

Text S1: Drug Analytical Quantification of Ivermectin and Piperazine**Ivermectin Assay (LC-MS/MS):**

Plasma samples were taken out of the -80°C freezer and allowed to come to room temperature. A 60 µL aliquot of each sample was assayed alongside plasma calibration curve standards (5-320 ng/mL) and quality control samples (low 15 ng/mL, medium 150 ng/mL and high 250 ng/mL). A 60 µL aliquot of each plasma sample was taken and placed into 1.5 ml Eppendorf tubes. 940 µL of acetonitrile containing doramectin 100 ng/ml was added as internal standard (IS). Samples were then mixed on a vortexer for 10 seconds and subsequently left to settle for 5 minutes. Following, the samples were centrifuged for 10 minutes at 14,000 rpm. The resulting supernatants were then taken and placed into clean and dry 10 mL glass test-tubes containing 1 ml of water. 3 mL of a 1/1/1 mix of dichloromethane/hexane/methyl-tert-butyl ether mix was added, which was subsequently vortexed for 10 seconds, followed by a 10-minute centrifugation at 4,000 rpm. The resultant supernatants were then removed and placed into clean and dry 7 mL glass test-tubes before being evaporated until dry using a gentle stream of nitrogen free air at 30°C. The dried down supernatants were then reconstituted in 60 µL of mobile phase and then vortexed for 10 seconds. The reconstituted samples were then transferred to clean glass insert vials and then centrifuged at 4,700 rpm for 5 minutes. Samples were then injected (20 µL) onto 100 x 2.1 mm, 1.9 (µm) particle size, Hypersil GOLD (Thermo Scientific) column using an isocratic gradient method of acetonitrile/0.1% formic acid (90/10) at a flowrate of 400 µL/min with a column oven set at 30°C. For the analysis and quantification of ivermectin from capillary plasma samples the above procedure was revalidated using a 30 µL aliquot of capillary plasma. The extraction procedure, calibration curve standards, quality control levels were kept the same except for the lower limit of quantification (LLOQ) which was changed from 5 ng/mL to 10 ng/mL. The method was then revalidated for the reduced aliquot of capillary plasma.

Piperazine Assay (LC-MS/MS):

Plasma samples were taken out of the -80°C freezer and allowed to come to room temperature. A 10 µL aliquot of each sample was assayed alongside plasma calibration curve standards (1.5-600 ng/mL) and quality control samples (low 4.5 ng/mL, medium 250 ng/mL and high 500 ng/mL). Using a 96-well filter plate, 300 µL of a perception solution (80/20 acetonitrile/methanol) was added to each well, containing 10 ng/ml piperazine-d6 as internal standard (IS). A 10 µL aliquot of each plasma sample was then added to a corresponding well. The filter plate containing the IS and plasma samples was then transferred to a 96-well vacuum manifold. A corresponding 96-well plate was then placed inside the vacuum manifold to catch the subsequent filtrate after the vacuum had been applied. The resultant filtrate was then evaporated until dry using a gentle stream of nitrogen free air at 30°C. The dried down supernatants were then reconstituted in 60 µL of mobile phase and then vortexed for 10 seconds. The reconstituted samples were then transferred to clean glass insert vials, followed by centrifuging at 4,700 rpm for 5 minutes. Samples were subsequently injected (20 µL) onto 50 x 2 mm, 3 (µm) particle size, Gemini (Phenomenex) column using an isocratic gradient of acetonitrile/2.5 mM ammonium bicarbonate (pH 7) (85/15) at a flowrate of 500 µL/min with a column oven set at 30°C. The capillary plasma samples were analysed and quantified using the exact same method as the venous samples.

Supplementary Equations:

$$\frac{dX_1}{dt} = -k_a \cdot X_1 \quad \dots\dots\dots \text{(Eq. S1)}$$

$$\frac{dX_2}{dt} = k_a \cdot X_1 - (k_e + k_{23} + k_{24}) \cdot X_2 + k_{32} \cdot X_3 + k_{42} \cdot X_4 \quad \dots\dots\dots \text{(Eq. S2)}$$

$$\frac{dX_3}{dt} = k_{23} \cdot X_2 - k_{32} \cdot X_3 \quad \dots\dots\dots \text{(Eq. S3)}$$

$$\frac{dX_4}{dt} = k_{24} \cdot X_2 - k_{42} \cdot X_4 \quad \dots\dots\dots \text{(Eq. S4)}$$

$$C_{venous} = \frac{X_2}{V_c} \quad \dots\dots\dots \text{(Eq. S5)}$$

$$C_{capillary} = \left(\frac{X_2}{V_c}\right) \cdot CapVen \text{ ratio} \quad \dots\dots\dots \text{(Eq. S6)}$$

$$k_e = \frac{CL/F}{V_c/F} \quad \dots\dots\dots \text{(Eq. S7)}$$

$$Q_1/F = k_{23} \cdot V_c/F \quad \dots\dots\dots \text{(Eq. S8)}$$

$$V_{P1}/F = \frac{Q_1/F}{k_{32}} \quad \dots\dots\dots \text{(Eq. S9)}$$

$$Q_2/F = k_{24} \cdot V_c/F \quad \dots\dots\dots \text{(Eq. S10)}$$

$$V_{P2}/F = \frac{Q_2/F}{k_{42}} \quad \dots\dots\dots \text{(Eq. S11)}$$

$$CL/F = CLi \cdot \left[\frac{\text{patient weight}}{60}\right]^{0.75} \quad \dots\dots\dots \text{(Eq. S12)}$$

$$V_c/F = V_{ci} \cdot \left[\frac{\text{patient weight}}{60}\right] \quad \dots\dots\dots \text{(Eq. S13)}$$

Where k_a is the absorption rate in hours. X_1 , X_2 , X_3 and X_4 respectively, represent the drug mass (in grams) in the gut (Dose compartment), the blood (central compartment), peripheral compartment 1, and for piperazine only peripheral compartment 2. k_e represents the elimination rate in hours and is calculated as Clearance (CL/F) divided by the central volume of distribution (V_c/F). Q_1/F , V_{P1}/F , Q_2/F , V_{P2}/F represent the exchange rates between the central and the two peripheral compartments (one for ivermectin) in hours. t represents time in hours, C the concentration in grams per liter, and CL/F and V_c/F are the weight adjusted individual clearance (CLi) and individual volume of distribution (V_{ci}), respectively. Q_1 , V_{P1} , Q_2 and V_{P2} values were assumed to be weight independent as weight scaling for these parameters resulted in similar or poorer PK predictions.

Capillary concentrations were modelled simultaneously with venous ones using **Equation S6**, where **CapVen** ratio represents the parameter that quantifies the ratio between capillary and venous concentration.

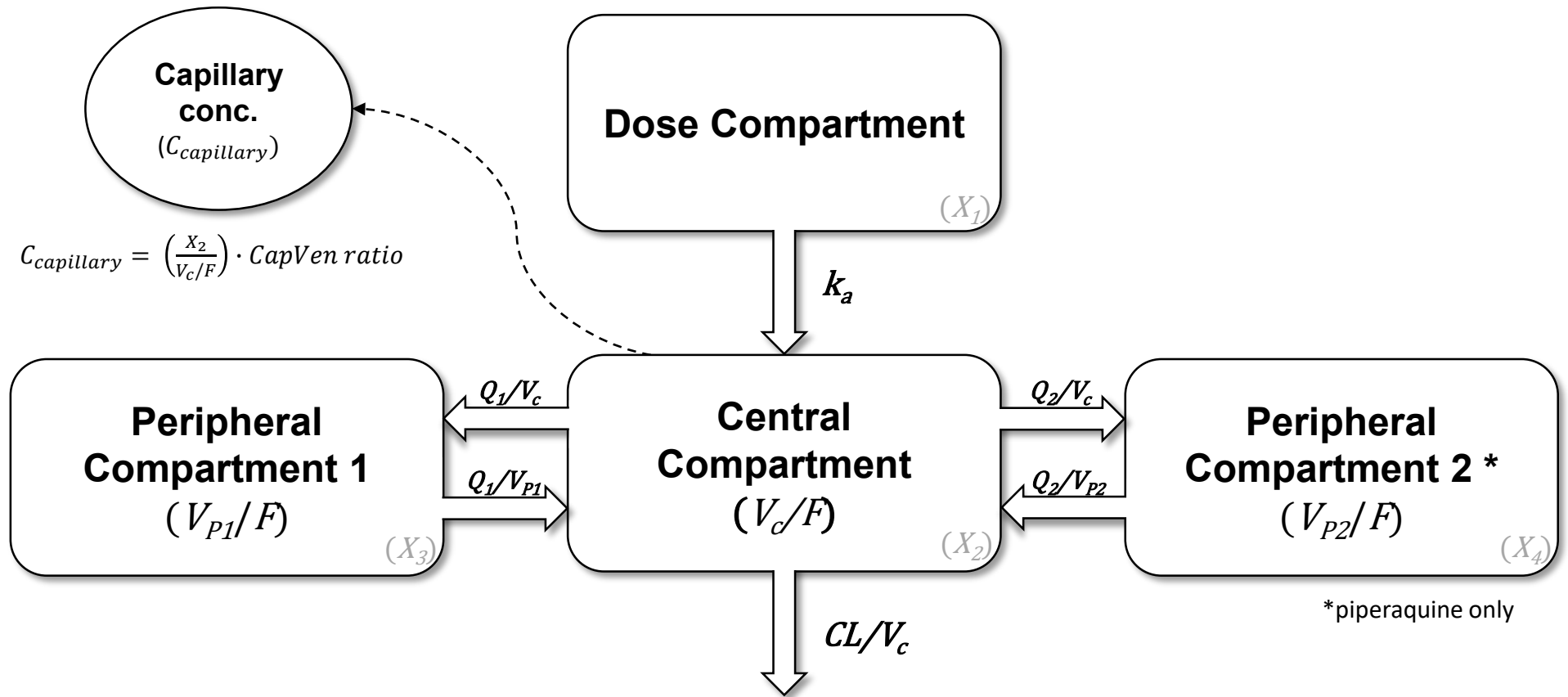
Absorption model

The absorption model used was a standard absorption model with an absorption rate k_a estimated for ivermectin and fixed for piperazine. No other absorption models (e.g. including transit

compartments and lag times) were attempted due to the limited number of concentrations at <2 hours post dosing.

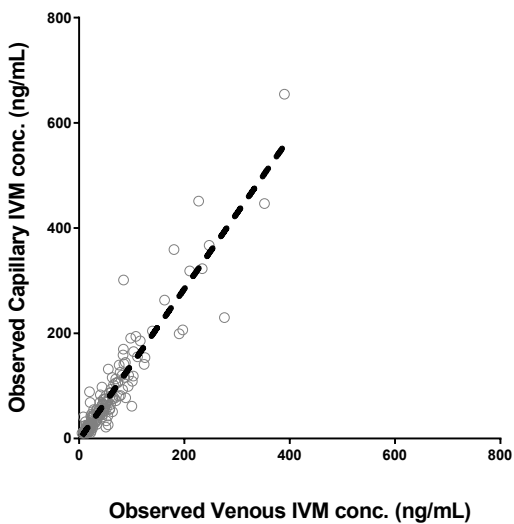
Error model

For ivermectin and piperazine we used a lambda error model (fixed-effect process noise multiplier of assay error), with a fixed L value set to 0.1 for all observations. The values (C0, C1, C2, C3) were used to estimate the error (standard deviation) of each observation for appropriate weighting in the fitting process, using the equation: $SD = C0 + C1*[obs] + C2*[obs]^2 + C3*[obs]^3$ where [obs] is the observation (Neely 2012). For ivermectin, C0 was set to 5 (equal to LOQ level) and C1 to 0.1 (assuming 10% intra-assay variability). For piperazine, C0 was set to 10 (equal to LOQ level) and C1 to 0.1. C2 and C3 were set to zero for both drugs.

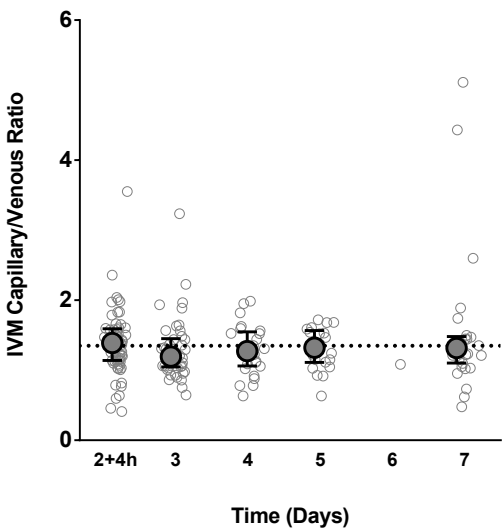


[Figure S2]

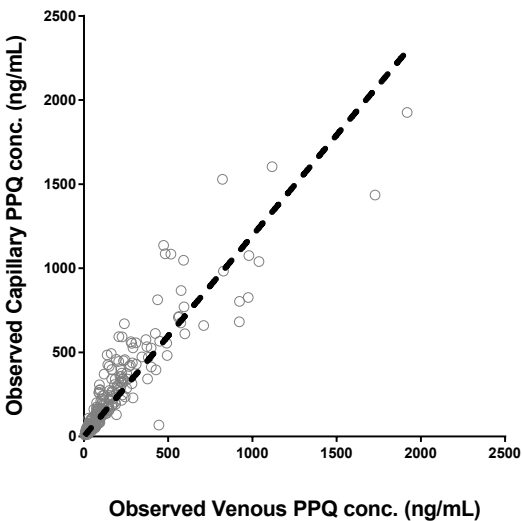
(a)

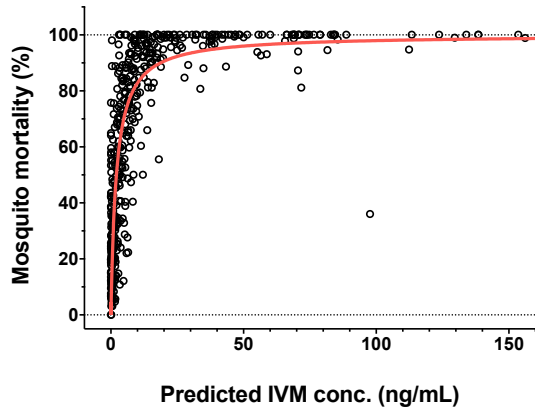
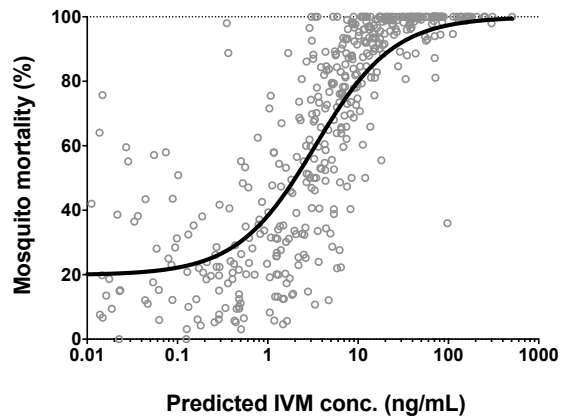
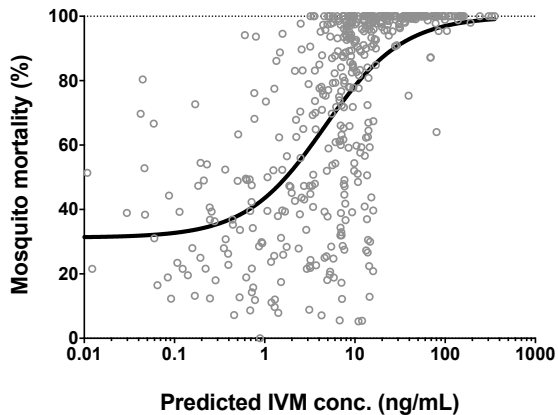
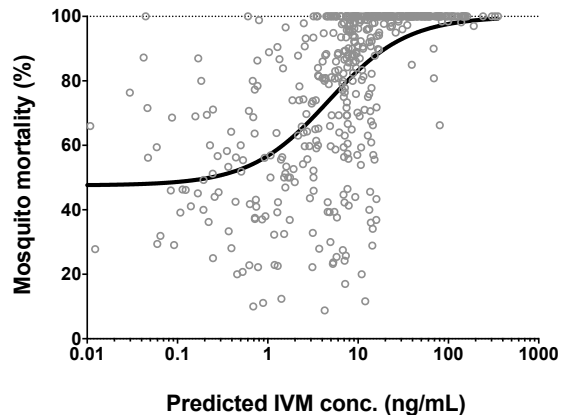


(b)



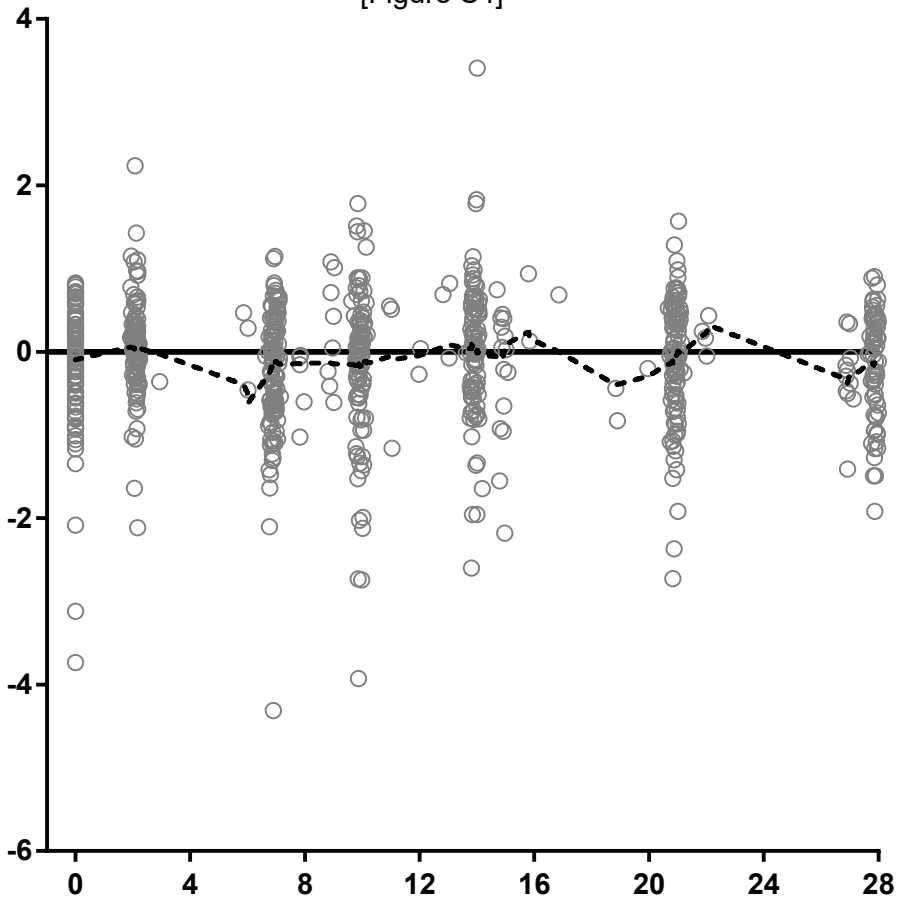
(c)



(a) 7-day- LC_{50} , unadjusted**(b) 7-day- LC_{50} , adjusted****(c) 10-day- LC_{50} , adjusted****(d) 14-day- LC_{50} , adjusted**

[Figure S4]

Weighted Residual Error



Time (Days)

[Figure S5]

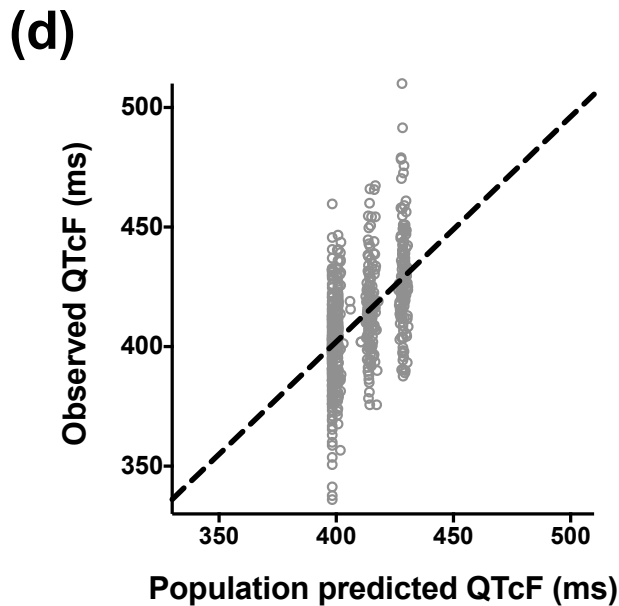
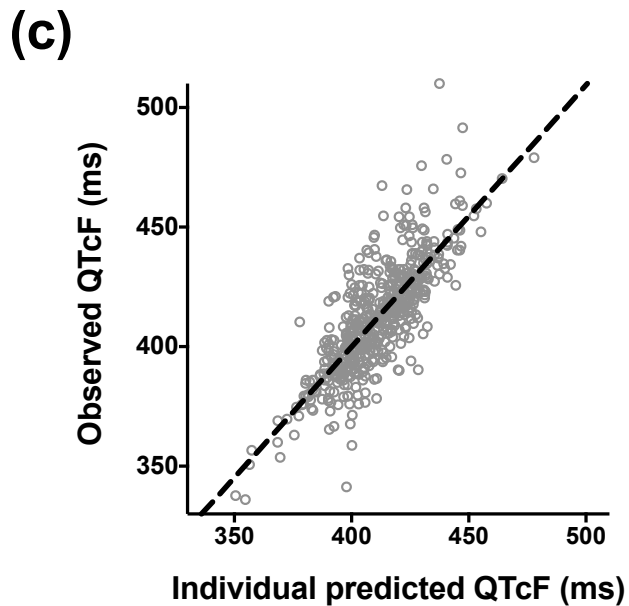
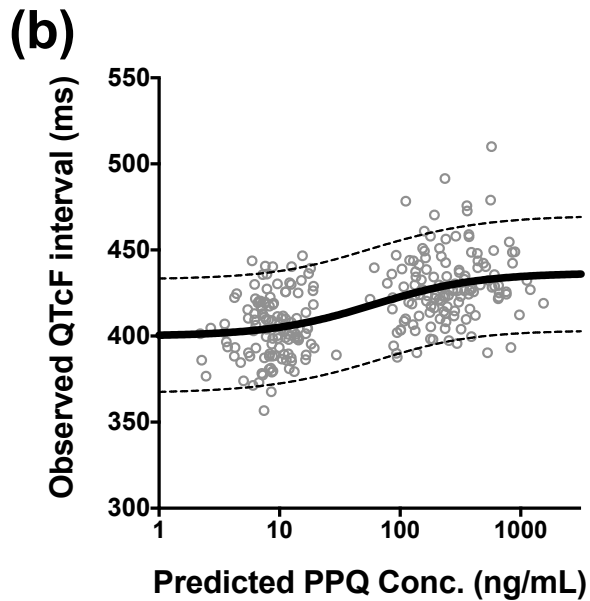
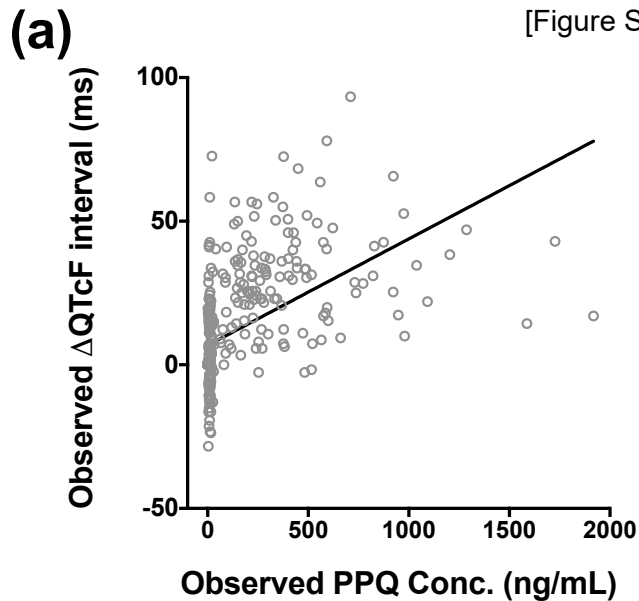


Table S1: Compartmental Model Fits for Ivermectin and Piperazine

	Ivermectin		Piperazine	
	One compartment	Two compartment	Two compartment	Three compartment
-2 log likelihood	6528.440	6202.912	17608.90	16375.37
AIC	6538.519	6217.061	17620.95	16391.45
BIC	6561.626	6249.373	17653.27	16434.52

Table S2: Number of observed concentrations and outcomes (ivermectin and piperazine)

Time (days)	plasma samples venous	plasma samples capillary	ivermectin venous	ivermectin capillary	mortality rate	ivermectin venous + mortality rate	ivermectin: venous + capillary	piperazine venous	piperazine capillary	QTcF	piperazine venous + QTcF	piperazine: venous + capillary
0	141	-	0	-	141	-	-	1	-	141	1	-
2	-	-	-	-	-	-	-	-	-	132	-	-
2+4h	133	86	88	58	128	86	58	133	87	133	133	87
7	128	85	70	30	128	70	29	128	85	-	-	85
10	118	-	52	-	112	52	-	118	-	-	-	-
14	122	-	29	-	119	28	-	122	-	-	-	-
21	117	-	8	-	111	7	-	115	-	-	-	-
28	118	-	3	-	111	3	-	117	-	118	117	-
Pop PK	516	162	284	93	-	-	90	512	161	-	-	160
Total	1,393	335	534	181	850	246	177	1,246	333	524	251	332

Pop PK= population pharmacokinetic samples which were drawn between days 0 to 28 and did not coincide with other outcomes.

Ivermectin observations for days 21 and 28 are mostly absent as they are below the limit of quantitation.

Table S3: Ivermectin LC₅₀'s by assay duration, and versus previous studies

Duration of mosquito follow-up post-feeding (days):	A. LC ₅₀ unadjusted to baseline mortality (CI95%) (ng/mL):	B. LC ₅₀ adjusted to baseline mortality (CI95%) (ng/mL):	C. Comparator in vivo study ¹⁵ LC ₅₀ adjusted to baseline mortality (CI95%) (ng/mL):	D. Comparator in vitro studies LC ₅₀ adjusted to baseline mortality (CI95%) (ng/mL):	Relative Difference:
1	1656 (1281-2287)	3883 (2378-9552)	1172 (499-N/A)		
2	19.71(17.5-22.3)	26.79 (22.64-31.93)	43.95 (36.30-54.87)		
3	7.89 (7.13-8.73)	10.29 (8.96-11.86)	20.94 (17.73-25.15)	6.1 (3.4-11.0) ¹⁵	D vs B: 0.6
4	5.07 (4.57-5.61)	6.92 (6.02-7.98)	15.40 (13.04-18.44)		
5	3.61 (3.24-4.01)	5.26 (4.56-6.09)	13.39 (11.24-16.18)	22.4 (18.0-26.9) ¹⁶	D vs B: 4.3
6	2.68 (2.39-32.99)	4.13 (3.57-4.79)	8.59 (7.09-10.52)		
7	2.08 (1.85-2.33)	3.35 (2.89-3.89)	7.92 (6.49-9.77)	15.9 (14.6-17.3) ¹⁷	D vs B: 4.7
8	1.74 (1.55-1.96)	2.97 (2.56-3.46)	7.43 (6.03-9.25)		
9	1.50 (1.32-1.70)	2.78 (2.38-3.25)	7.06 (5.69-8.86)	19.8 (14.3-25.3) ¹⁸	D vs B: 7.1
10	1.24 (1.08-1.41)	2.55 (2.17-3.01)	6.52 (5.22-8.23)		C vs B: 2.6
11	1.15 (0.99-1.32)	2.62 (2.22-3.11)			
12	0.97 (0.83-1.13)	2.60 (2.18-3.10)			
13	0.80 (0.67-0.95)	2.56 (2.13-3.08)			
14	0.62 (0.51-0.74)	2.53 (2.09-3.07)			
15	0.45 (0.36-0.56)	2.50 (2.04-3.07)			
16	0.31 (0.23-0.40)	2.45 (1.98-3.06)			
17	0.17 (0.11-0.25)	2.43 (1.95-3.06)			
18	0.012 (0.0054-0.027)	2.34 (1.85-2.98)			
19	<0.01	2.44 (1.90-3.16)			
20	<0.01	2.42 (1.85-3.18)			
21	<0.01	2.32 (1.74-3.12)			
22	<0.01	2.31 (1.69-3.18)			
23	<0.01	2.21 (1.58-3.13)			
24	<0.01	2.19 (1.52-3.20)			
25	<0.01	2.08 (1.40-3.14)			
26	<0.01	1.92 (1.23-3.06)			
27	<0.01	1.87 (1.14-3.12)			
28	<0.01	1.97 (1.15-3.44)			

LC₅₀'s using predicted concentrations and 3-parameter method; Hill's coefficient was fixed to 1. LC₅₀'s adjusted to baseline mortality are the concentrations required to kill 50% of mosquitoes that would have otherwise survived the assay without ivermectin exposure. Adjusted LC₅₀'s are more consistent during follow-up than unadjusted LC₅₀'s. Additionally, unadjusted LC₅₀'s cannot be determined over longer follow-up periods due to high baseline mortality. Comparator *in vivo* values were calculated using author's dataset.¹⁵ Comparator *in vitro* values as reported,^{16,17} except for one study that was converted from mol/L to ng/mL,¹⁵ and another study for which the SE was converted to CI95%.¹⁸ One study did not report whether it was adjusted to baseline, however probit analysis with control population was used, so baseline adjustment is assumed.¹⁸

Table S4: Piperazine concentration and QTcF interval (observed data)

Outcome	IVM-3x600 (N=47)	IVM-3x300 (N=48)	Placebo (N=46)	Mean ^{†Δ} or Risk [‡] difference (95% CI), p-value		
				IVM-3x600 vs Placebo	IVM-3x300 vs Placebo	IVM-3x600 vs IVM- 3x300
QTcF interval (Day 2+4h), change from baseline (ms)	27 (17) (n=42)	33 (17) (n=45)	29 (18) (n=44)	-0.8 (-8.0, 6.5), 0.84 [†]	4.7 (-2.6, 11.9), 0.21 [†]	-5.4 (-12.3, 1.5), 0.13 [†]
QTcF interval (Day 2+4h), ≥500 ms	0/42 (0%)	1/45 (2.2%)	0/44 (0%)	0.0% (-0.4%, 3.7%), 1.00 [‡]	2.2% (-1.4%, 5.8%), 0.23 [‡]	-2.2% (-5.9%, 1.5%), 0.24 [‡]
Piperazine plasma concentration (Day 2+4h) (ng/mL)	313 (208- 586) (n=43)	327 (179- 545) (n=45)	269 (169- 399) (n=45)	35.8 (-107.2, 178.7), 0.62 ^Δ	28.9 (-108.1, 165.9), 0.68 ^Δ	6.9 (-126.3, 140.0), 0.92 ^Δ

Data are mean (SD), median (IQR), or n/N (%), unless otherwise specified. IVM-3x600=ivermectin 600 mcg/kg/day for 3 days. IVM-3x300=ivermectin 300 mcg/kg/day for 3 days. QTcF=electrocardiogram QT interval, corrected for heart rate using Fredericia's formula.

Δ Mean difference (95% CI), p-value: obtained from GLM models.

† Mean difference (95% CI), p-value: obtained from GEE models adjusted for baseline measurement and repeated measures.

‡ Risk Difference (95% CI), p-value: obtained from GLM models.

Table S5: Piperavaquine concentration and QTcF interval (population fitted data)

Parameter	All Patients (N=141) [p5-p95]	IVM-3x600 (N=47) [p5-p95]	IVM-3x300 (N=48) [p5-p95]	Placebo (N=46) [p5-p95]
QTcF, baseline (E_{min}) (ms)	399.3 [377.5-416.3]	398.7 [371.9-413.2]	399.1 [379.5-415]	399.5 [379.8-416.5]
Δ QTcF, maximum possible change from baseline (E_{max}) (ms)	53.5 [31.1-122.9]	51.2 [32.2-119.6]	49.7 [31.2-123.3]	66.3 [27.2-118.3]
QTcF, maximum possible effect ($E_{max}+E_{min}$) (ms)	449.8 [415.1-520.0]*	445.2 [421.3-520.0]	447.8 [417.2-520.0]	464.1 [415.4-520.0]
Piperaquine concentration achieving half-maximal effect on QTcF (EC_{50}) (ng/mL)	181.7 [16.0-1200.0]	169.2 [16.0-1200.0]	199.0 [16.1-1200.0]	218.2 [15.9-1200.0]

Data are median [p5-p95]. IVM-3x600=ivermectin 600 mcg/kg/day for 3 days. IVM-3x300=ivermectin 300 mcg/kg/day for 3 days. QTcF=electrocardiogram QT interval, corrected for heart rate using Fredericia's formula.

* 18 subjects did not display a concentration-effect relationship for piperaquine and QT interval and their EC_{50} was estimated at the upper limit of the prediction, 1200 ng/mL. Upper limit for maximum possible effect ($E_{max}+E_{min}$) was set to be 520 ms which is 10 ms higher than the highest QT interval observed amongst all the patients.