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Four-year health-point surveillance of Human Gastro-intestinal parasites among patients attending a clinic in Bushenyi, Uganda

Agwu[*1] E, Tanayen[2] G and Moazzam[3] ML

1. Department of Microbiology, 2. Department of Medical Laboratory Sciences and 3. Dr. Moazzam Mohiuddin Lodhi, Department of Public Health, all at Kampala International University, Western Campus, Ishaka, Box 71, Bushenyi, Uganda.

Corresponding author: *agwu@spparenet.org and Phone: +256782101486, +256703129679

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ABSTRACT

Background: Despite advances in disease diagnosis, parasitic infection remains a public health challenge especially in developing countries with limited resources.

Objective: To determine the pattern of intestinal parasite prevalence in Bushenyi district Uganda.

Material and Methods: Standard parasitological methods were used to analyze stool samples for intestinal parasites for four years. Eight hundred and seventy-nine [(402 males and 477 female)] out-patients stool samples collected for four years between 2009 and 2012 were analyzed. Chi-square test was used to test for statistical significance of the result obtained (α=0.05).

Results: There was a 15.9% overall parasite prevalence in this study. The observed annual relative prevalences were: 68.4% in, 2009, 9.4%in 2010, 17.9% in 2011, and 6.2% in 2012 among the studied population respectively. The emergence of 0.5% Strongiloides stecoralis in 2012 alone and the absence of parasites co-colonization from 2010 to 2012 deserve research attention. Females were more relatively vulnerable to parasitic infestation compared to their male counterparts. Parasites prevalence decrease by about 70% between the years 2009 and 2012, has malaria control policy implications. The age group 21-30 years had the highest parasite prevalence (20.0%) in this study.

Conclusions: Pattern of parasites prevalence was significantly ($p<0.05$) dependent on age, year, and sex of participants. The observed downward trend in malaria spread between 2009 and 2012 has policy implications and prevalence (15.9%) of intestinal parasites may indicate the endemicity of associated diseases (anemia, dysentery, and diarrhea) in the Bushenyi District. Improved intervention is needed to reduce infection to the barest minimum

Keywords: Investigation, Prevalence, Helminthes, Protozoa, Flagellates, Uganda

INTRODUCTION

Parasitic diseases are widely distributed throughout the world and they continue to be problematic in developed, developing, and less developed countries. The disease-causing parasites are typically obligate organisms, dependent on vertebrate hosts, arthropod hosts, or both for survival and the parasites may produce serious infections and occasionally, the death of their hosts (1).

Three soil-transmitted helminth infections-ascariasis, hookworm infection, and trichuriasis are among the infections classified as neglected tropical diseases (NTD) and lead to long-term disability and poverty (2-3). NTD occurs primarily in rural areas and some poor urban settings of low-income countries in sub-Saharan Africa, Asia, and Latin America. These features contrast with those of emerging acute infections, such as avian influenza, *Ebola virus* infection, and West Nile virus infection (4). In aggregate, the NTD cause approximately 534,000 deaths annually (3). In terms of disability-adjusted life-years, the NTD together rank closely with diarrheal diseases, ischemic heart disease, cerebrovascular diseases, malaria, and tuberculosis as being among the most important health problems in the developing world (2-3). Besides, the effect of the NTD on worker productivity causes annual great losses of a significant magnitude capable impacting of the economy of any affected country (5-7).

Parasitic infestations represent one of the commonest problems in the Ugandan population. It has been reported that some intestinal parasites cause anemia which weakens the individuals and render them unable to do gainful activities further making the individuals succumb easily to other infections and infestations; makes the individuals more likely to respond slowly to treatment and which contributes to the development of complications of the disease and ultimately impoverishes them (8). The parasites may contribute to anemia in pregnancy and lead to poor pregnancy outcomes for both mothers and children. For school children, anemia reduces their cognitive ability and hence affects their academic performance (9). The upsurge of poverty; shortage of clean drinking water; poor-nutrition, health education, health facilities, personal and environmental hygiene in sub-Saharan Africa has raised infection due to human gastro-intestinal parasite to a public health dimension (10).

There is no surveillance system on intestinal parasitic infection in Bushenyi. Although the intestinal parasites are detected on routine diagnoses in the hospitals, they are not properly analyzed and published to help healthcare providers to take the necessary preventive, control, and curative measures. Even those detected on routine bases are not properly documented making information retrieval difficult and establishment of sentinel studies based on available data almost impossible.

The whole matter is worsened by the reluctance of patients attending clinics to give out stool samples for analysis for fear of the samples being used unethically even after getting ethics approval. A database is urgently needed to help predict the future trend of infection especially in this circumstance where there is a limited diagnostic facility. In this prospective laboratory-based health-point study, we there determined the prevalence of intestinal parasites among patients attending KIUTH Uganda for 4 years between 2009 and 2012 with the ultimate goal of establishing a trend of parasitic infections for use by healthcare providers and other stakeholders in this region with the ultimate goal of improving the diagnostic accuracy of parasitic infections with limited resources.

MATERIALS AND METHODS

This laboratory-based study was conducted in Kampala International University Teaching Hospital (KIUTH), in Bushenyi District Uganda. Informed consent from patients was sought and obtained and then the ethical review committee of KIUTH approved this study. Stool samples were collected in a sterile screw-capped, transparent, leak-proof wide-mouthed universal container, given to patients who came for stool analysis either as an in-patient, out-patient, or were referred to KIUTH from other clinics in Bushenyi. Eight hundred and seventy-nine (402 males and 477 female) patient's stool samples were analyzed over four years between 2009 and 2012. The stool samples were collected for analysis from patients with symptoms of lower abdominal pain and gastrointestinal discomfort at KIU-TH. Eight hundred and seventy-nine samples analyzed for this survey were obtained by using guidelines previously reported (8). Patients, who were on anti-parasitic medication were excluded. Chi-square tests were used to test the association between the degree or percentage of parasitic distribution and the demographic characteristics of participants per year (α=0.05).

Okpala et al. reported that direct light microscopy of stool samples is a reliable means of detecting the presence of parasites from stool samples (8). Therefore, direct microscopic (saline wet preparation for parasitic analysis) examination of stool samples for detection of intestinal parasites was conducted. Cheesbrough recommended staining the background of a stool smear with eosin to increase the visibility of the organism (11). A drop of commercially available eosin was therefore added to the wet mounts and re-examined with x10 and x40 low power objectives for the possible presence of cyst, ova, larva, trophozoites, or segments of parasites. Iodine wet mount for parasitic analysis was also made using the procedure reported previously (11). Iodine preparation was made to detect trophozoites and intestinal flagellates which might be stained by iodine to increase visibility and examined with x10 and x 40 low power objectives.

The stool samples soaked in sterile saline, iodine or eosin were incubated for 18-24 hours at 37^{o}C. After the incubation, saline, eosin, and iodine preparations were repeated as above and with x10 and x 40 low power objectives for the possible presence of cyst, ova, larva, trophozoites, or segments of parasites.

RESULTS

There was a 15.9 %(140 out of 879) overall prevalence of parasites in the 4 years survey. The annual prevalence was: 68.4% in 2009; 9.4% in 2010, 17.9% in 2011 and 6.2% in 2012 respectively [Table 1]. The prevalence of *Entamoeba histolytic* was highest in 2009, not seen in 2010, 5% 2011was detected and it was found in 1.4% of hospital attendees in 2012. Again, the prevalence of *Entamoeba coli* was ranked high up to 14.5% in 2009, *Entamoeba coli* showed 2.5% prevalence in 2010 and 2011 before disappearing completely in 2012. *Ascaris lumbricoides* were 9.2% prevalent in 2009 then decreased to 1.3% and 1.1% in 2010 and 2011 before going down to 0.3% in 2012. Hookworm was relatively stable in prevalence except in 2011 when it rose to 2.2% from 1.3% and 0.6% in 2009 and 2010 respectively before disappearing completely (0.0%) in 2012.

The emergence of 0.5% Strongiloides stecoralis in 2012 alone and the absence of parasites co-colonization from 2009 to 2011 deserve attention. Females were more vulnerable to parasitic infestation compared to their male counterparts throughout the four-year study period (Table 2). The percentage ratio of male/female parasites distribution recorded across the four year period include 25% / 43.4%, 6.2% / 8.8%, 8.3% / 8.6% and 1.1% / 4.6% for 2009, 2010, 2011, and 2012 respectively.

Generally, parasitic prevalence decreased from 2009 to 2012 in most age groups of the studied samples; e.g. females aged 21-30 years, showed the parasitic prevalence of 15.8% in 2009, 1.8% in 2010, 1.4% in 2011, and 0.8% in 2012. Again the prevalence of parasites among the females in the age group above 60 years gradually decreased to 3.9% in 2009, 0.6% in 2010, 0.4% in 2011, and 0.00% in 2012 respectively. On the other hand, males showed annual fluctuation in parasitic prevalence e.g. males aged 11-20 years in 2009 had 2.6% prevalence, 0.6% in 2010, 1.1% in 2011, and 0.3% in 2012 respectively.

Prevalence of parasites in female aged ≤10 years was low (2.6% in ten and below years of age and 3.9% in sixty and elderly aged females) in 2009, it remained the same for both aged groups in 2010, then it progressed in females aged ≤10 years up to 2.8% and 1.4% as compared to 0.4% to 0.0% in elderly ≤60 years aged group in the year 2011 and 2012 respectively (Table 2). The age group 21-30 years had the highest parasitic prevalence in this study.

The prevalence of intestinal parasites was significantly ($p<0.05$) dependent on age and sex.

Table 1: Prevalence of intestinal parasites among the studied population n=879

		Number (%) prevalence for 4 years		
Parasites	2009(n=76)	2010(n=159)	2011(n=278)	2012(n=366)
Entamoeba histolytica	17 (22.4%)	0 (0.0%)	14 (5.0%)	05(1.4%)
Entamoeba *coli*	11 (14.5%)	04 (2.5%)	07 (2.5%)	0 (0.0%)
Ascaris lumbricoides	7 (9.2%)	02 (1.3%)	3(1.1%)	1 (0.3%)
Giadia lamblia	2 (2.6%)	08 (5.0%)	18 (6.5%)	14 (3.8%)
Trichomonas hominis	2 (2.6%)	0 (0.0%)	02(0.7%)	1(0.3%)
Hookworm	1 (1.3%)	1 (0.6%)	06(2.2%)	0(0.0%)
Strongiloides stecoralis	0(0.0%)	0(0.0%)	0(0.0%)	2(0.5%)
Ascaris spp and *E-histolytica*	6 (7.9%)	0 (0.0%)	0(0.0%)	0 (0.0%)
Ascaris lumbricoides and *Giadia lamblia*,	3 (3.9%)	0 (0.0%)	0 (0.0%)	0(0.0%)
Trichomonas hominis and *Ascaris* lumbricoides	2 (2.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Schistosoma mansoni and Entamoeba histolytica	1 (1.3%)	0 (0.0%)	0(0.0%)	0(0.0%)

X^2=40.2 (P<0.05)

Table 2: Age and sex specific prevalence of intestinal parasites from 879 stool samples analyzed

n= 879

	2009, n=76				2010, n=159				2011, n=278				2012, n=366			
	[Male=27		Female=49]		[Male=83		Female=76]		[Male=116		Female=162]		[Male=176		Female=190]	
Age	NS	No (%)+	NS	No (%)+	NS	No (%)+	NS	No (%)+	NS	No (%)+	NS	No(%)+	NS	No (%)+	NS	No(%)+
≤10	1	0(0.0)	5	2(2.6)	19	4(2.5)	9	1(2.5)	28	3(1.1)	35	8(2.8)	35	1(0.3)	33	5(1.4)
11-20	2	2(2.6)	3	2(2.6)	15	1(0.6)	21	0(0.0)	33	3(1.1)	31	8(2.8)	38	1(0.3)	36	1(0.3)
21-30	6	5(6.6)	13	12(15.8)	17	1(0.6)	18	3(1.8)	20	6(2.2)	37	4(1.4)	26	2(0.5)	43	3(0.8)
31-40	5	3(3.9)	8	5(6.6)	12	1(0.6)	13	6(3.7)	7	6(2.2)	20	1(0.4)	20	0(0.0)	31	3(0.8)
41-50	5	4(5.3)	6	5(6.6)	9	0(0.0)	5	2(1.3)	9	2(0.7)	14	1(0.4)	16	0(0.0)	20	3(0.8)
51-60	3	2(2.6)	7	4(5.3)	7	2(1.3)	4	1(0.6)	6	2(0.7)	13	1(0.4)	17	1(0.3)	12	2(0.5)
≥60	5	3(3.9)	7	3(3.9)	4	1(0.6)	6	1(0.6)	13	1(0.3)	12	1(0.4)	24	1(0.3)	15	0(0.0)
Total	27	19(25.0)	49	33(43.4)	83	10(6.2)	76	14(8.8)	116	23(8.3)	162	24(8.6)	176	4(1.1)	190	17(4.6)

X^2=18.9; P<0.05; n=879; NS= number sampled; No (%) +=number percentage positive;

DISCUSSION

Developing countries in sub-Saharan Africa may continue to record increased cases of parasitic infection due to human gastro-intestinal parasites due to sustained acute shortage of clean drinking water; poor nutrition, health education, health facilities, and poor personal/ environmental hygiene (10). The 15.9% prevalence of intestinal parasites reported in this study (Table 1) is lower than 42.6% reported from Moyo district of Uganda, lower than 28.4% from Abeokuta Nigeria, lower than 44.8 % from Ethiopian HIV patients, and lower than two old reports of 89.5% and 70.8% from Ilorin and Lagos Nigeria respectively (13-17). Factors that define the endemicity of parasites in a given population include: environmental, parasitic, and host factors (14). In the Bushenyi district, good sanitary conditions are limited and sewage, sludge, and feces commonly contaminate water reservoirs, used as the main source of domestic water. We had earlier reported that: poor sanitary conditions; method of anal cleansing after defecation especially in females and periodic migration of people from one endemic location to another contribute to parasite transmission in resource-limited settings (8, 18-19).

High parasite prevalence was recorded when the result obtained in 2009 was presented as a poster during the 19th European congress of clinical microbiology and infectious disease (Abstract No: 1251), 19th ECCMID, at Helsinki, Finland. Unfortunately, when the survey continued for another 3 years (2010-2012), there was a dramatic decrease in prevalence and absence of parasite co-infections. This observation may be due to: a. Strengthened capacity to detect intestinal parasites at the laboratories of KIUTH and Bushenyi district health centers which usually serve as filter-clinics before samples are referred to KIUTH laboratories; b. Improved effectiveness of the existing national deworming exercise to reduce parasite burden over four years and c. Effective community-directed health education/promotion on reduction of intestinal parasites at the grass root.

This observed decrease may be explained by the fact that several public and private organizations have since 2010 introduced interventions aimed at reducing intestinal parasites burden in the Bushenyi District. One such example is the popular bi-annual health education and promotion training program/workshop called diagnostic portfolio for health care professionals in resource-limited settings, a five days workshop organized by the Special Pathogens Research Network Limited, Uganda. Uganda Ministry of health together with the center for disease control, world health organization, tropical disease research, and others now have better community-based working relationships whereby research consortia and multidisciplinary forums are encouraged instead of individual or single institutional research initiatives.

A thorough assessment of the data in (Table 1) reveals a constant trend in which prevalences of most identified parasites were high in 2009, and effectively reduced from 2010 to 2012. Although this study did not aim to monitor/evaluate the effectiveness of any intervention program, the data in (Table 1) showing a decreasing trend of infection rates over 4 years, clearly points towards an effective strategy in intestinal parasite intervention in the Bushenyi District. This decreasing trend in parasite distribution after the result of the 2009 short investigation was presented at the international conference may indicate an improvement in response to the published 20119 results. Strengthening of existing strategies in the study area is strongly recommended.

Dumba *et al* had suggested that poor hand washing, type of latrine, level of education, keeping of pigs, poor accessibility to clean water, and type of house floor were strongly associated with intestinal helminthic infection in Luweero District of Uganda (18). This explains the observed 15.9% parasitic prevalence in Bushenyi. Again, consumption of food (roasted meat, cooked rice/beans, fried potato chips and wheat flour) hastily and unhygienically prepared and served by the roadside, may explain the observed 15.9% prevalence of the intestinal parasite in this study. Other local stable food like maize and millet porridge (prepared by mixing maize and millet flour with water) commonly eaten in Bushenyi may also provide an avenue for parasitic infection while these food are prepared with the minimum sanitary condition.

Although the year 2009 report of 22.4 % prevalences of *Entamoeba histolytica* and 14.5% *Entamoeba coli* were higher than 4.0% prevalence reported from the United States and 5.7% *Entamoeba histolytica* and *Entamoeba coli* respective prevalences reported from Abeokuta Nigeria, years 2010 to 2012 reports were effective in line with the United States and Nigeria reports above (21, 14). Infection by *Entamoeba histolytica* and *Entamoeba coli* is common in communities with poor socioeconomic and inadequate sanitation. Since the endemicity of the intestinal parasites in an area is determined by the level of sanitation in that area, it is not clear why the prevalences of *Entamoeba histolytica* and *Entamoeba coli* in Bushenyi were in line with USA and Nigeria study (16). Compared to the USA and Abeokuta cities, Ishaka Bushenyi is a small university town that is growing at an alarming rate and so is the hygiene status improving as the presence of the KIUTH transforms Ishaka from a rural to a semi-urban area.

The actual frequency of amoebiasis due to *Entamoeba histolytica/Entamoeba coli* has been difficult to establish especially in resource-poor settings as there is a tendency to overestimate it in endemic areas where many cases of dysentery and bloody diarrhea are either misdiagnosed or under-diagnosed as insignificant gastrointestinal disorder (22). Clinical problems with intestinal parasites are usually related to the intensity of infection making only a few hosts with high worm burden go down with signs or symptoms. *Entamoeba dispar* was absent from the study population thus warranting further independent investigation to confirm the regional trend of infection

It is difficult to estimate the morbidity rate of intestinal parasites because it is hard to determine what makes up a clinical case and it is likely that the infection exacerbates the existing problem rather than cause problems themselves and an accurate and recording system is scarce in endemic regions (23). The detection of *Ascaris lumbricoides*, *Giardia lamblia, Trichomonas hominis,* and *Ancylostoma spp*, at a different level of prevalence, either underscores the endemicity of these parasites or points to a silent epidemic *of these parasites in this region. The s*ustained prevalence of *Giadia lamblia* from 2009 to 2012 is a probable indicator of ineffective control of the parasites or high regional endemicity of the parasite.

The detection of *Strongiloides stecoralis* only in 2012 may point towards emerging parasite infection and calls for surveillance to confirm or refute this prediction. Our data collection tool could not state whether the two patients whose samples were positive for *Strongiloides stecoralis* were residents of Bushenyi or visitors, this could have explained why the parasite did not show up from 2009 to 2011 but only appeared in 2012.

In an endemic intestinal parasite region, Crompton had suggested that growth-stunting, especially in children, should be considered as chronic diseases due to ascariasis, hookworm, and trichuriasis; iron deficiency anemia should be considered as hookworm disease and trichuriasis and rectal prolapsed should be considered as trichuriasis (23).

Most of the studied population presented with anemia and rectal prolapsed as recorded by the attending physician in the laboratory request form. In the Bushenyi district of Uganda, it is very common to see cases of growth stunting (in children) and iron deficiency anemia. The Association of intestinal parasites with the certain clinical condition may be substantiated by observation of 7.9% co-infection of *Ascaris spp* and *E-histolytica*, 3.9% of *Ascaris lumbricoides,* and *Giadia lamblia,* and 2.6% *Trichomonas hominis,* and *Ascaris lumbricoides* respectively in this study (23). This calls for further studies to establish parasite case definitions supported by laboratory findings.

The pattern of parasite prevalence recorded was high: in early adulthood, stabilized in late adulthood, and decreased in the elderly indicating an age-specific relevance ($p<0.05$) in the epidemiology of intestinal parasites in this region (Table 2). This may explain why prevalence was significantly ($p<0.05$) dependent on demographic (age and gender) characteristics of the studied population.

The high vulnerability of females to parasite infection compared to males depicts a shift in the pattern of gender-related infection which we reported earlier in which males were more affected than females due to more involvement in factory and construction works, prison, military barracks, farming, and other manual labor bringing males more in contact with parasites than females (Table 2) (24).

Again, the statistical significance ($p<0.05$) of gender-related parasite prevalence in this study imply other factors are responsible for the observed change. Poor socio-economic status, tendency to resort to herbal alternative for the treatment of infection, some culture, and resistance to conventional antimicrobial agents may add to the reason why male participants were less in number than females (22, 24, 25). This shift in gender-related infection needs further investigation.

In conclusion, intestinal parasites remain highly prevalent (15.9%) in the Bushenyi district over the 4 years of study. Parasitic prevalence was significantly ($p<0.05$) dependent on age and sex. Females being more infected than males indicate a shift infection pattern as earlier reported. The emergence of 0.5% *Strongiloides stecoralis* in 2012 alone and the absence of parasites co-colonization from 2010 to 2012, including the absence of *Entamoeba dispar* from this study, deserve attention. Female children aged ≤10 years harbored more parasites than the elderly aged ≥60 years. The age group 21-30 years had the highest parasite prevalence in this study. Multifaceted and interdisciplinary approaches in this region are invaluable.

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