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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

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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[1,2] and in 2007 in Italy.[3] 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 developed 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 bugdorferi*[15] 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*[20] and *An. darlingi*.[21]

Most studies of antibody responses to *Aedes* saliva have focused on allergic reactions with a view to identifying the allergenic salivary proteins[22] 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

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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 Senegal 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.[4] 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 ere 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 studies.[7–9,28] In brief, the exposure 1 parameter provides a measurement of the risk of immediate exposure to adult *Ae. aegypti.* It is defined as the number of L3 + L4 larvae + pupae counted in the prospection units of a given cluster divided by the number of persons in the cluster. This parameter, which was based on older immature stages, was used as a proxy of the adult mosquito density over the next few days. The exposure 2 parameter provides a measurement of the number of long-lasting breeding sites. It corresponds to the number of breeding sites containing L1 or L2 and L4 or pupae counted in a given cluster divided by the number of persons in the cluster.

Collection of *Aedes* salivary extracts. Salivary gland extracts (SGEs) were obtained from 10-day-old uninfected female *Ae. aegypti* bred in an insectary (Institut de Recherche pour le Développement, Montpellier, France). In brief, mosquitoes were sedated with CO_2, and salivary glands were dissected and transferred into a tube containing 20 μL of HEPES-NaCl buffer. Dissected glands were then pooled and frozen at –80°C before extraction. Soluble proteins were then extracted by centrifugation for 30 minutes at 20,000 $\times g$ at 4°C, and the supernatant was collected. The protein concentration was assayed by using the Bradford method (Pierce, Rockford, IL) after pooling and mixing of the various gland batches to generate SGE for immunologic testing. The SGE contained 870 μg/mL of salivary proteins. The SGE was then stored frozen at –80°C before use.

Evaluation of human IgG levels. An ELISA was conducted by using SGE from uninfected *Ae. aegypti*, and serum samples were tested for IgG. The SGE (1 μg/mL in 100 μL of carbonate/bicarbonate buffer) was coated onto 96-well plates (Nunc, Roskilde, Denmark) for 150 minutes at 37°C. Plates were blocked by using 200 μL of blocking buffer (Biorad, Marnes-la-Coquette, France) for one hour at room temperature. Individual serum samples were incubated overnight at 4°C at a 1:100 dilution in phosphate-buffered saline–Tween 1%. Optimal ELISA conditions had been determined in preliminary experiments. Mouse biotinylated monoclonal antibodies to human IgG (BD Pharmingen, San Diego, CA) were incubated at a 1:1,000 dilution (90 minutes at 37°C). Peroxidase-conjugated streptavidin (Amersham, Les Ulis, France) was then added (1:1,000 dilution for 60 minutes at 37°C). Colorimetric development was performed by using 2,2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid diammonium (Sigma, St Louis, MO) in 50 mM citrate buffer, pH 4, containing 0.003% H_2O_2, and absorbance (optical density [OD]) was measured at 405 nm.

Identical ELISAs were performed in parallel on a negative pool of serum samples from persons from Europe (n = 30) who had no known exposure to *Ae. aegypti* mosquito bites, and positive control serum samples (persons regularly exposed to *Ae. aegypti* bites) were used to validate each ELISA plate. Each sera sample was assessed in duplicate wells and in a blank well containing no antigen (ODn) to control for non-specific reactions between the serum and reagents. Individual results were expressed as ΔOD value calculated according to the formula ΔOD = ODx – ODn, where ODx represents the mean of individual ODs in both antigen wells. The reproducibility of OD-positive values from responders in the children studied was verified later in a random plate. A person was considered an immune responder if his or her ΔOD was higher than the mean ΔOD + (3 × SD) for an unexposed person (negative control). The

TABLE 1
Characteristics of the study population according to age and entomologic data, Bolivia*

Characteristic				
Age group (years)	No. males	No. females	Total	No. immune responders (%)
< 14	115	110	225	190 (84.4)
15–24	114	145	259	159 (65.2)
25–34	66	139	205	104 (50.7)
35–44	46	94	140	71 (50.7)
45–54	41	65	106	40 (37.7)
≥ 55	38	76	114	48 (42.1)
Exposure level to *Aedes aegypti*				
Exposure 1: immediate adult exposure (L3 + L4 + pupae/no. inhabitants)†				
Exposure group (n)	Cluster (n)	Individual (n)	Mean age years, (range)	
1 (0–5)	25	256	30.8 (3–83)	147 (57.4)
2 (6–9)	24	252	31.1 (4–94)	148 (58.7)
3 (10–14)	23	235	28.9 (4–87)	138 (58.7)
4 (15–21)	15	170	28.6 (6–75)	170 (61.8)
5 (≥ 21)	15	137	29.7 (4–79)	94 (68.1)
Exposure 2: long-lasting breeding site with L1 or L2 and L4 or pupae/no. inhabitants†				
1 (0–0.057)	25	258	29.6 (3–78)	141 (54.6)
2 (0.058–0.105)	23	252	31.1 (4–87)	143 (56.7)
3 (0.106–0.138)	27	272	28.8 (4–87)	163 (59.9)
4 (0.139–0.200)	14	155	30.4 (4–94)	93 (60.0)
5 (≥ 0.200)	11	113	30.4 (5–79)	82 (72.6)

*L = larvae.
†As indicated in the Materials and Methods.

threshold of positivity was ΔOD = 0.290 for IgG specific for *Ae. aegypti* SGE.

Statistical analysis. Data were analyzed by using GraphPad Prism software (GraphPad, San Diego, CA) and SAS statistical package software version 8.1 (SAS Institute, Cary, NC). The distribution of ΔOD was not normally distributed. Thus, non-parametric tests (Wilcoxon and Kruskall-Wallis) were used to compare ΔOD between males and females, age groups, and exposure groups. Both exposure parameters were categorized in five groups: the first three groups corresponded to the first three quartiles of the distribution; the last quartile was cut in half to assess the effect of different levels of high exposure. Six age groups were defined: < 14, 15–24, 25–34, 35–44, 45–54, and ≥ 55 years of age. Mixed logistic regressions (NLMIXED procedure) were used for modeling the probability of being an immune responder according to exposure groups, with adjustment for age and sex. The cluster unit was added in the model as a random effect to account for cluster sampling. After Box-Cox transformation of ΔOD, mixed linear regressions (MIXED procedure) were used to model the level of antibody response according to exposure with adjustment for age and sex. Similar to logistic regressions, the cluster unit was added as random effect.

RESULTS

Antibody response and age. The prevalence of immune responders decreased with age (Table 1): 84.4% of children (< 14 years of age) were responders, whereas only 40–50% of adults (> 25 years of age) had specific IgG. The level of IgG to saliva was also age dependent (Figure 1) ($P < 0.0001$). Despite considerable inter-individual variation within the same age group, the highest median level of specific IgG was observed in children (< 14 years of age), and specific IgG response decreased progressively with age. In persons > 25 years of age, antibody responses remained similar.

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

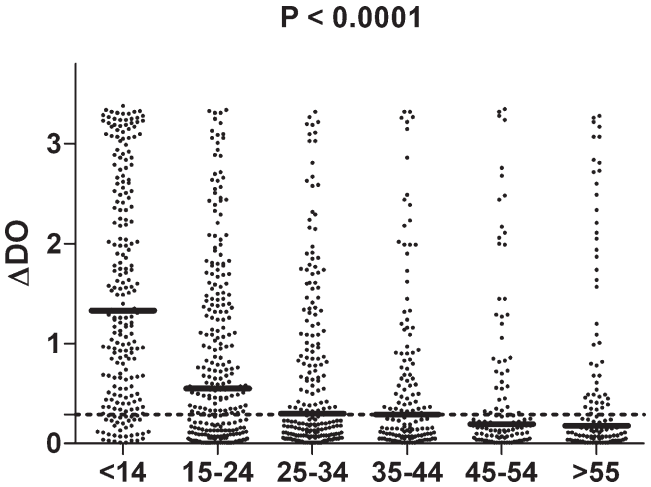

FIGURE 1. Levels of IgG against *Aedes aegypti* saliva according to age of the study participants. Bolivia. Individual optical density (ΔOD) results are shown for six age groups. Bars indicate the median value for each age group. The dotted line represents the threshold of the specific antibody response to *Ae. aegypti* (ΔOD = 0.290). The non-parametric Kruskall-Wallis test was used to compare all age groups ($P < 0.0001$). The Dunn's multiple comparison test showed that the IgG response to *Ae. aegypti* saliva was significantly different between persons ≤ 14 years of age and persons of all other ages (***). The test also showed significant difference between persons 15–24 and 45–54 years of age groups (**) and persons 15–24 and > 55 years of age (**).

TABLE 2

Probability of an immune responder according to exposure to *Aedes* with adjustment for age and sex, Bolivia*

Variables	Odds ratios	95% Confidence interval	*P*
Exposure 1: immediate adult exposure (L3 + L4 + pupae/no. inhabitants)†			
Exposure group			Global: 0.11
Group 2 vs. 1	0.9	0.6–1.3	0.62
Group 3 vs. 1	1.0	0.7–1.6	0.63
Group 4 vs. 1	1.3	0.8–2.0	0.23
Group 5 vs. 1	1.6	1.0–2.6	0.04
Age (years)			Global: < 0.0001
< 14 vs. ≥ 55	9.1	5.4–15.6	< 0.0001
15–24 vs. ≥ 55	2.8	1.8–4.5	< 0.0001
25–34 vs. ≥ 55	1.4	0.9–2.3	0.12
35–44 vs. ≥ 55	1.4	0.8–2.3	0.21
45–54 vs. ≥ 55	0.8	0.5–1.5	0.55
Sex (F vs. M)	1.8	1.3–2.3	< 0.0001
Exposure 2: long-lasting breeding site with L1 or L2 and L4 or pupae/no. inhabitants†			
Exposure group			Global: 0.009
Group 2 vs. 1	1.2	0.8–1.7	0.37
Group 3 vs. 1	1.3	0.9–1.8	0.20
Group 4 vs. 1	1.3	0.9–2.1	0.19
Group 5 vs. 1	2.6	1.5–4.3	0.0003
Age (years)			Global: < 0.0001
< 14 vs. ≥ 55	9.7	5.7–16.7	< 0.0001
15–24 vs. ≥ 55	3.0	1.9–4.7	< 0.0001
25–34 vs. ≥ 55	1.5	0.9–2.4	0.08
35–44 vs. ≥ 55	1.4	0.9–2.4	0.17
45–54 vs. ≥ 55	0.9	0.5–1.5	0.68
Sex (F vs. M)	1.8	1.3–2.3	< 0.0001

*L = larvae; P = pupae.
†As indicated in the Materials and Methods.

analysis indicated that the age-dependent difference in levels of specific antibodies was also not dependent on a cluster effect. A similar age effect was also observed when the specific antibody level was taken into account as a variable (continuous variable) ($P < 0.0001$, for exposure 1 and 2).

Antibody response and exposure to *Ae. 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 + L4 or 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 to *Ae. aegypti* (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

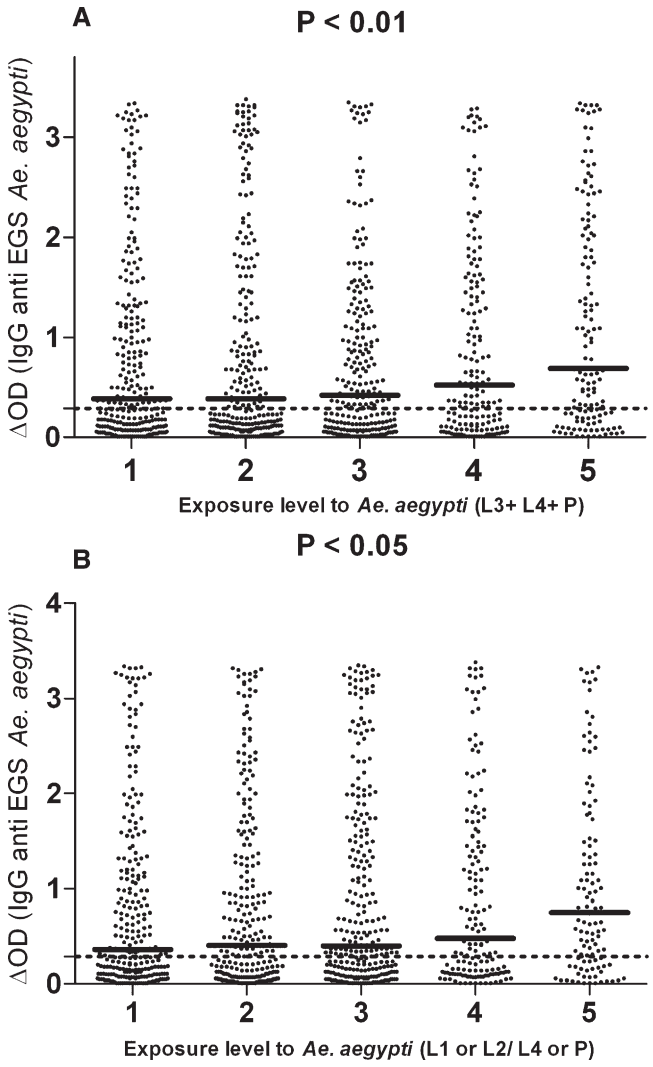

FIGURE 2. Levels of IgG antibodies against *Aedes aegypti* saliva according to *Ae. aegypti* exposure classes, Bolivia. Specific IgG levels are shown according to entomologic evaluation of exposure to *Aedes* bites, defined by two complementary parameters: Exposure 1 as immediate adult exposure (**A**) and Exposure 2 as long-lasting breeding site (**B**), as described in the Materials and Methods. Individual optical density (ΔOD) results are shown for five groups with increasing level of bite exposure. Bars indicate the median value for each exposure group. The dotted line represents the threshold of the specific antibody response to *Ae. aegypti* (ΔOD = 0.290). The non-parametric Kruskall-Wallis test was used to compare groups of exposure: *P* values were < 0.01 and < 0.05 in the Exposure 1 parameter and the Exposure 2 parameter, respectively. According to the Exposure 1 parameter, the Dunn's multiple comparison test showed a significant difference between groups of exposure 1 and 5 (*), 2 and 5 (*), 3 and 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).

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

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 ($\beta \pm SD = -0.32 \pm 0.15$, $P = 0.03$ and $\beta \pm SD = -0.40 \pm 0.15$, $P = 0.01$ for exposure parameters 1 and 2, respectively). Similar results were found for comparisons of group 2 and group 5 ($\beta \pm SD = -0.33 \pm 0.15$, $P = 0.02$ and $\beta \pm SD = -0.37 \pm 0.15$, $P = 0.02$ 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 stabilize 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*.[27] The same results were obtained for children exposed to *An. gambiae* bites.[19] 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.[29] Another study using the same method showed an association between biting rate and age of women (women > 15 years of age received more bites).[30]

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.[22]

With regard to the third hypothesis, potential natural desensitization that occurs in adults over time may be a factor.[31] A shift to production of IgG4 and IgE could be driven by chronic antigenic stimulation.[32] 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.[16,33,34] 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.[35] 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.[36] Thus, an adequate biomarker for exposure needs to be based on *Aedes*-specific immunogenic proteins or peptides, as has been developed for *An. gambiae*.[34,37] 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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