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Prevalence of Intestinal Parasites, and its Association with Severe Acute Malnutrition Related Diarrhoea

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Abstract
Intestinal parasites contribute to undernutrition with or without overt diarrhoea and diarrhoea remains one of the commonest illnesses leading to high mortality rates in children with severe acute malnutrition (SAM). The objective of this study was to determine the prevalence of intestinal parasites and its association with diarrhoea in children admitted with SAM. This cross-sectional study enrolled severely acute malnourished children 6-59 months. SAM was diagnosed basing on a very low weight for height (below -3 z scores of the median WHO growth standards) or mid upper arm circumference below 11.5cm (MUAC<11.5 cm) or presence of bilateral pitting edema based on WHO definition of SAM. From each child, fecal samples were also collected and screened for presence of intestinal parasites using direct microscopy and modified Ziehl-Neelsen methods. Bivariate and multivariate logistic regressions were used in data analysis. The overall prevalence of intestinal parasites was 32.8%. The prevalence of protozoa (20.9%) was higher than and helminth (13.9%) infections (p=0.354). *Giardia lamblia* had the highest prevalence at 15.4% followed by *hookworm* at 9%. All other intestinal parasites like *Entamoeba histolytica*, *Cryptosporidium species*, *Entamoeba coli*, and *Isospora species*, *Ascaris lumbricoides*, *Hymenolepis nana*, Schistosoma mansoni, *Trichuris trichura*, *Strongyloides stercoralis* and *Taenia species* had a prevalence ranging from 2.5 to 5%. Adjusted logistic regression analysis showed that children presenting with *Giardia lamblia* were 3.53 times most likely to present with diarrhoea (95% CI: 4.15-142.3, p<0.0001) and 19.8 times at risk of having chronic diarrhoea (95% CI: 7.3-53, p<0.0001) after controlling for age, sex, HIV status and fever. All other parasites were not significantly associated with any diarrhoea. Children with fever were 2.12 times likely to present with diarrhoea in adjusted logistic analysis (95% CI: 1.1 - 4.0, p= 0.02). The results suggest that intestinal parasitic infections are highly prevalent in SAM, and giardiasis is associated with SAM related diarrhoea, chronic type in particular. This implies that successful routine intestinal parasite screening could increase the diagnostic rate of parasitic diarrhoea and improve the treatment and prevention strategies of diarrhoea in SAM children.
Keywords: Severe acute malnutrition, diarrhoea, intestinal parasites

1. Introduction

Severe acute malnutrition (SAM) and diarrhoea remain leading causes of child morbidity and mortality in low income countries. Diarrhoea is the commonest illnesses leading to high mortality rates in severely acute malnourished children, despite following the World Health Organization guidelines for the management of diarrhoea in SAM (Irena et al. 2011). Diarrhoea and SAM are interconnected, in that one can be a cause or a consequence of the other (Brown 2003b). While the role of intestinal infections with or without overt diarrhea as a cause of under-nutrition in low income countries is well documented (Guerrant et al. 1992; Guerrant et al. 2009; Cervantes & San 2011; Brown 2003b), specific intestinal parasitic infection contribution to diarrhoea in severely malnourished children remains uncertain. However, comprehensive studies on the prevalence of intestinal parasites and its contribution to diarrhoea in severely acute malnourished children have not been conducted in Uganda. Intestinal parasitic diarrhoea has been previously recognized in well-nourished children but not in malnutrition (Ansari et al. 2012). Although Tumwine and colleagues did an intestinal parasitic study in Uganda, they only studied one parasitic species (*Cryptosporidium parvum)* in acute malnutrition (Tumwine et al. 2003), but not in severe acute malnutrition. The prevalence of intestinal parasitic infections is probably underestimated in SAM where many medical doctors do not routinely request for parasite stool testing, instead drugs mebendazole or albendazole are routinely given as blind treatment for helminth infections to all severely acute malnourished children (MOH Uganda 2010), though such treatment is not effective to all intestinal parasites including protozoa, Teania species and *Schistosoma mansoni*. This study investigated the prevalence of intestinal parasites and its association with diarrhoea in severe acute malnutrition.

2. Methods

2.1 Study setting

This study was conducted in the pediatric nutrition unit also called Mwanamugimu Nutrition Unit (MNU) of Mulago hospital, Kampala, Uganda's national referral and teaching hospital for Makerere University college of

health sciences; situated in the central region of the country. The unit admits and treats only children with SAM, with a mean monthly admission of 60 children, of which half of them present with diarrhoea (Nyeko et al. 2010).

2.2 Study participants, sample size and sampling strategy

This cross sectional study recruited severely acute malnourished children 6-59 months old admitted to the MNU inpatient unit**.** Severe acute malnutrition was diagnosed basing on a very low weight for height (below -3 z scores of the median WHO growth standards) or mid upper arm circumference below 11.5cm (MUAC<11.5 cm) or presence of bilateral pitting edema based on WHO definition of SAM. The formula below was used to calculate sample size.

$$N=\frac{z^2pq}{d^2}$$

Where N = minimum sample size, z = Standard deviation score at 95% = 1.96, p = Prevalence of intestinal parasites = 12% = 0.12 and q = Complimentary Probability (1- p) = 1- 0.12 = 0.88

d = Error Margin = 5% = 0.05

Substituting: $N = \frac{(1.96)^2 \times 0.12 \times 0.88}{(0.05)^2} = 162$

Assuming the prevalence of intestinal parasites to be 12% (Mcelligott et al. 2013), and a margin error of 5% and 95% confidence, a minimum sample size of 162 was calculated for determining the prevalence of intestinal parasites. Hence, I expected 12% prevalence with an error d of 5% for me to be confident that the prevalence of intestinal parasites will be from 7-17%. Participants whose caretakers were willing to provide their fecal samples were recruited into the study. Participants were consecutively enrolled until the required minimum sample size of 162 was obtained. The recruitment was conducted from 1st February to 30th April 2014.

2.3 Data collection procedure

Anthropometric measurements were taken by the principal investigator and trained nutritionists at the time of admission. Anthropometric measurements of mid-upper arm circumference (MUAC), height and weight were undertaken following the World Health Organization (WHO) guidelines and analyzed using AnthroPlus software.

2.4 Weight measurement

Weight of each naked child was measured to the nearest 100 grams using a UNISCALE. The average of the three measures in closest agreement was recorded.

2.5 Height/length measurement

The length for children aged below 2 years and height for children 2 years and above were measured using a stadiometer(height and length board) as described by the Uganda guidelines (MOH Uganda 2010). Length or height was measured to the nearest centimeter. The child stood or laid with back against the board, his/her heels, buttocks, shoulders and head touching a flat upright sliding head piece. The child's legs were placed together with the knees and ankles brought together. The headpiece was brought down onto the upper most point on the head and the height/length recorded to the nearest 0.1 cm at the examiner's eye level.

2.6 Measurement of mid upper arm circumference (MUAC)

The MUAC was taken using the MUAC tapes recommended by WHO and UNICEF. The MUAC was measured on the left arm at the level of the upper arm midpoint mark. The measurement was then taken to the nearest 0.1 cm.

2.7 Identification of pitting oedema

Bilateral pitting oedema is defined as the presence of a condition characterized by an excess of watery fluid collecting in the cavities or tissues of the body. Bilateral oedema in both feet (below the ankles) is mild or grade1; oedema in both feet and legs (below the knees) is moderate or grade 2 and severe or grade 3 if it is generalized oedema including both feet, legs, hands, arms and face. In order to diagnose bilateral pitting oedema, a gentle thumb pressure was applied on both feet for 3 to 5 seconds, in oedema the depression remained for some time if the thumb is withdrawn as described in the Ugandan guidelines (MOH Uganda 2010).

2.8 Identification of Severe Acute Malnutrition

Severe Acute Malnutrition (SAM) was assessed by determining Weight-for-Length/Height Z score (WLZ or WHZ) through comparing weight and height/length with the weight and height/length of the World Health Organization (WHO) reference population of the same age and sex. Children had SAM if WHZ was below -3SD or MUAC less than 11.5cm or had bilateral pitting edema of any grade.

2.9 Collection of stool samples and detection of intestinal parasites

Stool samples were collected from each child on admission and put in labeled and securely closed sterile containers. The caretakers were adequately instructed on how to get a little portion of their children's stool into the bottles. The fresh stool samples collected on each occasion were immediately examined in the MNU laboratory by a trained laboratory technician without preservation. Each fecal sample was examined as a smear stained with Lugol's iodine, as a direct wet smear in physiological normal saline and by modified Ziehl-Neelsen (ZN) staining method as described by Cheesbrough (Cheesbrough 2006) for identification of *Cryptosporidium spp*. and other protozoa. A giemsa of field's stained fecal smear was used to detect *Giardia lamblia* trophozoites. Diagnosis was based on the identification of helminth ova and protozoan trophozoites in the sample during microscopic analysis. The outcome variable was stool parasite status of the selected child, whether positive or negative for any intestinal parasite, which was determined from a stool sample. A child was considered to have a mixed infection if they were found to be positive for both helminths and protozoa.

2.1.0 Other measurement variables

A questionnaire with social demographic information was filled in an interview with children's parents/caretakers, in order to capture data on feeding practices, mothers education, fever (presence and duration), water source safety, fecal disposal practices, and diarrhoea (presence and duration). The HIV status data of recruited children was extracted from their medical records. HIV status was determined using routine antibody tests (DETERMINE1/2, START PARK and UNIGOLD) for children above 18 months and DNA PCR for those below 18 months.

2.1.1 Quality control

Training of research assistants and pre-testing of tools were done prior to the study. Weighing scales were checked and validated with standard weights every other day before actual weighing of the children commenced.

2.1.2 Data management and statistical analysis

During data collection, completed questionnaires were checked regularly to rectify any discrepancy, logical errors or missing values. For anthropometric data analysis, standard deviation (Z-scores) scores were obtained by WHO AnthroPlus software, version 3.2.2. Data were captured using Excel and analyzed using STATA version 12. Binary outcome variable (presence or absence of diarrhoea) was created. Exposure factors used included age, sex, HIV status, intestinal parasites, fever on admission, water safety, fecal disposal, and feeding options. Categorical variables are presented as numbers and percentages while continuous variables as ranges. Chi square test was used to examine differences for proportions and Odds Ratio (OR) calculated by logistic regression was presented to determine risk factors for diarrhoea. The association between independent variables and diarrhoea was tested using multivariate logistic regression. The level of statistical significance was set as p value less than 0.05 and for each statistically significant factor, an odds ratio and 95% confidence interval (CI) was computed by logistic regression analysis.

2.1.3 Ethical considerations

Permission to carry out the study was granted by the Uganda National Council for Science and Technology (UNCST). Prior to any enrollment of the children, permission was sought and obtained from caretakers/mothers. Caretakers/mothers had the right to accept or refuse their children to join the study without any consequences. Children who were found to have intestinal parasites got the appropriate treatment.

3. Results

3.1 Description of the study sample

Of the 203 children recruited for the study, 2 were excluded because of failure to provide stool specimens. For this reason a total of 201 children were included in the study. Of these, 107 (53.2%) were females, the mean (range) age of participants was 17.3 (6.0-54.1) months. 119 (59.2%) children had edematous malnutrition, of which 21 (17.5%), 29 (24.2%) and 69 (58.3%) had grade one, two and three oedema respectively. 101 (50.3%) of the study children had diarrhoea on admission. Of the diarrheic children, 54 (53.5%) had chronic diarrhoea while 47 (46.5%) had the acute type of diarrhoea. 9 (9.5%) of the recruited children were HIV positive, 42 (20.9%) and 159 (69.1%) were mixed and replacement fed respectively. 123 (61.2%) children had fever on admission. 176 (87.6%) and 75 (11.4%) of mothers/caretakers had access to safe (protected spring/well or tap water) and unsafe water for home use respectively, while 176 (87.6%) of mothers/caretakers reported to have adequate fecal disposal accessories (pit latrine or toilet). 186 (92.5%) of mothers /caretakers had attained some form of formal education (completed at least primary, secondary, high school or tertiary education).

3.2 Prevalence of intestinal parasites

Table 1 shows the prevalence of intestinal parasites. Sixty six (32.8%) of the study individuals were found to have at least a single intestinal parasitic infection. Four (2%) had a mixed (protozoa and helminth) infection. The intestinal protozoan infections were higher at 20.9 % in 42 positive samples compared to helminths at 13.9% in 28 positive samples but the difference was not significant (p=0.354). Different protozoans and helminths were detected from stool samples of study individuals. In the protozoa group, *Giardia lamblia* (*G. lamblia*) was the most common intestinal parasite (IP) with 31 positive samples (15.4%), followed by *Entamoeba histolytica* (*E. histolytica*) in 5 samples (2.5%), *Cryptosporidium species* (*Cryptosporidium spp)* in 3 samples (1.5%), *Entamoeba coli* (*E.coli*) in 2 samples (1%) and *Isospora species (Isospora spp)* in 1 sample (0.5%), in that order.

In the helminths group, hookworm was the most common IP present in 18 samples (9.1%), followed by *Ascaris lumbricoides* (*A. lumbricoides*) in 3 samples (1.5%). *Hymenolepis nana* (*H. nana*) and *Schistosoma mansoni* (*S.mansoni*) each were present in 2 samples (1%), while *Trichuris trichura* (*T.trichura*), *Strongyloides stercoralis* (*S.stercoralis*) and *Taenia species* (*Taenia spp*.) each were present in only 1 sample (0.5%).

Table 1: Prevalence of intestinal parasites among 201 children admitted with SAM at MNU, Mulago hospital

Variable	Parasite Positive, n	Prevalence %	x^2	P -value
Participants (n=201)	66	32.8		
Sex				
Boys(n=94)	29	14.4	3.41	0.065
Girls(n=109)	37	18.4		
Age, months				
6-<12 (n=57)	18	9.0	0.057	0.811
12-59 (n=44)	48	23.9		
Helminth	28	13.9		
Hookworm	18	9.0		
A.lumbricoides	03	1.5		
H.nana	02	1.0		
S.stercoralis	02	1.0		
S. mansoni	02	1.0		
T. trichuris	01	0.5		
Taenea spp	01	0.5		
Protozoa	42	20.9		
G.lamblia	31	15.4		
E. histolytica	05	2.5		
Isospora	01	0.5		
Crysporidum spp	03	1.5		
E. coli	02	1.0		
Mixed infection	04	2.0		

3.3 Association of intestinal parasites and diarrhoea

Out of the 201 study individuals, 101 (50.3%) had diarrhoea. Of the 66 samples that were positive for at least one intestinal parasite, 47 (69.7%) presented with diarrhoea. The association of all diarrhoea with different intestinal parasites is shown in tables 2 and 3. In unadjusted (bivariate) analysis (table 2), children who had parasites in their stool were 3.14 times more likely to present with diarrhoea compared to those with negative stool samples (95 CI: 1.69-5.85, p<0.0001).

Children with intestinal protozoan infections were 4.17 times at risk to present with diarrhoea (95% CI: 1.92-9.07, p<0.0001) compared to children presenting with helminths at a non-significant risk (OR 1.64, 95% CI: 0.72-3.69, p=0.236). *Giardia lamblia* was the only single intestinal parasite that was significantly associated with diarrhoea in unadjusted analysis, children with giardiasis were 12.4 times likely to have diarrhoea (95% CI: 3.63- 42.38, p<0.0001). All other intestinal parasitic infections were not significantly associated with diarrhoea in unadjusted analysis. Adequate fecal disposal methods and safe water for home use are protective against all diarrhoea though the protection is not significant in unadjusted logistic regression analysis (OR 0.53, 95% CI: 0.22-1.25, p= 0.14).

Table 2: Bivariate logistic regression analysis of factors associated with all diarrhoea among 201 SAM children admitted in MNU Mulago hospital

Variable	All Diarrhoea		OR	95% CI	P value
	Yes n (row %)	No n (row %)			
Sex					
Girls (n=107)	53 (49.5)	54 (50.5)	0.94	0.54-1.64	0.829
Boys (n=94)	48 (51.1)	36 (48.9)			
Age group, months					
6-<12 (n= 57)	26 (45.6)	31 (54.4)	0.77	0.41-1.43	0.408
12-59 (n= 144)	75 (52.1)	69 (47.9)			
Parasite	46 (69.7)	20 (29.3)	3.14	1.69-5.85	<0.0001*
Helminth	17 (60.7)	11(39.3)	1.64	0.72-3.69	0.236
Hookworm	11 (61.1)	8 (38.9)	1.63	0.60-4.37	0.334
A.lumbricoides	3 (100)	0 (0)	1.0		0.082
H.nana	2 (100)	0 (0)	1.0		0.157
S.stercoralis	1 (100)	0 (0)	1.0		0.319
S. mansoni	0 (0)	2 (100)	1.0		0.153
T. trichura	0 (0)	1(100)	1.0		0.314
Tinea spp	1(100)	0 (0)	1.0		0.319
Protozoa	32 (76.2)	10 (23.8)	4.17	1.92-9.07	<0.0001*
G.lamblia	28 (90.3)	3 (9.7)	12.4	3.63-42.38	<0.0001*
E. histolytica	2 (40.0)	3 (60)	1.65	0.11-3.99	0.643
Isospora	1 (100)	0 (0)	1		0.314
Cryptosporidium spp	2 (66.7)	1 (33.3)	2	0.18-22.4	0.567
E. coli	0 (0)	2 (100)	1		0.319
Fecal disposal					
Adequate (n=176)	85 (48.3)	91 (51.7)	0.53	0.22-1.25	0.141
Not adequate (n=25)	16 (64.0)	9 (36)			
Water source					
Safe (n=176)	85 (48.3)	91 (51.7)	0.53	0.22-1.25	0.142
Unsafe(n=25)	16 (64.0)	9 (36)			
Feeding					
Replacement (n=159)	80 (50.3)	79 (49.7)	1.01	0.51-1.20	0.971
Mixed (n=42)	21 (50.0)	21 (50)			
Fever					
Yes (n=123)	68 (55.3)	55 (44.7)	1.69	0.95-2.99	0.074
No (n=78)	33 (42.3)	45 (57.3)			
HIV status					
Negative (n= 182)	88 (48.4)	94 (51.6)	2.31	0.84-6.35	0.096
Positive (n=19)	13 (68.4)	6 (31.6)			

*Key: OR: Odds Ratio; n=number; * statistically significant (P<0.05)*

Any potential confounder that had at least a modest ($P < 0.10$) association with both diarrhoea and the predictor of interest, positive IPs with at least a number above 5, age and sex were included in final multivariate logistic models (tables 3 and 5). In multivariate (adjusted) logistic regression analysis (table3), *Giardia lamblia* remained highly significantly associated with all diarrhoea after controlling for age, sex, HIV status, and fever (AOR 3.53, 95% CI:4.15-142.3, p<0.0001). Children with fever were 2.12 times likely to present with all diarrhoea in adjusted analysis (95% CI: 1.1 - 4.0, p= 0.02).

3.4 Association of intestinal parasites and chronic diarrhoea

Tables 4 and 5 show the association of intestinal parasites and chronic diarrhoea. 101 of the study children presented with diarrhoea, of these 54 (53.5%) had chronic diarrhoea while 47 (46.5%) had acute diarrhoea on the time of admission.

Children who had Giardia lamblia were 16 times more likely to have chronic diarrhoea (95% CI: 6.31-40.54, P<0.0001) in unadjusted analysis (table 4). In adjusted analysis (table 5), Giardia lamblia remained highly significantly associated with chronic diarrhoea (AOR 19.8, 95% CI:7.3-53, p<0.0001) after controlling for age, sex, and HIV status (table 5). All other intestinal parasites were not significantly associated with chronic diarrhoea in both unadjusted and adjusted analysis.

Table 3: Multivariate logistic regression analysis of factors associated with all diarrhoea among 201 SAM children admitted in MNU Mulago hospital

Variable	All Diarrhoea		AOR	95% CI	P value
	Yes n (row %)	No n (row %)			
Sex					
Girls(n=107)	53(49.5)	4 (50.5)	1.35	0.71-2.55	0.356
Boys(n=94)	48 (51.1)	36 (48.9)			
Age group, months					
6-<12, (n= 57)	26 (45.6)	31(54.4)	0.67	0.33-1.33	0.254
12-59, (n= 144)	75 (52.1)	69 (47.9)			
Parasite	46 (69.7)	20 (29.3)	6.1	0.04-9.21	0.726
Helminth	17 (60.7)	11(39.3)	2.66	0.13-53.6	0.236
Hookworm	11 (61.1)	8 (38.9)	1.27	0.22-7.16	0.786
Protozoa	32 (76.2)	10 (23.8)	1.1	0.06-15.2	0.997
G.lamblia	28 (90.3)	3 (9.7)	3.53	4.15-142.3	<0.0001*
Fever					
Yes (n=123)	68 (55.3)	55 (44.7)	2.12	1.1-4.0	0.02*
No (n=78)	33 (42.3)	45 (57.3)			
HIV status					
Negative (n= 182)	88 (48.4)	94 (51.6)	2.31	0.7-7.6	0.1
Positive (n=19)	13 (68.4)	6 (31.6)			

Key: AOR: Adjusted Odds Ratio; n=number; * statistically significant (P<0.05)

Table 4: Bivariate logistic regression analysis of factors associated with chronic diarrhoea in study children with diarrhoea in Mulago hospital nutrition Unit.

Variable	Diarrhoea		OR	95% CI	P value
	Chronic n (row %)	Acute n (row %)			
Sex					
Girls (n=53)	30 (56.6)	23 (43.4)	1.14	0.61-2.13	0.689
Boys (n=48)	24 (50)	24 (50)			
Age group, months					
6-<12 (n= 26)	15 (57.7)	11(42.3)	0.96	0.48-1.93	0.96
12-59 (n= 75)	39 (52)	36 (48)			
Intestinal parasites					
Hookworm (n=11)	6 (54.5)	5 (55.5)	1.4	0.50-3.95	0.516
A.lumbricoides (n=3)	2 (66.7)	1 (33.3)	5.6	0.49-63.2	0.082
H.nana (n=2)	2 (100)	0 (00)	2.8	0.17-44.8	0.157
S.stercoralis (n=1)	0 (0)	1(100)	1		0.319
Tinea spp (n=1)	0 (0)	1(100)	1		0.319
G.lamblia (n=28)	24 (85.7)	4(14.3)	16	6.3- 40.5	<0.0001*
E. histolytica (n=2)	1 (50)	1(50)	1		
Isospora (n=1)	0 (0)	1(100)	1		
Cryptosporidium spp (n=2)	0 (0)	2 (100)	1		
Fecal disposal					
Adequate (n=85)	46 (54.1)	39 (55.9)	0.75	0.30-1.86	0.537
Not adequate (n=16)	8 (50)	8 (50)			
Water source					
Safe (n=85)	47 (55.3)	38 (54.7)	1.1	0.42-2.71	0.891
Unsafe (n=16)	07 (43.8)	9 (46.2)			
Feeding					
Replacement (n=80)	43 (53.8)	37 (52.2)	1.04	0.48-2.26	0.971
Mixed (n=21)	11 (52.4)	10 (47.6)			
Fever					
Yes (n=68)	37 (54.4)	31(53.6)	1.54	0.79-2.99	0.912
No (n=33)	17 (51.5)	16 (58.5)			
HIV status					
Negative (n= 88)	46 (52.3)	42 (47.7)	2.15	0.81-5.67	0.122
Positive (n=13)	8(61.5)	5 (38.5)			

Key: OR: Odds Ratio; n=number; * statistically significant (P<0.05)

Table 5: Multivariate logistic regression analysis of factors associated with chronic diarrhoea in study children admitted with diarrhoea in Mulago hospital nutrition unit.

Variable	**Diarrhoea**		**AOR**	**95% CI**	**P value**
	chronic n (row %)	Acute n (row %)			
Sex					
Girls (n=53)	30 (56.6)	23 (43.4)	1.9	0.9-4. 1	0.11
Boys (n=48)	24 (50)	24(50)			
Age group, months					
6-<12 (n= 26)	15 (57.7)	11 (42.3)	0.8	0.3-1.8	0.55
12-59 (n= 75)	39 (52)	36 (48)			
Intestinal parasites					
Hookworm (n=11)	06 (54.5)	5(55.5)	2.1	0.6-6.8	0.20
G.lamblia (n=28)	24 (85.7)	4(14.3)	19.8	7.3-53.4	<0.0001*
HIV status					
Negative (n= 88)	46 (52.3)	42 (47.7)	2.15	0.8-8.3	0.11
Positive (n=13)	08 (61.5)	5(38.5)			

Key: AOR: Adjusted Odds Ratio; n=number; * statistically significant (P<0.05)

4. Discussion

Intestinal parasitic infections remain leading causes of child undernutrition in low income countries, while diarrhoea in SAM is unacceptably killing many children in low income countries. Various studies in low income countries have shown intestinal parasites as risk factors of diarrhoea (Perera et al. 1999; Tinuade et al. 2006) and under nutrition (Mondal, Dinesh 2012; Lani S Stephenson 1994). The present study attempted to determine the prevalence of intestinal parasites in children admitted with SAM and their association with diarrhoea. Findings from this current study showed the occurrence of several intestinal parasites of nutritional and diarrhoea importance. The overall prevalence of intestinal parasites was 32.8%. The prevalence of intestinal parasites revealed in this study is higher than that reported among well-nourished under-fives in Kenya of 25.6% (Mbae et al. 2013). One explanation for the higher prevalence of intestinal parasites in SAM children is lower immunity in SAM compared to well-nourished children. There is always a reduction in T-lymphocyte function in SAM (Jassim 2005; Suliman et al. 2011; Chandra 1983) and the number of cells that produce IgA in the intestinal mucosa is greatly reduced (Kakai 1994; Jassim 2005; Suliman et al. 2011). A previous study, showed scarcity of intraepithelial lymphocytes in the intestinal mucosa of SAM children when intestinal parasites were found to be in contact on the same mucosal biopsy specimen (Green & Heyworth 1980b). This reduction in intestinal mucosa host defenses, which is common in SAM children, increases the pathogenic power of the intestinal parasites to adhere on the intestinal mucosa (Gendrel et al. 1992).

The lowest prevalence of helminthic infections as compared to protozoa infections in this study could be attributed to the Uganda's national policy of bi-annual deworming of all Ugandan children using anthelmintic drugs like mebendazole or albendazole, this program has been running in Uganda since 2003 (Lwanga et al. 2012). However, these drugs are not sensitive to other helminthic infections like *Schistosoma mansoni,* though it contributed to only 1% prevalence in this study.

The prevalence of giardiasis (20.9%) seen in this study is slightly lower than the 24.9% and 29.8% prevalence previously reported in undernourished Kenyan and Pakistan children respectively (Mehraj et al. 2008; Mbae et al. 2013). The reasons for this are not clear but it is possible that the application of direct microscopic examination of feces technique in the current study could have contributed to the lower detection rate. The direct examination of feces method detects motile trophozoites in diarrheic samples but not in more formed stool specimens. Furthermore, trophozoites of *Giardia lamblia* often attach themselves on intestinal walls; therefore several specimens are needed to increase the detection rate of trophozoites. However, we only investigated one stool sample for trophozoites in this study whereas the Malaysian and Pakistan study used both direct and concentration techniques to detect trophozoites and cysts with at least three fecal samples before reporting negative results.

The 50.2% prevalence of diarrhoea reported in this study population is nearly similar to that previously reported among SAM children in India (Rakesh Kumar, Jyoti Singh, Karan Joshi 2013) and Kenya (Talbert et al. 2012) but lower than the 67 % prevalence reported in Zambia (Irena et al. 2011). However, all these studies are in agreement that diarrhoea is a leading co-infection in SAM. Evidence of diarrhoea and its chronicity occurred more frequently in children with giardiasis than any other intestinal parasite, a finding consistent with that by Gendrel and colleagues (Gendrel et al. 2003). This might not be surprising and seems to reinforce the fact that diarrhoea due to giardiasis is symptomatic in immune compromised individuals like SAM children compared to

well-nourished individuals. SAM children tend to have significantly decreased anti-giardia secretory IgA in their intestinal mucosa (Suliman et al. 2011; Hughes & Kelly 2006; Green & Heyworth 1980a; Jassim 2005; Kakai 1994; Faubert et al. 2000), hence rendering overt diarrhoea in this population. In a study of infants less than 2 years of age in Libreville, Gendrel and colleagues showed that the rate of infection with parasites among well-nourished and SAM children was the same, but diarrhea caused by *Giardia lamblia* was common and chronic in the SAM children, while well-nourished children remained non diarrheic carriers (Gendrel et al. 2003). Furthermore, there is convincing evidence that *Giardia lamblia* invades and destroys the micro villi and villi of the small intestines to cause electrolyte, nutrient and water malabsorption which manifest as diarrhoea (Halliez & Buret 2013) as revealed in this present study.

The significant association of giardiasis with chronic diarrhoea in this study is in agreement with previous studies from Africa and Asia (Bhandari et al. 1999) which confirm that diarrhoea caused by *giardiasis* is particularly chronic and recurrent. Most children with acute dehydrating diarrhea can be managed with proper attention to fluid and electrolyte replacement, but those with chronic diarrhoea syndromes, often signal a path towards malnutrition, wasting and underweight (Lima et al. 2000; Black 1993).

The significant association between giardiasis and diarrhoea echoes negative effects on nutritional status because *Giardia lamblia* as cause of recurrent and prolonged form of childhood diarrhoea is well recognized (Muhsen & Levine 2012; Mondal, Dinesh 2012). It is important to mention that giardiasis alone (Carvalho-costa et al. 2007) has been linked to wasting while persistent diarrhoea due to giardiasis or any other cause has been consistently linked to undernutrition (Black et al. 1984; Brown 2003a; Cervantes & San 2011; David & Lobo 1995; Gracey 1995; Guerrant et al. 1992) and mortality in children with severe acute malnutrition (Irena et al. 2011; Talbert et al. 2012).

Although hookworm infestation was not significantly associated with diarrhoea in this present study, it contributed a prevalence of nearly14%. This prevalence is higher than the 10% prevalence previously reported in Ugandan school going children (Lwanga et al. 2012). The possible explanation is that the Uganda's national bi-annual anthelmintic campaign targets children from one year and above, including school going children. However this present study included a proportion of children from 6 months to less than one year, who are not a target group in the Uganda's national deworming campaign. Although this high hook worm prevalence seen in SAM children was not significantly associated with diarrhoea, it is of nutritional importance, since hookworm infestation has been consistently linked to iron deficiency anemia and poor growth (Easwaran, Variyam and John 1982; Lani S Stephenson 1994) and yet anemia is among the complications of SAM. However, hook worms may not impact much, since all SAM children are blindly dewormed using anthelmintic drugs (albendazole or mebendazole) during the rehabilitation phase as recommended by the Uganda guidelines for the management of SAM (MOH Uganda 2010). This blind treatment is important because of the convincing evidence that anthelmintic drug treatment improves nutritional status and linear growth (Hlaing 1993; Hall et al. 2008; Dickson et al. 2000).

Fever was significantly associated with diarrhoea in adjusted multivariate logistic regression analysis (table 3); this finding is similar to previous studies in Africa (SN,Okolo , C 2006; Suliman et al. 2011; Kakai 1994). One of the possible explanations is that fever is a possible cofounder. It is important to mention that SAM children usually have co- infections like malaria, viral and bacterial enteric infections (Anon 1998; Brown 2003b; Burpee & Duggan 2008) which might present with symptoms of fever and cause diarrhoea as well. However, although 55% of the study children presented with fever, we did not examine for its potential causes in this present study due to financial and time constraints.

4.1 Strengths and limitations

There are several strengths in this study. This study is unique in its nature; it investigated various intestinal parasites including both helminthic and protozoa infections with diarrhoea and SAM. Previous studies in Uganda and other low income countries have only investigated intestinal helminthic or protozoa infections alone but not both, while other studies looked at single intestinal parasites (Gendrel et al. 2003; Green et al. 2011; Crompton & Nesheim 2002; Dickson et al. 2000; Easwaran, Variyam and John 1982; Al-mekhlafi et al. 2005; Amare et al. 2013; Nguyen et al. 2012; Ahmed et al. 2012). Furthermore, most of these studies have been carried out in school going children and they explored stunting, underweight or wasting but not SAM. It is worthwhile to mention that this study used fairly simple and affordable laboratory methods to identify parasites in stool; such methods can easily be applied in resource limited settings of low income countries like Uganda. This study was also able to classify diarrhoea into acute and chronic; this was paramount since different parasites may cause specific types of diarrhoea with varying impact on nutrition status.

There are several limitations of this study. Diarrhoea on admission could have been as a result of other enteric pathogens including viruses and bacteria (Lama n.d.; Ahs et al. 2010; Georges et al. 1984; Anon 1998; Hodges & Gill 2010; Sodeinde et al. 1997; Youssef et al. 2000). However, this present study did not evaluate viral and bacterial enteric pathogens as an alternative explanation for diarrhoea occurrence in this study group. In

addition, the survey was cross sectional; therefore, causality cannot be examined. It is uncertain to establish whether it was the intestinal parasites that led to diarrhoea related SAM or it was SAM that predisposed these children to parasitic related diarrhoea. Furthermore, data on cofounding variables like fecal disposal and water source for home consumption were prone to information bias, since this data were reported by caretakers/mothers. It is worthwhile to mention that this study only investigated the qualitative but not quantitative (intensity) impact of parasites on diarrhoea, yet the intensity of parasites may affect diarrhoea differently. Recall bias is most likely in this present study, as the duration of fever and diarrhoea were reported by caretakers or mothers of the study children.

5. Conclusion

In conclusion, the results suggest that intestinal parasitic infections are prevalent in SAM, and *Giardia lamblia* is strongly associated with SAM related diarrhoea, chronic in particular. This implies that successful routine intestinal parasite screening could increase the diagnostic rate of parasitic diarrhoea and improve the treatment and prevention strategies of diarrhoea in SAM children.

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