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Effect of feeding of *Anopheles arabiensis* patton (Diptera: Culicidae) on ivermectin treated–rabbit blood

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Abstract
Anopheles mosquitoes (Diptera: Culicidae*)* are the main vectors of malaria. Innovative approaches are needed. The use of endectocides, *e.g.* ivermectin, could be a new addition of antimalarial measures. Some authors reported that *Anopheles* mosquitoes is particularly sensitive to very low concentrations of ivermectin relative to other vectors. The present work aimed to investigate the possibility of mortality of *An. arabiensis* adults after feeding on ivermectin-treated rabbits blood at different exposure periods and testing 3 doses (recommended =1ml/50kg body weight, 25% lower and 25% higher), determining the time required for mortality and to the effective dose. Mosquito were starved for 24 hr and fed on the rabbits. Rabbits were divided into group in 4 cages, 2 /cage one of the for the untreated control. To each cage 10 starved females were introduced and with the rabbits inside the cage for 1hr for feeding, then removed. The number of dead adults was recorded at 24, 48, 72, 96, 120 and 144 hr, and 2, 3 and 4 wk. Also, the Knock-down time (KdT) for the different treatments was calculated. The first part of the experiment (1st injection; phase 1) continued for 28 Days, followed by 21 days withdrawal, and the 2nd injection. The same parameters were taken. The experiment was repeated twice. The results showed that the recommended dose was more effective than the other 2 doses in terms of mortality rates during the 1st month, but in the 2nd month the 125% treatment resulted in better effect. However, it is concluded that ivermectin treatment on rabbits did not show promising effects.

Keywords: Rabbit, ivermectin, *Anopheles arabiensis*, the Sudan

1. Introduction
Ivermectin (endectocide) is used for treatment of many helminthic and ectoparasitic infections in animals and humans [1] and extensively used in the veterinary field. Its use in humans has recently increased in large programs to control lymphatic filariasis and onchocerciasis in endemic areas, specially in sub-Saharan Africa [2]. Due to its effectiveness, it has been referred to as a 'wonder drug' [3]. Ivermectin has anti-parasitic potentials, and broad -spectrum of activity against a wide- range of other invertebrates. Its effect in the environment, and on non-target aquatic and terrestrial organisms has been increasingly documented [4]. The drug is beneficial in Mass Drug Administration (MDA), including simultaneous curative effects on intestinal and skin parasitic infections [5]. Iakubovich *et al.* [6] used *An. stephensi* and fed it on ivermectin-treated rabbits. Their results revealed that death rates 4, 5 and 6 days after administration of ivermectin were 93, 70 and 79%, respectively. *In vitro* studies have shown that ivermectin also acts as an endectocide, causing the death of *Anopheles* that ingest sufficient doses in a blood meal [6, 7]. These results have also been confirmed in clinical studies using membrane [8] and direct-feeding [9] methodologies. Modeling based on these studies indicated that MDA with ivermectin has the potential to reduce malaria transmission [10, 11] mainly by negatively impacting mosquito survival [12]. Omura and Crump [13] stated that due to ivermectin therapeutic effectiveness and its broad-spectrum activity in controlling many tropical parasitic diseases, the scope and use of this drug may possibly expand in the near future.
An. gambiae adults were fed on 25 volunteers randomized to receive Ivermectin or nothing [14]. In mosquitoes feeding on volunteers given ivermectin the previous day, mean survival was 2.3 days, compared with 5.5 days in the control group. Mosquito mortality was 73%, 84%, and 89% on days 2, 3, and 4 in the ivermectin group.

After 14 days, no difference was found between groups. Ivermectin is safe and has significant short-term insecticidal properties.

The Solomon Islands the dominant vector is *Anopheles farauti* s. s. When administered to humans, ivermectin to control scabies, has been shown to have a mosquitocidal effect [15]. In this study they explored any incidental effect of ivermectin MDA conducted for scabies control on mosquitoes. here was a significant increase in 5-day mortality in anopheline mosquitoes caught post-MDA which was highest on the day of MDA itself (95%) and the following day (95%), compared to mosquitoes caught before MDA. This study showed a possible mosquitocidal effect of ivermectin MDA conducted for scabies control Malaria is a major public health problem in the Gezira state, central Sudan, and other states of the Sudan. *An. arabiensis* larvae and adults showed some levels of resistance to WHO recommended insecticides. The above-mentioned authors reported mortality of *Anopheles* adults after feeding on ivermectin- treated animals and human's blood. Those author concluded that the use of this drug is essential for interrupting mosquito life- cycle, and can be used as a new approach for mosquito control. The current study was designed to test the truth of this assumption by using the rabbits as a subject for feeding the *Anopheles* females.

2. Materials and Methods

2.1. Study design

This study was conducted as an experimental study in a completely randomized block (CRB) design males and females of rabbits of the same age and weight. Rabbits (Lagomorpha: Leporidae) [6] after treatment with the suggested doses (1ml/50 kg; 0.75 ml/ 50 kg and 1.25 ml/50 kg) were kept in cages under the conditions of rearing rabbits in houses and small farms. The rabbit is a standard laboratory animal in biomedical research [16].

2.2. Study area

The study was conducted in the laboratories of the Blue Nile National Institute for Communicable Diseases (BNNICD), University of Gezira (U of G), Wad Medani, Gezira State, Sudan.

2.3. Experimental procedure

The number of the rabbits used in this study was 4 males and 4 females. These 8 rabbits were divided into 4 groups (cages), *i.e.* 2/cage (1 male and 1 female), labeled with numbers indicating the treatment (doses). *Group I*: untreated control, *group II*: the recommended dose (RD 1ml/50kg) applied as subcutaneous (S.C.) [8]. Nichols. *et al* 1989); *group III*: 75% of the RD, and *group IV*: 125% of the RD. Rabbits were used to feed mosquitoes 10/ cage) for 1hr the removed. A total of 40 mosquitoes were divided into four groups, 10 individuals for each cage. The experiment started with the first injection, data collection for 4 wk, withdrawal period of 21 days, then the 2nd injection, followed by the same method of data collection. Mortality was recorded after 1, 2, 3, 4, 5, 6 and 24, 48, 72… and 144 hr n the first wk, the same for the rest of the 3 wk. After the withdrawal period, the 2nd injection was given to the treatments and the data was collected as above.

2.3.1 Data processing

Mortality was recorded as % then was corrected according to Abbott's formula [17, 18] (Abbott, 1925 and Finney, 1952).Corrected mortality% = (treatment% mortality – control% mortality) x 100 (100- control% mortality)

3. Results

3.1 First injection

3.1.1 First wk

None of the female mosquitoes fed on the rabbits blood treated with this drug died within the first 48 hr after feeding (Table 1). After 72 and 96 hr, the % mortality was 40% for each, when the rabbits were injected with 125% of the RD. However, for the rest of the first wk, no mortality was reported. On the other hand, when the rabbits were injected with 75% of the RD, 20% mortality was detected 144 hr after injection. No mortality was reported in the RD treatment.

3.1.2 Second wk

The RD did not show any mortality up to the end of the week. However, for the 75% and 125% treatments of the RD, 20% mortality was reported after 48hr. regarding the 75% treatment, no mortalities were detected up to the end of the 2nd wk. For the 125% treatment, 20 % mortality was found at 72 and 96hr; none was reported after that (Table 2)

3.1.3 Third wk

Twenty % mortality appeared after 24 hr in the RD and 125% treatments; no mortalities were found up to the end of the 3nd wk in the 125% treatments. Regarding the RD, 20% mortality was detected, but in the 75% treatments, 20% mortality occurred after48hr, 96hr and 120hr after treatment (Table 3).

3.1.4 Fourth wk

The 22nd day during 4th wk, after taking the 4th blood meal from the rabbits, the first impact of ivermectin appeared after 24 hr in the 125% treatment by killing 20% of the fed mosquitoes. The same mortality percentage appeared in the RD after 72 hr, none was reported after that (Table 4). However, no mortality in the 75% treatment up to the end of the 4th wk was detected. However, the 125%treatment caused 20% mortality after 96hr, and 60% after 120hr (Table 5).

Table 1: Percent mortality during 1st wk of the 1st injection.

Time after injection (hr)	Control% Mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1,2,3 and 6	0	0	0	0
24	20	0	0	0
48	0	0	0	0
72	0	0	0	40
96	0	0	0	40
120	0	0	0	0
144	20	0	20	0

Table 2: percent mortality during 2nd wk after the 1st injection.

Time of feeding (hr)	Control% Mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1, 2,3, 6 and 24	0	0	0	0
48	20	0	20	20
72	0	0	0	20
96	0	0	0	20
120	0	0	0	0
144	0	0	0	0

Table 3: Percent mortality during 3rd wk after the 1st injection.

Time after feeding (hr)	Control %mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1, 2,3, and 6	0	0	0	0
24	20	20	0	20
48	20	20	20	0
72	0	0	0	0
96	0	0	20	0
120	20	20	20	0
144	0	0	0	0

Table 4: Percent mortality during 4th wk after the 1st injection.

Time of feeding (hr)	Control % mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1,2,3 and 6	0	0	0	0
24	20	0	0	20
48	0	0	0	0
72	0	20	0	0
96	0	0	0	20
120	0	0	0	60
144	0	0	0	0

3.2 Second injection

3.2.1 First wk

According to table (5) 10% mortality was detected after 24 hr in the 75% treatment, however, after 48 hr and 72 hr in the 125% treatment , equal 20% , and 10% mortalities were detected, respectively, after 72 hr in the RD treatment the mortality was 20%,. However, 10% mortality after 96 hr and 120 hr in the RD was evident. Moreover, 20%, and 10% mortality occurred after 120 hr and 144 hr, respectively.

3.2.2 Second wk

After feeding on the 8th day from the 2nd injection, 10% mortality was detected after 6 hr in the RD and 125% treatments. After 24 hr, the mortality rate was 20% (RD), 10% (75% treatment), and 10% (for 125% treatment). After 48 hr, the respective doses caused 10%, 30%, and 10% mortalities, following the same order. No mortality was reported in the 75%treatment and 125% treatment up to the end of the wk, but 20% mortality in the RD occurred after 120 hr, and 144 hr (Table 6).

3.2.3. Third wk

At 15th day from the 2nd injection, the mortality was 10%, and 40% in the RD and 125% treatment, respectively, while no mortality appeared in the (75% treatment). After 120 hr from the injection 20% mortality was found at 72 hr and 96 hr, 10% mortality occurred in the (125% treatment) after 72 and 96 hr. The RD effected 20% mortality after 96 hr, and 10% after 120hr (Table 8).

3.2.4. Fourth wk

When the blood meal was taken on the 22nd day from the treatment; 20% mortality occurred after 24hr in the 75% treatment and 125%. After 48hr, 72hr and 120 hr, the RD effected10% mortality (Table 9). For 125% treatment, 10% mortality was registered after 48hr and 72hr. mortality was recorded (10%) at 72hr, and 144hr.

Table 5: Percent during 1st wk of the 2nd injection.

Time after feeding (hr)	Control % mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1,2,3 and 6	0	0	0	0
24	0	0	10	0
48	0	0	0	20
72	0	20	0	10
96	0	10	0	0
120	0	10	20	0
144	10	0	10	0

Table 6: Percent mortality during 2nd wk of the 2nd injection

Time after feeding (hr)	Control % mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1, 2 and 3	0	0	0	0
6	0	10	0	10
24	20	20	10	10
48	0	10	30	10
72	0	0	0	0
96	10	0	0	0
120	0	20	0	0
144	20	20	0	0

Table 7: Percent mortality during the 3rd wk of the 2nd injection.

Time after feeding (hr)	Control %mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1,2,3 and 6	0	0	0	0
24	0	10	0	40
48	0	0	0	0
72	0	0	0	10
96	10	20	0	10
120	10	10	20	0
144	10	0	0	0

Table 8: Percent mortality during 4th wk of the 2nd injection.

Time after feeding (hr)	Control %mortality	RD (%mortality)	75% of RD (%mortality)	125% of RD (%mortality)
1	0	0	0	0
2	10	0	0	0
3	0	0	0	0
6	0	0	0	0
24	10	0	20	20
48	10	10	0	10
72	10	10	10	10
96	0	0	0	0
120	0	10	0	0
144	20	0	10	0

4. Discussion

This piece of work was designed to investigate the possible effects, *viz*. mortality, of *An. Arabiensis* adult females after feeding on rabbit blood containing ivermectin at the RD, 75% lower than the RD and 125% of the RD, in addition to feeding at different intervals after 1st and 2nd injections. Several studies were conducted on human and different animals like dogs, mice, cattle, rats, Swiss mice, monkeys and rabbits using different methods and doses. Most of these studies calculated the mortality rate after 24 hr from treatment, but in the present study, mortality was calculated from 1 hr after the 1st injection up to 144 hr, then after 2,3 and 4wk. This was

Followed by a withdrawal period of 3 wk, then the same rabbits were subjected to the 2nd injection and another set of tests up to 4wk.

Regarding the rabbits, Iakubovich *et al.* [6] used ivermectin - treated rabbits. Their results for 4, 5 and 6 days after administration were 93, 70 and 79%, respectively. However, in the present study the mortality rate was taken after the first 24 hr up to 144 hr from the injection and within the first months. The rates were very low. This could be attributed to the species or strain differences, which might have different metabolic or physiological parameters or attributes. *In vitro* studies have shown that ivermectin also acts as an endectocide, causing the death of *Anopheles* mosquitoes that ingest sufficient doses in a blood meal [6, 7]. These results have also been confirmed in clinical studies using membrane [19] and direct-feeding [20] methodologies. Modeling based on these studies indicated that MDA with ivermectin has the potential to reduce malaria transmission [21] mainly by negatively impacting mosquito survival [22]. A study using *An. stephensi* [23], determined mortality rates of mosquitoes that took a blood meal on Swiss mice, Wistar rats and Cynomolgus monkeys that received oral doses of ivermectin (200-400 mg/kg), followed by feeding assays performed on 5 consecutive days after administration. Mortality was determined in the first 72 hr after feeding was 70-100% when mosquitoes fed on any of the animals 1-2 days after the last ivermectin administration. Jones *et al.* [24], used *An. quadrimaculatus* adults, fed with blood- on mixed breed dogs (orally fed) with the drug, after 4 hr from administration. Doses were 10, 500, 1,000, 2,500 mg/kg, in addition to untreated control. Moreover, females were fed on lambskin-membranes containing blood drawn from one dog in each treatment group. Data was collected at 24 and 48 hr post-feeding. Greater than 90% mortality was recorded in all treatment groups, except at the 24 hr post-feeding period at 10mg/kg dog, fed through the lambskin-membrane (65.4% mortality). The highest 2 doses resulted in 100% mortality at 48 hr post-feeding from either a dog or the *in vitro* system. From the 1 hr of the 1st injection in the present work, no mortality occurred; that is why it was calculated at 24, 48 and 72, etc. Mortality rate was highest in the 125% treatment, compared to the control.

The free-living population of *An. arabiensis* was allowed to forage on untreated or ivermectin-treated cattle in alternating nights [26]. Fresh blood- fed mosquitoes were collected in the morning and assessed for their blood meal digestion, egg-production, and survivorship. The residual activity of ivermectin-treated cattle was also determined by exposing mosquitoes to the same treatments after every 2 days up to day 21 post-treatments. The treated cattle reduced blood- meal digestion in the stomach of the mosquito, egg- production and survival over time. Blood- meal digestion was halved, egg-production was reduced up to 15 days. Survival was reduced 1–3 days compared o he untreated control. The daily mortality rates of mosquitoes fed on ivermectin-treated cattle increased by 5-fold relative to controls in the 1st wk, and it gradually declined up to 21st days after treatment

5. Conclusion

The findings showed that, in rabbits, under this experiment conditions, the results did not confirm what was reported by the others.

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