Original Article

Bangladesh Journal of Agriculture and Life Science
Faculty of Agriculture, University of Rajshahi
Rajshahi-6205, Bangladesh
Vol. 03 (1): 1-6, 2022
http://csa.ru.ac.bd/bjals/

Free-living Stage of Helminth Parasites Isolated from Common Vegetables Available in Local Markets of Rajshahi, Bangladesh

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ARTICLE INFO

Article History
Received: December 4, 2024
Accepted: January 10, 2025
Online: April 25, 2025

Keywords
Contamination
Helminth parasite
Public health
Vegetable

ABSTRACT

Vegetables are rich in critical vitamins, minerals, and antioxidants, as well as dietary fiber, which have numerous health benefits. Fresh veggies can carry a variety of deadly parasites, making people more prone to foodborne illness. This study aims to isolate and identify the free-living stage of helminth parasites in fresh vegetables. Nine different types of samples (30 each) were collected and washed scientifically. The wash materials were collected, sieved, centrifuged, and inspected under a light microscope to determine parasite contamination. Out of 270 samples, 186 (68.9%) tested positive for at least one parasite genus or species. Water spinach had the highest degree of parasite contamination (93.3%), while jute leaves had the lowest level (36.7%). *Strongyloides stercoralis* (51.1%) was the most commonly observed parasite, while *Stictodora fuscata* (3.7%) was the least prevalent. Other detected parasites were *Ascaris lumbricoides* (37.8%), hookworm (47.4%), *Enterobius vermicularis* (12.6%), *Schistosoma haematobium* (14.8%), and *Paragonimus* sp. (8.1%). There was a substantial association ($p<0.05$) between parasite contamination and several vegetable varieties. This finding highlights a critical public health hazard in which humans and animals are at great risk of being infected with different types of parasites. So, to avoid foodborne parasite infections, everyone should practice proper personal hygiene and carefully wash vegetables to ensure safe consumption.

Introduction

Vegetables are an important part of a healthy diet that humans and other animals eat as food. They provide nutritional fiber and critical vitamins, minerals, and trace elements, including antioxidants and vitamins A, C, and E. Vegetables can be consumed raw, semi-cooked, or cooked, and their inclusion in the diet has been linked to a lower incidence of cancer, stroke, cardiovascular disease, and other chronic illnesses (Buchner et al., 2010). Vegetable consumption offers numerous health benefits, but improper food safety practices can lead to health risks due to potential exposure to harmful microbes or parasites during cultivation, harvesting, processing, and marketing (Duedu et al., 2014).

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The probable source of contamination is the unsanitary quality of the environment that serves as a substrate for the growth, proliferation, and transmission of parasites (Taghipour et al., 2019). The heavy use of manure from cattle, poultry, and other animal sources increases the danger of parasite infection in the soil, water, vegetables, and people. This is due to the fact that eating vegetables directly or indirectly polluted with contaminated animal manure has been connected to a number of helminth parasites (Amissah-Reynolds et al., 2012). Many helminth parasites, such as *Ascaris lumbricoides*, *Toxocara* species, *Strongyloides stercoralis*, *Trichuris trichiura*, hookworm, *Fasciola hepatica*, *Hymenolepis nana*, and others, have been found in a variety of unwashed raw vegetables (Omar et al., 2017; Rostami et al., 2016; Benti and Gemechu, 2014). Accidental ingestion of infectious parasite eggs or larvae from the consumption of contaminated fresh vegetables can result in a wide spectrum of food-borne illnesses in humans (Nejadi and Abdoli, 2021). Vegetable contamination leads to outbreaks like strongyloidiasis, fascioliasis, fasciolopsiasis, giardiasis, amebiasis, cyclosporiasis, echinococcosis and cryptosporidiosis, causing higher morbidity and mortality rates in developing countries (Adamu et al., 2012; Hassan et al., 2012). In underdeveloped nations, particularly in tropical and sub-tropical areas, parasitic helminths are a major public health concern, affecting about 15 million people worldwide (Flammer et al., 2020). Malnutrition and stunted growth, particularly in children and pregnant women, are the most serious intestinal parasite consequences (Taghipour et al., 2021). Besides, these parasite infections can lead to a number of chronic issues, including iron deficiency anemia, severe cyst formation, and other physical and mental health issues (Alemu et al., 2019). They can also put a financial strain on people. The identification of parasites from fresh vegetables is not well documented in Bangladesh. This study accurately identified a prevalent parasite contamination in fresh vegetables that could potentially affect public health.

Materials and Methods

Study area and Sample collection

The study was carried out between December 2021 and November 2022 in Rajshahi district of Bangladesh. The following fresh vegetable samples were bought from various open markets: arum leaves (*Colocasia esculenta*), cabbage (*Brassica oleracea*), green amaranth (*Amaranthus viridis*), jute leaves (*Corchorus olitorius*), malabar night shade (*Basella alba*), radish (*Raphanus sativus*), red amaranth (*Amaranthus cruentus*), spinach (*Spinacia oleracea*), and water spinach (*Ipomoea aquatica*). After being collected, the samples were stored in an insulated cooler ice box and assigned a unique identification number before being delivered to the parasitological research laboratory of Veterinary and Animal Sciences Department, University of Rajshahi, Bangladesh.

Sample processing and identification of helminth parasite

Each collected sample weighed 125 grams and was washed individually in 1000 ml of phosphate-buffered saline (PBS) with tween 80 solution. It was shaken for 15 minutes using an electric shaker to detach free-living stages of helminth parasites, such as eggs and larvae (Punsawad et al., 2019). The washed solutions were sieved using medical gauzes. The mixture was then allowed to stand overnight to allow for sedimentation. The supernatants were carefully discarded without agitation, and the remaining sediments were centrifuged for 10 minutes at 2500 rpm. After draining the supernatant fluids, the leftover sediment was collected and thoroughly mixed using a vortex mixer. The final step was examining it with 10X and 40X objectives using a trinocular head light microscope equipped with a camera (Optica® Italy). Based on the keys and descriptions found in the reference books and literature, several foodborne helminth parasites were identified (Soulsby, 1982; Williams, 1992).

Statistical analysis

Data from research findings were compiled and analyzed with IBM SPSS Statistics version 25. The chi-square ($\chi 2$) test was performed to analyze the relationship between variables at a p-value < 0.05 with a 95% confidence interval.

Results

Parasitic contamination of fresh vegetables

A total of 270 fresh vegetable samples were tested for the presence of free-living helminth parasites, and 186 (68.9%) were positive for at

Table 1. Status of parasitic contaminations

Type of samples	Parasitic contamination					
	Positive (%)	Negative (%)	Total (n=270)	χ^2-value	p-value	Cramer's V value
Arum leaves	18 (60.0)	12 (40.0)	30			
Cabbage	18 (60.0)	12 (40.0)	30			
Green amaranth	23 (76.7)	7 (23.3)	30			
Jute leaves	11 (36.7)	19 (63.3)	30			
Malabar night shade	16 (53.3)	14 (46.7)	30	36.70	0.001[a]	0.37[b]
Radish	25 (83.3)	5 (16.7)	30			
Red amaranth	26 (86.7)	4 (13.3)	30			
Spinach	21 (70.0)	9 (30.0)	30			
Water spinach	28 (93.3)	2 (6.7)	30			
Total	186 (68.9%)	84 (31.1%)	270			

[a] p value < 0.05 were considered statistically significant, [b]Cramer's V value > 0.25 were considered very strong effect.

least one genus or species. The most contaminated vegetable was water spinach (93.3%), whereas the least contaminated vegetable was jute leaves (36.7%). This result was statistically significant (p = 0.001), and the types of samples and parasite contamination status were found to be strongly correlated (Table 1).

Prevalence of free-living stage of helminth parasite

Strongyloides stercoralis larvae (51.1%) were commonly observed in the tested samples. Other parasites that contaminate the samples include eggs of *A. lumbricoides* (37.8%), hookworm larvae (47.4%), eggs of *Enterobius vermicularis* (12.6%), eggs of *Schistosoma haematobium* (14.8%), eggs of *Paragonimus* sp. (8.1%), and eggs of *Stictodora fuscata* (3.7%). Water spinach and red amaranth were the vegetables most infested by *Strongyloides stercoralis* and hookworm, respectively. Arum leaves, cabbage, green amaranth, Jute leaves, Malabar night shade, radish, and spinach were all heavily contaminated with one or more helminth parasites. (Fig. 1-2, Table 2). The blue bar indicates contaminated samples, and the red bar indicates percentages of identified parasites. A total of 37.8% *Ascaris lumbricoides* was recovered from 102 contaminated raw vegetables. Similarly, *Enterobius vermicularis* 12.6% from 34, hookworm 47.4% from 128, *paragonimus* sp. 8.1% from 22, *Schistosoma haematobium* 14.8% from 40, *Stictodora fuscata* 3.7% from 10, *Strongyloides stercoralis* 51.1% were identified from 138 contaminated veggies.

Discussion

Helminths are common parasites associated with clinically relevant contamination from raw vegetable intake. Consuming unclean, uncooked, or improperly processed veggies can spread a variety of infectious diseases. Food safety concerns develop as a result of possible parasitic pathogens in raw produce, which pose serious health hazards to vulnerable groups such as pregnant women and small children. The issue has far-reaching worldwide ramifications for clinical and public health, particularly in low-income nations (Agbalaka et al., 2018).

This study's findings demonstrated 68.9% of parasitic contamination was similar to the predominance of 65.5% in Kenya (Nyarango et al., 2008), 63.7% in Iran (Salavati et al., 2017), 57.8% in Nigeria (Adenusi et al., 2015) and 61.5% in Bangladesh (Azim et al., 2018). However, differences were observed in certain prior investigations, including 48.7% in Ghana (Amissah-Reynolds et al., 2020), 35.1% in Thailand (Punsawad et al., 2019), 26.9% in Poland (Klapec and Borecka, 2012), and 16.2% in Saudi Arabia (Al-Megrin, 2010). It shows that differences in the prevalence of parasite infection in raw vegetables between studies can be attributed to geographical and climate conditions, vegetable type, sample size, sampling season, parasitological processing approaches etc. (Adenusi et al., 2015; Fallah et al., 2012).

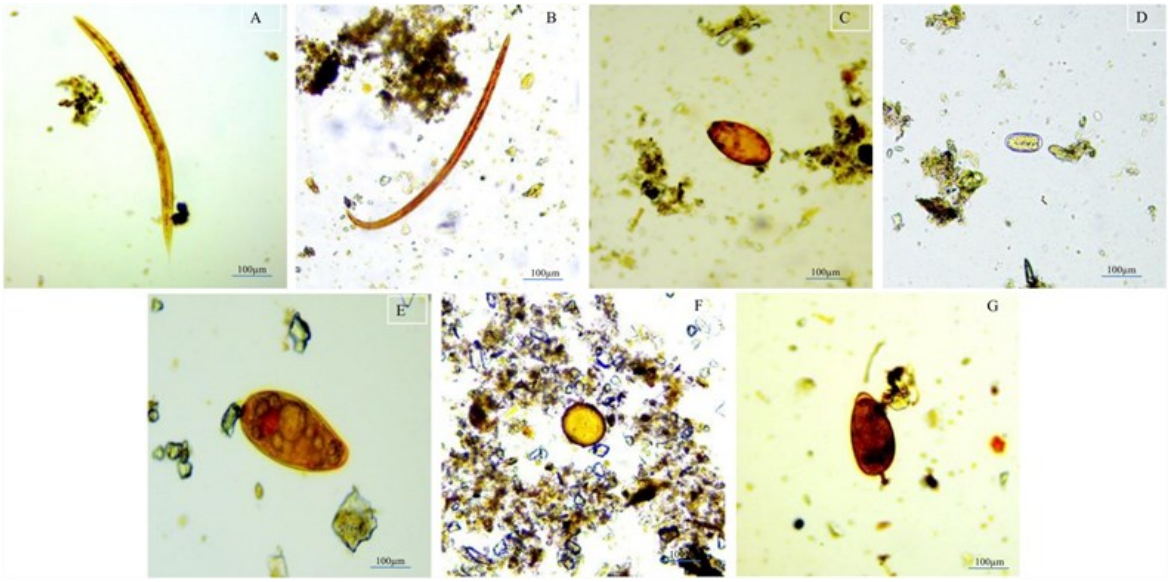

Fig. 1. Different types of identified free-living stages of helminth parasites (40X). (A) Hookworm larvae (B) *Strongyloides stercoralis* filariform larvae (C) *Paragonimus* sp. egg (D) *Enterobius vermicularis* egg containing developing embryo (E) *Stictodora fuscata* egg (F) *Ascaris lumbricoides* decorticated egg and (G) *Schistosoma haematobium* egg.

Table 2. Parasites isolated from different types of vegetable

Type of Parasites		Frequency of parasitic contamination in different samples								
		Arum leaves	Cabbage	Green amaranth	Jute leaves	Malabar night shade	Red amaranth	Radish	Spinach	Water spinach
Ascaris lumbricoides	+	14	4	12	6	12	14	12	12	16
	%	46.7	13.3	40.0	20.0	40.0	46.7	40.0	40.0	53.3
Enterobius vermicularis	+	4	2	6	0	0	4	4	6	8
	%	13.3	6.7	20.0	0.0	0.0	13.3	13.3	20.0	26.7
Hookworm	+	10	8	18	6	14	22	20	10	20
	%	33.3	26.7	60.0	20.0	46.7	73.3	66.7	33.3	66.7
Paragonimus sp.	+	2	0	2	0	2	4	4	2	6
	%	6.7	0.0	6.7	0.0	6.7	13.3	13.3	6.7	20.0
Schistosoma haematobium	+	8	2	4	0	6	8	4	2	6
	%	26.7	6.7	13.3	0.0	20.0	26.7	13.3	6.7	20.0
Stictodora fuscata	+	1	0	2	0	0	1	1	2	3
	%	3.3	0.0	6.7	0.0	0.0	3.3	3.3	6.7	10.0
Strongyloides stercoralis	+	14	12	20	8	12	18	14	18	22
	%	46.7	40.0	66.7	26.7	40.0	60	46.7	60.0	73.3

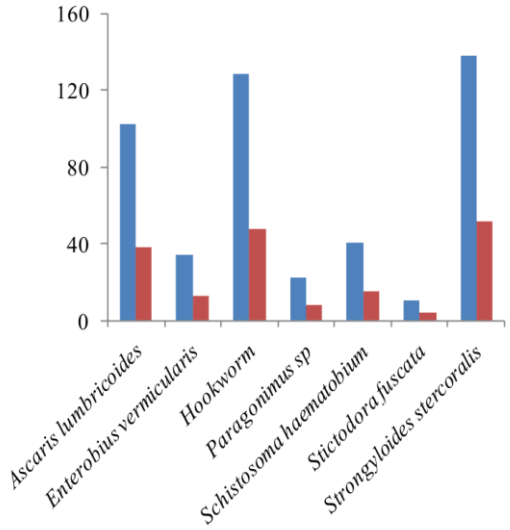

Fig. 2. Prevalence of identified helminth parasites

In this investigation, water spinach had the most contamination (93.3%), while jute leaves had the lowest (36.7%). The fluctuation of the maximum and lowest contamination was observed through the study of certain prior investigations. In Iran, leek (70%) was the most contaminated and parsley (10%) was the least contaminated vegetables (Asadpour et al., 2016). In Brazil, lettuce (61.1%) was the highest contaminated and green onion (44.4%) was the lowest contaminated (Luz et al., 2017) veggies. In the Philippines, maximum contamination was found in pechay (55%), whereas in lettuce (35%), the contamination was minimum (Su et al., 2012). In Egypt, root vegetables were the most contaminated, with 50.0% for radish whereas lettuce and dill had no parasite contamination (Hassan et al., 2012). This could occur as a result of diverse soil types, vegetable surfaces and shapes, irrigation and planting water quality, and hygienic methods employed throughout the marketing process, all of which have an impact on the degree of contamination.

In the vegetables examined in this study, *Strongyloides stercoralis* was the most prevalent parasite, occurring in 51.1%, but the prevalence of parasites may vary depending on the locality. In Nigeria, (Ogbolu et al., 2009) showed *Strongyloides stercoralis* (45.8%) was the most prevalent whereas *Taenia* spp. (4.2%) was the least prevalent helminth parasite found in vegetables. *Giardia lamblia* (19.93%) and *Ascaris lumbricoides* (26.94%) were the most common parasites in fresh vegetables found in Ghana (Amissah-Reynolds et al., 2020). *Toxocara* sp. eggs (75%) were the most common parasite found in raw vegetables in Iran, while *Chilomastix mesnili* (22%) eggs were the least common (Abdiet al., 2014). In Bangladesh, (Azim et al., 2018) found that *Ascaris lumbricoides* (36.5%) was the most common parasite, whereas *Trichuris trichiura* (1.5%) was the least prevalent. Many places and regions throughout the world engage in the unhygienic practice of fertilizing agricultural lands with human waste, animal dung, and wastewater, which leads to vegetable contamination. The aforementioned suggests that the parasites found in vegetables vary depending on the cultivation locations and irrigation techniques used.

Conclusion

The current study's findings suggest that raw vegetables sold in Rajshahi, Bangladesh, have the potential to spread helminth parasite diseases to consumers. According to the results, consumers should be made aware of preventative measures like properly cleaning or cooking vegetables before consuming. Additionally, media programs that promote health education and hygienic practices can be used to reduce exposure to vegetable-borne parasites.

Acknowledgments

The research work was supported in part by Grant for Advanced Research in Education (GARE), Ministry of Education, Government of Bangladesh (Grant Number LS20201467 to Moizur Rahman).

Authors' contributions

Conceptualization: M.R. and M.Z.U.; Methodology: M.Z.U., M.M.H. and M.M.R.; Statistical analysis: M.Z.U.; Investigation: M.Z.U., M.M.H. and M.M.R.; Draft preparation, review and editing: M.Z.U.; Supervision: M.R. and L.N. All authors have read and agreed to the published version of the manuscript.

Conflict of interests

The authors disclose no conflict of interests.

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