Original Paper

Correlation between iron deficiency and parasitic intestinal infection in children under 14 years of age from six urbanized indigenous cabildos in Colombia

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Abstract

Introduction: the concurrence of anaemia and intestinal parasitic infection continues to be a major public health problem in many developing countries, especially in economically disadvantaged communities. **Objective:** to assess the correlation between intestinal parasitic infection and iron deficiency in children aged less than 14 years from indigenous communities residing in urban districts of the city of Santiago de Cali.

Method: we conducted an observational and analytical study in a probability sample of indigenous children aged less than 14 years. Three serial stool samples and blood samples were analysed to establish the proportions of children with and without parasitic infections, anaemia and low iron stores. We used descriptive, univariate and bivariate statistical methods, finishing with multivariate principal component and hierarchical cluster analyses.

Results: 80% of the minors had parasitic infections, 17% by *Parascaris* and *Trichocephalon*. We did not find a statistically significant association between parasitic infection and sex; 5 children aged 5-7 years had anaemia. We identified three groups in the multivariate analysis.

Conclusions: We found a high overall prevalence of intestinal parasitic infection, and particularly of mild-to-moderate helminth infection. Overall, anaemia was not prevalent in the sample under study, and we did not find a significant association between anaemia and parasitic infection.

Key words:
- Anaemia
- Intestinal parasites
- Children
- Indigenous

Correlación entre deficiencias de hierro y enteroparasitismo en menores de 14 años de seis cabildos indígenas urbanizados de Colombia

Resumen

Introducción: las comorbilidades de anemia y enteroparasitismo siguen siendo un importante problema de salud pública en muchos países en desarrollo y en especial en comunidades con mayores desventajas económicas. **Objetivo:** evaluar la correlación entre enteroparasitismo y deficiencias de hierro en menores de 14 años de los cabildos indígenas urbanizados de la ciudad de Santiago de Cali.

Método: estudio observacional y analítico que involucró una muestra probabilística de indígenas menores de 14 años. Se analizaron tres muestras seriadas de materia fecal y sangre para establecer las proporciones de menores parasitados y no parasitados; anemia/ferropenia y depleción de las reservas de hierro. Se usó estadística descriptiva, univariada y bivariada, finalizando con los análisis multivariados de componentes principales y clúster jerárquico.

Resultados: el 80% de los menores estaban parasitados, el 17% con áscaris y tricocéfalo. No se halló asociación estadísticamente significativa entre parasitismo y género; cinco menores de entre 5-7 años presentaron anemia. Tres grupos se identificaron con el análisis multivariado.

Conclusiones: elevada prevalencia de parasitismo intestinal global y en particular una infestación significativa leve-moderada por helmintos; en términos generales, la anemia no predominó en la población de estudio y no se encontraron asociaciones significantes entre esta y parásitos patógenos.

Palabras clave:
- Anemia
- Escolares
- Indígena
- Parásitos intestinales

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INTRODUCTION

Anaemia is considered the most prevalent nutritional deficiency, as it affects approximately one fourth of the global population, especially children and women of reproductive age.[1] It usually results from a reduction in the iron stores of the body, the most common micronutrient deficiency in developing as well as developed countries.[2] Iron deficiency anaemia (IDA) has a serious impact on health and nutritional status, including impaired growth and neurodevelopment in children,[3] increased maternal mortality and frequency of low birth weight, and decreased productivity in adults[4]. It has also been associated with poor academic performance in schoolchildren and adolescents.[5]

There are different possible causes of IDA, ranging from a low intake of iron, folic acid and vitamin B_{12} to malabsorption and infectious diseases, specifically parasitic infections.[6] Parasitic infections are included in the 17 neglected tropical diseases identified by the World Health Organization (WHO), are the fourth leading cause of communicable diseases and are associated with a high proportion of disability.[7,8] The parasites involved most frequently are helminths, such as *Ascaris lumbricoides*, *Ancylostoma* hookworms and *Trichiuris trichiura*.[9] Helminth infections cause anaemia by reducing iron absorption in the gut through direct suction of blood, interfering in iron metabolism. Protozoans, too, can contribute to anaemia by destroying the intestinal mucosa, which affects the absorption of micronutrients such as iron.[10] Furthermore, as regards the association of these 2 types of infection, there is evidence that helminths and protozoans have a similar geographical distribution.[3]

It is estimated that approximately 2000 million people worldwide are infected by pathogenic parasites, which corresponds to 24.8% of the global population and accounts for about 10% of episodes of diarrhoea or health care visits.[11] They affect marginalised, low-income and low-resource regions disproportionately, including vulnerable communities such as indigenous peoples displaced from their ancestral lands and living in depressed settlements or neighbourhoods in urban and suburban areas.[3]

In Latin America, the indigenous peoples have historically been the collective suffering the greatest impact and prevalence of communicable infectious diseases, malnutrition, food insecurity, parasitic diseases such as helminthiasis and intestinal polyparasitism, and the highest child mortality rates.[12] In this context, we conducted a study in which the initial phase consisted in establishing the prevalence of intestinal parasitic infection and IDA in children aged less than 14 years residing in 6 indigenous *cabildos* (councils) in the city of Cali. The second phase involved the assessment of the correlation between intestinal parasitic infection and iron deficiency in children under 14 years living in urban indigenous cabildos and the spatial distribution of these diseases in the city relative to the socioeconomic characteristics of the administrative district where the minors resided with their families.

METHODS

Study design, sample size and variables

We conducted an observational and analytic study in children aged less than 14 years residing in 6 indigenous *cabildos* located in urban and suburban areas of the city of Santiago de Cali, Colombia. We calculated the necessary sample size for the first analysis for an expected proportion of anaemia in the under-14 years age group of 13%,[12] a precision of 10% and a level of confidence of 95%, which yielded a necessary size of 57 children.

We applied the same calculation to the second analysis, as the model used is not subject to requirements regarding the minimum sample size or a given ratio for the number of individuals and the number of variables included in the analysis.[13] The independent variables were the age, sex and indigenous community where the minor resided. We also included variables recorded in the system for the identification of potential beneficiaries of social programmes (Sistema de Identificación de Potenciales Beneficiarios de Programas Sociales [SISBEN]), which uses a specific score to classify residents based on socioeconomic variables and the socioeconomic status mode of the district of residence.[14] The dependent variables were: 1) intestinal parasitic infection, categorised into absence of intestinal parasites, presence of pathogenic intestinal parasites, presence of nonpathogenic intestinal parasites; and 2) anaemia, analysed as a dichotomous variable (iron deficiency/no iron deficiency).

Collection of stool samples and stool tests: we collected 3 serial stool samples consecutively for each minor. Samples were processed within 1 hour from submission following the protocols established by the manufacturer—the Fisher Diagnostic Kit protocol for microscopic examination and the Bio-Manguinhos Helm Test protocol for quantification of helminth ova. Collection of blood samples for haematology and blood chemistry tests: for each of the participants that provided the stool samples, we collected 2 fasting (8-10 hours) blood samples with the Venoject blood collection system (one in a tube with ethylenediaminetetraacetic acid [EDTA] and one in a plain tube) to obtain the serum marker levels and blood counts calculated with the automated haematology analyser and measure ferritin and transferrin levels. Table 1 presents the reference values used to define the thresholds for anaemia and iron deficiency.

Statistical analysis

The data were processed with the software SPSS version 25.0.0.0. We generated tables and charts with the software XLSTAT. We assessed differences between age groups, sexes and indigenous communities in ova and parasite exam, blood count and blood chemistry test results through the median test and the Kruskal-Wallis test. We assessed the correlations between age, haemoglobin level, haematocrit, ferritin and transferrin levels and haematological parameters (mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC] and red cell distribution width ancho de [RDW]) with the Pearson correlation matrix and confirmed the results with the Bartlett test of sphericity.

Since we found a strong correlation between the selected variables, we used principal component analysis (PCA) for the multivariate model, which allowed us to describe the data set in terms of new variables (components) that were not correlated without losing information on the variance contributed by each variable. We organised the data based on similarities between subjects and differences between groups by means of hierarchical cluster analysis (HCA), where Y represents the differences (dissimilarity) between the established groups of individuals.

The study was completed by georeferencing the included cases to analyse the distribution of comorbidities based on the SISBEN classification, the socioeconomic status mode of the district of the city (an administrative division known as *comuna*) where participants lived with their families, and the number of families per housing unit.

Ethical considerations

The study was approved by the Ethics Committee of the School of Health of the Universidad del Valle y and by each of the indigenous *cabildos*.

Table 1. Reference range for haemoglobin, ferritin and transferrin in children aged less than 14 years

Age range	Normal range		
5-7 years	Hb ≥ 11.5 g/dl	Ferritin > 12 µg/l	Transferrin < 400 µg/l
8-14 years	Hb ≥ 12 g/dl	Ferritin > 15 µg/l	Transferrin < 380 µg/l

Hb: haemoglobin.
Source: Concentraciones de hemoglobina para diagnosticar la anemia y evaluar su gravedad y valores de normalidad analítica según edad. In: World Health Organization [online] [accessed 24/11/2020]. Available at www.who.int/vmnis/indicators/haemoglobin_es.pdf

RESULTS

The mean age of the 57 minors included in the study was 9 years (standard deviation [SD], 3), 32 were male and 25 female. The distribution by indigenous *cabildo* was: 16 Yanacona, 13 Nasa, 13 Inga, 8 Quechua, 5 Misak and 2 Kofan.

The overall prevalence of intestinal parasite infection was 84%. Considering pathogenicity, we found that 19/48 of the involved parasites were pathogenic, among which the prevalence of helminths, *Ascaris* and *Trichocephalon* was 17%. We also detected pathogenic protozoans, such as the combination of *Entamoeba histolytica*/*E. dispar* and *Giardia*, in 21% of the samples. The prevalence of helminth infection was highest in children from the Inga, Nasa and Quechua communities (23%-25%), without significant differences (median test, $p > 0.092$). We found mixed infection (more than 1 type of parasite) in 16/48 samples, mainly in children aged 5 to 9 years in all communities except the Quechua. We compared the severity of infection by type of intestinal parasite and age group (5-7 years vs. 8-14 years) in the different indigenous communities and found no significant differences (Kruskal-Wallis test, $p = 0.77$ and $p = 0.13$ respectively). However, we found that 4 children aged 5 to 7 years had helminthiasis of mild (120 to 600 eggs per gram of faeces) to moderate severity (5400 to 17 600 eggs/g faeces), and another 4 children aged 8 to 14 years with mild infection (24-720 eggs/g faeces). We did not find a statistically significant association between parasite infection and the sex of the child (Kruskal-Wallis test, $p = 0.70$). Five children aged 5 to 7 years had anaemia (haemoglobin concentration below the reference threshold established by the WHO): 3 Ingas, 1 Nasa and 1 Quechua. Ferritin levels in these children were normal. Three children aged 8 to 14 years in the cabildo of Yanacona had mild anaemia (haemoglobin < 12 g/dl), associated with low iron stores in 1 (ferritin < 15 µg/l). The mean haemoglobin concentration in the overall sample was 12,8 g/dl (range, 11.0 to 14.6), the mean ferritin level was 45.6 µg/l (range, 11.2 to 94) and the mean transferrin level 317.8 µg/l (range, 97 to 388). One participant had anaemia associated with iron stores and mixed helminthiasis (*Ascaris* and *Trichocephalon*). Table 2 presents the average and range of blood count, blood chemistry and red blood cell indices, comparing them based on the presence and type of intestinal parasitic infection.

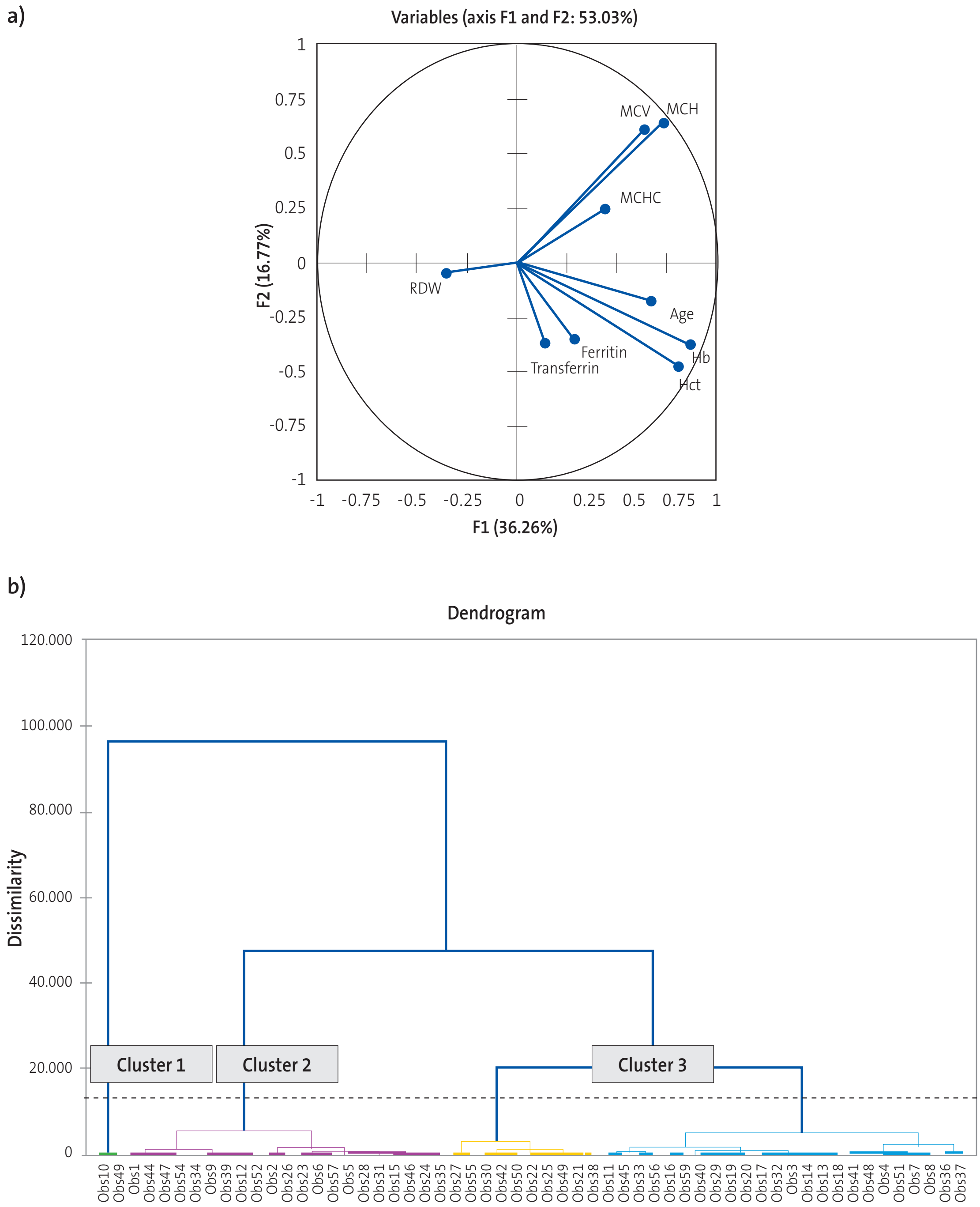
We found a strong correlation between variables based on the Pearson correlation matrix. We confirmed the correlation with the Bartlett sphericity test ($p < 0.0001$). The PCA generated 2 components that explained 53.03% of the total variance (Fig. 1A). The chart shows a first component derived from the MCV, MCH and CMCH indices, and a second component derived from the age, haemoglobin, haematocrit, ferritin, transferrin and RDW variables. On the other hand, HCA predefined 3 groups of minors based on the dissimilarity in variables defined by the dotted line (Fig. 1B), the dis-

Table 2. Distribution of red blood cell and blood chemistry values based on the presence of parasitic infection in children

		Hb	Hct	MCV	MCH	MCHC	RDW	Ferritin	Transferrin
Absence of intestinal parasites	Average	12.87	38.41	79.93	26.75	33.50	12.66	46.28	312.73
	Range	2.70	8.00	12.00	5.00	2.10	2.10	82.80	290.00
Presence of pathogenic intestinal parasites	Average	12.55	37.51	79.47	26.59	33.46	12.76	45.91	333.00
	Range	3.20	9.30	9.00	4.20	2.50	2.50	66.80	136.00
Presence of nonpathogenic intestinal parasites	Average	13.13	39.59	79.44	26.32	33.17	12.68	42.66	298.25
	Range	2.70	8.10	11.00	4.40	2.40	1.40	43.50	264.00
Total	Average	12.81	38.30	79.70	26.63	33.43	12.70	45.66	317.81
	Range	3.60	10.20	12.00	5.40	2.60	2.50	82.80	291.00

Hb: Haemoglobin; **Hct:** haematocrit; **MCH:** mean corpuscular haemoglobin; **MCHC:** mean corpuscular haemoglobin concentration; **MCV:** mean corpuscular volume; **Range:** maximum – minimum; **RDW:** red cell distribution width.

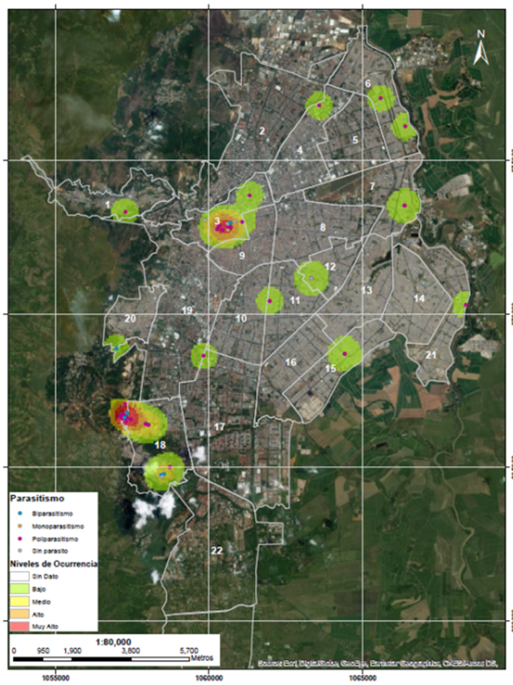
Figure 1. Distribution of the variables under study in the first plane of the principal component analysis (A) and grouping obtained through hierarchical cluster analysis (HCA) (B)



tributions of the MCV, MCH, CMCH and RDW variables in each group did not vary significantly (median test, p = 0.77), whereas we found differences in age, haemoglobin, ferritin and transferrin. For instance, in group 2 there were 2 participants aged 8 to 10 years with higher haemoglobin concentrations and biochemical marker values compared to group 3. The relative frequency distribution of pathogenic intestinal parasites was similar in groups 2 and 3, while pathogenic parasites were absent in group 1, a difference that was statistically significant relative to groups 2 and 3 (median test, p = 0.047).

Figure 2 represents the geographical distribution of the cases based on the residence of participants. Children of the Misak and Quechua communities were located in the centre and north-east of the city (districts 3, 18 and 20). Based on a report produced by the city planning department and a private university,[14] these 3 districts experience considerable economic disadvantage, reflected in aspects such as being beneficiaries of subsidised social welfare and public health programmes (> 75% covered by subsidised regimens 1 and 2) and considerable household crowding (number of households per residence), most frequently involving the presence of 2 households in a single housing unit. The Kofan resided in the centre (districts 10 and 11) in areas with better socioeconomic conditions, as reflected by the fact that most of the population fits SISBEN categories 2 (22% to 27%) and 3 (67% or more) and more than 80% of housing units are occupied by a single household. Children of the Inga and Yanacona communities resided in the centre and south-east of the city, and children of the Misak, Quechua and Nasa communities in the south-east (districts 3, 9, 11, 15, 18 and 19). The latter reside in areas of the city where there is a higher frequency of single households per housing unit, and the most frequent socioeconomic levels of households in these districts are 1 and 3. On the other hand, the population in these areas most frequently fall in SISBEN category 1, followed in frequency by categories 2 and 3.

Figura 1. Geographical distribution of cases included in the study in the indigenous communities of the city of Santiago de Cali (Colombia)



DISCUSSION

Five minors aged 5 to 7 years had haemoglobin and ferritin values below the threshold established for age by the WHO, and even below the values reported in studies conducted in children in indigenous reserves in Colombia.[12,15] Similarly, we found substantial differences in the development of anaemia in indigenous versus non-indigenous children based on international studies,[16] domestic studies[17] and the reports of the national survey of the nutritional status of children in Colombia (ENSIN 2015),[18] which reported that 7 out of 100 school-age children have chronic malnutrition. In contrast, the prevalence of chronic malnutrition in indigenous children is much higher, as 30 out of 100 have this problem. Along the same lines, the prevalence of parasitic infection varied significantly in the sample under study but was high overall. Similar results have been reported in indigenous children in Colombia[19], Paraguay[20] and Nigeria.[21] In all of these studies, the authors concluded that

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there were no statistically significant differences in the detection of pathogenic parasites based on age or sex. These findings were similar to those of our study, and contrasted with those of a study conducted in an indigenous community in Colombia's Amazon region.[22] The high prevalence of parasitic infection in each age group suggests a lack of knowledge or neglect of teaching basic hygiene to children and assimilating these practices in the culture.

We did not find an association between anaemia and infection by pathogenic parasites, findings that contrasted with those of a study conducted in Turbo in the Antioquia department,[12] which found an association of anaemia and low iron stores with helminthiasis. This association of diseases result in a relatively high frequency of chronic and acute malnutrition in children aged less than 5 or 6 years, who are at higher risk of parasitic infection, a situation that persists after age 6 years due to an inadequate intake of iron and recurrent microbial and parasitic infections that continue to affect their growth, an overall situation that worsens as these collectives migrate from one place to another, facing challenges in finding a spatial and territorial configuration in the receiving region.[21]

The cultural and political perception of these historically oppressed ethnic groups continues to be one of centralised, urban neocolonialism, characterised by the neglect by municipal authorities of the social and health care needs of indigenous peoples in the city.[23] However, it is important to remain aware that anaemia in children does not result exclusively from intestinal parasite infection, but depends on the type of parasite involved, the parasite load, the duration of infection, the body iron stores, dietary iron intake and absorption and the iron requirements of the individual.[24] A low parasite load may cause anaemia in children with a low iron intake with already depleted iron stores.[6] In our study, only one of the participants with moderate helminth infection had haemoglobin and ferritin levels before the lower limit established by the WHO, which is suggestive of recent infection or a high iron intake. The chronic and severe malnutrition found in these children calls for a comprehensive investigation of the dietary intake and anthropometric and metabolic parameters to elucidate the association of factors leading to nutritional deficiencies in the paediatric population.

The last analyses we carried out for this study, the PCA and HCA, provide a new perspective complementing previous studies in Colombian. These approaches were seldom used in the reviewed medical literature. They are statistical techniques that help identify the association between biochemical markers and the haematological parameters and indices under study. We found a similar study conducted outside of Colombia in non-indigenous children aged 1 to 12 years. However, the objective of that study was to assess the correlation between intestinal parasitic infection and social and environmental variables in children,[22] which we did not consider in our study. Gonzáles et al. conducted another exploratory analysis in 2 communities in Peru to establish the characteristics of anaemia in children aged 12 to 59 months.[25] The results showed three main patterns of anaemia, anaemia associated with soil-transmitted helminth infection, which corresponded to the highest prevalences (68.3% to 84.1%), iron-deficiency anaemia and anaemia associated with vitamin B_{12} deficiency. The frequency of the last two types varied between the 2 cities. The multivariate analysis in the study showed the association in the PCA between components derived from age, haemoglobin, haematocrit, MCV, MCH, CMCH, RDW, ferritin and transferrin values. In addition, the HCA allowed grouping individuals based on similarities in the distribution of the different variables. The sample size could be considered one of the weaknesses of our study. However, Formann[13] has proposed a specific subject-to-variables ratio for HCA. According to this expression, the minimum sample size for this type of analysis is twice the number of variables. Thus, based on this, in an analysis of 10 variables, the minimum sample size would be 20, which is far smaller than the sample in our study. Thus, we consider that the two statistical models used in the study are interesting and could be applied in future studies with larger sample sizes to assess not only trends but also statistically significant differences considering not individual variables, but components and the 2 outcome measures (intestinal parasite infection and iron-deficiency anaemia).

Attempting to analyse and discuss the environmental factors that may have contributed to the prevalence of parasitic infection in children based on the spatial distribution of cases and the socioeconomic characteristics of the districts where the children lived may be risky given the diversity found in the different areas of the city of Santiago de Cali and its political-administrative divisions, the *comunas*. Nevertheless, an official report from the city government shows that diseases transmitted in the context of inadequate food handling and deficient hygiene and sanitation are more frequent in poor districts, including the districts where several of the participants in our study lived.[26] We ought to highlight that the Nasa community, one of the most widespread in the city, resides both in areas where there is a good balance between the supply and demand of running water but also in areas where the water supply is deficient.[26] Thus, Nasa children that live in districts 3, 11 and 15 experience poor water quality and a reduced supply due to the methods used for water storage and pumping, and therefore restrictions in the access clean water.[27] This increases the risk of intestinal parasitic infection through deficiencies in the storage of the water supply.[25] In addition, family units of the Misak and Quechua communities in districts 3, 18 and 20 reside in sectors with socioeconomic status modes that range from 1 to 3.[26] Thus, these are families of reduced means, living in tenements, a type of dwelling that is very common in individuals of low socioeconomic status, or even in irregular settlements with dirt floors and walls made of all types of materials, and access to basic services through irregular channels not managed by the municipal administration.[14] These groups are doubly affected by their historical circumstances and their current circumstances in terms of their socioeconomic, ethnic and political status, material resources and the interaction between the city centre and the outskirts.[27]

This is the first study conducted in Colombia in children belonging to indigenous communities settled in the city of Santiago de Cali that assessed the frequency of anaemia, iron deficiency, intestinal parasitic infection and potential interactions of these variables with an observational and analytical design. The design of the study is relevant for the scientific community, as it allows proposing hypothesis regarding the causality of various associated factors, and highlights the need to increase our knowledge by considering variables concerning the household, neighbourhood and community environments, always keeping in mind that variables at each of these levels interact and promote individual, family and community health problems, with a higher frequency in socially vulnerable groups and the paediatric age group, including children and adolescents.[25] Another novelty of our study was that this far, indigenous communities have usually been approached as a homogeneous group without taking into account differences brought by the environment where families reside, which has resulted in the erroneous extrapolation of results, without determining the actual frequency of the event under study, its distribution and the interactions between variables.[27,28] The interdisciplinary makeup of the research group of academics, the participation of last-year students and leaders of the 6 indigenous collectives were an additional strength of the study. The leaders supported the study from the presentation of the project to the cabildos to the field work, including the collection of stool samples for parasite tests and blood samples for performance of blood counts and chemistry tests, which resulted in the development of trust and rapport between indigenous communities and the researchers.

In conclusion, we found a high prevalence of intestinal parasite infection overall, and specifically a high prevalence of mild-to-moderate helminth infection. We found a strong association between red blood cell index values and the levels of proteins directly associated with iron balance in the blood. Social factors such as crowding and socio-economic disadvantage defined based on the SI-BEN classification (1, 2 and 3 for the families of the minors included in the study) may be contributing to this problem.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare in relation to the preparation and publication of this article.

ABBREVIATIONS

EDTA: ethylenediaminetetraacetic acid • **HCA:** hierarchical cluster analysis • **IDA:** iron deficiency anaemia • **MCH**: mean corpuscular haemoglobin • **MCHC:** mean corpuscular haemoglobin concentration • **MCV:** mean corpuscular volume • **PCA:** principal component analysis • **RDW:** red cell distribution width • **SD:** standard deviation • **SISBEN:** Sistema de Identificación de Potenciales Beneficiarios de Programas Sociales (System for the Identification of Potential Beneficiaries of Social Programmes) • **WHO:** World Health Organization.

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