Original article

The prevalence of intestinal parasites in paediatric diarrhoeal and non-diarrhoeal patients in Addis Ababa hospitals, with special emphasis on opportunistic parasitic infections and with insight into the demographic and socio-economic factors

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Abstract

Background: Opportunistic intestinal parasitic infections cause severe diarrhoea specially in infants and in immuno-compromised people world wide.

Objective: The objective of this study was to assess and determine the prevalence of opportunistic intestinal parasites in paediatric patients with and without diarrhoea in selected hospitals in Addis Ababa.

Methods: A cross-sectional study was conducted on 222 children under five years of age who had diarrhoea and on 74 children who had no diarrhoea in selected Hospitals in Addis Ababa. Single stool specimens were collected and screened for intestinal parasitic infections by using direct and concentrated methods. The Modified Ziehl-Neelsen Staining and Modified Water-Ether Sendimentaion methods were used for detecting Coccidial parasites.

Results: Of the 222 paediatric diarrhoeal patients, 61(27.5%) were found to be infected with a variety of intestinal parasites and out of 74 children without diarrhoea 11(14.9%) were found to be infected. Among the emerging opportunistic parasites detected in diarrhoeal children were *Cryptosporidium parvum* (8.1%), *Isospora belli* (2.3%) and *Enterocytozoon bieneusi/ Encephalitozoon intestinalis* (0.5%). Other common intestinal parasites detected were *Ascaris lumbricoides* (0.5%), *Trichuris trichiura* (0.9%), *Giardia lamblia* (6.3%), *Entamoeba histolytica/ E. dispar* (1.4%), *Blastocystis hominis* (5.9%) and *Hymnolepis nana* (0.5%). Opportunistic parasites were found to be significantly associated with diarrhoeal and non-breastfed children ($p<0.001$). *C. parvum* and *I. belli* respectively were isolated from 83.3% and 80% of diarrhoeal children aged less than 12 months. *C. paravum* and *I. belli* infections were also higher in male children, with a prevalence of 72.2% for *C. paravum* and 80% for *I. belli*.

Conclusion: This study re-affirms and confirms the previously held view that opportunistic parasites can cause diarrhoea in paediatric patients and that it is more prevalent in male, non-breastfed children. The cause of diarrhoea in paediatrics in the absence of identifiable parasitic infections suggests that other infectious agents might be responsible for the diarrhoea. [*Ethiop.J.Health Dev.* 2005;20(1):39-46]

Introduction

Intestinal parasitic infections are among the most common infections worldwide. It is estimated that some 3.5 billion people around the world are affected as a result of these infections, the majority being children (1). The main clinical manifestation of the disease caused by these parasites is diarrhoea (2). Diarrhoea is defined as an increased loss of stool with a frequency and fluidity greater than the usual habit for each individual (3). It has a devastating effect particularly on infants; annually killing at least 4 to 5 million of those aged less than five years in Africa, Asia and Latin America (4).

Thus, diarrhoea caused by opportunistic intestinal parasites could be life threatening particularly for infants during the course of acute disease. 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 (5). For example, in 90% of paediatric diarrhoeal patients studied in the Gambia, the etiological agents isolated were opportunistic parasites (6). The parasites that emerged in recent years, thus, include members of the protozoan genera- *Cryptosporidium, Cyclospora, Isospora* and *Microsporidia* (7,8). These opportunistic parasites cause diarrhoeal diseases in children and are also considered as important AIDS-associated pathogens worldwide (9).

Cryptosporidium parvum has at present become one of the most commonly reported enteric parasites in both immuno-competent and immuno-compromised people worldwide. It occurs in up to 7% of children with diarrhoea in developed countries and up to 12% of children with diarrhoea in developing countries (10). In Ethiopia, the prevalence of Cryptosporidial infections in children with diarrhoea ranged from 3.3% in Jimma, and 5.6% in Addis Ababa to 9% in north-western Ethiopia (11,12,13).

Cyclospora cayetanensis was reported from Haiti in 11% of immuno-compromised adult patients with diarrhoea (7). In Tanzania, 1.8% of children with acute diarrhoea were found to have *C. cayetanensis* (8), while 5.6% of

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the children under 8 years of age in Peru (14) and 2.3% of children and adults in Guatemala (15) were found to be infected. In south-western Ethiopia 3.7% of adult AIDS patients have also been reported to be positive for *C. cayetanensis* (5).

Isospora belli affects immuno-competent and immuno-compromised individuals by causing self-limited and life threatening diarrhoea, respectively (16). In developing countries 8-40% of AIDS patients are infected (7,16). In Ethiopia, a prevalence of 7.4% was reported from the southwestern region (5) and among 5.4% of adult AIDS patients in Addis Ababa (17).

Human infections with *Microsporidia* have been reported from allover the world, and the majority of the cases have involved HIV- infected patients. The world prevalence of intestinal *Microsporidiosis* ranges between 7 and 50% (18, 19).

Intestinal parasites would be expected to be highly prevalent in Addis Ababa because of the shortage of clean water, problems in the sewage system and other unhygienic factors that increase the probability of infection by these parasites. Therefore, this study was aimed at determining the extent and association of opportunistic intestinal parasites in paediatric diarrhoea cases in Addis Ababa.

Materials and Methods

Study area and population

The study was carried out in selected hospitals in Addis Ababa namely: Police Hospital, Armed Forces General hospital, and Tikur Anbessa Specialized Hospital between January and March, 2004, (inclusive). The study Hospitals were selected using the random sampling method and by taking into consideration such factors as patient population size, location, and adequacy of facilities for sample collection. The study subjects were children of both sexes who are under five years of age and who had diarrhoea. The controls were age and sex matched children without diarrhoea who were brought to the hospitals, for immunization or illnesses other than diarrhoea. The parents or guardians of children were informed about the aims, methods and anticipated benefits of the study. Consenting parents or guardians were requested to sign a consent form.

Stool collection and processing

A total of 296 fresh stool samples were collected after obtaining the consent of parents or guardians in labeled vials and were processed within one hour of collection at the clinical parasitology laboratory, Ethiopian Health and Nutrition Research Institute (EHNRI). The following methods were used for the diagnosis of infections:

Direct and concentration methods

Specimens were examined as Saline-Iodine wet mounts. The wet mount faecal preparations were microscopically examined under a magnification of 400x to detect ova and/or parasites. The formol-ether concentration method was also carried out (20).

Modified ziehl-neelson staining method

Smears were prepared from the concentrated stool samples and were stained (6) with some modifications. Air-dried thin smears were fixed with absolute methanol for 5 minutes, air-dried and stained with carbol-fuchsin for 30 minutes. Smears were washed with tapwater and decolourised with 1% acid-alcohol for 1 minute; washed with tapwater and counterstained with 1% Methylene blue for 1 minute, rinsed again in tapwater and air-dried. The slides were then examined using a microscope at 1000x magnification for the detection of *Cryptosporidium, Isospora* and *Cyclospora* species oocysts.

Modified water-ether sedimentation method for detection of microsporidia

The stools (0.5-lg) were homogenised in 7ml of distilled water in a beaker and filtered through a sterile gauze; 3ml of Ether was added to the filtrate, the tube was then stoppered, and the Water-Ether mixture was shaken back and forth for 1 minute. It was then centrifuged at 2000 rpm for 5 minutes and the debris layer carefully poured away. The entire sediment was then transferred onto a slide and air-dried. The air-dried smear was then processed by the optical white technique (21). Briefly, the smears were fixed with absolute Methanol for 5 minutes, stained with Uvitex 2B for 10 minutes, washed with phosphate buffered saline, and counterstained with 1% Evans blue. The slides were then examined at 1000x magnification under a Leitz fluorescence microscope equipped with a 50 W Mercury high pressure lamp and an excitation filter that has a transmission range from 355 to 425 nm and a suppression filter of 460 nm to detect *Microsporidia* spores.

Survey on demographic and socio-economic/income/ status

Socio-demographic and economic data were obtained from parents or guardians by conducting interviews using a pre-structured questionnaire.

Statistical method

Statistical analysis was done by using the SPSS version 10.0 software. Data were summarized using frequency tables and bar charts. To analyse categorical risk factors for diarrhoea, contingency tables were used and the strength of association was measured using the Chi-square test statistics and its associated *P-value*. Observed differences in data were considered significant and noted in the text if a P value of 0.05 or less was obtained.

Results

The age and sex profile analysis of the children showed that there were almost equal numbers of both sexes and that the size of those below 12 months old and above was also similar (Table 1). Based on the parasitological examinations of the stool specimens, 9 species of intestinal parasites were detected in 26.1% (58/222) of the children with diarrhoea and in 14.8% (11/74) of the children without diarrhoea (Table 2).

Table 1: **Age and sex distribution of Cases and Controls in a study on opportunistic intestinal parasitic infections in paediatric patients in selected Addis Ababa Hospitals, 2004.**

Age group	Cases (n=222)		Controls (n=74)	
	Males n (%)	Females n (%)	Males n (%)	Females n (%)
< 6 months	27 (57.4)	20 (42.60)	11 (52.4)	10 (47.6)
6 - 12 months	24 (48)	26 (52)	10 (52.6)	9 (47.4)
>12 months	58 (46.6)	67 (53.6)	19 (55.9)	15 (44.1)
Total	**109**	**113**	**40**	**34**

Among all the emerging opportunistic parasites in all children with diarrhoea, *Cryptosporidium parvum* (8.1%), *Isospora belli* (2.3%) and *Entrocytozoon bieneusi/Ence. intestinalis* (0.5%) were detected. Other common parasites were also detected but were found to be rare. The prevalence of each parasite in the cases and the controls showed that *C. parvum* was more prevalent in children with diarrhoea, and the difference was highly significant (P<0.001) (Table 2). *A. lumbricoides* was significantly (P=0.001) more prevalent in children without diarrhoea. Although their significance could not be established *B. hominis, G. lamblia, I. belli* and the only *E. bieneusis/Ence. intestinalis* cases were detected in children with diarrhoea (Table 2).

Table 2: **The overall prevalence of intestinal parasites in paediatric diarrhoeal (n=222) and non-diarrhoeal (n=74) patients in selected Addis Ababa Hospitals, 2004**.

Parasites	Children with diarrhoea n (%)	Children with out diarrhoea n (%)
T. trichura	2 (0.9)	-
G. lamblia	14 (6.3)	-
E. histolytica/E. dispar	3 (1.4)	-
H. nana	1 (0.5)	2 (2.7)
A. lumbricoides	1 (0.5)	7 (9.4)*
B. hominis	13 (5.9)	-
C. parvum	18 (8.1)*	2 (2.7)
I. belli	5 (2.3)	-
E. bieneusis/ Ence. intestinais	1(0.5)	-
Total	**58 (26.1)**	**11 (14.9)**

Key * P<0.01

Out of all the diarrhoeal stools collected, 52.7% of the specimens were liquid/watery, 18.9% were bloody and 28.4% were mucoid. Association was observed between these types of diarrhoea and some kinds of parasitic infections, like *Cryptosporidium* with watery, *E. histolytica/E.dispar* with bloody and *G. lamblia* with mucoid diarrhoea. In parasite-negative stools, 161 (72.5%) samples of paediatric diarrhoeal patients were found to have many pus cells, yeast cells and red blood cells during microscopic examination.

Out of the total number of diarrhoeal children, 7.7% were non-breastfed, 91% were breastfed for 1 to 6 months, and only 1.4% were breastfed for more than 6 months.

This study also showed that co-infections were rare in children; but *A. lumbricoides* and *G. lamblia,* and *E. histolytica, Cryptosporidium parvum* and *E. bieneusi/Ence. intestinalis,* and *E. histolytic/E. dispar* and *B. hominis* detected in only four of the diarrhoeal children.

The prevalence of *Cryptosporidium* was found to be 55.6% in diarrhoeal children who weighed 1.00 to 5.0kgs and 38.9% in diarrhoeal children who weighed 5.1kgs to 10.0kgs showing significantly more cases in the low weight group (P<0.0001) (Table 3).

Height specific *Cryptosporidium* infections showed 10 (55.6%) children with diarrhoea to have a height of less than 55cms, 5 (27.8%) to have a height between 55cms and 71cms and 3 (16.7%) of the diarrhoeal children to have a height of more than 71cms. Therefore, *C. parvum* infections were more prevalent in shorter children than the taller ones (P<0.001) (Table 3). It was also much more prevalent (p<0.0001 in the younger age groups than in the relatively older ones, i.e., 50% in those aged less than 6 months; 33.3% in those aged between 6 and 12 months and 16.7% in those aged more than 12 months (Fig 1).

From the total number of *Cryptosporidium* infected children- 44.4% with diarrhoea and 100% children without diarrhoea- were non-breastfed. Among the breastfed, 38.9% of them were breastfed for 1 to 6 months only and 16.7% were breastfed for more than 6 months. *Cryptosporidium* infection was found to be significantly higher among non-breastfed children (P<0.001) than breastfed ones.

Among the 18 *C. parvum* positive diarrhoeal children, 72.2% were males and 27.8 were females (P=0.035) (Table 3). It is worth noting that the prevalence of *C.*

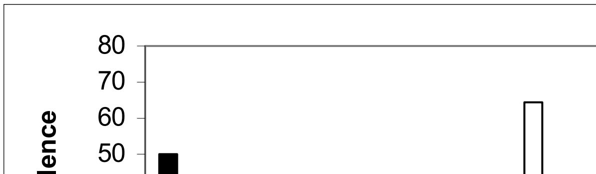

Figure 1: **The prevalence of diarrhoea causing intestinal parasites detected among children presenting with diarrhoea in selected Hospitals in Addis Ababa, 2004.**

Table 3: **Characteristics of diarrhoeal children (n=222) positive for *C. parvum, E. bieneusi/Ence. intestinalis* and *I. belli* in selected Hospitals in Addis Ababa, 2004.**

Criteria	*C. parvum*		*I. belli*		*E. bieneusi/Ence. intestinalis*	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Weight (in Kg)						
≤ 5	10 (55.6)	14 (6.9)	2 (40)	23 (10.6)	-	25 (100)
5.1-10	7 (38.9)	114 (55.9)	2 (40)	119 (54.8)	1 (0.8)	129 (99.2)
>10.1	1 (5.6)	76 (37.3)	1 (20)	75 (34.6)	-	76 (100)
Height (in Cm)						
<55	10 (55.60	22 (10.8)	3 (60)	29 (13.4)	1 (3.1)	31 (96.9)
55-71	5 (27.7)	82 (40.2)	1 (20)	87 (40.1)	-	88 (100)
>71	3 (16.7)	100 (49)	1 (20)	101 (46.5)	-	102 (100)
Breast feeding status (in months)						
Non-breastfed	8 (44.4)*	6 (2.9)	3 (60)	14 (6.5)	1 (5.9)	16 (94.1)
Breast fed for 1-6	7 (38.8)	198 (97.1)	2 (40)	200 (92.2)	-	202 (100)
Breast fed for > 6	3 (16.7)	-	-	3 (1.4)	-	3 (100)
Age (in months)						
<6	9 (50)	38 (18.6)	2 (40)	45 (20.7)	1 (2.1)	46 (97.9)
6-12	6 (33.3)	44 (21.6	2 (40)	48 (22.1)	-	50 (100)
>12	3 (16.7)	122 (59.8)	1 (20)	124 (57.1)	-	125 (100)
Sex of children						
Male	13 (72.2)**	96 (47.1)	4 (80)**	105 (48.4)	1 (0.9)	108 (99.1)
Female	5 (27.8)	108 (52.9)	1 (20)	112 (51.6)	-	113 (100)

* P<0.001 **P=0.035

parvum and *I.* belli in diarrhoeal children, from low income families, was 61.1% and 40%, respectively and that no children of high income families were infected (Fig 2). Although it was not significantly associated (P>0.05) in *Cryptosporidium* and *Isospora* infections, low maternal education was associated with *Cryptosporidium* and *Isospora* infections.

The prevalence of *I. belli* in children with diarrhoea was 2.3% whereas shorter and younger - less than 12 months old children-were more liable to *I. belli* infection than taller ones (P=0.013) while it is also more prevalent in non-breastfed than in the breastfed children (P<0.001) (Table 3). No significant difference was detected in *I. belli* infections among children who lived under parental care and in orphanages (Table 4).

Among the diarhoeal children infected with *C.parvum,* a significantly more (p=0.015) were living in orphanages and not with their parents (Table 4). An analysis of the living conditions of diarrhoeal children showed 49.1% to be living with their parents (Table 4); and among the parents, 7.2% of the mothers and 8.1% of the fathers were found to be suffering from diarrhoea.

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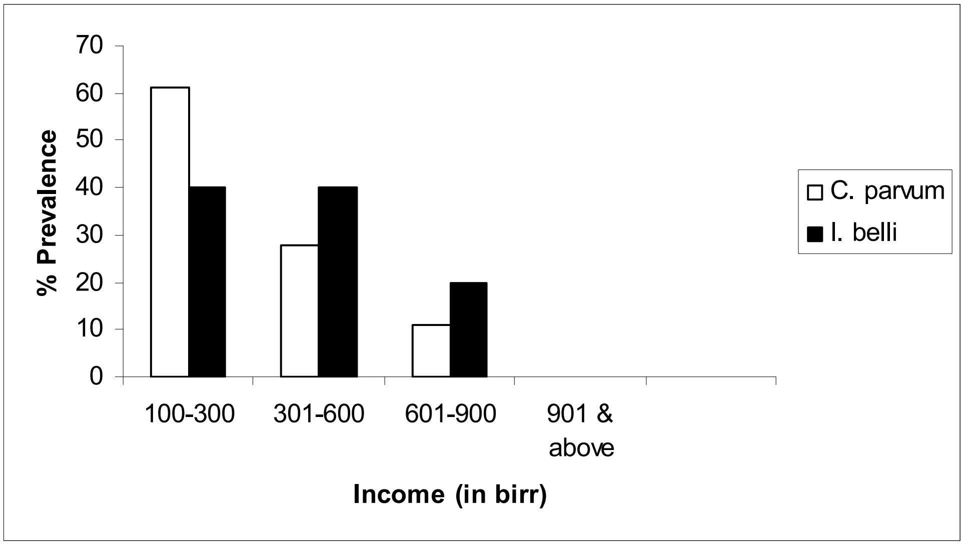

Figure 2: **Total monthly family income and *C. parvum* and *I. belli* infection in children in selected Hospital in Addis Ababa, 2004.**

Stool examination for Microsporidial parasites revealed the presence of *E. bieneusi/ Ence. intestinalis* spores with characteristic ovoid spores that have bright greenish white fluorescence in only one 2 months old male child with diarrhoea. The child was healthy in the past 7 weeks but his mother was sick with diarrhoea. He was 50cms tall and was co-infected with *Cryptosporidium parvum* (Table 3).

Table 4: **The living condition of diarrhoeal children and infection with *C. parvum* and *I. belli* in a study carried out on opportunistic parasitic infections in selected Addis Ababa Hospitals, 2004**

Parasite species	Children living with parents n (%)	 In orphanages n (%)	P. value
C. parvum			
Positive	4 (22.2)	14 (77.8)	0.015
Negative	105 (51.5)	99 (48.5)	
I. belli			
Positive	2 (40)	3 (60)	0.517
Negative	107 (49.3)	110 (50.7)	
Total	**109 (49.1)**	**113 (50.9)**	

Out of all the intestinal parasites detected in paediatric diarrhoeal patients, 55.2% were found to be opportunistic parasites (*C. parvum, I. belli, E. bieneusi/ Ence. intestinalis* and *B. hominis*). The steady increase in the prevalence of opportunistic parasites in Addis Ababa may be attributed to the use of more sensitive and specific diagnostic methods. Although the HIV/AIDS status of the study subjects was not determined, its immunosuppression is known to be a major factor that could convert asymptomatic low level parasitic infections into life-threatening diseases. *C. parvum* was found to be the most prevalent intestinal pathogenic parasite among children with diarrhoea in the selected Addis Ababa hospitals, while *Giardia lamblia* and *B. hominis* constituted the second important group.

Discussion

In is known that infection with *C. parvum* among children less than five years of age is more prevalent in developing countries (5% to 10%) (22). The present study, which shows an 8.1% prevalence of *C. parvum* in diarrhoeal children under five years of age, is similar to the 8% figure obtained in Brazil (23). The proportion of *C. parvum* infection associated with diarrhoea in this population is similar to that reported from north-western Ethiopia (9%) (11) and much lower than what was reported for adult diarhoeal AIDS patients (25.9%) from Addis Ababa Hospitals (17). This implies a low prevalence of HIV/AIDS in the study children.

The prevalence of *Cryptosporidiosis* in the present study was also found to be higher than that reported in another study by Assefa *et. al* (5.6%) (12). This indicates that *C. parvum* infection in children under five years of age in Addis Ababa is increasing. Because of the intermittent nature of the oocyst excretion of this parasite, it is possible that the infection rate observed in the present study would have been even higher, if more than a single stool specimen was collected from each child, especially in those who were symptomatic.

Age group and *C. parvum* infection were significantly associated ($P<0.001$) indicating that infants below 12 months of age and whose immune apparatus has not matured are more predisposed to infection by the parasite. This result is consistent with findings reported in different sub-Saharan African countries including Uganda and the Gambia (6, 24). This fact also confirmed an earlier report of the situation in Ethiopia (12). Age related prevalence of *Isosporiasis* had a similar pattern to that of *Cryptosporidiosis*, whereby the infection was more prevalent in children aged less than 12 months. A decrease in *I. belli* prevalence with increasing age of the

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children was also shown (25). This was explained by the interaction between *I. belli* infections and host immune maturation.

A majority of the children infected with *C. parvum* and a sizeable proportion of children infected with *I. belli* were non-breastfed, indicating that breastfed children might be protected from infections by *Cryptosporidiosis and Isosporiasis* (24). This explanation is strengthened by the finding that only a few number of children that were breastfed for more than six months were infected by *C. parvum* and there was no *I. belli* infection in children breastfed for more than 6 months. It is possible that the cause of infection of breastfed but *C. parvum* infected children may be due to the partially weaned state and it is also possible that the children might also get the infections from breastfeeding infected parents. This might support the findings of past studies which indicate the importance of affording protection to children against *Cryptosporidiosis* (26).

The higher prevalence of *C. parvum* and *I. belli* detected in the male than female children was similar to that observed in Guinea-Bissau (27) and among Bedouin infants in Israel (28). Assuming that immune competence is similar in males and females of this age, unmeasured intra-familial factors appear to have been functioning to expose infant boys or to protect infant girls in this study population. For example, differential rates or intensities of personal contact between the infants and the various caregivers or parents may be different for boys compared with girls. The investigators, who reported findings from Guinea-Bissau (27) and Israel (28), did not offer explanations as to why male infants and children were at higher risk of acquiring *Cryptosporidiosis* and *Isosporiasis* compared with females. Additional observational studies are needed to clarify the reasons for this association.

Studies show that clinically severe diarrhoea resulting from *Cryptpsporidiosis* is closely associated with malnutrition (29). The present study also revealed an association between *C. parvum* infection and underweight children ($P<0.001$). Although *I. belli* infection and light weightedness did not show a significant association, the fact that infection on the whole decreased as weight of the children increased could be an indication of the influence of nutritional status on infection.

The finding that infection prevalence of *C. parvum* and *I. belli* was much higher at lower household income and/or maternal illiteracy might be a reflection of poor hygiene, contaminated water supply and overcrowding in such families.

The possible explanation for the lack of detection of *Cyclosporiasis* during the dry study period (January to March) could be due to its marked seasonality (30), being associated with wet season (31). The detection of *E. bieneusi/Ence. intestinalis* infection among children less than five years of age is most likely the first record from Ethiopia. The low prevalence (0.5%) obtained is a reflection of the low sensitivity of the diagnostic method used. Such low prevalence was also reported from India (32) in studies that used similar diagnostic methods (microscopy). On the other hand, a prevalence of 17.4% was reported among paediatric patients in Uganda by using a highly sensitive, DNA analysis - Polymerase Chain Reaction method (33).

In HIV-AIDS-positive adult patients, infection with *E. bieneusi/ Ence. intestinalis* is strongly linked with chronic diarrhoea and wasting (33). However, although the *E. bieneusi/ Ence. intestinalis* positive male child was found to have severe diarrhoea, his HIV/AIDS status was not known. A high proportion of the children between 3 and 36 months of age acquire *E. bieneusi/ Ence. intestinalis* for the first time, as they do with other highly prevalent enteric infections, including *Cryptosporidiosis*. Therefore, the findings of this study underscore the importance of conducting carefully-designed studies, which should directly address questions on modes of transmission and the development of immune responses and the biology of *C. cayetanensis* which, in turn, may help elucidate the reasons for their seasonality.

Thus, the existence of diverse opportunistic parasitic infections in children with diarrhoea have been confirmed by this study. Therefore, since no effective and specific treatment to the diseases they cause exists to date, a more sensitive and careful diagnostic assessment is required for the prevention and control of these parasitic infections, which are known to be complicated with and result in severe consequences in HIV/AIDS patients.

Based on this study, the following are recommended for the proper care of paediatric diarrhoeal patients and for controlling the disease. There is need to determine the HIV status of children infected with opportunistic parasites; the stool specimens submitted for routine parasitological examination in children admitted because of diarrhoea should be routinely tested for these emerging pathogenic organisms; ongoing public health measures should continue to emphaisze the importance of personal hygiene as well as to provide and monitor the quality of drinking water in Addis Ababa.

In conclusion, even though these opportunistic parasites have been shown to be related with diarrhoea in children, the mere identification of oocystis in the present study may not necessarily mean that the parasites detected were the sole etiology of the disease.

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