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Investigation of *Cryptosporidium* Oocysts in Vegetables Collected from Rural Market in Selangor, Malaysia

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ABSTRACT

Cryptosporidiosis is a zoonotic protozoan parasitic disease caused by coccidial species of the genus *Cryptosporidium*. Oocysts from this parasite can be found and transmitted to human via contaminated raw vegetables, fruits and water. Hence, this study was carried out to investigate the presence of *Cryptosporidium* oocysts in vegetables collected from the rural market in Selangor, Malaysia. The study was carried out from May (dry season) until October 2017 (wet season). Approximately 250 grams of fresh leafy vegetables such as Centella (*Centella Asiatica*), water spinach (*Ipomoea aquatica*), celery (*Apium graveolens*), spring onion (*Allium fistulosum*) and Vietnamese coriander (*Persicaria odorata*) were collected separately and investigated for *Cryptosporidium* oocysts using the microscopic technique. Among all of the collected vegetables, water spinach (*Ipomoea aquatica*) showed the presence of *Cryptosporidium* oocysts during the wet season. Findings of this study depict the possibilities of this vegetable in the transmission of cryptosporidiosis among the consumers. Future study is needed to explore more vegetables from rural markets in Selangor to detect the presence of *Cryptosporidium* parasites as a public health safety precaution.

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INTRODUCTION

Vegetables are an essential part of a healthy human diet due to its nutritional value. Some vegetables such as salad are eaten raw to retain the natural taste and to preserve heat-labile nutrients. However, this poses the rick of contamination with enteric bacterial, viral and parasitic pathogens due to unhygienic irrigated water throughout the process from planting to consumption (Alhabbal, 2015).

Cryptosporidiosis is a diarrheal disease caused by *Cryptosporidium* protozoa that infects a wide range of vertebrate host including mammals, rodents, birds, reptiles and fish. In human, cryptosporidiosis causes mild to severe diarrhea, and it may be non-resolving among the immune-compromised individuals. The oocyte of *Cryptosporidium* (4.5-5.5µm in size) and is transmitted by the faecal-oral route, mainly through contaminated water, food or even animals to human contact (Smith et al., 2007).

The previous study carried out by Zeehaida et al. (2011) revealed the presence of *Strongyloides* larvae in Centella, Vietnamese coriander and water spinach from Kelantan, Malaysia. Another study by Afzan et al. (2017) in Kuantan, Pahang, Malaysia also showed the presence of *Strongyloides* larvae alongside with other common protozoa contaminating typical vegetables. Presence of these parasites is medically significant as they can be directly

transmitted to human via the consumption of fresh vegetables, causing foodborne diseases (Zeehaida et al., 2011). Since there are no previous reports on *Cryptosporidium* among Malaysian vegetables, this study was carried out to address this issue among different vegetable samples collected from the rural market in Selangor, Malaysia.

MATERIAL AND METHODS

Study Area

This study was conducted in the rural area of Hulu Langat, Selangor (2.9936° N, 101.7892°). The Hulu Langat District is located in the Southeastern corner of Selangor, between Kuala Lumpur and Negeri Sembilan with a population around 1.068 million. The samplings were carried out in several local markets located nearby indigenous villages in Hulu Langat, Selangor.

Collection of Vegetables and Parasite Identification

The study was carried out from May (dry season) till October 2017 (wet season). Several leafy vegetables like Centella (*Centella Asiatica*), water spinach (*Ipomoea aquatic*), celery (*Apium graveolens*), spring onion (*Allium fistulosum*), and Vietnamese coriander (*Persicaria odorata*) weighing approximately 250 grams were collected separately in a clean plastic zipper bag. According to the sellers, all of the vegetables were obtained directly from the farm.

For the isolation of parasites, the vegetables were washed by vigorous shaking with 0.95% normal saline. The samples were put through the sonication (water bath, 30 minutes) and subsequently, formalin ethylene sedimentation technique was used to isolate the parasite (Robertson et al., 2001). Firstly, the wash was filtered through wetted cheesecloth-type gauze placed using a disposable paper funnel into a 15ml conical centrifuge tube. Next, 10% formalin was added into the solution to bring the total volume to 15ml. The solution was centrifuged at 500 x*g* for 10 minutes and the supernatant was decanted. Approximately 10ml of 10% formalin was added to the sediment and mixed thoroughly with wooden applicator sticks. Next, 4ml of ethyl acetate was then added and shaken vigorously in an inverted position for 30 seconds. The solution was centrifuged again at 500 x*g* for 10 minutes and plug of debris was removed from the top of the tube and discarded with an applicator stick. The last layer containing parasites was added with several drops of 10% formalin prior to staining with modified Ziehl-Neelsen acid-fast stain. The slides were examined under oil immersion lens (100 x magnifications) using light microscopy.

RESULTS

Table 1 shows the overall results obtained from in this study. Among all of the vegetables inspected for *Cryptosporidium*, only water spinach was detected with the oocysts during wet seasons, between September and October (Table 1). Positive oocysts for *Cryptosporidium* appeared red to pink in colour measuring between 4.5 to 5.5µm in length (Figure 1).

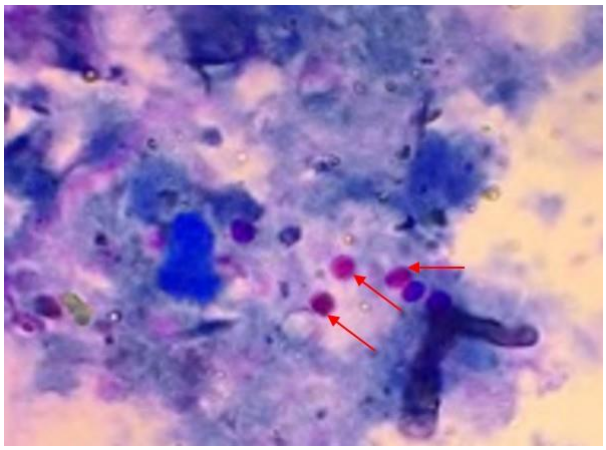

Figure 1: *Cryptosporidium* oocysts from "Water spinach" Red arrow indicate *Cryptosporidium* oocysts (→)

DISCUSSION

In this study, five types of vegetables that are commonly consumed by local Malaysians in Hulu Langat, Selangor were investigated for the presence of *Cryptosporidium* oocysts using sedimentation technique and by acid-fast staining. The presence of *Cryptosporidium* oocysts was detected in water spinach (*Ipomoea aquatica*) during the wet season. Presence of this parasite clearly indicated there was environmental contamination, most probably from the dirty water used in rinsing the vegetables prior to selling. This finding indicates the risk of intestinal parasitic infection among the community living in Hulu Langat areas.

Previous studies carried out in Malaysia indicated the presence of *Strongyloides stercoralis*, *Blastocyctis* spp., *Entamoeba* spp. and *Diphyllobothrium* eggs in commonly consumed raw vegetables in Kelantan and Pahang, Malaysia. Furthermore, in contrast to our study, the study from Pahang observed the parasites during the dry season and *Cryptosporidium* oocysts was not reported (Zeehaida et al., 2011; Afzan et al., 2017).

Cryptosporidium is ubiquitous in nature and water could be the main source of transmission of this oocyst. Similar to our study, Maikai et al. (2013) reported the presence of *Cryptosporidium* oocysts in

Table 1: The occurrence of *Cryptosporidium* oocysts on selected vegetables during the dry and wet season at a rural market in Selangor

Samples	Dry season			Wet season		
	May	June	July	August	September	October
Water spinach	Negative	Negative	Negative	Negative	*Positive*	*Positive*
Centella	Negative	Negative	Negative	Negative	Negative	Negative
Spring onion	Negative	Negative	Negative	Negative	Negative	Negative
Celery	Negative	Negative	Negative	Negative	Negative	Negative
Vietnamese coriander	Negative	Negative	Negative	Negative	Negative	Negative

common leafy vegetables like spinach, lettuce and cabbage from local markets in Kaduna State, Nigeria. They suggested that contaminated water used for irrigation, accidental entry of animal or human faeces from a nearby farm or human sewage could contribute to this parasite contamination (Maikai et al., 2013). We found that the water spinach sold in Hulu Langat market, are grown in a nearby land at an indigenous community village. Since there is lack of latrine facility in this village, we assume that the contaminated soil and water could have transmitted this parasite and trap them in the vegetable roots and washed out during monsoon season. Contaminated soil can play an important role in *Cryptosporidium* transmission during cultivation of water spinach (Fallah et al., 2012).

As compared to other studies that used floatation techniques to isolate the parasite, formalin ethylene sedimentation technique worked the best in our study. We found the presence of tiny soil debris increased the artefact interference during floatation technique. Therefore, all our work was standardized with a sedimentation method and stained with common acid-fast stain for *Cryptosporidium* oocysts detection.

CONCLUSION

The present study indicated contamination of water spinach with *Cryptosporidium* oocysts in Hulu Langat area of Selangor, Malaysia. We suggest that the contaminated soil and water from a nearby indigenous village could have transmitted these parasites which pose great public health concerns. We propose disinfection techniques and proper washing mechanism of the vegetables prior to selling to reduce vegetable contamination. Further study is needed to investigate the water and soil used to cultivate the water spinach for the presence of *Cryptosporidium*.

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