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Human Antibody Response to Aedes aegypti Saliva in an Urban Population in Bolivia: A New Biomarker of Exposure to Dengue Vector Bites

Souleymane Doucoure,*† François Mouchet,† Amandine Cournil, Gilbert Le Goff, Sylvie Cornelie, Yelin Roca, Mabel Guerra Giraldez, Zaira Barja Simon, Roxanna Loayza, Dorothée Misse, Jorge Vargas Flores, Annie Walter, Christophe Rogier, Jean Pierre Herve, and Franck Remoue
Institut de Recherche pour le Développement, Maladie Infectieuse et Vecteurs, Ecologie, Génétique, Evolution et Contrôle, Centre Institut de Recherche pour le Développement de Montpellier, Montpellier, France; Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Bolivia; Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo, Marseille, France

Abstract. Aedes mosquitoes are important vectors of re-emerging diseases in developing countries, and increasing exposure to Aedes in the developed world is currently a source of concern. Given the limitations of current entomologic methods, there is a need for a new effective way for evaluating Aedes exposure. Our objective was to evaluate specific antibody responses to Aedes aegypti saliva as a biomarker for vector exposure in a dengue-endemic urban area. IgG responses to saliva were strong in young children and steadily waned with age. Specific IgG levels were significantly higher in persons living in sites with higher Ae. aegypti density, as measured by using entomologic parameters. Logistic regression showed a significant correlation between IgG to saliva and exposure level, independently of either age or sex. These results suggest that antibody responses to saliva could be used to monitor human exposure to Aedes bites.

INTRODUCTION

Aedes mosquitoes are major vectors of re-emerging diseases including arbovirus infections (dengue, chikungunya, yellow fever). In Asia, Africa and South America, arthropod-borne diseases are major health problems, and some are viewed as re-emerging diseases. In addition, several diseases threaten to emerge in the developed world as a result of increasing exchanges with developing countries. Chikungunya outbreaks were recorded in 2005–2006 on Reunion Island and in 2007 in Italy.1 Dengue fever and more severe forms of dengue are also a major re-emerging infectious disease, and represent a risk in developed countries. The World Health Organization estimates that 50 million dengue infections occur every year worldwide. In South America, dengue infection is epidemic, especially in urban areas in Bolivia, where Ae. aegypti is the only known vector.4

These findings have prompted development of surveillance systems, including networks to monitor Aedes populations to identify the risks of transmission of dengue and other arboviruses.5,6 New epidemiologic tools for evaluating exposure to Aedes bites are thus needed in developing and developing countries. The level of exposure of human populations to Aedes bites is mainly evaluated by identification of breeding sites, capture of mosquitoes by trapping, aspirators, indoor spraying, and human landing catches. Some studies indicated that pupal monitoring could be useful for the epidemiologic surveillance of Ae. aegypti exposure.7,8 The indices of Breteau, adult productivity, house and adult density are the best current indicators for evaluating the abundance of adult Aedes.9 However, these entomologic methods have major limitations. Breeding site counting is long and difficult, and measurements are only accurate for high-density populations. Current entomologic methods are mainly applicable at the population level and cannot evaluate the heterogeneity of individual exposure to Aedes bites. In addition to their significant limitations for large-scale measurements in the field, there are ethical concerns, especially for human landing catches. These limitations appeared more considerable in the context of urban exposure. Much effort is now being devoted to develop new, simple, rapid and sensitive complementary indicators to evaluate exposure to Aedes bites and estimate the potential risk of arbovirus transmission in exposed populations.

Human exposure to arthropod vector bites can be assessed by monitoring human–vector contact. It has been previously demonstrated that the human antibody response to arthropod salivary proteins correlated with the intensity of exposure.10,11 At the time of biting, the female mosquito injects saliva containing bioactive molecules, including vasodilators and anticoagulants, which promote blood feeding.12,13 Human antibody responses to the saliva of Triatoma, the vectors of Chagas disease,14 and Ixodes tick vectors of Borrelia burgdorferi15 have been shown to be reliable immunologic markers for vector exposure. Poinsignon and others have shown that antibody responses to Glossina saliva could be a useful indicator of exposure with high diagnostic value.16 Antibody responses to saliva can also provide a measure of exposure to mosquitoes, such as Culex.17,18 Recently, it has been demonstrated that the IgG response to whole saliva from Anopheles gambiae is a reliable biomarker of exposure and the risk for developing malaria infection/morbidity.19 This association has been also observed for An. dirus and An. darlingi.20

Most studies of antibody responses to Aedes saliva have focused on allergic reactions with a view to identifying the allergenic salivary proteins22 and developing new diagnostic tests for Aedes-dependent allergy in the developed world (Finland and Canada). These studies demonstrated that the quantitative evaluation of antibody responses to saliva by enzyme-linked immunosorbent assay (ELISA) could be a useful biomarker for human exposure to Aedes bites.23,24 IgG4 to Aedes aegypti saliva was associated with intense exposure to Aedes bites.25

Recently, IgM and IgG responses to Ae. aegypti saliva were also considered to be a surrogate biomarker for exposure in
travelers, suggesting that antibody testing could be relevant to short-term exposure.26 Remoue and others showed that IgG responses to *Aedes* saliva could reflect the exposure of human populations in the developing world.27 IgE and IgG4 responses to *Ae. aegypti* saliva were detected in young children in Sengal living in an arbovirus-endemic area (dengue, yellow fever, chikungunya). The level of the specific antibody responses increased during the rainy season and varied according to villages studied. However, no entomologic data were available during this study, and no association could be made with antibody responses to saliva.

The objective of the present study was to evaluate the specific IgG response to whole *Ae. aegypti* saliva in persons living in an urban setting in Bolivia where this species is the only vector of dengue and dengue outbreaks are reported regularly.4 Immunologic results were analyzed according to: age (children and adults) and reference entomologic data, which estimate exposure levels to adult *Aedes*.

**MATERIALS AND METHODS**

**Study population.** The study was conducted in an urban area in the city of Santa Cruz de la Sierra, Bolivia, and was integrated to a large multidisciplinary study. *Aedes aegypti* is found in this area and several outbreaks of dengue had occurred in previous years. These outbreaks were caused by dengue virus serotype 3 (DENV-3) in 2003–2004 and DENV-1 in 2008. During the study period, a large dengue epidemic (DENV-2 and DENV-3) occurred in 2007 in Santa Cruz.

Households were selected by cluster survey. From maps and the last population census (2001), 100 city blocks (cluster) were chosen by using a selection probability proportional to population. In each cluster, a household was randomly chosen as a starting point by using an azimuth. Households were then sampled until 10 blood samples were obtained from residents. All residents from each selected house were invited to participate. When 10 serum samples were not directly obtained in the first house, the residents of the house on the left were also solicited. If a household refused to participate in the survey, the house on the left was solicited. Standardized questionnaires were given to the head of each household, and an individual questionnaire was given to each resident who gave a blood sample. Serum was ree collected in April–May 2007 from 1,049 persons 3–94 years of age, as shown in Table 1.

The study adhered to the ethical principles stipulated in the Edinburgh revision of the Helsinki Declaration, and was approved by the Bolivian Committee of Bioethics (September 2006). Informed consent was obtained from all adult participants and from the parents or legal guardians of minor subjects.

**Entomologic assessment.** Each household selected for the epidemiologic study was visited at the same time by two entomologic teams. Entomologic measurements were made every day for five weeks (April 23–May 30, 2007) in the morning (8:00 AM–noon) and afternoon (2:00 PM–6:00 PM). A total of 896 prospection units (households) were visited. In each prospection unit, all sites containing *Ae. aegypti* larvae and pupae were identified and characterized. All aquatic stages (L1–L4 larvae stage and pupae) were collected and counted.

According to the count of larvae and pupae, two entomologic parameters (exposure 1 and exposure 2) were defined to assess the level of exposure to *Ae. aegypti*. These entomologic parameters were defined according to those of previous stud-
ter unit was added as random effect. For age and sex. Similar to logistic regressions, the clus-

er regressions (MIXED procedure) were used to model the sampling. After Box-Cox transformation of added in the model as a random effect to account for cluster probability of being an immune responder according to exposure (NLMIXED procedure) were used for modeling the proba-

bility of being an immune responder according to exposure. Six age groups were defined: Exposure 1: immediate adult exposure (L3 + L4 + pupae/no. inhabitants) Exposure 2: long-lasting breeding site with L1 or L2 and L4 or pupae/no. inhabitants

<table>
<thead>
<tr>
<th>Exposure level to Aedes aegypti</th>
<th>Exposure group (n)</th>
<th>Cluster (n)</th>
<th>Individual (n)</th>
<th>Mean age years, (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure 1: immediate adult exposure</td>
<td>1 (0–5) 24 23 15</td>
<td>25 23 15</td>
<td>256 235 170</td>
<td>30.8 (3–83) 28.9 (4–87) 28.6 (6–75)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure 2: long-lasting breeding site with L1 or L2 and L4 or pupae/no. inhabitants</th>
<th>Exposure group (n)</th>
<th>Cluster (n)</th>
<th>Individual (n)</th>
<th>Mean age years, (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure 2: long-lasting breeding site with L1 or L2 and L4 or pupae/no. inhabitants</td>
<td>1 (0–0.057) 11 11</td>
<td>11 11</td>
<td>113 113</td>
<td>30.4 (5–79) 30.4 (5–79)</td>
</tr>
<tr>
<td></td>
<td>2 (0.058–0.105) 24 27</td>
<td>25 27</td>
<td>258 272</td>
<td>29.6 (3–78) 28.8 (4–85)</td>
</tr>
<tr>
<td></td>
<td>3 (0.106–0.138) 30 27</td>
<td>25 27</td>
<td>258 272</td>
<td>31.1 (4–87) 31.4 (4–87)</td>
</tr>
<tr>
<td></td>
<td>4 (0.139–0.200) 14 11</td>
<td>14 11</td>
<td>155 113</td>
<td>30.4 (5–94) 30.4 (5–94)</td>
</tr>
<tr>
<td></td>
<td>5 (≥ 0.200) 11 11</td>
<td>11 11</td>
<td>113 113</td>
<td>30.4 (5–79) 30.4 (5–79)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. males</th>
<th>No. females</th>
<th>Total</th>
<th>No. immune responders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 14</td>
<td>115</td>
<td>110</td>
<td>225</td>
<td>190 (84.4)</td>
</tr>
<tr>
<td>15–24</td>
<td>114</td>
<td>145</td>
<td>259</td>
<td>159 (65.2)</td>
</tr>
<tr>
<td>25–34</td>
<td>66</td>
<td>139</td>
<td>205</td>
<td>104 (50.7)</td>
</tr>
<tr>
<td>35–44</td>
<td>46</td>
<td>14</td>
<td>60</td>
<td>40 (37.7)</td>
</tr>
<tr>
<td>45–54</td>
<td>41</td>
<td>46</td>
<td>87</td>
<td>48 (42.1)</td>
</tr>
<tr>
<td>≥ 55</td>
<td>38</td>
<td>76</td>
<td>114</td>
<td></td>
</tr>
</tbody>
</table>

**Logistic regression analysis indicated that these differences between children and adults persisted after adjustment for exposure to Aedes (Table 2). Percentages of responders were significantly higher (P < 0.0001) in the < 14 and the 15–24 year age groups than in the > 55 year age group. This difference was also significant when both entomologic parameters (exposure 1 and 2) had been taken into account. These statistical results indicated that the age-related waning of the antibody response was not dependent on the level of Aedes exposure. Additional**

![Figure 1](image-url)
According to exposure level for the immediate adult exposure value of specific IgG level increased with exposure to exposure categorized in five arbitrary groups (1 to 5). The median of specific IgG level was higher in group 5 (Figure 2A) and long-lasting breeding site (Figure 2B) parameters. The median of specific IgG level was higher in group 5 than in the other exposure groups for both entomologic parameters (exposure 1 and 2).

**Antibody response and exposure to Aedes aegypti.** The specific IgG response was evaluated according to the intensity of exposure to *Ae. aegypti* vector as defined by two complementary entomologic parameters (Figure 2 and Table 1). The exposure 1 parameter represents a global picture of the risk to immediate exposure to *Ae. aegypti* adults and the exposure 2 parameter represents a picture of the long-lasting breeding site. Exposure 1 was calculated as the number of *L3 + L4 + pupae* per individual resident of the studied household, and exposure 2 was calculated as the number of breeding site containing *L1 or L2 + pupae* per resident. The percentage of immune responders (Table 1) and the level of the IgG response to saliva (Figure 2) were evaluated according to the level of exposure categorized in five arbitrary groups (1–5; Table 1).

For the entire population, 59% of persons were immune responders. According to entomologic exposure, no variation in percentage was observed between the first four exposure groups, regardless of the exposure parameter (exposure 1 or 2). A higher percentage of immune responders was observed in the highest exposure level group (group 5) than in the other groups (68.6% and 72.6% were immune responders in group 5 by exposure 1 and exposure 2 parameters, respectively). This trend was confirmed by the results for the level of IgG to saliva according to exposure level (Figure 2). The median value of specific IgG level increased with exposure to *Ae. aegypti*. Levels of IgG to saliva were significantly different according to exposure level for the immediate adult exposure (Figure 2A) and long-lasting breeding site (Figure 2B) parameters. The median of specific IgG level was higher in group 5 than in the other exposure groups for both entomologic parameters (exposure 1 and 2).

**Multivariate analysis of antibody response.** To assess the association between the probability of being an immune responder and *Aedes* exposure level independent of potential confounders, mixed logistic regressions were performed for the two exposure parameters (Table 2). Although the probability of being an immune responder increased with exposure (from group 1 to 5), the global effect of exposure was statistically

---

**Table 2**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratios</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposure group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2 vs. 1</td>
<td>0.9</td>
<td>0.6–1.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Group 3 vs. 1</td>
<td>1.0</td>
<td>0.7–1.6</td>
<td>0.63</td>
</tr>
<tr>
<td>Group 4 vs. 1</td>
<td>1.3</td>
<td>0.8–2.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Group 5 vs. 1</td>
<td>1.6</td>
<td>1.0–2.6</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 14 vs. ≥ 55</td>
<td>9.1</td>
<td>5.4–15.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>15–24 vs. ≥ 55</td>
<td>2.8</td>
<td>1.8–4.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>25–34 vs. ≥ 55</td>
<td>1.4</td>
<td>0.9–2.3</td>
<td>0.12</td>
</tr>
<tr>
<td>35–44 vs. ≥ 55</td>
<td>1.4</td>
<td>0.8–2.3</td>
<td>0.21</td>
</tr>
<tr>
<td>45–54 vs. ≥ 55</td>
<td>0.8</td>
<td>0.5–1.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Sex (F vs. M)</td>
<td>1.8</td>
<td>1.3–2.3</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

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*A* = larva; *P* = pupae.

†As indicated in the Materials and Methods.
significant only for the exposure 2 parameter (long-lasting breeding site). The risk of being an immune responder increased (odds ratio [OR] = 2.6) between the highest exposure group (group 5) and the lowest exposure group (group 1).

In both models, the effects of age and sex were highly significant (Table 2). Persons < 14 years of age had a much higher risk of being immune responders than persons > 55 years of age (OR = 9.1, 95% confidence interval = 5.4–15.6, P < 0.0001 and OR = 9.7, 95% confidence interval = 5.7–16.7, P < 0.0001 for exposure 1 and exposure 2 parameters, respectively). The random effect for cluster sampling was weak and not significant.

When we used level of antibody to saliva as a continuous variable, similar trends were found. The global effect of exposure was not statistically significant for either exposure parameter.

Two-by-two comparisons between exposure group 1 and exposure group 5 showed significant differences for both exposure indicators (β ± SD = −0.32 ± 0.15, P = 0.03 and β ± SD = −0.40 ± 0.15, P = 0.01 for exposure parameters 1 and 2, respectively). Similar results were found for comparisons of group 2 and group 5 (β ± SD = −0.33 ± 0.15, P = 0.02 and β ± SD = −0.37 ± 0.15, P = 0.02 for exposure parameters 1 and 2, respectively). In these models, the cluster effect was weak but significant and accounted for approximately 5% of the residual variance.

These results indicated that the IgG response to saliva increased with the level of Aedes exposure and was higher in group 5 (higher exposure) than in the other entomologic groups. This association was not dependent on age and sex and appeared particularly strong when the long-lasting breeding site method (exposure 2 parameter) was used to determine the potential exposure level.

A large dengue epidemic (DENV-2 and DENV-3) occurred in 2007 in Santa Cruz. In April–May 2007, the prevalence of IgM and IgG to dengue was 11.2% and 52.7%, respectively. The association between antibody response to saliva and seropositivity of persons for DENV was evaluated by comparing IgG levels to saliva with the percentage of responders either for IgM (recent infection) or IgG (previous infection). No significant difference was observed.

**DISCUSSION**

This study reports results of a large-scale epidemiologic study of antibody responses to Aedes saliva and entomologic data for Ae. aegypti exposure. The results showed that IgG responses to Ae. aegypti saliva were detected in many persons living in an urban area in Bolivia where Ae. aegypti is found. Despite disparate OD values, the IgG response to saliva was age dependent and identified most responders in the youngest age group. The specific antibody response decreased with age and stabilized in persons > 35 years of age. This study demonstrated a positive association between the IgG response to saliva and the level of exposure to Ae. aegypti as measured by the two entomologic parameters (immediate exposure to Ae. aegypti adults and long-lasting breeding site). Logistic regression confirmed that these associations were not dependent on age or sex. Age was not a confounding factor for the association between IgG response to saliva and level of human exposure to the mosquito vector.

The influence of age on the development of the antibody response to saliva has described. Levels of IgE and IgG4 against Aedes saliva were higher in the youngest children exposed to Ae. aegypti. The same results were obtained for children exposed to An. gambiae bites. However, no study has investigated the antibody response to saliva in children and adults. Our results showed that the IgG response to saliva was higher in children. Adults and children in our study lived in the same households, and their exposure to Ae. aegypti could be assumed to be similar. Three hypotheses might explain these results. The first hypothesis is that the antibody response to saliva correlated with the number of bites received, which implies that children are bitten more than adults in this area. The second hypothesis is that children react more strongly to Aedes bites than adults. The third hypothesis is that adults might show desensitization to saliva proteins and become immune tolerant to saliva antigens after long-term exposure.

With regard to the first hypothesis, it is generally accepted that Ae. aegypti is aggressive during the day and shows peaks early in the morning and at the start of the evening. At these times, children and adults probably had the same exposure to mosquitoes. Nevertheless, we cannot exclude the possibility that children may be more attractive to Aedes than adults. A study that analyzed blood meals of female Ae. aegypti by DNA fingerprinting showed that young adults are bitten more often than children. Another study using the same method showed an association between biting rate and age of women (women > 15 years of age received more bites).

With regard to the second hypothesis, the immune system of children would be more sensitive to antigenic stimulation than that of an adult. Aedes saliva is highly allergenic and induces a strong specific antibody response, which could explain why children could show development of stronger antibody responses to saliva than adults.

With regard to the third hypothesis, potential natural desensitization that occurs in adults over time may be a factor. A shift to production of IgG4 and IgE could be driven by chronic antigenic stimulation. The present work evaluated only IgG responses to Ae. aegypti saliva because previous studies have demonstrated that the IgG response is a useful biomarker for exposure to mosquito bites. Further investigations are therefore needed to establish whether the antibody response to saliva is age dependent.

The major result of this study is that the strength of the IgG response against Ae. aegypti saliva is positively associated with exposure to the vector, as confirmed by logistic regression analysis. On the basis of immature stage counts, two entomologic parameters were defined to evaluate exposure: immediate exposure to Ae. aegypti adults and long-lasting breeding sites. The population was then divided into five classes according to both parameters of exposure. We demonstrated that the percentage of IgG responders differed significantly between exposure groups. This difference was pronounced between the highest exposure and lowest exposure groups. It suggests that the evaluation of the IgG response to saliva might distinguish high-level exposure to Aedes bites. However, our study did not show a linear progression of antibody response to saliva according to exposure.

The reference entomologic methods measuring exposure in this study failed to distinguish such a progression. This lack of discrimination between low and high exposure represents a limitation, but this study clearly showed an increase in IgG response against Ae. aegypti saliva linked to both entomologic exposure parameters. Logistic regression analysis suggests...
that this association is significant for the long-lasting breeding site parameter. Other factors (human genetic background, concomitant infection, nutritional status) might have an effect. Nevertheless, this study suggests that the antibody response to saliva could be a useful complementary tool in the evaluation of human exposure to *Aedes*. In this study, no correlation between IgG response to saliva and dengue transmission was observed, probably because the rate of dengue seroconversion (IgM) was low. Additional studies should be carried out to address this specific point and define whether antibody responses to saliva could be used to assess the risk of dengue transmission.

We cannot exclude the possibility of cross-reactivity with other arthropod salivary proteins. Previous results evaluating the cross-reactivity between different *Aedes*, *Anopheles*, and *Culex* species have identified species-shared and species-specific antigens. Preliminary data on rabbits experimentally exposed to single species of mosquitoes have shown minor cross-reactivity between *Ae. aegypti*, *An. gambiae*, and *Culex quinquesfasciatus* (Mouchet F, unpublished data). Saliva composition depends on age, feeding, and infection. Thus, an adequate biomarker for exposure needs to be based on *Aedes*-specific immunogenic proteins or peptides, as has been developed for *An. gambiae*. The sialome of *Ae. aegypti* is currently being investigated by using an immune-proteomic approach to define antigenic candidates for a specific, sensitive, and reproducible biomarker of exposure to *Ae. aegypti*.

The present study is a first step toward being able to use human IgG responses to *Aedes* salivary proteins as a biomarker of individual exposure to bites. This procedure could provide a reliable measurement of human–vector contact in epidemic settings where *Ae. aegypti*-borne diseases are emerging or re-emerging. Further studies need to be conducted to design a sensitive biomarker for *Aedes* exposure. The present study indicates that use of antibodies to saliva could lead to development of a useful diagnostic tool. In addition, such an indicator could be also useful for monitoring the efficacy of vector control strategies.

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Authors’ addresses: Souleymane Doucoure, Francois Mouchet, Amandine Cournil, Gilbert Le Goff, Sylvie Cornelic, Mabel Guerra Giraldez, Dorothée Misce, Annie Walter, Jean Pierre Herve, and Franck Remoue, Maladie Infectieuse et Vecteurs, Ecologie, Génétique, Évolution et Contrôle, UMI-Centre National de la Recherche Scientifique 5290, Institut de Recherche pour le Développement de Montpellier, CP 34394, Montpellier, France. E-mails: souleymane.doucoure@ird.fr, francois.mouchet@ird.fr, amandine.cournil@ird.fr, gilbert.legooff@ird.fr, sylvie.cornelic@ird.fr, mabel.guerra@ird.fr, dorothée.misce@ird.fr, annie.walter@ird.fr, hervejp_ird@yahoo.fr, and franck.remoue@ird.fr.

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