Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam

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ABSTRACT

Helminth egg contamination of vegetables purchased at suburban market in Hanoi, Vietnam was examined. A total of 317 vegetables were examined and 82 (26%) were revealed to be positive for parasite eggs. Of the 15 varieties, 13 were positive except for horseradish and cucumber. Contamination was highest in leafy vegetables (31%), followed by root vegetables (17%) and fruit vegetables (3%). Throughout the survey, five species of parasite eggs were found: *Ascaris* sp., *Trichuris* sp., *Toxocara* sp., *Taenia* sp. and *Ascaridia galli.* In the interview with the villagers, 121 (81%) of 149 adult villagers stated that they usually use not only animal feces but also human feces as a fertilizer. Throughout the survey, a total of 453 eggs were recovered. Number of eggs recovered from vegetables was higher in the dry season (355 eggs) than in the rainy season (98 eggs). The study revealed that vegetables purchased at a market in suburban Hanoi (Vietnam) were highly contaminated with parasite eggs excreted by humans and animals. Considering the eating habits of the Vietnamese and the 17% embryonation rate of detected parasites, vegetables seem to play an important role in soil-transmitted helminth infection in this country.

Keywords: Parasite, Vegetable, Contamination, Vietnam.

INTRODUCTION

Soil-transmitted helminth (STH) infection is endemic in many areas of the world, principally in developing countries with poor environmental sanitation and personal hygiene. Since the mode of infection is fecal–oral, prevalence is high in people who live in areas contaminated with human feces. Vietnam is a high endemic area for STH infection[1,2] as shown by an epidemiological survey of STH by local institutions has been carried out in 50 of 64 provinces in Vietnam.[1] Of these provinces, 17 showed a greater than 50% prevalence of parasitic diseases. Uga *et al*.[2] investigated the intestinal parasites of school children in one province (not one of the 17 provinces mentioned above), using the centrifugal sedimentation technique, and revealed that 166 (76%) of 217 were positive for at least one of nine species of parasites. This study, together with that of Hoek *et al.*[1] indicates that prevalence of parasitic disease in Vietnam is relatively high throughout the country.

Since STH infection is mainly established by oral intake of infective eggs from the environment, numerous studies on the recovery of parasite eggs and/or oocysts from various sources have been reported, e.g. soil,[3] dust, hands, fingers, nails or water[4,5] and vegetables.[6-11] Of these, vegetables are thought to be the principal source of STH infection as they are consumed them daily.[12] Previous studies have revealed that many types of vegetables, purchased at markets in different regions, were contaminated with helminth eggs, as well as protozoan oocysts. Although these reports have emanated from many developing countries, no study has been performed in Vietnam, except one in Vietnamese in a local journal.[13] In addition, there are no reports from Vietnam discussing the relationship between parasitic infection of humans and contamination of vegetables by parasite eggs. We previously reported the intestinal parasitic infection in schoolchildren[2] from one village in suburban Hanoi. This study was also carried out in the same village to investigate vegetable contamination with parasite eggs and assess the parasitic fauna of the villagers.

MATERIALS AND METHODS

Survey area: This study was performed in a suburban village, located 10 km southwest of Hanoi, Vietnam. The village, surrounded by fields and isolated from other town or villages, has a population of 4,100 (1,010 households), 75% of whom are engaged in agriculture. There is a market (consisting of around 30 street stalls) in the village, which is open morning and evening.

Table-1: Contamination of vegetables with parasite eggs

Vegetable name in		No. of vegetable		Positivity	Positivity of parasite egg in: rainy season (%) / dry season (%)				
English	Vietnamese	examined	positive	(%)	*Ascaris* sp.	*Trichuris* sp.	*Toxocara* sp.	*Taenia* sp.	*Ascaridia galli*
Leafy vegetable									
Pumpkin (leaves)	Bi ngo	9	1	11	14/0	0/0	0/0	0/0	0/0
Water morning glory	Rau muong	49	23	47	24/54	4/21	4/17	0/8	12/4
Coriander	Rau mui	20	5	25	ND*/20	ND/10	ND/10	ND/0	ND/0
Sawtooth coriander	Rau mui tau	30	1	3	ND/3	ND/0	ND/0	ND/0	ND/0
Basil	Hung	32	10	31	19/31	6/25	6/0	0/0	0/0
Houttuynia sp.	Rau diep ca	34	19	56	29/65	29/24	6/6	0/0	6/6
Centella sp.	Rau ma	6	3	50	17/ND	0/ND	0/ND	0/ND	33/ND
Lettuce	Xa lach	30	5	17	13/13	13/0	0/0	0/0	0/0
King-guy-soy	Kinh gioi	29	6	21	7/20	0/0	0/7	0/0	14/0
Subtotal		239	73	31	19/28 (24)**	9/11 (10)	3/5 (4)	0/1 (1)	8/1 (4)
Root vegetable									
Taro	Khoai so	9	1	11	ND/11	ND/0	ND/0	ND/0	ND/0
Jicama	Cu dau	11	5	46	33/20	17/0	0/40	0/0	17/0
Carrot	Ca rot	18	2	11	22/0	0/0	0/0	0/0	0/0
Horseradish	Cu cai	10	0	0	0/0	0/0	0/0	0/0	0/0
Subtotal		48	8	17	18/8 (13)	5/0 (2)	0/8 (4)	0/0 (0)	5/0 (2)
Fruit vegetable***									
Tomato	Ca chua	18	1	6	0/11	0/0	0/0	0/0	0/0
Cucumber	Dua chuot	12	0	0	0/0	0/0	0/0	0/0	0/0
Subtotal		30	1	3	0/8 (3)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Total		317	82	26	16/23 (21)	7/8 (8)	2/6 (3)	0/1 (1)	6/1 (3)

* Not done.

**Percent positivity in rainy season/percent positivety in dry season, figure in parentheses is the mean.

*** Vegetables of which fruits are eaten.

Vegetables: A total of 15 varieties of vegetables were examined (Table-1): nine kinds of leafy vegetables, four kinds of root vegetables and two kinds of fruit vegetables. Vegetables were purchased at the village market, except water morning glory and *Houttuynia* sp., which were harvested directly from the village fields. Most vegetables were collected and examined in both the rainy (June–August) and dry season (December–February), but some were examined only in one season (dry: coriander, sawtooth coriander, taro; rainy: *Centella* sp.) owing to the seasonality of harvest.

Recovery and observation of eggs: Purchased vegetables were brought to the laboratory of the National Institute of Malariology, Parasitology, and Entomology. Then, 100g of leafy vegetables (chopped into small pieces) and the same weight of skins of root and fruit vegetables were soaked in 500 ml of 0.5% Tween-20 solution and left overnight. After removing bits of leaves or skins with a tweezers, about 300 ml of supernatant was removed and the remainder transferred to 50 ml test tubes and centrifuged at 2000 rpm for 10 min. After the centrifugation, the supernatant was removed and the sediment examined under a light microscope (×100–400) for parasite eggs. If positive for eggs, the number was counted and the developmental stage recorded. In the case of embryonated eggs, larval movement (viability) was also noted.

Questionnaire: During the survey period, a questionnaire survey and/or interview of the villagers was carried out to clarify vegetable consumption and disposal of feces. For the questionnaire survey, a total of 403 people (149 adult villagers and 254 schoolchildren) were involved and asked about consumption of vegetables. In the interview, adult villagers were questioned on the method of processing and disposal of feces, in particular, the usage of "night soil" as a fertilizer.

RESULTS

The results are shown in Table-1.0Of a total of 317 vegetables examined, 82 (26%) were positive for parasite eggs. Of the 15 varieties, 13 were positive, while horseradish and cucumber were negative for parasite eggs. Contamination was highest in leafy vegetables (31%), followed by root vegetables (17%) and fruit vegetables (3%). Among the leafy vegetable, approximately 50% of water morning glory, *Houttuynia* sp. and *Centella* sp. were contaminated. The questionnaire revealed that of the nine leafy vegetables, eight vegetables, excluding pumpkin, are eaten by raw, of which coriander, sawtooth coriander, basil, lettuce, and king-guy soy are eaten more frequently. In the case of root vegetables, contamination of jicama at 46% was noteworthy. No seasonal variation in contamination between the rainy and dry season were observed (Table-1).

In the survey, five species of helminth eggs, namely, *Ascaris* sp., *Trichuris* sp., *Toxocara* sp., *Taenia* sp., and *Ascaridia galli* were found but not protozoan cyst/oocyst: (Table-1). Regarding the frequency of helminths encountered, *Ascaris* sp. was the most prevalent (21%) followed by *Trichuris* sp. (8%). Although two unidentified eggs (1%) were found, further observation was not carried out. Five different species of parasite eggs were found from water morning glory. In the interview, 121 (81%) of 149 adult villagers stated that they usually use not only animal feces but also human feces as a fertilizer.

The number of eggs recovered from vegetables in the different seasons and percentage of embryonated egg are shown in Table-2. Data for *Taenia* sp. are excluded because the viability of cestode eggs is difficult to assess via light microscopic observation. Throughout the survey, a total of 453 eggs were recovered. Number of eggs recovered from vegetables was higher in the dry season (355 eggs; i.e. 78% of total number of egg recovered) than in the rainy season (98 eggs; 22%). In particular, the number of *Ascaris* sp. eggs recovered in the dry season was 4.8 times greater than in the rainy season. More than 90% (441/453) of the recovered eggs were from leafy vegetables. Of the 453 eggs recovered, 17% (77 eggs) had developed to embryonated stage: 86% of which were motile/alive (data not shown). Embryonation rates of egg varied from 0 to 50% throughout the year, with no significant difference between the rainy (15%) and dry (17%) seasons.

DISCUSSION

The study revealed that vegetables purchased at a market in suburban Hanoi (Vietnam) were highly contaminated with parasite eggs excreted by humans and animals. The cause of the vegetable contamination with STH eggs is the usage of feces as a fertilizer. In interviews, villagers have clarified that human and animal waste (especially from pigs) is used as a fertilizer for growing vegetables. They usually scatter the "night soil" onto the soil and, occasionally, on vegetables. The results clearly show that contamination was highest in leafy vegetables, followed by root and fruit vegetables. This finding agrees with that of Fueki,[14] who reported contamination with *Ascaris* sp. eggs only. To identify vegetables as a source of parasitic infection, it is necessary to provide the following three conditions proposed by Fueki[14]: firstly, they must be highly contaminated, secondly they must be cultivated in a warm season when the parasite eggs can develop, and thirdly they must be eaten uncooked. The following seven vegetables appear to be important sources of ascariasis and trichuriasis in the study area, satisfying all three conditions: water morning glory, coriander, basil, *Houttuynia* sp., *Centella* sp., lettuce, and king-guy soy. Skins of the root vegetable, jicama, were also highly contaminated but this is eaten raw after the skin is removed.

Fueki[14] reported that recovery rate of embryonation eggs from vegetable shows seasonality, i.e. more embryonated eggs were recovered in the rainy season (35 " 58%) than in the dry season (4 " 16%). Our result, however, showed no variation in embryonation rate between the seasons. The reason is unknown. The number of eggs recovered was higher in the dry than in the rainy season and it is assumed that eggs on the surface of vegetables are washed away by rain. This contrasts to the report of Anuar and Ramachandran[7] who noted that the eggs of *A. lumbricoides* are difficult to wash off due to their adhesive nature. *Toxocara* sp. eggs were found on a few occasions, proof of vegetable contamination by animal feces. Although few cases of toxocariasis have been reported from Vietnam,[15,16] attention should be paid to this zoonotic disease.

Since human feces are used as a fertilizer in Vietnam, the parasitic fauna in human feces should be reflected in the results of parasitic contamination of vegetables.

Table-2: Number of egg recovered from vegetables and their embryonation rates

Season	Vegetable	Total number of eggs recovered (% of embryonated eggs*)				Total
		Ascaris sp.	*Trichuris* sp.	*Toxocara* sp.	*Ascaridia galli*	
Rainy	Leafy	56 (18)	19 (11)	7 (43)	9 (0)	91 (17)
	Root	5 (0)	1 (0)	0 (0)	1 (0)	7 (0)
	Fruit	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Subtotal	61 (16)	20 (10)	7 (43)	10 (0)	98 (15)
Dry	Leafy	291 (22)	33 (9)	23 (0)	3 (0)	350 (17)
	Root	2 (50)	0 (0)	2 (0)	0 (0)	4 (25)
	Fruit	1 (0)	0 (0)	0 (0)	0 (0)	1 (0)
	Subtotal	294 (22)	33 (9)	25 (0)	3 (0)	355 (17)
	Total	355 (21)	53 (9)	32 (9)	13 (0)	453(17)

* Figures in parentheses are shown by percentage that devide the number of embryonated eggs by the total number of eggs recovered.

Uga *et al.*[2] have reported on parasitic infection among school children in this study area and revealed that 76% were positive for parasites. Of the helminths detected from feces, *T. trichiura* (67%) was the most frequent, followed by *A. lumbricoides* (34%). However, *A. lumbricoides* was dominant in the case of vegetables. This discrepancy can be explained from the difference of intensity of infection. The number of *A. lumbricoides* eggs excreted in this area was five times more than that of *T. trichuris*.[2] We previously reported *Cryptosporidium parvum* (10%) infection in Thailand,[17] *Cyclospora* sp. (0.4%) in Indonesia,[18] and *Isospora* sp. (6%) in Lao PDR,[19] but we could not find these protozoa either in the former study[2] or in present study in Vietnam. Previous studies from different countries where STH infection is endemic have shown that vegetables were highly contaminated with eggs, i.e. Korea,[6] Malaysia,[7,8] Thailand,[20] Philippines[10] and Turkey.[4]

Bier[9] studied the recovery of *T. trichuris* and/or *A. lumbricoides* eggs from vegetable and reported a 10% of recovery efficiency with his method. Although the recovery efficiency of the method used in this study was not evaluated, actual contamination of vegetable by parasite eggs is seem to be very high because both methods are similar. Although many potential agents for the dissemination of parasites have been reported,[21] infection through vegetable must be high.

Considering the eating habits of the Vietnamese plus the 17% embryonation rate of detected parasites, vegetables play an important role in STH infection. The result suggests the necessity for nationwide control measures against parasitic infection.

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