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Relationship between intestinal parasitic infections and anemia among school children of Yoro villages, Central region, Cameroon

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KEYWORDS

Anemia, intestinal infections, School children, Central region, Cameroon

A B S T R A C T

A cross-sectional study on hemoglobin and intestinal parasitic infections was conducted among 341 school children aged 1–15 years in Yoro Center and Bognangolo villages, in Central Cameroon. Helminths eggs and protozoan cysts were identified using the Kato Katz and concentration techniques. Prior to a finger prick, a drop of blood was collected for quantitative measurement of total hemoglobin. A total of 121 children (35.5%) were infected by any of the parasite species (*Schistosoma mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, *Entamoeba histolytica* and *E. coli*). Sixty-six (54.5%) had low hemoglobin concentration with majority of the cases registered for moderate anemia. Children from Yoro Center were significantly more infected for *S. mansoni* (P<0.05), same as high number of anemic infected children. High anemia prevalence were obtained for *N. americanus* (75.0%); *T. trichiura* (61.9%); *S. mansoni* (52.9%) and *A. lumbricoides* (50.0%). The mean Hb values varied from 7.4 ±0.9 g/dl to 9.9 ±2.5 g/dl. Infected children (1-5 years) had significantly low mean Hb values (4.7±0.0 g/dl: P < 0.05). Anemia prevalence of 50.0% -100% was observed for triple infections. This study highlights the importance of intestinal parasitic infections as contributors to reduced hemoglobin levels among schoolchildren and helps guide the implementation of integrated school health programmes in areas of different parasites transmission.

Introduction

Anemia remains one of the most intractable public health problems in Africa, contributing to a quarter of Africa's nutrition-related Disability Adjusted Life Years (DALYs) lost (WHO, 2002). It has a multifactorial aetiology and the major contributors include non-infectious causes such as malnutrition (Allen & Peerson, 2009) and infectious causes (Pullan *et al*., 2014). Several studies have highlighted the contribution of parasitic diseases to childhood anemia (Friedman *et al*., 2005; Kabatereine *et al*., 2007; Koukounari *et al*., 2008).

In Cameroon, the infection rate of different parasitic infections varies: schistosomiasis stands at more than 5 millions people at risk of infection, with 2 millions people currently infected and more than 10.000 cases declared each year; more than 10 million persons are infected by intestinal parasites (PNLSHI, 2005), including 5.6 millions persons infected by *A. lumbricoides*, 6.5 millions by *Trichuris trichiura* and 2.6 millions by *Necator americanus* (Brooker *et al*., 2000). Cases of polyparasitism have been observed in most endemic areas (Nkengazong *et al*., 2010; Aloho-Bekong *et al*., 2011). Recent studies conducted in some localities provide compelling evidence that parasitic infections (malaria, Soil transmitted Helminths and parasitic protozoan) contribute to anemia in infected children (Ebako *et al*., 2010; Makoge *et al*., 2012; Richardson *et al*., 2011). In contrast, little information is known on the contributory role of schistosomiasis. However, there are surprisingly few published studies describing the relative contribution of schistosomiasis and other intestinal infections to anemia in populations of school-aged children (Nkengazong *et al*., 2014).

In Yoro villages (Center region), infections caused by intestinal parasites (schistosomiasis, and STH) have been put into evidence (Moyou *et al*., 2003). However, these works did not take into account other intestinal parasites (protozoan) and related morbidities attributed to these infections in children.

The aim of the present study was to examine the relationships of hemoglobin (Hb) concentration and anemia with common parasitic infections, including *S. mansoni*, hookworm, *A. lumbricoides*, *T. trichiura* and amoeba species (*E. histolytica* and *E. coli*) in school children of Yoro villages in Mbam and Inoubou Division of the Center region by:

i) Assessing the prevalence of the different parasites species and anemia in the population studied,
ii) Evaluating the variation of anemia prevalence caused by each parasite species,
iii) Evaluating the level of co-infection related to anemia in infected children.
The results obtained will provide good evaluation indicators for control strategies of parasitic diseases in the different endemic areas nation wide.

Materials and Methods

Study area

The present study was conducted in two neighboring villages of Yoro village (Yoro center: 04°32.626'N, 11°09.496 E and Bognangolo: 04°33.628'N, 11°07.761 E) in Bokito town located in Mbam and Inoubou Division of center region. These villages were selected because of previous studies that showed endemicity for parasitic infections (*S. mansoni* and geohelminths) (Moyou *et al*., 2003). Two streams cross these villages: Assaga, which passes in Yoro center of about 100 m from Government school and homes of inhabitants; Gindigueldje, which flows towards other villages (Bougnangagne and Bongando). The population density of these villages is made up of 1000 inhabitants and the villagers practice mostly farm work, fishing and hunting. Despite the presence of forages in the villages, the human population still carryout their daily activities (fishing, swimming, laundry etc) in streams.

The two villages were characterized by almost the absence of toilets that makes the inhabitants to defecate in the bush or in the streams. Garbage piles on which children play on, were also found around the school premises.

Study subjects

The study was conducted from October 2014 to February 2015. Out of the 434 school children contacted, 341 (183 boys: 53.7% and 158 girls: 46.3%) participated in the survey. The sampled population was between the ages of 1 - 5, 6 – 10 and 11 - 15 years. An additional 34 volunteer parents aged 22 years and above participated in the study.

Subject consent

Administrative authorities (Chief of health District, school directors and traditional leaders) were informed about the project and they gave their verbal consent for the study to be undertaken. A written informed consent that met the standards of the National Ethical Commission was obtained from the pupils or the guardians of the young children that accepted to participate in the study.

Parasitological study

Following registration, two stool samples were collected from each participant in 50 ml screw-cap vials. In one of the srew-cap vials was added 10% formol to conserve the parasitic forms of the parasites. The samples were transported to the Parasitology laboratory (Nkomo) of the Medical Research Centre (IMPM, Yaounde) and examined using the quantitative Kato-katz technique (Katz *et al.*, 1970) for the identification of helminths eggs following their morphology (*A. lumbricoides, T. trichiura*, and hookworm), while the qualitative concentration formol ether technique (Cheesbrough, 1991) was used to identify helminths eggs and protozoan cysts. To minimize the measurement bias on the parasitological data, all slides were read within 24 h of preparation to avoid the degeneration of hookworm eggs. The eggs were counted under a light microscope at 10X magnification and their number in eggs per gram of stool (eggs/g) recorded, while the cysts of protozoan (Amoeba species) were observed at a magnification of 40X.

After a finger prick, a drop of blood was put on strips of URIT-12 haemoglobinometer for quantitative measurement of total hemoglobin in fresh whole blood. Children with values less than 11g/dl were considered to be anemic (WHO, 2001).

Data analysis

The Chi-square test was used to compare the prevalence of parasitic infections and anemia in relation to sex, age groups and villages while one – way ANOVA or Kruskal-Wallis tests were used to compare the parasite load in relation to sex, age groups, and villages. The Kruskal–Wallis test was used when the conditions of parametric ANOVA were not fulfilled. The level of statistical significance was at 95% ($P < 0.05$).

Results and Discussion

Parasitological results

A total of 341 (278 in Yoro Center versus 63 in Bognangolo) out of 434 school children were sampled giving an overall participation rate of 78.6%. Of the 341 participants, 121 (35.5%) were infected by any of the parasite species. Of this number, 72 (59.5%) children had single infections while 49 (40.5%) had multiple infections, with single infections being slightly higher in females (63.3%) than in males (58.6%) and multiple infection slightly higher in males (44.8%) than in females (36.7%) (Table 1). Of the total participants, 102 (36.7%) and 18 (28.6%) harbored any of the parasites identified respectively for Yoro Center and Bognangolo.

The overall infection rates for the different parasites species recorded using the two parasitological techniques (Kato katz and Formol ether) were 19.9%, 2.9%, 6.1%, 2.3%, 3.8% and 17.9% respectively for *S. mansoni*, *A. lumbricoides*, *T. trichiura*, *N. americanus*, *Entamoeba histolytica*, *and E. coli*. Children from Yoro center were more infected for all the parasites species compared to those from Bognangolo with a significant difference (P<0.05) observed for *S. mansoni* with a respective prevalence rate of 21.6% and 12.7%.

The infection rates recorded for the different techniques were 14.4 **%** and 19,9% for *S. mansoni*, 2.05% and 2.9% for *A. lumbricoides*, 5.3% and 6,1% for *T. trichiura*, 0.0% and 2,3 for *Necator americanus*, 0.0% and 3.8% for *E. histolytica*, and 0.0% and 17.9% for *E. coli* respectively for Kato Katz and formol ether techniques.

The infection rate stratified by sex was 33.3% (males) and 38.0% (females), while that of age group was 22.9% (1-5 years), 37.6% (6-10 years) and 35.4% (11-15 years) (Table 2). The mean egg loads of 380.6±455.0 e/g of stool, 199.0±306.8 e/g of stool and 384±859.5 e/g of stool were recorded respectively for *S. mansoni*, *A. lumbricoides*, and *T. trichiura*. The difference observed for infection rates and mean egg load did not vary significantly between sex and age groups (P>0.05).

Of the 34 volunteer parents who participated for the study, 12 (35.3%) were infected by *S. mansoni* with a mean egg load of 238±97.6 e/g of stool followed by *E. coli* (20.6%) (Table 3).

Anemia related to infections

Anemia constituted a major morbidity factor of parasitic infections in our study with the observed prevalence of 63.6%. Of the 121 participants infected by any of the parasite species, 66 (54.5%) had low concentration of hemoglobin ranging from severe to light anemia with majority of the cases registered for moderate anemia. High prevalence of anemic infected children was observed in Yoro Center (60.9%) compared to Bognangolo (21.1%), same as for single infections (50.0%) compared to double infections (24.2%) (Figure 1), but the difference observed was not significant (P>0.05).

As for each parasite species, the prevalence of anemia in children varies between 46.2% and 75.0% with high values obtained for those infected by *N. americanus* (75.0%) followed by *T. trichiura* (61.9%), *S. mansoni* (52.9%) and *A. lumbricoides (*50.0%) (Figure 2). The mean Hemoglobin (Hb) values of infected children for different parasite species varied from 7.4 ±0.9 g/l to 9.9 ±2.5 g/dl (Table 4).

Infected children aged between 1-5 years had significantly low mean values of Hb (4.7±0.0 g/dl) and high prevalence (37.5%) compared to other age groups (P < 0,05: Table 5).

Irrespective of the different parasitic infections and parasite species, anemia prevalence observed in infected children ranged from 42.0% to 100.0%. High prevalence of anemic infected children was observed for triple infections (50.0%-100.0%) (Table 6).

The findings from the current study confirm that Yoro villages are still endemic for most parasitic infections. The results show a high prevalence of parasitic infections (35.5%) in school children in the area. Though the prevalence of single infections (61.0%) was higher than that of multiple infections (40.7%), the co-existence of multiple parasites in same individuals could be explained by the fact that the different parasites identified have same transmission pattern, which is mostly linked to poor hygienic conditions. Similar results have also been observed in previous studies conducted in other localities (Koukounari *et al*., 2008; Nkengazong *et al*., 2014). The high prevalence (37.8%) observed for any infection in children aged 6- 10 years might reflect the active nature of children of this age group, what exposes them to high risk of infections (Nkengazong *et al*., 2010).

The infection rates of *S. mansoni*, *A. lumbricoides, T. trichiura* obtained during this study was low (19.9%, 2.9%, 6.1% respectively) compared to that obtained previously (54.7%, 33.9%; 6.2%, 10.7; 20.3%, 17.6% respectively) by Moyou *et al*. (2003) and Kengne (2010) respectively. This difference could be due to the impact of mass drug administration which has been going on in all schistosomiasis foci in the national territory. However, the infection rate for *N. americanus* was lower than that obtained in previous results: 6.2% versus 2.3% (Moyou *et al*., 2003) but higher (2.3% versus 0.9%) than the results of Kengne (2010). The decrease observed from 2010 could be due to the difference in methodology used during the two studies. The significant difference of *S. mansoni* infection observed in Yoro center compared to Bognangolo village is not unusual. Previous studies have demonstrated clearly high risk of schistosomes infections in people who live closer to the transmission sites (Saathoff *et al*., 2004; Nkengazong *et al*., 2013). The low prevalence and high egg load of *S. mansoni* observed is contrary to results of previous studies (Moyou *et al*., 2003). Our observations fall in line with that of previous authors in studies involving other helminths infections (Nkengazong *et al*., 2010). This differences could likely be linked to differences in host susceptibility (Flores *et al*., 2001), the number of adult female worms harbored or related to parasite accumulation in infected individuals (Nkengazong *et al*., 2010).

Our results showed that 35.3% of the volunteer parents were infected by *S. mansoni*. In a study conducted in other schistosomiasis focus in Cameroon, one parent was found to maintain high intensity of *schistosoma haematobium* infection at one and two years post treatment (Nkengazong *et al*., 2013). This may be a reflection of the observation that targeted school-age treatment can suppress transmission in some, but not all schistosomes infected communities, because one individual usually uses multiple water sites, and a single infected person can maintain local transmission for an indefinite period (Woolhouse *et al*., 1998).

During our study, we put into evidence the presence of other parasites (*E. histolytica* and *E. coli*) belonging to other biological groups (protozoan), compared to previous work conducted in the same area (Moyou *et al*., 2003; Kengne, 2010). This difference could be linked to a difference in methodology. During this study, we used the quantitative kato katz and the qualitative concentration formol ether technique to analyse our samples, while only the kato katz technique was used for previous studies. This implies the limitation of some parasitological techniques used for the identification of parasites. Despite the low prevalence of these amoeba species observed compared to results obtained in other localities of Cameroon (Mbuh *et al*., 2010; Richardson *et al*., 2011) and in other countries (Mazigo *et al*., 2011), their presence could reflect the existence of resistant cysts in the study area, what could consequently lead to much morbidities in infected children.

Parasitic infections have a large impact on the survival and quality of lives of school aged children living in Africa. Understanding the direct and indirect consequences of these infections on lower Hb levels is important, as findings may help guide the suite of school-based interventions in endemic areas where polyparasitism is the norm (Raso *et al*., 2004; Pullan and Brooker, 2008). In this study, anemia was found to be a public health problem among our study population. The prevalence was slightly lower than that observed by Nkengazong *et al*. (2014) who found 67.2%. However, this value was equally higher compared to results reported in other localities of Cameroon: 30.0% (Nkuo-Akenji *et al*., 2006), <50.0% (Richardson *et al*., 2011) and in Kenya: 13.5% (Koukounari *et al*., 2008).

Our analysis also found evidence that infections by quasi totality of the different parasites identified were significantly associated with lower mean Hb. This reflects the fact that, in the associations between parasitic infections and anemia, infection at any level may impose a significant burden on local health (King *et al*., 2005). It has clearly been documented that children infected by any of the parasite identified during our study have low hemoglobin concentration in blood (Richardson *et al*., 2011; Soares Magalhães *et al*., 2013), due to iron-deficiency anemia following the feeding mechanisms of the parasites that lead to blood loss, the worms also cause anemia of inflammation (Olsen *et al*., 2009).

The low mean Hb level observed in children infected by *S. mansoni* is not surprising, even though data involving the relationship of schistosomes infections and anemia are rare. However, previous studies have showed low concentration of hemoglobin in children infected by *S. mansoni* and *S. heamatobium* (Koukounari *et al*., 2008; Nkengazong *et al*., 2014). *S. mansoni* may contribute to anemia through blood loss caused by the rupture of blood vessels surrounding the intestine by the spined schistosome eggs, splenic sequestration, autoimmune hemolysis, and anemia of inflammation which is typically characterized by decreased RBC production induced by pro inflammatory cytokines (Friedman *et al*., 2005; Tolentino and Friedman, 2007). This observation could explain the high prevalence of anemic children in *S. mansoni* infected children inYoro center compared to Bognangolo village. It is well documented that, children with multiple parasitic infections experience more severe cognitive outcomes and other health problems such as malnutrition and anemia than those with only one single infection (Jardiam-Batelho *et al*., 2008). Our observations fall in line with these remarks, as the prevalence of anemia in almost 100% of double and multiple infections ranged from 46.0% to 100%.

The global prevalence of anemia in single infections was high compared to multiple infections. This observation is contrary to the results obtained in other localities where the prevalence of malaria (23.2%) recorded was not negligible (Nkengazong *et al*., 2014). This difference observed may reflect the exclusion of malaria parasite in this study as previous studies have clearly outlined the significantly associated of malaria parasitaemia with lower mean Hb (Koukounari *et al*.,2008; Makoge *et al*., 2012; Nkengazong *et al*., 2014).

The mean Hb levels same as anemia prevalence in the different age groups were globally low in infected children compared to the threshold below which anemia is said to be present proposed by the World Health Organization. These levels are <11 g/dl in children aged 6-59 months, <11.5 g/dl in children aged 5-11 years and 12 g/dl in older children (aged 12-14). Also, the prevalence of anemia observed in uninfected children was higher (68.6%) than that in infected children (54.5%). This could be explained by the fact that anemia level observed in our study population might likely involve other ethological factors like malnutrition that could lead to iron deficiency which was not assessed during the study, rather than only to parasitic infections. However, as observed in this study and many other studies, children of age 1-5 years had significant low Hb levels compared to those of any other age group (WHO, 2006; Sousa-Figueiredo *et al*., 2012; Nkengazong *et al*., 2014). This difference could probably be due to a difference in the defensive system of individuals. Young children have less developed immune system and are consequently more exposed to parasites morbidities than older ones.

In conclusion, this study revealed that *S. mansoni*, *A. lumbricoides*, *T. trichiura*, *N. americanus* are still prevalent with the appearance of parasitic protozoan (*E. histolytica* and *E. coli*) among school children in the study area, even though with low infection rates. The level of schistosomiasis infection in parents implies subsequent outbreak of the disease in future if appropriate control measures are not taken. The contribution of these parasitic infections to morbidities due to anemia in infected children is not negligible. The findings of the present study support the need to diagnose multiple parasitic groups in relation to different associated morbidities in human population of all age groups for a better approach in disease management.

Table.1 Distribution of study population and infection rates expressed in percentage in brackets

Variables	Boys (N)	Girls (N)	Total
Study group	183 (53.7)	158 (46.3)	341 (100.0)
Infected children	61 (33.3)	60 (38.0)	121 (35.5)
Uninfected children	122 (66.7)	98 (62.0)	220 (64.5)
Anemic children	123 (67.2)	94 (59.5)	217 (63.6)
Anemic children + infection	38 (62.3)	28 (46.7)	66 (54.5)
Anemic children without infection	85 (69.7)	66 (67.3)	151(68.6)
Single infection	34 (55.7)	38 (63.3)	72 (59.5)
Multiple infection	26 (42.6)	23 (38.3)	49 (40.5)
Single infection with anemia	20 (52.6)	13 (46.4)	33 (50.0)
Multiple infection with anemia	7 (18.4)	9 (32.1)	16 (24.2)

Table.2 Infected children by any of the parasite stratified by age among the study population

Age range	NE	NP	Prevalence (%)
1-5 years	**35**	**8**	**22.9**
Girls	15	4	26.7
Boys	20	4	20.0
6-10 years	**193**	**73**	**37.8**
Girls	89	38	42.7
Boys	104	35	33.7
11-15 years	**113**	**40**	**35.4**
Girls	54	18	33.3
Boys	59	22	37.3

NE= Number examined; NP= Number positive

Table.3 Infection rates of parasites species among volunteer parents

Parasite species	NE	NP (%)	Mean egg load±SD values
S. mansoni	34	12 (35.3)	238±97.6
A. lumbricoides	34	0(0.0%)	-
T. trichiura	34	1 (2.9)	96±0.0
N. americanus	34	1 (2.9)	-
E. histolytica	34	1 (2.9)	-
E. coli	34	7 (20.6)	-

Table.4 Mean Hb±SD values of infected children for different parasite species

Parasite species	N° infected with anemia	Hb±SD values
S. mansoni	36	7,4 ±0.9
A. lumbricoides	5	7,5 ±0.7
T. trichiura	13	7,4 ±1.0
N. americanus	6	7,6 ±0.6
E. histolytica	6	9,9 ±2.5
E. coli	30	7,5 ±0.8

Table.5 Anemic infected children stratified by age and sex among the study population

Age range	NP	Mean Hb +SD in g/dl	Prevalence (%)
1-5 years	**3**	**4.7**±0.0	**37.50**
Girls	1	4.7±0.0	6.70
Boys	2	4.7±0.0	10.0
6-10 years	**47**	**7.4±0.7**	**24.4**
Girls	21	7.5 ±0.6	23.4
Boys	26	7.3 ±0.8	25.0
11-15 years	**16**	**7.90±0.2**	**14.2**
Girls	6	7.9±2.0	11.1
Boys	10	7.9±2.0	16.9

SD = Standard deviation; g/l = gram per liter

Table.6 Prevalence of different parasite infections and anemia among the study population

Variables	Number of children infected (%)	Number of children infected +anemia (%)
Double infections		
S. mansoni +A. lumbricoides	2 (1,7)	1 (50,0)
S. mansoni + T. trichiura	7(5,8)	6 (85,7)
S. mansoni + E. histolytica	5 (4,1)	3 (60)
S. mansoni + E. coli	29 (24,0)	13 (44,8)
A. lumbricoides + T. trichiura	2 (1,7)	1 (50,0)
A. lumbricoides + E. histolytica	2 (1,7)	1 (50,0)
A. lumbricoides + E. coli	3 (2,5)	2 (66,7)
T. trichiura + N. americanus	1 (0,8)	0 (0,0)
T. trichiura +E. histolytica	2(1,7)	2 (100,0)
T. trichiura + E. coli	4 (3,3)	4 (100,0)
N. americanus + E. histolytica	1 (0,8)	1(100,0)
N. americanus + E. coli	2 (1,7)	2 (100,0)
E. histolytica + E. coli	13 (10.7)	6 (46,1)
Tripleinfections		
S. mansoni + A. lumbricoides + T. trichiura	1 (0,8)	1 (100,0)
S. mansoni + T. trichiura + E. coli	3 (2,5)	3 (100,0)
S. mansoni + E. histolytica + E. coli	5 (4,1)	3 (60,0)
S. mansoni + A. lumbricoides + E. coli	1 (0,8)	1 (100,0)
S. mansoni + A. lumbricoides + E. histolytica	1 (0,8)	1 (100,0)
S. mansoni + T. trichiura + E. histolytica	1 (0,8)	1 (100,0)
A. lumbricoides + T. trichiura + E. histolytica	2 (1,7)	1 (50,0)
A. lumbricoides + T. trichiura + E. coli	3 (2,5)	2 (66,7)
T. trichiura + E. histolytica + E. coli	2 (1,7)	2 (100,0)
N. americanus + E. histolytica + E. coli	1 (0,8)	1 (100,0)

Figure.1 Prevalence of anemia in single and multiple infections

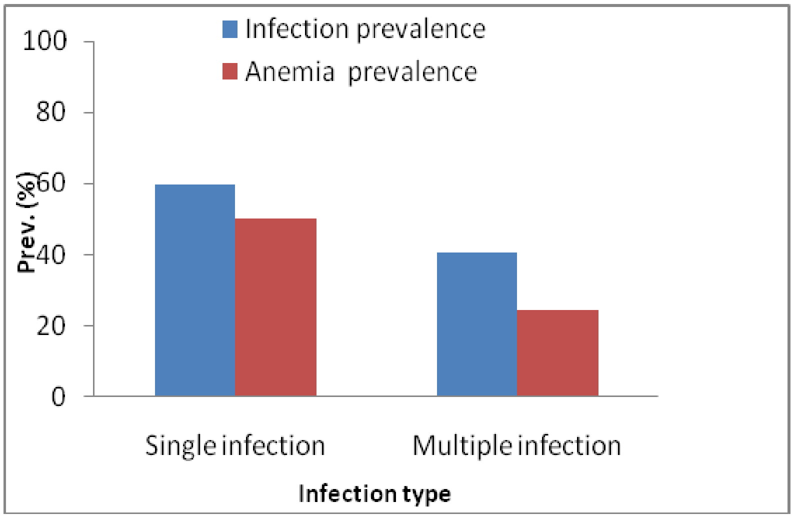
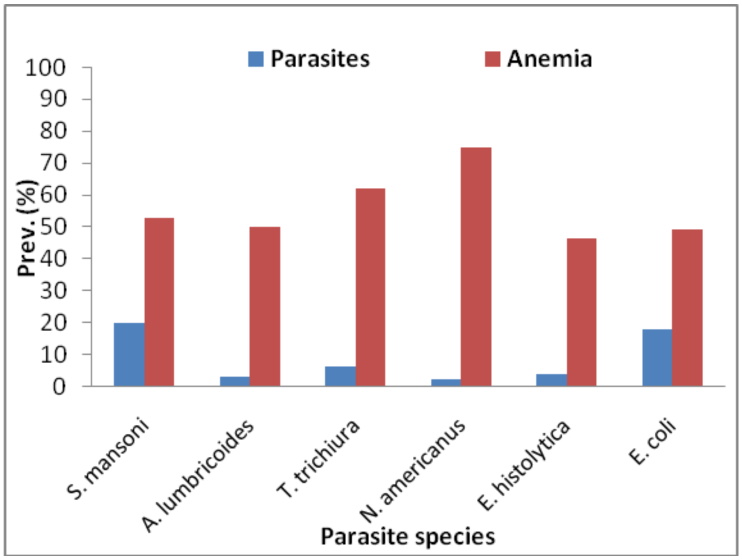


Figure.2 Infection rates of parasitic infections and anemia



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