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Occurrence of Intestinal Parasitic Contamination in Select Consumed Local Raw Vegetables and Fruits in Kuantan, Pahang

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Abstrak: Jangkitan parasit usus adalah salah satu penyakit manusia yang lazimnya menyebabkan masalah kesihatan yang serius dan mengakibatkan isu-isu ekonomi di negara-negara maju dan membangun. Sayur-sayuran mentah dan buah-buahan memainkan peranan yang penting sebagai medium jangkitan parasit ke dalam manusia. Oleh itu, tujuan kajian ini adalah untuk menyiasat pencemaran parasitologi daripada sayur-sayuran berdaun dan buah-buahan yang biasa dimakan serta dipilih penduduk tempatan di Kuantan, Malaysia. Sebanyak satu kilogram sayur-sayuran tempatan yang dimakan mentah dan buah-buahan telah dikumpulkan secara rawak dari pasar basah Kuantan (Pasar Tani) semasa musim tengkujuh (November 2014–Januari 2015) dan musim kering (Februari 2015–April 2015). Prosedur lazim "wet mount" dan teknik perwarnaan Ziehl-Neelsen yang dimodifikasi telah digunakan untuk mengesan parasit. Dalam kajian ini, pemeriksaan sayur-sayuran mendedahkan lima spesies parasite yang berbeza. Sampel sayur-sayuran yang diambil daripada pasar basah Kuantan adalah positif untuk kedua-dua helmin dan protozoa. Walau bagaimanapun, sampel buah-buahan adalah negatif untuk pencemaran parasit. Pegaga merupakan sayuran yang paling tercemar dalam kajian ini dan Strongyloides adalah parasit yang paling kerap ditemui. Tambahan pula, terdapat kepelbagaian yang tinggi dalam jenis parasit yang telah diperhatikan semasa musim kering berbanding musim tengkujuh. Oleh itu, tindakan lanjut perlu diambil untuk mengurangkan berlakunya pencemaran parasit didalam sayur-sayuran dengan melaksanakan prinsip-prinsip amalan pertanian yang baik serta keberkesanan rawatan air perlu dipertingkatkan.

Kata kunci: Buah-buahan, Sayur-sayuran, Parasit, Musim Tengkujuh, Musim Kering

Abstract: Intestinal parasitic infections are one of the most common causes of human diseases that result in serious health and economic issues in many developing and developed countries. Raw vegetables and fruits play an important role in transmitting

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parasites to humans. Hence, the aim of this study was to investigate the parasitological contamination of select commonly consumed local leafy vegetables and fruits in Kuantan, Malaysia. One kilogram of locally consumed raw vegetables and fruits were collected randomly from the Kuantan wet market (Pasar Tani) during the monsoon season (November 2014–January 2015) and the dry season (February 2015–April 2015). A standard wet mount procedure and modified Ziehl-Neelsen staining were used for the detection of parasites. In the present study, the examination of vegetables revealed five different parasite species. The vegetable samples collected from Kuantan's wet market were positive for both helminthes and protozoa. However, the fruits samples were negative for parasitic contamination. Pegaga was the most contaminated leafy vegetable in this study, and *Strongyloides* was the parasite found most frequently. Furthermore, there was a high diversity in the type of parasites observed during the dry season compared to the monsoon season. Therefore, further action should be taken to reduce the occurrence of parasitic contamination in vegetables by implementing the principles of good agricultural practice and improving water treatment efficacy.

Keywords: Fruits, Vegetables, Parasites, Monsoon Season, Dry Season

INTRODUCTION

Intestinal parasitic infections are considered to be one of the most common causes of disease in humans (Sehgal *et al.* 2010). It was reported that two billion individuals around the world are infected with pathogenic and nonpathogenic intestinal parasites (Gelaw *et al.* 2013). Protozoan parasites are capable of causing food-borne diseases, and some protozoan infections lead to serious health and economic issues in many developing and developed countries (Pepper *et al.* 2011). In the United States, 9.4 million food-borne illnesses are reported every year; of which, 0.2 million cases are caused by parasites (Scallan *et al.* 2011).

Many people regularly consume fresh vegetables. It has been reported that food-borne parasitic infections are associated with the consumption of contaminated fresh vegetables. In general, fruits and vegetables are considered to be vehicles that easily transmit parasites into individuals, especially when eaten raw or without peeling (Hassan *et al.* 2012). *Cryptosporidium, Cyclospora, Giardia, Entamoeba histolytica, Entamoeba coli,* and *Ascaris lumbricoides* are considered to be the most common parasitic contaminates of fruits and vegetables (Tefera *et al.* 2014). Vegetables and fruits become contaminated with different parasitic stages by means of three fundamental pathways, via the contaminated water used for irrigation or washing process, and through infected food handlers (Ishaku *et al.* 2013).

In developing countries, because of inadequate or even nonexistent systems for the routine diagnosis of food-borne pathogens, most disease outbreaks caused by contaminated vegetables go undetected, and the incidence of food-borne pathogens in food is underestimated (Dorny *et al.* 2009). High rates of intestinal parasite diseases have been reported in communities that eat raw vegetables, which indicates that the consumption of raw vegetables is an important route of intestinal parasite transmission (Abdalla *et al.* 2013).

Furthermore, consuming vegetables in a raw or slightly cooked form to protect or to preserve "heat-labile nutrients" may increase the risk of intestinal parasitic infections (Fallah *et al.* 2012).

Raw vegetables, also known as "ulam-ulaman" in Malay, are commonly consumed with rice. Because Malaysia is among the areas that have significant parasitic infections (WHO 2011), studies to identify the source of infections, methods of transmission, spread of such infections, and methods of prevention are all important. To date, only one study has investigated the occurrence of parasitic contamination in vegetables and fruits in Malaysia. Zeehaida *et al.* (2011) examined "cabbage, long beans, pegaga, carrot, water spinach, lettuce, cucumber, bean sprouts, daun kesum, ulam raja, apples, oranges, grapes, guava, rambutan and mangoes" in Kelantan, Malaysia. Their results revealed that none of the vegetables and fruits were contaminated with helminthes and protozoa except for pegaga, which was contaminated with *Strongyloides*.

Located on the east coast of Peninsula Malaysia, Kuantan is prone to flooding and is heavily influenced by the north-east monsoon wind, which brings the most rainfall during the monsoon season that runs from November until January (Malaysian Meteorological Department 2014). According to the World Health Organization (WHO 2011), the risk of parasitic infection is relatively low during floods unless there is a contaminated water source or significant population displacement. We investigated whether flooding affects parasite contamination in commonly consumed local raw leafy vegetables and fruits in Kuantan, Pahang, Malaysia. Therefore, our study is the first to address the parasitic contamination of select commonly consumed local raw leafy vegetables and fruits distributed in markets of Kuantan, Malaysia with seasonal variation (monsoon seasons and dry seasons).

MATERIALS AND METHODS

Study Area

The present study was carried out in Kuantan, Pahang. Kuantan is located on the East Coast of Peninsular Malaysia at a latitude of 3° 49' 0" North and a longitude of 102° 20' 0" East, with an estimated population of 607,778. Kuantan features a tropical rainforest climate with two seasons each year, monsoon season from November to January, with the most rainfall and dry season represented by the rest of the year. The climatic data were obtained from the Malaysian Meteorology Department (2014).

Sample Collection and Analysis

One kilogram of each of the following vegetables and fruits was collected monthly from November 2014 until April 2015 from the Federal Agriculture Marketing Authority markets (Pasar Tani) in Kuantan, Pahang: *Centella asiatica* (pennywort or pegaga), *Ipomoea aquatic* (water spinach or kangkung), *Persicaria odorata* (Vietnamese coriander or daun kesum), *Averrhoa carambola* (carambola or

belimbing), *Syzygium samarangense* (rose apple or jambu air) and *Psidium guajava* (guava or jambu batu). To successfully extract parasitic ova, larvae, and cysts from the random samples, each type of vegetable and fruit was rinsed and soaked with 100 mL of distilled water in a plastic container for 15 minutes (Zeehaida *et al.* 2011). After this, a sterile strainer was used to filter the suspension, and the filtrate was centrifuged at 1,500 rpm for 10 minutes. The supernatant was discarded, and the remaining sediment was transferred to glass slides for microscopic observation (Ebrahimzadeh *et al.* 2013).

Detection of Parasites

For wet smear preparation, a drop of the sediment was taken from the centrifuge tube and placed on a clean microscope slide. A clean cover slip was placed gently to avoid air bubbles and over flooding. The wet smear was observed under a light microscope using 10x and 40x magnification for the qualitative detection of helminth eggs and protozoan cysts or oocysts (El Said Said 2012). Modified Ziehl-Neelsen stained smears were prepared for the detection of *Cryptosporidium* and *Cyclospora* spp. oocysts (El Said Said 2012). The Ziehl-Neelsen stain was modified by replacing sulphuric acid with acid-alcohol decolourising agent (3% hydrochloric acid in 95% ethanol) and using Malachite green stain as a counterstain to provide differential staining for coccidian parasites such as *Cryptosporidium* oocysts as described by Casemore *et al.* (1985).

RESULTS

The results in Table 1 show the occurrence of helminthes and protozoan parasites on pegaga, kangkung, daun kesum, belimbing, jambu air, and jambu batu collected during the monsoon season (November 2014-January 2015) and the dry season (February 2015-April 2015). Trematodes (unidentified flukes) were detected in daun kesum samples collected during monsoon season. One nematode larva (Strongyloides) and one trematode (unidentified fluke) were detected in the water sample used to wash the pegaga collected in January. Neither helminthes nor protozoan parasites were detected in the kangkung, belimbing, jambu air, and jambu batu samples during the monsoon season. Moreover, Strongyloides larvae, cysts of Entamoeba spp., cysts of Blastocystis spp., and unidentified flukes were detected in pegaga samples collected during the dry season. Unidentified flukes were detected in daun kesum samples collected during February and March. On the other hand, the kangkung and the fruits samples were microscopically negative for the presence of helminthes and protozoan parasites. Only pegaga samples were positive for both helminthes and protozoa. Helminth parasites detected on pegaga were larvae of Strongyloides, eggs of Diphyllobothrium, and unidentified trematodes (flukes). Cysts of the Protozoan parasites *Entamoeba* and *Blastocystis* spp. were detected on pegaga.

Sample		Monsoon season	on		Dry season	
	November	December	January	February	March	April
	Negative	Negative	Unidentified fluke	Strongyloides	Entamoeba spp.	Entamoeba spp.
Pegaga			Strongyloides larva	larva	Blastocystis spp. Strongyloides larva Diphyllobothrium egg Unidentified fluke	Strongyloides larva
Kangkung	Negative	Negative	Negative	Negative	Negative	Negative
Daun kesum	Unidentified fluke	Unidentified fluke	Unidentified fluke	Unidentified fluke	Unidentified fluke	Negative
Belimbing	Negative	Negative	Negative	Negative	Negative	Negative
Jambu air	Negative	Negative	Negative	Negative	Negative	Negative
Jambu batu	Negative	Negative	Negative	Negative	Negative	Negative

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DISCUSSION

In the present work, three types of raw vegetables (pegaga, kangkung, daun kesum) and three types of fruits (belimbing, jambu air, jambu batu) that are commonly consumed among the population in Kuantan were examined. The five types of parasites reported in this study were primarily recovered from pegaga. *Strongyloides* rhabdatiform larvae were the most frequently identified parasite extracted from pegaga. *Strongyloides* rhabdatiform larvae of other hookworms by their very short buccal cavities (McPherson & Pincus 2016). Our finding was supported by Zeehaida *et al.* (2011), who reported the detection of *Strongyloides stercoralis* larvae in pegaga, kesum, and kangkung vegetables consumed in Kota Bharu, Kelantan, Malaysia, with pegaga being the most contaminated sample (152 larvae), followed by kangkung with 16 larvae.

This might be because the Strongyloides has a free-living state that does not require a host for its proliferation, in addition to its parasitic mode of life (Tefera et al. 2014). This result was in agreement with studies performed in Kota Bharu, Kelantan, Malaysia (Zeehaida et al. 2011), in Jimma Town, Southwest Ethiopia (Tefera et al. 2014), and in Kogi, Nigeria (Omowaye & Audu 2012). However, this finding is in contrast with studies performed in Alexandria, Egypt, and in Shahrekord, Iran, where Cryptosporidium spp. oocysts and Ascaris lumbricoides eggs were the most frequently isolated parasites in vegetables (Fallah et al. 2012). In our study, unidentified trematodes (flukes) were also detected on vegetable samples, with daun kesum being the most contaminated vegetable throughout the period of the study, which is similar to a study performed by Nadia (2014) who reported the presence of unidentified trematodes on the vegetables collected from the local markets around Bau campus, Bangladesh. The detection of unidentified flukes in daun kesum during almost all of the months of our study could be attributed to the equatorial climate of Malaysia that is characterised by high temperatures. Temperature has a strong influence on the emergence, survival, and infectivity of trematodes (Poulin 2006). Increases in environmental temperatures lead to a distinct increase in trematodes emergence (Mas-Coma et al. 2009). Diphyllobothrium eggs were also recovered from the vegetable samples examined in our study. This finding is in agreement with a study carried out in Seoul, Korea, that reported the detection of Diphyllobothrium eggs in Chinese cabbage (Park & Kim 1975).

In the present study, protozoan parasites recovered from the vegetables included *Entamoeba* spp. and *Blastocystis* spp. cysts. This finding is similar to results reported by Dabiri *et al.* (2014) and Ebrahimzadeh *et al.* (2013). The presence of the *Entamoeba* spp. in the vegetables samples could be due to inappropriate agricultural practices during cultivation, with cultivated vegetables coming into direct contact with soil and water that is contaminated with human and animal faeces (Silva *et al.* 2014).

In contrast there were no helminth ova or protozoa found on the fruits samples examined in this study. However, our result seems to differ from the results of previous studies performed by Hassan *et al.* (2013) and Alli *et al.* (2011), who reported the presence of intestinal protozoa and helminthes on the

fruit samples collected from a wet market in Nigeria. Variation in the detection of parasites on fruit samples between our studies and these previous studies could be due to differences in the geographical location and the type of fruit examined.

Variation in the occurrence of parasitic contamination among the different vegetable and fruit samples examined in this study could arise from differences in plant shape and surface area. Green leafy vegetables such as pegaga have a broad uneven surface that provides a large surface that allows parasitic eggs and cysts to easily attach to the vegetable. On the other hand, vegetables with long narrow leaves such as kangkung and fruits with smooth leathery surfaces like belimbing have the lowest occurrence of parasitic contamination because their narrow or smooth surfaces reduce the rate of parasitic attachment (Adamu *et al.* 2012; El Said Said 2012). The occurrence of more than one parasite type per sample in this study reflects the possibility of the multi-faecal contamination of vegetables, which could lead to multiple parasitic infections in humans. It might also indicate the persistence of intestinal parasitic infection in the area (Tefera *et al.* 2014).

For seasonal variation, five different species of parasites were found during the monsoon and dry seasons with two different parasites recorded during the monsoon season, and six different parasites recorded during the dry season. These results clearly indicate that the monsoon season has a low diversity of parasites compared with the dry season. Our findings are consistent with previous studies that reported higher rates of parasitic contamination and diversity during the dry season than during the monsoon season (Monge et al. 1996; Uga et al. 2009). Studies performed in Alexandria and Benha, Egypt, showed higher rates of parasitic diversity and contamination of raw vegetables during the warm season than during the cold season (El Said Said 2012; Eraky et al. 2014). The low diversity of parasites during the monsoon season could be explained by the parasitic eggs on the surface of the vegetables being washed away by the rain as assumed by Uga et al. (2009). S. stercoralis and flukes were found in both seasons, and this could be due to their environmental tolerance and resistance (Abagale et al. 2013). The high diversity of parasites during the dry season could be due to the high rate of parasitic excretion to the environment by humans or animals during the warm season (El Said Said 2012).

The contamination of vegetables and fruits occurs during either the harvesting stage, due to the absence of clean facilities for workers and dirty storage areas, or the post-harvest treatment, including during storage and transportation (Maqbool *et al.* 2014). Moreover, the contamination with parasitic pathogens could also be due to contact between the vegetables and contaminated soil or irrigation with contaminated water (Silva *et al.* 2014).

The habit of eating raw vegetables like pegaga and kangkung is commonly practiced in the study area. Hence, the findings of the present study may have implications for global food safety and public health. These findings highlighting the role raw vegetables may serve as a medium for transmitting parasites to humans in Kuantan, Pahang.

CONCLUSION

We found pathogenic helminth and protozoan parasites in the vegetables sold in Kuantan wet markets. Pegaga was the most contaminated leafy vegetables in this study, and *Strongyloides* was the most frequently isolated parasite. Furthermore, there was a high diversity in the type of parasites observed during the dry season compared to the monsoon season. Therefore, further action should be taken to reduce the occurrence of parasitic contamination in vegetables by implementing the principles of good agriculture practices and improving water treatment efficacy. The local health and environmental authorities should apply appropriate intervention methods to control the transmission of parasitic diseases through contaminated fruits and vegetables. These intervention methods include, improving sanitation in the areas where vegetables are grown and processed, implementing the proper treatment of wastewater used for irrigation, improving the hygiene of vegetable vendors and the cleanliness of markets, as well as increasing the consumers' awareness of the negative health effects of intestinal parasites especially those due to the improper washing of vegetables before consumption.

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