Studies on the Survival, Fecundity and Fertility of *Anopheles arabiensis* by feeding on LongRange™ Eprinomectin Treated Cattle

College of Natural Sciences, Addis Ababa University

MSc Thesis Submitted to Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University

BY

KEHULU BELAY

Advisors: Beyene Petros (DSc, Prof.), Microbial, Cellular & Molecular Biology Department, CNS, AAU

Meshesha Balkew (PhD), Aklilu Lema Institute of Pathobiology, AAU

Teshome Gebre-Michael (PhD), Aklilu Lema Institute of Pathobiology, AAU

April, 2017

ACKNOWLEDGEMENTS

First of all I would like to give my sincere gratitude to Dr. Teshome Gebre-Michael for supporting me to join the graduate program and for his unreserved help in developing the research proposal, for his constructive advice in the course of the field work and for commenting and editing the thesis. My deepest gratitude is also for Dr. Meshesha Balkew for his advice and support in the development of the proposal and the necessary materials for the field setup and the financial support. I sincerely thank Prof. Beyene Petros for his unreserved scientific and academic support during my course work, proposal development, research work and thesis write up. His timely responses for all my requests gave me the energy to complete my research work. My thanks also go to the Microbial, Cellular and Molecular Biology Department, for providing me the necessary support. I want to thank Ato Gudeta Gore and Woyama Gore for allowing me their calves for the experiment and helping me throughout feeding activity and also Dr. Asrat, the Veterinarian in Batu/Ziway town for measuring the calves' mass and administering the proper drug dose to the calves. I appreciate the Aklilu Lema Institute of Pathobiology, Vector Biology, Control & Research Unit Entomology assistants W/t Selamawit Yaregal and Ato Wossen Sisay for their help in mosquito colony maintenance and Ato Alemayehu Assefa in the field activity. Last but not least, I would like to thank all who helped me and made my dream come true financially, materially and morally.

DECLARATION

I, Kehulu Belay, declare that all the contents of this thesis contain my own original work. It is completed under the guidance of my advisors. All sources and materials used for the thesis have been properly acknowledged.

SUBMITTED BY

Kehulu Belay	_____	_____
Candidate	**Signature**	**Date**

APPROVED BY

1. Beyene Petros (DSc, Professor)	_____	_____
Advisor	**Signature**	**Date**
2. Meshesha Balkew (PhD)	_____	_____
Advisor	**Signature**	**Date**
3. Teshome Gebre-Michael (PhD)	_____	_____
Advisor	**Signature**	**Date**

Addis Ababa University, College of Natural Sciences, Microbial, Cellular and Molecular Biology.

April, 2017

ABBREVIATIONS

AAU - Addis Ababa University

ACT – Artemisinin-based Combination Therapy

CMM - Cellular Microbial and Molecular Biology

CNS - College of Natural Sciences

DEC- diethylcarbamazine

DMP - dimethyl phthalate

DMSO - dimethyl sulphoxide

IRS - Indoor Residual Spray

ITL - Insecticide Treated Livestock

ITNs - Insecticide Treated Nets

IVM - Integrated Vector Management

LLINs - Long Lasting Insecticidal Nets

MDA–Mass Drug Administration

MOH - Ministry of Health

PLGA - poly d, l-lactide-coglycolic acid

RDTs - Rapid Diagnostic Tests

WHO - World Health Organization

WHO/CHERG -World Health Organization Child Health Epidemiology Reference Group

WMR - World Malaria Report

TABLE OF CONTENTS

Contents **Page**

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Tables **Page**

LIST OF ANNEX

Annexes **Pages**

ABSTRACT

Malaria is a serious public health and economic problem in Ethiopia for about 68% of the population. The misuse of LLINs and resistance of the vectors to most of the insecticides used in LLINs and IRS necessitated the need for alternative and effective control methods. Reports showed that *Anopheles* mosquitoes die when they feed on endectocidal drug- (like ivermectin and eprinomectin) treated humans and animals. This study was designed to investigate the efficacy of LongRange™ eprinomectin against laboratory reared *Anopheles arabiensis* when fed on treated calves. Three local breed calves treated with a therapeutic dose of LongRange™ Eprinomectin (1ml / 50Kg body weight) and another three non-treated calves (control) were exposed to equal numbers of *An. arabiensis* mosquitoes. The mosquitoes were placed in paper cups, covered with meshed nylon cloth and then allowed to feed on calves' neck. Subsequently, their survival, fecundity, egg hatchability, larval development and adult emergence were recorded. Data were entered and analyzed by using the SPSS version 20. Kaplan-Meier survival analysis and, independent samples t-test were used. The survivorship of *An. arabiensis* that fed on LongRange™ Eprinomectin treated calves up to14 days post treatment was observed to be reduced significantly ($p < 0.001$). All of the mosquitoes that fed on the treated calves within 7 days following injection were observed to die. Although not statistically significant, fecundity and hatchability of *An. arabiensis* that fed on the treated calves were reduced. Hence, treatment of livestock with eprinomectin can be used to effectively control zoophagic *An. arabiensis* for 7days post- injection. Therefore, the drug can be used as part of a combination vector control tool against *An. arabiensis* effectively if a repeated MDA is given to cattle in malarious regions of Ethiopia.

Key words: *Anopheles arabiensis, cattle, LongRange Eprinomectin, malaria control, Ethiopia*

1. INTRODUCTION

Mosquitoes of the family Culicidae comprise two medically important subfamilies, Anophelinae (anophelines) and Culicinae (culicines) and the Toxorynchitinae, which is not medically important. They are found worldwide, except Antarctica and few islands (Service, 2004). Some species in the genus *Anopheles* transmit malaria parasites in the genus *Plasmodium*. In sub-Saharan Africa, where 90% of the world's malaria-infected people live, most transmissions are caused by three *Anopheline* species - *Anopheles gambiae* s.s*, An. arabiensis,* and *An. funestus,* the first being the most widespread (Collins & Paskewitz, 1995). They are also vectors of filariasis and few arboviruses (Gubler, 1998; Service, 2004). Culicine species transmit filariasis, a variety of arboviruses including yellow and dengue fevers (Service, 2004).

Human malaria is caused by infection with protozoan parasites of the genus *Plasmodium* and is by far the most important tropical disease (Collins & Paskewitz, 1995). Most female *Anopheles* mosquitoes depend on a blood meal for their egg production and comprise the species that are exclusive vectors of mammalian malaria (White *et al.,* 2011). Among about 500 *Anopheles spp*., two to three dozen are important vectors of human malaria, and the vast majority causes little or no effect on human health (Collins & Paskewitz, 1995).

The global malaria cases declined from an estimated 262 million in 2000 (range: 205-316 million), to 214 million in 2015 (range: 149-303 million), a decline of 18% (WHO, 2015). Malaria deaths lowered, in the WHO African Region, to 88% from 90% in the 2014. Out of this, 78% of the deaths were children under 5 years old (WHO, 2014). WHO South-East Asia Region (10%) was the second and followed by the WHO Eastern Mediterranean Region (2%).

The world malaria incidence is estimated to have decreased by 37% between 2000 and 2015. In the decade, malaria control efforts have been increased by the involvement of the respective governments and by global funding. This could have contributed to the decreasing trend of the disease throughout the world, including the tropical Africa region where the burden is heaviest (Mendis *et al.,* 2009).

1.1. Significance of the Study

The zoophilic feeding behavior of *An. arabiensis* can be exploited to find alternative control methods in conditions where it: 1) avoids biting on humans who reside inside houses with LLINs and IRS, 2) is resistant to insecticides used in LLINs and IRS and 3) cascades residual malaria transmission outdoors. Use of systemic LongRange™ eprinomectin in animals subcutaneously can kill malaria vectors directly and hence be used in the control of *An. arabiensis* in Ethiopia. Hence, the current research was undertaken to investigate survival, fecundity and fertility of *An. arabiensis* by feeding on LongRange™ eprinomectin treated calves.

1.2. The Research Question

Although the key malaria control interventions such as long lasting insecticidal nets (LLINs), artemisinin-based combination therapy (ACT), use of rapid diagnostic tests (RDTs) and targeted indoor residual spray (IRS) contributed to the decline of malaria transmission over the last 15 years, the development of insecticide resistance to chemicals used in LLINs and IRS threatened mosquito control programs. On the other hand, systemic endectocides like ivermectin which have been in use both in humans and animals for the treatment of filariasis and other helminthes

are also indicated to kill malaria vectors directly. Hence, this study was proposed to assess the potential of cattle treatment with LongRange™ eprinomectin in controlling zoophagic *An. arabiensis* in Ethiopia.

1.3. Objectives

1.3.1. General Objective

- To investigate the activity of LongRange™ eprinomectin (an endectocide) on *An. arabiensis* fed on calves treated with the drug in Ethiopia.

1.3.2. Specific Objectives

- To investigate the mortality of *An. arabiensis* that fed on LongRange™ eprinomectin treated calves
- To determine fecundity (eggs productivity) of *An. arabiensis* that fed on LongRange™ eprinomectin treated calves
- To determine fertility (egg hatchability) of *An. arabiensis* that fed on LongRange™ eprinomectin treated calves
- To evaluate the larvae development of *An. arabiensis* that fed on LongRange™ eprinomectin.

1.4. Ethical Considerations

The proposal was approved by Microbial, Cellular and Molecular Biology Department, College of Natural Sciences, Addis Ababa University and the Veterinary Drug and Animal Feed Administration and Control Authority of Ethiopia. Written informed consent was obtained from owners of calves.

2. LITERATURE REVIEW

2.1. Malaria in Ethiopia

In Ethiopia, malaria is a serious public health and economic problem for about 68% of the population although the risk differs in different areas of the country (Degefa *et al.,* 2015). The Ministry of Health reported that malaria accounted for 2 % of child mortality in the country, and 4 % of febrile children below five received antimalarial drugs since 2010 (MOH, 2014). The disease showed a declining trend over the last 15 years mainly due to the high coverage of key control interventions such as introduction of artemisinin-based combination therapy (ACT), use of rapid diagnostic tests (RDTs) at the remote health facilities, wide-scale distribution of long-lasting insecticidal nets (LLINs) and increased coverage of indoor residual spraying (IRS) since 2004/2005 (Deressa *et al.,* 2014a; Degefa *et al.,* 2015). Graves *et al.,* (2011) also reported that malaria in Ethiopia decreased from 4.1% in 2006 to 0.4% in 2007 following increased coverage of LLINs and ACTs. A recent malaria assessment by 2012 in the country has shown that there has been a 54% and 55 % reduction in malaria admission and death, respectively as compared to baseline period of 2001- 2004. A reduction in the malaria morbidity from 22% to 10% and malaria case fatality rate in age groups of 5 years and above from 4.5% to 2% and in the under 5 children from 5% to 2% (Biadgilign *et al.,* 2012). However, the high influx of non-immune people into malaria endemic areas for social and economic reasons, alternative income, and expansion of agricultural and other development projects could alter the trend (Degefa *et al.,* 2015).

Anopheles arabiensis, a member of the *An. gambiae* complex, is the main vector of the disease in the country, and *An. pharoensis, An. funestus* and *An. nili* serve are secondary vectors in some areas (White, 1974; Coluzzi, 1984; Balkew *et al.,* 2010; Kenea *et al.,* 2011; Animut *et al.,* 2012). The available malaria vector control programs and activities target mainly *An. arabiensis* as the species is generally responsible for transmitting *Plasmodium falciparum* and *P. vivax* in the country (Warrell & Gilles, 2002; Balkew *et al.,* 2010; Woyessa *et al.,* 2012; Massebo *et al.,* 2015).

In the country, *P. vivax, P. falciparum, P. ovale* and *P. malariae* cause human malaria. *P. falciparum* and *P. vivax* are most abundant and their prevalence varies with season and locality (Gari *et al.,* 2016), while the current prevalence of *P. ovale* and *P. malariae* could be very low as reports are absent or very rare (Endeshaw *et al.,* 2008).

2.2. Malaria Vector Control Methods

2.2.1. Larval Mosquito Control Methods

Larval control is indicated as a major method for vector control only if the anopheline breeding sites are within the vector's flight range and the community to be protected are few, fixed, findable and manageable (Karunamoorthi, 2011; Kenea *et al.,* 2011; WHO, 2014). Larval control is the main part of integrated vector management (IVM) program if malaria elimination is the goal. Biological agents are useful in controlling larval and pupa stages of mosquitoes (Karunamoorthi, 2011). The biological control agents that are in use against *Anopheles* mosquitoes include predators, particularly fish, and the bacterial pathogens *Bacillus thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs). Other organisms showing a promising effect are fungal

pathogens, the nematode *Romanomermis culcivorax*, and the aquatic plant Azolla (Walker, 2002; Chandra *et al.,* 2008). Use of biological control agents require a good understanding of the agents, the mosquitoes to be controlled and the breeding environment. The most widely employed biological control agents are larvivorous fish that feed on the larval stages and to some extent the bacteria *B. thuringiensis* (WHO, 1982). Fish requires suitable settings such as well-defined mosquito breeding places and suitable water conditions (WHO, 2013). Larvivorous fish can be used in both natural and constructed habitats such as water tanks, lakes, fountains, pools, ponds, swimming pools, water storage tanks, seepages, water storage sites, irrigation cisterns, canals, small dams, rice fields, slow moving small streams, swamps and temporary water collection sites (WHO, 2013).

Environmental modification and environmental manipulation are useful strategies that can be used in the control of malaria vectors at their larval stages. The term 'modification' refers to permanent or long-lasting physical transformation of land, water, and vegetation, including drainage, filling, land leveling and transformation, and multipurpose reservoir margins. Environmental manipulation is defined as 'any planned recurrent activity aimed at producing temporary conditions unfavorable to the breeding of vectors in their habitats (Esslinger, 1981). This includes the salinity fluctuation of aquatic habitat, over flooding of streams and reservoirs, dewatering or draining of swamps and reservoirs, vegetation removal, shading, and exposure to sunlight (Karunamoorthi, 2011). Source reduction is also a component of environment management which aims to modify the environment in order to deprive the vector population of its requirements for survival (mainly breeding, resting and feeding), thus reducing human-vector contact and transmission risks (Walker, 2002; Yohannes *et al.,* 2005). If such measures bring

about long-lasting or permanent changes on land, water or vegetation, they are referred to as environmental modification (e.g. filling, drainage, planting water loving trees such as eucalyptus trees in swampy areas, and closing or covering breeding sites).

Chemical larviciding for the control of malaria vectors is not feasible and effective unless mosquito breeding places are few or breeding sites are easily identified and treated (Kweka *et al.,* 2012); so that, it will be possible to reduce the adult *Anopheles* population which is responsible for subsequent malaria transmission.

2.2.2 Adult Mosquito Control Methods

Chemical control methods that target adult *Anopheles* species have been the most effective and widely used vector control methods since the 1940s. One of the most common practices is indoor residual house spraying (IRS), in which the inside walls, the ceiling, and sometimes the attic, veranda, and cattle sheds outside of residential houses are sprayed with a persistent insecticide. The IRS is used against the *Anopheles* species that land on the inside walls before or after biting humans (Walker, 2002). IRS was effective across a wide range of vectors and environmental conditions. However, problems with insecticide resistance and concerns about pesticide exposure risks suggest selective and limited applications (WHO, 1982).

Chemical repellents are used by people to avoid blood-feeding insects, ticks, mites, and other arthropods, thereby reducing arthropod-borne disease transmission (Brown & Hebert, 1997). Repellents provide protection for people who stay outdoors during the evening and go to bed late in the night, exposing themselves to early biting mosquitoes. A combination of mosquito

repellent with LLINs may be effective in Ethiopia where the primary malaria vector, *An. arabiensis,* is anthropophagic (Abose *et al.,* 1998). Use of mosquito repellents during the evening can improve the effectiveness of LLINs against malaria particularly in areas where people stay outdoors for some time in the evening. In addition, they could also help control outdoor biting vectors (Fradin, 1998; Moore *et al.,* 2007). The combined strategy for vector control interventions against malaria infection can thus be effective compared to a single strategy (Fullman *et al.,* 2013).

Controlling malaria transmission and possible future elimination and eradication efforts of the disease can be done by proper use of long-lasting insecticide treated mosquito nets (LLITNs) and IRS together with other methods (WHO, 2009). However, the scaling-up of the LLINs and IRS program resulted in a widespread resistance in *An. arabiensis* to most of the insecticides used in LLINs and IRS (Balkew *et al.,* 2010; Djogbenou *et al.,* 2011; Kabula *et al.,* 2013; Ochomo *et al.,* 2013). In addition, the use of insecticidal treated nets and their application during bed time may not prevent vectors that have early feeding behavior (Abose *et al.,* 1998). In addition, deposition of smoke and abrasions by net owners could reduce efficacy of the nets (Fettene *et al.,* 2009). Hence, repellents will be useful to prevent vectors that bite between dusk and bedtime while people are involved in different activities in outdoors and indoors (Deressa *et al.,* 2014b). A study by Abose *et al.,* (1998) revealed that the peak biting hours for the primary malaria vector in Ethiopia was from 18:00 - 20:00 (biting indoor) and 22:00 - 24:00 (biting outdoor) while a study by Kibret *et al.,* (2010) showed that, the peak time for indoor and outdoor activities of *An. arabiensis* were observed during the early period of the night, between 18:00-19:00 and 19:00-20:00 hours, respectively.

The use of LLINs during bed time together with the use of mosquito repellent during early evening reduces malaria transmission significantly. Burning of leaves of various plant species those produce smokes have been used to protect mosquitoes since ancient times (Karunamoorthi *et al.,* 2009). The majority of modern commercial repellents are prepared by using chemicals such as allethrin, N-N-diethyl-m-toluamide, dimethyl phthalate (DMP), N, N-diethyl phenyl acetamide, and N, N-diethyl mendelic acid amide, even though they may not be safe for public use (Zadikoff, 1979). As the efficacy of synthetic repellents reduces owing to sweating, expense, and allergic reactions, it requires impregnation of the repellent into cotton fabric strips for a more reasonable way of minimizing direct skin contact (Karunamoorthi, 2011). Repellents of plants origin are currently receiving massive attention, due to their environmental and user-friendly nature and they are used by poor societies that cannot afford the modern synthetic chemicals (Karunamoorthi *et al.,* 2009).

Environmental management was successfully applied early in the 20th. Its application depends on the local ecological, socioeconomic, political, and cultural setting (Walker, 2002). The method includes the building of settlements away from vector sources, mosquito-proofing of houses, personal protection and hygiene measures against vectors (WHO, 1982).

Integrated Vector Management (IVM) approach contains all kinds of vector control tactics. IVM make vector control program compatible with national health systems in addition to the integration of traditional control measures. IVM promotes sustainability by incorporating decision-making based on human and institutional resources, and engaging the community. IVM encourages integrative, multi-disease approaches and promotes the systematic application of different interventions in combination and in synergy with each other (WHO, 2013).

2.3 Challenges of Vector Control

WHO and different organizations encourage that, the use of pyrethroid-impregnated bed nets and promoting the reintroduction of DDT in IRS for malaria control will be helpful (Weissman, 2006; WHO, 2014). However, resistance of the malaria vectors to insecticides is a major challenge in malaria control programs (Djègbè *et al.,* 2011; Yewhalaw *et al.,* 2011). Particularly, the development of resistance to pyrethroids, the only class of insecticides available for use on LLINs and *An. arabiensis* resistance to DDT threatened the vector control program in the country (Toé *et al.,* 2014). Studies done in Southern Ethiopia, Gambella and in different localities have shown high levels of resistance to DDT (Balkew *et al.,* 2006; Balkew *et al.,* 2010; Yewhalaw *et al.,* 2011). Varying levels of pyrethroid resistance were also reported in Gorgora and the Ghibe River valley of the country (Balkew *et al.,* 2010).

The effectiveness of ITNs/LLINs against malaria, however, depends on their acceptability, operational feasibility and proper utilization (Deressa *et al.,* 2014a). Graves *et al.,* (2011) had documented the different factors that influence use of ITNs/LLINs in Ethiopia at the individual, household and community level. The age of nets may lead to misuse at the individual level since the average life span of LLINs (Perma Net® nets) may not be more than three years (Fettene *et al.,* 2009). Thus, if the distribution is not continuous nets will worn-out at 3-4 years and individuals will be forced to misuse nets. Gender also contribute for misuse of nets and perception of communities about the use of LLINs in killing mosquitoes will significantly be associated with increased ITNs use (Deressa *et al.,* 2011).

Lack of education and knowledge on malaria and ITNs use can also lead to misuse of nets (Deressa *et al.,* 2011). Besides, beliefs and risk perceptions, perceived benefits and disadvantages of nets, trust in health workers providing health education and LLINs, knowledge of appropriate net use/care practices, and net hanging skills do affect use and efficacy of LLINs (Deressa *et al.,* 2014a). At the household level, determinants of net use include household size and composition (Hwang *et al.,* 2010). The number of children fewer than five years of age in the household (women's use of ITNs was significantly associated with having a child under 5 years) also affects net use (Hwang *et al.,* 2010). Intra-household sleeping arrangements, household decision-making processes and power structures, and use of other vector control measures may also affect net use (Deressa *et al.,* 2011). In addition to that, social norms and values, cultural beliefs and practices; for instance, white nets may be associated with burial shrouds and death, and free nets may be regarded as toxic or even deliberately harmful to recipient groups also affect net use (Chuma *et al.,* 2010). Mechanisms of LLIN distribution and distance to LLIN suppliers, rumors about LLINs and social support and pressure all have the potential to influence net use by individuals within a community which may mislead the use of nets at the community level (Deressa *et al.,* 2014a).

The feeding behavior of malaria vectors has an important implication in the use of ITNs/LLINs (Deressa *et al.,* 2014b). LLINs are mostly used during bedtime. However, some vectors may bite before bedtime that allows them to escape from being exposed to LLINs or IRS (Yohannes & Boelee, 2012; Pooda *et al.,* 2015). Mosquitoes can also bite between dusk and bedtime while people are engaged in different activities both outdoors and indoors (Abose *et al.,* 1998). ITNs and/or IRS could also increase the importance of outdoor transmission of malaria and necessitate

new tools to target vectors that feed and/or rest outdoors (Ranson *et al.,* 2011). The zoophilic behavior of *An. arabiensis* also affects the use of LLINs and IRS. A study by Massebo *et al.,* (2015) shows that regardless of the three-fold higher number of humans in a village near Arba Minch, *An. arabiensis* showed a strong relative preference to bovine blood meal over humans which was 4.7 times higher on cattle than that on humans. This shows that available vector control tools are not effective enough to achieve widespread malaria elimination or eradication, and hence innovative approaches are needed.

2.4. Endectocides as Potential Malaria Control Tools

An. arabiensis, the major malaria vector in Ethiopia, is zoophagic and anthropophagic. Hence, keeping livestock between residential houses and vector breeding sites could bring a substantial reduction in malaria transmission (Kibret *et al.,* 2010). However, people prefer to keep the animals near their houses to prevent them from being stolen and to facilitate husbandry (Franco *et al.,* 2014). In conditions where the vectors exhibit substantial outdoor feeding and biting as well as crepuscular activity and a tendency to bite early at night, which limits the effectiveness of ITNs and IRS (Chaccour *et al.,* 2015), a vector control method that target hosts other than the human beings could be a better strategy to reduce the zoophilic vector populations.

Treatment of livestock with endectocides has beenreported to be effective against zoophilic malaria vectors (Naz *et al.,* 2013; Massebo *et al.,* 2015), other mosquitoes, tsetse flies, and tick in sub-Saharan Africa (Franco *et al.,* 2014). This technique helps to kill vectors that escape from interventions including LLINs and IRS. Ivermectin, which can be given as a drug to both

humans and animals (Chaccour *et al.,* 2015) and is toxic to *Anopheles* mosquitoes, kills the mosquitoes when they feed on the hosts treated with the drug (Steketee & ter Kuile, 2015). Researches done by Fritz *et al.,* (2009, 2012) showed that mass treatment of livestock with ivermectin could reduce zoophilic vector populations at the onset of the rainy season, precluding epidemics in malaria endemic regions. In addition, cattle owners would benefit by reducing parasite burden of the cattle, and thus improving the health, thereby increasing the productivity of cattle herds (Dimander *et al.,* 2003; Fritz *et al.,* 2012). A therapeutic dose of ivermectin, in a mosquitoes' blood meal, causes reduced survival, fecundity, and egg hatch rate in mosquitoes (Gardner *et al.,* 1993; Sulaiman *et al.,* 2007; Fritz *et al.,* 2009; Deus *et al.,* 2012; Pooda *et al.,* 2013; Ouédraogo *et al.,* 2015). Thus, it can be used as an alternative or supplementary approach to prevent malaria transmission by reducing the population of *Anopheles* mosquitoes (Ouédraogo *et al.,* 2015). It also affects the age structure of the vector population by changing/pulling it towards the younger age class which does not participate in transmission (Sylla *et al.,* 2010).

Endectocides, the macrocyclic lactones (avermectin/milbemycin) are drugs with endoparasitocidal and ectoparasitocidal activity (Chaccour *et al.,* 2013). They have been used in the battle against metazoan parasites and are effective against parasites from multiple phyla (Mousley *et al.,* 2004).

Ivermectins, the most effective and well-developed class of endectocides, are 16-membered ring macrocyclic lactones produced from the fermentation broth of an actinomycete (Omura, 2008; Chaccour *et al.,* 2013). Ivermectin is a semi-synthetic avermectin derivative that was first licensed in 1981 as a veterinary drug (Omura, 2008). The discovery of ivermectin has been

known for its successful history due to its high potency, long pharmacokinetic persistence in blood and lymph, harmless in vertebrates' physiology, its broad spectrum activity against endoparasites and arthropod ectoparasites, and nematodes (Fisher & Mrozik, 1992; Omura, 2008; Chaccour *et al.,* 2015). Ivermectins are glutamate-gated chloride channel activators that cause reduced motor activity and paralysis in both insects and nematodes (Fritz *et al.,* 2012). Unlike the adults, ivermectin can destroy immature filarial worms (Omura, 2008). It exhibits potent microfilaricidal activity against many major filarial parasites in humans, including *Wuchereria bancrofti, Brugia malayi, Loa loa* and *Manzonella ozzardi* (Chaccour *et al.,* 2013). Drug combinations (ivermectin/diethyl-carbamazine (DEC), ivermectin/albendazole, DEC/albendazole) proved highly effective (Sargison, 2012). Ivermectin is also active against *Ascaris lumbricoides, Trichuris trichuria* and *Enterobius vermicularis and Strongyloides stercoralis* (Omura, 2008). The broad activity of ivermectin is also evident from recent *in vitro* evidence that demonstrate its effect on viral replication of human immunodeficiency virus and several arthropod-transmitted Flaviviruses by targeting virus non-structural protein 3 (NS3) helicase activity or inhibiting nuclear import (Kobylinski *et al.,* 2012; Mastrangelo *et al.,* 2012).

Additionally, many other ivermectin derivatives have been developed by veterinary pharmaceutical companies for use in livestock and pets (e.g. selamectin, doramectin, eprinomectin, moxidectin, fipronil) which have enhanced pharmacokinetic profiles or reduced toxicity to certain breeds, and which have differential activity against malaria vectors (Foy *et al.,* 2011; Butters *et al.,* 2012). Fipronil has been used to control ectoparasites on domestic animals, administered as a pour-on or dip for cattle to control ticks with high efficacy (Lopes *et al.,* 2014). A single dose of fipronil caused 100% mortality in both adult and larval stages of *Phlebotomus argentipes* sand flies in India (Poché *et al.,* 2013).

Selamectin is a C13-monosaccharide-C5- oxime that was developed following reports of unusual IVM sensitivity of collies and the need for a broader spectrum anti-parasitic drug in companion animals (pets) (Butters *et al.,* 2012). Moxidectin is in the milbemycin drug class, which are also 16-membered macrocyclic lactone endectocides related to the avermectins, but they lack a disaccharide substituent at C-13 position of the macrolide ring and are derived from the fermentation broth of *Streptomyces cyanogriseus* (Prichard *et al.,* 2012). It is better known for its anthelmintic properties than its insecticidal properties. Nodulisporic acid A is a metabolite of the endophytic fungus *Nodulisporium spp.* and is structurally related to indole diterpenes. N-tert-butyl nodulisporamide is a derivative of nodulisporic acid A, and was developed as a long lasting oral systemic ectoparasitocide for flea and tick control in companion animals (Meinke *et al.,* 2009; Butters *et al.,* 2012).

Eprinomectin is also a highly effective broad-spectrum parasiticide developed as an endectocide for topical treatment of cattle of all classes and ages (Soll *et al.,* 2013). It is a semi-synthetic compound of the ivermectin family originally selected as a nematocide, insecticide, and miticide to be administered by the topical route (Shoop *et al.,* 1996; Shoop and Soll, 2002; Soll *et al.,* 2013). Eprinomectin is commercially used for control of endoparasites of livestock and was also demonstrated to be as effective as ivermectin at killing blood-feeding *An. gambiae* s.s. in the laboratory (Poché *et al., 2015).*

LongRange ™eprinomectin is incorporated in poly (d, l-lactide-coglycolic) acid (PLGA) (drug manual) and has been demonstrated to be a safe and effective biodegradable material which has been used as a drug delivery system for extended release (Lewis, 1990; Clark *et al.,* 2004).

According to Soll *et al.,* (2013), following subcutaneous injection, eprinomectin is released from the matrix formed with PLGA which degrades over time and provides a second plasma concentration peak more than70 days post dose. Hence, it is highly efficacious in protecting cattle from a variety of important nematode parasites when evaluated against single point challenges at 100, 120 or 150 days after treatment. An extended-release injection, which is administered at a rate of 1 mg eprinomectin/kg body weight, has been developed to provide up to 150 days control of parasites of cattle (Forbes, 2013). It protects the host against the negative impact of susceptible parasites in order to ensure control of disease and reduce parasite transmission.

2.4.1. The Mode of Action of Ivermectines

Many of the classical transmitters within invertebrate phyla act at receptors that are distinct pharmacologically from their vertebrate counterparts and many aspects of motor coordination in invertebrates are quite distinct from those seen in vertebrates provides a clear evidence that invertebrates motor function is a good drug target for parasite control (Mousley *et al.,* 2004). The mode of action of ivermectin in insect neurons is, hyperpolarizing the membrane potential of postsynaptic neurons and muscle fibers through agonization of the inhibitory glutamate-gated chloride channels uniquely used by insects to regulate neuromuscular transmission that causes flaccid muscle paralysis that can lead to death of the insect (Foy *et al.,* 2011). In vertebrates, it stimulates the release of gamma-amino butyric acid (GABA) in neurons, but as these are usually in the brain and thus protected by the blood/brain barrier, the drug is exceptionally safe for mammals **(**Omura, 2008; Foy *et al.,* 2011**)** (Figure 1.).

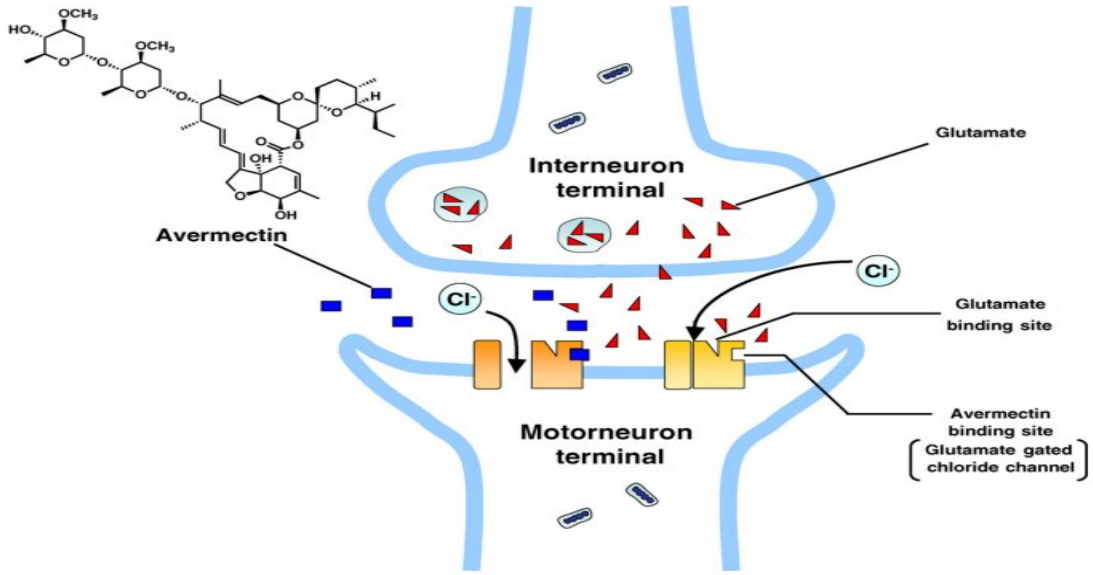


Figure 1. Mode of action of avermectin at nematode synapse (Omura, 2008).

2.4.2. Effect on Malaria Parasites

Ivermectin mass drug administration's (MDAs) can disrupt malaria parasite transmission, and as a systemic drug, ivermectin should be effective at targeting both endophagic and exophagic malaria vectors (Sylla *et al.,* 2010; Foy *et al.,* 2011; Butters *et al.,* 2012). A research by Sylla *et al* ., (2010) shows that endectocides affect malaria transmission basically in two ways: Firstly, the extrinsic incubation period (EIP), defined as the time it takes for *Plasmodium* parasites to develop from gametocytes in the blood meal into infectious sporozoites in the salivary glands is ≥9 days, hence a mosquito which has acquired *Plasmodium* gametocytes after biting an infected person will be able to spread these parasites to another person only after at least 9 days of parasite development, but in that same length of time, the mosquito will take blood meals from up to 5 more people without transmitting parasites. If only one of these five people has a mosquito-lethal concentration of endectocide circulating in their blood at the time of the bite,

their blood will kill the mosquito and the mosquito will never transmit parasites (Ouédraogo *et al.,* 2015). Secondly, ivermectin skew the age structure of vector population towards the younger age class that does not participate in transmission (Sylla *et al.,* 2010).

Ivermectin MDAs potentially inhibit the development of malaria parasite resistance to drugs targeting asexual and sexual stages in the human host which has a modulating effect on the mosquito host environment that is unfavorable for sporogonic development (Kobylinski *et al.,* 2012). Hence, if ivermectin MDAs were concomitantly deployed with MDAs of anti-malarias', any escapee parasites harboring drug-resistant alleles that must still develop in the mosquito to be spread to new hosts is likely to die in the mosquito host and they will never be propagated (Foy *et al.,* 2011). An experiment by Kobylinski *et al.,* (2012) shows that, ivermectin is sporontocidal to *P. falciparum* at concentrations sub-lethal to *An. gambiae* and *P. falciparum*-infectious; *An. gambiae* were slightly more susceptible to ivermectin than uninfected counterparts. These results further substantiate the utility of ivermectin MDA to reduce *P. falciparum* transmission.

2.4.3. Effect on Malaria Vectors

Initial ectoparasitocidal studies with the avermectins focused on biting flies, bot flies, fleas, lice, mange mites and ticks, and many were found susceptible to the drug (Foy *et al.,* 2011). Before 20 years ago Wilson (1993) reviewed the prospects of using avermectins in arthropod vector management and, he argued that the avermectins need to be assessed for their ability to affect all crucial variables of vectorial capacity from a vector population, especially reducing the probability of vector lifespan below the extrinsic incubation period and reducing the vector

feeding frequency through sub-lethal doses. Vertebrate blood meals, then, could deliver anti-vector drugs directly into the mid-guts of vectors to reduce the likelihood of pathogen transmission from the entire vector population (Foy *et al.,* 2011). It is an effective and safe endectocide that was approved for human use more than 30 years ago and it could be used in humans for malaria control (Foley *et al*., 2000). Recent studies also suggest that, it might become an effective and complementary strategy in malaria elimination and eradication efforts (Chaccour *et al*., 2013). In malaria endemic areas, timed mass treatments of livestock with ivermectin could reduce zoophilic vector populations at the onset of the rainy season, precluding epidemics (Foley *et al.,* 2000, Fritz *et al.,* 2009). Such MDAs could be highly effective tools for integrated malaria and helminthic control if administered more frequently. This human MDA strategy would likely benefit from alternative drugs that might be added to, or substituted for ivermectin to potentially relieve resistance pressure on both mosquitoes and helminthes. Despite differences in the routes and concentrations administered to different vertebrates *An. quadrimaculatus, An. stephensi, An. sacharovi, An. farauti, An. punctulatus,* and *An. gambiae* have all been shown to be susceptible to the avermectins when these mosquitoes ingest blood meals from treated vertebrates (Chaccour *et al.,* 2010; Butters *et al.,* 2012).

Ivermectin is toxic to all *Anopheles* species, has an excellent safety profile in humans and can be used in killing mosquitoes when they feed on treated humans (Chaccour *et al.,* 2013). Hence, Ivermectin MDA addresses the three main challenges identified by the Malaria Eradication Research Agenda (malERA) vector control group (malERACGVC, 2011): a) a different mode of action from current insecticides; b) it targets all biting vectors, regardless of their ecology and feeding behavior; and, c) it may be integrated into existing strategies to simultaneously control

malaria, filariasis and other neglected tropical diseases (Chaccour *et al.,* 2013; Alout *et al.,* 2014).

Attention is being given to ivermectins in controlling mosquitoes especially those that escape from current interventions, LLINs and IRS, because of their outdoor biting and outdoor resting behaviors. Thus, use of endectocidal drugs like ivermectin can provide an intriguing opportunity to kill the remaining mosquitoes that avoid or survive the existing vector control interventions (Steketee & ter Kuile, 2015; Chaccour *et al*., 2015),

Studies have demonstrated that, when imbibed in a blood meal, ivermectin causes a significant reduction in adult female mosquito survival, fecundity, and egg hatching rate (Foy *et al*., 2011; Deus *et al.,* 2012; Pooda *et al.,* 2013; Ouédraogo *et al.,* 2015) and reduce the population density of adult vectors. From an ivermectin MDA that was provided to a village in southeastern Senegal covering 80% of the villagers, a shift in the population age structure of mosquitoes towards younger age classes, for more than three weeks post-MDA has been observed (Sylla *et al.* 2010). This was due to a faster death of a significant proportion of the adult *An. gambiae* females. The mosquito abundance showed a temporary drop in the standing adult female mosquito population size that is most severe, one week post-MDA. The rebound effect and local environmental conditions were likely to explain the increased bites/person/month observed 28 days after a single MDA (Bockarie *et al.,* 1999.) However, given that the majority of the adult female mosquito population will be young in the month following MDA, regardless of the population size and local environmental conditions, there will be fewer infectious (sporozoite-transmitting) mosquitoes following treatment (Kobylinski *et al.*, 2011).

Eprinomectin is also a highly effective broad-spectrum parasiticide developed as an endectocide for topical treatment of cattle of all classes and ages (Soll *et al.,* 2013). It is a semi-synthetic compound of the ivermectin family originally selected as a nematocide, insecticide, and miticide to be administered by the topical route (Soll *et al.,* 2013). Eprinomectin is commercially used for the control of endoparasites of livestock and was also demonstrated to be as effective as ivermectin at killing blood-feeding *An. gambiae* s.s. in the laboratory (Poché *et al., 2015)*. LongRange™ eprinomectin is incorporated in poly (d, l-lactide-coglycolic) acid (PLGA) (Singh *et al.,* 2014). This polymer has been demonstrated to be a safe and effective biodegradable material which has been used as a drug delivery system for extended release (Lewis, 1990; Clark *et al.,* 2004). Based on Soll *et al.,* (2013), following subcutaneous injection, eprinomectin is released from the matrix formed with PLGA which degrades over time and provides a second plasma concentration peak more than 70 days post dose. Hence, it is highly efficacious in protecting cattle from a variety of important nematode parasites when evaluated against single point challenges at 100, 120 or 150 days after treatment. An extended-release injection, which is administered at a rate of 1 mg eprinomectin/kg body weight, has been developed to provide up to 150 days control of parasites of cattle (Forbes, 2013). It protects the host against the negative impact of susceptible parasites in order to ensure control of disease and reduce parasite transmission.

Different researches have been done on mosquitocidal effect of IVMs with in Sub-Saharan Africa (Pooda *et al.,* 2015). A study conducted in western Kenya to evaluate the potential of compounds in ivermectin, eprinomectin, and fipronil in Zebu cattle under semi-field conditions

to reduce the survival of blood feeding *An. Arabiensis* also showed that, all three compounds significantly reduced the survival time of *An. arabiensis* (Poché *et al.,* 2015). The mosquitocidal effect of LongRange™ eprinomectin has not been done anywhere including Ethiopia. Hence, the present study was proposed to investigate the activity of LongRange™ eprinomectin (an endectocide) on *An. arabiensis* fed on calves treated with the drug.

3. MATERIALS AND METHODS

3.1. The Study Area

The study was conducted in Edo Kontola (a rural village) which is located in about 4 to 5 kilometers north of Batu Town from February to May 2016. Batu (previously Ziway) town is located 167 KMs South of Addis Ababa, the capital of Ethiopia (Figure 2.**)**. The area lays at an average altitude of 1653m above sea level.

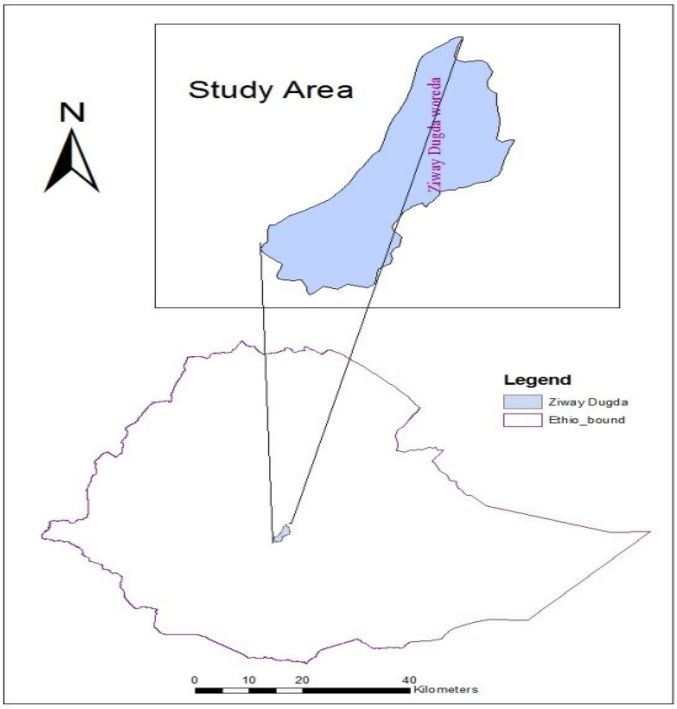


Figure 2. Location of Edo Kontola, near Batu Town, Central Ethiopia.

3.2. Experimental Insects/Mosquitoes

An. arabiensis maintained at the Aklilu Lemma Institute of Pathobiology, Addis Ababa University; since September 2001, was used for the study. It was originally collected from Bishoftu (Debrezeit) which is located at 45 kilometers east of Addis Ababa (Balkew *et al.,* 2010). The generation of these mosquitoes that the study began was F-165 (where F is for filial) and they were reared at 28–31^{0}C, 70-80% relative humidity and a 12:12 light dark cycle. Larvae were raised on a diet of ground Tetramin® fish food (fish meal) and adults on 10% sugar solution.

3.3. LongRange™ Eprinomectin

The LongRange™ Eprinomectin; "LongRange™", a trademark of Merial (registration pending in the United States of America) (Forbes, 2013), was imported, stored as recommended by the manufacturer and used in the study. It is a semi-synthetic compound of the ivermectin family drugs and was originally selected as a nematocide, insecticide, and miticide (Soll *et al.,* 2013) and control of endoparasites of livestock (Poché *et al., 2015).*

3.4. Treatment of calves with LongRange™ Eprinomectin

The experiment was performed following the method used by Naz *et al*., (2013) with some modifications. Six calves (1-3 year old) of local breed having approximately equal weights were divided into two groups (three calves per group).Calves were kept together with the herd in the field grazing grass around Lake Ziway, 5km North East of Batu (Ziway) town. Mass of calves was measured by tightly stretching the Heart Girth (mass measuring meter) (Milla *et al.,* 2012) behind the fore limbs from the ventral to dorsal (hump) area. After the weight of each calf was

known, the proper quantity of the drug were calculated for each calf (1 ml /110 lb, where 110 lb= 50 Kg) according to the instruction of the manufacturing company (Table 1). The drug was injected by a veterinarian to experimental calves subcutaneously under the loose skin in front of the shoulder by using 18 gauges, ½ inch needle after sanitizing the area with denatured alcohol as recommended by the manufacturer.

Table 1. Physical conditions of experimental calves and LongRange™ Eprinomectin doses given to calves at Edo Kontola, Batu, Central Ethiopia, Feb., 2016.

Group	Code	Age in months	Mass in Kg	Dose of LongRange™ Eprinomectin given (1 ml /50 KG)
LongRange™ Eprinomectin Treatment	Calf 1 (R1)	18	120	2.4 ml
	Calf 2 (R2)	36	160	3.2 ml
	Calf 3 (R3)	30	146	2.9 ml
Control Treatment	Calf 1 (R1)	36	188	0 ml
	Calf 2 (R2)	36	190	0 ml
	Calf 3 (R3)	36	190	0 ml

3.5. Bioassay of LongRange™ Eprinomectin against *An. arabiensis* that feed on calves treated with the drug

The Calves were checked for any former drug that may affect mosquitoes' life by feeding mosquitoes on all selected calves on the previous week of injection and a day before drug injection. But, no mortality was observed from the twice feeding. On the following day, the drug were given to experimental calves by a veterinarian doctor of Batu (Ziway) subcutaneously under the loose skin in front of the shoulder by using 18 gauges, ½ inch needle after sanitizing the area with denatured alcohol as recommended by the manufacturing company. The method for the experiment was based on Poche *et al*., (2013), with little modification. For the experiment, paper cups were covered with nylon netting on one side to facilitate blood feeding. On the following day (after 24 hours of injection), 20 unfed *An. arabiensis* females of 3-5 days old were put in each paper cup using mouth aspirator through a cotton plugged hole on the side of the paper cup (Kobylinski *et al.,* 2010).

A total of 40 unfed female *An. arabiensis* mosquitoes were allowed to feed on the neck of each calf for one hour in the evening (7:00 -8:00 PM) by dividing them into two cups containing 20 mosquitoes and wrapping on the left and right side of neck of the calves with a stripe of cotton cloth (Figure 3). The same number was done for the untreated control calves and replicated three times for both group of experiments.

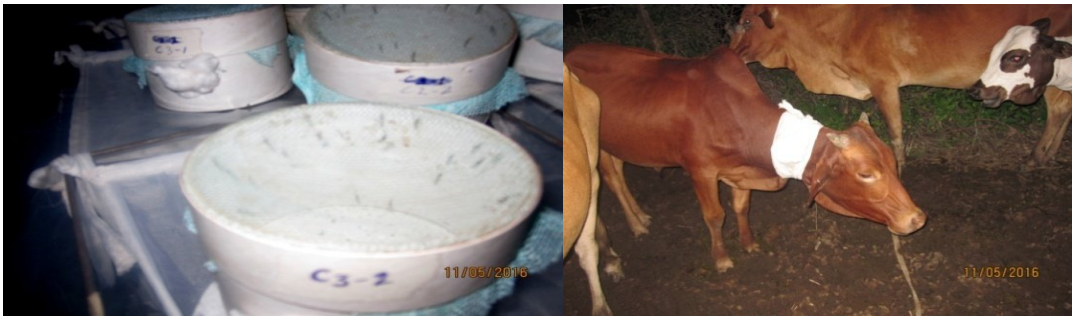


Figure 3. Mosquitoes ready to feed on calves (left) and mosquitoes feeding on the necks of calves (right), Edo Kontola, Batu, Central Ethiopia, May, 2016.

After the feeding session, the paper cups were carefully removed from the calves and mosquitoes were removed from the cups using mouth aspirator, a torch light and were transferred into small Barraud cages that were covered with a large polyethylene bag (Figure 4). In these Barraud cages the mosquitoes were provided with 10% sterile sugar solution soaked in cotton pads as a food source. On the following morning, blood fed females were counted, recorded and selected for observations, while the unfed females were disregarded and removed. The fed mosquitoes from the treated and untreated calves were followed up by daily changes of sterile sugar solution in the Barraud cages which were all placed in the polyethylene bag. Cotton pads soaked in water were also placed in the polythene bag to maintain high humidity (70-80% RH) during the observation period where they were then monitored for mortality (Figure 4). Mortality was recorded from each cage on every 24 hour difference. There should be a correction for treatment response for control response by using the Abbott's formula for the adjustment of insect mortality rates to consider the correction for natural mortality (Abbott, 1925). i.e,

X: the per cent mosquitoes surviving in the control.

Y: the per cent mosquitoes surviving in the treatment

Then X - Y: the per cent killed by the drug; LongRange™ Eprinomectin.

And the percent killed by the treatment (X -Y) divided by the per cent living in the control (X), gives the control or expressed by, an equation,

$$\% \text{ Mortality} = \frac{X - Y}{X} \times 100$$

But, Neal (1976) suggested that, Abbott's formula be applied to bioassay data whenever control response exceeded 10%. The control response values were <10% and had generally have only small effects on the value of the mean experimental treatment response corrected for control response. Therefore, using this formula had shown negligible effects and safely omitted.

Figure 4. Mosquito maintenance at field laboratory, Batu, Central Ethiopia, May 2016

In addition to mortality, observations on fecundity and fertility were recorded. Surviving females were monitored for fecundity and fertility (sections5.6 and 5.7). Since the drug provides a second peak concentration in the plasma after 70 days of a single dose treatment on calves (Soll *et al.*, 2013), such exposure of mosquitoes were conducted every week for about 3 months to the once treated calves and untreated calves (1 to 90 days).

3.6. Experiment to investigate the effect of LongRange™ Eprinomectin on *Anopheles arabiensis* fecundity

The method by Pooda *et al.*, (2015) was used to measure the effect of the experimental drug on the fecundity of *An. arabiensis* with some modification. After four days of observation on the fed mosquitoes, those survived were grouped into two of which half were dissected for fecundity (egg counting) and the remaining half were placed to hatch egg (egg hatchability). Ovarial dissection was done using fine mosquito needles under a 40x dissecting microscope and the number of eggs from each mosquito were counted and recorded.

3.7. Experiment to determine the effect of LongRange™ Eprinomectin on *Anopheles arabiensis* egg hatchability

Fertility of mosquitoes was determined based on the method by Derua *et al.,* (2015). This was done for each group (treatment and control) of the mosquitoes which were subjected to lay eggs (section 5.6). For experiments of weeks 2, 4 and 5:treatment group mosquitoes were placed in one cage and the control group mosquitoes in another cage and followed for egg lying for four days by providing egg laying substrate (a wetted filter paper on a Petri- dish). The dishes were left for 3 days and then transferred to rearing pans for incubation and hatching. The eggs laid were counted. Upon hatching, all first instars larvae were counted and removed daily. Larval count was made for 3 consecutive days (Fritz *et al.*, 2009). For experiments carried out from six to 12weeks, fertility was observed for individual mosquitoes. It was done by putting individual mosquitoes in a plastic cup covered with nylon netting and its substrate was maintained with wetted filter paper on which mosquitoes could lay eggs (Figure 5). Five of the survived

mosquitoes from each cage (12 cages; a total of 60 mosquitoes) were transferred singly into 60 plastic cups. Eggs laid from each cup were counted and transferred into individual plates/pans to maintain and follow larval development up to adult emergence during which the number of larvae, pupae and adults were counted and recorded.

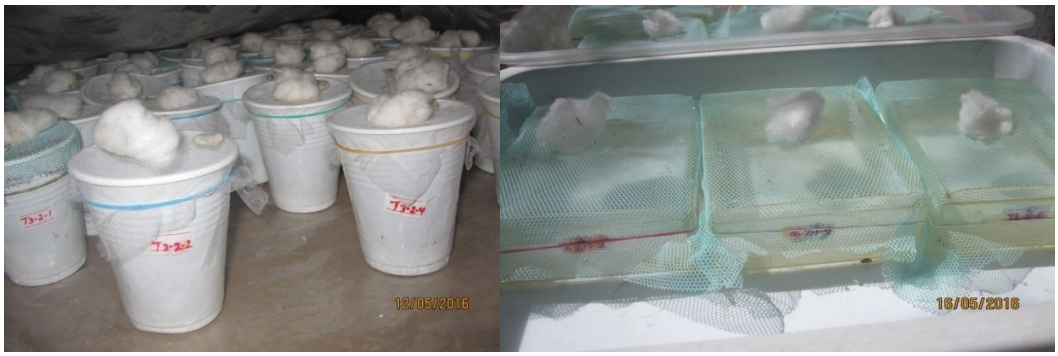


Figure 5. Mosquitoes ready to lay egg individually (left) and eggs transferred into respective trays for hatching (right), Batu, Central Ethiopia, May, 2016.

3.8. Experiment to evaluate the experimental drug on larval development and adult emergence

Up on hatching on the rearing pans the larva were raised at room temperature under the normal field condition; usually24–28 ^{0}C, 80% relative humidity, and a 12:12 light: dark cycle on a diet of ground Tetramin® fish food based on Kobylinski *et al.,* (2010), until new adult (F1) generation emerged (Figure 7).

Figure 6. Larval development (left) and adult emergence in field laboratory (right), Batu, Central Ethiopia, May, 2016

3.9. Data Analysis

Data were computerized using Microsoft Excel and analyzed using SPSS. Mortality data was compared between treatment and control arms using Kaplan-Meier estimator. The survival function curve produced by the Kaplan-Meier survival analysis shows the survivorship of *An. arabiensis* after taking blood meal for both groups of experiment. The fecundity, fertility and larval development data was compared between the two groups independent t-test. The comparison was based on the means of the target variables obtained from both treated and placebo/control group. Statistical significance was assumed whenever p-values <0.05.

4. RESULTS

4.1. Blood feeding and mortality rate of *Anopheles arabiensis*

A total of 3020 female *An. arabiensis* mosquitoes were allowed to feed on calves among which 1510 were on LongRange™ eprinomectin treated and the other 1510 on untreated controls (Table 2). During the study, a total of 2484 blood fed *An. arabiensis* were collected from treated calves and non-treated calves. From the 1510 *An. arabiensis* that were allowed to feed on LongRange™ eprinomectin treated calves, 81.3% (1228/1510) were blood fed and from those allowed to feed on the controls, 83.2% (1256/1510) were blood fed.

The percentage of *An. arabiensis* that fed on treated calves was not significantly different from the percentage that fed on the corresponding controls during each week. The mean feeding rate of *An. arabiensis* on LongRange™ eprinomectin treated calves was 102 (range: 81-117) which was 85% (range: 68%-98%) and the mean feeding rate on the control calves was 106 (range: 94-115) comprising to 88% (range: 78%-96%) feeding rate (Table 2).

Table2. Blood feeding rates of mosquitoes exposed to treated and untreated calves during the 12 weeks of experiments after 120 mosquitoes were exposed for each test at Edo Kontola, Ziway from February to June, 2016

Week*	Animal group	No. mosquitoes fed	Percent of fed mosquitoes	P-Value
0	Treated	27±6	67.50	0.800
	Control	26±2 **	78.0	
1	Treated	32±1	80.0	0.442
	Control	33±2.4	83.30	
2	Treated	27±4	67.50	0.116
	Control	35±2.6	87.50	
4	Treated	32±5	80.0	0.847
	Control	33±5	81.70	
5	Treated	35±3	86.70	0.368
	Control	37±2	91.70	
6	Treated	37±5	93.30	0.651
	Control	36±1	90.0	
7	Treated	38±0.7	94.20	0.116
	Control	37±0	92.50	
8	Treated	39±0.7	96.70	0.101
	Control	38±0.7	94.20	
9	Treated	35±4	86.70	0.468
	Control	37±2	91.70	
10	Treated	39±1	97.50	0.279
	Control	37±2	93.30	
11	Treated	33±4	83.30	0.116
	Control	38±2	95.80	
12	Treated	36±3.6	90.0	0.138
	Control	32±1	80.0	

* The week 3 feeding was disregarded from all experiments because of insecticide contamination of the cotton cages during the experiment.

**n =100

All the blood fed *An. arabiensis*, during each trial week, were followed for their mortality for four consecutive days. Their mortality differed at different weeks. The mortality among the first week fed and second week fed *An. arabiensis* in the controls were 5 % and 8 %, respectively. Neal (1976) suggested that, Abbott's formula be applied to bioassay data whenever control response exceeded 10%. The control response values were <10% and had generally shown negligible effects and safely omitted. On week 0, all of the *An. arabiensis* (100%) that fed on the treated calves died within 36 hours after feeding, while the mortality of *An. arabiensis* that fed on the control was 5 % (Table 3). On week 1, the mortality rate of *An. arabiensis* that fed on the treated calves was also 100%, but mortality started after two days (48 hours) of post feeding. The mortality difference between the treated and non-treated control groups was statistically significant ($p< 0.001$). On week 2, the mortality rate of *An. arabiensis* that fed on the treated calves was 35%. The week 3 trial was disregarded because of insecticide contamination. In the week 4 trial, the mortality rate of *An. arabiensis* that fed on the treated calves was 23%. The rate gradually declined from 10% on week 5 to 2% on week 11. Then, the mortality rate increased to 31% in week 12. The survival of *An. arabiensis* was very low when it fed on LongRange™ eprinomectin treated calves within seven days of post treatment with a very high statistical significance. The differences in the mortality of *An. arabiensis* between treated and the corresponding control group were statistically significant on weeks 2 to week 7 ($P\leq 0.008$) although the percent mortalities of these weeks were very low (ranges 35 % - 8 %, respectively). There was no difference between mortality of the treated and controls for the experiment of week 8, week 9, week 10 and week 11. But, mosquitoes that fed on day 85 also showed a highly significant mortality ($P < 0.001$).

Table 3. Mean mortality rate of *An. arabiensis* mosquitoes that fed on LongRange™ eprinomectin injected calves, Edo Kontola, Batu, Central Ethiopia, Feb. 2016 -May 2016

Group	Number fed (% mortality) of mosquitoes at different weeks from the start of experiment											
	0	1	2	4	5	6	7	8	9	10	11	12
Treated	100±0	100±0	33±19.8	23±20	10±6	8±7	8±3	4±3	5±2	2±1	2±1	31±12
Control	5±2	8±3	4±2	3±1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	2±1
Chi-Square	140	163	14	7	9	7	7	2	4	1	0.7	27
P-value	<0.001*	<0.001*	<0.001*	0.007*	0.003*	0.008*	0.007*	0.137	0.061	0.261	0.417	<0.001*

The Kaplan-Meier survival function showed the times at which individual mosquitoes of both the treated and control group died during the experiment, the curves represent the total number of 'death times', and the time intervals between these 'death times' (Figure 7A-7E). Hence, this survival curve can show the effect of the drug versus time in comparison with the control group.

Survival function result for week 0 (Figure 7A) showed that, 80 % of mosquitoes that fed on LongRange™ eprinomectin treated calves died within 24 hours of post feeding and the rest 20 % died at the 36th hour post feeding whereas dead mosquitoes in the control group were 5 % at the 36th hour. In week 1 (Figure 7B) about the 40% that fed on treated were censored at the 48th hours of post feeding and the other 40% died on the 72nd hours of post feeding while all the rest died after 120 hours of post feeding.

Figure 7. (A -E). Kaplan-Meier survival curves that show mortality of blood fed *An. arabiensis* mosquitoes on calves following a single injection of LongRange™ eprinomectin at Edo Kontola, Batu, Central Ethiopia, Feb., 2016 – May, 2016.

Figure 7A.Week 0 activity survival analysis

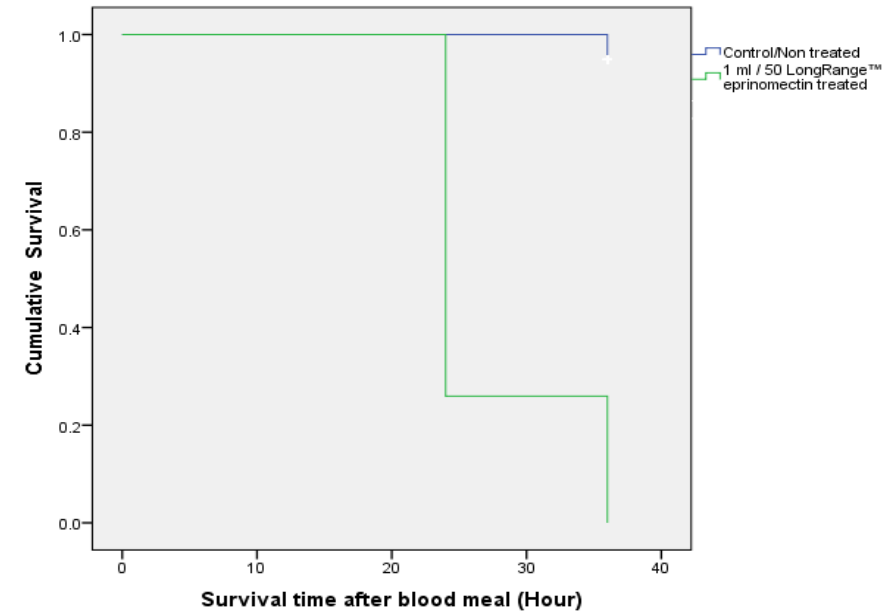


Figure 7B.Week one activity survival analysis

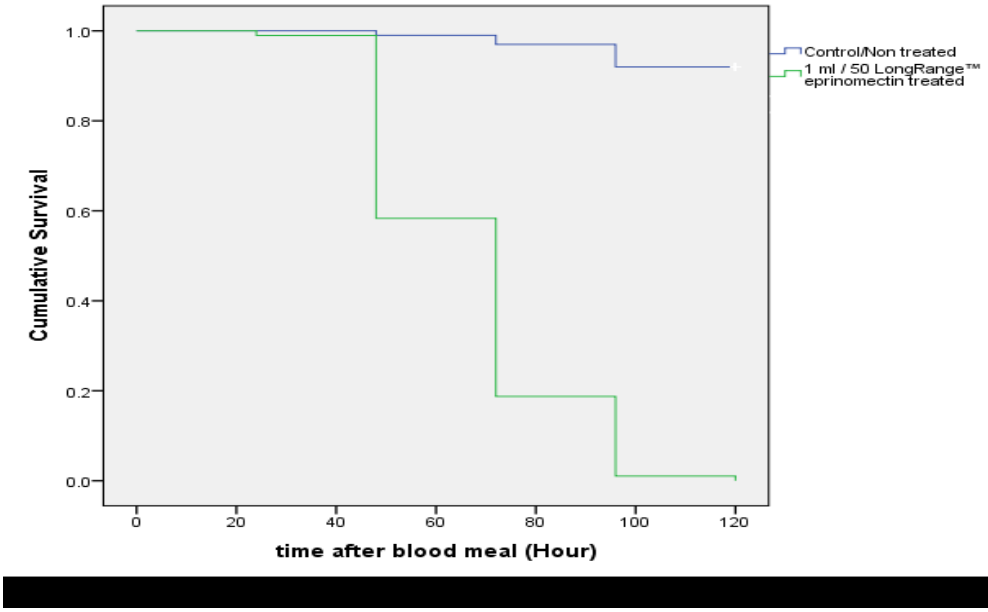


Figure 7C. Week two activity survival analysis

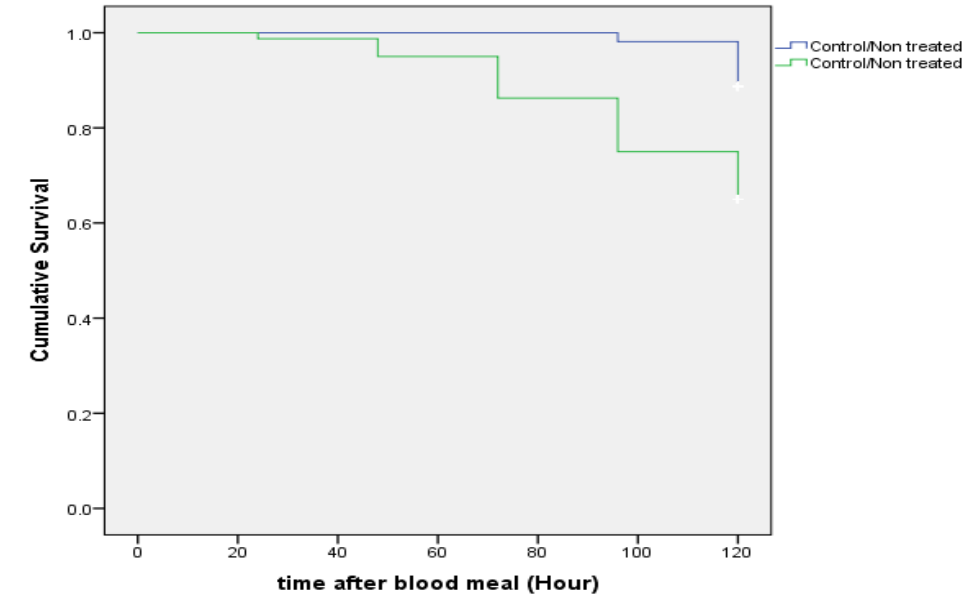


Figure 7D. Week four activity survival analysis

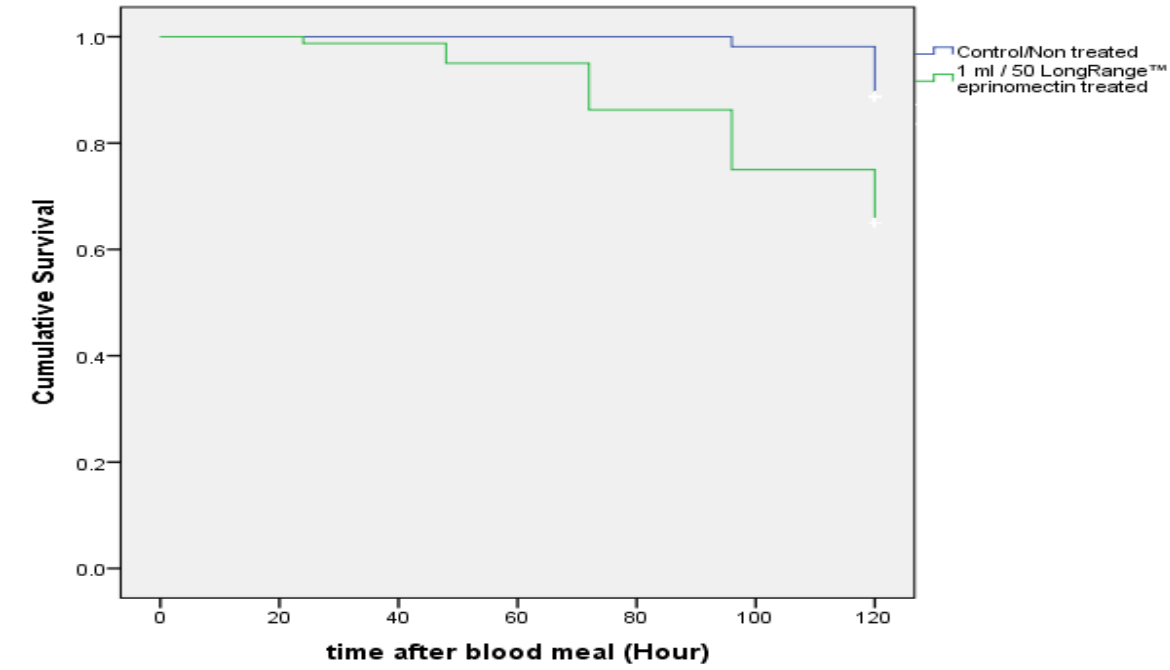
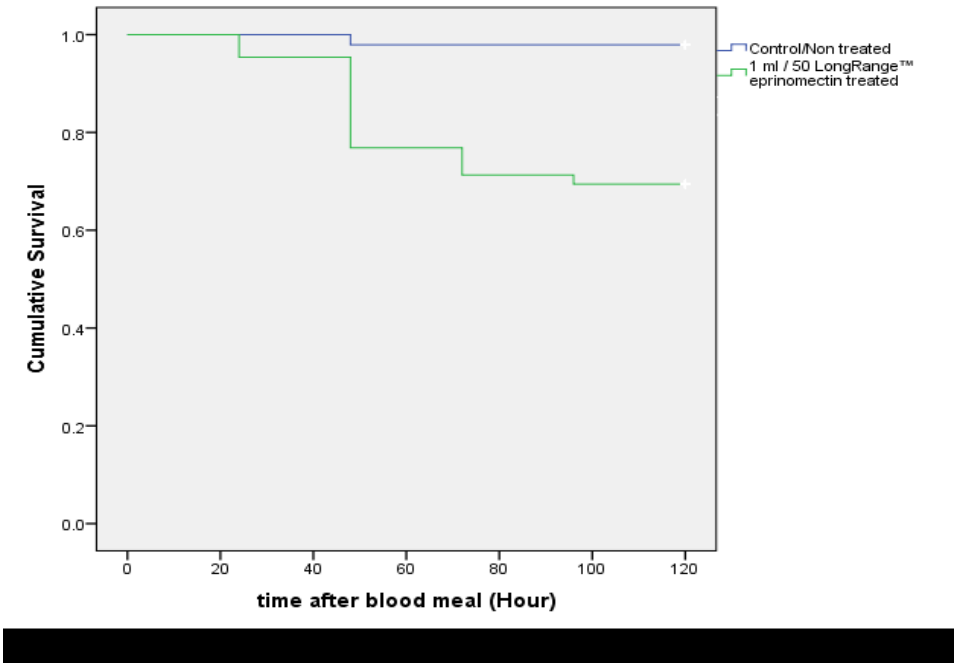


Figure 7E.Week twelve activity survival analysis



4.2. Fecundity of *An. arabiensis*

Among the *An. arabiensis* that fed on LongRange™ eprinomectin treated calves, 593 gravid mosquitoes were dissected and a total of 13,629 eggs were harvested (Table 4). Similarly, 573 gravid mosquitoes were dissected from the controls and a total of 18,388 eggs were obtained. The mean egg count of the mosquitoes that fed on LongRange™ eprinomectin treated calves was 23 (range: 9-37) eggs per mosquito while the corresponding value from the controls was 32 (range: 24-42) eggs per mosquito. The difference between the counts of the two groups was not statistically significant.

Table 4. Fecundity of *An. arabiensis* that fed on LongRange™ eprinomectin treated calves, Edo Kontola, Batu, Central Ethiopa, Feb. 2016-May 2016.

Animal group	Parameter	Weeks post Injecton									
		2	4	5	6	7	8	9	10	11	12
Treated	No. dissected	20	40	47	74	83	69	85	85	45	45
	Mean No of eggs/mosquito	35±4	27±6	38±3.4	17±2.6	33±3.6	24±3.6	20±5	10±3	21±3	21±6
Control	No. dissected	20	40	46	74	83	69	82	69	45	45
	Mean No of eggs/mosquito	40±2.6	31±2.6	44±3.6	25±4.6	37±2	27±6	24±7	26±2.3	42±2	42±4
P-Value		0.883	0.626	0.594	0.379	0.196	0.449	0.452	0.648	0.326	0.086

4.3. Fertility (Egg hatchability) of *An. arabiensis*

A total of 466 gravid *An. arabiensis* of which, 233 that fed on treated cattle and 233 on controls were placed for egg laying (Table 5). A total of 2,235 eggs were laid by the control group and 2,227 by the mosquitoes that fed on treated calves. 1,343 first instars were hatched (with 60 % hatchability) from the eggs of the treated group with mean of first instars of 64±8.6 hatched and 1,771 from the controls (with percent of hatchability of 79%) and mean of 83±8.9 first instars hatched. Rate of hatchability of eggs of LongRange™ eprinomectin treated calves fed mosquitoes was lesser by 19 % compared to those from the control ones. The drug was associated with a significantly ($p<0.001$) lower number of larvae on the week 4.

Table 5. Average No of 1st instars larvae hatched from eggs laid by *An. arabiensis* that fed on LongRange™ eprinomectin treated calves, Batu, Central Ethiopia, May, 2016.

Week*	Animal group	No. of Gravid mosquitoes	Total No of eggs laid	Average No of 1st instars larvae	Percent Hatch	P-Value
	Control	26	160	18±8	34	
2	Treated	26	166	16±9	29	0.335
	Control	40	850	222±7	78	<
4	Treated	40	550	67±8	36	0.001*
	Control	47	765	200±5	78	
5	Treated	47	534	127±7.6	71	0.194
	Control	30	120	40±5.9	99	
6	Treated	30	252	59±9	70	0.612
	Control	30	140	46±4.9	98	
7	Treated	30	94	26±8.6	82	0.495
	Control	30	152	50±9	98	
8	Treated	30	507	123±8.6	73	0.238
	Control	30	48	16±8	98	
11	Treated	30	124	31±9	75	0.437

*The week 9 and week 10 fed didn't produce egg**.**

4.4. Larval development and adult emergence

Three hundred seventy four pupae emerged from 1,771 larvae in the control arm and 316 pupae from 1,343 larvae in the treated group resulting in 21% and 24 % pupation, respectively. In addition to this, the probability of adult emergence from pupae in the control group was 42 % (6 % - 95%) and in the treated group was 41 % (4 % - 70 %).The drug significantly reduce the development of larvae and adult emergence on the week 4 (P=0.013), week 5 (P=0.019) and week 11(P<0.001) trials. However, on the other weeks; treating calves with LongRange™

eprinomectin did not significantly reduce the larval development and adult emergence of *An. arabiensis* mosquitoes that fed on calves treated with the drug.

Table 6. Average number of pupae and adult emergence from eggs of *An. arabiensis* that fed on LongRange™ eprinomectin treated calves and controls, Batu, Central Ethiopia, Feb., 2016-May, 2016 (85 days).

Week	Animal group	Average No of Pupae	Percent Pupation	Average No of Adult emergence	Percent Adult emerged	P-Value
	Control	11.3±1.15	63	11.3±1.15	63	
2	Treated	11.3±2.3	71	11.3±2.3	71	1
	Control	28.3±7.6	13	28.3±7.6	13	
4	Treated	9.7±0.58	15	9.7±0.58	15	0.013*
	Control	12.3±2.5	6	12.3±2.5	6	
5	Treated	6.7±0.58	5	6.7±0.58	4	0.019*
	Control	22.3±20.4	56	22.3±20.4	53	
6	Treated	27.7±25	47	27.7±25	56	0.663
	Control	29.7±22.8	65	29.7±22.8	95	
7	Treated	24.3±22	95	24.3±22	65	0.798
	Control	7.3± 6.7	15	7.3± 6.7	17	
8	Treated	16±13	13	16±13	15	0.362
	Control	13.3±0.58	85	13.3±0.58	45	
11	Treated	9.7±0.58	31	9.7±0.58	62	<0.001*

5. DISCUSSION

All the *An. arabiensis* that fed on LongRange™ eprinomectin treated calves, within a week of treatment, died. This shows that treatment of cattle with LongRange™ eprinomectin can help to reduce adult zoophagic *An. arabiensis* population significantly in south central Ethiopia and possibly in other areas of similar ecology. A previous study in Kenya also reported that eprinomectin mixed with bovine blood and provided to laboratory reared *An. arabiensis* in a membrane feeder killed *An. arabiensis* at low concentrations (Fritz *et al.,* 2012). In this study, the drugs effect was statistically significant up to 7 weeks of injection $P \leq 0.008$. However, mortality of *An. arabiensis* that feed on LongRange™ eprinomectin treated calves after a week was observed to reduce significantly. This indicates that treatment of cattle with LongRange™ eprinomectin may not be useful for a longer time control of *An. arabiensis*. This is in agreement with previous studies by Poché *et al.,* (2015) experiment in Western Kenya where all eprinomectin, fipronil and ivermectin were effective in killing *An. arabiensis* for at least 7 days at the lower doses of 0.25 and 0.5 mg/kg and at a concentration 0.2 mg/kg for up to 21 days post-treatment.

A similar study, using a related drug (ivermectin), by Derua *et al.,* (2015) also showed that all *An. gambiae* that fed on ivermectin treated human volunteers dead within 3 days post treatment. Hence, endectocides including LongRange™ eprinomectin can be used to control malaria transmitting vectors during epidemic seasons and also as a component of integrated vector management. In areas where there is continuous transmission, there could be a need for repeated treatment of cattle and humans with endectocides. In addition, eprinomectin was also observed to

be highly efficacious in protecting cattle from a variety of nematode parasites (Soll *et al.,* 2013; Forbes, 2013) which is an added advantage in countries like Ethiopia where malaria and other parasites of humans and animals are most common.

An. arabiensis was fed on LongRange™ eprinomectin treated and untreated calves with a similar preference. Treatment of calves with LongRange™ eprinomectin may not have any repellent effect on the mosquito. Thus treatment of cattle with LongRange™ eprinomectin can be implemented as a supplementary control of *An. arabiensis* in conditions where the mosquito is zoophagic, zoophagic and exophagic, crepuscular and resistant to the available insecticide based control methods such as LLITNs and IRS in particular and outdoor malaria transmission in general.

An. arabiensis that fed on LongRange™ eprinomectin were observed to lay relatively lesser number of eggs (not statistically significant) but their reproduction capacity was not affected. This is similar with a study by Fritz *et al.,* (2012) which showed no difference in the fecundity of eprinomectin mixed bovine blood fed *An. arabiensis* and the DMSO-treated blood (control) fed *An. arabiensis* in a membrane feeder system. However, the mean hatching rate of eggs laid in the untreated group exceeded the eggs in the treated group by 21% (with a respective of 83% and 62% hatchability). In addition to this, hatching time, larval development, pupation and adult emergence were also delayed by at least one day in the mosquitoes that fed on the treated calves than the controls (non-treated). A similar result was reported by Pooda *et al.,* (2013) that, a therapeutic dose of ivermectin delayed the first larviposition of *Glossina palpalis gambiensis*; however there was no significant effect on the hatching rate between the treatment and control

groups. According to previous reports, the effect of ivermectin on the fertility of flies can be explained by different factors: a delay in the ovulation process, an increase in the duration of gestation, and/or a disruption of pupation (Montasser *et al.,* 2011; Pooda *et al.,* 2013).

Although it was not consistent for every week trial, due to unknown reason, the presence of a sub-lethal dose of the drug affects the larval development of *An. arabiensis* that fed on calves treated with the drug. In addition to that, larval development, pupation and adult emergence were observed to highly rely on the daily weather condition. The weather fluctuation in the area affects the field laboratory air condition that may directly affect the larval development. In the beginning of the study, the daily temperature recorded was in the range between 24 - 28 ^{0}C while on week 4 and week 5 the daily temperature lowered to 17- 20 ^{0}C. Beck-Johnson *et al.,* (2013) also observed the effect of temperature on *Anopheles* mosquito population dynamics that, due to the fall in mean temperature below 18 ^{O}C, they didn't produce egg and there by their following life stages**.** Such temperature change might have affected the larval development and adult emergence on weeks 4, 5, 8, 9 and 10. Astudy on the temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *An. gambiae* in the laboratory revealed that, the lower temperature reduce the larval development there by their chance of emergence to adulthood (Bayoh & Lindsay, 2004). Yang *et al.,* (2009) also observed that, the increase in ambient temperature is a beneficial factor for the maintenance of vital physiological activities of the mosquito in order to maturate fertilized eggs.

6. CONCLUSIONS AND RECOMMENDATIONS

Treating calves with a therapeutic dose of LongRange™ eprinomectin can kill all of the *An. arabiensis* mosquitoes that fed on calves within 7 days of post-treatment. However, mortality of *An. arabiensis* that fed on these calves was reduced gradually after day 7 post-treatment. The drug showed no significant effect on fecundity and fertility of *An. arabiensis*. Based on the present study, it can be recommended that treating cattle with LongRange™ eprinomectin can be used together with IRS and LLINs for the control of zoophagic *An. arabienis* in Ethiopia. It can be used as a supplementary vector control tool against *An. arabiensis* if weekly mass drug administration (MDA) is recommended for cattle. MDA would fill the gap in mosquito control where the country is mainly using control measures targeting the human-vector contact, which would fail as mosquitoes shift their feeding behavior from humans to the domestic cattle. However, the present findings must be refined through future studies that are based on large sample size, using tent traps and allowing wild mosquitoes to feed and by conducting the study under different eco-epidemiological settings.

REFERENCES

Abose, T. Y., Olana, Y., and Alamirew, D. (1998). Re-orientation and definition of the role of malaria vector control in Ethiopiathe epidemiology and control of malaria with special emphasis on the distribution, behaviour and susceptibility of insecticides of anopheline vectors and chloroquine resistance in Zwai, Central Ethiopia and other areas (No. F/614.53236 R4).

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J Econ Entomol*. **18:** 265-267.

Alout, H., Krajacich, B. J., Meyers, J. I., Grubaugh, N. D., Brackney, D. E., Kobylinski *et al.*, (2014). Evaluation of ivermectin mass drug administration for malaria transmission control across different West African environments. *Malar J.* **13:** 417.

Animut, A., Gebre-Michael, T., Balkew, M., and Lindtjørn, B. (2012). Abundance and dynamics of *Anopheline* larvae in a highland malarious area of south-central Ethiopia. *Parasit Vectors*. **5:** 117.

Balkew M, Elhassen I, Ibrahim M, Gebre-Michael T, and Engers H. (2006). Very high DDT-resistant population of *Anopheles pharoensis* Theobald (Diptera: Culicidae) from Gorgora, Northern Ethiopia**.** *Parasit Vectors*. **13:** 327-329.

Balkew, M., Ibrahim, M., Koekemoer, L. L., Brooke, B. D., Engers, H., Aseffa *et al.,* (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of kdr mutation. *Parasit Vectors*. **3:** 40.

Bayoh, M. N., and Lindsay, S. W. (2004). Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Med Vet Entomol.* **18:** 174-179.

Beck-Johnson, L. M., Nelson, W. A., Paaijmans, K. P., Read, A. F., Thomas *et al.,* (2013). The effect of temperature on Anopheles mosquito population dynamics and the potential for malaria transmission. *PLOS one*, **8:** e79276.

Biadgilign, S., Reda, A., and Kedir, H. (2012). Determinants of ownership and utilization of insecticide-treated bed nets for malaria control in Eastern Ethiopia. *J Trop Med.* 2012.

Bockarie, M. J., Pedersen, E. M., White, G. B., and Michael, E. (2009). Role of vector control in the global program to eliminate lymphatic filariasis. *Annu Rev Entomol.* **54:** 469-487.

Brown, M., and Hebert, A. A. (1997). Insect repellents: an overview. *J Am Acad Dermatol.* **36:** 243-249.

Butters, M. P., Kobylinski, K. C., Deus, K. M., Da Silva, I. M., Gray, M., Sylla *et al.,*(2012). Comparative evaluation of systemic drugs for their effects against *Anopheles gambiae.* *Acta Trop.* **121:** 34-43.

Chaccour, C. J., Kobylinski, K. C., Bassat, Q., Bousema, T., Drakeley, C., Alonso *et al.,* (2013). Ivermectin to reduce malaria transmission: a research agenda for a promising new tool for elimination. *Malar J.* **12:**153.

Chaccour, C. J., Rabinovich, N. R., Slater, H., Canavati, S. E., Bousema *et al.,* (2015). Establishment of the Ivermectin Research for Malaria Elimination Network: updating the research agenda. *Malar J.* **14:** 243.

Chaccour, C., Lines, J., and Whitty, C. J. (2010). Effect of ivermectin on *Anopheles gambiae* mosquitoes fed on humans: the potential of oral insecticides in malaria control. *J Infect Dis.* **202:**113-116.

Chandra, G., Bhattacharjee, I., Chatterjee, S. N., and Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian J Med Res.* **127:** 13-27.

Chuma, J., Okungu, V., Ntwiga, J., and Molyneux, C. (2010). Towards achieving Abuja targets: identifying and addressing barriers to access and use of insecticides treated nets among the poorest populations in Kenya. *BMC Public Health.* **10:** 137.

Clark, S. L., Crowley, A. J., Schmidt, P. G., Donoghue, A. R., and Piché, C. A. (2004). Long-term delivery of ivermectin by use of poly (D, L-lactic-co-glycolic) acid micro-particles in dogs. *Am J Vet Res.* **65:** 752-757.

Collins, F. H., and Paskewitz, S. M. (1995). Malaria: current and future prospects for control. *Annu Rev Entomol.* **40:** 195-219.

Coluzzi, M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull World Health Org.* **62:** *107-13.*

Degefa, T., Zeynudin, A., Godesso, A., Michael, Y. H., Eba, K., Zemene, E., and Yewhalaw, D. (2015). Malaria incidence and assessment of entomological indices among resettled communities in Ethiopia: a longitudinal study. *Malar J.* **14:** 1-11.

Deressa, W., Fentie, G., Girma, S., and Reithinger, R. (2011). Ownership and use of insecticide-treated nets in Oromia and Amhara Regional States of Ethiopia two years after a nationwide campaign. *Trop Med Int Health.* **16:** 1552-1561.

Deressa, W., Yihdego, Y. Y., Kebede, Z., Batisso, E., and Tekalegne, A. (2014a). Individual and household factors associated with use of insecticide treated nets in southern Ethiopia. *Trans R Soc Trop Med Hyg.* **108:** 616-624**.**

Deressa, W., Yihdego, Y. Y., Kebede, Z., Batisso, E., Tekalegne, A., and Dagne, G. A. (2014b). Effect of combining mosquito repellent and insecticide treated net on malaria prevalence in Southern Ethiopia: a cluster-randomised trial. *Parasit Vectors.***7:** 132.

Derua, Y. A., Kisinza, W. N., and Simonsen, P. E. (2015). Differential effect of human ivermectin treatment on blood feeding *Anopheles gambiae* and *Culex quinquefasciatus*. *Parasit Vectors*. **8:** 130.

Deus, K. M., Saavedra-Rodriguez, K., Butters, M. P., Black IV, W. C., and Foy, B. D. (2012). The effect of ivermectin in seven strains of *Aedes aegypti* (Diptera: Culicidae) including a genetically diverse laboratory strain and three permethrin resistant strains. *J Med Entomol.* **49:** 356-363.

Dimander, S. O., Höglund, J., Uggla, A., Spörndly, E., and Waller, P. J. (2003). Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. *Vet Parasitol*. **11:** 193-209.

Djègbè, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., and Corbel, V. (2011). Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in *Anopheles gambiae* from West Africa. *Malar J.* **10:** 10-1186.

Djogbénou, L., Pasteur, N., Akogbeto, M., Weill, M., and Chandre, F. (2011). Insecticide resistance in the *Anopheles gambiae complex* in Benin: a nationwide survey. *Med Vet Entomol.* **25:** 256-267.

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P. M., Shargie, E. B., Ejigsemahu *et al.,* (2008). Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malar J.* **7:** 118.

Esslinger, J. H. (1981). Environmental Management for Vector Control. Third Report of the WHO Expert Committee on Vector Biology and Control World Health Organization Technical Report Series No. 649. *Am J Trop Med Hyg.* **30:** 1345-46.

Fettene, M., Balkew, M., and Gimblet, C. (2009). Utilization, retention and bio-efficacy studies of Perma Net in selected villages in Buie and Fentalie districts of Ethiopia. *Malar J.* **8:** 114.

Fisher, M. H., and Mrozik, H. (1992). The chemistry and pharmacology of avermectins. *Annu Rev Pharmacol Toxicol.* **32:** 537-553.

Foley, D. H., Bryan, J. H., and Lawrence, G. W. (2000). The potential of ivermectin to control the malaria vector *Anopheles farauti*. *Trans R Soc Trop Med Hyg.* **94:** 625-628.

Forbes, A. B. (2013). LongRange™ (eprinomectin 5%) extended-release injection parasiticide and the utility of extended-activity antiparasitics in cattle. *Vet Parasitol.* **192:** 308-312.

Foy, B. D., Kobylinski, K. C., da Silva, I. M., Rasgon, J. L., and Sylla, M. (2011). Endectocides for malaria control. *Trends Parasitol.* **27:** 423-428.

Fradin, M. S. (1998). Mosquitoes and mosquito repellents: a clinician's guide. *Ann Inter Med.* **128:** 931-940.

Franco, A. O., Gomes, M. G. M., Rowland, M., Coleman, P. G., and Davies, C. R. (2014). Controlling malaria using livestock-based interventions: a one health approach. *PloS one*, **9:** e101699.

Fritz, M. L., Siegert, P. Y., Walker, E. D., Bayoh, M. N., Vulule, J. R., and Miller, J. R. (2009). Toxicity of blood meals from ivermectin-treated cattle to *Anopheles gambiae*s.l. *Ann Trop Med Parasitol.* ***103*****:** 539-547.

Fritz, M. L., Walker, E. D., and Miller, J. R. (2012). Lethal and sub-lethal effects of avermectin/milbemycinparasiticides on the African malaria vector, *Anopheles arabiensis*. *J Med Entomol.* **49:** 326-331.

Fullman, N., Burstein, R., Lim, S. S., Medlin, C., and Gakidou, E. (2013). Nets, spray or both?The effectiveness of insecticide-treated nets and indoor residual spraying in reducing malaria morbidity and child mortality in sub-Saharan Africa. *Malar J.* **12:** 10-1186.Gardner, K., Meisch, M. V., Meek, C. L., and Biven, W. S. (1993). Effects of ivermectin in canine blood on *Anopheles quadrimaculatus, Aedes albopictus* and *Culex salinarius. J Am Mosq Control Assoc. Mosq News.* **9:** 400-402.

Gari, T., Kenea, O., Loha, E., Deressa, W., Hailu, A., Balkew, M., and Lindtjørn, B. (2016). Malaria incidence and entomological findings in an area targeted for a cluster-randomized controlled trial to prevent malaria in Ethiopia: results from a pilot study. *Malar J.* **15:**145.

Graves, P. M., Ngondi, J. M., Hwang, J., Getachew, A., Gebre, T., Mosher *et al.,* (2011). Factors associated with mosquito net use by individuals in households owning nets in Ethiopia. *Malar J.* **10:** 10-1186.

Gubler, D. J. (1998). Resurgent vector-borne diseases as a global health problem. *Emerg Infect Dis.* **4:** 442.

Habtewold, T., Prior, A., Torr, S. J., and Gibson, G. (2004). Could insecticide-treated cattle reduce Afro tropical malaria transmission? Effects of deltamethrin-treated Zebu on *Anopheles arabiensis* behaviour and survival in Ethiopia. *Med Vet Entomol.* **18:** 408-417.

Hwang, J., Graves, P. M., Jima, D., Reithinger, R., and Kachur, S. P. (2010). Knowledge of malaria and its association with malaria-related behaviors–results from the Malaria Indicator Survey, Ethiopia, 2007. *PLoS One*. **5:** e11692.

Kabula, B., Kisinza, W., Tungu, P., Ndege, C., Batengana, B., Kollo, D., and Magesa, S. (2014). Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in *Anopheles gambiae sensu lato* population of Tanzania. *Trop Med Int Health.* **19:** 331-341.

Karunamoorthi, K. (2011). Vector control: a cornerstone in the malaria elimination campaign. *Clin Microbiol Infect.* **17:** 1608-1616.

Karunamoorthi, K., Mulelam, A., and Wassie, F. (2009). Assessment of knowledge and usage custom of traditional insect/mosquito repellent plants in Addis Zemen Town, South Gonder, North Western Ethiopia. *J Ethnopharmacol.* **12:** 49-53.

Kenea, O., Balkew, M., and Gebre-Michael, T. (2011). Environmental factors associated with larval habitats of *Anopheline* mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia. *J Vector Borne Dis*. ***48*:** 85.

Kibret, S., Alemu, Y., Boelee, E., Tekie, H., Alemu, D., and Petros, B. (2010). The impact of a small-scale irrigation scheme on malaria transmission in Ziway area, Central Ethiopia. *Trop Med Int Health.* **15:** 41-50.

Kobylinski, K. C., Deus, K. M., Butters, M. P., Hongyu, T., Gray, M., da Silva *et al.,* (2010). The effect of oral anthelmintics on the survivorship and re-feeding frequency of anthropophilic mosquito disease vectors. *Acta Trop.* **116:** 119-126.

Kobylinski, K. C., Foy, B. D., and Richardson, J. H. (2012). Ivermectin inhibits the sporogony of *Plasmodium falciparum* in *Anopheles gambiae*. *Malar J.* **11:** 381.

Kweka, E. J., Munga, S., Mahande, A. M., Msangi, S., Mazigo, H. D., Adrias *et al.,* (2012). Protective efficacy of menthol propylene glycol carbonate compared to N, N-diethyl-methylbenzamide against mosquito bites in Northern Tanzania. *Parasit Vectors*. **5:**189.

Lewis, D. H. (1990). Controlled release of bioactive agents from lactide/glycolidepolymers. *Drug Pharm Sci.* **45:** 1-41.

Lopes, W. D. Z., Cruz, B. C., Teixeira, W. F. P., Felippelli, G., Maciel, W. G., Buzzulini *et al.,* (2014). Efficacy of fipronil (1.0 mg/kg) against *Rhipicephalus* (*Boophilus*) *microplus* strains resistant to ivermectin (0.63 mg/kg). *Prev Vet Med.* **115:** 88-93.

Lozano-Fuentes, S., Kading, R. C., Hartman, D. A., Okoth, E., Githaka, N., Nene *et al*., (2016). Evaluation of a topical formulation of eprinomectin against *Anopheles arabiensis* when administered to Zebu cattle (*Bosindicus*) under field conditions. *Malar J.* **15:** 324.

malERA Consultative Group on Vector Control. (2011). A research agenda for malaria eradication: vector control. *PLoS Med*. **8:** e1000401.

Massebo, F., Balkew, M., Gebre-Michael, T., and Lindtjørn, B. (2015). Zoophagic behaviour of *Anopheline* mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasit Vectors*. **8:** 1-9.

Mastrangelo, E., Pezzullo, M., De Burghgraeve, T., Kaptein, S., Pastorino, B., Dallmeier *et al.,* (2012). Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J Antimicrob Chemother.* **67:**1884-1894.

Meinke, P. T., Colletti, S. L., Fisher, M. H., Wyvratt, M. J., Shih, T. L., Ayer *et al.,* (2009). Discovery of the development candidate N-tert-butyl nodulisporamide: a safe and efficacious once monthly oral agent for the control of fleas and ticks on companion animals. *J Med Chem.* **52:** 3505-3515.

Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B., and Wernsdorfer, W. H. (2009). From malaria control to eradication: The WHO perspective. *Trop Med Int Health.* **14:** 802-809.

Milla, A. P., Mahagoub, M. M. M., and Bushara, I. (2012). Estimation of live body weight from heart girth, body length and condition score in Nilotic cattle–Southern Sudan. *J Anim Sci Adv*. **2:** 453-457.

Ministry of Health, Federal Democratic Republic of Ethiopia; and United Nations in Ethiopia. **MDG Acceleration Compact Accelerated Action Plan for Reducing Maternal Mortality,** February 2014.

Montasser, A. A., Marzouk, A. S., El-Alfy, S. H., and Baioumy, A. A. (2011). Efficacy of abamectin against the fowl tick, *Argas (Persicargas) persicus* (Oken, 1818) (Ixodoidea: Argasidae). *Parasitol Res.* **109:** 1113-1123.

Moore, S. J., Darling, S. T., Sihuincha, M., Padilla, N., and Devine, G. J. (2007). A low-cost repellent for malaria vectors in the Americas: results of two field trials in Guatemala and Peru. *Malar J.* **6:** 101.

Mousley, A., Marks, N. J., and Maule, A. G. (2004). Neuropeptide signalling: a repository of targets for novel endectocides? *Trends Parasitol.* **20:** 482-487.

Naz, S., Maqbool, A., Anjum, A. A., and Zaman, S. (2013). Efficacy of ivermectin for control of zoophilic malaria vectors in Pakistan. *Pakistan J Zool.* **45:** 1585-1591.

Neal, J. W., 1976. A manual for determining small dosage calculations of pesticides and conversion tables. Entomological Society of America, College Park, Maryland.

Ochomo, E., Bayoh, M. N., Brogdon, W. G., Gimnig, J. E., Ouma, C., Vulule *et al.,* (2013). Pyrethroid resistance in *Anopheles gambiae* s.s and *Anopheles arabiensis* in western Kenya: phenotypic, metabolic and target site characterizations of three populations. *Med Vet Entomol.* **27:** 156-164.

Omura, S. (2008). Ivermectin: 25 years and still going strong. *Int J Antimicrob Agents*. **31:** 91-98.

Ouédraogo, A. L., Bastiaens, G. J., Tiono, A. B., Guelbéogo, W. M., Kobylinski *et al.,* (2015). Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria transmission after treatment: a double-blind, randomized, clinical trial. *Clin Infect Dis.* **60**: 357-365.

Poché, R. M., Burruss, D., Polyakova, L., Poché, D. M., and Garlapati, R. B. (2015). Treatment of livestock with systemic insecticides for control of *Anopheles arabiensis* in western Kenya. *Malar J.* **14:** 351.

Poché, R. M., Garlapati, R., Singh, M. I., and Poché, D. M. (2013). Evaluation of fipronil oral dosing to cattle for control of adult and larval sand flies under controlled conditions. *J Med Entomol.* **50:** 833-837.

Pooda, H. S., Rayaisse, J. B., Hien, D. F., Lefèvre, T., Yerbanga, S. R., Bengaly *et al.,* (2015). Administration of ivermectin to peridomestic cattle: a promising approach to target the residual transmission of human malaria. *Malar J.* **14:** 496.

Pooda, S. H., Mouline, K., De Meeûs, T., Bengaly, Z., and Solano, P. (2013). Decrease in survival and fecundity of *Glossina palpalis gambiensis vanderplank* 1949 (Diptera: Glossinidae) fed on cattle treated with single doses of ivermectin. *Parasit Vectors*. **6:** 165.

Prichard, R. K., Basáñez, M. G., Boatin, B. A., McCarthy, J. S., García, H. H., Yang *et al.,* (2012). A research agenda for helminth diseases of humans: intervention for control and elimination. *PLoS Negl Trop Dis.* **6:** e1549.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., and Corbel, V. (2011). Pyrethroid resistance in African *Anopheline* mosquitoes: what are the implications for malaria control? *Trends Parasitol.* **27:** 91-98.

Sargison, N. D. (2012). Pharmaceutical treatments of gastrointestinal nematode infections of sheep—Future of anthelmintic drugs. *Vet Parasitol.***189:**79-84.

Service, M. W. (2004). *Medical Entomology for Students*. 3rd Edition. (pp 1-50). Cambridge University Press, UK.

Shoop, W. L., Egerton, J. R., Eary, C. H., Haines, H. W., Michael, B. F., Mrozik *et al.,* (1996). Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. *Int J Parasitol.* **26:** 1237-1242.

Shoop, W., and Soll, M. (2002). Ivermectin, abamectin and eprinomectin. In *Macrocyclic Lactones in Antiparasitic Therapy* (pp. 1-29). CABI Publishing Oxon, UK.

Singh, G., Kaur, T., Kaur, R., and Kaur, A. (2014). Recent biomedical applications and patents on biodegradable polymer-PLGA. *Int J Pharmacol Pharma Sci*, **1:**30-42.

Soll, M. D., Kunkle, B. N., Royer, G. C., Yazwinski, T. A., Baggott, D. G., Wehner *et al.*, (2013). An eprinomectin extended-release injection formulation providing nematode control in cattle for up to 150 days. *Vet Parasitol.* **192:** 313-320.

Steketee, R. W., and terKuile, F. O. (2015). Editorial commentary: ivermectin as a complementary strategy to kill mosquitoes and stop malaria transmission? *Clin Infect Dis.* **60:** 366-368.

Sulaiman, S., Fadhlina, K., and Hidayatulfathi, O. (2007). Evaluation of Pyrethrin Formulations on Dengue/Dengue Haemorrhagic Fever Vectors in the Laboratory and Sublethal Effects. *J Arthropod-Borne Dis.* **1:** 1-6.

Sylla, M., Kobylinski, K. C., Gray, M., Chapman, P. L., Sarr, M. D., Rasgon, J. L., and Foy, B. D. (2010). Mass drug administration of ivermectin in south-eastern Senegal reduces the survivorship of wild-caught, blood fed malaria vectors. *Malar J.* **9:** 10-1186.

Toé, K. H., Jones, C. M., N'Fale, S., Ismail, H. M., Dabiré, R. K., and Ranson, H. (2014). Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. *Emerg Infect Dis.* **20:** 1691-96.

Walker, K. (2002). A review of control methods for African malaria vectors. *Environ Health Project.*

Warrell, D. A., and Gilles, H. M. (2002). *Essential malariology*. (4th Edition). Arnold, Chicago. Pp 1-34.

Waters, A. P., Higgins, D. G., and McCutchan, T. F. (1993). Evolutionary relatedness of some primate models of *Plasmodium*. *Mol Biol Evol*. **10:** 914-923.

Weissmann, G. (2006). DDT is back: let us spray! *FASEB J.* **20:** 2427-2429.

White, B. J., Collins, F. H., and Besansky, N. J. (2011). Evolution of *Anopheles gambiae* in relation to humans and malaria. *Annu Rev Ecol Evol Syst.* **42:** 111-132.

White, G. B. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Trans R Soc Trop Med Hyg.* **68:** 278-301.

Wilson, M. L. (1993). Avermectins in arthropod vector management—prospects and pitfalls. *Parasitol Today*. **9:** 83-87.

World Health Organization (WHO). 1982. *Manual on environmental management for mosquito control with special emphasis on malaria vectors.* WHO Offset Publication No. 66, Geneva. Pp 28-150.

World Health Organization (2013). *Malaria entomology and vector control*. World Health Organization, Geneva. Pp 61-90.

World Health Organization (2014). *A global brief on vector-borne diseases*, World Health Organization, Geneva. Pp 1-15.

World Health Organization (2015). *World malaria report 2015*. World Health Organization, Geneva. Pp 1-53.

Woyessa, A., Deressa, W., Ali, A., Lindtjorn, B., (2012). Prevalence of malaria infection in Butajira area, south-central Ethiopia. *Malar J.* **11:** 84.

Yang, H. M., Macoris, M. L. G., Galvani, K. C., Andrighetti, M. T. M., and Wanderley, D. M. V. (2009). Assessing the effects of temperature on the population of *Aedes aegypti*, the vector of dengue. *Epidemiol Infec.* **137:** 1188-1202.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Denis *et al*., (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PloS One*. **6:** e16066.

Yohannes, M., and Boelee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Med Vet Entomol.* **26:** 103-105.

Yohannes, M., Haile, M., Ghebreyesus, T. A., Witten, K. H., Getachew, A., Byass *et al*., (2005). Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Trop Med Int Health.* **10:** 1274-1285.

Zadikoff, C. M. (1979). Toxic encephalopathy associated with use of insect repellant. *J Pediatr.* **95:** 140-142.

ANNEX 1 EXPERIMENT DRUG APPROVALS

The objective of this study is to investigate the effect of LongRange™ eprinomectin on *Anopheles arabiensis* after feeding on LongRange™ eprinomectin treated calves for no more than 3 months. The result obtained from this study will help for better control and management strategies of malaria vectors. Those who are willing to accept this idea will allow your compound to keep treated calves and normal calves for the overnight experiment. The sample will be collected by the principal investigator and technical assistants. Therefore, I am requesting you to allow your compound for this experiment. You have the right to refuse to allow your compound to the research and this will not affect your dignity.

I _____ here by giving my consent being willing to provide the compound for the research as the Investigator will do the best for my compound.

ANNEX 2 ANNEX BASIC EXPERIMENTAL SCHEDULE-

Day 0

- Preparing experimental and control group calves.
- Informed consent
- Measuring their weight and height
- Preparing the drug/ LongRange™ eprinomectin (calculating the concentration/ amount of endectocide to be injected as per the weight of the calf)
- Injecting LongRange™ eprinomectin to the experimental calves subcutaneously.

Day 1

- Exposing mosquitoes for the first round.
- Collecting mosquitoes and separating the blood meal engorged from both experimental and control groups separately and keeping in cages with wetted cotton wool, 30 % sugar solution with cotton and correct labeling with a maintained laboratory condition.

Day 2

- Observing the surviving and died mosquitoes and recording will be continued with 24 hrs interval for both experimental and control groups together with renewing sugar and maintaining humidity for a maintained laboratory condition.

Day 3

- Preparing petri-dishes with wetted filter papers with in a cage for oviposition and will be repeated for each blood fed mosquitoes after 2 days ago of blood meal with a maintained laboratory condition.

Day 4/5

- Feeding the fed mosquitoes with a restrained rabbit for blood meal source to their egg development and will be repeated within 4/5 days interval. Counting eggs laid in each cage and transferring them to pans with labeling to observing hatching and recording for hatchability for both experimental groups will be done and will be continued for observing larval condition and adult emergence capacity for both control and experimental groups with a maintained laboratory conditions.

Day 14

- Exposing new mosquitoes for the second time for both treated and control groups; which means blood feeding 14 days ago after LongRange™ eprinomectin injection.

Day 15

- Collecting those exposed mosquitoes and selecting the blood meal engorged mosquitoes separately and keeping in different cages with correct labeling together with wetted cotton wool for humidity and 30 % sugar solution with cotton wool in a maintained laboratory condition for survival. We will also record the died mosquitoes and will continue the whole that we did after the first day of experiment similarly and data will be recorded correctly for survivorship, oviposition and hatchability, larval condition and adult emergeability and abundance for both control and experimental groups.

Day 28

- Counting the same number of mosquitoes for the third phase feeding 28 days ago after LongRange™ eprinomectin treatment and all procedures that we did for the first(day 1) and second phase(day 14) experiment will be repeated for this round with in a maintained laboratory condition and data will be recorded correctly.

ANNEX 3 EXPERIMENTAL DRUG APPROVAL

የእንስሳት መድኃኒትና መኖ አስተዳደርና ቁጥጥር ባለሥልጣን

VETERINARY DRUG AND ANIMAL FEED ADMINISTRATION AND CONTROL AUTHORITY OF ETHIOPIA

Ref.No.: 02/NDFACA/103/1145

Date: 15/02/2016

To: Addis Ababa University

Department of Microbiology Cellular and Molecular Biology

Addis Ababa

Subject: Clinical Trial Approval

This is in response to your letter dated Dec 01, 2015 concerning a request to conduct a clinical trial based on a proposal entitled "Evaluation on the Effect of Long Range Eprinomectin on the Survival, Fecundity and Fertility of Anopheles arabiensis Fed on Cattle Treated with the Drug".

Accordingly, the Authority has reviewed the protocol submitted and found that the formulation and strength of the investigational drug are not registered (not listed in the National Veterinary Drug List) to be used in Ethiopia and that the study is of great significance with minimal risk on the animal population around the study area; consequently, the Authority grants its approval of the study without any change as it has been presented in the protocol.

Finally, you should note that the Authority expects to be informed about the progress of the study, any adverse effects during the course of the study, any change in the protocol and that you should provide a copy of the final study result report.

CC:

- Mr Kehulu Belay

With Best Regards,

Zelalem (Dr.)

Director

Standard

Veterinary Drug Registration & Licensing Team P.O.Box:31303 Tel: 251-11-5-523227 E-mail: vdrlt2005@gmail.com
Address: Kirkos Sub-City, Wereda 17/18, Kazanchis, Addis Ababa, Ethiopia
In reply please refer to our Ref. no. & Date