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Magnitude of Intestinal Parasitosis among Under Five Year Children Presenting with Acute Diarroheal Illness in South Ethiopian Hospital

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Abstract
Background: Diarrhoea is a leading cause of morbidity and mortality among children under 5 years in low-income and middle-income countries. Intestinal protozoa *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* are common causes of diarrhea in children in developing countries. The aim of this study was to investigate the incidence of intestinal parasites associated with acute diarrhea among children of five years of age and below.**Methods and materials:**A cross sectional study was conducted from Sep 2015 to Aug 2016 at Wolaita Sodo University Teaching and Referral Hospital from 423 under five year children. Convenient sampling method was applied for data collection. Stool specimen was collected and examined by using wet mount, iodine staining and formol-ether concentration technique. SPSS version 21 was applied and mean, median and frequency was calculated. The association has assessed using x2 –test. Variables that had a significant association have selected for further analysis using multiple logistic regression models with a p-value < 0.05 considered as statistically significant.**Result:**Twenty one percent, 87(21.2%) were positive for at least one intestinal parasite. Co-infection were observed in only 4(0.98%) of children. *G. lamblia* was the predominant parasite isolated 44(50.6%) followed by *E. histolytica/dispar/moshkovskii* 26. Out of the total 87 isolated parasites, 47 were from male children and the remaining 40 parasites were isolated from female children. There is no statistical significant association between gender and parasite infection (P=0.52). Children aged between 2.1-3 years showed higher intestinal parasites load with the prevalence of 28.4%. There was no statistical significance association observed between age and parasite infection (P=0.106). **Conclusion and recommendation:***G. lamblia* and *E. histolytica/dispar/moshkovskii*, were relatively prevalent among under-five children in the study area. Studies on causative organisms of childhood diarrhea should be carried out regularly and microbe-specific intervention strategies should be carried out for the control of childhood diarrhea.
Keywords: Intestinal parasites, under five, Children, WSUTRH, Diarrohea

Background

Diarrhoea is a leading cause of morbidity and mortality among children under 5 years in low-income and middle-income countries [1]. It accounts for 9% of all deaths among children under age 5 worldwide in 2015. Over 1,400 young children are dying of diarrhea each day, or about 530,000 children a year, despite the availability of simple effective treatment. Most deaths from diarrhoea occur among children less than 2 years of age living in sub-Saharan Africa and South Asia [2].

In Ethiopia, the child mortality rate in 2007 was 199 per 1,000 births with approximately one in every five deaths being due to diarrheal disease [3]. Diarrhoea accounted for 29.5% of deaths among infants aged 1 to 11 months in Ethiopia [4]. According to Ethiopia Demographic and health Survey (EDHS) of 2011, 13% of children under age of 5 years were reported to have had diarrhoea within two weeks of undertaking the survey, with wide variations among regions [5].

Although only bacteria and viruses were previously assumed to be the causes of diarrhoea, parasite-caused diarrhoea has also been found in 30-80% of patients [6]. Intestinal protozoa *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* are common causes of diarrhea in children in developing countries and asymptomatic infection is also common in this population [7]. Most of intestinal parasites are more severs in children than adults, which is associated with malnutrition, growth retardation, and poor care for children. The case is worth in under five year children because of poor maternal hygiene, play habitats of children, in the house in close proximity to one another that create an appropriate for the transmission and spread of the disease [8].

Most of the previous studies conducted in Ethiopia have focused on the prevalence and distribution of intestinal parasitic infections mainly among schoolchildren. Only few studies have been reported the magnitude of intestinal parasitic infections among under-five children [8, 9].The overall prevalence rates of the parasite and individual parasite infections vary considerably in different parts of the country. In Wolaita area of Ethiopia, there is no previous study conducted among children under age of 5 years. Thus, the aim of this study was to investigate the incidence of pathogenic parasitic protozoa associated with acute diarrhea among children of five years of age and below.

Materials and Methods

Study period and design

A cross sectional study was conducted from Sep 2015 to Aug 2016 at Wolaita Sodo University Teaching and Referral Hospital (WSUTRH), Sodo, located South Central Ethiopia. It's serving people in catchment's area of 2 million people. The hospital organized in department, case-teams and working hours are according to BPR standard. There are seven case teams, OPD, Emergency, Inpatient, Delivery, Dentistry and Ophthalmology. The hospital has 200 beds for inpatient service which is on medical, pediatrics, surgical, gynecology and obstetrics ward.

Sampling method

Convenient sampling method was applied for data collection. Individuals who come to donate blood during the study period and those who were volunteer to participate were included in this study.

Source population

All under five year children, who are attending Wolaita Sodo University Teaching Referral Hospital during the study period, were the source population.

Study population

Under five year children attending at Wolaita Sodo University Teaching Referral Hospital, who fulfill the inclusion criteria and selected during the study period were the study population.

Eligibility criteria

Inclusion criteria

- ✓ Patients whose guardians gave permission
- ✓ Under five children between

Exclusion criteria

- ✓ Those with any other acute/chronic disease causing immunosuppression
- ✓ Children who took anti-parasitic treatments drugs within one month before screening were excluded from the study.

Sample size

The sample size was calculated using the statistical formula for a single population proportion Prevalence of intestinal parasitosis 50%

Confidence level=95 %(α=0.05) z=1.96

Margin of error (desired level of absolute precision) d=5%

Non-response rate=10%

The calculated sample size with the addition of 10% non-response rate is 423.

Stool specimen collection and direct wet mount

A single stool specimen was collected from each patient. It was obtained from all assenting/permitting patients selected for the study. A direct saline and iodine wet mount of each sample was used to detect intestinal parasites microscopically. The wet mounts were examined under light microscope at 100X and 400X magnifications.

A small portion of the stool specimen was also preserved in 10% formalin for repeating the tests whenever required and further analysis [10].

Formol-Ether concentration method

A portion of each preserved stool specimen was taken and processed. Briefly, 1g of stool was placed in a clean conical centrifuge tube containing 7 mL 10% formol water by using applicator stick and shacked gently. The resulting suspension was filtered through a sieve into another conical tube. After adding 3-4 ml of diethyl ether to the formalin solution, the content was centrifuged at 3000 rpm for 1 minute. The supernatant was poured away and the tube was replaced in its rack. Finally smear was prepared from the sediment and observed under light microscope with a magnification of 100X and 400X [10].

Data Quality Assurance All laboratory analyses were carried out using standard operating procedures **Pre-analytical**: Adequate stool specimen (40 gram formed stool and 10 ml diarrheic stool) was collected using carefully labeled, dry, leak proof, grease free transparent stool caps. The specimen was kept free of contamination from water, soil, and urine. Specimens contaminated with water, urine and soil were rejected.

Analytical: Direct stool examination was performed within 30 minutes of collection and appropriate amount of stool sample was used to make a good smear devoid of air bubbles. After checking the contrast using 10X objective, the ova, larva, cyst and trophozoites of helminthes and protozoa were diagnosed by 40X objective. Each stool smears were examined for at least 10-15 minutes.

Post-analytical: All microscopic findings were encoded and reported appropriately.

Data analysis

The data entered in to Epi-Info 5.3.1soft ware and double checked before analysis and exported to SPSS version 21 for analysis. The descriptive statistics (means, percentages or frequency) have calculated & the bi-variant logistic regression analysis used to see the relation between dependent variable and independent variables. The

association has assessed using x2 –test. Variables that had a significant association have selected for further analysis using multiple logistic regression models with a p-value < 0.05 considered as statistically significant. Finally, the results presented on graphs and tables.

Ethical clearance

Ethical clearance was obtained from the Ethical Review Committee of Wolaita Sodo University. An Official letter was written from the Wolaita Sodo University to teaching referral hospital administrator and the hospital granted permission for study to be conducted. Written consent was obtained from the parents/guardians of each child. The objectives as well as the nature of the study were explained to the parents and study participants. Children positive for intestinal parasites were treated at the hospital according to national guidelines.

Result

Characteristics of the study participants

Out of 423 under five year children included in the study, a total of 410 children aged between 1month to 5 years presenting with diarrhea had participated in the study with a positive response rate of 96.9%, of which 234(57.1%) were male. The mean age is 2.96 with Std. Deviation of 1.459. Figure 1 shows sex distribution of study participants in different age categories.

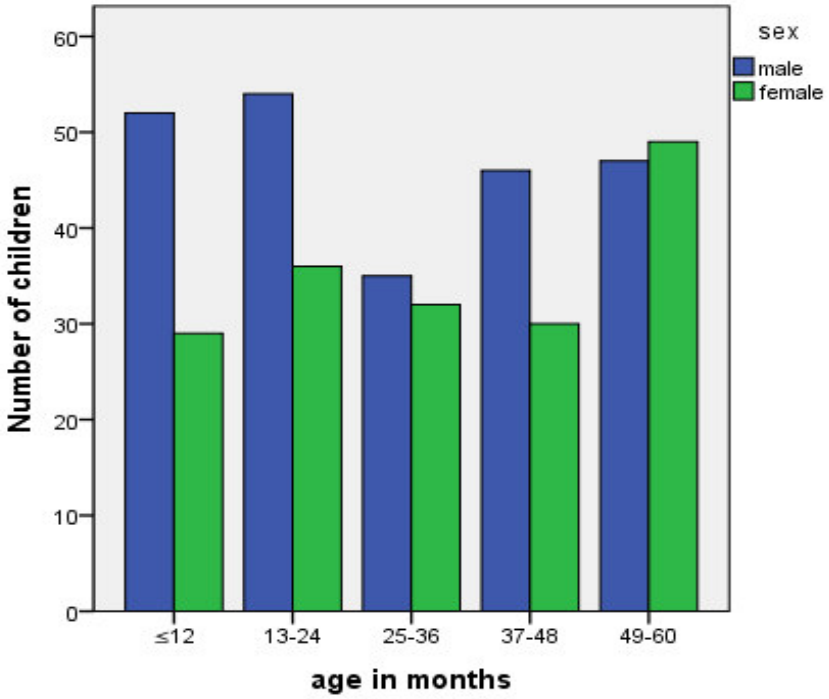
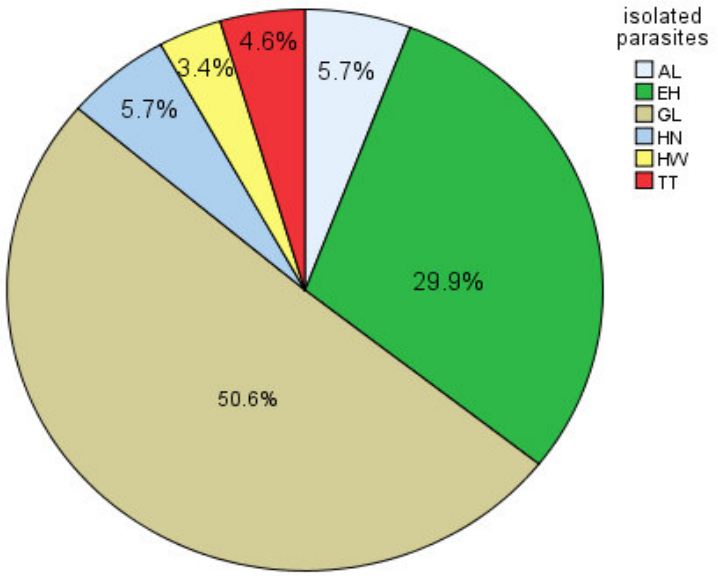


Figure 1. Age and sex distribution of the study participants at WSUTRH, 2016,wolaita Sodo Ethiopia

Prevalence of intestinal parasites

Twenty one percent, 87(21.2%) were positive for at least one intestinal parasite. Co-infection were observed in only 4(0.98%) of children. *G. lamblia* was the predominant parasite isolated 44(50.6%) followed by *E. histolytica/dispar/moshkovskii* 26(29.9%) (**Figure 2).**



Note- AL=*Ascaris lumbricoides*, **EH**= *Entamoeba histolytica*, **GL**=*Giardia lamblia*, **HN**= *Hymenolopsis nana*, **HW**= *Hook worm*, **TT**= *Trichuris trichiura*

Figure 2. Types and prevalence of intestinal parasites identified under five years of children presenting with diarrhea at WSUTRH, 2016, Wolaita sodo, Ethiopia

Table 1 shows distribution of each parasite among gender. Relatively higher prevalence of *G. lamblia*(11.9%) was observed in female children. Lowest prevalence of *H. nana* and *H. worm* were detected among female children with 0.6% prevalence each. *G. lamblia* showed 18.9% and 13.2% prevalence among age group of 49-60 and 37-48 months children respectively (Table 2). *T. trichuria* detected only from children aged from 13-24 months.

Table 1. Distribution of intestinal parasite species by sex among children under 5 years of age (n=410) with diarrheal diseases presenting to WSUTRH, 2016, Wolaita Sodo, Ethiopia

			Intestinal parasites					
			AL	**EH**	**GL**	**HN**	**HW**	**TT**
Sex	Male	Count	3	13	23	4	2	2
		%	1.3%	5.6%	9.8%	1.7%	0.9%	0.9%
	Female	Count	2	13	21	1	1	2
		%	1.1%	7.4%	11.9%	0.6%	0.6%	1.1%
Total		Count	5	26	44	5	3	4
		%	1.2%	6.3%	10.7%	1.2%	0.7%	1.0%

KEY- AL=*Ascaris lumbricoides*, **EH**= *Entamoeba histolytica*, **GL**=*Giardia lamblia*, **HN**= *Hymenolepis nana*, **HW**= *Hook worm*, **TT**= *Trichuris trichiura*

Table 2. Distribution of intestinal parasite by sex among 410 children below 5 years of age with diarrhoeal diseases presenting to WSUTRH, 2016, Wolaita Sodo, Ethiopia

			Isolated parasites					
			AL	**EH**	**GL**	**HN**	**HW**	**TT**
	≤12	Count	2	2	5	2	0	0
		%	2.5%	2.5%	6.2%	2.5%	0.0%	0.0%
	13-24	Count	0	6	3	0	1	4
		%	0.0%	6.7%	3.3%	0.0%	1.1%	4.4%
Age	25-36	Count	1	7	8	1	2	0
(months)		%	1.5%	10.4%	11.9%	1.5%	3.0%	0.0%
	37-48	Count	1	6	10	0	0	0
		%	1.3%	7.9%	13.2%	0.0%	0.0%	0.0%
	49-60	Count	1	4	18	2	0	0
		%	1.1%	4.2%	18.9%	2.1%	0.0%	0.0%
Total		**Count**	**5**	**25**	**44**	**5**	**3**	**4**
		%	**1.2%**	**6.1%**	**10.%**	**1.2%**	**0.7%**	**1.0%**

Note- AL=*Ascaris lumbricoides*, **EH**= *Entamoeba histolytica*, **GL**=*Giardia lamblia*, **HN**= *Hymenolepis nana*, **HW**= *Hook worm*, **TT**= *Trichuris trichiura*

Associated factors of intestinal parasitic infections

Out of the total 87 isolated parasites, 47 were from male children and the remaining 40 parasites were isolated from female children (Table 1). There is no statistical significant association between gender and parasite infection (P=0.52). Children aged between 25-36 months showed higher intestinal parasites load with the prevalence of 28.4% (Table 3). On the other hand, lower prevalence rate was observed in children ≤12 month of age (13.6%). There was no statistical significance association observed between age and parasite infection (P=0.106).

Socio-demographic characteristics		**Intestinal parasites**		**X^2 and P value**
		Negative n(%)	**Positive n(%)**	
sex	male	187(79.9%)	47(20.1%)	X^2=8.727
	female	136(77.3%)	40(22.7%)	P= .206
	≤12	70(86.4%)	11(13.6%)	
Age in months	13-24	76(83.5%)	15(16.5%)	X^2=5.315
	25-36	48(71.6%)	19(28.4%)	P= .091
	37-48	59(77.6%)	17(22.4%)	
	49-60	70(73.7%)	25(26.3%)	
Patient type	Outpatient	226(79.0%)	60(21.0%)	X^2 =4.981
	Inpatients	97(78.2%)	27(21.8%)	P=0.131
Residence	Urban	113(80.7%)	27(19.3%)	X2=45.1
	Rural	210(77.8%)	60(22.2%)	P=<0.001*
Parent education	Illiterate	158(81.0%)	37(19.0%)	X2 =3.261
	Literate	165(76.7%)	50(23.3%)	P=0.521

Discussion

A total of 21.2% prevalence of intestinal parasite in this study was comparable with studies conducted in Ethiopia and Senegal with isolation rate of 24.3%[9], 26.6%[11] and 26.2%[12].On the contrary to this, higher prevalence rate,36.5%[13],85.1%[14] and 41% [15], were noted in other studies conducted in south Ethiopia and Tanzania.

The lower rate of infection for this study may be due to improvement in sanitary environment and socioeconomic conditions though time and majority of the study participants are urban dwellers. However this finding is higher than the prevalence reported in Gondar Ethiopia (17.3%) and Nepal (10.7%) [8, 16].

G. lamblia and *E. histolytica/dispar/moshkovskii* were the two predominantly isolated parasites in this study. This finding was corroborated with previous finding [11]. Their cysts are highly resistant to environmental conditions, being able to survive in the environment for long periods of time. Acknowledging the

resilience of these cysts, it is possible that protozoan infections are much more frequent in poor settings than estimated, even in the absence of reported outbreaks and epidemiological surveys [17].

Giardiasis is a common childhood infection globally with reported prevalence, sources and modes of transmission varying among children from different geographic locations. In our finding, *G. lamblia* was the predominant parasite isolated with the prevalence of 10.8%. Giardiasis may produce severe acute diarrhea in children less than five years of age with chronic infections resulting in weight loss and growth retardation. *E. histolytica/dispar/moshkovskii* was the second most prevalent parasite isolated. Children with *E. histolytica*-associated diarrhoeal illnesses are more likely to be malnourished and stunted as documented by Mondal et al. [18].

Although age is not significantly associated with IPI in our finding, children aged between 25-60 months were the highest Giardiasis and Amebiasis infected group with the prevalence of 18.9%. Similarly, previous study from Kenya reported *Giardia lamblia* and *E. Histolytica* infections increased significantly with age with the most affected age group being the 37–48 months [17]. Considering that these children live in slum areas where the sanitation is poor, with open sewers, and limited access to clean water, they normally play in the soil which harbors these parasites and are less mindful of important personal hygiene practices such as washing of hands with soap and water before eating, after playing and after visiting the toilets.

Lower prevalence of helminthic infection observed in this study *Ascaris lumbricoides*(1.2%)**,** *Hymenolepis nana*(1.2%), *Hook worm*(0.7%), *Trichuris trichiura*(1%).This could be reason out by the ongoing deworming program of under-fives by the Ministry of Health (MoH) in the study area.

Conclusion and recommendation

In conclusion, the present study shows that intestinal parasitic infection, predominantly *G. lamblia* and *E. histolytica/dispar/moshkovskii*, were relatively prevalent among under-five children in the study area. Studies on causative organisms of childhood diarrhea should be carried out regularly and microbe-specific intervention strategies should be carried out for the control of childhood diarrhea. In addition early prevention method measures should be taken like provision of safe water supply and latrines, improvement of sanitation and health education on personal and environmental hygiene of the community.

Declarations

Ethical Considerations

The proposal was approved by the ethical review committee of Wolaita Sodo University. An Official letter was written from the university to Wolaita Sodo University teaching referral hospital administrator and the hospital granted permission for sample collection. The result of the study was communicated to the responsible bodies for any beneficiary or corrective measures.

Competing interest

All authors declare that they have no competing interest

Availability of data and materials

Anyone interested in the full data in excel format can have a data by writing to fisehawadilo@yahoo.com

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Author's contribution

FS: Conceived the study, FS, FW: Participated in the design of the study and performed the statistical analysis, FS, FW: Interpreted the data: FS: Obtained ethical clearance and permission for study: FW: Supervised data collectors: FS, FW: Drafting the article or revisiting it critically for important intellectual content. All authors read and approved the final manuscript.

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