PARASITIC CONTAMINATION OF STREET FOOD SAMPLES FROM SCHOOL-BASED FOOD VENDORS OF DHAKA CITY, BANGLADESH

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ABSTRACT: Street food is an old notion that arose from the need of quick access of light food to satisfy hunger. With the mention of street food in Bangladesh, a picture is visualized which includes open stalls with uncovered food, unawareness of vendors about hygienic practices, lack of safe source of water for cleaning and preparing food, increasing pollution in surrounding environment leading to infestation with various parasites and causing diarrhoeal diseases in people. The aim of the present study was to determine the prevalence of parasites in schoolbased street foods in Dhaka city. A total of 200 food samples were examined containing three representative types of street foods: tamarind water of snacks (79 samples), sliced fruits (71 samples) and fruit juices (50 samples) using Formalether concentration technique. Among them, 164 (82%) samples were contaminated with Entamoeba histolytica, Blastocystis hominis, Cystoisospora belli, Diphyllobothrium latum, Hymenolepis diminuta, Hymenolepis nana, Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura and Ancylostoma duodenale. C. belli was the most prevalent (38%), followed by A. lumbricoides (33%) and B. hominis (33%). While comparing the parasite prevalence in three types of street foods, the highest prevalence was observed for C. belli (21.5% in tamarind water of snacks, 6.5% in sliced fruits and 10% in fruit juices) followed by A. *lumbricoides*, *B. hominis* and *D. latum*. There were significant associations (p<0.05) among the different food types with B. hominis, C. belli, D. latum and A. lumbricoides positive samples. The study implicates that parasitic contamination of school-based street foods is alarming and it is urgent to take necessary steps to ensure the food safety.

Key words: Parasite, prevalence, street food, school-based vendors, mixed contaminations

INTRODUCTION

Street food refers to the ready-to-eat foods and beverages prepared and sold in the streets and other similar public places by vendors and hawkers from pushcarts, buckets, stalls, shops with less than four permanent walls (FAO 2005). Street food includes fast foods, snacks, sliced fruits, salads, junk foods,

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meals, beverages, and drinks but depending on the countries and culture, it varies greatly (Dawson and Canet 1991, Moy *et al.* 1997). These foods are acclaimed for their unique flavours, convenience as well as fulfilling the nutritional requirements of populations at affordable prices (Ackah *et al.* 2011, Muzaffar *et al.* 2009). Every day, approximately 2.5 billion people consume street food around the world (Ekanem 1998, Fellows and Hilmi (2011). People who regularly consume street food are more susceptible to food-borne illnesses such as food-poisoning, diarrhoea, typhoid fever and cholera (Rane 2011). Foodborne disease in humans includes a wide range of illness caused by more than 250 different bacteria, viruses, parasites, metals, poisons and prions (Tambekar *et al.* 2008). According to the World Health Organization (WHO), diarrhoea caused by food-borne diseases is one of the leading causes of death worldwide resulting into 1.6 million deaths per year (Baldursson and Karanis 2011).

Food vendors play a vital role in maintaining food safety at every phase of food manufacturing processes including processing, storage and preparation (Hedberg *et al.* 1994). Majority of street food vendors have no formal education, no training on food hygiene and even they do not have sufficient knowledge about causes of food-borne illness and they hardly realize the importance of knowing it (Barro *et al.* 2007). Food contamination done by food handlers is associated with 10-20% food-borne disease outbreaks (Zain and Naing 2002).

Parasites (protozoa and helminths) might spread to humans when one of the environmental transmission stages of parasites (cyst and oocytes of protozoa and egg and larva of helminths) are ingested through food and water (Slifko *et al.* 2000). Resistance to highly unfavourable environmental conditions and extreme proliferation ability are other reasons of transmission of parasites (Brooker *et al.* 2006). In the developing world, amoebiasis has been estimated to cause 450 million infections every year with an incidence of 50 million and 100,000 fatalities (Ravdin1988).

Common protozoan infections are frequently transmitted by food containing faecally contaminated soil or water, which may carry the environmentally resistant oocyst stage of the parasites (Hill and Dubey 2016). *Cryptosporidium* spp., *Giardia intestinalis* and *Blastocystis spp.*, *Entamoeba histolytica*, the enteric protozoans cause diarrhoeal disease worldwide. Synanthropic insects such as flies act as effective vectors for human intestinal protozoan parasites because of their feeding process and dirty breeding behaviour (Graczyk *et al.* 2005).

Fruits and vegetables play an important role in providing nutrients such as minerals, vitamins, nutritional fibres, and phytochemicals, particularly antioxidants, which protect the human body against a variety of infectious and non-infectious disorders (Poiroux-Gonord *et al.* 2010). Despite their nutritional benefits, fruits and vegetables act as vehicles of gastrointestinal parasites that cause a variety of disease to the people (Agbalaka *et al.* 2019). When raw fruits and vegetables are ingested without being washed properly, they are considered as a major route for transmission of soil-transmitted helminthiasis (STH) (Uneke 2007).

Bangladesh is a densely populated country where the greatest proportion of people depends on street foods for their easy availability and the cheapest price. A very common point for setting these food stalls is schools and colleges because the students are considered as a big section of their customers. In Bangladesh, approximately 30 million people experience food-borne diseases every year (Khairuzzaman *et al.* 2014). Enteric fever, hepatitis, and diarrhoeal illness are the three most prevalent food-borne illnesses in Bangladesh (FAO 2022). Children, pregnant women and people with compromised immune systems are the most serious victims of food-borne diseases in Bangladesh and other South-East Asian countries (WHO 2016). According to ICDDR,B, there were 501 hospital visits each day for diarrhoea caused by food and waterborne infections. Diarrhoea is responsible for one-third of child deaths in Bangladesh reported by the World Health Organization (WHO 2018).

There has been little research on parasites in street food. Most prior research has focused on microbiological results in street food, although there is a possibility for a large number of parasites to be present in these food items. In Bangladesh, this is for the first time when a study has been carried out to determine the prevalence of parasites in street foods which forms a baseline for parasitic study on this topic so that further work can be done in future.

MATERIAL AND METHODS

Study area: To determine the parasitic contamination of street foods, the current study was conducted near 50 schools of Dhaka city during 2021 to 2022. The sites were selected on the basis of dependency of students on street foods and 200 street food samples from those sites were collected. For the identification of parasites, laboratory tests were done in the Parasitology laboratory, Department of Zoology, University of Dhaka.

Types of food sample: There are various kinds of street foods sold in Dhaka city such as different snacks (e.g. fuchka, chotpoti, velpuri, puri, jhalmuri, haleem), fruit juice, sliced fruit, pickles etc. A total of 200 food samples were examined considering three representative types of street foods: Tamarind water of snacks (79 samples), sliced fruits (71 samples) and fruit juices (50 samples) (Table 1).

	Name	Number	Percentage (%)	Total
	Fuchka	48	24	
Snacks (tamarind	Velpuri	20	10	79 (39.5%)
water)	Chotpoti	11	5.5	
	Guava	25	12.5	
	Papaya	26	13	
Sliced fru	its Pineapple, Wood apple	9	4.5	71 (35.5%)
	Pomelo, hogplum, star fruit	8	4	
	Carrot, Cucumber	3	1.5	
	Sugarcane	30	15	
Fruit juic	e Lemon	10	5	
	Aloe vera	3	1.5	50 (25%)
	Wood apple	4	2	
	Mango	1	0.5	
	Mixed fruit	2	1	
	Total	200	100%	100%

Collection and preservation of food sample: About 20-25 ml of each food sample was collected from vendors of Dhaka city. Each sample was kept in a

Table 1: A list of food samples examined in this study

clean, dry, leak proof, transparent pots and labelled with the identification number (sample number and date of collection). After collection, the food samples were brought to the laboratory and preserved in 10% formalin.

Examination of food sample: Formal-ether concentration technique, a sedimentation technique was applied for detection of parasites' ova and cysts in food samples which is similar to previously published method (Cheesbourgh, 1987). At least six slides from each sample were examined for this study.

Identification of egg of different parasites: According to Chatterjee (2011), identification of cysts and oocysts of protozoans and egg and larva of helminths was performed through a microscope.

Statistical analyses: Chi-square (x^2) tests were performed using the statistical programming language R. Fisher's exact test was performed instead of chi-square test where the sample size was small.

Ethical approval: Ethical approval was obtained from the Faculty of Biological Sciences, University of Dhaka ethical review committee.

RESULTS AND DISCUSSION

The aim of the present study was to estimate the parasitic contamination of commonly consumed street foods sold in school-based areas of Dhaka city, Bangladesh. Out of 200 food samples comprising of tamarind water of snacks, fruit juices and sliced fruits, a total of 164 samples were found to be contaminated with at least one parasite. The overall parasitic contamination rate (82%) in the present study seems to differ from the results of previous studies performed by Alemu *et al.* (2020) in Northwest Ethiopia (39.1%) and Punsawad *et al.* (2019) in Southern Thailand (35.1%). In this study, a total of 10 parasite species, including three protozoans (*Entamoeba histolytica, Blastocystis hominis, Cystoisospora belli*), three cestodes (*Diphyllobothrium latum, Hymenolepis diminuta, Hymenolepis nana*) and four nematodes (*Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura* and *Ancylostoma duodenale*) were identified (Table 2, Figure 1).

In this study, protozoans and helminths were observed in 114 (57%) and 86 (43%) samples respectively (Table 3). Similarly, a study done by M'rad *et al.* (2020) in Pakistan, helminth eggs and protozoan oocysts were detected in 30 (12.5%) unwashed vegetables. The present study revealed that protozoan oocysts are more prevalent than helminth eggs, whereas, a study by Omowaye and Falola (2012) on 761 fruits in Nigeria found that helminthic contamination (4.3%) was higher than protozoans (2.5%). When the prevalence of protozoan and helminth parasites in three different types of street meals was compared, the highest prevalence of protozoans was found in fruit juices (80%) followed by tamarind water of snacks (56.96%) and sliced fruits (40.84%). Similarly, the prevalence of helminths was highest in fruit juices (64%), followed by tamarind water of snacks (45.57%) and sliced fruits (25.35%).

Out of 10 parasites, *Cystoisospora belli* was the most prevalent parasite (38%), followed by *Ascaris lumbricoides* (33%), *Blastocystis hominis* (33%), *Diphyllobothrium latum* (11.5%), *Trichuris trichiura* (6.5%) and the lowest prevalence was observed in *Enterobius vermicularis* (0.5%). The prevalence of *Hymenolepis diminuta, Ancylostoma duodenale* and *Entamoeba histolytica* was 1.5% each (Table 4).

Among the parasites identified in the present study, *Cystoisospora belli* was the most prevalent (38%) parasite. However, this finding is in contrast with the study conducted by Amissah-Reynolds *et al.* (2020), where *C. Belli* was recovered from >2% ready-to-eat salads in Accra Metropolis, Ghana. The oocysts of *C. belli* have been observed in the faeces of immunosuppressed people and the ingestion of faecally contaminated food and water with sporulated oocysts can lead to cystoisosporiasis (Dubey and Almeria 2019). Another possible explanation is that this parasite can cause self-limiting diarrhoea or asymptomatic infection in healthy people which may explain the high prevalence.

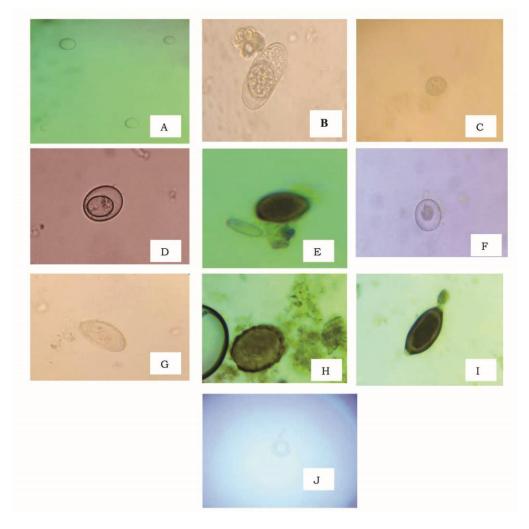


Fig.1: Parasitic stages contaminating food samples: (a) *Blastocystis hominis* cyst (40X), (b) *Cystoisospora belli oocyst* (40X), (c) *Entamoeba histolytica* cyst (40X), (d) *Hymenolepis nana* egg (40X), (e) *Diphyllobothrium latum* egg (40X), (f) *Hymenolepis diminuta* egg (40X), (g) *Enterobius vermicularis*

egg (40X), (h) Ascaris lumbricoides egg (40X), (i) Trichuris trichiura egg (40X), (j) Ancylostoma duodenale larva (10X).

Protozoa	Entamoeba histolytica
	Blastocystis hominis
	Cystoisospora belli
Cestoda	Diphyllobothrium latum
	Hymenolepis diminuta
	Hymenolepis nana
Nematoda	Ascaris lumbricoides
	Enterobius vermicularis
	Trichuris trichiura
	Ancylostoma duodenale

 Table 2: Name of parasites found in food samples

Blastocystis hominis are zoonotic microorganisms, transmitted through water contaminated with faeces, persist in the environment for a longer period and resistant to disinfectants and environmental conditions (WHO 2016, Zahedi and Ryan 2020, Martín-Escolano *et al.* 2023). Blastocystis hominis was the second most prevalent parasite in this study and observed in 33% samples. Similarly, a study by Al Nahhas and Aboualchamat (2020) in Syria, Blastocystis spp. had the second highest prevalence (29.5%). Efstratiou *et al.* (2017) also reported that 8.1% protozoan water outbreaks are produced by *B. hominis*.

The presence of helminth eggs in different fruits and vegetable may be due the contaminated soil and water (Kishk and Allam 2020). In this study, Ascaris lumbricoides, Trichuris trichiura and Ancylostoma duodenale were recovered from 33%, 6.5% and 1.5% samples respectively. In contrast, Punsawad *et al.* (2019) observed Ancylostoma duodenale (16.60%) as the most prevalent and Ascaris lumbricoides (2.64%) and Trichuris trichiura (2.64%) as the third most prevalent parasite. So, the presence of STHs in contaminated samples examined in the study could be attributed to the use of human waste-contaminated soil and water for irrigation and the poor hygiene practices of vendors during food processing.

The contamination rate of *Hymenolepis nana* (6%) and *Hymenolepis* diminuta (1.5) in this present study was lower than the study done by Bekele and Shumbej (2019), where they detected *Hymenolepis nana* and *Hymenolepis* diminuta in 11.9% and 10.7% fruits and vegetable samples respectively. In this study, *Diphyllobothrium latum* was found in 23 (11.5%) samples, this finding is **Table 3:** Cross-tabulation between prevalence of helminths and protozoans in three types of food samples

Food type	Number of examined	Number o sam	-	Prevalence (%)	
	sample	Helminth	Protozoa	Helminth	Protozoa
Tamarind water of snacks	79	36	45	45.57	56.96
Sliced fruits	71	18	29	25.35	40.84
Fruit juice	50	32	40	64	80
Total	200	86	114	43	57

Table 4: Prevalence of different species of parasites found in food samples

Name of parasites	No. of sample	No. of contaminated	Prevalence
		samples	(%)
Entamoeba histolytica		3	1.5
Blastocystis hominis		66	33
Cystoiospora belli		76	38
Diphyllobothrium latum		23	11.5
Hymenolepis diminuta	200	3	1.5
Hymenolepis nana		12	6
Ascaris lumbricoides		66	33
Enterobius vermicularis		1	0.5
Trichuris trichiura		13	6.5
Ancylostoma duodenale		3	1.5

similar to the studies carried out by Yusof *et al.* (2017) in Kuantan, Pahang where they detected *Diphyllobothrium* eggs in raw fruits and vegetables. Out of 79 tamarind water of snacks, *Cystoisopora belli* was found in 43 (21.5%) samples which was the highest prevalent, followed by *Ascaris lumbricoides* (21%), *Blastocystis hominis* (18%). Out of 71 sliced fruit samples, *Cystoisopora belli* was the most prevalent parasite (6.5%). *Entamoeba histolytica and Hymenolepis diminuta* (0.5%), *Hymenolepis nana*, *Diphyllobothrium latum* and

Trichuris trichiura (1%) (Table 5). This is somewhat similar to the findings of Lawal *et al.* (2015), who carried out a study in three open markets of Zaria Metropolis, Nigeria. They found 35.27% prevalence of parasites on the 360 fruits and vegetables examined in which 3.05% *Hymenolepis nana*, 4.17% *Entamoeba histolytica* and 2.5% *Ancylostoma duodenale* was observed. In this study, out of

Name of parasites	No. of sample	Tamarind v snacks	water of	Sliced fruit	S	Fruit juices		
	·	Contamina ted samples	Prevalen ce (%)	Contamina ted samples	Prevale nce (%)	Contaminat ed Samples	Prevale nce (%)	P value
Entamoeba		1	0.5	1	0.5	1	0.5	1*
histolytica								1
Blastocystis		36	18	12	6	18	9	0.000837
hominis								0.000037
Cystoisospora belli		43	21.5	13	6.5	20	10	0.000030
Diphyllobothrium		12	6	2	1	9	4.5	0.015054
latum								0.015054
Hymenolepis		0	0	1	0.5	2	1	0.184871*
diminuta								0.104071
Hymenolepis nana		6	3	2	1	4	2	0.335275*
Ascaris		42	21	9	4.5	15	7.5	8.3311E-7
lumbricoides								0.3311E-1
Enterobius		0	0	0	0	1	0.5	0.250000*
vermicularis								0.20000
Trichuris		8	4	2	1	3	1.5	0 000047*
trichiura								0.203347*
Ancylostoma		1	0.5	0	0	2	1	0.258553*
duodenale								0.200003"

Table 5: Comparison between j	prevalence of para	asites found in differe	ent food samples
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*Fisher's exact test has been done where the infected sample number is very low.

Table 6: Prevalence of mixed contaminations in food samples

No. of sample examined	No. of contaminated samples	Single contamination	Prevalence (%)	Mixed contaminatio ns	Prevalence (%)
200	164	86	43	78	39

50 fruit juice samples, the prevalence of Cystoisospora belli, Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, Entamoeba histolytica

were 10%, 7.5%, 1.5%, 1%, 0.5% respectively. All of the parasites were detected through formal-ether concentration technique. However, in Nigeria, Hassan *et al.* (2013) examined 264 fruits and vegetables in which *Cystoisospora* spp. (1.67%) was the lowest prevalent parasite while the prevalence of *Ascaris lumbricoides, Trichuris trichiura* was 38.09% and 4.55% respectively. Kinyoun acid-fast procedure was applied for identification of *Cystoisospora* spp. and wet mount technique for other parasites (Hassan *et al.* 2013).

The highest prevalence was observed for *Cystoisospora belli* in three types of street food (21.5% in tamarind water of snacks, 6.5% in sliced fruits and 10% in fruit juices). Prevalence of *Ascaris lumbricoides* was recorded 21% in tamarind water of snacks, 4.5% in sliced fruits and 7.5% in fruit juices. Prevalence of *Blastocystis hominis* was observed 18% in tamarind water of snacks, 6% in sliced fruits and 9% in fruit juices. Prevalence of *Diphyllobothrium latum* was found 6% in tamarind water of snacks, 1% in sliced fruits and 4.5% in fruit juices. There were significant associations (p<0.05) among the food types with *Blastocystis hominis, Cystoisospora belli, Diphyllobothrium latum and Ascaris lumbricoides* positive samples.

In the present study, 86 (43%) samples were found with mixed contaminations whereas the 78 (39%) samples were contaminated with one parasite (Table 6). This result contradicts the findings by Omalu *et al.* (2013) on 116 food samples (stew salad, soup, beans, salad) in Minna, Nigeria, in which single and mixed contamination were observed in 70 (67.31%) and 34 (32.69%) samples respectively. This finding also differs from the study done by M'rad *et al.* (2020), where they reported 63.3% single and 36.7% multiple contaminations.

CONCLUSION

Street food holds a favourable part of many developing countries like Bangladesh as it fulfils the nutritional need of food of a large number of people around the world at affordable prices and easy availability. Also, it makes a significant contribution to the economy of many countries. In spite of these advantages, street food poses a serious health risk to the consumers by leading to the spread of serious food-borne illness and deadly diseases. Besides, food vendors play a crucial role for contamination of street food during food handling practices such as food preparation, processing and storage as most of them do not have formal education, training on food hygiene and idea about the health risks their food imposes. In this study, *Cystoisospora belli, Ascaris lumbricoides* and *Blastocystis hominis* were predominant among the parasites identified. As the number of food-borne infection cases is increasing day by day, it is high time to take necessary steps to improve food safety and hygiene practices in Bangladesh. Extensive training methods of street food vendors about food safety should be arranged with the implication of policies to improve food safety knowledge, attitude and behaviour of vendors.

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