

Evaluating the potential of a long-acting injectable ivermectin formulation in cattle against Greater Mekong Subregion *Anopheles*

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Abstract

Background

Endectocide treatment of humans or livestock has been proposed as a novel control measure for malaria in the Greater Mekong Subregion (GMS). Ivermectin-treated hosts are lethal to blood-feeding GMS *Anopheles*. However, the mosquito-lethal effect of standard ivermectin in humans only lasts for about two weeks. A novel ivermectin formulation which achieves more sustained blood-level ivermectin concentrations in the treated host is desirable.

Methods

Three different formulations of a long-acting injectable ivermectin formulation (LAIF) (eg. mdc-STM-001, mdc-STM-002, mdc-STM-003) at three different doses (0.6, 1.0, 1.5 mg/kg) were administered to cattle in two different pre-clinical trials in Burkina Faso. Plasma was collected from the cattle at various timepoints for up to 112 days after injection (DAI) and then shipped to Thailand. Plasma samples were reconstituted with fresh cattle red blood cells and membrane fed to *Anopheles dirus* and *Anopheles minimus* mosquitoes. Mosquito survival was monitored daily for fourteen days post blood meal.

Results

Plasma collected in Trial 1 from cattle treated with mdc-STM-001 (0.6 mg/kg), mdc-STM-003 (0.6 mg/kg), or mdc-STM-003 (1.5 mg/kg) was lethal to *An. dirus* through DAI 70. However, plasma from cattle treated with mdc-STM-002 (0.6 mg/kg) was only lethal to *An. dirus* up to DAI 49. The mdc-STM-001 formulation was down-selected for further evaluation in the Trial 2, in which plasma from cattle treated with mdc-STM-001 at 1.0 mg/kg and 1.5 mg/kg was lethal to both *An. dirus* and *An. minimus* through DAI 112. The resulting *An. dirus* and *An. minimus* hazard ratios for mosquito mortality compared to baseline survival was greater than 4 through DAI 112 for both doses of mdc-STM-001. There were negligible improvements in mosquito-lethal effect between the 1.0 mg/kg and 1.5 mg/kg doses of mdc-STM-001, suggesting that the LAIF 1.0 mg/kg dose is ideal to balance efficacy, safety and cost considerations.

Conclusions

The novel LAIF mdc-STM-001 in cattle provides superior mosquito-lethal efficacy for up to 112 days after dosing against GMS *Anopheles* vectors. These pre-clinical findings support further evaluation of the LAIF for use in humans, to establish efficacy and safety. The LAIF in human mass drug administration might be a powerful tool to aid malaria elimination efforts in the GMS.

Background

Mass drug administration (MDA) of ivermectin to humans or livestock has been suggested as a novel malaria vector control tool as ivermectin-treated hosts are lethal to blood-feeding *Anopheles* mosquitoes. In the Greater Mekong Subregion (GMS), the two most important malaria vectors *Anopheles dirus* s.s. and *Anopheles minimus* s.s. have been shown to be susceptible to ivermectin [1-4]. In the GMS, many primary and secondary malaria vectors display more outdoor-feeding, outdoor-resting, and early-evening feeding behaviors [5,6]. Malaria in the GMS occurs primarily in or near forested habitats due to this being the preferred habitat of primary and secondary malaria vectors, combined with sporadic human entry into the forest for various agricultural and economic pursuits [7,8]. These vector behaviors limit the effectiveness of classical home-based vector control measures such as indoor residual spraying and long-lasting insecticide-treated nets. Ivermectin MDA to humans can directly target these outdoor-feeding and early-biting vectors making this vector control measure enticing for use in the GMS.

Standard oral ivermectin formulations in humans produce mosquito-lethal effects for approximately two weeks post-treatment [2]. This would require repeated MDAs over the duration of a malaria transmission season, increasing the cost, complexity of logistics and delivery, which risks decreasing compliance with the intervention over time. Developing a novel ivermectin formulation which can sustain blood-level ivermectin concentrations in humans for several months is desirable. An alternative to oral formulations would be an injectable long-acting formulation, for which injectable antimalarials are in clinical trials for malaria prophylaxis (NCT06558643, NCT07011511). In veterinary medicine, several injectable ivermectin formulations already exist, including long-lasting formulations. Previously, a long-lasting ivermectin formulation was evaluated in Indonesian cattle which, compared to standard ivermectin, had superior mosquito-lethal effect against wild *Anopheles*, demonstrating efficacy up to 72 days after injection (DAI) [9].

Using the BEPO[®] technology, which is a combination of tri-block and di-block copolymers to create a slower absorption from the injection site [10], several long-acting injectable ivermectin formulations (LAIFs) have been developed for evaluation in cattle. Two different LAIFs were administered to four cattle at 1.2 mg/kg, demonstrating mosquito-lethal effect up to five months post-treatment against the primary African malaria vector *Anopheles coluzzii* [11]. In a follow-on study a BEPO[®]-based LAIF at 2.4 mg/kg in seven cattle demonstrated mosquito-lethal effect for up to seven months post-treatment against *An. coluzzii* [12]. However, from a human treatment perspective, a LAIF with shorter duration of ivermectin exposure but sustained mosquito-lethal effect may be more desirable for human MDA. It was in this context that three novel LAIFs were designed for pre-clinical evaluation.

Two pre-clinical trials with three different LAIFs (mdc-STM-001, mdc-STM-002, mdc-STM-003) at various doses (0.6 mg/kg, 1.0 mg/kg, 1.5 mg/kg) were investigated in cattle against colonized *An. coluzzii* and *An. gambiae* s.s. (Trial 1) [13] and wild *Anopheles* spp. (Trial 2) [14] at two different sites in Burkina Faso. Plasma was collected from these LAIF-treated cattle and shipped to Thailand for further evaluation against colonized *An. dirus* and *An. minimus*.

Methods

Long-acting injectable ivermectin formulations

The long-acting injectable ivermectin formulations (LAIFs) tested in pre-clinical trials in cattle were developed using BEPO[®] technology [10], a proprietary long-acting injectable technology (Medincell, Jacou, France). Following subcutaneous injection, BEPO[®] forms an *in situ* bioresorbable polymeric depot via a solvent-exchange mechanism, leading to precipitation of a polymer matrix that enables sustained drug release. Each formulation consisted of three components: i) a biocompatible solvent to ensure injectability, ii) ivermectin as the active pharmaceutical ingredient, and iii) a mixture of two biocompatible and biodegradable polymers that control drug release kinetics. These polymers undergo progressive hydrolysis into bioabsorbable by-products over time due to a simple hydrolysis mechanism. Three ready-to-use LAIF solutions (mdc-STM) were evaluated in the Trial 1: mdc-STM-001, mdc-STM-002 and mdc-STM-003 [13]. The mdc-STM-001 formulation, selected as the lead candidate, was subsequently evaluated in Trial 2 [14].

Cattle trials in Burkina Faso

Two pre-clinical trials with three novel LAIFs (mdc-STM) at varied ivermectin doses were performed in cattle in Burkina Faso.

Trial 1 evaluated mdc-STM-001, mdc-STM-002, and mdc-STM-003 at 0.6 mg/kg, and mdc-STM-003 at 1.5 mg/kg, and a sham control. There were 25 male Sudanese Fulani or mixed Zebu breed cattle in total, with five cattle per group, so 20 cattle in total were treated with LAIFs. LAIFs were administered subcutaneously to cattle as a single dose. Colonized *An. coluzzii* and *An. gambiae* s.s. were fed directly on the treated cattle to evaluate the duration of mosquito-lethal effect. Plasma for mosquito-lethal evaluation was collected from the treated cattle at days after injection (DAI): 1, 2, 3, 4, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91. Trial 1 was performed near Bobo-Dioulasso in Burkina Faso. Mosquito survival and pharmacokinetic results from Trial 1 down-selected the mdc-STM-001 as the most effective formulation [13].

Trial 2 evaluated mdc-STM-001 at 1 mg/kg and 1.5 mg/kg, and a sham control. There were 36 male Taurine or Taurine-Zebu cross-breed cattle in total, with 12 cattle per group, resulting in 24 cattle treated with LAIFs. Cattle were exposed to wild *Anopheles* captured in net traps either indoors or outdoors to evaluate the duration of mosquito-lethal effect. Plasma for mosquito-lethal evaluation was collected from the treated cattle at DAI 0 (pre-dose), 1, 21, 28, 36, 42, 50, 56, 64, 71, 77, 84, 91, 112. Trial 2 was performed in Diebougou district in South-Western Burkina Faso [14].

In both trials, a 3-5 ml jugular vein blood sample was collected at each timepoint in sodium heparin anticoagulant tubes. Blood was centrifuged at $\times 2000g$ for 10 minutes at 4°C and 600 μ l aliquots of plasma were transferred to cryovials. Plasma was stored at -80°C for Trial 1 samples and at -20°C for

Trial 2 samples, shipped to Bangkok on dry ice, and stored at -80°C until mosquito membrane feeding evaluation.

***Anopheles dirus* and *An. minimus* mosquitoes**

Mosquitoes were reared at the Insecticide Research Unit at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University in Bangkok, Thailand. *Anopheles dirus* s.s. (Kaw Mai Khaw strain) and *Anopheles minimus* s.s. (Saraburi strain) were produced as described previously [4]. Mosquitoes were reared in the insectary at $28 \pm 2^\circ\text{C}$, $80 \pm 10\%$ relative humidity, and 12 h light: 12 h dark photoperiod.

Adult mosquitoes used for experiments were provided 5% sugar solution mixed with 5% multivitamin syrup solution for the first 48 hours post emergence and then 10% sucrose solution *ad libitum* until prepared for experiments. Mosquitoes for experiments were 5 to 7 days post emergence at the time of blood feeding. The mosquitoes were gently transferred via aspiration to 0.5 L cylindrical cardboard containers sealed with mesh screen on top, and each container held 40 mosquitoes. Mosquitoes were maintained in an upright incubator at $25 \pm 1^\circ\text{C}$ and $80 \pm 10\%$ humidity with a 12 h light:12 h dark photoperiod. Mosquitoes were sugar-starved with access to water from 16 to 20 h before their blood meal.

Mosquito blood meal preparation

The morning of each mosquito blood-feed, fresh blood samples were collected from cattle at the Rajamangala University of Technology Suvarnabhumi (RMUTSB) cattle farm located outside of Bangkok. Approximately 60 ml of jugular blood was collected in sodium heparin tubes from two cattle at each collection (*i.e.*, mosquito blood feed day). Tubes were labelled and gently inverted to mix the blood and anticoagulant. Tubes were placed on wet ice and transferred to MORU in central Bangkok.

Cattle plasma samples from the Burkina Faso trials were allowed to thaw at room temperature. Once thawed, 550 μl of plasma were transferred to a 1.5 ml centrifugation tube. In parallel, blood from RMUTSB Thai cattle was processed. RMUTSB Thai cattle blood was centrifuged in the sodium heparin tubes at $\times 3000g$ for 5 min at room temperature, and plasma removed. Approximately 1 ml of Roswell Park Memorial Institute 1640 (RPMI) media was added to 4 ml (1:4 ratio) of cattle red blood cells (RBCs) and mixed gently by inverting the tube. The RPMI and RBC mixtures were transferred by pipette to 50 ml Falcon tubes and equally distributed to the tubes to mix blood from both cows evenly. The RPMI and RBC mix was centrifuged at $\times 3000g$ for 5 min at room temperature, and the RPMI media removed. Approximately 1 ml of RPMI media was added to 2 ml of RBCs (1:2 ratio) and mixed gently by inverting the Falcon tube. The RPMI and RBCs mix was centrifuged at $\times 3000g$ for 5 min at room temperature, and the RPMI media removed. The RPMI-washed RBCs (300 μl) were gently added to each cattle plasma sample (550 μl) to achieve a final hematocrit of 35%. Cattle have an average hematocrit of 35% [15] and baseline evaluation of cattle hematocrit from Trial 1 confirmed these findings (data not shown). One negative control consisting of whole blood from RMUTSB Thai cattle was fed to mosquitoes to verify

that the RMUTSB source blood was not lethal to mosquitoes. Additional experiments from our lab have demonstrated that this RBC reconstitution of plasma is critical as plasma versus whole blood meals can dramatically alter mosquito mortality outcomes even at the same ivermectin concentrations [16].

For Trial 1 sample evaluation, plasma from an untreated Burkina Faso cow (control from sham-treated Trial 1 cattle) was prepared with RMUTSB RBCs as described above. For Trial 2 sample evaluation, pre-dose plasma sample from each Trial 2 Burkina Faso cow was prepared with RMUTSB RBCs as described above and was blood-fed at the same time as all post-treatment plasma samples. For *An. minimus* blood-fed Trial 2 plasma, an additional control from sham-treated Trial 2 cattle was evaluated at each blood meal to serve as an additional control.

Mosquito membrane feeding and mortality assays

For each blood meal, a membrane feeder was prepared by sealing the bottom of the glass membrane feeder with a piece of boudruche membrane (*i.e.*, dried flattened cattle intestine).

At each mosquito membrane feed, each cattle sample was provided to groups of 40 *Anopheles* mosquitoes via membrane feeders warmed to 37°C. Mosquitoes were allowed up to 30 minutes to membrane feed. After membrane feeding, up to 30 blood-fed mosquitoes per container were gently transferred via aspiration to clean cardboard containers (0.5 L). After the blood meal, mosquitoes were maintained in an incubator at 25 ± 1°C and 80 ± 10% humidity with a 12 h light: 12 h dark photoperiod, and provided 10% sucrose *ad libitum*. Mosquito survival was monitored daily for fourteen days and any dead mosquitoes were removed by aspiration and recorded. Fourteen days after the blood meal any remaining mosquitoes were recorded as alive and then frozen.

Statistical Analyses

Mosquito survival for each treatment regimen at each time point was compared to controls using Log-Rank survival-curve analysis (Mantel-Cox method). Mosquito mortality hazard ratios and 95% CI were calculated using the Mantel-Haenszel method. Analyses were performed with GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA).

Results

Mosquito survival results

A total of 7,434 blood-fed *An. dirus* were retained to evaluate Trial 1 plasma samples as follows: mdc-STM-001 (0.6 mg/kg) n = 1,769; mdc-STM-002 (0.6 mg/kg) n = 1,800; mdc-STM-003 (0.6 mg/kg) n = 1,675; mdc-STM-003 (1.5 mg/kg) n = 1,680; Control (Trial 1 Sham) n = 180; and RMUTSB (RBC donors) n = 210. Plasma samples from twenty cattle treated with mdc-STM formulations were evaluated. For one membrane feed round, plasma from four Trial 2 pre-dose cattle (n = 120) was used as controls for Trial 1 plasma evaluations. There were no significant survival differences ($\chi^2 = 25.57$, $P = 0.0604$) among *An.*

An. dirus blood-fed on whole blood samples from RMUTSB (RBC donors) or control plasma samples (i.e. Trial 1 sham, Trial 2 pre-dose) (Supplemental Figure S1). Thus, blood from RMUTSB cattle was demonstrated not to be lethal to *An. dirus* and control mosquito mortality data for Trial 1 comparison could be based on a combination of data from Trial 1 control cattle and Trial 2 pre-dose (n = 300). All plasma samples collected from days after injection (DAI) 1, 2, 3, 4, 21, 28, 35, 42, 49, 56, 63, 70 were evaluated in *An. dirus*. Due to limited mosquito numbers for experiments and dwindling efficacy (i.e. less than 20% mortality) at the 0.6 mg/kg dose by day 70 (Fig. 1), it was opted not to evaluate plasma samples from DAI 77, 84, 91. Several Trial 1 plasma samples were missing so could not be evaluated including: mdc-STM-001 (0.6 mg/kg) DAI 3 one sample, mdc-STM-003 (0.6 mg/kg) DAI 2 one sample, DAI 21 one sample, DAI 42 one sample, DAI 49 one sample, mdc-STM-003 (1.5 mg/kg) DAI 3 four samples.

Survival of *An. dirus* was significantly reduced ($P < 0.05$) at all DAIs compared to controls for mdc-STM-001 (0.6 mg/kg) and mdc-STM-003 (0.6 and 1.5 mg/kg). Survival of *An. dirus* was significantly reduced ($P < 0.05$) compared to controls for mdc-STM-002 (0.6 mg/kg) through DAI 49, but no reduction was observed at DAI 56 ($\chi^2 = 1.092$, $P = 0.2961$), DAI 63 ($\chi^2 = 1.736$, $P = 0.1877$), or DAI 70 ($\chi^2 = 0.206$, $P = 0.6948$) (Fig. 1; Supplemental Table S1). The mdc-STM-001 (0.6 mg/kg) was significantly more lethal than mdc-STM-002 (0.6 mg/kg) through DAI 63 ($P < 0.05$), but there was no difference at DAI 70 ($\chi^2 = 2.254$, $P = 0.133$). The mdc-STM-001 (0.6 mg/kg) was significantly more lethal than mdc-STM-003 (0.6 mg/kg) through DAI 49 ($P < 0.0001$), but there was no difference at DAI 56 ($\chi^2 = 2.460$, $P = 0.1168$) or DAI 63 ($\chi^2 = 0.006$, $P = 0.939$), while at DAI 70 mdc-STM-003 (0.6 mg/kg) was significantly more lethal ($\chi^2 = 4.638$, $P = 0.031$), but mortality effect had dropped substantially with both formulations. The mdc-STM-003 (0.6 mg/kg) was significantly more lethal than mdc-STM-002 (0.6 mg/kg) through DAI 70 ($P < 0.05$). The mdc-STM-003 (1.5 mg/kg) formulation was significantly ($P < 0.01$) more lethal than the other LAIFs (0.6 mg/kg) at all DAIs except for mdc-STM-001 (0.6 mg/kg) on DAI 56 ($\chi^2 = 1.389$, $P = 0.239$) (Fig. 1).

A total of 8,662 blood-fed *An. dirus* were retained to evaluate Trial 2 plasma samples as follows: mdc-STM-001 (1.0 mg/kg) n = 4,202; mdc-STM-001 (1.5 mg/kg) n = 4,220; and RMUTSB (donor) n = 240. Plasma samples from two cattle from the 1.0 mg/kg and two cattle from the 1.5 mg/kg treatments were fed to *An. dirus* mosquitoes, but there was a COVID infection in one of the staff, which per Mahidol University policy required the lab to be shut down so the mosquito survival monitoring could not be performed, these mosquitoes are not included in the counts above. Therefore, plasma samples from ten treated cattle from each 1.0 mg/kg and 1.5 mg/kg dose group were evaluated against *An. dirus*. There were no significant survival differences among *An. dirus* fed whole blood from RMUTSB (donor), or reconstituted plasma blood meals from pre-dose mdc-STM-001 at 1.0 mg/kg or 1.5 mg/kg ($\chi^2 = 32.93$, $P = 0.1993$) (Supplemental Figure S2). Pre-dose control *An. dirus* mortality results for Trial 2 are represented as a combination of results from all Trial 2 pre-dose plasma samples (blue lines) separately for either mdc-STM-001 at 1.0 mg/kg or 1.5 mg/kg doses. Survival of *An. dirus* was significantly lower ($P < 0.0001$) when blood-fed mdc-STM-001 at both doses (1.0 mg/kg or 1.5 mg/kg) compared to pre-dose controls though DAI 112 (Fig. 2; Supplemental Table S2).

A total of 9,317 blood-fed *An. minimus* were retained to evaluate Trial 2 plasma samples as follows: mdc-STM-001 (1.0 mg/kg) n = 4,546; mdc-STM-001 (1.5 mg/kg) n = 4,319; Control (Sham) n = 220; and RMUTSB (donor) n = 232. Plasma samples from twelve treated cattle from each 1.0 mg/kg and 1.5 mg/kg dose group were evaluated against *An. minimus*. There were no significant survival differences among *An. minimus* fed whole blood from RMUTSB (donor), or reconstituted plasma blood meals (i.e., Trial 2 Sham Controls, pre-dose mdc-STM-001 at 1.0 mg/kg and pre-dose mdc-STM-001 at 1.5 mg/kg ($\chi^2 = 47.26$, $P = 0.1708$) (Supplemental Figure S3). Pre-dose control *An. minimus* mortality results for Trial 2 are represented as a combination of results from all Trial 2 pre-dose plasma samples (blue lines) for each mdc-STM-001 dose (i.e. 1.0 mg/kg or 1.5 mg/kg). Survival of *An. minimus* was significantly lower ($P < 0.0001$) when blood-fed mdc-STM-001 at both doses compared to pre-dose controls through DAI 112 (Fig. 3; Supplemental Table S3).

For both *An. dirus* and *An. minimus*, the mean hazard ratio for mosquito mortality compared to baseline survival for both doses of mdc-STM-001 (1.0 mg/kg, 1.5 mg/kg) was greater than 4 through DAI 112 (Fig. 4). For *An. dirus*, the lower 95% confidence interval for the hazard ratio drops below 4 from DAI 71 and 64 for 1.0 mg/kg and 1.5 mg/kg doses, respectively.

Discussion

This is the first evaluation of the impact of LAIFs in pre-clinical development on the survival of GMS primary malaria vectors, *An. dirus* and *An. minimus*. Membrane feeding results with reconstituted Trial 1 cattle plasma blood-fed to *An. dirus* observed similar mosquito-lethal effect for mdc-STM-001 (0.6 mg/kg) and mdc-STM-003 (0.6 mg/kg), while mdc-STM-002 (0.6 mg/kg) was less effective with no significant mosquito mortality effect after DAI 49 (Fig. 1; Supplemental Table S1). The mdc-STM-003 (1.5 mg/kg) formulation induced the greatest mortality with *An. dirus*, but this was expected at the 2.5-fold higher dose (Fig. 1; Supplemental Table S1). Membrane feeding results with reconstituted Trial 2 cattle plasma with mdc-STM-001 against *An. dirus* significantly reduced survival through DAI 112 compared to pre-dose for both doses, but with less than 20% difference in reduced survival from controls at DAI 112 (Fig. 2; Supplemental Table S2). Membrane feeding results with reconstituted Trial 2 cattle plasma receiving mdc-STM-001 against *An. minimus* significantly reduced survival through DAI 112 compared to pre-dose for both doses, with approximately 80% difference in reduced survival from controls at DAI 112 (Fig. 3; Supplemental Table S3). Impressively, for both *An. dirus* and *An. minimus*, the mdc-STM-001 formulation achieved hazard ratios for mosquito mortality greater than 4 through DAI 112, although the *An. dirus* 95% CI drops below 4 at DAIs 71 and 64 for the 1.0 mg/kg and 1.5 mg/kg doses, respectively (Fig. 4). The WHO criteria set for novel endectocides is to achieve a mortality hazard ratio > 4 through 30 days post administration [WHO 2022]. For both species, there appears to be little, if any improved mosquito-lethal effect for mdc-STM-001 when increasing the dose from 1.0 mg/kg to 1.5 mg/kg, suggesting that there is no advantage to use doses above 1.0 mg/kg.

Across the globe, *An. dirus* has been demonstrated to be one of the most ivermectin-tolerant *Anopheles* species, while *An. minimus* is one of the most ivermectin-susceptible species [18]. Thus, these two

species represent the lower and upper bounds of ivermectin efficacy, and we would expect mdc-STM-001 induced mortality response of most *Anopheles* species across the globe to fall somewhere between that observed here (Figs. 2 & 3). Indeed, other primary and potential GMS vectors such as *An. maculatus*, *An. sawadwongporni*, and *An. campestris* display intermediate susceptibility to ivermectin [1, 9].

Malaria transmission in the GMS is seasonal in nature, thus it may be possible that one or two human MDAs with a LAIF would be sufficient for vector control for the duration of the transmission season. Malaria outbreaks in the GMS are sporadic and driven largely by shifting agro-economic demands, thus a vector control intervention may not require repeated deployment over several years, making human MDA with LAIFs attractive for rapid rollout and impact in affected areas. Outdoor-feeding of *Anopheles* is a major concern for vector control in the GMS [5, 6] and MDAs with LAIF is one of the few vector control options that could directly target these outdoor-feeding vectors. Since much of the malaria transmission in the GMS occurs in forested areas [7, 8] it may be possible to identify high risk groups and administer LAIFs prior to forest entry to deliver maximal impact to the *Anopheles* populations. These factors here, combined with the pre-clinical LAIF impacts observed against *An. dirus* and *An. minimus* (Figs. 2 & 3) support further development and evaluation of LAIFs in human trials against GMS vectors.

A major factor for the efficacy of ivermectin MDA to humans for malaria control is the host preference of local *Anopheles*. Variation in host preference has been observed across sites in the GMS for both *An. minimus* and *An. dirus*. *Anopheles minimus* was more attracted to humans than cattle in Western Thailand [19], northern Laos, and central Vietnam [6]. But in other locations, *An. minimus* was more attracted to cattle than humans along the Thai-Myanmar border [20], throughout Laos [21], northeastern Cambodia, northern Vietnam [6], and central Vietnam [22]. *Anopheles dirus* was more attracted to humans than cattle throughout Laos [21], central and southern Vietnam, northeastern Cambodia, and northern Laos [6], and similarly for *An. baimaii* (i.e., member of *An. dirus* Complex and primary malaria vector) along the Thai-Myanmar border [20]. But in Western Thailand *An. dirus* and *An. baimaii* were more attracted to cattle than humans [23]. In order to better understand the potential utility of human MDAs with LAIFs in the GMS, further assessment of *Anopheles* host choice preference is warranted, and field investigation should be applied with greater rigor with multiple host species. Simultaneous deployment of LAIFs in human MDAs combined with mass endectocide deployment in livestock may be ideal for GMS malaria control.

Conclusions

The novel mdc-STM-001 formulation administered to cattle in pre-clinical trials imparted substantial mosquito-lethal effect against two primary GMS *Anopheles* malaria vectors for up to nearly 4 months. Further development and evaluation of LAIFs in human clinical trials to assess impact on GMS malaria vectors is warranted. LAIFs for use in human MDAs in the GMS could be a useful addition to malaria control efforts in the region and warrants further exploration.

Abbreviations

Mass Drug Administration (MDA)

Long-Acting Injectable Ivermectin Formulation (LAIF)

Days After Injection (DAI)

Roswell Park Memorial Institute 1640 (RPMI)

Red Blood Cells (RBCs)

Greater Mekong Subregion (GMS)

Rajamangala University of Technology Suvarnabhumi (RMUTSB)

Declarations

Ethics approval and consent to participate

The pre-clinical Trial 1 in Burkina Faso was approved by Comité d’Ethique- Centre International de Recherche-Développement sur l’Elevage en zone Subhumide (CIRDES) (document reference number: 004–10/2020 CEB-CIRDES). The pre-clinical Trial 2 in Burkina Faso was approved by Comité d’Ethique Institutionnel Pour la Recherche en Sciences de la Sante (IRSS) (document reference number: 25-2021/CEIRES/ 06/15/2021). The Thailand animal study protocol was approved by the Animal Care and Use Committee of the Faculty of Tropical Medicine, Mahidol University (document reference number: FTM-ACUC-022-2021). All experiments were performed in accordance with the relevant guidelines and regulations of the above listed institutions.

Consent for publication

Not applicable.

Competing Interests

SL-D is currently an employee and shareholder of Medincell, and did not influence or contribute to sample collection, data collection, sample analysis, data analysis, evaluation, decision to publish, or conclusions presented in this paper. The other authors are not shareholders of Medincell and declare no competing interests.

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The funder had no role in the design of the study and collection, analysis, and interpretation of data or writing the manuscript.

Author Contribution

Wrote first draft of the manuscript KCK; designed research KCK, JT; performed membrane feeds and mosquito mortality observations NR, PK; provided mosquitoes TP, PSr; provided cattle blood PSa, SN; analyzed data KCK; designed the pre-clinical trials KM; conducted Trial 1 or Trial 2 LZ, COWO, SHP, KRD; provided administrative oversight PSr, PSa, SL-D, JT; all authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and/or analysed during the current study are available in the Harvard Dataverse repository, [<https://doi.org/10.7910/DVN/UYJY39>].

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Figures

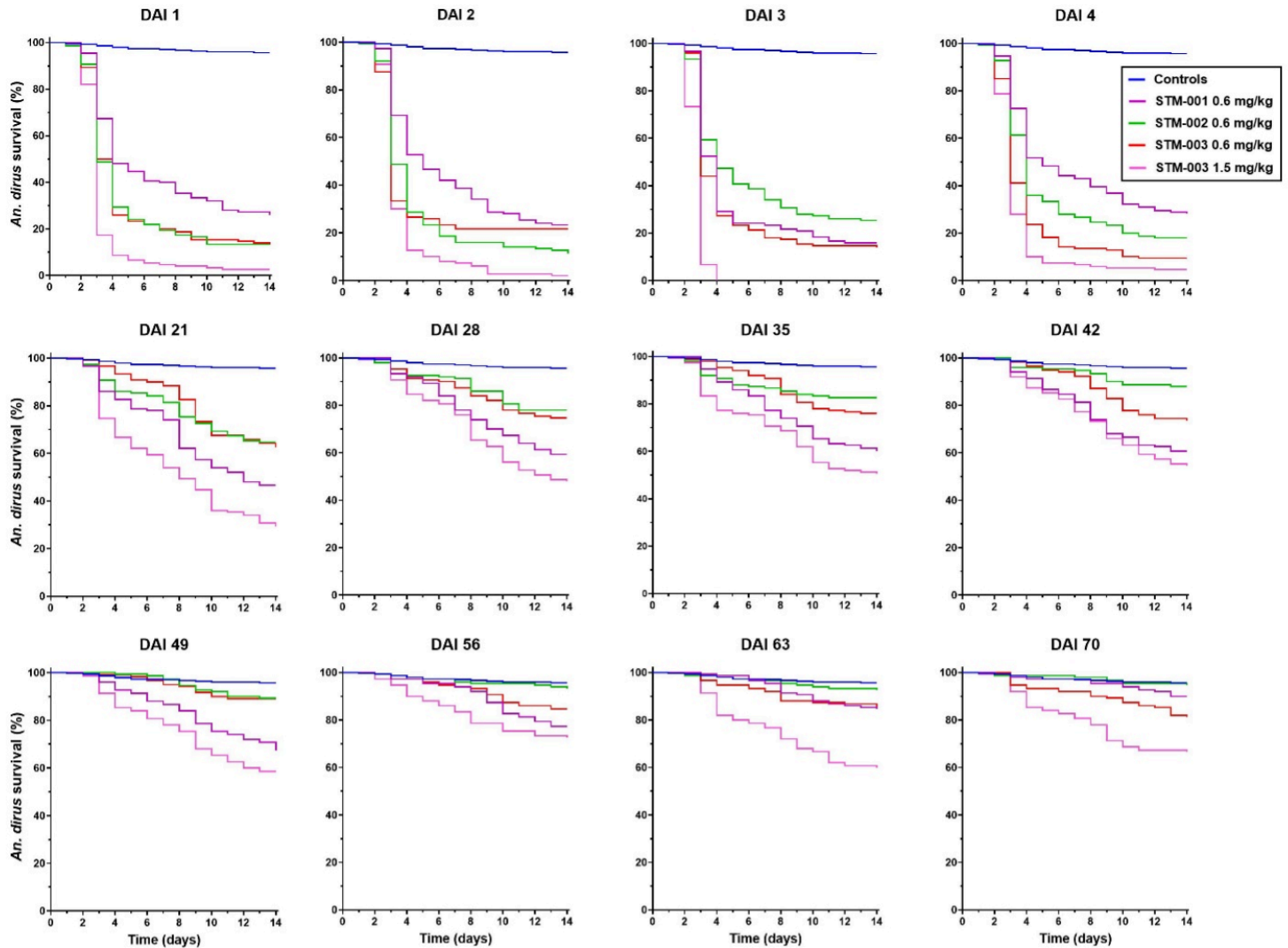


Figure 1

Survival results when *An. dirus* was blood-fed reconstituted Trial 1 blood meals from control cattle (blue lines) and cattle treated with mdc-STM-001 (0.6 mg/kg) (dark purple lines), mdc-STM-002 (0.6 mg/kg) (green lines), mdc-STM-003 (0.6 mg/kg) (maroon lines), mdc-STM-003 (1.5 mg/kg) (pink lines). Plasma was collected from the LAIF-treated cattle on days after injection (DAI) 1, 2, 3, 4, 21, 28, 35, 42, 49, 56, 63, 70 and survival results are shown by DAI in separate panels.

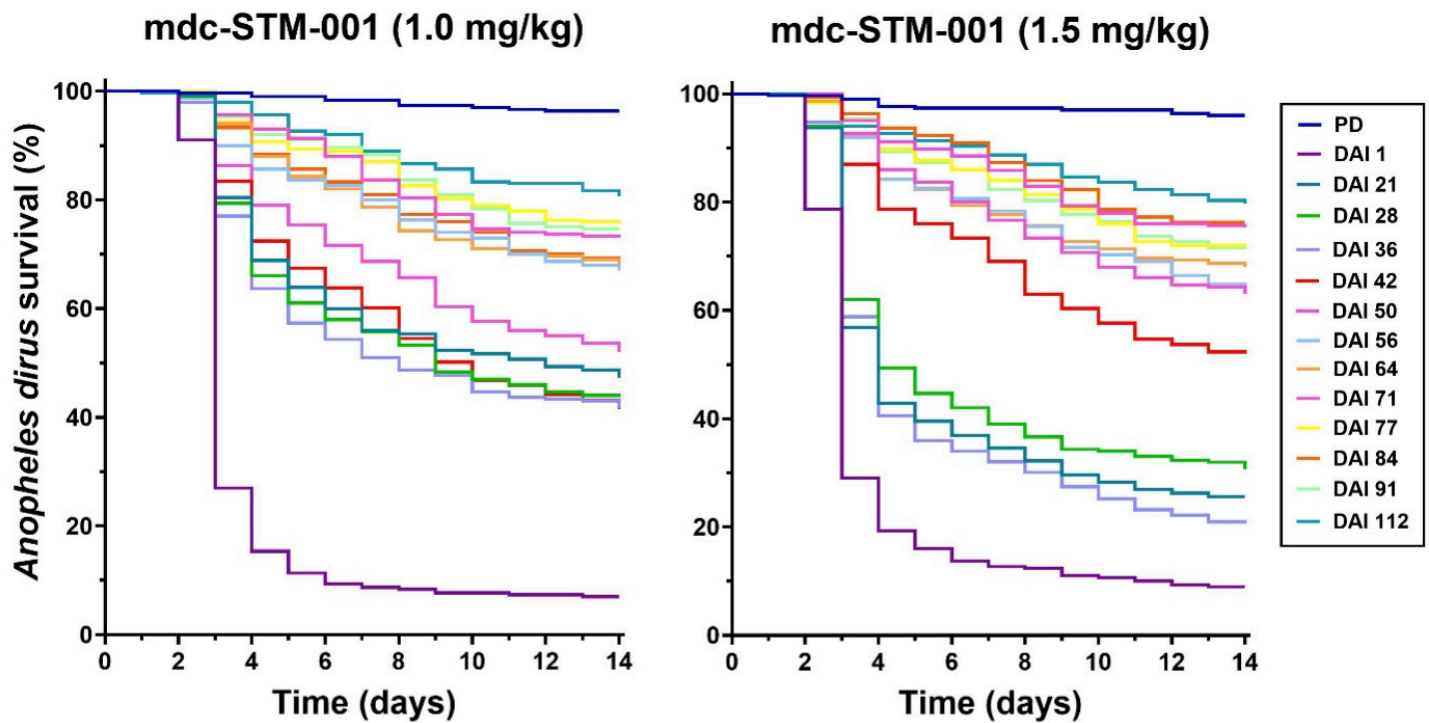


Figure 2

Survival results when *An. dirus* was blood-fed reconstituted Trial 2 blood meals from cattle treated with mdc-STM-001 at 1.0 mg/kg (left panel) or mdc-STM-001 at 1.5 mg/kg (right panel). Plasma was collected from the LAIF-treated cattle pre-dose (PD) (blue lines) and on days after injection (DAI) 1, 21, 28, 36, 42, 50, 56, 64, 71, 77, 84, 91, 112.

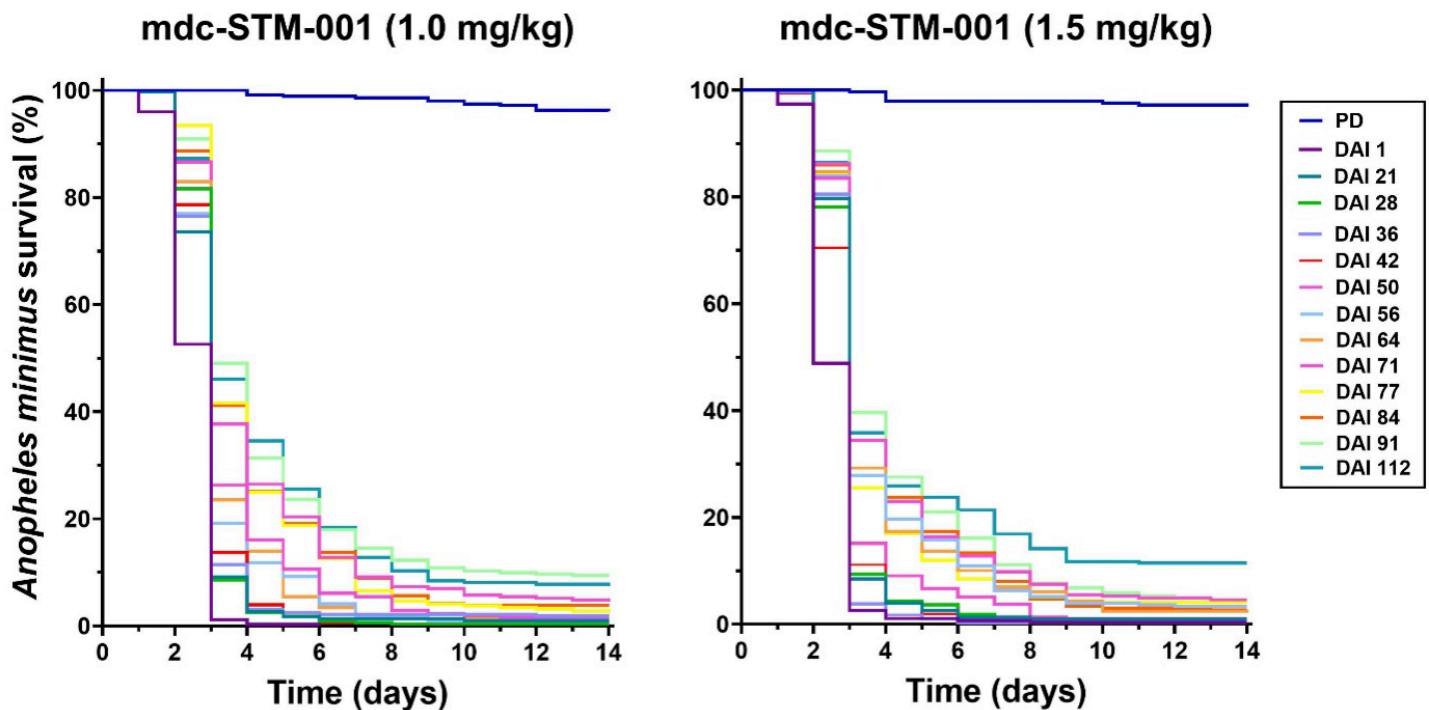


Figure 3

Survival results when *An. minimus* was blood-fed reconstituted Trial 2 blood meals from cattle treated with mdc-STM-001 at 1.0 mg/kg (left panel) or mdc-STM-001 at 1.5 mg/kg (right panel). Plasma was collected from the LAIF-treated cattle pre-dose (PD) (blue lines) and on days after injection (DAI) 1, 21, 28, 36, 42, 50, 56, 64, 71, 77, 84, 91, 112.

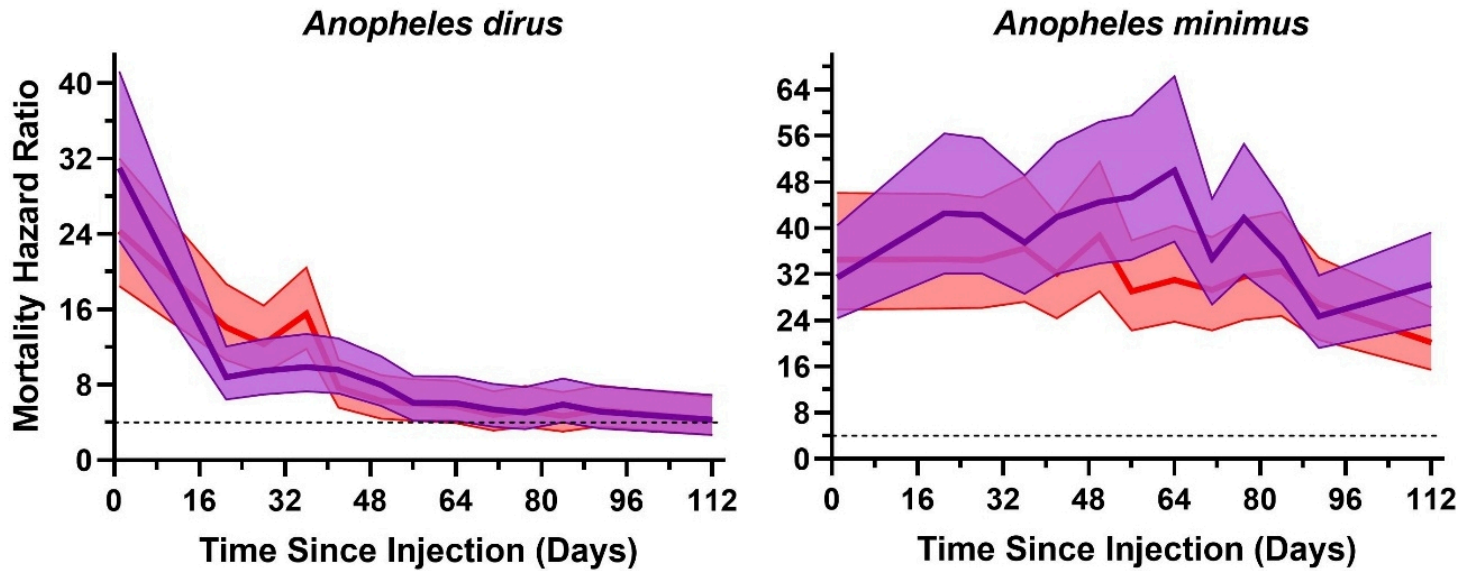


Figure 4

Anopheles dirus (left panel) and *Anopheles minimus* (right panel) Mantel-Haenszel hazard ratios when blood-fed reconstituted Trial 2 blood meals from mdc-STM-001 at 1.0 mg/kg (purple lines) or mdc-STM-001 at 1.5 mg/kg (red lines). Solid lines represent the mean hazard ratio for mortality at 14 days after blood meal ingestion and the shaded area represents the 95% confidence interval. The dashed lines indicate hazard ratios (HR) of 4, which is the standard set by the WHO Target Product Profile for new endectocides [17]. The *An. dirus* 95% confidence interval for mdc-STM-001 at 1.0 mg/kg crosses below the HR 4 at DAI 71 and at 1.5 mg/kg at DAI 64 but neither mean crosses the HR4 before DAI 112. The *An. minimus* 95% confidence interval for mdc-STM-001 at 1.0 or 1.5 mg/kg never crosses the HR4 through DAI 112.