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HELICOBACTER PYLORI SEROPREVALENCE IN PRESCHOOL-AGED CHILDREN BENEFITING FROM EDUCATIONAL AND NUTRITIONAL PROGRAMS IN COSTA RICA (2014-2016)

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Abstract – *Objective:* *Helicobacter pylori* infection is commonly associated with low-income settings and inadequate sanitation. Iron deficiency anemia, although a rare condition, has been linked to the widespread infection of *H. pylori*. This study aims to report the seroprevalence of *H. pylori* among Costa Rican preschool children enrolled in two national nutritional programs: the Education and Nutrition Centers (CEN) and the Children's Centers for Nutrition and Comprehensive Care (CINAI). Furthermore, we will investigate the correlation between *H. pylori* seropositivity and various hematological parameters, as well as specific socioeconomic and environmental factors within this cohort.

Materials and Methods: Based on a previous study, a subsample of 428 children, aged between 1 and 7 years, was selected from 13 Education and Nutrition Centers (CEN-CINAI) located in the Central Southern Region of Costa Rica. Socioeconomic data and baseline laboratory values – including iron indices, hemoglobin, hematocrit, and vitamin D levels – were sourced from the original study by Solano-Barquero et al (2018). In the present study, serum samples were analyzed for IgG antibodies against *H. pylori* using an ELISA.

Results: The overall seroprevalence of *H. pylori* infection was estimated at 15.0%. The infection was more frequent among children without proper sewage or septic tank systems (OR: 3.2, 95% CI: 1.3-8.2) and those living in households with inadequate floor material (OR: 4.0, 95% CI: 1.1-14.7). Co-protozoal infections also represented an additional risk factor (OR: 3.2, 95% CI: 1.5-7.5). Conversely, recent antiparasitic treatment was found to be a protective factor (OR: 0.3, 95% CI: 0.1-0.7). Anemia prevalence was lower in the subsample (4.5%) than in the original study (7.5%) and had no correlation with *H. pylori* infection. Serum iron levels, ferritin, TIBC, and vitamin D had no significant differences between infected and non-infected children.

Conclusions: In this cohort, no correlation was found between *H. pylori* infection and iron deficiency anemia. However, the observed seroprevalence of 15.0% is consistent with rates reported for children under 10 years of age in other Latin American countries. The findings suggest that environmental factors, such as sewage disposal and housing materials, along with co-infections and antiparasitic treatment, play a significant role in the epidemiology of *H. pylori* infection in this population.

Keywords: *Helicobacter pylori*, Preschool children, Seroprevalence, Anemia, Costa Rica.

INTRODUCTION

Helicobacter pylori is a Gram-negative, microaerophilic bacillus estimated to infect up to 50% of the world's population. The bacterium induces chronic gastric inflammation, which can lead to the development of a spectrum of clinically significant diseases, such as duodenal or gastric ulcers, acute and chronic gastritis, gastric cancer (GC), and gastric mucosa-associated lymphoid tissue lymphoma (MALT). If untreated, infection may persist for a lifetime[1,2].

H. pylori infection has been associated with extra-gastric manifestations such as impaired growth, iron deficiency, and iron deficiency anemia (IDA), potentially resulting from increased iron loss or reduced iron absorption. Several studies have reported that children infected with *H. pylori* exhibit lower body iron stores compared to uninfected[3,4].

H. pylori infection may impair iron assimilation and increase iron requirements, likely due to hypoacidity caused by pangastritis and reduced levels of ascorbic acid in the gastric environment, both of which can negatively affect iron absorption in the duodenum. Another plausible mechanism involves elevated levels of lactoferrin in the gastric mucosa, particularly in iron-deficient individuals infected with *H. pylori,* suggesting that increased lactoferrin sequestration may interfere with iron utilization. Finally, some scholars[5] have demonstrated that eradication of *H. pylori* can resolve iron deficiency anemia (IDA) in patients who do not respond to iron supplementation.

Vitamin D deficiency has also been associated with *H. pylori* infection, although findings across studies remain inconsistent. Emerging evidence suggests that vitamin D may play a role in the success of *H. pylori* eradication therapy. Sufficiency levels of this vitamin (above 20 ng/mL) are associated with higher success rates in treatment, while levels below 10 ng/mL are associated with therapy failure[6]. The incorporation of vitamin D3 in conjunction with *H. pylori* eradication therapy has been proposed; however, further research is required to substantiate its efficacy[7]. Moreover, there is a notable scarcity of studies specifically examining this relationship in pediatric populations.

Although the precise mechanisms of *H. pylori* transmission remain elusive and likely encompass multiple pathways, direct person-to-person contact *via* oral-fecal, gastro-oral, and oral-oral routes is regarded as the most likely mode of transmission. A range of factors associated with living conditions, particularly during childhood, have been linked to the infection. These factors include low socioeconomic status, an increased number of siblings, and lower height and weight[8,9]. While *H. pylori* is prevalent worldwide, its infection rates exhibit considerable variation both among and within countries, especially between developed and developing regions, as well as between urban and rural settings. This disparity is primarily attributed to differences in the rates of acquisition during childhood[10].

Seroprevalence of *H. pylori* in children <6 years old without gastrointestinal symptoms was reported to be 20% (95% CI: 14-25%) and 37% (95% CI: 25%-49%) for those 6-11 years old worldwide[10]. In a systematic review of 22 studies, a prevalence of 48.36% in people ≤18 years old in Latin America and the Caribbean between 1987 and 2012 was reported. No significant differences were observed between the different diagnosis tests used (serology and urea breath test)[11]. Another multi-study analysis showed a prevalence of 24.0% in children ≤6 years old and increased to 43.5% in the 13-18 years age group worldwide[12]. In Chile, a study found a prevalence of 20% among children aged 10 or younger, in both high- and low-risk groups for gastric cancer one[13]. Furthermore, in Brazil this prevalence goes up to 28.7% of children without dyspepsia[14]. Other Latin American studies[15,16] often involve children referred to gastrologic services and range from 26% (95%, CI, 20.7%-31.8%) in Buenos Aires, Argentina, up to 72.7% (95%, CI = 72.3-73.0%) in Coahuila, México.

The prevalence of *H. pylori* infection has been declining in most regions, particularly among younger age groups. However, data on age- and sex-specific prevalence remain limited in populations from countries with low or medium Human Development Index (HDI), as defined by the United Nations' four-tier HDI classification[17]. A major challenge is that many current studies depend on symptomatic patient samples and use different diagnostic methods, which hampers standardization and comparability results[13]. In Costa Rica, the most recent report on the prevalence of *H. pylori* in children was published in 2003, based on a sample of pediatric patients with dyspeptic symptoms. Using bacterial culture, the study found a prevalence of 31.8%[18].

This study aims to determine the seroprevalence of *H. pylori* and examine its association with hematological, sociodemographic, and environmental factors among Costa Rican preschool children enrolled in the Education and Nutrition Centers and Children's Centers for Nutrition and Comprehensive Care (CEN-CINAI) nutritional programs.

MATERIALS AND METHODS

Study Population

The study population consisted of children aged 1 to 7 years residing in the South-Central Region of Costa Rica who were enrolled in the Nutrition and Education Centers and Children's Centers for Nutrition and Comprehensive Care (CEN-CINAI). A total of 13 CEN-CINAI centers were randomly selected to conduct a population census. These included: CINAI Aserrí, CINAI Paso Ancho, CEN Río Azul, CINAI Uruca, CINAI La Facio, CEN Pavas, CEN San Miguel, CINAI Gravilias, CINAI Alajuelita, CEN Salitrillos, CINAI Santiago de Puriscal, CEN San Rafael, and CEN San Juan de Tibás. Serum samples were collected between August 2014 and May 2016 and stored at -80°C. For the present study, a randomly selected subsample comprising 50% of the total population (n = 428) was used, without compromising the representativeness of the original sample.

Parasitic infection, Anemia, Iron Indices, and Vitamin D

The information corresponding to these parameters was obtained from the databases of the previous studies conducted by Solano-Barquero et al[19]. Below is a brief description of the methodology employed, and the categorization carried out for each of the variables. Detailed protocols have been described by Solano-Barquero et al[20,21].

Sample Collection and Questionnaire

The questionnaire was completed by each child's parent or guardian and included selected sections from the National Homes Survey Extract (EENH), a condensed version of Costa Rica's National Homes Survey used in 2014 by the National Institute of Statistics and Census (INEC). The questionnaire covered topics such as housing conditions, access to water, household income, education, and health. Definitions of the variables derived from the questionnaire and used in this study are presented in Table 1. To examine associations with parasitic infections and digestive health, respondents were asked about episodes of diarrhea in the past six months and the use of antiparasitic treatment within the past year. These factors were included in the analysis as dichotomous variables. Informed consent was obtained from all participating parents or guardians, in accordance with the protocol approved by the Scientific Ethics Committee of the University of Costa Rica (VI-2884-2014, Resolution 16).

Parasitic infection

Stool samples were analyzed using direct fecal smear examination and the Kato method. The samples were classified as: a) negatives; b) positives for protozoa (positive for commensal or parasitic protozoa), and c) positive for helminthiasis.

TABLE 1. SOCIODEMOGRAPHIC AND ENVIRONMENTAL VARIABLE DEFINITIONS USED FOR THIS STUDY.

Variable	Definition	Possible outcomes
Housing	Characteristics of the infrastructure where the child lives at the moment of the study.	1. Adequate: conventional house, apartment building. 2. Inadequate: Room in shared facilities, indigenous house, slum, mobile home, orphanage, prison, pension. 3. Not specified: information unavailable.
Floor material	Characteristics of floor in the place where the child ives at the moment of the study.	1. Adequate: ceramic, mosaic, concrete, wood. 2. Inadequate: Earthen or dirt. 3. Not specified: information unavailable.
Parental education status	Parents last approved edu cational degree.	1. Primary or lower: Less than 6 years of schooling. 2. Secondary or higher: At least 6 completed years of schooling.
Parental work status	Profitable parental activity performed in the last week	1. Yes: one or both parents performed at least one hour of paid job in the last week. 2. No: none of the parents performed a paid job in the last week.
Water provider	Source of water in the child's house	1. Adequate: potable water from a direct connection to the public water system. 2. Inadequate: water from a river, well or water truck. 3. Not specified: information unavailable.
Sewage system	Implemented strategy for disposal of residual waters where the child lives	1. Adequate: sewage connected to the public system or septic tank. 2. Inadequate: sewage discharged in to water or soil without proper treatment. 3. Not specified: information unavailable.

Anemia

Hemoglobin (Hb) and hematocrit (Hc) determination were performed in blood samples with EDTA (ethylenediaminetetraacetic acid), using the Sysmex TX1800i (Sysmex, Japan). Anemia was defined according to WHO criteria: a) for children from six months to five years old (Hb ≤ 11.0 g/dL and/or Hc ≤ 33.0%), b) for 5 years old and older (Hb ≤ 11.5 g/dL and/or Hc ≤ 34.0%).

Serum Iron Profile

Serum iron (reference interval 50-170 µg/dL), total iron binding capacity (TIBC, reference interval 270-430 µg/dL), and ferritin (reference interval 24-336 ng/dL) were determined from serum samples using the COBAS C600 (Roche, Switzerland) according to the manufacturer's instructions.

Vitamin D

Vitamin D status was assessed through the measurement of 25(OH)D. Serum 25(OH)D concentrations were measured using the ARCHITECT 25-OH Vitamin D assay (Abbott, Chicago, USA), an electrochemiluminescence immunoassay performed on the ARCHITECT Plus i1000 instrument. Vitamin D status was classified according to the Endocrine Society (ES) guidelines: deficiency was defined as a 25(OH)D level below 20 ng/mL, insufficiency as 20–29 ng/mL, and sufficiency as 30–100 ng/mL. Participants with either vitamin D deficiency or insufficiency were considered to have hypovitaminosis D. Serum 25(OH)D levels (reference value ≥20 ng/mL) were measured using the ARCHITECT Plus i1000 analyzer (Abbott, Chicago, USA).

Anti-*Helicobacter pylori* Antibody Detection

Serum samples were stored frozen at -80°C until specific analyses were performed. Anti-*H. pylori* IgG levels were determined using an in-house Enzyme-Linked Immunosorbent Assay (ELISA) method as previously described by Mendez et al[2]. In brief, 45 serum samples were used to stablish an optic density (DO) cut-off value and categorize three groups in positive (95%, 1.49±0.47, CI 1.41-1.57), negative (95%, 0.32±0.26, CI 0.27-0.37) or indetermined (95%, 0.69±0.81 CI 0.11-1.27) and validated with the use of 185 adult serum samples. For this study, only one sample was in the indeterminate range.

Statistical Analysis

Descriptive statistics (mean, rank, standard deviation) were performed for continuous variables. A bivariate analysis between each independent variable and the dependent variable was performed to select the factors included in the logistic regression models. This model was built for *H. pylori* seroprevalence using forward stepwise regression and evaluated using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The association between outcomes and risk factors was estimated using an odds ratio with a 95% confidence interval. All the statistical analysis was performed using Stata version 8.0 (Stata Corporation, 2003, College Station, TX, USA). Statistical analysis was done considering that not all children had a complete dataset. Bivariate analysis for seroprevalence was done with an n=353.

RESULTS

Descriptive statistics for the studied variables are presented in Table 2. Parental education was generally low, with 67.7% of parents having completed only primary education or less, and 37.6% engaged in formal employment. Most children lived in adequate housing (88.5%), with access to proper water supply (90.6%) and sewage systems (82.2%). However, less than half of the children (47.2%) received antiparasitic treatment within the past year. Diarrhea was highly prevalent, with 51.4% of children reporting episodes in the previous six months.

Anti-*H. pylori* IgG antibodies were detected in 67 children, corresponding to a seroprevalence of 15.0%. Anemia, defined according to WHO criteria, was present in 4.9% (n = 21) of the children. Table 3 summarizes values of iron metabolism parameters (serum iron, total iron-binding capacity [TIBC], and ferritin), vitamin D, hemoglobin, and hematocrit stratified by *H. pylori* seropositivity. No significant association was observed between *H. pylori* seropositivity and anemia, with only one child presenting both conditions (OR: 0.30, 95% CI: 0.04-2.55). Similarly, no significant associations or trends were found between *H. pylori* status and serum iron, TIBC, ferritin, or vitamin D levels (all $p \geq 0.05$, Table 3). Specifically, no increased risk of vitamin D deficiency (<20 ng/mL) was detected in seropositive children (OR: 0.58, 95% CI: 0.13-2.57).

Two logistic regression models were conducted to evaluate factors associated with *H. pylori* seropositivity. The first model, which included sociodemographic and environmental variables (Table 4), identified inadequate floor material (OR: 3.99, 95% CI: 1.10-14.69, $p = 0.04$) and poor sewage disposal (OR: 3.32, 95% CI: 1.31-8.43, $p = 0.01$) as significant predictors of infection risk. This model explained 4.37% of the variability in *H. pylori* seropositivity.

The second model (Table 5), focusing on co-infection variables, demonstrated that protozoal infection increased the risk of *H. pylori* infection (OR: 3.00, 95% CI: 1.34-6.68, $p = 0.007$), while recent antiparasitic treatment was negatively associated with infection (OR: 0.34, 95% CI: 0.15-0.76, $p = 0.009$). This model accounted for 7.3% of the variability in *H. pylori* infection.

DISCUSSION

The seroprevalence of *H pylori* infection in this sample of preschool children was 15.0%, with no significant difference observed between children aged ≥5 years and those under 5

TABLE 2. SOCIODEMOGRAPHIC AND ENVIRONMENTAL CHARACTERISTICS OF CHILDREN INVOLVED IN THE STUDY (N= 428).

Study variables	N	%
Sex		
Male	215	50.2
Female	213	49.8
Housing		
Adequate	379	88.5
Inadequate	45	10.5
Not specified	4	0.9
Floor material		
Adequate	387	90.4
Inadequate	17	3.9
Not specified	24	5.6
Parental education		
Primary or lower	290	67.7
Secondary or higher	118	27.6
Not specified	20	4.7
Parental work situation		
Yes	161	37.6
No	244	57.0
Not specified	23	5.4
Water provider		
Adequate	388	90.6
Inadequate	8	1.9
Not specified	32	7.5
Sewage system		
Adequate	352	82.2
Inadequate	29	6.8
Not specified	57	11.0
Antiparasitic treatment in the last year		
Yes	202	47.2
No	202	47.2
Not specified	24	5.6
Diarrhea in the last six months		
Yes	220	51.4
No	183	42.8
Not specified	25	5.8
Anemia		
Yes	21	4.9
No	404	95.1
Stool test	248	
Protozoal infection		
Yes	62	25.0*
No	186	75.0*
Helminth infection		
Yes	5	2.0*
No	243	98.0*
H. pylori* serology*		
Positive	64	15.0
Negative	364	85.0

*Percentage according to stool samples; **Serology status.

years. However, seroprevalence was slightly higher among female children (16.4%) compared to males (13.5%) (OR: 0.83, 95% CI: 0.49-1.40). Globally, the reported prevalence of *H. pylori* infection in high-income countries, as classified by the World Bank, is approximately 21.1% (95% CI: 15.5-28.0), whereas in low- and middle-income countries it nearly doubles to 43.5% (95% CI: 37.5-49.6). A 2022 systematic review reported a prevalence of 24.0% (95% CI: 19.5-29.2) in children aged ≤6 years[12,22]. Most recent studies[23,24] show that

TABLE 3. ASSOCIATION BETWEEN IRON METABOLISM PARAMETERS, VITAMIN D AND *H. PYLORI* SEROPOSITIVE STATUS IN PRESCHOOL POPULATION (N=425).

Variable	Category	*H. pylori* Negative n=361	*H. pylori* Negative $\bar{x}$±SD	*H. pylori* Positive n=64	*H. pylori* Positive $\bar{x}$±SD	OR (CI 95%)	*p*
Haemoglobin (g/dL)	≥11/11.5* <11/11.5*	349 12	12.3±0.70 11.1±0.47	64 0	12.5±0.95 -	0.99 (0.19-4.16)	0.92
Hematocrit (%)	≥33/34%* <33/34%*	341 20	36.8±1.8 32.7±1.1	63 1	36.4±1.6 33.2±0.0	0.54 (0.12-2.37)	0.41
Serum iron (μg/dL)	≥50 <50	318 43	90.2±25.4 38.6±29.2	57 7	93.9±29.0 33.6±28.9	0.91 (0.39-2.12)	0.82
Total iron binding capacity (μg /dL)	≥270 <270	91 270	296.0±22 219.0±33	11 53	291.0±16 220.0±30	1.82 (0.89-3.72)	0.97
Ferritin (μg /L)	≥15 <15	356 5	49.0±25 13.0±2	64 0	48.0±27 -	0.85 (0.81-0.88)	0.34
Vitamin D (ng/mL)	≥20 <20	342 19	30.1±6.1 17.7±1.9	62 2	31.2±7.3 17.1±2.3	0.58 (0.13-2.57)	0.46

*Depending on age according to WHO criteria: for six months to 5 years old: Haemoglobin ≤ 11.0 g/dL and/or Hematocrit ≤ 33.0%, and for 5 years old and older, Haemoglobin ≤11.5 g/dL and/or Hematocrit ≤34.0%.

children between 4-5 years have a prevalence of 26.2% in Nepal (medium Human Developed Income (HDI) and 16.7% in Jordan (high HDI). As mentioned earlier, Latin American countries yield prevalences between 20.0% and up to 40.0% in children ≤10 years old without dyspeptic symptoms[3,11,13,14].

The seroprevalence observed in our study is comparable to the most recent data from high- and middle-income countries, though lower than that reported in other 2005-2020 Latin American studies. Costa Rica joined the Organization for Economic Co-operation and Development (OECD) in 2021[25] and is classified by the World Bank as an upper-middle-income country, with wide access to health services and well-established public health policies[26]. Although, the study period (2014-2016) predates Costa Rica's entry into the OECD, during this time the country was classified as having a High Human Development Index (HDI), positioned at the lower range of the very high HDI category[17].

TABLE 4. BEST REGRESSION MODEL FOR SOCIODEMOGRAPHIC AND ENVIRONMENTAL CHARACTERISTICS OF THE PRESCHOOL POPULATION AND *HELICOBACTER PYLORI* SEROPOSITIVITY (N= 353).

Characteristic	OR	95% CI	*p*
Sex			
Male	Ref	-	
Female	0.97	0.52-1.78	0.91
Age at the blood sampling			
≤5	Ref	-	
>5	1.22	1.10-14.69	0.56
Floor material			
Adequate	Ref	-	
Inadequate	3.99	1.10-14.69	0.04
Water provider			
Adequate	Ref	-	
Inadequate	0.64	0.06-7.35	0.72
Sewage system			
Adequate	Ref	-	
Inadequate	3.32	1.31-8.43	0.01

OR, odds ratio; CI, confidence interval.

TABLE 5. BEST REGRESSION MODEL FOR CO-INFECTION CHARACTERISTICS OF THE PRESCHOOL POPULATION AND *HELICOBACTER PYLORI* SEROPOSITIVITY (N= 232).

Characteristic	OR	95% CI	*p*
Sex			
Male	Ref	-	
Female	1.04	0.48-2.31	0.91
Age at the feces sampling			
≤5	Ref	-	
>5	0.95	0.39-2.32	0.92
Diarrhea in the last six months			
No	Ref	-	
Yes	0.64	0.29-1.43	0.28
Antiparasitic treatment in the last year			
No	Ref	-	
Yes	0.34	0.15-0.76	0.009
Protozoal infection			
Negative	Ref	-	
Positive	3.00	1.34-6.68	0.007

OR, odds ratio; CI, confidence interval

Comparative studies evaluating diagnostic methods for *H. pylori* have shown that serological testing exhibits lower sensitivity compared to the urea breath test and stool antigen assays. Nevertheless, serological testing remains valuable in epidemiological research due to its lower cost, ease of sample collection, and its unique advantage of being unaffected by proton pump inhibitors (PPIs). Furthermore, other diagnostic methods are generally recommended for pediatric patients only when complications are present[27-29].

Anemia remains endemic worldwide. According to the World Health Organization (WHO), in 2011, 43% of children aged 6 to 59 months were anemic, with 42% of these cases attributable to iron deficiency anemia (IDA)[30,31]. A more recent meta-analysis estimated the global pooled prevalence of IDA among children under five years of age to be 16.4% (95% CI: 10.82-22.01)[32] In Costa Rica, the prevalence of anemia among preschool children was reported as 7.6% in 2008-2009[33] and 8.6% among children attending nutrition centers in the South Central Region during 2014-2016. The subsample for the present study was drawn from this latter population[19].

The association of *H. pylori* infection with IDA is somewhat controversial. A 2017 meta-analysis showed an increased likelihood of IDA in *H. pylori*-infected children vs uninfected ones (95%, OR: 1.72, CI: 1.23-2.42), with some of those studies showing up to a two-fold increased IDA in infected children[34]. However, in this study, we found no correlation between anemia and *H. pylori* infection (95%, OR: 0.26, CI: 0.034-1.96).

Taking into consideration possible mechanisms of IDA induction by *H. pylori,* we analyzed serum iron, TIBC, ferritin, and vitamin D, but none of these variables showed an increased risk or statistically significant differences between infected and non-infected children. Actually, there was a tendency for higher mean values in *H. pylori* seropositive children for serum iron, ferritin, and vitamin D, whereas the mean value for TIBC was higher for noninfected children.

Some authors suggest that *H. pylori* infection has a minor association with the development of IDA; however, most infected patients do not develop this condition, possibly due to the absence of gastritis[35]. This is especially relevant in children, where the development of gastroduodenal mucosal lesions due to this infection is rare compared to adults, as is the decreased iron absorption caused by these lesions[36,37]. It has been hypothesized that the main mechanism for iron deficiency in infected children is direct iron competition and sequestration by bacteria in the upper gastrointestinal tract tract[38].

Risk factors associated with *H. pylori* seropositivity identified in this study included inadequate floor material, flush toilets not connected to a proper sewage system, and protozoal co-infections. Conversely, receiving antiparasitic treatment within the past year was found to be a protective factor. According to a housing survey conducted by the Costa Rican Na-

tional Institute of Statistics and Census (INEC), 73% of the country's population resides in urban areas, with only 9.12% of homes failing to meet quality standards. Additionally, 59.9% of houses were classified as good quality, with only minor improvements observed between 2010 and 2017. Access to potable water has significantly improved, increasing from 51% of homes in 1999 to 95.1% in 2014[39]. However, while 97.6% of homes reportedly have proper sewage connections – higher than the 82.2% observed in our study population – only approximately 3% of sewage undergoes treatment before being discharged into the environment[40]. Taking this into consideration, it is reasonable to observe a decreasing pattern in *H. pylori* seroprevalence.

Floor material, parents' education level, and parents' current occupation, flush disposal systems are good socioeconomic indicators and have been negatively associated with *H. pylori* infection in meta-analysis and Latin-American studies[11,13,14]. These indicators commonly differentiate countries and populations based on income levels. We hypothesize that these factors correlate with reduced household overcrowding, decreased interaction with extended family members such as grandparents, and overall improved hygiene and cleanliness.

Co-infection of *H. pylori* and protozoal parasites is prevalent due to shared transmission mechanisms, including gastro-oral and fecal-oral routes, utilized by these pathogens[41]. Although environmental factors, such as contaminated water and food, are plausible sources of infection, the evidence supporting these claims remains limited. While direct mechanisms facilitating the simultaneous establishment of these infections are not well characterized, their consequences (such as host tissue damage, impaired nutrient absorption, malnutrition, and immune modulation) may contribute to an environment conducive to co-infection[42]. Historically, infectious disease research has predominantly focused on identifying individual pathogens, often overlooking interactions among microorganisms. As a result, there is a growing consensus within the scientific community to explore the prevalence of concurrent infections and to identify shared risk factors that may promote the co-occurrence of multiple pathogens within a single host[43,44].

The majority of children in our study population were younger than five years old (71%). Several epidemiological studies have demonstrated that *H. pylori* infection typically occurs in early childhood, with infection rates increasing notably between the ages of five and seven. This period often coincides with children beginning group activities such as attending kindergarten or elementary school, which may facilitate person-to-person transmission and account for the accelerated infection rates observed from childhood into adulthood[12,22,27]. Although 72% of children in our study attended nutrition centers – potentially representing an early-life crowding factor – attendance did not appear to significantly influence *H. pylori* prevalence (95% CI, OR: 1.0; 0.85-1.84). This finding raises new questions about other mechanisms that may contribute to the increased infection rate during childhood.

CONCLUSIONS

This study gives an important insight into *H. pylori* prevalence in Costa Rican preschool children living in low-income urban zones. The observed prevalence was similar to that reported in other Latin American countries, and associated risk factors included poor living conditions and inadequate hygiene—findings consistent with previous regional studies. Co-infection with protozoal parasites was also significantly associated with *H. pylori* infection, likely due to shared transmission routes. However, microbial virulence factors may also contribute to creating a favorable environment for co-infection, while antiparasitic treatment appears to serve as a protective factor. Although previous research has suggested a link between *H. pylori* infection and anemia, iron deficiency, or vitamin D deficiency, our study did not find any significant associations with these conditions.

Ethics Approval

The study was conducted in compliance with the ethical standards of the institutional and national research committees and with the principles outlined by the Committee on Publication Ethics (COPE) and the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of the University of Costa Rica (approval No. VI-2884-2014 - Date of approval: 07/05/2014).

Informed Consent

Written informed consent was obtained from the child's parent or legal guardian prior to participation, in accordance with institutional ethical standards and the Committee on Publication Ethics (COPE) guidelines.

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Authors' Contributions

S.M.C., S.B.M., and V.R.M. had the idea for the manuscript. M.S.B. and M.G.Z. applied the survey, sample collection, parasitological and hematological analyses, the analysis of vitamin D and iron indices, as well as the creation of the databases. E.V.C. helped in the development and performed sample analysis for anti-H. pylori. V.R.M., J.R.C., and S.M.C. did the data analysis and interpretation. J.R.C. drafted the manuscript. All authors made substantial contributions to the conception and critically revised the manuscript for intellectual content. All authors revised the final manuscript and approved the final version.

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Conflict of Interest

The authors declare that they have no conflict of interest to declare.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Use of artificial intelligence

No generative AI or AI-assisted technologies were used during the writing or development of this manuscript.

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