Am. J. Trop. Med. Hyg., 83(1), 2010, pp. 115–121
doi:10.4269/ajtmh.2010.09-0684

Human Antibody Response to *Anopheles gambiae* Saliva: An Immuno-Epidemiological Biomarker to Evaluate the Efficacy of Insecticide-Treated Nets in Malaria Vector Control

Papa M. Drame,* Anne Poinsignon, Patrick Besnard, Jacques Le Mire, Maria A. Dos-Santos, Cheikh S. Sow, Sylvie Cornelie, Vincent Foumane, Jean-Claude Toto, Mbacké Sembene, Denis Boulanger, François Simondon, Filomeno Fortes, Pierre Carnevale, and Franck Remoue

Institut de Recherche pour le Développement (IRD), UR024 "Epidémiologie et Prévention" Unit, Dakar, Senegal; Institut de Recherche pour le Développement, UR016 "Contrôle et Caractérisation des Populations Vecteurs" Unit, Montpellier, France; Malaria Control Program, Société Nationale de Métallurgie (SONAMET), Lobito, Angola; Organisation de Coordination pour la Lutte contre les Endémies en Afrique (OCEAC), Laboratoire de Recherche sur le Paludisme, Yaoundé, Cameroun; Université Cheikh Anta Diop, Département de Biologie Animale, Dakar, Senegal; National Malaria Control Program, Ministry of Health, Luanda, Angola

Abstract. For the fight against malaria, the World Health Organization (WHO) has emphasized the need for indicators to evaluate the efficacy of vector-control strategies. This study investigates a potential immunological marker, based on human antibody responses to *Anopheles* saliva, as a new indicator to evaluate the efficacy of insecticide-treated nets (ITNs). Parasitological, entomological, and immunological assessments were carried out in children and adults from a malaria-endemic region of Angola before and after the introduction of ITNs. Immunoglobulin G (IgG) levels to *An. gambiae* saliva were positively associated with the intensity of *An. gambiae* exposure and malaria infection. A significant decrease in the anti-saliva IgG response was observed after the introduction of ITNs, and this was associated with a drop in parasite load. This study represents the first stage in the development of a new indicator to evaluate the efficacy of malaria vector-control strategies, which could apply in other arthropod vector-borne diseases.

INTRODUCTION

Most emerging and reemerging diseases are transmitted by arthropod vectors such as mosquitoes (malaria, dengue, and chikungunya), ticks (Lyme disease), and sandflies (leishmaniasis). Among these vector-borne diseases, malaria represents the greatest worldwide public health problem. The World Health Organization (WHO) estimates that 350–500 million people are infected by malaria annually, which causes 1.1–2.7 million deaths.[1] Most of these deaths occur in children from southern Saharan Africa and are caused by *Plasmodium falciparum*, which causes the most severe form of the disease. In these areas, the *Anopheles gambiae* complex is the major vector.[1]

Today, many preventive methods are available against both parasite (chemoprophylaxis) and vector (insecticide-based control of either larvae or adults) and are in widespread use. It has been well-documented that the use of synthetic insecticides can dramatically reduce the incidence of insect-borne diseases, notably malaria.[1–3] Among such strategies, insecticide-treated nets (ITNs) are currently the most efficient at reducing vector exposure, *Plasmodium* transmission, and malaria morbidity.[4–7] When correctly used, even moderate coverage of adults and children (35–65%) can result in greater community benefit than personal protection.[8–11]

Evaluating ITNs efficacy is currently based on entomological methods (mosquito abundance, blood feeding rates, mortality, etc.) and in humans, parasitological tests.[4,12,13] The reference WHO method for phase 3 trials is measurement of the density of *P. falciparum*.[12] However, these methods have certain limitations when it comes to large-scale field studies, especially where transmission rates and exposure levels are low (dry season, altitude, and urban context). For example, the evaluation of *Plasmodium* density requires a precise and active follow-up of populations in longitudinal studies. Entomological methods are mainly applicable at the population level and do not give a measure of the heterogeneity of individual exposure. Human-landing catch using adult volunteers is currently the best method for evaluating individual human exposure; however, this method raises ethical questions, and the results may not extrapolate to children.[14] In addition, as exposure levels dropped after the introduction of ITNs in endemic areas, these monitoring methods became less effective in National Malaria Control Programs.[15]

To improve vector control in line with the WHO recommendations, much effort is being devoted to developing new indicators and methods to evaluate—at the individual level—the efficacy of ITNs and other malaria vector-control strategies.

One approach is based on the idea that exposure to arthropod vector bites can be assessed by monitoring human–vector contact. It had been previously shown that the human antibody (Ab) response to arthropod salivary proteins could give a measure of exposure to vector bites.[16] At the time of biting, the female mosquito injects saliva containing bioactive molecules, including vasodilators and anticoagulants, that make the blood meal possible.[17,18] Human Ab responses to the saliva of *Triatoma*, vectors of Chagas disease,[19] *Ixodes* ticks, vectors of *Borrelia bugdorferi*,[20] phlebotomies,[21,22] vectors of *Leishmania*, *Glossina*,[23] and vectors of human African trypanosomiasis have been reported as reliable immunological markers for vector exposure. With respect to mosquitoes, anti-saliva Ab responses can give a measure of exposure to *Culex* and *Aedes*[24–27] as well as *An. gambiae*.[28]

This work addresses a potentially important application of this "saliva" biomarker as an immunological tool to evaluate the efficacy of ITN-based strategies. Human immunoglobulin G (IgG) responses to whole *An. gambiae* saliva were evaluated before and after the introduction of ITNs in children and adults living in a malaria-endemic and semi-urban area. The results were compared with the reference data derived using entomological and parasitological methods.

MATERIALS AND METHODS

Study population. This study was conducted in Lobito, a coastal city of western Angola, from March 2005 to October

*Address correspondence to Papa Makhtar Drame, IRD/CREC, 01 BP 4414 RP, Cotonou, Bénin. E-mail: papa-makhtar.drame@ird.fr

2006. The site is in the tropical savannah with a rainy season from October to May, and the area is typical of the humid tropical and intertropical regions of Africa with approximately 600–700 mm of rain per year. The duration of the malaria transmission season varies between 7 and 12 months with a peak between January and May. The most important malaria vector in this area is the *An. gambiae* s.l. complex.[29,30]

Twenty-one households of workers for the Société Nationale de Métallurgie (SONAMET) company were randomly selected from a malaria register of 2004 of the SONAMET in-patient clinic. These families lived in the same district of Bella Vista under the aegis of the SONAMET Malaria Control Program (MCP). The number of individuals in each household varied from 2 to 23. Two hundred thirty individuals of the twenty-one selected households, including all children and adults, were included for longitudinal follow-up, and evaluations occurred every 6 weeks over two periods: from March 2005 to January 2006 and from April 2006 to October 2006. The families were given ITNs (long-lasting insecticide net; LLIN) treated with deltamethrin (Permanet mosquito nets) in February 2006 (according to the number of rooms and beds per household). At each visit, thick blood smear and dried blood spot (on filter paper) samples were collected from each individual for parasitological measurements and immunological analysis, respectively. Parasite density (parasitaemia) was calculated as the number of *P. falciparum* parasites per microliter of blood; mean parasitaemia values ($x + 1$) were calculated by including positive and negative individual readings. Immunological tests were performed in a subsample of the whole study population ($N = 109$; 76 children aged from 0 to 10 years and 33 individuals aged more than 10 years) for whom blood spots were available for at least 12 of 14 visits; only 21 of 109 individuals missed one ($N = 13$) or two ($N = 8$) visits. Filter papers were kept at 4°C in Silicagel before testing.

The present study was conducted in accordance with the Edinburgh revision of the Helsinki Declaration and was approved by the ethical authorities (National MCP of the Ministry of Health of Angola, October 17, 2008).

Entomological analysis and survey of ITN use. Mosquitoes were collected every 6 weeks during the studied period at six reference households that were representative of the studied area. *An. gambiae* density was evaluated using capture by light traps (Center for Disease Control and Prevention [CDC] miniature light trap) placed from 19:00 hours to 07:00 hours for two consecutive nights. Polymerase chain reaction (PCR) was used to confirm genus and species to yield an estimate of the number of *An. Gambiae* per trap per night.

After the introduction of ITNs, their use was checked by local staff and through the administration of questionnaires every 6 weeks in all studied households. The questionnaires covered (1) the number of ITNs installed, (2) the number of exchanged ITNs, and (3) the number of damaged ITNs (holes, torn, etc.).

Method of *Anopheles* salivation. A mosquito salivation technique was improved to collect whole salivary extracts (WSE) from uninfected female *An. gambiae* bred in an insectarium (Institut de Recherche pour le Développement (IRD), Dakar, Senegal), as previously described.[28] Briefly, mosquitoes were sedated with CO_2, and their legs and wings were removed. Their proboscis was placed in 10 µL Hepes–NaCl buffer in a conventional plastic tip fixed on a glass slide. Salivation was stimulated by topical application of 0.25% malathion in acetone to the thorax region. After 1 hour of salivation at room temperature, the liquid in the tip (saliva + buffer) was collected and pooled with other mosquito saliva. Despite variability, this technique of salivation yielded 100–400 µg/mL of salivary proteins, which corresponded to 20–75 ng/mosquito.[28] The WSE batch was then pooled to obtain only one WSE that had been stored frozen at –20°C before use in immunological tests.

Evaluation of human IgG Ab levels by enzyme-linked immunosorbent assay. The standardized dried blood spots (blood fills one 1-cm circle) were eluted by incubation in 300 µl of phosphate buffer (PBS; Tween 0.1%, Sigma-Aldrich, St. Louis, MO) at 4°C for 24 hours.

A Maxisorp plate (Nunc, Roskilde, Denmark) was coated with 32 µL of the pooled WSE batch diluted (1/200) in carbonate/bicarbonate buffer for 2 hours and 30 minutes at 37°C. After washing, each eludate was incubated in duplicate at 4°C overnight at a 1/20 dilution (Tween 0.1%). This optimal dilution was determined after several preliminary experiments. Mouse biotinylated Ab to human IgG (BD Pharmingen, San Diego, CA) was incubated at a 1/1,000 dilution (1 hour and 30 minutes at 37°C), and peroxidase-conjugated streptavidin (Amersham, Les Ulis, France) was then added (1/1,000; 1 hour at 37°C). Colorimetric development was carried out using 2,2′-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) diammonium (ABTS; Roche Diagnostics, Lyon, France) in 50 mM citrate buffer (pH 4) containing 0.003% H_2O_2, and absorbance (OD) was measured at 405 nm. In addition, the absence of significant Ab detection was verified in wells without antigen (ODn = background Ab response). Individual results were expressed as the ΔOD value calculated according to the formula $\Delta OD = ODx - ODn$, where ODx represented the mean of individual OD values in antigen wells.

Statistical analysis. All data were analyzed with GraphPad Prism4 software (San Diego, CA). After verifying that values did not assume Gaussian distribution, the non-parametric Mann–Whitney *U* test was used for comparison of Ab levels of two independent groups, the Wilcoxon matched-pairs test was used for comparison of two paired groups, the non-parametric Kruskal–Wallis test was used for comparison of more than two groups, and the χ^2 test was used for comparison of two proportions. All differences were considered significant at $P < 0.05$.

RESULTS

Entomological and parasitological data. The level of exposure to *An. gambiae* s.l. is expressed by the mean number of *An. gambiae* per trap per night at each passage (Figure 1). Before the introduction of ITNs (March 2005 to January 2006), the intensity of exposure was low and varied by season with a peak during the rainy season (April to May). After the introduction of ITNs in 2006, a similar seasonal change was observed (not significant between May 2005 and April/May 2006). These results suggest that exposure levels to *An. gambiae* were similar before and after the introduction of ITNs.

Prevalence and intensity of *P. falciparum* infection in the total population ($N = 230$) and the immunological subpopulation ($N = 109$) (Figure 2) show no significant difference, indicating that the subpopulation seems to be representative of the total population. In 2005, prevalence peaked in May

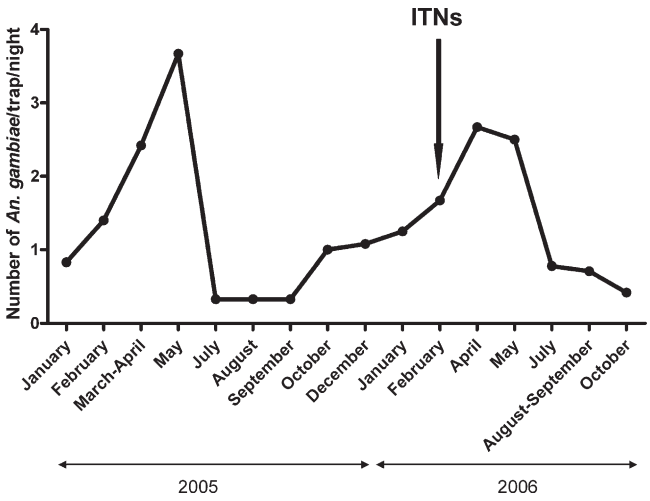

FIGURE 1. Evolution of *An. gambiae* exposure according to the studied period. Exposure to *An. gambiae* s.s. is presented as the mean of number of *An. gambiae* collected by trap and by night in six controlled households for each visit in 2005 and 2006. The arrow indicates the installation of ITNs in February 2006.

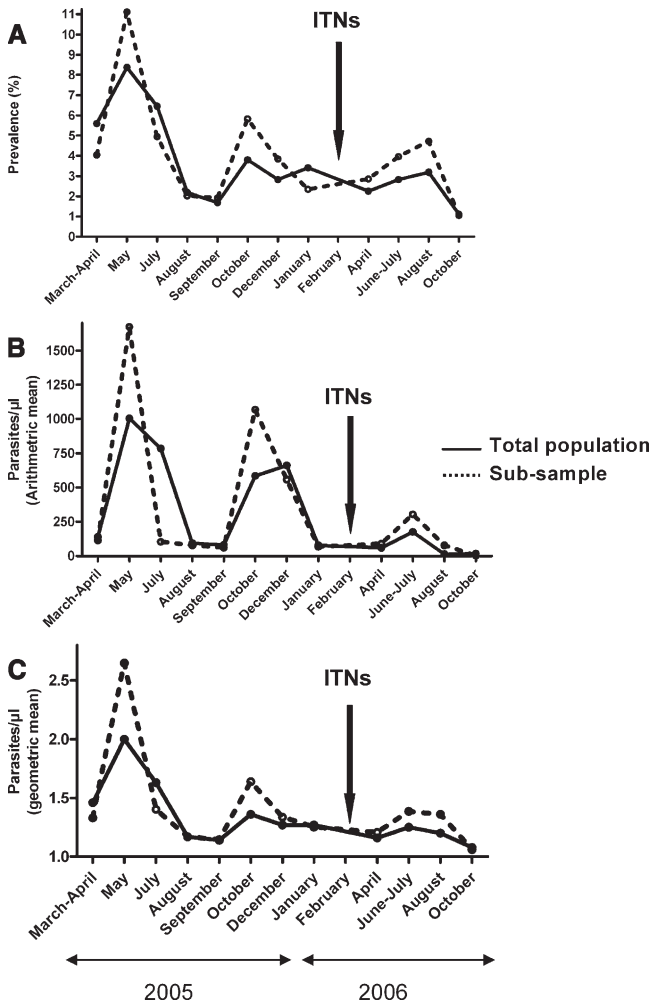

FIGURE 2. Prevalence and intensity of *P. falciparum* infection before and after the introduction of ITNs. The prevalence (**A**), arithmetic (**B**), and geometric (**C**) mean of infection intensity (parasitaemia) are presented in the whole population ($N = 230$; solid line) and the "immunological" subsample ($N = 109$; dotted line), which is described in *Materials and Methods*. The arrow indicates the installation of ITNs in February 2006.

and remained low throughout the study period. After the introduction of ITNs in February 2006, no significant peak was observed, and a reduction of 40% (subpopulation) was observed in April 2006 compared with May 2005 (Figure 2A). In terms of parasitaemia, the arithmetic (Figure 2B) and geometric (Figure 2C) means profoundly decreased in 2006 compared with 2005. In particular, the highest intensity, which was observed in May 2005, disappeared after the introduction of ITNs in 2006, although the difference was not significant ($P = 0.33$ between March/April 2005 and April 2006; $P = 0.07$ between May 2005 and April 2006). Taken together, these results suggest that the use of ITNs in this area effectively decreased malaria transmission, although the prevalence of *P. falciparum* was very low.

Results of ITN surveys. After the installation of ITNs ($N = 230$ for whole studied population) in February 2006, the loss rate and the correct-usage rate (damaged or not) were evaluated by means of two horizontal surveys (Table 1), one at the peak of the exposure season (April = survey 1) and the other at the beginning of the dry season (June/July = survey 2).

After just 2 months of use (survey 1), the rate of ITN loss was 21.5%, and this had increased to 26.9% after 4 months (survey 2; $P = 0.3$). The observed rate of use was 98.0% in April 2006, but it significantly decreased to 86.3% in June/July 2006 ($P = 0.004$). This loss of installed ITNs was accompanied by a significant increase in the use of damaged ITNs from 7% (survey 1) to 15.9% (survey 2; $P < 0.05$). Taken together, these results indicate that after just 4 months of installation (June/July), only 63% (82/130) of the ITNs were installed, and only 53% (69/130) were being used correctly (in use and undamaged).

Evaluation of anti-saliva IgG response before and after ITNs use. Changes in anti-saliva IgG Ab levels (median) and parasitaemia (geometric mean) are presented for the subpopulation (Figure 3). Specific IgG levels showed considerable seasonal variations before the introduction of ITNs in 2005 ($P < 0.0001$). The first peak was observed during the rainy season (May) and was associated with the peak of intensity of *P. falciparum* infection. After a decrease from July to September, a second anti-saliva IgG response peak was observed in October.

As observed for parasitaemia, the level of anti-saliva IgG Ab was considerably lower in 2006 than in 2005, regardless of month. In particular, a very low specific IgG level was observed from April to August 2006. Interestingly, the peak of specific IgG response in April/May 2005 disappeared in April 2006 ($P < 0.0001$), which was shortly after the introduction of ITNs (after 2–4 months of use). This effect was accompanied by a decrease in *P. falciparum* parasitaemia. In October 2006, anti-saliva IgG responses increased over the previous months, which was also observed in 2005; however, the Ab level remained significantly lower than in 2005 ($P < 0.001$).

The trend in anti-saliva IgG responses over the study period was also analyzed according to age (three age groups: 0–6 years [$N = 49$], 7–14 years [$N = 34$], and > 14 years [$N = 25$]). Changes in Ab levels in 2005–2006 were similar in all age groups (data not shown), suggesting that age did not affect specific Ab responses. In addition, it suggested that the considerable decrease in anti-

TABLE 1
Survey of ITNs use

Variable	Survey 1 (April 10–22, 2006)	Survey 2 (June 26–July 12, 2006)	*P*
	Loss rate		
Duration of ITN use (months)	2	4	
ITNs found	102	95	
ITNs lost	28	35	
Rate of loss	21.5%	26.9%	0.3
	Correct-use rate		
ITNs installed (%)	100/102 (98.0%)	82/95 (86.3%)	0.004
ITNs not damaged	93/100 (93.0%)	69/82 (84.1%)	< 0.05
ITNs exchanged	(0.0%)	(0.0%)	

Two sectional surveys evaluating ITNs use were performed in April (peak of the season of exposure to *An. gambiae*—survey 1) and June to July (beginning of dry season—survey 2), just after the installation of ITNs ($N = 130$) in February 2006. The "loss rate" and "correct-use rate" are indicated for each survey, and the *P* value indicates the significant percentage differences between surveys 1 and 2 (χ^2 test).

saliva IgG levels observed after the introduction of ITNs in 2006 was similar at all ages (young children, children, and adults).

Individual anti-saliva IgG response before and after ITNs use. Individual anti-saliva IgG Ab levels were compared before and after the introduction of ITNs; data is only presented for corresponding months in 2005 and 2006 (April/May, July, August, and October) (Figure 4). In April 2006, data were acquired April 10–22, whereas in 2005, they were acquired at the beginning of April (March 26 to April 12) and in mid-May (May 15 to June 4). For this reason, the IgG Ab level in April 2006 has to be compared with both April and May 2005.

In spite of intraindividual variations, a major drop in anti-saliva IgG responses was observed after the introduction of ITNs in all individuals. IgG levels were lower in 2006 than in 2005 for July ($P < 0.01$; Wilcoxon matched-pairs test), August ($P < 0.001$), and October ($P < 0.001$). More interestingly, individual specific Ab levels were very low in April 2006. Major decreases in anti-saliva IgG responses were, therefore, observed in all individuals within 2 months of ITN introduction ($P < 0.001$ for April 2006 compared with March/April 2005; $P < 0.0001$ for April 2006 compared with May 2005; Wilcoxon matched-pairs test).

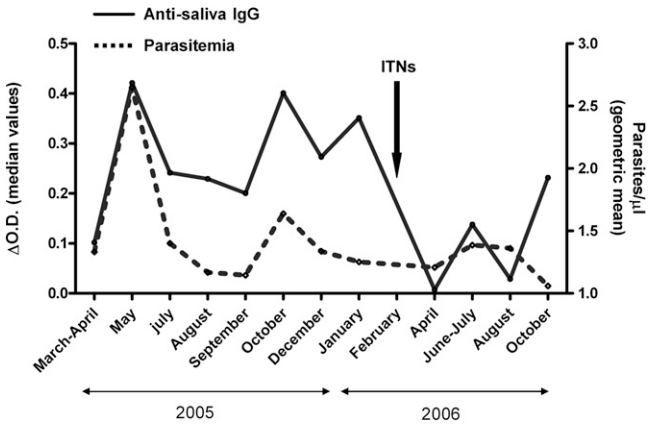

FIGURE 3. IgG antibody response to *An. gambiae* saliva before and after ITNs use. The median anti-saliva IgG level (solid line) and the intensity of parasite infection (the geometric mean of parasite densities is represented by the dotted line) are presented in the "immunological" subsample ($N = 109$) before (2005) and after (2006) the installation of ITNs. The arrow indicates the installation of ITNs in February 2006.

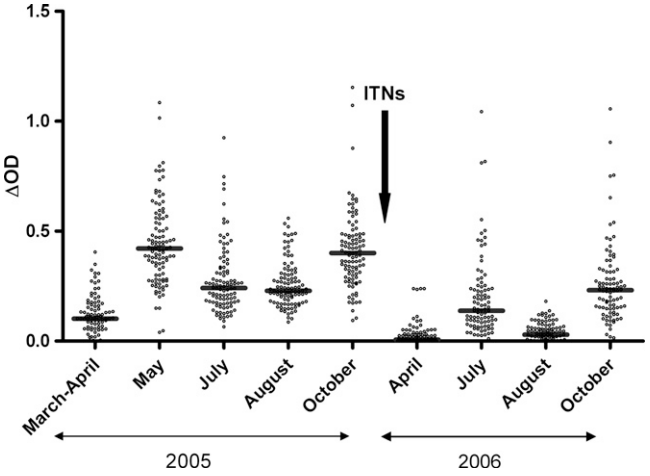

FIGURE 4. Individual IgG antibody levels to *An. gambiae* saliva according the season and the use of ITNs. Individual anti-saliva IgG levels for the "immunological" subpopulation ($N = 109$) before (2005) and after (2006) the installation of ITNs. The arrow indicates the installation of ITNs in February 2006. Bars indicate the median value for each studied month.

DISCUSSION

In this study, the human Ab response to *An. gambiae* whole saliva was investigated over 2 years in adults and children before and after the introduction of ITNs.

Before ITNs (2005), anti-saliva IgG responses varied seasonally. A major peak was observed in May after the rainy period (March to April), and it was associated with the peaks of (1) intensity of exposure to *An. gambiae* bites, as evaluated by entomological methods, and (2) parasite density. These results point to an association between the entomo-parasitological data and anti-saliva IgG Ab levels, confirming the validity of this anti-vector Ab parameter as an immuno-epidemiological marker for *An. gambiae* exposure and the risks of malaria transmission. It confirms previous results observed in Thailand with *An. stephensi* and *P. falciparum* and *P. vivax* complexes[31] and results seen in rural Senegal for *An. gambiae* exposure in children.[28] These results in Angola, covering both children and adults, suggest that this immunological marker could also be used to evaluate *Anopheles* exposure in adults.

The correct use of deltamethrin ITNs (from February 2006) was followed by a considerable decrease of *P. falciparum* prevalence and parasitaemia (the WHO reference criteria for evaluating ITN efficacy), indicating that ITN use was effective. In the area studied, the decrease in prevalence/intensity of malaria was not significant after the introduction of ITNs, although there seemed to be a real decrease between 2005 and 2006; the difference was not significant, probably because of the very low prevalence of malaria coupled with the smallness of the sample. For the purposes of evaluating the efficacy of ITNs, these results strengthen the special interest to evaluate the Ab response to *Anopheles* saliva (1) in a context of low malaria transmission and (2) in a subpopulation (around $N = 100$) compared with analysis with parasitemia referent criteria that needs a large population (around $N = 1{,}000$).

The ITNs surveys indicated that only 53% of ITNs were being correctly used and were undamaged after just 4 months of use (June/July); this result has been reported in several other endemic areas.[32] In concordance with these results, we

have shown that the anti-saliva IgG Ab level decreased considerably after the use of ITNs (2006) compared with 2005. This decrease was observed at both the population level and the individual level and was particularly marked in April 2006 compared with April/May 2005, corresponding to the peak of exposure/transmission. Surprisingly, a small increase in specific IgG levels was observed in October 2006, which had been observed before the introduction of ITNs in 2005. This could be caused by the incorrect use, which is suggested by the results of the ITN surveys. Taken together, the results showed that the evaluation of anti-saliva IgG Ab levels could be used to evaluate the efficacy of ITNs in such malaria hypo-endemic transmission areas. Interestingly, the entomological data indicated that the population density of *An. gambiae* was similar before and after the introduction of ITNs use, suggesting that exposure to *An. gambiae* was similar in the study periods (2005 and 2006; i.e., that the analysis of anti-saliva IgG responses could represent a real evaluation of human–vector contact). This individual approach seems to be complementary to entomological methods and also contributes information about the real intensity of bites by anthropophilic *Anopheles*. In addition, anti-saliva IgG responses showed similar trends in all three of the age groups studied (< 7 years, 7 –14 years, and > 14 years) before and after the introduction of ITNs. This result according to age is surprising in regard to the general immune response to specific antigens, which is known to be acquired progressively with age. This particular property of anti-saliva Ab could be caused by a particular short-time "life" of anti-saliva IgG response. Indeed, this kind of Ab immunity does not seem to accumulate with exposure during individual life but wanes rapidly, even with sustained exposure.[28,33] Nevertheless, these preliminary results according to age have to be confirmed, but they suggest that the use of this "saliva" biomarker could be relevant at all ages; this enhances the relevance of this indicator, because it is also suggested in other areas.[31] Deltamethrin, like most pyrethroid insecticides, has a powerful repulsive effect and is an effective mosquito killer (knockdown effect), but resistance has spread in the last decade in Africa.[34] In this study, ITNs were not observed to have any effect on *Anopheles* density, as evaluated by entomological methods (CDC catches). This could be caused by any or a combination of three factors: (1) the light traps were not installed in rooms containing ITNs (close to mosquito nets) but in yards,[35] (2) CDC traps are not a good method of evaluating small changes in *Anopheles* density in a low-exposure area,[36] or (3) insecticide resistance is high in the studied area. Recent studies (Toto J-C, unpublished data) have indicated that *An. gambiae* in Lobito largely comprises the Savannah (S) form (82%) rather than the Mopti (M) form (18%). Because S form is well-known to be preferentially associated with phenomena of resistances to insecticides,[37] we can hypothesize that insecticide resistance could occur in the studied area. A hypothesis related to Kdr resistance is currently under investigation. Nevertheless, it underlines that the use of anti-saliva IgG responses as an indicator of ITN efficacy could be particularly applicable to areas of high levels of insecticide resistance where current entomological methods have numerous limitations because of changes in vector behavior.[38,39]

The present study focused on the evaluation of IgG Ab level to whole saliva of *An. gambiae*. The sensitivity and specificity of this type of biomarker will have to be enhanced, especially to overcome possible cross-reactions between the salivary proteins of *An. gambiae* and those of other arthropod vectors.[40] The subsequent step will be to identify specific immunogenic *Anopheles* proteins and evaluate the corresponding IgG Ab levels. Several potential candidate biomarkers of exposure to *An. gambiae* have been identified by our team,[41] among which one peptide (P1) of the *An. gambiae* gSG6 protein, is proving to be a reliable immuno-epidemiological marker of the intensity of *Anopheles* exposure.[33] The evaluation of anti–gSG6-P1 IgG response as a marker of ITN efficacy could be planned in the same studied population.

CONCLUSIONS

To identify new biomarkers for malaria-control programs, an efficient and adapted indicator is being sought to evaluate the ITN efficacy. An approach based on measuring IgG Ab responses to *An. gambiae* salivary proteins represents a new way to evaluate the efficacy of vector-control strategies in malaria. This work has also showed that immunological tests can be carried out on blood spots collected on filter paper. This technique is ideal for investigating malaria, because it can be combined with thick blood smears that evaluate *Plasmodium* load. The development of a "saliva" biomarker could represent an alternative way to obtain new criteria for measuring the efficacy of ITNs, which could be integrated with (1) the evaluation of new vector-control strategies (ITN use, household spraying, curtains, etc.) in phase 3 trials and (2) the monitoring of selected strategies by National MCPs. Finally, the development of such a biomarker could be applied to vector-control strategies against other major mosquito-borne diseases such as arboviroses (dengue and chikungunya).

Received November 13, 2009. Accepted for publication January 8, 2010.

Acknowledgments: The authors gratefully acknowledge the population of the Bella Vista area (Lobito, Angola) for their participation in this study, the technical personal of the Malaria Control Program team, especially Maria Chilombo (Société Nationale de Métallurgie (SONAMET) Lobito), and Amady Ndiaye and Souleymane Doucoure from the Institut de Recherche pour le Développement (IRD)-Epidemiology of Vectors (EPIVECT) group for their technical support. The authors thank Dr. Jean-François Foucher from Acer-Energy (ACERGY) Company for his helpful contribution.

Financial support: Papa M. Drame was supported by a fellowship provided by Institut de Recherche pour le Développement (IRD) (Département Soutien et Formation). Anne Poinsignon was supported by fellowships provided by the Research Ministry of France and François Lacoste. Sylvie Cornelie was supported by a fellowship from the Inkerman Foundation. This work was supported by Acer-Energy (ACERGY)-Société Nationale de Métallurgie (SONAMET) Company (Malaria Control Program), the French Ministry of Defense (Délégation Générale pour l'Armement, DGA), and the IRD.

Authors' addresses: Papa Makhtar Drame, IRD/CREC, 01 BP 4414 RP, Cotonou, Bénin, E-mail: papa-makhtar.drame@ird.fr. Anne Poinsignon, IRD-UR016, BP 64501, 34394 Montpellier Cedex 5, France, E-mail: a.poinsignon@uq.edu.au. Patrick Besnard, Sonamet Company, 15 Rua 1° dezembro, CP 479, Lobito, Benguela Provincia, Angola, E-mail: prbesnard@yahoo.fr. Jacques Le Mire, Sonamet Company, 15 Rua 1° dezembro, CP 479, Lobito, Benguela Provincia, Angola, E-mail: drjlemire@yahoo.fr. Maria A. Dos-Santos, Sonamet Company, 15 Rua 1° dezembro, CP 479, Lobito, Benguela Provincia, Angola, E-mail: doctor.lobito@sonamet.com. Cheikh S. Sow, Espoir pour la Santé, St. Louis, Sénégal, E-mail: cheikh.sow@espoir-sante.org. Sylvie Cornélie, IRD-UR016, BP 64501, 34394 Montpellier Cedex 5, France, E-mail: sylvie.cornelie@ird.fr. Vincent Foumane, LRP-OCEAC, Yaoundé, Cameroun, E-mail: vfoumane@yahoo.fr.

Jean-Claude Toto, LRP-OCEAC, Yaoundé, Cameroun, E-mail: jctotofr@yahoo.fr. Mbacké Sembène, Département de Biologie Animale, BP 5005, FST-UCAD, Dakar, Sénégal, E-mail: sembene@ird.sn. Denis Boulanger, IRD-UMR145, BP 64501, 34394 Montpellier Cedex 5, France, E-mail: denis.boulanger@ird.fr. Francois Simondon, IRD-UMR145, BP 64501, 34394 Montpellier Cedex 5, France, E-mail: FSimondon@spmsd.com. Filomeno Fortes, National Malaria Control Program, National Institute of Public Health, Ministry of Health, Luanda, Angola, E-mail: filomenofortes@gmail.com. Pierre Carnevale, IRD-UR016, BP 64501, 34394 Montpellier Cedex 5, France, E-mail: pjcarnevale@gmail.com. Franck Remoue, IRD/CREC, 01 BP 4414 RP, Cotonou, Bénin, E-mail: franck.remoue@ird.fr..

REFERENCES

1. WHO-UNICEF, 2005. *World Malaria Report 2005*. Available at: www.rollbackmalaria.org. Accessed July 2009.
2. Lengeler C, 2004. Insecticide-treated nets for malaria control: real gains. *Bull World Health Organ 82:* 85–91.
3. Hemingway J, Bates I, 2003. Malaria: past problems and future prospects. After more than a decade of neglect, malaria is finally back on the agenda for both biomedical research and public health politics. *EMBO Rep 4:* S29–S31.
4. Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA, Curtis CF, 2002. Effect of community-wide use of insecticide-treated nets for 3–4 years on malarial morbidity in Tanzania. *Trop Med Int Health 7:* 1003–1008.
5. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczak MS, Hightower AW, 2003. Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *Am J Trop Med Hyg 68:* 121–127.
6. Carnevale P, Robert V, Snow R, Curtis C, Richard A, Boudin C, Pazart LH, Halna JM, Mouchet J, 1991. The impact of impregnated mosquito nets on prevalence and morbidity related to malaria in sub-Saharan Africa. *Ann Soc Belg Med Trop 71 (Suppl 1):* 127–150.
7. Carnevale P, Robert V, Boudin C, Halna JM, Pazart L, Gazin P, Richard A, Mouchet J, 1988. Control of malaria using mosquito nets impregnated with pyrethroids in Burkina Faso. *Bull Soc Pathol Exot 81:* 832–846.
8. Nevill CG, Some ES, Mung′ala VO, Mutemi W, New L, Marsh K, Lengeler C, Snow RW, 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Trop Med Int Health 1:* 139–146.
9. Killeen GF, Smith TA, Ferguson HM, Mshinda H, Abdulla S, Lengeler C, Kachur SP, 2007. Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. *PLoS Med 4:* e229.
10. Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude GH, Armah GE, Kajihara B, Adiamah JH, Smith PG, 1996. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Trop Med Int Health 1:* 147–154.
11. Binka FN, Indome F, Smith T, 1998. Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana. *Am J Trop Med Hyg 59:* 80–85.
12. Lengeler C, 2004. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev 2,* Art. No.: CD000363. Available at: www.cochrane.org/reviews/.../ab000363.html. Accessed July 2009.
13. Chouaibou M, Simard F, Chandre F, Etang J, Darriet F, Hougard JM, 2006. Efficacy of bifenthrin-impregnated bednets against *Anopheles funestus* and pyrethroid-resistant *Anopheles gambiae* in north Cameroon. *Malar J 5:* 77.
14. Smith T, Killeen G, Lengeler C, Tanner M, 2004. Relationships between the outcome of *Plasmodium falciparum* infection and the intensity of transmission in Africa. *Am J Trop Med Hyg 71:* 80–86.
15. Noor AM, Moloney G, Borle M, Fegan GW, Shewchuk T, Snow RW, 2008. The use of mosquito nets and the prevalence of *Plasmodium falciparum* infection in rural South Central Somalia. *PLoS One 3:* e2081.
16. Schwartz BS, Ribeiro JM, Goldstein MD, 1990. Anti-tick antibodies: an epidemiologic tool in Lyme disease research. *Am J Epidemiol 132:* 58–66.
17. Ribeiro JM, Makoul GT, Levine J, Robinson DR, Spielman A, 1985. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, *Ixodes dammini*. *J Exp Med 161:* 332–344.
18. Ribeiro JM, Francischetti IM, 2003. Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. *Annu Rev Entomol 48:* 73–88.
19. Nascimento RJ, Santana JM, Lozzi SP, Araujo CN, Teixeira AR, 2001. Human IgG1 and IgG4: the main antibodies against *Triatoma infestans* (Hemiptera: Reduviidae) salivary gland proteins. *Am J Trop Med Hyg 65:* 219–226.
20. Lane RS, Moss RB, Hsu YP, Wei T, Mesirow ML, Kuo MM, 1999. Anti-arthropod saliva antibodies among residents of a community at high risk for Lyme disease in California. *Am J Trop Med Hyg 61:* 850–859.
21. Rohousova I, Ozensoy S, Ozbel Y, Volf P, 2005. Detection of species-specific antibody response of humans and mice bitten by sand flies. *Parasitology 130:* 493–499.
22. Barral A, Honda E, Caldas A, Costa J, Vinhas V, Rowton ED, Valenzuela JG, Charlab R, Barral-Netto M, Ribeiro JM, 2000. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker? *Am J Trop Med Hyg 62:* 740–745.
23. Poinsignon A, Remoue F, Rossignol M, Cornelie S, Courtin D, Grebaut P, Garcia A, Simondon F, 2008. Human IgG antibody response to Glossina saliva: an epidemiologic marker of exposure to Glossina bites. *Am J Trop Med Hyg 78:* 750–753.
24. Reunala T, Brummer-Korvenkontio H, Palosuo K, Miyanij M, Ruiz-Maldonado R, Love A, Francois G, Palosuo T, 1994. Frequent occurrence of IgE and IgG4 antibodies against saliva of *Aedes communis* and *Aedes aegypti* mosquitoes in children. *Int Arch Allergy Immunol104*: 366–371.
25. Remoue F, Alix E, Cornelie S, Sokhna C, Cisse B, Doucoure S, Mouchet F, Boulanger D, Simondon F, 2007. IgE and IgG4 antibody responses to *Aedes* saliva in African children. *Acta Trop 104:* 108–115.
26. Peng Z, Rasic N, Liu Y, Simons FE, 2002. Mosquito saliva-specific IgE and IgG antibodies in 1059 blood donors. *J Allergy Clin Immunol 110:* 816–817.
27. Das MK, Mishra A, Beuria MK, Dash AP, 1991. Human natural antibodies to *Culex quinquefasciatus*: age-dependent occurrence. *J Am Mosq Control Assoc 7:* 319–321.
28. Remoue F, Cisse B, Ba F, Sokhna C, Herve JP, Boulanger D, Simondon F, 2006. Evaluation of the antibody response to *Anopheles* salivary antigens as a potential marker of risk of malaria. *Trans R Soc Trop Med Hyg 100:* 363–370.
29. Cuamba N, Choi KS, Townson H, 2006. Malaria vectors in Angola: distribution of species and molecular forms of the *Anopheles gambiae* complex, their pyrethroid insecticide knockdown resistance (kdr) status and *Plasmodium falciparum* sporozoite rates. *Malar J 5:* 2.
30. Calzetta M, Santolamazza F, Carrara GC, Cani PJ, Fortes F, Di Deco MA, della Torre A, Petrarca V, 2008. Distribution and chromosomal characterization of the *Anopheles gambiae* complex in Angola. *Am J Trop Med Hyg 78:* 169–175.
31. Waitayakul A, Somsri S, Sattabongkot J, Looareesuwan S, Cui L, Udomsangpetch R, 2006. Natural human humoral response to salivary gland proteins of *Anopheles* mosquitoes in Thailand. *Acta Trop 98:* 66–73.
32. Noor AM, Mutheu JJ, Tatem AJ, Hay SI, Snow RW, 2009. Insecticide-treated net coverage in Africa: mapping progress in 2000-07. *Lancet 373:* 58–67.
33. Poinsignon A, Cornelie S, Mestres-Simon M, Lanfrancotti A, Rossignol M, Boulanger D, Cisse B, Sokhna C, Arca B, Simondon F, Remoue F, 2008. Novel peptide marker corresponding to salivary protein gSG6 potentially identifies exposure to *Anopheles* bites. *PLoS One 3:* e2472.
34. Darriet F, Guillet P, N′Guessan R, Doannio JM, Koffi A, Konan LY, Carnevale P, 1998. Impact of resistance of *Anopheles gambiae* s.s. to permethrin and deltamethrin on the efficacy of impregnated mosquito nets. *Med Trop 58:* 349–354.
35. Mboera LE, Kihonda J, Braks MA, Knols BG, 1998. Short report: influence of centers for disease control light trap position,

relative to a human-baited bed net, on catches of *Anopheles gambiae* and *Culex* quinquefasciatus in Tanzania. *Am J Trop Med Hyg 59:* 595–596.

36. Mbogo CN, Glass GE, Forster D, Kabiru EW, Githure JI, Ouma JH, Beier JC, 1993. Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *J Am Mosq Control Assoc 9:* 260–263.
37. Chandre F, Manguin S, Brengues C, Dossou Yovo J, Darriet F, Diabate A, Carnevale P, Guillet P, 1999. Current distribution of a pyrethroid resistance gene (kdr) in *Anopheles gambiae* complex from West Africa and further evidence for reproductive isolation of the Mopti form. *Parassitologia 41:* 319–322.
38. N′Guessan R, Corbel V, Akogbeto M, Rowland M, 2007. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg Infect Dis 13:* 199–206.
39. Mbogo CN, Baya NM, Ofulla AV, Githure JI, Snow RW, 1996. The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. *Med Vet Entomol 10:* 251–259.
40. Poinsignon A, Cornelie S, Remoue F, Grebaut P, Courtin D, Garcia A, Simondon F, 2007. Human/vector relationships during human African trypanosomiasis: initial screening of immunogenic salivary proteins of Glossina species. *Am J Trop Med Hyg 76:* 327–333.
41. Cornelie S, Remoue F, Doucoure S, Ndiaye T, Sauvage FX, Boulanger D, Simondon F, 2007. An insight into immunogenic salivary proteins of *Anopheles gambiae* in African children. *Malar J 6:* 75.