Tropical Biomedicine 28(1): 188–193 (2011)

Research Note

Strongyloides stercoralis in common vegetables and herbs in Kota Bharu, Kelantan, Malaysia

Zeehaida, M.[1*], Zairi, N.Z.[1], Rahmah, N.[2], Maimunah, A.[2] and Madihah, B.[2]
[1] Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia;
[2] Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Penang, Malaysia
* Corresponding author email: zeehaida@kck.usm.my
Received 4 October 2010; received in revised form 24 December 2010; accepted 26 December 2010

Abstract. Transmission of soil-transmitted helminthes infection is by faecal oral route, and is influenced by food preference. Kelantanese love to consume *ulam* which are raw vegetables and herbs. Some of the herbs grow on grounds with high humidity and are abundant near drainage areas, these are also places with higher likelihood of harbouring viable parasite ova. The aim of this study was to determine the prevalence of soil-transmitted helminthes in vegetables, herbs and fruits found in our local setting. The results by microscopy showed that there was no helminthes ovum or protozoan parasite in the samples. However, *Strongyloides stercoralis* rhabdatiform larvae were identified in water samples used to wash pegaga, *kesum* and water spinach, and the number of larvae observed were 152, 9 and 16 respectively. Analysis by real-time PCR confirmed the microscopic observation of this helminth. This study highlighted that vegetables and herbs are likely sources of *Strongyloides stercoralis* infection in Kota Bharu, Kelantan. Thus vegetable sellers as well as the food handlers are the two important groups who are at high risk of acquiring the infection.

INTRODUCTION

Soil transmitted helminthes is still prevalent in developing counties. In Malaysia, a community study on the intensity and frequency distribution of *Ascris lumbricoides*, *Trichuris trichiura* and hookworm among Orang Asli children aged 1-13 years, showed that the overall prevalences of *A. lumbricoides*, *T. trichiura* and hookworm were 62.9%, 91.7% and 28.8%, respectively. (Norhayati *et al.*, 1997) as compared to Vietnam (44.4%, 23.1% and 28.6% respectively) (van der Hoek *et al.*, 2003).

Kota Bharu is the capital of the Kelantan, situated in the East cost of Peninsular Malaysia with a population of 1,560,5000 (Malaysian Population Census report, 2007). The common foods in Kelantan are *nasi kerabu*. *nasi ayam*, *laksa*, burger and *nasi lemak*, all of which contain raw vegetables such as lettuce, cucumber, *daun kesum* (Vietnames mint), cabbage, long bean, and bean sprouts. In addition, Kelantanese also love to consume *ulam* which are raw vegetables and herbs. Viable parasites such as soil-transmitted helminthes and protozoa may be found on these food items, and thus can be transmitted to human. Some of the herbs grow on grounds with high humidity and are abundant near drainage areas, these are also places with higher chances of

harbouring viable parasite ova. It is common practice in this area to eat local fruits without peeling the skin, thus another mode of parasite transmission may be by consumption of fresh contaminated fruits. Thus by faecal-oral route, diseases related to nematodes and protozoa infections are suspected to be prevalent in this area. The objective of this pilot study was to determine the parasite contaminating common vegetables, herbs and fruits consumed by people in Kota Bharu, Kelantan, Malaysia.

One kilogram of each of the following vegetables, herbs and fruits were purchased from the central wet market in Kota Bharu, Kelantan namely cabbage, long beans, *pegaga* (*Centella asiatica*), carrot, water spinach (*kangkung*), lettuce, cucumber, bean sprouts, *daun kesum*, *ulam raja*, apples, oranges, grapes, guava, rambutan and mangoes. Briefly, each kind of vegetable, herb or fruit was mixed well with 100 ml of distilled water. Then the water used for the washings was separated, divided into two portions (~50 ml each) and centrifuged at 1500 rpm for 10 minutes. The sediment was then mounted on glass slide and examined by light microscope (Nikon YS 100). Subsequently, real-time PCR was performed on the remainder of two sediment samples from the *pegaga*-washed water samples.

There was no helminthes ova or protozoan parasite found in the samples. However, *Strongyloides stercoralis* rhabdatiform larvae were identified in water samples used to wash pegaga, *kesum* and water spinach, and the number of larvae observed were 152, 9 and 16 respectively. Microscopic examination showed motile larva measuring about 390µm in length, 15 um in width, with short buccal cavity, presence of esophagus bulb, and the esophagus was short compared to the intestine (1:3), however the genital premodium was not well-visible.

DNA from the *pegaga*-washed water samples was extracted using the QIAamp Mini kit (Qiagen, Hilden, Germany) with minor modifications (Verweij *et al.*, 2001). In brief, approximately 200 µl of each sediment samples was suspended in 200 µL phosphate buffered saline (pH 7.2) containing 2% polyvinylpolypyrolidone (PVPP; Sigma, Steinheim, Germany). After heating for 10 minutes at 100°C, the suspension was treated with ATL buffer containing proteinase K for 3 hours at 55°C followed by DNA extraction. Phocine herpesvirus 1 (PhHV-1) at 10^3 PFU/mL was added to the AL lysis buffer as a control for the extraction process. It also serves as a control for the subsequent PCR reaction.

For each sample, a volume of 25 µL PCR mixture was prepared using PCR buffer (HotStar Taq master mix; Qiagen, Hilden, Germany), 5 mM $MgCl_2$ (MBI Fermentas Inc., Amherst, NY), 0.1 mg/mL bovine serum albumin (Sigma Aldrich Corp., St. Louis, MO), 5 µL of template DNA and varying amounts of primers according to the species and 250 nM of each probe. The primer concentrations were as follows: 40 nM for each *Ancylostoma duodenale* and *Ascaris lumbricoides*, 60 nM for *Strongyloides stercoralis* primers and 80 nM for each *Necator americanus* and PhHV-1. The primers and probes used for detection of *A. lumbricoides*, *N. americanus*, *A. duodenale* and *S. stercoralis* were as previously reported, except for some differences in the probe fluorophores (Wiria *et al.*, 2010). These primers and probes had previously been tested against DNA samples of a wide range of intestinal microorganisms and shown to be 100% specific (Verweij *et al.*, 2007, Verweij *et al.*, 2009).

The PCR amplification program comprised 15 minutes at 95°C, followed by 50 cycles of 9 seconds at 95°C and 60 seconds at 60°C using Rotor Gene 6000 (Rotorgene-Q, Hilden, Germany). Fluorescence was measured during the annealing step of each cycle at 555 nm, 510 nm, 610 nm, 710 nm, and 660 nm for *A. duodenale*, *N. americanus*, *A. lumbricoides*, *S. stercoralis*, and PhHV-1 respectively. Several kinds of controls were included in each PCR run namely PCR mixture without DNA template; DNA samples extracted from each helminth, and PhHV-1 DNA. The result was considered as

negative when the Ct value was more than 40 or when no amplification curve was obtained.

Real-time PCR on the two sediments from the pegaga-washed water samples using primers to detect four species of helminthes showed amplification for only *S. stercoralis* DNA, with Ct values of 35.32 and 34.81. Figure 1a & b showed the log and linear amplification plots respectively. Thus this confirmed the microscopic observation of strongyloides larvae from the pegaga samples.

The most important food borne parasite that has been implicated to be acquired by consumption of plant materials include *Giardia* spp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Fasciola* spp., *Fasciolopsis buski*, *Echinococcus granulosus* and *Echinococcus multilocularis* (Dawson, 2005; Dorny *et al.*, 2009). Washing solutions that has been used to isolate parasites from the plant materials include water (Ogbolu *et al.*, 2009), tris-buffered-saline (TBS) (Al-Binali *et al.*, 2006) and the cationic detergent, hyamine solution (Hyamin 1622 Fass Co) containing glass powder suspension in water (Damen *et al.*, 2007). Al-Binali *et al.* (2006) reported that the use of TBS significantly increased the parasite isolation rate (27.2%) compared

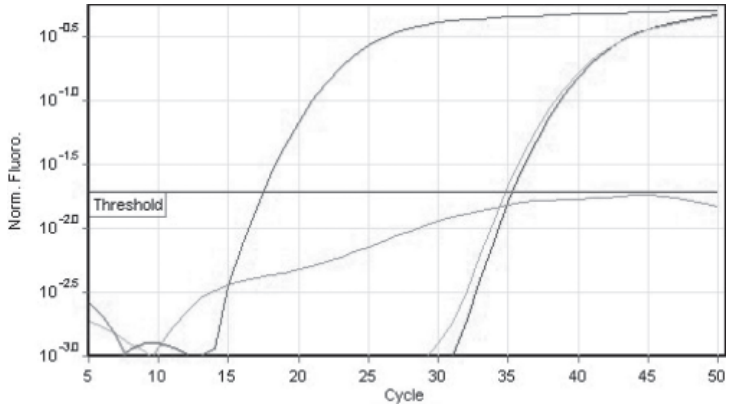
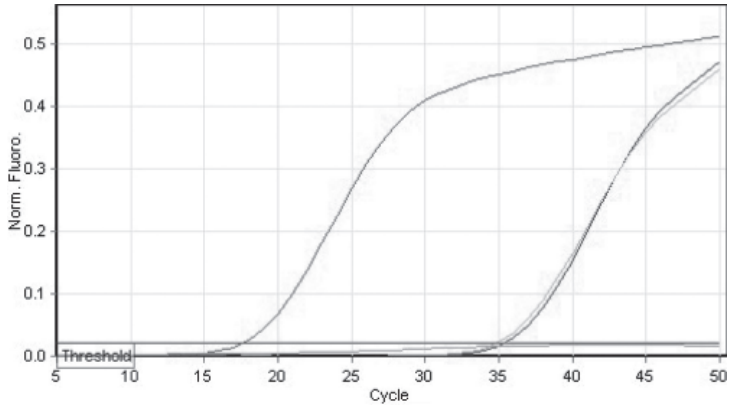


Figure 1a



Colour	Sample	Ct value
	Pegaga sediment 1	35.32
	Pegaga sediment 2	34.81
	Positive control	17.51
	No template control	-

Figure 1b

Log (Fig. 1a) and linear (Fig. 1b) amplification plots for detection of *Strongyloides stercoralis* in *pegaga*-washed water samples by real-time PCR

with tap water (7.8%) ($z=4.72$, $p<0.001$). Concentrations of the eggs were performed by magnesium sulphate floatation technique (Ogbolu *et al.*, 2009; Damen *et al.*, 2007) and centrifugation of the washing solution (Al-Binali *et al.*, 2006).

Based on the ecology of this area, we had predicted that helminthes ova would be prevalent in the samples collected. Thus it was rather unexpected that there was no detection of helminthes ova or protozoan cyst, and instead, numerous live *S. stercoralis* larva were observed on the vegetables and herbs. The larvae probably originated from the free-living life cycle of *S. stercoralis* in the humid areas which were used to cultivate these vegetables and herbs. The finding strengthened our suspicion that strongyloides is present in this area.

Strongyloides stercoralis is an intestinal nematode of humans. It is estimated that tens of millions of persons are infected worldwide, although no precise estimate is available. It is often considered a disease of tropical and subtropical areas and most infected individuals are asymptomatic (Keiser & Nutman, 2004). Several factors increase the risk for acquiring *Strongyloides* infection, such as low socioeconomic status, alcoholism, male gender and occupations like farming and coal mining (Keiser & Nutman, 2004). The prevalence of strongyloidiaisis differs among ethnic groups which may reflect behavioral or socioeconomic factors (Walzer *et al.*, 1982). In the Peruvian Amazon region, stool and serosurvey for *S. stercoralis* showed 8.7% and 72.0% positivity respectively (Yori *et al.*, 2006). The prevalence of infection among farm workers in a tourist region in Spain based on the detection of larvae of triple stool samples was 12.4% (Roman-Sanchez *et al.*, 2003). In Thailand, by stool examination and serologic tests, 11% had *S. stercoralis* in stool and 45% were seropositive (Douce *et al.*, 1987). In Malaysia, not much data on strongyloidiasis is available. The earliest prevalence of strongyloidiasis among fishermen community in Penang was reported to be 1.15% (Khairul *et al.*, 1978). In another study, *Strongyloides* larvae were detected in 7.1% of soil samples in the rural area of Kuala Lipis in the state of Pahang and urban area of Kuala Lumpur (Noor Azian *et al.*, 2008). A case report on hyperinfection of *S. stercoralis* in a diabetic patient with history of immunosuppression due to steroid and azathioprine therapy has recently been reported at HUSM (Azira & Zeehaida, 2010).

HUSM records in recent years did not document many cases of strongyloidiasis in this state. This could be due to under-detection due to the non-sensitivity of microscopic examination, and under-reporting among symptomatic cases. Other reasons could be due to asymptomatic presentation of the infected persons or non-specific symptoms among the chronically infected individual such as diarrhoea, asthma-like symptoms, nephrotic syndrome or dermatologic manifestation like urticarial rash (Keiser & Nutman, 2004). Thus more studies should be performed to map the infection in Kelantan. Investigation of this infection among chronically infected individuals with relevant symptoms should also be carried out. In addition, detection of *Strongyloides* infection should be performed in high risk groups such as immunosupressed cancer patients or patients receiving steroid therapy, in order to prevent fatal complication of strongyloidisis hyperinfection.

This study highlighted that vegetables and herbs are likely sources of *S. stercoralis* infection in Kota Bharu, Kelantan. Thus vegetable sellers as well as the food handlers are the two important groups who are at high risk of acquiring the infection. In view of the local preference for eating raw vegetables and herbs, the community members are probably constantly exposed to the transmission of the larva in their food chain, thus are at risk of acquiring strogyloidiasis. Thus, proper washing and cooking of vegetables should be stressed. A similar larger scale study and rigorous stool and serological screening of suspected or at-risk patients

should be performed to determine the actual prevalence of *Strongyloides* infection in Kota Bharu, Kelantan, Malaysia.

Acknowledgements. Funding for real-time PCR was provided by the research grant from European Commission grant (COINFECT, INCO-CT-2006-031714.). The fifth author received financial support from USM fellowship. We would like to acknowledge the technical assistance provided by Ms. Suhaila Che Musa, Ms. Fadilah Hussin and Ms. Syarifah Nadwah Md Yunus.

REFERENCES

Al-Binali, A.M., Bello, C.S., El-Shewy, K. & Abdulla, S.E. (2006). The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. *Saudi Medical Journal* **27**(5): 613–616.

Azira, N.M.S. & Zeehaida, M. (2010). *Strongyloides stercoralis* hyper-infection in a diabetic patient: Case report. *Tropical Biomedicine* **27**(1): 115–119.

Damen, J.G., Banwat, E.B., Egah, D.Z. & Allanana, J.A. (2007). Parasitic contamination of vegetables in Jos, Nigeria. *Annals of African Medicine*. **6**(3): 115–118.

Dawson, D. (2005). Foodborne protozoan parasites. *International Journal of Food Microbiology* **103**: 207–227.

Dorny, P., Praet, N., Deckers, N. & Gabriel S. (2009). Emerging food-borne parasites. *Veterinary Parasitology* 163: 196–206.

Douce, R.W., Brown, A.E., Khamboonruang, C., Walzer, P.D. & Genta, R.M. (1987). Seroepidemiology of strongyloidiasis in a Thai village. *International Journal for Parasitology* **17**: 1343–1348.

Keiser, P. & Nutman, T. (2004). *Strongyloides stercoralis* in the immunocompromised population. *Clinical Microbiology Review* **17(1)**: 208-217.

Khairul, A., Ramachandran, C.P. & Paran, T.P. (1978). Parasitic Disease Among Fishermen Living on Penang Island. *Medical Journal of Malaysia* **32**: 321-327.

Noor Azian, M.Y., Sakhone, L., Lokman Hakim, S., Yusri, M.Y., Nurulsyamzawaty, Y., Zuhaizam, A.H., Mohd Rodi, I. & Maslawaty, M.N. (2008). Detection of helminths infections in dogs and soil contamination in rural and urban areas. *Southeast Asian Journal of Tropical Medicine and Public Health* **39**(2): 205–212.

Norhayati, M., Zainudin, B., Mohammod, C.G., Oothuman, P., Azizi, O. & Fatmah, M.S. (1997). The prevalence of Trichuris, Ascaris and hookworm infection in Orang Asli children. *Southeast Asian Journal of Tropical Medicine and Public Health* **28**(1): 161–168.

Malaysia Population Census report (2007). http://www.scribd.com/doc/22081516/Stmp-Ge0-Asm-Kedah-2009. Last accessed on 04.10.2010.

Ogbolu, D.O., Alli, O.A., Ogunleye, V.F., Olusoga-Ogbolu, F.F. & Olaosun, I. (2009). The presence of intestinal parasites in selected vegetables from open markets in south western Nigeria. *African Journal Medicine and Medical Sciences* **38**(4): 319–324.

Román-Sánchez, P., Pastor-Guzmán, A., Moreno-Guille, S., Igual-Adell, N.R., Sun˜ Er-Generoso, S. & Banez, C.T. (2003). High Prevalence of *Strongyloides Stercoralis* among Farm Workers on the Mediterranean Coast of Spain: Analysis of The Predictive Factors of Infection in Developed Countries. *American Journal of Tropical Medicine and Hygiene* **69**(3): 336–340.

van der Hoek, W., De, N.V., Konradsen, F., Cam, P.D., Hoa, N.T., Toan, N.D. & Cong le, D. (2003). Current status of soil-transmitted helminths in Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**: Suppl 1: 1–11.

Walzer, P.D., Milder, J.E., Banwell, J.G., Kilgore, G., Klein, M. & Parker, R. (1982). Epidemiologic features of *Strongyloides stercoralis* infection in an endemic area of the United States. *American Journal of Tropical Medicine and Hygiene* **31**: 313–319.

Wiria, A.E., Prasetyani, M.A., Hamid, F., Wammes, L.J., Lell, B., Ariawan, I., Uh, H.W., Wibowo, H., Djuardi, Y., Wahyuni, S., Sutanto, I., May, L., Luty, A.J., Verweij, J.J., Sartono, E., Yazdanbakhsh, M. & Supali, T. (2010). Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). *BMC Infectious Diseases* **10**: 1–12.

Yori, P.P., Kosek, M., Gilman, R.H., Cordov, J., Bern, C., Chavez, C.B., Olortegui, M.P., Montalvan, C., Sanchez, G.M., Worthen, B., Worthen, J., Leung, F. & Ore, C.V. (2006). Seroepidemiology of strongyloidiasis in the Peruvian Amazon. *American Journal of Tropical Medicine and Hygiene* **74**(1): 97–102.