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Prevalence of Intestinal Protozoan Infections and the Associated Risk Factors among Children in Bushenyi District, Western Uganda

Ibrahim Ntulume[1], Julius Tibyangye[1,2*], Adamu Almustapha Aliero[1,3] and Barugahare John Banson[4]

[1]Department of Microbiology and Immunology, Kampala International University, P.O.Box 71, Ishaka – Bushenyi, Uganda.
[2]Department of Microbiology and Immunology, St. Augustine International University, P.O.Box 88, Kampala, Uganda.
[3]Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.
[4]Faculty of Health Sciences, Busitema University, Uganda.

Authors' contributions

This work was carried out in collaboration between all authors. Author IN designed the study, did the laboratory experiments, statistical data entry and analysis. Author JT participated in study design, wrote the first draft of the manuscript, critically supervised and reviewed the manuscript. Authors BJB and AAA participated in the study design, laboratory quality assurance and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the prevalence of intestinal protozoan infections and the associated risk factors in children.
Study Design: A cross sectional study.
Place and Duration of Study: The study was conducted in four selected health facilities in Bushenyi District, Western Uganda, between June 2016 and January 2017.

**Corresponding author: Email: jtibyangye@saiu.ac.ug, tibyangye1985@gmail.com;*

Methodology: A total of 200 children aged below one year to 12 years were enrolled. Stool samples were collected and analyzed according to the established standard methods.
Results: Of the 200 children enrolled, 73(36.5%); CI = 0.000-0.015, had intestinal protozoan infections. The prevalence of *Giardia lamblia* was the highest (16%) followed by *Entamoeba histolytica/dispar* (13%) compared to *Cryptosporidium* spp (4%) and *Cyclospora* spp (3.5%).The highest prevalence was in the age group 1-4 years (26.8%, OR; 2.601, *P = .015*), mothers with no formal education (44.4%, OR; 3.240, *P = .002*) and those with primary level education (23.5%, OR; 1.812,*P = .027*) were significantly associated with intestinal protozoan infections. There was no significant difference in the prevalence of infections between the females (22.3%) and males (16.5%).
Conclusion: The overall prevalence of intestinal protozoan infections was 73(36.5%), *Giardia lamblia* (16%) was the most prevalent intestinal protozoan infection in the study. The intestinal protozoan infections were most prevalent among children aged 1-4 years whose mothers had no formal education. Prevention strategies need to be tailored to the respective levels of education.

Keywords: Protozoan; infections; association; Giardia lamblia; Bushenyi District.

1. INTRODUCTION

Intestinal protozoan infections (IPIs) are common among children resulting in considerable malabsorption syndromes, gastrointestinal morbidity and mortality especially in developing countries [1,2]. Worldwide, IPIs have been recognized as one of the most significant causes of illnesses [3]. It is estimated that IPIs result in 450 million illnesses with an average prevalence rate of 50% in developed world, and almost 95% in developing countries [4]. It is estimated that *E. histolytica*, the etiological agent of amoebiasis, kills between 40000 and 100000 people per year hence considered one of the deadliest parasitic infections worldwide [2,5]. *Cryptosporidium* spp. is primarily affecting immunocompromised patients like HIV/AIDS patients [5,6]. These infections are ubiquitous and highly prevalent among the poor and socioeconomically deprived communities where overcrowding, poor environmental sanitation and hygiene, low level of education and lack of access to safe water are strong risk factors for IPIs more especially in Uganda [2,7].

Establishing the prevalence of IPIs in communities is essential for prioritizing public health interventions [8-10]. Since the diagnosis of IPIs in low income countries is usually limited, data on the prevalence rates is scarce. In addition, the risk factors for the IPIs in sub-Saharan Africa are heterogeneous [2,11]. Therefore, this study was conducted to determine the prevalence of IPIs and the associated risk factors among children in Bushenyi District, Western Uganda.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study was carried out in four selected health facilities (Ishaka Adventist Hospital, Comboni Hospital, Bushenyi Health Centre IV and Kyabugimbi Health Centre IV) in Bushenyi District, Western Uganda between June 2016 and January 2017.

2.1.1 Study area

Bushenyi is a district in Western Uganda boardered by Rubirizi District to the north west, Buhweju District to the north east, Sheema District to the east, Mitooma District to the south and Rukungiri District to the west. The coordinates of the district are; 00 32S, 30 11E. It covers an area of approximately 4,292.5 square kilometres (1,657.3 sq mi) with six sub-counties (i.e. Kyamuhunga, Kyabugimbi, Kyeizoba, Bumbaire and Nyabubare) and one Municipality (Bushenyi-Ishaka) (http://www.molg.go.ug) as shown in the map.

2.1.2 Inclusion criteria

The study included children aged below one year to 12 years attending out-and-in patient clinics confirmed to have intestinal protozoan infections signs and symptoms by the attending Clinician. Children with no history of treatment for intestinal parasites in the last two weeks and those who consented and assented to participate in the study.

2.1.3 Exclusion criteria

The study excluded the severely sick children that warranted admission in the Hospitals and Health facilities in the study area, those with history of treatment for intestinal parasites in the last two weeks and didn't consent/assent to participate in the study.

2.1.4 Consent/assent and counseling

A written consent/assent was sought from the patients who satisfied the inclusion criteria. The Self-administered questionnaire and interview guide was carried out to capture demographic data, predicting factors for intestinal protozoan infections, and counseling for

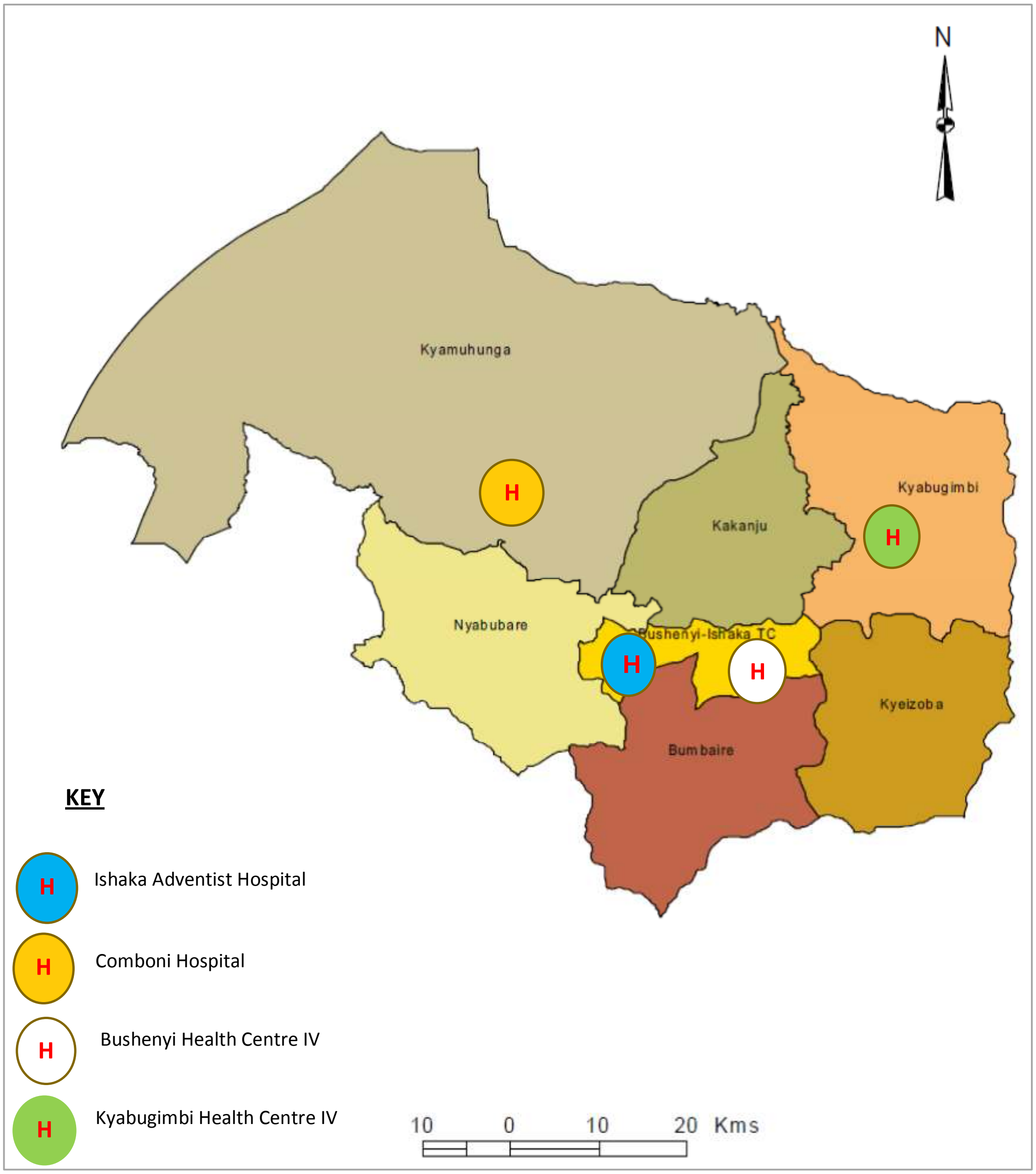

Fig. 1. Map of Bushenyi district showing the location of the study sites (Health facilities)
Source: www.ucc.co.ug

specimen collection. The study subject was then sent for specimen collection and the results were kept confidential.

2.1.5 Sample size

Two hundred stool samples were collected from in-and-out patients with the help of trained nursing staff. The sample size (n) was calculated using the standard formula,

$$n = \frac{Z\alpha PQ}{L} \text{ [12]}$$

Where n=sample size; Zα=Level of significance (1.96) for confidence interval of 95%; P = Estimated prevalence (26%) [10]; and L=Desired precision or allowable error=5% (0.05) Q = (1-P).

Note: The sample size that was analyzed is less compared to 296 sample size that was deduced from the formula above since some patients refused to consent/assent, some who had consented/assented failed to provide the samples within the study period and some withdrew from the study.

2.1.6 Collection of stool samples

All participants (parents/guardians) of children were given clear standard procedures prior to sample collection which stated the right sample, source, container, amount, how to collect and all necessary precautions necessary to avoid sample contamination. A stool sample container was given to participants and then given laboratory numbers for easy identification during sample processing and analysis. The samples were then transported on ice to the laboratory for standard parasitological analysis. The patients' biodata such as age, sex, and clinical history were recorded at the time of sampling.

2.2 Detection of Intestinal Protozoans

The stool samples were processed and analyzed immediately after collection by direct smear microscopy using normal saline and iodine wet preparations to detect trophozoites of *E. histolytica/dispar* and *G. lamblia* [13]. Concentrations methods (i.e. Formalin-ether, saturated sodium chloride, and Sodium acetate-acetic acid formalin (SAF)) were used to detect cysts of *E. histolytica/dispar* and *G. lamblia*; oocysts of *Isospora beli* and *Cyclospora cayentenesis* [13,14]. The Modified Zeihl Neelsen (ZN) was used to detect oocysts of *Cryptosporidium* spp. in stool samples following the procedure previously described by [15].

2.3 Data Analysis

The data obtained from the participants was recorded in a field book, then entered into computer MS Excel which was exported to SPSS-16 software for analysis. Bivariate and Multivariate analysis was performed to obtain descriptive statistics for the prevalence of infections and association of risk factors using the Chi-square tests at 95% confidence interval.

3. RESULTS

3.1 Socio-demographic Characteristics of Respondents

A total of 200 aged below one year to 12 years were enrolled in this study and grouped into four age groups. The largest category was 1-4 years, 112 (56.0%) and the least was <1 year, 20 (10%). Out of the enrollees, 103 (51.5%) children were females and 97 (48.5%) males. Most of the children drunk water from boreholes (41%) and taps (40%) while few consumed both pond water (10%) and tank water (9%).The largest percentage of the mothers had attained at most primary education level (45.5%) compared to those who had attained the University level (2%). The summary of these characteristics is indicated in Table 2.

3.2 Prevalence of Intestinal Protozoa Infections

A total of 73 (36.5%) children (CI = 0.000-0.015) had intestinal protozoa infections from various parasites including *Giardia lamblia* which was the most prevalent (16%) while *Cyclospora* spp was the least prevalent (3.5%). Of the 200 children, 14(7%) had double protozoa co-infections and 2(1%) had triple protozoa co-infections. Double infections of *G. lamblia* and *E. histolytica/dispar* (4%) were the most prevalent as shown in Table 1.

3.3 Risk Factors Associated with Intestinal Protozoa Infection among Children

From the Person's Chi- square tests, age *(P = .022)* and education level *(P = .013)* were the factors statistically significantly associated

Table 1. Prevalence of intestinal protozoan infections

Protozoa	Prevalence n(%)
Single infection	
G. lamblia	32(16)
E. histolytica/dispar	26(13)
Cryptosporidium spp	8(4)
Cyclospora spp	7(3.5)
Total	**73(36.5)**
Double infections	
G. lamblia + *E. histolytica/dispar*	8(4)
G. lamblia + *Cyclospora* spp	2(1)
E. histolytica/dispar + *Cryptosporidium* spp	2(1)
E. histolytica/dispar + *Cyclospora* spp	1(0.5)
Cryptosporidium spp+ *Cyclospora* spp	1(0.5)
Total	**14(7)**
Triple infections	
G. lamblia + *E. histolytica/dispar* + *Cryptospordium* spp	1(0.5)
E. histolytica/dispar + *Cryptospodium* spp+ *Cyclospora* spp	1(0.5)
Total	**2(1)**

with intestinal protozoa infections among children in the study area. Children aged between 1 to 4 years had the highest prevalence (26.8%), followed by those aged 9 to 12 years (15.2%) and least infected were aged 5 to 8 years old (5.7%). The children whose mothers had no formal education level (44.4%) were more prone to infections, followed by those with primary education level (23.5%) and least were those whose mothers had tertiary education level (6.9%). More females (22.3%) had infections compared to the males (16.5%).Interestingly a larger number of children with protozoa infections used borehole water (25.6%) as illustrated in Table 2.

In order to obtain the significant risk factors of the infection, binary logistic regression analysis (Odds ratios) was further done on age groups and education level of mother since they had a

Table 2. Association between socio-demographic characteristics with single protozoan infections in children

Variable	Number of Respondents n (%)	Positive n (%)	p-value	95% CI
Age group (years)				
Less than 1	20(10%)	2(10.0%)		
1-4	112(56.0%)	30(26.8%)	.022*	0.019-0.025
5-8	35(17.5%)	2(5.7%)		
9-12	33(16.5%)	5(15.2%)		
Gender				
Female	103(51.5%)	23(22.3%)	.372	0.345-0.386
Male	97(48.5%)	16(16.5%)		
Education level of mother				
No education	18(9.0%)	8(44.4%)		
Primary	85(42.5%)	20(23.5%)		
Secondary	64(32.0%)	8(12.5%)	.013*	0.011-0.015
Tertiary	29(15.5%)	2(6.9%)		
University	4(2.0%)	1(25.0%)		
Source of drinking water				
Borehole	82(41.0%)	21(25.6%)		
Tap	80(40.0%)	12(15.0%)	.342	0.333-0.351
Tank	18(9.0%)	3(16.7%)		
Pond	20(10.0%)	3(15.0%)		
Total	**200 (100%)**			

**Statistically significant factors influencing protozoa infections prevalence at 95% level of confidence (P≤ .05)*

P ≤ .05 in the bivariate analysis. The model used best fit the data *(P = .817)* and 81.5% of the children with protozoa infections were correctly classified. Children aged between1 to 4 year had a 2.6 times likelihood of being infected with intestinal protozoa compared to those aged below 1 year holding other factors constant. This relationship was statistically significant *(P < .05, CI; .001 - .024)*.The mothers who had attained primary education *(OR= 1.812, P < .05, CI; .006-.046)* and no education level *(OR=3.240, P <.05, CI; .000 - .012)* were also statistically significant predisposing risk factors for protozoa infections when other factors were held constant as shown in Table 3.

4. DISCUSSION

Overall prevalence of 36.5%; CI = 0.000-0.015, for IPIs among children aged below one year to 12 years in Bushenyi District, Uganda. This was in agreement with most previous studies on IPIs, 36% in South-Eastern Nigeria, 35% in Saudi Arabia and 34.2% in Northwest Ethiopia [16-18]. However, the prevalence was higher in comparison to similar previous studies carried out in other parts of Uganda (20.9%), and other countries i.e. Nepal (19.8%), Nigeria (27.6%), and Saudi Arabia (27.8%) and 30.6% among HIV/AIDs patients in Bahiri Dar, Ethiopia [3,19-23]. Nevertheless, we report a lower prevalence than the other reported studies from South Africa (64.8%), Pakistan (52%), Gondam Azezo (72.9%) and Ethiopia (83.8%) [10,24-26]. The lower prevalence obtained in the present study compared to other related studies could probably be explained by the Health sector's periodic campaign for anti-protozoa drug administration, health education given by different LNGOs, INGOs to school children in Uganda [27].

The prevalence of *G. lamblia* infections among children was higher than other protozoa; *E. histolytica/dispar, Cryptosporidium* spp and *Cyclospora* spp which was in agreement with similar previous studies [20,21]. Our study showed 16% prevalence of *G. lamblia* infections which is in agreement with the previous studies i.e. 12% and 15.4% respectively in Uganda [10,20]. However, our prevalence was higher than that obtained in a similar study conducted in Nigeria (1.3%) [28]. This outstanding result could be due to the differences in levels of education, social economic status and implementation of the government public health policies.

The prevalence of single infections of *E. histolytica/dispar* (13%) is close to the previous study among school going children in Uganda [7]. However, the prevalence in our study was higher than that obtained elsewhere Nepal 8%, and Ethiopia 8% [3,18]. This is evidence that the risk factors are heterogeneous and vary from community to community. There were low levels of *Cryptosporidium* spp (4%) and *Cyclospora* spp (3.5%) infections of which the latter was slightly higher than 2.0% prevalence reported among children in Tanzania [27]. The prevalence of double and triple protozoan infections was 7.0% and 1% respectively. This was in agreement with related studies conducted by [29], who reported 5.7% (double infection) and 0.7% (triple infections) respectively. However, the double infections obtained were contrarily higher than 2.6% reported by [18], but the same study reported a higher prevalence of triple infections (2%) compared to that reported in this study. This is because the single infections of *E. histolytica* and *G. lamblia* were far higher compared to others in our study. Secondly, the degree of co-infections seems to be proportional to the degree of the respective single infections.

Table 3. Binary logistic regression analysis for risk factors associated with intestinal protozoa infections

Variable	Odds ratio	P-value	95% CI
Age (years)			
1-4	2.601	.015*	0.001-0.024
5-8	0.513	.064	0.048-1.113
9-12	1.017	.058	0.021-1.104
Education level of mother			
No education	3.240	.002*	0.000-0.012
Primary	1.812	.027*	0.006-0.046
Tertiary	0.137	.476	0.082-0.349
University	0.411	.475	0.156-0.789

**Statistically significant risk factors associated with protozoa infections at 95% level of confidence (p≤ .05)*

The children's age group was statistically significant *(P < .05)* with higher infections among those aged 1-4 years (26.8%) which was in agreement with the previous study in Uganda (26.0%) [10]. The age group was statistically significant predisposing factor to protozoa infections because of the levels of engagement in activities characteristic of the age group (*OR = 2.601, P < .05*) [20]. However, this was contrary to the reported among Mexican children (20.0%) [27].

In this study, protozoan infections were higher in females (22.3%) versus males (16.5%). This was in agreement with other study findings from Nepal reporting a prevalence of 22.0% and 16.9% in both girls and boys respectively [21]. However, in a study conducted in Malaysia, a higher infection rate of protozoa in males (73%) and females (73.3%) was reported compared to the present study [30]. According to [3], reported a higher infection rate in females compared to males ((18.6% versus 18.4%). This indicates that the gender of children may or may not play a role in parasitosis depending on the region and other environmental or behavioral factors due to the reported statistical insignificance in these related studies [3]. It has been reported that the increased mobility and use of swampy ponds by the male increases their risk of infection while female have more soil contact during growing vegetables and eat raw vegetable with prepared food more often than males. Furthermore, infections among the males could probably be due to engagement in predisposing activities such as football, barefoot and playing in streams [28].

The findings of this study indicate that the prevalence of IPIs were significantly *(P < .05)* higher in children whose mothers had no formal education (44.4%) and primary level (23.5%) compared to other higher education levels. This was in agreement to studies conducted in Iraq, and in Northwest Ethiopia [18,24,29,31]. Therefore, parents' education level equipped them with knowledge about the necessary preventive precautions to guide their children accordingly.

The source of drinking water was insignificantly *(P > .05)* associated with the protozoan infections although higher prevalence were obtained for children who had drunk unboiled water from boreholes (25.6%), tank (16.7%), tap (15.0%) and ponds (15.0%). Similarly, related studies also showed the higher rate of infection among children who had drank such untreated water than those who boiled drinking water [13,30,31]. However, this was contrary to related studies which reported higher infections (66.7%) with usage of unboiled tap water [15,32]. This high prevalence may be due to contamination of water supplies with human waste, poor quality of water, faulty sewage line and insufficient level of chlorine. Multiple studies have addressed the risk factors and the prevalence of parasitic infestation among children in developing countries as a result of poor sanitary conditions in the homes, peridomestic conditions like waste disposal methods, garbage disposal systems, number of children in the household, number of persons per latrine and source of water supply, food contamination by food handlers and by the use of sewage water for irrigation of crops are factors in protozoan epidemiology [27]. Nevertheless, the level of education of the care takers has not been prominent as an equally important risk factor.

5. CONCLUSION

The overall prevalence of IPIs was 73(36.5%); CI = 0.000-0.015, the common intestinal protozoan was *G. lamblia* (10.5%) in the study. The IPIs were most prevalent among children aged 1-4 years and 9-12 years whose mothers were not educated and those with primary education.

Therefore, the Ugandan health sector should invest in education and sensitization of caretakers about these infections and the necessary preventive measures against IPIs. With the high prevalence of the IPIs detected in this study, there is need to carry out country wide survey to establish the overall burden of protozoan infections.

CONSENT

All authors declare that 'written informed consent was obtained from the patient.

ETHICAL APPROVAL

The ethical approval of the study was sought from Kampala International University (KIU), institutional research and ethics committee (IREC) on human research, and Uganda National Council for Science and Technology (UNCST). All experiments were examined and approved by the appropriate ethics committees and performed in accordance with the ethical standards of the committees on human experimentation laid down in the Helsinki declaration of 1975 as revised in 2000.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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