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RESEARCH ARTICLE

Anemia in 3 months to 6 years old Children infected with S. *mansoni* and intestinal Protozoans in the Western region (Tonkpi) of Côte d`Ivoire.

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ABSTRACT

Background: Anaemia is serious public health problem affecting nearly 42% of children less than 5 years of age globally. In Cote d`Ivoire, 72% of the children aged 6-59 months were anemic in 2019. Anemia is defined as a reduction in the hemoglobin level in the peripheral blood below the normal threshold set for a particular population. Very commonly in the subtropics, helminthes, protozoans or malaria co-infects the same person, causing morbidities that vary according to age and region. This research study aims to characterize the type of anemia observed in 6 years old children infected with helminthes and protozoans in the Western part of Côte d`Ivoire, in order to improve the recommended strategy of care.

Methods: The study was carried out from March 2020 to May 2021 in 22 villages of Biankouma and Man, in Tonkpi Region, with a cohort of 451 children, both male and female, aged from 3 months to 6 years. The children provided venous blood samples for the diagnosis and characterization of anemia (full blood count), urines and stool samples were used for the diagnosis of helminthiasis and protozoosis. Univariate analysis (Chi-2 test ($\chi 2$) and P: (Probability) were used for comparison between groups. Significant test was considered at a threshold of 0.05.

Results: Of the 451 children who completed the study, 221 (49.0%) were female and 230 (51.0%) were male. Helminthes infections (230, 51.0%) were more prevalent than the protozoans' infections (40, 8.9%). 50.0% of children infected by intestinal helminthes (*S. mansoni*) were anemics (33.9% mild, 64.4% moderate and 1.7% severe). The characterization of anemia revealed that hypochromic microcytic anemia (HMA) was the predominant type, being found in 87 (74.8%) children. It was followed by normochromic microcytic anemia (12 children, 10.4%), normochromic normocytic anemia (09 children, 7.8%), and, finally, hypochromic normocytic anemia (08 children, 7.0%).

Conclusion: *S. mansoni* infection was highly prevalent among 6 years old children in both sex and in different age groups, although the number of helminthes parasites present during infections was greatest in older children. Similarly, the prevalence of anemia was high, with moderate anemia and HMA being more prevalent in 6 years old children in the western region of Côte d'Ivoire.

Keywords: Prevalence, Characterization of anemia, 6 years old Children, Schistosomiasis, Protozoans, Côte d'Ivoire.

Introduction

Parasites responsible for diseases such as helminthiasis and or protozoosis are very common in the tropical and sub-tropical regions and have always cohabited with the human species for a very long time. However, they are to this day among the main causes of human misery and above all lead to a high morbidity and mortality, especially in children [1].

Helminthiasis alters the nutritional status of those affected, causing intestinal bleeding, loss of appetite, diarrhea and reduced micronutrient absorption. These infections are widespread in tropical and subtropical regions, with the greatest number of cases observed in sub-Saharan Africa, the Americas, China and East Asia, where there is a lack of adequate drinking water supplies and sanitary facilities [2-3]. Globally, schistosomiasis affects at least 236.6 million people of which approximately 800 million are at risk of infection in 78 countries [4-5].

As for protozooses, they are associated with diarrhea and dysentery [6]. According to the WHO, the prevalence of intestinal protozooses is high worldwide, with over 3 billion people infected, including 450 million in developing countries [7].

A common feature of helminthiasis and intestinal protozoan infections is that the causes are multifactorial, such as conditions of poverty, poor hygiene, lack of drinking water and sanitation [5]. One of the major consequences of all these infestations is anemia.

In Côte d'Ivoire, the prevalence of intestinal schistomiasis (*S. mansoni*) in 2016 was estimated at more than 22.1% and 23.2% in school-aged children and adults population respectively [8- 9] and that of anemia at 55.0% [10].

In most cases, the poly-parasitism is the rule and these conditions affect the same people, causing morbidities that vary according to age and region [11] (Darlan *et al.,* 2018). All of these conditions unfortunately alter the nutritional status of those affected by causing intestinal bleeding, loss of appetite, diarrhea and/or reduction in the absorption of micronutrients [4-5]. Children are the group most at risk with high morbidity and mortality. In addition, these diseases can lead to anemia, malnutrition, reduced aerobic capacity, growth retardation, cognitive and memory impairment [2]. Therefore, helminthes, protozoans, and soil-transmitted helminthiasis are a major public health problem, especially for school and pre-school children in sub-Saharan Africa where their occurrence as multi-species infections is known to be the norm [3, 12]. It seems therefore, appropriate, even essential to understand the epidemiology of these infections in school and preschool children and their joint contribution to lower the hemoglobin level and cause anemia.

This community-based research study aims to characterize the type of anaemia observed in 6 years old children infected with helminths and protozoans in two (2) departments of the "Tonpki Region" (Biankouma and Man), in the Western part of Côte d`Ivoire, in order to support the design of an integrated strategy for the control of this neglected diseases and recommend a better strategy of care.

Material and Methods

- **Study area and participants**

This observational clinical research study was carried out from March 2020 to May 2021 in 22 villages in Biankouma and Man, Tonkpi Region.

The sample size of 451 was obtained from Schwartz's formula: $n = z^2 \frac{p(1-p)}{e^2} * c$ Where: P is the expected prevalence of anemia in the target population estimated at 73.4% [13]; E an accuracy of 5%; C a corrective coefficient for the cluster effect of 1.5 and Z a confidence level of 95% (Z = 1.96).

Children aged 3 months to 6 years were enrolled and provided blood samples for the diagnosis of anemia and both stool and urine for diagnosis of intestinal schistosomiasis and protozoosis. All study participants belong to a variety of ethnic groups (Kwa, Gour, Mande, Krou and others from neighbouring countries such as Burkina Faso, Mali and Guinea) and reside in rural area with similar life style patterns and their parents earn their living either mostly as farmers or commercial traders.

- **Socio-demographic**

Socio-demographic data including age, sex, height (to the nearest centimeter), weight (to the nearest 0.5 kg) and parent's profession were also collected through a questionnaire administered to each enrolled participant who provided a signed written informed consent or gave a fingerprint (illiterates).

- **Blood collection**

A total of 2 ml of venous blood was drawn in the morning into ethylenediaminetetraacetic acid (EDTA)-treated evacuated tubes from each of the 451 consented study participants. Blood samples were kept on ice until they were transported to the central laboratory at the Centre Hospitalier Regional (CHR), Man, for the hematology testing [i.e., full blood count (FBC)].

- **Hemoglobin determination and classification of anemia**

The FBC was measured using the URIT 3000 PLUS analyzer (URIT Medical Electronic Co. Ltd, Guilin, China). The results were printed and analyzed further, and characterized on the basis of the mean corpuscular hemoglobin (MCH) (normal value 27–31 pg) and the mean corpuscular hemoglobin concentration (MCHC) (normal value 33–36 g/dl) as either normochromic or hypochromic, and on the basis of the mean cell volume (MCV) (normal value 80–100 fl) as normocytic, microcytic, or macrocytic.

Anemia was classified, depending on age and sex, according to hemoglobin (Hb) concentration, as per WHO guidelines [14]. Thus, children aged from 3 months to 6 years with a Hb level of< 11 g/dl were considered anemic, and those with a Hb level of ≥ 11 g/dl were considered normal (non-anemic). Anemia was also categorized as mild if the Hb level was< 10 g/dl, as moderate if Hb level was between 7 g/dl and 10 g/dl, and as severe if the Hb level was < 7 g/dl. The samples were further characterized based on the MCH and MCHC as either normochromic or hypochromic, and based on MCV as being normocytic, microcytic, or macrocytic.

- **Microscopy of the stool and urine for the diagnostic of *S. mansoni* and *S. hematobium***

Collected faecal (stool) and urine samples were transferred to the central laboratory of the Regional Health Center of Man (CHR Man) and worked upon the day of collection. For quality control, 10% of the Kato-Katz thick smears and the urine filtered slides were re-examined by senior technicians. In case of disagreement, the results were discussed with the concerned technician and discordant slides re-read until agreement was reached.

- **Stools microscopy (Kato-Katz test)**

For the diagnosis of *S. mansoni* and soil-transmitted helminthes, 2 stool specimens weighing 1-2 g each was collected in a 5-10mL capacity stool collection tube for each participant within three (3) days. Briefly, a duplicate Kato-Katz thick smear of approximately 41.7 mg was prepared from each collected stool sample as per Kato-Katz method [15]. The thick smears were allowed to clear for at least for 30 - 45 minutes and examined using light microscopy by experienced laboratory technicians for the presence of ova of *S. mansoni* and soil transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*). The intensity of the infection expressed in number of eggs per gram of stool (EPG) were counted and recorded for each species and individual separately. The arithmetic mean faecal egg count was determined for specimens with two positive slides. If there was only one positive slide in the sample, the single EPG value was used in the analysis. Diagnosis of positive *S. mansoni* was defined as the presence of 1 egg of *S. mansoni* in a stool count and categorized according to the WHO classification: mild (1-99 eggs per gram feces), moderate (100-399 eggs per gram of feces) and heavy/severe (≥ 400 eggs per gram of feces) [16].

- **Stools microscopy (Ritchie test)**

Furthermore, the remaining stool samples were subjected to the Ritchie's concentration method [17] for the presence of intestinal protozoan trophozoites and cysts in formaldehyde and ether solution. Briefly, 1 to 2 g stool sample was mixed in 10 mL of Ritchie's reagent (100 mL of formalin, 9g of NaCl, 900 mL of distilled water) and left to sediment for a few seconds. The mixture was then transferred to a centrifuge tube where ether was added (1/3 of ether for 2/3 of the mixture). Thereafter, the tube was capped and mixed by inversion for 30 seconds and centrifuged for 2 min at 1500 rpm. Finally, the centrifugation pellet was spread with an applicator stick and directly examined under a light microscopy with microscope slide, coverslip and oil immersion at x10 and x100 magnification. The exact number of eggs of each helminth species and the protozoan trophozoites and cysts was recorded. Protozoan parasite load or intensity was categorized as described by Utzinger *et al.*, as low infestation or "1^{+}" (corresponds to 1-5 parasites per microscopic slide analyzed), moderate infestation or "2^{+}" (1 parasite per field of view) and heavy infestation or "3^{+}" (more than 1 parasite per field of view) [18].

- **Urine filtration and microscopy for the diagnostic of *S. haematobium***

At least 10ml of urine sample were collected in a 10-15ml capacity urine collection tube only once for each participant after signing the consent form. Briefly, urine samples were vigorously shaken and 10 ml were pressed through a small-meshed filter (aperture: 30 µm) and a drop of Lugol's solution was added onto the filter paper and then examined under a microscope for the presence *S. haematobium* eggs as per Plouvier method [19]. The number of eggs was counted and recorded. The parasite load or intensity was defined according to WHO recommendation as light (if 1 - 49 eggs /10ml urine) or severe (≥ 50/eggs 10ml urine) [16].

- **Data management and analysis**

Data were entered into a database using the double-entry system in Epi-data version 3.1 (EpiData, Odense Denmark, 2004). Inconsistencies were cleaned and, after validation, the data were

exported for the analysis to SPSS version 20 (IBM Corp; 2011) and STATA 16 software (STATA Corporation, College Station, Texas, USA). Univariate analysis (Chi-2 test ($\chi 2$) and P: (Probability) were used for comparison between groups. Significant test was considered at a threshold of 0.05.

- **Ethical consideration**

This study was conducted after obtaining an ethical clearance from the "Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS) de la Côte d'Ivoire" (N/Ref : 024-21/MSHP/CNESVS-km). Further permission to conduct the study was obtained from each visited village chief`s. In essence, the full study details i.e. the aims, the procedures, the potential risks and the benefits were explained to the physicians, nurses, and assistant nurses of each involved Health Center and the villagers before the start of the study.

Since all the participants were minors, consent from one of the parent was sought and obtained before the study procedure was completed. Only voluntary consented participants were included in the study. Treatment was made available free of charge to all sick participants. Those who were diagnosed with a mild or moderate anemia with or without intestinal or urinanry schistosomiasis infection received an ambulatory treatment of antihelminthic (praziquantel at 40 mg/kg) and or anti anemic treatment as per their respective haemoglobin (Hb) level (ferric hydroxide polymatose complex sirups). Those who required further assistance were referred to the local health center for assistance.

Participants were further informed that their information will be anonymized (confidential) using a coding system instead of their names.

Results

- **Characteristics of the study populations**

A total of 451 children from 2 departments (Biankouma and Man) of the "Tonkpi region" composed of 22 villages (**Table I**) were enrolled in the study. Among them, 230 (51.0%) children were males and 221 (49.0%) children were females (**Table I**).

Table I : Distribution of the sampled population in the departments of Biankouma and Man, in Western Côte d'Ivoire.

Department	Sous-Préfecture	Villages	Male	Female	Participants n (%)
BIANKOUMA	Blapleu	Blapleu	20 (62,5)	12 (37,5)	32 (7,1)
	Biankouma	Kabakouma	19 (73,1)	7 (26,9)	26 (5,8)
	Kpata	Kpata	9 (39,1)	14 (60,9)	23 (5,1)
MAN	Gbangbégouiné-Yati	Bogouiné 1	13 (61.9)	8 (38.10)	21(4,7)
		Biakalé	7 (36,8)	12 (63,2)	19 (4,2)
		Kouitongouiné 2	32 (50,8)	31 (49,2)	63 (14,0)
		Douele Dimba	18 (41,9)	25 (58,1)	43 (9,5)
		Tiakeupleu	15 (45,5)	18 (54,5)	33 (7,3)
		Gouimpleu 2	11 (44,0)	14 (56,0)	25 (5,5)
		Gbangbegouiné Zélé	3 (60,0)	2 (40,0)	5 (1,1)
		Guiapleu	31 (45.6)	37 (54,4)	68 (15,1)
	Man	Botongouiné	5 (50,0)	5 (50,0)	10 (2,2)
		Guianle	7 (87,5)	1 (12,5)	8 (1,8)
		Bantegouin	8 (50,0)	8 (50,0)	16 (3,5)
		Kiélé	2 (40,0)	3 (60,0)	5 (1,1)
		Lamapleu	15 (51,7)	14 (48,3)	29 (6,4)
		Plouba	3 (60,0)	2 (4,0)	5 (1,1)
	Sangouiné	Bloleu	1 (100)	0	1 (0,2)
		Zoba	3 (75,0)	1 (25,0)	4 (0,9)
		Tiapleu	1 (50,0)	1 (50,0)	2 (0,4)
		Kpanzaopleu	1 (100)	0	01 (02)
		Guezon	6 (50,0)	6 (50,0)	12 (2,7)
Total	**6**	**22**	**230 (51,0)**	**221 (49,0)**	**451 (100)**

- **Prevalence of *S. mansoni* infected participants**

Out of the 451 children enrolled in the study, 230 (51.0%) of them were infected with *S. mansoni* (positive) following the Kato-Katz method testing. Amongst them, 45.2% were females and 54.8% males (**Table II**). Furthermore, in terms of infection intensity, 116 (50.4%) of the participants had mild, 59 (25.7%) moderate and 55 (23.9%) severe *S. mansoni* infection (data not shown). It was also noted that *S. mansoni* infection varies by age groups with the infants aged less than 6 months (0.0%) and younger children aged 6 months to 23 months (12.8%) being at a lower risk of *S. mansoni* infection compared to the older children (2 years to 6 years (60.0%) (**Table II**). In terms of parasitemia, the males and older children had the highest average 218.0 epg and 218.3 epg respectively (**Table II**).

Table II: Prevalence and intensity of *Schistosoma mansoni* infection by sex and age in the departments of Biankouma and Man, in Western Côte d'Ivoire.

	Examined*	**Infected**	**% (IC at 95 %)**	**χ2**	**P-value**	**DPM (IC at 95 %)**	**P-value**
Sex							
Male	230	126	54,8 (48,1-61,3)			218,0 (110,7-238,9)	
Female	221	104	45,2 (40,3-53,9)	2,69	0,101	136,8 (125,2-238,1)	0,060
Age Group							
< 6 M	6	0	0,0 (-)			0,0 (-)	
[6-23 M]	78	10	12,8 (6,3-22,3)			3,2 (0,6 - 5,8)	
[2-6 years]	367	220	60,0 (54,7-65,0)	63,49	**<0,001**	218.3 (166,7- 269,9)	**<0,001**
Total	451	230	**51.0 (46.3-55.7)**			178,2 (135,6 -220,9)	

* Kato-Katz, DPM = Mean Parasite Density (arithmetic mean), M = Month, χ2: Chi 2 test, P: Probability, %: Prevalence, CI: Confidence Interval.

- **Distribution of the type of anemia according to sex and age**

Males and females are almost equally affected (52.0% vs. 48.0%) with the moderate anemia having the highest prevalence (66.1%) followed by mild anemia (30.7%). In terms, of age group, infected infants aged less than 6 months showed the highest prevalence (100%) compared to older children aged 2 to 6 years (50.7%) (**Table III**). Overall, 248 (55.0%) children were anemic while 203 (45.0%) were not.

Table III: Prevalence and intensity of anemia by sex and age in the departments of Biankouma and Man, in Western Côte d'Ivoire.

	Testés	**Anemics**	**% (IC at 95 %)**	**χ2**	**P-value**	**Intensity of anemia**		
						Mild	**Moderate**	**Severe**
Sex								
Male	230	129	52,0 (49,4-62,6)			34 (26,4)	93 (72,1)	2 (1,6)
Female	221	119	48,0 (47,0-60,6)	0,23	0,633	42 (35,3)	71 (59,7)	6 (5,0)
Age Group								
< 6 M	6	6	100 (54,7-100)			2 (33,3)	4 (66,7)	0 (0,0)
[6-23 M]	78	56	71,8 (60,5-81,4)			10 (17,9)	41 (73,2)	5 (8,9)
[2-6 years]	367	186	50,7 (45,4-59,9)	16,56	**<0,001**	64 (34,4)	119 (64,0)	3 (1,6)
Total	451	248	**55,0 (50,3-59,6)**			76 (30,7)	164 (66,1)	8 (3,2)

*M: Month, χ2: Chi-2 test, P: Probability, %: Prevalence, CI: Confidence Interval, *Test significant at the 0.05 threshold, Mild (10 - <11 g/dL), Moderate (7 -10 g/dL), Severe (<7 g/dL).*

- **Prevalence of anemic participants with *S. mansoni* and protozoans infection**

Out of the 451 children enrolled in the study, a total of 248 (55.0%) children were anemic and, 230 (51.0%) were positive for *S mansoni* following the Kato-Katz testing method. In addition, Kato-Katz testing method identified 3 *Enterobius vermicularis*, 1 *Hymenolepis nana and* 2 Candidas sp. while the Ritchie testing method identified 40 types of intestinal protozoans (**Table IV**). And so, 115 (50.0%) children infected with S. *mansoni* (n = 230) and 25 (62.5%) children infected with intestinal protozoans (n = 40) were anemic. A characterization of the observed anemia and their severity showed that 40 (34.5%) had a mild anemia, 74 (63.8%) had a moderate anemia while 2 (1.7%) had a severe anemia (**Table V**). Among those 115 anemic children, the high majority of them *i.e.* 86 (74.8%) had a hypochromic microcytic anemia, 12 (10.4%) normochromic microcytic anemia, 9 (7.8%) normochromic normocytic anemia and 8 (7.0%) normocytic hypochromic anemia (**Table V**).

Table IV: Prevalence (%) of parasitic species observed in children aged 3 months - 6 years, in the two departments of Biankouma and Man, in Western Côte d'Ivoire.

	Infected (N=451)	**% (IC at 95 %)**
Helminths		
Schistosoma mansoni	230	51.0
Enterobius vermicularis	3	0.7
Hymenolepis nana	1	0.2
Protozoans		
Entamoeba coli	9	2
Entamoeba histolytica/dispar	4	0.8
Endolimax nana	3	0.7
Giardia lamblia	16	3.5
Blastocystis hominis	2	0.4
Coinfection (*E. coli* & *E. hytolitica*)	1	0.2
Coinfection (*E. coli* & *E. nana*)	2	0.4
Coinfection (*E. coli* & *G. lamblia*)	2	0.4
Coinfection (*E. coli* & *E. nana* & *G. Lamblia*)	1	0.2
Other parasites		
Candidas sp.	2	0.4

Table V: Prevalence of anemia and the type of anemia observed in children aged 3 months - 6 years infected with intestinal helminthes and or protozoans in the departments of Biankouma and Man, in Western Côte d'Ivoire.

Type of infection	**Severity of anemia**	**Type of anemia (n, %)**			
		Hypochromic microcytic anemia	Normochromic microcytic anemia	Normochromic normocytic anemia	Normocytic hypochromic anemia
***S. mansoni* (N = 230)**	**Mild**	28 (12.2)	6 (2.6)	5 (2.1)	0
	Moderate	56 (24.3)	6 (2.6)	4 (1.7)	8 (3.4)
	Severe	2 (0.8)	0	0	0
	Total	**86 (74.8)**	**12 (10.4)**	**9 (7.8)**	**8 (7.0)**
Protozoans (N = 40)	**Mild**	4 (10.0)	1 (2.5)	0	0
	Moderate	14 (35.0)	4 (10.0)	1 (2.5)	1 (2.5)
	Severe	0	0	0	0
	Total	**18 (72.0)**	**5 (20.0)**	**1 (4.0)**	**1 (4.0)**
Other infestations* (N = 6)	**Mild**	2 (33.3)	0	1 (16.6)	1 (16.6)
	Moderate	0	0	0	0
	Severe	0	0	0	0
	Total	**2 (50.0)**	**0**	**1 (25.0)**	**1 (25.0)**

*(Cestodosis, Candidiasis, Oxyuriasis)

Similarly, 5 (12.5%) of the 40 children infected with intestinal protozoans had a mild, 20 (50.0%) moderate and, 0% severe anemia. The characterization of the observed anemia showed that 18 (72.0%) of the 25 participants who were anemic had a hypochromic microcytic anemia, 5(20.0%) a normochromic microcytic anemia, 1(4.0%) normochromic normocytic anemia and 1(4.0%) normocytic hypochromic anemia (**Table V**). Lastly, other infestations (n = 4) made of candidiasis, cestodosis and oxyuriasis displayed a mild hypochromic microcytic anemia (50%) (**Table V**).

Discussion

The most prevalent helminthiasis was intestinal bilharzia (*S. mansoni* infestation) with a prevalence of 51.0%, of which half was anemic (50.0%). This prevalence is higher than those reported in the same region by Assare et al[8] to be at 22.1% even though some schools had 54% prevalence or the study of N'Zi et al reported[20] in Biankouma with a prevalence of 31.4%. In those studies, male children were the most infected, as in the present study (male: 24.3%; 53.06 % *vs.* 54.8% and female: 18.7%; 46.94 % *vs.* 45.2% respectively). *S. mansoni* infection was significantly associated with age ($\chi^2 = 63.49$; $p < 0.001$). This observation is consistent with studies by [21] in the Taï region and [22] in Biankouma, which showed low prevalence of 5.0% and 35.5% respectively with *S. mansoni* infection but correlated with male sex and advanced age. This observation is also shared in Agersew and co-worker`s study in Ethiopia [23]. Indeed, boys and girls do not generally share the risk in the same way, the former being significantly more infected than the latter. In our study, male and female prevalence were 54.8% and 45.2% respectively. This could be explained by the fact that girls are more likely than boys to be present at freshwater in the morning for predominantly domestic contacts (washing, swimming, other domestic usages). Boys, on the other hand, are mainly present from mid-day to evening for handy craft and leisure activities (fishing, swimming, accompanying their parents for market gardening, etc.) at the times of cercarial emissions of *S. mansoni* (i.e. close and repeated contact with water) [24-25]. Thus, the work of Mujumbusi et al[24] and Arinaitwe et al[25], which asserts that interactions with freshwater and swamps expose people to a high risk of catching *S. mansoni*, confirms the correlation between *S. mansoni* and male sex. Concerning the correlation between *S. mansoni* and advanced age, several studies have shown that adolescents always appear to be more parasitized than young children, as was the case in our study with 0% in children under 6 months, 12.8% in children aged [6-23 months] and 60% in children aged [2-6 years]. The work of N'Zi et al[20] also showed that children aged 2-6 years were more heavily infected with *S. mansoni* than those aged 1-2 years (OR = 14.24, 95% CI: 5.85-34.64). Likewise, the study by Kinung'hi et al[26] in Tanzania, which showed that older children (53.6%) were more infected than younger ones (20.1%) ($p < 0.001$). In our study, this can first be explained by the fact that the first two age categories of children (under 6 months and 6-23 months) have less frequent contact with water sources, unlike the oldest children [2-6 years], who are more frequent in the stream of water. And secondly, by a behavioral factors that have been shown to influence individual risk, particularly swimming, bathing, and washing clothes in open water sources. Indeed, some mothers during their swimming or bathing, or clothes washing time collect water in bucket and seat their infant less than 24 months in or at time seat the 1-2 years old near the shore which may have increased their infection risk [20, 27-28].

No soil-transmitted helminth (STH) were detected in our study population, even though under generally some positive cases have previously been reported in the community of the same study area [8, 22-21]. This absence may be explained by the fact that the Ministry of Health and Hygiene (MSHP) distributed Vitamin A and antihelminthic (Albendazole) via a national campaign from January 22 to 25, 2021, just one month before our data collection [29]. In Côte d'Ivoire, similar mass treatments gave very good results, with 96.1% efficacy in the same region (Adoubryn *et al.*, 2012).

When it comes to helminth control in pre-school children, the benefits of deworming are increasingly being reported. Elsewhere, in Ethiopia, Adugna et al reported[30] an 83.9% cure rate. Similar studies in Zanzibar indicate that antihelminthic treatment significantly reduced the prevalence of anemia and stunting in preschool children who received mebendazole every three months for one year [31]. Thus, based on the available evidence on treatment efficacy and the safety of providing young children with treatment, the WHO recommends treatment frequency and coverage levels as dependent on the prevalence of infection in a defined location. For this, current treatments are done once a year in high-prevalence settings (≥ 50%), once every 2 years in moderate-prevalence settings (10-50%) and once every 3 years in low-prevalence settings (<10%) [32].

Studies carried out in Africa and elsewhere on populations of similar age have shown the following

prevalences: in Ethiopia, 24.6% of *S. mansoni* and 15.4% of anemic [33]; in Uganda 71.0% *S. mansoni* infection and anemia not evaluated [24], and in India reaching 33.9% *S. mansoni* and anemia at 31.7% [34] (Al-Haidari *et al.,* 2021).

It was also noted, when analyzing the results of the prevalence and intensity of anemia in our study, that male participants (52.0%) were slightly more affected than female ones (48.0%), as well as the age category [2-6 years] compared to those under 6 months of age (50.7% *versus* 100%). The high rate of anaemia observed in children under 6 months of age, who were supposed to be more or less exclusively breastfed, could be attributed to the impact of their parents` precarious living conditions, particularly the mothers (from pregnancy through to the breastfeeding period). Indeed, studies carried out in India by Balarajan et al[35] and in Ethiopia by Vivek et al[36] showed that pregnant women from lower socio-economic classes were at greater risk of developing anemia than those from higher socio-economic classes. And so, the fact that pregnant women of lower socio-economic status would not have the ability to purchase the quality or quantity of food compared to those of higher socio-economic status exposed both the pregnant mother and her fetus [35-36]. Furthermore, maternal anaemia is strongly associated with infant anaemia, as malnourished pregnant women are more likely to be micronutrient and iron deficient [35]. Consequently, they would not be well nourished in order to maintain a good health and be able to cover the micronutrient and iron needs of their baby during the last trimester of pregnancy [37]. Indeed, the diet of lactating women should be balanced and show a moderate consumption of foods with a high heme iron content (meat, fish, eggs and dairy products) combined with a higher consumption of cereals, fats and vegetables in order to meet the iron needs of their children [38]. These impoverish conditions were observed in our study area. Several studies have confirmed the association of anemia with demographic, socio-economic and cultural factors [39-41]. Thus, the anemia observed in our study in children under 6 months of age could be explained by the fact that these children received an insufficient quantity of iron and micronutrients at birth (reserves), which would have been depleted before their first 6 months of life, thus confirming their anemic state.

The highest intensity was moderate anemia (66.1%), followed by mild anemia (30.7%), which was most pronounced in participants aged [6-23 months; 73.2%], followed by under 6 months; 66.7% and [2-6 years; 64.0%]. Finally, hypochromic microcytic anemia (HMA) (78.6%) was the most frequent ([2-6 years]; 55.6%], [6-23 months]; 21.0%]), followed by normochromic microcytic anemia (NMA) (11.7%). Similar anemia studies conducted in Cameroon by Koum *et al.,* (2013) [42] showed that 57.7% of boys versus 42.3% of girls were anemic with 87.3% in the age category [6-59 months]. Also, moderate anemia was the most frequent (67.1%) although it was followed by the severe form (27.6%). Finally, the hypochromic microcytic type was the most frequent (48.5%) followed by the normocytic normochromic type (22.6%) [42]. In addition, the study by Adebo and Yessoufou, (2018) in Benin [43], showed more cases of anemia in boys than in girls, with 68.83% of anemic children belonging to the age category under 24 months. A further 30% had severe anemia, followed by 56.47% with mild anemia. The most frequent type of anemia was hypochromic microcytic anemia (72.94%) followed by normochromic microcytic anemia (58.83%) [43].

The high frequency (71.8%) of hypochromic microcytic type anemia observed in participants aged [6-23 months] may be explained by an anomaly in hemoglobin synthesis in erythroblasts, due to the unavailability of iron for hemoglobinosynthesis (iron deficiency anemia) [44]. In fact, iron requirements are very high in young children, particularly those aged between 6 and 18 months. And so, once the birth iron stores are depleted, children`s iron status depends on complementary foods; unfortunately, in developing countries, traditional complementary foods are poor sources of bioavailable iron. As a result, children aged between 6 and 18 months are frequently deficient in this mineral [45]. Also, the majority of children surveyed were infected with parasites such as protozoans, intestinal helminths, etc., which can cause iron malabsorption in their bodies.

In the [2-6 years] age group, the prevalence of anemia was the lowest (50.7%) in the study. The most frequent intensity was moderate anemia (64.4%), followed by mild (34.4%) and severe (1.6%), with microcytic hypochromic anemia at a frequency of 55.6%. The observation of this anemia can be explained by the fact that at this age [2-6 years], children are most of the time walking or playing in various external environments where there are supplementary foods such as nuts and seeds, fruits and extra vegetables to grab in addition to the family food shared together usually [46]. These additional micronutrients would provide them with the iron balance needed to reduce their absolute iron deficiency. In fact, according to Gupta et al[47] and the PCRM guideline[48], iron deficiency anemia is generally preventable and highly

treatable with consumption of an iron-fortified diet or whole grains, vegetables, nuts and seeds, and fruits and vegetables.

It was also observed that the severity of the anemia decreased as the age of the participants increased [6-23 months; 5%] and [2-6 years; 3%]. This could be explained by the implementation of the National Multi-sectoral Nutrition Plan 2016 - 2020, which consisted in "guaranteeing the entire population an optimal nutritional status in order to improve its well-being and sustainably support inclusive growth and the country`s development". This program was an integrated fight against micronutrient deficiencies involving the fortification and fortification of widely consumed staple foods, micronutrient supplementation for vulnerable populations, nutrition education and the strengthening of nutrition-related health programs [29]. This involved the distribution of deworming medicines, vitamins and mineral supplements to adolescent children under 5 and pregnant women. Iron and folic acid supplements for teenage girls; vitamin A and zinc supplements for children aged 6 to 59 months. Finally, ensured 4 balanced meals per week at the level of each school canteen and the supply of health, social and community structures with nutritional inputs by the State of Côte d`Ivoire [29].

Our study has some limitations. Firstly, the small size of the children less than 6 months and or 6 to 23 months. Secondly, we did not measure serum ferritin levels to assess iron status or reticulocyte counts for confirming if the anemia is regenerative or not. Thirdly, we did not explore the eating habits and the nutritional deficiencies which represent the most frequent causes of anemia.

Conclusion

Based on our (a) Parasitological results the most common infestations found in the study area were the intestinal helminth, *Schistosoma mansoni* (51.0%) and various intestinal protozoan (8.9%). It is also important to note that no cases of *Schistosoma haematobium* were detected in this study. Other intestinal helminth and protozoa species were found at prevalences of less than 1.0%. And (b) Biological results, 55.0% of participants were anemic. The characterization of these anemia cases revealed four (4) types of anemia: hypochromic microcytic anemia (HMA) with a prevalence of 78.6%, followed by normochromic microcytic anemia (NMA) (11.7%), hypochromic normocytic anemia (HNA) (4.0%) and normochromic normocytic anemia (NNA) (5.7%). In terms of intensity, the moderate anemia was the most frequent (66.1%), followed by the mild anemia (30.7%) and then the severe anemia (3.2%). No macrocytic anemia was also observed. Because 100% of infants less than 6 months were anemic, it is therefore extremely important to continue through effective efforts and improve the general health of the high-risk population viz. the pregnant mothers that are in most needed.

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Conflict of interest statement

The authors have declared that no competing interests exist.

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