



High *Plasmodium* infection intensity in naturally infected malaria vectors in Africa



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ABSTRACT

The population dynamics of human to mosquito malaria transmission in the field has important implications for the genetics, epidemiology and control of malaria. The number of oocysts in oocyst-positive mosquitoes developing from a single, naturally acquired infectious blood meal (herein referred to as a single-feed infection load) greatly influences the efficacy of transmission blocking interventions but still remains poorly documented. During a year-long analysis of malaria parasite transmission in Burkina Faso we caught and dissected wild malaria vectors to assess *Plasmodium* oocyst prevalence and load (the number of oocysts counted in mosquitoes with detectable oocysts) and the prevalence of salivary gland sporozoites. This was compared with malaria endemicity in the human population, assessed in cross-sectional surveys. Data were analysed using a novel transmission mathematical model to estimate the per bite transmission probability and the average single-feed infection load for each location. The observed oocyst load and the estimated single-feed infection load in naturally infected mosquitoes were substantially higher than previous estimates (means ranging from 3.2 to 24.5 according to seasons and locations) and indicate a strong positive association between the single-feed infection load and parasite prevalence in humans. This work suggests that highly infected mosquitoes are not rare in the field and might have a greater influence on the epidemiology and genetics of the parasite, and on the efficacy of novel transmission blocking interventions.

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1. Introduction

In the effort to control malaria, understanding the processes governing transmission of wild parasites between vectors and vertebrate hosts is paramount. Quantitative information on the number of parasites in wild mosquito populations is lacking and very few studies have investigated either the infectivity of the human parasite reservoir to wild mosquitoes (Graves et al., 1988; Bonnet et al., 2003; Ouédraogo et al., 2016; Gonçalves et al., 2017) or malaria parasites in naturally infected wild mosquitos (Pringle, 1966; Billingsley et al., 1994; Hogg and Hurd, 1997; Gouagna et al., 2010).

Oocyst intensity describes the average number of visible oocysts in all mosquitoes fed on the same blood source (Medley et al., 1993). This quantity is easy to measure in experimental infections (where laboratory-reared mosquitoes can be given a single blood meal and maintained in an insectary), although it is difficult to quantify in the wild. This is because the number of detectable oocysts in wild caught mosquitoes might have been acquired over multiple blood meals and be at various stages of development. For example, wild mosquitoes might feed multiple times before oocysts developing from gametocytes ingested during the first blood meal become observable. Here we define the term oocyst load to be the average number of oocysts in wild caught mosquitoes with detectable oocysts (by microscopy), and single-feed infection load to be the average number of oocysts acquired during a single infectious blood meal that leads to oocyst development. Oocyst intensity, load and exposure are related to one

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another but only oocyst load is directly measurable in wild caught mosquitoes as the sources of previous blood meals are unknown.

Understanding the effectiveness of current and novel drugs and vaccines which target malaria and how best they should be evaluated and deployed in the field depends on a better understanding of human population infectivity (The malERA Consultative Group on Monitoring Evaluation and Surveillance, 2011; The malERA Consultative Group on Vaccines, 2011; Nunes et al., 2014). Vaccines which interrupt malaria parasite transmission by targeting sexual and sporogonic stages of the parasite are referred to as transmission-blocking vaccines. The efficacy of these vaccine candidates in reducing the number of infected mosquitoes shows a dose response to single-feed infection load (Churcher et al., 2012; Miura et al., 2016; Bompard et al., 2017) which depends primarily on the number of mature gametocytes in the blood, although many other factors are known to contribute. These include gametocyte maturity, parasite clone diversity, host blood component and environmental conditions (Price et al., 1980; Lensen et al., 1999; Drakeley et al., 2004; Nsango et al., 2012; Lefèvre et al., 2013; Bompard et al., 2017). The single-feed infection load therefore represents the realised infectiousness of a human host, assuming that the mosquito survives long enough for all oocysts to develop.

The current consensus is that oocyst load and single-feed infection load in the wild is very low, with each wild mosquito carrying less than five oocysts (Rosenberg, 2008). This might be true but very few studies have directly investigated single-feed infection load and values are likely to vary between locations and over seasons as environmental conditions and host factors affect the endemicity of *Plasmodium* transmission (Pringle, 1966; Endo and Eltahir, 2016). Field studies involving collection and dissection of wild caught mosquitoes are logistically difficult and time-consuming, so it is not practical to repeat these studies at every location where transmission blocking interventions may be used. Thus, to predict the intervention efficacy in the field and plan for deployment, we need to better understand the level of single-feed infection load in the wild and find measures of disease endemicity which are easier to collect and capable of predicting its changes.

A year-long field study was conducted in two high transmission villages in Burkina Faso, collecting information on *Plasmodium* parasites in wild mosquitoes caught in huts, and in the local human population. Wild mosquitoes were also collected and dissected to investigate *Plasmodium* parasites in two villages of Cameroon. It is not possible to reliably quantify the number of oocysts in a mosquito by dissection until the latest blood meal has digested, therefore mosquitoes collected in huts were kept in an insectary and dissected three and 7 days following collection (Gouagna et al., 2010) to detect oocysts and sporozoites. A novel mosquito transmission model was developed to support data analysis and estimate the single-feed infection load from the collected data.

2. Materials and methods

2.1. Data

Entomological and parasitological surveys were performed in rural endemic areas of Burkina Faso and Cameroon, to evaluate the *Plasmodium* human-mosquito spatial-temporal transmission. Two villages in each country served as sites for data collection: Klesso and Longo in Burkina Faso, and Mbelengui and Ekali in Cameroon. In Burkina Faso, data were collected during four different periods in 2014: the dry season (March to April), the start of the rainy season (July), the peak of the rainy season (September) and the end of the rainy season (November). In Cameroon, we were only able to collect data during one period in 2015 (May to July).

2.1.1. Mosquito collection

During each period, mosquito collection was uniformly organised: 10 people collected live mosquitoes each morning for at least 20 days using a map highlighting the collection sites in each village. Mosquitoes were caught using a mouth aspirator in the living rooms of human dwellings early in the morning (Anthony et al., 2000). In parallel, hut characteristics were reported, such as: presence of a bednet, type of roofing (straw, slab or metal), presence or not of paint on the walls, and localisation within locally defined quarters. Mosquitoes were counted, *Anopheles* females were identified, pooled in a cage per village and brought to the laboratory to be maintained in the insectary. During each period, a subset of collected mosquitoes was dissected to identify feeding status, parturition rate and gravidity at the time of collection.

2.1.2. Mosquito rearing and dissection

Mosquito females were maintained in the insectary and dissected following the work of Gouagna et al. (2010). For each collection date and location in Burkina Faso, mosquitoes were maintained in the laboratory (27–28 °C, 70–80% relative humidity, dark: light photoperiod 12:12 h), half of the population for 3 days and the rest for 7 days before dissection. Every mosquito midgut was dissected to count normal oocysts (as presented in the Results section) and report the number of broken oocysts (for further information on broken oocysts see Supplementary Data S1 and Supplementary Fig. S1). After dissection, we randomly selected 100 females from each village and season among mosquitoes without oocysts, and 100 females among mosquitoes with oocysts. The head and thorax of each of these mosquitoes were used for mosquito species identification via PCR (Fanello et al., 2002) and sporozoite detection via quantitative PCR (Boissière et al., 2013). The sporozoite rates were then adjusted using oocyst prevalence to reflect the population rate for each season and location. In Cameroon, mosquitoes were collected and then maintained in the laboratory under similar conditions. Dissections were only carried out on day 7 to assess sporozoite presence and oocyst number.

The single-feed infection load could not be deduced from the difference in oocyst numbers between day 7 and day 3 dissections in Burkina Faso, as in the method of Gouagna et al. (2010). To understand how many parasites were acquired during a single blood meal, we conducted a supplementary laboratory experiment on artificially infected mosquitoes to identify the speed at which oocysts become detectable when mosquitoes are reared in the laboratory (presented in Supplementary Data S2 and Supplementary Fig. S2–4), and fitted a transmission model to our data (presented in Section 2.2.).

2.1.3. Blood collection

At the end of each mosquito collection period, blood samples were collected from volunteers within the two Burkina Faso villages. Blood smears were carried out for 300 people per time point and village (100 per age class, <10 years old, 10–20 years old, >20 years old), and malaria-positive people received treatment (Ethical clearance 2014-040/MS/MRSI/CERS from the Comité Ethique pour la Recherche en Santé, Burkina Faso). Gametocytes, trophozoites and schizonts were counted and the *Plasmodium* spp. identified by microscopy. For each blood donor, one thick and one thin smear were made on the same slide. Gametocytes and schizonts were counted against 1000 white blood cells or on up to 100 fields on the thick smear; slides were declared negative after a minimum reading of 100 fields. Trophozoites were counted against 200 white blood cells, and counting was extended to 500 white blood cells if less than 10 parasites were found. Parasite numbers were converted to parasite densities assuming 8000 white blood cells/ μ l. Each slide was read three times by three independent qualified technicians, and results were compared two by

two to assess concordance. If the difference between two reading results was less than half of their mean, the readings were validated and the mean of the three results was kept as the parasite density. If the difference was greater between two of the three readings, the slide was re-read again until concordance was achieved.

2.2. Analysis

The data collected were first analysed using generalised linear mixed-effect models with the R package glmmADMB (<http://glmmadmb.r-forge.r-project.org/>). Prevalence data (for mosquitoes in houses, blood stage and mosquito stage parasites) was analysed using a binomial distribution, while intensity data presenting a moderate overdispersion (such as mosquito abundance in houses) was analysed using a zero-truncated Poisson distribution. Parasite loads in infected mosquitoes or blood samples displayed a high overdispersion, so we used the zero-truncated negative binomial distribution for their analysis. This distribution accounts for overdispersion by taking an arithmetic mean parameter together with an over-dispersion parameter: particular care should be made interpreting the results and estimating values with small datasets, but our dataset is large enough to overcome these issues (see [Supplementary Table S1](#)). P values below 5% were considered significant. The confidence intervals presented around the mean for parasite loads and intensity data were obtained by bootstrapping using the R package boot (<https://astrostatistics.psu.edu/su07/R/html/boot/html/00Index.html>).

We developed a compartmental differential equation model to track the mean oocyst load in the mosquito population and the proportion of susceptible, infected and infectious mosquitoes, as well as oocyst prevalence and load, and sporozoite prevalence in mosquitoes removed from the transmission cycle and reared for three to 7 days in the laboratory (programmed in Berkley Madonna, version 8.3.18, <https://berkeley-madonna.myshopify.com/>). The parameters of this model were then adjusted to match model results with the data collected in the field. It is described in the following sections.

2.2.1. Prevalence and load compartmental model

A simple Susceptible – Exposed – Infectious (SEI) model framework has been adapted to describe the prevalence of mosquitoes with different stages of infection, be it mosquitoes that have never been infected S_v , those that have been exposed and have developing oocysts (denoted $E_{v,1}, E_{v,2}$), exposed-infectious if they have oocysts and sporozoites (EI_v), and infectious if they only have sporozoites (I_v). Exposed mosquitoes are in $E_{v,1}$ if their oocysts are less than 7 days old and in $E_{v,2}$ if they are older. The total mosquito population is given by:

$$M = S_v + E_{v,1} + E_{v,2} + EI_v + I_v \tag{1}$$

Total mosquito abundance (M) remains the same throughout as the birth rate and death rate are equal. We assume that mosquitoes become infectious as soon as one oocyst breaks up and remain infectious until death. In order to account for a gradual break up of oocysts in the mosquito, we consider that oocysts develop for 7 days (Griffin et al., 2010), then start breaking at a constant rate, δ . The equations describing the mosquito population are:

$$\begin{aligned} \frac{dS_v}{dt} &= M\lambda - abX_h S_v - S_v\lambda \\ \frac{dE_{v,1}}{dt} &= abX_h S_v - E_{v,1}(t-7) * \exp(-7\lambda) - E_{v,1}\lambda \\ \frac{dE_{v,2}}{dt} &= E_{v,1}(t-7) * \exp(-7\lambda) - T_{E \rightarrow EI} - E_{v,2}\lambda \\ \frac{dEI_v}{dt} &= abX_h I_v + T_{E \rightarrow EI} - T_{EI \rightarrow I} - EI_v\lambda \\ \frac{dI_v}{dt} &= T_{EI \rightarrow I} - abX_h I_v - I_v\lambda \end{aligned} \tag{2}$$

Where λ is the mosquito birth and mortality rate, a is the number of mosquito bites per day per mosquito ($a = 0.33$), b is the probability that a mosquito develops an oocyst from biting a host with microscopically detectable parasites, X_h is the prevalence of malaria in the human population (determined by microscopy) and θ is the average number of oocysts acquired at each new blood-meal in those mosquitoes that develop oocysts (the single-feed infection load). Sub-patent infections (which have no detectable parasites by microscopy) will contribute substantially to transmission (Ouédraogo et al., 2016) so b should be interpreted as the transmission probability per patent infection not per infectious person. A full description of all parameters, their values and whether they were estimated as part of this work is provided in [Supplementary Table S2](#).

The total number of oocysts in the mosquito population ($O_{v,1}$ for oocysts younger than 7 days and $O_{v,2}$ for > or equal to 7 days) is given by :

$$\begin{aligned} \frac{dO_{v,1}}{dt} &= MabX_h\theta - O_{v,1}(t-7) * \exp(-7\lambda) - O_{v,1}\lambda \\ \frac{dO_{v,2}}{dt} &= O_{v,1}(t-7) * \exp(-7\lambda) - O_{v,2}\lambda\delta \end{aligned} \tag{3}$$

At each time unit, the number of mosquitoes that transfer from stage $E_{v,2}$ to stage EI_v is given by $T_{E \rightarrow EI}$ and from stage EI_v to stage I_v by $T_{EI \rightarrow I}$. As soon as one oocyst has broken, an exposed mosquito becomes exposed-infectious and transfers from stage $E_{v,2}$ to stage EI_v ; as soon as all oocysts have broken, a mosquito becomes infectious and transfers from stage EI_v to stage I_v if it hasn't been re-infected with new oocysts during that time. An infectious mosquito (I_v) can become infected-infectious (EI_v) if it develops oocysts following a new infection. The probability that at least one oocyst among the θ oocysts developing in a mosquito from a single blood-meal breaks up during a timestep is given by $1 - (1 - \delta)^\theta$; the average amount of time necessary for all oocysts to break up (less than one remaining) after an infection is:

$$h = 7 + \frac{\ln(\theta)}{\delta} \tag{4}$$

the average amount of time necessary to transfer from EI_v to I_v is:

$$h' = \frac{\ln(\theta)}{\delta} + 7 \left(\frac{abX_h I_v}{EI_v} \right) \tag{5}$$

Thus, $T_{E \rightarrow EI}$ and $T_{EI \rightarrow I}$ are given by :

$$\begin{aligned} T_{E \rightarrow EI} &= E_{v,2} \left(1 - (1 - \delta)^\theta \right) \\ T_{EI \rightarrow I} &= EI_v \frac{(1 - abX_h)^h}{h} \end{aligned} \tag{6}$$

Temporal fluctuations in mosquito emergence mean that mosquito infection typically fluctuates from day-to-day. Here data was collected and pooled over multiple days so the effect of these short-term fluctuations can be ignored. If we make the assumption that the prevalence of malaria in the human population remains constant over the time in which mosquitoes were collected, then Eq. (2) can be set at equilibrium (denoted with the superscript*) to describe the average mosquito infection status at a particular time and place:

$$\begin{aligned} S_v^* &= \frac{\lambda}{abX_h + \lambda} M \\ E_{v,1}^* &= \frac{abX_h S_v^*}{\exp(-7\lambda) + \lambda} \\ E_{v,2}^* &= \frac{E_{v,1}^* \exp(-7\lambda)}{1 - (1 - \delta)^\theta + \lambda} \\ EI_v^* &= h' \frac{abX_h (M - (S_v^* + E_{v,1}^* + E_{v,2}^*) + E_{v,2}^* (1 - (1 - \delta)^\theta))}{(1 - abX_h)^h + abX_h h' + \lambda h'} \\ I_v^* &= \frac{EI_v^* (1 - abX_h)^h}{(abX_h + \lambda) h'} \end{aligned} \tag{7}$$

Similarly, the average number of oocysts per mosquito can also be set at equilibrium:

$$O_{v,1}^* = \frac{abX_h\theta}{\exp(-7\lambda_1)+\lambda_1}M$$

$$O_{v,2}^* = \frac{O_{v,1}^*\exp(-7\lambda_1)}{\delta+\lambda_1}$$
(8)

2.2.2. Estimates on day 3 and day 7 after collection

We consider that the population is at equilibrium when taken to the laboratory. Inside the laboratory, conditions are more stable but the proportion of mosquitoes with oocysts and sporozoites will change. This is because there are no new infections or mosquitoes being born (susceptible) and because mosquitoes might die at a lower rate than they do in the wild (denoted with the subscript 1 in λ_1).

The infection status will depend on the day after collection that the mosquito is dissected. Let j denote the number of days in the laboratory. Oocysts can still mature and break up: at day j after collection and for $j < 7$, $T'_{E-EI}(j)$ infected mosquitoes become infected-infectious and $T'_{EI-I}(j)$ infected-infectious mosquitoes become infectious. The proportion of susceptible (S_{lab}), exposed (E_{lab}), exposed-infected (EI_{lab}) and infectious mosquitoes (I_{lab}) in the laboratory becomes:

$$S_{lab}(j) = S_v^*\exp(-j\lambda_1)$$

$$E_{lab}(j) = E_v^*\exp(-j\lambda_1) - T'_{E-EI}(j)$$

$$EI_{lab}(j) = E_v^*\exp(-j\lambda_1) + T'_{E-EI}(j) - T'_{EI-I}(j)$$

$$I_{lab}(j) = I_v^*\exp(-j\lambda_1) + T'_{EI-I}(j)$$
(9)

With :

$$T'_{E-EI}(j) = E_v^* \left(1 - (1 - \delta)^\theta\right) \sum_{i=1}^j \exp(-(8-i)\lambda + i\lambda_1)$$

$$T'_{EI-I}(j) = \frac{\delta}{\ln(\theta)} \sum_{i=1}^j (E_v^* + T'_{E-EI}(i)) \exp(-i\lambda_1)$$
(10)

Similarly, the number of developing oocysts in the mosquito population at day j after collection becomes:

$$O_{lab}(j) = O_v^* \exp(-\lambda_1 j) - O_v^* \delta \sum_{i=1}^j \exp(-(8-i)\lambda + i\lambda_1)$$
(11)

2.2.3. Oocyst detection in the laboratory

Oocysts can be difficult to detect by microscopy. In particular, oocysts that are only 3 days old are not always visible: to assess oocyst detection probability depending on the time p.i., we implemented a separate laboratory experiment detailed in [Supplementary Data S2](#) and [Supplementary Fig. S2–4](#). From this experiment, we assume that all oocysts are visible after 7 development days. No oocyst is detectable before day 3 due to remaining blood in the mosquito midgut impeding dissection. At day j after infection (for $j > 2$) the proportion of detectable oocysts ($det_I(j)$) and the proportion of mosquitoes with detectable oocysts ($det_P(j)$) depends on the single-feed infection load (θ):

$$det_I(j) = 1 - \exp(-0.257 * (j - 2) * (1 + 0.065 * \theta))$$

$$det_P(j) = \frac{(j - 2)(1 + 0.170 * \theta)^{3.089}}{((j - 2)(1 + 0.170 * \theta)^{3.089} + 19.742)}$$
(12)

(see [Supplementary Data S2](#) for full details)

In particular, 3 days after mosquito collection from the field, only a proportion of the mosquitoes in the E_{lab} compartment have detectable oocysts, and only a proportion of the oocysts in O_{lab} are detectable.

The proportion of mosquitoes in E_{lab} with detectable oocysts (D_E) 3 days after collection is given by:

$$D_E(3) = 1 - \frac{E_v^* \exp(-3\lambda_1)}{E_{lab}(3)} \sum_{i=0}^3 ((1 - det_P(i + 3)) \exp(-i\lambda))$$
(13)

where $\frac{E_v^* \exp(-3\lambda_1)}{E_{lab}(3)} (1 - det_P(x + 3)) \exp(-x\lambda)$ is the proportion of oocysts that have been infected x days before collection and are not detectable yet.

Similarly, the fraction of oocysts detectable in O_{lab} (D_O) 3 days after collection is:

$$D_O(3) = 1 - \frac{O_v^* \exp(-3\lambda_1)}{O_{lab}(3)} \sum_{i=0}^3 ((1 - det_I(i + 3)) \exp(-i\lambda))$$
(14)

2.2.4. Fitting day 3 and day 7 data

2.2.4.1. Model predictions. Seven days after infection the laboratory experiments indicate that all oocyst are detectable so the predicted prevalence of detectable oocysts (X_E) and sporozoites (X_I), and the predicted mean number of detectable oocysts per infected mosquitoes (X_O), is defined by:

$$X_E(7) = \frac{E_{lab}(7) + EI_{lab}(7)}{M * \exp(-\lambda_1 7)}$$

$$X_I(7) = \frac{I_{lab}(7) + EI_{lab}(7)}{M * \exp(-\lambda_1 7)}$$

$$X_O(7) = \frac{O_{lab}(7)}{E_{lab}(7) + EI_{lab}(7)}$$
(15)

Three days after infection, only a fraction of oocysts are detectable, so the predicted detectable prevalence and load can be given by:

$$X_E(3) = \frac{E_{lab}(3) * D_E(3) + EI_{lab}(3)}{M * \exp(-\lambda_1 3)}$$

$$X_I(3) = \frac{I_{lab}(3) + EI_{lab}(3)}{M * \exp(-\lambda_1 3)}$$

$$X_O(3) = \frac{O_{lab}(3) * D_O(3)}{E_{lab}(3) * D_E(3) + EI_{lab}(3)}$$
(16)

It is not possible to directly quantify the number of oocysts in mosquitoes fed the previous night. Nevertheless, this quantity is of interest as it allows the mosquitoes collected here to be compared with other populations. The estimated proportion of mosquitoes containing oocysts (E_0) and of mosquitoes with sporozoites (I_0) at the collection date in the wild population (day 0), irrespective of detectability, is:

$$E_0 = \frac{E_v^* + EI_v^*}{M}$$

$$I_0 = \frac{EI_v^* + I_v^*}{M}$$
(17)

If we consider bites and infections are occurring at random, the proportion of mosquitoes with superinfections (denoted SI) at any life stage is:

$$SI = abX_h \frac{E_{v,1}^* + E_{v,2}^* + EI_v^* + I_v^*}{M}$$
(18)

2.2.4.2. Fitting method. The model is fitted to data using maximum likelihood. We computed the log likelihood for oocyst prevalence (L_E), sporozoite prevalence (L_I) and the mean number of oocysts (L_O) for which the predicted data (denoted X_E, X_I, X_O , respectively) match the observed data (x_E, x_I, x_O , respectively). Oocyst and sporozoite prevalences are assumed to follow a binomial distribution whilst oocyst load is approximated to be a zero-truncated Poisson distribution:

$$L_E = \log(X_E) * x_E n_E + \log(1 - X_E) * n_E (1 - x_E)$$

$$L_I = \log(X_I) * x_I n_I + \log(1 - X_I) * n_I (1 - x_I)$$

$$L_O = n_O \log(\lambda_0) x_O - \lambda_0 - \log(1 - \exp(-\lambda_0))$$
(19)

where n_E, n_I, n_O are the numbers of mosquitoes dissected to observe oocysts and sporozoites, and λ_0 is the expected number of oocysts under the corresponding un-truncated Poisson distribution, given by the solution to $X_O = \frac{\lambda_0}{1 - \exp(-\lambda_0)}$ (computed numerically).

Log likelihoods were computed for day 3 and day 7 dissection dates, and we used the ‘optimise’ function of the Berkeley

Madonna software (version 8.3.18, <https://berkeley-madonna.myshopify.com/>) to find the model parameters that minimise the sum of the six log likelihoods for each season and location. Malaria prevalence in humans was allowed to vary between the confidence intervals of its observed data at each season and location, and initially set to its observed data average. Parameter estimates for the mosquito mortality rate in the laboratory and the overall density of mosquitoes were unidentifiable as they had no impact on the predicted oocyst and sporozoite prevalence and load, and were given arbitrary values. The mortality rate in the wild, the infection rate, single-feed infection load and oocyst breaking rate were allowed to vary between the bound described in the table of parameters (Supplementary Table S2). This was conducted for each Burkina Faso location and timepoint. For Cameroon locations, single-feed infection load, infection rate and day 0 sporozoite rates were estimated using Klesso parameters and Cameroon sporozoite and oocyst data collected at day 7.

3. Results

3.1. Parasites in humans

The prevalence of *Plasmodium falciparum* as determined by microscopy was particularly high in the study villages in Burkina Faso, with between 22–32% of people infected during the dry season (collections in March and April) and 35–55% during the rainy season (collections from July to November), depending on month and location (Fig. 1A, B). Asexual parasite prevalence was estimated from ~300 individuals at each time point and was significantly higher in Klesso than in Longo (dry season 32.3% (95% confidence intervals: 27–38%) versus 22% (17–26%), $P = 0.003$; rainy season: 49% (45–52%) versus 44% (40–47%), $P = 0.042$), although observed gametocyte prevalences were similar between locations. Both asexual parasite and gametocyte prevalences varied across seasons, reaching a maximum in September (at the peak of the rainy season) and a minimum during the dry season for asexual parasites and in November for gametocytes. The density of asexual parasites varied across season and location (Fig. 1A, B, Supplementary Table S2), but gametocyte density was independent of seasonal variations and similar between localities (Supplementary Table S3).

The prevalence of *P. falciparum* infections also varied with age class (0–5, 5–20, 20–50, and >50 years-old), with the highest asexual and gametocyte prevalences in the 0–5 year olds (61% (52–70%) asexual parasite carriers and 7% (2–11%) gametocyte carriers) and lowest for the 20–50 year olds (see Supplementary Table S3, Supplementary Fig. S5). *Plasmodium falciparum* composed over 98.9% (98.5–99.3%) of all *Plasmodium* infections; the remaining infections being split between *Plasmodium ovale* and *Plasmodium malariae*.

3.2. Mosquito abundance

The prevalence and abundance of mosquitoes in households, including all collected genera, varied substantially throughout the year and between locations in Burkina Faso (Fig. 1C, D), as did the mean number of *Anopheles* mosquitoes per household (Supplementary Table S4, Supplementary Fig. S6). Species composition among collected *Anopheles* differed between locations: *Anopheles gambiae* sensu stricto (s.s.) was the most prominent species in Klesso (55.8% (52–60%)) whereas *Anopheles coluzzii* was the most common in Longo (62.9% (59–67%)), see Supplementary Table S4). *Anopheles coluzzii* was the prominent *Anopheles* mosquito collected during the dry season at all locations (80% (75–85%)). Gravidity, parturition rates and feeding status of

mosquitoes were also reported (Supplementary Table S4). At the start of the rainy season, we found significantly more parous mosquitoes in Klesso than in Longo, suggesting a larger proportion of older mosquitoes and a more continuous reproductive cycle during the dry season in Klesso, in accordance with their higher observed prevalence during this season compared with Longo (Fig. 1C, D).

Bednet coverage was significantly higher in Longo than in Klesso all year round (85.1% (84.0–86.2%) versus 56.9% (55.3–58.4%), $P < 0.0001$, see Supplementary Table S4, Supplementary Fig. S7). Bednets were likely used in response to mosquito nuisance, as bednet coverage followed mosquito prevalence and abundance over time in each location. During the peak of the rainy season, bednets significantly reduced the number of mosquitoes in infested houses at all locations (–15.8% of mosquitoes in infested houses with bednets, $P < 0.0001$, Supplementary Fig. S7). Other houses characteristics (type of roof, presence of paint) were reported but had a very limited impact on mosquito prevalence and abundance (Supplementary Table S4).

3.3. Parasites in mosquitoes

Wild mosquitoes caught inside houses were kept for 3 days or 7 days in the insectarium in order to allow sufficient time for the last blood meal to be digested, enabling oocyst load to be quantified by microscopy after dissection. The difference in oocyst prevalence and abundance between day 3 and day 7 allows evaluation of the transmission dynamics (see Section 2 for more details).

3.3.1. Oocyst prevalence and oocyst load at day 7

The prevalence of oocyst-infected mosquitoes 7 days after collection varied significantly with season ($P < 0.0001$), with a maximum in September (25.9% (23.0–28.8%) in Klesso and 8.1% (6.4–9.9%) in July in Longo). During the dry season, prevalence dropped to 5.0% (2.5–7.2%) in Klesso and no oocysts were detected in Longo (Fig. 2A, B). The arithmetic mean of oocyst load among infected mosquitoes (the number of detectable oocysts in the midgut) during the rainy season was similar in both villages at day 7 with 13.8 (10.1–17.1) oocysts per mosquito in Klesso and 10.3 (7.8–12.5) oocysts/mosquito in Longo ($P = 0.73$). The oocyst load varied with season ($P < 0.0001$), reaching 14.3 (7.0–19.7) oocysts/mosquito in September in Klesso and 14.7 (8.9–20.3) in Longo, Fig. 2A, B). Oocyst distribution within mosquitoes was highly overdispersed (aggregated) with a median oocyst load of three oocysts/mosquito for the rainy season (four oocysts/mosquitoes in July) and 5.8% of infected mosquitoes presenting over 50 detectable oocysts (56 out of 977 mosquitoes examined). Two percent of infected mosquitoes had over 100 detectable oocysts (Fig. 2C, D) and the maximum detected oocyst load was 786. Mosquito species was not significantly associated with oocyst prevalence ($P = 0.23$) or load ($P = 0.26$).

Visible shells of oocysts that had already ruptured were also detected but this information was not included in the transmission model due to uncertainty about their persistence in the mosquito midgut after the release of sporozoites (see Supplementary Data S1). During the rainy season 3.4% (2.2–4.5%) of mosquitoes presented with ruptured oocysts, and among them the arithmetic mean of ruptured oocysts was 13.2 (10.3–15.8), while one mosquito displayed 127 detected ruptured oocysts.

3.3.2. Day 3 to day 7 changes and day 0 estimates

Oocyst prevalence 3 days after collection was not significantly lower than 7 days after collection (Longo: $P = 0.098$, Klesso: $P = 0.86$) as would have been expected if oocysts are undetectable 3 days after the infectious blood meal (Gouagna et al., 2010). Oocyst load even significantly decreased between day 3 and day 7 in Klesso (–24.9%, $P = 0.013$) while it displayed no significant

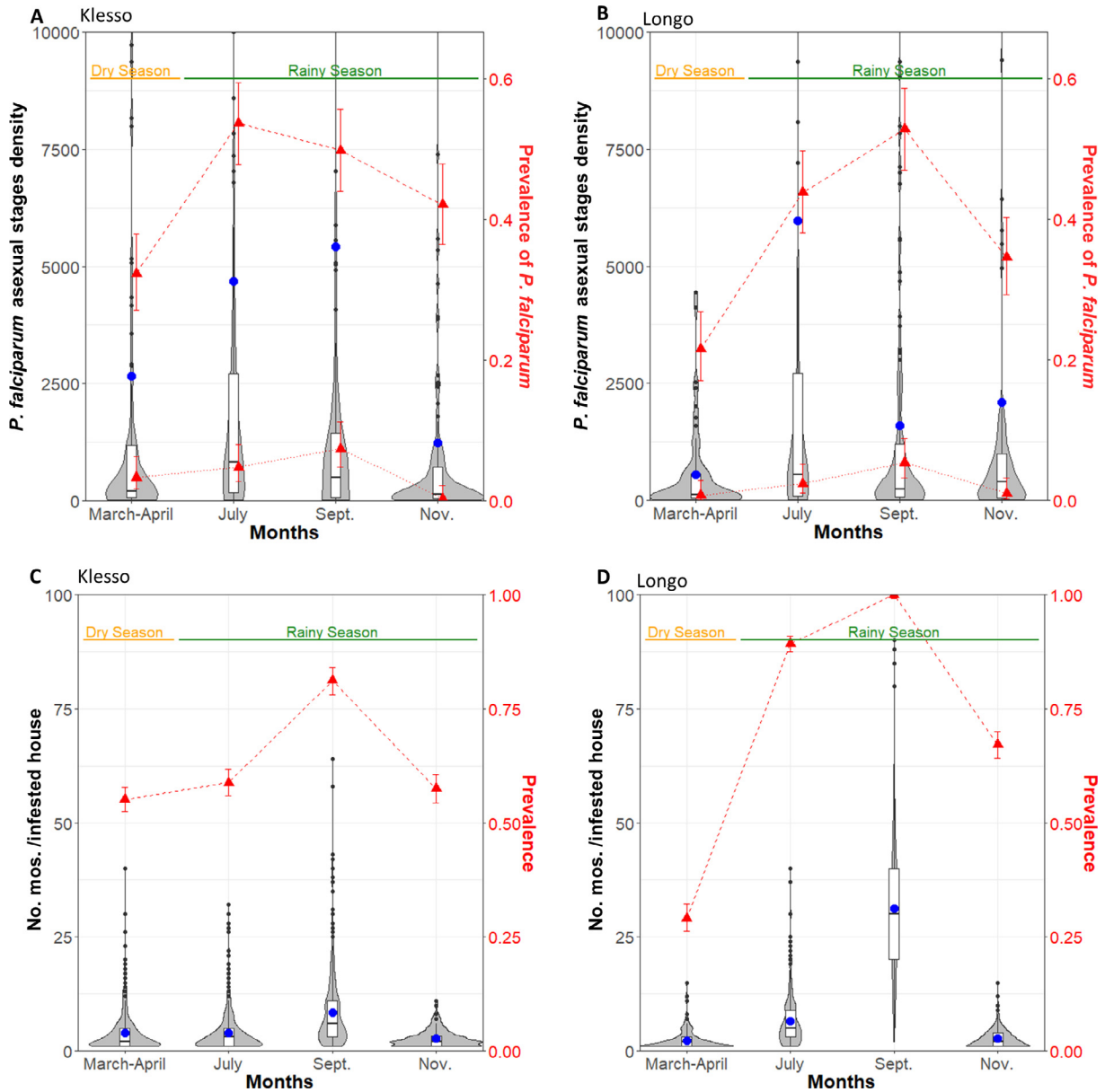


Fig. 1. Human parasite prevalence and density (A and B) and mosquito abundance (C and D) from two villages in Burkina Faso (Klesso and Longo) collected at four time points throughout the year. (A, B) *Plasmodium falciparum* asexual parasite density (trophozoite, measured as parasites/ μ l of blood) is shown on the left Y axis. Violin plot and point and whisker plots show the population density distribution with the mean as a blue dot. Right Y axes and red points show parasite prevalence, be it all parasite stages (triangular points, dashed line) or gametocytes (triangular points, dotted line), as measured by microscopy. (C, D) Number of mosquitoes (mos.; all genera) per infested houses (left Y axis: violin plot with mean as a blue dot) and prevalence of mosquitoes in houses (right Y axis: red line and points). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

difference in Longo ($P = 0.61$) (Fig. 2A, B). The median oocyst count remained the same between day 3 and day 7 (three oocysts/infected mosquito). In order to understand the evolution of oocyst prevalence and load between day 3 and day 7, we monitored the oocyst threshold of detectability in experimentally infected mosquitoes in the laboratory (see Supplementary Data S2, Supplementary Fig. S2–4). This supplementary experiment indicated that a substantial proportion of oocysts were already detectable 3 days after an infection: the prevalence of oocysts in mosquitoes dissected at day 3 was on average 60% of their prevalence at day 7 (with a range of 0 to 100% depending on the blood donor). The proportion of mosquitoes with detectable oocysts increased with the single-feed infection load (measured as the number of oocysts at day 7 after infection, as only one blood meal was taken and all oocysts are considered developed at that time).

This feature was then included in the transmission model (see Supplementary Data S2).

3.3.3. Sporozoite rates

During the the rainy season, from July to November, the prevalence of infectious mosquitoes (with sporozoites) 7 days after collection ranged between 27–38% in Klesso and between 12–34% in Longo (significantly lower in Longo, $P < 0.0001$, Fig. 3A, B). In both locations, sporozoite prevalence reached its maximum at the end of the rainy season. The prevalence of infectious mosquitoes was significantly lower at day 3 after collection (compared with day 7, $P < 0.0001$), ranging between 7–27% in Klesso and 11–23% in Longo across the season. We used the transmission model described in Section 2.2 to estimate the normalised prevalence of infectious mosquitoes in the wild, as would have been detected if

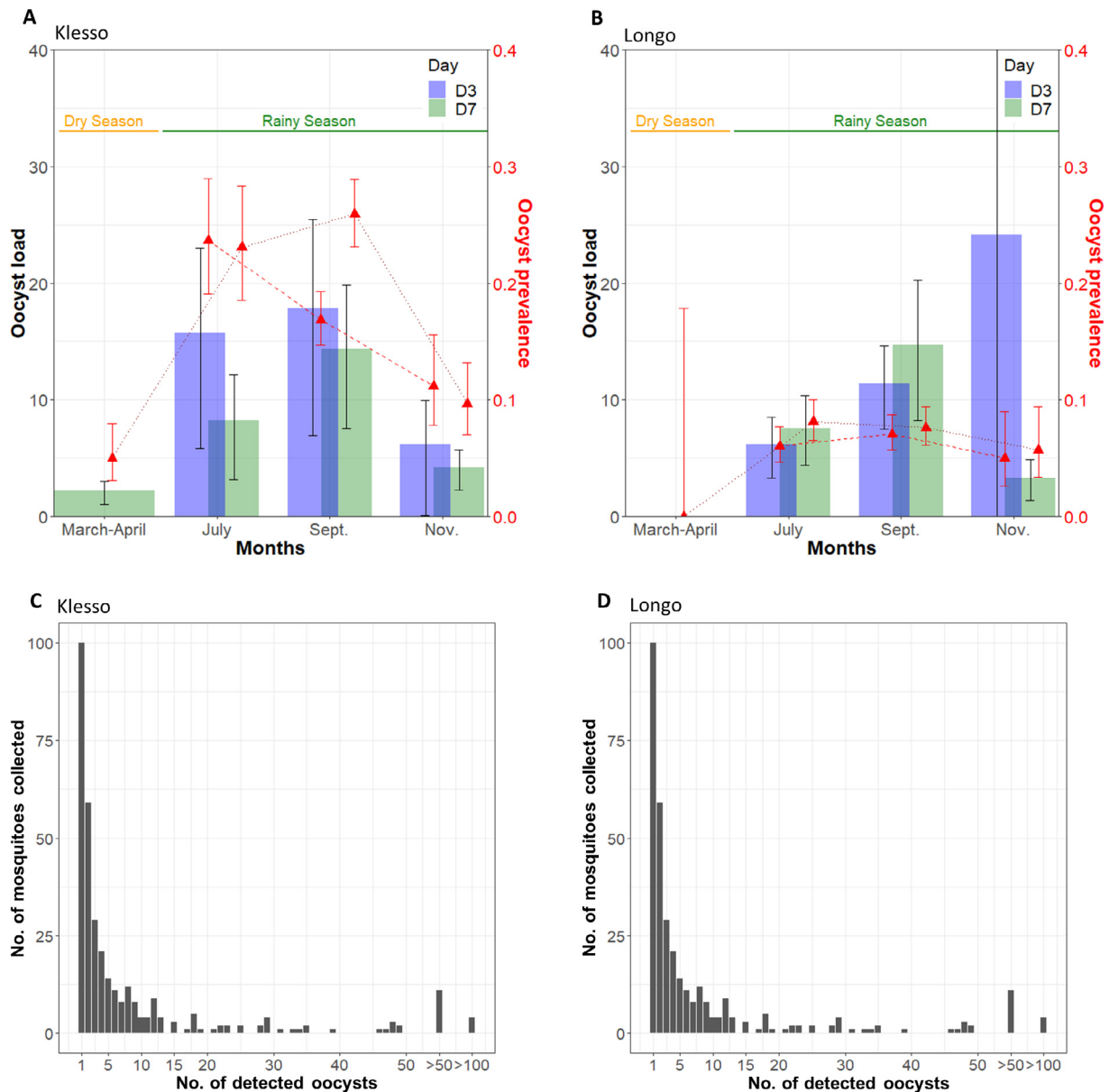


Fig. 2. *Plasmodium falciparum* oocyst prevalence and load in mosquitoes (*Culex* and *Anopheles*) from the villages of Klesso (A and C) and Longo (B and D) in Burkina Faso. (A, B) Arithmetic mean of oocyst load per mosquito with detectable oocysts 3 days (blue) or 7 days (green) after mosquito collection (barplot with 95% confidence intervals) and prevalence of oocysts 3 days (D3) and 7 days (D7) p.i. (right Y axis: blue triangle and dashed red line (D3) or green triangle and dotted red line (D7). No dissections were done at D3 during the dry season). (C, D) The distribution of the number of oocysts per infected mosquito (bar for uninfected mosquitoes has been omitted) measured 3 or 7 days after blood-feeding. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the mosquitoes had been dissected on the day of collection: these estimates range between 2–17% in Klesso and 2–7% in Longo (Fig. 3A, B). Differences between estimates of sporozoite rates on the day of collection and day 3/day 7 observations are likely to originate from parasite transmission dynamics and the high survival rate of mosquitoes in the laboratory. Mosquito species within the *Anopheles* group had no impact on sporozoite prevalence ($P = 0.672$).

3.3.4. Infection rate, single-feed infection load and superinfections

The infection rate (the probability of transmission between human and mosquito per blood meal on an infected host) and single-feed infection load were estimated during the rainy season by fitting a Susceptible-Exposed-Infectious compartmental deterministic *Plasmodium* transmission model to data on human and mosquito parasite prevalence and load (three and 7 days after

mosquito collection). Results are presented in Fig. 3 and Supplementary Fig. S8. Estimates of the probability of human to mosquito transmission (per blood meal taken on a person with detectable parasites of any stage by microscopy) showed little variation between seasons with an average estimate of 35% across all timepoints. Values were higher in Klesso (39–79%) than in Longo (9–15%) (see Supplementary Table S2). Overall estimates of single-feed infection loads across all villages and timepoints showed that infectious blood meal resulted in an average of 14 oocysts per mosquito. The mean single-feed infection load was consistent between villages, with an estimated number of 7.0–24.5 oocysts/infectious blood meal in Klesso and 3.2 – 18.3 oocysts/infectious blood meal in Longo. Exposure appeared to vary by season, being highest at the peak of the rainy season (September) and at a minimum at the end of the rainy season (November) in both locations. The average life expectancy of mosquitoes, as

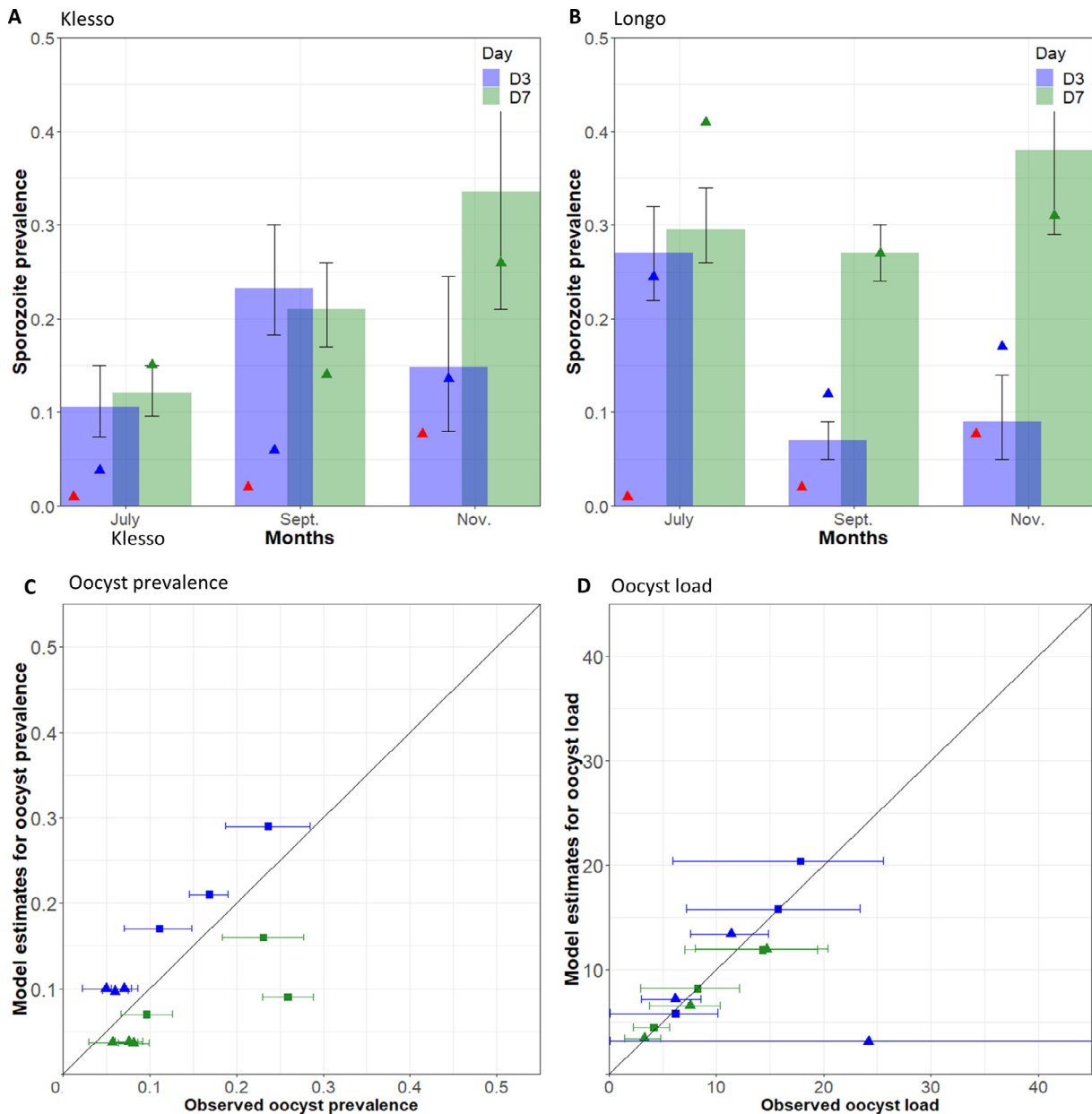


Fig. 3. Observed data on sporozoite prevalence and model predictions for *Plasmodium falciparum* in mosquitoes in the villages of Kesso and Longo, Burkina Faso. (A, B) Observed sporozoite prevalence at day 3 (D3; blue bars) and day 7 (D7; green bars) with vertical black lines indicating 95% confidence interval estimates. Model prediction for sporozoite prevalence in the mosquito population at the collection date (red triangles), at D3 (blue triangles) and D7 (green triangles) for Kesso (A) and Longo (B). (C, D) The ability of the model to capture oocyst prevalence and load. (C) Model prediction for oocyst prevalence at D3 (blue) and D7 (green) versus the observed oocyst prevalence at these times (with 95% confidence intervals on data) for Kesso (squares) and Longo (triangles). (D) Model prediction for oocyst load (the mean number of oocysts per mosquito) at D3 (blue) and D7 (green) versus the observed oocyst load at these times (with 95% confidence intervals) for Kesso (squares) and Longo (triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

estimated by the model, was similar between locations (approximately 8 days in Kesso, 7 days in Longo), and we estimated the proportion of mosquitoes infected at least twice to be between 2.3–3.8% in Kesso and between 0.4–0.9% in Longo.

3.4. Predicting single-feed infection load in mosquitoes from prevalence in humans

Gametocyte prevalence on slides of samples from humans was able to predict an estimate of the single-feed infection load, as evaluated by the transmission model, with good accuracy during the rainy season in Kesso and Longo ($R = 0.88$, $P = 0.033$, Fig. 4A) for a

relatively small dataset which had a low range of estimates of gametocyte slide positive individuals (0.01–0.08%). Malaria parasite prevalence of any parasite stage in humans was also a promising predictor of the single-feed infection load, although only of marginal significance ($R = 0.83$, $P = 0.058$, Fig. 4B). Gametocyte or trophozoite densities in humans were poor predictors of estimates of the single-feed infection load (gametocyte density: $P = 0.658$; trophozoite density: $P = 0.419$). The human-mosquito transmission probability (the probability that a mosquito becomes infected following a blood meal on a parasite carrier) was also estimated but could not be explained by the prevalence or density of either all blood stage parasites or gametocytes alone (as determined by microcopy).

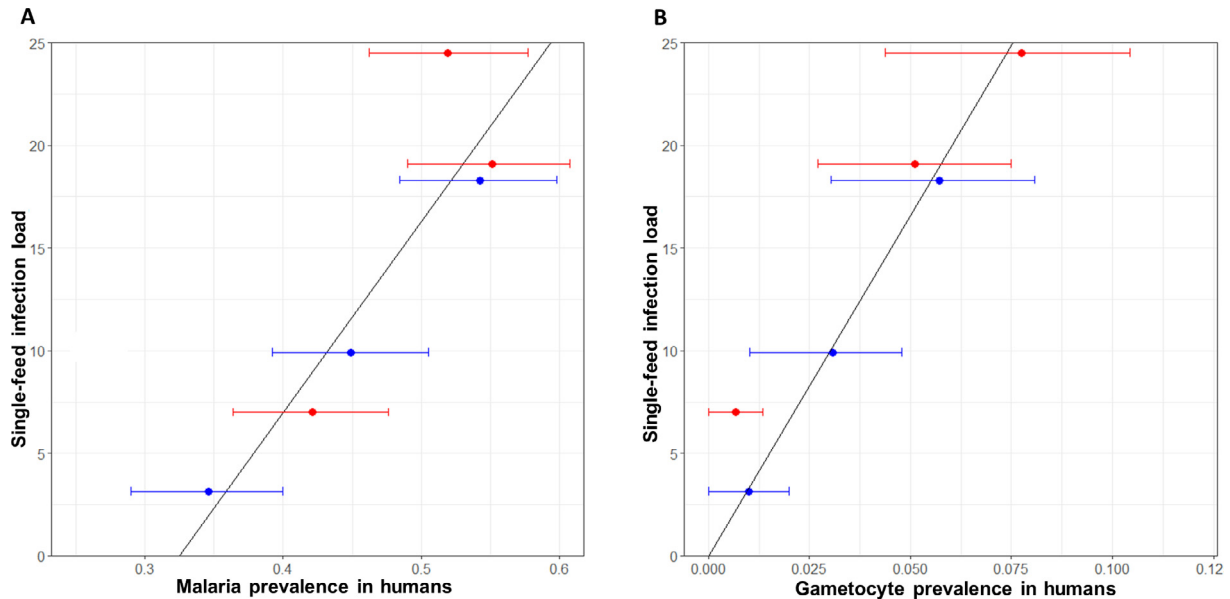


Fig. 4. The relationship between observed prevalence of any *Plasmodium falciparum* stage (A) or gametocytes (B) estimated by microscopy in the human population and model predictions for the single-feed infection load (the mean number of oocysts acquired per infectious blood meal). Red points are estimates for the village of Klesso (Burkina Faso) whilst blue points are estimates for the village of Longo (Burkina Faso) for each month during the rainy season. Horizontal lines indicate 95% confidence interval estimates for malaria prevalence.

3.5. Cameroon

Mosquito data were collected in two villages in Cameroon (Mbelengui and Ekali) during the rainy season (May–July), and mosquitoes were maintained for 7 days in the laboratory before dissection. Prevalence and abundance of *Anopheles* mosquitoes in Cameroonian houses were significantly lower than in Burkina Faso during the peak of the rainy season ($P < 0.0001$), and significantly higher in Mbelengui than in Ekali (prevalence: 41% (35–47%) versus 22% (18–26%), $P < 0.001$; abundance: 3.2% (2.6–3.8%) versus 1.8% (1.4–2.2) of mosquitoes per infested house, $P < 0.001$; Fig. 5A). *Anopheles gambiae* was the most prominent species (84% (81–88%)) and, contrary to Burkina Faso, *Anopheles funestus* was also frequently observed in Cameroon (8% (6–11%)) of all *Anopheles* spp., see Supplementary Table S4). Bednet coverage was significantly lower in Cameroon than in Burkina Faso during the peak of the rainy season ($P < 0.0001$; 34% (27–40%) in Ekali and 29% (25–33%) in Mbelengui) and houses with bednets had a significantly lower mosquito prevalence and abundance (prevalence –80.53%, $P_s < 0.0001$; abundance –38.04%, $P = 0.012$; see Supplementary Table S4 for further details).

Dissection showed that the prevalence of sporozoite-positive mosquitoes was 42% (37–47%) 7 days after collection, higher than Burkina Faso locations. Model estimates based on Klesso parameters (other than parasite prevalence) indicate sporozoite rates on the day of mosquito collection of approximately 16.5% (Mbelengui) and 15% (Ekali). The prevalence of oocyst-positive mosquitoes was similar to Klesso during the peak of the rainy season ($P = 0.23$, Klesso: 21% (19–24%), Mbelengui: 29% (20–39%) and Ekali: 24% (7–39%)), but the oocyst load in Cameroon locations was lower, with a mean 9.1 (3.2–12.9) oocysts per infected mosquito and a median of 2.5 oocysts per infected mosquito (Fig. 5B). Model estimates based on estimated parameters for Klesso indicate a Cameroon single-feed infection load of approximately 8.7 oocysts per infectious blood meal in Mbelengui and 8.1 oocysts per infectious blood meal in Ekali.

4. Discussion

Wild mosquito exposure to malaria parasites in two villages in Burkina Faso was substantially higher than had previously been assumed. Overall, model estimates indicate each infectious blood meal resulted in a mean of 14 oocysts per infected mosquito. The range of the single-feed infection load was large, although estimates at all time points were substantially greater than was previously assumed, even in high infection areas (Hogg and Hurd, 1997; Annan et al., 2007; Rosenberg, 2008). This result was driven by the high oocyst load observed in wild caught mosquitoes dissected three and 7 days post collection. In Burkina Faso there were on average over 10 oocysts per mosquito during the rainy season 7 days after collection, which is substantially more than previous work reported (less than five oocysts/infected wild mosquito (Rosenberg, 2008)). Estimates of sporozoite rates at day 0 (predicted using the mathematical model) were high, although similar to another recent study from Burkina Faso (Pombi et al., 2018; Epopa et al., 2019). Observed oocyst loads were only slightly lower in Cameroon than in Burkina Faso, and consistent with a previous Cameroon study (Annan et al., 2007). Parasite prevalence in humans was consistent with previous reports from the area (Ouédraogo et al., 2013), and showed a significant correlation to the single-feed infection load across seasons and locations. This suggests that the high single-feed infection load estimates in Burkina Faso are not atypical or specific to the area, but rather due to the high transmission pattern of these regions. There were some differences in the seasonality of parasite and mosquito abundance between the two villages in Burkina Faso (despite those being barely 100 km apart), although estimates of the distribution of oocysts per mosquito and the single-feed infection load were broadly similar.

The methods of Gouagna et al. (2010) for determining the single-feed infection load is to subtract the number of oocysts observed on day 3 from those observed on day 7 and therefore estimate the number of oocysts acquired the night before mosquito collection. This proved inadequate as there were significantly more

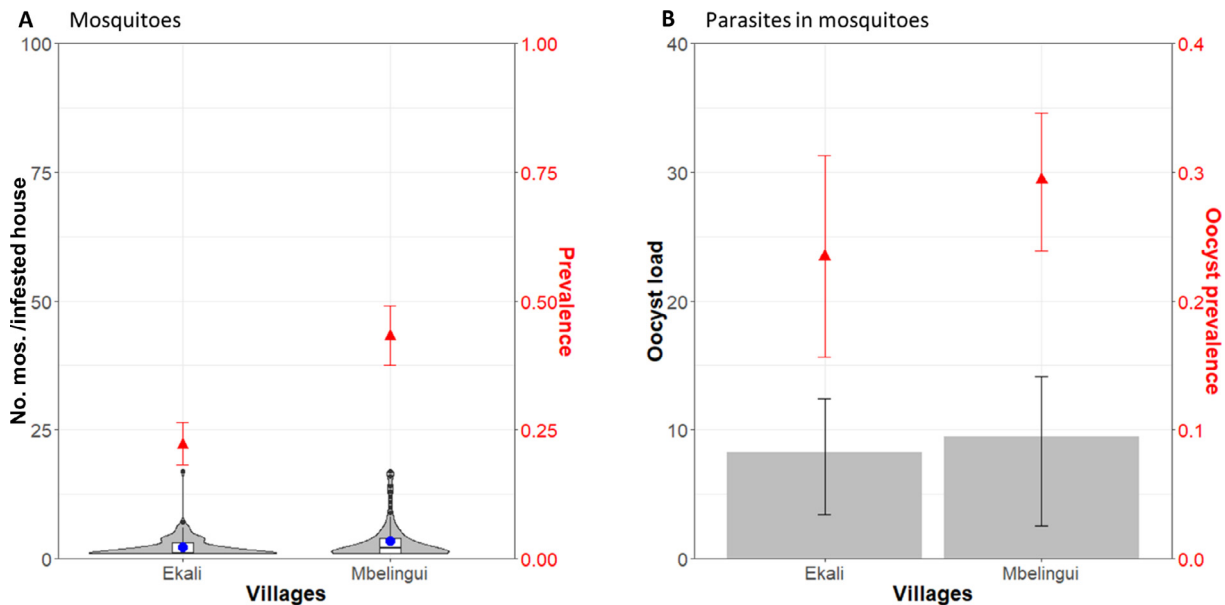


Fig. 5. Mosquito (A) and parasite (B) data collected in Cameroon during the peak of the rainy season. (A) Mosquito (mos.) abundance in an infested house (boxplot, left Y axis) and mosquito prevalence in houses (red triangles, right Y axis). (B) Arithmetic mean of oocyst load in infected mosquitoes dissected 7 days after collection (bars with confidence intervals) and oocyst prevalence in mosquitoes (red triangles, right Y axis). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oocysts at day 3 than at day 7 in some instances. Supplementary laboratory studies showed that this was due to the appearance of visible oocysts prior to day 3, with on average 60% of infected mosquitoes having visible oocysts on day 3. This might be due to high temperatures in the laboratories in Burkina Faso (Noden et al., 1995; Eling et al., 2001; Okech et al., 2004), or to a wild vector population which is particularly permissive to *Plasmodium* infections (Ohm et al., 2018), or to other environmental factors affecting the length of the extrinsic incubation period (Ohm et al., 2018). The proportion of mosquitoes with visible oocysts also appears to increase as oocyst exposure increases (Supplementary Data S2, Supplementary Fig. S2–4). Temperature has previously been reported to cause both an increase in the number of oocysts per infected mosquito and a decrease in the time to oocyst appearance (Okech et al., 2004) but this is the first time (to our knowledge) that a relationship between the latter and the number of oocysts has been observed at a constant temperature, and further studies are necessary to understand to what extent this could affect malaria parasite transmission. Biological causes for the faster appearance in more infected mosquitoes could be a higher investment in transmission stages due to oocyst competition for resources or higher mosquito immune response when infection intensity increases (Imwong et al., 2011; Ohm et al., 2018). This acceleration in oocyst development could be costly in resources and result in lower numbers of sporozoites (Sinden, 2002; Sinden et al., 2007; Rosenberg, 2008) but if oocysts that become mature also break and release sporozoites earlier, mosquitoes could become infectious more quickly (having a lower extrinsic incubation period) in high parasite exposure areas, exacerbating *Plasmodium* transmission.

In Burkina Faso and in Cameroon the single-feed infection load was shown to be higher than is usually assumed. The percentage of mosquitoes with visible oocysts is likely to be higher in mosquitoes caught and kept in the laboratory before dissection compared with being dissected on the day of collection as mortality in the laboratory is probably lower than in the wild. Nevertheless, a previous study in Tanzania reported that only 9% of naturally infected wild mosquitoes had over five oocysts per mosquito when dissected on day 6 after collection (Hogg and Hurd, 1997) and a study in Kenya

and Burkina Faso reported no mosquitoes with more than five oocysts in Kenya and 12.5% of mosquitoes with over 10 oocysts in Burkina Faso, a difference that was attributed to endemicity (Gonçalves et al., 2017). By comparison, in the present study in Burkina Faso, overall 65% of mosquitoes had 1–5 oocysts, 35% had over five oocysts, 21% over 10 and 2% over 100 oocysts. Over-dispersion in oocyst distribution has been documented before but this is the first time a substantial proportion of mosquitoes with very high oocyst numbers has been reported in nature (Pringle, 1966; Billingsley et al., 1994; Haji et al., 1996a, 1996b; Hogg and Hurd, 1997; Gouagna et al., 2014). It is interesting to note that very high oocyst loads are also observed in direct membrane feeding assays in the region where volunteer blood-donors with very high gametocyte densities are deliberately selected (Da et al., 2015). The occurrence of wild mosquitoes with over 100 broken oocysts indicates that it is likely these highly infected mosquitoes can survive long enough in the wild for the oocysts to release infectious sporozoites, and that even if some oocysts may fail to rupture (Vaughan et al., 1992; Gamage-Mendis et al., 1993), most do in this context. If these highly infected mosquitoes have in turn a higher sporozoite count, they would be particularly infectious, as more sporozoites in salivary glands might translate into a higher probability of infection (Churcher et al., 2017). However, whether sporozoite production per oocyst is maintained or decreases with oocyst load remains unclear (Rosenberg and Rungsiwongse, 1991; Sinden et al., 2007), so it is difficult to predict whether these highly infected mosquitoes will constitute a major component of transmission and a challenge for malaria elimination.

The consequences of an high single-feed infection load could be far-reaching for our understanding of malaria and how transmission-reducing interventions are assessed. Results of standard and direct membrane feeding assays (SMFA and DMFA, respectively) are often challenged because the single-feed infection load generated is seen as artificially high compared with the natural single-feed infection load (Rosenberg, 2008). However, the single-feed infection load we observed in high transmission area falls into a similar range as recent SMFA studies (Kapulu et al., 2015; Bompard et al., 2017; Eldering et al., 2017). Transmission-blocking interventions that show a dose–response to single-feed

infection load, such as transmission-blocking vaccines, might initially show lower efficacy when deployed in high-transmission areas. However, as recent results indicate, a transmission blocking vaccine (TBV) might reduce the single-feed infection load over time (Churcher et al., 2017), increasing efficacy. This positive feedback loop could substantially increase overall effectiveness of interventions which target the parasite component of human to mosquito transmission. More broadly, the work has implications for the understanding of the population dynamics of *P. falciparum* and the interaction between the malaria parasite and its vector.

The experiment outlined here to determine the single-feed infection load is complex, requiring large numbers of mosquitoes to be kept in the laboratory and dissected. Estimates of field single-feed infection load can be made by dissecting mosquitoes on the day of collection if they have not blood-fed. This does give an indication of the level of exposure, although it might be biased by malaria parasite endemicity, non-human blood-feeding and any impact the parasite may have on the mosquito in the wild. Here we compared single-feed infection load with the prevalence of the parasite in the human population to investigate whether this simpler to measure parameter can be used to predict exposure in the wild. The relationship between single-feed infection load and human *Plasmodium* prevalence is particularly promising, and supported by an earlier study which noticed a correlation between the number of very small, developing oocysts in *A. gambiae* and gametocyte prevalence in Tanzanian villages (Haji et al., 1996a). However, further work is needed in more locations with differing levels of parasite endemicity. Although gametocyte prevalence is a more precise predictor of single-feed infection load in our study, the prevalence of all stages of *Plasmodium* in humans would be an easier metric to collect and has greater ability to differentiate between single-feed infection loads as there is wider variability observed in the field and has fewer sub-microscopic infections. Variation in host natural immunity between locations could also affect this relationship so more data in various epidemiological settings would be needed to corroborate the relationship. If confirmed, a human *Plasmodium* prevalence- natural single-feed infection load curve could help predict initial and long-term TBV efficacy before clinical trial or deployment, as well as the minimum antibody titer necessary to achieve blockade (Bompard et al., 2017). The World Health Organization has recently stressed the need to target control in high burden countries such as Burkina Faso (WHO, 2019). The high single-feed infection load reported here might contribute to the resilience of malaria to ongoing control efforts in Burkina Faso and other high transmission settings.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2020.05.012>.

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