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Original Article

# Prevalence of anemia in pre-school tribal children with reference to parasitic infections and nutritional impact



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#### لملخص

أهداف البحث: فقر الدم هو مشكلة صحية عالمية وله معدل انتشار مرتفع الغاية في البلدان النامية والمتقدمة على حد سواء، وخاصة بين الأطفال والنساء. تقيم الدراسة الحالية مؤشرات أمراض الدم ونقص التغذية والالتهابات الطفيلية وارتباطها بانتشار فقر الدم يساعد هذا التحليل في تحديد حالة فقر الدم لدى أطفال ما قبل المدرسة في المناطق القبلية.

طرق البحث: أجريت دراسة مقطعية لدى 300 طفل في عمر 6 أشهر إلى 5 سنوات في قرية سانترامبور، غوجارات تم جمع الدم وإجراء تعداد الدم الكامل و تحليل إليزا (المقايسة المناعية المرتبطة بالإنزيم) لتقدير الفيريتين، الترانسفيرين، مستقبل الترانسفيرين القابل للذوبان، فيتامين ب12، وفيتامين ب9 (الفولات). تم جمع عينات البراز وتقييمها من أجل بكتيريا "المتحولة الحالة للنسج" و"الجياردية اللمبلية"، و"خفية الأبواغ" بواسطة طريقة إليزا. كما تم فحص الملاريا عن طريق الفحص المجهري.

النتائج: من بين 300 طفل، كان 87.7٪ من الأطفال مصابين بفقر الدم. كان 239 طفلا يعانون من فقر الدم الخفيف، بينما كان 20 منهم يعانون من فقر الدم المتوسط و 4 أطفال يعانون من فقر الدم الشديد. كان متوسط الهيمو غلوبين 9.49  $\pm 1.47$  جم / ديسيلتر حيث كان للذكور والإناث 9.39  $\pm 1.47$  جم / ديسيلتر و 4.58  $\pm 1.34$  جم / ديسيلتر، على التوالي. ومن بين هؤلاء، كان 26 طفلا مصابين بفقر الدم المنجلي و 5 أطفال مصابين بالثلاسيميا. أكثر من 50٪ من الأطفال يعانون من نقص فيتامين ب 12 و 19 إلى جانب 16٪ لديهم خلل في

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بروتين سي التفاعلي. العدوى الطفيلية لـ"خفية الأبواغ" ارتبطت بشكل أكثر إيجابية بفقر الدم يليه انتشار "الجياردية اللمبلية" و"المنحولة الحالة للنسج".

الاستنتاجات: يوصى بنشر الوعي حول تحسين المرافق الصحية وتقديم المشورة الغذائية للآباء والأمهات بشأن استهلاك الأطعمة الغنية بالحديد للوقاية من فقر الدم بين الأطفال في سن ما قبل المدرسة. لتقليل الإصابة بالطفيليات، يطلب إجراء تدبير دورى فعال للتخلص من الديدان.

الكلمات المفتاحية: سوء التغذية؛ فقر الدم؛ الثلاسيميا؛ عدوى طفيلية؛ الطفل؛ الصحة العالمية

# Abstract

**Objectives:** Anemia is a global health problem and has very high prevalence in developing as well as developed countries, particularly in children and women. The present study evaluates hematological predictors, nutrition deficiency, parasitic infections and their association with the prevalence of anemia. This analysis will help to identify the anemic status of tribal preschool children.

**Methods:** This was a cross-sectional study conducted in 300 children (age: 6 months to 5 years) in Santrampur village, Gujarat. Blood was collected and used to determine complete blood count (CBC); we also performed ELISA (enzyme-linked immunoassay) for the estimation of ferritin, transferrin, sTfR (soluble transferrin receptor), vitamin  $B_{12}$  and vitamin  $B_9$  (folate). Stool samples were also collected and assessed by ELISA for *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum*. Microscopy was used to screen samples for malaria.

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**Results:** Of the 300 children analyzed, 87.7% were anemic, 239 children were mildly anemic, 20 were moderately anemic and 4 were severely anemic. Mean Hb level was  $9.49 \pm 1.47$  g/dL; males and females had an Hb level of  $9.39 \pm 1.59$  g/dL and  $9.58 \pm 1.34$  g/dL, respectively. Twenty-six children had sickle cell anemia and five had thalassemia. Over 50% of the children had vitamin B<sub>12</sub> and B<sub>9</sub> deficiency and 16% had abnormalities in CRP (C-reactive protein) levels. Parasitic infection by *C. parvum* was positively associated the anemia followed by the prevalence of *G. lamblia* and *E. histolytica*.

**Conclusion:** An increased awareness of parents in the improvement of sanitary facilities and nutritional counselling with regards to iron-rich food consumption is recommended to if we are to prevent anemia among preschool children. To reduce parasitic infestation, effective periodic deworming measures are also recommended

**Keywords:** Anemia; Child; Global health; Malnutrition; Parasitic infection; Thalassemia

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#### Introduction

Anemia is common global health issue that is prominent in developing countries such as India and can exert a significant effect on public health and on social and economic growth. According to global estimations by the World Health Organization (WHO), 1.62 billion people are anemic and 293 million pre-school children are reported to represent the highest prevalence of anemia prevalence (47.4%); 89 million of these children are Indian. The fourth National Family Health Survey (NFHS) 2015-16, directed under the supervision of the Ministry of Health and Family Welfare (MoHFW; Government of India) discovered that at least 62.6% of Indian children aged 6—59 months were anemic. Furthermore, children from tribal areas have an estimated prevalence of 43%, thus representing a vital health problem.

In Gujarat the scheduled tribal population has been reported as being 89,17,714 (14.76% of the total population). The trends for malnutrition in the state of Gujarat, as reported by the NFHS, reports have shown that the situation continues to remain alarming. Several factors are associated with anemia in the tribal population, including socioeconomic, environmental, biological, and nutritional factors (WHO, 2017). Poor maternal health, poorer education, and insufficient dietary intake due to low economic status, can lead to anemia in tribal children which can subsequently result in prenatal mortality, impaired cognitive performance, delayed motor development and a delay scholarly achievements.

The three main causes of anemia are a lack of red blood cell (RBC) production, high rates of RBC destruction, and blood loss. The normal concentration of hemoglobin (Hb)

in children is 11–13 g/dL. According to the concentration of Hb in the blood, anemia is categorized as mild (10.0–10.9 g/dL), moderate (7.0–9.9 g/dL) and, severe (<7.0 g/dL) in children aged 5–60 months. Furthermore, anemia can be classified as microcytic, normocytic or macrocytic as based on the size of the RBCs; microcytic anemia is associated with low ferritin levels while macrocytic anemia is rarely detected in children.

Many other types of anemia have been described and there are three main reasons for the prevalence of anemia in low- or middle-income countries, including nutritional deficiency, genetic hemoglobin disorders and infectious diseases. Nutritional deficiency predominantly relates to the lack of iron, folic acid, vitamin B<sub>12</sub>, vitamin A, and protein energy malnutrition. Measuring levels of the serum transferrin receptor is considered to be a more reliable measure of iron status than measuring serum ferritin in areas with a high prevalence of infections and can distinguish between chronic anemia and iron deficiency anemia (IDA). Folate is required for the synthesis and malnutrition of RBCs whereas vitamin B<sub>12</sub> deficiency results in macrocytic anemia. Parasitic diseases, such as malaria, can increase in prevalence due to iron-folic acid supplementation as folate is essential for the growth of the parasites responsible for malaria, thus leading to anemia; this may also increase the rate of severe morbidity and mortality. Serum C-reactive protein (CRP) can also provide useful information about acute infections and chronic inflammation.<sup>10</sup>

Considering this information, we designed a study for preschool tribal children in Santrampur Taluka, District Mahisagar, Gujarat, India to investigate the possible association between the prevalence of anemia and intestinal infections in this population. The reasons for selecting this particular population related to age and tribal caste. The proportion of the population that is literate is lower than the state average. 11 This study aimed to demonstrate the epidemiology and pathophysiology of Furthermore, we investigated the impact of key etiological factors on the development of anemia, including malnutrition, infections, genetic disorders and sanitation.

#### **Material and Methods**

Study settings and participants

The study was carried out in Santrampur, Gujarat, between April 2017 and November 2018. The total population of Santrampur is 265,694 and approximately 92.7% of this population is tribal of which 16% (43,750) are between 0 and 6 years-of-age. 12 In order to investigate the prevalence of anemia in pre-school children, we selected 12 tribal villages; Ukhreli, Gadiya, Babri, Batakwada, Chittava, Godhara, Kherwa, Khedapa, Sarsan, Shanbar, Simaliya and Taladara. A total of 300 children were selected for this crosssectional study by simple random sampling and a household survey was conducted to analyze tribal pre-school children. The inclusion criteria were as follows: an informed consent form was signed by the parents or legal guardians of the child, the age of children was  $\geq 6$  months and  $\leq 5$  years, the children lived in Santrampur, Eastern Gujarat, and the children were generally healthy. Children were excluded if they were participating in another clinical study, were not part of a tribal community, were part of a migrant tribal community from Santrampur, or had severe co-morbidities including, heart, liver, and kidney diseases.

To acquire appropriate information from demographic and socioeconomic records, a predesigned family question-naire was applied. Socio-cultural and hygienic practice data were collected from the household survey and classified according to their housing plan. Anthropometric measurements, such as height and weight, were taken using standard techniques. The age of each child was obtained from the birth records available from the Anganvadi workers of each village, crosschecked with the parents and BMI (body mass index) was calculated based on the ratio of weight (kg) to height (square meters).

Sample collection and biochemical analysis

#### Blood and stool samples

Venous blood was collected and stored at -20 °C in EDTA coated plain tubes for the estimation of ferritin, transferrin, soluble transferrin receptor (sTfR), vitamin B12 and folic acid by commercially available ELISA kits (catalogue numbers: E-EL-H0168, E-EL-H6028 and E-EL-H6085, ElabScience; VB369B and FA370A, CalBiotech). The samples were also used to determine complete blood count (CBC) with an automated analyzer (MEK6420P, Nihon Kohden). Microscopy for malaria screening was performed following guidelines provided by the National Institute of Malaria Research and National Vector Disease Control Program. Stool samples were collected in a sterile and labeled container after counseling the mothers when antibiotic treatments had not been initiated in the child. Stool specimens

were first processed by gross examination; then, we determined the relative proportions of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* by commercially available ELISA kits (catalogue numbers: T5017/3040, TechLab; KT-838, KT-839, Epitope diagnostics, Inc.).

#### Data analysis

Data were entered into Microsoft Office Excel 2010 and SPSS version 20.0 was used for data analysis. Normal ranges were used for hematological indicators. Demographic details were analyzed by computing the prevalence. Percentage and descriptive characteristics, such as mean and standard deviation, were executed for each parameter separately. The Pearson's correlation test was attained to analyze the relationships between hematological indicators, biochemical parameters, parasitic infection, and anemia. The logistic regression method was used for predictive analysis.

# Results

This study included 300 children; male (N = 149) and female (N = 151) children were enrolled with ages ranging from 6 months to 5 years. The mean BMI (body mass index) of the male and female participants was 15.3 and 14.7, respectively. Logistic regression, depending on BMI, was used to calculate the unadjusted odds ratio (OR) and 95% confidence interval (CI). We used a two-sided 0.05 level of significance. Table I shows, that a rise in every unit of age led to a 1.047-fold higher odds of developing anemia; this was statistically significant when keeping other independent variables constant. There was no significant association between anemia and either BMI or gender.

Table 1: Logistic regression output depending on the BMI of male and female participants.						
	Significance of anemia (P-value)	(OR) 95% C.I. (Confidence Inter		fidence Interval)		
		Odds Ratio	Lower	Upper		
Age in months	0.000	1.047	1.021	1.074		
Gender- Male & Female	0.669	0.855	0.417	1.752		
BMI	0.155	1.138	0.952	1.359		

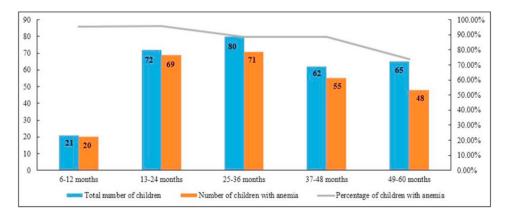


Figure 1: Age distribution of children with anemia.

Sociodemographic and household characteristics

Analysis revealed that the mean family size of the subjects was  $5.4 \pm 1.9$  family members and 75.3% participants had siblings. Educational profiling of the mothers revealed that 32.6% were illiterate and 22% had attended school up to 10th standard. More than 82% of participants lived in mud houses/huts which had mud floors and mud walls; 84% of families had two rooms in their house and only 39.3% had ventilation facilities. Astonishingly, 87% of houses had electrical facilities and the majority (85%) had animals in their houses, including cow, buffalo, sheep, goat, calf and ox; this was because families survive on animal husbandry work for their livelihood. With regards to the source of drinking water, approximately 48% participants used well water and 0.3% of used river water. The majority (51%) of families used water from wells to clean utensils. Surprisingly, more than half of families (58.3%) manually carried water from wells or hand pumps. Steel and mud utensils were used to store water at home in 26% and 92% participants respectively. Daily cleaning of water storage utensils was reported by 83% of participant families.

#### Sanitation and hygiene conditions

We recorded several details relating to sanitation and hygiene. According to our survey, 86% of families had no personal toilets and defecated outside, wherein 85% defecated >1 km from their home. The practice of hand and leg washing was noted in 98% and 52.6% of participants, respectively, while 84% used common cloths to wipe their hands and legs. Open drainage systems were reported in 91% of the participant families. Furthermore, more than 50% of respondents reported that drainage stems were 20 ft—30 ft away from the main home.

Hb concentrations and hematological parameters

Figure 1 shows the age distribution of children along with the prevalence of anemia. The proportion of anemia was 87.7%; most cases were found in children that were 13–24 months age, thus suggesting an extra nutritional requirement during this period of rapid growth.

Od the 300 children, only 37 (12.3%) had normal levels of hemoglobin (Figure 2).  $^4$  The mean Hb concentration in the studied population was 9.49  $\pm$  1.47 g/dL; the levels in males and females were 9.39  $\pm$  1.59 g/dL and 9.58  $\pm$  1.34 g/d, respectively.

Pearson's correlation test was used to analyze the relationship between distinct health indicators for children and established the degree of association (Table 2; range: +1 to -1). A value of 0 shows no association between two variables while a value above 0 suggests a positive association. Hb was positively correlated with white blood

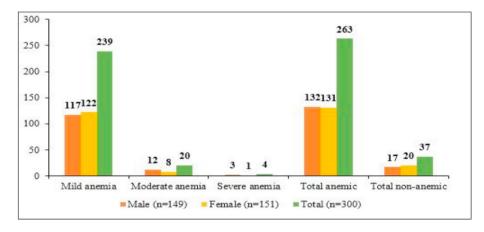


Figure 2: Distribution based on the hemoglobin concentration among children (n = 300).

Table 2: Pearson's correlation coefficients (r) of different parameters in children.												
	Hb	WBC	RBC	НСТ	MCV	MCH	MCHC	RDW	GRA	MON	LYM	PLT
Hb	1.000											
WBC	0.114*	1.000										
RBC	0.300**	0.274**	1.000									
HCT	0.842**	0.161**	0.416**	1.000								
MCV	0.617**	0.050	-0.195**	0.525**	1.000							
MCH	0.662**	-0.046	-0.219**	0.550**	0.787**	1.000						
MCHC	0.526**	0.024	-0.066	0.412**	0.518**	0.544**	1.000					
RDW	-0.548**	0.112	0.112	-0.508**	-0.577**	-0.600**	-0.444**	1.000				
GRA	0.139*	0.463**	0.082	0.173**	0.008	0.011	0.044	-0.065	1.000			
MON	-0.181**	0.284**	0.015	-0.146*	-0.158**	-0.198**	-0.132*	0.166**	-0.020	1.000		
LYM	-0.068	0.715**	0.079	-0.020	-0.069	-0.158**	-0.118*	0.214**	-0.014	0.340**	1.000	
PLT	-0.236**	0.082	0.012	-0.242**	-0.231**	-0.248**	-0.177**	0.320**	-0.059	0.135*	0.183**	1.000

Significance: \*p < 0.05, and \*\*p < 0.01; Hb = hemoglobin, WBC = white blood cell, RBC = red blood cell, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, GRA = granulocyte, MON = monocyte, LYM = lymphocyte, PLT = platelet.

	Ferritin	Transferrin	sTfR	VitB <sub>12</sub>	VitB <sub>9</sub>	CRP
Total (Anemic)	138	209	159	143	179	43
Percentage	52.5%	79.5%	60.5%	54.5%	68.1%	16.3%
r value	-0.007	-0.052	-0.381**	-0.038	-0.003	-0.023
Total	157	236	160	161	204	48
Percentage	52.3%	78.7%	53.3%	53.7%	68.0%	16.0%

cells (WBCs), RBCs, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and granulocytes, but negatively correlated with RBC distribution width (RDW). Granulocytes were negatively correlated with RDW, monocytes and platelet count. Correlations between other hematological indicators are shown in Table 2.

Table 3 shows the total number of children that were found to be deficient in ferritin, transferrin, sTfR, vitamin  $B_{12}$ , vitamin  $B_9$ , and those with abnormal CRP levels. For all parameters, the r value was significant at the p < 0.01 level.

# Parasitological analysis

Table 4 indicates a negative correlation between *E. histolytica* and RBC count whereas *G. lamblia* exhibits a negative correlation with WBC. We also found that *C parvum* had a significant effect on most hematological parameters and the highest rate of abnormality was for Hb level, MCV and MCH. The prevalence of *C. parvum* was most positively related to anemia; this was followed by the prevalence of *G. lamblia* and *E. histolytica*. Mild thinness was positively associated with infection by *E. histolytica* and *C. parvum* while severe thinness was negatively correlated with all three parasites. However, no absolute reason was found to be responsible for this correlation.

Table 4: Pearson's correlation of parasitic infections with anemia and related parameters.

Parasitic infection	E. histolytica	G. lamblia	C. parvum
Total N (%)	109 (36.3%)	145 (48.3%)	174 (58.0%)
Anemic (%)	101 (38.40%)	135 (51.30%)	169 (64.50%)
r value	0.115*	0.160**	0.340**
Hb	0.045	-0.028	0.129*
RBC	-0.127*	-0.009	-0.0102
WBC	-0.045	-0.114*	-0.073
MCV	0.085	-0.065	0.121*
MCH	0.102	-0.008	0.165*
RDW	0	0.026	-0.168**
MPV	-0.0114*	-0.063	-0.059
Anemia	0.115*	0.160**	0.340**
Mild thinness	0.162**	0.089	0.117*
Severe thinness	-0.177**	-0.139*	-0.284**
Mild anemia	-0.018	-0.138*	-0.074
Moderate	0.007	0.152**	0.009
anemia			

Significance: \*p < 0.05, \*\*p < 0.01, \*sTfR = soluble transferrin receptor, \*CRP= C- reactive protein.

Pearson's correlation coefficients of anemia with pathophysiological parameters are shown in Table 4; 101, 135 and 169 children were found to be anemic with *E. histolytica*, *G. lamblia* and *C. parvum* infections, respectively. In total, 245 children (81.7%) had co-infections; 99 children were infected by a single species (33.0%). No significant difference was found between the rates of *G. lamblia* infection in males (47.6%) and females (49%) and the rates of *E. histolytica* (36.9% in males and 35.8% in females). Microscopic analysis of stool samples revealed that 30.3% of samples contained pus cells, 42% contained RBCs, 50.7% contained macrophages and 10.3% of samples contained worms. The occult test was positive in 18.3% of samples.

#### Discussion

In this study, we aimed to assess the nutritional status and prevalence of anemia in 300 pre-school tribal children and found that 87.7% were anemic. The prevalence of anemia in male and female participants was 88.5% and 86.8%, respectively; there was no significant difference in the prevalence of anemia between the two genders. Most children were found to be mildly anemic. Similarly, a national representative survey of India reported that 70% of children between 6 and 59 months-of-age were anemic, including 26% who were mildly anemic, 40% who were moderately anemic and 3% who were severely anemic. 14

In the present study, only 12.3% of children had normal hemoglobin concentrations; the mean concentration for this group was  $9.49 \pm 1.47$  g/dL; male and female participants had concentrations of  $9.39 \pm 1.59$  g/dL and  $9.58 \pm 1.34$  g/dL, respectively, thus indicating that there were no significant differences with respect to gender. Similar results for mean hemoglobin concentrations among boys (110  $\pm$  10 g/L) and girls (110  $\pm$  9 g/L) were reported previously in a study based in the Marshall Islands. 15 Behera et al. (2016) reported that there was no difference in the hemoglobin (g/dL) concentrations of male and female preschool children in Odisha, India (10.57  $\pm$  3.01 and 10.27  $\pm$  2.99, respectively). 16 The size of the RBCs, reflected by mean corpuscular volume (MCV) was less than 80 fL; this was smaller than the normal size of RBCs. Almost all of the enrolled children (100%) were suffering from microcytic anemia. This shows the decreased production of hemoglobin due to the short supply of iron in the body. Engidaye et al. (2019) reported that 50.4% of preschool children in Ethiopia had microcytic hypochromic anemia and 12.2% had macrocytic anemia. Furthermore, these authors reported that the reason underlying the dominant type of microcytic hypochromic anemia was the greater consumption of cereals by the studied community; these cereals have a low iron content. <sup>17</sup>

Iron deficiency in children can be estimated by the levels of ferritin, transferrin and sTfR. The low levels of ferritin reflect a decline in the number of oxygen-carrying RBCs whereas high levels of transferrin and sTfR indicates iron deficiency. From the biochemical parameters, 52.3%, 78.7% and 53.3% of children were found to be deficient/abnormal for ferritin, transferrin, sTfR levels, respectively. Similar results were reported by Yoon et al. (2015) in which 78 children (aged 6–59 months) in an IDA group were found to have increased sTfR levels which correlated negatively with hemoglobin (Hb), ferritin and serum iron levels. 18

Infants between 6 and 12 months of age with vitamin  $B_{12}$ -deficient breast-feeding mothers or infants consuming low levels of animal food may be at risk of vitamin  $B_{12}$  deficiency. In our study, vitamin  $B_{12}$  and vitamin  $B_9$  deficiency was found in 53.7% and 68% children, respectively. In a previous study, Behera et al. (2016) also found vitamin  $B_{12}$  deficiency, as reflected by values above the reference levels for MCV and MCH (14.4% and 5.7%, respectively) in pre-school children. <sup>16</sup> The correlation coefficients for Vitamin  $B_{12}$  &  $B_9$  did not suggest any significant association with anemia.

Of the 300 children, 26 (8.7%) were found to have sickle cell anemia (SCA) with mean Hb concentrations of 9.62 g/dL and 5 children (1.7%) had thalassemia traits with mean Hb concentrations of 9.04 g/dL. These values indicated a lower prevalence of both diseases in children. A cross-sectional survey conducted by Foote et al. (2013) for preschool children (aged 6–35 months) in western Kenya identified Sickle cell trait (17.1%), sickle cell disease (1.6%), homozygous α-thalassemia (9.6%) and heterozygous α-thalassemia (38.5%). The high prevalence of SCA and thalassemia in Indian tribes were also reported by Colah et al. (2015).

Our results suggest the cause of reduced Hb in children is due to the enteric infection of parasites. This has a harmful effect on nutritional status and interferes with the use of many nutrients including iron, vitamin B<sub>12</sub>, folic acid, thus contributing to anemia. The main cause of disease transmission may be socioeconomic condition (education, occupation, income) along with personal hygiene (hand washing habit before food, after using the latrine, along with the type of water and water storage utensils).

Parasitic infections are governed by biological, socioeconomic, health system, behavioral, and environmental factors. Parasitic infections caused by E. histolytica, G. lamblia and C. parvum mainly occur through fecal contamination arising from poor drainage, sewage, and poor water quality. The efficacy of intestinal parasites depends on the species, the number of worms, and the duration of infection, while the intensity of infection is dependent on the size of the host and the nutritional status. Via predominating mechanisms, gastrointestinal parasites can cause damage in human hosts by feeding on host tissues, including the blood, thus leading to a loss of protein and iron. This may also provoke inflammatory responses which may affect appetite and food intake or alter the metabolism and storage of vital nutrients such as iron, thus causing the maldigestion of nutrients.<sup>21</sup>

We also detected a high prevalence of *C. parvum* in children, followed by *E. histolytica* infection. Chronic infection

by these parasites interferes with the growth of children by impaired nutrient digestion (vitamin and fat) and lactose intolerance in association with growth impairment and anemia.<sup>22</sup> One reason for the high parasitic infection observed in this study is the increased contamination of water sources with human excreta due to open defecation. In our study, 86% of families reported that they did not have toilets and defecated outside; 58.3% manually carried water from wells and hand pumps.<sup>23</sup> Moreover, the presence of blood in stool samples showed a correlation with C. parvum infection among the participants. The presence of macrophages was correlated significantly with 50.7% of positive occult blood tests. In a previous study, Petri et al. (2009) reported high numbers of RBCs and macrophages in stool samples in both children and adults that were positive for E. histolytica. Of all the enrolled children, not a single case was found with malarial parasitic infection.<sup>24</sup>

#### Conclusion

In the present study, we found that 87.7% of our study population had anemia. We also identified deficiencies for iron, vitamin B<sub>12</sub> and B<sub>9</sub>, thus indicating malnutrition. Lack of iron and other essential nutrients are a prime cause of anemia in children. Vitamin B<sub>12</sub> deficiency can be treated by hydroxocobalamin intramuscularly while folic acid tablets can be used to treat vitamin B<sub>9</sub> deficiency. <sup>25</sup> As hematological parameters are all interconnected, intervention strategies and constant monitoring is required; providing nutritional supplementation can also help to eradicate anemia. Increased awareness of safe drinking water, improvements in sanitary facilities and nutritional counseling to parents with regards to iron-rich food consumption is recommended to prevent anemia among preschool children. Effective periodic measurements and deworming is advised if we are to reduce parasitic infestation which also promotes the development of anemia. The critical need is to educate mothers through various government initiatives to raise awareness about the health status of their children. Complete health checkups of children and pregnant women should be scheduled on a regular basis to ensure that their health is not compromised. Exclusive breast feeding for 6 months and timely full vaccinations can also prevent malnutrition in children.

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#### Conflict of interest

The authors have no conflict of interest to declare.

#### Ethical approval

This study was approved by the Institutional Ethics Committee of Nirma University, Ahmedabad, Gujarat (India).

# **Authors contributions**

MM, JS and PS investigated the data. SSP supervised the study. UM provided resources and interpretated the data. SSP and SD conceptualized the research study. SSP, UM, CK and VK analyzed the data. SSP wrote the original draft of the manuscript. SSP, SD and VK reviewed and edited the data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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