Selection of the Optimal Long-Acting Injectable Formulation of Ivermectin for use in humans to Target Malaria Vectors in Western Africa: Evaluation of Pharmacokinetics and Mosquitocidal Efficacy in Cattle under Laboratory Conditions.

Lamidi Zela[1,2], Sié Hermann Pooda[1,3], Angélique Porciani[4], Samuel Beneteau[5], André Barembaye Sagna[4,6*], Sophie Le Lamer-Déchamps[7], Nicolas Moiroux[4,8], Cheick Oumar Wendpagnandé Ouédraogo[9,10], Anyirékun Fabrice Somé[9], Cédric Pennetier[4], Christophe Roberge[7], Adrien Marie Gaston Belem[2], Koumbobr Roch Dabiré[9], Guiguigbaza-Kossigan Dayo[1], Karine Mouline[4]**

Affiliations :

[1]Centre International de Recherche-Développement sur l'Elevage en zone Subhumide, Bobo-Dioulasso, Burkina Faso

[2]Université Nazi Boni, Bobo-Dioulasso, Burkina Faso

[3]Université Daniel Ouezzin COULIBALY, Dédougou

[4]MIVEGEC, University of Montpellier, IRD, CNRS, Montpellier, France

[5]Independant Statistician

[6]IRD, Représentation du Burkina Faso, Antenne de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso

[7]MedinCell, Montpellier, France

[8]Pôle de Zoologie Médicale, Institut Pasteur de Dakar, Dakar, Sénégal

[9]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Centre National de Recherche Scientifique et Technologique, Bobo-Dioulasso, Burkina Faso

[10]Université Cheikh Anta Diop, Dakar, Sénégal

* present affiliation: ASTRE, CIRAD-INRAE, Univ Montpellier, Centre de Recherche et de Veille sur les Maladies Vectorielles dans la Caraïbe (CRVC), Petit Bourg, Guadeloupe, France

** correspondence: karine.mouline@ird.fr

LZ: lamidase@yahoo.fr

SHP: poodasiehermann@yahoo.fr

AP: angelique.porciani@ird.fr,

SB: samuel.beneteau@ntymail.com

AS: andre.sagna@ird.fr

SLD: sophie.lelamer@medincell.com

NM: nicolas.moiroux@ird.fr

COW: cheickoumaroued92@yahoo.fr

AFS: afabricesome@yahoo.fr,

CP: cedric.pennetier@ird.fr

CR: christophe.roberge@medincell.com,

AMGB: belemadrien@yahoo.fr,

KRD: dabireroch@gmail.com,

G-KD: charlesdayo@yahoo.fr

KM: karine.mouline@ird.fr

Abstract

Background: Ivermectin, a semisynthetic endectocide, is widely used against parasitic nematodes in humans and animals. Its lethality to *Anopheles* mosquitoes after feeding on treated hosts represents a promising malaria control strategy, particularly against outdoor transmission. However, standard oral formulations for use in humans produce short-lived mosquitocidal blood concentrations, limiting epidemiological impact. To meet WHO Preferred Product Characteristics (PPC) for endectocides against malaria vectors (Hazard Ratios >4 for at least one month), three long-acting injectable ivermectin formulations (LAIFs) based on BEPO® depot technology were developed and compared in cattle to identify the most suitable candidate for future human use.

Methods: A cattle–*Anopheles* model was used under laboratory conditions in Bobo-Dioulasso, Burkina Faso. Three LAIF candidates (mdc-STM-001, mdc-STM-002, mdc-STM-003) were injected to calves (n=5 per formulation) at 0.6 mg/kg, with untreated calves as controls (n=5). Plasma ivermectin concentrations were measured over 130 days and analyzed using non-compartmental pharmacokinetics. Direct skin feeding assays were conducted at 15 timepoints (days 2–126 post-injection) using insecticide-susceptible (KIS) and wild-derived resistant (VK5) *Anopheles* colonies. Efficacy was assessed through 10-day cumulative mortalities, hazard ratios, 50% lethal concentrations (LC50), and duration of exposure above the 10-day LC50, accounting for the extrinsic incubation period of *Plasmodium falciparum*.

Results: All formulations were well tolerated. Mdc-STM-001 showed the most favorable pharmacokinetic profile, with a controlled peak concentration (Cmax = 34.5 ± 12.7 ng/mL) and the lowest inter-individual variability (12%). Ten-day hazard ratios exceeded 4 and cumulative mortalities were >50% for at least 60 days in both strains. Median mosquito lifespan remained below 10 days for at least 90 days post-injection. The 10-day LC50 for resistant mosquitoes (3.66 [2.69–4.97] ng/mL) was maintained for ≥126 days.

Conclusion: The Mdc-STM-001 was identified as the optimal candidate. A single injection induced sustained mosquitocidal efficacy for at least two months, achieving HR >4 against both susceptible and resistant *Anopheles* populations and meeting WHO PPC for malaria endectocides. Although extrapolation from cattle to humans requires caution, the favorable pharmacokinetic profile and robust entomological outcomes support progression to Phase 1 clinical trials. Ivermectin's established safety record further strengthens the rationale for clinical development.

Keywords: malaria vector control, residual transmission, *Anopheles* mosquitoes, insecticide resistance, ivermectin, long-acting injectable ivermectin, pharmacokinetics, mosquitocidal efficacy

Background

Malaria remains a leading global health concern with an estimated 263 million clinical cases and 597,000 deaths in 2023, over 95% of which occurred in Sub-Saharan Africa [1]. Significant gains in the fight against the disease have been made between 2000 and 2015 [2] mainly due to indoor residual spraying of houses and universal coverage with pyrethroid insecticide treated nets, two core vector control interventions recommended by the World Health Organization (WHO) [3]. Progresses have stalled since then[4], notably coinciding with widespread, growing, resistance in *Anopheles* vectors populations, due to the huge selection pressure exerted by continuous insecticide-based interventions and massive pesticide use in agricultural practices [5]. Resistance refers to both (i) physiological resistance, involving reduced susceptibility to the insecticidal molecule through target-site mutations or metabolic detoxification processes [6], and (ii) behavioral (qualitative) resistance, as defined by Carrasco et al. [7], whereby mosquitoes avoid insecticides exposure spatially (by biting outdoors), temporally (by biting early in evening or morning when

98 humans are outside their nets or housing), or trophically (by feeding on non-human hosts).
99 New generations of Long Lasting Impregnated Nets (LLINs) incorporating dual active
100 ingredients or synergists [8] [9] have been recently recommended by the WHO to circumvent
101 pyrethroid resistance [10] . However, these tools still primarily target nocturnal, indoor-biting
102 *Anopheles* mosquitoes, and are logically bypassed by behaviorally resistant vectors [11,12].
103 The resurgence in malaria cases in recent years [1] highlights the urgent need for novel and
104 complementary strategies to reinforce existing interventions and reverse this concerning
105 trend. In particular, tools targeting the diverse biting behaviors of *Anopheles* vectors, whether
106 innate or induced by interventions, are essential for addressing this challenge [7,13].
107 Mass drug administration (MDA) to humans of the ivermectin endectocide is a
108 proposed approach to overcome limits of current malaria vector control methods [14,15].
109 Acting systemically, the molecule targets internal parasites as well as external blood-feeding
110 arthropods, including *Anopheles* malaria mosquitoes. It thus primarily reduces *Anopheles*
111 survival probability, the most critical determinant of their vectorial capacity [16]. Because the
112 insecticide is delivered through the host's bloodstream, it functions regardless spatial or
113 temporal mosquitoes blood-feeding adaptations. Ivermectin is the leading endectocide
114 candidate for vector control notably because of its strong, well established, safety profile from
115 nearly 4 decades of extensive clinical use in MDA campaigns against lymphatic filariasis and
116 onchocerciasis [17]. This safety reflects its selective action on glutamate-gated chloride
117 channels unique to invertebrates [18]. Such mechanism of action is distinct from those of the
118 different insecticide classes used in public health against malaria vectors, so ivermectin is a
119 potent vector control tool that could mitigate current physiological resistance issues.
120 All *Anopheles* species and strains tested to date have been shown to be susceptible to
121 ivermectin, although the degree of susceptibility varies among species [19]. Moreoever sub-
122 lethal effects are also interesting to transmission control as they negatively impact vectors

123 fecundity and fertility [20,21], locomotor and biting behaviors [22,23], or *Plasmodium*
124 parasite development [24,25]. Mass drug administration of ivermectin to humans may
125 therefore effectively reduce residual malaria transmission.

126 Various oral drug dose regimens have been used during Randomized Controlled Trials
127 (RCTs) testing ivermectin MDAs against malaria. Standard oral ivermectin dosing for
128 parasitic disease treatment in humans (150–200 µg/kg) achieves lethal plasma levels for
129 *Anopheles* mosquitoes for fewer than seven days [26]. In field studies from Sub-Saharan
130 Africa, this regimen administered every three weeks during the rainy season to populations in
131 southern Burkina Faso resulted in a significant 20% reduction in malaria incidence among
132 children under five [27]. Increased dosing regimens (1x400 and 3x300 µg/kg) prolong
133 ivermectin persistence in the host bloodstream and mosquitocidal efficacy up to 14-21 with
134 good safety [28,29]. Modeling studies have predicted that 80% coverage of the target
135 population using such regimen has the potential to reduce malaria incidence by 30 to 70%
136 depending on the transmission settings [30].

137 In the fields, the BOHEMIA RCT in Mozambique and Kenya applied the 1X 400
138 µg/kg regimen monthly for three consecutive months across the rainy season [31]. In Kenya,
139 this led to reduced malaria incidence in children aged 5-15 years by 26% compared to the
140 control arm [32]. However, in Mozambique, the same regimen failed to produce significant
141 reduction [33]. Two other RCTs testing the 3 × 300 µg/kg dose administered monthly in
142 combination with either dihydroartemisinin–piperaquine (DHAP) in the Galápagos Islands
143 (MATAMAL [34]) or seasonal malaria chemoprevention (SMC) in Burkina Faso
144 (RIMDAMAL II [35]) also showed no significant epidemiological effect.
145 Possible explanations include biological factors related to vector populations such as poor
146 translation of mosquitocidal effects from laboratory studies to the field or overlooked aspects
147 of wild mosquito bionomics in the trial [36]. Pharmacokinetic data from the RIMDAMAL II

trial revealed also a shorter-than-expected residence time of lethal plasma ivermectin, as drug levels dropped below detection in nearly 50% of participants by day 14 [35]. In Mozambique, logistical challenges increased the administration rounds duration so that population coverage never reached the threshold expected for efficacy. All trials have however demonstrated safety of increased and repeated dosing of oral ivermectin.

Both pharmacokinetic limitations and operational challenges encountered in the fields are intrinsic to oral dosing and highlight the need for long-acting ivermectin formulations that can sustain mosquitocidal effects while allowing logistical flexibility.

To date, only a single human-targeted long-acting formulation has been developed a gastric-resident oral device with approximately 14-day efficacy [37].

In this context, using the BEPO® injectable technology [38], we developed three ivermectin LAIFs for human use in malaria endemic regions. These formulations were designed according to the efficacy targets set by the WHO recommendations for preferred product characteristics of endectocides in malaria vector control [39], which specify a hazard ratio (HR) greater than 4 for mosquitoes feeding on treated hosts for at least one month post-treatment.

We used a cattle-mosquito colony model [21,40], exposing both insecticide-susceptible and pyrethroid-resistant *Anopheles* strains through direct skin feeding assays, thereby assessing efficacy despite potential cross-resistance or tolerance mechanisms. Our main objective was to compare pharmacokinetic (PK), pharmacodynamic (PD), and mosquitocidal efficacy outcomes among the three candidate formulations to identify the optimal one for subsequent clinical evaluation in human.

Methods

Study site

All experiments were conducted in Bobo-Dioulasso, south-western Burkina Faso, through a collaboration between the Institut de Recherche en Sciences de la Santé (IRSS) and the Centre International de Recherche-Développement sur l'Élevage en zones Subhumides (CIRDES). Both institutes have controlled insectary facilities, and CIRDES also maintains stables for cattle husbandry.

Mosquito colonies

Two *Anopheles* mosquito colonies were used in this study:

(i) ***Anopheles (An.) gambiae* Kisumu (KIS)**: fully susceptible to all classes of insecticides and maintained in the IRSS insectaries. The colony is routinely monitored for contamination by other species and screened for insecticide resistance using molecular techniques [41,42]. For the experiments, mosquitoes were transported in meshed cages covered with moist cloth to the Centre International de Recherche-Développement sur l'Élevage en zone Sub-humide (CIRDES). Transport duration was approximately 10 minutes.

(ii) ***An. coluzzii* VK5 (VK5)**: This colony was established at CIRDES in November 2020, prior to the start of the experiments in January 2021. A total of 150 blood-fed females were collected from the village of VK5 in the Kou Valley (~50 km northwest of Bobo-Dioulasso), an irrigated rice growing area where different classes of pesticides are used for agriculture [43]. The use of pyrethroid impregnated bed nets is high due intense mosquito nuisance. The mosquito's bio-ecology is well characterized, with *An. coluzzii* representing more than 95% of *Anopheles* species, and permanently found breeding in the irrigated fields. *An. coluzzii* populations are there characterized by a high prevalence of resistance to all the insecticides classes, justifying the choice of this cite to found the insecticide

198 resistant colony. Collected females were allowed to individually oviposit. Species
199 identification was performed post-oviposition by PCR using established protocols
200 [41] and only progeny from confirmed *An. coluzzii* were retained. As expected,
201 based on the collection site, all females belonged to the target species.

202 Both colonies were housed in similar environmental conditions and maintenance
203 protocol [40]. The insectaries temperature was set to 26°C ± 1°C, relative humidity to
204 75% ± 5%, and photoperiod to 12-hour light/dark. The VK5 colony's pyrethroid
205 resistance status was routinely monitored by sampling approximately 40 females per
206 generation and screening for the knockdown resistance West African allele (KDR-W;
207 L1014F) of the voltage-gated sodium channel gene using quantitative polymerase chain
208 reaction (qPCR) [42]. For each direct skin-feeding assay time point, females were
209 randomly drawn from at least five different cages to minimize potential cage effects.

210 **Long acting ivermectin formulations**

211 The long-acting ivermectin formulations tested in this study were developed using BEPO®
212 technology [38], a proprietary long-acting injectable technology (Medincell, Jacou, France).
213 Following subcutaneous injection, BEPO® forms an *in situ* bioresorbable polymeric depot via
214 a solvent-exchange mechanism, leading to precipitation of a polymer matrix that enables
215 sustained drug release. Each formulation consisted of three components: i) a biocompatible
216 solvent to ensure injectability, ii) ivermectin, as the active pharmaceutical ingredient, and iii)
217 a mixture of two biocompatible and biodegradable polymers that control drug release kinetics.
218 These polymers undergo progressive hydrolysis into bioabsorbable by-products over time,
219 thanks to a simple hydrolysis mechanism. Three ready-to-use LAIFs solutions were
220 evaluated: mdc-STM-001, mdc-STM-002 and mdc-STM-003; the last one differed in
221 ivermectin loading and each differed in polymer composition and proportions.

222 All formulations were imported into Burkina Faso with clearance from the Direction Générale
223 des Services Vétérinaires of Burkina Faso (Permit No 2020/199) and were stored at room
224 temperature, protected from light, until administration to cattle. The study protocol was
225 approved by the Institutional Animal Ethics Committee of CIRDES (document reference
226 number: 004-10/2020 CEB-CIRDES).
227
228 **Cattle hosts and treatments**
229 Twenty-five male calves (Métis strain: Fulani zebus x Baoulé crossbreed) with a median
230 weight of 117 kg (interquartile range [IQR]: 106-129 kg), were purchased from villages in the
231 Valley du Kou area two months prior to the study to allow for acclimation. Upon arrival at the
232 CIRDES animal facility, calves were treated for trypanosomiasis and gastrointestinal parasites
233 using Berenil 2000® and Benzal®, respectively. Animals were housed outdoors under
234 screened enclosures to prevent exposure to or reinfestation with ectoparasites and
235 trypamosomoses. They were fed with rice straw and 1 kg/day of cottonseed cake, with water
236 and sea salt provided ad libitum.
237 Calves were randomly assigned to five experimental arms (n = 5 calves per arm) using
238 stratified randomization to balance body weight across groups. Four arms received LAIFs,
239 and one served as the untreated control. The formulations were administered *via* a single
240 subcutaneous injection into the loose skin anterior to the shoulder.
241 - mdc-STM-001 and mdc-STM-002 LAIFs were each injected at a unique dose of 0.6
242 mg/kg (referred to as mdc-STM-001-0.6 and mdc-STM-002-0.6, respectively).
243 - mdc-STM-003 was administered at two doses: 0.6 mg/kg and at 1.5 mg/kg (referred
244 to as mdc-STM-003-0.6 and mdc-STM-003-1.5).
245 The 1.5 mg/kg dose represents the upper limit of feasible subcutaneous volumes for
246 human application and was included to assess dose-dependent effects on

247 pharmacokinetics and mosquitocidal efficacy. Nominal injection volumes per animal
248 ranged from 0.62 to 2.14 mL (detailed in Table 1). The animals' identification numbers,
249 their weight upon arrival at CIRDES and on the day of injection, administered
250 formulations, and injected volumes are provided in the Additional file 1, Table S1.
251 Animal health was monitored throughout the study by assessing each day clinical signs,
252 local injection site reactions, body weight, and weight gain.
253
254 **Mosquito blood-feeding bioassays**
255 To ensure equivalent ivermectin exposure, mosquitoes from both KIS and VK5 colonies were
256 simultaneously exposed to the same cattle hosts during blood-feeding. This design enabled
257 direct comparison of susceptibility across colonies under identical conditions. Direct skin
258 blood-feeding assays were conducted following previously established protocols [40]. Prior to
259 feeding, mosquitoes were deprived of sugar for 12 hours, with access to water-soaked cotton
260 only. At each time point, approximately 100–130 females per colony were introduced into
261 net-covered bowls, and held against the shaved flank of the calf for 20 minutes using a rubber
262 band. Separate bowls were used for each colony. Fully engorged mosquitoes were distributed
263 into cups (10 females per cup), with a maximum of 120 cups per colony per time point. All
264 cups were maintained under controlled conditions in the CIRDES insectary. Mosquito
265 mortality was recorded daily between 10:00 and 11:00 a.m. for 30 days post-blood meal. To
266 mitigate positional bias, cups were randomly repositioned on shelves after each observation.
267 A total of 16 blood-feeding assays were conducted: once pre-treatment (baseline) and
268 at 2, 7, 17, 21, 28, 42, 56, 70, 84, 91, 98, 105, 112, 119, and 126 Days After Injection (DAI).
269 Due to limited mosquito availability, KIS mosquitoes were not tested at 7 and 91 DAI.
270 Surplus blood-fed mosquitoes were stored at –20°C for downstream analyses.
271

272 **Ivermectin bioanalysis and pharmacokinetics**

273 Jugular venous blood samples were collected from treated calves at each mosquito exposure
274 time point and at additional time-points (30 samples per animal) to characterize ivermectin
275 pharmacokinetics (PK). Sampling included pre-dose, 2h, 5h and 12h post-injection, then daily
276 sampling until DAI 4, every 2-4 days until DAI 21, and weekly until DAI 132-133. Blood
277 samples were also collected from control animals at pre-dose and at DAI 1, 14, 28, 56, 84 and
278 111. Blood samples were centrifuged at +4°C (2000 rpm for 10 min) to isolate plasma, which
279 was stored at -80°C until shipment for bioanalytical analysis. Ivermectin plasma
280 concentrations were quantified using a liquid-liquid extraction with qualified LC-MS/MS
281 methods. Two calibration ranges were used (0.2-20 ng/mL low range; 5-500 ng/mL high
282 range), using K_2-EDTA as anticoagulant and ivermectin-D_2 as internal standard. The quality
283 controls (0.6, 10, 16 ng/mL low range; 15, 250, 400 ng/mL high range) were within the
284 acceptance criteria (precision ≤ 15.00%, deviation ± 15.00%). Incurred sample reanalysis
285 validated ivermectin stability for at least 680 days at -20°C. Plasma samples collected from
286 cattle were stored at -80°C no longer than 162 days before analysis, validating the ivermectin
287 concentrations measured for PK analysis.

288 At study completion, control animals were returned to stock, while treated animals
289 were euthanized. Injection sites and depot with surrounding tissues were sampled to quantify
290 the residual ivermectin and copolymers. These analyses were performed using an ultra-
291 performance liquid chromatography (limit of quantification: 0.9 µg/mL) for ivermectin and
292 nuclear magnetic resonance analysis for copolymers. Both methods were developed for
293 exploratory purposes only.

294

295 **Outcomes and Analysis**

296 Unless specified otherwise, analysis were performed using the R version 4.4.3 (2025-02-
297 28) [44]. Models fit were assessed using the "performance" [45] and DHARMa [46]
298 packages. All estimates are reported with 95% confidence intervals (CI95%) and/or p-values.

299
300 *Cattle weight gain*
301 Differences in weight gain between treatment groups throughout the study were analyzed
302 using the Kruskal–Wallis rank sum test, followed by pairwise two-sided exact Wilcoxon rank
303 sum tests with 95% confidence intervals. *p*-values were adjusted using the Holm method
304 ([47]).
305
306 *Pharmacokinetics*
307 PK parameters of ivermectin were calculated for each LAIF using a non-compartmental
308 analysis (Phoenix® WinNonlin® version 8.1; Certara USA, Inc., Princeton, NJ). These
309 included the rate of systemic exposure (Cmax), the time to reach Cmax (Tmax), the extent of
310 systemic exposure over the study duration (aera under the curve, AUClast), over 28 DAI
311 (AUC0-28d) and over 91 DAI (AUC0-91d), concentrations at 28 DAI (C28d), 56 days
312 (C56d), 91 DAI (C91d) and at study end (Clast), and the apparent terminal phase half-life
313 (T1/2) when calculable. The systemic exposure parameters were normalized to the actual
314 administered dose, calculated for each cattle based on the weight of each syringe before and
315 after injection. The inter-individual variability in plasma concentrations and PK parameters
316 was expressed as the coefficient of variation (CV %) for each experimental group.
317
318 *Mosquito related outcomes*
319 Mosquito blood feeding rate

For each colony, we compared the mosquitoes' blood-feeding rates across treatment arms using a generalized linear mixed model (GLMM, [48]) with a binomial error distribution. The outcome variable was the number of blood-fed mosquitoes, with the total number of mosquitoes included as an offset. The model included an interaction term between treatment and DAI, as well as a random intercept for cattle. The model was fitted using restricted maximum likelihood.

Times post-feeding

Efficacy outcomes are provided for 4- 10 and 30-days post mosquito blood feeding. The 4-day period reflect the acute toxic effect, which typically manifests within 2-3 days post-ivermectin blood meal. The 10-day post-blood feeding period is used in relation the 10-day minimum extrinsic incubation period (EIP) needed for the parasite to develop in the mosquito and to reach the salivary glands [49], thereby rendering it infectious and active in transmission. The 30-day follow-up captures the residual effects of ivermectin on surviving mosquitoes, which may include both indirect sublethal effects and potential interactions with the mosquitoes' age. Only data related to the 10-day follow up period are presented and thoroughly discussed in the main text. Outcomes for the 4-day and 30-day follow-up period are given in the Additional file 2, Fig. S3 and S4.

Cumulative Mortality

Proportions of dead mosquitoes at 10-days post-feeding were computed for each colony, formulation, and DAI.

Survival Analysis and Hazard Ratios

344 Daily mosquito mortality was recorded for up to 30 days post blood-feeding. For each
345 mosquito colony and DAI, survival data were used to generate Kaplan–Meier survival curves,
346 stratified by treatment group.
347 Per colony, survival patterns at each time-point were compared to the control group
348 using Cox proportional hazards models (mixed-effects regression model [50]). The models
349 included "treatment group" and "DAI" as fixed effects, with random intercepts for individual
350 calf ID and mosquito cup (nested random effects) to account for variability within treatment
351 groups. Hazard ratios (HR) at different time points post-blood feeding are given to assess the
352 impact of ivermectin on mosquito mortality risks and HR variations over time. Post hoc
353 pairwise comparisons of HRs between time points were adjusted for multiple testing using the
354 Tukey method, implemented via the "emmeans" package [44]
355
356 Dose–Response Analysis and coverage duration
357 Dose–response relationships were analyzed using the "drc" package in R [51], modeling 4-
358 day and 10-day mosquito mortality as a function of host ivermectin plasma concentrations. A
359 four-parameter log-logistic model was fitted with the upper limit fixed at 1. Confidence
360 intervals were computed using the tfls method (t from logarithm scale).

361

362 # RESULTS

363 **Cattle follow up**

364 A mild reaction (agitation) was observed in most animals after the injection, regardless of
365 dose or LAIF administered (data not shown). There were no unscheduled cattle deaths, and no
366 clinical signs were observed in any of the treated groups throughout the study. The major
367 local reaction was swelling, which occurred in all animals, as expected with this LAIF
368 technology. Median weight gain throughout the study (i.e. 4 months) was unaffected by the

369 test item treatment (Kruskal-Wallis test, χ^2= 8.4892, df = 4, p-value = 0.075, Table 2). For
370 each experimental group, monthly weight measures are given in the Additional file 1, Table
371 S2.
372
373 **Ivermectin pharmacokinetics**
374 A total of 635 blood samples were collected, including 600 samples from LAIF-treated cattle
375 (4 treatment groups, 5 cattle per group, n=30 per cattle) and 35 samples from control cattle (1
376 control group, 5 cattle, n=7 per cattle). Concentration-time patterns were analyzed using data
377 until DAI = 132 for mdc-STM-001-0.6, mdc-STM-002-0.6 and DAI 133 for mdc-STM-003-
378 0.6 and mdc-STM-003-1.5. Ivermectin plasma concentrations were analyzed according to the
379 treatment (formulation and dose). Individual concentration-time profiles of ivermectin after
380 LAIFs injection are illustrated in Figure 1. The mean concentration-time profiles over the
381 study duration and for the first 7 days only to better visualize the burst (i.e., peak plasma
382 level), are provided in the Additional file 2: Fig. S1.
383 Ivermectin was not detected in any of the samples collected in the control group
384 during the study period nor in any samples collected before ivermectin injection in the treated
385 groups. Following sub-cutaneous injection, ivermectin plasma concentrations were generally
386 measured in all samples collected during the study, with quantifiable levels still present at the
387 final study time-point (*i.e.,* DAI 132 or 133).
388 After a single sub-cutaneous administration of LAIFs, the plasma levels of ivermectin
389 increased to reach the maximal level (*i.e.,* the burst), known to occur with LAIs [38]) at 24h
390 or 48h post-dose (median Tmax). This was followed by two phases of decrease: a first phase
391 until Day 56 (*i.e.,* 1344h post dose) then a slower phase with a regular decrease of ivermectin
392 plasma levels until the study end. Among the formulations, mdc-STM-001-0.6 showed the
393 lowest rate of decrease during the first phase and the steadiest levels during the second phase.

394 In contrast, the mdc-STM-002-0.6 exhibited the fastest decrease compared to other LAIFs.
395 The inter-animal variability in ivermectin plasma levels (expressed as coefficient of variation
396 CV%) was moderate. The lowest variability was seen with mdc-STM-001-0.6 (mean CV%:
397 41%, range: 12-70%), followed by mdc-STM-003-1.5 (mean: 54%, range: 22-87). The
398 highest variability was observed for mdc-STM-002-0.6 (mean CV%: 62%, range: 25-106%)
399 and mdc-STM-003-0.6 (mean: 72%, range: 34-98%).

400 The mean ivermectin PK parameters of each LAIF after injection are reported in Table
401 3. Additional parameters are given in the Additional file 1: Table S3.

402 At a dose of 0.6 mg/kg, PK differences emerged across formulations when considering
403 both the entire study duration or the 91-day period (*i.e.,* 3 months post-injection), which is the
404 best targeted release duration. These differences involved performance, quantification levels
405 over time, and inter-animal variability. The peak plasma concentration (Cmax) for mdc-STM-
406 001-0.6 was at least 30% lower compared to the other LAIFs, with mdc-STM-003-0.6
407 showing the highest Cmax. This suggests that mdc-STM-001-0.6 provided a safer burst
408 compared to the other LAIFs. The inter-animal variability in Cmax was moderate for all
409 formulations, with the lowest variability observed for mdc-STM-002-0.6 (CV% = 27%)
410 followed by mdc-STM-001-0.6 (CV%=36%) and mdc-STM-003-0.6 (CV%=43%). The
411 overall systemic exposure over DAI 91 ($AUC_{0\text{-}91d}$) was quite similar for all formulations, with
412 the lowest value for mdc-STM-002-0.6 and the highest value for mdc-STM-003-0.6. Inter-
413 animal variability in AUC was much lower for mdc-STM-001-0.6 (CV% = 12%), compared
414 to mdc-STM-002-0.6 (CV% = 42%) and mdc-STM-003-0.6 (CV = 66%). The mean
415 ivermectin plasma concentrations after each month post sub-cutaneous injection (C28d, C56d,
416 C91d, Clast) decreased slowly until the end of the study for all formulations, remaining close
417 to or above 3.5 ng/mL for mdc-STM-001-0.6 and mdc-STM-003-0.6, and around 1 ng/mL for
418 mdc-STM-002-0.6. There was moderate to high inter-animal variability, ranging from 53%

419 for mdc-STM-001-0.6 to 92% for mdc-STM-003-0.6. Additionally, the ratio of Cmax to the
420 level measured after each month (C28d, C56d, C91d) was up to 2-fold lower for mdc-STM-
421 001-0.6 compared to other LAIFs at the dose of 0.6 mg/kg.
422 For mdc-STM-003, both tested doses (0.6 and 1.5 mg/kg) exhibited a hypo-
423 proportional increase in Cmax and AUC, approximately 40% less than proportionality.
424 However, these findings should be considered cautiously due to the high inter-animal
425 variability observed, particularly at the low dose (Table 3 and Additional file1: Table S2).
426 Because the second phase of the PK profile was relatively flat, the apparent terminal
427 phase half-life (T1/2) was accurately estimated for only some animals, with a mean T1/2
428 ranging from 20 days for mdc-STM-003-1.5 to 33 days for mdc-STM-001-0.6. The remaining
429 amounts of ivermectin depot and copolymers were the lowest in animals treated with the mdc-
430 STM-001-0.6 formulation (Additional file tables S4 and S5).
431
432 **Mosquitocidal activity evaluation**
433 *Knock-down resistance allele (KDR-W) status of the VK5 mosquito lots*
434 Generations F2 to F8 of the VK5 colony were used in this experiment. Mosquito batches
435 exposed at DAI 2, 7, 17, 21, 28, and 42 consisted of 74–80% individuals exhibiting
436 pyrethroid-resistant phenotypes (i.e., homozygous or heterozygous for the KDR-W allele of
437 the voltage-gated sodium channel gene [42]). The remaining batches (exposed at DAI 56, 70,
438 84, 91, 98, 105, 112, 119 and 126) contained 50–54% resistant mosquitoes. Detailed qPCR
439 results by generation and DAI are given in Supplementary table S6.
440
441 *Blood feeding rate*
442 High blood-feeding rates were consistently observed across all time points for both KIS and
443 VK5 colonies (Table 4). No statistically significant differences in feeding success were

observed between treatment groups or formulations at any time point (data not shown), indicating uniform host attractiveness and feeding opportunity across experimental conditions.

Efficacy outcomes

The total number of mosquitoes monitored for survival by colony and treatment group is presented in Table 5. Their detailed distribution by individual cattle, cup, and day after injection (DAI) is provided in Supplementary Tables 6A (KIS) and 6B (VK5). Due to limited availability of KIS mosquitoes, no feeding assays were conducted at DAI 7 and DAI 28. Mosquito husbandry issues affected the same colony at DAI 105 and DAI 119; these data points were excluded from the analysis. Unexpected high mortality rates during the first 4 days post blood feeding (> 0.20) were detected for KIS mosquitoes fed on the control cattle B328 at DAI 42 (mortality rate = 0.437) and B321 at DAI 112 (mortality rate = 0.21). Therefore, these mosquito lots were excluded from efficacy analyses.

Mortality rates

The mdc-STM-001-0.6 showed the highest efficacy against KIS mosquitoes, comparable to mdc-STM-003-1.5 (Figure 2). From DAI 2 to approximately DAI 100, over 75% of KIS mosquitoes died within 10 days after feeding cattle treated with mdc-STM-001-0.6, and mortality rates remained above 50% beyond this period. For VK5 mosquitoes, mdc-STM-001-0.6 maintained ≥50% mortality between DAI 2 and 80, whereas other formulations at the dose of 0.6 mg/kg were less effective. The high-dose mdc-STM-003-1.5 achieved the longest duration of efficacy for VK5 mosquitoes, with mortality rates >50% from DAI 2 to 110.

468 Among all formulations, mdc-STM-001-0.6 exhibited the most consistent efficacy
469 profile. Mosquito mortality data for this LAIF showed the lowest inter-animal variability,
470 with fewer deviations from the mean trend across all sampling points.
471
472 Survival and Hazard Ratios
473 No significant differences in mosquito survival were observed across treatment groups prior
474 to LAIF administration for either KIS or VK5 colonies (Figure 3, Additional file 1: Table S8,
475 Additional file 2: Fig. S2 and S3), confirming no confounding effects from host variability.
476 Following treatment, all formulations administered at the dose of 0.6 mg/kg (mdc-STM-001,
477 mdc-STM-002, mdc-STM-003) and at 1.5 mg/kg (mdc-STM-003) induced a significant
478 mosquitocidal effect. The magnitude and duration of these effects varied according to
479 mosquito strain and formulation but remained statistically significant through the full follow-
480 up period (DAI 126).
481 The mdc-STM-002-0.6 formulation was clearly the least effective, with HRs ≥ 4 for
482 the shortest duration regardless of colony (Figure 3). It was therefore excluded from further
483 efficacy comparisons.
484 The mdc-STM-001-0.6 formulation induced sustained 10-days HR ≥ 4 through 70
485 days post-dose for both KIS and VK5 strains. The mdc-STM-003-06 display less sustained
486 efficacy, with HRs being superior to 4 at DAI 42 for both KIS and VK5 strains. The higher-
487 dose of the mdc-STM-003-1.5 showed a similar pattern to mdc-STM-001-0.6, with the
488 benefit of 10-day HRs >4 at an additional time point for KIS colony (DAI = 112).
489
490 Median survival times
491 Median survival times were consistently greater than 10 days for KIS and VK5 mosquitoes
492 fed on control calves (Figure 4) for all the DAI tested.

493 For KIS mosquitoes, median survival times remained consistently below 10 days
494 throughout the entire follow-up when fed on cattle treated with mdc-STM-001-0.6, mdc-
495 STM-003-0.6 and mdc-STM-003-1.5. In contrast, mdc-STM-002-0.6, induced median
496 survival times equal to or greater than the 10-day threshold from DAI 70 onward.
497 For VK5 mosquitoes, median survival times remained ≤ 10 days up to 91 days post-treatment,
498 and again at DAI 126, when exposed to cattle treated with mdc-STM-001-0.6, mdc-STM-
499 003-0.6, and mdc-STM-003-1.5 (Figure 4). The LAIF mdc-STM-002-0.6 maintained median
500 survival times below or equal to the EIP threshold for only 40 days post-dose.
501 Notably, mdc-STM-001-0.6 and mdc-STM-003-1.5 induced particularly short survival
502 durations, with median survival times <4 days for nearly 100 days in KIS mosquitoes and
503 approximately 60 days in VK5.
504
505 Lethal concentrations and coverage duration
506 Concentration/response relationships were examined for each colony and for each LAIF. No
507 significant differences in LC50 values were found between LAIFs, regardless of the mosquito
508 colony (Additional file 1: Tables S9–S12; Additional file 2: Fig. S4 and S5). A unified
509 concentration–response model, including mosquito colony as explanatory variable was thus
510 developed using the full dataset (Figure 5).
511 A significant difference in cumulative mortalities between KIS and VK5 was observed
512 in relation to ivermectin concentrations, with 10-day LC50 values lower for KIS than for VK5
513 (mean LC50 (95% CI) for KIS = 1.51 [1.1-2.07], for VK5 = 3.66 [2.69-4.97], t-value = -
514 6.3187, *P*-value < 0.0001). Ten-day LC50 values sufficient to kill KIS and VK5 mosquitoes
515 are sustained for the whole experiment duration for KIS and for more than 2 months for VK5,
516 except for the mdc-STM-002 formulation (table 6). Values for 4-day and 10-day LC50 and
517 LC90 are given in the Additional file1: Table S13.

518 Inter-animal variability was also assessed, as it directly informs the coverage potential
519 of each formulation, *i.e.* the proportion of treated animals maintaining mosquitocidal
520 ivermectin plasma concentrations above the LC50 over time. The mdc-STM-001-0.6 achieved
521 100% coverage of VK5 10-day LC50 for a duration of at least 2 months post-dose (data not
522 shown).
523

524 **Discussion**

525 This study represents the first evaluation of candidate long-acting injectable formulations of
526 ivermectin specifically developed for human use in malaria control. These formulations are
527 issued from unique long lasting delivery technology and are designed to meet, with a single
528 subcutaneous injection, the World Health Organization's minimum target product profile for
529 endectocides against malaria: achieving, with a single administration, at least one month of
530 efficacy, defined by HR > 4 for *Plasmodium* vectors survival. The primary objective of this
531 work was to identify the most promising formulation for further development, including
532 progression to Phase 1 clinical trial. To guide formulation selection, we employed a
533 comprehensive approach integrating multiple, complementary criteria related to
534 mosquitocidal efficacy, pharmacokinetic characteristics, and inter-host variability, all critical
535 for achieving effective and sustained vector control in real-world conditions and settings. The
536 mdc-STM-001 formulation met all the predefined criteria and was selected as the optimal
537 candidate for further evaluation in humans, based on the following key positive outcomes: i)
538 good local and systemic tolerability during and after injection, with no abnormal variation in
539 body weight; ii) optimal control of the initial burst, preventing systemic reactions during the
540 early post-injection period; iii) most suitable ivermectin PK and PD profiles, achieving LC50
541 levels over the target duration, resulting in the highest sustained mosquito mortality across
542 both insecticide susceptible and resistant *Anopheles* strains; iv) the lowest inter-host

543 variability in ivermectin exposure; v) appropriate clearance beyond the predefined release
544 duration, avoiding unnecessary systemic exposure.
545 In both magnitude and duration, the mosquitocidal efficacy of a single subcutaneous
546 injection of the mdc-STM-001 formulation is compelling. It provides efficacy durations of at
547 least 2 months, at least four times longer than those achieved with either gastric-resident or
548 conventional oral formulations, regardless of the dosing regimen considered. A sustained
549 reduction in mosquito survival rate lasting over two months is the factor expected to most
550 strongly and negatively influence vectorial capacity [52]. Furthermore, by consistently
551 shortening mosquito lifespan below the 10-day EIP, the mdc-STM-001 formulation has strong
552 potential to dramatically reduce the proportion of infectious mosquitoes, and therefore malaria
553 transmission, across most epidemiological contexts.
554
555 **Strengths and limitations of the experimental model for extrapolation to humans**
556 We acknowledge the limitation of extrapolating human pharmacokinetic and
557 pharmacodynamic parameters from data obtained following cattle treatment with the MDC-
558 STM-001, as plasma concentration dynamics can vary substantially across species [53].
559 Because ivermectin is a highly lipophilic compound, body fat content, which also varies
560 among species, represents a key determinant of its distribution within the organism [26].
561 Ivermectin tends to accumulate in adipose and dermal tissues surrounding capillaries [54]
562 from which mosquitoes obtain their blood meals. This may potentially lead to higher
563 ivermectin concentrations being ingested by mosquitoes than those measured in venous
564 plasma and used for PK/PD analysis [55]. Indeed, a clinical study reported ivermectin
565 concentrations to be approximately 1.33-fold higher in capillary than in venous blood [56]
566 even though this difference did not translate into measurable differences in mosquito
567 mortality. In addition, cattle-derived secondary metabolites may also modulate the

568 mosquitocidal effects of long-acting formulations, as has been suggested in humans [57] with
569 possible species-specific differences in both the nature and kinetics of these metabolites
570 (manuscript in preparation). For all the above reasons valid whatever the route of
571 administration of ivermectin, extrapolation of PK and PD data from cattle to humans must be
572 approached with caution and venous plasma ivermectin concentrations should be regarded
573 only as proxies for the actual levels of ivermectin and its metabolites ingested by mosquitoes.
574 Nevertheless, it is noteworthy that in a phase 1 human trial using oral ivermectin, the venous
575 plasma 10-day LC_{50} values lethal to *An. gambiae s.s.* fell within the same range as those
576 obtained in the present study [26,58].

577 Although our experimental cattle model, like any preclinical animal system, is subject
578 to criticism regarding pharmacokinetic extrapolations to humans, it was deliberately selected
579 to capture field relevant *Anopheles gambiae s.l.* fitness and ivermectin mosquitocidal effects
580 since we used mosquito lots derived from a recently founded colony from Bobo Dioulasso
581 surroundings. Indeed, throughout the Sudano-Sahelian zone, *Anopheles* mosquitoes
582 frequently feed on domestic animals, particularly calves, which represent major blood-meal
583 sources in areas of high bed net use [59,60]. Mosquitoes feeding on calves exhibit survival
584 rates and median lifespans comparable to those feeding on human [61], reinforcing the
585 biological relevance of this model. In addition, direct skin-feeding experiments on Burkinabé
586 local calves are highly practical and result in consistently high feeding success, as these
587 animals are natural alternative hosts for major malaria vectors. Our model therefore allows to
588 maximize the proportion of blood-fed mosquitoes and to reflect their true post-feeding
589 survival potential after feeding on treated or control hosts. Collectively, these parameters
590 provide robust and field transposable meaningful entomological endpoints that are essential
591 for predicting the performance of the long-acting ivermectin formulation in real settings.
592

593 **Mosquito resistance to ivermectin and mitigation plans**

594 Resistance to ivermectin exists in different arthropods species [62,63]. Equally to oral
595 formulation multiple dosing regimen, prolonged systemic exposure to ivermectin following
596 treatment with the LAIF may exert selective pressure on resistance mechanisms, not only on
597 its intended targets, human endoparasites, but also on *Anopheles* mosquitoes and other
598 ectoparasites. Pyrethroids and ivermectin cross-resistance has been shown in permethrin
599 resistant *Aedes aegypti* colonies [64]. Given the distinct molecular targets of these two
600 compound classes, the role of metabolic resistance pathways has been hypothesized. These
601 mechanisms often involve the overexpression of detoxification enzymes such as cytochrome
602 P450s and xenobiotic efflux pumps [65,66]. Supporting this, dual inhibition of P450s and
603 xenobiotic transporters was found to significantly increase ivermectin susceptibility in *An.*
604 *gambiae*, highlighting detoxification pathways as a credible resistance route [67]. Ivermectin
605 resistance in *Anopheles gambiae* could also arise due to existing alternative splicing of the
606 gene coding for the GluCl channel, producing protein variants with differing sensitivities to
607 ivermectin [68], which suggests that regulatory changes in splicing may contribute to reduced
608 susceptibility.

609 Our findings reveal clear differences in ivermectin susceptibility between mosquito
610 strains: the VK5 colony from *An. coluzzii* required higher plasma lethal concentrations than
611 the fully susceptible *An. gambiae* KIS strain, suggesting the presence of tolerance
612 mechanisms in VK5. Differences in susceptibility to ivermectin has been already shown
613 among different *Anopheles* species [22,69,70]. In this study, we monitored the frequency of
614 pyrethroid-resistance alleles in the VK5 mosquitoes lots, which was high throughout the
615 experiment; however, further individual-level characterization will be needed to determine
616 whether a direct association, if any, exists between pyrethroid-resistance genotypes and
617 ivermectin tolerance in VK5. Beyond metabolic processes, factors such as distinct genetic

backgrounds or morphological traits such as body size, may contribute to an intertwined tolerance phenotype in which multiple mechanisms interact, making it difficult to disentangle their respective roles.

The risk of selecting ivermectin resistant *Anopheles* populations during mass treatments campaigns does indeed exist, and could compromise sustained success of ivermectin-based malaria vector control strategies. In addition to physiological resistance, behavioral adaptations may further reduce the effectiveness of such interventions. Prolonged ivermectin exposure could also select for more zoophagic or opportunistic mosquito populations that preferentially feed on untreated animals or humans, thereby circumventing the drug's effects and diminishing overall intervention impact. Nonetheless, current MDA protocols for ivermectin include exclusion criteria that prevent treatment of pregnant or lactating women and children under 15 kg. Women of child bearing potential might further be excluded in case long-acting formulation deployment. These untreated subpopulations can act as genetic refuges, reservoirs of susceptible genotypes, which help maintain genetic diversity in vector and parasite populations and reduce the overall selection pressure for resistance [40,71,72]. Such refuges could contribute to the long-term sustainability of ivermectin-based interventions.

To preserve efficacy, it is critical that implementation strategies incorporate routine surveillance of both behavioral and physiological resistance [71]. However, there is currently no established and standardized assay for detecting ivermectin resistance in mosquitoes. While molecular markers will be instrumental in tracking the emergence and spread of resistance alleles, complementary phenotypic assays are urgently needed to detect functional resistance in the field. Traditional phenotypic methods rely on mosquitoes ingesting ivermectin via membrane feeding or direct feeding on treated hosts. These approaches, however, face practical limitations, membrane feeding often results in low blood uptake by

643 mosquitoes, and direct feeding raises ethical concerns. A promising alternative involves
644 delivering ivermectin-laced blood using filter papers [73].
645
646 **Coverage of blood sources**
647 The residence time of ivermectin at efficient mosquitocidal concentrations in human blood is
648 the principal determinant of its epidemiological impact, together with the proportion of the
649 population exposed at such concentrations [74]. This has been demonstrated using modeling
650 studies [30,75]. The target population for standard oral ivermectin administration excludes
651 pregnant women, nursing mothers, and children weighing under 15 kg [76,77], whereas the
652 prolonged duration of action of mdc-STM-001 may also necessitate excluding women of
653 childbearing potential to avoid fetal exposure. This would markedly reduce the target
654 population size and could therefore limit overall effectiveness. However, using simulated data
655 from theoretical formulations with constant efficacy durations of 14 to 90 days, models
656 demonstrate that, combined with SMC, longer lasting effects could lead to significant
657 decrease in malaria burden despite the reduced target population [78]. Further mathematical
658 modeling, building on previous work [79], is ongoing using the present data to explore the
659 potential of mdc-STM-001 under different implementation scenarios [80].
660 The specific safety concern regarding the inclusion of women of childbearing
661 potential, primarily related to possible fetal toxicity, is debated [81], as is the inclusion of
662 children weighing less than 15 kg [82]. Addressing these important matters will enable
663 accurate projections for future study designs and implementation strategies.
664 As already mentioned above, the effectiveness of long-acting ivermectin interventions
665 will strongly depend on achieving sufficient coverage of the blood sources available to
666 *Anopheles* mosquitoes, both human and animal. Detailed knowledge of mosquito bionomics
667 within potential implementation zones is therefore critical to success [36], particularly since

668 large regions of high malaria burden correspond to the Sudano-Sahelian belt, where vectors
669 are highly opportunistic in their feeding behaviour and often use domestic animals as
670 alternative blood sources [59,60]. In such settings, treating livestock with endectocidal
671 compounds has been proposed as a promising complementary strategy [83]. Commercial
672 veterinary injectable formulations have demonstrated efficacy during up to 1-2 months
673 against Asian vectors in buffalo and cattle, respectively [69]. A veterinary prototype long-
674 lasting formulation from the BEPO® technology extended this efficacy over 6 months for *An.*
675 *coluzzii* [40], while silicone implants achieved comparable results [84]. Beyond enhancing
676 impact on vector populations, targeting both human and animal hosts could also contribute to
677 mitigating the risk of ivermectin resistance if a distinct endectocide were used in animals.

678 **Acceptability and adherence**

679 The deployment of a long-acting injectable ivermectin formulation for malaria vector
680 control would represent an innovative addition to the portfolio of community-based malaria
681 interventions. Like other public health strategies relying on high population coverage, such as
682 vaccination programs, anti-helminthic MDAs, or malaria vector control campaigns, its
683 success will depend on strong acceptability and adherence. Conceptually aligned with
684 transmission-blocking vaccines (TBVs), the LAIF acts primarily through community
685 protection. Regarding the molecule, evidence from decades of oral ivermectin MDAs for
686 filarial control demonstrates high community acceptability, often above 90%, although
687 adherence tends to vary across rounds [85]. The same trend is observed when ivermectin is
688 proposed as a vector control tool against malaria [86]. Similarly, the 4 dose injectable vaccine
689 RTS,S/AS01 have achieved strong community acceptance (80–95%) when supported by
690 effective communication and community engagement [87,88]. Acceptability studies for TBV
691 concepts have also reported willingness- to-receive rates above 90% in high-burden settings
692 [89]. For the long-acting injectable ivermectin formulation, the anticipated subcutaneous

693 injection volumes (up to a maximum of 25 µL/kg) is relatively small, suggesting minimal
694 practical or acceptability constraints for large-scale implementation. Importantly, unlike
695 TBVs, an LAIF ivermectin intervention could also provide direct individual health benefits by
696 preventing or treating parasitic infections traditionally controlled by ivermectin, while
697 simultaneously reducing malaria transmission. Additional collateral benefits from ivermectin
698 MDAs against malaria have recently been reported, including reductions in childhood anemia
699 [35] and decreases in jigger infestations and bedbug nuisances [90]. This dual individual and
700 community benefit may substantially enhance perceived value, acceptability, and adherence
701 compared to TBVs. By combining infrequent dosing, a familiar and trusted product with a
702 well-known safety profile [91], and a clear community rationale, ivermectin MDA using
703 LAIF could become a socially acceptable and operationally feasible complement to existing
704 malaria control and vaccination platforms. Furthermore, such strategy could leverage the
705 logistical infrastructure and community trust established through vaccination programs,
706 facilitating delivery, adherence monitoring, and social acceptance.
707

708 **Conclusion**

709 This study demonstrates that among the three long-acting injectable ivermectin formulations
710 developed for human use, the mdc-STM-001 formulation exhibits the most favorable
711 combination of pharmacokinetic properties, pharmacodynamic efficacy, and tolerability in a
712 cattle-mosquito model. A single subcutaneous administration of mdc-STM-001 achieved
713 sustained mosquitocidal concentrations over two months, inducing high mortality in both
714 pyrethroid-susceptible and -resistant *Anopheles* strains, thereby meeting and exceeding the
715 WHO-defined minimum criteria for endectocides targeting malaria vectors. These findings
716 support the potential of mdc-STM-001 as a promising tool to complement existing vector
717 control strategies, particularly in areas with behavioral or physiological resistance to

conventional insecticides. While extrapolation from cattle to humans warrants caution, the observed pharmacological profile and robust entomological endpoints provide a strong rationale for advancing this formulation into Phase 1 clinical trials. Safety will remain a key consideration given the long-lasting systemic exposure; although the multiple administrations of high oral dose are safe which is supporting a favorable risk–benefit perspective for further human evaluation. The formulation will also retain the positive collateral benefits observed with oral ivermectin, such as reductions in parasitic infections, childhood anemia, and ectoparasite nuisances, underscoring the value of mdc-STM-001 as both a community- and individual-level intervention, potentially further enhancing community acceptability and adherence. Future investigations should leverage modeling approaches to optimize dosing regimens and explore synergistic integration with other vector control strategies, including seasonal malaria chemoprevention, long-lasting insecticidal nets, indoor residual spraying, or livestock-targeted endectocide interventions. Such models will also help anticipate the population coverage and logistical requirements needed to maximize epidemiological impact while ensuring operational feasibility. Given the potential for physiological or behavioral resistance to emerge, continued studies on mitigating resistance dynamics in vector populations will be essential to ensure sustainable efficacy. Overall, ivermectin MDA using the mdc-STM-001 long-acting injectable formulation based on BEPO® technology represents a potentially transformative adjunct to existing malaria control measures, with the capacity to reduce transmission by targeting both indoor- and outdoor-biting vectors over extended periods.

Supplementary information

Additional Tables. Additional file 1: **Table S1.** Injected volumes (mL) per animal and per formulation. **Table S2.** Median weight (Q1, Q3) in kg for each experimental cattle group at

743 each weighing date. **Table S3**. Additional Mean (SD) pharmacokinetic parameters of
744 ivermectin in cattle plasma. **Table S4**. Ivermectin extraction results in each depot collected at
745 study end. **Table S5.** Copolymers extraction results in each depot collected at study end.
746 **Table S6.** Number of mosquitoes homozygous or heterozygous for the mutated KDR-W
747 allele. Tables S7A and S7B. Number of KIS (A) and VK5 (B) blood fed mosquitoes followed
748 for their survival throughout the experiment. **Tables S8.** Hazard ratios (HRs), z-values, and
749 associated p-values derived from the Cox proportional hazards models for KIS and VK5
750 mosquitoes. **Tables S9-S12**: between formulations comparisons of lethal concentrations.
751 **Table S13.** Lethal concentration (LC50) for KIS and VK5 mosquitoes over 4-day follow up
752 period (4-day LC50).
753 Additional Figures. Additional file 2: **Fig. S1.** Additional mean concentration–time profiles of
754 ivermectin in cattle plasma. **Fig. S2.** Kaplan-Meier plots. **Fig. S3.** 4-day cumulative mortality
755 of KIS and VK5. **Fig. S4.** Heat maps illustrating the lower 95% confidence limit (LCL) of 4-day
756 and 30-day mortality hazard ratios (HRs) for KIS and VK5 colony mosquitoes. **Fig. S5.**
757 Relationship between ivermectin plasma concentration and 4-days (A and B) or 10-day (C
758 and D) cumulative mortality for each candidate formulation. Fig. S6. Relationship between
759 ivermectin plasma concentrations and 4-day cumulative mosquito mortality for KIS and VK5
760 colonies.
761

762 ## Abbreviations

763 CIRDES: Centre international de recherche-développement sur l'élevage en zones
764 subhumides; DAI: Day after injection; IRSS: Institut de Recherche en Sciences de la Santé;
765 K2-EDTA: Dipotassium Ethylenediaminetetraacetic acid; KDR: Knock Down Resistance;
766 LAIF: Long acting ivermectin formulation; LC: Lethal concentration; LC-MS/MS: Liquid
767 Chromatography – Tandem Mass Spectrometry; MDA: Mass drug administration; PD:

768 Pharmacodynamic; PK: Pharmacokinetic; qPCR: Quantitative polymerase chain reaction;
769 RCT: Randomized controlled trial; SMC: Seasonal malaria chemoprevention; TBV:
770 Transmission blocking vaccines; WHO: World health organization
771
772
773 ## Declarations

774 **Acknowledgements**

775 We are particularly grateful to Boly Saïdou for his dedicated care of the animals in the stable
776 and for his technical support during animal-related procedures. We thank Kambou Nourou
777 Ramzi for his valuable help in mosquito husbandry and for the assistance provided during the
778 experiments. We are also grateful to Da Firmin for his assistance with insectary husbandry,
779 and to Da Firmin and Millogo Léopold for their technical assistance during the molecular
780 work. We would like to thank the two anonymous reviewers for their advice and constructive
781 comments, which greatly improved our manuscript.
782
783 **Funding**

784 This study was funded by UNITAID under the IMPACT project consortium.
785

786 **Availability of data and material**

787 The datasets and code used for the analyses and figure generation reported in this study are
788 publicly available at https://doi.org/10.23708/PSJMLM
789
790 **Authors' contribution**

791 LZ: Investigation, preliminary statistical analysis, writing original draft. SHP, KM, NM, CP,
792 KRD, SLD: Methodology and protocol design. SHP: Supervision and coordination of
793 experimental work at CIRDES, conceptualisation. COW: Investigation, editing. AFS:
794 Supervision of COW, editing. AS: Molecular biology coordination and writing, editing. AP:

Formal analysis, data curation, and validation. SB: Visualization. SLD: Pharmacokinetic coordination, writing, and review. KM: Supervision, conceptualization, funding acquisition, administrative management, and writing, review and editing. CR: Conceptualization and funding acquisition. AMGB: Supervision and mentoring of LZ (thesis director). G-KD: Resources and administrative management. All authors read and approved the final manuscript.

Ethics approval

This study is part of IMPACT project that got approval from the CIRDES ethics committee under letter no. 004-10/2020 CEB-CIRDES. The study involving animals has been conducted ethically following the governance of animal care and use for scientific purposes in Africa and the Middle East [92].

Consent for publication

Not applicable

Competing interests

All authors and contributors currently or formerly employed at Medincell S.A. did not influence data collection, data analysis, manuscript writing, decision to publish, or conclusions presented in this paper. The other authors are not shareholders of Medincell S.A. and declare no competing interests.

References

1. World malaria report 2024 [Internet]. [cited 2025 Mar 22]. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024. Accessed 22 Mar 2025

2. World malaria report 2015 [Internet]. [cited 2025 Nov 5]. https://www.who.int/publications/i/item/9789241565158. Accessed 5 Nov 2025

3. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on \emphPlasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207–11.

4. World malaria report 2017 [Internet]. [cited 2025 Nov 5]. https://www.who.int/publications/i/item/9789241565523. Accessed 5 Nov 2025

5. Ranson H, Lissenden N. Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. Trends in Parasitology. Elsevier; 2016;32:187–96. https://doi.org/10.1016/j.pt.2015.11.010

6. Riveron Miranda J, Tchouakui M, Mugenzi L, Menze B, Chiang M-C, Wondji C. Insecticide Resistance in Malaria Vectors: An Update at a Global Scale. 2018. https://doi.org/10.5772/intechopen.78375

7. Carrasco D, Lefèvre T, Moiroux N, Pennetier C, Chandre F, Cohuet A. Behavioural adaptations of mosquito vectors to insecticide control. Current Opinion in Insect Science. 2019;34:48–54. https://doi.org/10.1016/j.cois.2019.03.005

8. Martin JL, Mosha FW, Lukole E, Rowland M, Todd J, Charlwood JD, et al. Personal protection with PBO-pyrethroid synergist-treated nets after 2 years of household use against pyrethroid-resistant *Anopheles* in Tanzania. Parasit Vectors. 2021;14:150. https://doi.org/10.1186/s13071-021-04641-5

9. Accrombessi M, Cook J, Dangbenon E, Yovogan B, Akpovi H, Sovi A, et al. Efficacy of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) and chlorfenapyr-pyrethroid LLINs compared with pyrethroid-only LLINs for malaria control in Benin: a cluster-randomised, superiority trial. The Lancet. 2023;401:435–46. https://doi.org/10.1016/S0140-6736(22)02319-4

10. WHO guidelines for malaria [Internet]. [cited 2025 Nov 5]. https://www.who.int/publications/i/item/guidelines-for-malaria. Accessed 5 Nov 2025

11. Sangbakembi-Ngounou C, Costantini C, Longo-Pendy NM, Ngoagouni C, Akone-Ella O, Rahola N, et al. Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be “out of control.” Proceedings of the National Academy of Sciences. Proceedings of the National Academy of Sciences; 2022;119:e2104282119. https://doi.org/10.1073/pnas.2104282119

12. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, et al. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. J Infect Dis. 2012;206:1622–9. https://doi.org/10.1093/infdis/jis565

13. Takken W, Charlwood D, Lindsay SW. The behaviour of adult *Anopheles gambiae*, sub-Saharan Africa's principal malaria vector, and its relevance to malaria control: a review. Malaria Journal. 2024;23:161. https://doi.org/10.1186/s12936-024-04982-3

14. Roadmappers TI. A Roadmap for the Development of Ivermectin as a Complementary Malaria Vector Control Tool. The American Journal of Tropical Medicine and Hygiene. The American Society of Tropical Medicine and Hygiene; 2020;102:3–24. https://doi.org/10.4269/ajtmh.19-0620

15. Foy BD, Kobylinski KC, da Silva IM, Rasgon JL, Sylla M. Endectocides for malaria control. Trends Parasitol. 2011;27:423–8. https://doi.org/10.1016/j.pt.2011.05.007

16. Macdonald G. The Epidemiology and Control of Malaria. Oxford University Press. 1957.

17. Campbell WC, Benz GW. Ivermectin: a review of efficacy and safety. Journal of Veterinary Pharmacology and Therapeutics. 1984;7:1–16. https://doi.org/10.1111/j.1365-2885.1984.tb00872.x

18. Campbell WC, editor. Ivermectin and Abamectin [Internet]. New York, NY: Springer; 1989 [cited 2025 Mar 22]. https://doi.org/10.1007/978-1-4612-3626-9

19. Maia M, Kobylinski K. Ivermectin for the Control and Elimination of Malaria. Practical Control of Mosquitoes Disease Vectors [Internet]. 2025 [cited 2025 Mar 22]. p. 139–65. https://doi.org/10.1079/9781789248845.0007

20. Mekuriaw W, Balkew M, Messenger LA, Yewhalaw D, Woyessa A, Massebo F. The effect of ivermectin((R)) on fertility, fecundity and mortality of *Anopheles arabiensis* fed on treated men in Ethiopia. Malar J. 2019;18:357. https://doi.org/10.1186/s12936-019-2988-3

21. Pooda SH, Hien DF de S, Pagabeleguem S, Heinrich AP, Porciani A, Sagna AB, et al. Impact of blood meals taken on ivermectin-treated livestock on survival and egg production of the malaria vector *Anopheles coluzzii* under laboratory conditions. PLoS One. 2024;19:e0308293. https://doi.org/10.1371/journal.pone.0308293

22. Kobylinski KC, Deus KM, Butters MP, Hongyu T, Gray M, da Silva IM, et al. The effect of oral anthelmintics on the survivorship and re-feeding frequency of anthropophilic mosquito disease vectors. Acta Trop. 2010;116:119–26. https://doi.org/10.1016/j.actatropica.2010.06.001

23. Sampaio V de S, Rivas GB da S, Kobylinski K, Pinilla YT, Pimenta PFP, Lima JBP, et al. What does not kill it makes it weaker: effects of sub-lethal concentrations of ivermectin on

887 the locomotor activity of *Anopheles aquasalis*. Parasites & Vectors. 2017;10:623.
888 https://doi.org/10.1186/s13071-017-2563-0
889 24. Pinilla YT, S CPL, V SS, Andrade FS, Melo GC, Orfano AS, et al. Promising approach to
890 reducing Malaria transmission by ivermectin: Sporontocidal effect against Plasmodium vivax
891 in the South American vectors *Anopheles aquasalis* and *Anopheles darlingi*. PLoS Negl Trop
892 Dis. 2018;12:e0006221. https://doi.org/10.1371/journal.pntd.0006221
893 25. Kobylinski KC, Foy BD, Richardson JH. Ivermectin inhibits the sporogony of
894 Plasmodium falciparum in *Anopheles gambiae*. Malar J. 2012;11:381.
895 https://doi.org/10.1186/1475-2875-11-381
896 26. Ouedraogo AL, Bastiaens GJ, Tiono AB, Guelbeogo WM, Kobylinski KC, Ouedraogo A,
897 et al. Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria
898 transmission after treatment: a double-blind, randomized, clinical trial. Clin Infect Dis.
899 2015;60:357–65. https://doi.org/10.1093/cid/ciu797
900 27. Foy BD, Alout H, Seaman JA, Rao S, Magalhaes T, Wade M, et al. Efficacy and risk of
901 harms of repeat ivermectin mass drug administrations for control of malaria (RIMDAMAL): a
902 cluster-randomised trial. Lancet. 2019;393:1517–26. https://doi.org/10.1016/S0140-
903 6736(18)32321-3
904 28. Smit MR, Ochomo EO, Aljayyoussi G, Kwambai TK, Abong'o BO, Chen T, et al. Safety
905 and mosquitocidal efficacy of high-dose ivermectin when co-administered with
906 dihydroartemisinin-piperaquine in Kenyan adults with uncomplicated malaria (IVERMAL): a
907 randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2018;18:615–26.
908 https://doi.org/10.1016/S1473-3099(18)30163-4
909 29. Kamau Y, Tuwei M, Wanjiku C, Ominde K, Ngama M, Karisa J, et al. Mosquitocidal
910 efficacy and pharmacokinetics of single-dose ivermectin versus three-day dose regimen for
911 malaria vector control compared with albendazole and no treatment: An open-label
912 randomized controlled trial. International Journal of Infectious Diseases [Internet]. Elsevier;
913 2024 [cited 2025 Mar 22];148. https://doi.org/10.1016/j.ijid.2024.107236
914 30. Slater HC, Foy BD, Kobylinski K, Chaccour C, Watson OJ, Hellewell J, et al. Ivermectin
915 as a novel complementary malaria control tool to reduce incidence and prevalence: a
916 modelling study. The Lancet Infectious Diseases. Elsevier; 2020;20:498–508.
917 https://doi.org/10.1016/S1473-3099(19)30633-4
918 31. Chaccour C, Casellas A, Hammann F, Ruiz-Castillo P, Nicolas P, Montaña J, et al.
919 BOHEMIA: Broad One Health Endectocide-based Malaria Intervention in Africa—a phase
920 III cluster-randomized, open-label, clinical trial to study the safety and efficacy of ivermectin

921 mass drug administration to reduce malaria transmission in two African settings. Trials.
922 2023;24:128. https://doi.org/10.1186/s13063-023-07098-2
923 32. Chaccour C, Maia M, Kariuki M, Ruiz-Castillo P, Wanjiku C, Kasiwa L, et al. Ivermectin
924 to Control Malaria — A Cluster-Randomized Trial. N Engl J Med. 2025;393:362–75.
925 https://doi.org/10.1056/NEJMoa2411262
926 33. Chaccour C, Nicolas P, Martinho S, Mundaca H, Elobolobo E, Ruiz-Castillo P, et al.
927 Ivermectin to Reduce Malaria Transmission - Safety and Efficacy Results from the
928 BOHEMIA Cluster Randomized Trial in Mozambique [Internet]. Rochester, NY: Social
929 Science Research Network; 2025 [cited 2025 Oct 31]. https://doi.org/10.2139/ssrn.5264477
930 34. Hutchins H, Pretorius E, Bradley J, Silva ET da, Vasileva H, Ndiath MO, et al. Adjunctive
931 ivermectin mass drug administration for malaria control on the Bijagos Archipelago of
932 Guinea-Bissau (MATAMAL): a quadruple-blinded, cluster-randomised, placebo-controlled
933 trial. The Lancet Infectious Diseases [Internet]. Elsevier; 2024 [cited 2025 Mar 22];0.
934 https://doi.org/10.1016/S1473-3099(24)00580-2
935 35. Somé AF, Somé A, Sougué E, Ouédraogo COW, Da O, Dah SR, et al. Safety and efficacy
936 of repeat ivermectin mass drug administrations for malaria control (RIMDAMAL II): a phase
937 3, double-blind, placebo-controlled, cluster-randomised, parallel-group trial. The Lancet
938 Infectious Diseases [Internet]. Elsevier; 2025 [cited 2025 Mar 22];0.
939 https://doi.org/10.1016/S1473-3099(24)00751-5
940 36. Mouline K, Costantini C. Is ivermectin surviving expectations in residual malaria control?
941 Lancet Infect Dis. 2024;S1473-3099(24)00652-2. https://doi.org/10.1016/S1473-
942 3099(24)00652-2
943 37. Bellinger AM, Jafari M, Grant TM, Zhang S, Slater HC, Wenger EA, et al. Oral, ultra-
944 long-lasting drug delivery: Application toward malaria elimination goals. Sci Transl Med.
945 2016;8:365ra157. https://doi.org/10.1126/scitranslmed.aag2374
946 38. Roberge C, Cros J-M, Serindoux J, Cagnon M-E, Samuel R, Vrlinic T, et al. BEPO®:
947 Bioresorbable diblock mPEG-PDLLA and triblock PDLLA-PEG-PDLLA based in situ
948 forming depots with flexible drug delivery kinetics modulation. J Control Release.
949 2020;319:416–27. https://doi.org/10.1016/j.jconrel.2020.01.022
950 39. Endectocide and ectocide products for malaria transmission control [Internet]. [cited 2022
951 July 12]. https://www.who.int/publications/i/item/9789240052512. Accessed 12 July 2022
952 40. Heinrich AP, Pooda SH, Porciani A, Zéla L, Schinzel A, Moiroux N, et al. An
953 ecotoxicological view on malaria vector control with ivermectin-treated cattle. Nat Sustain.
954 Nature Publishing Group; 2024;7:724–36. https://doi.org/10.1038/s41893-024-01332-8

41. Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J. 2008;7:163. https://doi.org/10.1186/1475-2875-7-163

42. Bass C, Nikou D, Donnelly MJ, Williamson MS, Ranson H, Ball A, et al. Detection of knockdown resistance (kdr) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods. Malar J. 2007;6:111. https://doi.org/10.1186/1475-2875-6-111

43. Toé KH, Jones CM, N'Fale S, Ismail HM, Dabiré RK, Ranson H. Increased Pyrethroid Resistance in Malaria Vectors and Decreased Bed Net Effectiveness, Burkina Faso. Emerg Infect Dis. 2014;20:1691–6. https://doi.org/10.3201/eid2010.140619

44. R: The R Project for Statistical Computing [Internet]. [cited 2024 Jan 25]. https://www.r-project.org/. Accessed 25 Jan 2024

45. Lüdecke D, Ben-Shachar M, Patil I, Waggoner P, Makowski D. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. JOSS. 2021;6:3139. https://doi.org/10.21105/joss.03139

46. Hartig F. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.3.3.0. 2020.

47. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. Scandinavian Journal of Statistics. [Board of the Foundation of the Scandinavian Journal of Statistics, Wiley]; 1979;6:65–70.

48. McGillycuddy M, Popovic G, Bolker BM, Warton DI. Parsimoniously Fitting Large Multivariate Random Effects in glmmTMB. Journal of Statistical Software. 2025;112:1–19. https://doi.org/10.18637/jss.v112.i01

49. Duvallet G, Fontenille D, Robert V, editors. Entomologie médicale et vétérinaire. IRD Editions; 2017.

50. Therneau TM. coxme: Mixed Effects Cox Models [Internet]. 2024 [cited 2025 May 21]. https://cran.r-project.org/web/packages/coxme/index.html. Accessed 21 May 2025

51. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. Xia Y, editor. PLoS ONE. 2015;10:e0146021. https://doi.org/10.1371/journal.pone.0146021

52. Macdonald G. The Epidemiology and Control of Malaria. Amen House, Warwick Square, London E.C.4.: Oxford University Press; 1957.

53. Takano K, de Hayr L, Carver S, Harvey RJ, Mounsey KE. Pharmacokinetic and pharmacodynamic considerations for treating sarcoptic mange with cross-relevance to

988 Australian wildlife. Int J Parasitol Drugs Drug Resist. 2023;21:97–113.
989 https://doi.org/10.1016/j.ijpddr.2023.02.004
990 54. Baraka OZ, Mahmoud BM, Marschke CK, Geary TG, Homeida MMA, Williams JF.
991 Ivermectin distribution in the plasma and tissues of patients infected with Onchocerca
992 volvulus. E J Clin Pharmacol. 1996;50:407–10. https://doi.org/10.1007/s002280050131
993 55. Chaccour C, Hammann F, Rabinovich NR. Ivermectin to reduce malaria transmission I.
994 Pharmacokinetic and pharmacodynamic considerations regarding efficacy and safety. Malaria
995 Journal. 2017;16:161. https://doi.org/10.1186/s12936-017-1801-4
996 56. Smit MR, Ochomo EO, Aljayyoussi G, Kwambai TK, Abong'o BO, Bousema T, et al.
997 Human Direct Skin Feeding Versus Membrane Feeding to Assess the Mosquitocidal Efficacy
998 of High-Dose Ivermectin (IVERMAL Trial). Clin Infect Dis. 2019;69:1112–9.
999 https://doi.org/10.1093/cid/ciy1063
1000 57. Kobylinski KC, Tipthara P, Wamaket N, Chainarin S, Kullasakboonsri R, Sriwichai P, et
1001 al. Ivermectin metabolites reduce *Anopheles survival*. Sci Rep. Nature Publishing Group;
1002 2023;13:8131. https://doi.org/10.1038/s41598-023-34719-2
1003 58. Smit MR, Ochomo EO, Waterhouse D, Kwambai TK, Abong'o BO, Bousema T, et al.
1004 Pharmacokinetics-Pharmacodynamics of High-Dose Ivermectin with Dihydroartemisinin-
1005 Piperaquine on Mosquitocidal Activity and QT -Prolongation (IVERMAL). Clin Pharma
1006 and Therapeutics. 2019;105:388–401. https://doi.org/10.1002/cpt.1219
1007 59. Lefevre T, Gouagna LC, Dabire KR, Elguero E, Fontenille D, Renaud F, et al. Beyond
1008 nature and nurture: phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae s.s.*
1009 when humans are not readily accessible. Am J Trop Med Hyg. 2009;81:1023–9.
1010 https://doi.org/10.4269/ajtmh.2009.09-0124
1011 60. Vantaux A, Yao F, Hien DF, Guissou E, Yameogo BK, Gouagna L-C, et al. Field
1012 evidence for manipulation of mosquito host selection by the human malaria parasite,
1013 *Plasmodium falciparum*. Peer Community Journal [Internet]. 2021 [cited 2025 Oct 31];1.
1014 https://doi.org/10.24072/pcjournal.13
1015 61. Vantaux A, Moiroux N, Dabiré KR, Cohuet A, Lefèvre T. Multiple hosts, multiple
1016 impacts: the role of vertebrate host diversity in shaping mosquito life history and pathogen
1017 transmission. Peer Community Journal. 2023;3:e54. https://doi.org/10.24072/pcjournal.288
1018 62. Amanzougaghene N, Fenollar F, Diatta G, Sokhna C, Raoult D, Mediannikov O.
1019 Mutations in GluCl associated with field ivermectin-resistant head lice from Senegal.
1020 International Journal of Antimicrobial Agents. 2018;52:593–8.
1021 https://doi.org/10.1016/j.ijantimicag.2018.07.005

1022 63. Furnival-Adams J, Kiuru C, Sagna AB, Mouline K, Maia M, Chaccour C. Ivermectin
1023 resistance mechanisms in ectoparasites: a scoping review. Parasitol Res. 2024;123:221.
1024 https://doi.org/10.1007/s00436-024-08223-z
1025 64. Deus KM, Saavedra-Rodriguez K, Butters MP, Black WC th, Foy BD. The effect of
1026 ivermectin in seven strains of Aedes aegypti (Diptera: Culicidae) including a genetically
1027 diverse laboratory strain and three permethrin resistant strains. J Med Entomol. 2012;49:356–
1028 63.
1029 65. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et
1030 al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria
1031 vector *Anopheles gambiae s.s.* Insect Mol Biol. 1998;7:179–84.
1032 66. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a
1033 point mutation in the voltage-gated sodium channel gene of Kenyan \emph*Anopheles*
1034 *gambiae* associated with resistance to DDT and pyrethroids. Insect Mol Biol. 2000;9:491–7.
1035 67. Nicolas P, Kiuru C, Wagah MG, Muturi M, Duthaler U, Hammann F, et al. Potential
1036 metabolic resistance mechanisms to ivermectin in *Anopheles gambiae*: a synergist bioassay
1037 study. Parasites & Vectors. 2021;14:172. https://doi.org/10.1186/s13071-021-04675-9
1038 68. Meyers JI, Gray M, Kuklinski W, Johnson LB, Snow CD, Black WC th, et al.
1039 Characterization of the target of ivermectin, the glutamate-gated chloride channel, from
1040 *Anopheles gambiae*. J Exp Biol. 2015;218:1478–86. https://doi.org/10.1242/jeb.118570
1041 69. Kobylinski KC, Satoto TBT, Nurcahyo W, Nugraheni YR, Testamenti VA, Winata IPBA,
1042 et al. Impact of standard and long-lasting ivermectin formulations in cattle and buffalo on
1043 wild *Anopheles* survival on Sumba Island, Indonesia. Sci Rep. 2024;14:29770.
1044 https://doi.org/10.1038/s41598-024-81743-x
1045 70. Dreyer SM, Morin KJ, Vaughan JA. Differential susceptibilities of *Anopheles albimanus*
1046 and *Anopheles stephensi* mosquitoes to ivermectin. Malar J. 2018;17:148.
1047 https://doi.org/10.1186/s12936-018-2296-3
1048 71. Sagna AB, Zéla L, Ouedraogo COW, Pooda SH, Porciani A, Furnival-Adams J, et al.
1049 Ivermectin as a novel malaria control tool: Getting ahead of the resistance curse. Acta
1050 Tropica. 2023;245:106973. https://doi.org/10.1016/j.actatropica.2023.106973
1051 72. van Wyk JA. Refugia--overlooked as perhaps the most potent factor concerning the
1052 development of anthelmintic resistance. Onderstepoort J Vet Res. 2001;68:55–67.
1053 73. Ominde KM, Kamau Y, Karisa J, Muturi MN, Kiuru C, Wanjiku C, et al. A field bioassay
1054 for assessing ivermectin bio-efficacy in wild malaria vectors. Malaria Journal. 2023;22:291.
1055 https://doi.org/10.1186/s12936-023-04718-9

74. Chaccour C, Rabinovich NR. Ivermectin to reduce malaria transmission II. Considerations regarding clinical development pathway. Malar J. 2017;16:166. https://doi.org/10.1186/s12936-017-1802-3

75. Slater HC, Walker PG, Bousema T, Okell LC, Ghani AC. The potential impact of adding ivermectin to a mass treatment intervention to reduce malaria transmission: a modelling study. J Infect Dis. 2014;210:1972–80. https://doi.org/10.1093/infdis/jiu351

76. Crompton DWT, World Health Organization. Preventive chemotherapy in human helminthiasis : coordinated use of anthelminthic drugs in control interventions : a manual for health professionals and programme managers. Chimioprévention des helminthiases chez l'homme : utilisation coordonnée des médicaments anthelminthiques pour les interventions de lutte : manuel à l'intention des professionnels de la santé et des administrateurs de programmes. Geneva: World Health Organization; 2006;62.

77. World Health Organization. WHO Community-Directed treatment with Ivermectin (CDTI) [Internet]. [cited 2025 Mar 26]. https://iris.who.int/bitstream/handle/10665/275546/275546-eng.pdf. Accessed 26 Mar 2025

78. Selvaraj P, Suresh J, Wenger EA, Bever CA, Gerardin J. Reducing malaria burden and accelerating elimination with long-lasting systemic insecticides: a modelling study of three potential use cases. Malaria Journal. 2019;18:307. https://doi.org/10.1186/s12936-019-2942-4

79. Dongmo FJ, Wondji CS, Tsanou B, Djidjou-Demasse R. Quantifying ivermectin's optimal strategy impacts on malaria transmission: a dual-structured mathematical model. Applicable Analysis. 2025;104:1526–64. https://doi.org/10.1080/00036811.2024.2429103

80. Dongmo FJ, Porciani A, Moiroux N, Pooda HS, Dabiré RK, Beneteau S, et al. Modeling the impact of ivermectin-based optimal strategies on malaria control: the role of formulation, coverage, and mosquitocidal efficacy timing.

81. Nicolas P, Maia MF, Bassat Q, Kobylinski KC, Monteiro W, Rabinovich NR, et al. Safety of oral ivermectin during pregnancy: a systematic review and meta-analysis. The Lancet Global Health. Elsevier; 2020;8:e92–100. https://doi.org/10.1016/S2214-109X(19)30453-X

82. Jittamala P, Monteiro W, Smit MR, Pedrique B, Specht S, Chaccour CJ, et al. A systematic review and an individual patient data meta-analysis of ivermectin use in children weighing less than fifteen kilograms: Is it time to reconsider the current contraindication? PLOS Neglected Tropical Diseases. Public Library of Science; 2021;15:e0009144. https://doi.org/10.1371/journal.pntd.0009144

1088 83. Imbahale SS, Montaña Lopez J, Brew J, Paaijmans K, Rist C, Chaccour C. Mapping the
1089 potential use of endectocide-treated cattle to reduce malaria transmission. Sci Rep.
1090 2019;9:5826. https://doi.org/10.1038/s41598-019-42356-x
1091 84. Chaccour CJ, Ngha'bi K, Abizanda G, Irigoyen Barrio A, Aldaz A, Okumu F, et al.
1092 Targeting cattle for malaria elimination: marked reduction of *Anopheles arabiensis* survival
1093 for over six months using a slow-release ivermectin implant formulation. Parasites Vectors.
1094 2018;11:287. https://doi.org/10.1186/s13071-018-2872-y
1095 85. Kumah E, Owusu P, Otchere G, Ankomah SE, Fusheini A, Kokuro C, et al. Factors
1096 influencing community acceptability of mass drug administration for the elimination of
1097 onchocerciasis in the Asante Akim South Municipal, Ghana. PLoS Negl Trop Dis.
1098 2023;17:e0011251. https://doi.org/10.1371/journal.pntd.0011251
1099 86. Aung PL, Sripoorote P, Rachaphaew N, Sattabongkot J, Parker DM, Nguitragool W, et al.
1100 Community acceptance of Ivermectin mass drug administration for malaria in Southern
1101 Thailand. Sci Rep. 2025;15:29026. https://doi.org/10.1038/s41598-025-14575-y
1102 87. Price J, Gurley N, Gyapong M, Ansah EK, Awusabo-Asare K, Gyasi SF, et al.
1103 Acceptance of and Adherence to a Four-Dose RTS,S/AS01 Schedule: Findings from a
1104 Longitudinal Qualitative Evaluation Study for the Malaria Vaccine Implementation
1105 Programme. Vaccines (Basel). 2023;11:1801. https://doi.org/10.3390/vaccines11121801
1106 88. Ansar F, Azzam A, Rauf MS, Ajmal Z, Asad Ullah G, Rauf S, et al. Global Analysis of
1107 RTS, S/AS01 Malaria Vaccine Acceptance Rates and Influencing Factors: A Systematic
1108 Review. Cureus. 16:e60678. https://doi.org/10.7759/cureus.60678
1109 89. McCoy KD, Weldon CT, Ansumana R, Lamin JM, Stenger DA, Ryan SJ, et al. Are
1110 malaria transmission-blocking vaccines acceptable to high burden communities? Results from
1111 a mixed methods study in Bo, Sierra Leone. Malaria Journal. 2021;20:183.
1112 https://doi.org/10.1186/s12936-021-03723-0
1113 90. Odongo T, Omondi I, Wanjiku C, Okoko M, Kiuru C, Kariuki M, et al. Toxicity of
1114 ivermectin to bed bugs (Cimex hemipterus) and risk factors associated with infestation in
1115 Kwale County, coastal Kenya. Parasit Vectors. 2025;18:269. https://doi.org/10.1186/s13071-
1116 025-06836-6
1117 91. Navarro M, Camprubí D, Requena-Méndez A, Buonfrate D, Giorli G, Kamgno J, et al.
1118 Safety of high-dose ivermectin: a systematic review and meta-analysis. J Antimicrob
1119 Chemother. 2020;75:827–34. https://doi.org/10.1093/jac/dkz524

92. Mohr BJ, Fakoya FA, Hau J, Souilem O, Anestidou L. The Governance of Animal Care and Use for Scientific Purposes in Africa and the Middle East. ILAR Journal. 2016;57:333–46. https://doi.org/10.1093/ilar/ilw035

Figures

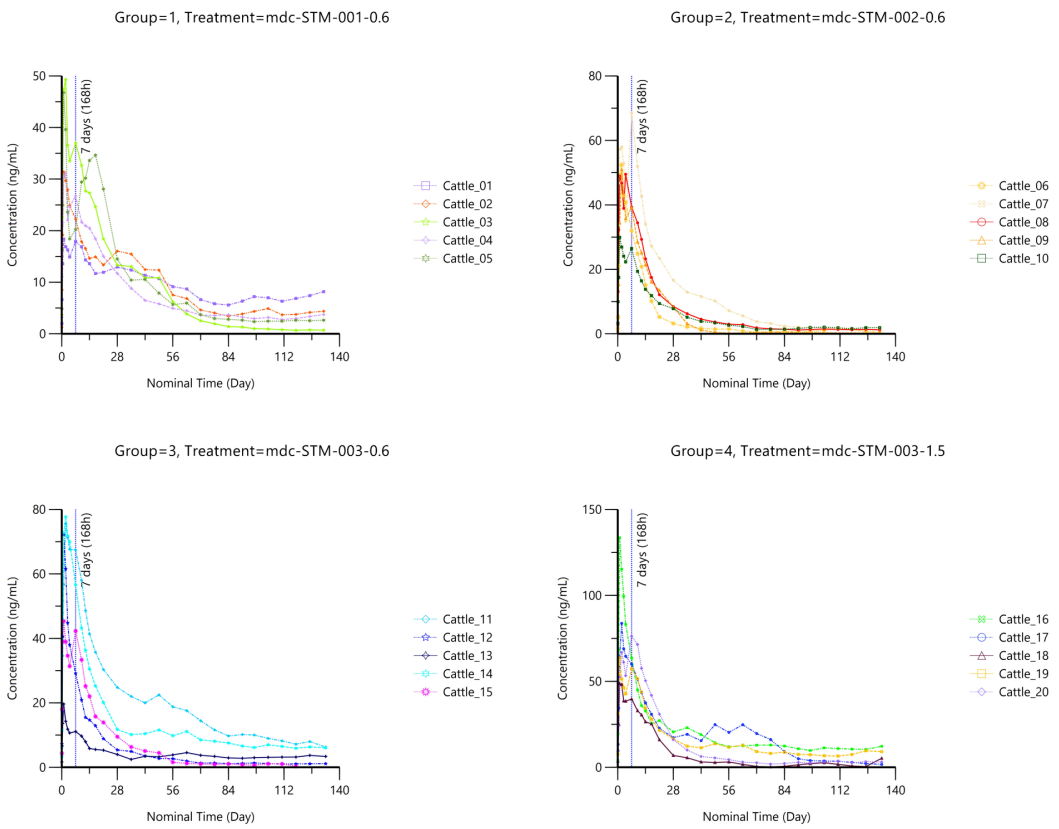


Figure 1: Individual kinetic profiles of ivermectin in cattle plasma after single sub-cutaneous administration of candidate long acting ivermectin formulations (LAIFs) at 0.6 or 1.5 mg/kg

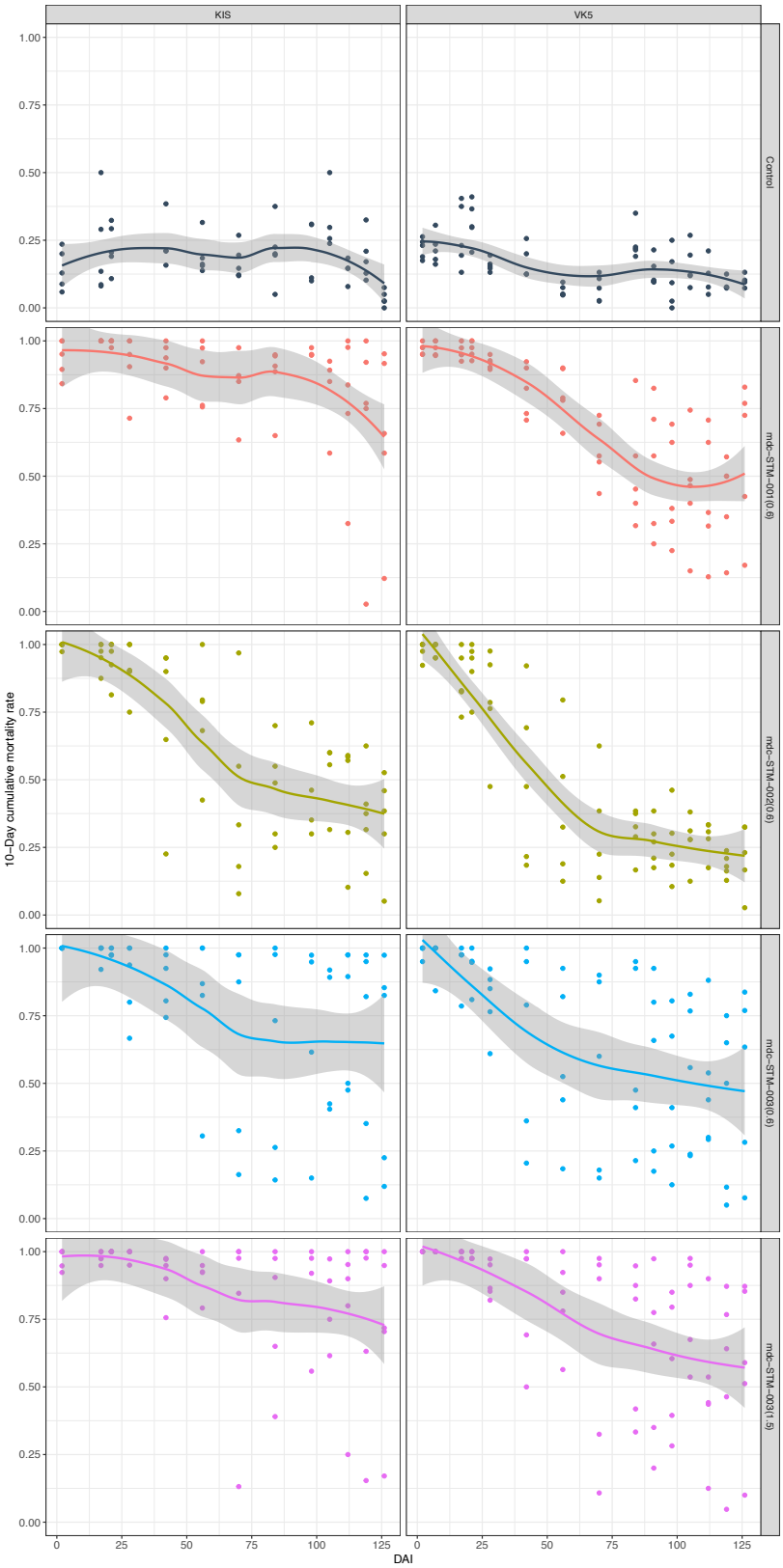


Figure 2. Ten-day cumulative mortality rates of KIS and VK5 mosquitoes fed at different days after injection (DAI) on control or treated cattle. Treated cattle received a single LAIF injection at a dose of 0.6mg/kg for the mdc-STM-001, mdc-STM-002, mdc-STM-003

formulations, and at an additional dose of 1.5 mg/kg for the mdc-STM-003. The smooth line represents mean values estimated by the LOESS (locally estimated scatterplot smoothing) method and grey area shows CI95% [44]. Results for the 4-day cumulative mortalities are shown in the Additional file1: Fig. S3.

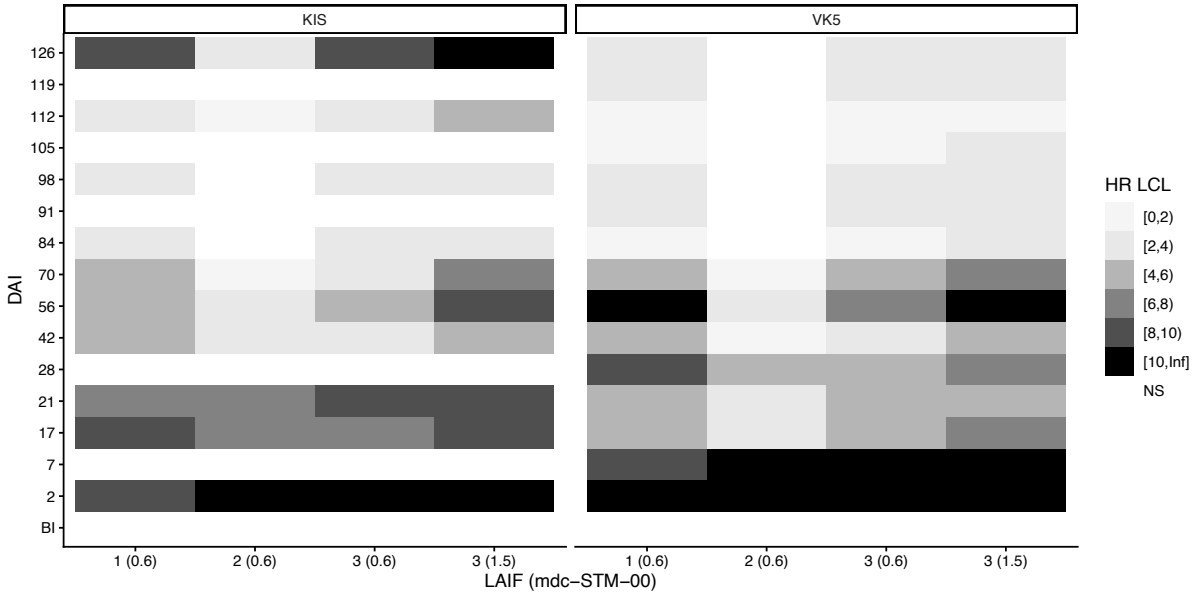


Figure 3: Heat maps illustrating the lower 95% confidence limit (LCL) of 10-day mortality hazard ratios (HRs) for KIS and VK5 colony mosquitoes fed at different DAIs on cattle treated with the formulations mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-003-0.6 or mdc-STM-003-1.5. HR and *P-values* for each DAI are available in Additional file 1: table S7. BI is the timepoint before injection. Results for 4- and 30-day HRs are given in the Additional file 1: Fig. S4.

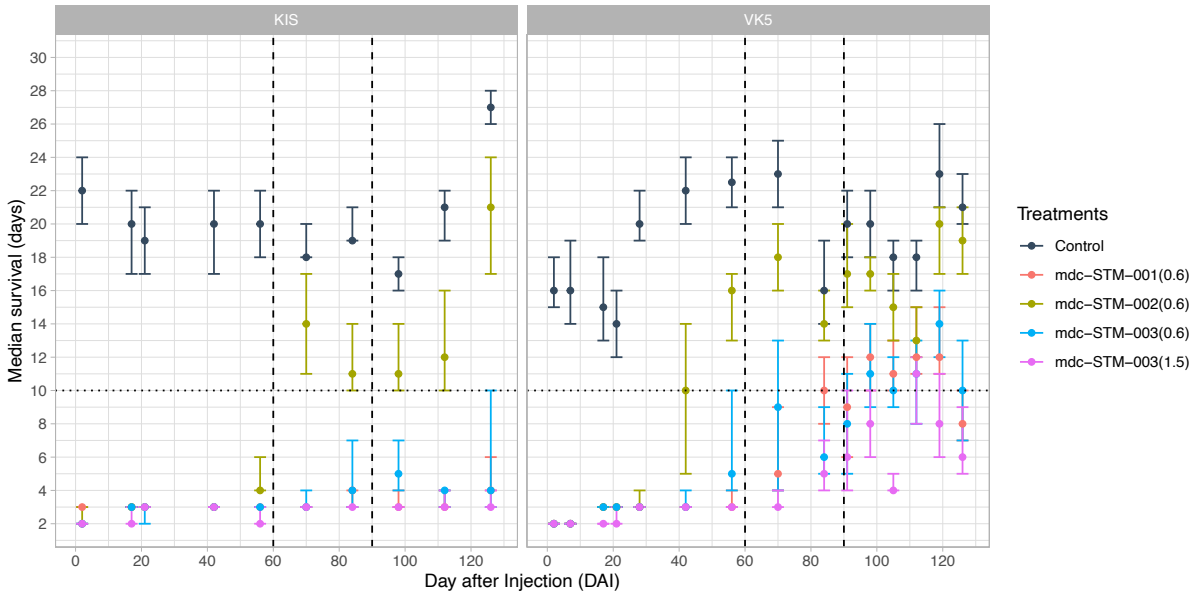
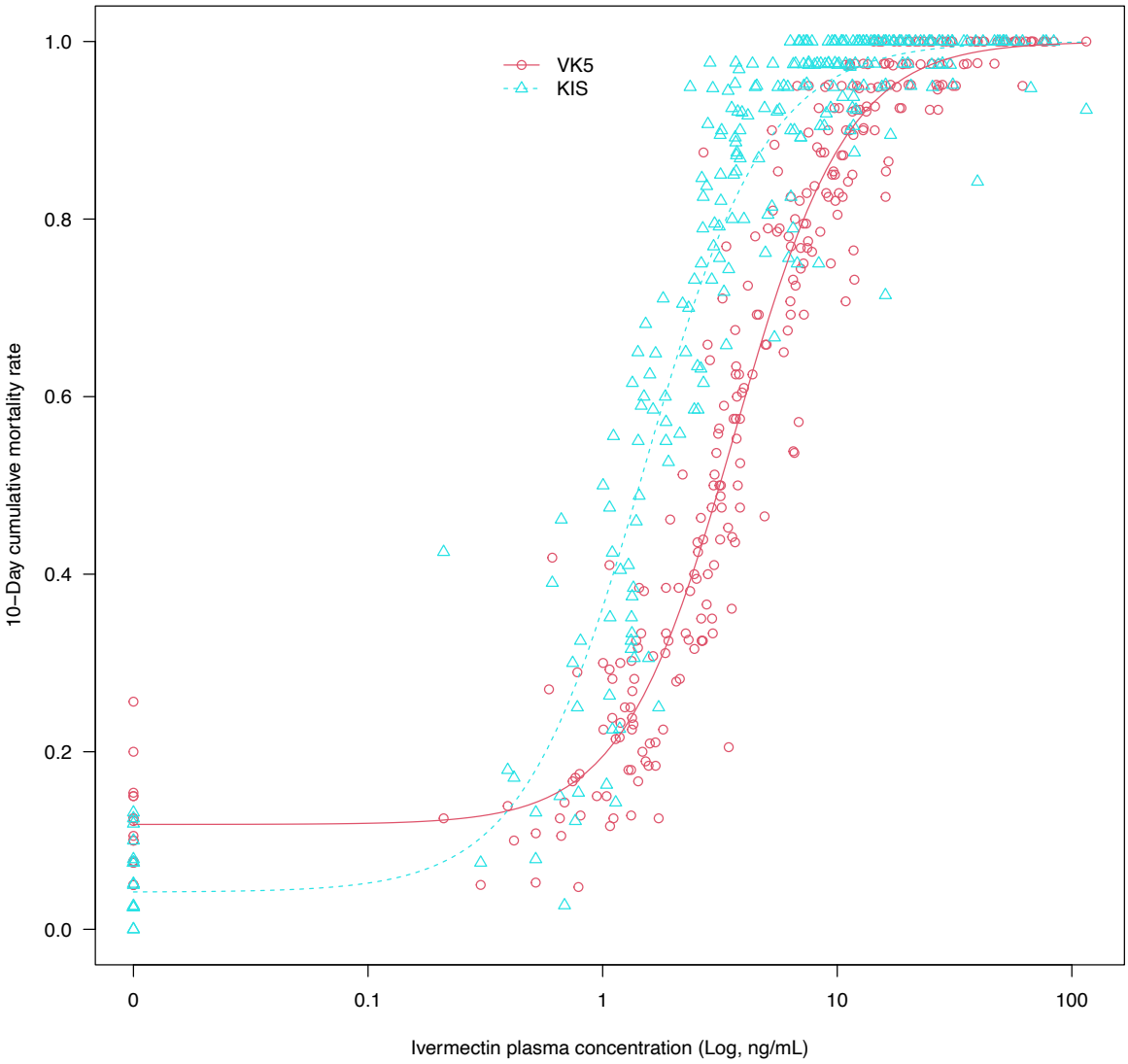


Figure 4: Median survival times of mosquitoes from both KIS and VK5 colonies fed at different days after injection of the LAIFs mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-003-06 and mdc-STM-003-1.5. The dashed horizontal line represents the average extrinsic incubation period (EIP) for *Plasmodium falciparum*. The dashed vertical lines represent 2- and 3-months post injections. Dots represent median values, +/- 95% CI are given.



1158

1159 Figure 5. Relationship between ivermectin plasma concentrations and 10-day cumulative

1160 mosquito mortality for KIS and VK5 colonies. Data for 4-day cumulative mortality are shown

1161 in the Additional file 2: Fig. S6.

1162

1163

1164

1165

1166

1167 # Tables

1168

1169 Table 1: Nominal treatment volumes per formulation and dose to be administered to cattle.

1170 N/A: not applicable.

Number of cattle	LAIF	Ivermectin dose (mg/kg)	Nominal volume (µL/kg)
5	mdc-STM-001	0.6	7.72
5	mdc-STM-002	0.6	7.80
5	mdc-STM-003	0.6	5.85
5	mdc-STM-003	1.5	14.6
5	N/A (control)	0	0

1171

1172
1173
1174
1175 Table 2: Median cattle weight gain per treatment group with interquartile range (IQR).

1176

	Control	mdc-STM-001-0.6	mdc-STM-002-0.6	mdc-STM-003-0.6	mdc-STM-003-1.5
Weight gain (kg) Median (Q1-Q3)	27.4 (25.8- 31.6)	37.2 (35.8-44.2)	39.6 (36.6-41.6)	35.3 (31.2- 36.2)	30.0 (28.6-40.0)

1177

1178

1179

1180 Table 3: Non compartmental pharmacokinetic parameters of ivermectin in cattle plasma after

1181 single sub-cutaneous administration of the different long acting ivermectin formulations

1182 (LAIFs) at a dose of 0.6 (mdc-STM-001, mdc-STM-002, mdc-STM-003) or 1.5 mg/kg (mdc-

1183 STM-003). For each parameter, values are expressed as mean ± standard deviation (SD).

1184 Tmax: time to reach Cmax. Cmax: maximal plasma concentration. Clast: last quantifiable

1185 plasma concentration. AUC(0-28d): area under curve from time 0 to 28 days. AUC(0-

1186 28d)/Dose: dose-normalized AUC(0-28d). AUC(0-91d): area under curve from time 0 to 91

1187 days. AUC(0-91d)/Dose: dose-normalized AUC(0-91d). C28d: plasma concentration 28 days
1188 post injection. C56d: plasma concentration 56 days post injection. C91d: plasma
1189 concentration 91 days post injection.
1190

Parameters	mdc-STM-001-0.6	mdc-STM-002-0.6	mdc-STM-003-0.6	mdc-STM-003-1.5
Tmax [a] (h)	24 (24; 48)	48 (24; 168)	24 (24; 48)	24 (24; 168)
Cmax (ng/mL)	35.40 ±12.70	50.30 ± 13.70	58.10 ± 25.20	81.20 ± 32.20
Cmax/Dose (ng/mL/mg/kg)	58.30 ± 22.00	84.30 ± 22.60	99.40 ± 42.20	56.10 ± 22.40
Clast (ng/mL)	3.93 ± 2.75	1.03 ± 0.70	3.43 ± 2.74	6.30 ± 4.35
AUC(0-91d) (h.ng/mL)	25055± 2936	21121 ± 8866	28890 ± 18668	41820 ± 13286
AUC(0-91d)/Dose (h.ng/mL/mg/kg)	40990 ± 5664	35479 ± 14839	49483 ± 31994	28903 ± 9300
C28d (ng/mL)	13.70 ± 1.60	8.90 ± 4.85	11.10 ± 8.30	15.50 ± 5.10
C56d (ng/mL)	6.70 ± 1.67	2.92 ± 2.63	7.34 ± 7.14	10.30 ± 6.90
C91d (ng/mL)	3.48 ± 1.85	1.11 ± 0.84	4.33 ± 3.99	5.55 ± 3.72

1191 [a] Median (min; max).

1192
1193
1194
1195
1196 Table 4: Blood-feeding rates of KIS and VK5 mosquitoes allowed to feed on cattle from the
1197 different treatment groups. Numbers in brackets indicate the number of fed mosquitoes out of
1198 the total exposed.

	Control	mdc-STM-001-0.6	mdc-STM-002-0.6	mdc-STM-003-0.6	mdc-STM-003-1.5	Total
KIS	0.900 (3908/4341)	0.921 (4128/4481)	0.897 (4206/4487)	0.892 (4080/4572)	0.831 (3675/4421)	0.889 (19817/22302)

VK5	0.966 (5047/5224)	0.945 (5005/5295)	0.935 (5091/5447)	0.961 (5160/5371)	0.968 (5174/5343)	0.955 (25477/26680)

Table 5. Number of KIS and VK5 mosquitoes followed for survival experiments for each treatment group

	KIS	VK5
Control	1,926	3,161
mdc-STM-001-0.6	2,122	3,206
mdc-STM-002-0.6	2,123	3,186
mdc-STM-003-0.6	2,135	3,207
mdc-STM-003-1.5	2,093	3,182
Total	10,399	15,942

Table 6: Time period during which the LAIF candidates reach ivermectin plasma concentration values above or equal to 10-day LC50 for KIS and VK5 colonies. Upper and lower confidence intervals (95%) are given in brackets.

LAIF	KIS	VK5
mdc-STM-001-0.6	132 [132-132]	132 [67-132]
mdc-STM-002-0.6	73 [65-119]	50 [39-58]
mdc-STM-003-0.6	132 [132-132]	128 [78-132]
mdc-STM-003-1.5	132 [132-132]	132 [132-132]

Supplementary tables

1218 Supplementary Table S1. Injected volumes (mL) per animal and per formulation, given the
1219 body weight (BW) measured the day of the treatment (kg)
1220

Animal ID	BW at arrival (kg)	BW at injection (kg)	Formulation ID	Injected volume (mL)
B122	141.6	153.4	mdc-STM-001	1.18
B126	116.8	140.2	mdc-STM-001	1.08
B128	109.4	119.8	mdc-STM-001	0.92
B332	119.0	126.6	mdc-STM-001	0.98
B335	99.2	115.2	mdc-STM-001	0.89
B121	107.2	120.8	mdc-STM-002	0.94
B130	128.8	137.6	mdc-STM-002	1.07
B324	97.4	115.2	mdc-STM-002	0.90
B330	138.0	144.6	mdc-STM-002	1.13
B334	142.0	151.4	mdc-STM-002	1.18
B123	101.2	122.3	mdc-STM-003	0.72
B124	124.4	130.6	mdc-STM-003	0.76
B125	124.6	142.2	mdc-STM-003	0.83
B326	140.8	160.8	mdc-STM-003	0.94
B333	94.2	106.6	mdc-STM-003	0.62
B322	127.4	146.8	mdc-STM-003	2.14
B323	108.6	120.0	mdc-STM-003	1.75
B327	129.4	144.6	mdc-STM-003	2.11
B329	99.8	115.8	mdc-STM-003	1.69
B331	106.4	124.8	mdc-STM-003	1.82

1221
1222

1223
1224 Supplementary Table S2. Median weight (Q1, Q3) in kg for each experimental cattle group at
1225 each weighing date.
1226

Experimental arm	Control N=5	mdc-STM-001 N=5	mdc-STM-002 N=5	mdc-STM-003 N=5	mdc-STM-004 N=5
Weighing date					
2020-11-25	114 (110,125)	117 (109,119)	129 (107,138)	124 (101,125)	109 (106,127)
2020-12-24	116 (116,126)	118 (114, 123)	134 (105,136)	121 (109,126)	113 (112,133)
2021-01-14	129 (123,140)	127 (120,140)	138 (121,145)	131 (122, 142)	125 (120, 145)
2021-02-15	138 (138,151)	135 (134,153)	157 (134,162)	146 (138,159)	139 (134,157)
2021-03-15	144 (141,152)	141 (141,158)	164 (134,169)	150 (138,162)	142 (140,163)
2021-04-15	162 (155,162)	160 (152,170)	177 (149,178)	166 (148,181)	163 (149,178)
2021-05-15	161 (155,167)	164 (161,177)	183 (157,186)	167 (158,182)	170 (149,173)

1227
1228

1229

1230 Supplementary Table S3. Mean (SD) pharmacokinetic parameters of ivermectin in cattle
1231 plasma after single subcutaneous administration of LAIFs at 0.6 or 1.5 mg/kg

1232

Parameters	mdc-STM-001-0.6	mdc-STM-002-0.6	mdc-STM-003-0.6	mdc-STM-003-1.5
Tmax [a] (h)	24 (24; 48)	48 (24; 168)	24 (24; 48)	24 (24; 168)
Cmax (ng/mL)	35.4 (12.7)	50.3 (13.7)	58.1 (25.2)	81.2 (32.2)
Cmax/Dose (ng/mL/mg/kg)	58.3 (22.0)	84.3 (22.6)	99.4 (42.2)	56.1 (22.4)
Clast (ng/mL)	3.93 (2.75)	1.03 (0.70)	3.43 (2.74)	6.30 (4.35)
AUC(0-28d) (h.ng/mL)	14227 (3853)	16253 (5465)	18085 (9789)	26248 (5534)
AUC(0-28d)/Dose (h.ng/mL/mg/kg)	23378 (6862)	27298 (9171)	30956 (16689)	18116 (3822)
AUC(0-91d) (h.ng/mL)	25055 (2936)	21121 (8866)	28890 (18668)	41820 (13286)
AUC(0-91d)/Dose (h.ng/mL/mg/kg)	40990 (5664)	35479 (14839)	49483 (31994)	28903 (9300)
AUClast (h.ng/mL)	28534 (2316)	22322 (9059)	32851 (21691)	47192 (15724)
AUClast/Dose (h.ng/mL/mg/kg)	46589 (3960)	37497 (15165)	56269 (37147)	32630 (11042)
T1/2 (h)	784 (244) (n=4)	731 (579) (n=4)	728 (399) (n=3)	489 (n=1)
C28d (ng/mL)	13.7 (1.6)	8.90 (4.85)	11.1 (8.3)	15.5 (5.1)
C56d (ng/mL)	6.70 (1.67)	2.92 (2.63)	7.34 (7.14)	10.3 (6.9)
C91d (ng/mL)	3.48 (1.85)	1.11 (0.84)	4.33 (3.99)	5.55 (3.72)

1233 SD: standard deviation.
1234 [a] Median (min; max).
1235 Tmax: time to reach Cmax. Cmax: maximal plasma concentration. Clast: last quantifiable plasma concentration.
1236 AUC(0-28d): area under curve from time 0 to 28 days. AUC(0-28d)/Dose: dose-normalized AUC(0-28d).
1237 AUC(0-91d): area under curve from time 0 to 91 days. AUC(0-91d)/Dose: dose-normalized AUC(0-91d).
1238 AUClast: area under curve from time 0 to last quantifiable plasma level. AUClast/Dose: dose-normalized
1239 AUClast. T1/2: apparent terminal phase half-life. C28d: plasma concentration 28 days post injection. C56d:
1240 plasma concentration 56 days post injection. C91d: plasma concentration 91 days post injection.
1241

1242 Supplementary Table S4. Ivermectin extraction results in each depot collected at study end

1243

Treatment Dose (mg/kg)	Animal ID	IVM injected dose (mg)	IVM recovered dose (mg)		IVM recovered dose (%)	
			Indiv.	Mean (SD)	Indiv.	Mean (SD)
mdc-STM-001-0.6	B122	100.4	38.6	19.4 (12.9)	38.5	23.0 (12.7)
	B126	85.0	18.3		21.6	
	B128	73.3	2.5		3.3	
	B332	76.3	20.6		27.0	
	B335	67.9	16.8		24.8	
mdc-STM-002-0.6	B121	76.1	39.0	32.3 (17.7)	51.3	39.1 (19.6)
	B130	84.7	6.1		7.2	
	B324	69.0	23.4		33.9	
	B330	87.7	41.7		47.6	
	B334	92.0	51.1		55.5	
mdc-STM-003-0.6	B123	70.6	5.4	17.8 (14.4)	7.7	23.1 (17.6)
	B124	76.4	14.3		18.7	
	B125	81.1	42.6		52.6	
	B326	96.4	12.0		12.5	
	B333	61.6	14.8		24.0	
mdc-STM-003-1.5	127.4	211.6	69.8	59.5 (35.5)	33.0	32.7 (20.0)
	108.6	173.8	55.5		32.0	
	129.4	212.3	0.5		0.2	
	99.8	164.8	79.7		48.4	
	106.4	182.9	91.8		50.2	

1244 IVM: ivermectin. Indiv.: individual. SD: standard deviation.

1245

1246

1247

1248

1249 Supplementary Table S5. Copolymers extraction results in each depot collected at study end

1250

Treatment Dose (mg/kg)	Animal ID	Copolymer injected (mg)	Copolymer recovered (mg)	Copolymer recovered (%)	
				Indiv.	Mean (SD)
mdc-STM-001-0.6	B122	442.2	120.1	27.2	18.8 (8.5)
	B126	374.4	90.0	24.0	
	B128	323.1	17.3	5.4	
	B332	336.0	55.5	16.5	
	B335	299.1	63.3	21.1	
mdc-STM-002-0.6	B121	217.6	105.8	48.6	34.1 (15.9)
	B130	242.2	19.5	8.0	
	B324	197.4	77.3	39.2	
	B330	250.8	108.8	43.4	
	B334	263.2	81.6	31.0	
mdc-STM-003-0.6	B123	155.6	25.0	16.1	25.9 (25.0)
	B124	168.4	11.4	6.8	
	B125	178.6	39.5	22.1	
	B326	212.4	32.0	15.1	
	B333	135.6	94.2	69.5	
mdc-STM-003-1.5	127.4	466.2	196.4	42.1	34.1 (18.8)
	108.6	382.8	164.3	42.9	
	129.4	467.6	3.5	0.8	
	99.8	363.0	167.4	46.1	
	106.4	403.0	155.6	38.6	

1251 Indiv.: individual. SD: standard deviation.

1252

1253
1254 Supplementary Table S6. Number of mosquitoes homozygous or heterozygous for the
1255 mutated KDR-W allele (resistant allele, R) of the voltage-gated sodium channel gene, which
1256 confers resistance to pyrethroids. In contrast, the wild-type allele is designated S
1257 (susceptible allele). RR and RS indicate homozygous and heterozygous genotypes for the
1258 KDR-W mutation, respectively. SS corresponds to homozygosity for the wild-type allele.
1259 Resistant phenotypes (RR and RS genotypes) among the tested mosquitoes (n) are given as
1260 percentages. Numbers are reported per generation (F2 to F9) and per day after injection
1261 (DAI) at which mosquitoes were exposed to cattle.
1262
1263

DAI	Generation (F)	RR (n)	RS (n)	SS (n)	Resistant phenotypes (%)	Moquitoes tested (n)
pre-dose	F2	10	15	8	73,52941176	33
2, 7, 17, 21, 28	F3	2	6	2	80	10
42	F5	12	20	9	74,41860465	41
56	F6				na	na
70, 84, 91, 98	F7	6	19	21	54,34782609	47
105,112, 119	F8				na	na
126	F9	5	14	17	52,77777778	36

1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282

1283 Supplementary Table S7A. Number of KIS blood fed mosquitoes followed for their survival

1284 before injection (BI) and at the different days after injection (DAI) for each cattle of the

1285 different treatment groups.

1286
1287
1288
1289

				Day after injection (DAI)														
Mosquito Colony	Treatment	Cattle ID	BI	2	7	17	21	28	42	56	70	84	91	98	105	112	119	126
kis	Control	B127	40	31	0	37	39	0	19	29	41	40	0	40	37	38	39	39
		B129	40	34	0	31	41	0	38	31	41	41	0	36	32	38	40	40
		B321	40	34	0	38	34	0	39	38	41	40	0	0	0	39	43	41
		B328	41	20	0	35	37	0	32	38	42	40	0	42	42	41	41	38
		B336	38	34	0	37	42	0	19	32	41	40	0	39	39	41	39	40
	mdc-STM-001-0.6	B122	40	38	0	39	40	20	40	37	40	39	0	40	37	40	41	42
		B126	39	40	0	40	40	21	40	38	38	36	0	40	40	42	38	36
		B128	38	28	0	39	41	9	40	41	41	40	0	0	0	40	37	41
		B332	40	41	0	34	39	21	38	42	40	44	0	38	40	43	39	38
		B335	40	38	0	40	40	24	32	39	39	43	0	39	41	41	40	41
	mdc-STM-002-0.6	B121	38	38	0	40	43	10	37	44	38	40	0	39	36	41	40	40
		B130	40	39	0	40	40	21	40	42	32	40	0	38	15	39	38	39
		B324	40	39	0	40	40	21	40	39	40	40	0	37	40	36	39	37
		B330	40	41	0	41	40	24	31	40	39	40	0	40	38	39	39	39
		B334	40	38	0	40	40	18	40	38	39	43	0	0	0	42	40	38
	mdc-STM-003-0.6	B123	40	40	0	41	38	10	40	40	40	39	0	38	37	40	39	38
		B124	38	39	0	37	39	6	40	40	40	42	0	39	42	38	37	40
		B125	40	40	0	38	40	25	39	38	40	41	0	0	0	38	39	41
		B326	40	37	0	39	40	16	40	38	40	42	0	39	37	39	40	40
		B333	40	42	0	39	39	4	41	36	43	38	0	40	33	40	40	42
	mdc-STM-003-1.5	B322	40	39	0	39	40	20	40	39	40	42	0	41	37	38	42	40
		B323	40	40	0	39	40	18	39	32	40	40	0	25	37	40	42	44
		B327	40	35	0	38	40	17	41	24	38	41	0	43	26	36	39	41
		B329	38	38	0	40	40	0	35	40	41	42	0	41	36	40	41	39
		B331	40	38	0	38	40	6	30	39	39	40	0	0	0	42	38	39
Total			990	921	0	959	992	311	910	934	993	1013	0	774	722	991	990	993

1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300

1301 Supplementary Table S7B. Number of VK5 blood fed mosquitoes followed for their survival

1302 before injection (BI) and at the different days after injection (DAI) for each cattle of the

1303 different treatment groups.

				Days after injection (DAI)														
Mosquito Colony	Treatment	Cattle ID	BI	2	7	17	21	28	42	56	70	84	91	98	105	112	119	126
vk5	Control	B127	40	41	31	39	41	38	39	40	38	40	42	40	41	39	40	41
		B129	40	39	34	40	40	39	40	39	41	41	41	40	41	38	39	39
		B321	40	38	36	42	34	36	40	41	37	40	39	41	40	39	39	38
		B328	40	40	38	38	39	37	40	42	40	42	39	42	41	39	40	41
		B336	41	37	39	36	37	41	40	42	37	42	42	43	42	40	41	42
	mdc-STM-001-0.6	B122	40	41	39	40	40	41	40	40	40	41	40	39	43	41	42	41
		B126	40	41	40	39	41	40	39	39	39	42	40	40	43	40	40	40
		B128	39	39	40	39	40	38	41	41	39	41	40	40	40	39	42	41
		B332	40	40	37	40	40	38	41	41	40	40	38	39	41	41	42	39
		B335	41	41	40	39	41	41	40	38	38	40	40	42	40	38	40	40
	mdc-STM-002-0.6	B121	38	41	40	41	40	40	38	37	38	38	37	38	40	39	42	42
		B130	40	38	37	40	40	41	38	39	40	46	39	40	45	39	39	39
		B324	40	40	40	40	40	42	39	41	39	42	40	43	42	39	39	40
		B330	40	39	39	40	39	40	37	40	36	40	40	38	43	40	43	37
		B334	40	39	41	41	40	38	40	40	40	39	38	39	43	39	43	40
	mdc-STM-003-0.6	B123	40	40	40	40	39	39	39	40	40	40	40	41	41	42	40	43
		B124	39	40	39	40	39	43	36	41	39	42	40	41	43	40	43	39
		B125	40	39	38	42	42	41	39	40	40	40	41	39	43	41	40	41
		B326	40	40	39	40	41	34	40	39	40	40	40	43	43	39	40	39
		B333	40	40	41	40	38	40	38	38	40	39	40	40	42	41	40	39
	mdc-STM-003-1.5	B322	40	39	40	40	40	41	39	39	40	38	38	40	40	40	39	39
		B323	39	39	39	38	39	37	41	40	40	40	41	43	40	43	39	41
		B327	40	40	40	40	40	39	38	39	37	43	40	39	40	40	42	40
		B329	40	41	40	39	39	37	37	40	41	40	40	39	40	41	43	41
		B331	40	40	39	40	39	41	39	41	40	39	40	38	41	39	41	39
Total			997	992	966	993	988	982	978	997	979	1015	995	1007	1038	996	1018	1001

1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318

1319 Supplementary table S8. Hazard ratios (HRs), z-values, and associated p-values derived from
1320 the Cox proportional hazards models. Values are given for 4, 10 and 30-days of survival follow-
1321 up after mosquitoes blood feeding, before injection (BI) and for each day after injection (DAI).
1322 A. KIS mosquitoes. B. VK5 mosquitoes.
1323
1324 **A.**
1325

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	z.ratio	p.value
(MDC-STM-001-0.6)	Control	BI	4	1 (0.1-17.2)	0.0018088	1.0000000
(MDC-STM-002-0.6)	Control	BI	4	1.9 (0.2-22.7)	0.6900508	0.9587058
(MDC-STM-003-0.6)	Control	BI	4	0.8 (0-14.3)	-0.1754490	0.9997884
(MDC-STM-003-1.5)	Control	BI	4	1.9 (0.2-22.4)	0.6751535	0.9618095
(MDC-STM-001-0.6)	Control	2	4	32.2 (9.8-106.3)	7.9378105	0.0000000
(MDC-STM-002-0.6)	Control	2	4	59.3 (18-195.1)	9.3486912	0.0000000
(MDC-STM-003-0.6)	Control	2	4	58.3 (17.7-191.8)	9.3098183	0.0000000
(MDC-STM-003-1.5)	Control	2	4	57.3 (17.4-188.9)	9.2598711	0.0000000
(MDC-STM-001-0.6)	Control	17	4	56 (17-184.3)	9.2185509	0.0000000
(MDC-STM-002-0.6)	Control	17	4	53.4 (16.2-175.7)	9.1132259	0.0000000
(MDC-STM-003-0.6)	Control	17	4	50.4 (15.3-165.8)	8.9748191	0.0000000
(MDC-STM-003-1.5)	Control	17	4	60.6 (18.4-199.5)	9.3992086	0.0000000
(MDC-STM-001-0.6)	Control	21	4	25.5 (9.3-70.3)	8.7248194	0.0000000
(MDC-STM-002-0.6)	Control	21	4	23.4 (8.5-64.4)	8.4758112	0.0000000
(MDC-STM-003-0.6)	Control	21	4	27.7 (10.1-76.4)	8.9365409	0.0000000
(MDC-STM-003-1.5)	Control	21	4	27.7 (10-76.2)	8.9388090	0.0000000
(MDC-STM-001-0.6)	Control	42	4	41.1 (10.2-165.9)	7.2625835	0.0000000
(MDC-STM-002-0.6)	Control	42	4	24 (5.9-97.5)	6.1919836	0.0000000
(MDC-STM-003-0.6)	Control	42	4	28.2 (7-114.1)	6.5248654	0.0000000
(MDC-STM-003-1.5)	Control	42	4	40.3 (10-162.9)	7.2196770	0.0000000
(MDC-STM-001-0.6)	Control	56	4	55.9 (14-223.3)	7.9184506	0.0000000
(MDC-STM-002-0.6)	Control	56	4	23.6 (5.8-95)	6.1826656	0.0000000
(MDC-STM-003-0.6)	Control	56	4	52.2 (13-209.7)	7.7629011	0.0000000
(MDC-STM-003-1.5)	Control	56	4	81.4 (20.3-326.2)	8.6502114	0.0000000
(MDC-STM-001-0.6)	Control	70	4	26.4 (8.2-84.9)	7.6620884	0.0000000
(MDC-STM-002-0.6)	Control	70	4	6.2 (1.9-20.7)	4.1257303	0.0003562
(MDC-STM-003-0.6)	Control	70	4	19.8 (6.1-64)	6.9522992	0.0000000
(MDC-STM-003-1.5)	Control	70	4	34.4 (10.7-110.5)	8.2674788	0.0000000
(MDC-STM-001-0.6)	Control	84	4	7.9 (2.9-21.6)	5.5987636	0.0000002
(MDC-STM-002-0.6)	Control	84	4	2.2 (0.8-6.2)	1.9916567	0.2699699
(MDC-STM-003-0.6)	Control	84	4	6.8 (2.5-18.8)	5.1726904	0.0000023
(MDC-STM-003-1.5)	Control	84	4	12.8 (4.7-34.9)	6.9245385	0.0000000
(MDC-STM-001-0.6)	Control	98	4	38.4 (8.7-169.4)	6.7085831	0.0000000
(MDC-STM-002-0.6)	Control	98	4	2.7 (0.5-13.6)	1.6602615	0.4587964
(MDC-STM-003-0.6)	Control	98	4	21.2 (4.7-94.6)	5.5628717	0.0000003
(MDC-STM-003-1.5)	Control	98	4	36.7 (8.3-162.4)	6.6104282	0.0000000
(MDC-STM-001-0.6)	Control	112	4	25.7 (6.4-103.6)	6.3494193	0.0000000
(MDC-STM-002-0.6)	Control	112	4	5.6 (1.3-23.6)	3.2765643	0.0092896
(MDC-STM-003-0.6)	Control	112	4	23.5 (5.8-95.2)	6.1673392	0.0000000
(MDC-STM-003-1.5)	Control	112	4	26.8 (6.6-108)	6.4270674	0.0000000
(MDC-STM-001-0.6)	Control	126	4	160.9 (9.3-2793.8)	4.8561169	0.0000118
(MDC-STM-002-0.6)	Control	126	4	29.5 (1.6-528.9)	3.1984047	0.0120573

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	z.ratio	p.value
(MDC-STM-003-0.6)	Control	126	4	156.2 (9-2712.6)	4.8271523	0.0000137
(MDC-STM-003-1.5)	Control	126	4	215.2 (12.4-3727.6)	5.1371852	0.0000028
(MDC-STM-001-0.6)	Control	BI	10	0.6 (0.2-2.3)	-0.9559426	0.8747113
(MDC-STM-002-0.6)	Control	BI	10	0.5 (0.1-1.8)	-1.5706514	0.5163317
(MDC-STM-003-0.6)	Control	BI	10	0.5 (0.2-1.9)	-1.3559929	0.6559403
(MDC-STM-003-1.5)	Control	BI	10	0.4 (0.1-1.5)	-1.9615231	0.2850212
(MDC-STM-001-0.6)	Control	2	10	22.3 (8.1-61.5)	8.3298780	0.0000000
(MDC-STM-002-0.6)	Control	2	10	44.4 (16.1-122.5)	10.2014974	0.0000000
(MDC-STM-003-0.6)	Control	2	10	44.1 (16-121.6)	10.1883342	0.0000000
(MDC-STM-003-1.5)	Control	2	10	40.8 (14.8-112.8)	9.9635258	0.0000000
(MDC-STM-001-0.6)	Control	17	10	22.1 (8.7-56.1)	9.0876376	0.0000000
(MDC-STM-002-0.6)	Control	17	10	19.9 (7.9-50.5)	8.7831843	0.0000000
(MDC-STM-003-0.6)	Control	17	10	19.5 (7.7-49.5)	8.7173277	0.0000000
(MDC-STM-003-1.5)	Control	17	10	22.9 (9-57.9)	9.1788002	0.0000000
(MDC-STM-001-0.6)	Control	21	10	19.4 (7.7-48.6)	8.8005338	0.0000000
(MDC-STM-002-0.6)	Control	21	10	16.7 (6.7-42)	8.3551000	0.0000000
(MDC-STM-003-0.6)	Control	21	10	20.7 (8.3-52)	8.9892405	0.0000000
(MDC-STM-003-1.5)	Control	21	10	21.3 (8.5-53.5)	9.0828836	0.0000000
(MDC-STM-001-0.6)	Control	42	10	12.1 (4.6-31.9)	7.0314926	0.0000000
(MDC-STM-002-0.6)	Control	42	10	7 (2.6-18.4)	5.4504723	0.0000005
(MDC-STM-003-0.6)	Control	42	10	8.9 (3.4-23.5)	6.1803507	0.0000000
(MDC-STM-003-1.5)	Control	42	10	12.3 (4.7-32.5)	7.0804487	0.0000000
(MDC-STM-001-0.6)	Control	56	10	14.4 (5.6-37.2)	7.6531475	0.0000000
(MDC-STM-002-0.6)	Control	56	10	7.2 (2.8-18.7)	5.6511352	0.0000002
(MDC-STM-003-0.6)	Control	56	10	14.5 (5.6-37.7)	7.6599087	0.0000000
(MDC-STM-003-1.5)	Control	56	10	21.2 (8.2-54.9)	8.7464041	0.0000000
(MDC-STM-001-0.6)	Control	70	10	12.3 (4.8-31.6)	7.2559840	0.0000000
(MDC-STM-002-0.6)	Control	70	10	3.2 (1.2-8.5)	3.2928640	0.0087904
(MDC-STM-003-0.6)	Control	70	10	9 (3.5-23.2)	6.3074379	0.0000000
(MDC-STM-003-1.5)	Control	70	10	15.7 (6.1-40.3)	7.9399520	0.0000000
(MDC-STM-001-0.6)	Control	84	10	8 (3.2-20.3)	6.1630229	0.0000000
(MDC-STM-002-0.6)	Control	84	10	2.4 (1-6.3)	2.5834060	0.0734027
(MDC-STM-003-0.6)	Control	84	10	5.7 (2.2-14.4)	5.0764744	0.0000038
(MDC-STM-003-1.5)	Control	84	10	10.1 (4-25.4)	6.8093812	0.0000000
(MDC-STM-001-0.6)	Control	98	10	10.1 (3.9-26.2)	6.5853039	0.0000000
(MDC-STM-002-0.6)	Control	98	10	2.1 (0.8-5.5)	1.9907963	0.2703929
(MDC-STM-003-0.6)	Control	98	10	5.7 (2.2-15.1)	4.9284663	0.0000082
(MDC-STM-003-1.5)	Control	98	10	9.6 (3.7-25)	6.4097776	0.0000000
(MDC-STM-001-0.6)	Control	112	10	10.9 (4-29.9)	6.4398595	0.0000000
(MDC-STM-002-0.6)	Control	112	10	3.3 (1.2-9.3)	3.1723213	0.0131335
(MDC-STM-003-0.6)	Control	112	10	10.7 (3.9-29.5)	6.3876542	0.0000000
(MDC-STM-003-1.5)	Control	112	10	11.5 (4.2-31.6)	6.5809723	0.0000000
(MDC-STM-001-0.6)	Control	126	10	34.9 (9.3-131.3)	7.3172285	0.0000000
(MDC-STM-002-0.6)	Control	126	10	10.5 (2.7-40.3)	4.7708941	0.0000181
(MDC-STM-003-0.6)	Control	126	10	33.2 (8.8-125.1)	7.2074558	0.0000000
(MDC-STM-003-1.5)	Control	126	10	45.5 (12.1-170.7)	7.8755092	0.0000000
(MDC-STM-001-0.6)	Control	BI	30	1.1 (0.5-2.3)	0.3311432	0.9974111
(MDC-STM-002-0.6)	Control	BI	30	1 (0.5-2.1)	-0.0125675	1.0000000
(MDC-STM-003-0.6)	Control	BI	30	0.8 (0.4-1.8)	-0.6516467	0.9663867
(MDC-STM-003-1.5)	Control	BI	30	0.9 (0.4-1.9)	-0.4079445	0.9941915
(MDC-STM-001-0.6)	Control	2	30	7.4 (3.5-15.7)	7.2400202	0.0000000

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	z.ratio	p.value
(MDC-STM-002-0.6)	Control	2	30	18.3 (8.6-38.9)	10.5353421	0.0000000
(MDC-STM-003-0.6)	Control	2	30	18.4 (8.7-39.1)	10.5658195	0.0000000
(MDC-STM-003-1.5)	Control	2	30	12.6 (5.9-26.8)	9.1477425	0.0000000
(MDC-STM-001-0.6)	Control	17	30	10.6 (5.1-22.3)	8.7066586	0.0000000
(MDC-STM-002-0.6)	Control	17	30	9.4 (4.5-19.7)	8.2714953	0.0000000
(MDC-STM-003-0.6)	Control	17	30	9.4 (4.5-19.6)	8.2404428	0.0000000
(MDC-STM-003-1.5)	Control	17	30	11.8 (5.6-24.7)	9.0870759	0.0000000
(MDC-STM-001-0.6)	Control	21	30	10.1 (4.8-21.2)	8.5489587	0.0000000
(MDC-STM-002-0.6)	Control	21	30	7.4 (3.5-15.4)	7.3709439	0.0000000
(MDC-STM-003-0.6)	Control	21	30	10.7 (5.1-22.4)	8.7404073	0.0000000
(MDC-STM-003-1.5)	Control	21	30	11.6 (5.6-24.3)	9.0550481	0.0000000
(MDC-STM-001-0.6)	Control	42	30	5.9 (2.7-12.7)	6.3153947	0.0000000
(MDC-STM-002-0.6)	Control	42	30	3.8 (1.8-8.2)	4.7956175	0.0000160
(MDC-STM-003-0.6)	Control	42	30	4.7 (2.2-10.2)	5.5638129	0.0000003
(MDC-STM-003-1.5)	Control	42	30	6.8 (3.2-14.6)	6.8245889	0.0000000
(MDC-STM-001-0.6)	Control	56	30	5.5 (2.6-11.7)	6.2391368	0.0000000
(MDC-STM-002-0.6)	Control	56	30	3.1 (1.5-6.6)	4.1792820	0.0002827
(MDC-STM-003-0.6)	Control	56	30	6.8 (3.2-14.3)	6.9961241	0.0000000
(MDC-STM-003-1.5)	Control	56	30	7.1 (3.4-15.1)	7.1113294	0.0000000
(MDC-STM-001-0.6)	Control	70	30	3.5 (1.7-7.2)	4.6389669	0.0000345
(MDC-STM-002-0.6)	Control	70	30	1.4 (0.7-2.9)	1.2618253	0.7146990
(MDC-STM-003-0.6)	Control	70	30	2.7 (1.3-5.7)	3.7237241	0.0018361
(MDC-STM-003-1.5)	Control	70	30	4.8 (2.3-9.9)	5.8158608	0.0000001
(MDC-STM-001-0.6)	Control	84	30	3.6 (1.7-7.6)	4.8051976	0.0000153
(MDC-STM-002-0.6)	Control	84	30	1.5 (0.7-3.2)	1.5836401	0.5079043
(MDC-STM-003-0.6)	Control	84	30	2.5 (1.2-5.1)	3.3574350	0.0070421
(MDC-STM-003-1.5)	Control	84	30	4.2 (2-8.7)	5.3227185	0.0000010
(MDC-STM-001-0.6)	Control	98	30	3.8 (1.8-8.1)	4.8907639	0.0000099
(MDC-STM-002-0.6)	Control	98	30	1.4 (0.6-2.9)	1.1222767	0.7948357
(MDC-STM-003-0.6)	Control	98	30	2.8 (1.3-6)	3.7662399	0.0015562
(MDC-STM-003-1.5)	Control	98	30	4.4 (2.1-9.3)	5.3605195	0.0000008
(MDC-STM-001-0.6)	Control	112	30	3.7 (1.8-7.8)	4.7881568	0.0000166
(MDC-STM-002-0.6)	Control	112	30	1.5 (0.7-3.2)	1.4979596	0.5637848
(MDC-STM-003-0.6)	Control	112	30	3.7 (1.8-7.9)	4.8328687	0.0000133
(MDC-STM-003-1.5)	Control	112	30	4.4 (2.1-9.3)	5.4279176	0.0000006
(MDC-STM-001-0.6)	Control	126	30	3.9 (1.9-8.3)	5.0130665	0.0000053
(MDC-STM-002-0.6)	Control	126	30	1.7 (0.8-3.6)	1.8970277	0.3188277
(MDC-STM-003-0.6)	Control	126	30	4.1 (2-8.7)	5.1720047	0.0000023
(MDC-STM-003-1.5)	Control	126	30	4.6 (2.2-9.7)	5.5941222	0.0000002

B.

Formulation	Treatment arm	DAI	Follow-up (days)	HR CI95%	z.ratio	p.value
mdc-STM-001(0.6)	Control	BI	4	0.4 (0.1-2.3)	-1.4525092	0.5935112
mdc-STM-002(0.6)	Control	BI	4	0.3 (0-1.9)	-1.7950230	0.3763965
mdc-STM-003(0.6)	Control	BI	4	0.6 (0.1-2.6)	-0.9988111	0.8560128
mdc-STM-003(1.5)	Control	BI	4	0.5 (0.1-2.7)	-1.0328193	0.8402120
mdc-STM-001(0.6)	Control	2	4	75.1 (22.9-246)	9.9245770	0.0000000
mdc-STM-002(0.6)	Control	2	4	72.9 (22.3-239)	9.8587828	0.0000000
mdc-STM-003(0.6)	Control	2	4	101.1 (30.9-331.1)	10.6117691	0.0000000
mdc-STM-003(1.5)	Control	2	4	91 (27.8-298)	10.3768245	0.0000000
mdc-STM-001(0.6)	Control	7	4	134.2 (28.1-640.2)	8.5530652	0.0000000
mdc-STM-002(0.6)	Control	7	4	169.1 (35.5-806.5)	8.9586219	0.0000000
mdc-STM-003(0.6)	Control	7	4	178.3 (37.4-850.7)	9.0478567	0.0000000
mdc-STM-003(1.5)	Control	7	4	255.2 (53.5-1217)	9.6791985	0.0000000
mdc-STM-001(0.6)	Control	17	4	53.1 (15.5-181.7)	8.8085244	0.0000000
mdc-STM-002(0.6)	Control	17	4	35.5 (10.4-121.7)	7.9027325	0.0000000
mdc-STM-003(0.6)	Control	17	4	50.7 (14.8-173.6)	8.7020934	0.0000000
mdc-STM-003(1.5)	Control	17	4	69.4 (20.3-237.1)	9.4072719	0.0000000
mdc-STM-001(0.6)	Control	21	4	62.4 (17.3-225.1)	8.7889633	0.0000000
mdc-STM-002(0.6)	Control	21	4	41.4 (11.4-149.7)	7.8924001	0.0000000
mdc-STM-003(0.6)	Control	21	4	45.2 (12.5-163.7)	8.0827196	0.0000000
mdc-STM-003(1.5)	Control	21	4	73.1 (20.3-263.7)	9.1269221	0.0000000
mdc-STM-001(0.6)	Control	28	4	97 (20.3-463.2)	7.9817790	0.0000000
mdc-STM-002(0.6)	Control	28	4	50.1 (10.5-240.3)	6.8120194	0.0000000
mdc-STM-003(0.6)	Control	28	4	51.5 (10.7-246.8)	6.8614600	0.0000000
mdc-STM-003(1.5)	Control	28	4	77 (16.1-368)	7.5763840	0.0000000
mdc-STM-001(0.6)	Control	42	4	25.3 (8.2-77.9)	7.8344226	0.0000000
mdc-STM-002(0.6)	Control	42	4	8.2 (2.6-25.8)	4.9966923	0.0000058
mdc-STM-003(0.6)	Control	42	4	16.7 (5.4-51.8)	6.7820428	0.0000000
mdc-STM-003(1.5)	Control	42	4	28.4 (9.2-87.5)	8.1068411	0.0000000
mdc-STM-001(0.6)	Control	56	4	78.8 (13.6-454.9)	6.7925446	0.0000000
mdc-STM-002(0.6)	Control	56	4	20.4 (3.4-121)	4.6202949	0.0000377
mdc-STM-003(0.6)	Control	56	4	55.9 (9.6-324.8)	6.2355142	0.0000000
mdc-STM-003(1.5)	Control	56	4	115.4 (20-665.7)	7.3936684	0.0000000
mdc-STM-001(0.6)	Control	70	4	14.3 (4.1-49.8)	5.8249608	0.0000001
mdc-STM-002(0.6)	Control	70	4	4.2 (1.1-15.4)	3.0047931	0.0223285
mdc-STM-003(0.6)	Control	70	4	14.7 (4.2-51.1)	5.8808958	0.0000000
mdc-STM-003(1.5)	Control	70	4	26.8 (7.8-92.5)	7.2458816	0.0000000
mdc-STM-001(0.6)	Control	84	4	8.8 (2.6-29.8)	4.8796195	0.0000105
mdc-STM-002(0.6)	Control	84	4	1.4 (0.4-5.7)	0.7288331	0.9498655
mdc-STM-003(0.6)	Control	84	4	13.7 (4.1-45.9)	5.9298691	0.0000000
mdc-STM-003(1.5)	Control	84	4	16.9 (5.1-56.1)	6.4215395	0.0000000
mdc-STM-001(0.6)	Control	91	4	43.8 (5.4-353.9)	4.9315684	0.0000081
mdc-STM-002(0.6)	Control	91	4	8.3 (0.9-73.3)	2.6608551	0.0599267
mdc-STM-003(0.6)	Control	91	4	52.4 (6.5-422.1)	5.1774268	0.0000022
mdc-STM-003(1.5)	Control	91	4	68.5 (8.5-550.5)	5.5334036	0.0000003
mdc-STM-001(0.6)	Control	98	4	8.7 (2.3-32.5)	4.4604133	0.0000801
mdc-STM-002(0.6)	Control	98	4	2.1 (0.5-8.9)	1.3968548	0.6296925
mdc-STM-003(0.6)	Control	98	4	8.5 (2.3-31.8)	4.4446592	0.0000861

Formulation	Treatment arm	DAI	Follow-up (days)	HR CI95%	z.ratio	p.value
mdc-STM-003(1.5)	Control	98	4	14.8 (4-54.2)	5.6493493	0.0000002
mdc-STM-001(0.6)	Control	105	4	7.1 (2.2-23.3)	4.5143215	0.0000623
mdc-STM-002(0.6)	Control	105	4	1.3 (0.3-4.9)	0.4836030	0.9888789
mdc-STM-003(0.6)	Control	105	4	6.8 (2.1-22.2)	4.4256915	0.0000940
mdc-STM-003(1.5)	Control	105	4	14.8 (4.6-47.2)	6.3098761	0.0000000
mdc-STM-001(0.6)	Control	112	4	5.6 (1.6-20.3)	3.6722060	0.0022378
mdc-STM-002(0.6)	Control	112	4	2.2 (0.6-8.8)	1.5855045	0.5066966
mdc-STM-003(0.6)	Control	112	4	7.1 (2-25.1)	4.2230989	0.0002335
mdc-STM-003(1.5)	Control	112	4	9.4 (2.7-33)	4.8654735	0.0000113
mdc-STM-001(0.6)	Control	119	4	15.5 (3.2-76.1)	4.6954432	0.0000262
mdc-STM-002(0.6)	Control	119	4	3.4 (0.6-18.7)	1.9371514	0.2975453
mdc-STM-003(0.6)	Control	119	4	18.3 (3.8-89.3)	5.0046730	0.0000055
mdc-STM-003(1.5)	Control	119	4	24.9 (5.1-120.6)	5.5529337	0.0000003
mdc-STM-001(0.6)	Control	126	4	42.1 (5.2-340.3)	4.8779078	0.0000106
mdc-STM-002(0.6)	Control	126	4	5.5 (0.6-51.2)	2.1031035	0.2186133
mdc-STM-003(0.6)	Control	126	4	36.3 (4.5-293.7)	4.6872015	0.0000273
mdc-STM-003(1.5)	Control	126	4	56.5 (7-454.2)	5.2765308	0.0000013
mdc-STM-001(0.6)	Control	BI	10	0.5 (0.2-1.4)	-1.8412832	0.3497023
mdc-STM-002(0.6)	Control	BI	10	0.6 (0.2-1.6)	-1.4389514	0.6023572
mdc-STM-003(0.6)	Control	BI	10	0.7 (0.3-1.8)	-1.1321622	0.7895059
mdc-STM-003(1.5)	Control	BI	10	0.7 (0.3-1.9)	-0.9502176	0.8771030
mdc-STM-001(0.6)	Control	2	10	23.7 (10.5-53.7)	10.5671244	0.0000000
mdc-STM-002(0.6)	Control	2	10	24.5 (10.8-55.4)	10.6674750	0.0000000
mdc-STM-003(0.6)	Control	2	10	34.7 (15.3-78.6)	11.8402697	0.0000000
mdc-STM-003(1.5)	Control	2	10	31.4 (13.9-71.1)	11.5091218	0.0000000
mdc-STM-001(0.6)	Control	7	10	21.8 (9.5-49.9)	10.1539733	0.0000000
mdc-STM-002(0.6)	Control	7	10	28.2 (12.3-64.4)	10.9963707	0.0000000
mdc-STM-003(0.6)	Control	7	10	29.5 (12.9-67.5)	11.1369242	0.0000000
mdc-STM-003(1.5)	Control	7	10	43.3 (18.9-99)	12.4087000	0.0000000
mdc-STM-001(0.6)	Control	17	10	12.4 (5.6-27.6)	8.6218624	0.0000000
mdc-STM-002(0.6)	Control	17	10	8.3 (3.7-18.5)	7.2272830	0.0000000
mdc-STM-003(0.6)	Control	17	10	12.4 (5.6-27.5)	8.6164337	0.0000000
mdc-STM-003(1.5)	Control	17	10	17.5 (7.9-38.8)	9.7930817	0.0000000
mdc-STM-001(0.6)	Control	21	10	11.1 (5.1-24.4)	8.3818904	0.0000000
mdc-STM-002(0.6)	Control	21	10	8 (3.6-17.6)	7.2161276	0.0000000
mdc-STM-003(0.6)	Control	21	10	9.1 (4.1-19.9)	7.6585295	0.0000000
mdc-STM-003(1.5)	Control	21	10	12.9 (5.9-28.4)	8.8957932	0.0000000
mdc-STM-001(0.6)	Control	28	10	19.3 (8.1-45.7)	9.3607437	0.0000000
mdc-STM-002(0.6)	Control	28	10	10.5 (4.4-25.1)	7.4189722	0.0000000
mdc-STM-003(0.6)	Control	28	10	10.6 (4.5-25.3)	7.4419849	0.0000000
mdc-STM-003(1.5)	Control	28	10	15.9 (6.7-37.6)	8.7267934	0.0000000
mdc-STM-001(0.6)	Control	42	10	10.4 (4.5-24.1)	7.6317937	0.0000000
mdc-STM-002(0.6)	Control	42	10	3.8 (1.6-8.9)	4.2488554	0.0002084
mdc-STM-003(0.6)	Control	42	10	7.2 (3.1-16.8)	6.3764610	0.0000000
mdc-STM-003(1.5)	Control	42	10	12.9 (5.6-29.7)	8.3139612	0.0000000
mdc-STM-001(0.6)	Control	56	10	28.4 (10.1-80.2)	8.8128648	0.0000000
mdc-STM-002(0.6)	Control	56	10	8.2 (2.8-23.6)	5.4098301	0.0000006
mdc-STM-003(0.6)	Control	56	10	19.1 (6.7-54.4)	7.7040423	0.0000000
mdc-STM-003(1.5)	Control	56	10	39.7 (14.1-111.8)	9.6938839	0.0000000
mdc-STM-001(0.6)	Control	70	10	12.9 (4.6-36)	6.8149255	0.0000000
mdc-STM-002(0.6)	Control	70	10	4.4 (1.5-12.6)	3.8067849	0.0013268

Formulation	Treatment arm	DAI	Follow-up (days)	HR CI95%	z.ratio	p.value
mdc-STM-003(0.6)	Control	70	10	12.5 (4.5-34.9)	6.7040907	0.0000000
mdc-STM-003(1.5)	Control	70	10	21.4 (7.7-59.5)	8.1851871	0.0000000
mdc-STM-001(0.6)	Control	84	10	2.9 (1.3-6.7)	3.5562395	0.0034569
mdc-STM-002(0.6)	Control	84	10	1.2 (0.5-2.9)	0.6589596	0.9650042
mdc-STM-003(0.6)	Control	84	10	4.2 (1.9-9.6)	4.8024106	0.0000155
mdc-STM-003(1.5)	Control	84	10	5.3 (2.4-12.1)	5.6207999	0.0000002
mdc-STM-001(0.6)	Control	91	10	5.7 (2.3-14)	5.3194529	0.0000010
mdc-STM-002(0.6)	Control	91	10	2.1 (0.8-5.3)	2.1260924	0.2088806
mdc-STM-003(0.6)	Control	91	10	6.6 (2.7-16.2)	5.7875928	0.0000001
mdc-STM-003(1.5)	Control	91	10	7.7 (3.2-18.8)	6.2545248	0.0000000
mdc-STM-001(0.6)	Control	98	10	5.4 (2.1-13.8)	4.9224411	0.0000085
mdc-STM-002(0.6)	Control	98	10	2.4 (0.9-6.3)	2.4565738	0.1007138
mdc-STM-003(0.6)	Control	98	10	5.4 (2.1-13.7)	4.9189045	0.0000086
mdc-STM-003(1.5)	Control	98	10	8.1 (3.2-20.3)	6.1510916	0.0000000
mdc-STM-001(0.6)	Control	105	10	3.8 (1.6-9.1)	4.1562642	0.0003123
mdc-STM-002(0.6)	Control	105	10	1.8 (0.7-4.3)	1.7014145	0.4329995
mdc-STM-003(0.6)	Control	105	10	4.2 (1.8-10.1)	4.5216255	0.0000602
mdc-STM-003(1.5)	Control	105	10	9.2 (3.9-21.7)	7.0669937	0.0000000
mdc-STM-001(0.6)	Control	112	10	3.6 (1.5-8.7)	3.8574151	0.0010845
mdc-STM-002(0.6)	Control	112	10	2.1 (0.8-5.2)	2.1245354	0.2095304
mdc-STM-003(0.6)	Control	112	10	4.2 (1.7-10.1)	4.3546434	0.0001299
mdc-STM-003(1.5)	Control	112	10	4.4 (1.8-10.8)	4.5611161	0.0000500
mdc-STM-001(0.6)	Control	119	10	6.2 (2.3-16.6)	5.0086104	0.0000054
mdc-STM-002(0.6)	Control	119	10	2.2 (0.8-6.2)	2.0603323	0.2375089
mdc-STM-003(0.6)	Control	119	10	6.2 (2.3-16.7)	5.0217955	0.0000051
mdc-STM-003(1.5)	Control	119	10	9.9 (3.7-26.5)	6.4010372	0.0000000
mdc-STM-001(0.6)	Control	126	10	8.6 (3.3-22)	6.2106643	0.0000000
mdc-STM-002(0.6)	Control	126	10	2.2 (0.8-6.1)	2.1991847	0.1799034
mdc-STM-003(0.6)	Control	126	10	6.9 (2.7-17.7)	5.5486393	0.0000003
mdc-STM-003(1.5)	Control	126	10	9.4 (3.6-24)	6.4624035	0.0000000
mdc-STM-001(0.6)	Control	BI	30	0.9 (0.5-1.7)	-0.4413259	0.9921471
mdc-STM-002(0.6)	Control	BI	30	0.8 (0.4-1.6)	-0.7137346	0.9534396
mdc-STM-003(0.6)	Control	BI	30	0.7 (0.4-1.4)	-1.3516314	0.6587202
mdc-STM-003(1.5)	Control	BI	30	0.7 (0.4-1.4)	-1.3346408	0.6695032
mdc-STM-001(0.6)	Control	2	30	9.5 (5-18.1)	9.5963295	0.0000000
mdc-STM-002(0.6)	Control	2	30	12.6 (6.7-24)	10.7821368	0.0000000
mdc-STM-003(0.6)	Control	2	30	15.2 (8-28.9)	11.5763541	0.0000000
mdc-STM-003(1.5)	Control	2	30	15.5 (8.2-29.5)	11.6599028	0.0000000
mdc-STM-001(0.6)	Control	7	30	9.9 (5.2-18.9)	9.7065456	0.0000000
mdc-STM-002(0.6)	Control	7	30	11.7 (6.1-22.2)	10.3959345	0.0000000
mdc-STM-003(0.6)	Control	7	30	13.1 (6.9-24.9)	10.8754247	0.0000000
mdc-STM-003(1.5)	Control	7	30	20.5 (10.7-39)	12.7590423	0.0000000
mdc-STM-001(0.6)	Control	17	30	7.1 (3.8-13.5)	8.3577625	0.0000000
mdc-STM-002(0.6)	Control	17	30	4.3 (2.3-8.2)	6.2506357	0.0000000
mdc-STM-003(0.6)	Control	17	30	6.5 (3.4-12.4)	7.9786739	0.0000000
mdc-STM-003(1.5)	Control	17	30	10.6 (5.6-20.2)	10.0472269	0.0000000
mdc-STM-001(0.6)	Control	21	30	7.6 (4-14.4)	8.6089502	0.0000000
mdc-STM-002(0.6)	Control	21	30	5 (2.6-9.5)	6.8561647	0.0000000
mdc-STM-003(0.6)	Control	21	30	5.2 (2.7-9.9)	6.9905957	0.0000000
mdc-STM-003(1.5)	Control	21	30	8.9 (4.7-16.9)	9.2674039	0.0000000
mdc-STM-001(0.6)	Control	28	30	6.8 (3.6-13)	8.1629184	0.0000000

Formulation	Treatment arm	DAI	Follow-up (days)	HR CI95%	z.ratio	p.value
mdc-STM-002(0.6)	Control	28	30	3.5 (1.8-6.7)	5.3356150	0.0000009
mdc-STM-003(0.6)	Control	28	30	3.5 (1.9-6.7)	5.3392816	0.0000009
mdc-STM-003(1.5)	Control	28	30	5.6 (2.9-10.6)	7.2901991	0.0000000
mdc-STM-001(0.6)	Control	42	30	5.5 (2.9-10.7)	7.0734936	0.0000000
mdc-STM-002(0.6)	Control	42	30	2.2 (1.1-4.2)	3.1845824	0.0126174
mdc-STM-003(0.6)	Control	42	30	4.4 (2.3-8.4)	6.0755004	0.0000000
mdc-STM-003(1.5)	Control	42	30	6.3 (3.2-12.1)	7.5618084	0.0000000
mdc-STM-001(0.6)	Control	56	30	4.8 (2.5-9.1)	6.6095855	0.0000000
mdc-STM-002(0.6)	Control	56	30	1.8 (0.9-3.4)	2.4143863	0.1113955
mdc-STM-003(0.6)	Control	56	30	3.4 (1.8-6.5)	5.1304999	0.0000029
mdc-STM-003(1.5)	Control	56	30	7 (3.7-13.4)	8.2612435	0.0000000
mdc-STM-001(0.6)	Control	70	30	2.8 (1.5-5.3)	4.3426810	0.0001371
mdc-STM-002(0.6)	Control	70	30	1.5 (0.8-2.8)	1.5709813	0.5161174
mdc-STM-003(0.6)	Control	70	30	2.5 (1.3-4.7)	3.8103196	0.0013084
mdc-STM-003(1.5)	Control	70	30	4 (2.1-7.6)	5.8398598	0.0000001
mdc-STM-001(0.6)	Control	84	30	1.6 (0.9-3.1)	2.1039597	0.2182455
mdc-STM-002(0.6)	Control	84	30	1.1 (0.6-2.2)	0.5889268	0.9767710
mdc-STM-003(0.6)	Control	84	30	2.4 (1.3-4.6)	3.8137967	0.0012905
mdc-STM-003(1.5)	Control	84	30	2.7 (1.4-5.2)	4.2572940	0.0002008
mdc-STM-001(0.6)	Control	91	30	1.9 (1-3.7)	2.7988980	0.0410041
mdc-STM-002(0.6)	Control	91	30	1.2 (0.6-2.2)	0.6465748	0.9673238
mdc-STM-003(0.6)	Control	91	30	2.3 (1.2-4.4)	3.5590342	0.0034214
mdc-STM-003(1.5)	Control	91	30	2.5 (1.3-4.9)	3.9686477	0.0006898
mdc-STM-001(0.6)	Control	98	30	2 (1.1-3.9)	3.0181363	0.0214296
mdc-STM-002(0.6)	Control	98	30	1.2 (0.6-2.3)	0.8332180	0.9203662
mdc-STM-003(0.6)	Control	98	30	2 (1.1-3.8)	2.9714118	0.0247237
mdc-STM-003(1.5)	Control	98	30	2.5 (1.3-4.8)	3.9617328	0.0007098
mdc-STM-001(0.6)	Control	105	30	1.4 (0.8-2.7)	1.5660574	0.5193176
mdc-STM-002(0.6)	Control	105	30	1.1 (0.6-2.1)	0.4346779	0.9925898
mdc-STM-003(0.6)	Control	105	30	1.7 (0.9-3.3)	2.3262059	0.1365363
mdc-STM-003(1.5)	Control	105	30	2.9 (1.5-5.4)	4.5183891	0.0000611
mdc-STM-001(0.6)	Control	112	30	1.6 (0.9-3.1)	2.1173510	0.2125465
mdc-STM-002(0.6)	Control	112	30	1.3 (0.7-2.4)	0.9586987	0.8735510
mdc-STM-003(0.6)	Control	112	30	1.7 (0.9-3.2)	2.1785290	0.1877897
mdc-STM-003(1.5)	Control	112	30	2.1 (1.1-4)	3.1795246	0.0128281
mdc-STM-001(0.6)	Control	119	30	1.9 (1-3.7)	2.7986785	0.0410296
mdc-STM-002(0.6)	Control	119	30	1.3 (0.7-2.5)	1.1645890	0.7716195
mdc-STM-003(0.6)	Control	119	30	1.9 (1-3.7)	2.7812962	0.0430913
mdc-STM-003(1.5)	Control	119	30	2.6 (1.4-5)	4.0242898	0.0005475
mdc-STM-001(0.6)	Control	126	30	2.2 (1.2-4.2)	3.3472055	0.0072961
mdc-STM-002(0.6)	Control	126	30	1 (0.5-2)	0.1792798	0.9997694
mdc-STM-003(0.6)	Control	126	30	1.9 (1-3.6)	2.7780905	0.0434810
mdc-STM-003(1.5)	Control	126	30	2.2 (1.1-4.1)	3.2770919	0.0092731

Supplementary table S9. Between formulations comparisons of 4-day LC50 and L90 values for KIS mosquitoes fed on treated cattle.

Comparison	Estimate	Std. Error	t-value	p-value
001-0.6/002-0.6:50/50	0.8867454	0.5245928	-0.2158904	0.8290732
001-0.6/003-0.6:50/50	0.9276367	0.5590509	-0.1294396	0.8970098
001-0.6/003-1.5:50/50	0.9299036	0.5664743	-0.1237415	0.9015200
002-0.6/003-0.6:50/50	1.0461138	0.3575767	0.1289621	0.8973877
002-0.6/003-1.5:50/50	1.0486704	0.3703614	0.1314131	0.8954485
003-0.6/003-1.5:50/50	1.0024438	0.3723224	0.0065637	0.9947629
001-0.6/002-0.6:90/90	1.3316348	0.7287634	0.4550651	0.6490624
001-0.6/003-0.6:90/90	1.1868356	0.5802706	0.3219801	0.7474678
001-0.6/003-1.5:90/90	0.9649750	0.4975064	-0.0704012	0.9438744
002-0.6/003-0.6:90/90	0.8912621	0.4205113	-0.2585850	0.7959554
002-0.6/003-1.5:90/90	0.7246544	0.3618711	-0.7608942	0.4467203
003-0.6/003-1.5:90/90	0.8130654	0.3533909	-0.5289739	0.5968236

001 = mdc-STM-001
002 = mdc-STM-002
003 = mdc-STM-003

Supplementary Table S10. Between formulations comparisons of 10-day LC50 and LC90 values for KIS mosquitoes fed on treated cattle.

Comparison	Estimate	Std. Error	t-value	p-value
001-0.6/002-0.6:50/50	0.8503147	0.4901133	-0.3054095	0.7600542
001-0.6/003-0.6:50/50	0.8484447	0.4406361	-0.3439467	0.7308864
001-0.6/003-1.5:50/50	0.9622920	0.5826786	-0.0647150	0.9484009
002-0.6/003-0.6:50/50	0.9978008	0.4234921	-0.0051931	0.9958566
002-0.6/003-1.5:50/50	1.1316892	0.5956824	0.2210728	0.8250358
003-0.6/003-1.5:50/50	1.1341835	0.5253544	0.2554152	0.7984025
001-0.6/002-0.6:90/90	0.9526083	0.5708619	-0.0830179	0.9338373
001-0.6/003-0.6:90/90	1.1594824	0.5445723	0.2928580	0.7696307
001-0.6/003-1.5:90/90	1.0292734	0.5106321	0.0573278	0.9542841
002-0.6/003-0.6:90/90	1.2171660	0.6993343	0.3105324	0.7561561
002-0.6/003-1.5:90/90	1.0804792	0.6443631	0.1248973	0.9006048
003-0.6/003-1.5:90/90	0.8877008	0.4136421	-0.2714888	0.7860151

001 = mdc-STM-001
002 = mdc-STM-002
003 = mdc-STM-003

Supplementary Table S11. Between formulations comparisons of 4-day LC50 and LC90 values for VK5 mosquitoes fed on treated cattle.

Comparison	Estimate	Std. Error	t-value	p-value
001-0.6/002-0.6:50/50	0.8629191	0.2962409	-0.4627344	0.6435547
001-0.6/003-0.6:50/50	0.9532895	0.2969513	-0.1573003	0.8750082
001-0.6/003-1.5:50/50	0.9981696	0.3163292	-0.0057865	0.9953830
002-0.6/003-0.6:50/50	1.1047263	0.3647694	0.2871029	0.7740335
002-0.6/003-1.5:50/50	1.1567359	0.3878491	0.4041157	0.6861277
003-0.6/003-1.5:50/50	1.0470792	0.3169059	0.1485588	0.8819018

Comparison	Estimate	Std. Error	t-value	p-value
001-0.6/002-0.6:90/90	0.8442851	0.4320073	-0.3604451	0.7185143
001-0.6/003-0.6:90/90	0.9480422	0.4348192	-0.1194928	0.9048849
001-0.6/003-1.5:90/90	1.0899361	0.4614506	0.1948987	0.8454723
002-0.6/003-0.6:90/90	1.1228935	0.5912852	0.2078414	0.8353528
002-0.6/003-1.5:90/90	1.2909575	0.6405087	0.4542600	0.6496417
003-0.6/003-1.5:90/90	1.1496704	0.5072949	0.2950363	0.7679661

001 = mdc-STM-001
002 = mdc-STM-002
003 = mdc-STM-003

Supplementary table S12. Between formulations comparisons of 10-day LC50 and LC90 values for VK5 mosquitoes fed on treated cattle.

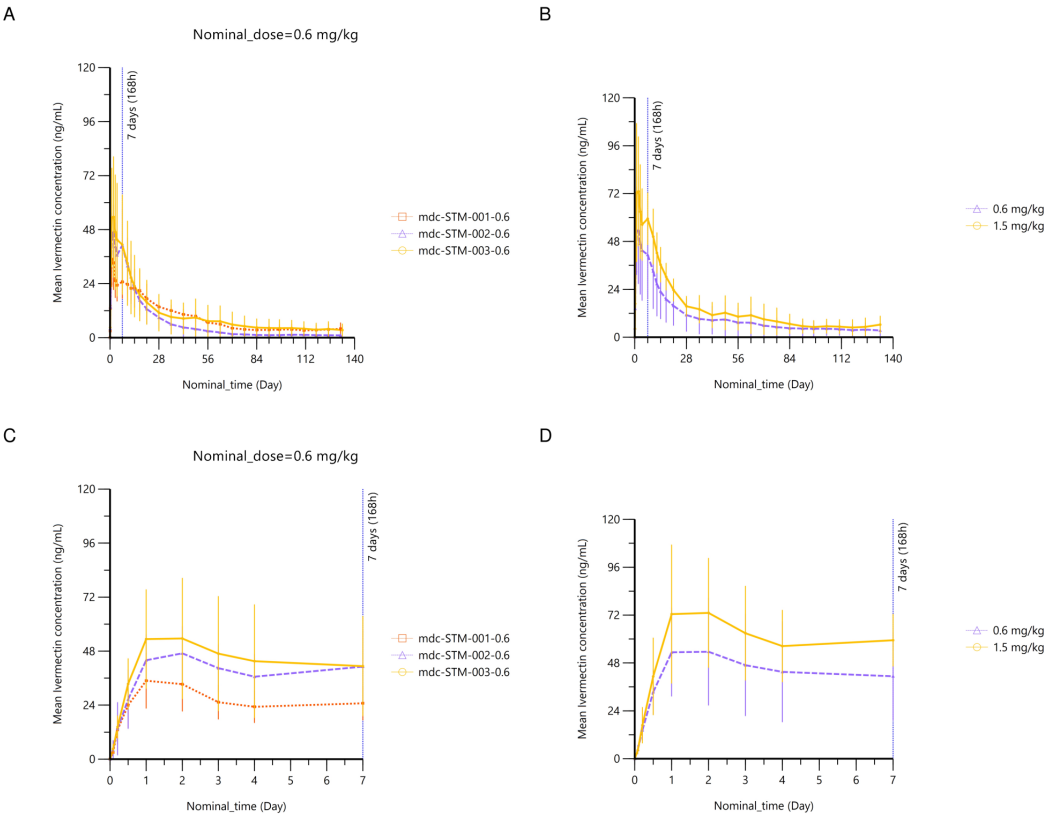
Comparison	Estimate	Std. Error	t-value	p-value
001-0.6/002-0.6:50/50	0.9513449	0.4334657	-0.1122468	0.9106277
001-0.6/003-0.6:50/50	0.9647061	0.4190361	-0.0842264	0.9328765
001-0.6/003-1.5:50/50	0.9823391	0.4071531	-0.0433767	0.9654013
002-0.6/003-0.6:50/50	1.0140446	0.4935095	0.0284586	0.9772964
002-0.6/003-1.5:50/50	1.0325793	0.4842848	0.0672731	0.9463643
003-0.6/003-1.5:50/50	1.0182780	0.4565668	0.0400337	0.9680663
001-0.6/002-0.6:90/90	0.9165698	0.5121319	-0.1629076	0.8705911
001-0.6/003-0.6:90/90	1.0141546	0.4742174	0.0298484	0.9761880
001-0.6/003-1.5:90/90	1.0997614	0.5007874	0.1992090	0.8420993
002-0.6/003-0.6:90/90	1.1064674	0.6179795	0.1722831	0.8632150
002-0.6/003-1.5:90/90	1.1998665	0.6578991	0.3037950	0.7612841
003-0.6/003-1.5:90/90	1.0844119	0.4934883	0.1710515	0.8641833

001 = mdc-STM-001
002 = mdc-STM-002
003 = mdc-STM-003

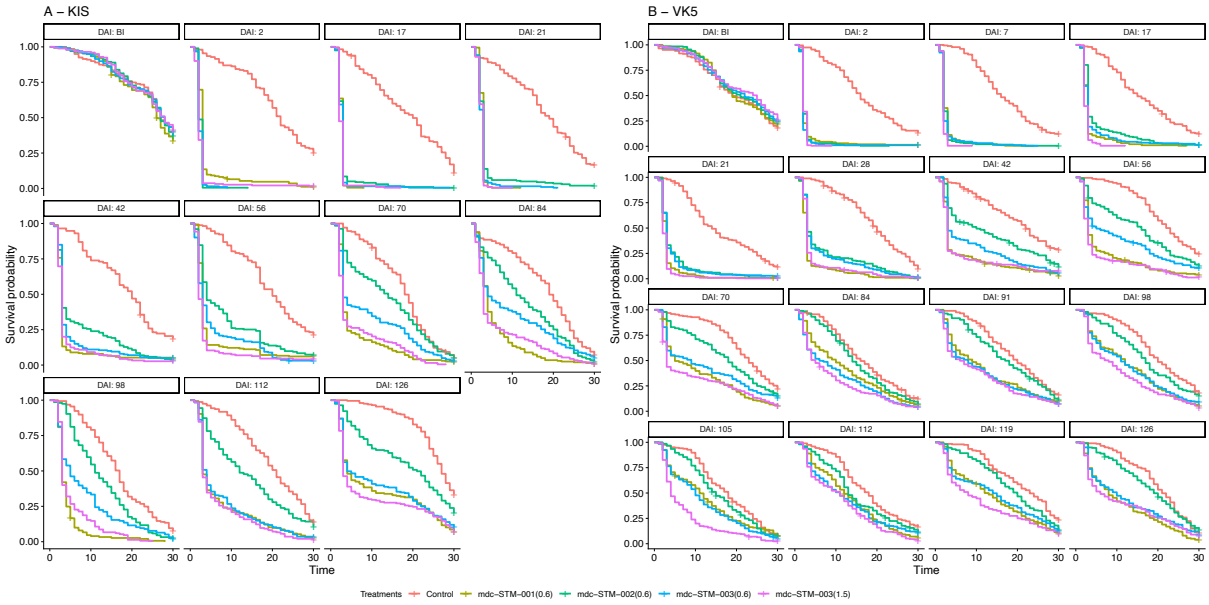
Supplementary Table S13. Lethal concentration (LC50) for KIS and VK5 mosquitoes over 4-day follow up period (4-day LC50). Mean concentrations are given with lower and upper confidence intervals (95% CI).

Cumulative mortality window (days)	Mortality %	KIS mosquitoes	VK5 mosquitoes	*P-value* (between colony comparison)
4	50	2.89 [2.27-3.67]	5.26 [4.36-6.35]	1.0e-07
4	90	9.2 [6.72-12.59]	17.65 [12.86-24.22]	0.0e+00

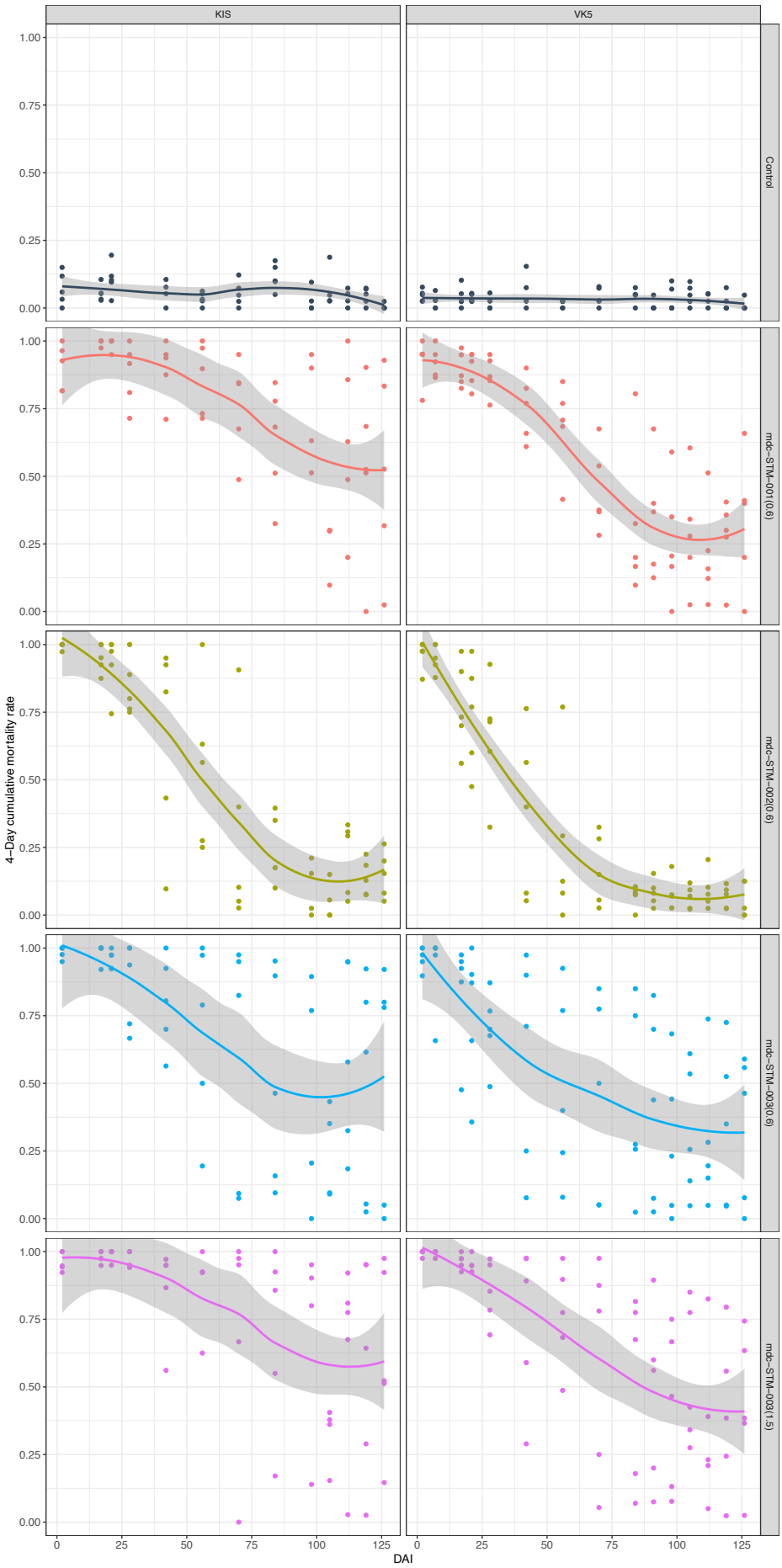
Supplementary figures



Supplementary Fig. S1: Additional mean concentration–time profiles of ivermectin in cattle plasma. Panels A and C: profiles by formulation at a dose of 0.6 mg/kg for the entire experiment (A) and during the first 7 days post-injection to visualize the initial burst (C). Panels B and D: profiles by dose for the entire experiment (B) and during the first 7 days post-injection (D)

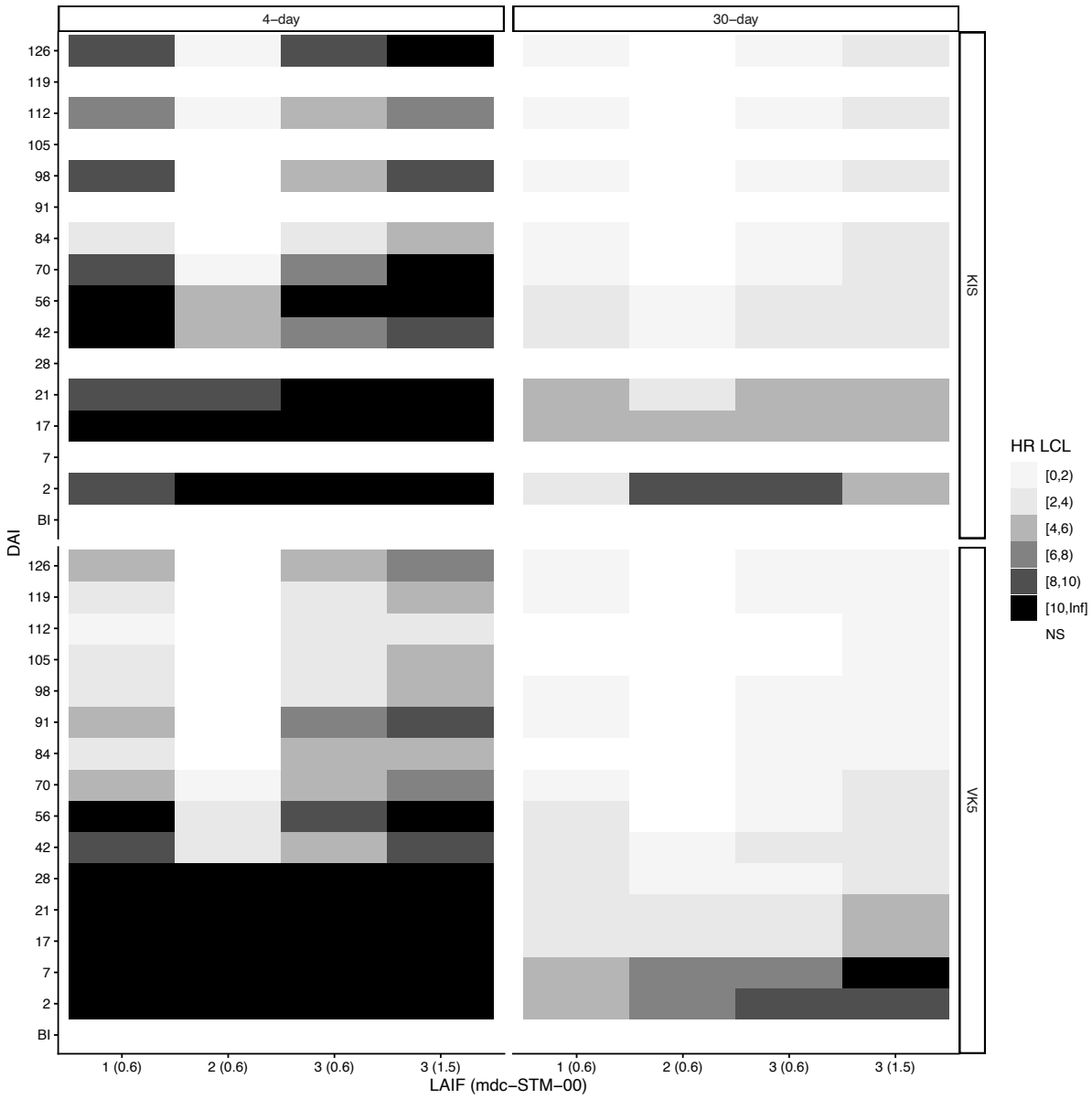


Supplementary Fig. S2. Kaplan-Meier plots illustrating the mosquitocidal effects of the candidate long-lasting ivermectin formulations administered to calves at doses of 0.6 mg/kg (mdc-STM-001, mdc-STM-002 and mdc-STM-003) or 1.5 mg/kg (mdc-STM-003 only). The plots depict the results from each direct skin feeding assay conducted at the indicated time points (Days After Injection, DAI). Mosquito survival was monitored daily for up to 30 days post-blood meal. Pannel A: KIS colony, Pannel B: VK5 colony.

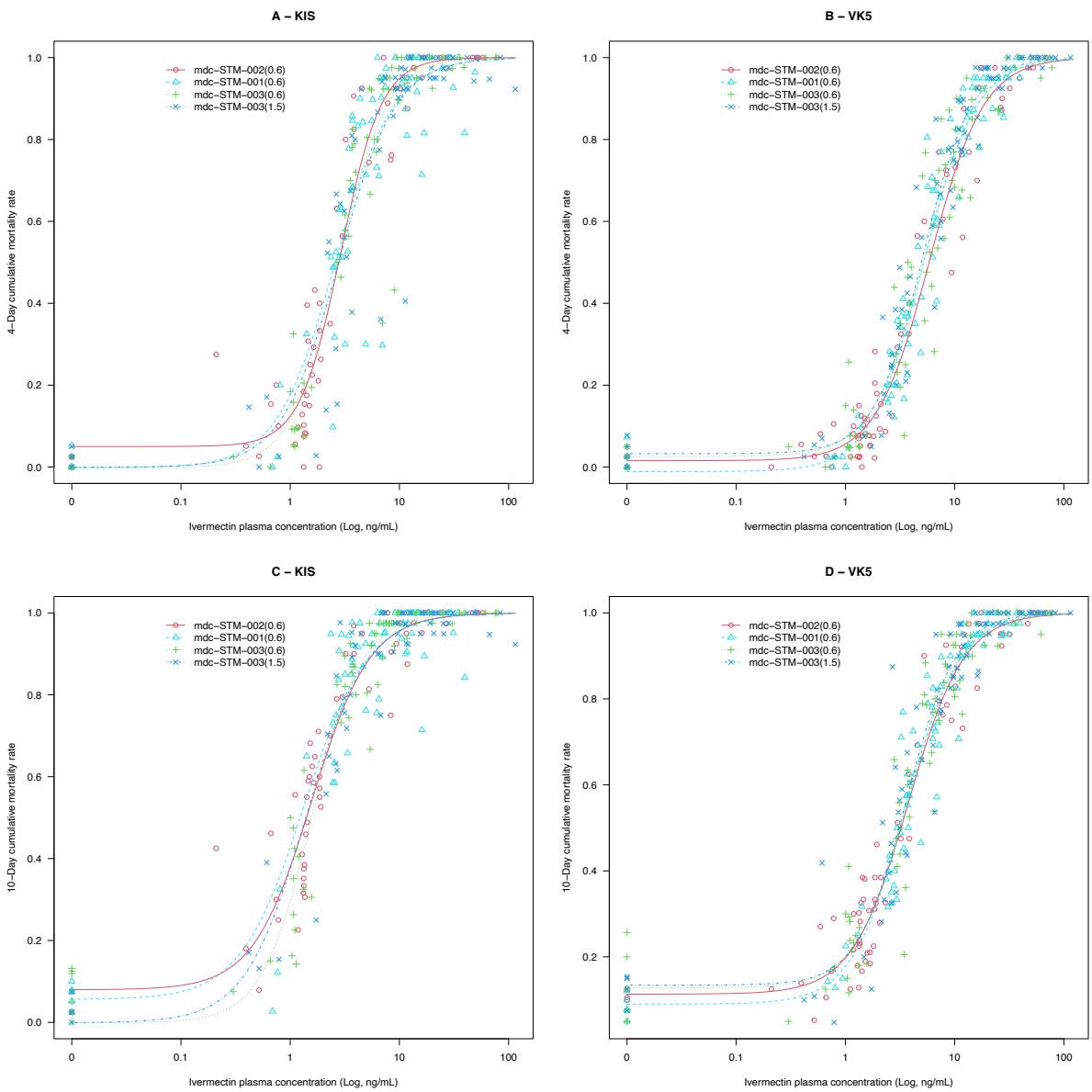

KIS
VK5
Control
mdc−STM−001(0.6)
mdc−STM−002(0.6)
mdc−STM−003(0.6)
mdc−STM−003(1.5)
4−Day cumulative mortality rate
DAI
1.00
0.75
0.50
0.25
0.00
0
25
50
75
100
125

1414

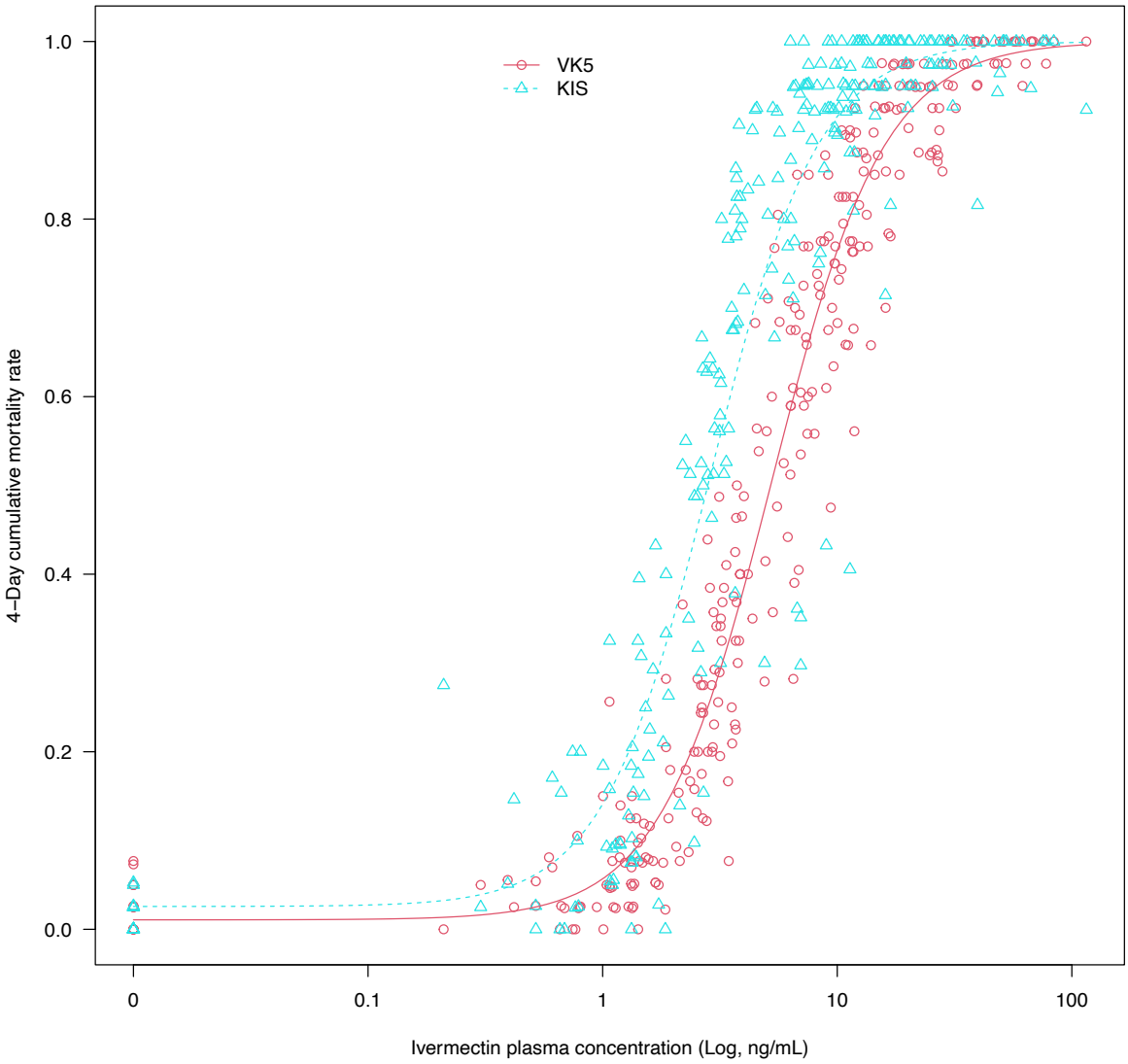
Supplementary Fig. S3: 4-day cumulative mortality of KIS and VK5 colony mosquitoes after feeding on calves treated using the candidate formulations. Treated cattle received a single LAIF injection at a dose of 0.6mg/kg for the mdc-STM-001, mdc-STM-002, mdc-STM-003 formulations, and at an additional dose of 1.5 mg/kg for the mdc-STM-003. The smooth line represents mean values estimated by the LOESS (locally estimated scatterplot smoothing) method and grey area shows CI95%. See the main text for further details.



Supplementary Fig. S4. Heat maps illustrating the lower 95% confidence limit (LCL) of 4-day and 30-day mortality hazard ratios (HRs) for KIS and VK5 colony mosquitoes fed at different DAIs on cattle treated with the formulations mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-003-0.6 or mdc-STM-003-1.5. HR and *P-values* for each DAI are available in Additional file 1: table S7. BI is the timepoint before injection.



Supplementary Fig. S5. Relationship between ivermectin plasma concentration and 4-days (A and B) or 10-day (C and D) cumulative mortality for each candidate formulation. A and C: KIS colony mosquitoes. B and C: VK5 colony mosquitoes



Supplementary Fig.6. Relationship between ivermectin plasma concentrations and 4-day cumulative mosquito mortality for KIS and VK5 colonies.