Selection of a Lead Long-Acting Formulation of Ivermectin to Target Major Malaria Vectors in Western Africa: Evaluation of Pharmacokinetics and Mosquitocidal Efficacy in Cattle under Laboratory Conditions.

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ABSTRACT

Background

Ivermectin, a semisynthetic endectocide from the avermectin family, has been used in mass drug administration (MDA) campaigns since the 1980s to control helminth infections. Its lethality to *Anopheles* mosquitoes after feeding on treated hosts offers a promising malaria control strategy, especially for outdoor transmission. However, existing oral formulations produce short-duration mosquitocidal blood concentrations, limiting epidemiological impact. The WHO's target product profile for endectocides calls for a hazard ratio >4 and sustained efficacy for at least one month. In this context, three long-acting injectable ivermectin formulations (LAIF) based on BEPO® depot technology were evaluated in cattle to evaluate the *in vivo* performance then to identify the most effective candidate.

Methods

The LAI candidates (mdc-STM-001, -002, and -003) were tested at 0.6 mg/kg, with mdc-STM-003 also evaluated at 1.5 mg/kg. Each treatment group consisted of five calves, each receiving single sub-cutaneous injection. Plasma samples intensively collected over 130 days enabled non-compartmental pharmacokinetic analysis of ivermectin. Two mosquito strains: *Anopheles coluzzii* (VK5-pyrethroid-resistant) and *Anopheles gambiae* (KIS-Kisumu strain) were allowed to simultaneously blood-feed on treated animals from 2 to 126 days post-dose. Mosquito survival was monitored for 30 days. Mortality within 4 and 10 days post-feeding was emphasized, targeting the window before *Plasmodium falciparum* becomes transmissible. Efficacy was assessed via hazard ratios, cumulative mortality, lethal concentrations (LC) 50 and 90 values, and duration of effective coverage.

Results

All formulations were well tolerated. mdc-STM-001 showed the most favorable pharmacokinetic profile with a controlled peak (Cmax= 34.5 ± 12.7 ng/mL) and the lowest inter-individual variability (12%). mdc-STM-001 and -003 produced hazard ratios >4 and cumulative mortalities >50% for at least 60 days in both mosquito strains. Surviving mosquitoes had median lifespans below 10 days for at least 90 days post-injection. The 10-day LC50 for resistant mosquitoes (3.66 [2.69-4.97]) was maintained for ≥126 days. mdc-STM-001 provided 100% coverage at this threshold for ≥2 months.

Conclusion

mdc-STM-001 is the lead candidate. A single injection allows killing >50% of biting mosquitoes before they become infectious, even in resistant populations, during at least 2 months. Given ivermectin's established safety profile, its repurposing in LAIFs offers a promising strategy for malaria control. This approach directly targets two major challenges: outdoor transmission and rising insecticide resistance, and warrants further investigation.

Keywords: residual malaria, outdoor transmission, mosquitoes resistance, ivermectin, repurposing, long-acting technology, pharmacokinetics, efficacy, impact

BACKGROUND

Progress in the fight against malaria has stagnated since 2015, following a remarkable 15-years of sustained decrease in disease incidence, largely driven by the widespread deployment of two core insecticide-based vector control tools: long-lasting insecticidal nets (LLINs) and insecticide residual spraying (IRS) [1]. While these methods remain effective,

93 their impact is increasingly undermined by the large-scale development of physiological
94 resistance in mosquito vectors to insecticidal compounds [2], as well as by behavioral
95 avoidance of treated surfaces [3]. New generations of LLINs incorporating dual active
96 ingredients or synergists have been introduced to address physiological resistances [4] [5].
97 However, these tools primarily target nocturnal, indoor-biting *Anopheles* mosquitoes are still
98 bypassed by vectors that feed outdoors and/or at times when people are not protected by
99 the nets [6][7], which leads to persistent residual malaria transmission.
100 Malaria therefore remains a leading global health concern with an estimated 263 million
101 clinical cases and 597,000 deaths in 2023, over 95% of which occurred in Sub-Saharan Africa
102 [8]. The resurgence in malaria cases in recent years highlights the urgent need for novel and
103 complementary strategies to reinforce existing interventions and reverse this concerning
104 trend [8]. In particular, tools targeting the diverse biting behaviors of *Anopheles* vectors,
105 whether innate or induced by interventions, are essential for addressing this challenge [9].
106 Mass drug administration (MDA) of endectocides, compounds active against both endo- and
107 ectoparasites, offers an approach that circumvent the limitations of current vector control
108 strategies, as it is independent of mosquito feeding time and location [10] : the insecticide is
109 delivered through the bloodstream of the treated host, making it effective regardless of the
110 vector's biting behavior. Ivermectin is the leading candidate among endectocides for vector
111 control, due to its extensive clinical use against lymphatic filariasis and onchocerciasis, with a
112 strong safety profile [11] [12]. Ivermectin binds to glutamate-gated chloride channels unique
113 to invertebrates, inducing neuromuscular hyperpolarization, paralysis and flaccid death [13].
114 This distinct mode of action makes ivermectin effective against mosquitoes physiologically
115 resistant to standard insecticides [14][15]. To date, it has shown to be lethal to all tested
116 *Anopheles* species [12], including nine Southeast Asian malaria vectors recently added to the

117 list of susceptible species [16]. Besides direct acute effects on survival induced by lethal
118 plasma concentrations, sub-lethal effects are also interesting to transmission control as they
119 negatively impact vectors fecundity and fertility [17][18], locomotor and biting behaviors
120 [19][20], or *Plasmodium* parasite development [21][22].

121 Standard oral dosing for parasitic disease treatment (150–200 µg/kg) achieves lethal
122 plasma levels for *Anopheles* for fewer than seven days [23]. The first community-level clinical
123 trial designed to test repeated ivermectin-MDA used this dosage, administered every 3
124 weeks during the transmission season [24]. This regimen resulted in a significant 20%
125 reduction of malaria incidence in children under five, however, it was subsequently
126 identified that extending ivermectin's duration of action could substantially improve impact
127 and logistics. Higher oral regimens (1x400 µg/kg or 3x300 µg/kg) have been demonstrated
128 safe and with extended mosquitocidal effects for at least 10-14 days on laboratory colonies
129 [25, 26, 27], with modelling suggesting up to 70% reduction in malaria incidence in seasonal
130 transmission settings [28]. A two-country trial using the 1x400 µg/kg is completed with
131 results pending [29]. However, two Phase 3 trials testing repeated ivermectin MDA (3x 300
132 µg/kg) in combination with either dihydroartemisinin-piperaquine (DHAP) [30] or seasonal
133 malaria chemoprophylaxis (SMC) [15] did not show significant epidemiological effects.
134 Explanatory factors discussed include underestimated wild mosquito tolerance, shorter-
135 than-expected ivermectin residence time (as demonstrated in the RIMDAMAL II trial, where
136 plasma levels dropped below detection in nearly 50% of participants by day 14) [15] and
137 overlooked aspects of mosquitoes bionomics, such as zoophagy [31].
138 Long-acting formulations that sustain effective plasma concentrations over extended periods
139 could overcome these limitations. Commercial veterinary injectables have demonstrated
140 mosquitocidal effects lasting 1–2 months [16], and BEPO®-based long-acting injectable

141 formulation (LAIF) prototypes [32] have maintained efficacy for over six months in cattle
142 after a single injection [33,34]. Comparable durations have been achieved with silicone
143 implants [35,36]. The only human-targeted long-acting formulation to date—a gastric-
144 resident oral device—has shown efficacy with ~14-day residence time in pigs [37].
145 We conducted a preclinical study to characterize the pharmacokinetic profile and
146 mosquitocidal efficacy of three LAIF based on BEPO® technology [32], developed for MDA
147 use in malaria endemic regions. The ivermectin formulations were designed to achieve a
148 molecule residence time of at least 2 months following a single injection, with sustained
149 plasma concentrations sufficient to ensure mosquitocidal efficacy, achieving a hazard ratio
150 (HR) > 4 as recommended by the World Health Organization (WHO) for endectocides in
151 malaria vector control [38]. Efficacy was further assessed via complementary entomological
152 endpoints, including mosquito median survival time, 4-day and 10-day cumulative mortality,
153 lethal concentrations 90 and 50 (LC90 and LC50) values, and corresponding duration of
154 coverage. A cattle-mosquito colony model was used [17][34], exposing both insecticide-
155 susceptible rand pyrethroid-resistant *Anopheles* strains. Comparative analysis of
156 pharmacokinetic and efficacy outcomes allowed us to identify the lead candidate for further
157 development toward clinical evaluation in humans.
158

159 ## METHODS

160 ### ***MOSQUITO COLONIES***

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

162 (i) ***Anopheles (An.) gambiae* Kisumu (KIS)**: This World Health Organization (WHO)
163 reference strain is fully susceptible to all classes of insecticides. It has been

maintained for over a decade in the insectary at the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo-Dioulasso, Burkina Faso. The colony is routinely monitored for contamination by other species and screened for insecticide resistance using molecular techniques [39,40]. 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) using an air-conditioned vehicle to maintain stable temperature. Transport duration was approximately 10 minutes.

(ii) ***An. coluzzii* VK5-IMPACT (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 area characterized by a high prevalence of insecticide resistance [41]. Collected females were allowed to individually oviposit. Species identification was performed post-oviposition by PCR using established protocols [39] and only progeny from confirmed *An. coluzzii*—the primary malaria vector in Burkina Faso—were retained. As expected based on the collection site, all females belonged to the target species [41].

Both colonies were housed in similar environmental conditions and maintenance protocol. The insectariums temperature was set to 26°C ± 1°C, relative humidity to 75% ± 5%, and photoperiod to 12-hour light/dark. Adult mosquitoes were maintained on 10% sterile glucose solution provided on cotton balls. For egg production, females were fed on rabbits two to three times per week. Eggs were placed in trays (32 x 13 x 5 cm) filled with tap water. After hatching, larvae were transferred to new trays (n=200 larvae /tray)

187 using Pasteur pipettes and fed ad libitum with commercial alevin food (TetraMin® Baby).
188 Breeding was conducted in 25 x 25 x 25 cages containing mixed-sex adults. The VK5
189 colony's insecticide resistance status was routinely monitored by sampling
190 approximatively 40 females from each generation and screening for the KDR-W mutation
191 using quantitative PCR (qPCR) ([40]). Routine maintenance included 10-15 cages
192 containing 100-200 females each to ensure a stable production of experimental
193 mosquitoes. For each direct skin-feeding assay time point, females were randomly drawn
194 from at least five different cages to minimize potential cage effects.

195

196 ***LONG ACTING IVERMECTIN FORMULATIONS***

197 The long-acting ivermectin formulations (LAIFs) tested in this study were developed using
198 BEPO® technology [32], a proprietary long-acting injectable technology (Medincell, Jacou,
199 France). Following subcutaneous injection, BEPO® forms an *in situ* bioresorbable polymeric
200 depot via a solvent-exchange mechanism, leading to precipitation of a polymer matrix that
201 enables sustained drug release. Each formulation consisted of three components: i) a
202 biocompatible solvent to ensure injectability, ii) ivermectin, as the active pharmaceutical
203 ingredient, and iii) a mixture of two biocompatible and biodegradable polymers that control
204 drug release kinetics. These polymers undergo progressive hydrolysis into bioabsorbable by-
205 products over time, thanks to a simple hydrolysis mechanism. Three ready-to-use LAIFs
206 solutions were evaluated: **mdc-STM-001**, **mdc-STM-002** and **mdc-STM-003**; the last one
207 differed in ivermectin loading and each differed in polymer composition and proportions.
208 All formulations were imported into Burkina Faso with clearance from the Direction
209 Générale des Services Vétérinaires of Burkina Faso (Permit No 2020/199) and were stored at

210 room temperature, protected from light, until administration to cattle. The study protocol
211 was approved by the Institutional Animal Ethics Committee of CIRDES (document reference
212 number: 004-10/2020 CEB-CIRDES).
213
214 ***CATTLE HOSTS AND FORMULATIONS' ADMINISTRATION***

215 Twenty-five male calves (Métis strain: Fulani zebus x Baoulé crossbreed) with a median
216 weight of 117 kg (interquartile range [IQR]: 106-129 kg), were purchased from villages in the
217 Valley du Kou area two months prior to the study to allow for acclimation. Upon arrival at
218 the CIRDES animal facility, calves were treated for trypanosomiasis and gastrointestinal
219 parasites using Berenil 2000® and Benzal®, respectively. Animals were housed outdoors
220 under screened enclosures to prevent exposure to or reinfestation with ectoparasites and
221 trypamosomoses. They were fed with rice straw and 1 kg/day of cottonseed cake, with
222 water and sea salt provided ad libitum.

223 Calves were randomly assigned to five experimental arms (n = 5 calves per arm) using
224 stratified randomization to balance body weight across groups. Four arms received LAIFs,
225 and one served as the untreated control. The formulations were administered *via* a single
226 subcutaneous injection into the loose skin anterior to the shoulder.

227 - mdc-STM-001 and mdc-STM-002 LAIFs were each injected at a unique dose of 0.6
228 mg/kg (referred to as mdc-STM-001-0.6 and mdc-STM-002-0.6, respectively).
229 - mdc-STM-003 was administered at two doses: 0.6 mg/kg and at 1.5 mg/kg (referred
230 to as mdc-STM-003-0.6 and mdc-STM-003-1.5).

231 The 1.5 mg/kg dose represents the upper limit of feasible subcutaneous volumes for
232 human application and was included to assess dose-dependent effects on
233 pharmacokinetics and mosquitocidal efficacy. Nominal injection volumes per animal
234 ranged from 0.62 to 2.14 mL (detailed in Table 1). The animals' identification numbers,
235 their weight upon arrival at CIRDES and on the day of injection, administered
236 formulations, and injected volumes are provided in the supplementary Table 1
237 (Appendix). Animal health was monitored throughout the study by assessing clinical
238 signs, local injection site reactions, body weight, and weight gain.
239
240 Table 1: Nominal treatment volumes per formulation to be administered to cattle. N/A:
241 not applicable.

Number of male cattle	LAI formulation name	Ivermectin dose	LAI nominal administered volume
5	mdc-STM-001	0.6 mg/kg	7.72 μL/kg
5	mdc-STM-002	0.6 mg/kg	7.80 μL/kg
5	mdc-STM-003	0.6 mg/kg	5.85 μL/kg
5	mdc-STM-003	1.5 mg/kg	14.6 μL/kg
5	N/A (control)	0 mg/kg	0 μL/kg

242
243 ***MOSQUITO BLOOD-FEEDING BIOASSAYS***

244 To ensure equivalent ivermectin exposure, mosquitoes from both KIS and VK5 colonies were
245 simultaneously exposed to the same cattle hosts during blood-feeding. This design enabled
246 direct comparison of susceptibility across colonies under identical conditions. Direct skin
247 blood-feeding assays were conducted following previously established protocols [34]. Prior
248 to feeding, mosquitoes were deprived of sugar for 12 hours, with access to water-soaked
249 cotton only. At each time point, approximately 100–130 females per colony were introduced

250 into net-covered bowls, and held against the shaved flank of the selected calf for 20 minutes
251 using a rubber band. Separate bowls were used for each colony. Fully engorged mosquitoes
252 were distributed into cups (10 females per cup), with a maximum of 120 cups per colony per
253 time point. All cups were maintained under controlled conditions in the CIRDES insectary.
254 Mosquito mortality was recorded daily between 10:00 and 11:00 a.m. for 30 days post-blood
255 meal. To mitigate positional bias, cups were randomly repositioned on shelves after each
256 observation.

257 A total of 16 blood-feeding assays were conducted: once pre-treatment (baseline) and at 2,
258 7, 17, 21, 28, 42, 56, 70, 84, 91, 98, 105, 112, 119, and 126 Days After Injection (DAI). Due to
259 limited mosquito availability, KIS mosquitoes were not tested at 7 and 91 DAI. Surplus blood-
260 fed mosquitoes were stored at –20°C for downstream analyses.

261 ***IVERMECTIN BIOANALYSIS AND PHARMACOKINETICS***

262 Jugular venous blood samples were collected from treated calves at each mosquito exposure
263 time point and at additional time-points (30 samples per animal) to characterize ivermectin
264 pharmacokinetics (PK). Sampling included pre-dose, 2h, 5h and 12h post-injection, then daily
265 sampling until DAI 4, every 2-4 days until DAI 21, and weekly until DAI 132-133. Blood
266 samples were also collected from control animals at pre-dose and at DAI 1, 14, 28, 56, 84
267 and 111. Blood samples were centrifuged at +4°C (2000 rpm for 10 min) to isolate plasma,
268 which was stored at -80°C until shipment for bioanalytical analysis. Ivermectin plasma
269 concentrations were quantified using a liquid-liquid extraction with qualified LC-MS/MS
270 methods. Two calibration ranges were used (0.2-20 ng/mL low range; 5-500 ng/mL high
271 range), using K_2-EDTA as anticoagulant and ivermectin-D_2 as internal standard. The quality
272 controls (0.6, 10, 16 ng/mL low range; 15, 250, 400 ng/mL high range) were within the

273 acceptance criteria (precision ≤ 15.00%, deviation ± 15.00%). Incurred sample reanalysis
274 validated ivermectin stability for at least 680 days at -20°C. Plasma samples collected from
275 cattle were stored at -80°C no longer than 162 days before analysis, validating the
276 ivermectin concentrations measured for PK analysis.
277 At study completion, control animals were returned to stock, while treated animals were
278 euthanized. Injection sites and depot with surrounding tissues were sampled to quantify the
279 residual ivermectin and copolymers. These analyses were performed using an ultra-
280 performance liquid chromatography (limit of quantification: 0.9 µg/mL) for ivermectin and
281 nuclear magnetic resonance analysis for copolymers. Both methods were developed for
282 exploratory purposes only.
283

284 ***OUTCOMES AND ANALYSIS***

285 Unless specified otherwise, analysis were performed using the R version 4.4.3 (2025-02-
286 28) [42]. Model fit was assessed using the "performance" [43] and DHARMa [44] packages.
287 All estimates are reported with 95% confidence intervals (CI95%) and/or p-values.

288
289 **Cattle weight gain**

290 Differences in weight gain between treatment groups were analyzed using the Kruskal–Wallis
291 rank sum test, followed by pairwise two-sided exact Wilcoxon rank sum tests with 95%
292 confidence intervals. *p*-values were adjusted using the Holm method ([45]).
293

294 **Pharmacokinetics**

295 PK parameters of ivermectin were calculated for each LAIF using a non-compartmental
296 analysis (Phoenix® WinNonlin® version 8.1; Certara USA, Inc., Princeton, NJ). These included
297 the rate of systemic exposure (Cmax), the time to reach Cmax (Tmax), the extent of systemic
298 exposure over the study duration (aera under the curve, AUClast), over 28 DAI (AUC0-28d)
299 and over 91 DAI (AUC0-91d), concentrations at 28 DAI (C28d), 56 days (C56d), 91 DAI (C91d)
300 and at study end (Clast), and the apparent terminal phase half-life (T1/2) when calculable.
301 The systemic exposure parameters were normalized to the actual administered dose,
302 calculated for each cattle based on the weight of each syringe before and after injection. The
303 inter-individual variability in plasma concentrations and PK parameters was expressed as the
304 coefficient of variation (CV %) for each experimental group.

305 **Mosquito related Outcomes**

306 Mosquito blood feeding rate

307 For each colony, we compared the mosquitoes' blood-feeding rates across treatment arms
308 using a generalized linear mixed model (GLMM [46, 47]) with a Poisson error distribution.
309 The outcome variable was the number of blood-fed mosquitoes, with the total number of
310 mosquitoes included as an offset. The model included an interaction term between
311 treatment and DAI, as well as a random intercept for cattle. The model was fitted using
312 restricted maximum likelihood.

313 Mosquitocidal efficacy

314 Multiple complementary outcomes were used to assess the efficacy of each LAIF and to
315 allow comparison between formulations and mosquito strains. Depending on the outcome,
316 values are given for a 4-day or a 10-day follow up period. Outcomes for 30-day follow-up
317 period are given in the Appendix when specified in the results section.

318 Outcomes provided for the 4 days post-blood feeding period reflect the acute effect of the
319 ivermectin concentration absorbed by the mosquitoes, as the direct lethal effect of the
320 molecule typically manifests within 2-3 days post-ivermectin blood meal. In relation to the
321 extrinsic incubation period of *Plasmodium* parasites, we also measured efficacy outcomes
322 for the 10-day post-blood feeding period, in order to assess the treatment's potential to
323 prevent the build-up of infectious mosquitoes' populations. Extended 30-day follow-up
324 captures the residual effects of ivermectin on surviving mosquitoes, which may include both
325 indirect sublethal effects and potential interactions with the mosquitoes' age.

326 *Cumulative Mortality*
327
328 Proportions of dead mosquitoes at 4- and 10-days post-feeding were computed for each
329 colony, formulation, and DAI.

330 *Survival Analysis and Hazard Ratios*
331
332 Daily mosquito mortality was recorded for up to 30 days post blood-feeding. For each
333 mosquito colony and DAI, survival data were used to generate Kaplan–Meier survival curves,
334 stratified by treatment group.
335 Per colony, survival patterns at each time-point were compared to the control group using
336 Cox proportional hazards models (mixed-effects regression model [48]). The models
337 included "treatment group" and "DAI" as fixed effects, with random intercepts for individual
338 calf ID and mosquito cup (nested random effects) to account for variability within treatment
339 groups. Hazard ratios (HR) at different time points post-blood feeding are given to assess the
340 impact of ivermectin on mosquito mortality risks and HR variations over time. Post hoc
341 pairwise comparisons of HRs between time points were adjusted for multiple testing using
342 the Tukey method, implemented via the "emmeans" package [42]
343

344 *Dose–Response Analysis and coverage duration*

345 Dose–response relationships were analyzed using the "drc" package in R [49], modeling 4-
346 day and 10-day mosquito mortality as a function of host ivermectin plasma concentrations.
347 A four-parameter log-logistic model was fitted with the upper limit fixed at 1. Confidence
348 intervals were computed using the tfls method (t from logarithm scale).

349

350 **RESULTS**

351 **Cattle follow up**

352 A mild reaction (agitation) was observed in most animals after the injection, regardless of
353 dose or LAIF administered (data not shown). There were no unscheduled cattle deaths, and
354 no clinical signs were observed in any of the treated groups throughout the study. The major
355 local reaction was swelling, which occurred in all animals, as expected with this LAIF
356 technology. Median weight intake was unaffected by the test item treatment (Table 2). For
357 each experimental group, monthly weight measures are given in the supplementary Table 2
358 of the Appendix.

359
360 Table 2: Median weight intake (in kg) per group with interquartile range (IQR). Q1 and Q3
361 values are given in brackets. The p-value is the result of the Kruskal-Wallis rank sum test.

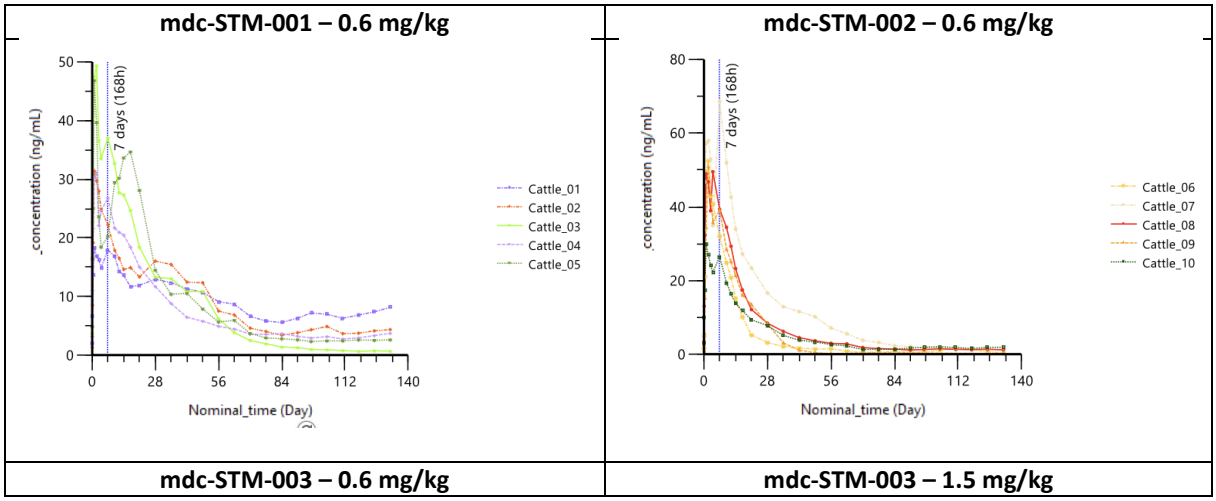
362

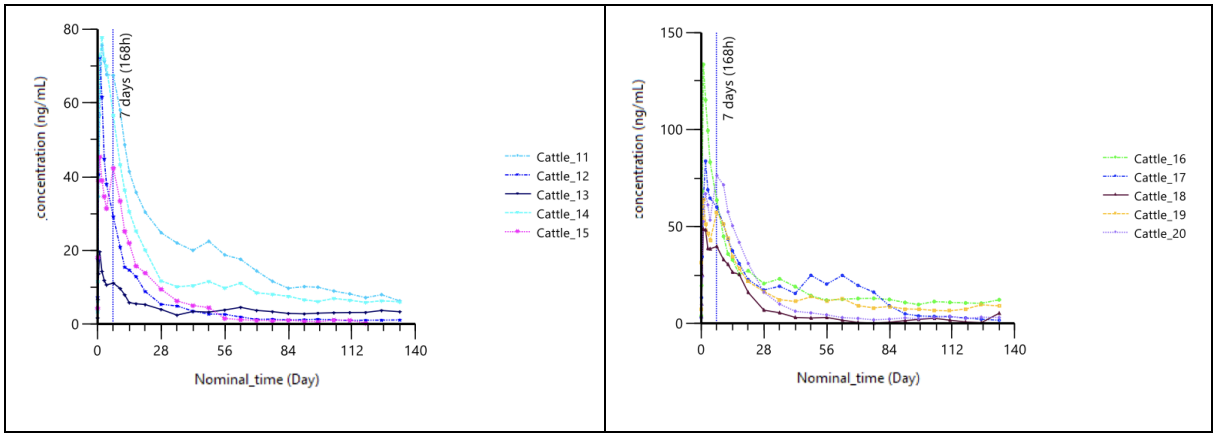
Treatment Group	**Control N = 5**	**mdc-STM-001-0.6 N = 5**	**mdc-STM-002-0.6 N = 5**	**mdc-STM-003-0.6 N = 5**	**mdc-STM-003-1.5 N = 5**	***p-value***
Median weight intake (kg)	27.4 (25.8, 31.6)	37.2 (35.8, 44.2)	39.6 (36.6, 41.6)	35.3 (31.2, 36.2)	30.0 (28.6, 40.0)	0.075

363

364 **Ivermectin pharmacokinetic**

365 A total of 635 blood samples were collected, including 600 samples from LAIF-treated cattle
366 (4 treatment groups, 5 cattle per group, n=30 per cattle) and 35 samples from control cattle
367 (1 control group, 5 cattle, n=7 per cattle). Concentration-time patterns were analyzed using
368 data until DAI = 132 for mdc-STM-001-0.6, mdc-STM-002-0.6 and DAI 133 for mdc-STM-003-
369 0.6 and mdc-STM-003-1.5. Ivermectin plasma concentrations were analyzed according to the
370 treatment (formulation and dose). Individual concentration-time profiles of ivermectin after
371 LAIFs injection are illustrated in Figure 1. Additional figures, such as the mean concentration-
372 time profiles over the study duration and those focused on the first 7 days to better visualize
373 the burst (i.e., peak plasma level), are provided in the supplementary figures 1A and 1B
374 (Appendix).
375
376 Figure 1: Individual kinetic profiles of ivermectin in cattle plasma after single sub-cutaneous
377 administration of candidate LAIFs at 0.6 or 1.5 mg/kg





Ivermectin was not detected in any of the samples collected in the control group during the study period nor in any samples collected before ivermectin injection in the treated groups. Following sub-cutaneous injection, ivermectin plasma concentrations were generally measured in all samples collected during the study, with quantifiable levels still present at the final study time-point (*i.e.,* DAI 132 or 133).

After a single sub-cutaneous administration of LAIFs, the plasma levels of ivermectin increased to reach the maximal level (*i.e.,* the burst, known to occur with LAIs [32]) at 24h or 48h post-dose (median Tmax). This was followed by two phases of decrease: a first phase until Day 56 (*i.e.,* 1344h post dose) then a slower phase with a regular decrease of ivermectin plasma levels until the study end. Among the formulations, mdc-STM-001-0.6 showed the lowest rate of decrease during the first phase and the steadiest levels during the second phase. In contrast, the mdc-STM-002-0.6 exhibited the fastest decrease compared to other LAIFs. The inter-animal variability in ivermectin plasma levels (expressed as coefficient of variation CV%) was moderate. The lowest variability was seen with mdc-STM-001-0.6 (mean CV%: 41%, range: 12-70%), followed by mdc-STM-003-1.5 (mean: 54%, range: 22-87). The highest variability was observed for mdc-STM-002-0.6 (mean CV%: 62%, range: 25-106%) and mdc-STM-003-0.6 (mean: 72%, range: 34-98%).

397 The mean ivermectin PK parameters of each LAIF after injection are reported in Table 3.

398 Additional parameters are given in the supplementary Table 3 of the Appendix.

399

400 Table 3: Mean (SD) pharmacokinetic parameters of ivermectin in plasma cattle after single

401 sub-cutaneous administration of LAIF at 0.6 or 1.5 mg/kg

402

Parameters	**mdc-STM-001-0.6**	**mdc-STM-002-0.6**	**mdc-STM-003-0.6**	**mdc-STM-003-1.5**
Tmax* (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(0-91d) (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)	13.7 (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)

403 *Median (min; max).

404

405 At a dose of 0.6 mg/kg, PK differences emerged across formulations when considering both

406 the entire study duration or the 91-day period (*i.e.,* 3 months post-injection), which is the

407 best targeted release duration. These differences involved performance, quantification

408 levels over time, and inter-animal variability. The peak plasma concentration (Cmax) for

409 mdc-STM-001-0.6 was at least 30% lower compared to the other LAIFs, with mdc-STM-003-

410 0.6 showing the highest Cmax. This suggests that mdc-STM-001-0.6 provided a safer burst

411 compared to the other LAIFs. The inter-animal variability in Cmax was moderate for all

412 formulations, with the lowest variability observed for mdc-STM-002-0.6 (CV% = 27%)

413 followed by mdc-STM-001-0.6 (CV%=36%) and mdc-STM-003-0.6 (CV%=43%). The overall

414 systemic exposure over DAI 91 ($AUC_{0\text{-}91d}$) was quite similar for all formulations, with the
415 lowest value for mdc-STM-002-0.6 and the highest value for mdc-STM-003-0.6. Inter-animal
416 variability in AUC was much lower for mdc-STM-001-0.6 (CV% = 12%), compared to mdc-
417 STM-002-0.6 (CV% = 42%) and mdc-STM-003-0.6 (CV = 66%). The mean ivermectin plasma
418 concentrations after each month post sub-cutaneous injection (C28d, C56d, C91d, Clast)
419 decreased slowly until the end of the study for all formulations, remaining close to or above
420 3.5 ng/mL for mdc-STM-001-0.6 and mdc-STM-003-0.6, and around 1 ng/mL for mdc-STM-
421 002-0.6. There was moderate to high inter-animal variability, ranging from 53% for mdc-
422 STM-001-0.6 to 92% for mdc-STM-003-0.6. Additionally, the ratio of Cmax to the level
423 measured after each month (C28d, C56d, C91d) was up to 2-fold lower for mdc-STM-001-0.6
424 compared to other LAIFs at the dose of 0.6 mg/kg.
425 For mdc-STM-003, both tested doses (0.6 and 1.5 mg/kg) exhibited a hypo-proportional
426 increase in Cmax and AUC, approximately 40% less than proportionality. However, these
427 findings should be considered cautiously due to the high inter-animal variability observed,
428 particularly at the low dose (Table 3 and Supplementary Table 2 of the Appendix).
429 Because the second phase of the PK profile was relatively flat, the apparent terminal phase
430 half-life (T1/2) was accurately estimated for only some animals, with a mean T1/2 ranging
431 from 20 days for mdc-STM-003-1.5 to 33 days for mdc-STM-001-0.6.

432 The analysis of each animal's depot collected at the end of the study (DAI 132 or 133)
433 showed that the remaining amount of ivermectin not released from the depot was the
434 lowest for mdc-STM-001-0.6 and mdc-STM-003-0.6 with an average of 23.0% of the injected
435 amount compared to 39% for mdc-STM-002-0.6 at the same dose of 0.6 mg/kg. A similar
436 trend was observed for the remaining amounts of copolymers which were the lowest mdc-
437 STM-001-0.6 (approximately 19% of the injected amount) compared to 26% for mdc-STM-

438 003-0.6 and 34% for mdc-STM-002-0.6. For mdc-STM-003 at the dose of 1.5 mg/kg, the
439 remaining amount of ivermectin and copolymers were 33% and 34% of the injected amount,
440 respectively (Supplementary Tables 4 and 5, Appendix).
441
442 **Mosquitocidal activity evaluation**
443 Knock-down resistance (Kdr) status of the VK5 mosquito lots
444
445 Generations F2 to F8 of the VK5 colony were used in this experiment. Mosquito batches
446 exposed at DAI 2, 7, 17, 21, 28, and 42 consisted of 74–80% individuals exhibiting
447 pyrethroid-resistant phenotypes (i.e., carrying RR or RS genotypes, [40]). The remaining
448 batches (exposed at DAI 56, 70, 84, 91, 98, 105, 112, 119 and 126) contained 50–54%
449 resistant mosquitoes. Detailed qPCR results by generation and batch are given in the
450 Appendix (p10).
451
452 Blood feeding rate
453 High blood-feeding rates were consistently observed across all time points for both *An.*
454 *gambiae* Kisumu (KIS) and *An. coluzzii* (VK5) colonies (Table 4). No statistically significant
455 differences in feeding success were observed between treatment groups or formulations at
456 any time point (data not shown), indicating uniform host attractiveness and feeding
457 opportunity across experimental conditions.
458
459
460
461

462 Table 4: Blood feeding rates and the number of fed mosquitoes out of the total exposed to

463 calves, either controls or those receiving the LAIFs.

	Cattle treatments					
Mosquito Colony	**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)

464

465

466 Efficacy outcomes

467 The total number of mosquitoes monitored for survival by colony and treatment group is

468 presented in Table 5. Their detailed distribution by individual cattle, cup, and day after

469 injection (DAI) is provided in Supplementary Tables 6A (KIS) and 6B (VK5). Due to limited

470 availability of KIS mosquitoes, no feeding assays were conducted at DAI 7 and DAI 28.

471 Mosquito husbandry issues affected the same colony at DAI 105 and DAI 119; these data

472 points were excluded from the analysis. Unexpected high mortality rates during the first 4

473 days post blood feeding (> 0.20) were detected for KIS mosquitoes fed on the control cattle

474 B328 at DAI 42 (mortality rate = 0.437) and B321 at DAI 112 (mortality rate = 0.21).

475 Therefore, these mosquito lots were excluded from efficacy analyses.

476

477 Table 5. Number of KIS and VK5 mosquitoes followed for survival experiments after blood-

478 feeding on cattle treated with the different LAIFs or on controls. Numbers in brackets

479 represent the proportion for each treatment relative to the total number of mosquitoes

480 followed.

481

Treatment	KIS Ntotal= 10.399	VK5 Ntotal = 15.942
Control	1.926 (19%)	3.161 (20%)
mdc-STM-001-0.6	2.122 (20%)	3.206 (20%)
mdc-STM-002-0.6	2.123 (20%)	3.186 (20%)
mdc-STM-003-0.6	2.135 (21%)	3.207 (20%)
mdc-STM-003-1.5	2.093 (20%)	3.182 (20%)

Mortality rates

A.

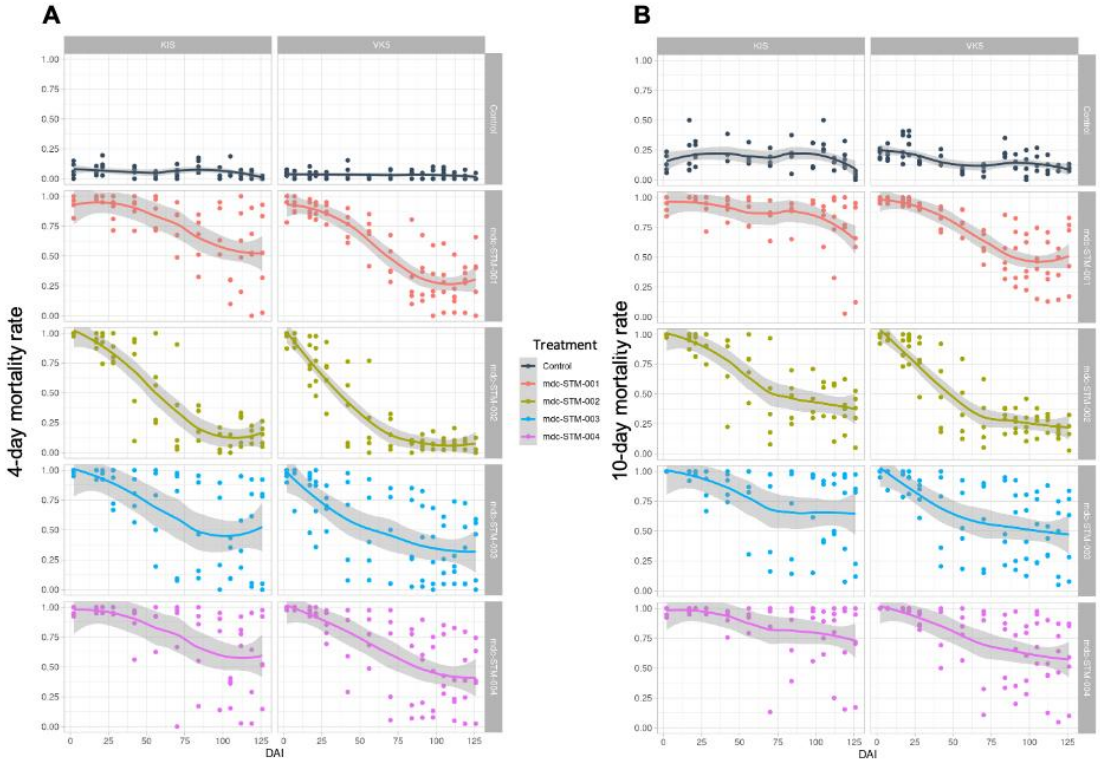


Figure 2.

Cumulative mortality rates of KIS and VK5 mosquitoes for the 4 day- (A) and 10 day- (B) periods post-feeding on cattle treated with three candidates of long-acting formulations at

491 the dose of 0.6 mg/kg (mdc-STM-001, mdc-STM-002, mdc-STM-003) or mdc-STM-003 at 1.5
492 mg/kg (i.e., mdc-STM-004 in the legend). Direct skin blood feeding experiments were
493 performed on different days after injection (DAI) of the formulations. For each plot, the
494 smooth line represents mean values estimated by the LOESS (locally estimated scatterplot
495 smoothing) method and grey area shows CI95% [42].
496

497 Acute toxic effects of ivermectin were assessed using 4-day cumulative mortality rates for
498 both *An. gambiae* Kisumu (KIS) and *An. coluzzii* VK5 mosquitoes (Figure 2A). For KIS
499 mosquitoes, mortality exceeded 50% for up to DAI = 50 with mdc-STM-002 and extended to
500 approximately 90 days with mdc-STM-001. For VK5 mosquitoes, mortality rates were
501 generally lower at equivalent DAIs. However, mdc-STM-001-0.6 and mdc-STM-003-1.5
502 maintained >50% mortality through 63 and 66 days post-injection, respectively. In contrast,
503 mdc-STM-002-0.6 and mdc-STM-003-0.6 exceeded the 50% mortality threshold only until
504 around DAI 30.

505 A similar trend was observed for 10-day cumulative mortality rates (Figure 2B). mdc-STM-
506 001-0.6 showed the highest efficacy in KIS mosquitoes, comparable to mdc-STM-003-1.5.
507 From DAI 2 to approximately DAI 100, over 75% of KIS mosquitoes died within 10 days after
508 feeding cattle treated with mdc-STM-001-0.6, and mortality rates remained above 50%
509 beyond this period. For VK5 mosquitoes, mdc-STM-001-0.6 maintained ≥50% mortality
510 between DAI 2 and 80, whereas other formulations at the dose of 0.6 mg/kg were less
511 effective. The high-dose mdc-STM-003-1.5 achieved the longest duration of efficacy for VK5
512 mosquitoes, with mortality rates >50% from DAI 2 to 110.

513 Among all formulations, mdc-STM-001-0.6 exhibited the most consistent efficacy profile.
514 Mosquito mortality data for this LAIF showed the lowest inter-animal variability, with fewer
515 deviations from the mean trend across all sampling points.

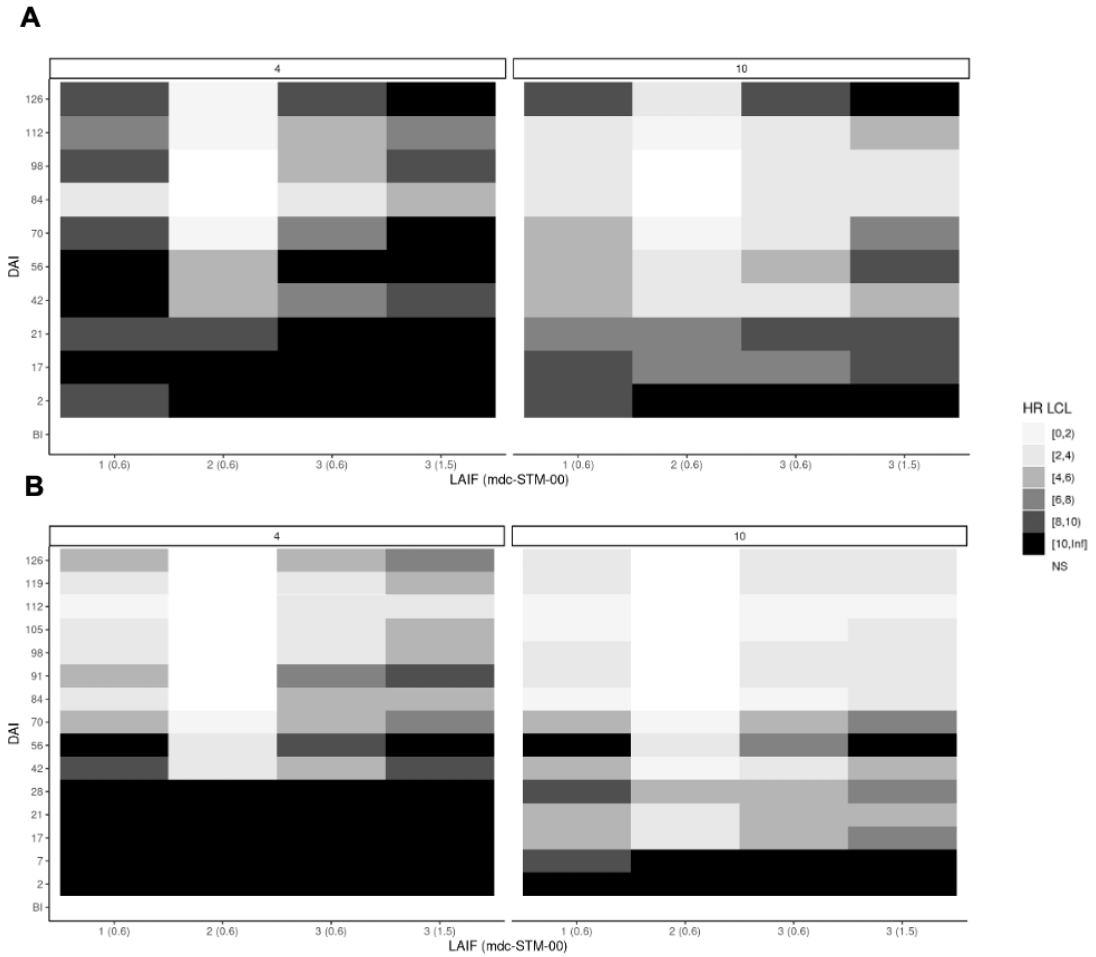
516

517 *Survival and Hazard Ratios*

518 No significant differences in mosquito survival were observed across treatment groups prior
519 to LAIF administration for either KIS or VK5 colonies (Supplementary Figures 2, 3;
520 Supplementary Table 7; Figure 3), confirming no confounding effects from host variability.
521 Following treatment, all formulations administered at the dose of 0.6 mg/kg (mdc-STM-001,
522 mdc-STM-002, mdc-STM-003) and at 1.5 mg/kg (mdc-STM-003) induced a significant
523 mosquitocidal effect. The magnitude and duration of these effects varied according to
524 mosquito strain and formulation but remained statistically significant through the full follow-
525 up period (DAI 126) in most cases.

526 Hazard ratios (HRs), derived from Cox proportional hazards models (Figure 3) quantified
527 treatment effects on mosquito survival relative to the control group. When benchmarked
528 against the WHO-recommended HR threshold of 4 for endectocidal efficacy against malaria
529 vectors [38], the different formulations—particularly mdc-STM-001-0.6 and mdc-STM-003-
530 1.5—met or exceeded this target across extended time intervals.

531



532

533

534 Figure 3: Heat maps illustrating the lower 95% confidence limit (LCL) of mortality hazard
535 ratios for KIS (A) and VK5 (B) colony mosquitoes fed on cattle treated with the formulations
536 mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-003-0.6, mdc-STM-003-1.5 relative to the
537 controls. Parallel direct skin feeding experiments were conducted for KIS and VK5 on each
538 cattle. Experiments were performed at different days after injection (DAI) of the
539 formulations. For each DAI, mosquito survival was monitored over a 30-day period following
540 blood feeding. Hazard ratios are provided for follow-up periods of 4 and 10 days. HR for 30-
541 days follow up periods are given in the supplementary Figure 2 of the Appendix. Colored
542 cells indicate a significant difference in survival between mosquitoes fed on treated versus

543 control calves ($p < 0.05$), while white cells represent time points where no significant
544 difference in survival was observed ($p > 0.05$). HR and *P-values* for each DAI are available in
545 supplementary table 7 of the Appendix. BI is the timepoint before injection.
546
547
548 The mdc-STM-002-0.6 formulation was clearly the least effective, with HRs ≥ 4 for the
549 shortest duration regardless of colony or follow-up period (4 or 10 days post blood-feeding).
550 It was therefore excluded from further efficacy comparisons.
551 For KIS mosquitoes, both mdc-STM-001-0.6 and mdc-STM-003-0.6 sustained 4-day HRs ≥ 4
552 across nearly the entire study period (up to DAI 126), except at DAI 84 (HRs between 2 and
553 4). Ten-days HR ≥ 4 was sustained through 70 post-dose for mdc-STM-001-0.6 and through
554 DAI 21 (and again at DAI 56 and 126) for mdc-STM-003-0.6. For both formulations, 30-day
555 HRs remained above 4 until DAI 21 (Supplementary Figure 3). The higher-dose formulation
556 mdc-STM-003-1.5 showed a similar pattern to mdc-STM-001-0.6, with the added benefit of
557 10-day HRs >4 at an additional time point (DAI 112). For DAI >21, its 30-day HRs remained
558 between 2 and 4.
559 For VK5 mosquitoes, both mdc-STM-001-0.6 and mdc-STM-003-0.6 maintained 4-day HR ≥ 4
560 through 91 days post-injection, except at DAI 84 (HRs was between 2 and 4 for mdc-STM-
561 001-0.6). Ten-day HRs remained >4 until DAI 70 and 30-day HRs until DAI 7 for both
562 formulations. The higher-dose LAIF mdc-STM-003-1.5 induced 4-day HRs >4 throughout the
563 experiment, except of DAI 112, where HR dropped slightly below the threshold (but
564 remained >2). Ten-day HRs were comparable in duration (up to DAI 70) to the smaller dose
565 of 0.6 mg/kg, and 30-day HRs exceeded 4 for a period of 21 days.
566

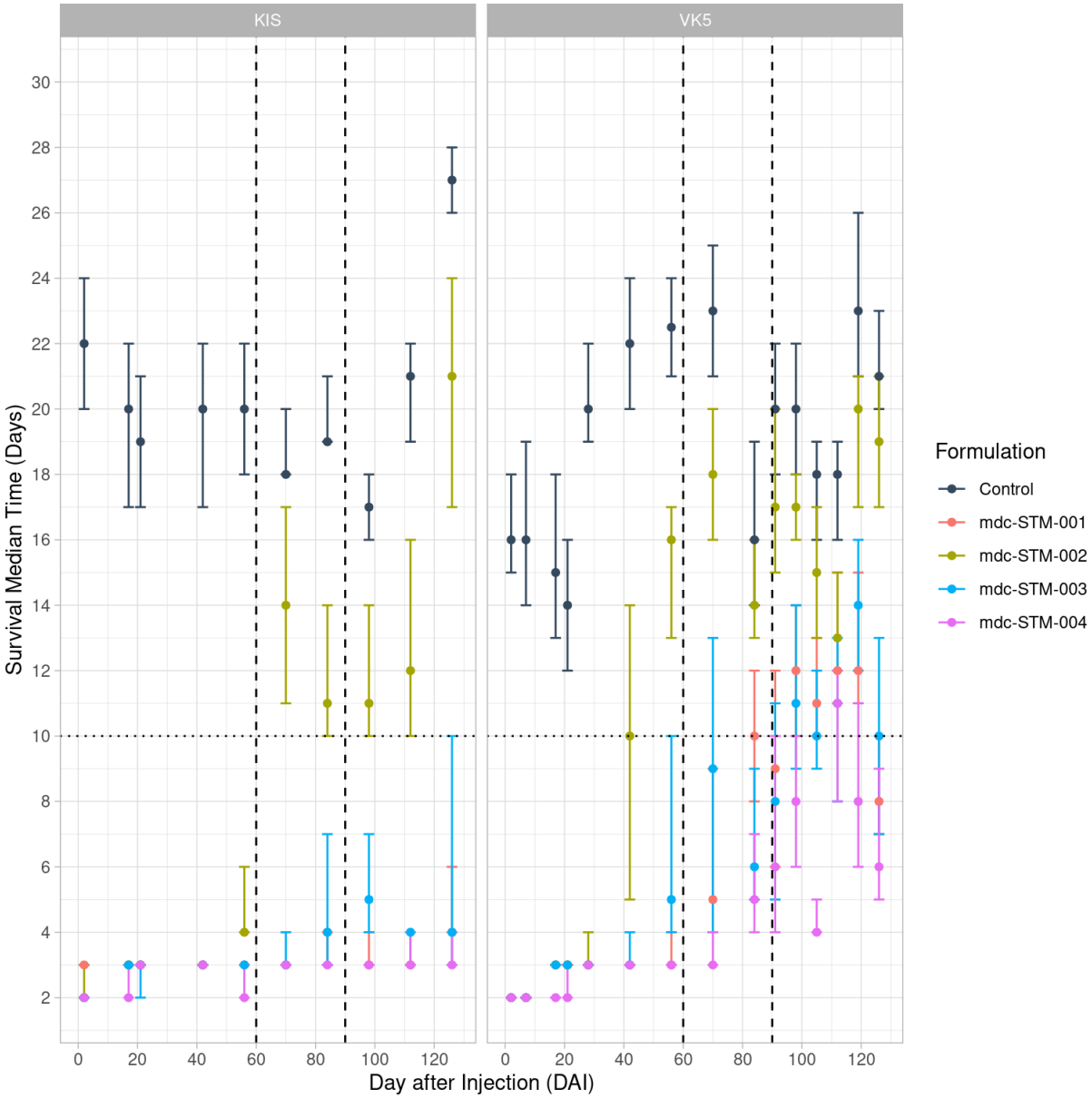
Median survival times

Median survival times were consistently greater than 10 days for KIS and VK5 mosquitoes fed on control calves (Figure 4) for all the DAI tested.

For KIS mosquitoes, median survival times remained consistently below 10 days (the minimum average extrinsic incubation period (EIP) of *Plasmodium falciparum*) throughout the entire follow-up (up to DAI 126) when fed on cattle treated with mdc-STM-001-0.6, mdc-STM-003-0.6 and mdc-STM-003-1.5 (Figure 4). In contrast, mdc-STM-002-0.6, induced median survival times equal to or greater than the 10-day threshold from DAI 70 onward (Figure 4).

For VK5 mosquitoes, median survival times remained ≤ 10 days up to 91 days post-treatment, and again at DAI 126, when exposed to cattle treated with mdc-STM-001-0.6, mdc-STM-003-0.6, and mdc-STM-003-1.5 (Figure 4). The LAIF mdc-STM-002-0.6 maintained median survival times below or equal to the EIP threshold for only 40 days post-dose.

Notably, mdc-STM-001-0.6 and mdc-STM-003-1.5 induced particularly short survival durations, with median survival times <4 days for nearly 100 days in KIS mosquitoes and approximately 60 days in VK5.



585

586

587 Figure 4: Median survival times of mosquitoes from both KIS and VK5 colonies fed at

588 different days after injection of the LAIFs mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-

589 003-06 and mdc-STM-003-1.5 (i.e, mdc-STM-004 in the legend). The dashed horizontal line

590 represents the average extrinsic incubation period (EIP) for *Plasmodium falciparum*. The

591 dashed vertical lines represent 2- and 3-months post injections. Median values +/- 95% CI

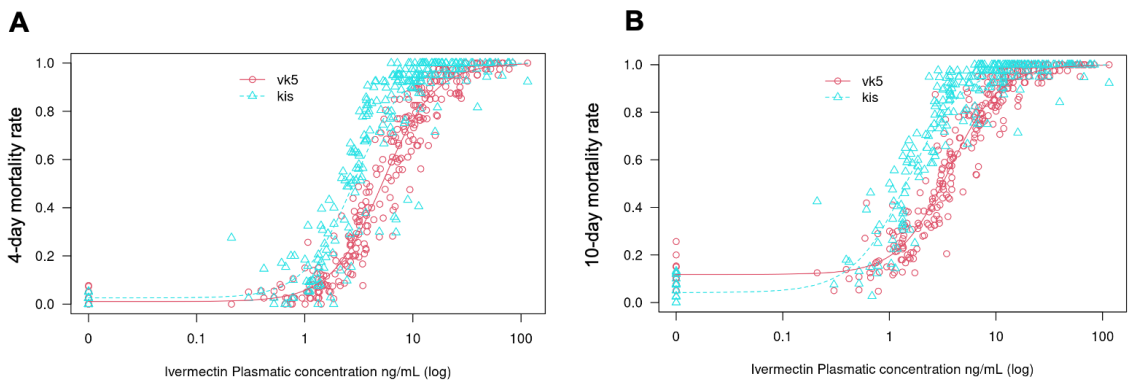
592 are given.

593

594 *Lethal concentrations and coverage duration*

595 Concentration/response relationships were examined for each colony and for each LAIF,
596 using both 4- and 10-day cumulative mortality outcome. No significant differences in LC50 or
597 LC90 values were found between LAIFs, regardless of the mosquito colony (Supplementary
598 Figures 4 and 5; Supplementary Tables 8–11 of the Appendix). A unified concentration–
599 response model, including mosquito colony as explanatory variable was thus developed
600 using the full dataset (Figure 5). LC50 and LC90 values are summarized in Table 6.

601



602

603

604

605 Figure 5. Ivermectin concentrations in relation to cumulative mortalities for KIS and VK5
606 colony mosquitoes. Cumulative mortalities are considered over either a 4-day (A) or a 10-day
607 period (B).

608

609

610 Table 6: Ivermectin concentrations inducing 50% and 90% KIS and VK5 mosquito mortalities
611 over a 4-day or a 10-day period. Mean concentrations are given with lower and upper

612 confidence intervals (95% CI). Concentration values for KIS and VK5, along with P-values for

613 comparisons between strains, were obtained using the drc model.

614

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
10	50	1.51 [1.1-2.07]	3.66 [2.69-4.97]	1.0e-07
10	90	5.63 [3.98-7.96]	12.28 [8.8-17.14]	1.5e-06

615

616 A significant difference in cumulative mortalities between KIS and VK5 was observed in

617 relation to ivermectin concentrations, with both LC50 and LC90 values consistently lower for

618 KIS than for VK5, irrespective of 4- or 10-day mortality window. As expected, LC90 values

619 were consistently higher than LC50, and lethal concentrations associated with 4-day

620 cumulative mortality were greater than those for the 10-day period. Table 7 summarizes the

621 time intervals during which each formulation maintained the mean, upper and lower bounds

622 of LC50 and LC90 values sufficient to kill KIS and VK5 mosquitoes. For consistency with

623 existing literature and to facilitate direct comparison with previous studies, only LC50 values

624 were presented in detail in the following sections.

625

626 Table 7: Time period during which the LAIF candidates reach Ivermectin Plasma

627 Concentration values above lethal concentrations inducing 50 and 90 % mortalities, over a 4-

628 day period or a 10-day period. The time period corresponding to mean LC values are given

629 and those corresponding to upper and lower confidence intervals (95%), respectively, are

630 given in brackets. KIS and VK5 are respectively the *An. gambiae* insecticide-susceptible and

631 the *An. coluzzii* resistant Anopheles colonies**.**

	KIS	VK5

FORMULATION	LC (%)	4-Day	10-Day	4-Day	10-Day
MDC-STM-001-0.6	50	132 [132-132]	132 [132-132]	65 [59-69]	132 [67-132]
	90	49 [32-55]	64 [52-73]	20 [8-31]	33 [21-50]
MDC-STM-002-0.6	50	56 [50-63]	73 [65-119]	38 [33-43]	50 [39-58]
	90	27 [21-33]	36 [30-47]	16 [12-20]	21 [16-28]
MDC-STM-003-0.6	50	132 [127-132]	132 [132-132]	75 [67-89]	128 [78-132]
	90	34 [25-65]	71 [53-111]	18 [13-25]	26 [19-49]
MDC-STM-003-1.5	50	132 [132-132]	132 [132-132]	132 [85-132]	132 [132-132]
	90	69 [38-83]	132 [76-132]	26 [20-37]	39 [26-71]

Inter-animal variability was also assessed, as it directly informs the coverage potential of each formulation, *i.e.* the proportion of treated animals maintaining mosquitocidal ivermectin plasma concentrations above the LC50 over time. For mdc-STM-001-0.6, 80% of treated calves sustained ivermectin concentrations above the KIS 10-day LC50 threshold for at least 4 months post-injection, compared to 60% for mdc-STM-003-0.6. The contrast was even more pronounced when considering VK5 mosquitoes: with mdc-STM-001-0.6, 60% of the calves maintained plasma concentrations above the VK5 10-day LC50 for at least 4 months, while the remaining animals sustained this threshold for at least 2 months (64 and 71 days, respectively). In contrast, for mdc-STM-003-0.6, only two calves maintained VK5 10-day LC50 levels for 4 months, while the remaining three dropped below this threshold after 29, 41 and 50 days post-dose. Taken together, these results indicate that mdc-STM-001-0.6 achieved 100% coverage of VK5 10-day LC50 for a duration of at least 2 months post-treatment.

DISCUSSION

This study represents the first evaluation of candidate long-acting injectable formulations of ivermectin specifically developed for human use in malaria control. These formulations are issued from unique long lasting delivery technology and are designed to meet, with a single

652 subcutaneous injection, the World Health Organization's minimum target product profile for
653 endectocides against malaria: achieving, with a single administration, at least one month of
654 efficacy, defined by hazard ratios (HR) for *Plasmodium* vector survival exceeding 4. The
655 primary objective of this work was to identify the most promising formulation for further
656 development, including progression to Phase 1 clinical trial. To guide formulation selection,
657 we employed a comprehensive approach integrating multiple, complementary criteria
658 related to mosquitocidal efficacy, pharmacokinetic (PK) characteristics, and inter-individual
659 variability, all critical for achieving effective and sustained vector control in real-world
660 conditions and settings.

661 **The lead formulation is mdc-STM-001**

662 All three LAI candidate formulations were well tolerated, and the increased dose of
663 mdc-STM-003 at 1.5 mg/kg did not produce any observable adverse effects. Based on
664 pharmacokinetics and efficacy results comparison, mdc-STM-001 emerged as the lead
665 formulation for further development toward human use. When administered at a single
666 dose of 0.6 mg/kg, mdc-STM-001 demonstrated several critical features associated with
667 optimal effectiveness:

668 **i)** An area under the curve (AUC) primarily driven by a longer duration above the
669 LC50 threshold rather than a higher Cmax. This indicates improved control of the
670 initial plasma concentration burst and resulted in the lowest observed Cmax
671 values (mean [range]: 23.0 ng/mL [11.9-32.0]), which is a favorable outcome from
672 a safety standpoint. Notably, this Cmax is at least eight times lower than the
673 average safe Cmax (248 ng/mL) reported in healthy volunteers receiving
674 escalating oral doses of ivermectin tablets up to 120 mg (equivalent to 1404-2000
675 µg/kg) ([50]);

676 **ii)** Lowest inter-individual variability in ivermectin plasma levels and systemic
677 exposure, enabling the highest proportion of treated animals to maintain LC50
678 plasma concentrations over a predefined period. This enhances the reliability of
679 achieving effective coverage at the population level;
680 **iii)** Mosquitocidal efficacy equal to or exceeding that of other formulations,
681 sustained for more than two months across all evaluated endpoints. Hazard
682 ratios (HRs) for both 4- and 10-day follow-up periods remained above 4 for at
683 least two months post-treatment in both insecticide-susceptible (KIS) and
684 resistant (VK5) mosquito strains. Median mosquito survival times remained
685 below 10 days post-blood feeding for a minimum of two months in VK5 and
686 throughout the entire 126-day observation period for KIS. Additionally, LC50
687 values were maintained for a mean duration of at least 132 days (95% CI: 67–132)
688 for VK5, and consistently for (at least) 132 days (95% CI: 132–132) for KIS.
689 In both magnitude and duration, the mosquitocidal efficacy of a single subcutaneous
690 injection of mdc-STM-001 formulation is compelling. It would likely reduce Anopheles vector
691 populations by at least 50% during the first 4 to 10 days after each blood meal, and this
692 would be sustained over 2 months (VK5) to at least 4 months (KIS) depending on insecticide
693 resistance status. Transmission potential would be further curtailed because infectious
694 mosquitoes typically require multiple blood meals across at least 2–3 gonotrophic cycles to
695 become competent vectors. Moreover, mosquitoes exposed to mdc-STM-001 had median
696 life spans insufficient to complete the average 10-day Plasmodium falciparum extrinsic
697 incubation period (EIP)**,** functionally rendering them unable to transmit the parasite.
698 Thus, mass drug administration (MDA) campaigns incorporating mdc-STM-001, as a
699 complement to existing malaria control strategies, could substantially and sustainably

700 reduce Plasmodium transmission, even in regions with high levels of insecticide resistance.
701 Additional sub-lethal effects of ivermectin—on vector capacity (e.g., fecundity, feeding
702 behavior) and competence (parasite development)—not assessed in this study, would likely
703 enhance this impact further and reinforce the formulation's epidemiological impact.
704 However, several concerns may arise regarding communities' mass treatments using
705 the lead long-lasting formulation.
706
707 **Exclusion Criteria and Targeted Population Coverage**
708 In ivermectin-based malaria control strategy, a cornerstone for impact lies in
709 achieving effective coverage of the human blood reservoir from which mosquitoes feed. This
710 requires that a sufficient proportion of the targeted population maintains ivermectin plasma
711 concentrations above mosquitocidal thresholds for a sustained period.
712 Currently, MDAs of ivermectin for onchocerciasis and lymphatic filariasis exclude
713 pregnant women, nursing mothers, and children under 15 kg from treatment [47,48].
714 Applying the same exclusion criteria to malaria-targeted interventions, modeling studies
715 have estimated that 70–80% population coverage is necessary to achieve meaningful
716 epidemiological impact with monthly high-dose oral regimens (300 µg/kg/day for 3 days,
717 maintaining efficacy for ~14 days)[28]. However, two recent Phase 3 randomized controlled
718 trials using this oral regimen failed to demonstrate reductions in malaria incidence, with
719 insufficient effective coverage cited as one likely explanation [15,30].
720 In the Rimdamal II study by Somé et al., PK data revealed that 50% of treated
721 individuals had plasma ivermectin concentrations below the limit of quantification just two
722 weeks post-treatment [15]. This indicates that effective drug exposure over the 14-day
723 period was substantially lower than anticipated, likely contributing to the lack of measurable

724 impact on malaria incidence. In comparison to oral regimens, long-acting injectable
725 formulations also require the exclusion of women of childbearing age for safety reasons [53],
726 further reducing the proportion of the population eligible for treatment. However, this
727 limitation is counterbalanced by the formulation's prolonged maintenance of mosquitocidal
728 plasma levels. The extended remanence of ivermectin, combined with low inter-individual
729 PK variability, increases the cumulative number of person-days with vector-lethal
730 concentrations. As a result, the effective population coverage during the intervention
731 window is enhanced, offering greater potential to interrupt transmission compared to oral
732 formulations [23]. Previous modeling studies have projected that long-lasting systemic
733 endectocides could significantly reduce malaria incidence across a range of epidemiological
734 contexts [54]. When administered alongside SMC, a formulation achieving at least two
735 months of efficacy could result in a 30–90% reduction in malaria burden, depending on the
736 achieved coverage (from 50% to full, excluding children under 15 kg and women of
737 childbearing age). Notably, the mdc-STM-001 formulation met and exceeded this two-month
738 efficacy threshold, depending on the resistance profile of the local mosquito population,
739 underscoring its high potential as a vector control tool. Ongoing modeling analyses based on
740 the present data will help assess the projected epidemiological impact of mdc-STM-001
741 across diverse transmission settings and deployment scenarios, including comparisons to
742 various oral regimens and incorporating the coverage limitations now documented.
743
744 **Resistances selection and mitigation**
745 Prolonged systemic exposure to ivermectin may exert selective pressure on both
746 endoparasites typically targeted by ivermectin treatments, as well as on Anopheles
747 mosquitoes and other ectoparasites [55,56]. However, since current exclusion criteria

748 prevent treatment of women of childbearing age, pregnant or lactating women, and children
749 under 15 kg, large untreated subpopulations would serve as genetic refuges. These refuges
750 help preserve parasite and vector genetic diversity, substantially mitigating the risk of
751 resistance selection [57]. As such, the presence of these refuges supports the long-term
752 sustainability of this long-acting strategy [34,55]. Nevertheless, behavioral resistance
753 remains a potential concern, particularly through the selection of more zoophagic or
754 opportunistic mosquito populations that avoid feeding on treated humans. This could
755 compromise the overall effectiveness of the intervention. Therefore, future implementation
756 should incorporate regular monitoring of mosquito biting behavior and the early
757 development of mitigation strategies to address possible shifts in vector ecology [55].
758
759 **Community acceptability considerations**
760 Mass drug administration campaigns involving an injectable product that provides
761 community-level protection against malaria, rather than direct individual benefit, may
762 encounter acceptability challenges. However, previous studies have shown high community
763 acceptance of altruistic strategies such as injectable transmission-blocking vaccines [58]. For
764 our approach, acceptability may be further reinforced by the additional health benefits of
765 ivermectin, which will remain effective against several neglected diseases still prevalent in
766 malaria-endemic regions. Notably, past MDAs have been associated with reductions in
767 childhood anemia [15], a previously unknown benefit, as well as decreases in jiggers
768 infestations and bedbug nuisances [59]. These positive side effect may contribute to
769 improved community acceptability and adherence.
770 The single injection regimen provides additional advantages for both acceptability and
771 compliance when compared to monthly oral regimen requiring repeated dosing. The

formulation volumes to be injected subcutaneously will be small in human (around 8 µL/kg, max 1.5 mL per human) and should not represent a limiting factor for the approach to be implemented. Nonetheless, proactive efforts to identify and address potential acceptability challenges specific to long-acting ivermectin MDA should be initiated early in the development process alongside further effectiveness evaluation. Community engagement will be essential; for sustained adoption, administration strategies must be co-developed with local stakeholders, including affected populations and health authorities [60].

Physiological resistance of mosquitoes and ivermectin mosquitocidal efficacy

Our findings reveal distinct differences in ivermectin susceptibility between the two mosquito colonies, with VK5 requiring higher plasma lethal concentrations than KIS. This suggests the presence of ivermectin tolerance mechanisms in the VK5 strain, potentially linked to genetic factors as species-specific traits or cross-resistance provided by the knockdown resistance (kdr) mutation, associated with pyrethroid resistance, identified in this colony. Variability in ivermectin susceptibility have also been documented among different Anopheles species (e.g. [16]), as well as among different pyrethroid-resistant Aedes strains, supporting the potential for shared or overlapping resistance mechanisms [61]. The persistence of metabolic resistance is unlikely, as such mechanisms typically vanish rapidly under laboratory conditions, especially in the absence of ivermectin exposure.

Tolerance mechanisms, possibly selected through repeated ivermectin MDA campaigns targeting lymphatic filariasis and onchocerciasis, have been proposed as a contributing factors to the limited success of some MDA interventions in reducing Anopheles vector densities [15]. While such underlying mechanisms have been observed in other species, they remained poorly studied in Anopheles [56]. Further investigations, including

796 experimental selection studies, are needed to better characterize the potential for
797 ivermectin tolerance in malaria vectors [62].
798 Differential susceptibility among malaria vector populations represents a significant
799 challenge to intervention effectiveness and must be considered when designing appropriate
800 dosing regimens for future strategies. For example, our data have shown that increasing the
801 dose of the mdc-STM-003 formulation extended its efficacy against VK5-tolerant
802 mosquitoes, achieving outcomes comparable to those observed in the more susceptible KIS
803 strain.
804 Additional threats to efficacy may arise from incomplete understanding of mosquito
805 bionomics in target areas, particularly regarding seasonal population dynamics and blood-
806 feeding behavior [31]. Incomplete coverage of mosquitoes blood sources, due to untreated
807 human individuals or the presence of domestic animals in the case of zoophagic behaviour,
808 could sustain vector populations at levels compatible with continued transmission.
809 Furthermore, subcutaneous ivermectin formulations may alter host odor profiles, potentially
810 affecting human attractiveness to mosquitoes. As children under 15kg and women of
811 childbearing age will not receive treatment, the possibility of their increased exposure to
812 mosquito bites warrants further investigation [55]. Ongoing studies are exploring potential
813 ivermectin-induced changes in the attractiveness of calves, but similar research addressing
814 this question in humans is needed.
815

816 **CONCLUSION**

817 The LAIF mdc-STM-001 emerged as the lead candidate formulation for further development
818 toward human use against malaria. A single injection can kill >50% of biting mosquitoes
819 before they become infectious, even in insecticide-resistant populations, for a duration of at

820 least 2 months. These findings strongly support the need for in-human pharmacokinetic and
821 pharmacodynamic studies to characterize the formulation's specific behavior in humans and
822 evaluate its real-world potential for malaria control.

823 To ensure relevance for vector control, mosquitocidal efficacy should be rigorously
824 assessed using hazard ratios, LC50 values, and mortality rates, with mosquito follow-up
825 limited to 10 days post-exposure. In other words, prioritizing acute ivermectin-induced
826 lethality in efficacy assessments is essential to suppress the buildup of infectious vector
827 populations. Epidemiological modeling studies must account for the most extreme
828 scenarios, notably regarding key variables such as human biting rate, human population
829 coverage and heterogeneous demographic structures. Crucially, understanding local
830 mosquito bionomics—including the diversity and proportion of blood sources—is essential
831 for optimizing deployment strategies and assessing whether complementary treatment of
832 domestic animals is warranted.

833 With the drug and the BEPO® long-acting delivery technology already available, ivermectin's
834 safety profile well established, and key challenges to ivermectin-MDA effectiveness both
835 identified and inherently addressed by the long-lasting formulations approach, we are
836 confident that mdc-STM-001 could be a game changer for controlling residual malaria when
837 used alongside existing tools. Crucially, it remains one of the very few approaches capable of
838 targeting outdoor transmission, a persistent gap in current malaria control strategies.

839 Evaluating the pharmacokinetics and efficacy of mdc-STM-001 in humans, following a single
840 injection across varying doses, is now the critical next step in determining its suitability for
841 broader deployment. Given the strength of these preliminary results—and the additional
842 public health value of ivermectin in treating co-endemic parasitic infections—it would be a

missed opportunity not to advance mdc-STM-001 into Phase 1 clinical trials. In our view, the case for further investigation is both timely and compelling.

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.

Funding

This study was funded by Unitaid under the IMPACT project consortium.

Authors' contribution

LZ performed the experiments at CIRDES facilities, preliminary statistical analysis and provided the first original version of the manuscript. SHP, KM, NM, CP, RKD designed the protocol. SHP coordinated experimental work at CIRDES facilities. AS managed the molecular biology and coordinated the manuscript drafting. SB and AP performed statistical analysis. SB produced the final figures. KM was the scientific coordinator for the IMPACT project. All co-authors contributed to revision of the manuscript, approved the final version of the manuscript for publication. All authors read and approved the final manuscript.

Data, code, and supplementary information availability

Supplementary information, data, analysis and figures are given in the Appendix.

Data and codes are available online (datasud link, ongoing)

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1059

Appendix

Selection of a Lead Long-Acting Formulation of Ivermectin to Target Major Malaria Vectors in Western Africa: Evaluation of Pharmacokinetics and Mosquitocidal Efficacy in Cattle under Laboratory Conditions.

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Table of contents

METHODS **3**

Injection volumes per cattle **3**
Supplementary table 1 3

RESULTS **4**

Cattle Weight **4**
Supplementary table 2 4

Pharmacokinetics **5**
Supplementary Figure 1 5
Supplementary table 3 6

Copolymers extraction at study end **7**
Supplementary table 4 7
Supplementary table 5 8

Mosquitocidal activity evaluation **9**
VK5 resistance status 9
Sample sizes 9
Supplementary Table 6A and 6B 9
Kaplan-Meier curves 11
Supplementary Figure 2 11
Supplementary Figure 3 12

HR values and corresponding p-values **12**
Supplementary table 7 12

Lethal Concentrations **23**
Supplementary Figure 4 23
Supplementary Figure 5 23
Supplementary table 8 24
Supplementary table 9 24
Supplementary table 10 25
Supplementary table 11 25

Methods

Injection volumes per cattle

Supplementary table 1

Injected volumes (mL) per animal and per formulation, given the body weight (BW) measured the day of the treatment (kg).

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

Results

Cattle Weight

Supplementary table 2

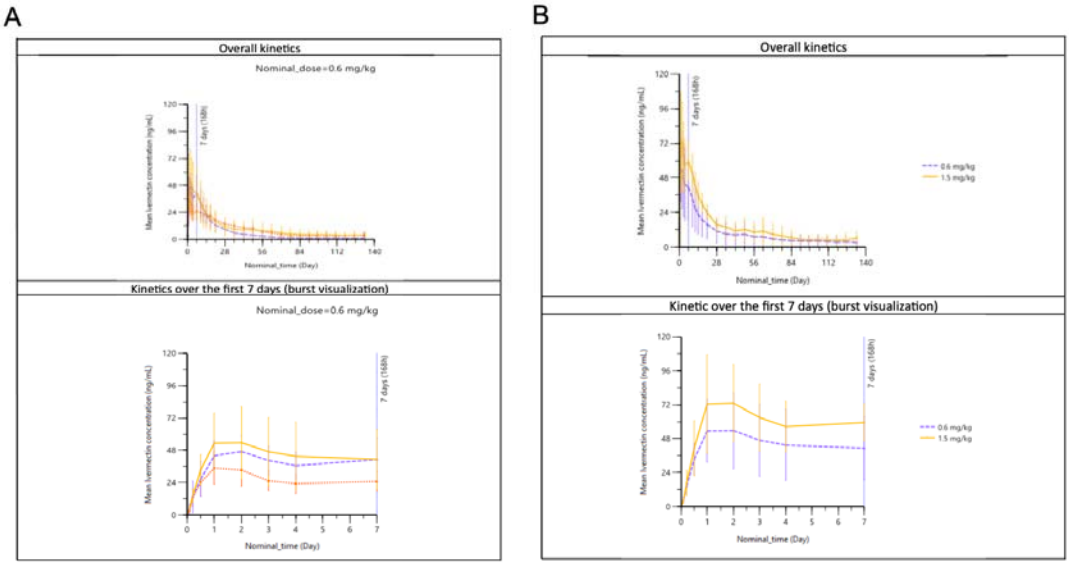
Median weight (Q1, Q3) in kg for each experimental cattle group at each weighing date.

Experimental arm	**Control N=5[1]**	**mdc-STM-001 N=5[1]**	**mdc-STM-002 N=5[1]**	**mdc-STM-003 N=5[1]**	**mdc-STM-004 N=5[1]**
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)

Pharmacokinetics

Supplementary Figure 1

A. Mean concentration-time profiles of ivermectin in plasma cattle after single subcutaneous administration of LAIFs at 0.6 mg/kg (Red: mdc-STM-001; purple: mdc-STM-002; yellow: mdc-STM-003).
B. Mean concentration-time profiles of ivermectin in plasma cattle after single subcutaneous administration mdc-STM-003 formulation at 0.6 and 1.5 mg/kg



Supplementary table 3

Mean (SD) pharmacokinetic parameters of ivermectin in plasma cattle after single subcutaneous administration of LAIFs at 0.6 or 1.5 mg/kg

Parameters	**mdc-STM-001** **0.6 mg/kg**	**mdc-STM-002** **0.6 mg/kg**	**mdc-STM-003** **0.6 mg/kg**	**mdc-STM-003** **1.5 mg/kg**
Tmax* (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)

SD: standard deviation.

*Median (min; max). Tmax: time to reach Cmax. Cmax: maximal plasma concentration. Clast : last quantifiable plasma concentration. AUC(0-28d): area under curve from time 0 to 28 days. AUC(0-28d)/Dose: dose-normalized AUC(0-28d). AUC(0-91d): area under curve from time 0 to 91 days. AUC(0-91d)/Dose: dose-normalized AUC(0-91d). AUClast: area under curve from time 0 to last quantifiable plasma level. AUClast/Dose: dose-normalized AUClast. T1/2: apparent terminal phase half-life. C28d: plasma concentration 28 days post injection. C56d: plasma concentration 56 days post injection. C91d: plasma concentration 91 days post injection.

Copolymers extraction at study end

Supplementary table 4

Ivermectin extraction results in each depot collected at study end

Treatment Dose (mg/kg)	Animal ID	IVM injected dose (mg)	IVM recovered dose (mg)		IVM recovered dose (%)	
			Indiv.	Mean (SD)	Indiv.	Mean (SD)
F31-0.6	1	100.4	38.6	19.4 (12.9)	38.5	23.0 (12.7)
	2	85.0	18.3		21.6	
	3	73.3	2.5		3.3	
	4	76.3	20.6		27.0	
	5	67.9	16.8		24.8	
F35-0.6	1	76.1	39.0	32.3 (17.7)	51.3	39.1 (19.6)
	2	84.7	6.1		7.2	
	3	69.0	23.4		33.9	
	4	87.7	41.7		47.6	
	5	92.0	51.1		55.5	
F73-0.6	1	70.6	5.4	17.8 (14.4)	7.7	23.1 (17.6)
	2	76.4	14.3		18.7	
	3	81.1	42.6		52.6	
	4	96.4	12.0		12.5	
	5	61.6	14.8		24.0	
F73-1.5	1	211.6	69.8	59.5 (35.5)	33.0	32.7 (20.0)
	2	173.8	55.5		32.0	
	3	212.3	0.5		0.2	
	4	164.8	79.7		48.4	
	5	182.9	91.8		50.2	

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

Supplementary table 5

Copolymers extraction results in each depot collected at study end

Treatment Dose (mg/kg)	Animal ID	Copolymer injected (mg)	Copolymer recovered (mg)	Copolymer recovered (%)	
				Indiv.	Mean (SD)
F31-0.6	1	442.2	120.1	27.2	18.8 (8.5)
	2	374.4	90.0	24.0	
	3	323.1	17.3	5.4	
	4	336.0	55.5	16.5	
	5	299.1	63.3	21.1	
F35-0.6	1	217.6	105.8	48.6	34.1 (15.9)
	2	242.2	19.5	8.0	
	3	197.4	77.3	39.2	
	4	250.8	108.8	43.4	
	5	263.2	81.6	31.0	
F73-0.6	1	155.6	25.0	16.1	25.9 (25.0)
	2	168.4	11.4	6.8	
	3	178.6	39.5	22.1	
	4	212.4	32.0	15.1	
	5	135.6	94.2	69.5	
F73-1.5	1	466.2	196.4	42.1	34.1 (18.8)
	2	382.8	164.3	42.9	
	3	467.6	3.5	0.8	
	4	363.0	167.4	46.1	
	5	403.0	155.6	38.6	

Indiv. : individual. SD: standard deviation.

Mosquitocidal activity evaluation
VK5 resistance status
Sample sizes
Supplementary Table 6A and 6B

Number of mosquitoes followed for their survival before injection (BI) and after blood feeding on the calves at different days either for the control arm or after injection (DAI) of the LAI formulations for the 4 treatment arms. A. KIS mosquitoes B. VK5 mosquitoes

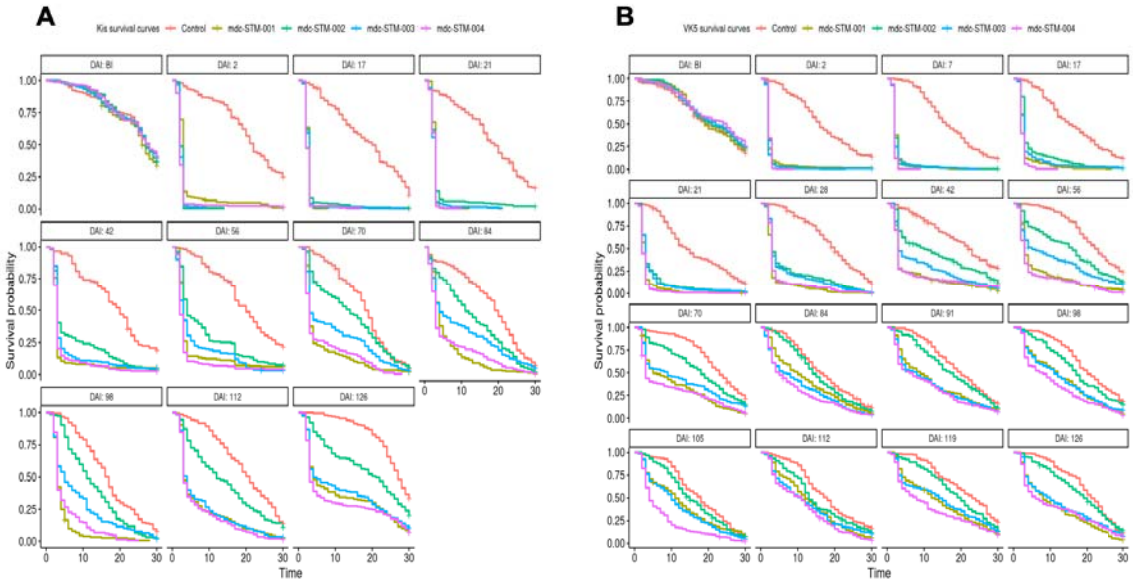
A.

				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**

B.

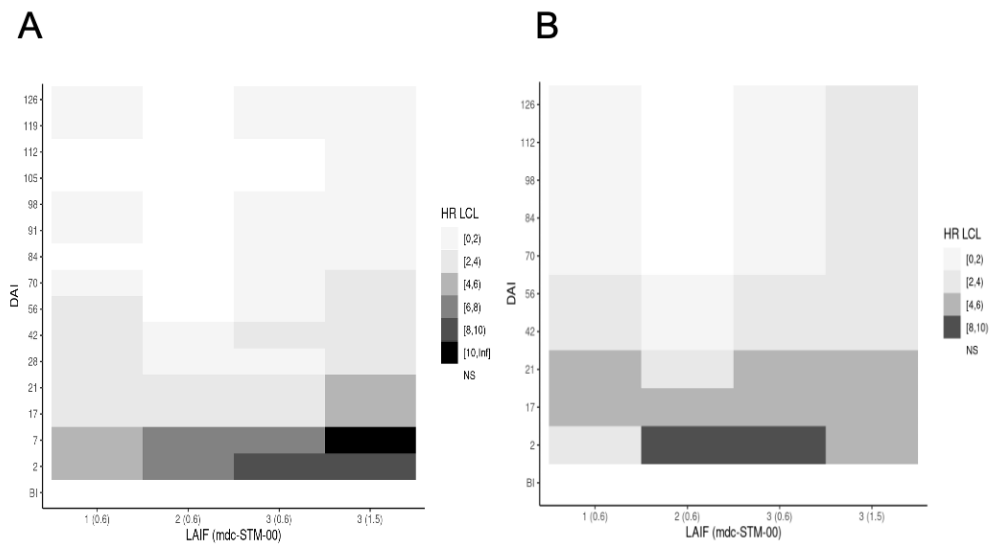
				Days after injection (DAI)														
Mosquito Colony	**Treatment**	**Cattle ID**	**BI**	**2**	**7**	**17**	**21**	**28**	**42**	**56**	**70**	**84**	**91**	**98**	**105**	**112**	**119**	**126**
vk5	Control	B127	40	41	31	39	41	38	39	40	38	40	42	40	41	39	40	41
		B129	40	39	34	40	40	39	40	39	41	41	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
	F31-0.6	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
	F35-0.6	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
	F73-0.6	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
	F73-1.5	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**

Kaplan-Meier curves



Supplementary Figure 2

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.



Supplementary Figure 3

Heat maps illustrating the lower 95% confidence limit (LCL) of mortality hazard ratios for KIS (A) and VK5 (B) colony mosquitoes fed on cattle treated with the formulations mdc-STM-001-0.6, mdc-STM-002-0.6, mdc-STM-003-0.6, mdc-STM-003-1.5 (here mdc-ST004) relative to the controls. Parallel direct skin feeding experiments were conducted for KIS and VK5 on each cattle. Experiments were performed at different days after injection (DAI) of the formulations. For each DAI, mosquito survival was monitored over a 30-day period following blood feeding. Hazard ratios are provided for follow-up periods of 30 days. Colored cells indicate a significant difference in survival between mosquitoes fed on treated versus control calves ($p < 0.05$), while white cells represent time points where no significant difference in survival was observed ($p > 0.05$). HR and *P-values* for each DAI are available in the supplementary table 6. BI is the timepoint before injection.

HR values and corresponding p-values

Supplementary table 7

Hazard ratios (HR) and their 95% CI with the corresponding p-values for mosquitoes fed on cattle treated with LAIFs of ivermectin and on controls. HR were computed for 4, 10 and 30-days of survival follow-up after mosquitoes blood feeding, before injection (BI) and for each day after injection (DAI). Hazard ratios and p-values are given for mosquitoes fed on the calves treated with LAIFs, relatively to the controls. A. KIS mosquitoes. B. VK5 mosquitoes.

A.

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-001-0.6)	Control	BI	4	1 (0.1-17.2)	1.0000000
(MDC-STM-002-0.6)	Control	BI	4	1.9 (0.2-22.7)	0.9587058
(MDC-STM-003-0.6)	Control	BI	4	0.8 (0-14.3)	0.9997884

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-003-1.5)	Control	BI	4	1.9 (0.2-22.4)	0.9618095
(MDC-STM-001-0.6)	Control	2	4	32.2 (9.8-106.3)	0.0000000
(MDC-STM-002-0.6)	Control	2	4	59.3 (18-195.1)	0.0000000
(MDC-STM-003-0.6)	Control	2	4	58.3 (17.7-191.8)	0.0000000
(MDC-STM-003-1.5)	Control	2	4	57.3 (17.4-188.9)	0.0000000
(MDC-STM-001-0.6)	Control	17	4	56 (17-184.3)	0.0000000
(MDC-STM-002-0.6)	Control	17	4	53.4 (16.2-175.7)	0.0000000
(MDC-STM-003-0.6)	Control	17	4	50.4 (15.3-165.8)	0.0000000
(MDC-STM-003-1.5)	Control	17	4	60.6 (18.4-199.5)	0.0000000
(MDC-STM-001-0.6)	Control	21	4	25.5 (9.3-70.3)	0.0000000
(MDC-STM-002-0.6)	Control	21	4	23.4 (8.5-64.4)	0.0000000
(MDC-STM-003-0.6)	Control	21	4	27.7 (10.1-76.4)	0.0000000
(MDC-STM-003-1.5)	Control	21	4	27.7 (10-76.2)	0.0000000
(MDC-STM-001-0.6)	Control	42	4	41.1 (10.2-165.9)	0.0000000
(MDC-STM-002-0.6)	Control	42	4	24 (5.9-97.5)	0.0000000
(MDC-STM-003-0.6)	Control	42	4	28.2 (7-114.1)	0.0000000
(MDC-STM-003-1.5)	Control	42	4	40.3 (10-162.9)	0.0000000
(MDC-STM-001-0.6)	Control	56	4	55.9 (14-223.3)	0.0000000
(MDC-STM-002-0.6)	Control	56	4	23.6 (5.8-95)	0.0000000
(MDC-STM-003-0.6)	Control	56	4	52.2 (13-209.7)	0.0000000
(MDC-STM-003-1.5)	Control	56	4	81.4 (20.3-326.2)	0.0000000
(MDC-STM-001-0.6)	Control	70	4	26.4 (8.2-84.9)	0.0000000
(MDC-STM-002-0.6)	Control	70	4	6.2 (1.9-20.7)	0.0003562
(MDC-STM-003-0.6)	Control	70	4	19.8 (6.1-64)	0.0000000
(MDC-STM-003-1.5)	Control	70	4	34.4 (10.7-110.5)	0.0000000
(MDC-STM-001-0.6)	Control	84	4	7.9 (2.9-21.6)	0.0000002
(MDC-STM-002-0.6)	Control	84	4	2.2 (0.8-6.2)	0.2699699

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-003-0.6)	Control	84	4	6.8 (2.5-18.8)	0.0000023
(MDC-STM-003-1.5)	Control	84	4	12.8 (4.7-34.9)	0.0000000
(MDC-STM-001-0.6)	Control	98	4	38.4 (8.7-169.4)	0.0000000
(MDC-STM-002-0.6)	Control	98	4	2.7 (0.5-13.6)	0.4587964
(MDC-STM-003-0.6)	Control	98	4	21.2 (4.7-94.6)	0.0000003
(MDC-STM-003-1.5)	Control	98	4	36.7 (8.3-162.4)	0.0000000
(MDC-STM-001-0.6)	Control	112	4	25.7 (6.4-103.6)	0.0000000
(MDC-STM-002-0.6)	Control	112	4	5.6 (1.3-23.6)	0.0092896
(MDC-STM-003-0.6)	Control	112	4	23.5 (5.8-95.2)	0.0000000
(MDC-STM-003-1.5)	Control	112	4	26.8 (6.6-108)	0.0000000
(MDC-STM-001-0.6)	Control	126	4	160.9 (9.3-2793.8)	0.0000118
(MDC-STM-002-0.6)	Control	126	4	29.5 (1.6-528.9)	0.0120573
(MDC-STM-003-0.6)	Control	126	4	156.2 (9-2712.6)	0.0000137
(MDC-STM-003-1.5)	Control	126	4	215.2 (12.4-3727.6)	0.0000028
(MDC-STM-001-0.6)	Control	BI	10	0.6 (0.2-2.3)	0.8747113
(MDC-STM-002-0.6)	Control	BI	10	0.5 (0.1-1.8)	0.5163317
(MDC-STM-003-0.6)	Control	BI	10	0.5 (0.2-1.9)	0.6559403
(MDC-STM-003-1.5)	Control	BI	10	0.4 (0.1-1.5)	0.2850212
(MDC-STM-001-0.6)	Control	2	10	22.3 (8.1-61.5)	0.0000000
(MDC-STM-002-0.6)	Control	2	10	44.4 (16.1-122.5)	0.0000000
(MDC-STM-003-0.6)	Control	2	10	44.1 (16-121.6)	0.0000000
(MDC-STM-003-1.5)	Control	2	10	40.8 (14.8-112.8)	0.0000000
(MDC-STM-001-0.6)	Control	17	10	22.1 (8.7-56.1)	0.0000000
(MDC-STM-002-0.6)	Control	17	10	19.9 (7.9-50.5)	0.0000000
(MDC-STM-003-0.6)	Control	17	10	19.5 (7.7-49.5)	0.0000000
(MDC-STM-003-1.5)	Control	17	10	22.9 (9-57.9)	0.0000000
(MDC-STM-001-0.6)	Control	21	10	19.4 (7.7-48.6)	0.0000000

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-002-0.6)	Control	21	10	16.7 (6.7-42)	0.0000000
(MDC-STM-003-0.6)	Control	21	10	20.7 (8.3-52)	0.0000000
(MDC-STM-003-1.5)	Control	21	10	21.3 (8.5-53.5)	0.0000000
(MDC-STM-001-0.6)	Control	42	10	12.1 (4.6-31.9)	0.0000000
(MDC-STM-002-0.6)	Control	42	10	7 (2.6-18.4)	0.0000005
(MDC-STM-003-0.6)	Control	42	10	8.9 (3.4-23.5)	0.0000000
(MDC-STM-003-1.5)	Control	42	10	12.3 (4.7-32.5)	0.0000000
(MDC-STM-001-0.6)	Control	56	10	14.4 (5.6-37.2)	0.0000000
(MDC-STM-002-0.6)	Control	56	10	7.2 (2.8-18.7)	0.0000002
(MDC-STM-003-0.6)	Control	56	10	14.5 (5.6-37.7)	0.0000000
(MDC-STM-003-1.5)	Control	56	10	21.2 (8.2-54.9)	0.0000000
(MDC-STM-001-0.6)	Control	70	10	12.3 (4.8-31.6)	0.0000000
(MDC-STM-002-0.6)	Control	70	10	3.2 (1.2-8.5)	0.0087904
(MDC-STM-003-0.6)	Control	70	10	9 (3.5-23.2)	0.0000000
(MDC-STM-003-1.5)	Control	70	10	15.7 (6.1-40.3)	0.0000000
(MDC-STM-001-0.6)	Control	84	10	8 (3.2-20.3)	0.0000000
(MDC-STM-002-0.6)	Control	84	10	2.4 (1-6.3)	0.0734027
(MDC-STM-003-0.6)	Control	84	10	5.7 (2.2-14.4)	0.0000038
(MDC-STM-003-1.5)	Control	84	10	10.1 (4-25.4)	0.0000000
(MDC-STM-001-0.6)	Control	98	10	10.1 (3.9-26.2)	0.0000000
(MDC-STM-002-0.6)	Control	98	10	2.1 (0.8-5.5)	0.2703929
(MDC-STM-003-0.6)	Control	98	10	5.7 (2.2-15.1)	0.0000082
(MDC-STM-003-1.5)	Control	98	10	9.6 (3.7-25)	0.0000000
(MDC-STM-001-0.6)	Control	112	10	10.9 (4-29.9)	0.0000000
(MDC-STM-002-0.6)	Control	112	10	3.3 (1.2-9.3)	0.0131335
(MDC-STM-003-0.6)	Control	112	10	10.7 (3.9-29.5)	0.0000000
(MDC-STM-003-1.5)	Control	112	10	11.5 (4.2-31.6)	0.0000000

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-001-0.6)	Control	126	10	34.9 (9.3-131.3)	0.0000000
(MDC-STM-002-0.6)	Control	126	10	10.5 (2.7-40.3)	0.0000181
(MDC-STM-003-0.6)	Control	126	10	33.2 (8.8-125.1)	0.0000000
(MDC-STM-003-1.5)	Control	126	10	45.5 (12.1-170.7)	0.0000000
(MDC-STM-001-0.6)	Control	BI	30	1.1 (0.5-2.3)	0.9974111
(MDC-STM-002-0.6)	Control	BI	30	1 (0.5-2.1)	1.0000000
(MDC-STM-003-0.6)	Control	BI	30	0.8 (0.4-1.8)	0.9663867
(MDC-STM-003-1.5)	Control	BI	30	0.9 (0.4-1.9)	0.9941915
(MDC-STM-001-0.6)	Control	2	30	7.4 (3.5-15.7)	0.0000000
(MDC-STM-002-0.6)	Control	2	30	18.3 (8.6-38.9)	0.0000000
(MDC-STM-003-0.6)	Control	2	30	18.4 (8.7-39.1)	0.0000000
(MDC-STM-003-1.5)	Control	2	30	12.6 (5.9-26.8)	0.0000000
(MDC-STM-001-0.6)	Control	17	30	10.6 (5.1-22.3)	0.0000000
(MDC-STM-002-0.6)	Control	17	30	9.4 (4.5-19.7)	0.0000000
(MDC-STM-003-0.6)	Control	17	30	9.4 (4.5-19.6)	0.0000000
(MDC-STM-003-1.5)	Control	17	30	11.8 (5.6-24.7)	0.0000000
(MDC-STM-001-0.6)	Control	21	30	10.1 (4.8-21.2)	0.0000000
(MDC-STM-002-0.6)	Control	21	30	7.4 (3.5-15.4)	0.0000000
(MDC-STM-003-0.6)	Control	21	30	10.7 (5.1-22.4)	0.0000000
(MDC-STM-003-1.5)	Control	21	30	11.6 (5.6-24.3)	0.0000000
(MDC-STM-001-0.6)	Control	42	30	5.9 (2.7-12.7)	0.0000000
(MDC-STM-002-0.6)	Control	42	30	3.8 (1.8-8.2)	0.0000160
(MDC-STM-003-0.6)	Control	42	30	4.7 (2.2-10.2)	0.0000003
(MDC-STM-003-1.5)	Control	42	30	6.8 (3.2-14.6)	0.0000000
(MDC-STM-001-0.6)	Control	56	30	5.5 (2.6-11.7)	0.0000000
(MDC-STM-002-0.6)	Control	56	30	3.1 (1.5-6.6)	0.0002827
(MDC-STM-003-0.6)	Control	56	30	6.8 (3.2-14.3)	0.0000000

Formulation	Treatment arm	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-003-1.5)	Control	56	30	7.1 (3.4-15.1)	0.0000000
(MDC-STM-001-0.6)	Control	70	30	3.5 (1.7-7.2)	0.0000345
(MDC-STM-002-0.6)	Control	70	30	1.4 (0.7-2.9)	0.7146990
(MDC-STM-003-0.6)	Control	70	30	2.7 (1.3-5.7)	0.0018361
(MDC-STM-003-1.5)	Control	70	30	4.8 (2.3-9.9)	0.0000001
(MDC-STM-001-0.6)	Control	84	30	3.6 (1.7-7.6)	0.0000153
(MDC-STM-002-0.6)	Control	84	30	1.5 (0.7-3.2)	0.5079043
(MDC-STM-003-0.6)	Control	84	30	2.5 (1.2-5.1)	0.0070421
(MDC-STM-003-1.5)	Control	84	30	4.2 (2-8.7)	0.0000010
(MDC-STM-001-0.6)	Control	98	30	3.8 (1.8-8.1)	0.0000099
(MDC-STM-002-0.6)	Control	98	30	1.4 (0.6-2.9)	0.7948357
(MDC-STM-003-0.6)	Control	98	30	2.8 (1.3-6)	0.0015562
(MDC-STM-003-1.5)	Control	98	30	4.4 (2.1-9.3)	0.0000008
(MDC-STM-001-0.6)	Control	112	30	3.7 (1.8-7.8)	0.0000166
(MDC-STM-002-0.6)	Control	112	30	1.5 (0.7-3.2)	0.5637848
(MDC-STM-003-0.6)	Control	112	30	3.7 (1.8-7.9)	0.0000133
(MDC-STM-003-1.5)	Control	112	30	4.4 (2.1-9.3)	0.0000006
(MDC-STM-001-0.6)	Control	126	30	3.9 (1.9-8.3)	0.0000053
(MDC-STM-002-0.6)	Control	126	30	1.7 (0.8-3.6)	0.3188277
(MDC-STM-003-0.6)	Control	126	30	4.1 (2-8.7)	0.0000023
(MDC-STM-003-1.5)	Control	126	30	4.6 (2.2-9.7)	0.0000002

B.

Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
(MDC-STM-001-0.6)	Control	BI	4	1 (0.1-17.2)	1.0000000
(MDC-STM-002-0.6)	Control	BI	4	1.9 (0.2-22.7)	0.9587058
(MDC-STM-	Control	BI	4	0.8 (0-14.3)	0.9997884

Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
003-0.6)					
(MDC-STM-003-1.5)	Control	BI	4	1.9 (0.2-22.4)	0.9618095
(MDC-STM-001-0.6)	Control	2	4	32.2 (9.8-106.3)	0.0000000
(MDC-STM-002-0.6)	Control	2	4	59.3 (18-195.1)	0.0000000
(MDC-STM-003-0.6)	Control	2	4	58.3 (17.7-191.8)	0.0000000
(MDC-STM-003-1.5)	Control	2	4	57.3 (17.4-188.9)	0.0000000
(MDC-STM-001-0.6)	Control	17	4	56 (17-184.3)	0.0000000
(MDC-STM-002-0.6)	Control	17	4	53.4 (16.2-175.7)	0.0000000
(MDC-STM-003-0.6)	Control	17	4	50.4 (15.3-165.8)	0.0000000
(MDC-STM-003-1.5)	Control	17	4	60.6 (18.4-199.5)	0.0000000
(MDC-STM-001-0.6)	Control	21	4	25.5 (9.3-70.3)	0.0000000
(MDC-STM-002-0.6)	Control	21	4	23.4 (8.5-64.4)	0.0000000
(MDC-STM-003-0.6)	Control	21	4	27.7 (10.1-76.4)	0.0000000
(MDC-STM-003-1.5)	Control	21	4	27.7 (10-76.2)	0.0000000
(MDC-STM-001-0.6)	Control	42	4	41.1 (10.2-165.9)	0.0000000
(MDC-STM-002-0.6)	Control	42	4	24 (5.9-97.5)	0.0000000
(MDC-STM-003-0.6)	Control	42	4	28.2 (7-114.1)	0.0000000
(MDC-STM-003-1.5)	Control	42	4	40.3 (10-162.9)	0.0000000
(MDC-STM-001-0.6)	Control	56	4	55.9 (14-223.3)	0.0000000
(MDC-STM-002-0.6)	Control	56	4	23.6 (5.8-95)	0.0000000
(MDC-STM-003-0.6)	Control	56	4	52.2 (13-209.7)	0.0000000
(MDC-STM-003-1.5)	Control	56	4	81.4 (20.3-326.2)	0.0000000
(MDC-STM-001-0.6)	Control	70	4	26.4 (8.2-84.9)	0.0000000
(MDC-STM-002-0.6)	Control	70	4	6.2 (1.9-20.7)	0.0003562
(MDC-STM-003-0.6)	Control	70	4	19.8 (6.1-64)	0.0000000
(MDC-STM-003-1.5)	Control	70	4	34.4 (10.7-110.5)	0.0000000
(MDC-STM-001-0.6)	Control	84	4	7.9 (2.9-21.6)	0.0000002
(MDC-STM-	Control	84	4	2.2 (0.8-6.2)	0.2699699

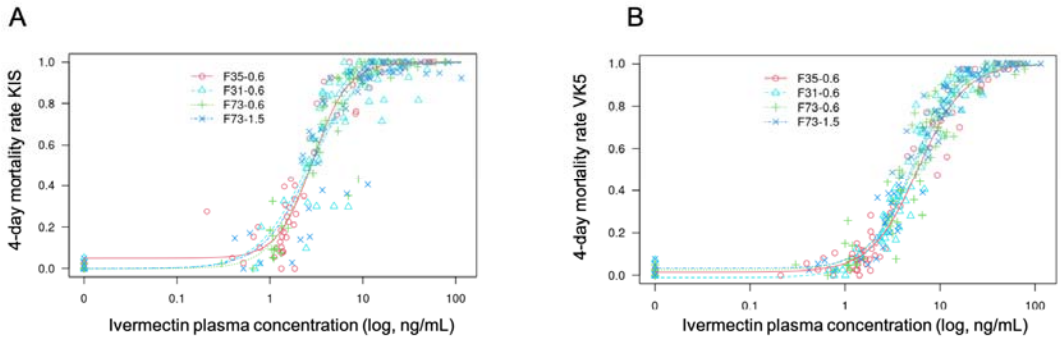
Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
002-0.6)					
(MDC-STM-003-0.6)	Control	84	4	6.8 (2.5-18.8)	0.0000023
(MDC-STM-003-1.5)	Control	84	4	12.8 (4.7-34.9)	0.0000000
(MDC-STM-001-0.6)	Control	98	4	38.4 (8.7-169.4)	0.0000000
(MDC-STM-002-0.6)	Control	98	4	2.7 (0.5-13.6)	0.4587964
(MDC-STM-003-0.6)	Control	98	4	21.2 (4.7-94.6)	0.0000003
(MDC-STM-003-1.5)	Control	98	4	36.7 (8.3-162.4)	0.0000000
(MDC-STM-001-0.6)	Control	112	4	25.7 (6.4-103.6)	0.0000000
(MDC-STM-002-0.6)	Control	112	4	5.6 (1.3-23.6)	0.0092896
(MDC-STM-003-0.6)	Control	112	4	23.5 (5.8-95.2)	0.0000000
(MDC-STM-003-1.5)	Control	112	4	26.8 (6.6-108)	0.0000000
(MDC-STM-001-0.6)	Control	126	4	160.9 (9.3-2793.8)	0.0000118
(MDC-STM-002-0.6)	Control	126	4	29.5 (1.6-528.9)	0.0120573
(MDC-STM-003-0.6)	Control	126	4	156.2 (9-2712.6)	0.0000137
(MDC-STM-003-1.5)	Control	126	4	215.2 (12.4-3727.6)	0.0000028
(MDC-STM-001-0.6)	Control	BI	10	0.6 (0.2-2.3)	0.8747113
(MDC-STM-002-0.6)	Control	BI	10	0.5 (0.1-1.8)	0.5163317
(MDC-STM-003-0.6)	Control	BI	10	0.5 (0.2-1.9)	0.6559403
(MDC-STM-003-1.5)	Control	BI	10	0.4 (0.1-1.5)	0.2850212
(MDC-STM-001-0.6)	Control	2	10	22.3 (8.1-61.5)	0.0000000
(MDC-STM-002-0.6)	Control	2	10	44.4 (16.1-122.5)	0.0000000
(MDC-STM-003-0.6)	Control	2	10	44.1 (16-121.6)	0.0000000
(MDC-STM-003-1.5)	Control	2	10	40.8 (14.8-112.8)	0.0000000
(MDC-STM-001-0.6)	Control	17	10	22.1 (8.7-56.1)	0.0000000
(MDC-STM-002-0.6)	Control	17	10	19.9 (7.9-50.5)	0.0000000
(MDC-STM-003-0.6)	Control	17	10	19.5 (7.7-49.5)	0.0000000
(MDC-STM-003-1.5)	Control	17	10	22.9 (9-57.9)	0.0000000
(MDC-STM-	Control	21	10	19.4 (7.7-48.6)	0.0000000

Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
001-0.6)					
(MDC-STM-002-0.6)	Control	21	10	16.7 (6.7-42)	0.0000000
(MDC-STM-003-0.6)	Control	21	10	20.7 (8.3-52)	0.0000000
(MDC-STM-003-1.5)	Control	21	10	21.3 (8.5-53.5)	0.0000000
(MDC-STM-001-0.6)	Control	42	10	12.1 (4.6-31.9)	0.0000000
(MDC-STM-002-0.6)	Control	42	10	7 (2.6-18.4)	0.0000005
(MDC-STM-003-0.6)	Control	42	10	8.9 (3.4-23.5)	0.0000000
(MDC-STM-003-1.5)	Control	42	10	12.3 (4.7-32.5)	0.0000000
(MDC-STM-001-0.6)	Control	56	10	14.4 (5.6-37.2)	0.0000000
(MDC-STM-002-0.6)	Control	56	10	7.2 (2.8-18.7)	0.0000002
(MDC-STM-003-0.6)	Control	56	10	14.5 (5.6-37.7)	0.0000000
(MDC-STM-003-1.5)	Control	56	10	21.2 (8.2-54.9)	0.0000000
(MDC-STM-001-0.6)	Control	70	10	12.3 (4.8-31.6)	0.0000000
(MDC-STM-002-0.6)	Control	70	10	3.2 (1.2-8.5)	0.0087904
(MDC-STM-003-0.6)	Control	70	10	9 (3.5-23.2)	0.0000000
(MDC-STM-003-1.5)	Control	70	10	15.7 (6.1-40.3)	0.0000000
(MDC-STM-001-0.6)	Control	84	10	8 (3.2-20.3)	0.0000000
(MDC-STM-002-0.6)	Control	84	10	2.4 (1-6.3)	0.0734027
(MDC-STM-003-0.6)	Control	84	10	5.7 (2.2-14.4)	0.0000038
(MDC-STM-003-1.5)	Control	84	10	10.1 (4-25.4)	0.0000000
(MDC-STM-001-0.6)	Control	98	10	10.1 (3.9-26.2)	0.0000000
(MDC-STM-002-0.6)	Control	98	10	2.1 (0.8-5.5)	0.2703929
(MDC-STM-003-0.6)	Control	98	10	5.7 (2.2-15.1)	0.0000082
(MDC-STM-003-1.5)	Control	98	10	9.6 (3.7-25)	0.0000000
(MDC-STM-001-0.6)	Control	112	10	10.9 (4-29.9)	0.0000000
(MDC-STM-002-0.6)	Control	112	10	3.3 (1.2-9.3)	0.0131335
(MDC-STM-003-0.6)	Control	112	10	10.7 (3.9-29.5)	0.0000000
(MDC-STM-	Control	112	10	11.5 (4.2-31.6)	0.0000000

Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
003-1.5)					
(MDC-STM-001-0.6)	Control	126	10	34.9 (9.3-131.3)	0.0000000
(MDC-STM-002-0.6)	Control	126	10	10.5 (2.7-40.3)	0.0000181
(MDC-STM-003-0.6)	Control	126	10	33.2 (8.8-125.1)	0.0000000
(MDC-STM-003-1.5)	Control	126	10	45.5 (12.1-170.7)	0.0000000
(MDC-STM-001-0.6)	Control	BI	30	1.1 (0.5-2.3)	0.9974111
(MDC-STM-002-0.6)	Control	BI	30	1 (0.5-2.1)	1.0000000
(MDC-STM-003-0.6)	Control	BI	30	0.8 (0.4-1.8)	0.9663867
(MDC-STM-003-1.5)	Control	BI	30	0.9 (0.4-1.9)	0.9941915
(MDC-STM-001-0.6)	Control	2	30	7.4 (3.5-15.7)	0.0000000
(MDC-STM-002-0.6)	Control	2	30	18.3 (8.6-38.9)	0.0000000
(MDC-STM-003-0.6)	Control	2	30	18.4 (8.7-39.1)	0.0000000
(MDC-STM-003-1.5)	Control	2	30	12.6 (5.9-26.8)	0.0000000
(MDC-STM-001-0.6)	Control	17	30	10.6 (5.1-22.3)	0.0000000
(MDC-STM-002-0.6)	Control	17	30	9.4 (4.5-19.7)	0.0000000
(MDC-STM-003-0.6)	Control	17	30	9.4 (4.5-19.6)	0.0000000
(MDC-STM-003-1.5)	Control	17	30	11.8 (5.6-24.7)	0.0000000
(MDC-STM-001-0.6)	Control	21	30	10.1 (4.8-21.2)	0.0000000
(MDC-STM-002-0.6)	Control	21	30	7.4 (3.5-15.4)	0.0000000
(MDC-STM-003-0.6)	Control	21	30	10.7 (5.1-22.4)	0.0000000
(MDC-STM-003-1.5)	Control	21	30	11.6 (5.6-24.3)	0.0000000
(MDC-STM-001-0.6)	Control	42	30	5.9 (2.7-12.7)	0.0000000
(MDC-STM-002-0.6)	Control	42	30	3.8 (1.8-8.2)	0.0000160
(MDC-STM-003-0.6)	Control	42	30	4.7 (2.2-10.2)	0.0000003
(MDC-STM-003-1.5)	Control	42	30	6.8 (3.2-14.6)	0.0000000
(MDC-STM-001-0.6)	Control	56	30	5.5 (2.6-11.7)	0.0000000
(MDC-STM-002-0.6)	Control	56	30	3.1 (1.5-6.6)	0.0002827
(MDC-STM-	Control	56	30	6.8 (3.2-14.3)	0.0000000

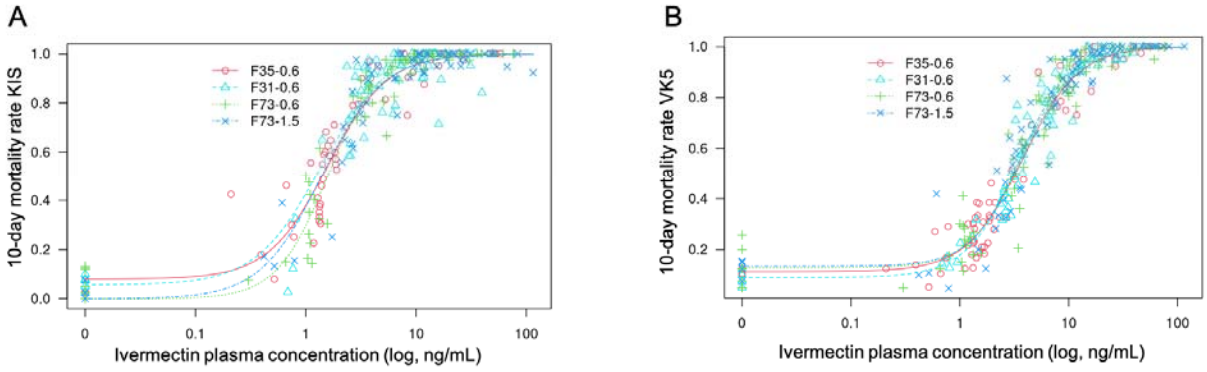
Formulation	Reference	DAI	Follow-up (days)	HR (CI 95%)	p.value
003-0.6)					
(MDC-STM-003-1.5)	Control	56	30	7.1 (3.4-15.1)	0.0000000
(MDC-STM-001-0.6)	Control	70	30	3.5 (1.7-7.2)	0.0000345
(MDC-STM-002-0.6)	Control	70	30	1.4 (0.7-2.9)	0.7146990
(MDC-STM-003-0.6)	Control	70	30	2.7 (1.3-5.7)	0.0018361
(MDC-STM-003-1.5)	Control	70	30	4.8 (2.3-9.9)	0.0000001
(MDC-STM-001-0.6)	Control	84	30	3.6 (1.7-7.6)	0.0000153
(MDC-STM-002-0.6)	Control	84	30	1.5 (0.7-3.2)	0.5079043
(MDC-STM-003-0.6)	Control	84	30	2.5 (1.2-5.1)	0.0070421
(MDC-STM-003-1.5)	Control	84	30	4.2 (2-8.7)	0.0000010
(MDC-STM-001-0.6)	Control	98	30	3.8 (1.8-8.1)	0.0000099
(MDC-STM-002-0.6)	Control	98	30	1.4 (0.6-2.9)	0.7948357
(MDC-STM-003-0.6)	Control	98	30	2.8 (1.3-6)	0.0015562
(MDC-STM-003-1.5)	Control	98	30	4.4 (2.1-9.3)	0.0000008
(MDC-STM-001-0.6)	Control	112	30	3.7 (1.8-7.8)	0.0000166
(MDC-STM-002-0.6)	Control	112	30	1.5 (0.7-3.2)	0.5637848
(MDC-STM-003-0.6)	Control	112	30	3.7 (1.8-7.9)	0.0000133
(MDC-STM-003-1.5)	Control	112	30	4.4 (2.1-9.3)	0.0000006
(MDC-STM-001-0.6)	Control	126	30	3.9 (1.9-8.3)	0.0000053
(MDC-STM-002-0.6)	Control	126	30	1.7 (0.8-3.6)	0.3188277
(MDC-STM-003-0.6)	Control	126	30	4.1 (2-8.7)	0.0000023
(MDC-STM-003-1.5)	Control	126	30	4.6 (2.2-9.7)	0.0000002

Lethal Concentrations



Supplementary Figure 4

4-days mortality rates of mosquitoes fed on cattle treated with different LAIFs as a function of Ivermectin Plasmatic concentration. A. KIS colony mosquitoes. B. VK5 colony mosquitoes.



Supplementary Figure 5

10-days mortality rates of mosquitoes fed on cattle treated with different LAIFs as a function of Ivermectin Plasmatic concentration. A. KIS colony mosquitoes. B. VK5 colony mosquitoes.

Supplementary table 8

Between formulations comparisons of 4-day LC50 and L90 values for KIS mosquitoes fed on treated cattle. Model outputs are given by the drc package (ref)

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 9

Between formulations comparisons of 10-day LC50 and LC90 values for KIS mosquitoes fed on treated cattle. Model outputs are given by the drc package (ref)

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

Supplementary table 10

Between formulations comparisons of 4-day LC50 and LC90 values for VK5 mosquitoes fed on treated cattle. Model outputs are given by the drc package (ref).

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
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

Supplementary table 11

Between formulations comparisons of 10-day LC50 and LC90 values for VK5 mosquitoes fed on treated cattle. Model outputs are given by the drc package (ref).

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