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Comparison of field-based xenodiagnosis and direct membrane feeding assays for evaluating host infectiousness to malaria vector *Anopheles gambiae*

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ABSTRACT

Several techniques are currently being used to study host infectiousness to mosquitoes, including the experimental possibility of laboratory reared mosquitoes acquiring infections through membrane feeders or directly on host skin. Here, the relative performance of the laboratory-based membrane feeding method (DMFA) and the field-based xenodiagnosis (XD) of malaria infectious hosts using wild *Anopheles* mosquitoes were compared. A cross-sectional survey involving a sample of 70 children (aged 3–12 years) living in a malaria endemic area in Western Burkina Faso, was carried out to measure their infectiousness to Anopheles mosquitoes using two approaches. The first approach used the xenodiagnostic procedure in which children were exposed to mosquito bites overnight, being sleeping individually in different sentinel huts from 6 pm to 6 am (4 nights per child). *Anopheles* sp that had acquired blood-meal on each child were subsequently collected early in the morning, and examined for *Plasmodium falciparum* oocyst infection on day 7 post-feeding. In the second approach, the infectiousness of the same children was estimated by whole-blood membrane feeding procedure using F0 *An. gambiae s.l.* that emerged from field-collected larvae cohorts. In the DMFA, 41.4% of the children successfully infected at least one mosquito with the mean oocyst prevalence of only 4.6 ± 1.1% in the 2171 mosquitoes that were examined (mean oocyst intensity: 2.0 ± (std error of mean) 0.3 oocysts per infected midgut). Comparatively 78.6% of children yielded oocysts infection in mosquitoes during the XD approach (Chi square = 20.11, df = 1; $p < 0.001$), with a mean rate of 19.6 ± 2.0 in the 3752 wild caught mosquitoes (mean intensity: 3.93 ± 0.2 oocysts per infected mosquito). The DMFA failed to reveal a portion ($n = 26$) of infectious individuals that were sharply evidenced by the XD, particularly at low gametocyte densities or at levels that could not be detected by the classical microscopic examination of blood smears. As opposed to the resource consuming DMFA, which is often mined by technical constraints, using the XD method could be an advantage in experimental investigations of host infectiousness in areas where anopheline species cannot be conveniently reared for the experimental studies. Ethical aspects of this approach, mainly related to exposure of the human subjects to potentially infectious mosquito bites are discussed.

1. Background

Malaria remains one of the world's most burdensome parasitic diseases and a major focus for international biomedical research. Malaria transmission requires that susceptible vector mosquitoes must feed upon a human host carrying sexual stage of *Plasmodium* infection (Nedelman, 1989; Carter and Graves, 1988). However, the key factors that determine overall transmission intensity and contribute to ecological variation in transmission patterns are vectorial capacity of vector populations and the potential infectiousness of the human population to mosquitoes (Killeen et al., 2000, 2006; Koella, 1991).

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The force of infection from human to vector in malaria is poorly understood but is obviously one of the transmission factors under consideration for control via such strategies as transmission blocking vaccines (Targett and Sinden, 1985; Kumar, 2007), drugs (White, 2008), and the introduction of Plasmodium-refractory genes into wild vector populations (Marshall and Taylor, 2009; Alphey et al., 2002). The vast majority of mosquito bites taken from infected humans do not always result in transmission to the vector, even in holoendemic settings (Beier et al., 1999), where a high fraction of the human population carries patent sexual parasites (Drakeley et al., 2006; Bousema et al., 2004). Despite its importance, the extent of such natural variation and susceptibility to intervention remain unclear because it is an extremely difficult parameter to measure in practice (Killeen et al., 2006).

It has long been widely established that the best way to estimate host infectiousness is to detect gametocytes on blood smear and subsequently test their infectivity with a method that can allow measuring key parameters. First, the traditional method for detection of potential infectious individuals has been based on microscopic observation of the gametocytes in their blood using Giemsa-stained thick smear tests (Bonnet et al., 2002, 2003). Recent evidences suggest that this parasitological method may not adequately reflect an individual's infectious status since natural infections are often characterized by levels of circulating parasites far below the threshold for microscopic detection (Shekalaghe et al., 2007; Okell et al., 2009). The quantitative nucleic acid sequence-based amplification (QT-NASBA) is a recent molecular diagnostic technology for detecting submicroscopic gametocyte infections in host blood (Schneider et al., 2007). While this method offers a good degree of sensitivity, it is cumbersome, expensive as it requires special facilities. In addition, it is important to perform definite diagnostic experiments to yield complementary results of the infectivity of such submicroscopic gametocyte the mosquito vectors. Second, the most physiological method for measuring host infectiousness is termed "direct skin feeding" and involves a direct exposure of laboratory-reared mosquitoes on blood samples from pre-selected human gametocyte carriers or upon sufficient samples from each age category within the human population (Githeko et al., 1992; Muirhead-Thomson, 1954). This approach has certain practical advantages over others (Bousema et al., 2012; Robert and Boudin, 2003). Alternatively, the direct membrane feeding assay (DMFA), which relies upon feeding reared mosquitoes on blood samples from gametocyte positive donors under a given set of laboratory conditions is a strategy which is being pursued for many experimental studies of vector–parasite interactions as possible substitute for the direct skin feed of inbred mosquitoes. Available literatures indicate that the results obtained with the DMFA are fully consistent with those yielded by a reference skin feeding test (gold standard) (Bousema et al., 2012; Diallo et al., 2008; Awono-Ambene et al., 2001; Bonnet et al., 2000), suggesting that this approach could be used to monitor the efficacy of specific malaria transmission blocking measures. However, the DMFA involves much more technically difficult procedures that require the drawing of larger blood samples for feeding through thermo-regulated membrane apparatus to laboratory-reared mosquitoes (Harada et al., 1996; Graves, 1980), the expertise for which is not available in most remote areas where field works are undertaken. This approach is also mined by stresses associated with careful husbandry/or breeding of mosquitoes. Under circumstances where there are multiple potential vectors, some anopheline species cannot be conveniently reared under laboratory conditions for the experimental study (Benedict, 1997; Higgs and Beaty, 1996), and some cannot be amenable to feed through artificial membrane for experimental purposes (Boudin et al., 1993; Tchuinkam et al., 1993). Another difficulty is that related to the lack of reproducibility in repeated measurements as observed with the NF54 gametocyte strain (Van der Kolk et al., 2006), and technical expertise is required to obtain reproducible results in routine standardized membrane feeding assay.

A third established method is a simple field-based xenodiagnosis (XD) of naturally acquired infection in wild anthropophilic mosquitoes. Its principle consists essentially in letting wild mosquitoes to bite selected hosts on any night and later examining blood-fed mosquitoes for the presence of malaria parasites. If gametocytes were present in the exposed human host, the only sign of presumption of their transmissibility resides in the presence of oocysts on the mosquito midgut following a 7 days incubation period. This approach offers an advantage in that it allows humans, the parasite they carry, and the naturally occurring vectors that feed upon them, to be identified individually under full field conditions that reliably reflect human biting rates. This XD approach is not novel (Robert et al., 1998; Lines et al., 1991; Muirhead-Thomson, 1957), but it has never been compared with the membrane feeding assay in the field. Here, we sought to extend the existing research by comparing the DMFA with an exploratory field-based XD using randomly selected hosts exposed to adult female Anopheles mosquitoes. Both approaches used oocyst infection rates and infection intensity as indicators of host infectivity. Information from this study may be useful for the development of more convenient field evaluation strategies to quantify infectiousness of the human reservoir and its implications for malaria transmission dynamics.

2. Methods

2.1. Study site

This cross-sectional study was conducted at the Soumousso (11°00′46″N, 4°02′45″W), a rural locality endemic for malaria situated at about 20 km southeast of Bobo-Dioulasso, Western Burkina Faso. This area has perennially high mosquito densities and large tracts of uninhabited swampy land within the village. The area has a rainy season lasting from May to November and a dry season from December to April. Malaria transmission is perennial with large seasonal fluctuations in intensity. The main malaria vectors including *Anopheles gambiae* and *An. funestus* exhibit intense seasonal fluctuations (Dabiré et al., 2008a; Robert et al., 1985). In Soumousso village where the geographical distribution of domestic animals overlaps with human dwellings, both *An. gambiae* and *An. funestus* are known to be anthropophagic and biting in the middle of the night, and strictly indoor-resting species. The average exposure rate is about 50–100 infectious bites per person per year. *P. falciparum* is the predominant malaria parasite with prevalence varying from 58 to 65%, particularly in young children (Dabiré et al., 2008b).

2.2. Participants

The computerized data was retrieved retrospectively from a dataset obtained during a previous study (Gouagna et al., 2010), which provides the parasitological and entomological complete status of host–vector–parasite relationships at in Soumousso. This previous study mostly aimed at determining the influence of host genetic on host infectiousness (irrespective of parasitological status) to local *Anopheles* malaria vectors. Data of a series of 70 children were selected on the basis of criterions that are outlined as follows: human subjects enrolled in study were resident asymptomatic children (age: 3–12 years); they were selected on the basis of their genetic profile and regardless of their gametocytes status (Gouagna et al., 2010); none of them were using bednet either before or during a 5 month study period. They were therefore continuously exposed to risks of natural transmission in area where they live. Exclusion