Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*

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Abstract

Whether the malaria parasite *Plasmodium falciparum* can manipulate mosquito host choice in ways that enhance parasite transmission toward human is unknown. We assessed the influence of *P. falciparum* on the blood-feeding behaviour of three of its major vectors (*Anopheles coluzzii, An. gambiae* and *An. arabiensis*) in Burkina Faso. Host preferences assays using odor-baited traps revealed no effect of infection on mosquito long-range anthropophily. However, the identification of the blood meal origin of mosquitoes showed that females carrying sporozoites, the mature transmissible stage of the parasite, were 24% more anthropophagic than both females harbouring oocysts, the parasite immature stage, and uninfected individuals. Using a mathematical model, we further show that this increased anthropophagy in infectious females can have important epidemiological consequences with up to 123% increase in parasite transmission at low mosquito to human ratios. This increase in transmission potential highlights the importance of vector control tools targeting infectious females.

Introduction

There is mounting evidence that malaria parasites affect phenotypic traits of their vectors and hosts in ways that increase contacts between them, hence favouring parasite transmission (Hurd 2003, Lefèvre and Thomas 2008, Koella 2005). In addition to increased vertebrate attractiveness to mosquito vectors (De Moraes et al. 2014, Cornet et al. 2013, Lacroix et al. 2005, Batista, Costa, and Silva 2014, Busula et al. 2017, Emami et al. 2017), another frequently reported parasite-induced change is the alteration of vector motivation and avidity to feed (Cator et al. 2012, Stanczyk, Mescher, and De Moraes 2017). Mosquitoes infected with *Plasmodium*

sporozoites (the mosquito to human transmission stage) can indeed display increased (i) response to host odours (Rossignol, Ribeiro, and Spielman 1986, Cator et al. 2013), (ii) landing and biting activity (Rossignol, Ribeiro, and Spielman 1984, 1986, Wekesa, Copeland, and Mwangi 1992, Anderson, Koella, and Hurd 1999, Koella, Rieu, and Paul 2002, Smallegange et al. 2013), (iii) number of feeds (Koella, Sorensen, and Anderson 1998) and (iv) blood volume intake (Koella and Packer 1996, Koella, Sorensen, and Anderson 1998, Koella, Rieu, and Paul 2002). In contrast, mosquitoes infected with oocysts (the immature non-transmissible stage of the parasite), are less likely to attempt to feed (Anderson, Koella, and Hurd 1999, Koella, Rieu, and Paul 2002, Cator et al. 2013). Since biting is risky (e.g., host defensive behaviours can kill the vector and its parasite), reduced feeding attempts seems beneficial to the parasite (Schwartz and Koella 2001).

These "stage-dependent" behavioural alterations likely increase parasite transmission (Cator et al. 2014, Dobson 1988), provided that mosquito feeds are taken on a suitable vertebrate host species for the parasite. While malaria vectors can usually feed on a range of different vertebrate species (Takken and Verhulst 2013), the malaria parasites they transmit are often highly host-specific, infecting only one or a few vertebrate species (Perkins 2014). For example *P. falciparum*, which causes the most severe form of human malaria, displays an extreme form of specificity and can develop and reproduce in hominids only (predominantly in human and to a lesser extent in chimpanzee, bonobo, and gorilla) (Rayner et al. 2011, Prugnolle et al. 2011, Ngoubangoye et al. 2016), such that any mosquito bite on another vertebrate species would be a dead-end for the parasite. In contrast, the vectors of *P. falciparum* can feed on a wide range of vertebrate hosts species in the wild depending on the geographic area and the relative abundance of human and other vertebrates (Costantini et al. 1999, Takken and Verhulst 2013). Accordingly,

P. falciparum could modify its vector choice in ways that enhance transmission toward human and/or reduce mosquito attraction to other unsuitable host species (i.e. specific manipulation). A previous study testing this hypothesis found no effect of *P. falciparum* infection on host preference of three major vector species, *An. coluzzii*, *An. gambiae,* and *An. arabiensis* (Nguyen et al. 2017). However, this study examined the odour-mediated mosquito host preference in laboratory conditions using a Y-olfactometer, not the final realised host choice which is of primary importance for parasite transmission.

Here, we assessed the influence of *P. falciparum* on *An. coluzzii*, *An. gambiae* and *An. arabiensis* blood-feeding behaviour in three villages of Burkina Faso. First, inherent mosquito host preferences were determined using odor-baited traps, set side by side in a choice arrangement, releasing either human or calf odors. Patterns of host selection were then assessed by the identification of the blood meal origin of indoor-resting samples.

Results

Anthropophily index – To assess the inherent mosquito host preference of field populations of mosquitoes, we used two odour-baited entry traps (OBETs) and two odour-baited double net traps (BNTs) releasing either calf or human odours (Figure supplement S1). The anthropophily index (AI) was expressed as the number of *Anopheles gambiae s.l.* caught in the human-baited trap over the total number of mosquitoes caught in both human- and calf- baited traps. The infection status was successfully determined in 584 out of the 674 mosquitoes (86.6%) collected in the OBETs (383 individuals) and BNTs (201 individuals). Uninfected, oocyst-infected and sporozoite-infected females displayed similar host preferences (X^2_2 = 3.6, P = 0.17, Figure supplement S2), with a significant attraction toward human odours (uninfected females: 63.3 ±

4%, N=531, OR=0.58, 95% CI = 0.53-0.63, P <0.0001; oocyst-infected females: 55.2 ± 18 %, N=29, OR=0.81, 95% CI = 0.56-0.81, P=0.58; sporozoite-infected females: 45.8 ± 20 %; N=24, OR=1.18, 95% CI = 0.78-1.78, P=0.7). There was no effect of collection method on AI (OBETs: 64 ± 5%, BNTs: 59 ± 7%; $X^2_1 = 1.5$, P = 0.21), indicating that both methods are comparable to assess mosquito host preference. There was no interaction between mosquito infection and collection method ($X^2_2 = 0.26$, P = 0.9; Figure supplement S2).

Human blood index – To assess the realized host selection of *Anopheles gambiae s.l.*, the blood meal origins of females collected indoors between 7 am and 9 am were identified. The human blood index (HBI) was expressed as the number of females fed on human (including mixed human-animal bloodmeals) over the total number of blood-fed females. Of the 3447 blood-fed *Anopheles gambiae s.l.* collected indoors, the blood meal origin was successfully identified in 2627 samples (76%). Among these 2627 samples, infection status was successfully determined in 2328 mosquitoes (88.6%). The following analyses are restricted to these 2328 females. HBI was significantly affected by mosquito infection status ($X^2_2 = 13.007$, P = 0.0015; Figure 1) with a 24% increase in HBI in sporozoite-infected females compared to oocyst-infected and uninfected counterparts (sporozoite-infected: 77 ± 5.7%; N=209, deviation from random feeding: OR=0.3, 95% CI = 0.25-035, P <0.0001; oocyst-infected females: 63.6 ± 5.7%, N=118, OR=0.57, 95% CI = 0.47-0.69, P =0.004; uninfected females: 61.1 ± 2.1%; N=2001, OR=0.64, 95% CI = 0.61-0.66, P <0.0001). HBI was also significantly influenced by shelter type ($X^2_2 =$ 145.92, P < 0.0001) and villages ($X^2_2 = 139.5$, P < 0.0001; see supplementary material for details). The HBI of sporozoite-infected mosquitoes was higher than that of oocyst-infected and uninfected females regardless of the village considered (infection status: village interaction: X^2_4

= 2.3, P = 0.68, Figure 1) or the shelter type in which mosquito females were collected (infection status: shelter type interaction: $X^2_4 = 0.7$, P = 0.95, Figure supplement S3).

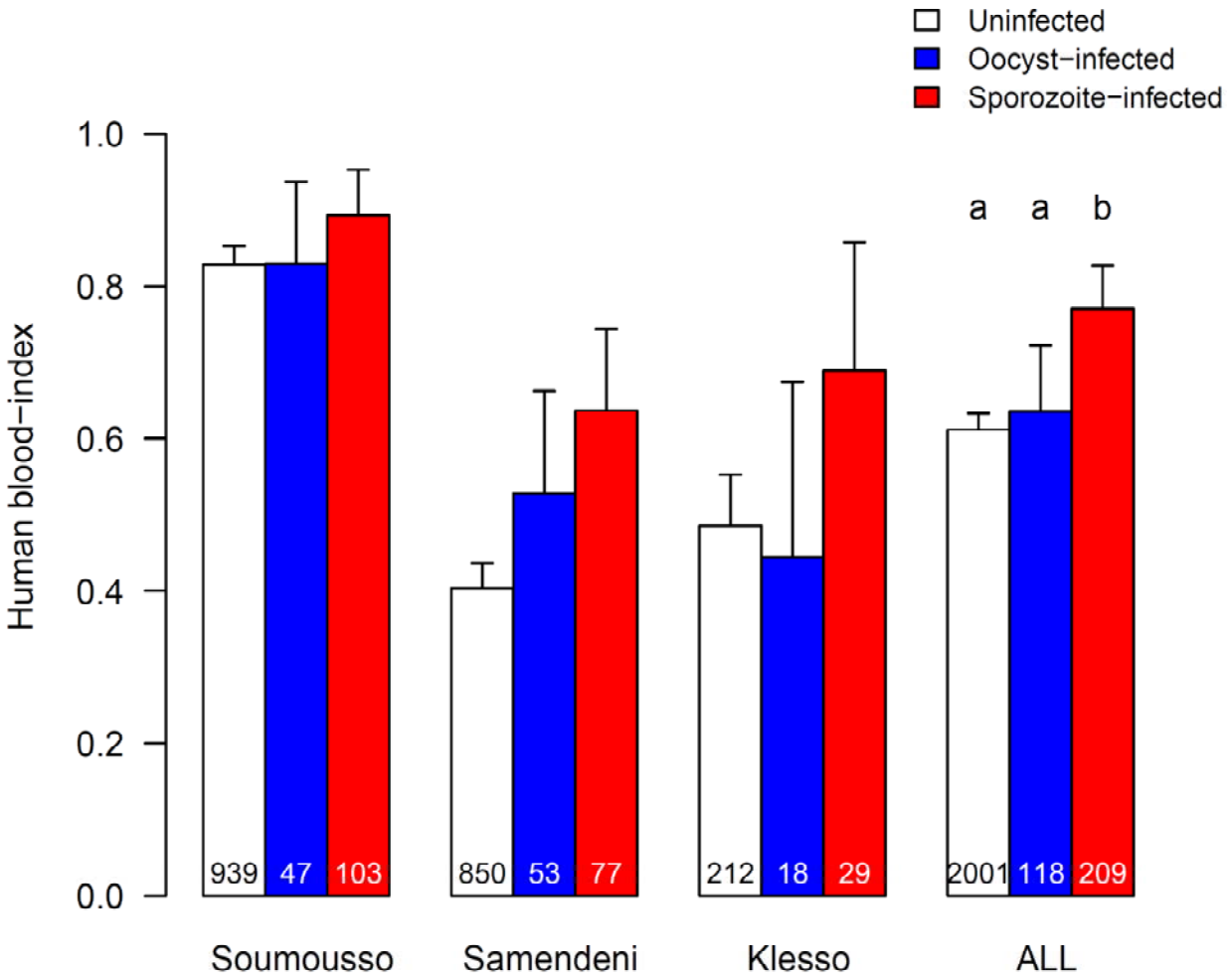

Figure 1. Effect of infection status on the human-blood index of *Anopheles gambiae s. l.* females expressed as the number of females fed on human out of the total number of blood-fed females for the three sampled villages. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes. Different letters indicate differences between infection status (Chi-square *post-hoc* tests: sporozoite-infected *vs.* oocyst-infected females X^2_1=6.1, P=0.013; sporozoite-infected *vs.* uninfected females X^2_1=19.4, P<0.0001; oocyst-infected *vs.* uninfected females X^2_1=0.18, P= 0.67).

120

121 A significant species variation in HBI was observed (X^2_2 = 10.2, P = 0.006; Figure 2)
122 with *Anopheles arabiensis* being significantly less anthropophagic (22.2 ± 15%, N=27, OR=3.5,
123 95% CI = 2.2-5.56, P = 0.007) than *An. gambiae* (54.8 ± 7.1%; N=186, OR=0.82, 95% CI =
124 0.71-0.95, P = 0.19) and *An. coluzzii* (55.1 ± 6.8%; N=205, OR=0.81, 95% CI = 0.71-0.94,
125 P=0.14). Although HBI varied among mosquito species, sporozoite-infected individuals
126 displayed highest anthropophagy regardless of the species considered (infection status: species
127 interaction: X^2_4 = 4, P = 0.42; Figure 2 and supplementary material).

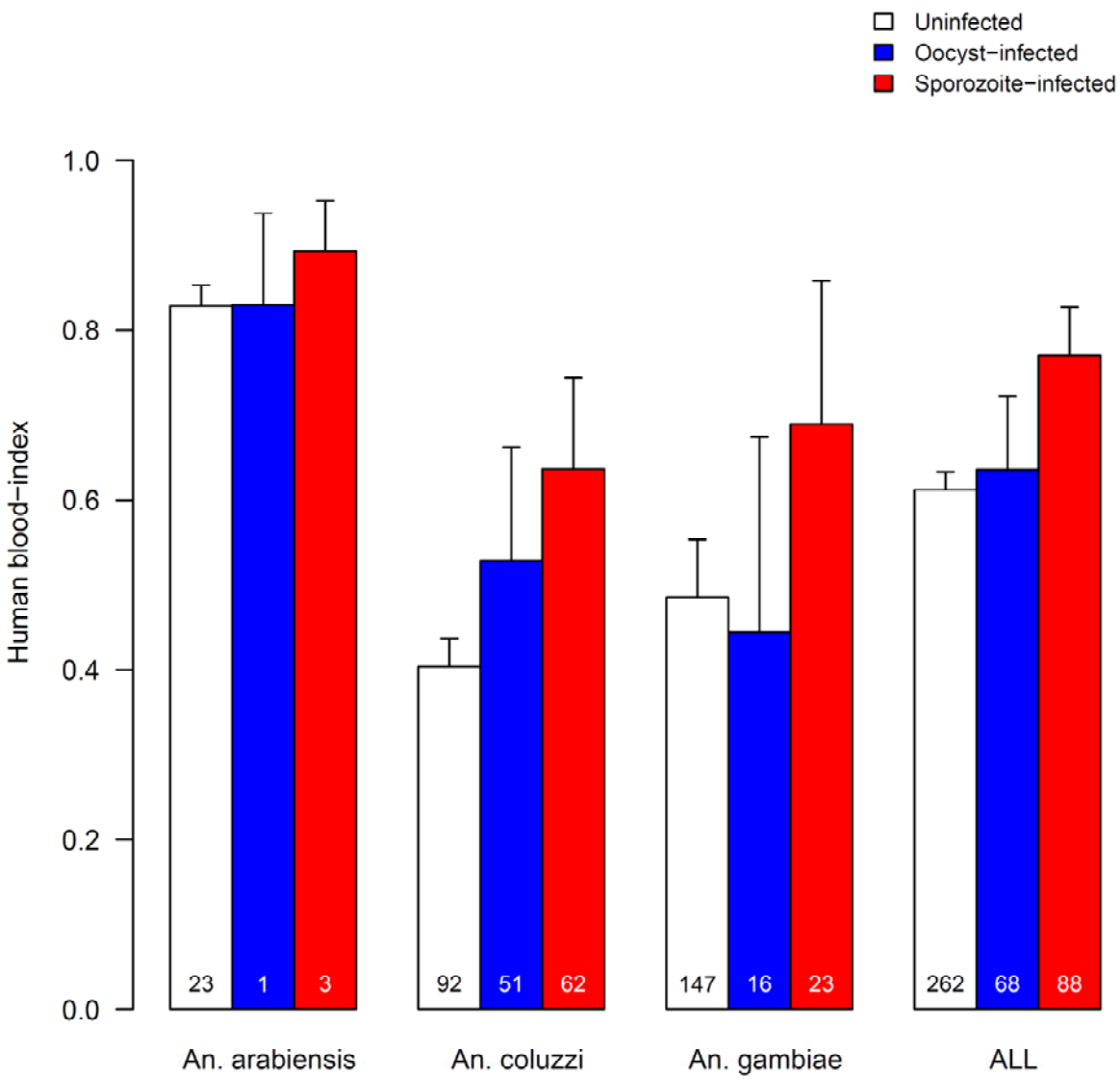

Figure 2. Effect of infection status and *Anopheles* species *sensu stricto* on the human-blood index expressed as the proportion of females fed on human or human and animal out of the total of blood-fed females. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes.

Finally, HBI was not significantly affected by parity (nulliparous females: 49.53 ± 9%, parous females: 45.6 ± 7.5%; $X^2_1 = 0.4$, P = 0.52).

Epidemiological consequences – To investigate the epidemiological impact of a higher HBI in infectious females compared to oocyst-infected and uninfected females, we built a mathematical model based on the experimental values observed in this study. This model assessed the impact of different HBIs on the Entomological Inoculation Rate (EIR, number of infectious bites received by a person during one year) at different mosquito densities. The HBI of susceptible mosquito was fixed at 0.62 (as in uninfected and oocyst-infected mosquitoes) and the impact of HBI variation in infectious (sporozoite-infected) mosquitoes on parasite transmission potential was explored at different mosquito-to-human ratios (Figure 3). At a low ratio of 1 (1 mosquito per human), an HBI of infectious mosquitoes of 0.62 (similar to that of susceptible mosquitoes) resulted in an EIR of 17, while an HBI of 0.77 (as observed here in infectious mosquitoes) resulted in an EIR of 38. In other words, a 24% increase in HBI resulted in a 123% increase in EIR, everything else being equal. Transmission consequences are less striking when the human-to-mosquito ratios were higher (52 *vs.* 65 with a ratio of 10, i.e. a 25% increase in EIR; and 65 *vs.* 71 with a ratio of 100, i.e. a 9% increase in EIR).

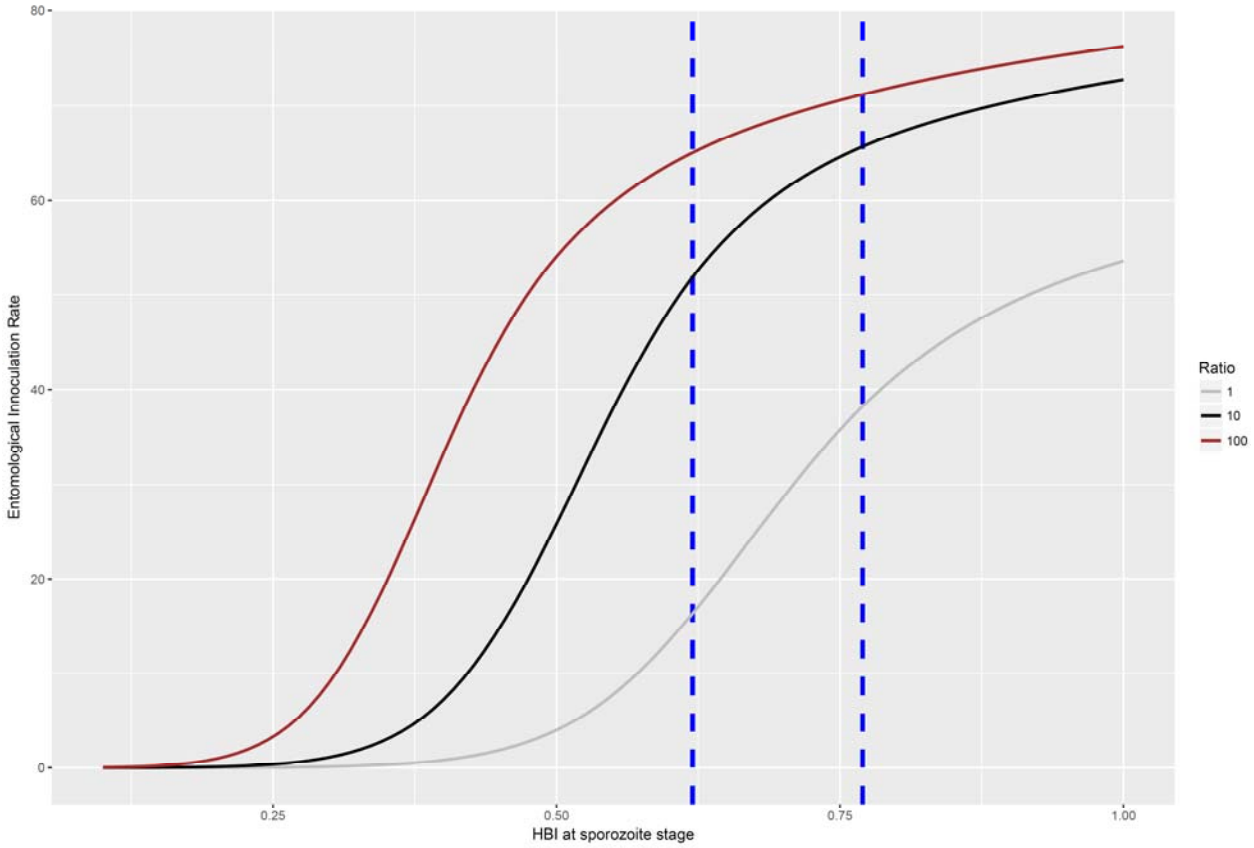

Figure 3. Expected epidemiological consequences of HBI variation. X axis represents the range of values considered for the HBI of infectious (sporozoite-infected) mosquitoes and the Y axis is the Entomological Inoculation Rate (EIR, number of infectious bites received by a person over one year) when the HBI of susceptible (uninfected and oocyst-infected) is 0.62. The plain lines show the evolution of EIR according to HBI of sporozoite infected mosquitoes for different values of mosquito-to-human ratio. The dashed lines represent the value considered for susceptible mosquitoes (0.62) and the value measure for sporozoite-infected mosquitoes (0.77).

Discussion

Consistent with the hypothesis of specific manipulation, the patterns of mosquito host selection showed that sporozoite-infected *An. coluzzi*, *An. gambiae* and *An. arabiensis* females were more

likely to have fed on human than oocyst-infected and uninfected individuals. By distinguishing sporozoite and oocyst infection, we ruled out the potential confounding effect of a mere intrinsic mosquito characteristic. Infected mosquitoes may indeed exhibit increased anthropophagy not because of being infected but just because of an innate preference for human, thus making these mosquito individuals infected (Lefèvre and Thomas 2008). The preference assays (OBETs and BNTs) showed that infected mosquitoes displayed similar long-range attraction toward human odour as uninfected individuals regardless of parasite developmental stages (oocyst vs sporozoite), confirming previous laboratory results (Nguyen et al. 2017).

The precise mechanisms responsible for increased anthropophagy in sporozoite-infected mosquitoes is not yet clear, but at least three hypotheses can be proposed. First, malaria parasites might manipulate mosquito short-range behaviours only, whereas at longer range when mosquitoes rely mainly on CO_2 and other volatile odours, sporozoite-infected mosquitoes display similar preference as uninfected and oocyst-infected individuals. At short range, mosquitoes rely on other cues including visual stimuli, moist, heat and skin emanations (Takken and Verhulst 2013). These stimuli can be host-specific and inform of host suitability for parasite development before the mosquito engage in selection and eventually in feeding. In addition to a possible preferential short-range attraction of sporozoite-infected mosquitoes toward host species suitable for parasite development, there could also be short-range repellence by unsuitable host species.

Second, the parasite may induce changes in the vector such as an alteration of microhabitat choice to spatially match the habitat of the suitable host. This could be achieved through parasite manipulation of mosquito endophagic/philic behaviours resulting in a higher degree of indoor -feeding and -resting of sporozoite-infected females. For example, infectious mosquitoes may exhibit an enhanced tendency to enter house interstices regardless of emitted

187 odors. Such changes could also be mediated through a modification of mosquito phototactic
188 response: while uninfected mosquitoes would respond normally causing the natural fraction of
189 the population to leave the house following a blood-meal, infectious mosquitoes may display a
190 negative phototactic response increasing their chances of staying inside human dwellings. Future
191 experiments exploring the phototactic response of infected and uninfected mosquitoes would
192 help addressing this hypothesis.

193 Third, the parasite may induce changes in the vector such as an alteration of time
194 activity in order to temporally match the time rest or activity of the suitable host. Mosquitoes
195 exhibit circadian rhythms in many activities such as flight, host-seeking, swarming, egg-laying,
196 etc. There is mounting evidence that, following bed-nets introduction, malaria vectors can
197 display increased tendency to feed outdoors (Russell et al. 2011) or bite earlier in the evening or
198 later in the morning (Moiroux et al. 2012). Accordingly, *P. falciparum* could manipulate
199 mosquito host-seeking rhythm in a way that increases bites on unprotected people. Testing this
200 hypothesis would require sampling mosquitoes at distinct period and comparing the proportion
201 of uninfected, oocyst-infected and sporozoite-infected vectors among samples.

202 *Plasmodium falciparum* takes about 10 to 18 days to complete its development
203 (depending on temperature) (WHO manual 2014). Therefore, there is an increased likelihood of
204 sporozoite infection as mosquitoes become older which means that mosquito age could be a
205 confounding factor of infection. In other words, infected mosquitoes may display increased HBI
206 not because they harbour sporozoites but because they are older. Such an age effect could be
207 mediated by specific physiological requirements in old mosquitoes or by a positive
208 reinforcement (learning / memory) of feeding on human. Our data does not support an age effect

as we did not find a significant effect of parity on HBI. However, parity is only a rough proxy of female age and more precise techniques are needed to firmly rule out the potential role of age.

Sporozoite-induced change in mosquito host selection occurred in three major and related mosquito vectors, namely *An. coluzzii*, *An. gambiae* and *An. arabiensis*. This suggests that manipulation likely already occurred in the common ancestor of these three species and that the parasites might exploit a physiological pathway common to all three mosquito species to modify its vector host choice.

Transmission models generally assume that uninfected and infected vectors have similar preference for human. This study suggests that this assumption may not be valid and that these models possibly underestimate transmission intensity. Our modelling approach confirms that HBI increase in infectious mosquitoes can have dramatic impact on disease transmission, especially when the mosquito-to-human ratio is low. At a ratio of 1, there was a 123% increase in transmission potential when the HBI of infectious mosquitoes was set similar to our experimental value. Operationally, this suggests that manipulation of vector host choice may boost parasite transmission during the early rainy season when mosquito density is still low and hence that control measures targeting old infectious females early in the transmission season would likely be efficient in limiting disease transmission.

In conclusion, our results suggest that the human malaria parasite *P. falciparum* evolved the ability to enhance transmission toward human, the appropriate host species, by increasing mosquito anthropophagy (or decreasing zoophagy) with potentially profound public health consequences. Future laboratory and field studies will be essential to confirm these results and to better understand the epidemiological, ecological and evolutionary consequences of parasite manipulation of vector behaviours.

Material and methods

Collection sites

The study was conducted in three villages of South-Western Burkina Faso: Soumousso (11°23'14"N, 4°24'42"W), Klesso (10°56'40.5"N, 3°59'09.9"W) and Samendeni (11°27'14.3"N, 4°27'37.6"W) (Figure supplement S4). The three villages are located in an area characterized by wooded savannah, where *Anopheles* females only have access to temporary, rain-filled puddles and quarries that permit larval development during the rainy season from June to November. The dry season extends from December to May. In these rural villages, domestic animals (including cattle, goats, sheep, pigs, chickens, donkeys, dogs) are usually kept in compounds in open conditions but a few households use separate roofed shelters for sheep, goats, pigs and chicken. Most houses are mud-walled with roofs of iron sheets or thatch, but a few houses are made of bricks.

Field testing of host preference

Two odour-baited entry traps (OBETs as in Lefèvre et al. 2009, Costantini et al. 1996, Costantini et al. 1998) and two odour-baited double net traps (BNTs as in Tangena et al. 2015) baited with calf and human odours were used to assess the host preference of field populations of mosquitoes in Samandeni and Klesso villages (Figure supplement S1).The two OBETs were connected to a tent (Lxlxh: 250x150x150 cm) by air vent hoses (Scanpart®, DxL=10*300cm; Figure supplement S1a). The odours of the two hosts were drawn by a 12-V fan from the tents and into the OBETs by the air vent hoses, coming out of the traps at a speed of 15cm/s (±2cm/s), as measured with a Testo 425-Compact Thermal Anemometer (Testo, Forbach, France) equipped with a hot wire probe [range: 0 to + 20m/s, accuracy: ± (0.03 m/s + 5% of mv)]. Host-seeking

mosquitoes responding to the host cues flew up the odour-laden streams and entered one of the two traps.The two odour-baited double net traps (BNTs) consisted of an untreated bed net (Lxlxh: 300x250x185 cm) from which each corner was raised 20 cm above ground and a smaller untreated bed net (Lxlxh: 190X120X150 cm) protecting the human volunteer in the human baited trap (Figure supplement S1b).

In both OBETs and BNTs, the human volunteers rested on a metal-framed bed (Lxl: 190x80 cm) and were protected from mosquito bites. OBETs and BNTs were operated from 19:00 to 05:30 hours, for 3 nights in June 2013, and 13 nights in September 2013 in Samendeni. Only the BDNTs were set-up for 6 nights in September in Klesso. Trapped mosquitoes were retrieved in the morning using mouth aspirators. They were kept in a 20X20X20 cm cage with a humid towel on top and brought back to the laboratory for further processing (see below).OBETs and BNTs were operated from 19:00 to 05:30 hours. Trapped mosquitoes were retrieved in the morning using mouth aspirators, placed in a 20 x 20 x 20 cm cage with a humid towel on top, and brought back to the laboratory for further processing.

Indoor resting mosquitoes sampling

Indoor resting mosquitoes were collected between 7 am and 9 am by insecticide pyrethroid spray catches. All sample collections were carried out during the rainy and dry seasons for Soumousso village, whereas collections were only carried out in the rainy season for Samendeni and Klesso villages Collections were carried out over 26 days between January and November 2009 in Soumousso, over 4 days in June 2013, and 13 days in September 2013 in Samendeni, and 6 days in September 2015 in Klesso. In Soumousso, human dwellings (from 10 neighbourhoods) only were sampled whereas animal sheds and unoccupied houses were also sampled in Samandeni and Klesso. A total of 27 human dwellings, 7 unoccupied houses and 20 animal sheds were

sampled in Samendeni. A total of 7 human dwellings, 7 unoccupied houses and 9 animal sheds were sampled in Klesso. All mosquitoes were kept in petri dish with a humid paper towel to facilitate later dissection and brought back to the laboratory for further processing (see below).

Laboratory processing of samples

Anopheles gambiae sl. females were dissected in a drop of phosphate buffered saline (PBS) (pH 7.2). Blood-fed midguts were gently squeezed to get the blood out, which was mixed with PBS, absorbed on a filter paper, and then kept at -20°C until identification by an enzyme-linked-immunosorbent assay (ELISA) for Soumousso and Samendeni samples and by multiplex PCR as in Kent and Norris (2005) with minor modifications (see supplementary material) for Klesso samples. Each blood meal was discriminated between human, cattle, goat/sheep, chicken, dog, pig, and horse/donkey origins. The extracted midguts were then stained with 1% mercurochrome to detect with a microscope (20× magnification) the presence and number of *Plasmodium* spp. oocysts. PCR on a subset of oocyst-infected individuals confirmed that these oocysts belonged to *P. falciparum*. Head and thorax of individual mosquitoes were stored at −20°C in 1.5 mL Eppendorf tubes. Sporozoite infection with *P. falciparum* was determined by ELISA for the Soumousso samples (Wirtz et al. 1987) and by qPCR for Samendeni and Klesso samples (Boissière et al. 2013).

This protocol allowed us to gather the following information for each collected individual mosquito: immature *Plasmodium* infection status (presence of oocysts in the midgut); mature *P. falciparum* infection status (presence of sporozoites in salivary glands); source of blood meal or trap (calf/human) chosen; shelter type (human dwellings, unoccupied houses, animal sheds). A total of 3447 blood-fed *Anopheles gambiae s.l.* collected indoors and 674 females collected in the choice traps were processed. In addition, a subset of 276 females collected indoors was used

to determine parity (parous versus nulliparous) based on the condition of ovarian tracheoles. Similarly, a subset of 418 individuals was used to distinguish between the *Anopheles* species sensu stricto (*Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae*) using routine PCR-RFLP as described in Santolamazza *et al*. (2008).

A total of 3447 blood-fed *Anopheles gambiae s.l.* collected indoors and 674 females collected in the choice traps were processed. In addition, a subset of 276 females collected indoors was used to determine parity (parous versus nulliparous) based on the condition of ovarian tracheoles. A subset of 418 individuals was used to distinguish *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae* using routine PCR-RFLP as described in (2008).

Statistical analyses

The anthropophily index (AI) was expressed as the number of *Anopheles gambiae s.l.* caught in the human-baited trap over the total number of mosquitoes caught in both human- and calf-baited traps. We tested the effect of infection status, collection method (OBET vs. BNT), and their interaction on AI using a general linear model (GLM) with a binomial error structure.

The human blood index (HBI) was expressed as the number of *Anopheles gambiae s.l.* fed on human including mixed human-animal bloodmeals over the total number of blood-fed *Anopheles gambiae s.l.*. We tested the effect of *Plasmodium* infection status (uninfected, oocyst-infected, sporozoite-infected individuals - 25 individuals with both oocysts and sporozoites were included in the sporozoite infected group and excluding these individuals from the analysis yielded similar results), village (Soumousso, Samendeni, Klesso), shelter type (human dwelling, unoccupied house, animal shed) and relevant two-ways interactions (infection status by shelter type and infection status by village) on HBI using a general linear model (GLM) with a binomial error structure. The effect of species (*Anopheles gambiae*, *An. coluzzii* and *An. arabiensis*),

infection status, shelter type, and their interactions on HBI was assessed using the subset of females identified to the molecular level using a GLM with a binomial error structure. The effect of parity (nulliparous *vs.* parous) on HBI was assessed on a subset of females using a GLM with a binomial error structure.

We also verified for both AI and HBI whether choice significantly differed from a random distribution between humans and animals or whether mosquitoes displayed a statistically significant attraction to one type of blood meal or trap.

For model selection, we used the stepwise removal of terms, followed by likelihood ratio tests (LRT). Term removals that significantly reduced explanatory power ($P<0.05$) were retained in the minimal adequate model (Crawley 2007). All analyses were performed in R v.3.0.3.

Mathematical model

In order to explore the epidemiological consequences of such variation in HBI, we built a SIR model (Keeling and Rohani 2008):

$$\frac{dS_m}{dt} = \mu N_m - ab\frac{S_m}{N_m} I_h \varepsilon_s - \mu S_m$$

$$\frac{dE_m}{dt} = ab\frac{S_m}{N_m} I_h \varepsilon_s - (\mu + \gamma)$$

$$\frac{dI_m}{dt} = \gamma E_h - \mu I_m$$

$$\frac{dS_h}{dt} = -ac\frac{S_h}{N_h} I_m \varepsilon_i + \delta$$

$$\frac{dI_h}{dt} = ac\frac{S_h}{N_h} I_m \varepsilon_I - \delta$$

Susceptible mosquitoes (S_m) born at rate μ and become exposed (E_m) according to their biting rate (a), their probability to get infected (b) and the HBI of susceptible mosquitoes (ε_s). Then,

exposed mosquitoes become infectious (I_m) according to their extrinsic incubation period (γ). Each mosquito population die at rate (μ). Susceptible humans (S_h) get infected according to mosquito biting rate, probability to develop infection (c) and HBI of infectious mosquitoes (ε_i). Then, infectious humans remain infectious (I_m) during their infectious period equals to $1/\delta$ on average.

See parameters values in table supplement S1. We have fixed HBI of susceptible mosquitoes (ε_s) to the value that has been experimentally measured in this study and we explored the impact of HBI of infectious mosquitoes (ε_i, during the sporozoite stage) on the Entomological Inoculation Rate (EIR, representing the number of infectious bites received by a human during one year (Smith and Ellis McKenzie 2004), as defined by:

$$EIR = ma\frac{I_h}{N_h}$$

where m is the ratio between mosquitoes and humans, and other parameters are similar as above. We assumed different ratios (m) between mosquitoes and humans (low: m=1, medium: m=10 and high: m=100) to explore the impact of different HBIs on the EIR in relation to mosquito densities.

Ethics

Ethical approval was obtained from the Centre Muraz Institutional Ethics Committee under agreement no. 0003-2009/CE-CM and A0003-2012/CE-CM.

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Competing interests

We have no competing interests.

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Supplementary Material

Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*

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Supplementary Results:

HBI was also significantly influenced by shelter type (X^2_2 = 145.92, P < 0.0001). Females collected in animal sheds were significantly less likely to have fed on human host (22.3 ± 4%) than females collected in unoccupied houses (40.9 ± 6.8%; Chi-square post-hoc test: X^2_1 = 21.6, P < 0.0001) or in human dwellings (74.5 ± 2%; Chi-square post-hoc test: X^2_1 = 385, P < 0.0001). Females collected in human dwellings were also significantly more likely to have fed on human host than females collected in unoccupied houses (Chi-square post-hoc test: X^2_1 = 96, P < 0.0001). HBI was significantly affected by the village (X^2_2 = 139.5, P < 0.0001). However, in Soumousso human dwellings only were sampled confounding the effect of village and shelter type in this case. Therefore, we carried out an analysis on the human dwellings only to compare HBIs in the three villages. Mosquitoes were significantly less anthropophagic in Samendeni (56.5± 4%), compared to Soumousso (83.5±2.2%; Chi-square test: X^2_1 =138.8, P < 0.0001) and Klesso (77.3±9 %; Chi -square test: X^2_1 = 12.7, P = 0.0004). HBIs in Soumousso and Klesso were not significantly different (83.5±2.2% *vs*. 77.3±9 % respectively; Chi-square test: X^2_1 = 1.8, P = 0.18).

Species subset

HBI was significantly affected by mosquito infection status (X^2_2 = 8.5, P = 0.014) with sporozoite-infected females being significantly more anthropophagic (71.6 ± 9.4%) than oocyst-infected females (50 ± 11.9%; Chi-square post-hoc tests: X^2_1 =6.7, P = 0.0096) and

uninfected females (47.3 ± 6%; Chi-square post-hoc tests: $X^2_1 = 14.6$, P =0.0001). There was no significant differences between oocyst-infected and uninfected females (Chi-square post-hoc test: $X^2_1 = 0.07$, P = 0.8). HBI was significantly affected by the shelter type ($X^2_2 = 50.8$, P < 0.0001). In particular, the HBI in human dwellings females (73.7 ± 6.3%) was significantly higher than the HBI in unoccupied houses (34.7 ± 9.4%; Chi-square post-hoc tests: $X^2_1 = 39$, P < 0.0001) and animal sheds (37.3 ± 8.2%; $X^2_1 = 40.1$, P < 0.0001). The HBIs of unoccupied houses and animal sheds were not significantly different (Chi-square post-hoc test: $X^2_1 = 0.07$, P = 0.8). There was no significant interactions (infection status*shelter type: $X^2_4 = 2.4$, P = 0.66; shelter types*species: $X^2_4 = 2.3$, P = 0.67; three-way interaction: $X^2_5 = 8$, P = 0.15).

Supplementary Material and methods:

PCR-based determination of the females' blood meals origin targeting the vertebrate host cytochrome B has been performed as described by Kent and Norris, 2005, with the following modifications: *(i)* Three more primers were designed from available Genbank sequences to target the following potential hosts: chicken470F (Genbank accession number: AB044986.1), sheep695F (KY662385.1), donkey574F (FJ428520.1). *(ii)* For each individual, two multiplex reactions were performed to avoid cross-reactions between primers and to optimize the determination. In the multiplex reaction #1, UNREV1025, Chicken470F, Sheep695F, Goat894F and Donkey574F primers were used at an amplification temperature of 49.2 °C. In the multiplex reaction #2, UNREV1025, Dog368F, Human741F, Cow121F and Pig573F primers were used at an amplification temperature of 58°C. Blood meal origin diagnostic was based on the PCR products expected sizes as follow: donkey (460bp), sheep (340bp), chicken (290bp), goat (150bp), dog (680bp), cow (561bp), pig (453bp), human (334bp).

Supplementary Table

Table S1: Parameters used in the mathematical model.

Parameter	Unit	Value
a (biting frequency)	days.ind-1	4
b (mosquito probability to get infected)	%	0.5
ε_s (human biting rate of susceptible mosquitoes)	%	0.62
γ (extrinsic incubation period)	days.ind-1	14
μ (mosquito population dying rate)	days.ind-1	30
c (human probability to develop infection)	%	0.5
ε_i (human biting rate of infectious mosquitoes)	%	variable
$1/\delta$ (human infectious period)	day	30

Supplementary figures

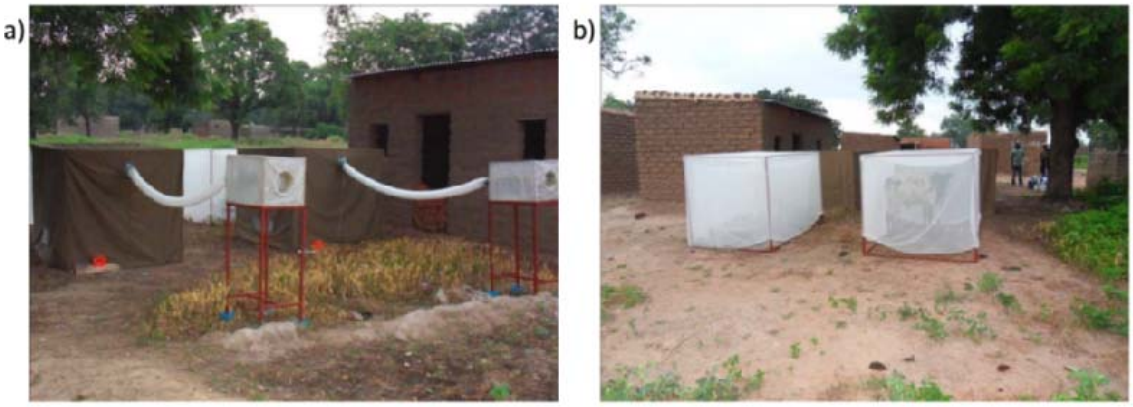

Figure S1. Traps baited with calf and human odours used to assess the host preference of field populations of mosquitoes in Samandeni and Klesso villages. **a)** Two odor-baited entry traps (OBETs) were connected to a tent by air vent hoses. **b)** Two odor-baited double net traps (BNTs).

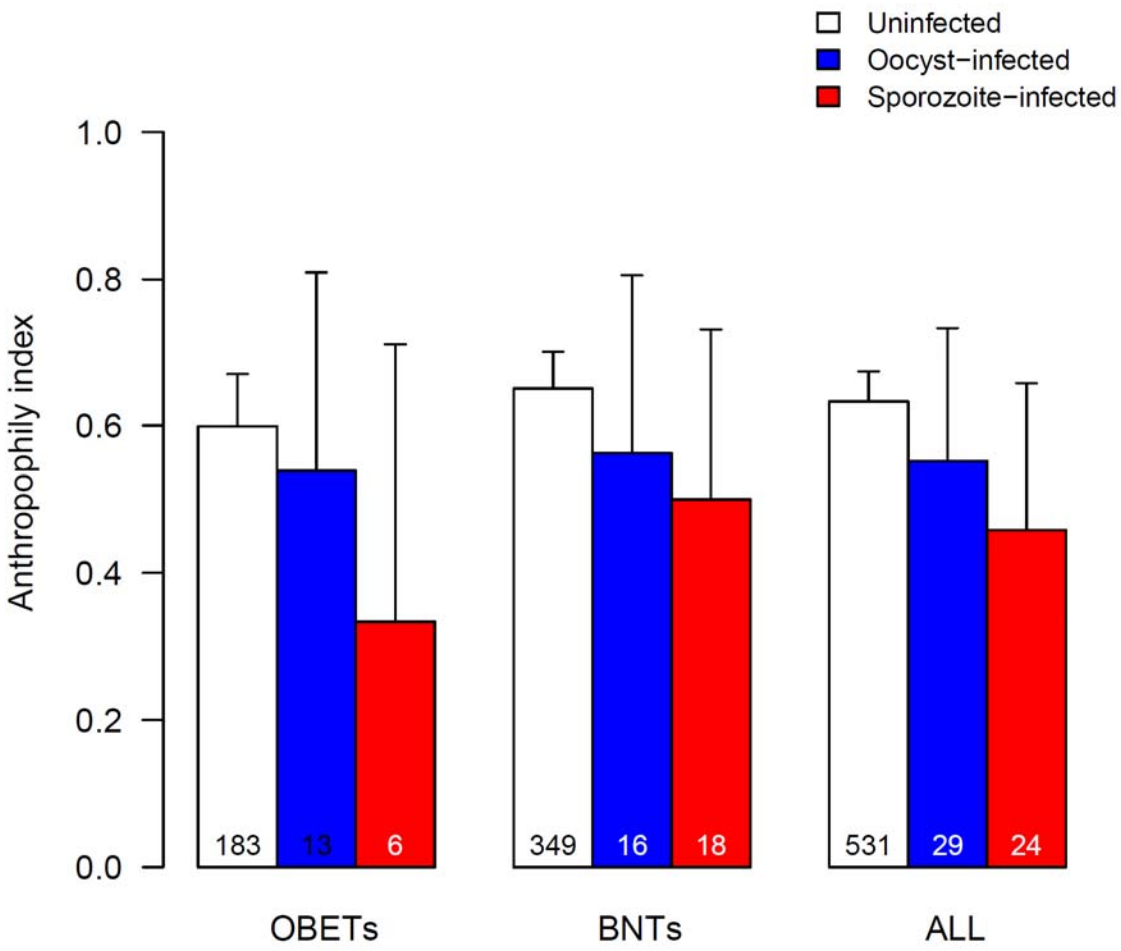

Figure S2. Effect of infection status on the anthropophily index of *Anopheles gambiae s. l.* females expressed as the proportion of females caught in the human-baited traps out of the total number retrieved from both human- and calf- baited traps. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes in both traps.

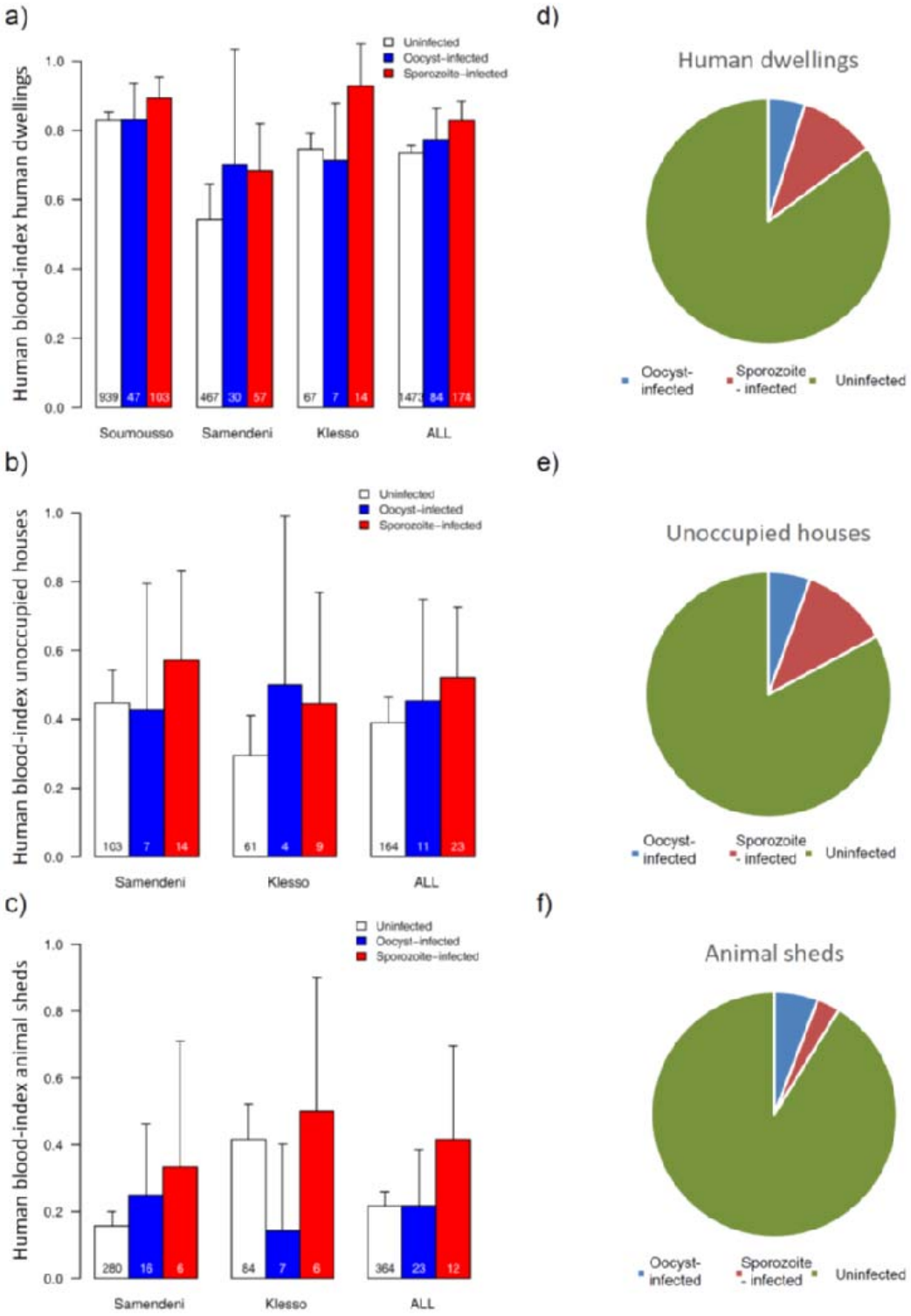

Figure S3. Effect of infection status on the human-blood index of *Anopheles gambiae s. l.* females expressed as the number of females fed on human or human-animal mixed blood meal over the total number of blood-fed females in the different villages samples in a) human

dwellings, b) unoccupied houses and c) animal sheds. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes in both traps. Relative proportions of females according to their infection status in d) human dwellings, e) unoccupied houses and f) animal sheds.

Figure S4. Study collection sites: Soumousso (11°23’14”N, 4°24’42”W), Klesso (10°56’40.5”N, 3°59’09.9”W), Samendeni (11°27’14.3”N, 4°27’37.6”W)