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Faculty of Health Sciences
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Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe.

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF

DOCTOR OF PHILOSOPHY (PHD) IN ENVIRONMENTAL HEALTH

by

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DECLARATION

I declare that this thesis, which I hereby submit for the degree Doctor of Philosophy in Environmental Health at the University of Pretoria's School of Health Systems and Public Health of the Faculty of Health Sciences, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Mulamuli Mpofu

DEDICATION

This thesis is dedicated to my wife Tackler Moyo and my children Kelsie Sipho Bubelebenkosi, and Kayla Nonhle Mpofu.

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TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

Introduction

Successful control of malaria vectors requires the control of the larval and the adult stages. There is currently enough evidence on effectiveness of adult control methods through indoor residual spraying and insecticide treated nets, and these remain the main vector control methods in both Botswana and Zimbabwe. However, the growing resistance to insecticides used for indoor residual spraying and for treating long lasting insecticide treated nets is threatening the successes towards malaria elimination in the two countries. There is therefore need for implementation of other complimentary interventions such as larviciding. Products for implementing larviciding are available but the implementation is affected by insufficient evidence on the effectiveness of this strategy, particularly in rural areas. Both Botswana and Zimbabwe implement larviciding to some extent but the national malaria control programs of the two countries have not quantified the contribution of the intervention towards the overall malaria response. This study was conducted to assess the effectiveness of larviciding in selected rural areas of Botswana and Zimbabwe, particularly on larval density and adult mosquito density.

Materials and Methods

An experimental study was conducted in Molalatau and Mathathane villages of Botswana and Ward 33 (also known as Birchnough Bridge) of Zimbabwe. In Botswana, Mathathane was used as an intervention village while Molalatau was a control village. In Birchnough Bridge of Zimbabwe, the northern side of the ward (also known as Pfupi village) was used as an intervention while the southern part (also known as Tamanikwa village) was used as the control. The two villages in Zimbabwe were separated by the irrigation fields which acted as a buffer. Implementation of the intervention and data collection started at the end of May in Zimbabwe and in July in Botswana. Within both the intervention and control areas, all larval habitats were identified and mapped using portable hand-held geographic positioning system devices. Habitats in the intervention areas were treated using the commercial larvicide VectoBac® (*Bacillus thuringiensis* var. *israelensis [Bti])* through community volunteers identified with the help of the local national malaria control

programs. Every fortnight, larval counts were made from selected breeding habitats in both the intervention and control villages/wards to determine the effectiveness of the larvicide on the larvae. Within the same interval, adult mosquito sampling was conducted using exit window traps to capture mosquitoes which would enter houses for a blood meal and then want to rest outdoors. Pyrethrum spray catches were used to capture indoor resting mosquitoes. All sampled larvae and captured adult mosquitoes were identified to genera. Additionally, interviews were conducted with members of the community to understand their perceptions on effectiveness and acceptability of larviciding. Random-effects Poisson regression was used to compare intervention with control areas with respect to larval and adult mosquito counts. This was done using Stata Release 13, (StataCorp, College Station, TX: StataCorp LP). The incidence rate ratio (IRR) for treatment was of primary importance. Thematic analysis was used to analyse qualitative data from the interviews.

Findings

There was a significant overall effect of 92% and 65% on mosquito larvae in study sites of Botswana and Zimbabwe respectively following the application of larvicide ($p<0.001$). The effect on the early and late larval stages was 77% ($P<0.001$) and 91% ($P<0.001$) respectively for both countries combined. The average marginal effect of larviciding on the mosquito larvae taking interaction with time (period) into account, was -1.94 (95% CI: -2.42 to -1.46) with incidence rate ratio of 0.14, thus, an 86% larval effect attributable to the intervention for both countries combined. The estimated effect on the adult mosquitoes was 70% (IRR=0.303, $p<0.001$) in the intervention areas relative to the control for both countries combined, and was 77% (IRR=0.233, $p<0.001$) and 63% (IRR=0.369, $p<0.001$) for Botswana and Zimbabwe respectively. The volunteers who conducted larviciding also demonstrated to have competency in implementing larviciding. Additionally, larviciding was found to be an acceptable intervention in both countries, and factors influencing acceptability included; its importance as a supplementary method to IRS and LLINs; the desire to be protected from mosquito bites; known effectiveness; as well as willingness to support it. Identified strengths included: ability to kill mosquitoes early; safety; and ease of implementation.

Conclusion

This study demonstrated that larviciding using Bti is an effective vector control intervention in semi-arid rural areas of Botswana and Zimbabwe and the two countries should consider scale-up of the intervention, only in areas that have few, fixed and findable mosquito breeding habitats. Additionally, as part of the broader roll-out, local communities should be mobilised and utilised to ensure sustainability. Implementation should also be accompanied by detailed operational manuals.

THESIS STRUCTURE

This thesis is presented in six chapters that include peer-reviewed articles in various stages (under review to published) originating from this research work.

1. ***Chapter one***: Presents the general introduction of the study and it covers extensive review of literature which guided this study. It includes literature on vector control and specifically on larviciding; and historical implementation and successes of larviciding. The review also separately presents successes of larviciding on larval stages of mosquitoes under laboratory and field conditions; reported impact on adult mosquito and vector density; impacts on malaria incidence; as well as community perceptions on larviciding. This chapter also presents the study rationale and justification, study objectives, and an overview of research methods adopted in addressing each objective.
2. ***Chapter two:*** Presents an original paper published in the Malaria Journal and focuses on the effectiveness of larviciding on the larval stages of the mosquito observed during this study.
3. ***Chapter three***: Presents a manuscript which has been submitted to Plos One and currently awaiting feedback. The manuscript focuses on the observed impacts of larviciding on the adult mosquito populations in Botswana and Zimbabwe.
4. ***Chapter four:*** Presents focuses on paper on community perceptions and opinions on larviciding.
5. ***Chapter five***: Presents competencies of community larviciders, and this chapter is also in the format of a manuscript. The abstract for this chapter was accepted and presented as a poster at the 3rd Malaria Research Conference 2017, Johannesburg, South Africa.
6. ***Chapter Six:*** Presents the overall general discussion of this research, the recommendations, and proposed areas of further research.

CHAPTER ONE

1. GENERAL INTRODUCTION

This chapter introduces the context within which this thesis was focused. It starts by providing some historical background in malaria control and narrowing down to historical and current implementation of larviciding. The background includes a detailed review of literature on the subject area. The literature review highlights some of the most recent successes through laboratory and field trials on larviciding using bio-larvicides and *Bti* in particular. Later in the literature review is a discussion on community perceptions on larviciding as well as their willingness to support the intervention. The last section is focused on some key consideration on larviciding from different authors and the World Health Organisation. Throughout the literature review, is specific reference to typical geographic locations where larviciding trials were conducted and found appropriate, and where it is being recommended.

Later in his section, a justification and/or motivation is provided detailing the rationale behind researching in this subject area including the objectives. Methods that were used to meet the objectives are also detailed in this section.

1.1. LITERATURE REVIEW

1.1.1. Global Malaria trends

In 2017, an estimated 219 million cases of malaria occurred worldwide (203–262 million)[1], an increase of almost two million compared to 2016 and of these, 90% were in the WHO African Region (92%), followed by the South-East Asia Region (5%), and then the Eastern Mediterranean Region (2%). The World Health Organisation (WHO) has targeted malaria for elimination which can be achieved through strengthening of country health systems such as surveillance, diagnosis, case management and vector control[2,3]. Implementation of proven vector control interventions of long lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) is currently at the core of successful malaria vector control[4-6], responsible for reductions in malaria morbidity and mortality. Vector control remains one of the pillars for malaria elimination[7].

LLINs protect their occupants by diverting host-seeking vectors to look for a blood meal elsewhere and by killing those that attempt to feed[8,9]. An estimated 552 million

ITNs were distributed by NMPs globally, with most (459 million or 83%) being delivered in sub-Saharan Africa over the period 2015–2017. Globally, 85% of ITNs were distributed through free mass distribution campaigns, 8% in antenatal care facilities and 4% as part of immunization programmes[1]. in 2017, 50% of the population was protected by this intervention, an increase from 29% in 2010. Furthermore, the percentage of the population with access to an ITN increased from 33% in 2010 to 56% in 2017[1].

IRS provides some small amount of individual house protection by repelling and reducing the number of vectors that come into a house. However, the greatest impact takes place after feeding when the mosquito is more likely to rest on a sprayed surface and pick up a lethal dose of insecticide[10]. IRS coverage has declined rapidly, attributable to cessation of spraying with pyrethroids particularly in the WHO Africa Region[1]. The proportion of the population at risk protected by IRS declined from a peak of 5.7% globally in 2010 to 2.9% in 2016.

1.1.2. Mosquito Biology

Mosquitoes (Order Diptera, Family Culicidae) are some of the most adaptable and successful insects on Earth and are found in some extraordinary places. Virtually any natural or man-made collection of water can support mosquito production. There are than 3,000 species known throughout the world but only a few of these species are important as carriers of disease, but many more are important nuisance species that dramatically affect peoples' quality of life.

While all mosquitoes need standing water to reproduce, different mosquito species are found in different habitats[11]. Some mosquitoes are considered "floodwater" species that breed in temporary water habitats, while others are considered "permanent water" mosquitoes and breed in water sources that remain for long periods of time. Regardless, all mosquitoes undergo the same four-stage life cycle: egg, larva, pupa, and adult, with the larval and pupal stages always being aquatic (**Figure 1.1**).

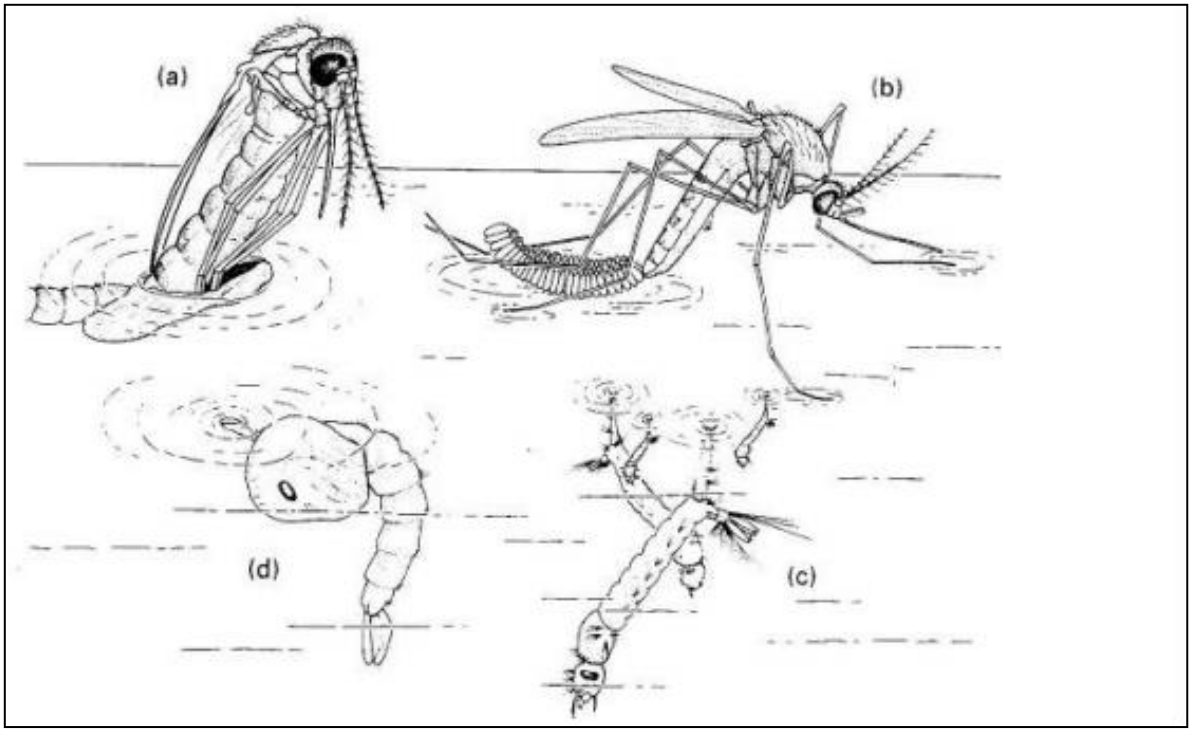

Figure 1.1: Life cycle of *Culex Pipiens.*

(a) Emerging adult **(b)** Female adult ovipositing egg raft on water surface

(c) Representative of each larval instar using siphon to breathe at water surface

(d) Comma-shaped pupae breathing using trumpet at water surface.

Diagram. *Gullan, P.J and Cranston, P.S. 2005. The Insects. 3rd Edition. Blackwell Publishing.*

Mosquito Eggs: The female mosquito lays her eggs either individually or in attached groups called rafts. The eggs are placed either directly on the surface of still water, along its edges, in tree-holes, or in other areas that are prone to flooding from rain, irrigation, or flooding. In some species, the eggs may hatch within a few days of being laid, with the exact amount of time dependent on temperature. But if the egg is laid out of water and is subject to intermittent flooding, the embryo may lay dormant for several years until the ideal natural hatching conditions are met. Mosquitoes frequently overwinter in the egg stage, but some species may also overwinter as larvae or adults.

The Larval Stage: Once the egg hatches, the larval stage begins. The larvae of most mosquito species hang suspended from the water surface because they need air to breath (**Figure 1.2**). An air tube, called a siphon, extends from the larva's posterior to the water surface and acts as a snorkel. Larvae filter feed on aquatic microorganisms near the water's surface. As a defense mechanism, when alarmed, the larvae can dive deeper into the water by swimming in a characteristic "S" motion, which has earned them the nickname "wigglers" or "wrigglers". As they feed, larvae outgrow their exterior covering and form a new exoskeleton, casting off the old ones. The stages between these molts are called instars. The larval stage has four instars. The length of the larval stage ranges from 4 to 14 days, varying with species, water temperature, and food availability.

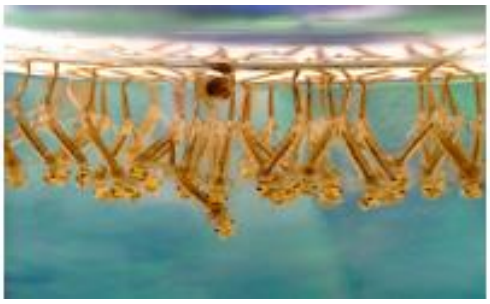

Figure 1.2: *Hanging culex larvae*

The Pupal Stage: In the pupal stage, no feeding occurs, however the pupa must still breathe air at the water's surface and is sensitive to light, shadows, and other disturbances (**Figure 1.3**). Pupae are also physically active and employ a rolling or tumbling action to escape to deeper water, which is why they are commonly referred to as "tumblers". The pupal stage lasts from 1 1/2 to 4 days, after which the pupa's skin splits along the back allowing the newly formed adult to slowly emerge and rest on the surface of the water.

Figure 1.3: *Mosquito Pupal Stage*

Adult Mosquitoes: The male adult mosquito will usually emerge first and will linger near the breeding site, waiting for the females. Mating occurs quickly after emergence due to high adult mortality rates. As much as 30% of the adult population can die per day. The females compensate for this high rate by laying large numbers of eggs to assure the continuation of the species. Male mosquitoes will live only six

or seven days on average, feeding primarily on plant nectars, and do not take blood meals. Females with an adequate food supply can live up to five months or longer, with the average female life span being about six weeks. To nourish and develop her eggs, the female usually must take a blood meal in addition to plant nectars. She locates her victims by the carbon dioxide and other trace chemicals exhaled, and the temperature patterns they produce. Mosquitoes are highly sensitive to several chemicals including carbon dioxide, amino acids, and octenol. The average female mosquito's flight range is between 1 and 10 miles, but some species can travel up to 40 miles before taking a blood meal. After each blood meal, the female will oviposit (lay) her eggs, completing the life cycle. While some species oviposit only once, others may lay eggs several times over the course of their lives.

All stages in the life cycle of a mosquito are dependent upon several environmental factors for their survival and development. Some common and measurable environmental factors, such as wind, light, temperature, rainfall, and humidity, have a known relationship to the survival of mosquitoes and can be used as the basis of an index for use in surveillance and control[12].

1.1.3. Malaria Vectors in Southern Africa

Over 100 anopheline mosquito species can transmit human malaria parasites but there are important differences among these species that influence their role in malaria transmission. Many of these species belong to a sibling complex; a complex is a taxonomic group of morphologically identical, closely related species. In the past, sibling species have been hard to distinguish, and complexes have often been treated as a single entity despite important differences among sibling species. In Africa, *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae* from the Gambiae complex and *Anopheles funestus* from the Funestus subgroup are undoubtedly the most important vectors transmitting both *Plasmodium falciparum* and *Plasmodium vivax* parasites to humans[13,14]. Within the Gambiae complex, *Anopheles melas* and *Anopheles merus* are also considered dominant vectors ("dominant" is defined as a vector species that has been identified as the main, dominant or important vector in at least one region) whereas there is no strong evidence that other species from this complex play any role in malaria transmission[13].

1.1.4. History of Malaria Control

Malaria was endemic in most countries early in the 19th century, affecting about 90% of the world's population[15]. At the 8th World Health Assembly of 1955, the Global Malaria Eradication Campaign was launched for all malarias countries except Madagascar and sub-Saharan Africa, with indoor residual spraying (IRS) primarily with DDT being recommended as the main vector control intervention[15-17]. The campaign led to the elimination of malaria in 37 of the 143 countries by 1978, with 27 of these being in Europe and the Americas. However, significant decreases in morbidity and mortality was archived in all other countries[15, 18] though the emphasis was changed to long-term integrated control programmes because of the realisation that a 'time-limited eradication program was impractical'[19]. During the same period, the following were set as priorities for countries pursuing malaria elimination[20]:

1. Identification and treatment of malaria patients and all people carrying parasites, including those carrying gametocytes, ensuring that they become non-infectious as early as possible; and to
2. Sustainably reduce human–vector contact and the vectoral capacity of the local anopheles mosquito population to prevent new infections from occurring.

1.1.5. Malaria vector control and integrated vector management

Vector control is defined as measures of any kind directed against a vector of disease and intended to limit its ability to transmit the disease[21]. On the other hand, integrated vector management (IVM) is a rational decision-making process to optimize the use of resources for vector control, by making vector control more efficient, cost effective, ecologically sound and sustainable[22, 23]. The WHO has for long, advocated for integrated vector management which is cost effective. More than one and a half decades ago, Konradsen *et al.*[24] reported that much remained unknown about the impact of a fully developed IVM programme on malaria transmission; while Keiser *et al*.[25]and Utzinger *et al.*[26] viewed IVM as an approach with great promise for disease control in Africa. All this was despite earlier reports of the 1970s by Bang *et al*.[27] that programmes with IVM elements have historically brought about significant reductions in vector populations and malaria transmission across a range of transmission settings. However, a report by *Chanda et al.*[28] in

2008, described how a comprehensive and highly successful IVM program implemented over a relatively short time period, expanded coverage of vector control interventions and leveraged additional resources to build national capacity to the point where they have successfully reduced malaria-related morbidity and mortality. Their conclusion was that the successful implementation of IVM and integrated malaria control in Zambia serves as a prominent success story for all of Africa. This was consistent with earlier reports by Killen *et al.*[29] that there is evidence that IVM can complement other existing malaria control strategies (ITN use, access to effective treatment) by avoiding reliance on any single intervention to reduce the burden of malaria.

By re-orientating to IVM, vector control programmes will make changes in roles, responsibilities and organizational links making them better able to meet the growing challenges in the control of malaria and other vector-borne diseases in the face of dwindling public sector human and financial resources.[23] Key elements of an integrated vector management (IVM) strategy are [23]:

1. ***Advocacy, social mobilization & legislation: -*** Promotion and embedding of IVM principles in designing policies in all legislation relevant agencies, organizations and civil society; establishment or strengthening of regulatory and legislative controls for public health; and empowerment of communities.
2. ***Collaboration within the health sector and with other sectors***: - Consideration of all options for collaboration within and between public sector and with other sectors and private sectors; application of the principles of subsidiarity in planning and decision-making; strengthening channels of communication among policy-makers, vector-borne disease programme managers and other IVM partners.
3. ***Integrated approach: -*** Ensure rational use of available resources by addressing several diseases, integrating non-chemical and chemical vector control methods and integrating with other disease control methods
4. ***Evidence-based decision making: -*** Adaptation of strategies and interventions to local ecology, epidemiology decision--making and resources, guided by operational research and subject to routine monitoring and evaluation
5. ***Capacity-building: -*** Provision of the essential material infrastructure, financial and human resources at national and local level to manage IVM strategies based

on a situational analysis knowledge about the vectors, diseases and disease determinants.

Vector control is one of the four basic technical elements of the global malaria control strategy (GMCS) because it remains the most generally effective measure to prevent malaria transmission[30] and remains a key component of the three pillars of the Global Technical Strategy for Malaria: 2016-2030[7]. Malaria vector interventions using only ITNs and/or IRS successfully reduced transmission intensity and the burden of malaria in many situations[22], but these interventions alone are no longer expected to achieve those critical low levels that result in malaria elimination. To meet this need and to ensure sustainability of control efforts, malaria control programmes are encouraged to strengthen their capacity to use data for decision-making with respect to evaluation of current vector control programmes, employment of additional vector control tools in conjunction with ITN/IRS tactics, case-detection and treatment strategies, and determine how much and what types of vector control and interdisciplinary inputs are required to achieve malaria elimination[22]. Despite these efforts, integrated management of malaria vectors in African countries such as Uganda remains an underdeveloped component of malaria control policy, requiring cooperation between the health and other sectors[31]. Malaria vector control, targeting both larval and adult mosquitoes has lately received a lot of attention because of its potential in control and elimination of malaria[5, 6]. The interest in larval control led the WHO to issue an *Interim Position Statement on Larviciding in Sub-Saharan Africa* in 2012[4]; and subsequently developed and launched the *Larval Source Management (LSM) guidelines* in 2013[32].

1.1.6. Insecticide resistance in malaria control

There has been a growing resistance to insecticides used in IRS and for treating LLINs[33, 34], the frontline malaria vector control interventions. Pyrethroids remain the main vector control insecticides used in most countries in Sub-Saharan Africa. Other available insecticides used include organochlorines, carbamates, and organophosphates in form of Fenithrothion, Malathion, and Priphos-methyl[35]. In May 2012, WHO and Roll Back Malaria (RBM) released the *Global Plan for Insecticide Resistance Management in malaria vectors*[36] to help countries monitor and manage insecticide resistance. Resistance to at least one insecticide used for malaria control

was reported by 68 countries in 2017, an increase over 2016 due to improved reporting and three new countries reporting on resistance for the first time[1]. In total, 80 malaria endemic countries had provided insecticide resistance monitoring data[1]. The same year, resistance to two or more insecticide classes was reported in 57 countries, with pyrethroid resistance being widespread and detected in at least one malaria vector in more than two thirds of the sites. Resistance to organochlorines was detected for at least one malaria vector in almost two thirds of the sites and was highest in the WHO South-East Asia Region. Resistance to carbamates and organophosphates was less prevalent and was detected in 33% and 27% of the tested sites, respectively.

Resistance to insecticides is widespread in *Anopheles* mosquitoes in sub-Saharan Africa, especially to pyrethroids which are used on all long-lasting insecticidal nets. Mathematical models predict this drop in effectiveness could lead to increased malaria incidence [37]. However, little evidence has been reported of an epidemiological effect resulting from resistance. One of the known examples of control failure due to resistance was in KwaZulu-Natal, South Africa in the late 1990s, where despite good indoor residual spraying coverage, a ten-times increase in malaria cases was reversed when pyrethroid spraying was replaced with dichlorodiphenyltrichloroethane (DDT) spraying in response to reported pyrethroid resistance in a local malaria vector, *An. funestus*[38]. This apparent control failure was with indoor residual spraying, not insecticide-treated nets, and could have been confounded by other factors.

Studies from Malawi, Kenya and Sudan have showed that insecticide-treated nets can provide protection against malaria infection in areas with substantial amounts of pyrethroid resistance[37].

1.1.7. Larval Source Management in malaria vector control

The elimination of malaria vector larval habitats can be a cost-effective and long-term solution [39-41] in well-defined settings where it is feasible. Unlike LLINs and IRS, which target the adult mosquito vector, LSM targets the immature, aquatic stages of the mosquito (the larvae and pupae), thereby reducing the emergence and abundance of adult vectors. There are four types of LSM:

1. Habitat modification: a permanent alteration to the environment, e.g. land reclamation;
2. Habitat manipulation: a recurrent activity, e.g. flushing of streams;
3. Larviciding: the regular application of biological or chemical insecticides to water bodies
4. Biological control: the introduction of natural predators into water bodies[32].

While LLINs and IRS remain the backbone of malaria vector control, larval source management is recommended as a complimentary strategy in Africa[40,42-50]. Elimination of all potential breeding sites has been found to reduce the number of infective bites per person per year (the Entomological Inoculation Rate [EIR]), thereby reducing malaria transmission[51,52], with field trials having shown that larviciding can reduce the density of adult vectors and consequently malaria transmission and morbidity[44, 45, 53]. However, there are also other field trials where it has been shown that LSM does not work, for instance in areas with extensive flooding[46].

Mosquito larval control is cost-effective in areas where larval habitats are well-defined possibly seasonal or relatively few, where habitats are accessible by ground crews, and in cooler parts where larval development is prolonged[30,54]. These conditions occur frequently in sub-Saharan Africa and are common in urban environments, desert fringe communities[6], highland settlements and rural areas with high population densities. However, there are instances where larval control and larviciding have been successful and should be considered as part of vector control. Castro *et al*.[55] described a successful intervention in Dar es Salaam, Tanzania, from the late 1980's through the 1990's while Gilroy *et al.*[56] reported decreases in malaria incidence and sporozoite prevalence rates in Nigeria using a variety of larval source management techniques. In coastal flood plains of Haiti, Schliessmann *et al.*[57] used a combination of water drainage techniques and larviciding to reduce the number of malaria cases by 98% as early as 1969.

1.1.8. Larviciding for malaria vector control

Larviciding is a form of LSM which involves the regular application of biological or chemical insecticides to water bodies[32]. The different classes of larvicides include the following:

- ***Surface oils and films,*** e.g. highly refined oils and biodegradable ethoxylated alcohol surfactants, or "monomolecular films" (MMF) that suffocate larvae and pupae;
- ***Synthetic organic chemicals***, e.g. organophosphates that interfere with the nervous system of immature larval stages, such as chlorpyrifos, fenthion, pirimiphos-methyl and temephos;
- ***Bacteria,*** e.g. Bacillus thuringiensis subsp. israelensis (Bti), and Bacillus sphaericus (Bs) that produce insecticidal crystal proteins which, when ingested by larvae, attack the gut lining causing cessation of feeding and subsequent mortality;
- ***Spinosyns,*** e.g. metabolites extracted from the bacterium Saccharopolyspora spinosa, that act as nicotinic acetylcholine receptor (nAchR) allosteric activators and can cause mortality through both contact and ingestion***;***
- ***Insect growth regulators***, e.g. diflubenzuron, methoprene, novaluron and pyriproxyfen that prevent emergence of adults from the pupal stage

Each of the different classes of larvicides listed above have varying advantages and disadvantages. Table 1.1 shows the advantages and disadvantages of the different forms of larvicides.

Table 1.1: Advantages and disadvantages of different types of larvicides

Larvicide Type	Advantages	Disadvantages
Surface oils and films	• The oil is visible on the water surface and so it is possible to see whether it has been applied properly. • For small surfaces such as borrow-pits, pools, latrines, drains and soakaway pits, it is a relatively cheap method and easy to apply. • Mosquitos cannot develop resistance against this method.	• For large surfaces the method is costly. • It is not very effective in the presence of vegetation and floating debris, which therefore has to be removed before the oil is applied. • The effect usually lasts only a few days. • The oil coats vegetation, tree trunks

	• At recommended dosages there is no toxicity to mammals, fish and most other non-target organisms.	and so on.
Synthetic organic chemicals	• Operations can be carried out quickly; • Larvicides can be applied by hand for small-scale treatments; • For large-scale treatments, agricultural sprayers or IRS hand-compression spray pumps may be used.	• Control is temporary and frequent reapplication may be required; • Some larvicides are harmful to non-target organisms including the natural predators of larvae; • Larvicides may be toxic to humans, therefore precautions are necessary
Spinosyns	• Operations can be carried out quickly; • Harmless to fish, birds, mammals and humans at the recommended doses; • Relatively safe for use in multiple habitats including drinking water and on irrigated crops; • Effective where mosquitoes have developed resistance to synthetic chemical larvicides	• Product can be used to also control agricultural pests; • Not as target-specific as bacterial larvicides; • Toxic to non-target aquatic invertebrates as well as other beneficial arthropods (e.g. bees)
Bacterial larvicides	• Operations can be carried out quickly; • Harmless to other insects, fish, birds, mammals and humans at the recommended doses; • Safe for use in multiple habitats including drinking water and on irrigated crops; • Effective where mosquitoes have developed resistance to synthetic chemical larvicides; • Extensive bacterial larvicide formulation options allow for various efficacy and residual objectives at the IVM programme level	• The window of time for application is narrower, relative to that for synthetic chemicals; • Larvae must be feeding when the bacterial larvicide is present for it to be effective (for mosquitoes, this is the 1st to the middle 4th instar; very late 4th instar larvae cease feeding as they prepare for pupation); • In open, natural habitats, Bti breaks down quickly in the environment, so more frequent applications may be needed.
Insect growth regulators	• Operations can be carried out quickly; • Long-lasting residual impact from 2 weeks up to 6 months in specific habitats reduces re-treatment cycles; • Highly effective at extremely low dosages; • Relatively safe for use in drinking water and irrigated crops that have been treated, can be safely eaten • Effective where mosquitoes have developed resistance to synthetic chemical larvicides;	• High dosages (e.g. when accidentally overdosed for mosquito control) can be toxic to the immature aquatic stages of some non-target insects and to some crustaceans; • The impact of the treatment with hormone mimics is very difficult to monitor for the immature stages because larvae develop normally, and the impact can only be observed after evaluating adult emergence from

	• Very low toxicity to mammals, birds, fish and adult insects.	pupae; monitoring systems therefore need to be set up for IGRs than for other larvicides that kill larvae within 48h.

Large-scale larviciding was the main vector malaria vector control intervention in the early 20th century and was disbanded in favour of IRS with dichloro-diphenyl-trichloroethane (DDT) despite being a highly effective method of malaria control[58-61]. Before the discovery of DDT, controlling anopheline vectors was mainly directed towards the larval stage with high level of community participation needed to ensure slow but often sustainable progress[30]. This was successful in countries such as Brazil and Egypt[62-65]. In each situation, the solution of a local malaria problem required an in-depth study by a multi-disciplinary team to design a multi-sectoral programme, often including environmental sanitation, modification or manipulation, the use of larvivorous fish as predators, petroleum oils and Paris green[30]. However, despite these documented successes, there have been arguments that these examples are misleading because *An. gambiae* had colonized areas to which it was not well adapted[66].

As malaria declines in many African countries, there is a growing realization that new interventions need to be added to the front-line vector control tools of LLINs and IRS that target adult mosquitoes indoors. Many argue that LSM is not feasible in Africa due to the high number of small and temporary larval habitats for *An. gambiae* that are difficult to find and treat promptly[58].

1.1.9. Safety of bio-larvicides for malaria control

Bacillus thiringiensis var Israelensis (Bti) products are available for vector control and do not pose any hazard to humans or other vertebrates or to the great majority of non-target invertebrates owing to their specific nature[67]. *Bti* products are safely used for the control of insect pests of agricultural and horticultural crops as well as forests, and they are also safe for use in aquatic environments including drinking-water reservoirs for the control of mosquito, black fly and nuisance insect larvae. *Bti* has not been observed to adversely affect birds, fish or any other non-target aquatic vertebrates tested in a large number of laboratory and field studies. The field application of *Bti* can result in considerable aerosol and dermal exposure of workers

and result in contamination of potable water and food. With the exception of case reports on ocular and dermal irritation, no adverse health effects have been documented after occupational exposure, and human volunteers have ingested and inhaled large quantities of a *Bti* formulation but experienced no adverse health effects. Antibody titers to the vegetative cells, spores and spore-crystal complexes have been demonstrated in workers who spray *Bti* products; however, no adverse health effects were reported. There have been some case reports on the occurrence of *Bti* in patients with different infectious diseases. However, none of these studies unequivocally demonstrates an actual risk to human health from the use of Bti. Additionally, *Bti* has not been reported to cause adverse effects on human health when present in drinking-water or food[67].

1.1.10. Effectiveness of larviciding on larval densities

Anopheles larvae are considered 'sitting ducks'; because of their relatively immobile characteristic and accessibility, argues Fillinger et al[58]. Unlike adult mosquitoes, they cannot change their habitat to avoid control activities and targeting the larval stages, mosquitoes are killed '*wholesale*' before they disperse to human habitations[58]. In view of the increasing resistance to insecticides used for the frontline interventions of LLINs and ITNs, larval control through larviciding has lately gained attention. The WHO, recognising the growing importance of larviciding recommended larviciding as a supplementary intervention in 2013[32].

Larviciding, using biological larvicides has shown effectiveness in reducing larval density under laboratory and field conditions. As early as 1999, trials in Germany with *Bti WG* implemented with minimum dosages caused 100% mortality within 24–48 hours after application under laboratory assays[68]. Similarly, 100% larval mortality within 24 hours was observed in Kenya, with *Bti* demonstrating a residual effect lasting up to 11 days. Not a single larva survived during this period[48]. In a separate study, *Bti* formulations were found more effective against larvae of Aedes and Culex species than Anopheles spp. Among the two anopheline species tested in the laboratory, *An. stephensi* was more susceptible than *An. culicifacies* to different *Bti* formulations[69]. However, *A. gambiae* the common vectors in sub-Saharan Africa had in earlier years been found to have a high sensitivity to *Bti* formulation when compared with the data from tests in polluted water and targeting Culex[70, 71].

Seyoum and Abate[72] also found comparable sensitivities for *An. arabiensis*. In particular, late instars were found susceptible to *Bti* in malaria endemic areas [72], an observation which was seen as promising and encouraging for malaria vector control.

During field trials in rural highlands of Kenya, Fillinger *at al.* used a Bti commercial product VectorBac and reduced larval density by 95% when compared with pre-intervention levels and 97% when compared with post-intervention levels[73], a promising result towards consideration of larviciding for vector control. The same study, showed a similar sharp decline in the prevalence of late instar larvae in aquatic habitats resulting in a corresponding decline in abundance of adult vectors[73]. Still in East Africa, Fillinger *at al.* reduced overall anopheline larval abundance by 96% using *Bti* in the intervention wards compared to controls in urban Dar es Salem, Tanzania, which consequently resulted in a significant reductions of malaria transmission by *An. gambiae s.l.*[45].

In 2009, larviciding was associated with a 91.1% reduction in the mean number of late instar anopheline larvae in Kenya[6], with reported reduction of late instar densities also reported as high as 99%[73]. Suppression of late instars and the resulting pupae from open field trials was achieved with low dosages of 200 g/ha (2700 ITU/mg) using *Bti WDG*. Such low application dosages offered the possibility of keeping operational costs low even if weekly treatments, caused by the absence of residual activity, have to be considered[48]. After 24 hours exposure of third instar larvae of *An. gambiae s.s*. to *Bti WDG* concentrations of 0.039 mg/l (117 ITU/l) and 0.132 mg/l (396 ITU/l 50 and 95% of the larvae were killed respectively[74]. Reduced late instar densities were recorded eight to ten days after application despite only statistically significant up to day five. Late instar larvae and pupae developed in increasing numbers five to six days after *Bti* application, demonstrating a low residual effect. However, weekly treatment intervals are believed to reduce pupae production by 64–94%[74].

1.1.11. Effectiveness of larviciding on malaria vector densities

The effect of larviciding on the larval stages of the vector mosquitoes has a corresponding effect on the adult mosquito population, and in 2009, Fillinger *at al*[6]. found that it was associated with an 85.9% reduction in adult mosquitoes resting

indoors in Kenyan highlands comparing vector densities at pre-intervention, during intervention and after the intervention. Additionally, there was a 92% reduction in the density of blood-fed malaria vectors per person (resting inside houses during the intervention period compared with the pre-intervention period. Despite the observed effectiveness in reduction of vector densities, the population of adult vectors returned to pre-treatment levels following increased rainfall, approximately 8 weeks after the last treatment[73].

The regulation of larval survival in breeding sites through larviciding and other larval source management methods is considered the primary ecological factor controlling *An. gambiae s.l.*[75]. As early as 1995, Kroeger *et al.*[76] in a study where there was application of *Bti* on a weekly basis over a period of 7 – 10 weeks, a reduction of *Anopheles* adult density (bites per person per hour on human baits) by an average of 70% and up to 50% in Peru and Ecuador was observed. In Tanzania, larviciding substantially suppressed annual mean densities of both secondary vectors in Dar es Salaam, namely *An. Funestus* and *An. coustani*, although no significant suppression of the primary vector *An. Gambiae* was observed over the course of the year. Total entomological inoculation rate calculated from the combined annual mean densities and sporozoite prevalence of all three malaria vectors was reduced by 32%[77].

Larviciding using biological larvicides is currently not used exclusive of other vector control interventions of IRS and LLINs. However, White *at al.*[75] have modelled the impact of *Bti* in larval breeding sites where mosquito larvae and pupae experience increased mortality, and they estimated that that an 88% reduction in the number of observable larvae corresponds to a 99% reduction in the number of pupae emerging as adults, as the observed larvae are young and unlikely to survive until pupation[75].

1.1.12. Effect of larviciding on human malaria cases

Larval control of vector mosquitoes was used to eliminate malaria in the early 20th century, and this experience was successful repeated in supressing malaria in Egypt and around a Zambian copper mine[60]. There is a correlation between larval density, adult vector density and overall malaria transmission. In Tanzania, Fillinger *et al.*[45] found that overall anopheline larval abundance reduction of 96% in the intervention areas using *Bti* resulted in a significant reduction of 31% of malaria transmission by

An. gambiae s.l. Furthermore, analyses of parasitological surveys showed that the larviciding was associated with an overall reduction of 40% ($p < 0.001$) of *P. falciparum* infection prevalence in the study population and that the highest impact was achieved during the dry season of 2006[45].

In one cluster-RCT from Sri Lanka, larviciding reduced parasite prevalence by almost 90%, while five other controlled before-and-after trials in Greece, India, the Philippines, and Tanzania, LSM resulted in an average reduction in parasite prevalence of around two-thirds. The interventions in these five trials included dam construction to reduce larval habitats, flushing of streams, removal of domestic water containers, and larviciding. The larviciding trials conducted in Tanzania were community-based, but centrally managed by the urban malaria control program (UMCP) of Dar-es-Salam. The intervention archived maximum effectiveness during the dry season and had synergistic effects with other protective measures such as use of LLINs, houses with window screens, and houses with complete ceilings[78]. In the randomized cross-over trial in the flood plains of the Gambia River, larviciding by ground teams did not significantly reduce parasite prevalence[79].

For larviciding to be effective in reduction of malaria incidence, it should be implemented at the right time. In Kenya, Minakawa *et al.* reported that larviciding showed to be more effective at lowering the prevalence of malaria infection during the dry season than during the rainy season[40]. This was particularly an interesting result since 49% of malaria cases were sampled during the dry season but remains a key finding and highlights one of the key aspects of successful larviciding programs consistent with the recommendation that the intervention should be implemented in areas where breeding sites are few, fixed and findable. However, in Kenya, Fillinger *at el.*, were able to report declines in incidence of new infections during long rains due to larviciding implemented together with LLINs[6].

The WHO recently developed the global vector control response 2016-2030[80] which proposes a vector response framework (Figure 1.4) whose aim is to reduce the burden and threat of vector-borne diseases through effective locally adapted sustainable vector control. This framework applies to malaria as it applies to all other vector-borne diseases, calling for improved public health entomology (and malacology) capacity and capability, a well-defined national research agenda, better

coordination within and between sectors, community involvement in vector control, strengthened monitoring systems and novel interventions with proven effectiveness.

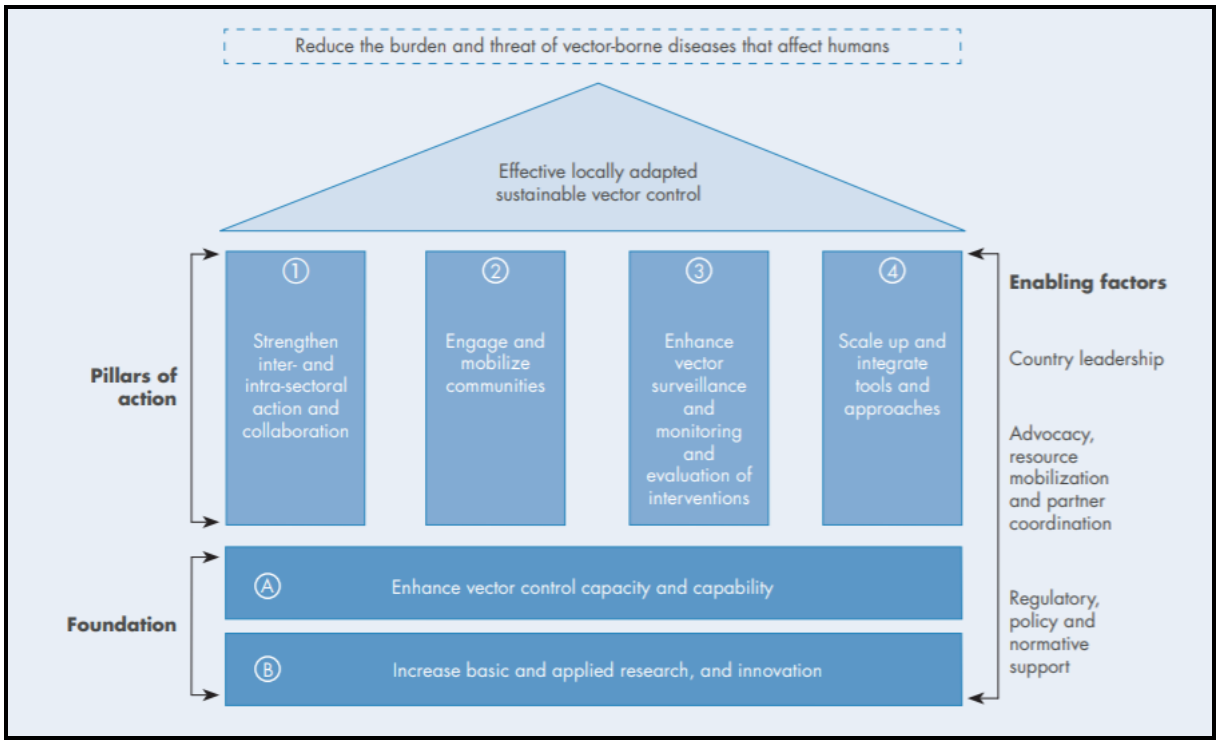

Figure 1.4: Malaria vector control response framework. *Source: WHO, 2017*[80]

The four pillars of success proposed in the framework include strengthening inter and intra-sectoral action and collaboration; engaging and mobilising communities; enhancing vector surveillance and monitoring and evaluation interventions and scaling up and integrating tools and approaches.

1.1.13. Acceptability of larviciding

The success of larviciding is dependent on its feasibility, acceptance and positive perceptions from the population, and this has been demonstrated during recent field trials in sub-Saharan Africa. During a cross-sectional malaria survey in Western Kenya where malaria prevalence is moderate (3.2–6.5%), Imbahale *et al.*[81] found that the residents perceived malaria as their major health risk, with 68% of all respondents fully knowing that mosquitoes are responsible for the transmission of malaria. An additional 67% knew about mosquito breeding and that such sites could be found close to their homes though correct knowledge of habitat characteristics was poor. Man-made pools, drainage channels and burrow pits were rarely

mentioned as potential breeding sites for malaria mosquitoes which were later acknowledged after explaining. While larval source management was the most frequently mentioned as a tool for malaria control, less than 5% practiced it, indicating the low level of utilisation of the intervention at household level[81].

During the evaluation of community acceptability of larviciding through surveys, focus group discussions and in-depth interviews in a rural district in East-central Tanzania, Mboera *et al.*[82] found that larviciding safety was well trusted by as much as 73% of the respondents, while at least 90% trusted that the intervention can reduce mosquitoes and malaria infection. In general, larviciding was acceptable though the community members stressed the importance of sensitization before its implementation, with more than 73% indicating a willingness to make a contribution towards larviciding[82].

A study of community awareness of mosquitos and related subjects in Dar es Salaam and Tanga in Tanzania, Stephens *et al.*[83] found that residents were well aware of mosquitos, and all claimed to use some form of domestic mosquito control product for their personal protection. The residents could not separate the problems of nuisance-biting and malaria transmission, and the persistence of nuisance-biting made residents sceptical and dissatisfied with vector control interventions making the residents' priorities evidently not the same as those of the health authorities. While larviciding was being appreciated, they wanted it implemented in all kinds of standing water, some of which are of little importance for mosquitos of any kind such as water associated with rubbish dumps[83]. Still in Tanzania, the implementation of larviciding through local community-based staff was found to have led to high community acceptance and support[45].

In another study that included focus group discussions and in-depth interviews in wester Kenya, Matuku *et al*[84] reported the extent of knowledge of the village residents of larval habitats, mosquito sources in the local environment, and what might be done to prevent mosquito breeding. While the residents did not associate specific habitats with anopheline larvae, they expressed reluctance to eliminate water habitats because they were sources of domestic water supply, though they indicated willingness to participate in a source reduction program if support were available[84].

1.1.14. Key considerations for larviciding

During implementation of larviciding interventions, making it community based and involving the local health committees in recruiting individual program staff is required to optimize community participation, particularly to improve access to fenced compounds. A simpler, more direct, less extensive community-based surveillance system in the hands of a few, less burdened, better paid and maintained program personnel may improve performance and data quality[85]. One example of an important innovative approach for involving the community is reported by van den Berg[86], and is a participatory education tool known as the "Farmer Field Schools", which makes the connections between health, vector-borne disease control and agricultural productivity. Work by Maheu-Giroux and M.C. Castro [78] has important implications for malaria control in sub-Saharan Africa as it provides evidence that a community-based application of microbial larvicides was effective in reducing malaria transmission in urban Dar-es-Salaam. The bio-larvicides are specific in action and highly effective in killing Anopheles larvae under field conditions a demonstration of environmental safety reported by Majambere *et al*, Fillinger *et al*, and van de Berg *et al.*[46, 48, 87].

From the Kenyan study, Fillinger and Lindsay[78] shared the following lessons learnt for consideration when implementing larviciding:

i. Breeding habitats can, and should, be mapped at high resolution using low-cost technology
ii. Locally relevant entomological information should be collected to inform operational activities,
iii. Monitoring and evaluation systems should be implemented to ensure effective and appropriate delivery and fine-tuning of interventions, and
iv. Community involvement and sensitization can be beneficial to programmatic activities. Other strategies included in an IVM approach could facilitate the use of larviciding. In this context, the use of environmental management can reduce the area to be covered with larviciding e.g in urban areas, restoring the functionality of drains would result in fewer breeding habitats.

The WHO has supported the integration of larviciding into vector control and has provided guidance[32]. One of its key recommendations is that additional research still

needs to be conducted to support large scale-up of larviciding. Another WHO sanctioned systematic literature review conducted by Tusting *et al.*[79] was also inconclusive but, recommended that additional research around larviciding still needs to be done. However, Maheu-Giroux, M. and M.C. Castro[78] still content that the projected increases in urban population in sub-Saharan Africa, the behavioural adaptation of vector mosquitoes to current control strategies, and the already recorded emergence of resistance to pyrethroid insecticides justifies the consideration of larval source management, and larviciding in particular by malaria control program[78]. Additionally, Maheu-Giroux, M. and M.C. Castro have demonstrated that larviciding is a cost-effective intervention[88].

1.2. STUDY RATIONALE, JUSTIFICATION AND MOTIVATION

1.2.1. Rationale

With the detection of resistance to pyrethroids[89] there is a demand for alternative technology and products to help in the control and subsequent elimination of malaria. Pyrethrum based insecticides are currently widely used in indoor household residual spraying and for treating nets. Additionally, behavioural adaptation of adult mosquito vectors gives them the ability to avoid LLINs and walls treated through IRS[90,91]. Larval stages of mosquitoes are of relatively low mobility compared with flying adults and interventions like larviciding will help in malaria control because it will target the less mobile stages of the mosquitoes. However, larviciding interventions should be guided by scientific evidence on effectiveness which is currently lacking, and this study was well timed to provide information on the effectiveness of larviciding in rural areas.

There are arguments that LSM is not feasible in Africa due to the high number of small and temporary larval habitats which are difficult to find and treat promptly[5], and a lack of rigorous evaluation in areas with extensive habitats[92]. Although the WHO has recommended larviciding, it identifies it as a useful supplement to core interventions of LLINs and IRS only in some specific locations, where breeding sites are ‘few, fixed and findable’; where the density of the human population to be protected is sufficiently high to justify the necessary resources[32]; areas of low to medium transmission intensity; and areas of focal transmission or epidemic prone areas[5], while research to determine field effectiveness are conducted. Such

conditions are common in urban environments, desert fringe communities, highland settlements and rural areas with high population densities. A 2013 Cochrane review, while proposing larviciding as a policy option, also recommended additional research to be conducted to determine its effectiveness in rural areas and its role in malaria elimination[54]. The Malaria Policy and Advocacy Committee (MPAC) also recognizes the existence of research gaps, and that it may be some time before there is sufficient evidence for a comprehensive policy statement on LSM[33].

1.2.2. Justification

There are well-documented accounts of successful larviciding programmes, and there are also numerous examples of it failing in situations where the intervention was incorrectly applied or applied in inappropriate ecological settings, resulting in a waste of resources[32]. Large-scale larviciding was a highly effective method of malaria control in the first half of the twentieth century, but was largely disbanded in favour of IRS with DDT[5,93]. The complete eradication of accidentally introduced *An. Gambiae* from the north east coast of Brazil[94] and the Nile Valley of Egypt[95] six decades ago, are campaigns that were executed almost exclusively by ruthless, well-managed larval control[94,95].

Recent evidence of larviciding effectiveness presents an opportunity in sub-Saharan Africa, particularly in urban areas because of the rapidly growing urban centres[96]. Studies have shown effectiveness of larviciding in urban areas[78,93,96], highlands[97], when combined with LLINs[4-6], and where there is the opportunity to eliminate all or a large proportion of the breeding sites with little effort[4,33]. However, none of these studies were in semi-arid regions of Sub-Saharan Africa.

Reports received from national programmes indicate that 27 malaria-endemic countries worldwide use larval control in certain specific foci of malaria transmission[33]. In 2011, nine countries reported activities involving habitat manipulation (temporary changes to vector habitats) and nine reported some form of habitat modification (long-lasting physical transformations to reduce vector larval habitats). Larval control through chemical larviciding was reported by 16 countries, while 13 reported biological larviciding activities[33]. Botswana and Zimbabwe are some of the countries where larviciding is recommended as a policy option. Reports from these countries give an indication of the range of larval control methods

employed, but the scale of efforts are not quantified and the impact on individual country malaria burden is not easily measured. Through this study, national malaria control programs of Botswana and Zimbabwe will get an understanding of the effectiveness of this intervention in malaria control.

This study will lead the scientific advancement and generation of knowledge on the effectiveness of larviciding in semi-arid regions of Sub-Saharan Africa. These areas in Botswana and in Zimbabwe also happen to have advanced towards malaria elimination and requiring additional malaria tools and interventions to compliment the front-line vector control interventions of LLINs and IRS. The host national malaria control programs of Botswana and Zimbabwe will benefit by understanding the impact and effectiveness of community-based larviciding which is more sustainable considering the labour-intensive nature of the intervention. The study provided recommendations which can be used to guide policy development and advocacy for the intervention in Botswana and Zimbabwe, potentially leading to improvements in funding. The study villages continued to receive all the other vector control interventions of LLINs and indoor residual spraying as implemented by the national malaria control program. However, the intervention villages/wards also benefited from larviciding.

1.2.3. Motivation

This study was conducted to provide evidence on the effectiveness of larviciding using *Bti* in selected rural areas of Sub Saharan countries of Botswana and Zimbabwe. Such information will help in the enrichment and completion of the WHO manual on larval source management which was not conclusive because of insufficient evidence. Since studies have also shown that the costs of larviciding compare favourably with those of IRS and LLINs[98], this study will seek to guide policy on recognition and implementation of larviciding consistent with the identified effectiveness.

Research on other potential malaria control interventions has not been successful. These include transmission-blocking vaccines and genetically modified mosquitoes which will not be available for several years and their chances of success have been seriously questioned.[99] Larviciding is therefore the closest intervention that can be considered for scale-up in malaria vector control because effective larviciding

products already exist but their effectiveness at field level remain unknown. This study investigated the effectiveness of larviciding in selected rural areas of Botswana and Zimbabwe, using a *Bti*-based biological larvicide.

1.3. AIM, OBJECTIVES, HYPOTHESIS AND EXPECTED OUTCOMES

1.3.1. Aim

The aim of the study was to assess the effectiveness of winter larviciding as an additional malaria vector control intervention in selected rural areas of Botswana and Zimbabwe.

1.3.2. Specific objectives

1) To determine the effect of winter larviciding on larval density of mosquito vectors in selected rural areas of Botswana and Zimbabwe.
2) To establish larval survival post-larviciding, measured as the proportion of the late instar (3^{rd} and 4^{th}) stages to the total larvae sampled from the breeding points.
3) To assess the effectiveness of winter larviciding in reducing malaria vector densities by measuring the difference in adult mosquito densities between the intervention and control sites.
4) To determine the effect of winter larviciding on human malaria cases by measuring the difference between cases occurring in intervention and control sites.
5) To establish community perceptions on the benefits of winter larviciding in malaria control.
6) To make appropriate recommendations to the national malaria control programs of Botswana and Zimbabwe for the development of larviciding policies.

1.3.3. Hypothesis

Winter larviciding can reduce larval density, vector density and subsequently contribute towards the reduction of malaria morbidity and mortality in selected rural areas of Botswana and Zimbabwe that also receive IRS and LLINs.

1.3.4. Outcomes

Entomological outcome measures were used to assess the impact of winter larviciding in the two countries. The primary entomological outcomes were the differences in larval density by genera and stage between the intervention and control areas of both Botswana and Zimbabwe; and adult mosquito densities in houses in the intervention and the control villages.

1.4. MATERIALS AND METHODS

1.4.1. Study Design

An experimental study was conducted in Botswana and Zimbabwe. In Botswana, the study was conducted in Mathathane and Molalatau villages of Bobirwa district. Mathathane Village was used as the experimental village while Molalatau was used as the control village. In Zimbabwe, the study was conducted in Ward 33 or Birchnough Bridge Ward of Buhera District. Within the ward, the northern part of the ward also known as Pfupi Village was used as the intervention area while the southern part, also known as Tamanikwa Village was used as the control. The two villages in Ward 33 are separated by irrigation fields.

1.4.2. Study Areas

The study was conducted in two neighbouring villages, Molalatau and Mathathane which are 25 km apart and near Bobonong in Botswana; and Ward 33 of Buhera District also known as Birchnough Bridge Ward of Zimbabwe (Figure 5).

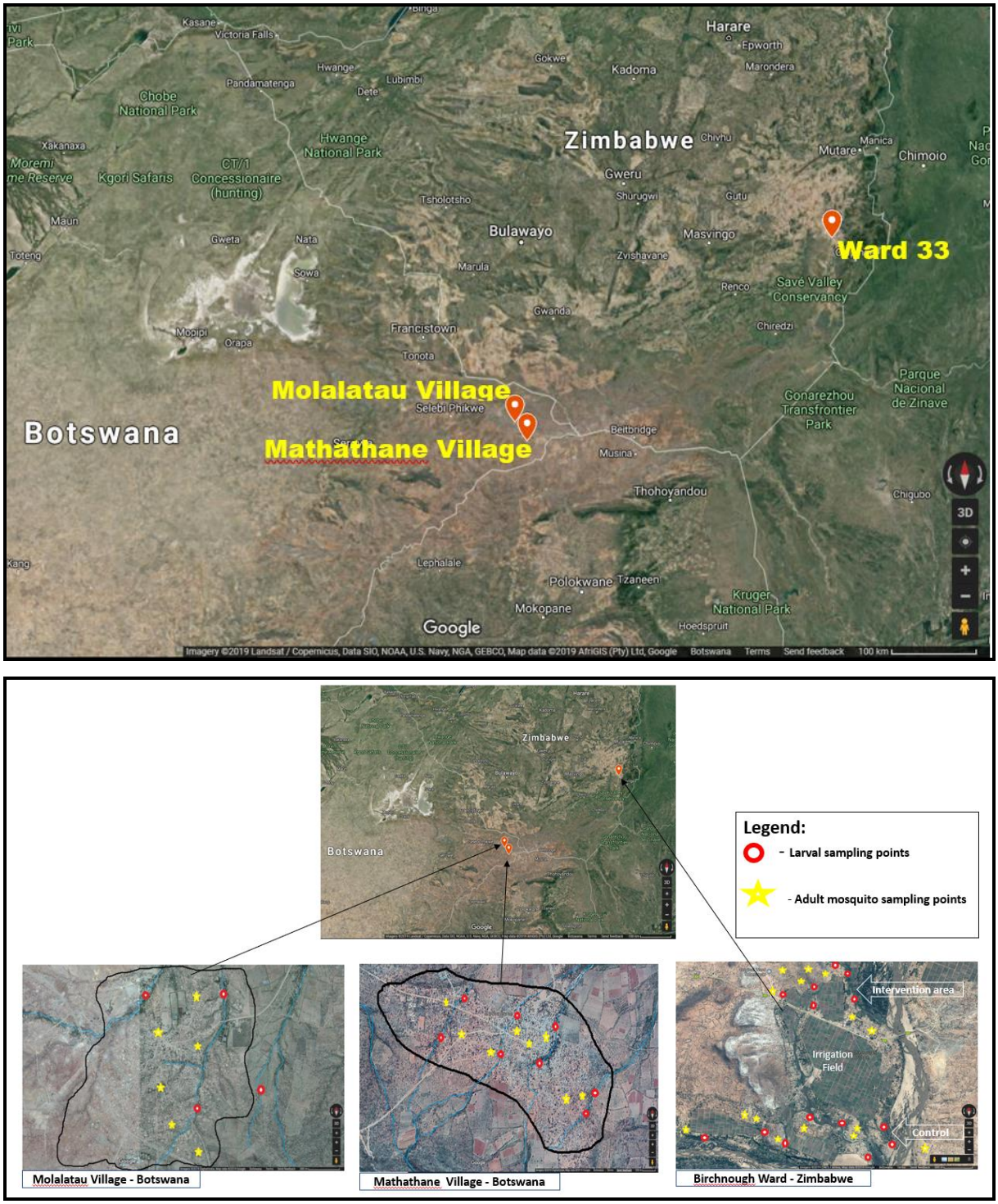

Figure 1.5: Study locations in Botswana and Zimbabwe

Table 1.2: Characteristics of study locations

Characteristic	Botswana		Zimbabwe
	Molalatau	Mathathane	Ward 33- Birchnough Bridge
Number of Households	534	752	2,188
Population	1,788	1,845	9,594
Average Rainfall	340mm	339mm	447mm
Altitude	685m	673m	500m
Average temperature	21.7^{0}C (Min=8^{o}C: Max 36^{o}C)	21.6^{0}C (Min=8^{o}C: Max 36^{o}C)	22.5^{o}C (Min =16^{o}C: Max = 27^{o}C)
Human activities	Small-scale agriculture (Seasonal)	Small-scale agriculture (seasonal)	Large-scale agriculture (irrigation and throughout the year)
IRS Coverage			88%
LLINs coverage			100%

Source: *Zimbabwe Statistics Agency[100], Statistics Botswana[101], Provincial Medical Director-Manicaland*

The villages/ward were selected with the help of the national malaria control programs for both countries. Botswana is in malaria pre-elimination and the selected villages are in a district that experiences low transmission but also reports sporadic outbreaks. The country has been implementing larviciding since 2013, but the intervention has been inconsistently implemented. Zimbabwe is moving towards malaria pre-elimination, with one province bordering Botswana and South Africa already in pre-elimination phase. This is also the only province receiving larviciding as part of the national programme while the rest of the country has no larviciding programme. The selected ward (Birchnough bridge) in Buhera district reports the lowest cases of malaria in Manicaland province. Birchnough Bridge is characterised by agricultural activities through irrigation.

1.4.3. Data collection

Data collection activities were categorised as follows:

Preparatory activities – these were pre-intervention/larviciding activities which included mapping of the breeding sites and the collection of baseline data (Figure 1.6).

Intervention activities- the activities included treatment of the identified larval habitats and the conduction of entomological surveillance activities (larval and adult mosquito sampling).

Post-intervention activities- activities included interviews with community members.

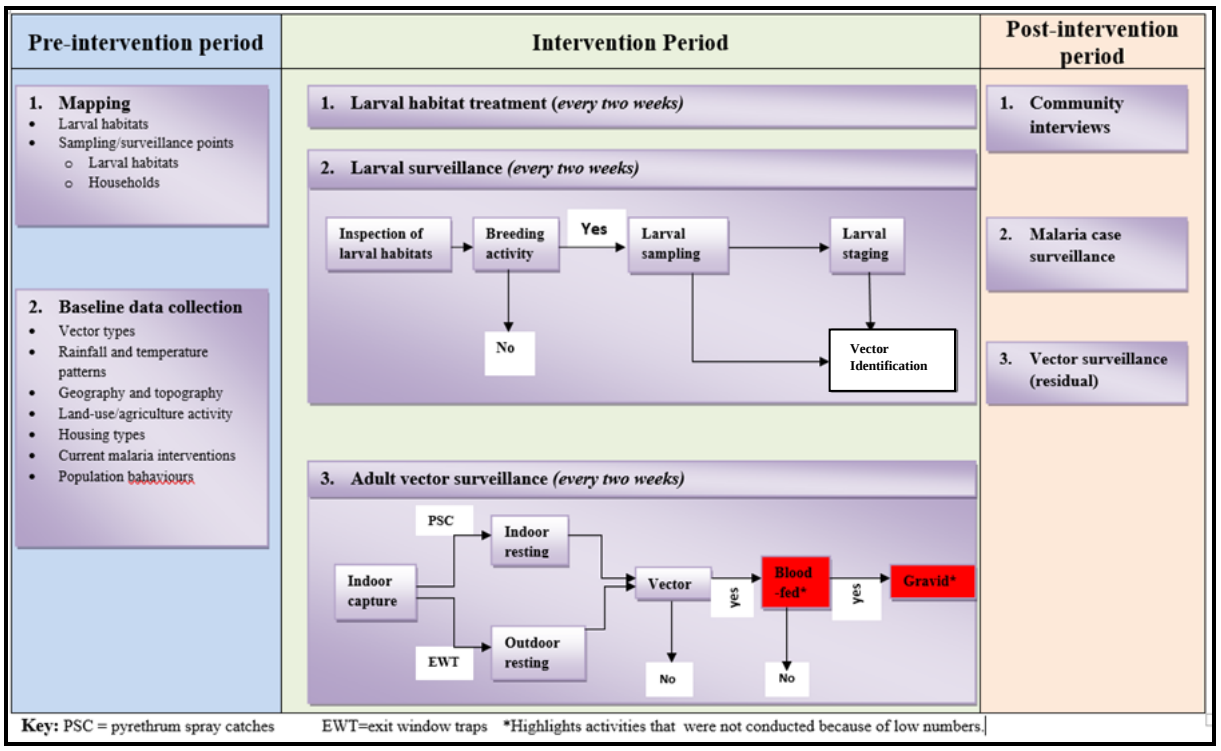

Figure 1.6: Data collection flow-chart

1.4.3.1. Pre-intervention data collection

1.4.3.1.1. Larval habitat surveying and mapping

The location and types of larval breeding habitats were surveyed in the two study villages in Botswana and the ward in Zimbabwe. All semi-permanent and permanent habitats were mapped using handheld geographic positioning system receivers and given a unique identification name and number to allow for quick reference during field operations. They were also described by type, size, presence and type of vegetation as these characteristics have an impact on breeding activity and the application of control strategies.[32] The larval surveying points were along river beds in Botswana because all temporary water points had dried up. In Zimbabwe, the larval sampling points included river beds and other stand-alone water points which

consistently have water from seepage from the irrigation fields. Mosquito breeding activity and the ability of the same sampling point to have water throughout the study period were used as the main factors in selection of larval sentinel sites. Additionally, their distribution within the study villages in Botswana and the ward in Zimbabwe was also considered to ensure adequate geographic spread.

1.4.3.2. Baseline data

Baseline data collection included assessment of receptivity characteristics which indicate the extent to which local conditions favour malaria transmission[102]. Varied factors influencing malaria transmission include:

- The mosquito vector species, their abundance and behaviour
- Temperature and rainfall
- Geography and topography of the land
- Amount and type of agriculture or land-cover in the area
- Other vector control interventions and their coverage
- Average annual malaria cases
- Quality of housing in which people live
- How people spend their time in the places and times when vectors are feeding

Baseline data collection also included information on malaria trends in the study areas and reports on similar studies done locally.

1.4.3.3. Intervention period

1.4.3.3.1. Treatment of larval habitats

Treatment of the larval habitats was done during winter, starting in the month of May in Zimbabwe and June 2015 in Botswana. It was completed at the end of October after 16 weeks of implementation in each of the countries. Treatment was only done to all habitats within the intervention villages using *Bacillus thuringiensis israelensis* (*Bti*), a WHO recommended bio-larvicide[103]. Larvicide was applied once every fortnight in all mapped water habitats in the intervention areas using handheld pressure spray pumps for eight treatment periods.

1.4.3.3.2. Selection and training of larviciders

Community larviciders were identified with the help of the local NMCP focal point in consultation with the local community and health facilities. These were permanently resident within the study villages/wards. They received a two-day training on the study. In Botswana, they were trained by the PhD student while in Zimbabwe they were trained with the help of the Provincial Malaria vector control focal point. The training components included identification of breeding sites, identification of larva and staging, larval sampling, application of larvicide, installation of exit window traps and conducting pyrethrum spray catches.

1.4.3.3.3. Entomological surveys

Entomological surveys included larval sampling from selected breeding sites and adult mosquito surveillance from selected houses. These activities were conducted fortnightly and in Botswana they were conducted by the PhD student with the assistance from three community volunteers while in Zimbabwe the Provincial Field Officer who was seconded by the Ministry of Health to support the study primarily led the entomological surveys. Because of the potential spill over of vectors from untreated areas outside the intervention area,[32] sampling points (breeding sites and houses) were located at least 2500m inside the boundary of the intervention area.

1.4.3.3.4. Larval sampling

During larval sampling, data on both habitat occupancy and larval density was collected:

***Habitat occupancy*:** The presence or absence of larvae in a breeding site was determined by visual observation. If a habitat is positive (i.e. larvae are present), the next step was to determine larval density.

***Larval density*:** The presence or absence of larvae was scored after a minimum of 10 dips per site[73], taken with a standard 250 ml capacity mosquito dipper (Clarke Corporation, IL, USA). For larger breeding sites, one dip was taken per square metre of surface, up to a maximum of 30 dips as per the WHO recommendations[32]. The following information was recorded for every site during the surveys: (i) the presence or absence of water in the habitat, (ii) the presence or absence of Anopheles early larvae stages (stages I and II instars), (iv) and late larvae stages (stages III and IV instars). The proportion of late instar larvae was calculated as an indicator of larval

survival, adult mosquito emergence and appropriateness of application by the Larvicider.

Larval surveys were conducted purposely to maximize sensitivity of collections, with most dips being made at the water edges and close to tufts of vegetation where larvae can be expected. These surveys addressed objectives 1) and 2) of the study.

1.4.3.3.5. Adult mosquito monitoring

Adult mosquito monitoring involved surveying host-seeking vectors as well as indoor and outdoor-resting. Window traps and pyrethrum spray catches were used to capture adult mosquitoes every fortnight in both the intervention and the control villages and this was done in houses within a 500m radius from the breeding sites because of the expectation that mosquitoes will fly and settle within the houses closest from the breeding points. These activities addressed objective 3) of the study.

Exit window traps: Exit window traps were used to trap exophagic mosquitoes which will enter the room for a blood meal but go and rest outside. The traps were fitted just before sunset which was usually at around 1800 hrs in winter and removed at sunrise which was around 0600 hrs.

Pyrethrum Spray Catch (PSC): This method took advantage of the indoor resting tendency of mosquitoes. White cloth sheeting would be laid down in a house, and pyrethrum insecticide applied as an aerosol (PSC; WHO 1992). The mosquitoes killed and knocked down by the spray were collected. Pyrethrum spray catches were conducted in the morning just after the removal of exits traps[103].

1.4.3.4. Post intervention activities

1.4.3.4.1. Community interviews

In both countries, interviews were conducted at the end of the intervention period to establish community acceptability, perceptions and opinions on the effectiveness of larviciding. A questionnaire with both open and closed-ended questions was administered to heads of households (Appendix 9). This activity addressed objective 5) of this study.

1.4.4. Sample size and sampling

Breeding sites were identified in four areas, two villages in Botswana and two wards in Zimbabwe, with one village/ward being used as an intervention and the other as a control. In each of the study villages/wards eight sampling water points were selected because it was logistically possible to handle 7-10 breeding sites in each area. At these points, larval sampling was repeatedly conducted to measure changes in density over time. The sampling points were also selected based on their ability to continuously have water throughout the study period without drying up.
Eight houses were selected for adult mosquito surveillance in each of the four study areas. Households within a 500m distance to the breeding sites where larval sampling was conducted were conveniently sampled to assess relationship between larval densities and adult mosquito densities indoors. The first eight head of households that agreed to have their households participate in the study were included.

1.4.5. Statistical analyses

From each breeding habitat selected for sampling, larval counts were conducted at each of eight-time points 14 days apart. The primary analysis assessed larval counts for intervention and control sites, and the analysis employed random-effects Poisson regression to compare intervention with control sites with respect to larval counts, and the factors included in the model were treatment (larvicided; not larvicided), country (Botswana; Zimbabwe) and relevant covariates. The incidence rate ratio (IRR) for treatment was of primary importance. Data analysis was done using Stata Release 13, (StataCorp, College Station, TX: StataCorp LP).
Similar analysis was conducted to adult mosquito counts.

1.4.6. Data management

While field data collection was underway, paper-based data (annotated field notebooks and completed tools) were stored in a locked briefcase. Electronic data was stored in password-protected data files on a password-protected computer. All raw data will be kept for 15 years after the presentation of the final report, thereafter it will be destroyed.

1.4.7. Ethical considerations

Approval for the study was obtained from the Ethics Committee of the Faculty of Health Sciences, University of Pretoria (South Africa) (Appendix 1 & 2); The Human Research Development Committee (HRDC) of the Botswana Ministry of Health (Appendix 3); and the Medical Research Council of the Zimbabwe Ministry of Health and Child Care (Appendix 4). Additionally, written approvals were obtained from the local Ministry of Health authorities for both Botswana and Zimbabwe (Appendix 5 & 6). In Zimbabwe, approval was also obtained from the Provincial Medical Director-Manicaland Province, Zimbabwe (Appendix 7).

The larvicide which was used for the experimental study received an experimental importation permit from the Ministry of Agriculture in Zimbabwe (Appendix 8). Local community leaders were used as the entry point to the study communities and community consent for the implementation of mosquito larval control was sought through community meetings. Household consent was obtained from heads of household for adult mosquito collection and for participation in a questionnaire (Appendix 10 and 11).

Community larviciders received BWP100 and USD15 weekly reimbursements in Botswana and Zimbabwe respectively for meals during the weekly larviciding activities.

1.4.8. Partnership with National Malaria Control Programs (NMCPs)

The study was conducted in collaboration with the national malaria control programs of Botswana and Zimbabwe for ownership of the results. The two programs also supported some of the activities and logistics as indicated in Table 1.3

Table 1.3: Support activities by NMCPs- Botswana and Zimbabwe

NMCP supported activities	
Botswana	**Zimbabwe**
Community sensitisation on the study	Community sensitisation on the study
Identification of the community larviciders	Secondment of a Provincial Field Officer who supported the following activities: • Training of community larviciders

	• Supervision of the larviciders • Fortnightly entomological surveillance
	Identification of the community larviciders

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CHAPTER TWO

2. FIELD EFFECTIVENESS OF MICROBIAL LARVICIDES ON MOSQUITO LARVAE IN MALARIA AREAS OF BOTSWANA AND ZIMBABWE[1]

This chapter presents results of the experimental study in both Botswana and Zimbabwe focusing on the primary outcome which is the impact on the larval stage of the vector mosquito. It presents findings on the impact of microbial larviciding using a Bti based larvicide on larvae of different mosquito species (the anopheles and the culex), and different larval stages (early and late larval stages).

2.1. Abstract

Background

The successful control of malaria vectors requires the control of both the larval and adult stages. The adult control methods through indoor residual spraying (IRS) and use of long-lasting insecticidal nets (LLINs) continue to be widely used with some high measure of success. Larval control methods are also being used by a number of National Malaria Control Programmes (NMCPs) with limited understanding of its contribution. Larval control might be needed in some areas to move from malaria control to elimination. This experimental study was conducted to assess the field effectiveness of winter larviciding on the larval stages of the mosquito in Botswana and Zimbabwe.

Methods

Two villages were selected in each of the two countries, one as an intervention and the other as the control. Water bodies in the intervention villages were treated using the commercial product VectoBac® WG (Valent BioSciences Corporation, IL, USA) containing the active ingredient *Bacillus thuringiensis var. israelensis* (*Bti*), a WHO recommended bio-larvicide, applied at a rate of 300g per hectare. Random-effects Poisson regression was employed during data analysis to compare intervention with control sites with respect to larval counts.

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Results

The average marginal effect of larviciding on the mosquito larvae taking interaction with time (period) into account, was -1.94 (95% CI:-2.42 to -1.46) with incidence rate ratio of 0.14, thus an 86% larval reduction attributable to the intervention for both countries combined. There was a 92% and 65% effect for Botswana and Zimbabwe respectively. The effect on the early larval and late stages was 77% (P<0.001) and 91% (P<0.001), respectively. Overall, intervention larval sampling points had five more larvae than the control at baseline and 26 less after 16 weeks. The effect on the different species also showed similar trends.

Discussion/Conclusion

Larval control using *Bti* showed a high effect on the population of the mosquito larvae. The reduction of the early and late larval stages can lead to reduced adult mosquito emergence and low adult mosquito densities. Larviciding can be used to control mosquito vector population by suppressing the larval stages thereby reducing adult emergence and malaria risk.

Key words: Larviciding, microbial larvicides, Botswana, Zimbabwe, malaria vector control

2.2. Background

The World Health Organization (WHO) has targeted malaria for elimination which can be achieved through strengthening of country surveillance, diagnosis, case management and vector control activities [1, 2]. Implementation of proven vector control interventions of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) is currently at the core of successful malaria vector control [3-5]. LLINs protect their occupants by diverting host-seeking vectors and by killing those that attempt to feed [6, 7], but the number distributed annually since 2005 has remained below the target required to reach universal access [8-10]. In sub-Saharan Africa early malarial eradication pilot projects showed that malaria is highly responsive to vector control by IRS [11, 12], with the first trial being carried out in

1931 in KwaZulu-Natal, South Africa using pyrethrum. In the 1950s, IRS with DDT had become the main vector control method in South Africa [11].

Integrated vector management (IVM), targeting both larval and adult mosquitoes has lately received a lot of attention because of its potential in control and elimination of malaria [4, 5]. The interest in larval control led the WHO to issue an *Interim Position Statement on Larviciding in Sub-Saharan Africa* in 2012 [3]; and subsequently developed and launched the *Larval Source Management (LSM) guidelines* in 2013 [13]. Recent evidence of larviciding effectiveness presents an opportunity in sub-Saharan Africa, particularly in urban areas because of the rapidly growing urban centres [14]. Studies have shown effectiveness of larviciding in urban area [14-17], in highlands [18], and when used in combination with LLINs [3-5]. Based on available data, larviciding has been recommended as a complementary intervention to IRS and LLINs [13], and to be utilized in areas with water bodies which are few, fixed and findable while additional research is being conducted to measure the effectiveness of the intervention [19].

Reports received from national malaria programmes indicate that 48 malaria-endemic countries worldwide use larval control in certain specific foci of malaria transmission of which 18 are in Sub-Saharan Africa [10]. These reports give an indication of the range of larval control methods employed, but the scale of efforts is not quantified and the impact on individual country malaria burden is not easily measured. With the increasing trends of resistance to pyrethroids used for IRS and for treating LLINs, [20-24] there is a demand for alternative technology and products to help in the control and subsequent elimination of malaria. Behavioural adaptation of adult mosquito vectors gives them the ability to avoid LLINs and walls treated through IRS [25-28], while research on other potential malaria control interventions such as transmission-blocking vaccines and genetically modified mosquitoes have not been successful [29]. There is current discussion that relying solely on IRS and LLINs may be insufficient to achieve malaria elimination in much of sub-Saharan Africa [15], and larviciding would have to be part of an integrated vector management (IVM) approach [30] that could help hinder malaria transmission.

This study was conducted in selected semi-arid rural areas of Botswana and Zimbabwe to establish the effectiveness of winter larviciding as an additional vector control intervention. The study involved assessment of the effectiveness of larviciding on larval density as well as adult mosquito density. This paper presents a comprehensive analysis of the effectiveness of winter larviciding on larval density in two semi-arid regions.

2.3. Methods

Study design and setting

An experimental study was conducted in two neighbouring countries, Botswana and Zimbabwe. In Botswana, Mathathane and Molalatau villages in Bobirwa District, which are 25 km apart were selected as the intervention and the control villages, respectively. In Zimbabwe, Birchnough Bridge which is an irrigation area was used as the study village, with the northern part of the village being used as the intervention area and the section of the village south of the irrigation scheme being used as the control. The irrigation fields acted as the buffer between the intervention and the control area, and distance between the two study arms was 5km. With north to south bound winds, there was minimal expectation that mosquitoes will fly from the control areas to the intervention area.

The villages in the two countries were selected with the help of the national malaria control programs for the assessment of larviciding in supporting elimination efforts in similar localities. The whole of Botswana is in the pre-elimination phase and experiencing concentrated malaria with low transmission. Mathathane and Molalatau villages in Botswana have previously reported sporadic malaria outbreaks and entomological surveys have yielded positively on malaria vectors. In Zimbabwe, Birchnough Bridge is in Buhera District, reports the lowest number of malaria cases in Manicaland province and has the potential to be considered for malaria pre-elimination.

Data collection

Larval habitat surveying and mapping

The location and type of larval breeding habitats was surveyed in May 2015 in both the intervention and the control villages. All semi-permanent and permanent aquatic mosquito habitats in the intervention and control villages were mapped using handheld geographic positioning system receivers. Mapped habitats were given a unique identification name and number to allow quick reference during field operations, including global positioning system (GPS) coordinates [13].

In both countries, the study arms were characterized by few larval habitats as all of the temporary habitats had dried up. Breeding was mainly along river beds, with a lot of animal activity which was creating thousands of minute breeding points. However, these hoofmarks were not mapped as separate breeding points.

In the intervention area of Zimbabwe, larval habitats were tributaries draining from Save river and the irrigation area through seepage. The two tributaries referred as Bonda Mud and Bonda Sand permanently have water throughout the year, each stretching for almost 500m. At the start of the data collection, the two tributaries including their collection ponds had an estimated combined water surface area of 0.5 hectares with bonda sand being approximately 0.3 hectares and bonda mud being 0.2 hectares. In Zimbabwe, the control area was downstream and south of the irrigation. Permanent breeding was along five irrigation drains feeding into a stream that later drains into the Save river a further three kilometres from the human settlements. Additional breeding was along a drain from a borehole that supplies potable water to the local residents.

In Mathathane, the intervention village in Botswana, breeding occurred along river beds of Selepye and Mathathane that pass through the village. The village lies on an aquifer and on the southern part of the village water naturally comes out, flows and settles along the beds of the two rivers. It flows and covers a distance of 400 m along Mathathane river with a water surface area of approximately 0.5 hectares while along Selepye river it covers a surface of approximately 1.5 hectares along its 2 km stretch. In the control village of Molalatau, the water source to the river where permanent breeding occurs is the borehole drilled on the aquifer where water flows out under pressure into the river.

Treatment of larval habitats

Treatment of the larval habitats was done in winter of 2015, from June-October, using the commercial product VectoBac® WG (Valent BioSciences Corporation, IL, USA) containing the active ingredient *Bacillus thuringiensis var. israelensis* (*Bti*), a WHO recommended bio-larvicide [31]. Application of biolarvicide was conducted in intervention areas/villages at two-week intervals for eight time periods. Though mapped, water bodies in the control villages were not treated. In both Botswana and Zimbabwe, larviciding was conducted by community volunteers who were identified with the help of the local community leadership and through the local health facilities.

In Zimbabwe, two community volunteers participated in the study and worked under the full supervision of an entomologist seconded to the study by the Ministry of Health. In Botswana, three community volunteers participated throughout the study. The main responsibilities of the community volunteers was to identify breeding habitats, conduct the larviciding and assist the entomologist in larval sampling. They received training from the study coordinator and the entomologist on how to identify breeding sites and complete the habitat survey forms and on how to apply larvicide. Through their interaction with the entomologist and the study coordinator, they also learnt how to sample larvae and the determination of larval density by type and stage. However, these activities were the responsibility of the study coordinator and the entomologists, and the volunteers were conducting these activities under supervision. Knap sack sprayers were used for application of the larvicide at a rate of 300 grams per hectare surface of water.

Intervention timelines

Implementation of the intervention started early June in Zimbabwe (Figure 2.1) and seven weeks later in Botswana. Implementation continued for 16 weeks at each of the two countries at two-week intervals.

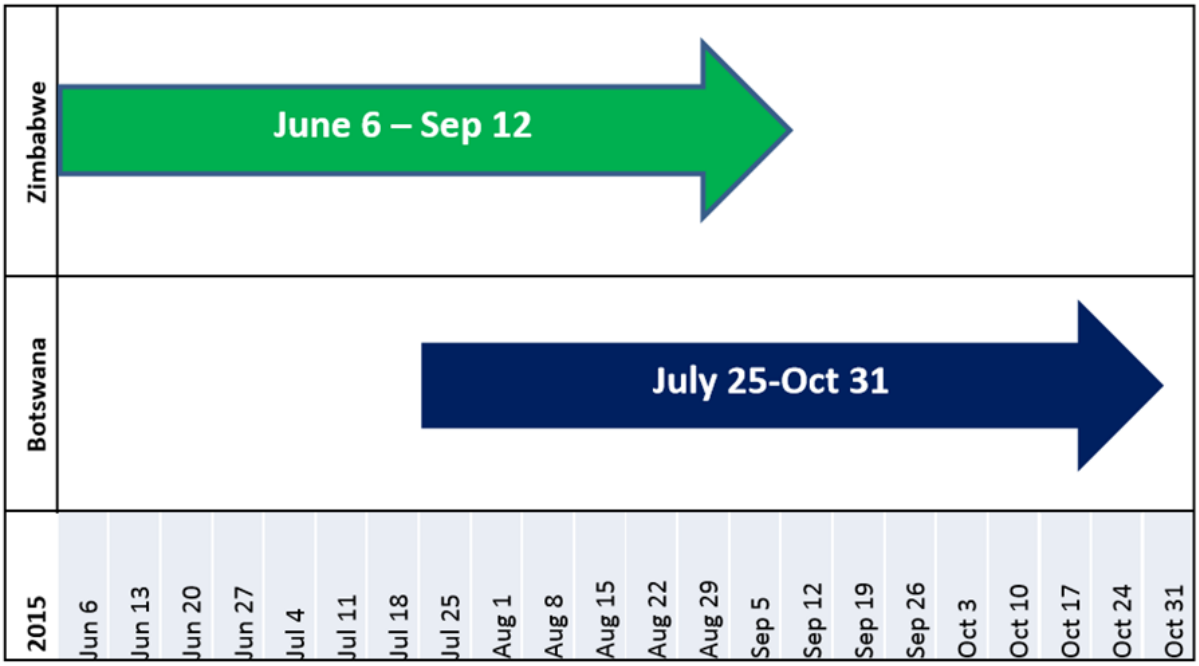

Figure 2.1. Study timelines in Zimbabwe and Botswana

2.3.1.1. Entomological surveys

Larval inspections and sampling was conducted consistently at two-week intervals before the next treatment in intervention areas, and the next day in the control villages. VectoBac® WG product has previously demonstrated to have a high effectiveness within the first 24-48 hours on low doses [32]. In this study, larval sampling was repeatedly conducted at the same sampling points, and 14 days after treatment and just before the next treatment. During larval sampling, data on both habitat occupancy and larval density was collected.

Habitat occupancy: The presence or absence of larvae in a breeding site was determined by visual observation. If a habitat was positive (i.e. larvae are present), the next step was to determine larval density.

Larval density: The presence or absence of larvae was scored after a minimum of 10 dips per site [33], taken with a standard 250 ml capacity mosquito dipper (Clarke Corporation, IL, USA). The following information was recorded for every site during the surveys: (i) the presence or absence of early larvae stages (stages I and II instars), (ii) late stages (stages III and IV instars). The larvae were also disaggregated by specie, either *Anopheles* or *Culex*. The proportion of late instar larvae was calculated as an indicator of larval survival, adult mosquito emergence and appropriateness of application by the larvicider. Morphological features used to

identify *Culex* larvae were a rounded head, presence of a long siphon tube, and a resting position which is at an angle to the water surface. *Anopheles* features included a long head, a short and at times invisible siphon tube, and a resting position which is parallel to the water surface.

Statistical analyses

From each larval sampling site, larval counts at each of eight time periods 14 days apart were done. The time periods for the two countries did not coincide exactly, the Zimbabwe arm ran for periods one through eight and the Botswana arm from period four to 11. The analyses assessed larval counts for intervention and control sites. Random-effects Poisson regression was employed to assess the relationship between larval counts and the fixed-effects treatment (larvicided; not larvicided), country (Botswana; Zimbabwe), time period, the interaction between treatment and time period and covariate baseline count. Sites were specified as the random-effects component with an intercept and takes care of the repeated measures within sites. The incidence rate ratio (IRR) for treatment was of primary importance. Data analysis was done using Stata Release 13 &14, (StataCorp, College Station, TX: StataCorp LP).

Ethical considerations

Approval for the study was obtained from the Ethics Committee of the Faculty of Health Sciences, University of Pretoria (South Africa, Protocol number 289/2014); The Human Research Development Committee (HRDC) of the Botswana Ministry of Health; and the Medical Research Council of the Zimbabwe (Approval number MRCZ/A/1898). Support to conduct the study was obtained from the National Malaria Control Programmes of both Zimbabwe and Botswana. Local chiefs were used as the entry point to the study communities and community consent for the implementation of mosquito larval control was obtained during community meetings initiated through the local area chief and village heads.

2.4. Results

Larval density

Table 2.1 below shows the average larval density per dip at visit one through visit eight in both Botswana and Zimbabwe. Data collection started early in Zimbabwe on

the 4th of June 2015, ending on 12th of September 2015, while in Botswana data collection and the interventions started on the 25th of July 2015 and ending on the 30th of October 2015.

Table 2.1: Larval density by visit and country

	Average number of larvae per dip by country and visit					
	Botswana			**Zimbabwe**		
	Calendar date (2015)	**Intervention**	**Control**	**Calendar date (2015)**	**Intervention**	**Control**
Visit 1	July 25	32	33	June 6	22	10
Visit 2	Aug 08	16	32	June 20	16	3
Visit 3	Aug 22	3	36	July 04	7	8
Visit 4	Sep 05	2	35	July 18	5	3
Visit 5	Sep 19	3	32	Aug 01	4	5
Visit 6	Oct 03	3	32	Aug 15	1	14
Visit 7	Oct 17	1	31	Aug 29	3	25
Visit 8	Oct 31	2	32	Sep 12	2	22

NB: The average number of larvae was rounded to the next whole number.

Botswana started with high larval counts for both intervention and control which were comparable. On average, there were 32 and 33 larvae per dip in the intervention and control area respectively. Zimbabwe had an average larval count of 22 per dip at baseline (visit 1) in the intervention area, and 10 larvae in the control. The control areas in Botswana maintained larval density at more than 30 while in Zimbabwe, there was a reduction to an average of 3 larvae. These counts are the average for all the sampling points in each of the two countries and study arms.

Average change in larval density

Table 2.2 and Figure 2.2 show the predicted difference/change in number of larvae in the intervention areas relative to the control by visit and country attributable to larviciding.

Table 2.2: Average marginal effects (CI) in larval counts between intervention and control

	Botswana		**Zimbabwe**	
Visit	Marginal effect (CI)		Marginal effect (CI)	
Visit 1	0.45	(-6.61 ; 7.52)	13.77	(9.22 ; 18.33)
Visit 2	-18.76	(-25.83 ; 11.69)	9.20	(5.60 ; 12.81)
Visit 3	-30.53	(-37.10 ; 23.96)	1.61	(-1.47 ; 4.70)
Visit 4	-34.29	(-40.30 ; 28.27)	-5.09	(-8.01 ; -2.18)
Visit 5	-33.25	(-38.98 ; 27.51)	-10.45	(-13.27 ; 7.63)
Visit 6	-29.93	(-35.60 ; 24.26)	-14.80	(-17.86 ; 11.75)
Visit 7	-25.82	(-31.44 ; 20.19)	-18.56	(-22.50 ; 14.62)
Visit 8	-21.76	(-27.31 ; 16.21)	-22.01	(-27.54 ; 16.48)

Note: Marginal effect for factor levels is the discrete change from the base level.

From Table 2.2 above, the average difference in the total number of larvae as a result of intervention ranged from 0.45 at visit one to 21.76 at visit eight, and 13.77 and 22.01 for Botswana and Zimbabwe, respectively. At baseline or visit 1, the intervention and control sites in Botswana were comparable with an average difference in number of larvae of less than one, while in Zimbabwe intervention sites had an average of 13.77 more larvae relative to the number in the control though not statistically significant. From the analysis, the effect of the intervention demonstrated a reduction in larval density in the intervention areas of Botswana relative to the control, while in Zimbabwe the predicted effect through visits is rather an increase in larval population in the control areas, while the intervention remained stable and low due to the introduction of larviciding.

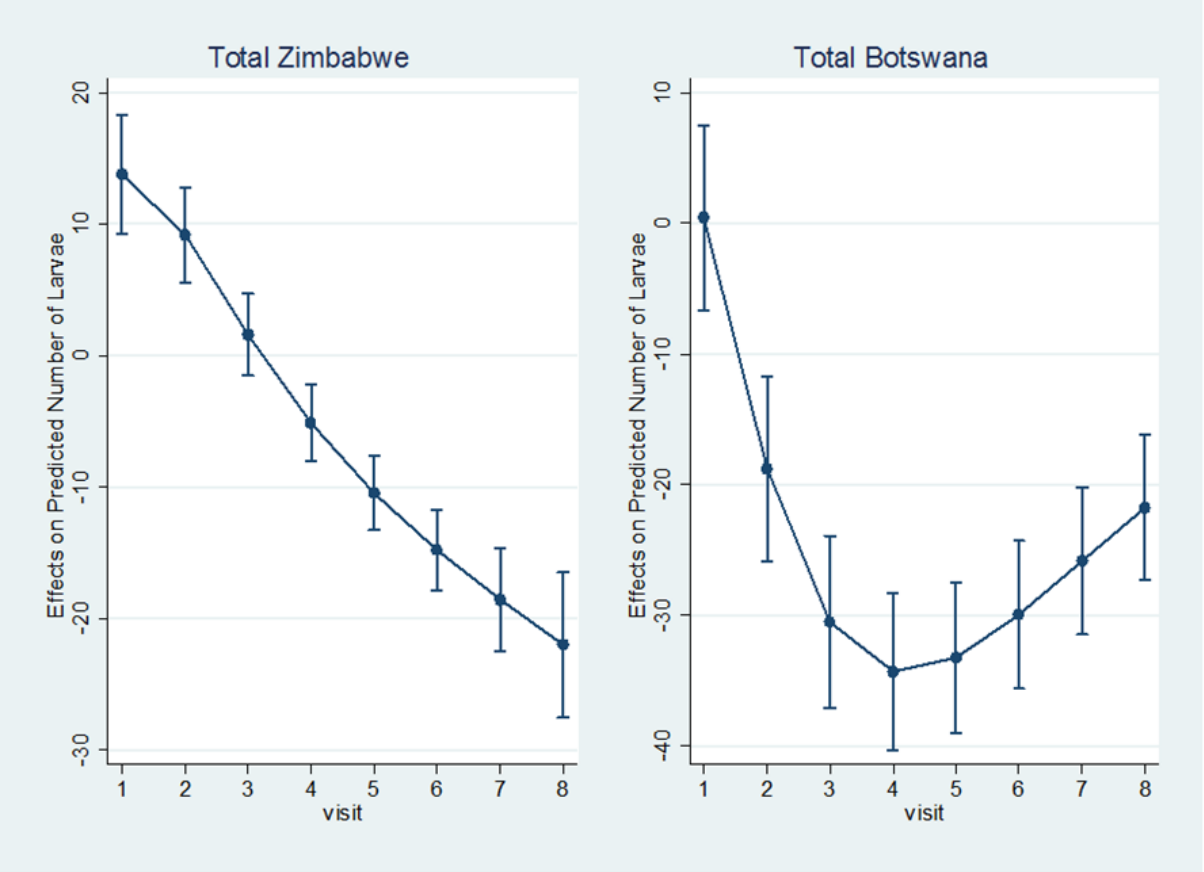

Figure 2.2: Average change in mosquito larval density over time from baseline

From Figure 2.2 above, there was a steady increase in change from baseline in larval density between the intervention and the control over time in Zimbabwe. In Botswana, there was a sharp increase in change from baseline after the introduction of the intervention, which then evened out from visit 5 until the end of data collection at visit eight.

Effect of intervention on larvae

Table 2.3 below shows the marginal effect of larviciding on the larvae, its effect on the different larval stages and the larval specie, as well as the incidence rate ratio.

Table 2.3: Marginal effect of larviciding in Zimbabwe and Botswana

Larvae type	Marginal effect	IRR (CI)	p-value	% reduction
All larvae				
Both Countries	-1.94	0.14 (0.09 ; 0.23)	<0.001	86

Zimbabwe	-1.06	0.35 (0.24 ; 0.51)	<0.001	65
Botswana	-2.51	0.08 (0.06 ; 0.11)	<0.001	92
Larval Stage				
Early instar				
Both Countries	-1.47	0.23 (0.17 ; 0.32)	<0.001	77
Zimbabwe	-1.23	0.29 (0.17 ; 0.50)	<0.001	71
Botswana	-1.83	0.16 (0.12 ; 0.22)	<0.001	84
Late instar				
Both Countries	-2.40	0.09 (0.04 ; 0.19)	<0.001	91
Zimbabwe	-0.56	0.57 (0.31 ; 1.03)	0.062	43
Botswana	-4.04	0.02 (0.01 ; 0.04)	<0.001	98
Larvae Species				
ALL *Anopheles*				
Both Countries	-1.63	0.20 (0.05 ; 0.76)	0.019	80
Zimbabwe	-0.64	0.53 (0.30 ; 0.92)	<0.025	47
Botswana	-3.01	0.05 (0.02 ; 0.13)	<0.001	95
ALL *Culex*				
Both Countries	-1.03	0.36 (0.10 ; 1.23)	0.102	64
Zimbabwe	-0.86	0.42 (0.26 ; 0.68)	<0.001	58
Botswana	-2.21	0.11 (0.05 ; 0.27)	<0.001	89

The average marginal effect of larviciding on the mosquito larvae, taking interaction with time (period) into account, was -1.94 (95% CI:-2.42 to -1.46) with incidence rate ratio of 0.14, thus an 86% larval reduction attributable to the intervention for both countries combined. There was a 92% and 65% effect for Botswana and Zimbabwe respectively. The reduction (%) on the early and late larval stages was 77% (P<0.001) and 91% (P<0.001) respectively (Table 2.3).

The effects of larviciding were also significant for the different larval species, with 95% and 47% reduction of *Anopheles* larvae for Botswana (p<0.001) and Zimbabwe (p=0.025), respectively. The seemingly low reduction in Zimbabwe is due to small

denominators and numerators because of low breeding activity during winter. The average marginal effects on the *Culex* larvae were -2.21 (89% reduction) and -0.86 (58% reduction) for Botswana and Zimbabwe and were both statistically significant ($p<0.001$).

2.5. Discussion

The study has demonstrated that larviciding using *Bti* is an effective vector control intervention in low transmission malaria areas of Botswana and Zimbabwe because of the reduction of the larval stages of the mosquito. *Bti* functions as a stomach poison in the mosquito larval midgut, and its effect on larvae is largely due to protoxins in parasporal crystals and the spore coat, rather than the actual infection [34, 35], and is usually active for one to two weeks generally requiring fairly clean water to be effective [35, 36]. All treated water bodies in the intervention areas of both Botswana and Zimbabwe were fresh water points, with minimal pollution due to animal activity, and larviciding happening every two weeks. *Bacillus thuringiensis var. israelensis* and *Bacillus sphericus* based microbial larvicide products have lately been assessed and found to be effective in reducing malaria vector mosquito larvae under field conditions, and subsequent reduction in malaria vector population densities [32,37-40]. Efficacy trials have also illustrated that *Bti* can reduce malaria transmission when implemented at a large scale [14], and when delivered as a supplementary measure alongside LLINs [5].

While most field studies have been conducted in highlands and urban areas [5,14, 41, 42], this is probably the first field study in semi-arid malaria transmission areas of sub-Saharan Africa, experiencing low transmission and in pre-elimination of malaria. The demonstrated effectiveness of *Bti* in inhibiting the progression from the early stages to the late larval stages is an indicator on its effect on adult mosquito emergence and risk of malaria transmission. In the control areas, larval development progressed uninterrupted to the late stages, while larviciding effect on the more sensitive early instars resulted in fewer larva progressing to the late stages. Studies have shown that early instars are more susceptible than the late instars to various formulations of *Bti* [43, 44], which reduces the occurrence of the later during habitat treatment periods [33].

Implementation of larviciding started at the beginning of winter in Zimbabwe, and later towards the end of winter in Botswana. While the study areas in the two countries have comparable environmental conditions, larval density was lower at baseline in Zimbabwe compared to Botswana. This is understandable considering that breeding activity is usually lower when temperatures are at their lowest early in winter. However, despite the different levels at baseline, the intervention showed high effectiveness at first treatment, which was sustained. For Botswana, later during the study, the difference in the larval density in the intervention and the control reduced due to an increase in larval density in the intervention. Since the intervention continued to be implemented at 14-day intervals despite increasing temperatures, this could be due to the effect of rising temperatures on the microbial larvicides. At high temperatures, solar inactivation has been found to affect microbial larvicidal products [41]. Elsewhere, *Bti* has shown a residual effect of up to 10 days in standardized field tests implemented during the dry season, providing complete protection when applied weekly [44]. Low doses of 200 g/ha is required to effectively suppress late instars, but can also lead to the absence of residual activity [45]. For this study, we applied doses of 300 g/ha. Despite the start of the intervention at different times of the year in the two countries, *Bti* still demonstrated a significant effect on the larval population when comparing the intervention and the control areas in both countries.

The effectiveness of *Bti* in both Botswana and Zimbabwe was consistent for both the *Anopheles* and *Culex* larva, and for the different stages of both species. While the *Culex* species are not vectors of human malaria, the reduction in its population reduces human exposure to mosquito bites which is also important in the general population's assessment of the effectiveness of a malaria vector control intervention. *Bti* formulations are known to have a large activity spectra covering larvae from many Culicidae (mosquito) genera: Culex, Aedes, and Anopheles [43, 44, 46], which reduces overall adult mosquito emergence and human exposure to bites and transmission [5, 33].

2.6. Conclusion

The use of the microbial larvicide *Bti* has shown to have an impact on larval densities in low malaria transmission areas of Botswana and Zimbabwe, which can lead to a reduction in adult mosquito densities and malaria transmission. The results of this study presents an opportunity for strengthening integrated vector management by including larviciding. Mosquito larvae, unlike adults are relatively immobile and cannot change their habitat to avoid control activities making larviciding an effective vector control intervention which can be used in semi-arid malaria areas with low transmission such as Botswana and parts of Zimbabwe. It can be considered as an additional intervention for malaria elimination in such areas.

List of abbreviations

LLINs: long-lasting insecticide-treated nets; **NMCP:** National Malaria Control Programme; ***Bti*****:** *Bacillus thuringiensis var. israelensis*; **WHO:** World Health Organization; **IRS:** indoor residual spraying; **DDT:** Dichloro-diphenyl-trichloroethane; **IVM:** Integrated vector management.

2.7. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MM acted as the study coordinator and designed the study, conducted data collection, analysis and report writing. KM and CdJ supervised and reviewed the design, data collection, analysis and reviewed the manuscript. PB designed the methodology, conducted data analysis and reviewed the manuscript.

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CHAPTER THREE

3. SUPPRESSION OF ADULT MOSQUITO DENSITIES USING MICROBIAL LARVICIDES CREATES PUBLIC HEALTH OPPORTUNITIES TOWARDS MALARIA ELIMINATION IN SEMI-ARID RURAL AREAS OF BOTSWANA AND ZIMBABWE.

This chapter focuses on the secondary outcome which is the effect of winter larviciding on the adult mosquito density. The previous chapter demonstrated that larviciding is effective in reducing larval densities which is expected to translate to reductions in adult mosquito densities. This chapter highlights what the impact was on adult mosquitoes and explores the implications of the results in vector control and efforts towards malaria elimination in both Botswana and Zimbabwe.

3.1. Abstract

Background

Malaria continues to be a major public health problem particularly in Sub-Saharan Africa despite successful vector control through indoor residual spraying and the use of long lasting insecticidal-nets. Mosquito populations can be suppressed by managing larval populations which are restricted to aquatic habitats. The effect of bio-larviciding on adult mosquito populations was examined in semi-arid villages of Botswana and Zimbabwe and used as a proxy for vector effectiveness.

Methods

In each country, experiments were conducted in two paired rural locations. In the intervention group all semi-permanent and permanent surface water bodies were mapped and treated with a commercial bio-larvicide containing the active ingredient *Bacillus thuringiensis var. israelensis* at two-week intervals from May to October 2015. During this period, adult mosquitos were surveyed at houses within 500m of the water bodies. Mosquito populations between the control and intervention houses were compared using random-effects Poisson regression.

Results

On average, intervention rooms in Botswana had four (range = 0 - 12) mosquitoes throughout the study compared to an average of 15 (0 - 43) in the control. In Zimbabwe, it was one (0 - 3) and three (0 - 15) mosquitoes in the intervention and control rooms respectively. For both Botswana and Zimbabwe combined, adult mosquito populations in the intervention areas were significantly suppressed by 70% (IRR=0.303, p<0.001) compared to the control, and suppression was77% (IRR=0.233, p<0.001) and 63% (IRR=0.369, p<0.001) for Botswana and Zimbabwe respectively.

Conclusion

The study found that bio-larviciding can effectively reduce adult mosquito densities, and Botswana and Zimbabwe should consider the intervention as a key component of integrated vector management towards malaria elimination in their semi-arid locations.

Keywords: larviciding, malaria, integrated vector management, vector control, Botswana, Zimbabwe

3.2. Background

Intense or moderate malaria transmission can be reduced to low levels by controlling mosquito vectors [1]. Controlling mosquito vectors targeting the last foci of transmission in the later stages of elimination, reduces outbreak risk and defends against malaria reemergence [2]. Some countries have maintained long term intensive and successful vector control operations, while many countries have encountered serious technical and operational challenges to sustainable vector control. One of the most serious challenges is insecticide resistance to key interventions including indoor residual spraying and long-lasting insecticidal-treated nets [2]. As an intervention, larval control was the only method available early in the 20th Century [3], until dichlorodiphenyltrichloroethane (DDT) was developed and used for indoor residual spraying (IRS) to target adult mosquitoes. Indoor residual spraying was the main vector control intervention used during the malaria eradication

campaigns of the 1950s and 1960s, with supplemental chemical larviciding in some areas [4].

Controlling the larva of Anopheles mosquitoes is a well-proven, but neglected, preventive method for controlling mosquito populations and deserves renewed consideration for malaria control programs in the 21st Century [5, 6]. Effective larval control requires highly specialized expertise, substantial investment and constant effort. Mosquito larva can be effectively controlled when breeding sites are few, fixed, and easy to identify [7, 8]. Recently, biological larval control using *Bacillus thuringiensis var. israelensis* (Bti) and *Bacillus sphaericus* (Bs) in highlands, urban areas and selected rural areas of sub-Saharan Africa has been successful [9-14]. The bio-larvicides *Bti* and *Bs* are live bio toxin-producing strains of bacteria belonging to the Bacillus group [12, 15]. The advantages of bio-larvicides include their effectiveness at relatively low doses, safety to humans and non-target wildlife (including natural predators of mosquito larvae), low-cost of production in some cases and lower risk of resistance development [16, 17].

Botswana and Zimbabwe are members of the Elimination Eight (E8) group of countries which have targeted malaria elimination in Member States by 2030 [18]. Botswana is one of the four frontline E8 members targeting elimination by 2020 despite an increase in malaria cases in 2014 [19]. Botswana and Zimbabwe mainly use indoor residual spraying (IRS) and long lasting insecticide nets (LLINs) [19] to control adult mosquito populations. Both these countries have larviciding as a vector control policy option but there in not enough evidence in support of using larviciding to control adult mosquito populations in these settings. There is a need for alternative control strategies because mosquitoes are developing resistance to pyrethrum based products [19] which are mainly used for treating LLINs and for IRS. Biological larviciding products are readily available but data on their field effectiveness is insufficient to promote the establishment of intense and robust larviciding programs [7, 20].

A larviciding experimental study was conducted in selected rural areas of Botswana and Zimbabwe to assess its field effectiveness as a vector control intervention. This paper presents the effectiveness of larviciding on adult mosquito densities during the larviciding experimental study.

3.3. Methods

The study was conducted in Botswana and Zimbabwe. In Botswana, Mathathane and Molalatau villages in the Bobirwa District, and 25 km apart, were chosen for the intervention and the control, respectively. In Zimbabwe two wards were selected in the Birchnough Bridge village, 5 km apart, separated by irrigation fields which acted as the buffer between the control and the intervention. The study villages/wards were selected with the assistance of the local national malaria control programs taking the low seasonal malaria transmission and the suitability for larviciding into account.

3.3.1. Data Collection

Pre-intervention period

All larval breeding habitats were surveyed in both the intervention and the control areas as described [21]. All breeding sites or open water bodies were identified while walking on foot through the villages and mapped using handheld geographic positioning system receivers. A unique identification number was allocated to each site to allow quick reference during field operations. Houses within 500 m of the breeding sites were selected as sampling points because we assumed that mosquitoes would fly and settle within houses nearest to the breeding points. Written consent was obtained from the head of households, and mosquitoes were only sampled in rooms where a person sleeps.

Intervention period

From May to October 2015, all water bodies in the intervention areas were treated with the commercial product VectoBac® WG (Valent BioSciences Corporation, IL, USA) containing the active ingredient Bacillus thuringiensis var. israelensis (Bti), a WHO recommended bio-larvicide [31]. Treatment of water bodies in the intervention area was fortnightly, amounting to eight treatments over the time period. Water bodies in the control villages were not treated.

<u>Adult mosquito monitoring</u>

Exit window traps were used to trap mosquitoes that have endophagic and exophilic tendencies, and will enter the room for a blood meal and go and rest outside. Traps were fitted at sunset and removed at sunrise after extracting the mosquitoes (Figure 3.1).

Figure 3.1:

3.1a: Early morning inspection of an exit window trap fit through a broken window.

3.1b. Extraction of mosquitoes captured through a window trap

Mosquitoes which choose to rest indoors after a blood meal were captured using the pyrethrum spray catch (PSC) method. Early in the morning before sunrise, a white cloth was laid down covering the entire floor (Figure 3.2a) below, and pyrethrum insecticide applied as an aerosol (PSC; WHO 1992) to knock down all mosquitoes in the house.

Figure 3.2.

3.2a. White clothing fully laid out on the floor of a room in preparation for pyrethrum spray catches.

3.2b. Inspection, separation and counting of adult mosquitoes under a portable lighted magnifying glass

Pyrethrum spray catches were conducted early in the morning just after the removal of exit window traps, before house cleaning was done which allowed for an accurate determination of vector density [22]. All mosquitoes captured through exit window traps and pyrethrum spray catches were immediately classified to genus level under a portable lighted magnifying glass (Figure 3.2b) above.

Statistical analyses

At each sampling house, mosquitos were counted fortnightly, coinciding with the application of the bio-larvicide. The sampling periods for the two countries did not coincide exactly, as sampling in Zimbabwe started earlier than in Botswana. The analyses compared mosquito counts for intervention and control sites. We used random-effects Poisson regression to assess the relationship between adult mosquito counts and the fixed-effects treatment (larvicided; not larvicided), country (Botswana; Zimbabwe), time period, the interaction between treatment and time period and covariate baseline count. Sites were specified as the random-effects component, taking care of the repeated measures within sites. The incidence rate ratio (IRR) for treatment was of primary importance. Data were analyzed using Stata Release 14, (StataCorp, College Station, TX: StataCorp LP). Since the analysis was sensitive to too many zeros, we did not compare adult mosquito densities using multiple dis-aggregations.

3.3.2. Ethical considerations

This study was approved by the Ethics Committee of the Faculty of Health Sciences, University of Pretoria, South Africa (Reference number 289/2014); The Human Research Development Committee (HRDC) of the Botswana Ministry of Health; and the Medical Research Council of Zimbabwe (Approval number MRCZ/A/1898). The National Malaria Control Programs of both Zimbabwe and Botswana supported the study. Written consent was obtained from heads of households to access houses for adult mosquito capture.

3.4. Results

3.4.1. Characteristics of the study sites

Table 3.1 shows the characteristics of the study areas in the two countries.

Table 3.1: Characteristics of study sites

Country		Intervention	Control
Number of sampling rooms			
Botswana		6	6
Zimbabwe		8	8
Total number of mosquitoes captured			
Botswana		206	753
Zimbabwe		50	228
Total number of mosquitoes by type			
Botswana	Culex	206	753
	Anopheles	0	0
Zimbabwe	Culex	47	227
	Anopheles	3	1
Mean number of mosquitoes per room per collection (Range)			
Botswana		4 (0 – 12)	15 (1-43)
Zimbabwe		1 (0-3)	3 (0-16)
Total number of mosquitoes captured by method			
Botswana	Exit window traps	0	0
	Pyrethrum spray catches	206	753
Zimbabwe	Exit window traps	1	16
	Pyrethrum spray catches	49	212

Mosquitos were repeatedly captured in 12 rooms in Botswana and 16 rooms in Zimbabwe, equating to 28 homes in total. Data from four rooms (two in intervention and two in control) in Botswana were excluded due to inconsistent data collection. Despite being comparable at the start, at the end of the study, control rooms in

Zimbabwe had three times more mosquitoes compared to the intervention sites in both countries.

From Table 3.1, all mosquitoes captured in Botswana were Culex spp. In Zimbabwe, very few Anopheles spp were caught in the intervention (6%, n=3) and control (4%, n=1). The Provincial Field Officer who was part of the study had this to say, "*This is the first study to manage to capture Anopheles gambiae in this area*". All the mosquitoes in Botswana were captured through pyrethrum spray catches in both the intervention and control areas. In Zimbabwe, 17 (6%) of all catches were through exit window traps.

Adult mosquito numbers at baseline were comparable between the control and intervention sites in Botswana (Figure 3.3a). Over time, the average number of mosquitoes increased in the control areas reaching approximately 25 per room in week five. In the intervention area, the average number of adult mosquitoes remained low at under five throughout the data collection period.

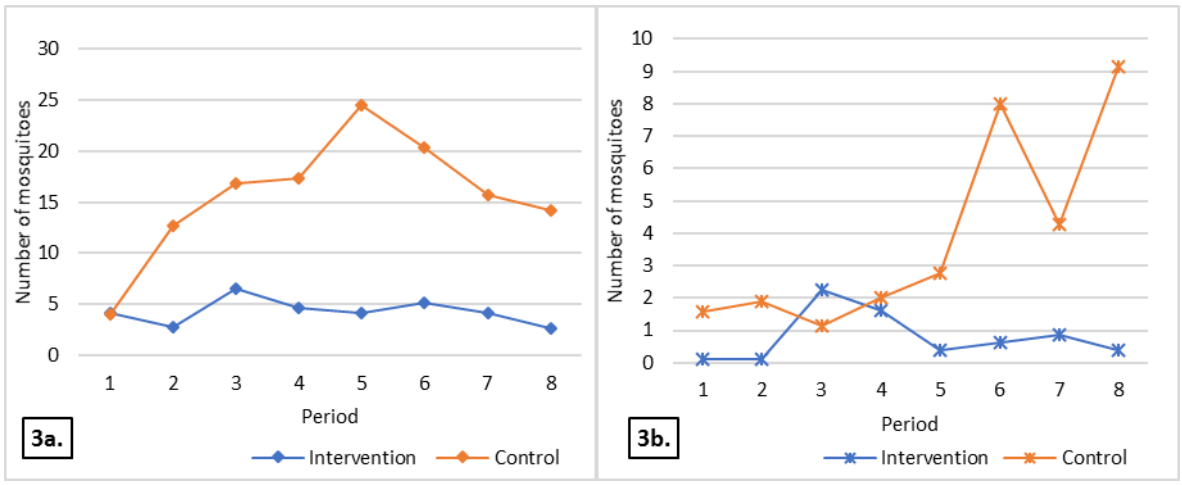

Figure 3.3

3.3a. Mean mosquito density in Botswana

3.3b. Mean mosquito density in Zimbabwe

In Zimbabwe (Figure 3.3b), the number of mosquitoes were very low at baseline at almost zero in both the intervention and the control. At the end of data collection, the number of mosquitoes reached a maximum of nine mosquitoes per room in the

control area when temperatures started increasing. Mosquito numbers remained low in the intervention areas, at less than one.

3.4.2. Effect of larviciding on adult mosquito density, by country

Figure 3.4 & 3.5 below show the effect of larviciding on mosquito density between the intervention and the control over time.

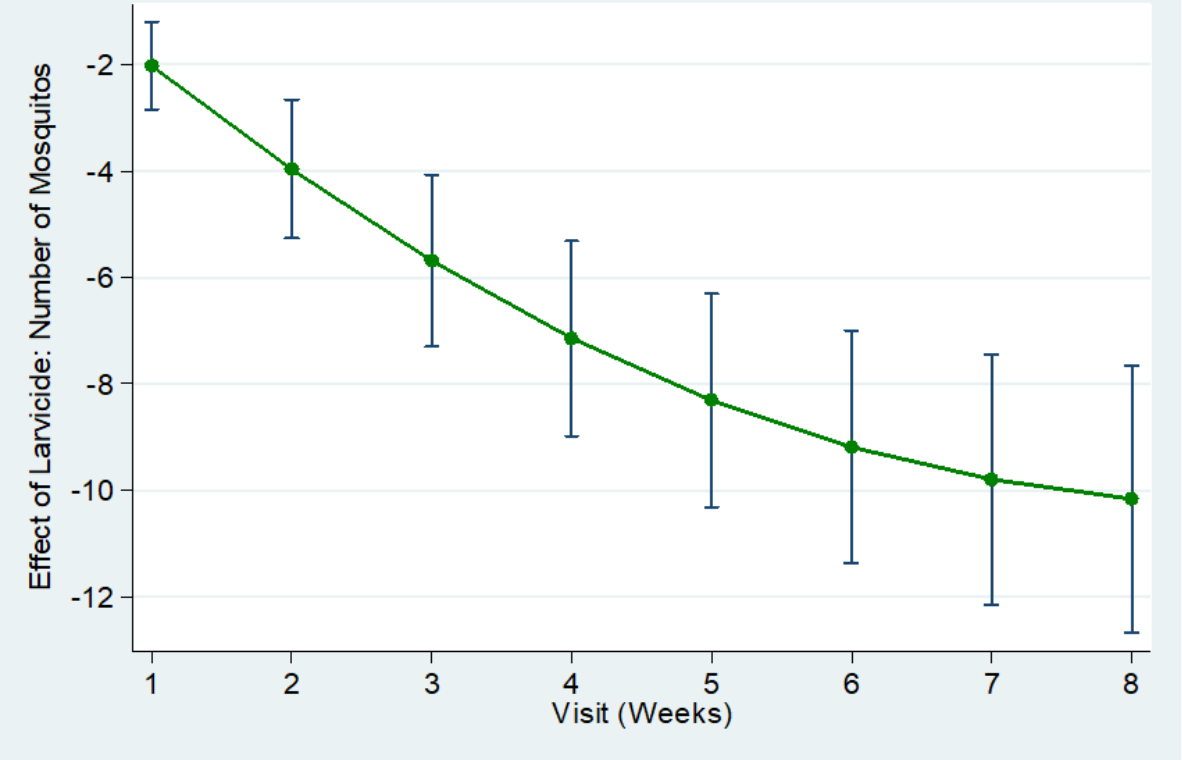

Figure 3.4: Overall (pooled) effect of larviciding from baseline in adult mosquitoes between intervention and control for Botswana and Zimbabwe combined.

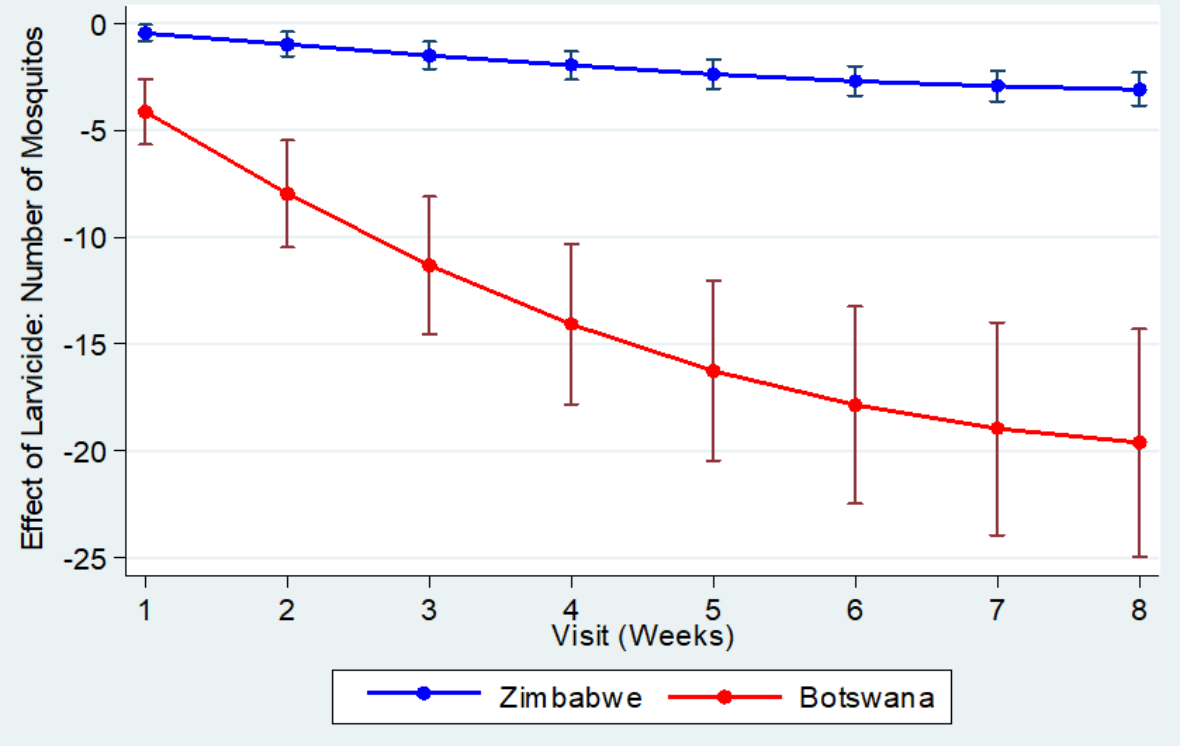

Figure 3.5: Effect of intervention from baseline in adult mosquitoes between intervention and control for Botswana and Zimbabwe

In Botswana, at visit one (baseline), rooms in the intervention arm had significantly (p<0.001) fewer adult mosquito densities than control arm (-4.126). Over time, at visit eight, with larviciding, the difference between intervention and control increased to 18 less mosquitoes in the intervention (p<0.001).

Results in Zimbabwe also showed similar trends. At visit one, the houses in the intervention arm had similar mosquito numbers to the houses in the control arm with 0.437 less mosquitoes on average (p=0.023). With subsequent visits, the difference increased though it was not significant during the second and third visit. Table 3.2 summarizes the overall effect of larviciding in Botswana and Zimbabwe.

Table 3.2: Overall marginal effect of larviciding in Botswana and Zimbabwe

Country	Marginal effect	IRR*	% reduction	
Combined	-1.194	0.303	70%	P<0.001
Botswana	-1.457	0.233	77%	p<0.001
Zimbabwe	-0.997	0.369	63%	P<0.001

*Incidence rate ratio

The overall effect of larviciding on adult mosquito densities in the intervention areas relative to the control taking into account interaction with time was -1.457 in Botswana and 0.997 in Zimbabwe. Adult mosquito densities were reduced by 77% and 63% in Botswana and Zimbabwe, respectively.

3.5. Discussion

Bio-larviciding has demonstrated that it effectively reduces adult mosquito densities. In a larger study [21], larviciding reduced mosquito larvae by up to 86%, and the effect was highest in the late instars at 91% in the intervention areas relative to the control. Reduction of late instars is known to reduce the emergence of adult mosquitos [23, 24], supporting our findings. Other studies in Sub-Saharan Africa have also found larviciding to reduce adult mosquito densities leading to reductions in exposure to bites [10]; substantial reduction in new infections [11] and overall reduction in malaria prevalence [12].

The WHO, while recognizing the potential of larviciding, recommends it as a supplementary intervention to IRS and LLINs while its field effectiveness is being determined [7, 20, 25]. This study showed that bio-larviciding substantially reduced the numbers of adult mosquitoes caught. Despite catching a large number of Culex spp., which are non-vectors, the effectiveness of larval control is significant to justify inclusion as part of the IVM package in semi-arid rural areas of Botswana and Zimbabwe. Bio-larviciding has shown to reduce the numbers of Anopheles larva by up to 80% [21], which is likely to have retarded adult emergence.

Larviciding is recommended in Botswana and Zimbabwe [7], but the extent of the effectiveness of the intervention in the two counties is unknown [26]. Zimbabwe recognizes larval control as a vector control intervention for focal control [27] and has guidelines for larviciding. The NMCP has identified the lack of mapping of perennial mosquito breeding sites and the lack of basic training for larviciding as major barriers to the intervention [27]. It is hoped that the findings of this study will support

informed decision making for the Botswana and Zimbabwe NMCPs, allowing them to consolidate larviciding as an important part of their vector control strategy.

This study was conducted in winter, the driest season in the two countries and when larviciding is most suitable. This is also time of low breeding activity, possible resulting in low numbers of mosquitoes and the predominance of Culex genus in our sample. The low numbers of mosquitoes caught in Zimbabwe may be due to the timing of study which was early in winter, at time when temperatures were at their lowest the reason a number of houses had no mosquitoes despite being close to breeding habitats. However, there was an increase in mosquito numbers over time, with increasing temperatures, in the control area. In the intervention area mosquito numbers remained low despite rising temperatures. The effect of the intervention on the adult mosquito numbers reported here is used as a proxy for the effect on malaria vectors because we could not trap enough Anopheles genus vectors to support a statistical analysis. *An. arabiensis* and Anopheles gambiae, the vectors that have been identified in Zimbabwe [28] and Botswana have previously demonstrated sensitivity to *Bti* products [29, 30]. The principal vector *An. arabiensis* has demonstrated exophilic tendencies in most parts of Zimbabwe [28], making it difficult to find indoors, demonstrating the limitation of the adult capture methods used. Despite this limitation, larviciding using *Bti* has been found to significantly reduce mosquito populations even where outdoor human bait collections are conducted [31]. The exophilic behavior of the *An. arabiensis* provides justification for intensifying larviciding as a way of decimating their larval stage leading to limited numbers of the adult mosquito.

While the benefits of larviciding in controlling adult mosquito densities have been observed even when applied in low doses [32, 33], considerable research still needs to be conducted to understand the full potential and limitations of the intervention [12]. Still, that does not stop countries like Botswana and Zimbabwe in implementing the intervention in rural areas where breeding sites are few, fixed and findable as a supplement to IRS and LLINs. This will require an intensive surveillance and treatment system to maintain coverage of all potential larval habitats. Furthermore

Bti has been assessed by the WHO Programme on Chemical Safety [34], and its products are considered safe for use in drinking water [7].

3.6. Conclusion

The use of microbial larvicide *Bti* has demonstrated effectiveness in the suppression and reduction of adult mosquitoes, a proxy indicator for the potential effect on malaria vectors. Results of this study and previous studies [21] recommends that countries such as Botswana and Zimbabwe, that are targeting malaria elimination, include larviciding as part of the IVM package. This will complement IRS and LLINs which are becoming less efficient due to increasing insecticide resistance. These control methods may also not be efficient because the principal vector *An. arabiensis*, displays exophilic behaviors and does not rest indoors. Bio-larviciding may target these mosquitos in the early stages of their development.

List of abbreviations

LLINs: long-lasting insecticide-treated nets; **WHO**: World Health Organization; **IRS**: indoor residual spraying; **IVM**: Integrated Vector Management; **Bti:** *Bacillus thuringiensis israelensis*; **Bs**: *Bacillus sphaericus*

3.7. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MM acted as the study coordinator and designed the study, conducted data collection, analysis and report writing. KM and CdJ supervised and reviewed the design, data collection, analysis and reviewed the manuscript. PB designed the methodology, conducted data analysis and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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CHAPTER FOUR

4. COMMUNITY KNOWLEDGE, PERCEPTIONS AND ACCEPTABILITY OF MICROBIAL LARVICIDING FOR MALARIA CONTROL IN SELECTED RURAL AREAS OF BOTSWANA AND ZIMBABWE.

This chapter builds on the experimental findings presented in chapter three and four and focusing on the acceptability of larviciding at community level as an additional vector control intervention. Despite demonstrated effectiveness on the mosquito larva and the impact on adult mosquito densities, acceptability of the intervention is critical for its success. This chapter presents findings of the assessment on the acceptability of larviciding in the study villages/wards of Botswana and Zimbabwe respectively

4.1. Abstract

Background

Indoor residual spraying with pyrethroids and long-lasting insecticidal nets are the main malaria vector control interventions in Botswana and Zimbabwe. Increasing resistance of mosquitos to pyrethroids is threatening successful malaria control leading to increasing demand for additional interventions such as larviciding. While larviciding field trials have shown promising results, success at program level requires community participation and support. In this paper, we report on community knowledge, perceptions and acceptability of bio-larviciding in selected rural areas of Botswana and Zimbabwe.

Methods

Thirty-two heads of households were interviewed using a semi-structured interview guide with both closed and open-ended questions. Participants came from two villages and two wards in Botswana and Zimbabwe, respectively. The villages and wards were study arms of an ongoing larviciding experimental study in the two countries.

Results

Bio-larviciding was known to 81% and 31% of the respondents in Botswana and Zimbabwe respectively. All the participants, from both countries, knew about indoor residual spraying and long-lasting insecticidal nets. Interviewed community members felt that larviciding was acceptable and were willing to support it because it could effectively supplement IRS and LLINs. They also had a desire to be protected from mosquito bites, and they perceived that larviciding was able to kill mosquitoes early, and that it was safe and easy to implement. Community members perceived that the weaknesses of larviciding included the inability to control all mosquitoes, that larvicides were not always available and that finding all the breeding sites would be difficult.

Conclusion

Community members, from Botswana and Zimbabwe, felt that larviciding using microbial larvicides is acceptable. National mosquito control initiatives should take advantage of the communities' willingness to support and include larviciding into an integrated vector management (IVM) package for selected rural areas where breeding habitats are few, fixed and findable.

Key words: Larviciding, vector control, integrated vector management, Botswana, Zimbabwe

4.2. Background

Frontline vector control interventions such as indoor residual spraying (IRS) and long lasting insecticidal-nets (LLINs) [1] need to be applied consistently and correctly to be effective. Microbial larviciding is less prone to the vagaries of human behaviour such as uptake and consistent use but is less commonly used for malaria control in Sub-Saharan Africa despite significant potential as part of an integrated vector management (IVM) strategy [2]. It is an appropriate intervention [3] where breeding sites are few, fixed and findable [4] ,though some breeding sites are hidden [5], while some are small and scattered all over due to animal activity which leaves hoof marks suitable for vector breeding. Mosquito breeding sites in semi-arid regions of sub-Saharan Africa have these characteristics where microbial larviciding has proven efficacious [6].

Microbial larviciding involves targeting mosquito larvae in their breeding habitats and applying an anti-larval agent. The common agents are of bacterial origin and include *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) [7], with well-documented efficacy [4, 8-12] in rural highlands [8, 10, 13], urban areas [14, 15] and semi-arid rural areas [12] in east and sub-Saharan Africa. Larviciding targets the larval and immobile stages of the mosquitoes which cannot escape the bacteria in water, a concern with IRS and LLINs [1]. Mosquitoes are also becoming increasingly resistant to pyrethrum-based insecticides used for IRS and for treating LLINs. Pyrethrum-resistant mosquitoes may survive up to a 1000 times the concentration of insecticide that kills susceptible mosquitoes [16]. In an effort to minimise the impact, stakeholders developed the global plan for insecticide resistance management in malaria vectors [17] which also identifies larval source management including larviciding as providing an opportunity for insecticide resistance management, in addition to insecticide rotation. This makes integrating larval control into the IVM package attractive in circumventing pyrethroid resistance. The benefits of microbial larviciding may be enhanced with support from local communities, and could accelerate progress towards malaria elimination [18]. The knowledge and attitudes of local communities towards larviciding have been investigated in Sub-Saharan Africa [19-21] in conjunction with field trials and have in certain circumstances resulted in scaling up larviciding interventions.

Botswana and Zimbabwe are members of the Elimination 8 (E8) group of countries in the Southern Africa Development Community (SADC) region. The E8 initiative facilitates collaboration and data-sharing across four malaria-eliminating countries at the front-line—Botswana, Namibia, South Africa and Swaziland—and their second line northern neighbours working to reduce transmission and achieve subnational elimination—Angola, Mozambique, Zambia and Zimbabwe. The frontline countries aim to achieve elimination by 2020 [16], a goal that is threatened by increasing resistance to pyrethrum based insecticides [22-24]. To explore other supplemental vector control interventions, a larviciding experimental study was established in Mathathane and Molalatau villages in Botswana and Birchnough bridge village in Zimbabwe in 2015 [12]. In this paper, we report on community knowledge, acceptability and perceptions about larviciding as part of the experimental study.

4.3. Methods

4.3.1. Study areas

This study was conducted in Molalatau and Mathathane villages of Bobirwa District in Botswana, and Birchnough Bridge village of Buhera District in Zimbabwe. The whole of Botswana including the two villages are in malaria pre-elimination, while Buhera District is characterised by low malaria transmission reporting the lowest number of malaria cases in Manicaland Province [25]. Larviciding is recommended in both countries and Botswana adopted the intervention as a national policy in 2012 [26], but implementation has been inconsistent over the years.

Molalatau and Mathathane villages are 25 km apart and they have 534 and 752 households and a population of 1,788 and 1,845 respectively[27]. The two villages had ongoing IRS and LLINs interventions during the year of this study. A combination of DDT and pyrethroids were being used for IRS in the two villages with the former being applied to traditional mud houses and the later on modern plaster and painted houses. Birchnough Bridge in Zimbabwe has 2,188 and 9,594 households and residents respectively[28]. The village is an agriculture area and had ongoing IRS using pyrethroids and LLINs vector control interventions during this study.

4.3.2. The larviciding experimental study

The participating villages were part of an ongoing experimental study to assess the field effectiveness of larviciding using microbial larvicides [12]. During the experimental study, Mathathane village and one ward in Birchnough Bridge were used as intervention areas while the other were used as controls. During the experimental study, all permanent and semi-permanent water bodies were mapped using handheld GPS machines in both the intervention and the control areas. However, those in the intervention areas were treated using the commercial larvicide VectoBac® WG (Valent BioSciences Corporation, IL, USA) containing the active ingredient *Bacillus thuringiensis var. israelensis* (Bti), a WHO recommended larvicide[29] at two-week intervals. A day before the next treatment period, larval sampling was conducted from the same 32 breeding points while adult mosquito sampling was also conducted from 32 households (8 from each of the study arms per country).

4.3.3. Data collection

Semi-structured interviews were held with heads of households where the adult mosquito surveillance and sampling was conducted. In the absence of the head of household, an adult representative was interviewed. The heads of households who participated were conveniently selected because their households were already enrolled into the study. The interview guide used had both closed and open-ended questions and was administered by an experienced interviewer.

4.3.4. Ethical considerations

The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of Pretoria (South Africa, Reference number 289/2014), The Human Research Development Committee (HRDC) of the Botswana Ministry of Health and the Medical Research Council of Zimbabwe (Approval number MRCZ/A/1898). The study was supported by National Malaria Control Programmes of both Zimbabwe and Botswana. Each head of household, who agreed to participate, gave written consent.

4.3.5. Data analysis

Quantitative data from the questionnaire were entered into an excel data base, exported and analysed in SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The qualitative data, from the open-ended questions, were analysed using a general inductive approach. Textual data were coded manually, and common themes were identified to condense the data.

4.4. Results

4.4.1. Characteristics of respondents

Thirty-two questionnaires were administered, 16 in Botswana and 16 in Zimbabwe. In each of the two countries, eight questionnaires were administered in the intervention villages and eight questionnaires in the control villages. The majority of the respondents were female (17/32), and twenty-eight had lived in the study villages for more than five years, while 26 (78.1%) had ever contracted malaria during their lifetime (68.8% in Botswana and 87.5% in Zimbabwe). Of those who had contracted malaria, 12.6% and 36.6% reported their most recent event in the past five years for Botswana and Zimbabwe respectively.

4.4.2. Knowledge and access to malaria vector control interventions

Respondents were asked to identify and share the vector control interventions familiar to them, and the ones which they had access to. Figure 4.1 shows the malaria vector control methods known to respondents in both Zimbabwe and Botswana. All the respondents were familiar with IRS and LLINs, while 81% and 31% of the respondents in Botswana and Zimbabwe respectively were familiar with larviciding.

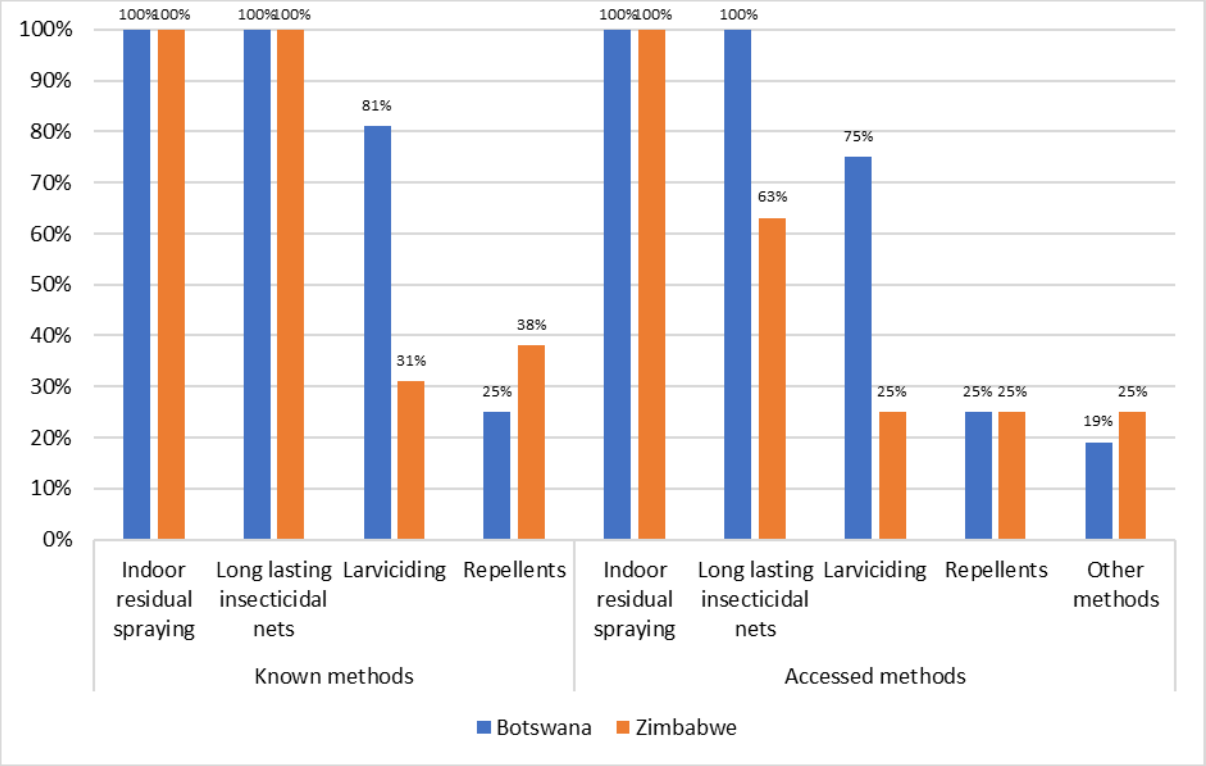

Figure 4.1: Malaria vector control methods known and accessed by heads of households from Botswana and Zimbabwe.

From Figure 1 above, all respondents in both countries indicated having access to IRS. All of the participants from Botswana, reportedly, had access to LLINs while it was 63% in Zimbabwe. Seventy-five percent of respondents from Botswana reported having access to larviciding, and 25% of participants from Zimbabwe. In terms of knowledge, all the respondents knew at least two vector control methods in both countries (Figure 4.2).

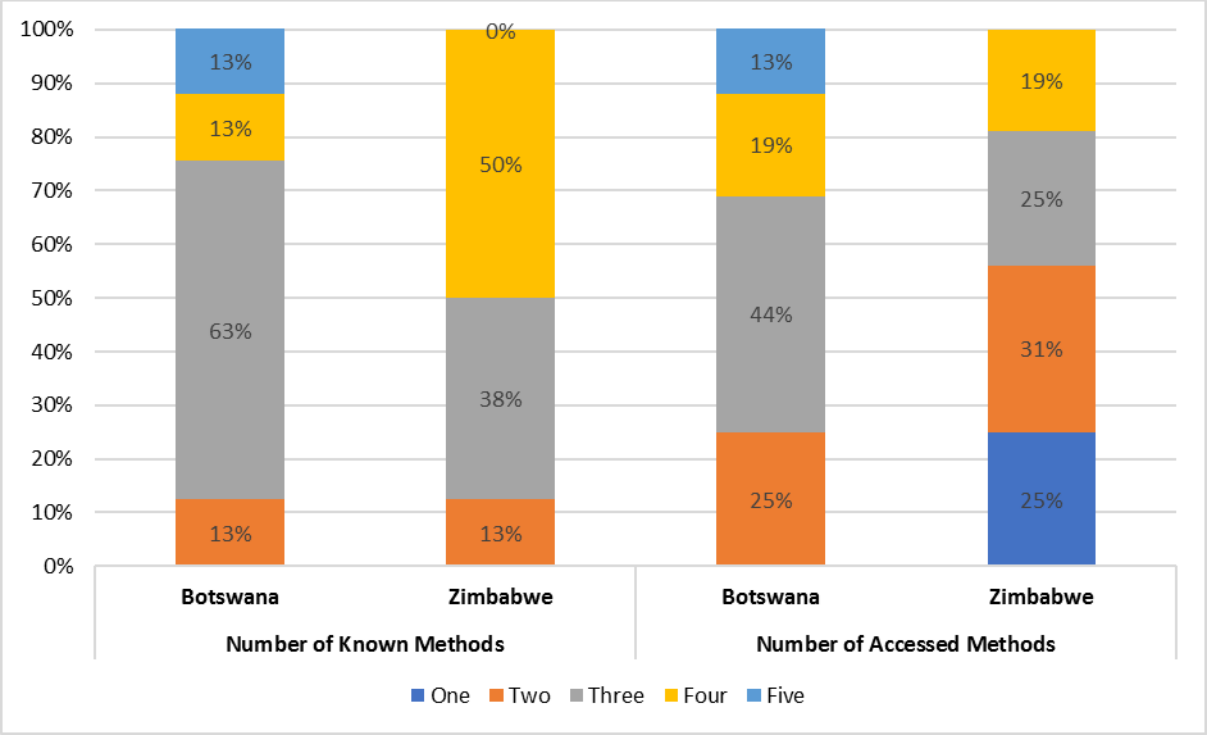

Figure 4.2: Number of known and accessed vector control methods

From Figure 4.2 above, 25% of participants in Botswana reported having access to at least two vector control methods, which was the minimum, with 69% having access to three methods. Thirteen percent of participants indicated having access to five methods which also includes some traditional methods such as burning cow dung. In Zimbabwe, 25% of participants indicated having access to only one method which is IRS, and 19% indicated having access to four methods, the highest number of vector control methods accessed.

4.4.3. Acceptability and opinions on larviciding

Participants were asked to indicate if they would accept larviciding as a vector control method and share their perceived strengths and weaknesses of the intervention. Table 4.1 summarises the indicated factors of acceptability and perceptions about larviciding.

Table 4.1: Acceptability factors, and perceived strengths and weaknesses of larviciding

	Specific factors & perceptions
Acceptability factors	Larviciding is important to complement other methods
	Desire to be protected
	Observed effectiveness
	Willingness to support larviciding
Strengths	Larviciding kills mosquitoes early before they can bite
	Safety
Weaknesses	Unavailability of chemical
	Does not kill all mosquitoes
	Difficult to identify all breeding sites

4.4.4. Acceptability factors

Larviciding as a complementary method

Respondents in both countries highlighted that larviciding is a necessary intervention able to complement IRS and LLINs, which are known to have deficiencies. The following points were raised by participants:

> "*It can complement other methods and reduce mosquitoes*"
>
> "*Larviciding is necessary because it reduces the number of adult mosquitoes which bite us*"

Participants were able to attach importance to the intervention demonstrating the value and acceptability of larviciding. One participant said:

> "*Anything that controls mosquitoes is acceptable*"

Desire to be protected

Participants expressed a need and desire to be protected from mosquito bites, which would protect them from malaria and facilitate the acceptability of larviciding.

Respondents indicated that anything that would protect them from malaria will never be objected to. The participants made the following comments:

"*I think there will be no problem if it is done. We want to be protected from malaria*"

"*In our community we support programs that benefit us*"

Observed effectiveness

Participants in the intervention villages were familiar with larviciding and had experienced the effectiveness which they felt was of benefit to them. Based on this, they found the intervention acceptable.

"*It brings benefits - now there are few mosquitoes*"

Not all participants knew about larviciding but still indicated that larviciding would be acceptable if there are benefits to the community:

"*This (larviciding) hasn't been implemented much but once people realise the benefits it will be acceptable*"

Willingness to support

The willingness to support larviciding demonstrates its acceptability. Multiple participants indicated numerous ways in which they would support larviciding if called upon:

"*We can report to the local clinic when we see water points with breeding happening*"

"*If given the chemical, I can spray in my yard and areas close. I have my own pump*"

4.4.5. Perceived strengths of larviciding

It kills mosquitoes early

The ability to kill mosquitoes before they become adults was one of the identified strengths of larviciding. Even participants who were unsure about how larviciding worked had this to say:

"*I understand it kills mosquitoes early".*

The belief that larviciding would reduce mosquito bites by reducing the number of adult mosquitoes was one of the perceived strengths of larviciding.

Safety

Larvicide is applied to water bodies where animals drink and where water is drawn for domestic purposes. The assurance that the microbial larvicides applied to water sources are safe to both humans and animals was seen as a strength compared to other methods of larviciding such as pouring used oil over water sources. One of the participants said:

"*No one has ever objected and we have always been told that it is safe*" and

"*As long as it does not affect other animals, it is fine*"

4.4.6. Perceived weaknesses of larviciding

Unavailability of chemical

Some participants raised the concern that microbial larvicides are not readily available, which was seen as a weakness. Some participants speculated that the unavailability of the chemical may lead to inconsistent implementation as highlighted by the following comment:

"It *(larviciding) is not consistently done*"

Does not kill all mosquitoes

Participants raised concerns that larviciding, just like the other interventions, does not lead to full protection and mosquitoes remain present though reduced in population. Some participants had this to say:

"*It (larviciding) does not kill all mosquitoes*", and

"*I think it's acceptable, but we still get bitten by mosquitoes*"

Difficult identifying all breeding points

Because mosquitoes breed in any water point, some of which are temporary, the participants cited the difficulty in identifying all the breeding points as a weakness because breeding activity can still occur in un-identified water points. One participant observed:

"*It may be difficult to identify all the breeding points*"

4.4.7. Perceived effectiveness and importance of larviciding

Participants were asked to rank mosquito abundance prior to the intervention and during the intervention. Table 4.2 shows rankings disaggregated by intervention (larviciding) or control (no larviciding).

Table 4.2: Perceived effectiveness and importance of larviciding

	Control N=16		**Intervention N=16**		**Total N=32**	
	n	**%**	**n**	**%**	**n**	**%**
Aware Larviciding						
Yes	6	38	16	100	22	69
No	10	63	0	0	10	31
Ranking mosquito abundance in the past						
Very Few	0	0	0	0	0	0
Few	0	0	0	0	0	0
Don't Know	6	38	0	0	6	19
Abundant	5	31	9	56	14	44
Extremely Abundant	5	31	7	44	12	38
Ranking mosquito abundance now *						
Very Few	0	0	2	13	2	6
Few	2	13	12	75	14	44
Don't Know	10	63	0	0	10	32
Abundant	4	25	2	13	6	19
Extremely Abundant	0	0	0	0	0	0
Perceived importance of larviciding *						
Not Important						
Some Importance	1	6	0	0	1	3
Don't Know	6	38	0	0	6	19
Important	6	38	3	19	9	28
Very Important	3	19	13	81	16	50

*Significant at p<.05 using Pearson chi-square tests

All the participants in the intervention villages were aware of larviciding activity while 38% of participants in the control villages were aware of some larviciding activity happening. Participants in both the intervention and control areas ranked the past mosquito abundance as either abundant or extremely abundant. During the intervention period, there was a significant difference in ranking of mosquito abundance in the intervention villages compared to the control villages ($p<0.05$). Most of the participants from the intervention villages ranked abundance as either few or very few (13% and 75% respectively), while those in the control areas were mostly unsure of the abundance. Most participants in the intervention villages ranked larviciding as very important (Table 4.2), whilst significantly fewer participants ranked larviciding as very important ($p<0.05$).

4.5. Discussion

Currently, malaria vector control relies on insecticides used in IRS and for treating LLINs, but the distribution and strength of insecticide resistance has increased in recent years threatening the success of control programs [30]. While measures have been put in place counter insecticide resistance [17, 31], a shift to alternative non-insecticidal methods has been encouraged where feasible [22]. One of the alternatives is microbial larvicide, which have shown successes during field trials [3, 8, 12, 32, 33], yet less commonly used despite their significant potential as part of an IVM strategy [34]. Microbial larviciding costs have also shown to be relatively low [35] though cost-effectiveness in combination with other vector control strategies in rural settings still needs to be investigated further.

For larviciding to succeed as part of an IVM strategy, target communities need to acknowledge, accept and support the intervention, as well as generate service demand. In this study, heads of household, particularly from the intervention rural communities reported having experienced a reduction in number of mosquitoes. While those from the control communities had no experience of the benefits, they demonstrated optimism that the intervention will be able to reduce mosquito densities.

The knowledge of larviciding as a malaria vector control intervention demonstrated in this study is a good starting point in promoting and ensuring acceptability within the community. In general, participants had an adequate knowledge of different vector

control interventions, similar to findings from rural districts of Tanzania where general knowledge of malaria transmission, breeding sites and prevention measures was good among communities [21]. Indoor residual spraying and LLINs have been used extensively in both countries [26],. From different studies, community and user acceptability and participation in vector control interventions was affected by inadequate knowledge about the reasons for, and safety of the interventions [36-38]. In this study, participants from both countries demonstrated an awareness of larviciding despite acknowledging that it is not readily available because of inconsistent application. However, some of this knowledge could have been acquired during this study while some could have been referring to other methods of larviciding other than microbial larviciding.

While larviciding is promoted for malaria control, protection from mosquito bites is probably one of the expected primary benefits to the ordinary person in the community. The benefit of a reduction in mosquito bites has also been reported for new interventions where acceptability of insecticide treated wall linings was found to be motivated by decreases in mosquito nuisance and biting in general, and other annoying insects post-installation [39].

In both countries, participants were willing to support any intervention that would reduce mosquito densities and accompanying bites. This acceptability factor could be leveraged upon to promote larviciding for malaria control. Participants indicated their willingness to help with the identification of breeding points, while others were prepared to conduct larviciding itself in areas within their jurisdiction. Similar findings were observed in Burkina Faso where acceptability was influenced by a high perceived success rate of larviciding in reducing the number of malaria vector mosquitoes and malaria cases [19], while community members in Tanzania indicated willingness to contribute financially towards the implementation of larviciding [20]. The study in Tanzania was also conducted amongst participants living in an intervention area where a larviciding experimental study was ongoing, and who reported a significant decline in mosquito populations compared to corresponding periods in previous years. There are however concerns that efficacy of larviciding in controlling mosquito nuisance may lead to complacency and reduced used of other vector control interventions, such as the non-use of bed nets [39].

Highlighting the safety of microbial larvicides to humans and animals is critical for addressing the concerns raised in this study. In Burkina Faso, most of the participants who were interviewed declared not knowing anything about microbial larvicides yet they considered it safe for humans and animals [19], a trust which they bestowed on health workers promoting it. Even in Botswana and Zimbabwe, while some safety concerns were raised, participants trusted that health workers would not introduce harmful interventions. Both Bti and Bs are safe, and to date, neither Bti nor Bs has been shown to have any negative effects on non-targeted organisms, including humans [4].

Participants were concerned about availability of the larviciding, a genuine concern considering that the two countries have not been able to implement larviciding consistently. Additionally, participants from the intervention areas acknowledged that being bitten by mosquitoes despite larviciding activity, a genuine concern, and consistent with the intervention because it does not provide full protection.

This study was limited by the likelihood of social desirability bias where participants, despite constant encouragement to be truthful in their responses, may have supplied answers that they believed the researcher wanted to hear. Additionally, the number of interviews were few to warrant drawing conclusions from the quantitative data. However, the qualitative data provided useful insights on the perceptions and opinions of community members in the study villages.

4.6. Conclusion

This study indicates that larviciding is acceptable in rural communities of Botswana and Zimbabwe. There is already some information on larviciding, which is an important step in planning scale-up. Participants desired to be protected from mosquito bites, which still occurred despite extensive IRS and use of LLINs. This desire may be leveraged upon to get community support. Community members are willing to help identify breeding points and conduct larviciding, which is a key step towards community based larviciding and sustainable implementation. However, to sustain community confidence in larviciding, both Botswana and Zimbabwe need to implement the intervention consistently in priority areas. Microbial larviciding could be another tool in IVM strategies to move towards elimination in the two countries.

List of abbreviations

LLINs: long-lasting insecticide-treated nets; **WHO**: World Health Organization; **IRS**: indoor residual spraying; **IVM**: Integrated Vector Management; **Bti:** *Bacillus thuringiensis israelensis*; **Bs**: *Bacillus sphaericus*

4.7. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MM was the study coordinator and designed the study, conducted data collection, analysis and led the writing of the manuscript. KM and CdJ supervised and reviewed the design, data collection, analysis and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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CHAPTER FIVE

5. COMPETENCIES OF COMMUNITY VOLUNTEERS IN BIOLOGICAL LARVICIDING FOR MALARIA VECTOR CONTROL IN BOTSWANA AND ZIMBABWE[2].

The previous chapters have demonstrated the effectiveness of the larviciding intervention and this chapter is focusing on the competencies of the community volunteers who were used to implement the intervention.

5.1. Abstract

Background

Malaria continues to be a major public health problem in Botswana and Zimbabwe despite successful vector control using indoor residual spraying and long-lasting insecticidal-treated nets. A larviciding experimental study using community volunteers was conducted in selected villages in Botswana and Zimbabwe to establish its effectiveness in vector control. In this paper we present the competencies of the community larviciders in implementing biological larviciding.

Methods

The larviciding experimental study was conducted between May and November 2015 in two villages in Botswana and two wards in Zimbabwe. The community volunteers received training at the beginning of the study on how to identify breeding points; how to conduct larval sampling and identification; and how to prepare and apply larvicide. At the start and end of the study, the volunteers were assessed for their ability to independently: a) identify and estimate size of breeding sites, b) conduct larval sampling and differentiate between different larval species and stages, and c) mix and apply larvicide to breeding points. Additionally, the quality of data in the forms which were completed by the volunteers during the study was assessed.

[2] The abstract for this chapter was accepted and presented as a poster at the 3rd Malaria Research Conference 2017, Johannesburg, South Africa.

Results

Five volunteers participated in the study, two in Zimbabwe and three in Botswana. At the start of the study, all volunteers in both countries could not identify breeding habitats nor accurately identify larvae in the water. However, by the end of the study, they could mix and apply larvicide independently, and do larval sampling. Their accuracy in identifying larvae increased from 0% to 87%, and 20% to 100% for *Culex* in Botswana and Zimbabwe, respectively. For *Anopheles*, the accuracy increased from 0% to 83%, and 19% to 97% in Botswana and Zimbabwe respectively. In both countries combined, their accuracy in identifying the different larval stages increased from 13% at the start of the study to 88% at the endof the study. Data quality was at least 85% for key attributes of completeness, reliability and validity at the end of the study.

Conclusions

Community volunteers can make a significant contribution in malaria vector control interventions. With adequate supervision, countries like Botswana and Zimbabwe targeting elimination and considering larviciding should consider using volunteers because of their ability to implement the intervention after receiving training.

Key words: Malaria, vector control, larviciding, Botswana, Zimbabwe

5.2. Introduction

Malaria continues to have a devastating impact on people's health and livelihoods, with 216 million cases reported in 2016worldwide despite 44 countries moving towards elimination compared to 37 countries in 2010.[1]. Long-lasting-insecticidal nets (LLINs) and indoor residual spraying (IRS) are currently the front-line vector control interventions, and though promising, vaccines haven't been overly successful yet [2].

Recent successes in malaria prevention and control are fragile because of growing resistance to insecticides used to treat LLINs and for IRS, and further progress will depend on investments in innovation and research amongst others [3]. Control of aquatic-stages of vector mosquitoes is one of the oldest and most historically successful interventions to prevent malaria, but it has seen little application in Africa

[4]. It is a labour intensive undertaking requiring unusual specialist skills, particularly for the most common Africa vectors such as *Anopheles gambiae* that colonize a large variety of habitats distributed widely over space and time, [4-7] and are best tackled with rigorous searches on foot [8]. However, the growing resistance to pyrethrum-based insecticides used for IRS and ITNs, [9, 10] and the outdoor feeding and biting behaviours of malaria vectors [11-15] justifies the need to consider larval control as a vector control intervention. While larval control of has shown some promising results,[16-19] it is currently recommended as a supplementary intervention to IRS and LLINs [20, 21] while further research is conducted to determine its full effectiveness.

Community health workers and related cadres have important preventive, case management and promotive roles in malaria interventions,[22] and have led to promising outcomes in malaria programs.[22, 23] The potential for using community health workers (CHW) for administering timely and effective treatment for presumptive malaria attacks has previously been evaluated [24], though the willingness to define the form of participation in consultation with the community itself is seen as the pinnacle of successful community interventions [25]. The scope and extent of community participation in health has usually remained poorly defined [26, 27]. However, community-participation which involves highly-localized task of detection and management of mosquito larval habitats within public and private facilities is considered vital to the cost-effectiveness and sustainability of vector control.[11, 25, 28]

A larviciding experimental study using community volunteers was conducted in selected villages in Botswana and Zimbabwe in 2015 to establish its effectiveness in vector control [16]. This paper presents the competencies of the community volunteers in implementing larviciding in the two countries.

5.3. Methodology

The larviciding intervention and the study areas

The larviciding experimental study was conducted in Molalatau and Mathathane villages of Botswana, and Birchnough Bridge in Zimbabwe. Bio-larviciding was

conducted every two weeks in all identified water points in the intervention areas using community volunteers.

Selection and training of larviciders

A select few community volunteers were identified with the help of the local national malaria control programs (NMCP) and used as community larviciders. In Botswana, the NMCP through the local health facility staff facilitated meetings with the local community and its leadership at a *Kgotla* (a traditional community meeting) where the study was introduced, and the role of the community clarified. On approval from the community, the health facility helped identify from its registered list, three volunteers.

In Zimbabwe, the NMCP through the local health facility identified volunteers who had traditionally participated in health events. All identified volunteers in the two countries were permanently resident in the villages where the study was conducted and had no intention of migrating out of the villages during the study period.

In each of the two countries, the community larviciders worked as a team when conducting their activities, with the assessment of their competencies also being team-based.

Characteristics of volunteers

In total, five community volunteers participated in the experimental study, two in Zimbabwe and three in Botswana. All the volunteers in Zimbabwe were male while in Botswana, two were female. The volunteers in Zimbabwe had some experience in malaria programs having previously participated during indoor residual spraying for a number of seasons as sprayers. They had some experience mixing vector control products and using pressure pumps. The volunteers in Botswana had no prior experience in malaria vector control programs, with two of them doing voluntary work for the first time.

Training of the community larviciders

In Botswana, the volunteers were trained by the study coordinator while in Zimbabwe they were trained with the help of the provincial malaria field officer. Volunteer trainings were hands-on, and they included how to identify mosquito breeding sites;

how to conduct larval sampling and determining larval density; differentiating larvae for *Anopheles* and *Culex* mosquitoes as well as by larval stage. Additionally, they were trained on how to estimate the size of the breeding sites, how to mix the bio-larvicide; and the technique of applying the larvicide in the breeding sites using pressure pumps. Community larviciders worked under supervision throughout the study and their competencies were assessed at the start of the study during training and throughout the study. During assessments, the study coordinator and the field officer would independently conduct larval counts and differentiation from the same scoop/sample.

Entomological surveys

Entomological surveys included larval sampling from selected breeding sites and conducted fortnightly. While this was entirely the responsibility of the field officer and the study coordinator, the volunteers were also given an opportunity to conduct this activity and were assessed. They were assessed for their ability to differentiate between *Culex* and *Anopheles* larvae as well as differentiating between the different stages of larvae.

Data quality

While conducting their work, the volunteers were responsible for completing the following study tools; the mosquito larval habitat survey form, and the larval inspection and sampling form. The mosquito larval habitat survey form was used to list every potential breeding site/habitat found with water while surveying walking on foot within the study villages. The form captured information on the type of habitat, its geographic positioning system (GPS) coordinates, description, vegetation coverage as well as information on habitat occupancy. The larval inspection and sampling form was used to capture information on quantity of larvae found at the larval sampling points, by type (*Anopheles* or *Culex*) and stage (early of late stage). Larval sampling was conducted at the same points every two weeks.

During training, volunteers were given the forms to complete on their own as part of baseline data collection. All the forms that were completed by volunteers were subjected to data quality checks by the study coordinator and/or the provincial field

officer (for Zimbabwe). During the final week of data collection, the study coordinator and the provincial field officer completed their own set of forms independent to what the volunteers had completed which were used to compare with that of the volunteers.

The following attributes of data quality were assessed:

Completeness - were all the data fields in the different forms completed?

Validity - are all data entered into the different forms done so accurately representing a true picture of what was observed?

Reliability - is there consistency in the collection of data?

5.4. Results

Larvicider competencies

Qualitative assessment

The community larviciders' ability to implement larviciding improved over time. During the first week when they were undergoing training for the study the community larviciders in Botswana couldn't identify mosquito breeding activity nor mix larvicide, Table 5.1 below.

Table 5.1: Competencies of community larviciders.

Competency assessed	**Ability of community larviciders to accurately conduct activity**	
	Start of study	**End of study**
Identification of breeding sites		
Botswana	Yes, partly	Yes, entirely
Zimbabwe	Yes, partly	Yes, entirely
Mixing of larvicide		
Botswana	No, not at all	Yes, entirely
Zimbabwe	Yes, partly	Yes, entirely

Application of larvicide			
	Botswana	No, not at all	Yes, entirely
	Zimbabwe	Yes, partly	Yes, entirely
Identifying larvae in water			
	Botswana	No, not at all	Yes, entirely
	Zimbabwe	Yes, partly	Yes, entirely

By the end of the study after 16 weeks, they were able to accurately identify breeding activity in water points. Larviciders in Zimbabwe had experience in malaria programs through their participation in IRS activities. Despite being their first involvement in larviciding activities, they were able to adapt and mix larviciding products and operate pressure pumps. They were also able to identify breeding activity even though they were ignoring small water points such as hoof marks and disregarding them as potential malaria vector breeding habitats.

Differentiation of mosquito larvae

Table 5.2 shows the ability of the community volunteers to accurately identify larvae for *Culex* and *Anopheles* mosquitoes as well as accurate classification by larval stage.

Table 5.2: Ability of community larviciders to differentiate larval species and stages

	Larvae accurately identified n (%)	
	Start of study	**End of study**
Larval species		
Culex		
Botswana	0 (0)	260 (87)
Zimbabwe	41 (20)	302 (100)
Anopheles		
Botswana	0 (0)	82 (83)
Zimbabwe	83 (19)	83 (97)
Larval stage		
Early Instar		
Botswana	0 (0)	173 (62)
Zimbabwe	221 (41)	202 (87)
Late Instar		
Botswana	0 (0)	162 (94)
Zimbabwe	74 (0)	201 (100)

From Table 5.2, volunteers in Botswana admittedly didn't know how mosquito larvae looked like and were not asked to identify by specie *(Culex* or *Anopheles*) nor by stage (first or late stage). On the other hand, volunteers in Zimbabwe had understanding of the difference in larvae for the two species, but only managed to accurately identify at most 20% at the beginning of the study. After 16 weeks of participation in the study, the volunteers in both countries could accurately classify to genus at least 80% of the larvae, and by stage- at least 62% of the larvae.

Data Quality

The completeness, validity and reliability of data on both the mosquito larval habitat survey form and the larval inspection and sampling form were at least 87% for both countries at the end of the study, Figure 5.1.

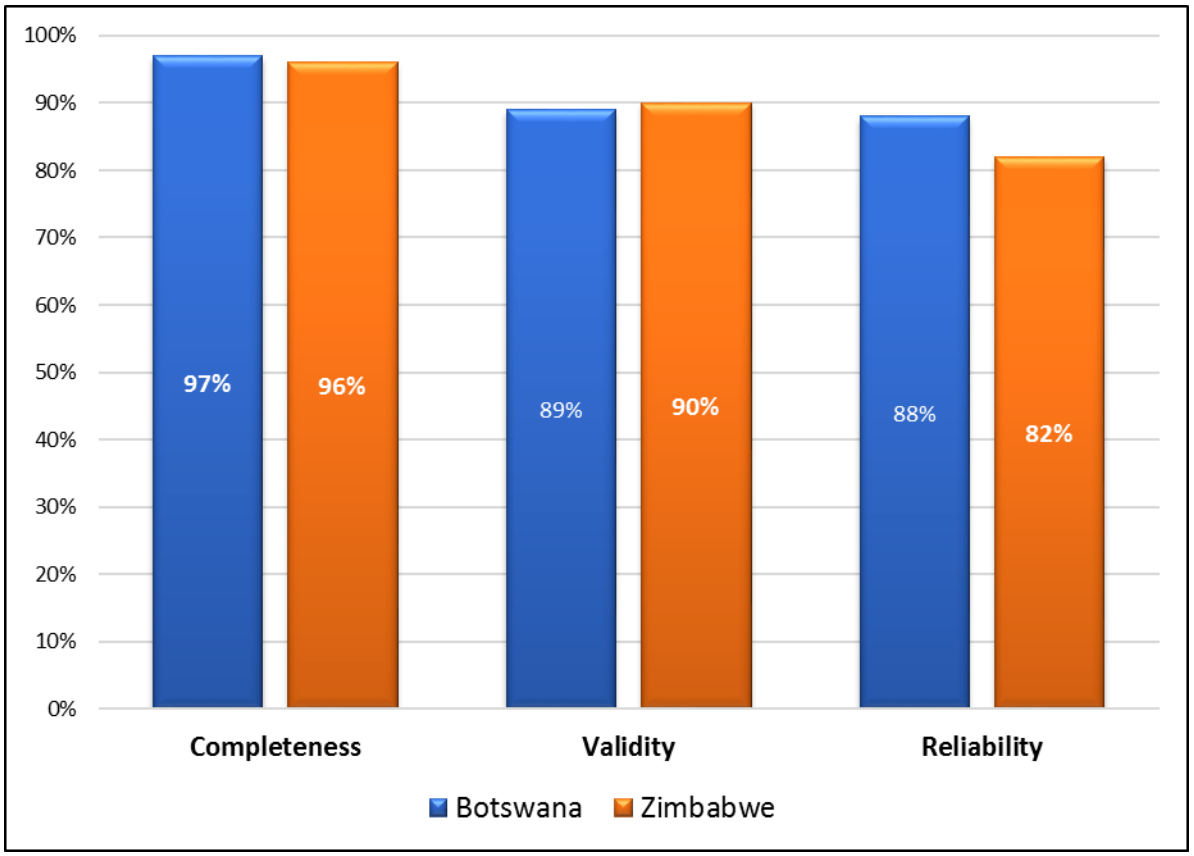

Figure 5.1: Percentage data quality in the larval habitat survey form and the larval inspection and survey form

The attribute of completeness had the highest performance at more than 95%, with reliability being the lowest for both countries though still at more than 80%.

5.5. Discussion

The experimental study in Botswana and Zimbabwe has demonstrated that community volunteers can be a potential resource in the implementation of biological larviciding for malaria vector control. They can play a role in the identification of breeding sites and the application of the larvicide. The volunteers showed improvements in their skills and could confidently identify the breeding sites, assess for breeding activity, mix and apply biological larvicides. There is an

acknowledgement that larval control for malaria prevention is a labour intensive intervention [21] requiring rigorous searches of breeding sites on foot.[8] It is even difficult for the common African vectors which colonize a large variety of habitats distributed widely over space and time.[4-7] These attributes make local volunteers who know the local setting, as well as the distribution of permanent and semi-permanent water points key in implementation of larviciding interventions. In Tanzania, one of the reasons why some breeding points could not be identified is because they were within enclosed private units.[29] Such access issues can be of concern, and local community members can be key in locating the water points; and their relationship with the rest of the community members can facilitate identification and treatment of all water points including those in private units.

The World Health Organisation recommends larviciding in areas where breeding sites are few, fixed and findable [21]. This is characteristic of several semi-arid areas in sub-Saharan Africa which are in pre-malaria elimination, requiring regular and appropriate treatment of the breeding sites for a successful larviciding intervention. The competencies in application of bio-larvicides demonstrated by community volunteers in Botswana and Zimbabwe after minimal training presents an opportunity for mobilising communities to support and own the intervention, consistent with one of the recent nine vector control priority recommendations for 2017-2030 [30]. Larval control requires unusual specialist skills at all levels from community volunteers up to PhD level individuals because of accompanying entomological activities which include characterisation of the larva. However, while diverse skills are necessary, the basics of a successful larval control program is the successful identification and treatment of all breeding sites. With all water points in malaria transmission areas being recommended to be treated as potential vector breeding sites at any time of the year and exhaustively targeted in any larval control intervention, [4] such wholesale treatment will require community participation where members/volunteers who know the location of the water points can assist with the implementation of the intervention.

There is already a precedent set where community volunteers have successfully been utilised in malaria programs primarily in diagnosis and treatment [23]. Despite

showing improvements in their skills, it must be noted that the volunteers in both countries worked under supervision from the provincial malaria field officer (in Zimbabwe) and the study coordinator (in Botswana)..The improvement in skills of volunteers is consistent with observations in Tanzania where there was an improvement in the ability of the community-owned resource persons (CORPs) in identifying breeding sites, from baseline [29, 31]. In the Tanzania study, despite improvements in the skills of the CORPs, their accuracy in identifying the breeding sites still remained low. However, the study in Tanzania was also in an urban area where there are more access concerns in private properties unlike in the study areas of Botswana and Zimbabwe which were rural, with breeding sites mainly along river beds. Broadly, involvement of communities in finding solutions to their health problems including in the implementation of preventative interventions such as larviciding is considered part of community empowerment and ownership which ultimately contributes to sustainable development.

5.6. Conclusion

Community volunteers can make a significant contribution in malaria vector control interventions, particularly for larviciding because of their local knowledge and ability to identify vector breeding points, and application of larvicide. Larviciding is a labour-intensive intervention which requires regular surveillance of breeding points and frequent application of larvicide and community volunteers can alleviate some of the labour challenges with minimal training. With adequate supervision, countries like Botswana and Zimbabwe targeting elimination and considering larviciding as an additional vector control intervention should also consider using volunteers because of their potential and ability to competently implement the intervention.

List of abbreviations

CORPs: Community-owned resource persons; LLINs: long-lasting insecticide-treated nets; NMCP: National Malaria Control Programme; CHW: Community Health Worker; WHO: World Health Organization; IRS: indoor residual spraying; GPS: Geographic positioning system.

5.7. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MM acted as the study coordinator and designed the study, conducted data collection, analysis and led the writing of the manuscript. KM and CdJ supervised and reviewed the design, data collection, analysis and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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CHAPTER SIX

6. GENERAL DISCUSSION

Botswana and Zimbabwe are members of the Elimination 8 group of countries which initially targeted malaria elimination by 2020. Botswana is amongst the frontline states initially targeting elimination by 2018[1], while Zimbabwe had targeted elimination by 2020[2]. In both countries, malaria vector control has relied heavily on IRS and LLINs, but the progress towards malaria elimination has been threatened by insecticide resistance which has increased dramatically in recent years[3]. This has led to calls and encouragement for a shift to alternative non-insecticidal methods being where feasible[4]. Larviciding, which is one of the supplementary malaria vector control interventions has effective microbial products which have shown success in different settings[5-9], yet the intervention is less commonly used[10].

As presented in Chapters two and three of this thesis, larviciding using *Bti* has demonstrated to be an effective intervention in controlling the larval and adult stages of the mosquito in low malaria transmission areas of Botswana and Zimbabwe. During the experimental study, all water bodies in the intervention areas of both countries were treated every fortnight using a commercial *Bti* based larvicide. These were fresh water points, with minimal pollution due to animal activity. Over the 16-week study period, there was a significant decrease in larval density by stage in the intervention areas in both countries a demonstration of the effectiveness of larvicide.

These results are similar to findings from other field studies conducted in other countries in Sub-Saharan Africa[11-14]. This is probably the first field study in semi-arid malaria transmission areas of sub-Saharan Africa, experiencing low transmission and in pre-elimination stage. Botswana and Zimbabwe have malaria transmission areas typically suitable for larviciding such as the North and North-Eastern parts of Botswana and the Southern parts of Zimbabwe. These have few water bodies which can easily be identified and treated, consistent with the World Health Organisation recommendation that larviciding should be conducted in areas where breeding sites are few, fixed and findable[15]. During the study, larviciding reduced mosquito larvae by up to 86%, and the effect was highest amongst the late instars at 91% in the intervention areas relative to

the control. Reduction of late instars leads to low adult mosquito emergence[16,17], which also impacts on adult mosquito emergence and is also demonstrated in Chapter three. Other experimental studies in Sub-Saharan Africa have also found larviciding to reduce adult mosquito densities leading to reductions in exposure to bites[18]; substantial reduction in new infections[12], and overall reduction in malaria prevalence[19]. While few adult mosquitoes were captured and generally all being non-vectors, the effectiveness on adult mosquitoes shown in Chapter three is significant particularly to the general public because of the impact it has on mosquito bites. This study was conducted in winter, the reason why very few mosquitoes were captured despite sampling rooms closer to the breeding sites. *An. Arabiensis* and *An. Gambiae*, the known vectors in Zimbabwe[20] and Botswana have previously demonstrated sensitivity to *Bti* products[21,22]. *An. Arabiensis* has demonstrated exophilic tendencies in most parts of Zimbabwe[20], a possible reason we had challenges finding it indoors, demonstrating the limitation of the adult capture methods used, which are exit window traps and pyrethrum spray catches. Despite this limitation, larviciding using *Bti* has been found to significantly reduce mosquito populations even where outdoor human bait collections are conducted[23].

For larviciding to be successful, it is important to have the target communities to be part of the roll-out of the intervention. Extensive consultations will be necessary, but most importantly, the intervention has to demonstrate effectiveness once it is scaled up. During this study, the effectiveness of *Bti* in both Botswana and Zimbabwe was consistent for both the Anophelene and Culex larva, and for the different stages of both genera. While the culex genera is not a family of malaria vectors, the reduction in its population reduces human exposure to human bites which makes it a “hygiene” factor and is also important in the general population’s assessment of the effectiveness of the intervention. The activity spectra of *Bti* formulations covers many culicidae (mosquito) genera[10,24,25], which reduces overall adult mosquito emergence and human exposure to bites and transmission[12,18].

Larviciding is a labour intensive intervention because of the need to identify all breeding points, both permanent and semi-permanent[15,26]. The frequency of application of the larvicide and the behaviour of local vectors which colonize a large variety of habitats

distributed widely[27-30] makes larviciding even more labour intensive. This cannot be sustained when using high skilled teams, especially because of the need for wholesale treatment of breeding sites for larviciding to be successful[30]. In this experimental study we used community volunteers in both countries who demonstrated good competencies in supporting the intervention as shown in Chapter four, and pursuant of one of the recent nine vector control priority recommendations for 2017-2030[31]. They played a key role in the identification of breeding sites and the application of the larvicide despite receiving minimal training. Over time and with repeated exposure their skills improved, and they could confidently identify the breeding sites, assess for breeding activity, mix and apply biological larvicides. In Tanzania, one of the reasons some breeding points could not be identified was because they were within enclosed private units[32]. Such access issues can be of concern, and locally known community members can be key in locating the breeding sites. The use of community volunteers in larviciding will not be a new phenomenon in malaria programs, these have successfully been utilised primarily in diagnosis and treatment[33].

The knowledge of larviciding as a malaria vector control intervention demonstrated in this study and presented in Chapter five is a good starting point in promoting and ensuring acceptability within the community. Inadequate knowledge about the reasons for, and safety of the interventions[34-36] is known to affect user acceptability and participation by the community. There was an overwhelming acceptance of larviciding in both Botswana and Zimbabwe. Acceptability was also influenced by the perceived effectiveness in reducing mosquitos and the desire to be protected against mosquito bites. In both countries, there was a willingness to support any intervention (including larviciding), that will reduce mosquito densities and accompanying bites, an acceptability factor which can be leveraged upon in promoting larviciding for malaria control. In South Africa acceptability of insecticide treated wall linings was influenced by the reduction of mosquito nuisance and biting in general, and other annoying insects post-installation[37]. Chemical safety of the larvicides to both the humans and their domestic animals is also an important factor to be addressed and assured to get support from the general community. This came up as an issue during interviews with

the community members. *Bti* has been assessed by the WHO Programme on Chemical Safety[38], and its products are considered safe for use in drinking water[39]. One of the weaknesses raised by the participants is the concern about availability of the microbial larvicides, a genuine concern considering that the two countries haven't been consistently implementing larviciding.

6.1. Recommendations

Larviciding using microbial larvicide *Bti* has demonstrated to be an effective intervention in reducing the larval stages of the mosquitoes as well as the adult mosquitoes. In several other studies it has also shown similar results including reduction in malaria incidence and prevalence when used as part of the vector control package. Based on these results, the following are recommendations for the national malaria control programs of Botswana and Zimbabwe.

- The two countries should consider larviciding as an additional vector control intervention. Zimbabwe has larviciding as a policy option and has limited implementation happening in Matabeleland South, while Botswana implements larviciding in Bobirwa district, all areas which are semi-arid and suitable for larviciding. However, in these locations larviciding should be scaled up and implemented consistently during the dry season for it to contribute effectively to malaria prevention and towards elimination.
- Larviciding is an expensive intervention that should be implemented consistently every two weeks. For sustainability, the two countries should use the community-based approach, utilising volunteers identified by the community. The national malaria control programs will continue to provide training, commodities and technical supervision. Extensive consultations will be required and the NMCP should develop policies and operational plans to promote larviciding before engaging the community and ensure that implementation will be conducted uninterrupted.
- The two countries should develop operational manuals and SOPs, including for community based larviciders. The SOPs should include monitoring and

evaluation of larviciding which is currently weak in most national malaria control programmes. These will help in roll-out and standardisation of implementation of larviciding.

- The use of community volunteers should go beyond larviciding but also cover other larval source management interventions such as habitat modification and destruction to obstruct mosquito breeding at the community level.

6.2. Limitations

I acknowledge that the conduction of the intervention for a prolonged period and for multiple seasons would have further strengthen the results. However, this could not be sustained because of the costs and distance between the two study sites which were at least 1,100 km apart and I (the student) staying 450km away from the nearest study site. Despite using community volunteers to implement the intervention, alternate weekly travel to either of the two sites for data collection and for collecting entomological measures was a physically demanding and expensive undertaking.

Additionally, because of the low residual effect of the intervention, it was difficult to assess the impact on the malaria cases in the two cases. *Bti* has a low residual effect of less than 14 days, and when larviciding ended in October, it was pointless to assess its impact on malaria events happening three months later especially when implementation was localised in a few villages within the targeted districts.

6.3. Areas for further research

Larviciding is an effective intervention but with a low residual effect. However, in areas where DDT is used for IRS, because of its long residual effect, understanding the impact of overlapping the timing of larviciding activities and IRS will be necessary. While larviciding still remains a supplementary intervention to IRS and LLINs, delaying its implementation so that its timed to overlap with start dates for IRS will likely provide insights of the synergistic effect of combined larval and adult mosquito control.

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APPENDIX 1: RESEARCH PERMIT-UNIVERSITY OF PRETORIA FACULTY OF HEALTH SCIENCES ETHICS COMMITTEE

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

31/07/2014

Approval Certificate
New Application

Ethics Reference No.: 289/2014

Title: Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe.

Dear Mr Mulamuli Mpofu

The **New Application** as supported by documents specified in your cover letter for your research received on the 2/07/2014, was approved, by the Faculty of Health Sciences Research Ethics Committee on the 30/07/2014.

Please note the following about your ethics approval:
- Ethics Approval is valid for 3 years.
- Please remember to use your protocol number (**289/2014**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

♦ Tel:012-3541330 ♦ Fax:012-3541367 Fax2Email: 0866515924 ♦ E-Mail: fhsethics@up.ac.za
♦ Web: //www.healthethics-up.co.za ♦ H W Snyman Bld (South) Level 2-34 ♦ Private Bag x 323, Arcadia, Pta, S.A., 0007

APPENDIX 2: RESEARCH PERMIT EXTENSION-UNIVERSITY OF PRETORIA FACULTY OF HEALTH SCIENCES ETHICS COMMITTEE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

31/08/2017

Mr Mulamuli Mpofu
Department of School of Health Systems and Public Health (SHSPH)
University of Pretoria

Dear Mr Mulamuli Mpofu

RE.: 289/2014 ~ Letter dated 21 July 2017

PROTOCOL NUMBER.	**289/2014**
STUDENT	**Name & Surname:** Mr Mulamuli Mpofu, **Dept:** School of Health Systems and Public Health (SHSPH),School of Health Systems and Public Health University of Pretoria. **Cell:** 00267 74283812 **E-Mail:** mulampofu@gmail.com
TITLE OF RESEARCH PROJECT	Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe.

We hereby acknowledge receipt of the following document:

- Extension for study until end of December 2018.

This will be processed in due course and filed.

With regards

Dr R Sommers; MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

☎ 012 356 3085 **fhsethics.up.ac.za** http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4-59, Gezina, Pretoria

APPENDIX 3: RESEARCH PERMIT-HUMAN RESEARCH DEVELOPMENT COMMITTEE- BOTSWANA

TELEPHONE: 363 2766
FAX: 391 0647
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD

Republic of Botswana

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

REFERENCE NO: PPME 13/18/1 PS V (333) 24 November 2014

Health Research and Development Division

Notification of IRB Review: New application

Mr Mulamuli Mpofu
University of Pretoria
Faculty of Health Sciences
School of Health systems and Public Health
5 Floor, HW Synyman Building North
31 Bophelo Road
Gezina
0031

Protocol Title:	EFFECTIVENESS OF WINTER LARVICIDING AS A MALARIA VECTOR CONTROL INTERVENTION IN SELECTED RURAL AREAS OF BOTSWANA AND ZIMBABWE
HRU Approval Date:	21 November 2014
HRU Expiration Date:	21 November 2015
HRU Review Type:	HRU reviewed
HRU Review Determination:	Approved
Risk Determination:	Minimal risk

Dear Mr Mpofu

Thank you for submitting new application for the above referenced protocol. The permission is granted to conduct the study.

This permit does not however give you authority to collect data from the selected sites without prior approval from the management. Consent from the identified individuals should be obtained at all times.

The research should be conducted as outlined in the approved proposal. Any changes to the approved proposal must be submitted to the Health Research and Development Division in the Ministry of Health for consideration and approval.

1

Furthermore, you are requested to submit at least one hardcopy and an electronic copy of the report to the Health Research, Ministry of Health within 3 months of completion of the study. Approval is for academic fulfillment only. Copies should also be submitted to all other relevant authorities.

Continuing Review

In order to continue work on this study (including data analysis) beyond the expiry date, submit a Continuing Review Form for Approval at least three (3) months prior to the protocol's expiration date. The Continuing Review Form can be obtained from the Health Research Division Office (HRDD), Office No. 7A.7 or Ministry of Health website: www.moh.gov.bw or can be requested via e-mail from Mr. Kgomotso Motlhanka, e-mail address: kgmmotlhanka@gov.bw As a courtesy, the HRDD will send you a reminder email about eight (8) weeks before the lapse date, but failure to receive it does not affect your responsibility to submit a timely Continuing Report form

Amendments

During the approval period, if you propose any change to the protocol such as its funding source, recruiting materials, or consent documents, you must seek HRDC approval before implementing it. Please summarize the proposed change and the rationale for it in the amendment form available from the Health Research Division Office (HRDD), Office No. 7A 7 or Ministry of Health website: www.moh.gov.bw or can be requested via e- mail from Mr. Kgomotso Motlhanka, e-mail address: kgmotlhanka@gov.bw . In addition submit three copies of an updated version of your original protocol application showing all proposed changes in bold or "track changes".

Reporting

Other events which must be reported promptly in writing to the HRDC include:

- Suspension or termination of the protocol by you or the grantor
- Unexpected problems involving risk to subjects or others
- Adverse events, including unanticipated or anticipated but severe physical harm to subjects.

If you have any questions please do not hesitate to contact Mr. P. Khulumani at pkhulumani@gov.bw, Tel +267-3914467 or Lemphi Moremi at lamoremi@gov.bw or Tel: +267-3632754. Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours sincerely

P. Khulumani
For Permanent Secretary

APPENDIX 4: RESEARCH PERMIT – MEDICAL RESEARCH COUNCIL OF ZIMBABWE

Telephone: 791792/791193
Telefax: (263) - 4 - 790715
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazoe Street
P. O. Box CY 573
Causeway
Harare

APPROVAL

REF: MRCZ/A/1898 **6 February, 2015**

Mulamuli Mpofu
University Of Pretoria
School Of Health Systems and Public Health
South Africa

RE: -Application For Ethical Review And Approval Of Study Titled:-Effectiveness Of Winter Larviciding As A Malaria Vector Control Intervention In Selected Rural Areas Of Botswana And Zimbabwe

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review:-

a) Completed MRCZ form 101
b) Research Protocol, version 1.0 dated 30 January, 2015
c) Informed Consent Form (English and Shona), version 0.1 dated 30 January 2015
d) Data collection tools (English and Shona)
e) Certificate of registration of Pesticide
f)

- **APPROVAL NUMBER : MRCZ/A/1898**

This number should be used on all correspondence, consent forms and documents as appropriate.

- **EFFECTIVE APPROVAL DATE : 06 February, 2015**
- **EXPIRATION DATE : 05 February, 2016**

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

- **SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.
- **MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.
- **MCAZ APPROVAL:-Please note that this clinical trial can only be initiated after obtaining MCAZ approval.**
- **QUESTIONS**: Please contact the MRCZ on Telephone No. (04) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours Faithfully

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
2015 -02- 06
APPROVED
P.O. BOX CY 573 CAUSEWAY, HARARE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

APPENDIX 5: RESEARCH APPROVAL-MINISTRY OF HEALTH BOTSWANA

TELEPHONE: 363 2500
FAX: 391 0647
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD

Republic of Botswana

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

Ref: MH 13/18 XXIV (36)

10 February 2015

Mulamuli Mpofu
Box ACH46
ACH Riverwalk
Gaborone

Dear Mpofu

RE: REQUEST TO CONDUCT RESEARCH AT PhD LEVEL IN BOTSWANA

Reference is made to your letter dated 21 January 2015 on the above subject matter.

Permission is hereby granted to interview Ministry of Health malaria program officers.

This research is very much relevant to us as a country as we move towards eliminating malaria by 2015.

Please contact the office of the Director, Public Health at 3632069 to arrange your meetings.

Thank you.

Yours sincerely

S.El-Halabi
For/PERMANENT SECRETARY

APPENDIX 6: RESEARCH APPROVAL-MINISTRY OF HEALTH AND CHILD CARE-ZIMBABWE

Telephone 730011

Telegraphic Address
"MEDICUS", Harare
Fax: 791557/793634
Telex: MEDICUS 22211ZW

ZIMBABWE

Reference:

MINISTRY OF HEALTH AND
CHILD WELFARE
P.O. Box CY1122
Causeway
Zimbabwe

28th April 2014

To: PMD Manicaland

RE: LARVICIDING FOR MALARIA CONTROL AT BIRCHNOUGH BRIDGE AREA IN BUHERA DISTRICT

Mr. Mulamuli Mpofu, a PhD student with Botswana University has been granted permission by Ministry of Health and Child Care to conduct larviciding study for malaria control at Birchnough Bridge starting from 2 May 2014. The study will present baseline data in support of the NMCP prior to the intended integrated vector management (IVM).

Please note that Mr. Mpofu proposes to meet PMD in the morning and DHE in Buhera in the afternoon on Friday 2 May 2014, before proceeding to the study area. Your office is requested to provide the necessary support for the benefit of the community at Birchnough Bridge.

Thank you.

02 MAY 2014

BRIGADIER GENERAL (DR) G GWINJI
SECRETARY FOR HEALTH AND CHILD CARE

Cc: DMO Buhera district

APPENDIX 7: RESEARCH PERMISSION-PROVINCIAL MEDICAL DIRECTOR MANICALAND

Telephone: 63355/60655
Fax: 60698/64401

ZIMBABWE

Reference:

PROVINCIAL MEDICAL DIRECTOR
MANICALAND
P.O. Box 323
Mutare

08 October 2014
The Secretary
Medical and Research Council of Zimbabwe
P O Box CY573, Causeway,
Harare

Ref: Permission to carry out study in Manicaland by Mulamuli Mpofu entitled Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe.

Mr Mpofu Mulamuli is a PHD Public Health student at Pretoria University Student number **13273567** .He wishes to conduct a study in partial fulfillment of his PHD studies in Manicaland to evaluate the effectiveness of winter biolarviciding. The province is in support of the study as the results will assist in guiding future malaria control interventions.
Kindly assist him to obtain the necessary clearances in order for him to carry out the studies.
Regards

Dr P T Mafaune
Provincial Medical Director - Manicaland

APPENDIX 8: IMPORTATION OF EXPERIMENTAL PESTICIDE PERMIT

$1 NO 578629

ZIMBABWE

№ 1798 F

DEPARTMENT OF RESEARCH AND SPECIALIST SERVICES

Ministry of Lands, Agriculture and Rural Resettlement,

Plant Protection Research Institute, P.O. Box 8100, Causeway, Harare.

9/4/2015

MULAMULA MPOFU (UNIVERSITY OF PRETORIA)

Dear Sir/Madam

IMPORTATION OF EXPERIMENTAL PESTICIDE

The above named firm is hereby authorized to import the following pesticide named .....
20KG VECTOBAC WG (BACILLUS THURINGIENSIS ISRAELENSIS for purely experimental use in Zimbabwe.

A condition of the authorization of this import into Zimbabwe is that the Registering Officer must be supplied with full details of proposed experiments and with full details at the end of experiments.

Your attention is drawn to the fact that plants treated with an experimental pesticide should not be sold or disposed of for consumption without authority of the Registering Officer. The fee for importation of an experimental pesticide is $...20,00...

MINISTRY OF AGRICULTURE
09 APR 2015
P.O. BOX CY 550 CAUSEWAY
ZIMBABWE

Yours faithfully,

Pesticide Registering Officer.
For: HEAD
Plant Protection Research Institute

APPENDIX 9: INFORMED CONSENT FORM FOR ANONYMOUS QUESTIONNAIRES

MRCZ FORM 109

MRCZ No.|**1898**

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
INFORMED CONSENT FORM FOR ANONYMOUS QUESTIONNAIRES

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
School of Health Systems and Public Health

***PROJECT TITLE*: Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe**

Principal Investigator: **Mulamuli Mpofu**
Phone number(s) : **00267 74283812**

What you should know about this research study:

- We give you this consent so that you may read about the purpose, risks, and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help future programming.
- We cannot promise that this research will benefit you.
- You have the right to refuse to take part, or agree to take part now and change your mind later.
- Whatever you decide, it will not affect your regular care.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your participation is voluntary.

PURPOSE

You are being asked to participate in a research study of larviciding, which is the use of chemicals and other poisons to spray water points where mosquitoes lay their eggs. It is done during the cold season and it's for the purposes of killing the mosquitoes before they are able to fly and bite human beings.

The purpose of the study is to investigate whether spraying water points where mosquitoes lay their eggs can be used to control mosquitoes and malaria in selected rural areas of Botswana and Zimbabwe. Although spraying of water points has been used in many parts of the world, its proof in controlling mosquitoes and malaria is limited in Southern Africa when compared to other methods like treated mosquito nets and spraying of houses inside. This study will help us to get an understanding of the use of spraying of water points in your village and the country.

I would like you to complete a questionnaire, which is a list of questions. About 10 other heads of households in this village will complete the same list of questions. In total, 40 heads of household will participate in completing the list of questions, 20 in Botswana and 20 here in Zimbabwe.

Page 1 of 3

PROCEDURES AND DURATION
Your participation in this study is voluntary. You can refuse to participate or stop at any time without giving any reason. As you do not write your name on the questionnaire, you give the information anonymously which means that no one will be able link the responses to you. Once you have given the questionnaire back to us, you can't recall your consent. We will not be able to trace your information; therefore, you will also not be identified as a participant in any publication that comes from this study.
The completion of the list of questions may take about 20-30 minutes. The completed questions will be collected from you immediately after completion. It will be kept in a safe place to ensure confidentiality. Please do not write your name on the questionnaire. I will be available to help you with the questions or to fill it on your behalf if you need assistance.

RISKS AND DISCOMFORTS
There are no risks expected from you participating in this study. However, if you feel uncomfortable participating in research activities, you are free to decline even after you have started completing the list of questionnaire. If you are not comfortable completing the list of questions in English language, there is a Shona version which asks the same questions like the English. This research represents no risks at all to pregnant women and unborn children.

BENEFITS AND/OR COMPENSATION
There will be no direct benefits to your participation in the study but the information obtained from you will be used to inform the national malaria control programs of Botswana and Zimbabwe. It will be used to inform the development and/or revision of larviciding policies to help fight malaria within your communities

CONFIDENTIALITY
The completed questions will be collected from you immediately after completion. It will be kept in a safe and locked place to ensure confidentiality. Please do not write your name on the questionnaire. This will ensure confidentiality. I will be available to help you with the questions or to fill it on your behalf if you need assistance.

ADDITIONAL COSTS
Participating in this study will not have any financial implications to you, as such, you will not incur any expenses.

IN THE EVENT OF INJURY
There are no risks associated with this study and as such, no injury is expected resulting from your participation in this study.

VOLUNTARY PARTICIPATION
Participation in this study is voluntary. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty.

SIGNATURE PAGE

***PROJECT TITLE*:** Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe

Protocol Version Number/date-December 2014

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

You are making a decision whether or not to participate in this study. Your signature indicates that you have read and understood the information provided above, have had all your questions answered, and have decided to participate.

_____ _____

Signature of Participant or legally authorized representative Time

_____

Relationship to the Participant if authorised representative

Name of Staff Obtaining Consent Signature _____ Date

Name of Witness (*if required)* Signature _____ Date

YOU WILL BE OFFERED A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research participant or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe (MRCZ) on telephone (04)791792 or (04) 791193 and cell phone lines 0772 433 166 or 0779 439 564. The MRCZ Offices are located at the National Institute of Health Research premises at Corner Josiah Tongogara and Mazowe Avenue in Harare.

APPENDIX 10: INFORMED CONSENT FORM FOR MOSQUITO CAPTURE

MRCZ FORM 109

MRCZ No.|**1898**

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
INFORMED CONSENT FORM-MOSQUITO CAPTURE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences
School of Health Systems and Public Health

***PROJECT TITLE*: Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe**

Principal Investigator: **Mulamuli Mpofu**
Phone number(s) : **00267 74283812**

What you should know about this research study:

- We give you this consent so that you may read about the purpose, risks, and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help communities.
- We cannot promise that this research will benefit you.
- You have the right to refuse to take part, or agree to take part now and change your mind later.
- Whatever you decide, it will not affect your regular care.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your participation is voluntary.

PURPOSE

You are being asked to participate in a research study of larviciding, which is the use of chemicals and other poisons to spray water points where mosquitoes lay their eggs. It is done during the cold season and it's for the purposes of killing the mosquitoes before they are able to fly and bite human beings.

The purpose of the study is to investigate whether spraying water points where mosquitoes lay their eggs can be used to control mosquitoes and malaria in selected rural areas of Botswana and Zimbabwe. Although spraying of water points has been used in many parts of the world, its proof in controlling mosquitoes and malaria is limited in Southern Africa when compared to other methods like treated mosquito nets and spraying of houses inside. This study will help us to get an understanding of the use of spraying of water points in your village and the country.

I would like to request you to allow us access to your household. Within the household, we wish to capture mosquitoes inside and outside rooms where members of the household sleep. The purpose of the study is not in any way meant to interrupt your family daily routines, nor interfere with your sleeping schedule. Window traps will be put up at around 1800pm and removed the next morning around 0600hrs. Pyrethrum spray catches will also be conducted around 0600 in the morning. These activities will be repeated every fortnight from the month

of June 2015 until September 2015. The methods that we will be using are safe and do not pose any harm to you or any of your family members.

PROCEDURES AND DURATION

Your participation in this study is voluntary. You can refuse to participate or stop at any time without giving any reason. As you do not write your name on the consent form, you give the information anonymously which means that no one will be able link the data collected to you.

RISKS AND DISCOMFORTS

There are no risks expected from you participating in this study. However, if you feel uncomfortable participating in research activities, you are free to decline even after capturing of mosquitoes has started. This research represents no risks at all to pregnant women and unborn children. However, some people with sensitive skin usually experience minor irritation due to pyrethrum which will be applied during spraying. To combat that, family members are expected to enter the room at least 30 minutes after spraying to avoid the irritation.

BENEFITS AND/OR COMPENSATION

There will be no direct benefits to your participation in the study but the information obtained from your household will be used to inform the national malaria control programs of Botswana and Zimbabwe. It will be used to inform the development and/or revision of larviciding policies to help fight malaria within your communities. The use of pyrethrum sprays indoors will kill mosquitoes that would have had the potential of biting you and your family.

CONFIDENTIALITY

The data collected from your household will be kept in a safe and locked place to ensure confidentiality. Please do not write your name on the consent form. This will ensure confidentiality. I will be available to help you clarify issues and concerns that may not be clear to you.

ADDITIONAL COSTS

Participating in this study will not have any financial implications to you, as such, you will not incur any expenses.

IN THE EVENT OF INJURY

There are no risks associated with this study and as such, no injury is expected resulting from your participation in this study.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty.

SIGNATURE PAGE

***PROJECT TITLE*:** Effectiveness of winter larviciding as a malaria vector control intervention in selected rural areas of Botswana and Zimbabwe

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Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

You are making a decision whether or not to participate in this study. Your signature indicates that you have read and understood the information provided above, have had all your questions answered, and have decided to participate.

_____ _____

Signature of Participant or legally authorized representative Time

_____

Relationship to the Participant if authorised representative

_____ _____ _____

Name of Staff Obtaining Consent Signature Date

_____ _____ _____

Name of Witness (*if required)* Signature Date

YOU WILL BE OFFERED A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research participant or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe (MRCZ) on telephone (04)791792 or (04) 791193 and cell phone lines 0772 433 166 or 0779 439 564. The MRCZ Offices are located at the National Institute of Health Research premises at Corner Josiah Tongogara and Mazowe Avenue in Harare.

APPENDIX 11: COMMUNITY LEVEL QUESTIONNAIRE

Effectiveness of winter larviciding as a malaria vector control intervention in rural Botswana and Zimbabwe.

COMMUNITY LEVEL QUESTIONNAIRE:

*This questionnaire has two pages and 15 questions. Please take your time to complete **ALL** the questions. It should take you between 20-30 minutes to complete. Please be as honest as possible with your responses. The responses will not be linked to you at all.*

Date of questionnaire administration: __/__/_____

Demographic Information

1. Sex: ☐ Male ☐ Female
2. Age: ____
3. Number of years staying in the village: Tick. ☐ <1 ☐ 1-2 ☐ 3-4 ☐ 5+
4. Have you suffered from malaria before? ☐ Yes ☐ No
   a. If yes, when was the last time? Indicate the year and month.___/_____

Malaria and mosquito control methods

5. What methods of mosquito control do you know? Tick all that apply.
   ☐ Spraying of houses inside ☐ Treated mosquito nets
   ☐ Spraying water points where mosquitoes lay eggs
   ☐ Using materials whose smell chase away mosquitoes
   ☐ Other. Specify: _____

6. Which of these methods do you have access to in your village?
   ☐ Spraying of houses inside ☐ Treated mosquito nets
   ☐ Spraying water points where mosquitoes lay eggs
   ☐ Using materials whose smell chase away mosquitoes
   ☐ Other. Specify: _____

The following questions apply to larviciding only.

Larviciding is defined as the application of chemicals or poisons to water points where mosquitoes lay their eggs for the purposes of killing mosquitoes before they are able to fly and bite humans.

7. Are you aware of the larviciding program being implemented in your area? ☐ Yes ☐ No

8. How would you rate the number of mosquitoes before larviciding was started in your village?
   ☐ Very few ☐ Few ☐ Don't know ☐ Plenty ☐ Very plenty

Page 1 of 3

9. How would you rate the number of mosquitoes after larviciding was started in your village?
   ☐ Very few ☐ Few ☐ Don't know ☐ Plenty ☐ Very Plenty

10. What do you think is the importance of larviciding in the control of mosquitoes that cause malaria?
    ☐Not important ☐ some Importance ☐ Don't know ☐ Important ☐ Very important

    a. Support your answer._____
    _____
    _____
    _____

11. Do you think larviciding should continue being conducted in your village?
    ☐ No, it should not continue ☐ I don't know ☐ Yes, it should continue

    a. Support your answer._____
    _____
    _____
    _____

12. Do you think members of your village accept larviciding?
    ☐No, they do not accept it at all ☐ Others accept while others don't
    ☐ Yes, they accept it ☐ I don't know

a. Support your answer._____
_____
_____
_____

13. As an individual, what do you think you can do to support larviciding in your village?
_____
_____
_____

14. What do you consider as the strengths of larviciding compared to other methods of mosquito control? List
    a. _____
    b. _____
    c. _____
    d. _____

15. What do you consider as the weaknesses of larviciding compared to other methods of mosquito control? List

a. _____
b. _____
c. _____
d. _____