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DETECTION OF PATHOGENS IN WASTEWATER AND SOIL BY TAQMAN ARRAY CARD (TAC) SYSTEM

Fahmida Sarkar[1], Hamida Khanum*, Rimi Farhana Zaman, Tahmina Ahmed[1], Md. Khalid Eakbal Anik and Rashidul Haque[1]

Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

Abstract: The study was conducted in an a urban slum area of Dhaka city. The environmental samples (soil and water) were collected from Mirpur Bihari camp area including Madrasha camp, Muslim camp and ADC camp. The main areas of sample collection were near the sewerage lines, chicken coop, goat house etc. In the present investigation, out of 28 wastewater samples, *Entamoeba, Shigella, Aeromonas, Campylobacter, Vibrio cholerae, Blastocystis, Salmonella, Trichuris, Ancylostoma, Plesiomonas, Bacteroides fragilis* and Rota virus pathogens were recorded. In wastewater samples, 14.29% had single infection, 85.71% double infection, whereas, observation on 20 soil samples by TAC system, total 12 types of pathogens were recorded such as *Shigella, Aeromonas, Campylobacter, Vibrio cholerae, Blastocystis, Salmonella, Plesiomonas, Bacteroides fragilis* and Rota virus including *Entamoeba* sp*., Ancylostoma* sp. and *Trichuris* sp*.*

Key words: Soil, Water, Wastewater, Parasites, Bacteria and virus

INTRODUCTION

The prevalence of intestinal parasite in Bangladesh is very high. Parasitic infestation is a common health problem around the globe especially in the developing countries which imposes a continual and unacceptable threat to the well-being of millions of people in the tropics and subtropics; the cost of parasites in terms of human misery and economic loss is incalculable (Cox 2002, Mondal *et al*. 2012). In Bangladesh, infestation with protozoa and helminthes such as *Giardia intestinalis*, *Ascaris lumbricoides, Entamoeba histolytica* and *Trichuris trichuira* are major public health problem both in rural and urban areas with wide spread endemically. It was stated that health problems related to diarrhea and helminthes are mostly water borne (Kramer *et al*. 1998, Sultana *et al.* 2007, Krkoset *et al.* 2016).

Intestinal parasitic infections are among the most common infections worldwide. Most intestinal parasites are heterogeneously distributed in host populations; according to a frequently quoted estimate, 20% of hosts harbor 80% of the intestinal helminthes. Adolescents and children are at high risk of

*Author for correspondence: <hamida_khanum@yahoo.com> [1]icddrb, Mohakhali, Dhaka-1212, Bangladesh.

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parasitic infection because of their behavioral aspects, general hygiene knowledge, socio-economic status (SES), environmental contamination, etc. The intestinal parasite may present asymptotically or may cause mild or severe diseases, generally producing symptoms like abdominal pain and vomiting. Besides this there are other symptoms like anorexia, nausea, diarrhea, indignation etc. Some of them may produce severe clinical manifestation like anemia, obstruction, perforation of gastrointestinal tract through peptic ulcer which causes secondary infection of bacteria (Greenberg and Estes 2009, Hudson 2002, Khanum *et al.* 2008). In Bangladesh, one in 30 children die of diarrhea or dysentery by his or her fifth birthday. In Bangladesh, one third of the total child death burden is due to diarrhea (Haque *et al.* 2003, WHO 2013).

In Bangladesh, incidence of intestinal parasites is high due to moist, hot climate, poor hygienic habit, ignorance, poverty and mostly importance in the lack of health education. Various studies have been carried out to find the prevalence of intestinal parasites in different rural and urban areas in Bangladesh. But the prevalence rate varied from place to place. The findings of the present investigation will also help in developing awareness among the people infected with parasites (Khanum *et al.* 2009). In Bangladesh intestinal parasitic infestation endemically wide spread all over due to low standard of living condition, poor personal hygiene practices (Khanum *et al.* 2008).

MATERIAL AND METHODS

The study area was Mirpur (Sector-11, avenue-5), an urban slum of Dhaka city. This area was selected as the living condition is unhygienic and impoverished children are mostly affected by diarrheal disease than the others. The majority of the inhabitants of the Mirpur site are of Bihari ethnic origin. This site is densely populated with more than one lakh people. The environmental samples (soil and water) were collected from Mirpur Bihari camp area including Madrasha camp, Muslim camp and ADC camp. Samples of soil and water were collected from the areas where children had diarrhea often through diarrhea surveillance system at this site conducted by ICDDR, B. Moreover, the main areas of sample collection were near the sewerage lines, chicken coop, goat house etc.

Soil samples were collected from the site by a spatula in a falcon tube (5 mg) and the water samples were collected from the site by handled pot (5 ml) and kept in zip lock water pack. The study period was July, 2016 - June, 2017. Total 48 samples were collected (28 water and 20 soil samples) from Mirpur. Samples were examined by TaqMan Array Card (TAC) system. Soil samples that were collected from near the sewerage line were muddy as they were mixed with

sewerage water and other samples that were collected from near the chicken coop and goat house were silty and dry. Wastewater samples were collected from sewerage line was muddy (Table 1).

Table 1. The places and types of collected soil samples

Sample	Collection area	Nature
Soil	Near sewerage line	Muddy
	Near goat house	Silt
	Near chicken coop	Silt
Wastewater	Sewerage line	Muddy

RESULTS AND DISCUSSION

In the present observation on wastewater samples by TAC system, total ten types of pathogens were recorded such as *Shigella, Aeromonas, Campylobacter, Vibrio cholerae, Blastocystis, Salmonella, Plesiomonas, Bacteroides fragilis* and Rota virus including *Entamoeba* sp. Out of 28 water samples, 4 (14.29%) had single infection and 24 (85.71%) double infection, 16 (57.71%) triple infection, 20 (71.42%) quadruple infection with four different species. The presence of five or more parasite species at a time in a single host was considered as multiple infections and thus 12 (42.86%) had multiple infection. Among 3 study areas, the single infection was highly prevalent (33.33%) near Muslim camp, lowest (0%) in both Madrasha camp and ADC camp. Double, triple and quadruple infections were highest (100%) in both Madrasha camp and ADC camp, whereas, lowest (66.66, 33.33 and 33.33%) in Muslim camp. Multiple infections were found highest (100%) in ADC camp and lowest (33.33 and 33.33%) in both Madrasha camp and Muslim camp (Table 2).

Observation on Soil samples by TAC system: In the present observation on soil samples by TAC system, total 12 types of pathogens were recorded such as *Shigella, Aeromonas, Campylobacter, Vibrio cholerae, Blastocystis, salmonella, Plesiomonas, Bacteroides fragilis* and Rota virus including *Entamoeba* sp., *Ancylostoma* sp. and *Trichuris* sp. (Table 3).

Out of 20 soil samples, there was no single infection (0%) while, 20 (100%) double infection, 20 (100%) triple infection, 16 (80%) quadruple infection with four different species. The presence of five or more parasite species at a time in a single host was considered as multiple infections, 12 (60%) had such multiple infection. Among 3 study areas (Muslim camp, Madrasha camp and ADC camp) no single infection was found. Double and triple infection were highest (100%) among 3 study areas (Madrasha camp, ADC camp and Muslim camp).

Quadruple and multiple infections were found highest (100%) in both ADC camp and Muslim camp and lowest (66.66 and 33.33%) in Madrasha camp (Table 4).

Table 2. Prevalence of pathogensin 28 wastewater samples

Pathogen	Number of tested water samples	Number of positive samples	Prevalence (%)
Entamoeba	28	20	71.42
Shigella	28	8	28.57
Aeromonas	28	16	57.14
Campylobacter	28	24	85.71
Vibrio cholerae	28	12	42.85
Blastocystis	28	8	28.57
Bacteroides fragilis	28	8	28.57
Adenovirus	28	4	14.29
Sapovirus	28	4	14.29
Plesiomonas	28	4	14.29

Table 3. Prevalence (%) of other pathogens in soil samples

Pathogen	Number of tested soil samples	Number of positive samples	Prevalence (%)
Entamoeba	20	16	80
Shigella	20	4	20
Aeromonas	20	12	60
Campylobacter	20	20	100
Vibrio cholerae	20	16	80
Blastocystis	20	8	40
Salmonella	20	4	20
Ancylostoma	20	8	40
Trichuris sp.	20	4	20
Plesiomonas	20	12	60
Bacteroides fragilis	20	4	20
Rota virus	20	4	20

Diarrhea was acquired from food or water that has been contaminated by stool, or directly from another person who is infected. Environmental conditions also make human beings vulnerable to parasites, and 1000 of people in developing countries live in below standard condition like, lacking safe water supplies and proper sanitation. Under these conditions parasitic diseases are common due to environmental pollution by human and animal excreta (Victora *et al.* 1993). So, overcrowding, lack of personal hygienic and sanitary conditions of the area, contaminated drinking water, may be responsible for higher

Table 4. Prevalence of different double infections in total wastewater samples

Pathogen	Total no. of soil samples examined	No. of positive samples with two parasites	Prevalence (%)
Cryptosporidium + Entamoeba	28	12	42.86
Cryptosporidium + Campylobacter	28	12	42.86
Cryptosporidium + Aeromonas	28	8	28.57
Cryptosporidium + Blastocystis	28	8	28.57
Cryptosporidium + Shigella	28	4	14.29
Entamoeba + Campylobacter	28	20	71.42
Entamoeba + Blastocystis	28	12	42.86
Entamoeba + Aeromonas	28	16	57.14
Entamoeba + Plesiomonas	28	4	14.29
Entamoeba + Sapovirus	28	4	14.29
Entamoeba + Adenovirus	28	4	14.29
Entamoeba + Virio cholera	28	12	42.86
Entamoeba + Shigella	28	8	28.57
Entamoeba + Bacteroides fragilis	28	4	14.29
Campylobacter + Aeromonas	28	16	57.14
Campylobacter + Plesiomonas	28	4	14.29
Campylobacter + Vibrio cholerae	28	8	28.57
Campylobacter + Sapovirus	28	4	14.29
Campylobacter + Shigella	28	8	28.57
Campylobacter + Blastocystis	28	8	28.57
Campylobacter + Bacteroides fragilis	28	8	28.57
Vibrio cholerae + Aeromonas	28	12	42.86
Vibrio cholerae + Plesiomonas	28	4	14.29
Vibrio cholerae +Shigella	28	8	28.57
Vibrio cholerae + Sapovirus	28	4	14.29
Vibrio cholerae +Blastocystis	28	8	28.57
Vibrio cholerae + Bacteroides fragilis	28	8	28.57
Shigella + Sapovirus	28	4	14.29
Shigella + Adenovirus	28	4	14.29
Shigella + Plesiomonas	28	4	14.29
Shigella + Bacteroides fragilis	28	8	28.57
Blastocystis + Aeromonas	28	8	28.57
Blastocystis + Plesiomonas	28	4	14.29
Blastocystis + Bacteroides Fragilis	28	8	28.57
Blastocystis + Adenovirus	28	4	14.29

prevalence of infections. However, the prevalence of the parasites the present study was contradictory with previous studies (Ngan *et al.* 1992, Verle *et al.* 2003). This difference may be due to diagnostic techniques.

About 1.7 to 5 billion cases of diarrhea occur per year (Abdelmalak and Doyle 2013). Diarrhea is a major public health problem which is most common in developing countries, including Bangladesh where young children get diarrhea on average three times a year (WHO 2013). Walker *et al*. (2013) estimated high rate of diarrhea mortality among young children in low and middle income countries. Diarrheal disease may have a negative impact on both physical fitness and mental development. "Early childhood malnutrition resulting from any cause reduces physical fitness and work productivity in adults, and diarrhea is a primary cause of childhood malnutrition (Guerrant *et al*.1992, WHO 1987).

The most common cause is an infection of the intestines due to either a virus, bacteria, or parasite; a condition known as gastroenteritis. According to Wright *et al.* (1991) environmental factors influence the prevalence of diarrheal causing agent. They stated that house structure (28%), water usage (24%), toilet and bathing area (12%), animal management (11%), food preparation area (10%), hygiene (8%) and wastewater management (6%) also influence their presence which is similar to the findings of the present study.

In the present study, soil samples were also collected from chicken coop, goat house and out of total 20 soil samples 4 were positive for *Cryptosporidium* spp. (14.29%). There was no single infection 0%, 100% double infection, 100% triple infection, 80% quadruple infection and 60% had multiple infections. Water samples were wastewater. Out of total 28 water samples 24 samples were positive for *Cryptosporidium* (85.71%). In the present study, in soil and wastewater samples rotavirus, adenovirus, *Campylobacter* spp., *Salmonella* spp. and *Shigella* spp. were also found. There was 14.29% single infection, 85.71% double infection, 57.71% triple infection, 71.42% quadruple infection and 42.86% multiple infections (Tables 5, 6, 7).

There are many causes of infectious diarrhea, which include viruses, bacteria and parasites (Navneethan and Gianella 2008, Abrahams 2002). Along with *Cyptosporidium* spp. and *Giardia lamblia*, rota virus is the most common cause in children under five years old (Greenberg and Estes 2009) and Adenovirus (Uhnoo *et al.* 1990) cause a significant number of infections (Rose 1990, Rose *et al*. 1991). *Campylobacter* spp. is a common cause of bacterial diarrhea but infections by *Salmonella* spp., *Shigella* spp. are also a frequent cause (Viswanathan *et al.* 2009). Soil ingestion is also associated with child diarrhea. Environmental characteristics and behavioral practices have been

Table 5. Prevalence of different triple infections in total wastewater samples

Pathogen	Total no. of soil samples examined	No. of positive samples with three parasites	Prevalence (%)
Cryptosporidium + Entamoeba + Blastocystis	28	8	28.57
Cryptosporidium + Entamoeba + Campylobacter	28	16	57.14
Cryptosporidium + Entamoeba + Shigella	28	4	14.29
Cryptosporidium + Entamoeba + Aeromonas	28	4	14.29
Entamoeba + Campylobacter +Blastocystis	28	12	42.86
Entamoeba + Campylobacter + Aeromonas	28	16	57.14
Entamoeba + Campylobacter + Vibrio cholerae	28	12	42.86
Entamoeba + Shigella + Bacteroides fragilis	28	8	28.57
Entamoeba + Adenovirus + Aeromonas	28	4	14.29
Entamoeba + Adenovirus + P	28	4	14.29
Entamoeba + Aeromonas + Plesiomonas	28	4	14.29
Entamoeba + Aeromonas + Sapovirus	28	4	14.29
Entamoeba + Aeromonas + Vibrio cholerae	28	12	42.86
Shigella + Campylobacter + Sapovirus	28	4	14.29
Shigella + Campylobacter + Adenovirus	28	4	14.29
Shigella + Campylobacter + Blastocystis	28	8	28.57
Shigella + Blastocystis + Bacteroides frigilis	28	8	28.57
Shigella + Aeromonas + Plesiomonas	28	4	14.29
Blastocystis + Campylobacter + Vibrio cholerae	28	8	28.57
Blastocystis + Aeromonas + Bacteroides fragilis	28	8	28.57
Blastocystis + Aeromonas + Plesiomonas	28	4	14.29
Blastocystis + Adenovirus + Plesiomonas	28	4	14.29
Blastocystis + Sapovirus + Vibrio cholera	28	4	14.29
Campylobacter + Vibrio cholera + Aeromonas	28	12	42.86
Adenovirus + Plesiomonas + Aeromonas	28	4	14.29

Table 6. Prevalence of different quadruple infections in total wastewater samples

Pathogen	Total no. of soil samples examined	No. of positive samples with four parasites	Prevalence (%)
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Blasocystis*	28	8	28.57
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Aeromonas*	28	8	28.57
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Vibrio cholera*	28	4	14.29
Cryptosporidium + *Entamoeba* + *Bacteroides fragilis* + *Shigella*	28	4	14.29
Cryptosporidium + *Entamoeba* + *Aeromonas* + *Sapovirus*	28	4	14.29
Cryptosporidium + *Blastocystis* + *Shigella* + *Vibrio cholerae*	28	4	14.29
Entamoeba + *Blastocystis* + *Campylobacter* + *Sapovirus*	28	4	14.29
Entamoeba + *Blastocystis* + *Campylobacter* + *Adenovirus*	28	4	14.29
Entamoeba + *Blastocystis* + *Shigella* + *Bacteroides fragilis*	28	8	28.57
Entamoeba + *Blastocystis* + *Aeromonas* + *Plesiomonas*	28	4	14.29
Entamoeba + *Campylobacter* + *Aeromonas* + *Vibrio cholerae*	28	8	28.57
Shigella + *Campylobacter* + *Sapovirus* + *Bacteroides fragilis*	28	4	14.29
Shigella + *Campylobacter* + *Bacteroides fragilis* + *Blastocystis*	28	8	28.57
Shigella + *Plesiomonas* + *Aeromonas* + *Vibrio cholerae*	28	4	14.29
Campylobacter + *Plesiomonas* + *Aeromonas* + *Vibrio cholerae*	28	4	14.29

identified as risk factors for diarrhea in developing countries as people living in slums or in the lower socio-economic stratum have little or no access to services such as water sanitation, proper drainage and waste disposal and as a result also become polluted by diarrhea causing agents. Worldwide in 2004, approximately 2.5 billion cases of diarrhea occurred, which resulted in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia (WHO 2009). This is lower from a death rate of 4.5 million in 1980 for gastroenteritis (Mandell *et al.* 2004). Diarrhea remains the second leading cause of infant mortality (16%) after pneumonia (17%) in this age group (WHO 2009).

Table 7. Prevalence of different multiple infections in total wastewater samples

Pathogen	Total no. of soil samples examined	No. of positive samples with five parasites	Prevalence (%)
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Blastocystis* +*Shigella*	28	4	14.29
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Blastocystis* + *Bacteroides fragilis*	28	4	14.29
Cryptosporidium + *Entamoeba* + *Campylobacter* + *Shigella* + *Sapovirus*	28	4	14.29
Entamoeba + *Campylobacter* + *Shigella* + *Vibrio cholera* + *Sapovirus*	28	4	14.29
Entamoeba + *Campylobacter* + *Aeromonas* + *Plesiomonas* + *Bacteroides fragilis*	28	4	14.29
Entamoeba + *Vibrio cholera* + *Aeromonas* + *Plesiomonas* + *Shigella*	28	4	14.29
Entamoeba + *Vibrio cholera* + *Aeromonas* + *Plesiomonas* + *Adenovirus*	28	4	14.29
Campylobacter + *Aeromonas* + *Plesiomonas* + *Bacteroides fragilis* + *Adenovirus*	28	4	14.29
Campylobacter + *Bacteroides fragilis* + *Shigella* + *Sapovirus* + *Blastocystis*	28	4	14.29

CONCLUSION

In ICDDR,B, Dhaka, 1000s of diarrheal patients admit every month. It is a matter of great sorrow that death from diarrheal infection decreased but levels of morbidity have not been declined in comparison to historical levels. For this reason diarrhea is still a major cause of morbidity and mortality among children in developing countries. Absolute requirement for rapid and cost effective diagnostic methods are essential of detection for the intestinal protozoan parasites which causes diarrhea. Regarding the socio-economic condition of Bangladesh, it can be said that that PCR (Polymerase Chain Reaction) is one of the best method for detection of intestinal parasites. PCR test and TAC system were demonstrated to be accurate and useful tool in the detection of parasites and other diarrhea causing agent in human stool and environmental samples (soil and wastewater) that can be transmitted by fecal-oral route.

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