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ORIGINAL ARTICLE

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

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Background: Anaemia is a public health problem in Peru. In the Loreto region of the Amazon, ≥50% of children may be anaemic, although insufficient information exists for rural villages.

Methods: To generate more data about childhood anaemia in the Peruvian Amazon, haemoglobin was measured as part of a trachoma survey in 21 randomly selected villages. All children 1–9 y of age from 30 randomly selected households per village were recruited. Anaemia was classified according to the World Health Organization guidelines and a socio-economic 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 haemoglobin data, 25.4% (95% confidence interval [CI] 21.2 to 30.1) had mild-or-worse anaemia and 22.1% (95% CI 15.6 to 30.3) had moderate-or-worse anaemia. Mild-or-worse anaemia was more common among children whose primary source of drinking water was surface water (prevalence ratio [PR] 1.26 [95% CI 1.14 to 1.40], p<0.001) and who were in the lowest SES tercile (PR 1.16 [95% CI 1.02 to 1.32], p=0.021). Moderate-or-worse anaemia was more common among boys (PR 1.32 [95% CI 1.09 to 1.60], p=0.005). No evidence of geospatial clustering was found.

Conclusions: Remote villages of the Amazon would benefit from interventions for childhood anaemia and the poorest households would have the most to gain. Integrating anaemia screening into neglected tropical diseases surveys is an opportunity to use public health resources more efficiently.

Keywords: anaemia, global health, Peru, prevalence, rural health

Introduction

Anaemia is a public health problem in the South American country of Peru.[1] The nationwide prevalence of childhood anaemia 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 anaemia has been reported to exceed 50% for each of the past 5 y.[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 healthcare than other parts of the region and thus the burden of anaemia, as well as the risk factors for anaemia, may be different in such communities.

Information on anaemia prevalence; which children are most likely to be anaemic; associated factors such as gender, socio-economic status and access to drinking water; and geographic distribution of anaemia could help public health programs better target interventions in anaemia prevention and control.

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

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 2020 in the 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 (Balsapuerto, Lagunas, Santa Cruz, Jeberos, Teniente César López Rojas and Yurimaguas).[4] According to the 2017 census, 39 141 (31.9%) people lived in rural areas and 12 756 (15.1%) of those ≥12 y of age identified as indigenous.[4]

A 2018 report by Peru's National Institute of Statistics and Informatics found that of the 196 provinces in Peru, Alto Amazonas ranked as the 82nd poorest, with 32.6–39.4% 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 20.4–23.9% of the population below the poverty line, while the Lima province was ranked 170th, with 12.4–14.1% 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 (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 of <100 people were excluded because 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 1–9 y of age were eligible for this substudy.[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 socio-economic 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 socio-economic status (SES) instead of information on consumption, expenditures or income.[7] Questions about mobile phone or stereo ownership were included, as these could influence access to information about government-sponsored anaemia interventions.[8] Households were also asked about water, sanitation, and hygiene (WASH) access, adapted from the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) core questions for monitoring WASH in households.[9] After the trachoma survey, children 1–9 y of age from each household were invited to a central location for haemoglobin measurement using a HemoCue Hb-301 (HemoCue, Brea, CA, USA).

Definitions and conventions

A principal component analysis (PCA) was performed to create an SES score for each household. Haemoglobin values were adjusted for altitude following WHO recommendations. Classification of anaemia was based on WHO guidelines, which define anaemia as <11.0 g/L for children ages 6–59 months (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 ages 5–11 y (severe: <8.0 g/L; moderate: 8.0–10.9 g/L; mild: 11.0–11.4 g/L).[10] The centre 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 anaemia via iron supplementation) and employment status of the head of household (dichotomized as employed vs unemployed; selected because unemployment may prevent adequate nutrition in the household and thus lead to anaemia). The final model was created using a forward stepwise procedure, requiring a p-value <0.05 for all covariates, and separate models were constructed to evaluate mild-or-worse anaemia and moderate-or-worse anaemia. Standard errors were clustered at the district level. The significance level was set to 5% for this exploratory analysis.

Table 1. General characteristics of the study population

Characteristics	Children with haemoglobin data (n=678)		Children with complete data for association analysis (n=545)	
	n	% (95% CI)	n	% (95% CI)
Gender				
Female	335	49.4 (43.8 to 55.0)	266	48.8 (42.4 to 55.2)
Male	343	50.6 (45.0 to 56.2)	279	51.2 (44.8 to 57.6)
Age (years)				
1–4	299	44.1 (39.3 to 49.0)	245	45.0 (40.3 to 49.7)
5–9	379	55.9 (51.0 to 60.7)	300	55.1 (50.3 to 59.7)
Anaemia				
None	356	52.5 (46.1 to 58.9)	298	54.7 (46.7 to 62.4)
Mild	172	25.4 (21.2 to 30.1)	129	23.7 (20.4 to 27.3)
Moderate	148	21.8 (15.3 to 30.2)	116	21.3 (15.1 to 29.1)
Severe	2	0.3 (0.1 to 0.9)	2	0.4 (0.1 to 1.1)

CIs account for district-level clustering.

Spatial autocorrelation (e.g. clustering) of village-level prevalence of mild-or-worse and moderate-or-worse anaemia was determined using Moran's I, which was calculated using the APE package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria). 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 anaemic children tended to cluster. Households were classified as cases if they had one or more children with mild-or-worse anaemia, 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 anaemic children (i.e. cases) and households without anaemic 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 relabelled as cases or controls and then the difference in K functions was calculated. This was repeated 999 times to generate confidence envelopes. Values that lay above the upper limit of the 95% confidence interval (CI) 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 m. This analysis was repeated with households with one or more children with moderate-or-worse anaemia.

Association analyses were performed with Stata version 16.0 (StataCorp, College Station, TX, USA), while geospatial analyses were performed using R version 3.6.0.

Results

The leader of one 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 anaemia are depicted in Supplementary Fig. 1. The flow of participants through recruitment, data collection and analysis is shown in Supplementary Fig. 2. A total of 873 children 1–9 y of age were enumerated at the home, of whom 106 (12%) refused to provide a blood sample. Of the 767 remaining children, 89 (11%) had no haemoglobin data due to HemoCue failure.

A total of 678 children from 345 households had complete haemoglobin data. Of these, 335 (49.4%) were female and 299 (44.1%) were in the 1- to 4-year-old age group (Table 1). The prevalence of any anaemia (i.e. mild, moderate or severe) in all ages was 47.5% (95% CI 41.1 to 53.9), with the highest burden among children 1 y of age (Supplementary Fig. 3). The prevalence of any anaemia was estimated to be 48.2% (95% CI 45.5 to 50.8) among children ages 1–4 y and 47.0% (95% CI 36.1 to 58.1) among children ages 5–9 y. Overall, 25.4% (95% CI 21.2 to 30.1) of children had mild anaemia and 22.1% (95% CI 15.6 to 30.3) had moderate-to-severe anaemia (Table 1).

Of the 678 children with haemoglobin data, 545 (80.4%) had complete SES data. Age, gender and anaemia status were similar in the populations with and without complete SES data (Table 1). The results of the SES survey are shown in Table 2, stratified by

Table 2. Characteristics of the study population and results of the SES survey stratified by anaemia severity

	No anaemia (n=298)		Mild anaemia (n=129)		Moderate-to-severe anaemia (n=118)	
Characteristic	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Gender of children						
Female	150	50.3 (39.2 to 61.4)	66	51.2 (41.5 to 60.7)	50	42.4 (37.1 to 47.8)
Male	148	49.7 (38.6 to 60.8)	63	48.8 (39.3 to 58.5)	68	57.6 (52.2 to 62.9)
Child age (years)						
1–4	130	43.6 (34.9 to 52.8)	78	60.5 (53.3 to 67.2)	37	31.4 (18.4 to 48.1)
5–9	168	56.4 (47.2 to 65.1)	51	39.5 (32.8 to 46.7)	81	68.6 (51.9 to 81.6)
HOH employment						
Employed	284	95.3 (78.4 to 99.1)	120	93.0 (86.8 to 96.4)	111	94.1 (78.4 to 98.6)
Unemployed	14	4.7 (0.9 to 21.6)	9	7.0 (3.6 to 13.2)	7	5.9 (1.4 to 21.6)
Drinking water source						
Piped, spring, well	96	32.2 (8.7 to 70.3)	30	23.3 (7.1 to 54.7)	30	25.4 (4.5 to 71.1)
Surface water	202	67.8 (29.7 to 91.3)	99	76.7 (45.3 to 92.9)	88	74.6 (28.9 to 95.5)
Radio/stereo at home						
No	222	74.5 (60.0 to 85.0)	103	79.8 (61.6 to 90.7)	84	71.2 (48.9 to 86.4)
Yes	76	25.5 (15.0 to 40.0)	26	20.2 (9.3 to 38.4)	34	28.8 (13.6 to 51.1)
Mobile phone						
No	253	84.9 (58.9 to 95.7)	118	91.5 (74.4 to 97.5)	103	87.3 (58.6 to 97.1)
Yes	45	15.1 (4.3 to 41.1)	11	8.5 (2.5 to 25.6)	15	12.7 (2.9 to 41.4)
Breeding animals						
No	15	5.0 (0.8 to 26.0)	9	7.0 (0.9 to 38.5)	6	5.1 (1.0 to 21.8)
Yes	283	95.0 (74.0 to 99.2)	120	93.0 (61.5 to 99.1)	112	94.9 (78.2 to 99.0)
Wealth index terciles						
1 (poorer)	100	33.6 (12.7 to 63.7)	54	41.9 (17.0 to 71.7)	35	29.7 (10.5 to 60.3)
2	95	31.9 (22.8 to 42.6)	45	34.9 (26.7 to 44.1)	48	40.7 (33.4 to 48.4)
3 (wealthier)	103	34.6 (9.8 to 72.1)	30	23.3 (6.9 to 55.4)	35	29.7 (8.8 to 64.7)

HOH: head of household.

anaemia 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 who raised animals at home and 389 (71.4%) came from families consuming surface water as their primary source of drinking water.

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

Moran's I analysis did not find any spatial autocorrelation for the village-level prevalence of mild-or-worse or moderate-or-worse anaemia ($p=0.624$ 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 anaemia, respectively). The results of Ripley's K analysis for mild-or-worse and moderate-or-worse anaemia are summarized in Figure 1. There was no evidence of household clustering of mild-or-worse (Figure 1A) or moderate-or-worse anaemia (Figure 1B).

Discussion

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

Other studies have found a high prevalence of anaemia 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 anaemia is plausible given the increased risk of ingesting pathogens when consuming surface water, such as *Entamoeba histolytica* and

Table 3. Multivariable regression analysis. The association between various sociodemographic factors and moderate-to-severe anaemia is shown for children 1–9 y of age in Alto Amazonas (N=545)

	Mild-or-worse anaemia			Moderate-or-worse anaemia		
Characteristics	PR	95% CI	p-Value	PR	95% CI	p-Value
Gender of child						
Female	Ref.			Ref.		
Male	1.10	0.94 to 1.29	NS	1.32	1.09 to 1.60	0.005
Age of child (years)	0.97	0.89 to 1.04	NS	1.07	0.97 to 1.18	NS
Employment, head of household						
Employed	Ref.			Ref.		
Unemployed	1.20	0.99 to 1.50	NS	1.03	0.65 to 1.64	NS
Drinking water source						
Piped, spring, well[a]	Ref.			Ref.		
Surface water	1.26	1.14 to 1.40	<0.001	1.22	0.79 to 1.91	NS
Mobile phone or stereo ownership						
Yes	Ref.			Ref.		
No	1.01	0.92 to 1.11	NS	0.80	0.61 to 1.05	NS
Ownership of animals						
No	Ref.			Ref.		
Yes	0.88	0.59 to 1.32	NS	1.14	0.53 to 2.45	NS
SES tercile						
1 (poorer)	1.16	1.02 to 1.32	0.021	1.01	0.66 to 1.54	NS
2	1.27	1.09 to 1.49	0.003	1.38	0.98 to 1.93	NS
3 (wealthier)	Ref.			Ref.		

[a]Including protected and unprotected spring sources of water, such as artisanal system designed to avoid water contamination and village artisanal wells.
NS: not significant; Ref.: reference.

Giardia duodenalis, which have been associated with iron deficiency anaemia.[17,18]

Our study found male gender to be associated with a higher prevalence of moderate-or-worse anaemia, which has been reported in some studies on childhood anaemia in the Amazon basin.[19,20] The probable lack of menstruating females in our sample due to only including children ≤9 y of age may explain why there is a relatively lower prevalence of anaemia among girls. The higher anaemia prevalence in males could be explained by higher rates of hookworm infection in males, which predisposes to iron deficiency anaemia.[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 anaemia 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 anaemia in this study, and households with an unemployed head of household also had a higher prevalence of anaemia—although this latter relationship did not reach statistical significance. While we did not observe a linear relationship between wealth and anaemia, these findings are generally consistent with other studies examining the effect of SES on anaemia in indigenous populations in Peru.[13,23] Results from neighbouring Brazil paint a similar picture, with a higher anaemia prevalence in the poorest social classes and in those with a lower SES.[24,25] This could be due to the relationship between socio-economic 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 anaemic.[27] The lack of a linear relationship between anaemia prevalence and SES may indicate a reduced risk of anaemia above a certain SES threshold or could be due to misclassification error.

Our study found no evidence of clustering of anaemia prevalence at the village level or clustering of households with anaemic children. In contrast, a study based on the Ethiopian DHS concluded that there was slight but significant clustering of childhood anaemia in 2005, 2011 and 2016.[28] Similarly, a nationwide survey in India found a high level of anaemia clustering.[29] In Peru, analysis of nationwide healthcare data showed district-level spatial autocorrelation of anaemia in children <5 y of age.[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 anaemia while the present study did not. It also could be the case that there is insufficient heterogeneity in anaemia at smaller geographic scales.

This study found a 47.5% prevalence of mild or worse anaemia in children 1–9 y of age in remote villages of the Alto Amazonas

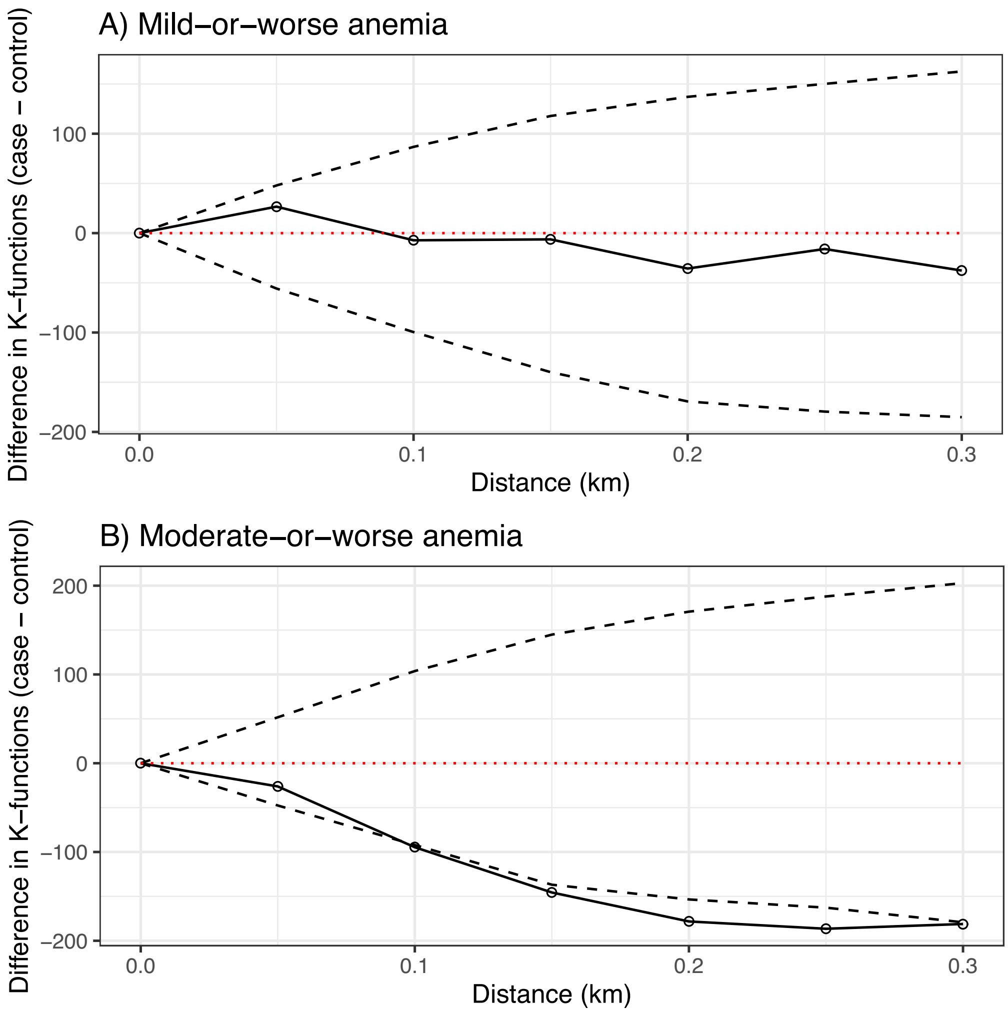

Figure 1. K plots for **(A)** mild-or-worse and **(B)** moderate-or-worse anaemia. The solid line represents the difference in K function for cases (i.e. households with one or more anaemic children) and controls (i.e. households without anaemic children), the dashed lines represent the upper and lower limits of the CIs generated via 999 Monte Carlo simulations and the red dotted line indicates a difference in K functions of zero.

region, which is similar to the prevalence of anaemia that has been reported in the Loreto region more generally.[2] This level of anaemia 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 anaemia.[31] Iron supplementation or micronutrient fortification and scheduled deworming are recommended for areas where anaemia 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 aetiology of anaemia 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 anaemia). 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 anaemia.

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 anaemia 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 contexts. They are usually excluded from national health and nutrition surveys due to the high operational costs and difficulties in accessing them. Hence, integrated approaches should be leveraged to leave no one behind.

Our study has limitations. Although we estimated the prevalence of anaemia, we did not collect information on variables such as childhood feeding practices, iron supplementation, infections or recent fevers, anthropometrics and maternal anaemia, 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 haemoglobin concentrations reported by the HemoCue Hb-301 were within ±7% of the reference test and thus within the acceptable range 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 haemoglobin concentrations. 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 (18 [5.2%]) were missing GPS data, but this was not associated with anaemia nor did it impact results.

In summary, nearly half of children 1–9 y of age in Alto Amazonas had mild-or-worse anaemia. Anaemia 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 anaemia in rural villages of the Amazon Basin and highlights groups that would benefit the most from intervention.

Supplementary data

Supplementary data are available at *Transactions* online.

Authors' contributions: NM-A and AQ-L contributed equally to this work. NM-A, AQ-L, JMN, JDKlausner and AGL conceived the study. NM-A, JMN, MM, ST, EMH-E, HAH-M, SD and CAC-A contributed to data collection. JLC-C, OAE, MIS-D, JDKlausner, JDKeenan and AGL contributed to the data analysis and interpretation. NM-A, AQ-L, JMN, JLC-C and OAE drafted the initial manuscript. JDKlausner, JDKeenan and AGL critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript. JDKlausner and AGL are the guarantors of the paper.

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