summary, these studies have provided valuable insights into the impact of ivermectin-DHP MDA for malaria and has highlighted areas for future studies to elaborate and expand the knowledge needed for successful implementation of IVM as an additional tool for malaria control and optimal effect on off-targets of such an MDA.

Chapter 10 Where does an ivermectin-DHP MDA position itself in the broader context of malaria control?

Ivermectin, especially in combination with DHP, shows great potential for malaria vector control, comparable to SMC and LLITNs. However, this is based on modelling assumptions and the real world can be quite different(23–26).

An intervention that is primarily targeted at seasonal malaria transmission that requires at least a coverage of > 70% over several days per month throughout a season(24,25) might be challenging in regard to infrastructure, logistics and monetary support. This maybe especially the case for large areas with poor road infrastructure, long distances and geographical obstacles delaying the delivery of medical interventions. Data for this scenario will be available after some of the ongoing studies have been finished, and more details on the feasibility of IVM-MDA will be available (27,28).

Aside from the logistical challenges, IVM-DHP offers benefits outside of malaria control, as IVM is highly effective against a variety of parasites including soil-transmitted helminths, ectoparasites and other filarial nematodes and hence could be combined with other already existing control programmes.

On top of this, DHP has been shown to be an effective, albeit more expensive drug for SMC(29). Hence, IVM-DHP could be used as another way to deliver SMC to areas where SMC is already an established programme.

However, one last point to consider is the large-scale application of IVM in areas without good sewage systems. Ivermectin itself is metabolized via the liver and metabolites are being excreted via the faecal route(30), therefore, it is not impossible that an MDA could release IVM or its metabolites in high enough quantities into the environment to cause damage to biome, specifically dung feeding invertebrates but also aquatic animals(31–33).

Chapter 11 Future Research Questions and Topics:

The Luminex data that was analysed in my PhD and the serological data already known (1–4) shows the need for answering several question:

- What is optimal timing of samples for malaria serology in relation to intervention?

- What are the malaria antibody kinetics of short-term antibodies post treatment/MDA from day 1 – 6 months?
- What are the malaria antibody kinetics in people with repeated infections under DHP MDA to evaluate the impact on the antibody kinetics?

The last question is under the assumption that DHP does not protect from infection, hence the 1st generation of merozoites exiting the liver into the bloodstream could expose antigens to the immune system and cause a short boost of an existing immune response.

The results for the NTD part of my PhD were not as expected, including the absence of bed bugs and headlice, which left several questions unanswered and added other questions for future work.

- What is the most optimal and practical approach to control scabies in an open geographic setting lacking natural boundaries and high population movement?
  - What is the best strategy to successfully impact scabies control using ivermectin for malaria vector control?
- What is the effect of ivermectin MDA for malaria vector control on bed bugs, head lice and if present body lice?
- What is the effect of ivermectin MDA for malaria vector control or repeated ivermectin MDA itself on hookworm species in an area with medium to high prevalence?

Consideration of these questions will help move forward the field of ivermectin as a tool for both malaria and NTD control.

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