METHODS

Study setting

We conducted our study in two coastal regions of Peru: Lima and Tumbes. Pampas de San Juan de Miraflores is a peri-urban shanty town with high-population density centered on a heavily-trafficked avenue. Pampas currently has 57,000 inhabitants, many of whom are highland immigrants (1,2). Average ambient temperatures in Lima range from 17^{o}C to 30^{o}C. Relative humidity ranges from 55% to 80%. Mean annual precipitation is 50 mm per year. The Tumbes site is located along the Tumbes River near the Peru-Ecuador border and has low traffic and low migration. We conducted our study in 23 rural villages situated 3 to 35 kilometers from Tumbes city. Average ambient temperature in Tumbes ranges from 25^{o}C to 33^{o}C, relative humidity ranges between 55% and 80%, and precipitation can reach 2200 mm per year.

Study subjects

Our target population was adolescents 13 to 15 years of age. We identified participants from censuses available in both communities. In Lima, we invited an age and sex random stratified sample of subjects from the census to participate in our study. In Tumbes, we invited all 13 to 15 year old adolescents in the first 23 villages along the Tumbes River to participate.

Adolescents were eligible to participate if they were capable of understanding or performing procedures, if their parents or guardians were capable of providing written informed consent and they were capable of providing assent; if they had no ocular, abdominal, or thoracic surgery in the last three months; and if they were not hospitalized for cardiac reasons in the last three months. Children were ineligible to participate if they had a chronic respiratory condition other than asthma such as cystic fibrosis or chronic lung disease of prematurity, if they were

pregnant, or if they had pulmonary tuberculosis or were currently receiving treatment for pulmonary tuberculosis. We recruited only one adolescent per household.

Study design

We conducted a cross-sectional study of the prevalence of asthma in two regions of Peru between January 2009 and February 2010. We carried out home visitations to enroll participants, administer the survey, and perform physical testing. During the first visit, we asked about asthma and allergy symptoms using the previously validated Spanish ISAAC questionnaire in Peru (3). We also asked about use of asthma medications, asthma severity, biomass fuel exposures and sociodemographics.

We conducted all other tests during a second visit. We measured height with a locally-made stadiometer and weight with a digital scale (Seca, Hamburg, Germany). We conducted spirometry pre- and post-bronchodilator in accordance with joint guidelines from the European Respiratory Society and the American Thoracic Society (4). We used portable, handheld spirometers (SpiroPro, Jaeger/CareFusion, San Diego, USA). We allowed for a maximum of eight forced expiratory maneuvers to achieve three maneuvers that met acceptability and reproducibility criteria (4). We then administered four doses of inhaled salbutamol (100µg each) and repeated spirometry 15 minutes later. All maneuvers were performed seated upright and with a nose clip. Participants who did not meet quality criteria were revisited up to two more times on a different day and asked to repeat spirometry. We asked participants to withhold any short-acting bronchodilators within 8 hours and long-acting bronchodilators for 24-48 hours of testing unless clinically necessary; however, we did not have instances where this occurred. We

revisited participants who reported having a respiratory infection in the last two weeks on a later date.

We measured eNO levels in parts per billion (ppb) using the NIOX MINO portable eNO monitor (Aerocrine, New Providence, USA). We applied allergy skin prick tests for cockroach, dust mite mix, cat hair, dog epithelium, mouse epithelium, and mixed molds (ALK-Abello, Round Rock, USA) using the Multi-Test II allergen applicator (Lincoln Diagnostics, Decatur, USA). After 20 minutes, we recorded induration and erythema dimensions, presence of pruritis and pseudopodia. We interpreted results with an algorithm designed according to the manufacturer's recommendations. Briefly, an allergy skin test was considered positive if the sum of the vertical and horizontal dimensions of the induration was $\geq$3 mm than the negative control and if the sum of the vertical and horizontal dimensions of erythema was $\geq$5 mm larger than the negative control.

We measured 48-hour PM concentrations ($\mu g/m^3$) on weekdays with a portable nephelometer (pDR-1000, Thermo Scientific, Franklin, MA, USA) operated in the passive mode, i.e., without an air-sampling pump. This device incorporates a pulsed, high output, near-infrared light emitting diode source (880 nm). The intensity of the light scattered over the forward angle by particles passing through the sensing chamber is linearly proportional to airborne PM concentration. The pDR-1000 detects particles of 0.3 μm ($PM_{0.3}$) to PM_2 in size more efficiently than PM_2 to PM_{10} (5–7); however, there is a high level of agreement between PM determined by the pDR-1000 and gravimetric measurements of $PM_{2.5}$ (5, 7). Therefore, PM measured with the pDR-1000 is a good approximation for $PM_{2.5}$. We placed the pDR-1000 approximately 1.0 to 1.5 meters above the floor in the living room or main room in the house where adolescents spent most of their time. We measured relative humidity (RH) and temperature using the HOBO Data

Logger (Onset Corp., Bourne, USA) and PM measurements were adjusted by RH as previously described (8). In Lima, we randomly selected 100 participating households from 11 geographic zones based on 300-meter intervals from the main avenue. In Tumbes, we randomly selected 70 participating households from all 23 villages. Outdoor PM data in Lima were provided by DIGESA (www.digesa.sld.pe).

This study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD, USA, and A.B. PRISMA in Lima, Peru.

Definitions

We defined participants as having current asthma symptoms if they reported wheezing in the past 12 months or if they used asthma medications in the past 12 months. We defined atopy as a positive test to one or more skin test allergens. We defined reversibility as a ≥12% increase in FEV_1 from pre- to post-salbutamol (4). We defined asthma severity as per National Asthma Education Prevention Program guidelines (9).

Biostatistical methods

We used Chi-square tests or Fisher exact tests to compare differences in proportions in dichotomous or categorical outcomes between sites. We compared values of lung function between sites using linear regression adjusting for height and age in sex-stratified models. We used standard techniques to calculate unadjusted odds ratios for each risk factor to assess the odds of asthma stratified by site and used the Mantel-Haenszel procedure to estimate an overall summary odds ratio. We used logistic regression stratified by study site to assess the odds of current asthma symptoms as a function of multiple variables. We conducted a pooled

multivariable logistic regression to determine the contribution of peri-urban environment on the odds of asthma after controlling for all other exposures. We used a generalized linear model with a log-normal distribution stratified by site to determine factors that affected eNO. We compared the distribution of indoor PM concentrations using a Wilcoxon signed-tank test. We conducted our analyses in R (www.r-project.org) and STATA (StataCorp, College Station, USA).

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