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Spatial Distribution of *Giardia intestinalis* in Children up to 5 Years Old Attending Out-patient Clinic at Provincial General Hospital, Embu, Kenya

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ABSTRACT

Intestinal parasitic infections are prevalent in developing countries especially giardiasis causing serious pathological consequences including diarrhoea in young children. Although, *G. intestinalis* is recognized as a major worldwide contributor to diarrhoeal disease in humans, reliable data on its distribution and frequency in Kenya are simply lacking. Factors including unhygienic conditions, improper disposal of sewage, low socio-economic status may account for intestinal parasitic infections. Fresh stool samples from 376 children aged five years and below were collected in polypots and direct wet preparations prepared using normal saline and iodine. Formal-ether concentration method was also used and the preparations were examined microscopically for intestinal parasites. *Giardia intestinalis* was detected in 12.8% of the total study cases. Other intestinal parasites were frequently encountered and included *Entamoeba histolytica* (5.3%), *Ascaris lumbricoides* (2.9%), hookworm species (1.6%), *Hymenolepis nana* (0.8%), *Enterobius vermicularis* (0.5%) and non pathogenic amoeba. *G. lamblia* was significantly associated with diarrhoea ($\chi^2 = 67.35$, $p = 0.0001$). It was frequently found in children between 3 and 4 years while helminthes were detected in children above the age of 3 years. Sex, constipation, lack of appetite and other parasites did not show statistically significant association. Giardiasis in children under the age of 5 years is common and should be promptly treated and regimen subsequently followed up to curtail re-infection. Identification of risk factors should be prioritized and a combination of public health control strategies including improved sanitation, health education, treatment and follow up that would enable improved living condition of children enhanced.

Key words: Amoeba, diarrhoea, helminthes, parasitic infections, young children

INTRODUCTION

Infection by the Eukaryote *Giardia intestinalis* aka *Giardia lamblia* and *Giardia duodenalis* is one of the most frequent worldwide and causes diarrhoeal diseases in humans and other

mammals (Ankarklev *et al*., 2010). In developing countries, diarrhoea causes more than 2.2 million deaths of children under the age of five years (WHO, 2003; Rayan *et al*., 2010). Factors including unhygienic conditions, improper disposal of sewage, low socio-economic status and unavailability of safe water have been identified as contributing factors. There are an estimated 280 million symptomatic human cases per year (Lane and Lloyd, 2002), prompting its inclusion as part of the WHO Neglected Disease Initiative since, 2004 (Ankarklev *et al*., 2010). Estimates vary but about 20% of the world's population have giardiasis, most of whom are asymptomatic (De Regnier *et al*., 1989; Kappus *et al*., 1994; Backer, 2000). The number of infective cysts shed in feaces is highly variable but has been estimated as high as 900 million a day for a human (Yoder *et al*., 2007). Human giardiasis has acute and chronic phases whereby acute phase is usually short-lived, characterized by flatulence with sometimes sulphurous belching and abdominal distension with cramps. The disease is characterized by watery diarrhoea but later becomes bulky, sometimes frothy, greasy and offensive (Feachem *et al*., 1983). Other signs and symptoms include epigastric pain, nausea, vomiting and weight loss which appear 6-15 days after infection and the clinical impact is stronger in young children and in undernourished or immunodeficient individuals (Ankarklev *et al*., 2010); treatment is usually with metronidazole or other nitroimidazoles. In chronic cases malaise, weight loss and other features of malabsorption become prominent and stools are usually pale or yellow, frequent and of small volume. Malabsorption of vitamins and other essential growth factors may also occur. Other pathogenic amoeba especially *Entamoeba histolytica* can also be found in young children. Helminthes including *Ascaris lumbricoides,* hookworms, *Enterobius vermicularis* and *Hymenolepis nana* also infect children with equal measure due to poor sanitary and environmental conditions (Kappus *et al*., 1994; Phiri *et al*., 2000; Okyay *et al*., 2004). In this study, we investigated possible occurrence and distribution of *G. intestinalis,* other amoeba and helminthes in children up to five years of age.

MATERIALS AND METHODS

The study was conducted at the outpatient Department whereby children up to the age of 5 years were examined for the presence of *G. intestinalis* and other intestinal parasites. Informed consent was obtained from the mothers and a questionnaire was filled up with pertinent data. Mothers were then given clean poly pots and shown how to introduce fresh stool samples of their children. Direct and formal ether concentration techniques of stool examinations were done using standard methods previously described (Cheesbrough, 2004). Briefly, wet mounts were prepared separately on glass slides by emulsifying little amounts of stool specimens in normal saline and Lugol's iodine using applicator sticks. A cover glass of 22×22 mm was then used to cover the preparations to avoid drying up. Concentration preparations were also made using 10% formal saline and were then examined using a binocular microscope. In brief, the concentration technique employed 2-3 g of fresh stool sample that were emulsified in 7 mL of formal saline. The resultant suspension was filtered in a centrifuge tube using three layers of wet cotton gauze and 3 mL of diethyl ether added, vigorously shaken and span for 3 min at 2500 RPM using a centrifuge. The supernatant was poured off by dislodging the layer of detritus using an applicator stick and a smear made from the sediment at the bottom of the tube and examined under a microscope. This was a one-time stool examination collected in a poly pot and immediately examined for presence of trophozoites, cysts, helminthe larvae and/or ova. Data obtained through the questionnaire were entered into Ms excel and analysed using SPSS Version 16.0. Tests included Chi-square (χ^2), cross tabulation and ANOVA in relation to the stool examination results.

RESULTS

The results of this study showed that infection with *G. intestinalis* is apparent in children aged 5 years and below. A total of 48 cases (12.8%) were diagnosed positive for the parasite out of a population sample size of 376. *Giardia* cases were 30 (7.98%), 14 (3.72%) and 4 (1.06%) from those using piped and self-treated water, untreated water and in those using unknown/all water sources, respectively. However, there were no significant differences in these three categories of water sources ($p = 0.3811$, $\chi^2 = 0.77$; $p = 0.5168$, $\chi^2 = 0.42$ and $p = 0.9636$, $\chi^2 = 0.002$) (Table 1). *Giardia* cases in males and females were 27 (7.2%) and 21 (5.6%), respectively. Other pathogenic parasites were also shown to be more in males than females (Fig. 1). The ages mostly affected were those between 3 and 4 years, however, no significant ($p>0.05$) differences were observed in infection between age and sex. Significant association was observed between *Giardia* and multiple infections, those attending pre-unit centres (day-care) and diarrhoea ($p<0.001$). The parasite has been found together with other amoeba and helminthes in some instances.

Commensals, other pathogenic amoeba and helminthes were also identified in the specimens. Amoeba included *Entamoeba coli* with 61 cases (16.2%), *Trichomonas hominis* (11; 2.9%), *Endolimax nana* (5; 1.3%), *Iodamoeba butschilli* (6; 1.6%), *Blastocystis hominis* (6; 1.6%), *Chilomastix mesnilli* (10; 2.7%) and *Entamoeba histolytica* which had 20 (5.3%). Helminthes identified included *Ascaris lumbricoides* (11; 2.9%), *Hymenolepis nana* (3; 0.8%), *Enterobius vermicularis* (2; 0.5%) and hookworm species (6; 1.6%) (Table 2). Infection by more

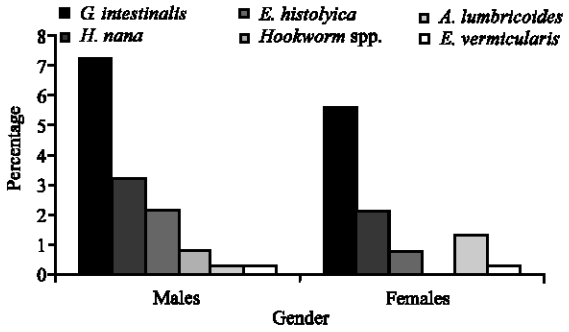

Fig. 1: Comparative pathogenic parasites infection trends in males and females in the study samples

Table 1: Significance relations of *Giardia intestinalis* and other corresponding parameters

	Overall		Outcome		
Parameter	n	%	χ^2	p	Frequency*
Multiple infection	27	07.2	48.25	0.0001	0.287±0.053
Diarrhoea	122	32.4	67.35	0.0001	1.301±0.097
Constipation	18	04.8	0.05	0.8262	0.191±0.044
Piped water	181	48.1	0.77	0.3811	1.193±0.103
Untreated water	125	33.2	0.42	0.5168	1.33±0.0097
Lack of appetite	94	25.0	2.20	0.1381	1.00±0.0009
Vomiting	40	10.6	1.26	0.2611	0.425±0.064
Self treated water	42	11.2	0.03	0.8525	0.448±0.065
All water sources	24	06.4	0.002	0.9636	0.256±0.051
Presence of cats	123	32.7	67.00	0.1482	0.992±0.462
Pre-unit attendance	312	82.9	88.77	0.0001	1.329±0.099

*Mean±SE; Total N = 376

Table 2: Prevalence of *Giardia duodenalis* and other intestinal parasites identified in stools of children less than 5 years attending Embu provincial general hospital laboratory

Type of parasite	Frequency	Percentage
G. duodenalis	48	12.8
E. coli	61	16.2
E. histolytica	20	5.3
B. hominis	6	1.6
T. hominis	11	2.9
C. mesnilli	10	2.7
E. nana	5	1.3
A. lumbricoides	11	2.9
E. vermicularis	2	0.5
H. nana	3	0.8
Hookworm	6	1.6

Table 3: Prevalence by age stratum and the type of intestinal parasite (n = 376)

	Parasite											
	A l	H/w	Ev	Hn	Eh	Ec	Gi	Ib	Th	En	Bh	Cm
Age bracket												
<1 year	-	-	-	-	-	3 (0.8)	3 (0.8)*	-	-	-	-	-
1-2 year	-	-	-	-	-	12 (3.2)	3 (0.8)	1 (0.3)	-	-	-	1 (0.3)
2.1-3 year	2(0.5)	1 (0.3)	-	-	6 (1.6)	15 (3.9)	12 (3.2)	1 (0.3)	6 (1.6)	1 (0.3)	2 (0.5)	3 (0.8)
3.1-4 year	6(1.6)	3 (0.8)	1 (0.3)	2 (0.5)	8 (2.1)	19 (5.1)	12 (3.2)	3 (0.8)	3 (0.8)	3 (0.8)	3 (0.8)	6 (1.6)
4.1-5 year	3(0.8)	2 (0.5)	1 (0.3)	1 (0.3)	6 (1.6)	12 (3.2)	18 (4.8)	1 (0.3)	2 (0.5)	1 (0.3)	1 (0.3)	-
Total	11(2.9)	6 (1.6)	2 (0.5)	3 (0.8)	20 (5.3)	61 (16.2)	48 (12.8)	6 (1.6)	11 (2.9)	5 (1.3)	6 (1.6)	10 (2.7)

*:Values in parenthesis represent prevalence in percentages AL: *Ascaris lumbricoides*, Hw: Hookworm species, Ev: *Enterobius vermicularis*, Hn: *Hymenolepis nana*, Eh: *Entamoeba histolytica*, Ec: *Entamoeba coli*, Gll: *Giardia intestinalis*, Ib: *Iodamoeba butschilii*, Th: *Trichomonas hominis*, En: *Endolimax nana*, Bh: *Blastocystis hominis*, Cm: *Chilomastix mesnilli*

than one parasite was evident with 27 cases (7.2%) out of the total stool samples examined. The helminthes were noticeably present in children in the age bracket of 3 years and above (Table 3). In relation to giardiasis, significant difference was only observed in hookworm infection ($\chi^2 = 5.69$, $p = 0.0171$) and multiple infection ($\chi^2 = 6.02$, $p = 0.0142$).

Among the parameters employed, diarrhoeal cases were the highest reported by the mothers of the children with 122 cases (32.4%), followed by lack of appetite with 94 cases (25%). Others included vomiting with 40 cases (10.6%), constipation with 18 (4.8%) and the presence of cats in the households, 59 cases (17.1%). The number of children in the household, family social economic status and care at pre-unit centres (day care centres) were also considered. Those who reported presence of cats in their homesteads numbered 123, but there were no significant relations to *Giardia* infection. Similarly the number of children in the homes did not have significant correlation to *Giardia* infection however. Analysis on the use, type and quality of domestic water in their homes revealed that 181 (48.1%) homes used tap water, 42 (11.2%) of the respondents personally treated and/boiled drinking water, 125 (33.2%) used untreated water while 24 (6.4%) used water from all sources, treated or not. Generally, diarrhoea, multiple infection and attendance of day care centers were significantly co-related with giardial infection (Table 1).

DISCUSSION

The aim of present study was to establish cases of *Giardia intestinalis* in children of 5 years and below attending out patient hospital services with or without the history of intestinal disturbances. Although, we used direct and formal ether concentration methods, the former was ideal for the demonstration of motile trophozoites while the latter was the best in quantifying ova and cysts (Dhanurkar, 2005). Motile trophozoites of *T. hominis* and *Giardia* were demonstrated in some of the specimens. Out of the 12.8% infection with *Giardia,* 10% had trophozoite stages and were found in watery stools. Those affected most by the infection were from three years and above. This is in agreement with another study conducted by Dhanurkar, 2005 in India, although the total percentage was higher than in this study (25.61%). Despite the fact that *Giardia* infection was found to be more in males than females, other studies involving older children have shown different findings (Kurup and Hunjan, 2010). Diarrhoea cases featured significantly in giardial infection (42 out of 48 cases) and supports a study in North-East Poland conducted by Skorochodzki *et al.* (1998) but contradicts a study conducted in a Thailand orphanage by Janoff *et al.* (1990) which shown that diarrhoea was not a prominent feature in children less than 61 months (5 years) infected with *Giardia.* Another study done in Guatemala to children aged 24 to 61 months revealed elevated giardial infection of 22% (Gupta and Urrutia, 1982). The prevalence of infection among children world-wide has been found to range from 1% to 36% and occasionally may be as high as 72% depending on the age group and country (EPA, 1999). Several other studies have demonstrated high prevalence in young children (Guimaraes and Sogayar, 1995; Cardoso *et al.*, 1995; Prado *et al.*, 2003; Huang and White, 2006). In this study, there was no significant association of the family social-economic status and *Giardia* infection and supports a study in Santiago, Chile which shown that 33% of the children in seven nursery schools and one primary school, were infected with *G. intestinalis* but had no association to economic status (Goldin *et al.*, 1990). Although, a sizeable number of children infected with *Giardia* were found in those who used piped and self treated water, there was however no direct significant association with those who used untreated water and the infection. Several other factors seem to have perpetrated and maintained giardial and other intestinal infections in this instance since even helminthic infections did not show any significance in the treated, untreated or usage from all water sources. Poor nutrition, unhygienic conditions and some cultural practices may have contributed significantly to recurrent or persistent infection and may therefore be important to break the circle by identifying and treating infection sources in such children even when there are risks of re-infection. Other important sources may include contaminated water accidentally swallowed when swimming, in picnics and campsites with irregularly treated or unfiltered surface water as was found in cases in the USA (EPA, 1999).

Giardia cysts are emitted intermittently and could easily been have missed since only one stool sample was examined. Examination of several stool samples on different days has been found useful and increase greatly recovery of cysts and ova (Guimaraes and Sogayar, 1995). Newer techniques such as ELISA, immunofluorescence and counter immune electrophorese that may well complement the low sensitive traditional diagnostic techniques have varied specificity and sensitivity (Flanagan, 1992). Although immunodeficiency and malnourishment are also critical factors, origin and outcome of the latter are often difficult to distinguish (Christie, 1987). While Giardiasis is reported to be non-invasive and secrete no known toxin, malabsorption of electrolytes,

solutes and water and the inhibition of brush-border enzymes do occur due to the many parasite elusive factors involved (Buret, 2007). The true prevalence and morbidity of giardial infection in children in most parts of Kenya is not documented. Most of the information available usually comes from laboratory-based surveillance systems which provide information mainly on infection in those who visit clinics and does not naturally include asymptomatic cases unless detected in suggested routine medical check up.

CONCLUSIONS AND RECOMMENDATIONS

This study therefore concludes that since random samples have shown that *Giardia* and other intestinal infections are widespread in young children, combined advanced control measures are in dire need. There is also need for improved sanitary conditions, harmonized health education programs, scheduled examination and treatment of intestinal infections and follow-up of effectiveness of recommended regimen. Combination of such actions would enable improved living condition of children and the community in general (Tashima *et al*., 2009; Banke *et al*., 2006).

A comprehensive nationwide campaign is needed to map *Giardia* and other intestinal parasitic infection trends in young children for a successful and sustainable scheduled control program in Kenya.

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