Intestinal parasitosis, anaemia and risk factors among pre-school children in Tigray Region, Northern Ethiopia.

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Abstract

Background

Intestinal parasitic infections (IPIs) and anaemia are major health problems. This study assessed the prevalence of IPI, anaemia and associated factors among pre-school children in rural areas of the Tigray region, northern Ethiopia.

Methodology/Principal Finding

A community based cross-sectional study was conducted among 610 pre-school children in rural communities of Northern Ethiopia from June 2017 to August 2017. Stool specimens were examined for the presence of trophozoites, cysts, oocysts, and ova using direct, formal-ethyl acetate concentration, Kato–Katz, and Ziehl-Neelsen techniques. Haemoglobin was measured using a HemoCue spectrometer. Among the 610 pre-school children participated in the study, prevalence of IPIs and anaemia were 58% (95% CI: 54.1–61.9%) and 21·6% (95% CI: 18·5% to 25·1%), respectively. Single, double, and triple parasitic infections were seen in 249 (41%, 95% CI: 37% to 45%), 83 (14%, 95% CI: 11% to 17%), and 22 (3.6%, 95% CI: 2.4% to 5.4%) children, respectively. Of the seven intestinal parasitic organisms recorded from the participants, *Entamoeba histolytica/dispar* was the most prevalent 220 (36.1%) followed by *Giardia lamblia* 128 (20.1%), and *Hymenolepis nana* 102 (16.7%). Mixed infections were common among *G. lamblia, E. histolytica/dispar* and *Cryptosporidium* spp. oocyst. Age 48-59 months prevalence ratio (PR = 1·078, P=0·009), child deworming (PR= 1.2; 95% CI=1.00-1.4, p= 0.045), and having two or more children aged under five (PR=0.76, 95% CI= 0.61-0.95, p=0.015) were independent predictors for IPIs. Anaemia was associated with proper disposal of solid waste (PR= 1.5, 95% CI=0.1.1-2.10, p=0.009). Eating raw meat (PR=0.49, 95% CI=0.45-0.54, p=0.000), any maternal education (PR=0.64 95% CI=0.52-0.79, p=0.000), and household water treatment (PR=0.75, 95% CI=0.56-1.0, p=0.044) were associated with lower prevalence of anaemia.

Conclusions

More than half of the children were infected with intestinal parasites and one in five were anaemic. This study has identified a number of potentially modifiable risk factors to address the significant prevalence of IPIs and anaemia in these children. Improvements in sanitation, clean water, hand hygiene, maternal education could address both short and long-term consequences of these conditions in this vulnerable population.

Keywords: Intestinal parasite; anaemia; pre-school age children; risk factors, Tigray

Author Summary

Intestinal parasitic infection and anaemia are public health problems in developing counties. Children due to their immature immune systems and frequent exposure to unhygienic environments are at high risk which in turn put them at an increased risk of malnutrition and growth deficits. Similarly, childhood anaemia impairs physical growth, impairs immune function and weakens motor development. The finding of this study reveals more than half of the children were infected. *Entamoeba histolytica/dispar*, *Giardia lamblia* and *Hymenolepis nana* were dominant parasites. Multiple infections was common among *Giardia lamblia, Entamoeba histolytica/dispar* and *Cryptosporidium* spp. Oocyst. Children aged 48- 59 months were more infected with intestinal parasites. Soli transmitted helminths in this study was low. 21.5% of the children were anaemic and was associated with disposal of solid waste and presence of domestic animals. However, Eating raw meat, maternal education and household water treatment were found preventive of anaemia in the study. It seems worth understanding the prevalence and effects of parasitic infection and anaemia in this vulnerable group to design an appropriate interventions. Finally, if parasite transmission and anaemia is to be significantly prevented control programs such as improving sanitation, clean water, maternal education may be critical in this vulnerable age groups.

Introduction

Intestinal parasitic infections (IPIs) are an important cause of morbidity and mortality worldwide (1,2) affecting about 3.5 billion people globally (3). IPIs are endemic in resource-limited regions due to high population density, low access to improved water sources, low latrine availability, poor hygiene conditions, low health awareness, and limited economic resources (4,5). Helminths such as *Ascaris lumbricoides,* Hookworm*, Strongloide stercolaris* and *Trichuris trichiura,* and enteric protozoan parasites such as *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* spp. cause high incidences of health problems especially in children in low to middle income countries (6–9).

Children due to their immature immune systems and frequent exposure to unhygienic environments are at high risk for IPI including helminths (10), and protozoa (11). These infections are common during the period of life most critical for physical and cognitive development, hence are linked with an increased risk of childhood malnutrition and growth deficits (12). Poor health in children also results in deficits in cognitive development and educational achievements (13–15).

As with IPIs, anaemia remains a public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (16). Globally, 2011 data indicate that 43% of children under-five were anaemic, with a higher prevalence in the developing world, specifically South Asia and East Africa, being 58% and 55%, respectively (17). Sub-Saharan Africa is one of the most affected regions with 53.8% of children under-five suffering from anaemia (18). Causes of anaemia include folate and iron deficiencies (19,20), malaria (21–23), infections (e.g., intestinal helminths), and diarrhoea (23). Childhood anaemia has many irreversible impacts; it impairs physical growth (24,25), impairs immune function*,* increases susceptibility to infections (26,27), and weakens motor development leading to reduced cognitive ability (16,28–31), poor school performance (32), and short or long term mortality in acute severe cases (20).

The 2016 Demographic and Health Survey of Ethiopia (EDHS) report showed that the national prevalence of anaemia among children aged 6 to 59 months was 57% (33), which exceeds the 40% threshold set by the World Health Organization (WHO) classification of

anaemia as a severe public health problem (18). The prevalence of anaemia in the Tigray regional state in the EDHS report 54% is marginally below the national average (33).

Most studies conducted in Ethiopia on the prevalence of IPIs are on school-age children. Studies conducted among pre-school age children have shown wide variance in IPI prevalence from 21.2 % to 85.1% (34–40). Similarly, national studies on anaemia prevalence report prevalence from 32% to 37.3 % (41–43). These studies, however, are focused on either soil transmitted helminths alone (34,35,38) or symptomatic hospitalised children (39), or investigated anaemia alone. The association of IPI and anaemia has not been well addressed. Furthermore, none of the above studies used modified Ziehl-Neelsen techniques to detect *Cryptosporidium* spp., the second most causative agent of diarrhoea among children under five next to rotavirus (44).

There is scarcity of data on the prevalence of IPI, anaemia, and associated risk factors among pre-school children in the study area. However, the prevalence is expected to be high given the poverty, poor hygiene, hot/humid tropical climate and lack of access to potable water. Establishing baseline prevalence and elucidating potentially modifiable risk factors for IPI and anaemia would help public health planners, policy makers and implementers to plan and design appropriate intervention strategies to reduce associated morbidity and mortality among pre-school children.

Materials and Methods

Study area, design, study population, setting and period

Study design, study population and data collection sections have been previously described elsewhere (45) .

Sample size and sampling technique

The sample size of the study was determined using a single population proportion formula, considering an estimate of 24.3% expected prevalence of IPIs among children younger than 5 years old (35). Assuming any particular outcome to be within a 5% marginal error and a 95% confidence interval of certainty, the final sample size with a design effect of two and a 90% response rate was determined to be 610 mothers/children pair.

We used a multistage stratified sampling technique to identify study participants after the kebelles ((a kebelle is the smallest local government administrative unit in Ethiopia) were stratified. In the selected kebelles, 2,674 children aged 6–59 months were identified with

their respective households using the registration at health posts and through the health extension workers (HEW).

We allocated the calculated sample proportionally to the selected kebelles based on the total number of households with children aged 6–59 months in each kebelle. Study participants were then identified using simple random sampling of the households. In cases where households had more than one eligible child, the eldest child was included. Accordingly, the distribution of households with respect to the kebelles was, 133 from Tsawnet, 142 from Harena, 158 from Serawat, and 177 from Mynebri.

Data collection

After written consent was obtained from mothers or guardians of eligible children, socioeconomic, environmental, behavioural and health related data were collected using a structured questionnaire (translated from English and printed in the local Tigrigna language). Data were collected using a face-to-face administrated questionnaire and an observation method by trained data collectors, under the supervision of the principal investigators. Child hand cleanness and nail status in addition to toilet availability were assessed by direct observation.

Variables

Dependent variables

IPIs and anaemia among children aged 6-59 months (pre-school children).

Independent variables

Socio-economic variables: Gender and age of the study child, mother's/guardian's educational status and occupation, family size, family income, number of children 6-59 months in the household.

Environmental and Behavioural variables: Consumption of raw vegetables, child contact with pet animals, child deworming, habit of playing in soil, shoe wearing habit, child hand cleanliness and fingernail status. Use of soap for hand washing, water source, use of household water treatment, latrine availability, and type of drinking water source.

Faecal sample collection

Following the completion of consent and questionnaire, a clean, wide screw capped plastic stool cup, labelled with names was provided to each mother/ guardian. They were requested to bring about 10 g (thumb size) fresh stool from their child to the nearby health posts the following day within 10-30 min of passage. Participants who were not able to provide a sample on the first day were asked again on the following day.

Parasitological analysis

Stool specimens were analysed at the respective health posts by three trained laboratory technicians using direct saline wet mount, formalin ethyl acetate concentration technique (46), and single Kato–Katz technique (thick smear 41.7 mg) (47). For the detection of *Cryptosporidium* spp. oocysts, modified Ziehl-Neelsen (MZN) staining was performed (48). Kato–Katz, wet mount preparations and modified Ziehl-Neelsen were analysed within 1h of preparation in each respective health posts to detect hookworm eggs, protozoa parasites (*E. histolytica/dispar* and *G. lamblia*), and *Cryptosporidium* spp., respectively. The remaining stool specimens were transported in screw-capped cups in 10 % formalin to Mekelle University Medical Microbiology Laboratory and were examined using the concentration method within 8 hours after collection. After 72 hours, Kato–Katz preparations were re-examined to detect helminth ova. A child was categorised as infected if the stool sample was positive for any parasite by any of the methods used. To ensure quality, each slide was examined twice by two of the three experienced laboratory technician independently.

Quality control

To control our data quality, 10% of the total positive specimens were randomly selected and re-examined by three experienced laboratory technicians who did not have any information about the previous results. The results of the new laboratory examinations were therefore used as quality control.

Haemoglobin (Hb) measurement

Finger-prick blood specimens were obtained from participants to assess Hb levels using a HemoCue analyser in the health post (HemoCue Hb 201z, Sweden) (49). The apparatus was calibrated daily using the reference micro cuvettes as indicated by the manufacturer. Definition and classification of anaemia were according to the WHO cut-offs (50).

Data analysis

Data were analysed with Stata Release 15. Confidence intervals for prevalence were calculated using the Agresti-Coull formula. Negative binomial regression was used to model **prevalence rate ratios**. Prevalence rate ratios have several advantages over odds ratios. The first is that they are simple to interpret - they directly compare prevalence, so a prevalence ratio of 2 means that prevalence is twice as high. Second, prevalence ratios, but not odds ratios, have a mathematical property called collapsibility; this means that the size of the risk ratio will not change if adjustment is made for a variable that is not a confounder

(51)(52). All reported p-values were two-tailed, and statistical values were considered significant when p < 0.05.

Ethical Clearance

Ethical clearance was obtained from Mekelle University; College of Health Science Institutional Review Board (IRB) [ERC 0844/2016]. Permission was obtained from the Tigray Regional Health Bureau and respective district Health Bureau. Written consent was obtained from mothers / guardians. Children with IPIs and anaemia were referred to the nearby health institutions and treated from the project fund.

Results

Prevalence of intestinal parasitosis and anaemia

Among the 610 participating pre-school children, 354(58%) (95% CI: 54.1–62.1%) were infected by one or more parasitic organism. Single, double, and triple parasitic infections were seen in 249 (40.8%), 83(13.6%), and 22 (3.6%) children, respectively. Seven different intestinal parasitic organisms were detected with *E. histolytica/dispar* the most prevalent 220(36.1%) followed by *G. lamblia* 128 (20.1%), and *H. nana* 102(16.7%). Mixed infections were common among children positive for *G. lamblia, E. histolytica/dispar* and *Cryptosporidium* spp. The prevalence of any type of anaemia was 21.6% (95% CI: 18.5% to 25.1%) (**Table 1**).

Table 1. Intestinal parasite and anaemia among 610 pre-school children in the Tigray region of Northern Ethiopia, 2017

VARIABLES	No (%)
Children with intestinal parasite(s) detected	354(58)
Number of single parasitic infections	249(40.8)
Number of double parasitic infections	83(13.6)
Number of triple parasitic infections	22(3.6)
Haemoglobin level	
Normal	478(78.4)
Anaemia	132(21.6)
Infection type	
Protozoa	
E. histolytica/dispar	125(20.5)
G. lamblia	52(8.5)
Cryptosporidium spp.	11(1.8)
E. vermicularis	2(0.3)
Helminths	
H. nana	59(9.7)
A. lumbricoides	1(0.2)
S. stercoralis	1(.2)
Double infection	
G. lamblia + E. histolytica/dispar	44(7.2)
G. lamblia + H. nana	8(1.3)
E. histolytica/dispar + H. nana	27(4.7)
G. lamblia + Cryptosporidium spp. Oocyst	2(0.3)
E. histolytica/dispar + E. vermicularis	2(0.3)
Triple infection	
G. lamblia + E. histolytica/dispar + H. nana	8(1.3)
E. histolytica/dispar +G. lamblia+ Cryptosporidium spp.	14(2.3)

Prevalence of intestinal parasitic infection rose from 50% in children aged under 2 years, rising to 66% in children aged 4 to 5 years. The prevalence rate increased by 7·8% per year over the age range studied (negative binomial regression, IRR = 1·078, p=0·009). Children with unclean hands were 63.4% times more infected with IPIs than their counter parts. Similarly, prevalence of anaemia rose from 28% in those aged 6 months to one year to 44% in children aged 1 year. Thereafter it declined sharply with age to reach 7% in those aged 4 years. Because prevalence declined from one year onwards but was lower in children aged under one year, we calculated the effects of risk factors adjusting for age coded as two variables: age in years, and a binary variable indicating that the child was aged under one year (**Table 2**).

Table 2. Distribution of IPIs and Anaemia among 610 pre-school children in Tigray, Ethiopia, 2017.

Risk factors	Categories	No (%) (n=610)	IPIs No (%)(n=354)	Anaemia No(%)(n=132)
Gender	Male	326(53.4)	191(59.6)	76(23.3)
	Female	284(46.6)	163(57.4)	56(19.7)
Age (months)				
	6-11	32(5.3)	15(47)	9(28)
	12-23	108(17.7)	54(50)	47(44)
	24-35	160(26.2)	87(54)	43(27)
	36-47	149(24)	91(61)	22(15)
	48-59	161(26.4)	107(66)	11(7)
The habit of a child playing in soil				
	Never	23(3.8)	12(52.2)	7(30.4)
	Sometimes	135(22.1)	73(54.1)	35(25.9)
	Regularly	452(74.1)	269(59.5)	90(19.9)
Child shoe wearing habit				
	Never	49(8)	29(63.3)	11(22.5)
	Sometimes	202(33.1)	114(56.4)	41(20.3)
	Regularly	359(58.9)	211(58.8)	11(22.5)
Family monthly income in Ethiopian birr				
	<500 birr	99(16.2)	62(62.6)	80(22.3)
	500-2000birr	451(73.8)	260(57.5)	25(25.3)
	>2000 birr	60(9.8)	32(53.3)	101(22.4)
Consumption of raw vegetables				
	Never	106(17.4)	62(58.5)	6(10)
	Sometimes	504(82.6)	292(57.9)	18(17)
	Always			106(22.5)
Child fingernail status				
	Trimmed	315(51.6)	179 (56.8)	8(25)
	Untrimmed	295(48.4)	175(53.2)	75(23.8)
Child hand cleanness				
	Clean	367(60.2)	200(54.5)	57(19.3)
	Unclean	243(39.8)	154(63.4)	92(25)
Child hand washing habit with soap before a meal				
	Never	80(13.1)	48(60)	40(16.5)
	Sometimes	344(56.4)	197(57.3)	27(33.6)
	Always	186(30.5)	109(58.6)	75(21.8)
Number of families in the house				
	<4	144(23.6)	77(53.5)	30(16.2)
	≥4	466(76.4)	277(59.4)	27(18.8)
Number of under five children in the house				
	1	482(79)	294(61)	100(20.7)
	2 and more	128(21)	60(46.9)	32(25)

Mother's educational level				
	Illiterate	354(58)	218(61.6)	105(22.5)
	Primary school	193(31.6)	104(53.9)	90(25.4)
	Secondary school	54(8.9)	27(50)	36(18.7)
	Diploma and above	9(1.5)	5(55.6)	5(9)
Mother's/guardian's Occupation				
	House wife	562(92.1)	327(58.2)	1(11)
	Governmental employee	19(3.1)	8(42)	101(18)
	Self-employed	29(4.8)	19(65.5)	7(38)
Household water source				
	Protected	140(23)	77(55)	34(24.3)
	Unprotected	470(77)	277(58.9)	98(20.9)
Solid waste disposal				
	Proper	51(8.3)	32(62.8)	8(15.7)
	Improper	559(91.7)	322(57.6)	124(22.2)
Use household water treatment				
	Yes	150(24.6)	98(65)	25(16.7)
	No	460(74.4)	256(55.7)	107(23.3)
Toilet availability				
	Yes	364(59.7)	211(58)	15[52]
	No	246(40.3)	143(58)	86(23.6)
Child contact with pet animals				
	Never	366(60)	205(56)	88(24)
	Sometimes	149(24.4)	93(62.4)	29(19.5)
	Regularly	95(15.6)	56(59)	15(15.8)
Child deworming (n=578)				
	Yes	395(68.3)	263(61.6)	82(20.8)
	No	183(31.7)	91(49.7)	50(27.3)
Diarrhoea in the last 14 days				
	Yes	173(28)	119(68.8)	38(22)
	No	437(71.6)	235(53.8)	94(21.5)
Current GI symptoms				
	Yes	135(22)	98(72.6)	30(22)
	No	475(77.9)	256(53.9)	102(21.5)

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Factors associated with IPIs among pre-school age children

Because prevalence of parasites is associated with age, we used negative binomial regression adjusted for age to calculate prevalence rate ratios associated with each of the associated risk factors. Adjusted for age, prevalence was higher in children with current GI symptoms (p= **0.001),** who had diarrhea in the previous 14 days (p= **0.000**), and who had been de-wormed (p= **0.045**). Prevalence was however, lower in households with two or more children aged under 5 (p=**0.015**) (**Table** 3). In multivariable analysis, having two or more children aged under five and child deworming remained significantly associated with parasite prevalence (p< 0.05).

Table 3. Associated Risk factors for prevalence of IPIs among 610 pre-school children in the Tigray, Ethiopia, 2017.

Risk factor	Prevalence ratio	95% CI	p value
2+ under five children in the house	0.76	0.61–0.95	0.015**
Any education	0.89	0.74–1.10	0.186
Child's hands clean	0.89	1.0–0.80	0.129
Solid waste disposed of properly	0.91	0.76–1.10	0.327
4 or more children	0.93	0.72–1.20	0.611
Water from improved source	1.0	0.85–1.10	0.382
Child's fingernails trimmed?	1.0	0.89–1.10	0.588
Eats fruit or veg raw	1.0	0.82–1.20	0.798
Presence of domestic animals in house	1.0	0.78–1.30	0.939
Soap used in handwashing	1.0	0.89–1.10	0.988
Latrine availability in the house	1.0	0.92–1.10	0.949
Male sex	1.0	0.89–1.20	0.714
Habit of raw meat eating of the child	1.0	0.75–1.40	0.855
Child in contact with animals	1.1	1.0–1.20	0.230
Any skin disease	1.1	0.95–1.20	0.258
House hold water treatment	1.2	1.0–1.40	0.057
Did the child get dewormed*	1.2	1.0–1.40	0.045**
Diarrhea in the last 14 days	1.3	1.2–1.40	0.000**
Current GI symptoms	1.3	1.1–1.60	0.001**

**= statistically significant, *=this item is labelled "for above 2 years, but there are data for all children. All prevalence ratios are adjusted for age using negative binomial regression

Factors associated with anaemia among pre-school age children

Prevalence of anaemia rose from 28% in those aged 6 months to one year to 44% in children aged 1 year. Thereafter it declined sharply with age to reach 7% in those aged 4 years. Because prevalence declined from one year onwards but was lower in children aged under one year, we calculated the effects of risk factors adjusting for age coded as two variables: age in years, and a binary variable indicating that the child was aged under one year. Eating raw meat (p= 0.000), any maternal education (p= 0.000), and household water treatment (p= 0.044), were associated with lower prevalence of anaemia, when adjusted for age. On the other hand, presence of domestic animals in the house (p=0.006), and proper disposal of solid waste (p=0.009) were both associated with increased risk (Table 4).

Table 4 Risk factors for anaemia among 610 pre-school children in the Tigray, Ethiopia, 2017.

Risk factors	Prevalence ratio	95% CI	P- value
Habit of raw meat eating of the child	0.49	0.45–0.54	0.000**
4 or more children	0.64	0.39–1.00	0.075
Any maternal education	0.64	0.52–0.79	0.000**
House hold water treatment	0.75	0.56–1.00	0.044**
Soap used in handwashing	0.85	0.64–1.10	0.279
Child in contact with animals	0.87	0.6–1.20	0.399
Any skin disease	0.95	0.56–1.60	0.839
Diarrhea in the last 14 days	1.0	0.66–1.40	0.832
Current GI symptoms	1.0	0.59–1.60	0.898
Did the child get dewormed*	1.0	0.86–1.20	0.891
Water from improved source	1.0	0.82–1.30	0.752
Child's fingernails trimmed	1.1	1.0–1.20	0.077
Male sex	1.1	1.0–1.30	0.134
Child's hands clean	1.2	0.87–1.60	0.280
Latrine availability in the house	1.2	0.84–1.70	0.308
Number of under-five children in the house	1.3	0.92–1.90	0.129
Eats fruit or veg raw	1.4	0.91–2.00	0.133
Presence of domestic animals in house	1.5	1.1–2.00	0.006**
Solid waste disposed of properly	1.5	1.1–2.10	0.009**

= statistically significant, *** This item is labelled "for above 2 years, but there are data for all children. All prevalence ratios are adjusted for age using negative binomial regression

Discussion

Locally relevant epidemiological data on IPI and anaemia prevalence among pre-school children in rural communities and identification of potentially modifiable predisposing factors, is essential to design appropriate intervention strategies. We report an overall prevalence of IPIs and anaemia of 58 % and 21.6%, respectively and have identified inadequate sanitation and clean water supplies and a requirement for family hand hygiene education as potentially modifiable associated factors. Improvements in these areas could address both short and long-term consequences of these conditions in this vulnerable population.

IPI prevalence (58 %) in our study was comparable with previous reports from Karachi, Pakistan 52.8% (53), and Benue, Nigeria 51.4% (54), though lower than studies conducted in Wondepgent, South Ethiopia 85.1% (40), Matanzas, Cuba 71.1%(55), and West Malaysia 76.5% (56). However, our prevalence is higher than previous similar studies conducted in other parts of Ethiopia such as: Wonji, 24.3% (35), Butajira, South Ethiopia 4.9% (57); Gonder, North West Ethiopia 17.3% (39), Dessie 15.5% (58), Jimma, West Ethiopia 49.6% (59), Addis Ababa 27.5% (60), Shoa, Ethiopia 17.4% (61), Dembiya, Northwest Ethiopia 25.8% (62), Hawassa, South Ethiopia 26.6 % (63), and Yirgalem Hospital, South Ethiopia 36.52% (37). Others have reported lower IPI prevalence in Islesa, Nigeria 23.3% (64), Ebonyi, Nigeria 13.7% (65), Gonbad-e Karus, Iran 26.6 % (66), Infaka, Tanzania 9.1%(67), Hoima district, rural Western Uganda 26.5% (68), Riyadh, Saudi Arabia 17.7% (69), Gaza, Palestine, 16.6%(70), and Sana'a city, Yemen, 30.9% (71). These reported differences in IPI prevalence might be due to the difference in parasitological methods used, geographical location, level of environmental sanitation, drinking water source, season, family education, personal hygiene, parental socioeconomic, and cultural difference of the study participants.

One explanation for the high IPI prevalence in this study could be because of seasonal variation. Data collection for our study took place during the rainy season in Ethiopia, other studies [35, 52, 54, 56- 58, 61, 64, 69] were conducted in the dry season. Seasonal variation may be explained by increased contamination of water sources (e.g., rivers, streams, and wells) with human excreta from open defecation which is the main risk factor for diarrhoeal disease and IPIs, especially children who routinely play in the an unhygienic environment (45). In addition, as 77% of households in our study used

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unprotected water sources, the main factor for faecal-oral transmitted disease such as IPIs, this may also contribute to the high IPI prevalence.

Another possible reason for the high prevalence of IPIs in our study is the laboratory methodology we employed. We used the modified Ziehl Nelson method to detect *Cryptosporidium* spp, whereas the other studies except [37] and [66] did not. Furthermore, some studies isolated only the soil-transmitted helminths(STH) and not protozoa which would artificially decrease IPI prevalence(57)(35)(68) (56).

E. histolytica, G. lambilia and *C. parvum* were the most prevalent protozoan parasites in our study cohort. The high prevalence of *E. histolytica/dispar* (36.1%) in our study is in agreement with previous reports (54,58,72). Consequences of childhood *E. histolytica* infection include malnourishment, anaemia and stunted growth (25). However, others have reported *G. lamblia* (55,69–71,73,74), and *C. parvum* (67) as the dominant parasites. The difference in prevalence of enteric protozoa may be due to differences in contamination of drinking water sources, availability of toilets, handwashing and consumption of raw vegetables of the study participants.

Helminthic intestinal parasites, particularly soil transmitted helminths (STHs), commonly infect children in low to middle-income countries. In our study, helminthic infections were identified in 104 (17.1 %) of the children with *H .nana* 102 (16.7%) being dominant while *Ascaris*, hookworm and *Trichuris* infections were very rare or absent. The low STH incidence in our study might be due to the initiation of a national deworming program, as the majority, 68.3% of the children were dewormed during the data collection time and deworming is given on a regular basis. The relatively high prevalence of wearing shoes either regularly or sometimes and low prevalence of consumption of raw vegetables may also partially explain the low STH prevalence. In addition, the prevalence of STH might also be due to differences in environmental factors such as climate, topography (75), surface temperature, altitude, soil type and rainfall (76).

Our study shows that dewormed children were more infected with IPIs than their counter part which is against the established fact. This could be due to the fact that the prevalence of STHs, where deworming is given for, in our study was very small, and the protozoan infection which deworming has less effective against, was the most dominant. A lower prevalence of STH compared with *H. nana* has been previously reported in Peru (77) and in Eastern Ethiopia in elementary school children receiving regular albendazole deworming

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treatment (78). This could be probably due to the fact that albendazole has little effect on *H. nana* unlike *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (79).

Children aged 48-59 months (PR=1.078, p=0.009) were more likely to be infected by IPIs compared with younger children. This likely reflects increased risk of IPI exposure from playing activity of the older, more mobile children within unhygienic external environments and hence exposed to faecal-contaminated soil.

Only a quarter of households used household water treatments, mainly chlorination and boiling. Though not statistically significant, children whose family did not treat their water were 1.2 times (p= 0.057) more likely to be infected by IPI. Chlorination is less expensive, less time consuming and provides residual disinfection to protect against recontamination and significantly reduces the risk of diarrhoea (80). However, few families in our study performed chlorination because of the associated smell and taste and informed the investigators that their children prefer to drink untreated water instead. This was consistent with reports from elsewhere (81) (82). Likewise, sufficient heating, consistent and correct boiling of water reduces the childhood diarrhoea. Nevertheless, this increases fuel consumption, is time consuming, and because of lack of residual protection, treated water can be re-contaminated following the immersion of unclean fingers, other fomites, or through storage in a dirty container (83). Hence a cheap, point of use technology which overcomes these limitation is required by such communities to tackle the consumption of unsafe drinking water.

The prevalence of childhood anaemia among pre-school children in our study was 21.6%, which is comparable with studies from South central Ethiopia 28.2% (84), Western province, Kenya 25% (85), West Malaysia 26.2% (56), and Northeast Brazil 28.9% (86). Our study was, however, lower than other reports from Ethiopia 37.3% -42.2% (43) (87), the 2016 Ethiopian Health Demographic Survey, 57 % [33], the EDHS anaemia prevalence of the Tigray regional state, 54% [33] and the WHO classification for anaemia 40% [18]. Likewise, studies performed outside Ethiopia have reported higher anaemia prevalence including those conducted in Pernambuco, Brazil 32.8% (88), North Western Uganda 37.2% (21), Uganda 72% and 60% (23), Edo state, Nigeria 38.6% (89), Bangladesh 51.9% (90), Senegal 53.4% (91), Arusha District, Tanzania 84.6% (17), Cape Verde, West Africa 51.8% (92), Simanjiro district, Tanzania 47.6% (93), Indonesia 56.1% (94), Namutumba district, Uganda 58.8% (95), sub-Saharan Africa 59.9% (96), and Brazil 45.1% (88).

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Possible reasons for the variation in anaemia prevalence could be due to differences in maternal education [84,69,78], concomitant childhood malaria superinfection [21-23,77,81], family income [21,23,43,69,76,81,85], drinking water source [78, 81], personal hygiene (97), and STH infections [23,69,80,84]. For example, the high anaemia rate in the studies such as Arusha District, Tanzania, Kenya, Senegal, Uganda, South central Ethiopia, was due to concomitant malaria infection. Seasonality may have also affected the anaemia prevalence in our study as malaria prevalence is usually low during the rainy season of the country (June-August). In addition, our study participants were from the community rather than from health institutions, and the prevalence of STH as the main cause of anaemia [23, 69, 80, 84,(98) was very low in our study. Our multivariate logistic regression analysis shows that children aged 12-23 months were more likely to have anaemia compared with younger age groups. This association of age and anaemia has been previously reported [43, 76, 81, 83].

Limitation of the study

One of the limitations of our study was that differentiation between the morphologically identical species of *Entamoeba* was not within the scope of this study, as only conventional microscopy was used to detect the amoebae.

Conclusions

This study revealed the significant burden of IPI and anaemia in preschool children in a rural community in Northern Ethiopia. More than half of the children were infected with intestinal parasites and one in five were anaemic. Age of the child, child deworming and having two or more children aged under five were significant predictors for the IPIs in the study areas. More investment and commitment to addressing the problem of childhood IPIs by designing and implementing prevention strategies, such as integrating mothers'/guardian's education on personal and environmental hygiene into existing national health extension program.

While anemia was comparably lower, it was associated with age, mother's/guardians educational status, and household water treatment. Given the identified burden of childhood IPI and anaemia during the period of life most critical for physical and cognitive development (i.e., the preschool years), this study underlines the need for interventions focusing on the identified modifiable risk factors to prevent long-term morbidity and give these children the maximal opportunity for health in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Author Contributions

AGW, TA, MT, LN, DY, KMG, JM designed the study. AGW, HTA, RC worked on the analysis and interpretation of the data. AGW, prepared the manuscript for publication. AGW, TA, MT, HTA, JM, FF, KMG, RC reviewed the manuscript for publication. All authors read and approved the final paper.

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Legend 1. Supporting Information Legends

Title : Raw data working