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Prevalence and Association of Parasitic Helminths among the Cross Section of Male and Female Gender Groups at University of Guyana, Georgetown, Guyana

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ABSTRACT

The acquisition of epidemiological information on the type and prevalence of helminths in any geographic location would be very relevant in the development of control techniques that would be advantageous as a contributing factor on the general health status of the population. The purpose of this study was to determine the prevalence and association of helminths among the two gender groups at the University of Guyana. A cross-sectional study was conducted with a total of 36 participants that provided stool samples to be examined for helminths. The stool samples were examined using the normal saline wet mount and formalin ether sedimentation technique. The study was carried out during February-July, 2014. The data was analyzed for statistical significance using the chi-square test. The study found that 72.2% of the sample population investigated were positive for at least one helminth, with the most prevalent helminth identified being *Enterobius vermicularis* (55.5%). Of the total positive stool sample, 41.6% had single infection, followed by 25.0% with double infection and triple and quadruple infection had 2.8%. The gender distinction showed 52.8% females and 47.2% males, however, it is statically insignificant. Hence, based on these findings it is clear to say that there is a prevalence of helminths among the student population at University of Guyana.

Key words: Helminths, stool samples, gender, health status, examination

INTRODUCTION

There are more than a dozen different species of soil-transmitted helminths that infect humans, mainly found in the tropical and subtropical parts of the developing world. However, they are four nematodes in particular stand out because of their widespread prevalence and distribution that result in millions of human infections. These include the large roundworm, *Ascaris lumbricoides*, the whipworm, *Trichuris trichiura* and two species of hookworm, *Necator americanus* and *Ancylostoma duodenale* (Hotez *et al.*, 2003).

The World Health Organization (WHO) estimates that almost 2 billion people are infected with one or more of these soil transmitted helminths, accounting for 40% of the global morbidity from infectious diseases, exclusive of malaria. This global phenomenon over time has led to much research into the health of individuals living in tropical and sub-tropical regions in relation to parasitic helminths. The science of parasites and their interactions with their host is generally known as parasitology (Cox *et al.*, 1983). Parasites are a source of continued ailments and threat to the health of humans and animals within many urban and rural communities, more significantly

within tropics and sub tropics areas (Hotez, 2006). Parasites in humans are found to spend part of their life cycle or complete life cycle in the human host (Nath, 2012).

WHO views helminths as a broad range of organism inclusive of intestinal parasitic worms which are characterized by their modes of transmission, that is, via ingestion of eggs or larvae in contaminated food or through penetration of the skin by infective larvae in soil or water. It was also found that the prolonged expression of these infections may lead to infestation which can cause morbidity and sometimes death by compromising nutritional status, affecting cognitive process, including tissues reactions, such as granulosa and provoking intestinal obstruction or rectal prolapsed. Control of helminthiasis is based on drug treatment, improved sanitation and health education (Hotez *et al.*, 2008).

Human parasites are separated into blood-borne and intestinal parasites (Cuomo *et al.*, 2009). However, the scope of this research focused mainly on parasites found in the intestine which are categorized as protozoa and helminths, more specifically helminths and their presence and prevalence in students of the University of Guyana.

Helminths that are associated with human host are thought to have evolved more than 100 million years ago to live in their intestines and other organelles. However, the colonization with humans only became universal in the early 20th century (Legesse and Erko, 2004). Over the years helminths have grown to adapt and evolve with mankind creating a neighborhood of livelihood in their intestine to feed and reproduce; some of these intestinal helminths of importance to human are *E. vermicularis* (pinworm), Soil-Transmitted Helminths (STH) including *A. lumbricoides* (round worm), *T. trichiura* (whip worm), *N. americanus*, *A. duodenale* (hookworm) and *S. stercoralis* (threadworm) (Norhayati *et al.*, 2003).

It is estimated that approximately one-third of the almost three billion people that live in developing regions of sub-Saharan Africa, Asia and the Americas are infected with one or more helminths. The most common helminthiasis are those caused by infection with intestinal helminths; Ascariasis, Trichuriasis and hook-worm, followed by Schistosomiasis and Lymphatic Filariasis. This practically illustrates that the inhabitants of thousands of rural, impoverished villages throughout the tropics and subtropics are often chronically infected with several different species of parasitic worm, that is, they are polyparasitized (Hotez *et al.*, 2008).

Helminths and parasitic worms are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis (Hotez *et al.*, 2008). This is also supported by literature by Hotez *et al.* (2008), which estimated that approximately one third of the almost three billion people that live in developing regions of sub-Saharan Africa, Asia and the Americas are infected with one or more helminths. The most common helminthiasis are those caused by infection with intestinal helminths; such as Ascariasis, Trichuriasis and hook-worm, followed by Schistosomiasis and Lymphatic Filariasis. Hence, the acquisition of the epidemiological data on the various helminthic infection in the sample population of university of Guyana students acts as a pre-requisite for the implementation of control measures and follow up research to reduce or eradicate such conditions.

Helminths pose a significant long term threat to the people of a nation, however, it is highly overlooked and taken for granted which makes it a silent threat. This in turn grasped the researcher attention since, the University of Guyana holds the future leaders of Guyana and maybe at risk to this silent threat. Hence, this led the researcher to venture on researching the prevalence and identification of helminths in the students of the University of Guyana between the ages of 19-25. This epidemiological data can be used to implement control measures to better enhance the population health status of the University of Guyana and the nation at large. This study was carried out as to investigate the types of helminths infecting students of the University of Guyana.

This study also aimed at spreading awareness of the importance of being tested for helminths and the long term adverse impact, it may have on the nutrition of individuals.

MATERIALS AND METHODS

The analytical tests for the presence of helminth were carried out at the University of Guyana. Sterile cups were given for collection of stool sample on request of returning the sample the next day during the morning period. Each sample collected was labeled and tested individually for the presence of helminth. Microscopic examination of stool was done by preparing slide using Normal Saline and Lugol's Iodine to observe the ova of different parasites. First we used low power lens and afterward the high power lens. Then we observed ova, cyst of parasites. The microbiological examination was done as follows.

Laboratory procedure:

- The stool samples were collected with sterile cups
- With an applicator stick 1.0 g of faeces was added to 10 mL of formalin in a centrifuge tube and stirred to form a suspension
- The suspension was then strained through two layers of wet surgical gauze into a different centrifuge tube and the gauze was discarded
- The addition of more 10% formalin was added to the suspension to make it 10 mL
- The 3.0 mL of ethyl acetate was then added in the tube which was then covered with a plastic cover and shaken vigorously for 10 sec
- The tube was then placed in the centrifuge then the tube was balanced and centrifuge for at 1000 g for 1.5 min
- The tube was then removed from the centrifuge with an observed 4 layers
- The debris was gently loosened with an applicator stick by a spiral movement
- The top 3 layers were then poured off in a single movement with the tube being inverted for 5 sec
- A drop of saline was then added to the fluid sediment using a pipette and mixed to form a suspension
- A drop of the suspension was placed on a glass slide
- The suspension was then gently spread into a thin film on the slide
- A drop of iodine was then added to the smear on the slide and heat fixed
- The slide was then left to cool for 5 min
- After cooling DPX mount was added to the slide and gently covered with a cover slip
- The slide was then left to dry overnight and examined under the microscope with 10X objective or 40X objective lens for helminths
- Pictures were taken of helminths identified (egg, larva and adult stages) and size recorded which was used to identify them using lab manuals and the internet sources

Data analyses: The chi-square test was used to determine whether, there was statistical significant difference between the sexes, male and female being infected by helminths. The chi-square test was also done to decipher statistical significance between male and female with multiple-helminth infection. This degree of freedom being 1 was tested at a critical value of 0.05 with a 95% probability was found to prove that there is no statistical significance between male and female being infected by helminths in the sample population.

RESULTS

The investigation and microscopic analysis of the stool samples from the tested subjects led to the revelation of various helminthic infections. A total of 36 subjects were tested with 26 (72.2%) of them yielding positive result for helminthic infection including one or more parasitic helminths. The gender distinction showed 19 (52.8%) females and 17 (47.2%) males. The mean age of the study was 21.6 with a minimum age of 19 and a maximum age of 25.

The researcher observed that the identification of helminths involves a tedious process focusing keen attention of various characteristic features which distinguishes various helminths based on genus and species. The researcher placed emphasis on specific characteristics of each helminth seen to aid in the verification of the type of species, such as the stage of development, size, shape, colour, other distinguishing characteristics and pictorial representation of helminths mentioned in literature. However, greater emphasis was placed on the physical features visible through the microscope. The characteristics observed for each of the species identified are summarized in the Table 1.

Table 1: Characteristic features of parasites identified

Helminths	Distinguishing features	
	Ova	Adult
<i>Enterobius vermicularis</i>	Size: 48-60 μ m long and 20-35 μ m wide Shape: Oval, one-side flattened Embryo: Stage of development varies, may be unembryonated, embryonated, mature Shell: Double-layered, thick, colorless	Male Length: 2-4 mm Width: 0-3 mm Colour: Yellowish-white Tail: Pointed, resembles pin head Female Length: 7-14 mm Width: 0-0.5 mm Colour: Yellowish-white Tail: Pointed
<i>Ascaris lumbricoides</i>	Unfertilized Ova Size: 85-95 μ m by 38-45 μ m Shape: Varies Embryo: Unembryonated, amorphous mass of protoplasm Shell: Thin Other features: Usually corticated Fertilized Ova Size: 40-75 μ m by 30-50 μ m Shape: Rounder than unfertilized version Embryo: Undeveloped unicellular embryo Shell: Thick, chitin Other features: May be corticated or decorticated	Female Length: 22-35 cm Colour: Creamy-white pink tint Other features: Pencil-lead thickness Male Up to 30 cm Colour: Creamy-white pink tint Other features: Prominent incurved tail
<i>Ancylostoma duodenale</i>	Size: Length: 55-60 μ m Width: 35-40 μ m Embryonic cleavage: 2-, 4-, or 8-cell stage Shell: Smooth, colorless Rhabditiform larva Size: Newly hatched: 270 by 15 μ m 5 days old: 540-700 μ m long Buccal cavity: Long Genital primordium: Small Filariform larva Length of esophagus: Short Tail: Pointed	General characteristics: Color: Grayish-white to pink Cuticle: Somewhat thick Anterior end: Conspicuous bend/"hook" Buccal capsule characteristics: Contains actual teeth Female Length: 9-12 mm Width: 0.25-0.50 mm Male Length: 5-10 mm long Width: 0.2-0.4 mm Other features: Prominent posterior copulatory bursa
<i>Taenia</i> spp.	Length: 25-45 μ m Width: Main diagnostic feature:	

Table 1: Continue

Helminths	Distinguishing features	
	Ova	Adult
<i>Strongyloides stercoralis</i>	Non-operculated and contains six hooked hexacanth embryo	
	Shell: Thick, brown, radially striated embryophore or composed of blocks.	
	Size: 48 by 35 μm	Size: 2 by 0.4 mm
	Typical growth phase: Contains well-developed larvae	Other features: Colorless, transparent body
	Embryonic cleavage: When present, 2-, 4-, or 8-cell stage	Finely striated cuticle
	Shell: Thin, hyaline	Short buccal cavity
	Rhabditiform larva	Long and slender esophagus
	Size: 220 by 15 μm	
	Buccal cavity: Short	
	Genital primordium: Prominent	
<i>Trichuris trichiura</i>	Filariform larva	
	Length of esophagus: Long	
	Tail: Notched	
	Size: 50-55 by 25 μm	Size: 2.5-5 cm long
	Shape: Barrel/football, hyaline polar plug at each end	Anterior end: Colorless, resembles a whip handle, contains slender esophagus
	Embryo: Unicellular, undeveloped	Posterior end: Pinkish-gray, resembles whip itself, contains digestive and reproductive systems, males possess prominent curled tail
	Shell: Smooth, yellow-brown color due to bile contact	

Table 2: Distribution of parasitic infestation among study population

Helminths identified	No. of subjects with positive results (eggs)	No. of helminths found (eggs) (g L^{-1})
<i>Ancylostoma duodenale</i>	0	0
<i>Ascaris lumbricoides</i>	4	36
<i>Enterobius vermicularis</i>	4	58
<i>Taenia</i> sp.	2	28
<i>Trichuris trichiura</i>	0	0
Total number of ova/egg of parasite = 122		

Table 3: Prevalence of parasitic infestation among study population

Helminths identified	No. of subjects with positive results (adults)	No. of helminths found (larva/adult)/(g L^{-1})
<i>Ancylostoma duodenale</i>	4	6
<i>Ascaris lumbricoides</i>	8	11
<i>Enterobius vermicularis</i>	20	52
<i>Strongyloides stercoralis</i>	1	1
<i>Taenia</i> sp.	2	4
<i>Trichuris trichiura</i>	2	2
Total number of adult/larva parasite = 76		

The groups of helminths identified during the investigation included, *Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia saginata*, *Trichuris trichiura* and *Strongyloides stercoralis*. The prevalence of infection with the identified helminths are shown in Table 1. The prevalence of *Enterobius vermicularis* was recorded as being the highest with 20 (55.5%), followed by *Ascaris lumbricoides* with 8 (22.2%), *Ancylostoma duodenale* (adult) 4 (11.1%), *Taenia* sp. 2 (5.6%), *Trichuris trichiura* 2 (5.6%) and *Strongyloides stercoralis* 1 (2.7%). The remaining 10 (27.8%) were found to be negative.

The total number of helminths identified at their early stage of development, i.e, ova/egg was 122 (Table 2), with *Enterobius vermicularis* being most prevalent with 47.5%, followed by *Ascaris lumbricoides* (29.5%) and *Taenia* sp. (22.9 %) at later developmental stage larva to adult was 76 (Table 3).

The prevalence of parasitic infestation was seen higher in males than females as illustrated in Table 4. However, using the chi-square test, it was found that there is no statistical significance

Table 4: Distribution of parasitic infestation among males and females in study population

Sex	Total (n)	+ve (n)	%
Male	17	15	88.24
Female	19	11	57.89
Total	36	26	72.22

Table 5: Distribution of at least double parasitic infestation among males and females in study population

Sex	Total (n)	+ve(n) for double infection	%	Overall (%)
Male	17	5	29.41	13.9
Female	19	4	21.05	11.1
Total	36	9	25.00	

Table 6: Prevalence of single and mixed infection among study population of the University of Guyana

Type of infection	Total frequency n (%)	Most prevalent intestinal parasite in each type of infection	
		Species	Total frequency n (%)
Single	15 (41.6)	<i>Enterobius vermicularis</i>	8 (25)
Double	8 (25.0)	<i>Ascaris lumbricoides</i>	8 (25)
Triple	1 (2.8)	<i>Ancylostoma duodenale</i>	1 (2.7)
Quadruple	1 (2.8)	<i>Trichuris trichiura</i>	1 (2.7)

between males and females being infected by helminths of the university of Guyana. It was found that the prevalence of multiple infections in male and female was almost equal, with 5 double infection occurring in males with 4 in females, given an infection prevalence in males tested 29.41 and 21.05% in females and an overall percentage for study population as 13.1% in males and 11.1% in females giving an almost 1:1 ratio for multiple infection in males and females (Table 5).

On investigation based on the types of helminths in subjects, it was found that out of the 72.2% of subjects infected with helminths, 41.6% had single infection, followed by 25.0% with double infection and triple and quadruple infection (2.8%), respectively giving an occurrence of 30.6% polyparasitism in the sample population as illustrated by Table 6, which also evaluated the most common helminths in each type of infection, resulting in *Enterobius vermicularis* being dominant throughout each.

DISCUSSION

This study is the first, to the knowledge of the researcher to explore the prevalence of helminths infection among the student population of the University of Guyana. It practically involved the identification of helminths in human stool sample based on visible physical characteristics observed using the microscope. In this study, it was found that almost three-quarters (72.2%) of the sample population had at least a single helminth infection indicated by stool analysis. This is relatively higher than that of previous studies carried out in different regions of Guyana (Lindo *et al.*, 2002; Wani *et al.*, 2007). This study found that there is a prevalence of helminth within the study area, with *Enterobius vermicularis* being most common and the least being *Strongyloides stercoralis*.

It was found that findings in this study had similarities in the study done previously by Wani *et al.* (2007) based on the fact the prevalence of *Enterobius vermicularis*, *Ascaris lumbricoides*, *Taenia* spp. and *Trichuris trichiura* with the only exception being *Ancylostoma duodenale* and *Ancylostoma duodenale* being present in this study only with *Enterobius vermicularis* and *Ascaris lumbricoides* being highly prevalent into both study population. This gives indication that these helminth populations are existent in various regions of Guyana. An even greater discovery in this research was the occurrence of polyparasitism which occurred in 30.6%

of the study population. This possess greater detriment since studies have indicated individuals infected with multiple helminth species may have the most intense infections and may also have a greater impact on morbidity than the sum of single-species with added impact on the human body, rendering it more prone to acquire life threatening ailments such as Malaria and HIV (Lustigman *et al.*, 2012).

Analysis of data found that there was no statistical predilection of gender with any of the parasites identified, this finding was also supported by previous literature (Adhikari *et al.*, 2010; Sah *et al.*, 2013). Based on previous research, it is presumed that some of the factors responsible for this prevalence may include; not wearing of shoes or footwear when walking around at home since this expose the skin to penetration by soil transmitted helminths infective larvae, e.g., *Ancylostoma duodenale* and *S. stercoralis*, biting of nails and failure to wash hands with soap and water, may also result in helminths entering the body via the oral-fecal route, e.g., *Ascaris lumbricoides* (Sah *et al.*, 2013) and eating of raw or unprepared vegetable (Ayalew *et al.*, 2011). Poor environmental sanitation may also have resulted in this prevalence of helminths (Ayalew *et al.*, 2011), since, these organisms thrive well in unhealthy environs and are very opportunistic in invading the human body e.g., *Ascaris lumbricoides*.

The level of harm that may be caused by intestinal parasite resulting in degradation to the health of individual and communities depend on the parasite species, the nature of the interaction between the parasite and the concurrent infections along with the intensity and course of infection and nutritional and immunological status of the population (WHO., 2006; Hadidjaja *et al.*, 1998). The compound impact of these factors and a high prevalence of helminths over times may be a detriment to many over time. Hence, this emphasizes the importance of this study since, the findings of this study provide preliminary information for the implementation of environmental and public health measures to reduce or eradicate soil transmitted helminths, however, consideration must be made for external risk factors and limitations. This study may also be used to predict risk for communities under consideration.

CONCLUSION

This study has provided conclusive evidence, that there is a prevalence of helminths among students of the University of Guyana. Based on research some of the causative agents in this situation may be as a result of poor environmental conditions, habitual behavior of not wearing footwear on walking around home environs or failure to wash hands before meals and also the consumption of raw or improperly prepared foods. Even as adults, one might fail to practice many of the latter mentioned and the result is evident. Hence, this result should advocate the relevancy of further investigative study and an intervention program which is necessary for the control or eradication of these helminths, aiding in enhancement of the health status of the community.

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