

The effect of cattle-administered ivermectin and fipronil on the mortality and fecundity of *Anopheles arabiensis* Patton.

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Research Article

Keywords: malaria, vector control, endectocides, ivermectin, fipronil, *Anopheles arabiensis*, cattle, livestock

DOI: <https://doi.org/10.21203/rs.3.rs-425743/v1>

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Abstract

Background

Malaria control primarily depends on two vector control strategies; indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLIN). Both IRS and LLIN target indoor-biting mosquitoes. However, some of the most important malaria vectors have developed resistance against the chemical compounds used in IRS and LLIN's. Insecticides-induced behavioural changes in vectors, such as increased outdoor feeding on cattle and other animals also limit the effectiveness of these strategies. Novel vector control strategies must therefore be found to complement IRS and LLIN's. A promising tool is the use of cattle-applied endectocides. Endectocides are broad-spectrum systemic drugs effective against a range of internal nematodes parasites and blood-feeding arthropods. The aim of this study was to investigate the effect of two endectocide drugs, ivermectin and fipronil, on the survival and fecundity of zoophilic *Anopheles arabiensis*.

Methods

Laboratory-reared mosquitoes were allowed to feed on cattle treated with either ivermectin (0.2 mg/kg), fipronil (1.0 mg/kg) or saline (Control) on day 0, 1, 4, 7, 13, 21 and 25 post-treatment and mortality and egg production were recorded daily.

Results

Compared to controls, the mortality of *An. arabiensis* increased 3.52 and 2.43 times with ivermectin and fipronil, respectively. The overall fecundity of mosquitoes that fed on both ivermectin- and fipronil-treated cattle was significantly reduced by up to 90% and 60%, respectively, compared to the control group. The effects of both drugs attenuated over a period of 3 weeks. Ivermectin was more effective than fipronil and increased mosquito mortality by a risk factor of 1.51 higher than fipronil. Similarly, both drugs significantly reduced the fecundity of *An. Arabiensis*.

Conclusions

This study demonstrated that ivermectin and fipronil are able to suppress *An. arabiensis* density and could help to reduce outdoor malaria transmission. The data from the current study as well as other similar studies suggest that current-generation endectocides have limited-duration action and are expensive. However, new-generation sustained-release formulations of ivermectin have multi-week high mortality impact on vector populations, thus holding promise of effective reduction of outdoor malaria transmission.

Background

Malaria is a preventable and treatable disease that infects millions of people globally every year. The World Health Organization (WHO) reported the global malaria burden for 2018 as an estimated 228 million cases associated with approximately 405 000 deaths [1]. This constitutes a significant increase from 219 million cases reported for 2017 [2]. Africa is the most malaria-burdened continent, accounting for 93% of the cases in 2018 [1]. Following notable declines in global malaria indices between years 2000 and 2015, these gains have reached a plateau and malaria cases have once again increased although the mortality has declined [1–3]. South Africa has shown similar trends, with significant decreases over the past two decades [4]. In the 1999/2000 malaria season, there were over 60 000 malaria cases that decreased to less than 13 000 in the 2013/2014 malaria season [5]. However, a steep increase in malaria cases and associated mortality in recent years (2017/18) has revealed the fragile nature of control efforts and the ease with which malaria can resurge [2, 3]. This multi-year trend of stagnating malaria control is an indication that the current malaria control strategies are no longer adequate and new or supplementary measures must be developed.

Human malaria is caused by five *Plasmodium* parasites which are transmitted by females of certain *Anopheles* mosquitoes [6]. In Africa, malaria vector species are mainly from two taxonomic clusters: the *Anopheles gambiae* complex and the *Anopheles funestus* group [7]. The most significant species from the *An. gambiae* complex are *An. arabiensis* and *An. gambiae*, and *An. funestus* from the *An. funestus* group [8, 9]. However, a number of other, less efficient, secondary vectors are also capable of transmitting malaria [10]. The *An. funestus* group is broadly distributed across Africa, with its major species, *An. funestus*, widely distributed over subtropical and tropical Africa where it breeds in permanent large water bodies with emergent vegetation [7, 11]. *Anopheles funestus* is highly anthropophilic (human biting) and exhibits endophilic (indoors) feeding and resting behaviours [7, 12]. The major malaria vector species from the *An. gambiae* complex, *An. arabiensis* and *An. gambiae* are widely distributed across Africa [13]. Members of the *An. gambiae* complex prefer to breed in temporary bodies of water that are clean and shallow [14, 15]. While *An. gambiae* is highly anthropophilic [12], *An. arabiensis* is zoophagic, feeding readily on animals in most areas, particularly cattle [15–17]. Studies have shown that *An. arabiensis* prefers to feed outdoors even in areas where it mostly feeds on humans [18, 19]. *Anopheles arabiensis* is therefore less impacted by indoor vector control strategies [16]. The different behaviours of the major malaria vectors makes it challenging to control malaria transmission [20]. In South Africa, *An. arabiensis* is widely acknowledged as the main vector although other species such as *An. merus*, *An. rivulorum* and *An. funestus* become locally important [21].

The primary control of malaria for decades in most malaria-endemic regions of the world has been the implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated bednets (LLIN) [22, 23]. LLIN's primarily utilise pyrethroid insecticides while a range of pyrethroids, organophosphates, carbamates as well as the organochloride DDT are applied to internal walls and ceilings of housing structures during IRS programmes [24]. Both pyrethroid insecticides and DDT have the same target on the voltage-gated sodium channel found on the mosquitoes' neurons therefore vectors become resistant to

both strategies [25]. Furthermore, these resistance alleles have spread at a rapid rate throughout Africa, requiring urgent action to prevent an increase in malaria [24, 26]. The increasing resistance developed within various *Anopheles* species to these insecticides poses a major challenge to the effectiveness of these key vector control methods [20, 24, 27].

Aside from escalating insecticide resistance affecting the value of IRS and LLIN's indoor interventions, additional challenges are emerging [28]. The major malaria vector species historically preferred feeding indoors [29, 30]. However, some recent studies have shown a shift in behaviours of *An. gambiae* and *An. funestus* to feeding outdoors instead of indoors in some areas where IRS and LLIN are implemented [29, 31]. Zoophilic characteristics in some of the major vectors are also a challenge [15]. Yet another challenge is a temporal shift in feeding behaviour with the malaria vector species biting in the early evenings and mornings when people are not under their protective nets [32, 33]. Given these limitations of IRS and LLIN to curb transmission, the challenge of residual malaria poses a serious hurdle in reaching malaria elimination objectives, and the number of malaria cases and deaths remains unacceptably high [34].

The use of cattle-administered endectocides is a promising strategy for outdoor vector control that could complement IRS and LLINs [35, 36]. Several endectocide drugs are effective against a wide range of both endo- and ectoparasitic nematodes and arthropods in humans and cattle [20, 35]. These drugs include ivermectin, eprinomectin, fipronil and diflubenzuron [37]. Ivermectin was the first endectocide to be used in humans and continues to be used to treat river blindness through mass drug administration (MDA) [36, 38]. Ivermectin is a lipophilic drug belonging to the avermectin class of macrocyclic compounds [39] and is also used to treat onchocerciasis, strongyloidiasis, lymphatic filariasis, scabies and head lice [20, 38]. Endectocides are also of veterinary importance as they are used to control parasites in animals such as cattle and goats [40].

Endectocides utilize a different mode of action against insects to that of IRS and LLIN [35] and can thus complement traditional control measures. In the vector, ivermectin primarily targets the glutamate gated chloride channels, which are neurotransmission inhibitors through their 16-membered macrocyclic lactone [20, 45–47]. Binding to the channels leads to an influx in chloride ions leading to neuromuscular junction and hyperpolarization [48]. In contrast, fipronil is a phenylpyrazole compound that works by blocking the GABA-gated ion channels, which are also in the central nervous system of arthropods [48–50]. Both ivermectin and fipronil result in flaccid paralysis and eventually death in the target parasites [20, 49]. These chloride gated ion channels are not present in vertebrates, therefore, ivermectin and fipronil are non-toxic to humans and livestock [51]. Ivermectin MDA in humans is a promising malaria control tool that targets mosquitoes with control-avoidance biting behaviours and those that have developed physiological insecticide resistance [35, 47]. Several studies have shown that ivermectin-treated human blood decreases survival, feeding frequency, blood meal digestion and fecundity of mosquitoes [52–54]. Ivermectin (brand name Mectizan®) has been used successfully in humans for river blindness since 1987 and does not have toxic side effects at recommended doses [55, 56]. Several studies have shown that ivermectin and several other endectocides applied to cattle or other livestock also decrease the

survival and fecundity of malaria vector mosquitoes [16, 35, 51, 57]. Fipronil has been approved for use on domestic animals in many countries and is used to control arthropods such as ticks, cockroaches and fleas [58]. In addition, fipronil has been used in cattle to control leishmaniasis vectors [59, 60]. Similarly, fipronil is effective against all life stages of *Anopheles* mosquitoes [16]. However, field studies on its use against mosquitoes are limited.

The effectiveness of endectocides is linked with their pharmacokinetics, which vary across different species [41]. The route of administration also has a significant effect on the pharmacokinetics of endectocides. In previous studies, ivermectin pharmacokinetics studies were conducted in cattle to compare subcutaneous and oral routes of administration [16, 42]. Ivermectin injected subcutaneously in cattle was effective against *An. arabiensis* mosquitoes for longer than oral or topical treatment [16]. Higher ivermectin plasma concentrations were produced with subcutaneous treatment than with oral administration. High concentrations produce an enhanced systemic availability which results in higher efficacy against the targeted parasites [42]. For formulations typically used in recent years, the maximum concentration of subcutaneously injected ivermectin was reached at day 1 while the minimum was reached after 25 days [42]. Similarly, fipronil injected in cattle reached its maximum and minimum concentrations rapidly within 24 hours [43]. In a study where the pour-on fipronil was administered in cattle to investigate its effect against ticks, the mean plasma concentration values over time varied and maximum concentration was 73.7 g/L reached after 2.5 days [44]. The pour-on fipronil concentration reached its half-life at day 19 and decreased slowly until minimal level at day 40 [44]. Topical treatment results in exposure to environmental degradation such as mechanical removal by rain (Cid *et al.* 2016). Factors such as body weight, nutrition type and physiological status also lead to variation in drug concentrations within individuals of the same species [41].

Currently there are no studies investigating the impact of cattle-administered endectocides on mosquitoes in South Africa. Studies that have conducted this type of research in other countries have mostly focused on ivermectin only. The present study included an additional potential endectocide, fipronil. This study also considered the pharmacokinetics profiles of the two drugs and conducted feeding trials at various points including at the minimum and maximum concentrations. The aim of this study was to investigate the effectiveness of two endectocides, namely: ivermectin and fipronil for control of *An. arabiensis* in South Africa. The specific objectives were: 1) to demonstrate that ivermectin and fipronil reduce adult survival and fecundity of *An. arabiensis*, 2) to compare the efficacy of ivermectin against that of fipronil and 3) to assess the duration efficacy of each endectocide. We predicted that endectocides-treatment would result in a significant increased mortality of *An. arabiensis* and a reduction in the egg production. We also predicted that injected ivermectin would be more effective than pour-on fipronil. We further predicted that the efficacy of both endectocides would last for a month based on the manufacturer's instructions on their duration effect against other parasites and data from previous related studies.

Methods

Insectary-rearing *An. arabiensis* mosquitoes

Anopheles arabiensis eggs were obtained from the Vector Control Laboratory of the South African National Institute for Communicable Diseases in Johannesburg, South Africa. Colonies of *An. arabiensis* were established and maintained in an insectary at the University of Pretoria, Faculty of Health Sciences. The insectary is kept at a constant temperature of $25 \pm 2^\circ\text{C}$, $75 \pm 5\%$ humidity and has a 12 hours light: 12 hours darkness photoperiod. Eggs were placed in containers (2–5 litres) filled with water and the larvae fed a mixture of powdered dog biscuits and yeast mixture at a ratio of 75/25. Growing larvae were subdivided into separate containers with water and allowed to mature. Mesh-netting covers over the larval basins prevented emerging adults from escaping. A small slit was cut into each cover to allow access for suction capture of adults daily. A mouth aspirator was used to transfer emerged adults into bucket-cages (5–20 litres). A circular hole was made on the side of each bucket and fitted with a netting sleeve to allow transfer of adult mosquitoes with a mouth aspirator and enable regular replacement of sugar water. Adult mosquitoes were provided permanent access to a 10% sugar solution by way of soaked cotton wool in a small plastic container. Male and female mosquitoes were kept together in these buckets for reproduction, and sample specimens were removed periodically for experimental purposes as required. For egg production, female mosquitoes were provided with a bloodmeal three times a week. Bloodmeals were provided by human volunteers placing their exposed arms against the netting at the top of the lid, mosquitoes then feeding through the netting. All mosquitoes in the colony were maintained in conditions that do not enable contamination with malaria or other parasites that could infect humans. There was therefore no risk of disease transmission to or between humans. For collection of eggs, small plastic trays with water were placed on the floor of the mosquito bucket-cages containing adults. Adult *An. arabiensis* were harvested from this colony for use in the cattle-feeding experiments and were generally obtained two to five days post-emergence from pupae. Adults that had been fed on treated and control cattle were treated as described below.

Cattle treatment

Six cattle (Pinzyls strain, Nguni crossbreed with Pinzgauer) were housed and cared for at the Experimental farm of the University of Pretoria, in Pretoria. This is a cross-breed of the dominant cattle breed, Nguni, in South Africa's malaria endemic areas [61]. In this facility, animals are kept outdoors in groups, where they graze freely and have permanent unimpeded access to water and shade. When required for experimental purposes, the animals are restrained in crushes (Fig. 1.1 A) after which they are returned to their paddocks. None of these had been treated with any insecticides or acaricides for at least three months prior to the commencement of the experiments described in this paper. The weight of each experimental animal was determined (Fig. 1.1 A) before the cattle were treated. Cattle weights ranged from 570 kg to 793 kg (see appendix). Two endectocide drugs; 1% ivermectin (Noromectin®, Norbrook Laboratories, Centurion, South Africa) and 0.9% fipronil (Attila®, Ascendis Health, Sandton, South Africa) were used. We were unable to obtain injectable fipronil and hence opted for the pour-on form. Two of the cattle were treated with ivermectin (0.2 mg/kg, subcutaneous injection), two with fipronil (1.0 mg/kg,

pour-on) while the other two animals served as control (saline, subcutaneous injection and pour-on) with all six cattle receiving each of the treatments. Ivermectin and fipronil were administered at the manufacturer's recommended dosages and applications. Fipronil was sprayed in two lines on both sides of the spinal cord from the base of the head to the tail root. Experiments were conducted in three replicates spaced one month apart to allow drugs to be eliminated from the cattle or to decline to undetectable levels before the next trial. The order in which individuals received the treatments was randomized.

Mosquito Bioassays

Mosquito blood-feeding bioassays were conducted at day 0, 1, 4, 7, 13, 21 and 25 post-treatments. The days were chosen based on the pharmacokinetic profiles of injectable ivermectin and pour-on fipronil in cattle [42, 44]. Day 0 post-treatment represents the day when the experimental cattle individuals were treated, the feeding experiments were initiated 2–3 hours after treatment. The days were standardized for pour-on fipronil to be the same as for ivermectin for comparative purposes. All mosquito exposure experiments were performed in duplicate for every individual cattle. Two circular areas, slightly larger than the size of a paper cup opening (70 mm), were shaved on all experimental cattle individuals on the upper back. A mouth aspirator was used to transfer female mosquitoes (n = 30 mosquitoes per cup) to polystyrene cups (25 ml) covered by netting (Fig. 1.1 B). Female mosquitoes were distinguished from the males by visual observations. The cups with mosquitoes were exposed to the shaved spots of cattle individuals for 15 minutes to allow adequate time for feeding (Fig. 1.1 C). Different cups with new batches of mosquitoes were used for each feeding experiment.

After the feeding experiments on each day post-treatment, unfed mosquitoes were separated from the blood-fed specimens using a mouth aspirator after visual inspection. The abdomen of a blood-fed mosquito becomes distended and red-coloured for several hours after feeding. Unfed mosquitoes were excluded from the experiments. Blood-fed mosquitoes were kept in polystyrene cups covered by netting for observation in the insectary and provided with a permanent access to a 10% sugar source. Dead mosquitoes were counted and mortality was recorded once a day for successive days until the death of all mosquitoes from each cup.

Egg production

Additional *An. arabiensis* mosquitoes were placed in six polystyrene cups (n = 10 mosquitoes per cup) and each cup was assigned and coded to a particular cattle individual for blood-feeding. The mosquitoes for fecundity analysis fed from the cattle after the mosquitoes for survival analysis. The blood-fed mosquitoes were placed individually into separate glass vials for egg production. Wet filter paper was placed at the bottom of each vial to encourage mosquitoes to lay eggs. The glass vials were covered with netting and each mosquito was provided with a 10% sugar source. Mosquitoes were monitored for egg production on a daily basis. Filter papers in glass vials with mosquitoes that had laid eggs were placed under a dissecting microscope (Olympus SZ51, Olympus Corporation, Tokyo, Japan) and the eggs counted daily from the first day the mosquito laid the eggs until her death. Eggs were destroyed after

being counted to avoid double-counting. The proportion of *An. arabiensis* mosquitoes that laid eggs were used to determine the mean number of eggs for the three treatments.

Statistical Analysis

A non-parametric Kruskal Wallis test was used to compare the proportion of mosquitoes that fed between treatments (control, ivermectin- or fipronil-treated). The survival data was not normally distributed (Kolmogorov-Smirnov test: 0.102, $df = 252$, $p < 0.001$). The effects of treatment (control, ivermectin or fipronil) on the survival of *An. arabiensis* were determined using the Cox Proportional Hazard Model (coxph) package in the statistical software R version 3.6.1. The coxph model is a semiparametric model that uses the hazard ratio to measure the risks between the treatments and control by comparing the survival curves. The variables; treatments, days post-treatment (i.e. 0, 1, 4, 7, 13, 21 and 25) and days post-feeding (i.e. from day 1 until all mosquitoes died) as well as all 2- and 3-way interactions were included as independent variables. The mortality of mosquitoes was the dependant variable. The cattle identity and replicate number (i.e. first, second or third) were included as random effects. The Kruskal Wallis test was further used to investigate whether treatment had a significant effect on the proportion of mosquitoes that laid eggs. The data of the *An. arabiensis* egg production was not normally distributed (Kolmogorov-Smirnov test: 0.229, $df = 197$, $p < 0.001$). Thus, analysis was conducted using generalized linear mixed model (GLMM) with a Poisson distribution that uses the log-link function to test the effect of treatment on the egg production of *An. arabiensis*. The number of eggs produced was the dependant variable while the treatments, days post-treatment and days post-feeding were the independent variables. The effect of treatment on the proportion of mosquitoes that laid eggs was also evaluated. This analysis was carried out using IBM SPSS version 25. Results for both the survival and egg production sections are reported as means \pm SE.

Ethical clearance

Ethical clearance for use of cattle was obtained from the University of Pretoria Animal Ethics Review Committee (Ethics reference number: EC063-18, 180000035).

Results

Survival

Analysis to investigate the effects of ivermectin and fipronil on *An. arabiensis* survival was conducted on a total of 4940 mosquitoes blood-fed from the experimental cattle. Treatment did not significantly affect the proportion of mosquitoes that fed on cattle for the three treatments ($H = 5.04$, $df = 2$, $p = 0.08$). The overall proportion of mosquitoes that fed was 65%, 64% and 67% for ivermectin, fipronil and control, respectively. The Cox Proportional hazard model showed that treatment ($X^2 = 1182$, $df = 2$, $p < 0.001$) had significant effect on the survival of *An. arabiensis*. The mortality of *An. arabiensis* was 3.52 higher for ivermectin ($X^2 = 1082$, $df = 1$, $p < 0.001$) and 2.43 higher for fipronil ($X^2 = 578.4$, $df = 1$, $p < 0.001$) compared

to the control group, respectively. Ivermectin increased the mosquito mortality by a risk factor of 1.51 compared to fipronil ($\chi^2 = 133.7$, $df = 1$, $p < 0.001$). The day post-treatment ($\chi^2 = 196.1$, $df = 6$, $p < 0.001$) had significant effect on the mortality of *An. arabiensis*. Post-hoc comparisons showed significant effects at day 7, 13 and 21 post-treatments where the effects were 0.729, 0.769 and 0.762 higher than at day 0 post-treatment, respectively ($\chi^2 = 2746$, $df = 20$, $p < 0.001$). Furthermore, the interaction between treatment and day's post-treatments also significantly affected survival ($\chi^2 = 2746$, $df = 12$, $p < 0.001$).

Table 1.1
Hazard ratios and their 95% confident intervals comparing the risks between the treatments (ivermectin and fipronil) and control at different days post-treatments.

Days post-treatment	Treatment	Hazard ratio: exp (coeff) \pm se	Lower, upper 0.95 CI
0	Ivermectin	1.22 \pm 0.09	1.02, 1.46
	Fipronil	1.06 \pm 0.09	0.88, 1.27
1	Ivermectin	14.80 \pm 0.13	11.47, 19.09
	Fipronil	2.02 \pm 0.10	1.67, 2.45
4	Ivermectin	18.49 \pm 0.16	13.64, 25.07
	Fipronil	10.87 \pm 0.15	8.11, 14.56
7	Ivermectin	11.52 \pm 0.13	8.94, 14.85
	Fipronil	4.17 \pm 0.11	3.37, 5.17
13	Ivermectin	7.82 \pm 0.12	6.16, 9.91
	Fipronil	3.82 \pm 0.11	3.08, 4.76
21	Ivermectin	4.67 \pm 0.11	3.76, 5.79
	Fipronil	3.39 \pm 0.10	2.78, 4.15
25	Ivermectin	2.26 \pm 0.11	1.83, 2.79
	Fipronil	2.59 \pm 0.11	2.09, 3.21

Treatment had no significant effect on the survival of *An. arabiensis* on day 0 post-treatment ($\chi^2 = 4.84$, $df = 2$, $p = 0.09$, Fig. 1.2 a). The effect of treatment was significant from day 1 post-treatment ($\chi^2 = 439.8$, $df = 2$, $p < 0.001$, Fig. 1.2 B). Ivermectin reduced the survival of *An. arabiensis* to a greater extent than fipronil from day 1 until day 21 post-treatment (Fig. 1.2 B – Fig. 1.2 F, Table 1.2). The mortality risk of *An. arabiensis* mosquitoes was highest for both ivermectin and fipronil at day 4 post-treatment with hazard ratio of 18.49 \pm 0.13 and 10.87 \pm 0.15, respectively (Fig. 1.2 C, Table 1.1). At Day 4 post-treatment, the mortality of mosquitoes that fed on ivermectin-treated cattle was up to 60% higher than those that fed on the control group 4 days after exposure and achieved a 100% mortality within 8 days. The mortality of

mosquitoes that fed on fipronil-treated cattle reached 60% within 6 days and 100% within 10 days. From day 7 post-treatment, the mortality of *An. arabiensis* that fed from the treated cattle gradually decreased and the hazards ratio was 4.67 ± 0.11 and 3.39 ± 0.11 at day 21 post-treatment for ivermectin and fipronil, respectively (Table 1.1). Although the treatment effect had gradually decreased at day 25 post-treatment, it was still significant ($\chi^2 = 75.68$, $df = 2$, $p < 0.001$) with 20% or fewer differences between treatment and control.

Egg production

Analysis for egg production was conducted on a total of 198 *An. arabiensis* mosquitoes that blood-fed from control, fipronil and ivermectin-treated cattle. The proportion of mosquitoes that laid eggs was not significantly affected by treatment ($H = 4.268$, $df = 2$, $p = 0.118$). It was 46.67%, 57.71% and 65.17% for ivermectin, fipronil and control, respectively. The GLMM showed that treatment had a significant effect on the number of eggs laid ($F = 49.98$, $df = 2$, $p < 0.001$). Post-hoc comparisons showed that after feeding on both ivermectin (7.53 ± 0.96 eggs, $t = 6.835$, $df = 102$, $p < 0.001$) and fipronil (12.46 ± 1.54 eggs, $t = 5.798$, $df = 102$, $p < 0.001$) treated cattle, the number of eggs laid was significantly lower compared to the control group (29.88 ± 1.85 eggs, Fig. 1.3). There was no significant difference between the number of eggs laid by mosquitoes that fed on ivermectin and fipronil-treated cattle ($t = 1.749$, $df = 102$, $p = 0.083$). Overall, days post-treatment did not significantly affect the number of eggs produced ($F = 1.75$, $df = 6$, $p = 0.118$). However, the interaction between treatment and days-post treatment was significant ($F = 2.59$, $df = 12$, $p = 0.005$).

The significant effect of treatment on the number of eggs laid was persistent throughout the days post-treatment from day 0 until day 25. At day 4 post-treatment, there was a significant effect for all comparisons amongst the three groups; ivermectin vs control ($t = 4.10$, $df = 102$, $p < 0.001$), ivermectin vs fipronil ($t = 2.15$, $df = 102$, $p = 0.034$) and fipronil vs control ($t = 2.50$, $df = 102$, $p = 0.014$). Both drugs significantly affected the number of eggs laid at day 7 (ivermectin: $t = 3.08$, $df = 102$, $p = 0.003$, fipronil: $F = 2.94$, $df = 102$, $p = 0.004$) and day 13 (ivermectin: $t = 3.37$, $df = 102$, $p = 0.001$, fipronil: $t = 2.48$, $df = 102$, $p = 0.015$) post-treatment (Fig. 1.4). All post-hoc comparisons for day 21; ivermectin vs control ($t = 4.30$, $df = 102$, $p < 0.001$), ivermectin vs fipronil ($t = 3.30$, $df = 102$, $p = 0.001$) and fipronil vs control ($t = 2.04$, $df = 102$, $p = 0.044$) were significant. The analysis for day 25 post-treatment showed a significant effect for ivermectin vs control ($t = 3.45$, $df = 102$, $p = 0.001$) and fipronil vs control ($t = 2.91$, $df = 102$, $p = 0.005$) but no significant effect between ivermectin and fipronil ($t = 0.72$, $df = 102$, $p = 0.471$) (Fig. 1.4).

Ivermectin led to a reduction in egg production by up to $\pm 90\%$ while fipronil decreased the egg production by up to $\pm 60\%$ (Fig. 1.3). Days post-treatments contrasts showed significant differences in the overall number of eggs laid between day 4 and 25 ($t = 2.405$, $df = 90$, $p = 0.018$) and between day 7 and 25 ($t = 2.709$, $df = 90$, $p = 0.008$) post-treatments (Fig. 1.4). At day 21 post-treatment, both ivermectin and fipronil were still highly effective ($F = 9.70$, $df = 2$, $p < 0.008$, Fig. 1.4). The post-hoc comparisons for interactions between treatment and days post-treatments showed significant effects at day 1 ($t = 2.396$, $p = 0.019$)

and 21 ($t = 2.331, p = 0.022$) (Fig. 1.4). The effect of the treatment was still significant at day 25 post-treatment and reduced egg production by more than 50% ($F = 12.50, df = 2, p < 0.002$, Fig. 1.4).

Discussion

In the current study, the effect of two endectocides (ivermectin and fipronil) on the survival and fecundity of *An. arabiensis* was investigated. In accordance with the first prediction, this study demonstrated that both ivermectin and fipronil are able to reduce the survival of *An. arabiensis*. Mortality was increased 77% and 70% times higher with ivermectin and fipronil, respectively, compared to the control group. Mosquitoes that did survive the treatment furthermore exhibited a significantly reduced fecundity by up to 90% and 60%, for ivermectin and fipronil, respectively. The results for the effect of ivermectin on the survival of *An. arabiensis* found in this study are comparable to results reported for this species in other studies. Pooda *et al.* 2015 reported a mortality reduction by 75% in the third week and 45% in the fourth week [20]. In a study by Lyimo *et al.* 2017, survival and fecundity of *An. arabiensis* were reduced by 52.5% and 64.6%, respectively [35]. Both Lyimo *et al.* 2017 and Pooda *et al.* 2015 used the same subcutaneous injection administration method for ivermectin and at the same concentration to treat cattle in a semi-field setting as used in the present study. Although Pooda *et al.* 2015 used a sibling species of *An. arabiensis* (*Anopheles coluzzii*), the results for mortality are still comparable. Ivermectin was also found to be effective in other livestock such as pigs [62]. Pasay *et al.* 2019 treated pigs with ivermectin to investigate its effect on *Anopheles farauti* survival and fecundity which were reduced by 75% and 50%, respectively [62]. In comparison to ivermectin, research into the use of fipronil for malaria control is limited. Dreyer *et al.* 2019 investigated the impact of topical fipronil and injectable ivermectin on the survival of *An. albimanus* and reported opposite results from the present study (Dreyer *et al.* 2019). In the study, fipronil was found to be more effective than ivermectin at day 2, 5 and 7 as it killed the *An. albimanus* at faster speed than ivermectin (Dreyer *et al.* 2019). The observed opposite effects between Dreyer *et al.* 2019 and the present study suggest that the two drugs seem to affect the two mosquito species differently. Poché *et al.* 2017 investigated the efficacy of oral fipronil against *An. arabiensis* in cattle and found that the mosquito indoor resting density (number of mosquitoes resting on walls and other surfaces inside houses) was reduced by 89% [49]. Data on the overall effect of cattle- or livestock-administered fipronil on *Anopheles* mosquito's fecundity could not be obtained from the literature. To the best of our knowledge, the present study is the first to measure the effect of fipronil on fecundity. Fipronil significantly reduced *An. arabiensis* fecundity and this is comparable to the effects of other endectocides, ivermectin in particular, on *An. arabiensis* or other vectors.

As predicted for the second objective, injected ivermectin was more effective than the topical fipronil. The ivermectin treatment suppressed the survival of *An. arabiensis* quicker and this effect lasted for longer than the fipronil treatment although both chemicals were similarly effective during most of the measurement. As mentioned earlier, subcutaneous injections seem to yield better results than the topical or pour-on treatment. The different administration methods for ivermectin and fipronil are a limitation in this study, as a result, we could not distinguish between the effects of the drug itself and the effect of the application methods. The application methods differ in their efficacy, injected fipronil might produce

better results. However, in a malaria endemic region, with higher numbers of livestock that would require treatment, the pour-on method could be a better option. The results in this study suggest that different routes of administration should be considered and additional endectocides rather than just injectable ivermectin could also have the potential to be added into the malaria control toolbox. Oral [16, 49] and pour-on [48] fipronil have been found to be effective against malaria vectors (Table I). In general, subcutaneous treatment has been shown to be more effective than oral and topical applications. Subcutaneous injection leads to a higher distribution of drugs and increases their duration of residence in the lipids [20, 63]. Although injectable endectocides would be preferable due to their quick absorption, they are more costly to administer because they require needles and an experienced person [44]. The uptake of topical fipronil has been investigated previously by Cochet *et al.* 1997. Similar to what was observed in this study, they reported a delay that fipronil is not immediately absorbed from the site of application but translocates dermally and gets confined in sebaceous glands and lipids of hair follicles [64]. This might be the reason fipronil showed its effectivity at a later stage than the injected ivermectin.

The reduced-survival effect of both endectocides on mosquitoes lasted for a period of about 3 weeks, which was a week less than we had predicted for the third objective. The largest effect was at day 4 post-treatment for ivermectin and at day 7 post-treatment for fipronil. Ivermectin showed its efficacy from day 1 post-treatment while the fipronil only started being effective from day 4 post-treatment. Surprisingly, treatment effects on egg production were already apparent at day 0 post-treatment as opposed to effects on survival, this may be attributable to the time delay in feeding of mosquito batches used for survival and egg production. Although the effects of both endectocides had significantly decreased at day 25 post-treatment, fipronil had a larger effect on the mosquito survival than ivermectin at this day post-treatment. The mosquitoes that were used for egg production analysis were fed later after the ones used for survival analysis. The additional time elapsed between feeding of mosquitoes for survival and fecundity was different between the groups and could be one possible reason for the observed differences. The drugs might also have a greater and faster physiological effect on the fecundity of *An. arabiensis* than the survival, which might not be surprising since the nutrients from the blood meals are incorporated into the eggs. There are many factors that affect the fecundity of mosquitoes such as blood-meal source and size [65, 66]. Some control mosquitoes laid relatively small numbers of eggs compared to the others, resulting in a lower than the expected mean number of eggs for the control group. It has been previously observed that in the field, some *Anopheles* mosquitoes require multiple blood-meals to produce larger batches of eggs [67]. The effect of both ivermectin and fipronil on fecundity was persistent over the 25 days period which could be an advantage for malaria control. Unlike the present study, most studies that conducted similar type of research did not investigate the effect of endectocides on fecundity at all the days post-treatment [16, 20]. The differences in duration effect between the present study and the others might be due to various factors such as the strains of the mosquito vector and the cattle breed. The different strains of the *Anopheles* mosquitoes will develop resistance to endectocides in different ways [48, 68], this should be considered before implementation of the strategy.

The short duration effect of current-generation drugs has been noted previously and this question the validity of cattle-administered endectocides for malaria control and thus, modifications might be necessary. Ivermectin was effective for a longer period in a study where slow-release ivermectin implants formulations were used [57]. In this study, *An. arabiensis* fed from cattle that had received subcutaneous high dose ivermectin from slow-release implants. These implants significantly reduced mortality for up to 40 weeks compared to the control group [57, 69]. Ivermectin implants in livestock could serve as a long-lasting malaria outdoor strategy. However, it will be crucial to also determine their safety, cost and practicality of use in malaria endemic regions. The use of long-lasting endectocides in livestock, particularly cattle, could enhance agricultural production and lead to food security [70]. However, the overuse of endectocides may also lead to resistance in cattle and parasites, hence be more detrimental than beneficial. Worms, ticks and other parasites that are the main cause for disease and productivity loss in livestock [70]. The safety of cattle meat consumption should also be considered. Cattle treated with ivermectin and fipronil should not be slaughtered for human consumption within 28 and 105 days of treatment, respectively [71, 72]. Furthermore, ivermectin should not be administered in lactating cattle if the milk products are used for human consumption [71]. It is crucial for most endectocides to be administered at their safe doses and not frequently as this may lead to poor effects and sometimes to the death of the animals [73]. The use of IRS and LLINs in areas dominated by zoophagic vectors will not aid in malaria elimination. An integrated approach whereby various strategies are implemented for malaria control is required if malaria is to be eliminated [74, 75].

Conclusion

This study shows that *An. arabiensis* mosquitoes exhibit increased mortality and reduced fecundity after feeding on cattle treated with ivermectin or fipronil at their manufacturer's recommended dosages. Ivermectin used in this study proved to be a more effective endectocide than fipronil, possibly as a result of difference in their application method. Both endectocides were only effective for a period of up to three weeks. This limited period of efficacy has resulted in some doubts in literature about the practical value of the method. However, given the current lack of effective vector control tools against outdoor-biting mosquitoes, this method may still provide a realistic option for significantly impacting outdoor-biting vectors given the large number of mosquitoes known to feed on cattle at night. More advanced forms of endectocides administration to cattle, such as a slow-release formulation, could lead to higher and prolonged concentrations of endectocides in the blood. In addition, strategic use of these endectocides at the beginning of the malaria season when vector populations are low may have a significant impact on malaria incidence.

Declarations

Ethics Approval

Ethical clearance for use of cattle was obtained from the University of Pretoria Animals Ethics Review Committee (Ethics reference number: EC063-18, 180000035).

Consent for publication

Not applicable.

Availability of data and materials

The datasets that were used are available and can be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

TM was the recipient of a Dr Sylvia Meek scholarship from the Malaria Consortium, which enabled this study. We also acknowledge the financial support received from the National Research Foundation of South Africa.

Authors' contributions

TM, LB and HL designed the study. TM conducted the field, insectary and laboratory experiments. TM, LB and HL wrote and revised the paper. All authors approved the final manuscript.

Acknowledgements

We would like to thank Corlia Swanepoel and the Diary Unit team at the Experimental Farm for assisting with the animal experiments. We also thank Cyril Ndongane, the insectary manager, who assisted with the mosquitoes' colony rearing as well as the animal experiments. The University of Pretoria Institute for Sustainable Malaria Control (UP ISMC, MRC-collaborating Centre) is acknowledged for providing its insectary for rearing mosquitoes and for the experimental facilities.

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Note

The Appendix and Table 1.2 are not available with this version

Figures



Figure 1

1 An overview of the mosquito blood-feeding experiments; A) Cattle were kept in crushes and their weights were measured before treatment, B) Duplicate cups with 30 mosquitoes each covered by netting and C) Mosquitoes were blood-fed by applying the cups against the cattle on the shaved spots for 15 minutes.

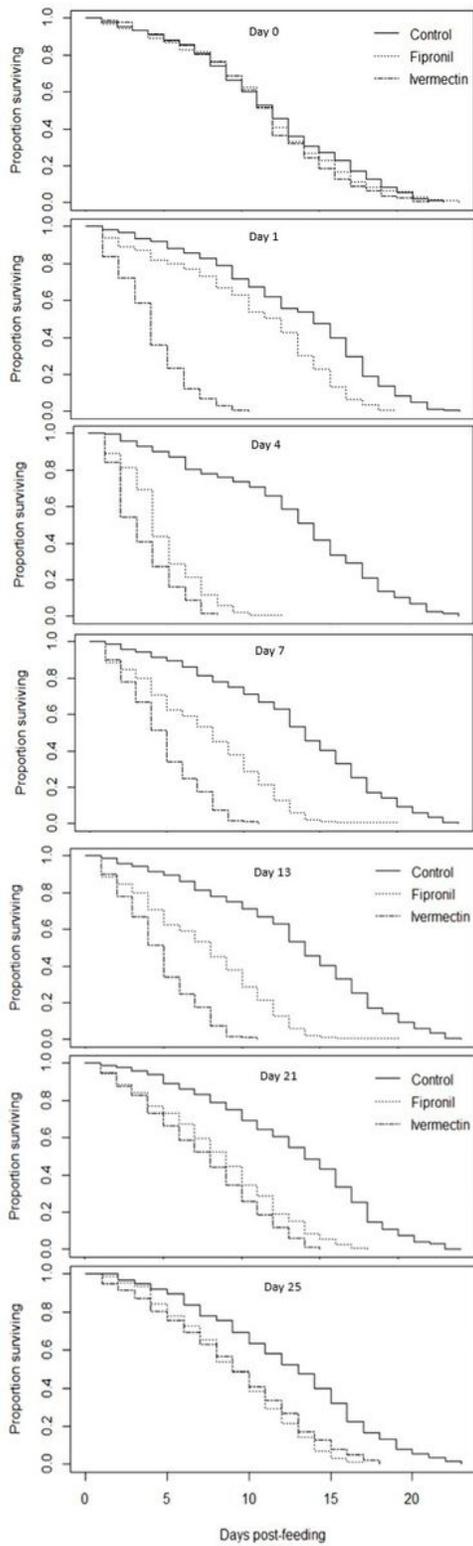


Figure 2

[1.2] Estimates of *An. arabiensis* survival after blood feeding on control, ivermectin-, fipronil-treated and control cattle at different days post-treatments: a) Day 0, b) Day 1, c) Day 4, d) Day 7, e) Day 13, f) Day 21 and g) Day 25 post-treatments. The lines represent survival curves from the Cox proportional hazard model regression.

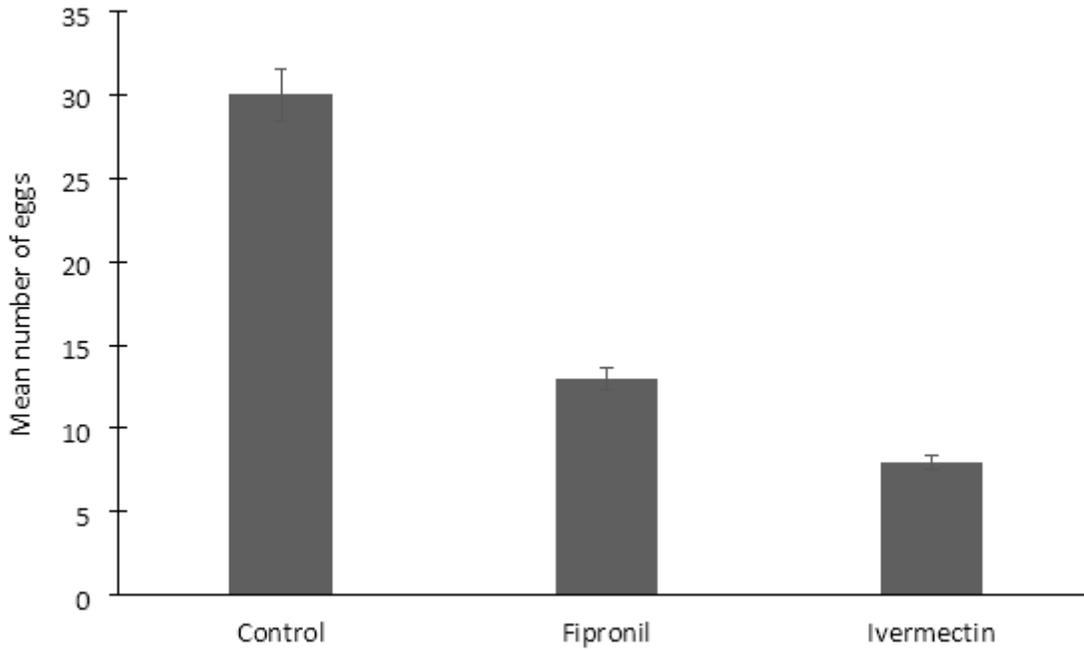


Figure 3

[1.3] Effect of treatment on the number of eggs laid by *An. arabiensis*, displayed as means \pm SE.

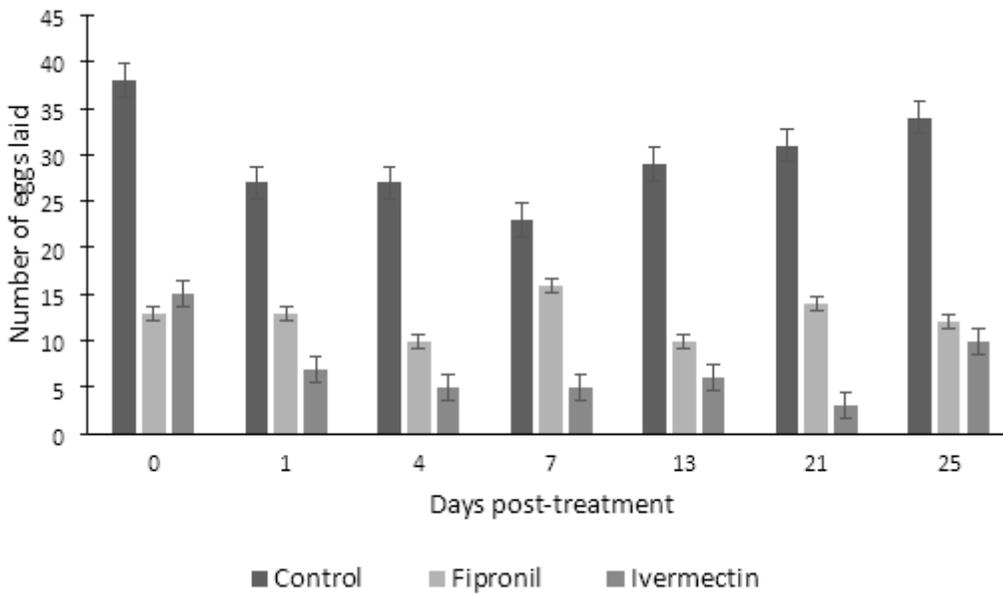


Figure 4

[1.4] Estimates of mean number of eggs laid by *An. arabiensis* mosquitoes that blood-fed from control, fipronil and ivermectin-treated cattle at 0, 1, 4, 7, 13, 21- and 25-days post-treatments.

Supplementary Files

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- [Graphicalabstract.pdf](#)