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Original Article

Prevalence of Enteric Parasitic Diseases among Patients Referred at a Teaching Hospital in Kenya

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Abstract

Aim: Enteric parasitic diseases pose a serious public health problem worldwide and yet are neglected. To refocus attention on these diseases, a cross-sectional study was conducted to assess the prevalence of enteric infections in patients referred to referral hospital in Kenya. **Materials and Methods:** This study was conducted from April to December 2015 and involved a randomly selected sample of 185 patients. Fecal specimens were collected and delivered to laboratory for analysis. Preliminary macroscopic assessment of specimens for segments, larvae, and adult stages was done. To confirm the presence of ova, trophozoites, cysts, and oocysts, direct wet smear, formol–ether concentration, and modified Ziehl–Neelsen techniques were used. **Results:** Overall prevalence of 46.5% of enteric parasitic diseases was confirmed. Highest and lowest prevalence was due to protozoans and helminthes, respectively. Protozoan parasite prevalence was *Entamoeba histolytica* (23.9%), *Cryptosporidium parvum* (13%), *Entamoeba coli* (6.5%), *Giardia lamblia* (6.5%), and *Iodamoeba butschlii* (6.5%). Helminth prevalence was *Ascaris lumbricoides* (1.6%), *Hymenolepis nana*, *Trichuris trichiura*, and *Ancylostoma duodenale* each (0.5%). There was no significant difference in prevalence in age groups and gender ($P = 0.05$). Females were at the highest risk of *C. parvum* infection. Polyparasitism was prevalent among protozoans than helminthes. **Conclusion:** High prevalence of protozoan infections was observed among referred patients in comparison to helminthiasis. Based on reported multiple infections, deworming programs targeting helminthiasis should be restructured to incorporate diagnosis and treatment of enteric protozoan infections to reduce prevalence of enteric parasitic infections.

Keywords: *Amoebiasis, Ascariasis, Geohelminths, Giardiasis, Helminths, Protozoans*

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Introduction

Intestinal parasitic infections caused by protozoa and helminthes are among the most widespread of human infections worldwide. These constitute the greatest single cause of illness and disease and are important threats to healthy living in both developed and developing countries.[1,2] The world health organization estimates that approximately 60% of the world's population is infected with intestinal parasites known to play a significant role in morbidity and mortality.[3] The infections, therefore, constitute one of the greatest single worldwide causes of illness and disease.

The prevalence is high in Sub-Saharan Africa due to poor sanitary habits, lack of access to safe water, and improper hygiene, and hence, these infections are often referred to as diseases of poverty.[4] In many Africa countries, the prevalence of infections varies from one region and community to another due to

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various factors. This is in most cases is associated with contaminated environment and the sociocultural habits of communities.[5-7]

The parasites involved are single-celled protozoans and multicellular helminthes of various species known to infect humans since prehistoric times and have evolved with man throughout history.[8] The clinical presentation of diseases associated with the parasites varies depending on species but generally includes diarrheal illness caused by protozoans such as *Entamoeba histolytica*, *Cryptosporidium parvum, Giardia lamblia,* and intestinal helminthiases caused by geohelminths including nematodes and some trematodes.

Enteric protozoan infections are prevalent and constitute a major global infectious disease burden. The most common being amoebiasis caused by intestinal amoeba, *E. histolytica*, is an important parasitic disease worldwide, with the highest impact reported in developing countries.[9] *C. parvum* has been consistently associated with diarrhea in HIV/AIDS worldwide.[10,11] *G. lamblia*, causative agent of giardiasis, is prevalent worldwide infecting an estimated 200 million people.[12-14] *Blastocystis hominis* whose parasitic status is not clearly known has been reported in humans and its prevalence is not adequately documented.[15]

The global prevalence of intestinal geohelminth infections is estimated to be over 1 billion cases of *Ascaris lumbricoides,* 740 million cases of *Necator americanus* and *Ancylostoma duodenale,* and 795 million cases of *Trichuris trichiura*. The four intestinal geohelminths occasionally occur concurrently in the community, resulting in multiple infections over a period of time, especially in children with serious adverse effects.[16]

Intestinal parasitic infections have serious consequences on human health, such as swollen liver and spleen and intestinal bleeding.[16] In spite of the current intervention strategies in Kenya and many other countries, the infections remain a major but neglected health problem, and yet, in most of the endemic areas, there is continued exposure.[17] There is a need to encourage renewed interest and focus on these neglected tropical diseases.

Adverse effects including disabilities due to various intestinal parasitic infections are prevalent among patients who seek medical attention at county health facilities in Kenya that lack modern laboratory equipment and are inadequately staffed.[17] The patients often present with nonspecific clinical manifestations and diagnosis based on clinical observations alone are often misleading and may lead to wrong treatment. Such inadequately managed infections, therefore, persist and most of the cases end up as referral cases at teaching hospitals for appropriate laboratory-based diagnosis and disease management. The aim of the study was to identify the specific enteric parasite species and assess the gender- and age group-related disease prevalence among patients referred to a teaching and referral hospital. The purpose of the study was to encourage renewed interest and focus on these neglected tropical diseases some of which have emerged as important opportunistic infections in the current AIDS pandemic and requires specific diagnostic testing and treatment approaches. Prevalence findings provide a basis for targeted approach for treatment based on evidence-based diagnostic test results. This is important in improving patient treatment outcome, rational use of drugs, and setting up of appropriate community-based specific intervention programs.[18,19]

Materials and Methods

Study site and setting

The study was conducted at referral hospital, located along Nandi road in Eldoret town, 310 km northwest of Nairobi city, Kenya. It is the second largest national hospital and the main referral facility in Uasin Gishu County and in the North Rift region of western Kenya. The hospital has 800-bed capacity and is a teaching and referral facility that receives patients from western Kenya, parts of eastern Uganda, and southern Sudan. The hospital offers a wide range of specialized services to both outpatients and inpatients. The hospital has modern state of the art clinical and diagnostic equipment manned by qualified and experienced medical, paramedical, and support staff from the hospital and the college of health sciences.

Study design, population, and sample size

This was an analytical cross-sectional study conducted from April 1 to December 31, 2015. The study population consisted of all consenting age groups and sexes who were referred to the laboratory for stool analysis. All willing participants were advised on how to collect fresh stool specimen without contamination and were provided with the polypots. They were instructed to collect fresh stool and deliver it immediately to the parasitology diagnostic laboratory.

Basing on previous related studies, the sample size was calculated at 95% confidence level and 5% marginal error. The study sample size (n) was estimated using modified Fisher's formula as used by Mugenda and Mugenda.[20]

$n = z^2pq/d^2$

n = Desired sample size

z = Standard normal deviate (1.96)
p = Prevalence of intestinal parasites from previous study of 13.7%
$q = 1.0 - p$
d = Degree of accuracy
$n = (1.96)^2\ (0.14)\ (1.0\text{–}0.14)/(0.05)^2$
n = 185 patients.

Therefore, the minimum sample size aimed at was 185 patients.

The study participants were categorized into the age groups: <9 years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, and 50 and beyond years.

Inclusion and exclusion criteria

All patients who were sent to the laboratory for stool analysis and consented by signing the provided form were included in the study. Patients of unsound mind and those whose parents/guardians did not consent were excluded from the study.

Ethical considerations

The study protocol was approved by the Institutional Research and Ethics Committee approval reference number 0001601. The purpose and benefit of the study were explained to the patients through informed verbal consent before signing the consent form. For participants below the legal age of 18 years, consent was obtained from parents/guardians before considered for enrollment in the study.

For confidentiality, all participants were identified by specific codes and none of them was identified by name. There was no monetary benefit or any form of inducement for participation in the study. However, patients diagnosed positive with intestinal parasites were referred to a clinician for treatment and management. All individuals in the population were recruited regardless of age, ethnic origin, education, marital status, or social status so long as consent was obtained.

Collection and preservation of stool specimens

All consenting patients were given a dry, clean, leak-proof plastic container labeled with the serial code age, date and gender for identification, and a wooden scoop for the collection of stool specimen. They were guided on how to collect the specimen appropriately. In the case of children, stool was collected immediately after defecation and specimen put into the sample bottle. They were advised to fill half the container and safely discard the scoop after use. The stool specimens were delivered to the laboratory for processing.

Once specimens were received in the medical parasitology laboratory, they were either processed or preserved in 10% formalin until Formol-ether concentration technique was performed. Preservation of the specimens was essential for maintenance of protozoal morphology and also to prevent further development of helminthic eggs and larvae and thus render the specimens safe.

Specimen processing and identification of parasites

Immediately after delivery in laboratory, all stool specimens were examined macroscopically for adult and the larval stages of helminth parasites. Further, the specimens were analyzed microscopically for the presence of trophozoites, ova, oocysts, and cysts using both direct saline and iodine mounts on clean grease-free slides. Slides were then prepared directly for wet mount in saline as well as in iodine and were microscopically examined for helminth cysts or eggs and protozoan parasites.

Detection and identification of protozoal cysts and helminth eggs were achieved by formol–ether concentration technique.[21] One gram of stool specimen was fixed by emulsifying in 7 ml of 10% formal saline and kept for 10 min. It was then strained through a wire gauge and the filtrate was collected in a centrifuge tube. Three milliliters of ether was added to it and the mixture was shaken vigorously for 1 min. It was then centrifuged at 2000 rpm for 2 min and then allowed to settle. The debris was loosened with a stick; the upper part of the test tube was cleared of fatty debris; and the supernatant fluid was decanted, leaving 1 or 2 drops. The deposit, after shaking, was poured on to a glass slide, and a cover slip placed over it and the specimen was examined microscopically.

Modified Ziehl–Neelsen (Z-N) technique was used to identify coccidian oocysts in stool specimens. Stool smears were prepared from the concentrated stool specimen; air dried and stained by the modified Z-N staining technique for identification of oocysts of *Cryptosporidium* species, *Isospora belli*, and *Cyclospora cayetanensis* following the method described by Cheesbrough (1985).[22] The smears were fixed with methanol for 10 min and 7 drops of carbol fuchsin were flooded for 3 min. Decolorization was done with 5% sulfuric acid for 30 s. Then, it was counterstained with methylene blue for a minute. The smear was rinsed, drained, air-dried, and examined under oil immersion power. This diagnostic technique is the most suitable for demonstration of oocysts of the protozoans. Microscopy was done first with power (×40 = 400 times magnification) to determine the distribution then power (×100 = 1000 times magnification) bright field for identification. For each batch of smears which was processed through the modified Z-N stain, positive control was included for quality assurance. Each sample was observed microscopically by two other technologists for confirmation and verification before declaring the final result.

Quality assurance and data analysis

To ensure quality results, only trained, qualified, and experienced research assistants were engaged in the study. It was mandatory for slide preparations to be checked by three different observers before declared negative. A third of the slides was randomly selected and sent to the Department of Medical Microbiology and Parasitology, School of Medicine, for results' verification quality assurance.

All data were checked for accuracy before it was entered into and analyzed using SPSS incorporation for windows, version 16.0. (Chicago, USA). Descriptive and inferential statistics such as mean median, standard deviations, and ranges were carried out for continuous data while frequency listing and percentages were used to explore categorical data. Prevalence was calculated for each identified parasite species and association between categorical variables such as the gender status assessed using Chi-square test. In all analyses, $P < 0.05$ was considered statistically significant.

Results

Participant's characteristics and identified parasites

A total of 185 participants were enrolled in the study translating into 104 (56.2%) females and 81 (43.8%) males. The participants had an age range of between 2 and 70 years with a mean age of 24. The total number of patients who were diagnosed positive for parasitic infections was 86 (46.5%). The number of positive cases due to protozoans was 103 (56%) while those diagnosed with intestinal helminthiases was 7 (6.4%). The identified protozoan and helminth parasite species are presented in Table 1.

Enteric parasitic infection distribution in age groups

Amoebiasis was the most prevalent infection across all age groups in all referred cases. The prevalence was low (3.7%–12.9%) in the lower age groups than in the elderly (25%). However, there was no significant difference in prevalence between the age groups (P = 3.525). Cryptosporidiosis had moderate prevalence but sufficient to cause concern because of its current status as important opportunistic infection in HIV/AIDS patients. Both cryptosporidiosis and giardiasis occurred as low prevalence infections in all age groups except the elderly and there was no significant difference between age groups (P = 1.160). The age group-related protozoal and helminth infection prevalence distribution is presented in Table 2.

Comparison of age group- and gender-related parasitic infections

Overall, of the two categories of enteric infections (protozoal and helminth infections), protozoal infections were the most prevalent in all age groups and both genders. Under this category, amoebiasis was the most common infection in all ages and gender, while cryptosporidiosis and giardiasis were the least prevalent. However, cryptosporidiosis had a higher infection rate in females than males whereas amoebiasis was more prevalent in males than females.

Compared to protozoal infections, intestinal helminth infections were least prevalent in age groups and both genders. Whereas protozoal infections were spread across all age groups, no positive cases of helminthiasis

Table 1: Identified parasite species and positive cases

Parasites	Stage identified	Associated infection	Diagnostic test	Number of positive cases and percentage
Protozoans				
Entamoeba histolytica	Cysts	Am	Formol ether	43 (23.9)
Cryptosporidium parvum	Cysts	Cr	Modified Ziehl–Neelsen	24 (13)
Entamoeba coli	Cysts	Am	Formol ether	12 (6.5)
Giardia lamblia	Cysts	Gi	Formol ether	12 (6.5)
Iodamoeba buetschlii	Cysts	Am	Formol ether	12 (6.5)
Subtotal				103 (55.7)
Helminthes				
Ascaris lumbricoides	Ova	As	Formol ether	3 (1.6)
Hymenolepis nana	Ova	Hy	Formol ether	1 (0.5)
Trichuris trichiura	Ova	Tr	Formol ether	1 (0.5)
Ancylostoma duodenale	Ova	HWD	Wet smear	2 (1.1)
Subtotal				7 (3.8)
Total				110 (59.5)

Am: Amoebiasis, Gi: Giardiasis, Cr: Cryptosporidiosis, As: Ascariasis, Tr: Trichuriasis, HWD: Hookworm disease, Hy: Hymenolepiasis were recorded in lower and upper age groups. This implies that helminthiasis was only prevalent in middle age groups (10–39 years) of the referred cases. Low-level prevalence of helminthiasis was recorded in both genders. No cases of giardiasis, cryptosporidiosis, and helminthiasis were detected in referred patients' age >50 years. In general, the prevalence of the parasitic infections was higher in males than females, but this difference was not statistically significant ($P > 0.05$). Comparative age group- and gender-related prevalence is presented in Table 3.

Intestinal polyparasitism

Multiple intestinal infections were 6.4% prevalent in the referred cases with the most common combinations being protozoan species *E. histolytica* and *Entamoeba coli*, *E. histolytica* and *Iodamoeba butschlii.* A rare combination of *A. duodenale* and *I. butschlii* was also recorded. There was no statistically significant difference in the number with single and multiple infections ($P = 0.562$). Polyparasitism was not specific to a particular age group or gender.

Discussion

A variety of protozoan and helminth parasite species were identified from referred patients and confirmed as causative agents of gastrointestinal problems and general ill health. The study findings confirm that overall, protozoan parasites were the main cause of enteric infections among referred cases. The parasite species *E. histolytica*, *E. coli*, *I. butschlii*, and *G. lamblia* accounted for over 55% of all infections associated with protozoans compared to intestinal helminths at 7%. This is comparable to related studies.[23,24] In these studies, the prevalence of intestinal parasitic infection by protozoa ranged 33%–53%. On the other hand, our findings are in disagreement with studies done elsewhere. The findings in these studies indicate protozoan prevalence as low as 13.2% to 34% and that of helminth infections as 26.9%.[6,25,26] The variance could be explained by application of different diagnostic tests and variations in sample size and selection procedures and participant inclusion and exclusion criteria.

Table 2: Age group-related enteric parasitic infections

Parasite infection	Infection prevalence in age groups (years) (%)					
	<9	10-19	20-29	30-39	40-49	>50
Protozoal infections						
Am	24 (12.9)	22 (11.8)	24 (12.9)	21 (11.4)	7 (3.7)	20 (25)
Gi	1 (1.8)	1 (1.8)	2 (3.7)	2 (3.7)	1 (1.8)	0
Cr	10 (5.4)	6 (3.2)	9 (4.8)	3 (1.6)	8 (4.3)	0
Subtotal	35 (100)	29 (90.6)	35 (94.6)	26 (89.7)	16 (100)	20 (100)
Helminthic infections						
As	0	1 (1.8)	1 (1.8)	1 (1.8)	0	0
Tr	0	0	0	1 (1.8)	0	0
HWD	0	1 (1.8)	1 (1.8)	1 (1.8)	0	0
Hy	0	1 (1.8)	0	0	0	0
Subtotal	0	3 (9.4)	2 (5.4)	3 (10.3)	0	0
Total	35	32	37	29	16	20

HWD: Hookworm disease, Am: Amoebiasis, Gi: Giardiasis, As: Ascariasis, Tr: Trichuriasis, Cr: Cryptosporidiosis, Hy: Hymenolepiasis

Table 3: Comparison of age group- and gender-related prevalence

Variable	Prevalence of protozoal infections (%)			Prevalence of helminthic infections (%)				*P*
	Am	Gi	Cr	As	Tr	HWD	Hy	
Age groups (years)								
<9	24 (12.9)	1 (1.8)	10 (5.4)	0	0	0	0	1.160
10-19	22 (11.8)	1 (1.8)	6 (3.2)	1 (1.8)	0	1 (1.8)	1 (1.8)	
20-29	24 (12.9)	2 (3.7)	9 (4.8)	1 (1.8)	0	1 (1.8)	0	
30-39	21 (11.8)	2 (3.7)	3 (1.6)	1 (1.8)	1 (1.8)	1 (1.8)	0	
40-49	7 (3.7)	1 (1.8)	8 (4.3)	0	0	0	0	
>50	20 (25)	0	0	0	0	0	0	
Gender								
Males	67 (36.2)	3 (1.6)	10 (5.4)	1 (0.5)	0	1 (0.5)	0	0.667
Females	51 (27.6)	4 (2.2)	27 (14.6)	2 (1.1)	1 (0.5)	1 (0.5)	0	

Am: Amoebiasis, Gi: Giardiasis, Cr: Cryptosporidiosis, As: Ascariasis, Tr: Trichuriasis, HWD: Hookworm disease, Hy: Hymenolepiasis

Amoebiasis caused by *E. histolytica* was the most prevalent protozoal infection in all age groups (3.7%–25%) and both genders (27.6%–36.2%). This suggests that all ages and genders are susceptible to infection. The findings are in agreement with related studies in developing and developed countries.[27-29] Further, related studies reveal similar trend in amoebiasis gender-related prevalence, for instance, Nepal (17%–22%), Brazil (26%–30%), and Ethiopia (32%–36%).[25,30,31] Exceptionally higher rates of amoebiasis infection in males and females have been reported elsewhere in Africa (60%–64%) and other regions of Kenya (48%–52%).[32,33] In these studies, fairly large samples collected for longer period were used. This is also suggestive of sustained environmental contamination and poor hygiene which enhances continuous infection and re-infection.

The high prevalence of amoebiasis suggests that the infection transfer between and among persons through food or water is high. This is an indication of high-level fecal contamination by animal reservoir hosts and humans. The human role in this aspect is further supported by reported high number of asymptomatic cases in general population that constitute source of infection to others without suffering ill health.[34] In addition, the high prevalence of *E. histolytica* could be due to the double-walled resistant cysts of the parasite which can withstand and survive adverse environmental including chemical water treatment.[4]

The 13% prevalence of cryptosporidiosis was comparable with other related studies in Africa.[35,36] However, this prevalence was lower compared to 32%–67% reported in immunosuppressed and symptomatic subjects.[37,38] This re-emphasizes the importance of *C. parvum* as an intestinal opportunistic infection. The high prevalence rate of 33% recorded in the females in the 40–49 years' age group may to some extent be attributed to age-related weakened immunity and autoinfection due to repeated exposure to infective oocysts. Further, in African setting, it is the women who take care of the sick including HIV/AIDS victims at home. In this regard, close contact while taking care of patients enhances probability of transmission through accidental ingestion of oocysts in contaminated water or food. It is possible that cases of cryptosporidiosis are underestimated and therefore not adequately documented because modified Z-N test is not routinely used in most of the hospital laboratories at county level. Such cases including healthy carriers remain continuous source of infection in a community unless referred to facilities with modern diagnostic facilities.

Compared to other enteric protozoal infections, giardiasis has the lowest prevalence (1.6%–3.7%) across all age groups and gender except patients >50 years. This is low prevalence compared to similar study in which prevalence of 20%–30% was reported. However, in both studies, there was evidence of giardiasis persistence in the community. This phenomenon could be attributed to animal reservoirs, cross-infectivity between animals and humans, and asymptomatic individuals and possibly involvement of several parasite species and strains. The net effect is increased risk of transmission in all age groups and gender and therefore a possible explanation for the cosmopolitan nature of giardiasis which may in the near future emerge as an opportunistic infection.[39,40] The nonpathogenic protozoa found in all age groups were *E. coli* and *I. butschlii*.

The prevalence of ascariasis and trichuriasis was low (0.5%–1.6%) comparable with several similar studies.[41-44] It is possible that the ova of these nematodes require further development outside the host before becoming sufficiently infective. In harsh environment (dry soil contaminated with pesticides and chemical fertilizers), many of the ova die and become noninfective even if ingested. The few ova that survive are probably the ones responsible for the low infection prevalence.[45] In related studies, either none or very few cases of the two parasites have been reported.[5,46] However, isolated reports of prevalence exceeding 12% have been documented.[47,48] The overall low prevalence of intestinal helminthiasis in the study is possibly indicative of a positive outcome of ongoing school-based deworming program at county level. Diagnosis and treatment of school children accompanied with community health education could explain the low rate of intestinal helminthiasis reported elsewhere.[49]

The present study findings indicate an equal exposure of both genders to enteric parasitic infections. This suggests that these infections may be associated with everyday activities of individuals rather than gender. Therefore, under shared similar environmental conditions, gender has no influence on the prevalence of enteric parasitic infections. Nevertheless, age has a profound effect on infections. Children who most often have a tendency to eat food without hand washing unless reminded or may lick contaminated fingers end with higher rates of infection compared to adults. Furthermore, the age group comprises individuals who are increasingly involved in outdoor activities including handling items likely to be fecal contaminated which predispose them to parasitic infections.

Cases of polyparasitism were more prevalent among intestinal protozoa and rare between intestinal helminthes and protozoans. Even though the parasites in the study share similar external and internal environmental conditions and all are feco-orally transmitted, the rare occurrence of polyparasitism involving helminthes and protozoa needs further investigation.

Conclusion

Based on the study findings, *E. histolytica* and *G. lamblia* were the most prevalent pathogenic intestinal protozoa and major contributors to ill health and diarrheal disease while *A. lumbricoides, A. duodenale,* and *T. trichiura* were less common in patients referred to teaching and referral hospital.

All age groups were susceptible to enteric parasitic infections but at different rates. The most prevalent parasitic infections were amoebiasis, cryptosporidiosis, and giardiasis.

Both genders were susceptible to infection with protozoal and helminth parasites, though among the protozoal infections, cryptosporidiosis was more prevalent in females than males.

Cases of polyparasitism involving protozoans were common while a combination of protozoa and helminthes was rare.

Considering that all the parasites in the study are feco-orally transmitted and confirmation of polyparasitism, we conclude that faecally polluted environment and poor sanitary conditions lead to continuous infection and re-infection in the community. These together with the reported prevalence rates of enteric parasitic infections among the referred cases suggest that the infections constitute a major a public health problem.

Recommendations

We recommend that since provision of health services is a devolved function in Kenya since 2010, the regional governments should focus more attention on: community-based health promotions with a component on regular checkups and treatment of enteric parasitic infections; provide modern and appropriate diagnostic equipment and laboratories and scale up deworming programs; provide clean water for domestic use; and improve on environmental hygiene and human waste disposal.

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Conflicts of interest

There are no conflicts of interest.

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