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Intestinal Parasitic Contamination in Commonly Consumed Vegetables from Wet Markets in Pulau Pinang: A Pilot Study

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ABSTRACT

Eating raw vegetables is one of the parasites' modes of transmission to humans. This study aimed to screen and investigate the occurrence of parasitic intestinal contamination in commonly consumed vegetables among Malaysian from local wet markets in Pulau Pinang. In this study, the vegetables selected were *Brassica oleracea* (cabbage), *Centella asiatica* ('pegaga'), *Daucus carota* (carrot), *Ipomea aquatica* (water spinach or 'kangkung'), and *Oenanthe javanica* ('selom'). One kilogram of each vegetable was purchased randomly from two wet markets in the Northeast and Southeast districts of Pulau Pinang, respectively. Specimens were processed using the sedimentation technique and standard wet mount microscopy. The specimens were examined for the presence of ova (parasite's egg) or larvae under an Olympus optical microscope. The results revealed that vegetables from both wet markets were contaminated with soil-transmitted helminths (STH) and other intestinal parasites, with pegaga being the most contaminated vegetable. Out of the 200 slides examined, these vegetables were contaminated with at least one type of parasite: 'pegaga' 35 % (n=14), selom 17.5 % (n=7), cabbage 10 % (n=4), carrot 5 % (n=2) and water spinach or 'kangkung' 2.5 % (n=1). Of these, the parasites that were found in the specimens were *Ascaris* sp. (70.59 %), followed by hookworm (17.65 %), *Fasciolopsis* sp. (8.82 %), and *Strongyloides* sp. (2.94%). This study highlighted the importance of raising awareness among the community to prevent STH infections. This can be done by promoting good hand hygiene practices and educating on how to prepare uncooked vegetables properly.

Keywords: *Intestinal parasite, screening, malay salad, local market, seasonal month*

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INTRODUCTION

Intestinal parasitic infections are considered one of the most widespread neglected tropical diseases, primarily affecting low and middle-income communities in poor and developing countries. It is reported that more than 1.5 billion people worldwide are infected with soil-transmitted helminths (STH) [1]. The transmissions usually occur when an individual comes in contact with soil or consumes vegetables or fruits that must be adequately prepared, cooked, and washed. Past studies show that a high incidence of intestinal parasitic illnesses in a community that consumes raw vegetables has been recorded, indicating a significant route of intestinal parasite transmission [2, 3]. Globally, the main species that infect humans are Nematode worms, including roundworms *(Ascaris lumbricoides),* hookworms *(Ancyclostoma duodenale* or *Necator americanus*), and whipworm *(Trichuris trichiuria)* [4]. A recent review of the global burden of STH infection showed that 70% of infections occur in Asian countries [5]. Furthermore, Southeast Asia (SEA) has had the highest prevalence of STH infections in previous years, as reported by Silver *et al.* [5].

Pulau Pinang, Malaysia, for instance, has been known as the Food Heaven of Malaysia or also known as Asia's food paradise consists of a wide variety of signature dishes, namely *Asam Laksa, Curry Mee, Char Koay Teow, Pasembor, Rojak* and many more. All these dishes include uncooked or pre-cooked vegetables as their main ingredients. The dishes are very popular among the locals as well as tourists, in which they can be obtained conveniently either at restaurants or at street food hawkers. Besides, raw or uncooked vegetables are also served as main dishes during meal times. Traditionally, vegetables are commonly eaten raw and fresh during meal times and are known as 'ulaman' or traditional Malaysian salads, served in bunches with a wide range of dipping sauces.

Considering the nature of favorite Malaysian food, ensuring a high quality of personal hygiene practices or habits among food handlers is becoming a huge challenge and problem, especially in dealing with a large quantity of food preparation and services. Good personal hygiene practices or habits in handling and preparing food can prevent food poisoning, contamination, and the spreading of infection. Thus, this condition would increase the risk of STH and other intestinal parasitic infections if the food is improperly handled to maintain hygiene and cleanliness [6, 7]. According to past studies, in a community where consuming raw vegetables are common, there is a high incidence of intestinal parasitic illnesses documented [3]. Furthermore, there is evidence from a study that environmental factors, such as the amount of rainfall received, played a significant role in the presence of soil-transmitted helminths in a certain area [4].

In this study, screening on STH and other intestinal parasites in Malay salads ('ulaman') and commonly consumed vegetables among Malaysian were conducted in local wet markets in two different district areas of Pulau Pinang. It is a short study within four months of sampling collection, from December 2021 until March 2022. The Malay salads ('ulaman') and vegetables selected in this study were *Brassica oleracea* (cabbage), *Centella asiatica* ('pegaga'), *Daucus carota* (carrot), *Ipomea aquatica* (water spinach or 'kangkung'), and *Oenanthe javanica* ('selom'). This study aims to detect the presence of intestinal parasites in the contaminated raw vegetable specimens and to relate their occurrence with the dry and wet climate.

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EXPERIMENTAL

Sampling Collection

All vegetables were purchased randomly from two local wet markets in Pulau Pinang from December 2021 until March 2022. These two localities are situated in the Northeast and Southwest districts of Pulau Pinang, respectively. This sampling procedure was done once every four months for each market. After each sampling, the vegetables were processed immediately for sedimentation technique and microscopy examination. Five vegetable species were selected, such as viz. *Brassica oleracea* (cabbage), *Centella asiatica* ('pegaga'), *Daucus carota* (carrot), *Ipomea aquatica* (water spinach or 'kangkung'), and *Oenanthe* ('selom'). A total of 500-1000 g of each vegetable species was sampled throughout this study. The vegetable specimens were separated into different plastic bags according to their species to avoid cross-contamination. The specimens were returned to the School of Biological Sciences laboratory for sample processing using sedimentation technique and microscopy examination [8].

Sedimentation Technique

Collected vegetable specimens were cut and divided into eight (8) portions before being washed and soaked in 100 mL of distilled water in a 1000 mL transparent container for 5 minutes using a cleaned, sterilized knife and different gloves for each type of vegetable to avoid cross-contamination. Later, the container was shaken vigorously for 10 minutes. The water from the container was poured into a 20 mL centrifugal tube and centrifuged for 10 minutes at 4,000 rpm. The supernatant was removed, and the remaining sediment was kept at room temperature (30.9 - 32 °C) for parasites examination [8] within 48 hours.

Microscopy Examination

Two hundred slide specimens were prepared for microscopy examination, with 40 slides per vegetable specimen. Two types of wet smear slide preparation were used in this microscopy examination, i.e., using saline and Lugol's iodine. A standard wet smear using saline, also known as the unstained wet mount, was used to observe the presence of any motile protozoa (i.e., amoebae) or ova (eggs), while Lugol's iodine smear, which was also known as the stained wet mount was used to characterize the eggs (i.e., giving yellow-brown to brown color of the *Ascaris* ova). When added to the wet smear, the Lugol's iodine dye contrast will give a brown color to the ova (eggs) and clear transparent color for the rest of the slide sample. A few drops of the sediment from the centrifuge tube were placed onto a clean microscope glass slide. Then, it was covered with a coverslip to prevent the specimen slide from drying out while examining the slide and protecting the microscope lens. Light microscopy model OLYMPUS BX43, attached with the camera, was used for observation and qualitative identification of either helminthic eggs or protozoan cysts or oocysts. The slides were examined under 10x and 40x magnification [8].

SCIENCE LETTERS

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Data Analysis

All data obtained were calculated and analyzed with Statistical Product and Service Solutions (SPSS) v 27.0. *Z* scores were calculated to observe the mean positive samples of all prepared slides of the vegetable specimens.

RESULTS AND DISCUSSION

Table 1 shows the percentage of parasites found from the 200 slides examined on the selected vegetable species. All vegetable species were contaminated with at least one type of parasite. Of these, 'pegaga' gives the highest number of positive slides with parasites, 35 %, followed by 'selom' 17.5 %, cabbage, 10 %, carrot 5 %, and 'kangkung' 2.5 %, respectively. Thus, the total percentage of slides positive for parasites was 65.5%, with *Z* score calculations (mean ± SD) of 5.6 ± 5.22 for the positive samples with parasites. Similarly, 'pegaga' being the most contaminated vegetable was agreeable with a previous study by Yusof *et al.* [10]. These findings agreed with another researcher who reported that leafy-green vegetables were more likely to harbor intestinal parasites that can cause illnesses [11]. Besides, a report also showed that vegetables with uneven and more exposed surfaces were more likely to be contaminated because parasitic eggs, cysts, and oocysts attached more quickly to the leafy vegetables than those with smooth and small surface areas [9].

Table 1: The presence of parasites in the selected vegetables

Vegetable species	No. of slides prepared and examined	No. of slide positive with parasites	Slide positive with the parasite (%)	**Z* score for positive slide
Brassica oleracea (cabbage)	40	4	10	-0.31
Centella asiatica ('pegaga')	40	14	35	1.61
Daucus carota (carrot)	40	2	2	-0.69
Ipomoea aquatica ('kangkung'-water spinach*)*	40	1	1	-0.88
Oenanthe javanica ('selom')	40	7	17.5	0.27
TOTAL	200	28	65.5	

* mean Z score is 5.60 ± 5.22
minus Z score indicates observation is below the mean
positive Z score indicates observation is above the mean

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Table 2 and the pie chart in Figure 1 show the distribution of parasites in vegetable specimens and the percentage of the types of parasites obtained during the sampling months, respectively. As shown in the pie chart in Figure 1, *Ascaris* spp. was found to be the most common species of 70.59% with the approximately moderate distribution of 24 corticated/decorticated eggs on every selected vegetable in every month of the sampling collection period. On the other hand, ten other parasites were less commonly found, including unidentified hookworm species 17.65%, *Fasciolopsis* sp. 8.82%, and *Strongyloides* sp. 2.94%. There is no protozoan recovered from any of the vegetable specimens. *Ascaris* is the most common cause of worm infestation in humans due to its primary habitat in the human intestine. The eggs (infective stage) are passed in the feces of infected people. Thus, the frequent presence of *Ascaris* spp. eggs (Figure 2 (a) and (b) in contaminated vegetables could be likely due to the possible usage of human or pig feces as fertilizers [12].

Table 2. Distribution of parasites in selected vegetables from December 2021 until March 2022

Vegetable species	**December 2021**	**January 2022**	**February 2022**	**March 2022**
Brassica oleracea (cabbage)	1 free-living rhabditiform *Strongyloides* sp.	3 *Fasciolopsis* spp. eggs	-	-
Centella asiatica (‘pegaga’)	6 *Ascaris* spp. eggs 1 filariform unidentified hookworm	-	1 corticated *Ascaris* sp. egg 2 decorticated *Ascaris* spp. eggs 1 unidentified hookworm egg	2 decorticated *Ascaris* spp. eggs 2 unidentified hookworm eggs
Daucus carota (carrot)	-	2 corticated *Ascaris* spp. eggs	-	-
Ipomoea aquatica (water spinach or ‘kangkung’)	1 *Ascaris* sp. egg	-	-	-
Oenanthe javanica (‘selom’)	2 *Ascaris* spp. eggs 1 hookworm egg	1 corticated *Ascaris* sp. egg	6 decorticated *Ascaris lumbricoides* eggs	1 unidentified hookworm egg 1 decorticated *Ascaris* sp. egg
TOTAL	12	6	10	6

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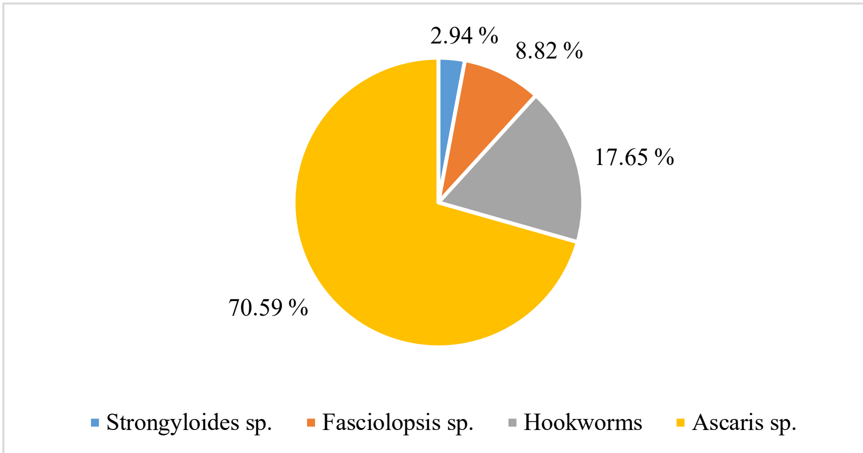

Figure 1: Percentage (%) of parasite species obtained in specimens

Nevertheless, contamination might also occur at any point along the chain, such as types of soil used during planting, the quality of water used for irrigation, harvesting, transportation, and hygienic practices during marketing [3]. In this study, there were an average of two types of parasites recovered from each vegetable sampled. For instance, rhabditiform *Fasciolopsis* sp. eggs (Figure 4) and rhabditiform larva of *Strongyloides* sp. (Figure 5) were from *cabbage.* Rhabditiform *Strongyloides* larva possesses a short buccal cavity [13] that can be distinguished microscopically from hookworms, as shown in Figure 3(A) and Figure 3(B).

Notably, the presence of *Fasciolopsis* eggs in January 2022 would be more likely due to the contaminated water from watering plant activities near sewers [14]. This finding agrees with a study by Achra *et al.* [15] in Bihar, India, where pigs and cows are reservoir hosts in the endemic area for this parasite. It is crucial to distinguish different types of helminthic ova and larvae, as every helminth has a different infective stage. For example, ova are the infective stage for *Ascaris lumbricoides* and *Trichuris trichiura*, filariform larvae for both *Strongyloides stercoralis* and hookworm (*Necator americanus* and *Ancylostoma duodenale*), and lastly metacercariae for *Fasciolopsis buski*.

During this study, December 2021 was the wettest month, while January 2022 was the transition between wet and dry months. Whereas February and March 2022 were considered dry months in which the average rainfall decreased compared to December 2021 and January 2022, as shown in Figure 6. In this study, the highest number of parasites (12) were found in December 2021, followed by February 2022 (10 parasites), January 2022 (6 parasites), and March 2022 (2 parasites). These results were contradictory with many other previous studies in which dry months give a higher number of parasites found in tested vegetables [10, 16]. Statistical analysis using Pearson's correlation shows no correlation between wet or dry months with the number of parasites obtained ($P>0.05$; $P=0.253$).

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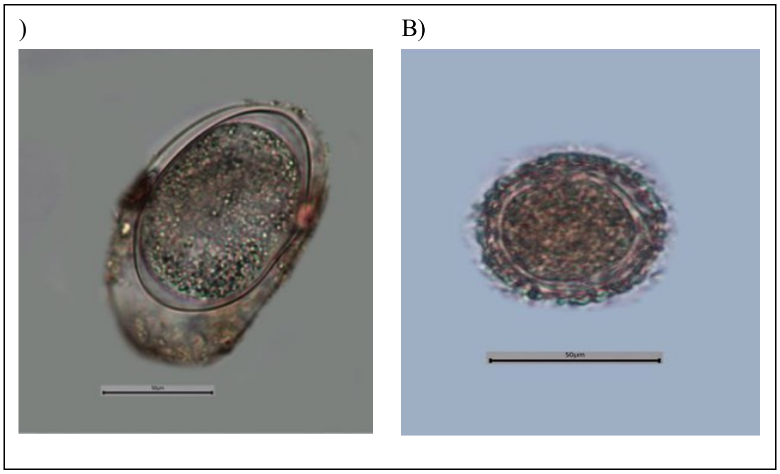

Figure 2: *Ascaris* sp. egg (A) decorticated (B) corticated

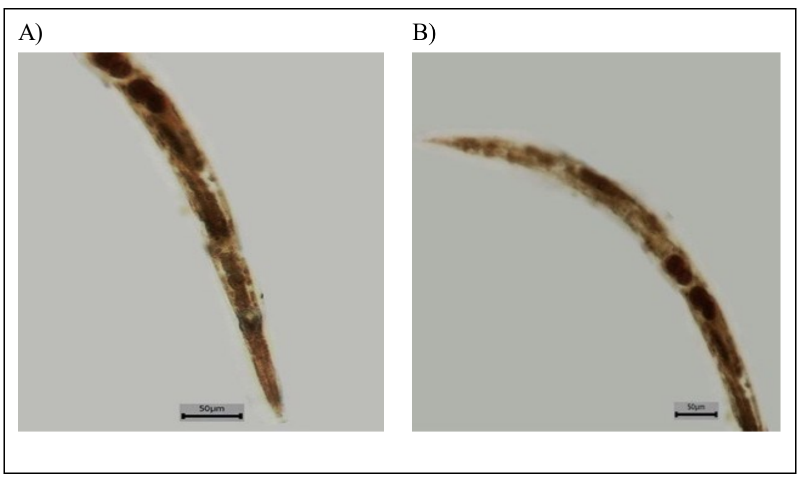

Figure 3: Hookworm larva (unidentified species) (A) anterior and (B) posterior

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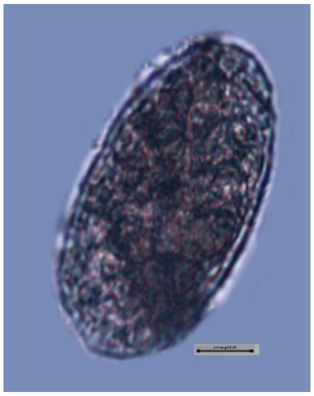

Figure 4: *Fasciolopsis* sp. egg

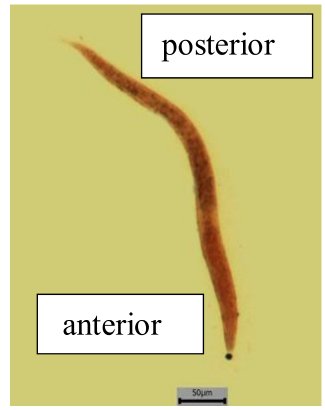

Figure 5: Rhabditiform *Strongyloides* sp.

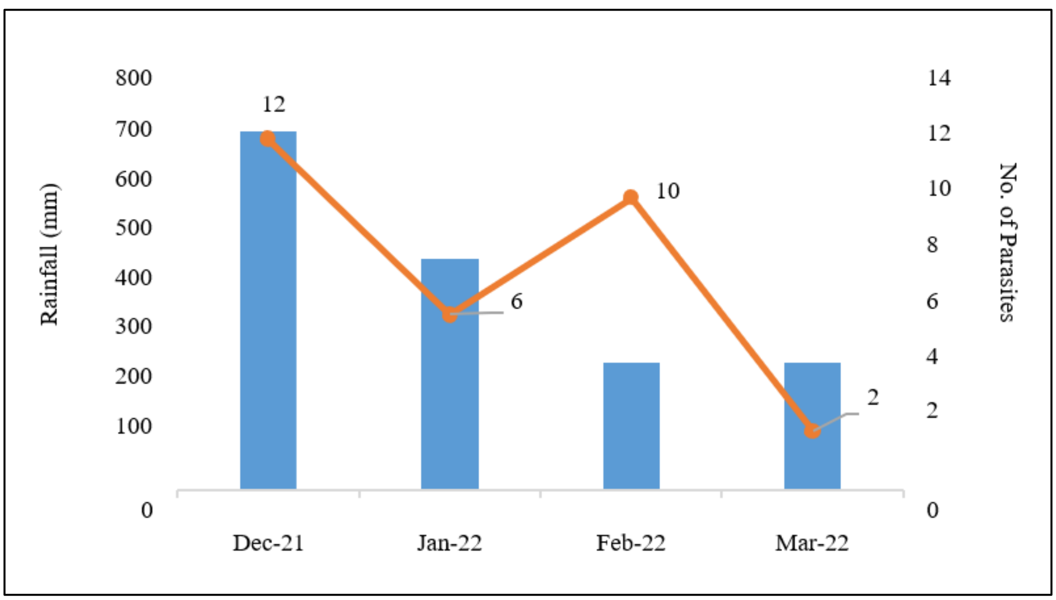

Figure 6: Average rainfall (mm) in a month and the number of parasites obtained

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CONCLUSION

In conclusion, the results from this study show that all five (5) selected vegetables from both wet markets were positive for intestinal parasites such as *Ascaris* sp. egg, *A. lumbricoides,* hookworm eggs, rhabditiform *Strongyloides* larva*, and Fasciolopsis* sp. eggs. *Ascaris* spp. eggs were the most abundant parasite found, and 'pegaga' was the most contaminated vegetable with the highest number of parasites obtained. These findings concluded that leafy-green vegetables with more surface area were more susceptible to parasite contamination. The correlation between rainfall and the number of parasites was insignificant, possibly due to the short study period and small sample size. Briefly, this study suggests that all vegetables from these wet markets were at high risk of contamination with various intestinal parasites. Among the preventive measures that the health authorities can take is implementing good hygiene habits (i.e., sanitation and personal hygiene, washing vegetables thoroughly, and health education) among the vendors and food handlers, especially those involved in the food servicing activity. Besides, periodical deworming, especially for children, must be strengthened. Health education is also essential to prevent re-infection and to improve sanitation further to prevent soil contamination with infective eggs or larvae. Creating awareness among these essential networking groups in the food industry would play a huge role in achieving this objective and reducing the burden of intestinal parasitic disease in Malaysia.

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AUTHOR'S CONTRIBUTION

Balkis Ballina Ukhra Yunus carried out the research and analyzed the results. Azlinda Abu Bakar conceptualized the central research idea, provided the theoretical framework, designed the research, supervised the research progress, funded the research, wrote the article, anchored the review, and approved the submission. Zeti Norfidiyati Salmuna reviews and revises the article.

CONFLICT OF INTEREST STATEMENT

The author(s) declare no conflict of interest.

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