Prevalence of and factors associated with childhood anemia in remote villages of the Peruvian Amazon: a cross-sectional study and geospatial analysis

Running head: Childhood anemia in remote Peruvian Amazonian villages

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ABSTRACT

Background: Anemia is a public health problem in Peru. In the Loreto region of the Amazon, 50% or more of children may be anemic, although insufficient information exists for rural villages. **Methods:** To generate more data about childhood anemia in the Peruvian Amazon, hemoglobin was measured as part of a trachoma survey in 21 randomly selected villages. All children aged 1-9 years from 30 randomly selected households per village were recruited. Anemia was classified according to the World Health Organization guidelines and a socioeconomic status (SES) index was created for each household using principal component analysis. Spatial autocorrelation was determined using Moran's I and Ripley's K function. **Results:** Of 678 children with complete hemoglobin data, 25.4% (95% CI 21.2–30.1%) had mild-or-worse anemia and 22.1% (95% CI 15.6–30.3%) had moderate-or-worse anemia. Mild-or-worse anemia was more common among children whose primary source of drinking water was surface water (PR=1.26, 95% CI 1.14–1.40, $p<0.001$) and who were in the lowest SES tercile (PR=1.16, 95% CI 1.02–1.32, $p=0.021$). Moderate-or-worse anemia was more common among boys (PR=1.32, 95% CI 1.09-1.60, $p=0.005$). No evidence of geospatial clustering was found. **Conclusions:** Remote villages of the Amazon would benefit from interventions for childhood anemia, and the poorest households would have the most to gain. Integrating anemia screening into neglected tropical diseases surveys is an opportunity to use public health resources more efficiently.

Keywords: anemia, global health, Peru, prevalence, rural health.

Introduction.

Anemia is a public health problem in the South American country of Peru.[1] The nationwide prevalence of childhood anemia was reported to be approximately 40% in 2019, with a higher prevalence found in rural regions (58%) than urban ones (42% nationwide; 31% in the largest city, Lima).[2] In the Loreto region of the Peruvian Amazon, the prevalence of childhood anemia has been reported to exceed 50% for each of the past five years.[2] However, only a single study has included data from rural communities in the Amazon basin.[3] These remote settlements have higher rates of poverty and more limited access to health care than other parts of the region, and thus the burden of anemia, as well as the risk factors for anemia, may be different in such communities.

Information on anemia prevalence, which children are most likely to be anemic, the factors associated as gender, socioeconomic status, access to drinking water and others; geographic distribution of anemia could help public health programs better target interventions in anemia prevention and control.

The objective of the present study was to determine the prevalence of anemia in the Alto Amazonas region of Peru. We further aimed to identify socio-demographic and water access and hygiene factors associated with anemia as well as to identify any geospatial clustering of mild and moderate-to-severe anemia.

Materials and methods.

Study design and setting. This ancillary study was conducted as part of a cross-sectional trachoma prevalence survey carried out from January to March of

2020 in Alto Amazonas Province, Loreto District, Peru. Peru is organized into 26 regions, which are further subdivided into provinces and districts. Alto Amazonas is one of eight provinces in the region of Loreto, covering 18,764 km^2 and containing a population of 122,725 in its six districts (i.e., Balsapuerto, Lagunas, Santa Cruz, Jeberos, Teniente César López Rojas, and Yurimaguas).[4] According to the 2017 census, 39,141 (31.9%) of the total population was rural and 12,756 (15.1%) of those aged 12 years and above identified as indigenous.[4]

A 2018 report by Perú's National Institute of Statistics and Informatics found that out of the 196 provinces in Perú, Alto Amazonas ranked as the 82nd poorest with 32.6% to 39.4% of the population living below the poverty line. For comparison, the Maynas province, which contains the capital city of the Loreto region, was ranked 144th with 20.4% to 23.9% of the population below the poverty line, while the Lima province was ranked 170th with 12.4% to 14.1% of the population below the poverty line.[5]

Sampling for the study was based on 2019 census records provided by the Alto Amazonas health network. The most urban district in the province (i.e., Yurimaguas) and the capital cities of each of the remaining five districts were excluded for the parent study given the low likelihood of trachoma in urban areas.[6] Settlements <100 people were excluded since they were not considered large enough to constitute a village for this study. From the remaining 105 eligible villages, 22 were randomly selected using probability-proportional-to-size sampling. Permission was requested from the leader of each village so that the inhabitants could participate in the study. Within each enrolled village, roughly 30 households were randomly selected for participation either from village administrative records or a hand-drawn map. All members of each selected household were invited to participate in the study and all children aged 1 to 9 years were eligible for this sub-study.[6]

Data collection. The Global Positioning System (GPS) coordinates of each randomly selected household were recorded using a mobile device. Each household was then administered a socioeconomic survey of questions modified from Peru's Demographic and Health Survey (DHS), with input from local healthcare workers and researchers. Since the study was planned for a rural and relatively resource-limited population, the questionnaire focused on asset-based measures to capture socioeconomic status (SES) instead of information on consumption, expenditure, or income.[7] Questions about mobile phone or stereo ownership were included as these could influence the access to information about government-sponsored anemia interventions.[8] Households were also asked about Water, Sanitation, and Hygiene (WASH) access, adapted from the World Health Organization and United Nations Children's Fund (UNICEF) core questions for monitoring WASH in households.[9] After the trachoma survey, children aged 1-9 years from each household were invited to a central location for hemoglobin measurement using a HemoCue Hb-301 (HemoCue, Brea, CA, USA).

Definitions and conventions. A principal component analysis (PCA) was performed to create a SES score for each household. Hemoglobin values were adjusted for altitude following World Health Organization (WHO) recommendations. Classification of anemia was based on WHO guidelines, which define anemia as <

11.0 g/L for children 6-59 months old (severe: < 7.0 g/L; moderate: 7.0-9.9 g/L; mild: 10.0-10.9 g/L) and < 11.5 g/L for children 5-11 years old (severe: < 8.0 g/L; moderate: 8.0-10.9 g/L; mild: 11.0-11.4 g/L).[10] The center of each village was defined as the median latitude and longitude coordinates of the randomly selected households belonging to that village.

Statistical considerations. Adjusted prevalence ratios were estimated from a generalized linear model. Several covariates were defined *a priori* as potential confounders and included in the final model regardless of statistical significance, including gender, age in years, animal ownership (since meat consumption could improve anemia via iron supplementation), and employment status of the head of household (dichotomized as employed versus unemployed; selected because unemployment may prevent adequate nutrition in the household and thus lead to anemia). The final model was created using a forward stepwise procedure, requiring a $p < 0.05$ for all covariates, and separate models were constructed to evaluate mild-or-worse anemia and moderate-or-worse anemia. Standard errors were clustered at the district level. The significance level was set to 5% for this exploratory analysis.

Spatial autocorrelation (e.g., clustering) of village-level prevalence of mild-or-worse and moderate-or-worse anemia was determined using Moran's I, which was calculated using the APE package in R statistical software. This value reports the overall spatial autocorrelation in a dataset as a single value ranging from -1 (i.e., dispersed) to +1 (i.e., clustered) with 0 suggesting complete spatial randomness.[11] Since the estimate of spatial autocorrelation can be biased if the data are not normally distributed, the analysis was repeated with prevalence estimates converted to the empirical logit scale.

Ripley's K function was employed to determine if households with mildly or moderately anemic children tended to cluster. Households were classified as cases if they had ≥1 child with mild-or-worse anemia, otherwise they were classified as controls. The K function is designed for detection of point clusters and is defined as the expected number of events a distance *d* from an arbitrary event.[12] The function was calculated using the spatstat package in R. As apparent clustering of cases could be driven by the underlying tendency of households to cluster, we took the difference in K-functions for households with anemic children (i.e., cases) and households without anemic children (i.e., controls). Under the null hypothesis of no clustering, the cases and controls are independent samples of the same population at risk and therefore the difference in their K-functions should equal zero. Statistical evidence of clustering was determined using a Monte-Carlo simulation to generate confidence envelopes under the assumption of complete spatial randomness. Specifically, households were randomly relabeled as cases or controls and then the difference in K functions calculated. This was repeated 999 times to generate confidence envelopes. Values that lay above the upper limit of the 95% confidence envelope were considered indicative of significant clustering. The distance, *d*, at which clustering occurs provides an idea of the size of individual clusters. The maximum value of *d* for this analysis was half the average village diameter, approximately 300 meters. This analysis was repeated with households with one or more children with moderate-or-worse anemia.

Association analyses were performed with Stata (v.16.0, StataCorp, College Station, TX, USA) while geospatial analyses were performed using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Ethics. The study was evaluated and approved by the Ethics Committees of the Universidad Peruana Cayetano Heredia (UPCH), University of California San Francisco (UCSF), and the Pan American Health Organization (PAHO). Written informed consent was obtained from the child's parent or legal guardian prior to enrollment in the study.

Results

The leader of a village refused the participation of its inhabitants in the study. The geographic distribution of the remaining 21 study villages and the prevalence of mild-or-worse and moderate-or-worse anemia are depicted in Supplementary Figure 1. The flow of participants through recruitment, data collection, and analysis is shown in Supplementary Figure 2. 873 children aged 1-9 years were enumerated at the home, of whom 106 (12%) refused to provide a blood sample. Of the 767 remaining children, 89 (11%) had no hemoglobin data due to HemoCue failure.

678 children from 345 households had complete hemoglobin data. Of these, 335 (49.4%) were female and 299 (44.1%) were in the 1-4-year-old age group (Table 1). The prevalence of any anemia (i.e., mild, moderate, or severe anemia) in all ages was 47.5% (95% CI 41.1–53.9%), with the highest burden among children 1 year of age (Supplementary Figure 3). The prevalence of any anemia was estimated to be 48.2% (95% CI 45.5–50.8%) among children aged 1-4 years and 47.0% (95% CI

36.1–58.1%) among children aged 5-9 years. Overall, 25.4% (95% CI 21.2–30.1%) of children had mild anemia and 22.1% (95% CI 15.6–30.3%) had moderate-to-severe anemia (Table 1).

[Insert Table 1]

Of the 678 children with hemoglobin data, 545 (80.4%) had complete SES data. Age, gender, and anemia status were similar in the populations with and without complete SES data (Table 1). Results of the SES survey are shown in Table 2, stratified by anemia status. Of the 545 children with complete data, 515 (94.5%) came from households whose heads had remunerated jobs, 515 (94.5%) were from families that raised animals at home, and 389 (71.4%) came from families consuming surface water as their primary source of drinking water.

[Insert Table 2]

The multivariable analysis found that children whose primary source of drinking water was surface water had a significantly higher prevalence of anemia (PR = 1.26, 95% CI 1.14–1.40, $p < 0.001$). Furthermore, compared to those children in the highest SES tercile, anemia was more common in children in the lowest tercile (PR = 1.16, 95% CI 1.02–1.32, $p = 0.021$) and middle tercile (PR = 1.27, 95% CI 1.09–1.49, $p = 0.003$). The magnitude of these relationships was similar for the outcome of moderate-or-worse anemia, although the associations did not achieve statistical significance except for male sex, which became associated with a higher prevalence moderate-or-worse anemia (Table 3). Omission of the mobile phone or stereo ownership variable did not significantly alter the results of the multivariable analysis (Supplemental Table 1).

[Insert Table 3]

Moran's I analysis did not find any spatial autocorrelation for the village level prevalence of mild-or-worse or moderate-or-worse anemia ($p = 0.624$ and 0.280, respectively). The results did not change when the analysis was repeated on the logit-transformed prevalence estimates ($p = 0.676$ and 0.241 for prevalence of mild-or-worse and moderate-or-worse anemia, respectively). The results of Ripley's K analysis for mild-or-worse and moderate-or-worse anemia are summarized in Figure 1. There was no evidence of household clustering of mild-or-worse (Figure 1, Panel A) or moderate-or-worse anemia (Figure 1, Panel B).

[Insert Figure 1]

Discussion

Consistent with other studies from the Peruvian Amazon, the present study found a high prevalence of childhood anemia in Alto Amazonas, Peru.[13,14] The factors most associated with anemia were the source of drinking water, gender, and SES.

Other studies have found a high prevalence of anemia in children who have poor access to safe drinking water.[15,16] Poor access to water is an indicator of poverty, and thus the observed relationships may not be causal. However, an association between consumption of surface water and anemia is plausible given the increased risk of ingesting pathogens when consuming surface water, such as *Entamoeba histolytica* and *Giardia duodenalis*, which have been associated with iron deficiency anemia.[17,18]

Our study found male gender to be associated with a higher prevalence of moderate-or-worse anemia, which has been reported in some studies on childhood anemia in the Amazon basin.[19,20] The probable lack of menstruating females in our sample due to only including children 9 years of age or younger may explain why there is a relatively lower prevalence of anemia among girls. The higher anemia prevalence in males could be explained by higher rates of hookworm infection in males which predisposes to iron deficiency anemia.[21] It is also possible that higher rates of malaria among boys, although this has not been found in other studies,[22] or differences in upbringing could contribute to the observed difference in anemia prevalence; however, further research is needed to evaluate these hypotheses.

Belonging to the lowest or second-lowest SES tercile was associated with a significantly higher prevalence of anemia in this study, and households with an unemployed head of household also had a higher prevalence of anemia—though this latter relationship did not reach statistical significance. While we did not observe a linear relationship between wealth and anemia, these findings are generally consistent with other studies examining the effect of SES on anemia in indigenous populations in Perú.[13,23] Results from neighboring Brazil paint a similar picture, with a higher anemia prevalence in the poorest social classes and in those with a lower SES.[24,25] This could be due to the relationship between socioeconomic factors and the care children receive, including proper diet, access to health services, and living conditions.[26] Children from poor families are more likely to be malnourished and thus anemic.[27] The lack of linear relationship between anemia prevalence and SES may indicate a reduced risk of anemia above a certain threshold of SES or could be due to misclassification error.

Our study found no evidence of clustering of anemia prevalence at the village level or clustering of households with anemic children. In contrast, a study based on the Ethiopian DHS concluded that there was slight but significant clustering of childhood anemia in 2005, 2011 and 2016.[28] Similarly, a nationwide survey in India found a high level of anemia clustering.[29] In Peru, analysis of nationwide healthcare data showed district-level spatial autocorrelation of anemia in children under 5 years.[30] These studies were conducted with larger samples and geographic areas than the present study, which may explain why they detected significant spatial autocorrelation of anemia while the present study did not. It also could be the case that there is insufficient heterogeneity in anemia at smaller geographic scales.

This study found a 47.5% prevalence of mild or worse anemia in children 1-9 years of age in remote villages of the Alto Amazonas region, which is similar to the prevalence of anemia that has been reported in the Loreto region more generally.[2] This level of anemia meets the criteria to be classified as a severe public health problem (i.e., a prevalence ≥ 40%) according to the WHO classification for public health severity of anemia.[31] Iron supplementation or micronutrient fortification and scheduled deworming are recommended for areas where anemia is a moderate to severe public health problem.[31] As the Control de Crecimiento y Desarrollo (CRED) program is already addressing micronutrient deficiencies, future studies on the etiology of anemia in this population may enable more precise interventions (e.g., regular deworming programs if intestinal parasites are found to be contributing to the burden of anemia). Furthermore, the present study identified lower SES groups, those whose primary source of drinking water is surface water, and male children as especially at-risk groups who may benefit from interventions to reduce anemia.

A 2016 paper evaluated barriers to CRED implementation in Loreto and two other regions of Peru and found that factors associated with CRED compliance varied across regions.[32] Within Loreto, being left unattended once was a risk factor for CRED non-compliance while receiving information on CRED during the child's first year of life and being a beneficiary of a government safety net program were associated with increased CRED compliance. Future iterations of the CRED program might improve compliance by increasing education and outreach about the program and minimizing interruptions in attendance, especially in areas with low compliance.

The present study shows that the integration of anemia screening into neglected tropical diseases surveys is an opportunity to efficiently use finite public health resources in rural and remote areas such as those in the Amazon Basin. These populations are in urgent need of integrated health services tailored to their specific needs, risk factors, and context. They are usually excluded from national health and nutrition surveys due to the high operational cost and difficult access to reach them. Hence, integrated approaches should be leveraged to leave no one behind.

Our study has limitations. Although we estimated the prevalence of anemia, we did not collect information on variables such as childhood feeding practices, iron supplementation, infections or recent fevers, anthropometrics, and maternal anemia, as these were outside the scope of the main objective of the survey (i.e., trachoma prevalence) and financial and temporal constraints precluded their collection. While the HemoCue Hb-301 has been reported to have moderate validity and suboptimal diagnostic capacity in community-based settings,[33] a 2019 review found that the hemoglobin concentrations reported by the HemoCue Hb-301 were within ±7% of the reference test and thus within the range of acceptable difference as set by the College of American Pathologists.[34] Furthermore, the remoteness of the study communities and lack of readily available laboratory testing precluded the use of more sophisticated methods of measuring hemoglobin concentration. A portion of children were missing data on SES or refused to participate in the study. While measured characteristics of participants and non-participants appeared similar, this still may have led to some degree of selection bias. A small proportion of households (5.2%, N = 18) were missing GPS data, but this was not associated with anemia nor impacted results.

In summary, nearly half of children aged 1 to 9 years in Alto Amazonas had mild-or-worse anemia. Anemia was more common in children drinking surface water, those belonging to lower SES terciles, and males. This study adds to the body of evidence showing a considerable burden of childhood anemia in rural villages of the Amazon and highlights groups that would benefit the most from intervention.

Authors' contributions: NMA, AQL, JN, JK and AL conceived the study; NMA, JN MM, ST, EHE, HHM, SD and CCA contributed to data collection; JCC, OE, MSD, JK, JK and AL contributed to the data analysis and interpretation; NMA, AQL, JN, JCC and OE drafted the initial manuscript; JK, JK and AL critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript. JK and AL are the guarantors of the paper.

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