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# Social Stressors, Arboviral Infection, and Immune Dysregulation in the Coastal Lowland Region of Ecuador: A Mixed Methods Approach in Ecological Perspective

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*Abstract.* *Aedes aegypti*, the mosquito that transmits arboviral diseases such as dengue (DENV), chikungunya (CHIKV), and Zika viruses (ZIKV), is present in tropical and subtropical regions of the world. Individuals at risk of mosquito-borne disease (MBD) in the urban tropics face daily challenges linked to their socio-environment conditions, such as poor infrastructure, poverty, crowding, and limited access to adequate healthcare. These daily demands induce chronic stress events and dysregulated immune responses. We sought to investigate the role of socio-ecologic risk factors in distress symptoms and their impact on biological responses to MBD in Machala, Ecuador. Between 2017 and 2019, individuals ($\geq$ 18 years) with suspected arbovirus illness (DENV, ZIKV, and CHIKV) from sentinel clinics were enrolled (index cases, $N = 28$). Cluster investigations of the index case households and people from four houses within a 200-m radius of index home (associate cases, $N = 144$) were conducted (total $N = 172$). Hair samples were collected to measure hair cortisol concentration (HCC) as a stress biomarker. Blood samples were collected to measure serum cytokines concentrations of IL-10, IL-8, TNF-$\alpha$, and TGF-$\beta$. Univariate analyses were used to determine the association of socio-health metrics related to perceived stress scores (PSS), HCC, and immune responses. We found that housing conditions influence PSS and HCC levels in individuals at risk of MBD. Inflammatory cytokine distribution was associated with the restorative phase of immune responses in individuals with low-moderate HCC. These data suggest that cortisol may dampen pro-inflammatory responses and influence activation of the restorative phase of immune responses to arboviral infections.

## INTRODUCTION

Mosquito-borne diseases (MBDs) are a significant public health concern in Latin America and the Caribbean (LAC).[1,2] In Ecuador, the persistent transmission of MBDs is associated with complex historical social, economic, environmental, and cultural determinants.[3–6] The *Aedes aegypti* mosquito is the primary vector transmitting dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses (collectively referred to as DCZ in this study) in the coastal lowland region of Ecuador. Studies in the area have indicated that vector dynamics are influenced by seasonally variable socio-ecological and environmental drivers.[5] Household- and community-level characteristics in the peri-urban areas of Machala (e.g., household conditions, crowding, abandoned properties, interruptions in water supply, stagnant rain water, social status, and financial constraints that hinder an individual's ability to adhere with vector control programs) influence MBD transmission and persistence.[3,7,8] These factors increase the risk of MBD illness and contribute to higher levels of perceived stress that may affect immune responses to infections.[9,10] Stressors can culminate in psychological and physical health problems, such as anxiety, depression, metabolic diseases, and immune dysfunction.[11–14]

The sum of lifetime social stressors—such as those related to the risk of arboviral infection—may predispose individuals to immune dysregulation and an increased risk of developing (or prolonging) infectious diseases. This phenomenon has been attributed to the activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, and subsequent elevated cortisol output—a biomarker of stress.[15–17] Cortisol, the prominent human glucocorticoid hormone, is an important biomarker of stress that has been widely used to study the body's response to adverse events.[15] The release of a cascade of stress hormones is a result of HPA stimulation, which mediates a series of bidirectional communication responses between the brain and immune system.[13,18,19] Therefore, studying the effect of glucocorticoid hormones, such as cortisol, is important because it can influence a variety of immune-related cells through glucocorticoid receptors (GR) and, in turn, influence cell responsiveness to physiologic, immune, and inflammatory responses.[17,20–22]

In this study, we sought to evaluate the impact that peri-urban living conditions have on cortisol production, subsequently impacting innate immune responses in individuals at risk of arboviral infections. The stress hormone cortisol can be measured in various body fluids including blood, saliva, and urine, and more recently has been shown to be measurable in hair.[23] The effectiveness of measuring hair cortisol concentrations (HCC) has already been demonstrated as a practical method to measure cortisol levels because its collection is noninvasive and is a field-friendly sample collection method.[24]

We hypothesized that individuals living in impoverished conditions are associated with higher levels of distress symptoms (perceived and physiological), which are associated with

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deficient inflammatory responses in those individuals positive for arboviral disease. These results describe, for the first time, the evidence of how socio-ecological risk factors known to increase DCZ transmission also influence distress symptoms.

## METHODS

**Study site.** This research study was conducted in Machala, Ecuador (population 283,037, capital of El Oro Province), the commercial heart of Ecuador's banana-producing region known for its agricultural and mining economic productivity.[25,26] The city is a mid-sized coastal port city in southern Ecuador settled through a process of rapid unstructured urbanization and agricultural industrialization.[27,28] Machala continues to experience hyperendemic DENV transmission, reporting 1,195 cases in 2014, 2,781 cases in 2015, 2,100 cases in 2016, and 1,175 cases in 2017, the start of this study.[29] Moreover, with the introduction of CHIKV and ZIKV in subsequent years, the province reported 1,209 cases of CHIKV infection (as of December 2018) and 78 cases of ZIKV infection (as of December 2018).[29,30] Similar to other coastal areas of other Latin American countries, the risk of infection and vector dynamics in the area are driven by climate and socio-ecological and environmental risk factors.[31] The unprecedented emergence of epidemic arboviral infections, along with the social-ecological characteristics that constitute the city of Machala, have changed the perception of researchers and scientists regarding the incidence and distribution of these diseases by increasing interest in applying multi- and interdisciplinary approaches.[4,32,33]

This research study was conducted as part of an ongoing collaboration with the Ministry of Health of Ecuador (MoH), the Technical University of Machala, State University of New York (SUNY) Upstate Medical University, and other public health partners in Machala. SUNY and the MoH have partnered in Machala and other coastal areas of Ecuador for more than a decade, focusing on the synergistic influence of climate and social-ecological drivers of DCZ transmission.[3,5,6] The city of Machala began a process of urban renewal and gentrification in the mid-2000s that became stagnant by 2015 because of economic decline.[26,34,35] The research team continues exploring how these changes and processes impact MBDs transmission from a socio-ecological perspective.

**Research framework.** We conducted a mixed-method, retrospective, population-based cohort study adapting McElroy and Townsend's[36] Medical Ecological Framework (MEF; Figure 1) to explore if cortisol responses associated with socio-ecological challenges impact the distribution of inflammatory responses. We integrated epidemiologic and basic science methods into our approach to assure adequate examination of this priority. Previous studies in the area had described the household and community socio-environmental drivers leading to increased DCZ transmission.[3,6,33,37] To further explore these associations, we implemented the MEF, a model that emphasizes the intersection of sociocultural, biological, abiotic, and biological processes in influencing health and disease (Figure 1).[36,38,39] This framework, therefore, serves as an explanatory model to help conceptualize the factors influencing DCZ transmission.

Implementing the MEF allowed us to examine how household- and community-level characteristics from the sociocultural and abiotic elements synergistically impact individual levels of biological (stress) response. Understanding these links is essential for raising awareness among public and private sectors about the importance of implementing programs that tackle health-damaging conditions, which put the most vulnerable populations at risk. This approach, however, requires an integrated, analytical framework that evaluates and recognizes the different factors and levels that impact health.

**Human subject's protection.** The study was reviewed and approved by the University of Rochester Research Subjects Review Program (RSRB); study ID: 00001719. Preexisting approvals were also established with the institutional review boards or ethical review committees at the SUNY Upstate Medical University, the Luis Vernaza Hospital, and the Ecuadorean MoH.

**Study design.** The study was conducted in Machala, Ecuador, from 2017 to 2019. Participants were excluded at baseline if they were younger than 18 years of age or pregnant. We calculated sample size using an open source software (OpenEpi version 3.01) for epidemiologic statistics[40] and published literature identifying distress symptom levels in Ecuador, and applying stratified analysis for cohort studies with two-sided significance of 5% and power of 80%. The analysis was conducted with the most conservative estimate, and total sample size as inflated to 200 to accommodate for loss of samples, errors, or missing participant information. Since missing values were below 5%, missing data were excluded from analysis of data.

**Data collection.** The study design has been described previously in published research, and is summarized here.[33] From 2017 to 2019, individuals with suspected arboviral illness from four MoH sentinel clinics and the central hospital in Machala were referred by MoH clinicians to the SUNY Upstate Medical University study site facility and were invited to take part in the study. These individuals were identified as index cases.

*Index cases* included individuals with clinical diagnosis of a febrile illness who tested positive for DENV by NS1 rapid test. These individuals were randomly selected to initiate a cluster investigation. Cluster investigations were conducted in the households of positive index cases, and for all individuals from four households who resided within a 200-m radius of the index home, which is the typical flight range of the *Ae. aegypti* mosquito. This latter group was classified as associate cases.[33] The combination of the four associate residences with the initiate index case are referred to as a cluster.

After consent was obtained, data were collected from index and associate cases by trained technicians using a customized database on an iPad (FileMaker Pro Advanced 13.0v5; FileMaker Inc., Santa Clara, CA).[33] The following individual-, household-, and community-level data were collected: patient demographics, medical history, symptoms within the last 7 days, date of onset of fever, aural (ear) temperature, access to water services, overall household characteristics, presence of abandoned houses, stagnant water, and community road conditions. Data were uploaded and saved in a protected cloud-based server (GoZync). Furthermore, a 20-mL blood specimen was obtained from each participant by venipuncture. The samples were processed at the SUNY–MoH laboratory in Machala, where the NS1 rapid test was also conducted

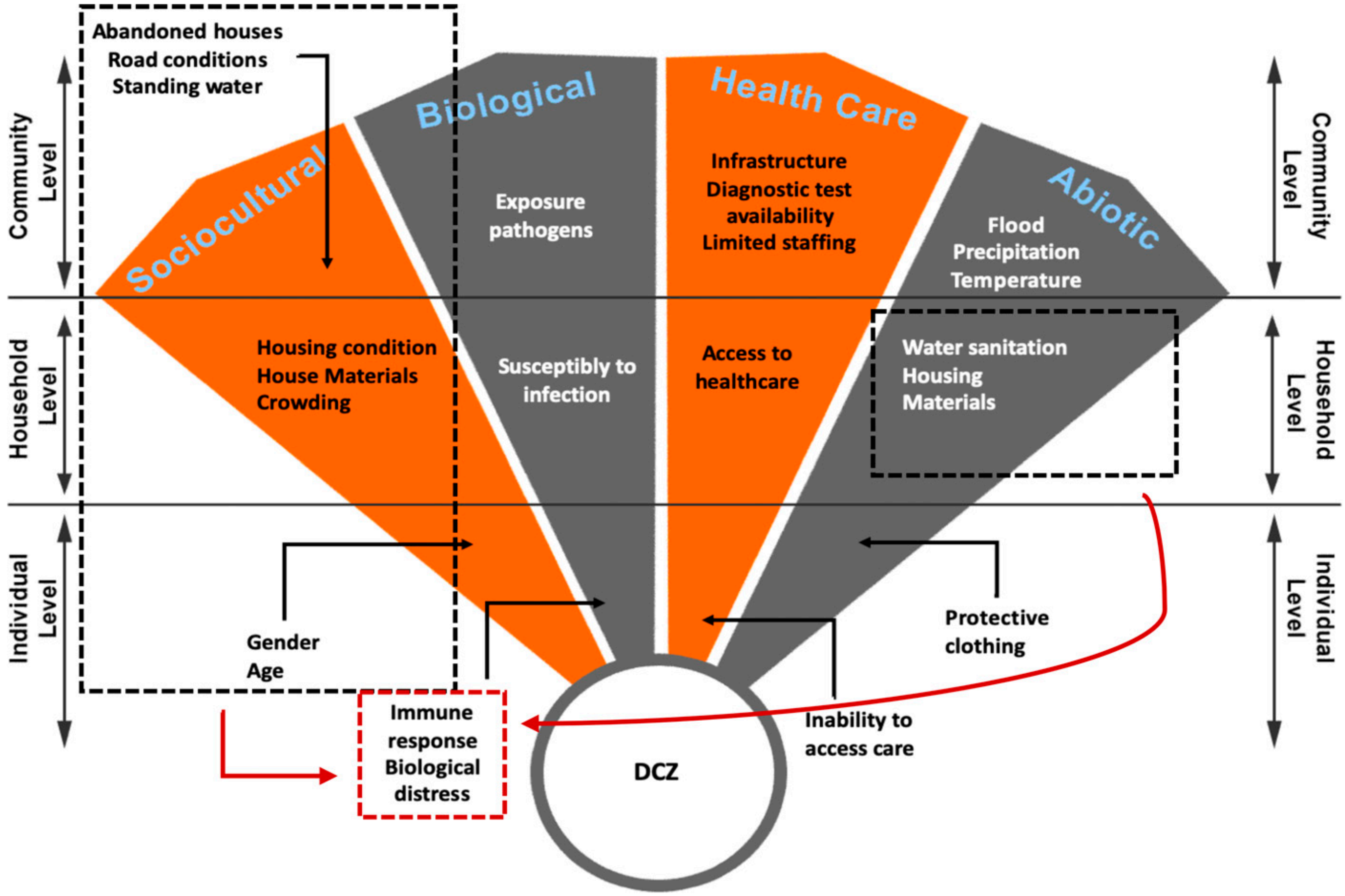


FIGURE 1. Conceptual framework for study. This figure appears in color at www.ajtmh.org.

(PanBio Dengue Early Rapid Test).[33] Hair samples were also collected (3 cm of hair closest to scalp). Perceptions of stress were measured using the Perceived Stress Score (PSS) psychological instrument.[41] The Ecuadorian Spanish version was used in this study and has been used previously in multiple studies in the country.[41]

**Hair cortisol concentration.** Three centimeters of hair closest to the scalp was collected and stored at room temperature in dry and dark conditions. Hair was wetted with isopropanol, minced, and washed with 1 mL of isopropanol at room temperature for 2 minutes to remove external contamination. Hair was then dried under a nitrogen stream and weighed. Cortisol was extracted with 1 mL of methanol overnight at 55°C, 1 mL acetone for 5 minutes, and then 1 mL of methanol overnight at 55°C one more time. Pooled solvent fractions were removed under a nitrogen stream. Samples were dissolved in phosphate-buffered saline (PBS), randomly distributed on different plates to avoid a batch effect, and analyzed in quadruplicate using Salimetrics cortisol enzyme-linked immunosorbent assay (ELISA) (SALIMETRICS ASSAY #1-3002). HCC measurements were conducted at SUNY Oswego State University.[42]

**Diagnostic testing.** Serum specimens from 2017 to 2019 were shipped to the University of Rochester for testing using qualitative reverse transcription polymerase chain reaction (RT-PCR) assays for DENV1-4, CHIKV, and ZIKV. Total RNA was extracted from 140 μL of human serum specimens using the QIAmp Viral RNA Mini Kit (Catalog number 52906; Qiagen) following the manufacturer's protocol. Viral RNA was stored at −80μC for further PCR amplification. A total of 5 μL of RNA was used in a 10-μL reverse transcriptase reaction. All samples and controls were analyzed in duplicates in a multiplex RT-PCR (BioRad CFX Connect Real-Time PCR Detection System) for 42 cycles. Samples were classified as positive according to a suggested C(t) value of $\leq$ 37.0, which coincides with a cutoff based on Centers for Disease Control and Prevention (CDC) recommendations.[43] Both primer and probe sets were specific for their respective viral targets and did not detect other viruses transmitted by the same vector (DENV1–4, YFV, JEV, ZIKV, and CHIKV). Primers and probes for DENV, CHIKV, and ZIKV are represented in Supplemental Table 1.

Further diagnostic testing for DENV, CHIKV, and ZIKV was conducted using serum samples and qualitative RT-PCR assays for each virus respectively, and commercial ELISA kits to test for DENV and ZIKV IgM concentrations (Abcam Human Anti-Dengue virus IgM and InBios ZIKV Detect 2.0 IgM). Each test was conducted following the manufacturer's suggested protocol. Samples identified as positive for either DENV, CHIKV, or ZIKV in either RT-PCR or IgM were classified as laboratory-confirmed cases for each respective disease. Samples that were positive for two or more diseases ($N$ = 10) were removed from the study. Confirmed cases for any diseases are collectively referred to as DCZ infection.

**Inflammatory cytokines.** A multiplex bead immunoassay (Luminex Performance Assay; Human Cytokine Base Kit A;

Catalog number LUH000) was used to simultaneously test cytokine expression as well as High Sensitivity Quantikine ELISA kits (IL-8, TNF-α, and IL-10; R&D systems, catalog numbers HS800, HSTA00E, and HS100C, respectively). Quantikine ELISA was used for TGF-β (R&D systems, catalog number DB100B). Each assay and dilution were conducted following the manufacturer's protocol.

**Data analysis.** Statistical analysis was conducted using IBM SPSS Statistics for Macintosh, Version 25.0[44] A $P$ value of $< 0.05$ was considered significant in all statistical tests.

*Stress data.* We dichotomized the variables used for PSS and HCC based on previous literature. The variables of PSS can range from 0 to 40 with higher scores indicating higher perceived stress. Using the cutoff values, the three categories were dichotomized into two new variables: (0–26) was considered low to moderate perceived stress and (27–40) was considered higher levels of perceived stress.

Because the HCC variable was not normally distributed, we therefore dichotomized the variables into two categories (low to moderate and high). Using median values (9.0 pg/mg) and standard deviation (SD) values (7.7 pg/mg,) two cutoff scores were used to classify HCC into two categorical variables. The cutoff scores were $\leq 9$ for low to moderate HCC and $\geq 10$ for higher HCC.

*Arboviral sample data.* Confirmed cases of DENV, CHIKV, and ZIKV were also grouped together to discover commonalities between infections and converted into a new variable called "DCZ" cases, representative of any arbovirus infection. Similar to the classification described in Stewart-Ibarra et al., 2018,[45] a participant was categorized as having an acute or recent DCZ infection if tested positive (RT-PCR or IgM), allowing for a broader spectrum of infection. Analysis was conducted using the DCZ variable, as a representation of overall current and recent arbovirus infection.

*Bivariate analysis.* Association between a particular social risk factor (exposure) and distress symptoms (health outcome) in individuals with a laboratory-confirmed positive DCZ infection was conducted using univariate analysis to assess magnitude of the association and $\chi^2$ statistical test analysis to assess statistical significance. Because the method uses dichotomous variables, data were stratified into two or more levels and a series of two-by-two tables showing the association is presented. Fisher's exact test was used where more than 20% of the cells had expected cell counts less than five.

*Regression analysis.* Logistic regression was used to examine the association of cortisol levels (dependent variable) and perceived stress (independent variable) when controlled for age and gender. In our adjusted models, we estimated odds ratio (95% confidence intervals [CI]) for binary outcomes in perceived stress (PSS), age (AG), and gender (GE) (Equation 1). An additional variable was constructed evaluating the simultaneous interaction of age and gender (AgeGen) in hair cortisol responses; HCC was classified as the dependent variable and AG, GE, PSS, and AgeGen were classified as the independent variables ($X_1$–$X_4$).

$$\boldsymbol{P}(\boldsymbol{HCC}) = \frac{1}{1+\boldsymbol{e}^{-(\beta_0+\ \beta_1 \boldsymbol{PSS}_1+\beta_2\boldsymbol{AG}_2+\beta_3\boldsymbol{GE}_2+\beta_4\boldsymbol{AgeGen}_2)}} \quad (1)$$

**Analysis of inflammatory markers.** Before analysis, continuous variables (inflammatory cytokines: IL-10, IL-8, TNF-α, and TGF-β) were tested for normality using the Kolmogorov–Smirnov test. Variable significance was less than 0.05, indicating that the data were non-normally distributed. Therefore, assumptions for analysis of variance (ANOVA) were not met and a nonparametric test for independent samples (Mann–Whitney $U$ test) was used to compare the median differences between the two independent groups. The Mann–Whitney $U$ test is a counterpart of the $t$ test and provides the most accurate estimates of significance, especially when sample sizes are small and/or when the data do not approximate a normal distribution.[46] All data for independent samples were represented as medians.

## RESULTS

**Sample description.** A total of 172 participants (28 index cases and 144 associate cases) were enrolled from 2017 to 2019 in Machala, Ecuador. Participants were 39.5 years of age on average (SD = 16.0, median = 36.5): 75% were female and 25% were male. Index cases represented 16.3% and associate cases represented 83.7% of the participants. The total number of individuals positive for DCZ infection accounted for 40.7% (70 cases); the mean PSS score was 19.3 (SD = 5.9, median = 19.5) and the mean HCC score was 10.5 pg/mg (SD = 7.7, median =8.9). The following inflammatory cytokines were measured: IL-8 (mean ± SD: 71 ± 47), TNF-α (mean ± SD: 66 ± 47), TGF-β(mean ± SD: 2,100 ± 1,700), and IL-10 (mean ± SD: 63 ± 56) (Data not shown).

*DCZ diagnostic results.* The majority of cases we identified positive for ZIKV (32%), followed by 9.3% of cases identified as positive for CHIKV, and 5.2% cases identified as positive for DENV (data not shown). A total of nine positive cases were removed from the analysis because of cross-reactivity; for a total of 70 (40.7%) cases positive for DCZ infection. There were no significant differences in the relative frequency distribution of infection between index and associate cases. Cases were defined as individuals with laboratory-confirmed DCZ infection.

*Measurements of association.* Chi-square ($\chi^2$) statistical analysis was implemented to assess the magnitude of association between a particular social risk factor (exposure) and health outcomes (distress symptoms and DCZ infection).

**Association between household and community characteristics and suspected DCZ cases.** We identified a total of 149 participants with complete data and evaluated the association of household and community characteristics known to be risk factors for DCZ transmission (Tables 1 and 2, respectively). We found that, in general, individuals with poor housing conditions were not more likely to have a DCZ infection.

Of individuals negative for DCZ, 61.5% lived in house conditions classified as "neglected," 81.8% lived in houses made of bamboo material, 58.3% did not have window screen protectors, 59.0% resided in houses where there was $< 1$ room per person, and 63.3% of participants lived in residences with $\leq 3$ people per house. A significant association was only found between those negative for DCZ and the number of rooms per household ($P < 0.05$).

Community-level characteristics are represented in Table 2. No significant association was found between community conditions and the presence/absence of DCZ infections.

**Psychological distress.** A total of 160 individuals had PSS and HCC measured; 12 participants were lost due to refusal to give hair sample. Overall, 87.5% of participants were

TABLE 1
Distribution of household characteristics by DCZ infection status, perceived stress, and hair cortisol concentrations

| | DCZ | | | | PSS | | | | HCC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive, $N = 64$ | Negative, $N = 85$ | Unadjusted OR (95% CI) | *P* | High (27–40), $N = 17$ | Low to moderate (0–26), $N = 124$ | Unadjusted OR (95% CI) | *P* | High (≥ 10), $N = 74$ | Low (≤ 9), $N = 74$ | Unadjusted OR (95% CI | *P* |
| House conditions | | | | | | | | | | | | |
| Neglected | 37 (38.5%) | 59 (61.5%) | 0.60 (0.31, 1.19) | 0.14 | 15 (16.9%) | 74 (83.1%) | 5.07 (1.11, 23.13) | **0.02** | 48 (50.0%) | 48 (50.0%) | 1.00 (0.51, 1.96) | > 0.99 |
| Good | 27 (50.9%) | 26 (49.1%) | Referent | | 2 (3.8%) | 50 (96.2%) | Referent | | 26 (50.0%) | 26 (50.0%) | Referent | |
| House material | | | | | | | | | | | | |
| Bamboo | 2 (18.2%) | 9 (81.8%) | 0.30 (0.03, 2.52) | 0.22* | 1 (10%) | 9 (90%) | Referent | 0.96 | 3 (27.3%) | 8 (72.7%) | Referent | 0.28 |
| Brick | 59 (45.0%) | 72 (55.0%) | 1.09 (0.24, 5.08) | | 15 (12.1%) | 109 (87.9%) | 1.21 (0.14, 10.77) | | 67 (51.5%) | 63 (48.5%) | 2.84 (0.72, 11.17) | |
| Wood | 3 (42.9%) | 4 (57.1%) | Referent | | 1 (14.3%) | 6 (85.7%) | 1.50 (0.08, 28.90) | | 4 (57.1%) | 3 (42.9%) | 3.55 (0.48, 26.28) | |
| Screen | | | | | | | | | | | | |
| No | 48 (41.7%) | 67 (58.3%) | 0.81 (0.37, 1.74) | 0.58 | 17 (15.9%) | 90 (84.1%) | 12.84 (0.75, 219.) | **0.01** | 57 (50%) | 57 (50%) | 1.00 (0.46, 2.15) | > 0.99 |
| Yes | 16 (47.1%) | 18 (52.9%) | Referent | | 0 (0%) | 34 (100%) | Referent | | 17 (50%) | 17 (50%) | Referent | |
| Rooms per person* | | | | | | | | | | | | |
| < 1 | 48 (41%) | 69 (59%) | 0.65 (0.29, 1.44) | 0.29 | 13 (11.8%) | 97 (88.2%) | 0.87 (0.26, 2.90) | 0.80 | 53 (53.3%) | 64 (54.7%) | 0.35 (0.15, 0.84) | **0.02** |
| ≥1 | 16 (51.6%) | 15 (48.4%) | Referent | | 4 (13.3%) | 26 (86.7%) | Referent | | 21 (70.0%) | 9 (30.0%) | Referent | |
| People per house | | | | | | | | | | | | |
| ≤3 | 18 (36.7%) | 31 (63.3%) | 0.65 (0.32, 1.32) | 0.24 | 9(19.1%) | 38 (80.9%) | 2.55 (0.91, 7.10) | 0.07 | 23 (47.9%) | 25 (52.1%) | 0.88 (0.44, 1.76) | 0.73 |
| ≥4 | 47 (40.0%) | 53 (53.0%) | Referent | | 8 (8.5%) | 86 (91.5%) | Referent | | 51 (51.0%) | 49 (49.0%) | Referent | |
| Rooms in house | | | | | | | | | | | | |
| 1 | 7 (21.9%) | 25 (78.1%) | 0.18 (0.05, 0.63) | **0.03*** | 3 (9.7%) | 28 (90.3%) | 0.44 (0.11, 1.71) | 0.11* | 8 (25.0%) | 24 (75%) | 0.27 (0.08, 0.91) | **0.02*** |
| 2 | 27 (45.0%) | 33 (55.0%) | 0.52 (0.18, 1.53) | | 11 (19.6%) | 45 (80.4%) | 2.85 (0.74, 11.01) | | 34 (56.7%) | 26 (43.3%) | 1.05 (0.36, 3.02) | |
| 3 | 19 (48.7%) | 20 (51.3%) | 0.60 (0.19, 1.88) | | 3 (7.9%) | 35 (92.1%) | 2.74 (0.13, 58.01) | | 22 (57.9%) | 16 (42.1%) | 1.10 (0.36, 3.41) | |
| ≥4 | 11 (61.1%) | 7 (38.9%) | Referent | | 0 (0%) | 16(100%) | Referent | | 10 (55.6%) | 8 (44.4%) | Referent | |

CI = confidence interval; DCZ = dengue, chikungunya, and Zika; HCC = hair cortisol concentration; OR = odds ratio; PSS = perceived stress scores.

TABLE 2

Distribution of community-level characteristics by DCZ infection status, perceived stress, and hair cortisol concentrations

| Community variables | DCZ Positive, $N = 64$ | DCZ Negative, $N = 85$ | Unadjusted OR (95% CI) | $P$ | PSS High (27–40) $N = 17$ | PSS Low to moderate (0–26), $N = 124$ | Unadjusted OR (95% CI) | $P$ | HCC High (≥ 10), $N = 74$ | HCC Low (≤ 9), $N = 74$ | Unadjusted OR (95% CI) | $P$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Road conditions | | | | | | | | | | | | |
| Dirt road | 10 (34.5%) | 19 (65.5%) | 0.64 (0.28, 1.50) | 0.32 | 4 (13.0%) | 25 (86.2%) | 1.22 (0.37, 4.06) | 0.73 | 11 (37.9%) | 18 (62.1) | 0.54 (0.24, 1.25) | 0.16 |
| Paved road | 54 (45.0%) | 66 (55.0%) | Referent | | 13 (11.6%%) | 99 (88.4%) | Referent | | 63 (52.9%) | 56 (47.1%) | Referent | |
| Abandoned houses | | | | | | | | | | | | |
| Yes | 25 (37.9%) | 41 (62.1%) | 0.69 (0.36, 1.33) | 0.27 | 7 (10.9%) | 57 (89.1%) | 0.82 (0.29, 2.30) | 0.72 | 27 (40.9%) | 39 (59.1%) | 0.52 (0.23, 1.00) | **0.05** |
| No | 39 (47.0%) | 44 (53.0%) | Referent | | 10 (13.0%) | 67 (87.0%) | Referent | | 47 (57.3%) | 35 (42.7%) | Referent | |
| Standing water | | | | | | | | | | | | |
| Yes | 32 (37.2%) | 54 (62.8%) | 0.57 (0.30, 1.11) | 0.10 | 8 (9.8%) | 74 (90.2%) | 0.60 (0.22, 1.66) | 0.32 | 40 (46.5%) | 46 (53.5%) | 0.72 (0.37, 1.38) | 0.32 |
| No | 32 (50.8%) | 31 (49.2%) | Referent | | 9 (15.3%) | 50 (84.7%) | Referent | | 34(54.8%) | 28 (45.2%) | Referent | |

CI = confidence interval; DCZ = dengue, chikungunya, and Zika; HCC = hair cortisol concentration; OR = odds ratio; PSS = perceived stress scores.

considered to have low to moderate levels of perceived stress and 12.1% of all participants reported higher levels of perceived stress. Although not significant, females were more likely to report higher perceived stress compared with males (Supplemental Table 1), those between the age of 18 and 38 years old (odd ratio [OR]: 2.9, 95% CI: 1.01−8.46, $P = 0.04$) reported a higher perceived stress (Supplemental Table 1). No significant association was observed between cortisol concentrations and age and gender.

**Association between household and community characteristics and distress symptoms.** The association between PSS or HCC and household-level characteristics was evaluated in 141 participants (Table 1); individuals with missing data were excluded ($N = 31$). In Table 1, a significant association was observed between housing condition and PSS; an individual exposed to neglected housing conditions had higher odds of reporting a high PSS (OR: 5.07, 95% CI: 1.11−23.13, $P = 0.02$) compared with individuals living in good housing conditions. Similarly, individuals living in household without screen protectors on windows or doors presented a greater odd of high PSS (OR: 12.84, 95% CI: 0.75−219.8, $P = 0.01$); 100% of individuals with high PSS ($N = 17$) lived in homes without screens. We found a positive association between household-level characteristics and HCC levels (Table 1). Individuals living in households with more than one room per person (OR: 0.35, 95% CI: 0.15, 0.84, $P = 0.02$) and having two or more rooms per house (OR: 1.05, 95% CI: 0.36, 3.02, $P = 0.02$) had significantly higher levels of cortisol concentrations.

In Table 2, the associations between PSS, HCC, and community-level characteristics was also measured. No significant association was observed between PSS and community characteristics. For HCC, living in communities with no abandoned houses was associated with higher levels of HCC (OR: 0.52, 95% CI: 0.23–1.00, $P = 0.05$).

Logistic regression analysis assessed the association of HCC and PSS when controlling for age and gender (Table 3). A significant association was found between HCC and PSS (model 1), indicating that PSS is positively associated with cortisol concentrations (OR: 4.0, 95% CI: 1.23–12.81, $P = 0.02$). Controlling for age, gender, and the simultaneous interaction of age and gender did not impact the relationship between HCC and PSS (model 2–4, Table 3).

**Distribution of inflammatory cytokines.** Among DCZ positive cases, median serum concentrations of TGF-β (median = 2,021.0; $P = 0.04$) were significantly higher compared with those who tested negative (median = 1,527.0). No significant difference was found in median serum concentrations of IL-10 ($P = 0.17$), TNF-α ($p = 0.45$), or IL-8 ($P = 0.75$) between those who tested positive (median = 66.8; median = 62.5; median = 74.5) and those who tested negative (median = 45.3; median = 66.0; median = 65.0), respectively (Supplemental Figure 1).

**Distribution of HCC.** The distribution of inflammatory markers by levels of cortisol, measured by HCC, was evaluated. No significant difference was found in median serum levels of IL-10 ($P = 0.77$), TNF-α ($P = 0.84$), TGF-β ($P = 0.93$), and IL-8 ($P = 0.73$) between those with high to moderate HCCs (median = 52.6; median = 79.0; median = 1,784.0; median = 72.5) and those with lower HCCs (median = 44.0; median = 69.0; median = 1,810.0; median = 75.0), respectively (Figure 2).

TABLE 3
Logistic regression of perceived stress and cortisol concentration controlling for potential confounders

| | Odds ratio (95% CI) | P |
|---|---|---|
| Model 1: PSS v. HCC | | |
| PSS | 4.0 (1.23–12.81) | 0.02 |
| Model 2: PSS v. HCC, controlling for age | | |
| Age | 4.05 (1.24–13.26) | 0.02 |
| Model 3: PSS v. HCC, controlling for gender | | |
| Gender | 4.18 (1.27–13.76) | 0.02 |
| Model 4: PSS v. HCC, controlling for AgeGen | | |
| Age × Gender | 4.26 (1.28–14.14) | 0.02 |

CI = confidence interval; HCC = hair cortisol concentration; PSS = perceived stress scores.

The median distribution of inflammatory markers by HCC levels, within infection status, was evaluated. Median serum IL-10 ($P = 0.03$) was significantly higher in individuals who tested positive (high: median = 59.7; low: median = 68.9) versus those who tested negative (high: median = 60.7; low: median = 41.7) for DCZ when stratified by HCC level. No significant difference was found in the levels of TNF-$\alpha$ ($P = 0.67$), TGF-$\beta$ ($P = 0.10$), and IL-8 ($P = 0.41$) (Figure 3).

## DISCUSSION

The aim of this study was to evaluate whether socio-ecological determinants related to DCZ transmission was associated with higher physiological and perceived stress, and in turn, impact innate immune responses in individuals at risk of arboviral infections. To our knowledge, no previous studies have been conducted seeking to examine the relationships between social risk factors, distress symptoms—both psychological and biological—and its impact in innate immune responses in communities at risk of DCZ infection.

We found that socio-ecological determinants related to DCZ transmission is associated with higher perceived stress but have an inverse relationship to cortisol responses. Moreover, moderate levels of cortisol concentration might confer protection against severe stages of DCZ, by favoring reparative immune responses.

**Socio-health metrics and distress symptoms.** Previous studies have shown that social risk factors, such as household and community characteristics, play an important role in the incidence and prevalence of DCZ infection and stress.[3,5,6,14,31,32,47–49] In this study, we observed that perceived stress was more prevalent in characteristics related to household characteristics (household conditions, screen protectors). However, a similar pattern was not observed in relation to HCC. Indeed, previous studies indicated that environmental conditions do not always accurately represent higher levels of stress.[50,51] Previous studies have described the complexity of perceived stress and cortisol responses, indicating that individuals continually exposed to stressful situations are at risk of allostatic load, causing damage to the endocrine system that results in overproduction of cortisol. Another consequence of overexposure to stress is "hypo-response"—a flattening effect that causes individuals to not produce cortisol even when it is needed. Furthermore, perceived stress is both subjective and contextual and has a wide range of causative and conductive factors that can be influenced by cultural and social environmental factors.[52,53] Further studies in the area are needed to evaluate the long-term effect of stress exposure on physiological responses in those testing positive for DCZ.[11]

Our logistic regression analysis demonstrated that perceived stress is associated with cortisol concentrations. Further analysis assessed the effect of age and gender and the simultaneous interaction of age and gender; we found that the interaction of these variables does not impact the relationship between cortisol concentrations and perceived stress, indicating that age and gender do not have strongly confounding effects on the relationship between perceived stress and cortisol concentration in our sample. Based on our results, we speculate that distress symptoms are associated with external factors such as cumulative chronic experiences, historical sociocultural contexts, coping abilities, and cultural behaviors toward an illness (not measured in this study).[14,54]

**Cortisol role in inflammatory responses.** We sought to determine whether the distribution of cytokine concentrations would be impacted by cortisol concentrations, using hair cortisol as a surrogate marker. We found no significant difference in median serum concentrations of inflammatory cytokines in relation to levels of cortisol. These results suggest that in this population cortisol concentration in hair samples has no effect on the distribution of inflammatory cytokines without the stimulus of a pathogen. The distribution of cytokines by DCZ infection status and cortisol concentration was further evaluated. When stratified by infection status we found a significant association of IL-10 median serum levels of positive DCZ cases compared with those negative for DCZ. This indicates that lower to moderate cortisol concentrations shift cytokine

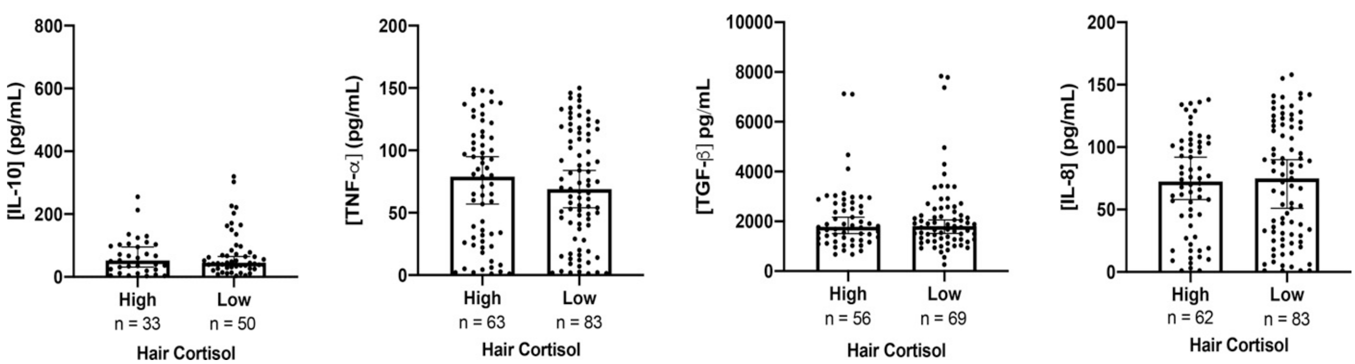


FIGURE 2. Distribution of inflammatory cytokines in serum samples.

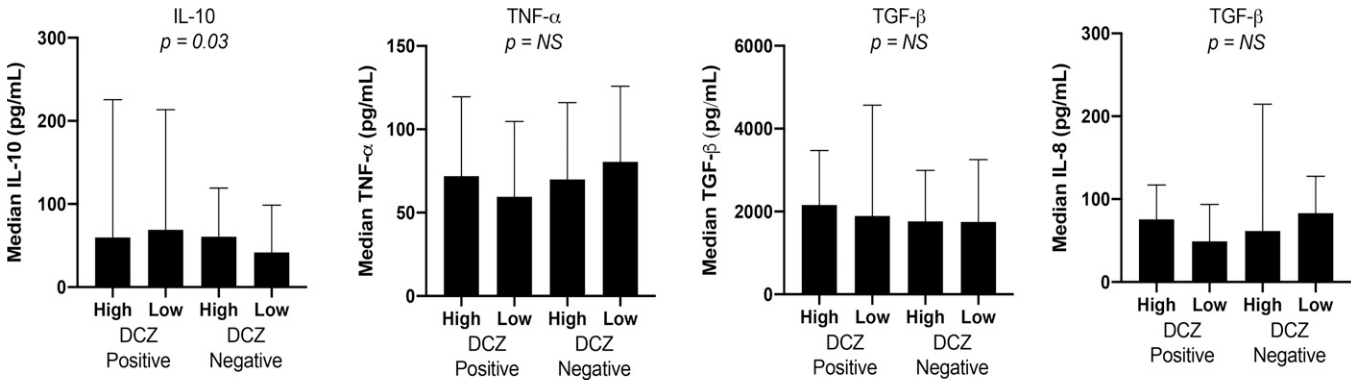


FIGURE 3. Median distribution of inflammatory cytokine by cortisol levels stratified by dengue, chikungunya, and Zika (DCZ) cases.

release in favor of anti-inflammatory cytokine secretion by dampening pro-inflammatory responses in individuals at risk of DCZ infection. The dampening of pro-inflammatory responses could potentially provide protection against overstimulation of the immune system and, in turn, prevent progression toward severe cases, such as dengue hemorrhagic fever (DHF). Previous studies have described that production of glucocorticoids can inhibit transcription control pathways necessary for secretion of pro-inflammatory responses.[21,55–58] Because our site study is hyperendemic to arboviral infection, previous flavivirus infection could significantly influence the observed results. More research is needed to understand the specific mechanisms involved in cortisol response and immune activity in individuals who test positive for DCZ and the role previous infection may play in conferring protection.

*Limitations.* There are a number of limitations inherent in the current study. It is difficult to generalize these results to other populations, as our sample consisted primarily individuals of lower income in peri-urban communities. These findings may or may not vary along a gradient of socioeconomic statuses. This study did not assess additional outcomes such as possible coinfections and comorbidities at the time of study and preexisting conditions that could have affected the study results. Our study also did not take into consideration previous infection with DCZ as well as the number of days of illness during the course of infection. Furthermore, this study did not evaluate cumulative chronic experiences, including childhood adverse effects, predisposition to violence, and mental health illness (e.g., anxiety and depression). These experiences could elicit alterations in the production of cortisol levels, affect biological stress responses, and increase emotional response to perceived stress. The analysis/models did not take nonindependence into account. There is potential for spatial clustering of housing conditions and DCZ status because some participants are from the same household. Lastly, our sample size was limited by a decrease in arboviral transmission in the area from 2018 to 2019. Larger and preferably longitudinal studies are needed to confirm and further explore these findings.

## CONCLUSION

Psychological stress has been described to have adverse effects in health by influencing individual's immunological responses. Traditional stress models, such as those described by McEwen (1998) and Cohen (2016) are linear models that target the relationship between stress and health[59,60] These theories, however, have been challenged by multiple authors that describe stress as a multilevel, emergent, and complicated event that is influenced by an individual's age, gender, genetic makeup, and historical sociocultural context.[61–63] Individuals at risk of arboviral infection often live in communities with a higher risk of socio-ecological challenges and health disparities, which in turn also act to increase distress symptoms. This initial study demonstrated that stress can manifest differently among populations and perceived stress does not always align with biological responses (e.g., cortisol levels measured in hair specimens). The initial phase of this research should serve as the foundation for other researchers to further explore the impact of distress and well-being in populations endemic to MBDs. Further research needs to be conducted in this population to investigate the effect of chronic stress from early life and whether sustained stress may lead to HPA axis "fatigue" and in turn cause glucocorticoid resistance. Moreover, further research needs to be conducted to evaluate the multiple molecular mechanisms contributing to immune dysregulation and glucocorticoid resistant in populations endemic to MBDs. Studies should implement an integrated, multifactorial approach that takes into consideration historical, cultural, and biological factors involved in stress responses. Understanding the causes of distress symptoms, their impact on physical health, and cultural idioms toward diseases can help practitioners grant culturally responsive therapies and interventions adequate to meet community needs.

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