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OCCURRENCE OF PROTOZOA AND SOIL TRANSMITTED HELMINTH (STH) IN HUMAN FECES USED AS MANURE IN AGRICULTURE

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Abstract

To assess the health risk association of soil contamination with parasite, a total number of 168 faecally contaminated night soil was studied. The presence of four protozoan parasites, *viz. Cryptosporidium* sp., *Entamoeba histolytica*, *Giardia* sp., *Trichomonas hominis* and eight different helminthes, *viz. Diphyllobothrium latum*, *Hymenolepis* sp., *Taenia* sp., *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Strongyloides stercolaris* and *Trichuris trichiura* were identified. Among the protozoans, *Giardia* sp. was the highest (46.43%) and most abundant (339.87 ± 17.01 cysts/g); among helminthes the prevalence of *Ascaris lumbricoides* was the highest (80.95%) and *Strongyloides stercoralis* was mostly abundant (409.31 ± 47.53 larvae/g). Highest percentage of mild infections was found in *Giardia* sp. (43.45), and lowest in *Trichomonas hominis* (8.33). Whereas, among helminthes, highest percentage (64.29), of mild infections was found in *Ascaris lumbricoides* and lowest (11.90) in *Taenia*. In case of protozoa, moderate infection found in *Giardia* sp. (2.98%) and *Entamoeba histolytica* (3.57%), but in case of helminth only *Ascaris lumbricoides* showed moderate infections (16.67%).

Introduction

Parasitic infestation is one of the causes of anaemia[1,2]. Shah and Baig[3] reported that anaemia significantly related with helminth infection. Actually parasitic infections may reduce competitive fitness[4,5] thus influence population cycles[6] and regulate host population abundance[7]. Often, parasitic infections tend to be over dispersed, to where many individual hosts have low parasite intensities and a few individuals have high intensities of parasites[5,8,9]. Thus, many animals may maintain low levels of infection whereas a few actually succumb to disease. The sanitary condition in rural communities of Bangladesh was unsatisfactory, more than 75% families have insanitary latrines which were mostly infected with helminthes[10]. Contaminated food and water, inadequate sanitation and poor personal hygiene may be the major sources of intestinal parasite infection.

Adult parasites and their eggs may inhabit soil; therefore, poor nutrition and sanitation may foster the spread of infections. From the hygienic point of view, any exposure to faeces constitutes a risk[11] and thus faeces should always be considered potentially to contain pathogens. Most pathogenic or potentially intestinal pathogenic microorganism enter a new host by ingestion or through the lungs or through the eye, when eyes are rubbed with contaminated fingers,[11] while others may also enter through the skin or wounds.

Fecal wastes from domestic animals, wildlife and humans are applied to the soil surface and to varying extents are incorporated into the soil. These fecal wastes can also enter water systems by direct contamination of the water or through seepage or surface run off. Raw sewage is processed to varying degrees before it is distributed on soil or discharged into water systems. Failure to appropriately process human sewage probably poses the greatest threat to human health, however it is often impossible to identify sources of food and water contamination.

Actually using night soil solves environmental and sanitary problems systematically[12]. It helps to return resources to the environment through the resource recovery process of the nutrient cycle and also build a bridge between sanitation and agriculture[13]. But parasites can contaminate crops through various routes, for example, via water contaminated by faeces that used for irrigation or spraying of crops. Intestinal parasites are producing detrimental effects on health of millions of people mainly children and adolescents in Bangladesh.[14] Several studies showed that intestinal parasitic infections are present all the time everywhere in Bangladesh.[15] In Bangladesh, among the helminths and protozoan parasites, *A. lumbricoides*, *A. duodenale, T. trichura*, *Enterobius vermicularis* and *Etaboeba histolytica*, *Giardia lamblia* are common.[16]

In developing countries, excreta-related diseases are very common, and human faeces contain correspondingly high concentrations of excreted pathogens - the bacteria, viruses, protozoa, and helminths (worms) that cause gastro-intestinal infections in man. So the main objectives of this work were to estimate the actual risks to the public health that occur through night soil use in agriculture; to enumerate prevalence of protozoa and helminth; and to measure the single, double and multiple infections.

Materials and Methods

The present study was conducted by collecting 168 samples from selected areas in rural Tangail, Bangladesh and laboratory analysis was done in the Parasitology laboratory of Zoology, Dhaka University. The study period extends from February, 2013 to March, 2014. For analysis, firstly faecal sludge on the samples was dewatered on a drying bed. Dewatering was used to separate faecal sludges from on site sanitation. This process does not treat the sludge, it only separates solid from liquid part. Then, dewatered sludges were mixed at a aludge: solid organic material (1 : 3) volume ratio[17]. The active composting process lasted for one month and then collected for laboratory analysis and stored at a 4^0C until analysis. This manure was used in growing vegetables and plants, such as, cauliflower (*Brassica oleracea*), cabbage (*Brassica oleracea capitata*), bottle gourd (*Lagenaria siceraria*), brinjal (*Solanum melongena*), snake gourd (*Trichosanthes* *cucumerina*), bitter gourd (*Momordica chanantia*), and mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), orange (*Citrus sinensis*), wood apple (*Aegle marmelos*) etc.

Samples were collected in a small sterile, clean and leak proof plastic container or fresh transparent polythene bag. All the samples were preserved with most suitable and available preservative (10% formalin). After collection, samples were kept in a cool dry place and examination was carried in Parasitology Lab, Dhaka University. From each sample, at least two slide were examined separately through the processing and technique, and the results were recorded accordingly.

The formol-ether concentration technique[18] was applied for the diagnosis of protozoa and soil-transmitted helminthes. The units for measuring the intensity of infection at the individual level are "eggs per gram of faeces" (EPG), for determining the number of parasite's eggs per gram of feces in order to estimate the worm burden material on the slides. Firstly, weight out 3 grams of feces and measure out 42 ml of water and place it into a dish. Using a tongue depressor, push the 3 grams of feces through a sieve into the water, lift the sieve and hold over the dish. While stirring the water-feces mixture, take 0.15 ml of the suspension and spread over 2 slides. Cover each slide with a long coverslip. Examine both slides for worm eggs, the total number of eggs counted × 100 represents the number of eggs per gram of feces. The mathematics: 0.15 ml is 1/300 of 45 ml (42 ml water and 3 gm feces) so the number of eggs in 0.15 ml × 100 is equal to 1/3 of the total number of eggs in the original 3 g and thus equal to eggs per gram (EPG). This technique is known as stool's egg counting technique. The advantage of this technique is that it requires no specialized equipment and the disadvantage is the counting takes a long time because of the amount of extra (nonegg) material on the slides.

When the occurrence of the range of parasite number in the form of cyst or ova or larvae were from 300 to 599, from 600 to 1099, from 1100 to 1300/g of human feces in each sample infestation level would be marked positive and designated as mild, moderate and heavy, respectively. The level of infection cases were classified as mild, moderate and heavy infection according to WHO[19] cut off values.

Results and Discussion

Twelve intestinal parasite species were identified from the human feces, these include four protozoans, *viz. Cryptosporidium* sp., *Entamoeba histolytica*, *Giardia* sp. and *Trichomonas hominis*; three cestodes, *viz. Diphyllobothrium latum, Hymenolepis* sp. and *Taenia* sp.; and five nematodes, *viz. Ascaris lumbricoides*, *Ancylostoma duodenale, Enterobius vermicularis*, *Strongyloides stercoralis* and *Trichuris trichiura* (Table 2).

The level of prevalence of helminth was mostly similar to the findings of Tu *et al.*[20], who reported 90% prevalence of helminths in their study population. Actually eggs of helminths including *Ascaris* spp., *Trichuris* spp. and hookworms need a period of time, outside the host body to develop and attain infective stage. Presence of these parasites in the environment can be a public health indicator[21]. High prevalence (96.42%) of these helminths in the present study indicates higher contamination of soil samples (Table 1).

Table 1. Prevalence of intestinal parasites in the observed night soil samples collected from Tangail from February, 2013 to March, 2014.

Parasites	No. of total sample	Positive (Total)	Prevalence (%)	Positive (only)	Prevalence (%)	No. of protozoa and helminth parasite	Preva-lence (%)
Protozoa	168	128	76.19	06	3.57		
Helminth	168	162	96.42	40	23.80	122	72.62
	Cestode	75	46.30	08	4.94		
	Nematod	123	75.93	32	19.75		

Table 2. Prevalence, dominance, intensity, and CPG/EPG of intestinal parasites in observed samples.

Parasites	No. of contaminated samples	Prevalence	Dominance (%)	Cyst/egg per gram (CPG/EPG)	Intensity (±SD)
Protozoa					
Trichomonas hominis	14	8.33	10.93	306.43	306.43 ± 6.16
Entamoeba histolytica	76	45.24	59.38	274.75	274.75 ± 6.44
Giardia sp.	78	46.43	60.94	339.87	339.87 ± 17.01
Cryptosporidium sp.	22	13.09	17.19	314.54	314.54 ± 10.63
Cestode					
Hymenolepis sp.	50	29.76	30.86	343.60	343.60 ± 40.11
Taenia sp.	20	11.90	12.35	355.30	355.30 ± 21.05
Diphyllobothrium latum	62	36.90	38.27	307.60	307.60 ± 6.30
Nematode					
Ascaris lumbricoides	136	80.95	83.95	333.16	333.16 ± 19.84
Trichuris trichiura	34	20.24	20.99	311.65	311.65 ± 4.01
Hookworm	64	38.10	39.51	376.41	376.41 ± 52.99
Strongyloides stercoralis	58	34.52	35.80	409.31	409.31 ± 47.53
Enterobius vermicularis	26	15.48	16.05	316.08	316.08 ± 64.26

Giardia sp. (46.43%), *Entamoeba histolytica* (45.24%), *Ascaris lumbricoides* (80.95%), and Hookworm (38.10%) were highly prevalent; other parasites were comparatively less frequent (Table 2). The prevalence of hookworm was 38.10% which showed close to the findings of Khanum *et al.*[22]. They observed that the prevalence of hookworm infection was higher in Kutumbopur (30%) than that of in Gazirchat (26.7%). The prevalence of *T. trichiura* (20.24%) also revealed similarity (Table 2) to Khanum *et al.*[23], who carried out a study among the children in two slum areas in Dhaka city and reported the higher prevalence of *T. trichiura* (18.80%) in Agargaoan than Mirpur slum area (16.17%).

The highest percentage of mild infections was due to *Giardia* sp. (43.45%), and lowest due to *Trichomonas hominis* (8.33%). Whereas, among helminthes, highest percentage of mild infections was caused by *Ascaris lumbricoides* (64.29%) and lowest by *Taenia* (11.90). In case of protozoa, moderate infection was due to *Giardia* sp. (2.98%) and *Entamoeba histolytica* (3.57%). But in case of helminth only *Ascaris lumbricoides* caused moderate infections (16.67%) (Table 3).

Table 3. Prevalence of mild and moderate infections positive samples.

Parasites	No. of mild infection positive samples	Prevalence (%)	No. of moderate infection samples	Prevalence (%)
Protozoa				
Trichomonas hominis	14	8.33		
Entamoeba histolytica	70	41.67	06	3.57
Giardia sp.	73	43.45	05	2.98
Cryptosporidium sp.	22	13.10		
Cestode				
Hymenolepis sp.	50	29.76		
Taenia sp.	20	11.90		
Diphyllobothrium latum	62	36.90		
Nematode				
Ascaris lumbricoides	108	64.29	28	16.67
Trichuris trichiura	34	20.24		
Hookworm	64	38.10		
Strongyloides stercoralis	58	34.52		
Enterobius vermicularis	26	15.48		

Willets[24] reported 54.37% for hookworm infestation in over 4000 samples and Walker *et al.*[25] found 48.27% hookworm infestation in 58 persons at Mindoro. Hookworm infestation in both the findings was slight higher than the present observation which was 38.10% (Table 3). The defecation manner in open space by animal or human may be the possible source of intestinal parasites. Intestinal parasites found in these samples indicate faecal contamination of the soil. However, the lower prevalence (11.84%) of *Ascaris lumbricoides* was observed by Reinthaler *et al.*[26] who conducted survey in South-west Nigeria, whereas present investigation revealed 80.95% prevalence of *Ascaris lumbricoides*. This might be due to different climate condition that persists in Nigeria. The potential risk of contracting helminthic ova and larvae through ingestion of unwashed, raw or uncooked fruits and vegetables obtained from farmlands.

Enhanced disease surveillance, epidemiological systems and techniques are needed to improve our current understanding of the significance of these organisms in primary production and throughout the food chains. None-the-less, the number of infections can be used to give an idea of the national scale of the effort required to control protozoa and soil transmitted helminthes (STHs). The economy of Bangladesh largely depends on agriculture; livestock and about 80% people of the country are directly or indirectly depend on agriculture[27]. Assessment of the degree of infection of parasite will help to recommend possible ways and means to control and prevention of protozoan and helminthic parasite and thus improve socio-economic conditions, sanitary practices which help to control the extent of parasitic infestation and anaemia. Furthermore, results of the study will allow the planners, professionals and researchers of the country to take appropriate preventive and remedial measures to control parasitic infestation and henceforth improve the extent of anaemia and overall nutritional status, health and well-being of population of the country. Poverty and poor hygiene were implicated as an important risk factor for intestinal parasitic infestations. Improving the sanitation level, creating awareness about health and hygiene and promotion of de-worming therapy program could reduce intestinal parasitic infestations.

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