Ivermectin Mda For Malaria Control And Plasmodium Species Diversity In Burkina Faso

Julia Ellman
julia.ellman@yale.edu

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Ivermectin MDA for Malaria Control and *Plasmodium* Species Diversity in Burkina Faso

Julia Ellman  
Yale School of Public Health  
Master of Public Health 2024  
Epidemiology of Microbial Diseases

Primary Adviser  
Dr. Sunil Parikh, Epidemiology of Microbial Diseases

Secondary Advisor  
Dr. Joshua Warren, Biostatistics
ABSTRACT

Malaria is a major cause of mortality globally, but particularly in Africa and among children. As the growth of drug resistance threatens current malaria control and treatment strategies, novel interventions are needed to combat the recent increase in cases. Ivermectin is an endectocide that has been historically used to treat neglected tropical parasitic diseases through mass drug administration (MDA). Recently, it has been demonstrated to have a lethal effect on mosquitos, suggesting its potential as a valuable vector control strategy. RIMDAMAL II is a cluster randomized control trial that was conducted in southwestern Burkina Faso in 2019-2020 to evaluate the impact of ivermectin MDA on malaria transmission. Villages randomly assigned to the treatment arm were given monthly ivermectin MDA during the transmission season, July-October of both years. Blood samples from active case detection by monthly sampling of 206 children <10 years of age in both arms were screened for Plasmodium infection and then speciated using multiplex real-time PCR. Ivermectin MDA had a small, insignificant impact on malaria infections in children. The odds of detecting malaria infection in children in the control villages are 12% higher than those in the villages receiving ivermectin (OR = 1.12, 95% CI [0.701, 1.786]). 8% of all samples tested identified a mixed infection with P. falciparum and at least one non-falciparum species. This is a higher contribution of non-falciparum species to overall infections than expected, though the proportion of each species present did not not differ by treatment ($X^2 = .31$, df = 2, $p= 0.856$). Additionally, while the proportion of species detected cannot be statistically compared between individual villages, there was notable variation in the amount of mixed infections and each species observed by village. This surprising diversity of Plasmodium spp. infections elucidates the need for further sampling and speciation to determine the true prevalence of non-falciparum infections across Burkina Faso, as there is currently very limited data. Accurate identification of P. ovale is particularly important, as this may have implications for the inclusion of primaquine in treatment guidelines. Although this study did not find ivermectin MDA to significantly reduce malaria infections in children, additional research is needed to investigate potential environmental confounders and methods to optimize implementation of ivermectin MDA.
ACKNOWLEDGEMENTS

I would like to thank the researchers behind the Repeat Ivermectin Mass Drug Administrations for MALaria Control II (RIMDAMALL II) study and all the work that went into its planning and management. Without RIMDAMALL II and the samples that came from it, this project would not exist. Specific thanks to Dr. Sunil Parikh and Dr. Brian Foy from Colorado State University, as well as Dr. Roch Dabiré and Dr. Fabrice Somé from Institut de Recherche en Sciences de la Santé in Burkina Faso.

Thank you to Dr. Sunil Parikh and Dr. Joshua Warren for their guidance as thesis advisors. I’m also thankful for Dr. Parikh for his mentorship during my time in the Parikh Lab over the past year. This study would not have been possible without the countless hours spent organizing and processing samples by others in the Parikh Lab- Hannah Sproch, Elizabeth Zhang, and Ijeamaka Ichebe. A very important thank you to Martina Wade and her help around the lab, for without whom no one would be able to find anything.

Finally, I would like to thank my friends and family for their support during these past two years at the Yale School of Public Health, as well as everything that came before it.
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INTRODUCTION

Malaria is a major cause of morbidity and mortality globally. This is particularly true for countries in Africa with high transmission, such as Burkina Faso. Preliminary evidence suggests ivermectin (IVM) mass drug administration (MDA) as a promising new vector control strategy to reduce malaria transmission. Utilizing existing drugs in novel ways has the potential to reinvigorate the fight against malaria and improve quality of life and health equity around the globe. The principal objectives of this study are to further the understanding of how ivermectin MDA impacts risk of malaria infection in children within a cluster randomized control trial (RIMDAMAL II), as well as to fill a gap in the literature regarding the current landscape of Plasmodium species diversity in Burkina Faso.

Aims

The first aim of this study is to investigate the impact of ivermectin mass drug administration on malaria infection among children under 10 years of age in the treatment villages as compared to control villages. Blood samples collected through routine monthly sampling of children are screened for positive Plasmodium infection using molecular diagnostics. It is expected that there will be fewer children who test positive in the treatment villages receiving ivermectin MDA.

The second aim is to describe Plasmodium species diversity within the study site in southwestern Burkina Faso. The contribution of mixed infections to each treatment arm as well as the overall malaria burden will be evaluated. Because the majority of surveillance and control efforts are focused on Plasmodium falciparum, there is limited knowledge on the current prevalence of non-falciparum species, though it is expected to be very low.

Background

The Malaria Burden

Malaria is a parasitic disease transmitted by the Anopheles mosquito and is one of the most significant causes of death in children worldwide. In 2022, there were an estimated 249 million total malaria cases, an increase from the year prior and demonstrating the concerning
trend reversal in the global fight against malaria.\(^1\) Though there has been success in reducing the number of cases and deaths since 2000, progress stalled around 2015 and in recent years cases have been growing globally.\(^2\) Current interventions and control measures are no longer as effective due to a variety of factors including insecticide resistance, artemisinin partial resistance, and mutation-driven rapid diagnostic test (RDT) evasion.\(^3\) Context-specific and novel approaches in malaria prevention and control are urgently needed in order to revitalize the fight against malaria.

There is an extremely unequal distribution of malaria worldwide, with the majority of cases in Africa. In 2022, 94% of cases and 95% of all malaria deaths (580,000) worldwide were attributed to the WHO African Region.\(^4\) In countries where malaria is endemic, the disease is one of the greatest contributors to morbidity and mortality in children under five years of age. Burkina Faso, a country in West Africa, is one of six countries contributing the most deaths worldwide.\(^5\)\(^6\) As a highly endemic country, there is both a pressing need for innovative malaria control measures and opportunity for lives to be saved in Burkina.

---

**Figure 1: Malaria \((P.\ falciparum)\) incidence rate per thousand in Burkina Faso (2010-2020)**\(^7\)

[https://tinyurl.com/malariaatlasproject](https://tinyurl.com/malariaatlasproject)

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\(^1\) ([World Malaria Report 2023, 2023](https://www.who.int/malaria/world-malaria-report-2023/en/))

\(^2\) ([World Malaria Report 2023, 2023](https://www.who.int/malaria/world-malaria-report-2023/en/))

\(^3\) (Khaligh et al., 2021)

\(^4\) ([Fact Sheet about Malaria, 2023](https://www.who.int/malaria/factsheets/en/))

\(^5\) ([World Malaria Report 2023, 2023](https://www.who.int/malaria/world-malaria-report-2023/en/))

\(^6\) ([Hanboonkunupakarn et al., 2022](https://www.who.int/malaria/publications/world-malaria-report-2023/9789242798805-en))

\(^7\) ([Malaria Atlas Project | Data, n.d.](https://www.who.int/malaria/atlas/en/))
Life Cycle

The life cycle of *Plasmodium* is complex, and while the only vector is the mosquito, there are many different *Anopheles* species capable of transmission. Malaria is transmitted to humans through the bite from a mosquito infected with the *Plasmodium* parasite. There are six distinct *Plasmodium* species, with *P. falciparum* making up the large majority of cases.\(^8\) When a person is bitten, *Plasmodium* sporozoites from the mosquito salivary glands enter the skin and then blood vessels, making their way to the liver. The liver stage of infection is asymptomatic and can last for varying lengths of time depending on the *Plasmodium* species. The different species and our disparate understanding of each is one of the challenges to malaria control initiatives. During the liver stage, mitotic division produces many more merozoites which exit the liver and quickly find red blood cells, which is when the infection becomes symptomatic. During the erythrocytic stage, some merozoites develop into gametocytes which enter into the circulatory system. Time spent in this stage ranges from 24-72 hours by species.\(^9\) The transmission cycle continues when a mosquito feeds and picks up at least one female and one male gametocyte. In the mosquito midgut, fertilization occurs which then leads to ookinetes that pass through the midgut cell wall and become oocysts. Oocysts produce sporozoites which can then lead to a new infection and continuation of the cycle.

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\(^8\) *World Malaria Report 2023, 2023*

\(^9\) *Sato, 2021*
Figure 2: Malaria life cycle
(https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(13)60024-0/fulltext)

The Importance of Species Identification

*Plasmodium falciparum* is the most prevalent species of malaria in Africa. As a result, the large majority of diagnostic and treatment strategies have been focused on this species. However, there are six recognized *Plasmodium* species that infect humans, as the species formerly known as *P. ovale* has recently been divided into two distinct species: *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*.10 *P. vivax*, *P. malariae*, and *P. knowlesi* are the remaining three species. There is currently relatively limited knowledge of the prevalence and geographic distribution of the minor *Plasmodium* species across Africa due in part to a lack of reliable diagnostic methods.

The parasite life cycle can vary greatly between species, which has implications for effective treatment and prevention measures, as well as successful eradication campaigns. One such differentiation is the time it takes for the parasite to complete the erythrocytic stage. *P. knowlesi* is the shortest at 24 hours, *P. falciparum* and *P. ovale curtisi* and *P. ovale wallikeri* at 48

10 (Snounou et al., 2024)
hours, and *P. malariae* at 72 hours.\(^{11}\) Additionally, *P. vivax* and the formerly *P. ovale* species have been documented to produce hypnozoites, cells that lie dormant in the liver and can persist for an extended period of time.\(^{12}\) These hypnozoites in the liver have the ability to reactivate weeks and even months after clearance of the initial infection.\(^{13}\) The resulting relapses contribute to overall mortality and pose a challenge to preventing transmission.

While *P. vivax* is not a concern in Burkina Faso, *P. malariae* and the *P. ovale* species are known to be rare but present, in addition to *P. falciparum*. Quantifying the true burden of these minor species, particularly *P. ovale curtisi* and *P. ovale wallikeri*, is important to inform the inclusion of primaquine in treatment guidelines. Without the addition of primaquine in the treatment regimen, hepatocytes in the liver can survive artemisinin-based combination therapy (ACT) and cause relapses.\(^{14}\) Additionally, mixed infections with multiple *Plasmodium* species is a growing topic of importance. Individuals with mixed *Plasmodium* species infection were found to have a greater proportion of multiple organ failure and other complications as compared to those with *P. falciparum* mono-infection.\(^{15}\) Identification and further study of these mixed infections to better understand the prevalence and impact on therapeutics is needed.

**Current State of Malaria Treatment and Prevention Strategies**

There are a variety of strategies currently in the toolbox for malaria treatment and elimination. This includes treatment with artemisinin-based combination therapy (ACT), though the emergence of artemisinin resistance is a serious threat to the successful treatment of malaria, as ACTs are first-line treatments.\(^{16}\) Drug-based prevention with intermittent preventive treatment for pregnant women (IPTp), seasonal malaria chemoprevention (SMC) for children, and the RTS,S and R21 vaccines are important as well. SMC is a valuable method of reducing the malaria burden among children under 5 in endemic countries with highly seasonal transmission.\(^{17}\) The SMC drug combination consists of sulfadoxine-pyrimethamine and amodiaquine, and is often

\(^{11}\) (Sato, 2021)  
\(^{12}\) (Sato, 2021)  
\(^{13}\) (Commons et al., 2020)  
\(^{14}\) (*Fact Sheet about Malaria*, 2023.)  
\(^{15}\) (Kotepui et al., 2020)  
\(^{16}\) (Ashley Elizabeth A. et al., 2014)  
\(^{17}\) (*Updated WHO Recommendations for Malaria Chemoprevention among Children and Pregnant Women*, 2022)
given in monthly intervals during the rainy, high-transmission season.\textsuperscript{18} In Burkina Faso, SMC is given between 3 to 5 cycles from June and October during the rainy season.\textsuperscript{19} This established infrastructure is useful for the integration of additional drug-based interventions, with delivery to be performed by community health workers as it is with SMC. However, peak transmission season varies across the country due to diversity in geographic regions, including difference in rainfall.\textsuperscript{20} In the south and southwest regions of the country, the rainy season and thus high malaria transmission begins in June.\textsuperscript{21} This complicates the optimal timing for distribution of SMC and other MDA interventions like ivermectin.

\textbf{Vector Control- Triumphs and Challenges}

There are also various forms of vector control, with the most common being indoor residual spraying (IRS) and insecticide-treated bed nets (ITN) which have contributed greatly to the reduction of malaria cases worldwide. Vector control initiatives seek to manage and/or kill the mosquitos that transmit malaria to humans rather than targeting the parasite. Because malaria cannot be transmitted human-to-human and is dependent on mosquitos to complete the transmission cycle, vector control is a strategy that limits the number of new cases. However, vector control often utilizes pyrethroid insecticides, for which there is growing resistance. This is the only insecticide class approved for ITNs, and resistance has been documented in numerous vector species across much of Sub-Saharan Africa.\textsuperscript{22} Many vector control-based interventions are shaped by the parameters that make up the Ross-Macdonald vectorial capacity equation (Box 1). The probability of daily survival of adult mosquitos (p) is a very important component of the model as it appears twice and thus is a popular target for interventions.\textsuperscript{23}

\textbf{Box 1: Vectorial Capacity Equation and Parameter Definitions}

\textsuperscript{18} (Cola et al., 2022)
\textsuperscript{19} (Malaria Consortium - Malaria Consortium Burkina Faso, 2021)
\textsuperscript{20} (U.S. President’s Malaria Initiative Burkina Faso Malaria Profile, 2022)
\textsuperscript{21} (Malaria Consortium - Malaria Consortium Burkina Faso, 2021)
\textsuperscript{22} (Ranson et al., 2011)
\textsuperscript{23} (Brady et al., 2016)
**Vectorial Capacity (V)** = \( \frac{ma^2 p^n}{-\ln(p)} \)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>Ratio of mosquitoes to humans</td>
</tr>
<tr>
<td>a</td>
<td>Human biting rate</td>
</tr>
<tr>
<td>p</td>
<td>Probability of mosquito daily survival</td>
</tr>
<tr>
<td>n</td>
<td>Parasite extrinsic incubation period, number of days for sporogony (EIP)</td>
</tr>
<tr>
<td>-1/ln(p)</td>
<td>Represents life expectancy of infected mosquitoes</td>
</tr>
</tbody>
</table>

(Brady et al., 2016. Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination)²⁴

**Ivermectin- Novel Use of a Familiar Endectocide**

Ivermectin (IVM) is an antiparasitic drug that has historically been used to treat a variety of neglected tropical diseases through mass drug administration campaigns. As such, the drug has been thoroughly studied since it was approved for use over 30 years prior, and the safety profile is well established.²⁵ IVM is also a type of endectocide, meaning it kills both endoparasites like nematodes and ectoparasites such as blood-feeding arthropods. The drug’s toxicity towards all *Anopheles* species has been demonstrated through a variety of studies.²⁶ A systematic review of endectocide use as a complementary intervention for malaria control in 2021 found that *Anopheles* mosquito survivorship was notably decreased after exposure to ivermectin-containing blood meals across multiple experiments, both with artificial feeding in the lab and in the field.²⁷ There was an observed reduction in daily *Anopheles gambiae* mosquito survival rate within the ivermectin MDA treatment arm and a subsequent reduction in vectorial capacity in a study conducted in multiple West African countries.²⁸ Additionally, an earlier

²⁴ (Brady et al., 2016)
²⁵ (Chaccour et al., 2013)
²⁶ (Chaccour et al., 2013)
²⁷ (Khaligh et al., 2021)
²⁸ (Alout et al., 2014)
double-blind, placebo controlled trial found a lower likelihood of malaria transmission after a single or repeated dose of IVM at 200 mg/kg, which is at the low end of the dosage spectrum.\textsuperscript{29}

Furthermore, some data suggest ivermectin may be active against the \textit{Plasmodium} parasite as well. Growth-inhibiting effects were observed in different \textit{Plasmodium falciparum} strains, though it appears dosage must be sufficiently high for any significant impact.\textsuperscript{30} While the antiparasitic effects are seemingly minimal, this is an interesting potential dual effect on both vector and parasite for further study. Ivermectin appears to be a promising new form of vector control for multiple reasons. It provides a new means for controlling malaria transmission in the face of demonstrated resistance to other insecticides which as a result are no longer reliable.\textsuperscript{31} It also has the potential to kill both indoor and outdoor-feeding mosquitoes, while IRS and ITNs only target indoor transmission, which is likely insufficient to achieve eradication.\textsuperscript{32} While many malaria interventions are focused on preventing or treating malaria for an individual, ivermectin MDA works through indirect protection by decreasing the odds of being bitten by an infected mosquito. This is achieved through the reduction in size of the mosquito population due to the lethal or impairing effects on a mosquito that feeds on a person taking IVM. While the person bitten is not protected, breaking the cycle of transmission through indirect community protection is a valuable strategy for the control and elimination of malaria.

\textbf{Rationale}

In the face of increasing insecticide resistance that threatens the efficacy of many vector control strategies, novel use of ivermectin through high-dose MDA has the potential to provide a valuable tool to prevent malaria transmission. While prior studies show promising results, further data is needed to evaluate the real-world efficacy of this intervention in endemic regions. In Burkina Faso, SMC is given between 3 to 5 cycles from June and October during the rainy season.\textsuperscript{33} This established infrastructure is useful for the integration of additional drug-based interventions like ivermectin, with delivery to be performed by community health workers as it is with SMC.

\begin{itemize}
\item \textsuperscript{29} (Ouédraogo et al., 2015)
\item \textsuperscript{30} (de Carvalho et al., 2019)
\item \textsuperscript{31} (Chaccour et al., 2013)
\item \textsuperscript{32} (Govella & Ferguson, 2012)
\item \textsuperscript{33} (Malaria Consortium - Malaria Consortium Burkina Faso, 2021)
\end{itemize}
As this use of IVM to kill and shorten the lifespan of mosquitoes is new, it is important to understand how the impact may vary across different *Anopheles* vectors and *Plasmodium* parasite species. Preliminary data from the RIMDAMAL II trial in Burkina Faso indicated a potential disparity in species makeup between treatment arms. It appeared that ivermectin villages had a higher percentage of minor species than control villages. Though this initial observation was not supported by analysis of the remaining samples, it sparked interest in the investigation of the proportion of non-falciparum malaria cases in children in the study villages and the importance of evaluating any heterogeneous impact of ivermectin MDA on species-specific transmission.

Furthermore, there is a gap in the literature regarding the current presence of non-falciparum species. Although *P. falciparum* is the predominant cause of malaria in Sub-Saharan Africa, including Burkina Faso, non-falciparum species have been reported in the region. Because *P. falciparum* is assumed to be virtually the sole contributor to malaria infection here, surveillance and diagnostic testing for non-falciparum species is very limited. As a result, there is limited data on the true prevalence and resulting impact on transmission and overall clinical burden of malaria. It is valuable to describe the *Plasmodium* species diversity detected among infected individuals in the study region to address the knowledge gap.

**METHODS**

**RIMDAMALL II**

Repeat Ivermectin Mass Drug Administrations for MALaria Control II (RIMDAMAL II) is a double-blind, cluster randomized control trial led by Brian Foy, PhD from Colorado State University and Sunil Parikh, MD, MPH from the Yale School of Public Health. The primary objective is to assess the impact of repeated ivermectin mass drug administration in conjunction with the standard provision of SMC on seasonal malaria incidence in children. The primary outcome for this trial is the incidence of malaria episodes in children <10 years of age assessed through active case surveillance.

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34 (Sendor et al., 2023)
35 (Foy et al., 2023)
Study Design

All the data presented in this thesis are sourced from samples collected through RIMDAMAL II which was conducted during consecutive rainy seasons in Burkina Faso from 2019-2020. The study is organized by fourteen villages located throughout southwestern Burkina. Seven villages are assigned to the treatment arm—ivermectin and the other seven make up the placebo arm. Villages are chosen with believed sufficient distance in between to decrease the likelihood of travel of mosquitos. In the active case detection cohort, samples were collected monthly August through November during which a study nurse visited each household and took blood samples from children <10 years. Some individuals also had a baseline sample taken in July prior to administration of the first IVM dose.

Table 1: Village Grouping Summary

<table>
<thead>
<tr>
<th>Ivermectin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG</td>
<td>Bamako-Gourée</td>
</tr>
<tr>
<td>DK</td>
<td>Djinkargo</td>
</tr>
<tr>
<td>DT</td>
<td>Dolo Toundja Djegero</td>
</tr>
<tr>
<td>GB</td>
<td>Goumbori</td>
</tr>
<tr>
<td>KP</td>
<td>Kolepar</td>
</tr>
<tr>
<td>KS</td>
<td>Konsabla</td>
</tr>
<tr>
<td>SG</td>
<td>Segri</td>
</tr>
</tbody>
</table>

Administration of ivermectin does not directly prevent infection for the individual taking the drug, but protection is theoretically garnered indirectly, through a community-wide decrease in exposure to infectious mosquitoes bites. For the intervention to be successful, ivermectin must persist at a sufficient level in the blood of enough members of each treatment village to shorten the lifespan of mosquitoes after taking a blood meal, therefore reducing the population size and limiting the potential for children to be bitten and infected. As a result, children who do not meet
the height inclusion criteria to be given ivermectin should still experience a protective effect through the reduction in overall community transmission.

Ivermectin MDA is an oral regimen of generic ivermectin given in a 3-day course of 300 µg/kg/day each month from July through October. Inclusion criteria specifies individuals who are healthy, not pregnant or newly breastfeeding, and height ≥90 cm. IVM doses are delivered through an existing distribution program for SMC, conducted by community health workers.

Study Setting

RIMDAMAL II was conducted in southwestern Burkina Faso. All 14 villages are located within the Diébougou Health District in the Bougouriba Province, which is in the Sud-Ouest Region of the country. The rainy season typically spans from June through September, with this region receiving much more rainfall than the northern part of the country.36

Figure 3: Malaria prevalence in Burkina Faso among children <5 as determined by microscopy (2017-2018)37


36 (Burkina Faso Climate: Average Weather, Temperature, Rain - Climates to Travel, n.d.)
37 (U.S. President’s Malaria Initiative Burkina Faso Malaria Profile, 2022)
Figure 4: Location of Study Site Diébougou

Subset of Data Used

The active case detection (ACD) cohort consists of children ≤10 years of age from each of the fourteen villages (n=206). Each individual has a set of monthly samples collected throughout the rainy/high-transmission season for both 2019 and 2020 which should ideally total 10 samples across both years (including the July baseline samples). The baseline sample was taken in July of 2019 and 2020, prior to the onset of ivermectin MDA for that transmission season. Samples were then collected monthly August through November, during and right after the period of ivermectin dissemination. Due to logistic complications, not all participants had a complete set of samples collected for each month as planned.

38 (“Bougouriba Province,” 2022)
**Laboratory Protocol**

**DNA Extraction**

Samples were collected in the field in the form of dried blood spots on filter paper. In order to perform DNA extraction in the lab, the Kingfisher Flex (Thermo Fisher Scientific) was used with the Mag-Bind Blood & Tissue DNA HDQ Kit (Omega Bio-Tek). DNA was isolated from two 3mm punches per sample taken using a sterile biopsy punch. During instances when there was not sufficient dried blood spot for two full punches, as much as possible was used and the inconsistency was noted.

**Multiplexed Real Time PCR**

Positive samples in the ACD cohort were identified using a multiplexed real-time PCR assay adapted from Shokoples et al.\(^{39}\) Two rounds of non-nested PCR were conducted with different sets of primers and probes. The first “screening” round used conserved primers and a probe in order to detect all *Plasmodium* species. Original DNA from samples that were *Plasmodium* positive in the screening round was then amplified a second, separate time in the next round for speciation. This speciation step used species-specific forward primers and probes and a conserved reverse primer for a multiplexed reaction. Detection of non-falciparum species in mixed infections is a recognized challenge due to insufficient sensitivity of many existing assays. By using a conserved reverse primer and species-specific forward primers for *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale* (developed prior to the distinction of separate species), the Shokoples reaction improves the ability to detect mixed infections.

**Statistical Analysis**

All analyses were conducted as intention to treat with respect to the ivermectin and placebo arms. Microsoft Excel and R Studio 4.2.1 were used to store and analyze data, as well as produce figures. A logistic regression model adjusted for potential confounders, was used to determine whether there was a significant relationship between malaria infection and treatment arm. This model was chosen to accommodate for the categorical outcome of species. A

\(^{39}\) (Shokoples et al., 2009)
chi-square test was used to evaluate the association between treatment arm and species detected in the positive samples. Small sample size was not a concern because P. vivax was excluded as there were no positives detected, and variations of mixed infections were grouped together.

RESULTS

Insignificant Observed Impact of Ivermectin

A total of 206 children in the ACD cohort were sampled at monthly intervals during the transmission season and over both study years (2018 and 2019). Both sex and age were distributed relatively equally across treatment arms, with the control arm containing a slightly higher proportion of males (54% male to 46% female) (Table 2). The number of blood samples successfully speciated (or found to be negative) using molecular diagnostics varied somewhat between treatment arms, with a total of 837 samples tested in the ivermectin arm and 1084 samples tested in the control arm (Table 2).

The impact of ivermectin MDA on malaria infection among children under 10 years of age in the treatment villages as compared to control villages was assessed by comparing the proportion of samples that were found to be positive for at least one *Plasmodium* spp. among those tested. Because the direct impact of ivermectin MDA on malaria transmission is very complicated to measure, the focus is the primary outcome of malaria infection among children. Overall, molecular testing identified 444 (53%) samples with a positive *Plasmodium* infection among children in the treatment arm, while the remaining 393 (47%) were negative (Table 2). In the placebo arm, which had a slightly higher sample size, 593 (55%) of samples tested positive, and 491 (45%) were negative (Table 2).
Ivermectin did not appear to have an impact on malaria cases in children in the ACD cohort, as there is no reduction in the proportion of *Plasmodium* positives in the ivermectin arm (Table 2). There is no significant difference observed by a logistic regression model controlling for potential confounding variables including “year”, “age”, “sex”, and “month”. The odds of detecting a malaria infection for children in the placebo arm are 12% greater as compared to those in the IVM arm (OR = 1.12, 95% CI [0.701, 1.786]). This slight reduction in odds for children in villages receiving ivermectin MDA is not statistically significant, as the confidence interval contains 1.

Although the amount of infections cannot be statistically compared between villages due to the cluster randomized study design, the difference in the proportion infected is interesting. The highest total proportion of positive samples among those tested per village is in DB (Dangbara), with 66% of all samples testing positive for at least one *Plasmodium* species (Figure 5). On the other extreme, in NB (Niaba), only 38% of all samples tested in that village were

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**Table 2: Description of study sample according to treatment arm (IVM and control)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ivm, N = 838</th>
<th>placebo, N = 1,085</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>410 (49%)</td>
<td>501 (46%)</td>
</tr>
<tr>
<td>M</td>
<td>428 (51%)</td>
<td>584 (54%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>5 (3, 7)</td>
<td>5 (3, 7)</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mixed (pf-pm)</td>
<td>36 (4.3%)</td>
<td>42 (3.9%)</td>
</tr>
<tr>
<td>mixed (pf-po-pm)</td>
<td>4 (0.5%)</td>
<td>8 (0.7%)</td>
</tr>
<tr>
<td>mixed (pf-po)</td>
<td>26 (3.1%)</td>
<td>32 (2.9%)</td>
</tr>
<tr>
<td>neg</td>
<td>393 (47%)</td>
<td>491 (45%)</td>
</tr>
<tr>
<td>pf</td>
<td>378 (45%)</td>
<td>507 (47%)</td>
</tr>
<tr>
<td>pm</td>
<td>0 (0%)</td>
<td>4 (0.4%)</td>
</tr>
</tbody>
</table>

1 n(%); Median (IQR)
positive (Figure 5). Interestingly, both of these villages are assigned to the control arm. This demonstrates a pretty large range of infection burden across villages, and a difference of greater than 20% is observed in both treatment arms.

**Species Distribution Across Treatment Arms**

Additionally, the proportion of mixed infection with both *P. falciparum* and *P. malariae* differs greatly between villages both within and across treatment arms. Village GB (Goumbori) in the treatment arm has the greatest proportion of the *P. falciparum* and *P. malariae* mixed infection at 10% of all samples tested, with MT (Moutori) in the control arm as a close second (Figure 5). Mixed infection with a combination of *P. falciparum*, *P. malariae* and *P. ovale* was only observed in four villages, with KS (Konsabla) in the treatment arm having the highest proportion at 4% (Figure 5). Mono-infections of non-falciparum species are relatively uncommon, with the only *P. ovale* infections detected occurring in BG (Banako-Gourée), an ivermectin village, and TJ (Tedjia), a control village. The only *P. malariae* mono-infections were detected in three placebo villages, with none found in the IVM villages.
When species presence in each positive sample is categorized by percent of each individual species detected, the results are similar. A chi-square goodness of fit test was used to determine whether the proportions of each of the three *Plasmodium* species identified in ACD positive samples varies between treatment arms. Because there were no *P. vivax* species detected in any of the blood samples, the chi-square test was conducted with only *P. falciparum*, *P. malariae*, and *P. ovale*. The proportion of each species present did not not differ by treatment ($X^2 = .31$, df = 2, p= 0.856). *P. malariae* and *P. ovale* were each detected in 5% and 4% of all positive samples respectively (Figure 6).

Similarly to the test for difference between individual species detected, there was no significant relationship observed in a chi-square test for independence between the treatment arm and status of positive samples as a *P. falciparum* mono-infection or mixed-infection with non-falciparum species ($X^2 = .14$, df = 1, p-value = 0.704). Despite the insignificance of *P. falciparum* mono vs. mixed-infection with the treatment arm, the contrast in the number of mono...
and mixed infections between villages is interesting. For example, Figure 7 shows that in placebo village NB (Plot d), there are nearly double the number of negative samples as there are *P. falciparum* mono-infections. However, in placebo village DB (Plot a), the reverse is nearly true, with the amount of negative results almost half that of *P. falciparum* mono-infections (Figure 7, Figure 8). In the treatment arm, villages GB (Plot D) and KS (Plot F) have close to twenty mixed-infections, while KP (Plot E) has virtually none. These discrepancies between villages within the same treatment arm may potentially be obscuring the true relationship between ivermectin and the species causing malaria cases, as well as an understanding of an accurate proportion of the malaria burden each species is responsible for.

**Figure 7: Species characterization of ivermectin villages**

Plot A = BG, Plot B = DK, Plot C = DT, Plot D = GB, Plot E = KP, Plot F = KS, Plot G = SG

*Y-axis limit and scales are not the same*
**DISCUSSION**

**Negligible Impact of Ivermectin**

Despite promising earlier studies, ivermectin did not appear to provide any protection for children under 10 from being infected with malaria. Despite a relatively successful implementation of the ivermectin MDA as planned, there was no reduction in *Plasmodium* positives in villages in the ivermectin arm as compared to those in the placebo arm. While it is very possible that there are confounding factors that are concealing a true effect, further research would be required to support this hypothesis. Some potential confounders consist of differences in the environment, including proximity to mosquito breeding sites. This is in addition to
behaviors of study participants that lead to increased risk/exposure, such as certain children playing outside more, or farther away from the community where they would be more likely to be bitten by mosquitoes not impacted by the ivermectin MDA.

**Diversity of Plasmodium Species Distribution**

The proportion of non-falciparum *Plasmodium* species detected, particularly in a mixed infection with *P. falciparum*, was higher than expected. Non-falciparum species were detected in 9% of infected samples, demonstrating a noteworthy burden that the minor species play in malaria prevalence and transmission. Additionally, mixed infections generally were observed in close to 8% of all samples tested, regardless of treatment arm. With 53% and 55% of positive samples having a mixed infection in the IVM and control arm respectively, it is apparent that mixed infections are contributing a consequential amount. This reinforces the importance of testing for non-falciparum species and improving the reliability of diagnostics for identifying mixed infections. Although there was no difference detected between ivermectin and control treatment arms, the large variation between villages is an interesting finding. There may be factors contributing to these small-scale trends that have not been considered, and further research should be done to investigate. Given the higher proportion of non-falciparum species than expected, additional sampling is needed in order to provide an accurate and current estimate of the prevalence of minor species in Burkina Faso. The heightened level of *P. ovale* infections in particular has potential implications for treatment guidelines regarding use of primaquine to ensure sufficient clearance of hepatocytes for successful treatment.

**Limitations**

Some limitations of this study include the potential impact that the varying number of malaria cases per village might have had on the analysis. The smaller sample size of the non-falciparum species makes having sufficient statistical power to detect significance difficult. Additionally, there were some missing samples from KP (treatment arm) that may have partially contributed to the lower number of samples tested in the ACD cohort. The number of malaria cases in the second year of the trial (2020) was severely impacted by distribution of insecticide treated bednets by the Ministry of Health to individuals within the study area. Furthermore, the nature of repeated monthly sampling may lead to an over-representation of infections if the same
infection in an individual was not successfully cleared out by treatment. With the currently available data, it is difficult to discern whether repeated positive samples in the same individual are representative of the same persistent infection, or new infections as a function of high transmission, as malaria is endemic in Burkina Faso.

The limited sensitivity of diagnostics both RDTs used in the field and molecular assays conducted after the fact in the laboratory. Multiplex real time PCR has a lower sensitivity threshold for detecting the minor species, especially as part of a mixed infection. Additionally, the Ct curves used in conjunction with Ct values from PCR assays to validate *Plasmodium* positives are at times relatively arbitrary. Finally, the quality of the dried blood spot sample was somewhat variable, with some not having enough blood for the two full size punches needed for DNA extraction.

Another limitation is the uncontrollable nature of mosquito travel. There is a possibility of contamination between clusters/villages if there was mixing between mosquitoes living and feeding in ivermectin villages and placebo villages. Additionally, if people receiving ivermectin MDA in treatment villages traveled to placebo villages and were then fed on by mosquitoes there, this would constitute contamination and make any differential effect very difficult to detect. Furthermore, the sheer transmission intensity may have overshadowed a smaller effect size. Even if some mosquitoes were being exposed to ivermectin through bloodmeals and successfully killed, there may have been too many other mosquitoes that immediately took their place for any effect to be measurable. Finally, with such a dynamic study and so many moving parts, it is possible that there are other factors impacting the outcomes that we are not aware of - we don’t know what we don’t know.

**Next steps**

Given the higher prevalence of *P. ovale* cases than expected, it would be valuable to gain a better understanding of which species these truly are, *P. ovale curtisi* and *P. ovalewallikeri*. Additionally, further analysis is currently underway to determine whether both species were even accurately detected by the qPCR primers used in molecular diagnostics. Further investigation of the specific villages with a much higher proportion of minor species detected in positive samples would be valuable to determine if it was a fluke or a regular pattern.
Additional spatial analysis should be done to incorporate environmental factors that might impact malaria transmission. This could be the proximity of households to breeding sites or other mammals such as cattle. Comparison of hyper-local differences in environmental and weather data between study arms would be valuable in order to determine whether this was a confounding factor. Longer term climate data such as yearly rainfall and temperature could be investigated to reveal any relationship between changing climate data and species distribution, particularly with mixed infections.


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