Multiple hosts, multiple impacts: the role of vertebrate host diversity in shaping mosquito life history and pathogen transmission

Amélie Vantaux[1,2*], Nicolas Moiroux [2,3], Kounbobr Roch Dabiré[1,3], Anna Cohuet[2,3], Thierry Lefèvre[1,2,3]

[1] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso
[2] MIVEGEC, Université de Montpellier, IRD, CNRS, Montpellier, France
[3] Laboratoire Mixte International sur les Vecteurs (LAMIVECT), Bobo-Dioulasso, Burkina Faso

*Corresponding author
Correspondence: amelie.vantaux@gmail.com

ABSTRACT
The transmission of malaria parasites from mosquito to human is largely determined by the dietary specialization of *Anopheles* mosquitoes to feed on humans. Few studies have explored the impact of blood meal sources on the fitness of both the parasite and the mosquito. Our study investigated the effects of 3-4 consecutive blood meals from one of four vertebrate species (human, cattle, sheep, or chicken) on several fitness traits, including mosquito feeding rate, blood meal size, susceptibility to wild isolates of *Plasmodium falciparum*, survival, fecundity, F1 offspring development time, and size. Our findings revealed no significant effect on parasite development. Similarly, parasite exposure had no overall effects on mosquito fitness. However, blood meal type did have a strong impact on mosquito feeding rate, survival, lifetime fecundity, and offspring size. Specifically, mosquitoes that were fed successive chicken blood meals produced fewer eggs and fewer and smaller F1 adults compared to those fed human blood. Combining our results in a theoretical model, we show a decrease in the vectorial capacity of mosquitoes fed chicken or cow blood and an increase in the capacity of those fed sheep blood compared to those fed human blood. These findings emphasize the importance of considering the diversity of blood meal sources in understanding mosquito ecology and their role in the transmission intensity of malaria parasites.

Keywords: *Plasmodium*, transmission, fitness, competence, vectorial capacity

Introduction

The diet of female *Anopheles* mosquitoes, including those species capable of transmitting human malaria parasites, is characterized by the ingestion of vertebrate blood during each ovarian cycle to sustain vitellogenesis and egg production, while plant carbohydrates are mainly used for energy and maintenance reserves (Clements 1992). With a rather short gonotrophic cycle, which can be as fast as 48h between two egg-lays, mosquito females are recurrently looking for a blood meal during which they can transmit malaria

parasites. The successful transmission of the malaria parasite is highly dependent of the diet specialization of the *Anopheles* vector and, in particular, its degree of anthropophagy (propensity to feed on human). Female mosquitoes must bite a human host twice to potentially transmit malaria parasites. Therefore, the higher the human feeding rate, the greater the transmission potential (Smith and McKenzie 2004) . Furthermore, other key parameters of pathogen transmission such as mosquito longevity (Smith and McKenzie 2004) can be influenced by blood meal source (Lyimo *et al.* 2012, Lyimo *et al.* 2013). Consequently, dietary specialization on human could be associated with fitness benefits for mosquito females which could increase parasite transmission rates.

Comparison of host use among 111 *Anopheline* mosquito populations drawn from 52 species showed that 82% of the populations exhibits some level of dietary specialism ('≥ 50% bloodmeals taken from one host type'; Lyimo and Ferguson 2009). Dietary specialization assumes a trade-off between exploitation of different diets which results in fitness benefits when a specialist feeds on specialized resource and costs when exploiting sub-optimal resources. On the contrary, generalism would be expected when the chances of optimal host encounter are low and the costs of waiting are high (Lyimo and Ferguson 2009) and only small differences between resources would be observed with no optimal use of one type of diet. For example, *Anopheles arabiensis* is rather an opportunistic vector displaying either anthropophilic or zoophilic preferences depending on the geographic area and the relative abundance of humans and cattle (Costantini *et al.* 1999, Takken and Verhulst 2013). On the other hand, in *Anopheles coluzzii*, considered as strongly anthropophagic, environmental changes such as the widespread usage of bed nets can induce mosquitoes to feed on more accessible although less preferred host species (Lefèvre *et al.* 2009). Only a handful of studies have investigated the fitness of anopheline mosquitoes fed on different vertebrate blood (Lyimo *et al.* 2012, Lyimo *et al.* 2012, Lyimo *et al.* 2013, Phasomkusolsil *et al.* 2013, Emami *et al.* 2017). While all studies observed some effects of host type on mosquito fitness traits, the exploitation of less preferred hosts did not seem to strongly impact mosquito fitness so that the predicted relationship between host-preference and fitness benefits was not always confirmed (Lyimo *et al.* 2013) or could be offset by a second blood meal even on a different non –preferred host (Lyimo *et al.* 2012).

Blood meal type is also likely to directly impact parasite fitness since parasite growth is fueled by host resources (Shaw *et al.* 2022). For example, xanthurenic acid, a gametocytogenesis activation factor is synthesized by the mosquito host (Billker *et al.* 1998), and essential amino acids such as valine, histidine and methionine and leucine are incorporated by parasite oocysts (Beier 1998). Similarly, host lipids are taken up by malaria parasites, probably to sustain its membrane biogenesis (Atella *et al.* 2009) while these lipids are also central to mosquito immune defenses and reproduction (Briegel *et al.* 2002, Atella *et al.* 2006, Cheon *et al.* 2006, Rono *et al.* 2010). As vertebrate host species vary in these haematological characteristics (Wintrobe 1933, De Smet 1978, Hawkey *et al.* 1991), they are likely important drivers of both mosquito and parasite fitness. It has also been shown that the provision of a second blood meal to infected females can increase the rates and amount of sporozoites (the mosquito to human infective stage) in salivary glands (Ponnudurai *et al.* 1989, Emami *et al.* 2017, Pathak *et al.* 2022), accelerate parasite growth and shorten the extrinsic incubation period (Brackney *et al.* 2021, Habtewold *et al.* 2021, Kwon *et al.* 2021, Shaw *et al.* 2021, Pathak *et al.* 2022), thereby enhancing the transmission potential of malaria-infected mosquitoes. Thus, the nutritive quality of the mosquito blood meals following malaria parasite invasion might affect parasite fitness, competition for resources between the parasite and its mosquito host as well as mosquito fitness and ability to cope with infection (Shaw *et al.* 2022).

To our knowledge, only two studies have investigated the effects of blood meal sources taken from different vertebrate host species on mosquito competence for malaria parasites by providing a second blood meal 4 or 8 days post-infectious blood meal, using laboratory colonies of mosquitoes, and cultured clones of parasites (Emami *et al.* 2017, Pathak *et al.* 2022). Both studies revealed that the development of the malaria parasite can be influenced by the source of blood consumed following the infection.

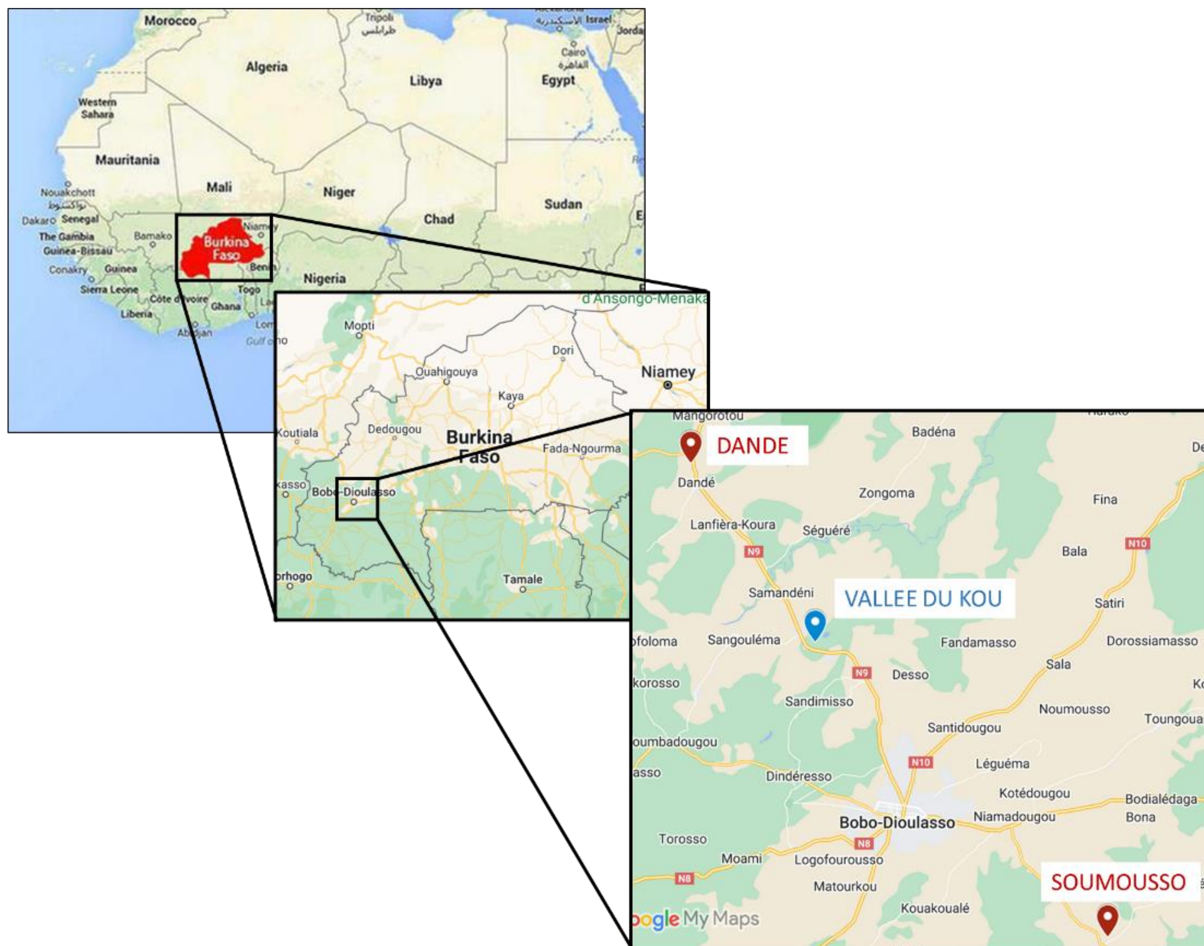
While *Anopheles coluzzii* is generally considered highly antropophilic, it can also feed on a wide range of other vertebrate hosts (Lemasson *et al.* 1997, Sousa *et al.* 2001, Caputo *et al.* 2008, Lefèvre *et al.* 2009). The current study investigated the effect of blood meals from four different vertebrate hosts on malaria parasite development and mosquito vector life history traits using field isolates of the parasite *P. falciparum* and a natural population of the mosquito *An. coluzzii* (previously *An. gambiae* M molecular form, (Coetzee *et al.* 2013). Previous studies on females fed on different blood meal sources were carried out after one or two blood meals (Lyimo *et al.* 2012, Lyimo *et al.* 2012, Lyimo *et al.* 2013, Phasomkusolsil

et al. 2013, Emami *et al.* 2017). In nature, females can be exposed to a wide range of host species and seek a blood meal every 2 to 4 days. Therefore we here used four vertebrate species, provided multiple blood meals (3 to 4) and measured multiple fitness-related traits (feeding rate, blood meal size, competence to parasites, survival, fecundity, F1 development time and wing length) to obtain a thorough picture of the effect of blood-meal diversity on mosquito and parasite fitness. Mosquito females were first fed an infectious or a non-infectious blood meal. They then received up to three subsequent blood meals from either human, chicken, cow or sheep. We predicted that blood type would affect parasite development and mosquito traits such as survival and fecundity and hence vectorial capacity and that effects would add up with subsequent blood meals. Our results were combined into a theoretical model to predict the relative contribution of different vertebrate hosts to overall malaria transmission.

Methods

Mosquito colony

Laboratory-reared females of *An. coluzzii* were obtained from an outbred colony established in 2008 and repeatedly replenished with F1 from wild-caught mosquito females collected in Kou Valley (11°23'14"N, 4°24'42"W), 30 km from Bobo Dioulasso, south-western Burkina Faso (West Africa, Fig 1). Females were identified by species diagnostic PCR (Santolamazza *et al.* 2008). Mosquitoes were maintained under standard insectary conditions (27 ± 2°C, 70 ± 5% relative humidity, 12:12 LD). The larvae were reared in spring water under insectary conditions and fed with Tetramin® Baby Fish Food *ad libitum*. Adults were reared in mesh cages (30x30x30cm) and provided with 5% glucose and water on imbibed cottons *ad libitum*. Female mosquitoes were starved for sugar 24h prior access to a blood meal to ensure willingness to feed.

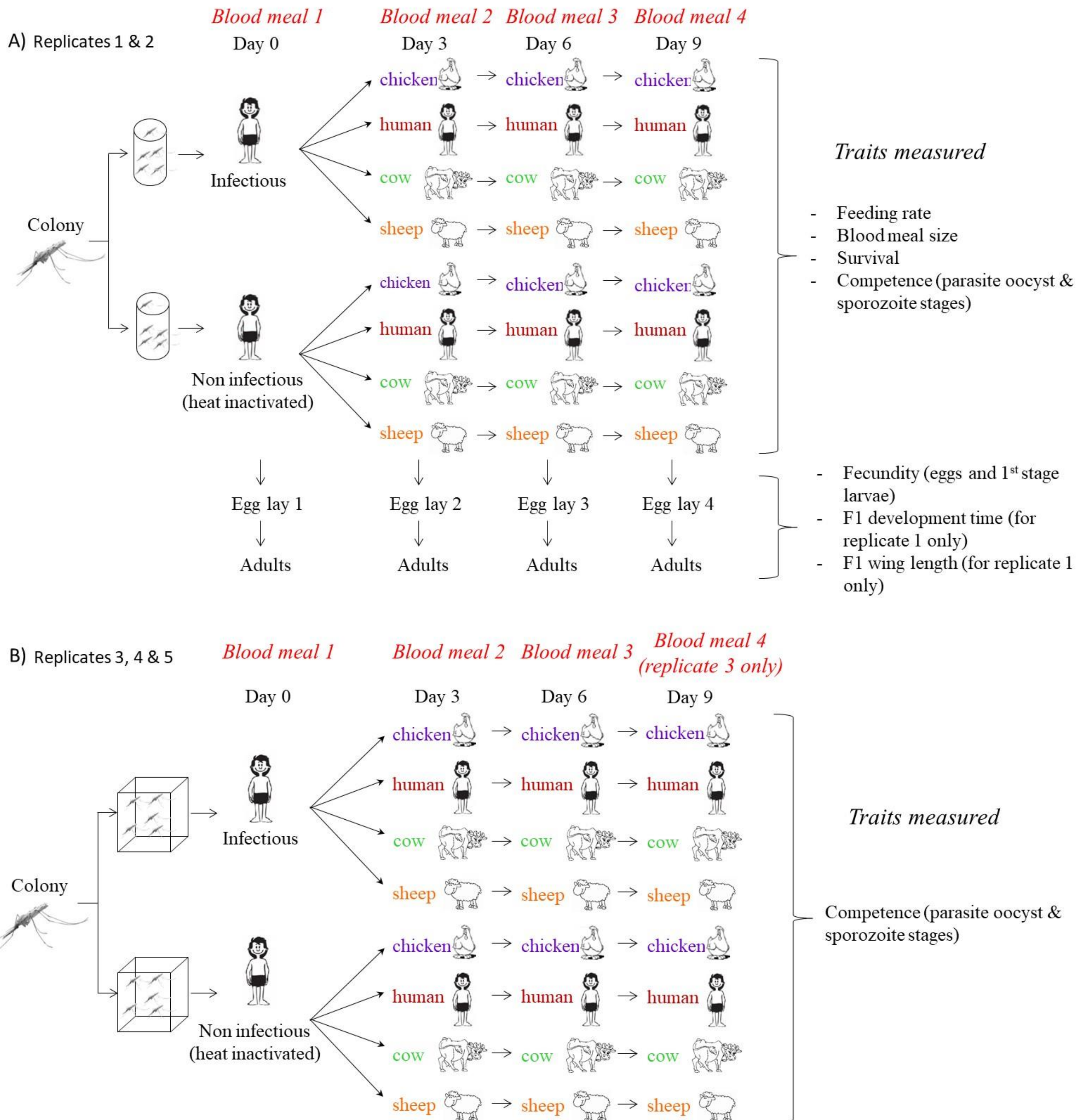


116 Figure 1: Geographic situation of Burkina Faso in West Africa, and of our sites around Bobo Dioulasso where
117 mosquitoes (Vallée du Kou, blue marker) and parasites (Dande and Soumousso, red markers) were collected.

118

119 **Mosquito infection**

120 Experimental infections were carried out as described in (Bousema *et al.* 2012, Ouédraogo *et al.* 2013,
121 Roux *et al.* 2015, Vantaux *et al.* 2015, Vantaux *et al.* 2016). Briefly, 3-to-5-day-old females were fed through
122 membranes on *P. falciparum* gametocyte-infected blood taken from malaria parasite carriers in Burkina
123 Faso. Gametocyte carriers were selected by examining thick blood smears from children aged between 5
124 and 11 from two villages in southwestern Burkina Faso (Dande and Soumousso, located 60km north and
125 40km southeast of Bobo-Dioulasso, respectively, Figure 1). Malaria positive individuals were treated
126 according to national recommendations. Venous blood from gametocyte carriers was collected in
127 heparinized tubes. As a negative control (uninfected mosquitoes), females were fed on the same blood in
128 which gametocytes were heat-inactivated. This heat-inactivation inhibits the infection and does not affect
129 the blood nutritive quality (Sangare *et al.* 2013). This was done to avoid the potential confounding effects
130 of different blood origins on fitness of infected and control mosquitoes (Sangare *et al.* 2013, Alout *et al.*
131 2014, Hien *et al.* 2016, Vantaux *et al.* 2016). Parasite inactivation was performed by placing the blood in a
132 thermo-mixer and heated at 43°C for 15 min and 900 rpm while the remaining blood was maintained at
133 37°C. Three hundred µl of blood were distributed in membrane feeders maintained at 37°C by water
134 jackets. Cups containing 80 mosquitoes were placed under the feeders to allow blood feeding through
135 Parafilm® membranes for 2 hours. Unfed females were discarded and fed females had access to water
136 only. Five experimental replicates using six distinct parasite isolates were performed (Appendix 1-Table
137 S1, Fig 2). Owing to the high malaria endemicity of Burkina Faso and the resulting high probability of
138 multiplicity of infection (Grignard *et al.* 2018, Sondo *et al.* 2020, Barry *et al.* 2021), although not genotyped
139 we are considering the six isolates as six biological replicates likely having different clonal compositions.



140 Figure 2. **Schematic representation of the experimental design and list of traits measured** for the two replicates in
141 cups (A) and the three replicates in cages (B). For design A, after feeding an infectious blood meal females were distributed
142 in 94 cups and females fed a non infectious blood meal were distributed in 27 cups. Afterwards they received successive
143 blood meals on different vertebrate hosts using membrane feeders. This cup design is schematically represented by
144 cylinders. For design B, after feeding an infectious blood meal or a non infectious blood meal, females were distributed in
145 32 cages (4 biological replicates by 4 different vertebrates by 2 status of exposure to infection). Afterwards they received
146 successive blood meals on different vertebrate hosts using membrane feeders. This cage design is schematically
147 represented by cubes.

148

149 Ethical approval was obtained from the Centre Muraz Institutional Ethics Committee under agreement
150 no. A003-2012/CE-CM. The protocol conforms to the declaration of Helsinki on ethical principles for

151 medical research involving human subjects (version 2002) and informed written consent were obtained
152 from all volunteers.

153 **Multiple blood meals on different hosts**

154 In addition to the first infectious/uninfectious feed, mosquitoes received two to three additional blood
155 meals every three days through membranes on venous blood drawn from one of four different vertebrate
156 species ("blood type" hereafter): human, cow, sheep or chicken (Fig 2)). After each blood meal, unfed
157 females were discarded and lost to follow-up. As a result, two different experimental designs were
158 employed: one utilizing cups (Fig 2A), which enabled the tracking of small groups of females and the
159 measurement of several life history traits (see below), and a second design using cages (Fig 2B), which
160 allowed for the monitoring of a larger number of females but without measuring all life-history traits. This
161 second design was solely used for measuring mosquito competence and was analyzed separately (see
162 details below). Mosquitoes were fed on the same vertebrate species for either three successive blood
163 meals resulting in a total of four blood meals (replicates 1 to 3) or two successive blood meals resulting in
164 a total of three blood meals (replicates 4 & 5, Fig 2). Membrane feeders were maintained at a specific
165 temperature corresponding to each vertebrate body temperature: 37°C for human blood, 38.5°C for cow
166 blood, 39°C for sheep blood and 41.5°C for chicken blood. For each blood meal episode, three different
167 vertebrate individuals were used per species. The correspondence between mosquito cups and vertebrate
168 individuals was organized so that mosquitoes fed on different individuals of the same host species at each
169 blood meal episode and a total of 14 human volunteers, 8 sheep, 12 cows and 15 chickens were used.
170 Mosquitoes in cages (Fig 2B) were randomly fed on individuals of the same host species at each blood meal
171 and a total of 14 human volunteers, 7 sheep, 8 cows and 18 chickens were used. Fitness costs are more
172 commonly observed in stressful environmental conditions (Lalubin *et al.* 2014, Sangare *et al.* 2014, Roux
173 *et al.* 2015) and sugar feeding strongly affects mosquito survival and fecundity (Foster 2022). Therefore,
174 we did not provide a sugar solution to the mosquitoes during the whole experiment as it could hide or
175 compensate for the fitness effects of the different blood types.

176 The absence of malaria parasite in human blood donors at feeding episodes 2, 3 and 4 was confirmed
177 by a blood smear prior to blood collection. This study was carried out in strict accordance with the
178 recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of
179 Health. Animals were cared for by trained personnel and veterinarians.

180 **Traits measurements**

181 The effect of blood type on a series of mosquito life-history traits, namely (i) competence for *P.*
182 *falciparum* (oocyst / sporozoite prevalence and intensity), (ii) feeding rate, (iii) blood-meal size, (iv) survival,
183 (v) fecundity, (vi) progeny developmental time, and (vii) progeny body size, was assessed.

184 For replicates 1 and 2, following the first human (infectious or non-infectious) blood meal, a total of
185 1818 fully-fed *An. coluzzii* females were randomly distributed in 121 paper cups (10 cm height X 7.5 cm
186 down diameter X 9.5 cm up diameter) by group of 9 to 22 (median 12) per cup. Cups were divided in four
187 groups, of which the first three groups of cups contained females fed on infectious blood (thus containing
188 both exposed-infected and exposed-uninfected females) and the fourth group females fed on non-
189 infectious blood. The first (32 cups) and second group (32 cups) were used to investigate the effect of blood
190 type on vector competence, respectively at the parasite oocyst stage 8 days post-bloodmeal (dpbm: days
191 post-bloodmeal) and the sporozoite stage 14 dpbm. The third (30 cups) and fourth group (27 cups) were
192 used to investigate the effect of blood type and *P. falciparum* exposure on mosquito survival. All other life-
193 history traits were measured on mosquitoes from all the cups (n = 121 cups). *F1* traits were measured on
194 offspring from replicate 1. Mosquitoes from replicates 3 to 5, maintained in cages, were used to measure
195 competence traits only (Fig 2).

196 *Competence* – Oocyst prevalence (i.e. proportion of females harboring at least one oocyst on their
197 midgut) and intensity (i.e. number of *P. falciparum* oocysts in the midgut of infected females) were
198 determined at 8 dpbm. At this time, females had received two additional bloods meal post-infection.
199 Midguts were dissected in 1% Mercurochrome® stain and the presence and number of oocysts were
200 determined under a microscope (at 20× objective). A total of 119 individuals were used to determine oocyst
201 prevalence derived from 13 cups (4 cups from human and sheep blood, 3 cups from cow blood, 2 cups from
202 chicken blood – between 1 to 5 females per cup) in replicate 1 and 16 cups (4 cups per type of blood –

203 between 2 to 11 females per cup) in replicate 2. A total of 757 individuals were used to determine oocyst
204 prevalence in replicates 3 to 5 (202 females in replicate 3, 215 females in replicate 4 and 340 females in
205 replicate 5). Sporozoite prevalence was determined at 14 dpbm. At this time, females had received three
206 (replicates 1 to 3) or two (replicates 4 and 5) additional blood meals post-infection. For each individual, the
207 abdomen was removed and the head and thorax were stored at -20°C. Prevalence was determined using
208 PCR assays on the crushed head and thorax (Morassin *et al.* 2002). Sporozoite prevalence was determined
209 on 34 individuals derived from 6 cups (2 cups from human, cow and sheep) in replicate 1 and 10 cups in
210 replicate 2 (3 cups from human and cow and 4 cups from sheep). Sporozoite prevalence was assessed on
211 235 individuals in replicates 3 and 5 (175 individuals from replicate 3 and 60 individuals from replicate 5).
212 *Feeding rate*. The feeding rate of females during meals 2 to 4 was calculated as the number of fully-fed
213 females over the total number of females. This trait was assessed on 121 cups for blood meal 2, on 119
214 cups for blood meal 3 and on 71 cups for blood meal 4. Feeding rate was not measured for the first meal
215 on infectious or non-infectious human blood.
216 *Mosquito blood meal size* was estimated following each meal (1 to 4) by measuring the amount of
217 haematin (a by-product of the decomposition of haemoglobin) excreted in each paper cup and averaging
218 it by the number of females in the cup (Briegel 1990). One ml of 1% lithium carbonate solution was
219 distributed in each cup to elute faeces and the absorbance of the resulting solution was read at 387nm,
220 using $LiCO_3$ solution as a blank, and compared with a standard curve made with porcine serum haematin
221 (Sigma-Aldrich). Blood meal size was assessed on 116 cups for the first blood meal, on 119 cups for the
222 second blood meal, on 74 cups for the third blood meal and on 60 cups for the fourth blood meal.
223 Mosquito *survival* was recorded daily at 8:00 by counting and removing the number of dead individuals
224 in the cups. *Survival* was derived from a total of 855 individuals followed from 57 cups.
225 *Fecundity* - At each gonotrophic cycle (n=4), petri dishes containing humid cotton covered with a piece
226 of Whatmann® paper were placed in the cups two days post-bloodmeal. Eggs laid on the Whatmann® paper
227 were recovered the following morning, pictured and placed in a plastic weighing pan with 25 ml of water.
228 Two days later, pictures of the 1st instar larvae were taken. The number of eggs were counted using the
229 Egg Counter software (Mollahosseini *et al.* 2012) and the number of first instar larvae with ImageJ
230 (Abramoff *et al.* 2004). *Fecundity* was estimated using six parameters : (i) the *egg-laying rate* corresponding
231 to the proportion of cups containing at least one egg, (ii) the *average number of eggs* corresponding to the
232 number of eggs counted in a positive cup (i.e. a cup with at least one egg) divided by the number of females
233 in the cup, (iii) the *lifetime fecundity* corresponding to the sum of the average number of eggs of
234 gonotrophic cycles 2, 3 and 4, (iv) *the hatching rate* corresponding to the number of 1st instar larvae divided
235 by the number of eggs placed in water, (v) the *average number of larvae* corresponding to the number of
236 1st instar larvae in a plastic weighing pan divided by the number of females in the cup used to collect the
237 eggs placed in that pan, and (vi) the *lifetime production of larvae* corresponding to the sum of the average
238 number of 1st instar larvae of gonotrophic cycles 2, 3 and 4. These six parameters were assessed on a
239 maximum of 121 cups (egglay 1) and a minimum of 41 cups (egglay 4), accounting for mosquito mortality
240 between egglay 1 and 4.
241 *F1 development time* was assessed by introducing 1st instar larvae (median number of larvae = 9, range
242 = 1-13) randomly selected from each weighing pan in a plastic cup with 50ml of water. Mosquito larvae
243 were provided with Tetramin® Baby Fish Food ad libitum once a day, and excess food was removed to avoid
244 water pollution. Development time was calculated as the duration from egg-lay to emergence and was
245 measured for a total of 1,035 individuals from the four egg-lays of the first replicate (464 individuals from
246 56 cups in egg-lay 1, 303 individuals from 36 cups in egg-lay 2, 142 individuals from 18 cups in egg-lay 3,
247 126 individuals from 15 cups in egg-lay 4).
248 *F1 wing length* was used as a surrogate of body size and was measured from the alula to the wing tip,
249 excluding scales (Van Handel and Day 1989). One wing per F1 individual was dissected on the day following
250 emergence on a subset of individuals. The wing was pictured with a stereomicroscope and measured with
251 ImageJ software (Wayne Rasband, rsb.info.nih.gov/ij/). Wing length was measured on 656 individuals of
252 the first replicate (300 individuals from 55 cups in egg-lay 1, 176 individuals from 34 cups in egg-lay 2, 100
253 individuals from 18 cups in egg-lay 3, 80 individuals from 15 cups in egg-lay 4).

254 **Statistical analyses**
255 *Competence* – Parasite prevalence (oocyst or sporozoite stages) and intensity (oocyst stage only) were
256 analysed using Generalized Linear Mixed Models (GLMMs) with a binomial and a zero-truncated negative
257 binomial error structure respectively. The replicates in cups and the replicates in cages were analyzed
258 separately. In these GLMMs, blood type (four levels: cow, sheep, chicken or human blood), gametocytemia
259 and their interaction (only for replicates 3-5) were coded as fixed factors, and cup and parasite isolate
260 nested in replicate (for replicates 3-5) as random factors.
261 *Feeding rate* was analysed using a GLMM with a binomial error structure. In this model, blood type, *P.*
262 *falciparum* exposure (two levels: mosquito previously fed an infectious blood meal vs fed the same heat-
263 inactivated blood), blood feeding episode (three levels: 2 to 4) and their interactions as well as parasite
264 isolate were coded as fixed factors and cup as a random factor.
265 For the following traits, data from the first gonotrophic cycle (resulting from infectious vs. non-
266 infectious human blood) were analysed separately from data from gonotrophic cycles 2-4 for which
267 mosquitoes were fed on four different types of blood (human, cow, sheep, chicken). Data analyses and
268 results from the first gonotrophic cycle are presented in the supplementary material.
269 *Mosquito blood meal size* – Data from the blood meals 2 to 4 were log-transformed before being
270 analyzed with a GLMM with a Gaussian distribution. In this model, blood type, mosquito exposure, blood-
271 feeding episode and their interactions as well as parasite isolate were coded as fixed factors and cup as a
272 random factor.
273 *Survival* data were analysed using Cox proportional hazard mixed models (coxme package) with
274 exposure to infectious blood, blood type, parasite isolate and their interactions coded as fixed factors and
275 mosquito cup as a random factor. Since unfed females from blood meals 2 to 4 were removed, they were
276 given a censoring status of 0 indicating that the individual was alive when last seen.
277 *Fecundity* – Egg-laying rate, hatching rate, average number of eggs (log-transformed), and average
278 number of 1st instar larvae (log-transformed) over gonotrophic cycles 2-4 were analysed using GLMMs with
279 binomial or Gaussian error structures. Blood type, exposure, isolate, blood meal size and gonotrophic cycle
280 were coded as fixed factors and cup as a random factor. In addition, GLMs with quasipoisson structure (to
281 correct for overdispersion) were used to analyze the effect of blood type, exposure, isolate and their
282 interactions on the lifetime fecundity and lifetime production of larvae corresponding to the sum of the
283 average number of eggs and 1st instar larvae over gonotrophic cycles 2-4.
284 The *development time* of larvae from gonotrophic cycle 2-4 was analyzed using a a Cox proportional
285 hazard mixed effect model with maternal exposure, maternal blood type, gonotrophic cycle, larval density
286 and mosquito sex coded as fixed factors, and rearing cup as a random factor. The effect of blood type on
287 the sex ratio of the progeny was analyzed using a binomial GLMM with blood type coded as a fixed factor
288 and rearing cup as a random factor.
289 *F1 wing length*– A Gaussian GLMM was used to explore the effects of maternal blood type, maternal
290 exposure, egg-lay episode, larval density and mosquito sex on log-transformed wing length of the progeny
291 from gonotrophic cycles 2 to 4.
292 For model selection, we used the stepwise removal of terms, followed by likelihood ratio tests (LRT).
293 Term removals that significantly reduced explanatory power (P<0.05) were retained in the minimal
294 adequate model (Crawley 2007). All analyses were performed in R v. 3.0.3 (R Core Team 2020). Results are
295 presented as mean ± standard error (se) and proportion ± confidence interval (CI).

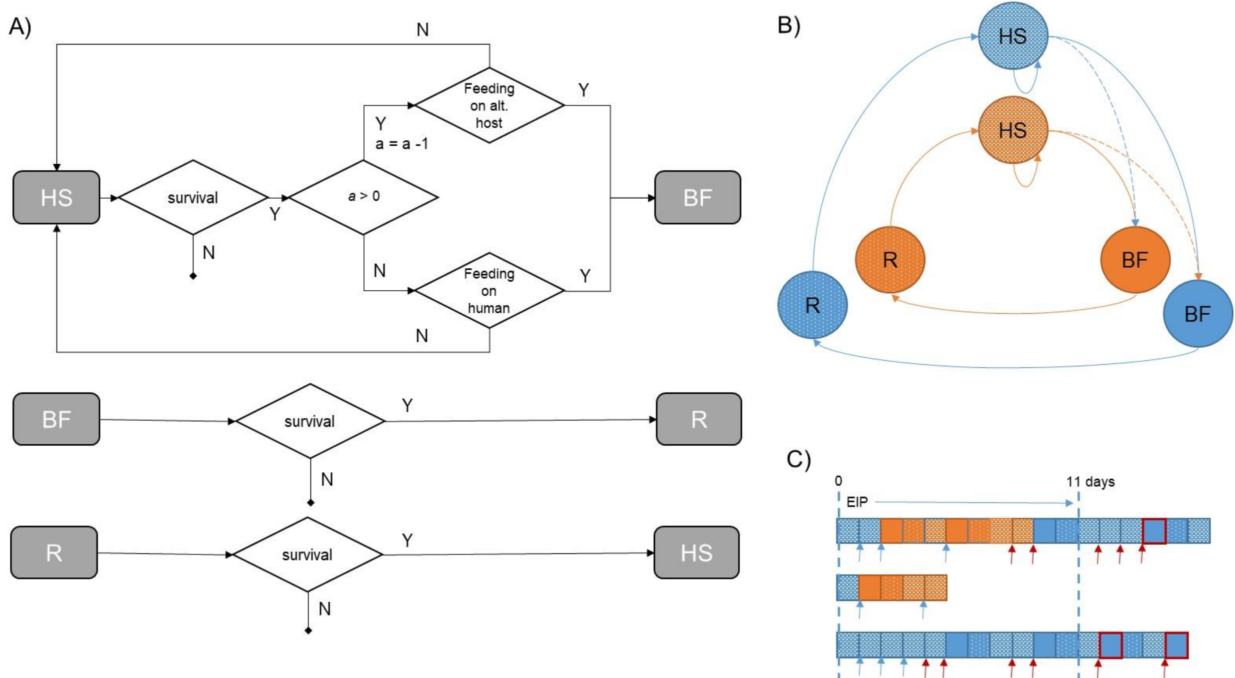
296 **Theoretical modelling**
297 We explored the relative contribution of the blood type on mosquito mean individual vectorial capacity
298 (Saul *et al.* 1990). Individual vectorial capacity (IC) is the mean number of infectious bites given by an
299 infected vector (i.e. the number of bites it gives after the *Plasmodium* extrinsic incubation period is
300 completed). Therefore, IC expresses the efficiency with which individual mosquitoes transmit malaria. To
301 estimate IC, we developed a model that simulates the daily life history of individual mosquito vectors after
302 taking an infectious blood meal on a human under various scenarios (Fig 3). The environment (= scenario)
303 was characterized by the presence of humans and an alternative host (either chicken, cow or sheep) with
304 varying availability (0 to 3 consecutive possible feeding attempts during *Plasmodium* incubation period).
305 There was therefore 12 scenarios tested (3 alt. host x 4 availability levels). 250 000 individuals (representing
306 500 populations of 500 individuals) were simulated per scenario. The model allowed to track daily

physiological states (either Host-Seeking, HS; Blood-Fed, BF; or Resting, R) of individuals. Daily transitions from one state to another depended on survival probability (related to the origin of the previous blood meal) and blood-feeding success probability (related to the host that the mosquito is attempting to bite: human, chicken, cow or sheep; only for transition from HS to BF). A binomial GLMM of feeding success and a COXPH model of survival were fitted to the data presented in the manuscript and used to calculate host-specific probabilities of feeding success and daily survival. For each individual simulation, the number of days spent in state BF (= number of successful feeding attempts) following the duration of *Plasmodium* extrinsic incubation period (n = 11 days) was counted and the mean (= IC) was calculated for each population. The model was implemented in R with the use of the tydiverse, furr, glmmTMB, coxme and emmeans packages (Brooks *et al.* 2017, Wickham *et al.* 2019, Therneau 2020, Vaughan and Dancho 2021, Lenth 2022). The detailed description of the model following the ODD (overview, design concepts and details) protocol for describing individual- and agent-based models (Grimm *et al.* 2010) is as follow:

Purpose: The purpose of the model is to explore the effect on the *Anopheles* mean individual vectorial capacity (1) of various number of feeding attempts on alternative animal hosts during the *Plasmodium* extrinsic incubation period.

Entities, state variables and scales: The entity of the model is a female *Anopheles coluzzi.* having taken an infectious blood meal on a human. The female *Anopheles* is characterized by its physiological state (Host-seeking, HS; Blood fed, BF; or Resting, R), the source of its last blood meal (human or animal) and the remaining number of feeding attempts to be done on the alternative host. The environment is characterized by the presence of humans and an alternative host (either chicken, cow or sheep) with varied availability (x consecutive possible feeding attempts). One time step of the model corresponds to one day. Simulation is run for 40 days or until the female dies. Every simulation (individual *Anopheles*) is independent (i.e. no interaction).

Process overview and scheduling: Every time step, the physiological state of the female mosquito is updated according to its state at the previous time step, survival probability (depending on the origin of the previous blood meal) and blood-feeding success probability (depending on the host that the mosquitoes is attempting to bite: human, chicken, cow or sheep; only for transition from HS to BF). An HS female, if survives, attempts to feed and becomes BF (if it successes to feed) or stay HS (if it fails to feed) at next time step. A BF female, if survives, becomes R. An R female, if survives, becomes HS. Firsts feeding attempts are made on the alternative animal host (the total number of possible attempts is defined by the scenario: 0, 1, 2 or 3). Then, all attempts are made on humans. The sequence of successive daily physiological states is stored.



341 Figure 3: **Schematic representations of the model and its outputs.** (A) Daily transitions between physiological states
342 (HS: Host-Seeking; BF: Blood-Fed; R: Resting) and the process which cause these transitions (Y: Yes; N: No; alt: Alternative;
343 *a*: remaining number of feeding attempts to be done on the alternative host). (B) Diagram showing possible successions
344 between physiological states together with the origin of blood-meal (blue: human blood, orange: alternative host). At the
345 start of a simulation, the mosquito is always HS with a previous blood meal taken on human (blue). If $a > 0$, it attempts to
346 feed on an alternative host. If successful (blue doted arrow), it enters the orange cycle where daily survival probabilities
347 depends on the type of alternative host bitten, until $a = 0$. Then, the mosquito attempts to feed on human and if it
348 succeeds (orange doted arrow), it enters the blue cycle, with corresponding daily survival probabilities, until death. (C)
349 Three possible sequences of physiological states for single mosquitoes under a scenario of up to 3 possible feeding
350 attempts on an alternative host, until death. Each box corresponds to one day. Colors and texture of boxes relates to panel
351 B. Blue and red arrows indicate feeding attempts on alt. host and human, respectively. Boxes with red borders indicate
352 days in state BF following EIP (External Incubation Period) of the malaria parasite, that are counted to compute Individual
353 Vectorial Capacity (IC).

354

355 *Design concept*: Mosquito-to-human transmission may occur during each bite after the pathogen
356 incubation period is completed. Transmission is therefore dependent on both the longevity of the
357 mosquito and the duration of its gonotrophic cycle (i.e. the mean time between two consecutive bites).
358 Longevity vary according to the daily survival probability, duration of the gonotrophic cycle vary according
359 to feeding success probability (as feeding is delayed in case of failure).
360 *Stochasticity* occurs for daily transition between physiological states since feeding success and survival
361 are Bernoulli trials with host-specific probabilities of success calculated from (i) a binomial GLMM of
362 feeding success and (ii) a Cox proportional hazard model of survival (see sub-model section below),
363 respectively.
364 *Observation*: For each individual simulation, the number of days spent in state BF (= number of
365 successful feeding attempts) following the duration of *Plasmodium* extrinsic incubation period (n = 11 days)
366 is counted and population mean is calculated. Populations are made of 500 independent individuals.
367 *Initialization*: Initially, a female vector is in physiological state "Blood-Fed" with previous blood meal
368 taken on human (the infectious blood meal).
369 *Sub-models*: Probabilities used in the Bernoulli trial of feeding success were provided by a binomial
370 mixed effect model of feeding success fitted on data from the current study. Feeding success was modelled
371 according to blood meal source and with blood meal episodes and cup of origin (of the mosquito batch) as
372 crossed random effect, using the *glmmTMB* function. Feeding success probabilities according to blood
373 meal source were extracted using the *emmeans* function and are shown in Table 1. Probabilities used in
374 the Bernoulli trial of survival were derived from the result of a Cox Proportional Hazard mixed effect model
375 of survival fitted on data from the current study. Death events were modelled according to blood meal
376 source and replicates with cups of origin (of the mosquito batch) as random effect, using the *coxme*
377 function. Hazard ratios (relative to human blood source) were extracted using the *emmeans* function (Table
378 1). In the simulations, we set daily survival probability with a previous blood meal taken on human to be
379 0.8. Survival probabilities for mosquitoes having taken their previous blood meal on animals was therefore
380 0.8 exponentiated by the corresponding Hazard ratio (Table 1).

381 Table 1: Hazard ratio, corresponding daily survival probabilities, and feeding success probabilities used in the individual
382 simulations.

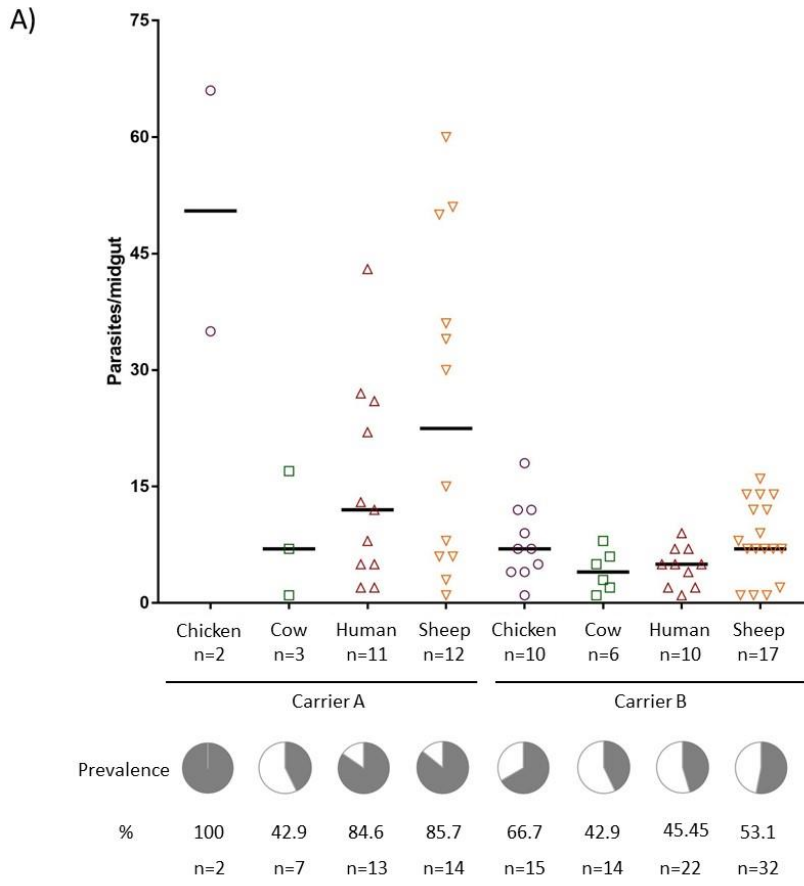
Host	Hazard Ratio	Daily survival probability	Feeding success probability
Human	1 (ref)	0.8	0.753
Chicken	3.254	0.484	0.796
Cow	1.392	0.733	0.717
Sheep	0.833	0.83	0.774

383

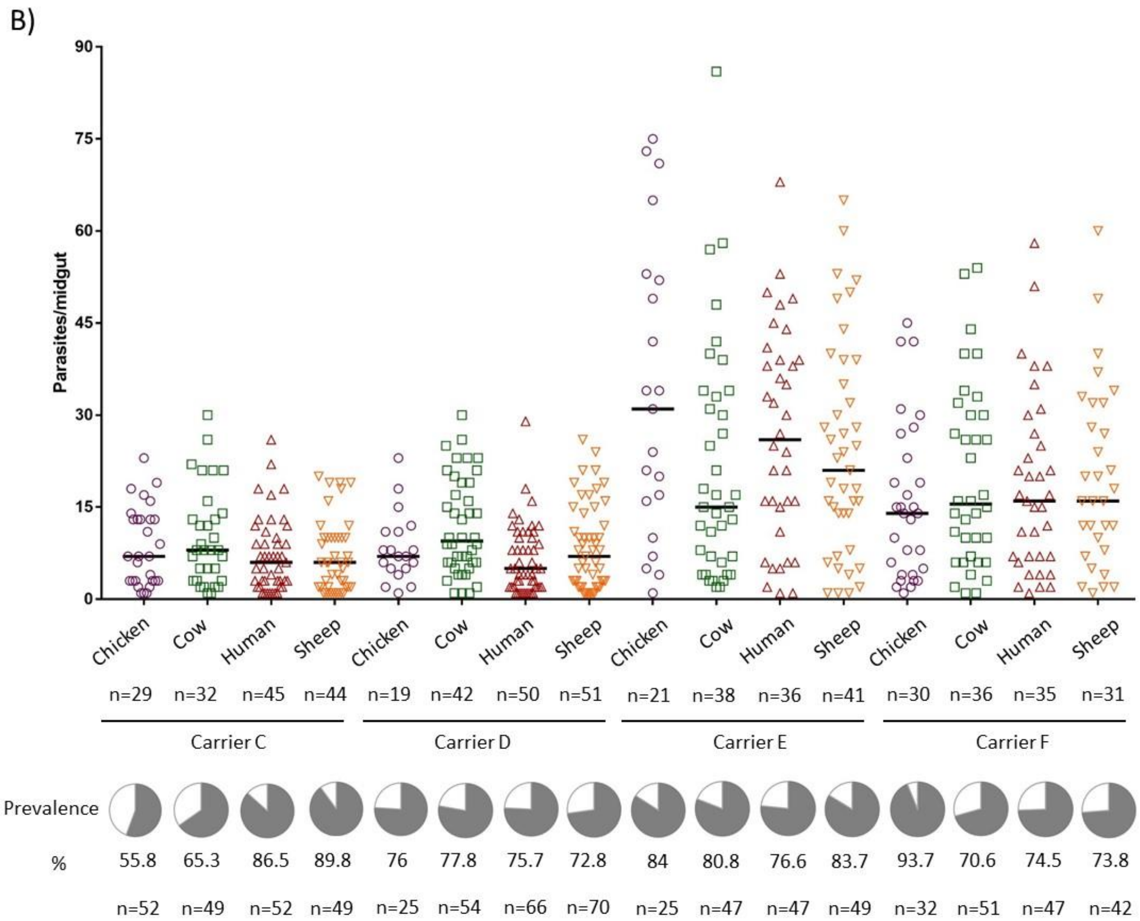
384 Results

385 *Competence* – In replicates 1 and 2, among the 119 females dissected eight days post-infectious blood
386 meal, 71 (59.7 ± 9 %) harboured parasites. Parasite prevalence, intensity and gametocytemias for each
387 parasite isolate are given in the supplementary material (Appendix 1-Table S1). Although blood type did

388 not significantly influence oocyst prevalence (X^2_3 = 5.02, P = 0.17; Fig 4A), oocyst intensity varied among
389 blood type (X^2_3 = 10.55, P = 0.01; Fig 4A). However, none of the multiple post-hoc comparisons were
390 significant. As expected, the parasite isolate with the highest gametocytemia (A: 232 gametocyte/µl)
391 caused higher parasite prevalence and intensity than that with the lowest gametocytemia (32
392 gametocyte/µl) (prevalence: 77.8 ± 14% and 51.8 ± 11%, X^2_1 = 6.92, P = 0.009; intensity: 21.1 ± 3.6 oocysts
393 and 6.7 ± 0.7 oocysts; X^2_1 = 24.89, P < 0.0001). In replicates 1 and 2, prevalence at the sporozoite stage was
394 determined in individuals fed on cow, human and sheep only since all the females fed on chicken blood
395 were dead by 14 dpi. Blood type and parasite isolate did not influence sporozoite prevalence (X^2_2 = 0.62,
396 P = 0.73 and X^2_1 = 2.09, P = 0.15, respectively). The prevalence at the oocyst and sporozoite stages were
397 similar for both isolate A (77.8 ± 13% *vs*. 90 ± 19%, respectively; Fisher's exact test: n=46, P = 0.66) and B
398 (51.8 ± 11% *vs*. 66.7 ± 19%, respectively; n=107, Chi square test: X^2_1 = 1.12, P = 0.29).



399



400

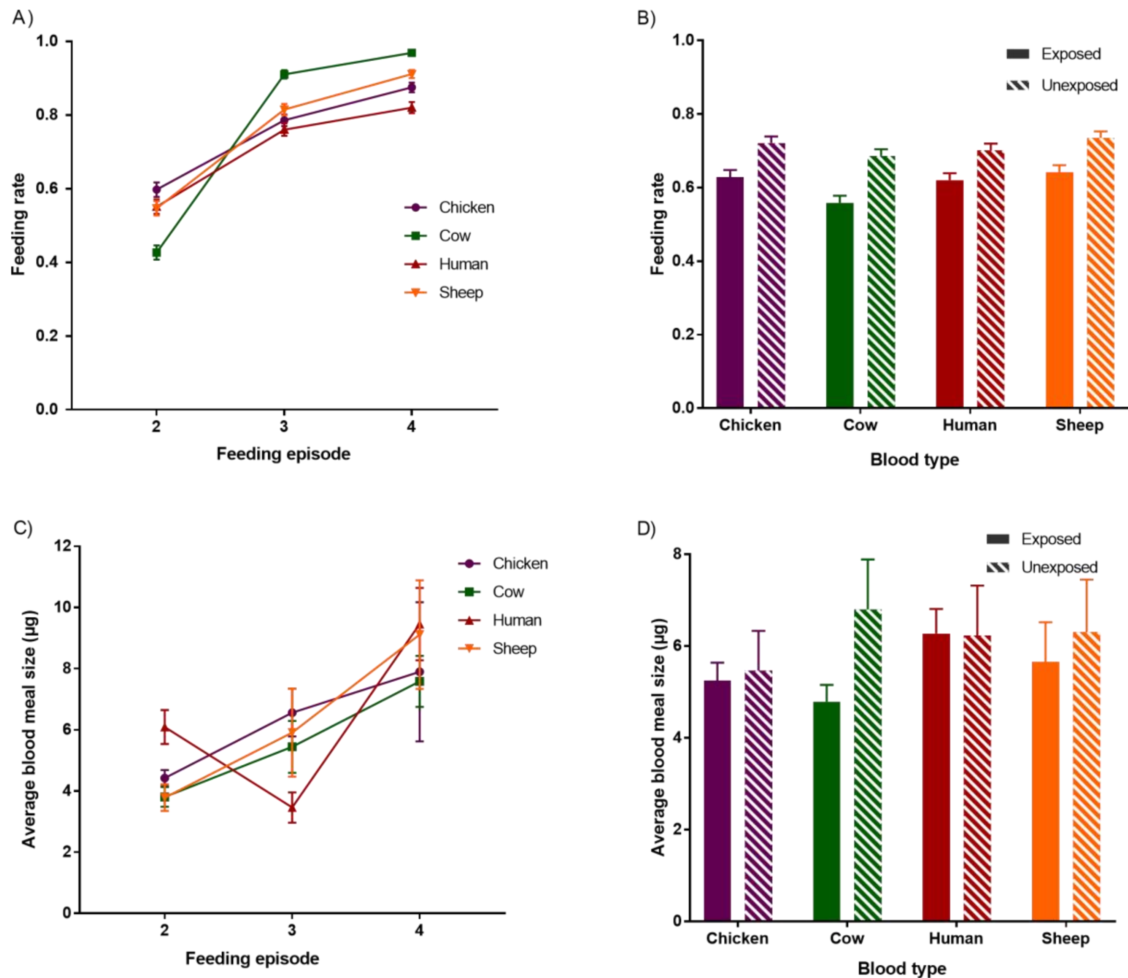
401

402 Figure 4. **Effects of blood type on parasite (oocyst stage) prevalence and intensity for each parasite isolate** in (A)
403 replicates 1 (isolate A) & 2 (isolate B) and in (B) replicates 3 (isolate C), 4 (isolate D) & 5 (isolates E and F). Horizontal bars
404 show the median values. Each colored point represents a *P. falciparum*-infected mosquito individual. Pies show the
405 infection prevalence (grey area). Numbers indicate the sample size (n = total number of mosquito females for parasite
406 prevalence or number of infected females for parasite counting) for each treatment and isolate.

407

408 In replicates 3 to 5, among the 757 females dissected eight days post-infectious blood meal, 580 (76.6
409 ± 3%) harboured oocysts (Appendix 1-Table S1). Neither blood type (X^2_3 = 3.14, P = 0.37) nor gametocytemia
410 (X^2_1= 1.37, P = 0.24), nor their interaction (X^2_3= 1.27, P = 0.74) affected oocyst prevalence (Fig 4B). Parasite
411 intensity did not significantly vary among blood type (X^2_3= 4.99, P = 0.17; Fig 4B). Gametocytemia was
412 positively correlated to parasite intensity (X^2_1=11.09, P < 0.001) and there was a significant blood type by
413 gametocytemia interaction (X^2_3= 11.06, P = 0.01). Parasite prevalence at the sporozoite stage was not
414 significantly affected by the blood type (X^2_2 = 0.65, P = 0.72), nor by gametocytemia (X^2_1 = 1.3, P = 0.25) nor
415 by their interaction (X^2_2 = 0.33, P = 0.85). Parasite prevalence at the oocyst and sporozoite stages were
416 similar for isolate C (74.3 ± 6% *vs*. 81.7 ± 6% respectively; n=377, Chi square test: X^2_1 = 2.6, P =0.11), E (80.9
417 ± 6 % *vs*. 71.4 ±14 %, respectively; n=210, Chi square test: X^2_1 = 1.31, P =0.25), and F (76.7 ± 6% *vs*. 66.7
418 ±22 %, respectively; Fisher's exact test: n=190, P = 0.39).
419 *Feeding rate* – Blood type significantly affected mosquito feeding rate (X^2_3 = 14.4, P = 0.002) with
420 highest overall feeding on sheep blood (66.32±1.8%) followed by chicken blood (64.6±1.9%), human blood
421 (64.1±1.9%) and cow blood (58.8±1.9%). There was a significant interaction between blood type and
422 feeding episode (X^2_6 = 37.8, P< 0.0001; Fig 5A), with cow blood providing lowest feeding rate during the
423 second blood-meal and highest rate during blood-meals three and four. Mosquitoes that received an
424 infectious blood meal displayed lower feeding rate than mosquitoes previously fed an uninfectious blood-
425 meal (61.4 ± 1.9% *vs*. 71.1 ± 1.8%; X^2_1 = 9.09, P = 0.003, Fig 5B), regardless of the blood type (exposure
426 :blood type: X^2_3 = 0.38, P=0.94, Fig 5B) and of the feeding episode (exposure:feeding episode: X^2_2 = 0.03,
427 P=0.99). Feeding rate consistently increased over the successive feeding episodes (53.1 ± 2, 81.2 ± 1.5, and
428 89.1 ± 1.2% at the 2nd, 3rd and 4th episode, respectively; X^2_2 = 160.2, P< 0.0001, Fig 5A). No other effects
429 were found (Appendix 2-Table S2).

430



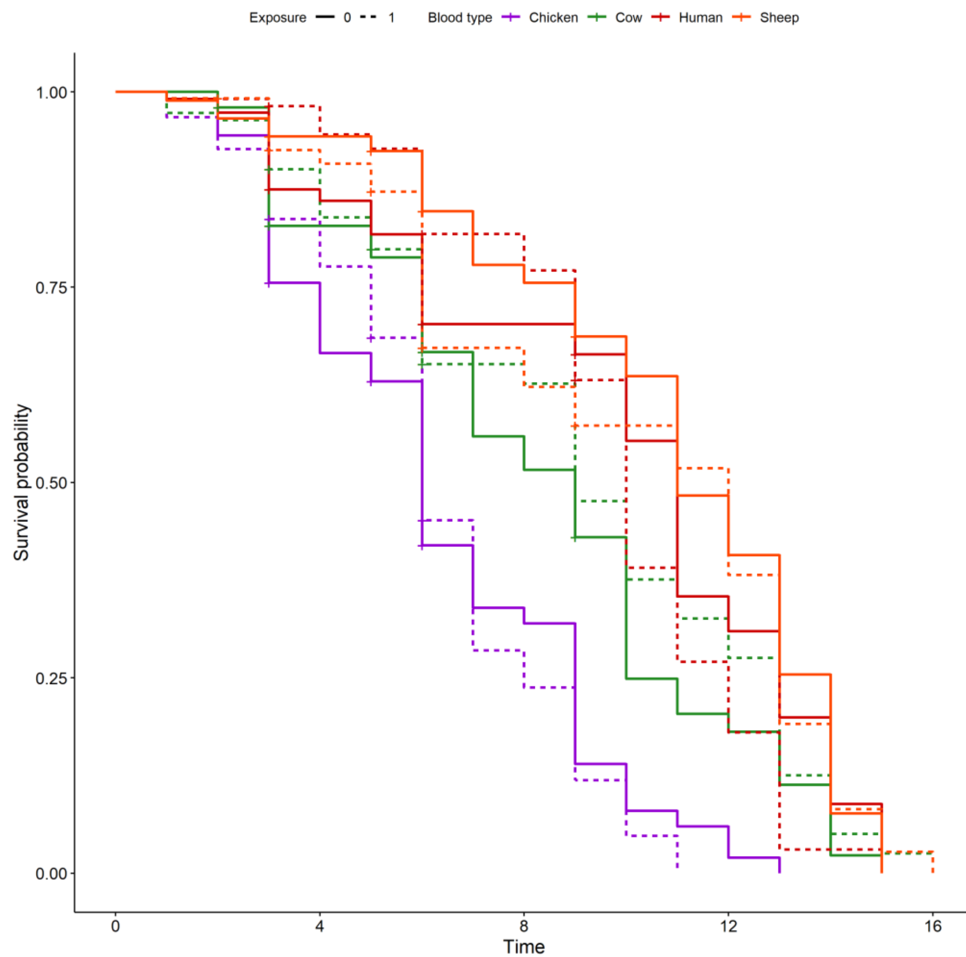
431

432 Figure 5. **Effects of blood type on mosquito feeding rate and blood meal size**. (A) Feeding rate (number of fed
433 females/number of alive females) ± 95% CI as a function of blood feeding episode and blood meal type. B) Feeding rate as
434 a function of blood meal type and infection group (females exposed vs. females unexposed to an infectious blood meal on
435 feeding episode 1). Bars show the average feeding rate across feeding episodes 2 to 4 + 95% CI. C) Average blood meal size
436 ± se as a function of blood feeding episodes and blood meal type. D) Average blood meal size + se as a function of blood
437 type and infection group (females exposed vs. females unexposed to an infectious blood meal). Bars show the average
438 meal size across feeding episodes 2 to 4.

439

440 *Mosquito blood meal size* – Blood type did not significantly affect meal size ($X^2_3 = 4.2$, P = 0.24, Fig 5C).
441 Meal size varied among feeding episodes ($X^2_2 = 54.04$, P < 0.0001) with biggest size observed for the fourth
442 bloodmeal. There also was a significant blood type by feeding episode interaction ($X^2_6 = 23.7$, P = 0.0006;
443 Fig. 5C) such that blood type providing highest or lowest meal size were not always the same across feeding
444 episodes. Meal size was not influenced by the previous exposure of mosquitoes to parasites ($X^2_1 = 0.18$, P
445 = 0.67) regardless of the blood type (exposure: blood type: $X^2_3 = 4.7$, P = 0.19; Fig 5D) or the feeding episode
446 (exposure: feeding episode: $X^2_2 = 0.8$, P = 0.67). No other effects were found (Appendix 2-Table S2).
447 *Survival* – Mosquito survival over the duration of the experiment was strongly influenced by the blood
448 type ($X^2_3 = 68.26$, P < 0.0001; Fig 6), with lowest survival observed when females were successively fed with
449 chicken blood (median survival time: chicken: 6 days, cow: 9 days, human: 10 days and sheep 11 days).
450 Females fed on isolate B during the first feeding episode survived significantly longer than females fed on
451 isolate A (10 and 6 days respectively; $X^2_1 = 24.38$, P < 0.0001, Appendix 3-Fig S1). Mosquito exposure to *P.*
452 *falciparum* gametocytes did not significantly influence mosquito survival (9 days for both unexposed and
453 exposed mosquitoes, $X^2_1 = 0.03$, P =0.87) regardless of the blood type (exposure: blood type: $X^2_3 = 2.38$,

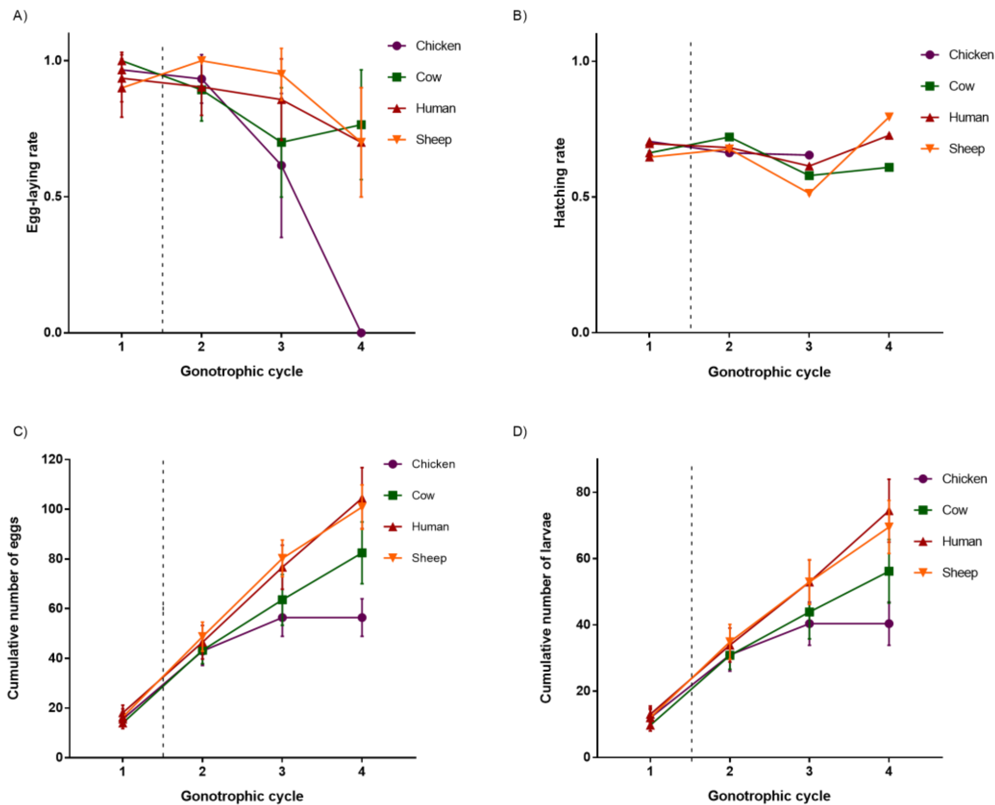
454 P=0.50; Fig 6) or the isolate (exposure: isolate: X^2_1 =0.27, P =0.96). No other effects were found (Appendix
455 2-Table S2).
456



457
458 Figure 6. Effects of blood type (colored lines) and infection group (dashed lines: females exposed vs. solid lines:
459 females unexposed to an infectious blood meal on feeding episode 1) on mosquito survivorship.

460

461 *Fecundity* – Blood type had no effect on egg-laying rate (X^2_3 = 5.6, P = 0.13; Fig 7A), the average number
462 of eggs per female (X^2_3 = 0.85, P = 0.84; Appendix 3- Fig S2A), the hatching rate (X^2_3 = 5.26, P = 0.15; Fig 7B),
463 nor on the average number of 1st instar larvae (X^2_3 = 1.4, P = 0.70; Appendix 3-Fig S2B).
464



465

466 Figure 7. **Effects of blood type on mosquito fecundity and fertility**. (A) Egg-laying rate (number of cups with eggs/total
467 number of cups) ± 95% CI as a function of gonotrophic cycles and blood type. B) Hatching rate (number of larvae/number
468 of eggs) ± 95% CI as a function of gonotrophic cycles and blood type. C) Cumulative average number of eggs ± se as a
469 function of gonotrophic cycle and blood type. D) Cumulative average number of larvae ± se as a function of gonotrophic
470 cycles and blood type.

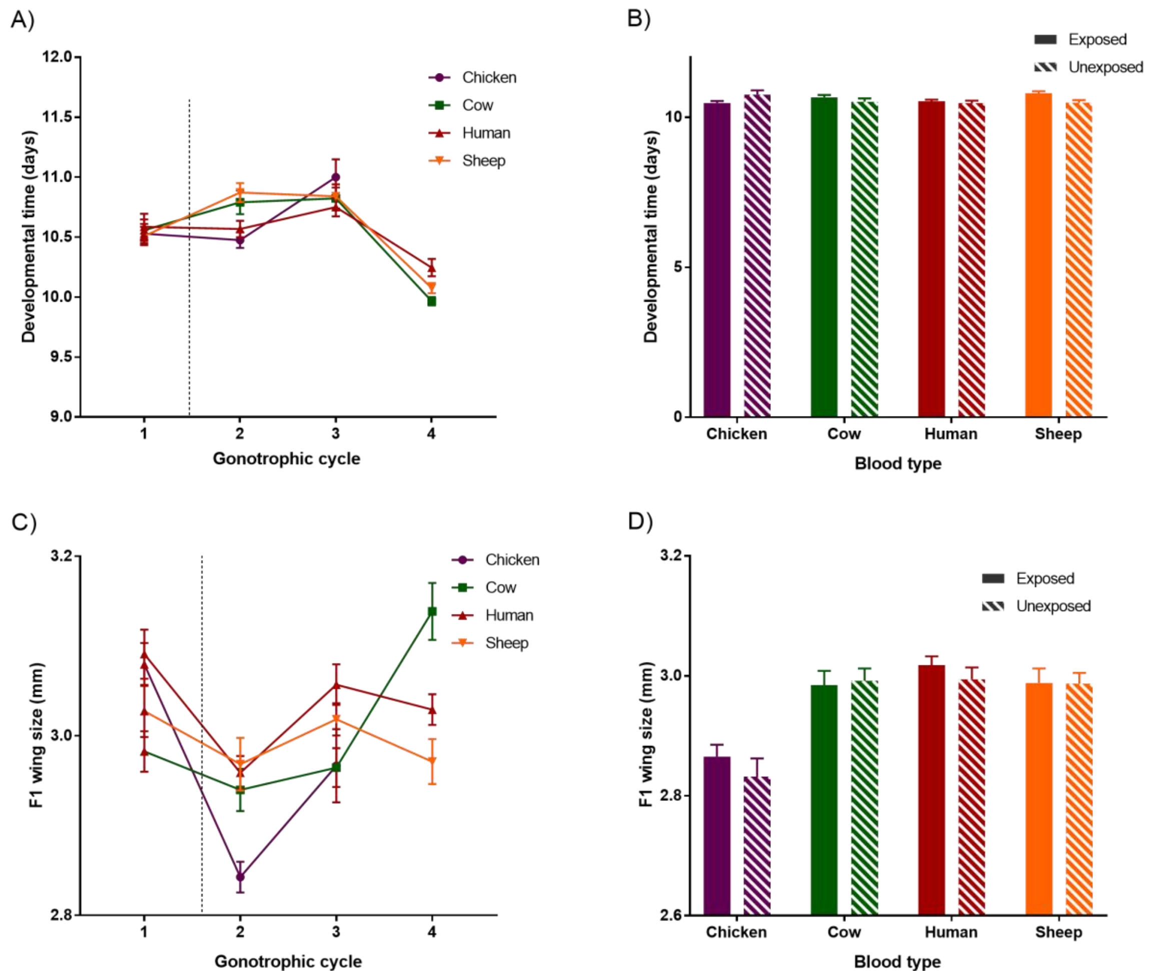
471

472 Importantly, blood type significantly influenced mosquito lifetime fecundity i.e. the cumulative
473 average number of eggs at the 4th gonotrophic cycle ($F_{1,88}$=4.79, P=0.004; Fig 7C as well as the lifetime
474 production of larvae i.e. the cumulative average number of larvae at the 4th gonotrophic cycle ($F_{1,88}$=3.86,
475 P=0.012; Fig 7D), with females successively fed on chicken having a lower lifetime fecundity and lifetime
476 production of larvae than females fed on human blood (z=3.29, P=0.005 and z=3.03, P=0.01) or on sheep
477 blood (z=3.17, P=0.008 and z=2.72, P=0.03). All other post-hoc comparisons were not significant. The effect
478 of blood type on lifetime fecundity and on lifetime production of larvae was independent of the isolate
479 used during the first feeding episode (blood type: parasite isolate: $F_{1,76}$=0.58, P=0.63 and $F_{1,80}$=0.31, P=0.82)
480 and of exposure (blood type: exposure: $F_{1,80}$=0.67, P=0.57 and $F_{1,80}$=0.86, P=0.46).

481 Parasite exposure did not influence egg-laying rate (X^2_1 = 0.08, P = 0.78), the average number of eggs
482 (X^2_1 = 0.0005, P = 0.98; Appendix 3-Fig S2C), the lifetime fecundity ($F_{1,84}$=0.005, P=0.94), the hatching rate
483 (X^2_1 = 2.06, P = 0.15), the average number of 1st instar larvae (X^2_1 = 0.97, P = 0.32, Appendix 3-Fig S2D) nor
484 the lifetime production of larvae ($F_{1,84}$=0.07, P=0.79).

485 The successive gonotrophic cycles influenced the egg-laying rate (X^2_2 = 53.6, P < 0.0001; Fig 7A) with
486 93.3± 4% of egg-positive cups following egg-lay 2, 79.7±9% following egg-lay 3 and 68.3±12% following egg-
487 lay 4. This reduction was likely associated to mosquito mortality causing decreased mosquito number in
488 the cups overtime. Indeed, over the four gonotrophic cycles the probability of laying eggs was positively
489 associated to the survival rate (X^2_1 = 32.9, P < 0.0001; Appendix 4-Supplementary methods and results;
490 Appendix 3-Fig S3A). This was particularly true for the chicken blood treatment for which there were only
491 3 cups left at cycle 4. Similarly, the hatching rate varied over the successive gonotrophic cycles (X^2_2 = 501,
492 P < 0.0001) with maximum rate observed for egg-lay 4 (71.7 ± 0.5 %) followed by egg-lay 2 (68.4 ± 0.5 %)
493 and egg-lay 3 (57.8 ± 0.5 %; all post-hoc comparisons: P <0.0001, Fig 7B). Although the average number of

494 eggs per female was not affected by gonotrophic cycle ($X^2_2 = 3.48$, P =0.17; Appendix 3-Fig S2A), the average
495 number of larvae varied among gonotrophic cycles ($X^2_2 = 10.1$, P = 0.006), with the highest average in
496 gonotrophic cycle 4 (30 ± 3 larvae per female) followed by gonotrophic cycle 3 (25 ± 3 larvae per female)
497 and gonotrophic cycle 2 (22 ± 1 larvae per female; Tukey's post-hoc tests significantly different only for the
498 comparison between egg-lay 2 and 4, Appendix 3-Fig S2B). In addition, the survival rate was negatively
499 correlated with the average number of eggs laid ($X^2_1 = 5.5$, P = 0.02; Appendix 4-Supplementary method
500 and results; Appendix 3-Fig S3B).
501 The parasite isolate had no effect on egg-laying rate ($X^2_1 = 1.8$, P = 0.28). However, females fed blood
502 from isolate A during the first feeding episode laid on average more eggs during gonotrophic cycles 2-4
503 than females fed blood from isolate B (41±3 and 28±1 eggs respectively; $X^2_1 = 9.12$, P = 0.0025. Appendix
504 3- Fig S4A), the hatching rate of their eggs was also higher (isolate A: 71.5 ± 0.5 % vs. isolate B : 62.9 ± 0.5
505 %; $X^2_1 = 11.58$, P <0.001. Appendix 3-Fig S4B) and they produced on average more larvae than females fed
506 on blood from donor B (isolate A: 32 ± 2 and isolate B: 19 ± 1 larvae; $X^2_1 = 23$, P < 0.0001; Appendix 3-Fig
507 S4C).
508 The blood meal size had no significant effect on egg-laying rate ($X^2_1 = 0.63$, P = 0.43), the average
509 number of eggs ($X^2_1 = 0.1$, P = 0.75), and the average number of 1st instar larvae ($X^2_1 = 1.09$, P = 0.3) but was
510 negatively correlated to the hatching rate ($X^2_1 = 33.43$, P <0.0001).
511
512 *F1 development time* –Neither the blood type nor the parasite exposure of the mothers significantly
513 influenced the development time of their progeny ($X^2_3 = 3.7$, P =0.3, Fig 8A and $X^2_1 = 0.07$, P=0.79, Fig 8B,
514 respectively). The larval density in the rearing cup had no effect on the development time ($X^2_1 = 1.47$,
515 P=0.22). F1 males developed significantly faster than F1 females (10.65 ± 0.04 days *vs*.10. 52 ± 0.04 days;
516 $X^2_1 = 3.98$, P = 0.046). The gonotrophic cycle significantly influenced mosquito's development time ($X^2_2 =$
517 64.4, P < 0.0001; Fig 8A). In particular, individuals from gonotrophic cycle 4 developed significantly faster
518 (10.12 ± 0.04 days) than the ones from gonotrophic cycles 2 and 3 (10.67 ± 0.04 days and 10.82 ± 0.05 days,
519 respectively; Tukey's *post-hoc* tests <0.001, no significant difference between gonotrophic cycles 2 and 3).
520 The sex ratio was not affected by the blood type ($X^2_3 = 0.28$, P = 0.96).
521



522

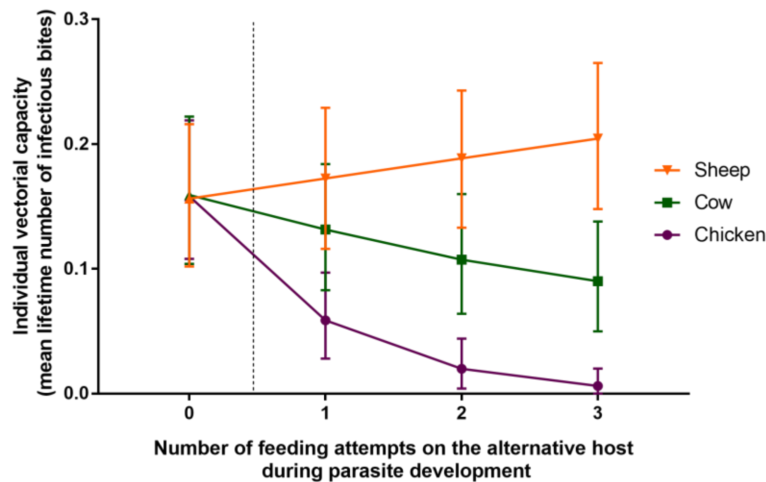
523 Figure 8. **Effects of blood type on progeny development and wing size**. A) Effects of blood type on the development
524 time of the progeny for gonotrophic cycles 1, 2, 3 and 4. B) Effects of blood type and infection group (females exposed vs.
525 females unexposed to an infectious blood meal on feeding episode 1) on the development time of the progeny averaged
526 from gonotrophic cycles 2, 3 and 4. C) Effects of blood type on the wing size of the progeny from gonotrophic cycles 1, 2, 3
527 and 4. D) Effects of blood type and infection group on the average progeny wing size ± se, averaged from gonotrophic
528 cycles 2, 3 and 4.

529

530 *F1 wing length* – Mosquito wing length was significantly affected by the blood type (X^2_3 = 12.9, P =
531 0.005; Fig 8C). The wing lengths of progeny from females fed on and chicken blood (2.86 ± 0.02mm) were
532 significantly shorter than the progeny from females fed on human blood (3.009 ± 0.01mm; Tukey's *post-*
533 *hoc* test: z=3.7, P = 0.001), on cow blood (2.99± 0.02mm; Tukey's *post-hoc* test: z=2.7, P = 0.04,) and on
534 sheep blood (2.99 ± 0.01mm; Tukey's *post-hoc* test: z=2.7, P = 0.03; all other comparisons being non-
535 significant). F1 females were significantly bigger than F1 males (3.03 ± 0.01 *vs.* 2.9 ± 0.01mm; X^2_1=132,
536 P<0.0001). Gonotrophic cycle significantly influenced mosquito wing length (X^2_2=30, P<0.0001) with
537 significantly bigger individuals in gonotrophic cycle 3 and 4 compared to gonotrophic cycle 2 (Tukey's *post-*
538 *hoc* tests: P<0.001 and P <0.0001, respectively; no significant difference between gonotrophic cycle 3 and
539 4: Tukey's *post-hoc* tests: P = 0.26). There was a negative effect of density on F1 size (X^2_1=9.6, P<0.01) and
540 maternal parasite exposure did not significantly affect the progeny wing length (X^2_1 = 0.43, P =0.51; Fig 8D).
541

542 **Theoretical modelling**
543 Our simulations showed an average IC (the average number of infecting bites transmitted by an
544 infected mosquito during its lifetime) of 0.16 infectious bites when females fed on human hosts only

545 (corresponding to the values with zero feeding attempts on the alternative host in Fig. 9). Compared to a
546 human blood meal, feeding on chicken blood drastically reduced the individual vectorial capacity. There
547 was 2.6 times fewer infectious bites after a single potential blood meal on chicken (with a 0.796 probability
548 of feeding success; mean IC=0.06) and even 26 times fewer after 3 potential blood meals on chicken (mean
549 IC=0.006; Fig. 9). Although less marked, a similar decrease was observed when females obtained blood
550 meals on cow with almost halved of the individual vectorial capacity after 3 potential blood meals on cow
551 (with a 0.717 probability of feeding success; mean IC=0.09; Fig.9). On the contrary, feeding on sheep during
552 *Plasmodium* development increased the vectorial capacity by 25% after 3 potential blood meals on sheep
553 (with a 0.774 probability of feeding success; mean IC=0.2; Fig. 9).



554
555
556 Figure 9. **Theoretical modeling of the individual vectorial capacity** (mean lifetime number of infectious bites) at the
557 population level depending on the number of feedings attempted on an alternative host during the parasite development
558 time with zero corresponding to blood meals taken on human host only. The individual vectorial capacity was estimated
559 with a model simulating the daily life history of individual mosquito vectors after taking an infectious blood meal on a
560 human under various scenarios. The scenario was characterized by the presence of humans and an alternative host (either
561 chicken, cow or sheep) with varying availability (0 to 3 consecutive possible feeding attempts during *Plasmodium*
562 incubation period).

563 ## Discussion

564 The relationships between blood meal type, and mosquito and parasite fitness were explored using a
565 total of 2810 *An. coluzzii* females initially fed an infectious or a non-infectious blood meal and then followed
566 by up to three subsequent blood meals from either human, chicken, cow or sheep. We found no significant
567 effect of blood type on malaria parasite development both at the non-transmissible (oocyst) and the
568 transmissible (sporozoite) stages for either parasite prevalence or intensity. No effects at the oocyst stage
569 or negative effects at the sporozoite stage were also found in *An. gambiae s.s*. females consuming a second
570 blood meal on cow compared to human blood or unfed controls, whereas higher oocyst and sporozoite
571 prevalences were observed in *An. arabiensis* females having a second blood meal on cow compared to
572 human blood or unfed controls (Emami *et al.* 2017). In another study, a second blood meal shortened
573 parasite development in *P. falciparum* with no group specific differences, while a marginal increase was
574 observed with human blood compared to unfed controls for *P. berghei* development (Pathak *et al.* 2022).
575 Thus, the effects of blood type on *Plasmodium* sp. development seem to be variable both between and
576 within *Plasmodium* species and our results with sympatric field strains do not seem to confirm those
577 previous findings. Findings obtained in the laboratory using unnatural host-parasite associations or long-
578 time derived strains do not always reflect natural interactions and an increasing number of studies
579 highlights the importance of confirming laboratory observations with more natural systems for studying
580 disease ecology and evolution (Aguilar *et al.* 2005, Tripet *et al.* 2008).

581 Exposure to malaria parasite with wild isolates resulted in 78% (isolate A) and 52% (isolate B) infected
582 females and had, overall, no effect on female fitness traits. Parasite exposure did not affect female's
583 survival regardless of the host type they fed on nor did it affect female fecundity nor their progeny
584 development time and wing size. The only indirect cost we observed was a lower feeding rate in the
585 following blood meals of exposed females compared to unexposed females. The existence of fitness costs
586 of malaria parasite infection in the mosquito host has long been debated and seem to depend on the
587 environmental conditions under which the fitness traits are measured. First, mortality is more commonly
588 reported in unnatural parasite-vector combinations (Ferguson and Read 2002). Second, fitness costs are
589 also more commonly observed in stressful environmental conditions (Lalubin *et al.* 2014, Sangare *et al.*
590 2014, Roux *et al.* 2015) and can depend on the genetic background (Alout *et al.* 2016). In our experiment,
591 we used a natural parasite-host combination and did not provide any sugar-meals to not alleviate potential
592 fitness costs. Indeed, sugar feeding can affect mosquito competence, survival and fecundity ((Ferguson and
593 Read 2002, Lambrechts *et al.* 2006, Foster 2022) and could compensate for the fitness costs of the different
594 blood types. One explanation could be that the cost of infection are minimal in our system and that
595 infection might only be costly for exposed-infected females. Following the infectious blood meal, our setup
596 did not separate exposed-infected and exposed-uninfected females, thus we were not able to measure the
597 cost of infection in exposed-infected females only. Another possibility is that those costs are quickly offset
598 by the following blood meals the females received, although a study on *Plasmodium relictum* and *Culex*
599 *pipiens* observed fecundity costs following the infection which lasted for three consecutive gonotrophic
600 cycles (Pigeault and Villa 2018).
601 Blood type strongly affected mosquito survival. In particular, chicken blood reduced mosquito
602 survivorship by 40% (Fig 6). The larger and nucleated red blood cells of chicken compared to human might
603 be more difficult to digest for *An. coluzzii* (Wintrobe 1933). In addition, although anopheline mosquitoes
604 can reduce their body temperature while blood feeding (Lahondère and Lazzari 2012), chicken host
605 temperature might be too high for evaporative cooling as we saw increased mortality after each blood
606 meal. Interestingly, females fed on sheep blood had the highest survival followed by human which
607 translated in an increased vectorial capacity in our transmission model (Fig 9). Indeed, the type of host the
608 female feeds on strongly increase or decrease the average number of infectious bites in the population (Fig
609 9). Modeling of malaria transmission showed that mosquito survival is the factor with the biggest impact
610 on transmission (MacDonald 1957). Indeed, parasite development is relatively long (10-14 days) compared
611 to mosquito survival (2-3 weeks). Consequently, females will be infectious for a limited period of time only
612 and any small changes in mosquito longevity will dramatically affect malaria transmission (Smith and
613 McKenzie 2004).
614 Vectorial capacity is also very sensitive to feeding rates (Brady *et al.* 2016). Our model highlights how
615 even minute differences in survival and feeding rates, such as those observed between females fed with
616 human and sheep blood (Fig 5B & 6), can cause large variation in vectorial capacity (Fig.9). Mosquito
617 feeding rate was highest on sheep blood followed by chicken, human and cow blood. Although membrane
618 feeders were maintained at a temperature corresponding to each vertebrate body temperature, the
619 difference is likely linked to the blood characteristics for the females as sheep blood was maintained at
620 39°C which is close to cow blood temperature, 38.5°C. Comparison of feeding rates between several
621 vertebrates with blood maintained at the same temperature showed large variation depending on the
622 mosquito species (Phasomkusolsil *et al.* 2013, Al-Rashidi *et al.* 2022). Contrary to our results, sheep blood
623 tended to have a negative impact on survival in *An. dirus*, *An. cracens* and *An. minimus* female and on
624 fecundity in the five mosquito species investigated (Phasomkusolsil *et al.* 2013). Future studies linking
625 fitness measurements and detailed vertebrate blood composition would be useful to determine the
626 chemical and physical characteristics influencing blood digestion and utilization in different mosquito
627 species.
628 We measured several individual fecundity traits (egg laying rate, average number of eggs, average
629 number of 1st instar larvae, eggs and larvae prevalence, hatching rate) and found no effect of blood type.
630 However, the lifetime fecundity and production of larvae corresponding to the sum of the average number
631 of eggs or larvae of gonotrophic cycles 2, 3 and 4, were affected by the blood type which was lower for
632 chicken compared to human (Fig 7C, D). Thus, our proxy of the lifetime fecundity showed that the fitness
633 of females fed on chicken or cow blood was lower than the fitness of females fed on human or sheep blood.
634 Indeed, the small differences observed at each gonotrophic cycle were not significantly different, but the

635 accumulation of all those small differences gave overall a difference when looking over the lifetime
636 production of eggs and larvae.
637 We observed a donor effect on fecundity with females fed on blood from donor A on their first blood
638 meal laying more eggs and having a higher hatching rate and more larvae than females fed on donor B. The
639 difference between the two donors in the average number of eggs and larvae in blood meals 2, 3 and 4
640 tended to blur over the successive gonotrophic cycles (Fig S4A & S4D), such that the successive blood meals
641 slowly offset the difference in donor blood quality.
642 The survival rate was negatively associated with the average number of eggs laid (Fig S3B) and this
643 association was stronger for the females fed on human blood compared to the other blood types. The life
644 history traits of an organism are constrained by the total amount of resources available (Stearns 1992) such
645 as the tradeoff observed here between the energy allocated to reproduction and survival. Similar results
646 were observed in *Culex pipiens* for which the higher the number of eggs laid, the lower was their
647 subsequent survival (Vézilier *et al.* 2012). Such trade-off between reproduction and survival has been
648 extensively characterized in many organisms with *Drosophila melanogaster* being one of the main model
649 system (Zera and Harshman 2001, Flatt 2011, Flatt 2020, Hsu *et al.* 2021).
650 Although blood type did not inluence progeny development time, progeny from females fed on chicken
651 blood was smaller than the progeny from females fed on all other vertebrates blood (Fig. 8), which
652 highlights the fitness cost of feeding on chicken blood. Larger females generally take larger blood meals,
653 lay more eggs, live longer and are more competent (Briegel 1990, Kittayapong *et al.* 1992, Lyimo and Koella
654 1992, Lyimo and Takken 1993, Barreaux *et al.* 2016), but the local environmental conditions can modulate
655 this pattern (Barreaux *et al.* 2016, Barreaux *et al.* 2018). Thus, the bloodmeal type-mediated differences in
656 mosquito progeny size could have strong effects on malaria parasite transmission in the following
657 generations, through increases in mosquito density, competence and survival, three traits that play key
658 role in transmission.
659 Although we measured several life-history traits simultaneously, our experimental set-up has several
660 limitations. First, we measured averaged fecundity traits per cup since multiple blood-feeding over the
661 lifetime of each female individually would have been technically challenging (i.e. using a single membrane
662 feeder for each individual female), and at the minimum would have strongly reduced our sample size.
663 Second, the laboratory colony used is replenished with wild mosquitoes, however processes such as
664 genetic drift or selection by artificial feeding in the laboratory can happen on a very short time frame
665 especially in small population sizes and those could have eroded the specialization on human blood. Third,
666 specialization on humans could be linked to other ecological or behavioural factors which might exert
667 stronger selection pressures than blood characteristics. While we investigated the effects of the blood type,
668 this was disconnected from the effects of the host type as a whole since other characteristics were not
669 taken into account such as e.g. defensive behavior (Edman and Scott 1987, Lyimo *et al.* 2012), host
670 availability (Lyimo *et al.* 2013, Fikrig *et al.* 2021), parasite manipulation of host behaviour (Vantaux *et al.*
671 2021) or female individual experience (Vantaux *et al.* 2014). In addition, even though we observed a lower
672 feeding rate of exposed females compared to unexposed females in the following blood meals, all
673 successive blood meals were carried out on membrane feeders and results could be different on whole-
674 body hosts. Fourth, our laboratory setting did not take into account the natural blood foraging rhythm of
675 the vector nor the circadian rhythm of the parasite which have both been shown to influence mosquito
676 and parasite fitness (Schneider *et al.* 2018, O'Donnell *et al.* 2019, Habtewold *et al.* 2022). Fifth, we
677 measured mosquito competence at two time points classically illustrating the non-transmissible and the
678 transmissible parasite stages. It would be interesting to measure the effects of multiple blood meals on
679 different hosts on a more continuous time line as studies showed that multiple blood meals accelerate
680 parasite development (Shaw *et al.* 2021) and growth (Habtewold *et al.* 2021, Kwon *et al.* 2021) and this
681 could have tremendous effects on pathogen development and disease transmission (Brackney *et al.* 2021).
682 Here, successive meals were taken from one of the four vertebrate species used. Under natural conditions,
683 mosquitoes can shift from one host species to another between their gonotrophic cycles. It would be of
684 particular interest to examine the effect of successive meals taken from different host species on the traits
685 measured here. The type and frequency of blood meals not only has consequences on reproduction,
686 survival and epidemiology but also on many other physiological and ecological aspects such as e.g. the
687 maintenance of insecticide resistance phenotype for longer period (Oliver and Brooke 2014, Oliver *et al.*
688 2022) or an increased ivermectin susceptibility in previously bloodfed females (Seaman *et al.* 2015). Our

689 findings emphasize that considering the diversity of vertebrate blood-meal sources is important to better
690 understand the ecology of mosquitoes as well as their capacity to transmit malaria parasites.
691 Overall, blood type had a significant impact on mosquito survival and feeding rates, leading to
692 considerable variation in vectorial capacity and differences in progeny sizes. These findings imply that the
693 diversity of vertebrate hosts (including both the number of species and their relative abundance) within
694 villages could influence the transmission of malaria parasites. Specifically, transmission may decrease when
695 chickens or cows make up the majority of available blood sources, while it may increase when a relatively
696 large number of sheep are present. However, the host selection patterns of mosquitoes are not solely
697 driven by vertebrate abundance, but are also influenced by mosquito innate preferences and host
698 defensive behavior (Lefèvre *et al.* 2009, Lyimo and Ferguson 2009). The human blood index (number of
699 females fed on humans, including mixed human-animal bood meals, over the total number of blood fed
700 females) was highly variable between villages and mosquito species in this area of Burkina Faso, with
701 anthropophagy ranging from 56.5± 4% to 83.5 ± 2.2% in different villages (Vantaux *et al.* 2021). Among the
702 2627 fed *An. gambiae s.l.* for which the blood meal origin was determined during this study, only 10 (0.38±
703 0.24%) individuals fed on chicken, 63 (2.4± 0.6%) individuals fed on sheep and 951 (36.2± 1.8%) fed on cow
704 (A. Vantaux personnal communication). Although those numbers represent only a limited period of the
705 year, they are a reminder that *An. gambiae s.l.* females also feed on non-human hosts and that the local
706 composition of domestic animals could influence parasite transmission in the village. Limiting or increasing
707 access to particular non-human hosts in the village could help improve malaria control during the peak of
708 transmission. Since zooprophylaxis, which involves using livestock to draw mosquitoes away from humans
709 and decrease malaria transmission, is dependant on specific conditions such as the optimal distance
710 between humans and livestock sleeping areas and the characteristics of the local mosquito populations
711 (Donnelly *et al.* 2015, Hasyim *et al.* 2018), our findings suggest that it may be feasible to further potentiate
712 zooprophylaxis effects by considering the use of endectocides (Pooda *et al.* 2015, Khaligh *et al.* 2021)
713 and/or application of formulation on animal fur to divert mosquitoes from their prefered host to less
714 prefered hosts (Kemibala *et al.* 2020).

715 ## Acknowledgements

716 We would like to thank all volunteers for participating in this study as well as the local authorities for their
717 support. We are very grateful to the IRSS staff in Burkina Faso for technical assistance. We thank Diego
718 Santiago-Alarcon, Francisco C. Ferreira and an anonymous reviewer for their fruitful comments.

719 ## Data, scripts, code, and supplementary information availability

720 Data and script for the statistical analyses are available online: https://zenodo.org/record/7940843

721 Data and scripts for the theoretical modeling are available online: https://zenodo.org/record/7645483

722 ## Conflict of interest disclosure

723 The authors declare that they comply with the PCI rule of having no financial conflicts of interest in
724 relation to the content of the article.

725 ## Funding

726 This study was supported by the ANR grant no. 11-PDOC-006-01.

727 ## References

728 Abramoff, M. D., P. J. Magalhaes and S. J. Ram (2004). "Image processing with ImageJ." Biophotonics
729 International **11**(7): 36-42.

730 Aguilar, R., Y. M. Dong, E. Warr and G. Dimopoulos (2005). "*Anopheles* infection responses; laboratory models
731 versus field malaria transmission systems." Acta Tropica **95**(3): 285-291. DOI:
732 10.1016/j.actatropica.2005.06.005.
733 Al-Rashidi, H. S., K. M. Alghamdi, W. M. Al-Otaibi, H. M. Al-Solami and J. A. Mahyoub (2022). "Effects of blood
734 meal sources on the biological characteristics of *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae)." Saudi
735 Journal of Biological Sciences **29**(12): 103448. DOI: https://doi.org/10.1016/j.sjbs.2022.103448.
736 Alout, H., R. K. Dabiré, L. S. Djogbénou, L. Abate, V. Corbel, F. Chandre and A. Cohuet (2016). "Interactive cost
737 of *Plasmodium* infection and insecticide resistance in the malaria vector *Anopheles gambiae*." Scientific
738 Reports **6**: 29755. DOI: 10.1038/srep29755.
739 Alout, H., B. Yameogo, L. S. Djogbénou, F. Chandre, R. K. Dabiré, V. Corbel and A. Cohuet (2014). "Interplay
740 between malaria infection and resistance to insecticides in vector mosquitoes." Journal of Infectious Diseases.
741 DOI: 10.1093/infdis/jiu276.
742 Atella, G. C., P. R. Bittencourt-Cunha, R. D. Nunes, M. Shahabuddin and M. A. C. Silva-Neto (2009). "The major
743 insect lipoprotein is a lipid source to mosquito stages of malaria parasite." Acta Tropica **109**(2): 159-162. DOI:
744 10.1016/j.actatropica.2008.10.004.
745 Atella, G. C., M. A. C. Silva-Neto, D. M. Golodne, S. Arefin and M. Shahabuddin (2006). "*Anopheles gambiae*
746 lipophorin: characterization and role in lipid transport to developing oocyte." Insect Biochemistry and
747 Molecular Biology **36**(5): 375-386. DOI: http://dx.doi.org/10.1016/j.ibmb.2006.01.019.
748 Barreaux, A. M. G., P. Barreaux, K. Thievent and J. C. Koella (2016). "Larval environment influences vector
749 competence of the malaria mosquito *Anopheles gambiae*." Malaria World Journal **7**(8).
750 Barreaux, A. M. G., C. M. Stone, P. Barreaux and J. C. Koella (2018). "The relationship between size and
751 longevity of the malaria vector Anopheles gambiae (s.s.) depends on the larval environment." Parasites &
752 Vectors **11**(1): 485. DOI: 10.1186/s13071-018-3058-3.
753 Barry, A., J. Bradley, W. Stone, M. W. Guelbeogo, K. Lanke, A. Ouedraogo, I. Soulama, I. Nébié, S. S. Serme, L.
754 Grignard, C. Patterson, L. Wu, J. J. Briggs, O. Janson, S. S. Awandu, M. Ouedraogo, C. W. Tarama, D. Kargougou,
755 S. Zongo, S. B. Sirima, M. Marti, C. Drakeley, A. B. Tiono and T. Bousema (2021). "Higher gametocyte
756 production and mosquito infectivity in chronic compared to incident *Plasmodium falciparum* infections."
757 Nature Communications **12**(1): 2443. DOI: 10.1038/s41467-021-22573-7.
758 Beier, J. C. (1998). "Malaria parasite development in mosquitoes." Annual Review of Entomology **43**(1): 519-
759 543. DOI: doi:10.1146/annurev.ento.43.1.519.
760 Billker, O., V. Lindo, M. Panico, A. E. Etienne, T. Paxton, A. Dell, M. Rogers, R. E. Sinden and H. R. Morris (1998).
761 "Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito." Nature
762 **392**(6673): 289-292.
763 Bousema, T., R. R. Dinglasan, I. Morlais, L. C. Gouagna, T. van Warmerdam, P. H. Awono-Ambene, S. Bonnet,
764 M. Diallo, M. Coulibaly, T. Tchuinkam, B. Mulder, G. Targett, C. Drakeley, C. Sutherland, V. Robert, O. Doumbo,
765 Y. Touré, P. M. Graves, W. Roeffen, R. Sauerwein, A. Birkett, E. Locke, M. Morin, Y. Wu and T. S. Churcher
766 (2012). "Mosquito feeding assays to determine the infectiousness of naturally infected *Plasmodium falciparum*
767 gametocyte carriers." PLoS ONE **7**(8): e42821.
768 Brackney, D. E., J. C. LaReau and R. C. Smith (2021). "Frequency matters: How successive feeding episodes by
769 blood-feeding insect vectors influences disease transmission." PLOS Pathogens **17**(6): e1009590. DOI:
770 10.1371/journal.ppat.1009590.
771 Brady, O. J., H. C. Godfray, A. J. Tatem, P. W. Gething, J. M. Cohen, F. E. McKenzie, T. A. Perkins, R. C. Reiner,
772 Jr., L. S. Tusting, M. E. Sinka, C. L. Moyes, P. A. Eckhoff, T. W. Scott, S. W. Lindsay, S. I. Hay and D. L. Smith
773 (2016). "Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination."
774 Trans R Soc Trop Med Hyg **110**(2): 107-117. DOI: 10.1093/trstmh/trv113.
775 Briegel, H. (1990). "Fecundity, metabolism, and body size in *Anopheles* (Diptera, Culicidae), vectors of malaria."
776 Journal of Medical Entomology **27**(5): 839-850.
777 Briegel, H., A. Hefti and E. DiMarco (2002). "Lipid metabolism during sequential gonotrophic cycles in large and
778 small female *Aedes aegypti*." Journal of Insect Physiology **48**(5): 547-554. DOI: 10.1016/s0022-1910(02)00072-
779 0.
780 Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. M. and B.
781 M. Bolker (2017). "glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized
782 Linear Mixed Modeling." The R journal **9**: 378-400.
783 Caputo, B., D. Nwakanma, M. Jawara, M. Adiamoh, I. Dia, L. Konate, V. Petrarca, D. J. Conway and A. della
784 Torre (2008). "*Anopheles gambiae* complex along The Gambia river, with particular reference to the molecular
785 forms of *An. gambiae* s.s." Malaria Journal **7**: 182. DOI: 10.1186/1475-2875-7-182.

786 Cheon, H. M., S. W. Shin, G. W. Bian, J. H. Park and A. S. Raikhel (2006). "Regulation of lipid metabolism genes,
787 lipid carrier protein lipophorin, and its receptor during immune challenge in the mosquito *Aedes aegypti*."
788 Journal of Biological Chemistry **281**(13): 8426-8435. DOI: 10.1074/jbc.M510957200.
789 Clements, A. N. (1992). The biology of mosquitoes. Development, nutrition and reproduction. London,
790 Chapman & hall.
791 Coetzee, M., R. H. Hunt, R. Wilkerson, A. Della Torre, M. B. Coulibaly and N. J. Besansky (2013). "*Anopheles*
792 *coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex." Zooataxa **3619**(3): 246-
793 274.
794 Costantini, C., N. Sagnon, A. della Torre and M. Coluzzi (1999). "Mosquito behavioural aspects of vector-human
795 interactions in the *Anopheles gambiae* complex." Parassitologia **41**(1-3): 209-217.
796 Crawley, M. J. (2007). The R book. The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England,
797 John Wiley & Sons Ltd.
798 De Smet, W. H. (1978). "The total protein content in the blood serum of vertebrates." Acta Zool Pathol
799 Antverp(70): 35-56.
800 Donnelly, B., L. Berrang-Ford, N. A. Ross and P. Michel (2015). "A systematic, realist review of zooprophylaxis
801 for malaria control." Malaria Journal **14**(1): 313. DOI: 10.1186/s12936-015-0822-0.
802 Edman, J. D. and T. W. Scott (1987). "Host defensive behavior and the feeding success of mosquitoes." Insect
803 Science and Its Application **8**(4-6): 617-622.
804 Emami, S. N., L. C. Ranford-Cartwright and H. M. Ferguson (2017). "The transmission potential of malaria-
805 infected mosquitoes (*An. gambiae*-Keele, *An. arabiensis*-Ifakara) is altered by the vertebrate blood type they
806 consume during parasite development." Scientific Reports **7**: 40520. DOI: 10.1038/srep40520.
807 Ferguson, H. M. and A. F. Read (2002). "Genetic and environmental determinants of malaria parasite virulence
808 in mosquitoes." Proceedings of the Royal Society of London Series B-Biological Sciences **269**(1497): 1217-1224.
809 DOI: 10.1098/rspb.2002.2023.
810 Ferguson, H. M. and A. F. Read (2002). "Why is the effect of malaria parasites on mosquito survival still
811 unresolved?" Trends in Parasitology **18**(6): 256-261.
812 Fikrig, K., E. Martin, S. Dang, K. St Fleur, H. Goldsmith, S. Qu, H. Rosenthal, S. Pitcher and L. C. Harrington
813 (2021). "The effects of host availability and fitness on *Aedes albopictus* blood feeding patterns in New York."
814 Am J Trop Med Hyg **106**(1): 320-331. DOI: 10.4269/ajtmh.21-0157.
815 Flatt, T. (2011). "Survival costs of reproduction in Drosophila." Exp Gerontol **46**(5): 369-375. DOI:
816 10.1016/j.exger.2010.10.008.
817 Flatt, T. (2020). "Life-history evolution and the genetics of fitness components in *Drosophila melanogaster*."
818 Genetics **214**(1): 3-48. DOI: 10.1534/genetics.119.300160.
819 Foster, W. A. (2022). Chapter 7: Behavioural ecology of plant-mosquito relations Sensory ecology of disease
820 vectors. R. Ignell, C. Lazzari, M. G. Lorenzo and S. R. Hill, Wageningen Academic Publishers**:** 171-218.
821 Grignard, L., B. P. Goncalves, A. M. Early, R. F. Daniels, A. B. Tiono, W. M. Guelbeogo, A. Ouedraogo, E. M. van
822 Veen, K. Lanke, A. Diarra, I. Nebie, S. B. Sirima, G. A. Targett, S. K. Volkman, D. E. Neafsey, D. F. Wirth, T.
823 Bousema and C. Drakeley (2018). "Transmission of molecularly undetectable circulating parasite clones leads
824 to high infection complexity in mosquitoes post feeding." Int J Parasitol **48**(8): 671-677. DOI:
825 10.1016/j.ijpara.2018.02.005.
826 Grimm, V., U. Berger, D. L. DeAngelis, J. G. Polhill, J. Giske and S. F. Railsback (2010). "The ODD protocol: A
827 review and first update." Ecol Model **221**(23): 2760-2768. DOI:
828 https://doi.org/10.1016/j.ecolmodel.2010.08.019.
829 Habtewold, T., A. A. Sharma, C. A. S. Wyer, E. K. G. Masters, N. Windbichler and G. K. Christophides (2021).
830 "*Plasmodium* oocysts respond with dormancy to crowding and nutritional stress." Scientific Reports **11**(1):
831 3090. DOI: 10.1038/s41598-021-81574-0.
832 Habtewold, T., S. Tapanelli, E. K. G. Masters, N. Windbichler and G. K. Christophides (2022). "The circadian
833 clock modulates Anopheles gambiae infection with Plasmodium falciparum." PLOS ONE **17**(12): e0278484.
834 DOI: 10.1371/journal.pone.0278484.
835 Hasyim, H., M. Dhimal, J. Bauer, D. Montag, D. A. Groneberg, U. Kuch and R. Müller (2018). "Does livestock
836 protect from malaria or facilitate malaria prevalence? A cross-sectional study in endemic rural areas of
837 Indonesia." Malaria Journal **17**(1): 302. DOI: 10.1186/s12936-018-2447-6.
838 Hawkey, C. M., P. M. Bennett, S. C. Gascoyne, M. G. Hart and J. K. Kirkwood (1991). "Erythrocyte size, number
839 and hemoglobin content in vertebrates." British Journal of Haematology **77**(3): 392-397. DOI: 10.1111/j.1365-
840 2141.1991.tb08590.x.
841 Hien, D. F. d. S., K. R. Dabiré, B. Roche, A. Diabaté, R. S. Yerbanga, A. Cohuet, B. K. Yameogo, L.-C. Gouagna, R.
842 J. Hopkins, G. A. Ouedraogo, F. Simard, J.-B. Ouedraogo, R. Ignell and T. Lefevre (2016). "Plant-mediated

843 effects on mosquito capacity to transmit human malaria." PLoS Pathog **12**(8): e1005773. DOI:
844 10.1371/journal.ppat.1005773.
845 Hsu, C. H., O. Posegga, K. Fischbach and H. Engelhardt (2021). "Examining the trade-offs between human
846 fertility and longevity over three centuries using crowdsourced genealogy data." PLoS One **16**(8): e0255528.
847 DOI: 10.1371/journal.pone.0255528.
848 Kemibala, E. E., A. Mafra-Neto, T. Dekker, J. Saroli, R. Silva, A. Philbert, K. Nghabi and L. E. G. Mboera (2020). "A
849 zooprophylaxis strategy using l-lactic acid (Abate) to divert host-seeking malaria vectors from human host to
850 treated non-host animals." Malaria Journal **19**(1): 52. DOI: 10.1186/s12936-020-3136-9.
851 Khaligh, F. G., A. Jafari, E. Silivanova, M. Levchenko, B. Rahimi and S. Gholizadeh (2021). "Endectocides as a
852 complementary intervention in the malaria control program: a systematic review." Systematic Reviews **10**(1):
853 30. DOI: 10.1186/s13643-021-01578-9.
854 Kittayapong, P., J. D. Edman, B. A. Harrison and D. R. Delorme (1992). "Female body size, parity, and malaria
855 infection of *Anopheles maculatus* (Diptera, Culicidae) in peninsular Malaysia." Journal of Medical Entomology
856 **29**(3): 379-383.
857 Kwon, H., M. L. Simões, R. A. Reynolds, G. Dimopoulos and R. C. Smith (2021). "Additional feeding reveals
858 differences in immune recognition and growth of *Plasmodium* parasites in the mosquito host." mSphere **6**(2):
859 e00136-00121. DOI: doi:10.1128/mSphere.00136-21.
860 Lahondère, C. and Claudio R. Lazzari (2012). "Mosquitoes cool down during blood feeding to avoid
861 overheating." Current Biology **22**(1): 40-45.
862 Lalubin, F., A. Delédevant, O. Glaizot and P. Christe (2014). "Natural malaria infection reduces starvation
863 resistance of nutritionally stressed mosquitoes." Journal of Animal Ecology **83**(4): 850-857. DOI:
864 https://doi.org/10.1111/1365-2656.12190.
865 Lambrechts, L., J. M. Chavatte, G. Snounou and J. C. Koella (2006). "Environmental influence on the genetic
866 basis of mosquito resistance to malaria parasites." Proceedings of the Royal Society B-Biological Sciences
867 **273**(1593): 1501-1506. DOI: 10.1098/rspb.2006.3483.
868 Lefèvre, T., L. C. Gouagna, R. K. Dabire, E. Elguero, D. Fontenille, C. Costantini and F. Thomas (2009).
869 "Evolutionary lability of odour-mediated host preference by the malaria vector *Anopheles gambiae*." Tropical
870 Medicine & International Health **14**(2): 228-236. DOI: 10.1111/j.1365-3156.2009.02206.x.
871 Lefèvre, T., L. C. Gouagna, R. K. Dabire, E. Elguero, D. Fontenille, F. Renaud, C. Costantini and F. Thomas (2009).
872 "Beyond nature and nurture: phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae* s.s. when
873 humans are not readily accessible." The American Journal of Tropical Medicine and Hygiene **81**(6): 1023-1029.
874 DOI: 10.4269/ajtmh.2009.09-0124.
875 Lemasson, J. J., D. Fontenille, L. Lochouarn, I. Dia, F. Simard, K. Ba, A. Diop, M. Diatta and J. F. Molez (1997).
876 "Comparison of behavior and vector efficiency of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae) in
877 Barkedji, a Sahelian area of Senegal." Journal of Medical Entomology **34**(4): 396-403.
878 Lenth, R. V. (2022). "emmeans: Estimated Marginal Means, aka Least-Squares Means." R package version
879 1.7.2. https://CRAN.R-project.org/package=emmeans.
880 Lyimo, E. O. and J. C. Koella (1992). "Relationship between body size of adult *Anopheles gambiae* s.l. and
881 infection with the malaria parasite *Plasmodium falciparum*." Parasitology **104 (Pt 2)**: 233-237. DOI:
882 10.1017/s0031182000061667.
883 Lyimo, E. O. and W. Takken (1993). "Effects of adult body size on fecundity and the pre-gravid rate of
884 *Anopheles gambiae* females in Tanzania." Medical and Veterinary Entomology **7**(4): 328-332. DOI:
885 10.1111/j.1365-2915.1993.tb00700.x.
886 Lyimo, I. N. and H. M. Ferguson (2009). "Ecological and evolutionary determinants of host species choice in
887 mosquito vectors." Trends in Parasitology **25**(4): 189-196. DOI: 10.1016/j.pt.2009.01.005.
888 Lyimo, I. N., D. T. Haydon, K. F. Mbina, A. A. Daraja, E. M. Mbehela, R. Reeve and H. M. Ferguson (2012). "The
889 fitness of African malaria vectors in the presence and limitation of host behaviour." Malar J **11**(425): 1475-
890 2875.
891 Lyimo, I. N., D. T. Haydon, T. L. Russell, K. F. Mbina, A. A. Daraja, E. M. Mbehela, R. Reeve and H. M. Ferguson
892 (2013). "The impact of host species and vector control measures on the fitness of African malaria vectors."
893 Proceedings of the Royal Society B: Biological Sciences **280**(1754). DOI: 10.1098/rspb.2012.2823.
894 Lyimo, I. N., S. P. Keegan, L. C. Ranford-Cartwright and H. M. Ferguson (2012). "The impact of uniform and
895 mixed species blood meals on the fitness of the mosquito vector *Anopheles gambiae* s.s: does a specialist pay
896 for diversifying its host species diet?" Journal of Evolutionary Biology **25**(3): 452-460. DOI: 10.1111/j.1420-
897 9101.2011.02442.x.
898 MacDonald, G. (1957). The epidemiology and control of malaria. London, Oxford University Press.

899 Mollahosseini, A., M. Rossignol, C. Pennetier, A. Cohuet, A. d. Anjos, F. Chandre and H. Shahbazkia (2012). "A
900 user-friendly software to easily count *Anopheles* egg batches." Parasites & Vectors **5**(1): 122.
901 Morassin, B., R. Fabre, A. Berry and J. F. Magnaval (2002). "One year's experience with the polymerase chain
902 reaction as a routine method for the diagnosis of imported malaria." American Journal of Tropical Medecine
903 and Hygiene **66**(5): 503-508.
904 O'Donnell, A. J., S. S. C. Rund and S. E. Reece (2019). "Time-of-day of blood-feeding: effects on mosquito life
905 history and malaria transmission." Parasites & vectors **12**(1): 301-301. DOI: 10.1186/s13071-019-3513-9.
906 Oliver, S. V. and B. D. Brooke (2014). "The effect of multiple blood-feeding on the longevity and insecticide
907 resistant phenotype in the major malaria vector Anopheles arabiensis (Diptera: Culicidae)." Parasites & Vectors
908 **7**(1): 390. DOI: 10.1186/1756-3305-7-390.
909 Oliver, S. V., C. L. Lyons and B. D. Brooke (2022). "The effect of blood feeding on insecticide resistance intensity
910 and adult longevity in the major malaria vector *Anopheles funestus* (Diptera: Culicidae)." Scientific Reports
911 **12**(1): 3877. DOI: 10.1038/s41598-022-07798-w.
912 Ouédraogo, A. L., W. M. Guelbéogo, A. Cohuet, I. Morlais, K. J. G., B. P. Gonçalves, G. J. H. Bastiaens, M.
913 Vaanhold, J. Sattabongkot, Y. Wu, M. Coulibaly, B. Ibrahima, S. Jones, M. Morin, C. Drakeley, R. R. Dinglasan
914 and T. Bousema (2013). "A protocol for membrane feeding assays to determine the infectiousness of *P.*
915 *falciparum* naturally infected individuals to *Anopheles gambiae*." MWJ **4**: 16.
916 Pathak, A. K., J. C. Shiau, C. R. S. de Freitas and D. E. Kyle (2022). "Blood meals from 'dead-end' vertebrate
917 hosts enhance transmission potential of malaria-infected mosquitoes." bioRxiv: 2022.2012.2024.521872. DOI:
918 10.1101/2022.12.24.521872.
919 Phasomkusolsil, S., J. Tawong, N. Monkanna, K. Pantuwatana, N. Damdangdee, W. Khongtak, Y. Kertmanee, B.
920 P. Evans and A. L. Schuster (2013). "Maintenance of mosquito vectors: effects of blood source on feeding,
921 survival, fecundity, and egg hatching rates." Journal of Vector Ecology **38**(1): 38-45. DOI: 10.1111/j.1948-
922 7134.2013.12006.x.
923 Pigeault, R. and M. Villa (2018). "Long-term pathogenic response to *Plasmodium relictum* infection in *Culex*
924 *pipiens* mosquito." PLOS ONE **13**(2): e0192315. DOI: 10.1371/journal.pone.0192315.
925 Ponnudurai, T., A. H. W. Lensen, G. J. A. Vangemert, M. P. E. Bensink, M. Bolmer and J. Meuwissen (1989).
926 "Sporozoite load of mosquitoes infected with *Plasmodium falciparum*." Transactions of the Royal Society of
927 Tropical Medicine and Hygiene **83**(1): 67-70. DOI: 10.1016/0035-9203(89)90708-6.
928 Pooda, H. S., J.-B. Rayaisse, D. F. d. S. Hien, T. Lefèvre, S. R. Yerbanga, Z. Bengaly, R. K. Dabiré, A. M. G. Belem, I.
929 Sidibé, P. Solano and K. Mouline (2015). "Administration of ivermectin to peridomestic cattle: a promising
930 approach to target the residual transmission of human malaria." Malaria Journal **14**: 496. DOI:
931 10.1186/s12936-015-1001-z.
932 R Core Team (2020). R: A language and environment for statistical computing. Vienna, Austria, R Foundation
933 for Statistical Computing.
934 Rono, M. K., M. M. A. Whitten, M. Oulad-Abdelghani, E. A. Levashina and E. Marois (2010). "The major yolk
935 protein vitellogenin interferes with the anti-*Plasmodium* response in the malaria mosquito *Anopheles*
936 *gambiae*." Plos Biology **8**(7): e1000434. DOI: 10.1371/journal.pbio.1000434.
937 Roux, O., A. Vantaux, B. Roche, K. B. Yameogo, K. R. Dabiré, A. Diabaté, F. Simard and T. Lefèvre (2015).
938 "Evidences for carry-over effect of predator exposure on pathogen transmission potential." Proceedings of the
939 Royal Society of London Series B-Biological Sciences **282**(1821): 20152430. DOI: 10.1098/rspb.2015.2430.
940 Sangare, I., R. Dabire, B. Yameogo, D. F. Da, Y. Michalakis and A. Cohuet (2014). "Stress dependent infection
941 cost of the human malaria agent *Plasmodium falciparum* on its natural vector *Anopheles coluzzii*." Infection,
942 Genetics and Evolution **25**: 57-65. DOI: http://dx.doi.org/10.1016/j.meegid.2014.04.006.
943 Sangare, I., Y. Michalakis, B. Yameogo, R. K. Dabire, I. Morlais and A. Cohuet (2013). "Studying fitness cost of
944 *Plasmodium falciparum* infection in malaria vectors: validation of an appropriate negative control." Malar J
945 **12**(1): 2. DOI: 10.1186/1475-2875-12-2.
946 Santolamazza, F., E. Mancini, F. Simard, Y. Qi, Z. Tu and A. della Torre (2008). "Insertion polymorphisms of
947 SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms." Malaria Journal
948 **7**(1): 163. DOI: 10.1186/1475-2875-7-163.
949 Saul, A. J., P. M. Graves and B. H. Kay (1990). "A cyclical feeding model for pathogen transmission and its
950 application to determine vectorial capacity from vector infection rates." J Appl Ecol **27**(1): 123-133. DOI:
951 10.2307/2403572.
952 Schneider, P., S. S. C. Rund, N. L. Smith, K. F. Prior, A. J. O'Donnell and S. E. Reece (2018). "Adaptive periodicity
953 in the infectivity of malaria gametocytes to mosquitoes." Proceedings of the Royal Society B: Biological
954 Sciences **285**(1888). DOI: 10.1098/rspb.2018.1876.

955 Seaman, J. A., H. Alout, J. I. Meyers, M. D. Stenglein, R. K. Dabiré, S. Lozano-Fuentes, T. A. Burton, W. S.
956 Kuklinski, W. C. Black and B. D. Foy (2015). "Age and prior blood feeding of *Anopheles gambiae* influences their
957 susceptibility and gene expression patterns to ivermectin-containing blood meals." BMC Genomics **16**(1): 797.
958 DOI: 10.1186/s12864-015-2029-8.
959 Shaw, W. R., I. E. Holmdahl, M. A. Itoe, K. Werling, M. Marquette, D. G. Paton, N. Singh, C. O. Buckee, L. M.
960 Childs and F. Catteruccia (2021). "Multiple blood feeding in mosquitoes shortens the *Plasmodium falciparum*
961 incubation period and increases malaria transmission potential." PLOS Pathogens **16**(12): e1009131. DOI:
962 10.1371/journal.ppat.1009131.
963 Shaw, W. R., P. Marcenac and F. Catteruccia (2022). "*Plasmodium* development in *Anopheles*: a tale of shared
964 resources." Trends Parasitol **38**(2): 124-135. DOI: 10.1016/j.pt.2021.08.009.
965 Smith, D. and F. E. McKenzie (2004). "Statics and dynamics of malaria infection in *Anopheles* mosquitoes."
966 Malaria Journal **3**(1): 13. DOI: 10.1186/1475-2875-3-13.
967 Sondo, P., K. Derra, T. Rouamba, S. Nakanabo Diallo, P. Taconet, A. Kazienga, H. Ilboudo, M. C. Tahita, I. Valéa,
968 H. Sorgho, T. Lefèvre and H. Tinto (2020). "Determinants of *Plasmodium falciparum* multiplicity of infection
969 and genetic diversity in Burkina Faso." Parasites & Vectors **13**(1): 427. DOI: 10.1186/s13071-020-04302-z.
970 Sousa, C. A., J. Pinto, A. P. G. Almeida, C. Ferreira, V. E. Do Rosari and J. D. Charlwood (2001). "Dogs as a
971 favored host choice of *Anopheles gambiae* sensu stricto (Diptera : Culicidae) of Sao Tome, west Africa." Journal
972 of Medical Entomology **38**(1): 122-125. DOI: 10.1603/0022-2585-38.1.122.
973 Stearns, S. C. (1992). The evolution of life histories. Oxford, Oxford University Press.
974 Takken, W. and N. O. Verhulst (2013). "Host preferences of blood-feeding mosquitoes." Annual Review of
975 Entomology **58**: 433-453. DOI: 10.1146/annurev-ento-120811-153618.
976 Therneau, T. M. (2020). "coxme: Mixed Effects Cox Models. R package version 2.2-16." http://CRAN.R-
977 project.org/package=coxme.
978 Tripet, F., F. Aboagye-Antwi and H. Hurd (2008). "Ecological immunology of mosquito-malaria interactions."
979 Trends in Parasitology **24**(5): 219-227. DOI: 10.1016/j.pt.2008.02.008.
980 Van Handel, E. and J. F. Day (1989). "Correlation between wing length and protein content of mosquitoes." J
981 Am Mosq Control Assoc **5**(2): 180-182.
982 Vantaux, A., D. F. de Sales Hien, B. Yaméogo, K. R. Dabiré, F. Thomas, A. Cohuet and T. Lefèvre (2015). "Host-
983 seeking behaviors of mosquitoes experimentally infected with sympatric field isolates of the human malaria
984 parasite *Plasmodium falciparum*: no evidence for host manipulation." Frontiers in Ecology and Evolution **3**(86).
985 DOI: 10.3389/fevo.2015.00086.
986 Vantaux, A., T. Lefèvre, A. Cohuet, K. R. Dabiré, B. Roche and O. Roux (2016). "Larval nutritional stress affects
987 vector life history traits and human malaria transmission." Scientific Reports **6**: 36778. DOI:
988 10.1038/srep36778.
989 Vantaux, A., T. Lefèvre, R. Dabiré and A. Cohuet (2014). "Individual experience affects host choice in malaria
990 vector mosquitoes." Parasites & Vectors **7**: 249.
991 Vantaux, A., I. Ouattarra, T. Lefèvre and K. R. Dabiré (2016). "Effects of larvicidal and larval nutritional stresses
992 on *Anopheles gambiae* development, survival and competence for *Plasmodium falciparum*." Parasites &
993 Vectors **9**(1): 1-11. DOI: 10.1186/s13071-016-1514-5.
994 Vantaux, A., F. Yao, D. F. Hien, E. Guissou, B. K. Yameogo, L. C. Gouagna, D. Fontenille, F. Renaud, F. Simard, C.
995 Constantini, F. Thomas, K. Mouline, B. Roche, A. Cohuet, K. R. Dabire and L. T (2021). "Field evidence for
996 manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*." Peer
997 Community Journal **1**: 13. DOI: 10.24072/pcjournal.13.
998 Vaughan, D. and M. Dancho (2021). "furrr: Apply Mapping Functions in Parallel using Futures." R package
999 version 0.2.3 **https://CRAN.R-project.org/package=furrr**.
1000 Vézilier, J., A. Nicot, S. Gandon and A. Rivero (2012). "*Plasmodium* infection decreases fecundity and increases
1001 survival of mosquitoes." Proceedings of the Royal Society B: Biological Sciences **279**: 4033-4041. DOI:
1002 10.1098/rspb.2012.1394.
1003 Wickham, H., M. Averick, J. Bryan, W. Chang, L. D'Agostino McGowan, R. François, G. Grolemund, Hayes A., L.
1004 Henry, J. Hester, M. Kuhn, T. L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D. P. Seidel, V.
1005 Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo and H. Yutani (2019). "Welcome to tidyverse." J Open Source
1006 Softw **4**: 1686. DOI: 10.21105/joss.01686.
1007 Wintrobe, M. M. (1933). "Variations in size and hemoglobin content of erythrocytes in the blood of various
1008 vertebrates." Folia Haematol **51**: 32-49.
1009 Zera, A. J. and L. G. Harshman (2001). "The Physiology of Life History Trade-Offs in Animals." Annual Review of
1010 Ecology and Systematics **32**(1): 95-126. DOI: 10.1146/annurev.ecolsys.32.081501.114006.

1011

1012

1013

Supplementary files

1014

Appendix

1015 Appendix 1 - Table S1: Parasite prevalence and intensity in females.

Replicate	Parasite isolate	Gametocyte Density (µl)	Blood meal type	Prevalence ± 95% CI	Parasite/midgut ± se	n
1	A	232	Chicken	1 ± 0	50.5 ± 15.5	2
			Cow	0.43 ± 0.37	8.3 ± 4.7	7
			Human	0.85 ± 0.2	15 ± 3.9	13
			Sheep	0.86 ± 0.18	25 ± 6.1	14
2	B	32	Chicken	0.7 ± 0.24	7.9 ± 1.6	15
			Cow	0.43 ± 0.26	4.2 ± 1	14
			Human	0.45 ± 0.21	4.7 ± 0.8	22
			Sheep	0.53 ± 0.17	8.2 ± 1.2	32
3	C	136	Chicken	0.56 ± 0.13	8.6 ± 1.2	52
			Cow	0.65 ± 0.13	10 ± 1.4	49
			Human	0.87 ± 0.09	7.2 ± 0.9	52
			Sheep	0.9 ± 0.08	6.8 ± 0.9	49
4	D	72	Chicken	0.76 ± 0.17	8.4 ± 1.3	25
			Cow	0.78 ± 0.11	11.7 ± 1.2	54
			Human	0.76 ± 0.1	6.4 ± 0.8	66
			Sheep	0.73 ± 0.1	8.5 ± 1	70
5	E	192	Chicken	0.84 ± 0.14	33.5 ± 5.3	25
			Cow	0.81 ± 0.11	21.2 ± 3.1	47
			Human	0.77 ± 0.12	26.6 ± 2.9	47
			Sheep	0.84 ± 0.1	24.1 ± 2.7	49
	F	136	Chicken	0.94 ± 0.08	15.6 ± 2.3	32
			Cow	0.71 ± 0.13	19.6 ± 2.5	51
			Human	0.74 ± 0.12	18.4 ± 2.5	47
			Sheep	0.74 ± 0.13	19.7 ± 2.6	42

1016

1017

1018

1019 Appendix 2 - Table S2- Statistical analyses results

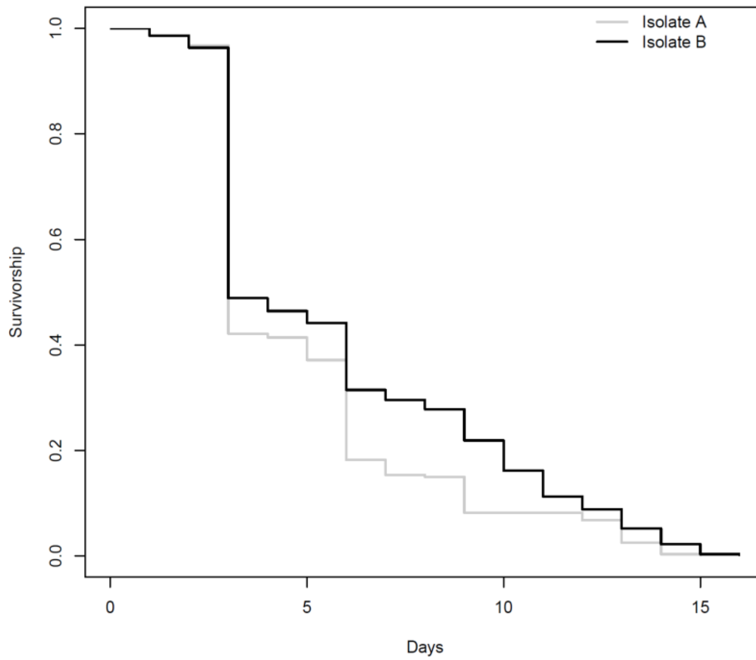
Life-history traits	Response variable	Model	Effect Test Outputs
Competence	Oocyst prevalence, design 1	GLMM, binomial distribution	Blood type: LRT X^2_3 = 5.02, P = 0.17 **Parasite isolate: LRT X^2_1 = 6.92, P = 0.009**
	Oocyst intensity, design 1	GLMM, zero-truncated negative binomial distribution	**Blood type: LRT X^2_3 = 10.55, P = 0.01** **Parasite isolate: LRT X^2_1 = 24.89, P < 0.0001**
	Sporozoite prevalence, design 1	GLMM, binomial distribution	Blood type: LRT X^2_2 = 0.62, P = 0.73 Parasite isolate: LRT X^2_1 = 2.09, P = 0.15
	Oocyst prevalence, design 2	GLMM, binomial distribution	Blood type: LRT X^2_3 = 3.14, P = 0.37 Gametocytemia: LRT X^2_1= 1.37, P = 0.24 Blood type*Gametocytemia: LRT X^2_3= 1.27, P = 0.74
	Oocyst intensity, design 2	GLMM, zero-truncated negative binomial distribution	Blood type: LRT X^2_3= 4.99, P = 0.17 **Gametocytemia: LRT X^2_1=11.09, P =< 0.001** **Blood type*Gametocytemia: LRT X^2_3= 11.06, P = 0.01**
	Sporozoite prevalence, design 2	GLMM, binomial distribution	Blood type: LRT X^2_2 = 0.65, P = 0.72 Gametocytemia: LRT X^2_1 = 1.3, P = 0.25 Blood type*Gametocytemia: LRT X^2_2 = 0.33, P = 0.85
Feeding rate	Proportion of fed females during blood meal 2 to 4	GLMM, binomial distribution	**Blood type: LRT X^2_3 = 14.4, P = 0.002** **Feeding episode: LRT X^2_2 = 160.2, P< 0.0001** **Exposure: LRT X^2_1 = 9.09, P = 0.003** Parasite isolate: LRT X^2_1 = 1.17, P = 0.28 **Blood type*Feeding episode: LRT X^2_6 = 37.8, P< 0.0001** Blood type*Exposure: LRT X^2_3 = 0.38, P=0.94 Exposure*Feeding episode: LRT X^2_2 = 0.03, P=0.99 Exposure*Feeding episode*Blood type: LRT X^2_6 = 12.29, P=0.06
Mosquito blood meal size	Size blood meal 1	ANOVA	**Parasite isolate: $F_{1,115}$=34.6, P < 0.0001** Exposure: $F_{1,113}$=0.48, P = 0.49 Isolate*Exposure: $F_{1,113}$=0.14, P = 0.71
	Size blood meal 2 to 4	GLMM, Gaussian distribution	Blood type: LRT: X^2_3 = 4.2, P = 0.24 **Feeding episode: LRT X^2_2 = 54.04, P < 0.0001** Exposure: LRT X^2_1 = 0.18, P = 0.67 Parasite isolate: LRT X^2_1 = 2.4, P = 0.12 **Blood type*Feeding episode: LRT X^2_6 = 23.7, P = 0.0006** Blood type* Exposure: LRT X^2_3 = 4.7, P = 0.19 Feeding episode*Exposure: LRT X^2_2 = 0.8, P = 0.67 Exposure*Feeding episode*Blood type: LRT X^2_6 = 2.5, P = 0.87
Survival	Survival	Cox proportional hazard mixed models	**Blood type : LRT X^2_3 = 68.26, P < 0.0001** **Parasite isolate: LRT X^2_1 = 24.38, P < 0.0001** Exposure : LRT X^2_1 =0.03, P=0.87 Blood type*Exposure: LRT X^2_3=2.38, P=0.50 Parasite isolate*Exposure: LRT X^2_1=1.25, P=0.26 Blood type*Parasite isolate : LRT X^2_3 =0.27, P=0.96 Blood type*Parasite isolate*Exposure : LRT X^2_3=4.79, P =0.19
Fecundity	Egg-laying rate gonotrophic cycle 1	GLM, binomial distribution	Parasite isolate: LRT X^2_1 = 3.2, P = 0.07 Exposure : LRT X^2_1 = 3.07, P = 0.08 Meal size : LRT X^2_1 = 0.73, P = 0.39 Parasite isolate*Exposure: X^2_1 = 1.3E-8, P = 0.99
	Egg-laying rate gonotrophic cycle 2 to 4	GLMM, binomial distribution	Blood type: LRT X^2_3 = 5.6, P = 0.13 Meal size: LRT X^2_1 = 0.63, P = 0.43

			Gonotrophic cycle: LRT X^2_2 = 53.6, P < 0.0001 Exposure: LRT X^2_1 = 0.08, P = 0.78 Parasite isolate: LRT X^2_1 = 1.8, P = 0.28
	Hatching rate gonotrophic cycle 1	GLM, quasi binomial distribution	Exposure: LRT $F_{1, 102}$= 0.99, P = 0.32 Meal size: LRT $F_{1, 100}$= 0.84, P = 0.36 **Parasite isolate : LRT $F_{1, 102}$=67.59, P < 0.0001** Exposure*Parasite isolate: LRT $F_{1, 100}$=0.76, P = 0.38
	Hatching rate gonotrophic cycle 2 to 4	GLMM, binomial distribution	Blood type: LRT X^2_3 = 5.26, P = 0.15 Exposure: LRT X^2_1 = 2.06, P = 0.15 Meal Size : X^2_1 = 33.43, **P < 0.0001** **Gonotrophic cycle: LRT X^2_2 = 501, P < 0.0001** **Parasite isolate: LRT X^2_1 = 11.58, P <0.001**
	Average number of eggs gonotrophic cycle 1	GLM, quasipoisson distribution	Exposure: LRT $F_{1, 106}$= 0.22, P = 0.85 **Meal size: LRT $F_{1, 108}$= 6.29, P = 0.014** **Parasite isolate: LRT $F_{1, 108}$=41.54, P < 0.0001** Parasite isolate*Exposure: LRT $F_{1, 106}$= 0.01, P = 0.92
	Average number of eggs gonotrophic cycle 2 to 4	GLMM, Gaussian distribution	Blood type: LRT X^2_3 = 0.85, P = 0.84 Meal size: LRT X^2_1 = 0.1, P = 0.75 Gonotrophic cycle: LRT X^2_2 = 3.48, P =0.17 Exposure: LRT X^2_1 = 0.0005, P = 0.98 **Parasite isolate: LRT X^2_1 = 9.12, P = 0.0025**
	Average number of 1st instar larvae gonotrophic cycle 1	GLM, quasipoisson distribution	Exposure: LRT $F_{1,100}$=0.02, P = 0.88 **Meal size : LRT $F_{1,102}$=4.46, P = 0.04** **Parasite isolate: LRT $F_{1,102}$=56.98, P < 0.0001** Parasite isolate* Exposure: LRT $F_{1, 100}$=0.09, P=0.76
	Average number of 1st instar larvae gonotrophic cycle 2 to 4	GLMM, Gaussian distribution	Blood type : LRT X^2_3 = 1.4, P = 0.70 Exposure: LRT X^2_1 = 0.97, P = 0.32 Meal size: LRT X^2_1 = 1.09, P = 0.30 **Gonotrophic cycle: LRT X^2_2 = 10.1, P = 0.006** **Parasite isolate: LRT X^2_1 = 23, P < 0.0001**
	Average lifetime fecundity of eggs over gonotrophic cycle 2-4	GLM, quasipoisson distribution	**Blood type: LRT $F_{1,88}$=4.79, P=0.004** Parasite isolate: LRT $F_{1,84}$=0.02, P=0.90 Exposure: LRT $F_{1,84}$=0.005, P=0.94 Blood type*Parasite isolate: LRT $F_{1,76}$=0.58, P=0.63 Blood type*Exposure: LRT $F_{1,80}$=0.67, P=0.57 Exposure*Parasite isolate : LRT $F_{1,80}$=0.06, P=0.81 Blood type*Exposure*Parasite isolate : LRT $F_{1,76}$=1.11, P=0.35
	Average lifetime fecundity of 1st instar larvae over gonotrophic cycle 2-4	GLM, quasipoisson distribution	**Blood type: LRT $F_{1,88}$=3.86, P=0.012** Parasite isolate: LRT $F_{1,84}$=0.63, P=0.43 Exposure: $F_{1,84}$=0.07, P=0.79 Blood type*Parasite isolate: $F_{1,80}$=0.31, P=0.82 Blood type*Exposure: LRT $F_{1,80}$=0.86, P=0.46 Exposure*Parasite isolate: LRT $F_{1,76}$=0.0059, P=0.94 Blood type*Exposure*Parasite isolate: LRT $F_{1,76}$=0.9, P=0.44
Development time	Development time of larvae from gonotrophic cycle 1	Cox proportional hazard mixed models	Mosquito sex: X^2_1 = 1.23, P = 0.27 Maternal parasite exposure: X^2_1 = 0.15, P = 0.7 Maternal parasite exposure*Mosquito sex : X^2_1 = 0.5, P = 0.48
	Development time of larvae from gonotrophic cycle 2-4	Cox proportional hazard mixed models	Blood type: X^2_3 = 3.7, P =0.3 Density: X^2_1 = 1.5, P =0.23 Maternal parasite exposure: X^2_1 = 0.07, P=0.79 **Mosquito sex : X^2_1 = 3.98, P = 0.046** **Gonotrophic cycle : X^2_2 = 64.4, P < 0.0001**

F1 wing length	F1 wing length gonotrophic cycle 1	GLMM, Gaussian distribution	M**osquito sex: X^2_1=68, P<0.0001** Maternal parasite exposure: X^2_1 = 1.1, P = 0.29 Maternal parasite exposure*Mosquito sex : X^2_1 = 0.24, P =0.62
	F1 wing length gonotrophic cycle 2-4	GLMM, Gaussian distribution	**Blood type: X^2_3 = 12.9, P = 0.005** **Mosquito sex: X^2_1=132, P<0.0001** **Gonotrophic cycle : X^2_2=30, P<0.0001** **Density: X^2_1=9.64, P=0.0019** Maternal parasite exposure X^2_1 = 0.43, P =0.51

1020
1021

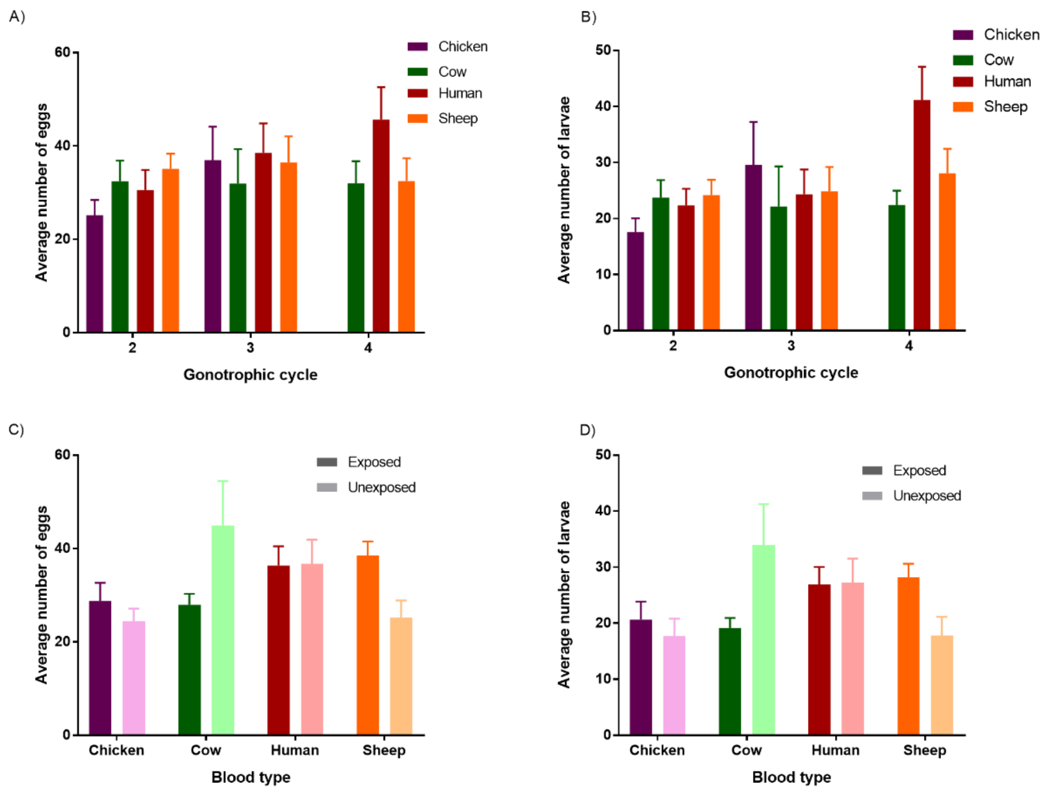
1022 Appendix 3 – Supplementary Figures
1023



1024

1025 Figure S1. Effects of isolates used for the first infectious blood meal on mosquito survivorship.

1026

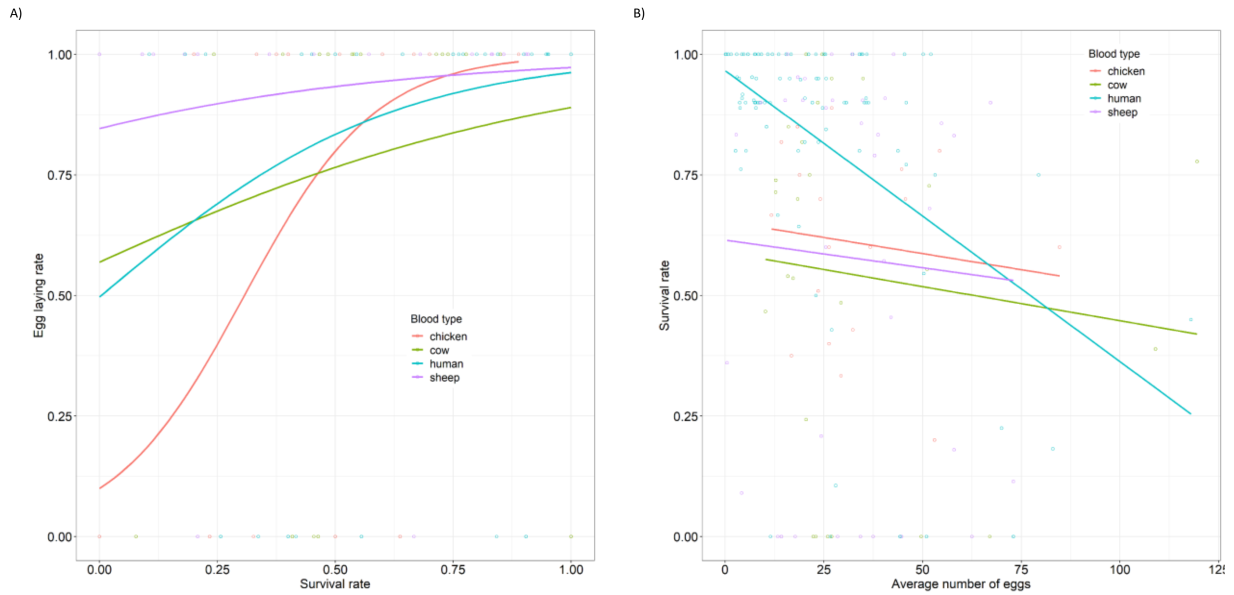


1027

1028 Figure S2. Effects of blood type on A) the average number of eggs per female ± se and B) the average
1029 number of larvae per female ± se, from gonotrophic cycles 2, 3 and 4. Effects of blood type and infection

1030 group (females exposed vs. females unexposed to an infectious blood meal on feeding episode 1) on C)
1031 the average number of eggs per female ± se and on D) the average number of larvae per female ± se
1032 averaged from gonotrophic cycles 2, 3 and 4.

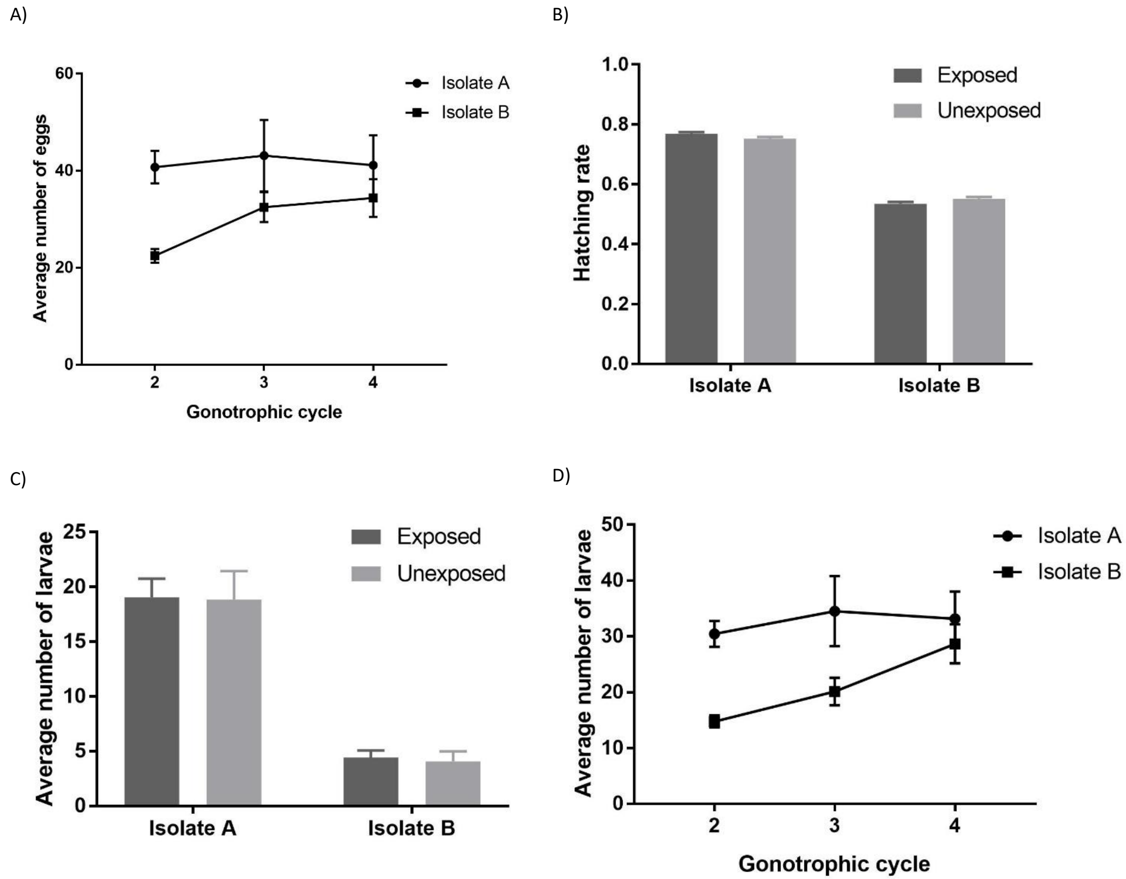
1033



1034

1035 Figure S3. Effect of blood type on A) the relationship between egg laying rate and mosquito survivorship
1036 and on B) the relationship between mosquito survivorship and the average number of eggs.

1037



1038

1039 Figure S4. A) Effects of isolates used for the first infectious blood meal on the average number of eggs per
1040 female ± se for gonotrophic cycles 2, 3 and 4. B) Effects of isolates used for the first infectious blood
1041 meal and infection group (females exposed vs. females unexposed to an infectious blood meal on feeding
1042 episode 1) on the hatching rate (number of larvae/number of eggs) ± 95% CI averaged from gonotrophic
1043 cycles 2, 3 and 4. C) Effects of isolates used for the first infectious blood meal and infection group
1044 (females exposed vs. females unexposed to an infectious blood meal on feeding episode 1) on the
1045 average number of larvae per female ± se averaged from gonotrophic cycles 2, 3 and 4. D) Effects of
1046 isolates used for the first infectious blood meal on the average number of eggs per female ± se for
1047 gonotrophic cycles 2, 3 and 4.

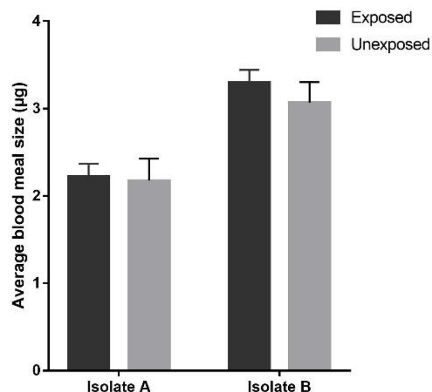
1048

1049 Appendix 4 – Supplementary method and results

1050 **Method**

1051 *Mosquito blood meal size* – Data from the first blood meal were reduced centered (aka centered and scaled)
1052 and analyzed using an ANOVA with parasite isolate, mosquito exposure and their interaction as factors.
1053 *Fecundity* – The effects of parasite exposure, isolate and their interaction on egg laying rate , hatching rate,
1054 average number of eggs, and average number of 1st instar larvae at the first gonotrophic cycle were analysed
1055 using General Linear Models (GLMs) with binomial, quasi binomial, and quasipoisson error structure,
1056 respectively.
1057 The effects of blood type, survival rate and their interaction on the egg-laying rate over the four gonotrophic
1058 cycles were analyzed with a binomial GLMM with rearing cup as a random factor.
1059 The effects of blood type, the average number of eggs and their interaction on the survival rate over the four
1060 gonotrophic cycles were analyzed with a binomial GLMM with rearing cup as a random factor.
1061 The *development time* of larvae from the first gonotrophic cycle was analysed using a Cox proportional hazard
1062 mixed effect model with maternal exposure, sex and their interaction coded as fixed factors, and rearing plastic
1063 cup as a random factor.
1064 *F1 wing length*–Wing length of the progeny from gonotrophic cycle 1was analysed using a Gaussian GLMM with
1065 maternal exposure, mosquito sex and their interactions as fixed factors and rearing cup as a random factor.
1066
1067
1068 **Results**

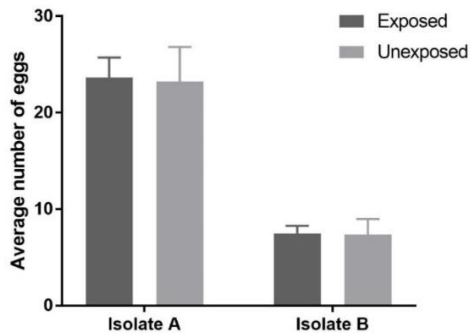
1069 *Mosquito blood meal size* – Following the first feeding episode on human blood, females exposed to
1070 parasite isolate A had smaller blood meals than the ones fed on isolate B (2.21 ± 0.13 µg *vs*. 3.25 ± 0.12 µg
1071 respectively; $F_{1, 115}$=34.6, P < 0.0001, Fig S5). The meal size of females fed on infectious blood was similar to that
1072 of females fed a noninfectious blood (2.76 ± 0.12µg *vs*. 2.61± 0.19 µg respectively; $F_{1, 113}$=0.37, P = 0.49, Fig S5),
1073 regardless of the isolate (parasite isolate*infection status: $F_{1, 113}$=0.14, P = 0.71, Fig S5).



1074

1075 Figure S5. Effects of exposure and parasite isolate on the average blood meal size ± se of the first feeding
1076 episode.

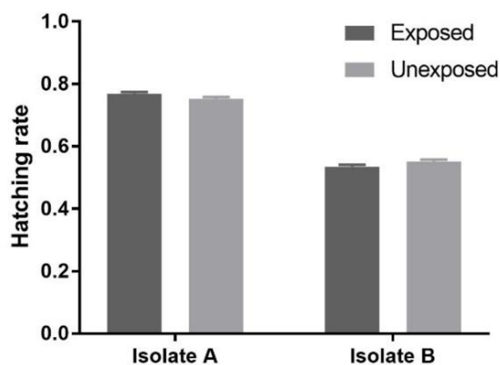
1077
1078 *Fecundity* – The egg-laying rate following the first gonotrophic cycle was high with eggs present in 95 ± 4%
1079 (Fig 7A) of all cups (n=121). There was no effect of blood meal size (X^2_1 = 0.73, P = 0.39), of isolate (X^2_1 = 3.2, P =
1080 0.07), nor of parasite exposure (X^2_1 = 3.07, P = 0.08) nor of isolate by parasite exposure interaction (X^2_1 = 1.3E-8,
1081 P = 0.99) on egg-laying rate.
1082 The average number of eggs laid on the first gonotrophic cycle was 16 ± 1 eggs per female (min = 0.1 eggs
1083 per females and max=52.1 eggs per female). Females exposed to an infectious blood meal on the first
1084 gonotrophic cycle laid as many eggs as unexposed control females (16 ± 1 and 16 ± 3 eggs respectively; $F_{1, 106}$=
1085 0.99, P = 0.32; Fig S6). The average number of eggs laid was negatively correlated to the average blood meal size
1086 ($F_{1, 108}$= 6.29, P = 0.014). There was a strong effect of isolate, with females fed blood from isolate A laying more
1087 eggs than females fed blood from isolate B (24 ± 2 and 7 ± 1 eggs respectively; $F_{1, 102}$= 67.59, P <0.0001),
1088 regardless of parasite exposure (exposure* parasite isolate: $F_{1, 106}$= 0.01, P = 0.92; FigS6).



1089

1090 Figure S6. Effects of exposure and parasite isolate on the average number of eggs ± se of the first feeding
1091 episode.

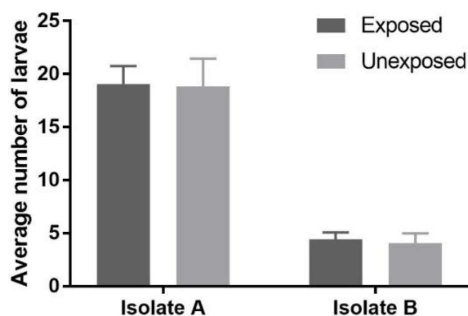
1092 On the first gonotrophic cycle 68.1 ± 0.6% of the eggs hatched. Eggs from females exposed to an infectious
1093 blood meal had a similar hatching rate as eggs from unexposed females (71 ± 0. 6 % and 68 ± 0.6 % respectively;
1094 $F_{1, 102}$= 0.99, P = 0.32, Fig S7). Eggs from females fed on isolate A had a higher hatching rate than eggs from
1095 females fed on isolate B (76.4 ± 0.6 % and 53.9 ± 0.7 % respectively; $F_{1, 102}$=67.59, P < 0.0001), regardless of
1096 parasite exposure (exposure* parasite isolate: $F_{1, 100}$=0.76, P = 0.38, Fig S7). There was no effect of the average
1097 blood meal size ($F_{1, 108}$= 6.29, P = 0.014).
1098



1099

1100 Figure S7. Effects of exposure and parasite isolate on the hatching rate ± 95% CI of the first feeding
1101 episode.

1102 The average number of 1st instar larvae on the first gonotrophic cycle was 12 ± 1 larvae per female. There
1103 was no effect of parasite exposure on the average number of larvae ($F_{1,100}$=0.02, P = 0.88, Fig S8). Females fed
1104 on isolate A had significantly more larvae per female than females fed on isolate B (19 ± 1 and 4 ± 1 larvae per
1105 female respectively; $F_{1,102}$=56.98, P < 0.0001), regardless of parasite exposure (exposure*parasite isolate $F_{1,}$
1106 $_{100}$=0.09, P = 0.76, Fig S8). The average number of 1st instar larvae laid was negatively correlated to the average
1107 blood meal size ($F_{1, 108}$= 6.29, P = 0.014).



1108

1109 Figure S8. Effects of exposure and parasite isolate on the average number of larvae ± se of the first
1110 feeding episode.

1111 The egg-laying rate decreased with the survival rate (X^2_1 = 32.9, P < 0.0001; Fig S3A). Neither the blood type
1112 (X^2_3 = 7.7, P = 0.052) nor the two-way interaction (X^2_3 = 7.6, P = 0.054) significantly affected the egg-laying rate.
1113 The survival rate was negatively associated with the average number of eggs laid (X^2_1 = 5.5, P = 0.02; Fig
1114 S3B) and this association was stronger for the females fed on human blood compared to the other blood types
1115 (X^2_3 = 10.2, P = 0.017; Fig S3B). No effect of the interaction was found (X^2_3 = 3.45, P = 0.33).
1116 *F1 development time* –the F1 development time on the first gonotrophic cycle (10.55±0.04 days) was not
1117 affected by mosquito sex (X^2_1 = 1.23, P = 0.27), nor by maternal parasite exposure (X^2_1 = 0.15, P = 0.7), nor by
1118 their interaction (X^2_1 = 0.5, P = 0.48).
1119 *F1 wing length* – On the first gonotrophic cycle, F1 females were significantly bigger than F1 males (3.14 ±
1120 0.02 *vs.* 2.97 ± 0.02mm; X^2_1=68, P<0.0001). Neither maternal parasite exposure (X^2_1 = 1.1, P = 0.29) nor the two-
1121 way interaction (X^2_1 = 0.24, P =0.62) significantly affected the progeny wing length of gonotrophic cycle 1.
1122
1123