LETHAL AND SUB LETHAL EFFECTS OF IVERMECTIN ON *ANOPHELES ARABIENISIS* UNDER LABORATORY CONDITION: AN APPROACH FOR MALARIA ELIMINATION

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The Ivermectin treated cattle blood induced high mortality in treatment groups compared to control at all test groups (p ≤ 0.001).The mortality rate significantly increased with increasing in concentration. The LC50, 95 and 99 were 16.46, 75.7 and 141.8 ng/ml respectively. There were no egg production at the concentration of >10ng/ml Ivermectin. The egg produced at 5ng/ml and 10ng/ml has significant difference between treatment and control group (p=0.019 and 0.001 respectively). Among egg produced from survived An. arabiensis; the hatchability, pupae and Adult emergence were lower in treatment group compared to control. The mean survival time was 8, 7.5, 6.8, 5.7, 4.9 days for An. arabiensis those ingested 5, 10, 20, 40, and 80ng/ml respectively and it was significantly shorter compared to control (p<0.001). The median survival time also statistically shorter among treatment group compared to control (p<0.001).

Abstract Reference

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BACKGROUND

Ivermectin is a combination of two semi-synthetic analogs of the fermentation products of Sterptomycesavermitilis. It's an endectocide approved for the treatment of different human and animal parasitic infections for more than 30 years (Hussain *et al.*, 2014, Chaccour *et al.*, 2015). Ivermectin is the safest drug in humans and used in mass drug administration campaigns to reduce the burden of *onchocerciasis* and *lymphatic filariasis* in Africa (Dadzie*et al.*, 2018) and is also active against different species of mosquitoes(Lyimo *et al.*, 2017, Dreyer *at al.*, 2018).

Throughout the world, malaria continues to have a devastating impact on people's health and livelihood. Even though vector control has played a central role, the problem still remains unacceptably high (Chaccour*et al.*, 2015). The world takes a stance towards malaria eradication since 2007 using different approaches. However, reaching and sustaining zero transmission is unlikely without innovation of new strategies due to the insecticide resistance, outdoor feeding and change in host preference(Degarege and Erko, 2016, Benelli and Beier, 2017)

Malaria vector control tools such as the long-lasting insecticide-treated nets (LLIN) and indoor residual spraying (IRS) have controlled malaria transmission in Africa by targeting mosquitoes that feed on humans blood (anthropophagy, anthrophily), and rest inside houses (endophagy, endophily) (Bhatt *et al.*, 2015, Stone and Gross, 2018). Specifically, these indoor interventions reduced feeding frequency, density, and survival of *Anopheles gambiae s.s.*(Russell *et al.*, 2011, Abreha *et al.*, 2014) and *Anopheles funestus (*Mutuku*et al.*, 2011). However, transmission risk and burden of malaria in Africa is yet much too high even in areas with high coverage of LLIN and IRS(Russell *et al.*, 2011, Taylor, 2018, Sherrard-smith *et al.*, 2019) because of feeding on animals and resting in cattle shelters (zoophagy, zoophily) (Mutuku *et al.*, 2011, Killeen, 2014, Massebo *et al.*, 2015, Charlwood *et al.*, 2018),

An. arabiensis continue transmitting malaria in East Africa (Sinka*et al.*, 2010, Emami *et al.*, 2017). They feed on cattle and remains to transmit malaria outside houses since cattle serve as adequate hosts when humans are unavailable, allowing vector populations to persist (Massebo *et al.*, 2015, Mwangangi *et al.*, 2013, Njenga *et al.*, 2016).

Therefore, a novel vector-control strategy capable of reducing the density and survival of outdoor biting, and zoophilic *An. arabiensis* mosquitoes are urgently required in Ethiopia to complement LLIN and IRS by controlling residual transmission of malaria. Thus, this study aimed to evaluate the effects of cattle blood treated with Ivermectin on survival, egg production and developmental stage of *An. arabiensis* under laboratory conditions for the possible elimination of malaria transmission by *An. arabiensis*.

METHODS

Adult Mosquitoes

The 3-5 days old adults *An. Arabiensis* for the test were obtained from the insectary of Tropical and Infectious Diseases Research Center (TIDRC) of Jimma University.

preparation of Ivermectin solution

An Ivermectin with doses equal to 0ng/mL (control), 5ng/ml, 10ng/ml, 20ng/ml, 40ng/ml and 80ng/ml were used for the experiment. These concentrations of Ivermectin were prepared from Ivermectin solution of 10 mg/ml that was commercially available, which received from Jimma University Veterinary clinic Type B

Mosquito blood-feeding

Each of the cattle blood samples with Ivermectin was presented to 50 female *An. Arabiensis* mosquitoes of 3–5 day-olds. The mosquitoes were starved of sugar solution for 24hrs before blood feeding. They were allowed to feed using an artificial membrane feeder which maintained at the temperature of 37 ^{0}C. The mosquitoes were transferred to paper cups from 30cm×30cm×30cm cages (Fritz *et al.*, 2009, Yamada *et al.*, 2013) thirty minutes before starting blood feeding and each blood sample was offered to mosquitoes for 30 min (Mekuriaw *et al.*, 2019). After blood-feeding was completed, unfed mosquitoes were removed from each cage using a mouth aspirator, and the total numbers of blood-feed mosquitoes were recorded and followed for evaluating the lethal and sub-lethal effects of Ivermectin on *An. arabiensis*. The whole experiment was repeated three times for accuracy of the finding

Mosquito mortality

The numbers of dead mosquitoes were recorded every 24 hrs. post blood feeding for 9 days(Fritz *et al.*, 2009) and dead mosquitoes were removed (Yamada *et al.*, 2013). Throughout the study, cages of adults were provided cotton moistened with 10% sugar solution. Mosquitoes were considered dead if they were lying on the bottom of the cage and unable to move. If a mosquito was unable to fly yet it was able to stand on its legs, it was considered alive and for survival data the mosquito existed after day 9 were considered as censored (WHO, 2018).

Egg production and developmental stages

Petri dishes lined with cotton batting, a filter paper, and moistened with 25ml deionized water were placed in each cage as an oviposition substrate (Mekuriaw*et al.*, 2019). The egg papers were replaced daily starting from 3 to 5 days post blood feeding (Chaccour *et al.,* 2010, Kisinza *et al*., 2015, Dreyer *et al*., 2018). The eggs laid were counted, recorded and placed in plastic pans filled distilled water at low density and the room temperature was maintained at 30 ^{0}C for hatchability test and from 2-4 days hatched larvae were counted and recorded (Pooda *et al.*, 2015, Dreyer *et al.*, 2018). Hatched larvae were supplied with instant dry yeast daily in larval pans and examined its development to pupae for 7days and then emerged Pupae were collected in cups and placed in 30 × 30 × 30 cm cages to examine its emergence to adults for 3 days (kobylinski, 2011, Pooda *et al.*, 2015). The eggs were counted under the dissecting microscope at an ocular magnification of 10x (Kobylinski, 2011).

Data Analysis

Data was recorded through appropriately designed forms, analysis was done by using Microsoft excel and SPSS version 22 software. Probit analysis was used to calculate LC50, 95 and 99s. Two independent sample T-test was used to compare mean feeding rate, mortality and Egg production of mosquitoes between treatment and control groups and One way ANOVA was used to compare mean mortality rate and egg production among the treatment groups. Descriptive statistics were used to calculate the hatching rates of eggs, emergence of pupae and adults. Survivorship of mosquitoes was analyzed using Kaplan–Meier survival curves and mean and median survival days were compared among treatment and control group by log Rank test. Cox regression was used to predict the proportional hazards probability. Statistical significance was assumed whenever p-values <0.05.

RESULTS

Effect of Ivermectin on *An. arabiensis* Mortality

The *An. arabiensis* morality was detected in all tests and mean mortality rate of *An. arabiensis* was higher at 80ng/ml. In all trials, mortality rate was significantly higher among the treatment groups compared to control (p≤0.001) (Table 3). According to One Way ANOVA and Post hoc multiple comparison test 9 days mean mortality rate among different the treatment groups were significantly different from each other(p <0.005).

The 9 days LC 50, 95 and 99 were 16.46 ng/ml, 75.7 ng/ml and 141.8 ng/ml respectively. 5 days and 7day LC 50 were 50.7 ng/ml and 27.6 ng/ml respectively.

Effect of Ivermectin on Egg production and hatchability of *An. arabiensis*

An. arabiensis produce egg only at test group one (5ng/ml) and two (10ng/ml) among the treatment groups and the egg produced at this concentration were statistically different were compared to the control (p=0.019 and 0.01 respectively). The mean number of egg laid were statistically different between the treatment group one and group two (p > 0.001) and there were no any laid egg at concentration of Ivermectin >10ng/ml. The hatchability rate was lower in the treatment compared to control and it's decreased with increasing in concentration

Effect of Ivermectin on emergence of pupae and Adult of An. arabiensis

Effect of Ivermectin on emergence of pupae and Adult of An. arabiensis

the emergence of pupae from larvae were 38% and 27.3% in group one & group two the treatment groups and it was smaller than their respective control group and the emergence of adult from pupae of *An. arabiensis* was 39.8% and 32.4% among the treatment group one and two respectively, which shows Adult emergence was decreasing in an increase concentration and its lower than their respective control

Effect of Ivermectin on Survivorship of *An. arabiensis*

Kaplan-Meier survival analysis curve shows the comparison between the treatment and control groups.

The mean survival time was 8, 7.5, 6.8, 5.7, 4.9 days for An. arabiensis those ingested 5, 10, 20, 40, and 80ng/ml respectively and it was significantly shorter compared to control (p<0.001). The median survival time also statistically shorter among treatment group compared to control (p<0.001).

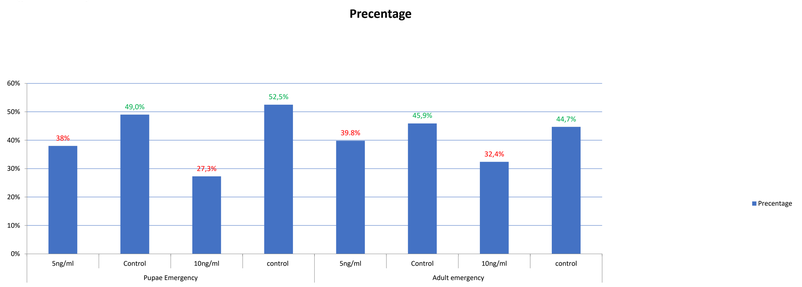

Figure 1 shows the emergence rate of pupae and Adults *of An. arabiensis* at Jimma University TIDRC from Apr-June 2020

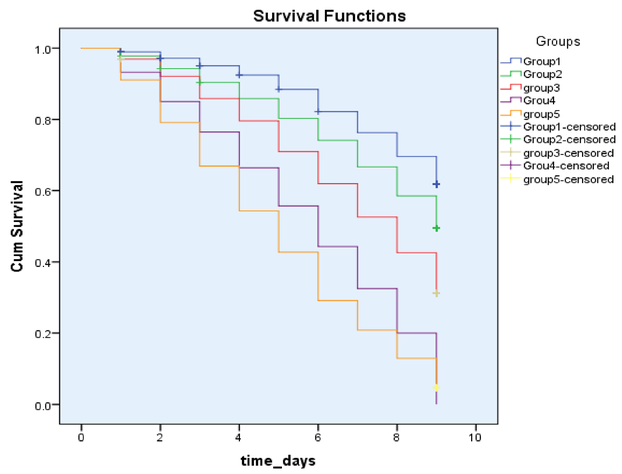

Figure 2 shows Kaplan–Meier survival curve of *An. arabiensis* during 9 days of follow-up of different concentration of Ivermectin (5, 10, 20, 40, and 80ng/ml)

CONCLUSIONS

Ivermectin treated cattle blood has an effects on survival, mortality, egg production and developmental stage of *An. arabiensis* under laboratory condition. At lower concentration Ivermectin could killed 50% of exposed group (feed blood contained Ivermectin) at 9 days post blood feeding. This provides evidence that treatment of cattle with Ivermectin can result an impact on malaria vector survival and reduce the number of infectious vector

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MORE INFORMATION / REFERENCES

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