Parasitology

cambridge.org/par

Research Article

Cite this article: Paller VGV, Macalinao-Ramirez CA, Bandal MZJr. (2022). Environmental contamination with parasites in selected rural farms in the Philippines: impacts of farming practices on leafy greens food safety. *Parasitology* **149**, 482–489. https://doi.org/10.1017/S0031182021002031

Received: 22 August 2021
Revised: 8 November 2021
Accepted: 23 November 2021
First published online: 6 December 2021

Keywords:
Farming practices; food safety; foodborne parasites; public health; zoonosis

Author for correspondence:
Vachel Gay V. Paller,
E-mail: vvpaller@up.edu.ph

Environmental contamination with parasites in selected rural farms in the Philippines: impacts of farming practices on leafy greens food safety

Vachel Gay V. Paller, Constance Aurelle Macalinao-Ramirez and Modesto Z. Bandal Jr.

Parasitology Research Laboratory, Animal Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, College 4031, Laguna, Philippines

Abstract

Fresh vegetables are an important part of a healthy diet, however, they can also be agents of transmission of intestinal parasites. This study aimed to evaluate the presence of parasite contamination in vegetable farms as a source of transmission in selected rural communities in Laguna, Philippines. A total of 168 vegetable, 55 soil and 15 water samples collected from four selected farms and a reference farm were processed through various standard parasitological techniques. Of these, 17.3% of vegetables, 47.3% soil and 73.3% water samples were found contaminated with parasites' eggs or cysts. Interestingly, leafy vegetables, such as lettuce, were found to be more contaminated. Results showed that strongylids/hookworms (egg/larva) showed the highest prevalence in farm soil (38.2%) and vegetable (13.1%); other helminth parasite eggs were also recovered such as *Toxocara* sp., *Ascaris* sp., *Trichiuris*, *Trichostrongylus* sp. and protozoan cysts of *Balantidium coli*. *Cryptosporidium* sp. oocysts of and *Giardia* sp. cysts were observed in all water samples in the farms. Furthermore, results revealed that some farming practices such as the use of improperly treated animals manure as fertilizers, unhygienic practice of farmers and sanitation issues were factors that contribute to parasite contamination in the farms. These findings have implications on food safety in poor-resource communities posing public health risks. Recommendations were discussed in the study for the control and prevention of parasite contamination at the farm level.

Introduction

A safe food supply is essential to the survival of humankind. As people have multiplied and technology has advanced, the food supply has evolved from a base of local consumer production to large-scale integrated production and distribution practices. Consumers are unaware of the origin and processing of their food. Similarly, most producers are unaware of the variety of potential public health hazards that are transmissible by food. Although information regarding foodborne hazards is widely available from many sources, especially regarding hazards such as bacteria and chemical residues, there is limited information on foodborne parasites (Dorny *et al*., 2009; Gupta *et al*., 2009). Both consumers and producers rely on government regulations and oversight for guidance and protection. However, there has been complacency which could be due to a lack of technological know-how for control strategies and further aggravated by the complacency in standards and policies for food safety.

Several studies mentioned that environmental contamination with parasites *via* animal host defecation contributes significantly to ongoing transmission in agricultural settings. Animals play an important role in the transmission of parasites to humans, in fact, animal hosts play an obligatory part in the lifecycles of certain human helminths. Recently, molecular tools for interrogating relationships between morphologically similar helminths infecting humans and animals have revealed zoonotic infection reservoirs (Torgerson and Macpherson, 2011). For example, the transmission of *Ascaris suum* from pigs to humans occurs worldwide in agricultural settings (Betson *et al*., 2014). Also, a high proportion of human hookworm infections in Asia are attributable to canine hookworm species (Jourdan *et al*., 2018). However, the contribution of foodborne zoonotic transmission to the overall burden of parasitic infection and the relative importance of different animal and environmental reservoirs remains poorly understood. Zoonotic transmission is likely to be important in the Philippines, where many people depend on livestock production and integrated farming. This has significant implications for zoonotic parasite transmission, especially in rural communities, where poor agricultural practices provide a high risk of disease transmission (Betson *et al*., 2020).

Moreover, foodborne diseases (FBD) have been on the rise globally in the past recent years and parasites have been implicated as one of the aetiological agents. Eating green leafy vegetables is vital to a healthy and balanced diet. However, these have been linked to foodborne parasitic diseases. Most leafy greens are grown outdoors and are exposed to soil, animals and water, all of which can be a source of parasite contamination. In addition, leafy greens, such as lettuce, are eaten raw and the cooking process to kill potential biological hazards is eliminated in the process, thus, further posing health risks. In the Philippines, the contribution of fresh produce in FBD is poorly understood, thus the true dimension of the problem is unknown. This study aimed at providing science-based evidence on the need to address issues concerning farming practices that pose threats to farmers and consumers' health particularly in poor-resource communities in the country.

Material and methods

Study site and sampling design

The study sites were selected farms in Laguna, Philippines which is part of the CALABARZON (Region IV-A) region and located 30–60 km south of Metro Manila, Philippines. It is the third-largest province in Region IV-A and known to produce and supply large quantities of fresh produce. Four farms were selected study sites based on the following criteria: (a) must be within the range of 200–20 000 m^2; (b) fresh produce is for commercial purposes and (c) fresh produce include green leafy vegetables such as lettuce. Some of the selected farms were practising organic farming while others were conventional but also used animal manure as fertilizers. A certified organic farm located outside the sampling district area was included to serve as a reference farm for comparison. Hence, a total of five farms were considered in the study.

Vegetable collection and processing

The farm produce samples collected include lettuce varieties, cabbages, cucumbers and tomatoes. One sample unit is equivalent to 100 g of each vegetable type randomly collected from the farms. The number of vegetable samples collected from each farm was based on the size of the vegetable farming area. The vegetable planting area was divided into plots measuring 1 m^2 and were numbered from 1 to 100. The five points were randomly selected based on the number generated by a calculator. For example, ten vegetable samples were collected from a farm which approximately measures 200 m^2. The samples were placed individually in properly labelled sealed bags and were brought immediately to the laboratory for processing and analysis. Samples were then processed through the sedimentation technique. Briefly, the vegetable samples were cut into small pieces and put into a 1000-mL beaker containing 400 mL of 0.95% NaCl solution. The solution was stirred for about 10 min and the vegetable samples were removed and discarded. Subsequently, the remaining saline solution was left overnight for sedimentation to take place; after which, the supernatant was discarded carefully using suction until about 100 mL of the residue remained in the beaker. The remaining residue was transferred into test tubes and centrifuged for 10 min at 3000 rpm. After centrifugation, the supernatant was discarded and about 1 mL remained in the test tube. The remaining residue at the bottom of the tube was then mixed thoroughly and a drop was placed on a glass slide, dyed with Lugol's iodine and examined microscopically in 200× and 400× magnification. Three readings were done for each sample (Uga *et al*., 2009).

Soil collection and processing

Soil samples were also collected from the same farms. The number of samples collected from each farm were also based on the size of the vegetable farming area as mentioned above. Soil samples were processed through a modified floatation technique developed by Horiuchi and Uga (2016). Approximately 200 g of a sample at 5 cm depth (topsoil) were collected from the farms and were contained in sealable plastic bags. These were transported to the laboratory for analysis. Soil samples were then air-dried before processing. The dried soil was strained using a 125-μm mesh sieve. Two grams of fine soil were transferred in a calibrated test tube and added 6 mL of distilled water, mixed thoroughly using a vortex mixer (Seoulin Bioscience, Korea), then centrifuged at 1800 rpm for 10 min. The supernatant was decanted after centrifugation. Then 8 mL of 1.2 specific gravity sucrose solution was added to the soil sediments, mixed, then centrifuged at 1800 rpm for another 10 min. After which, using a 10-mL syringe, 1.3 specific gravity of sucrose solution was slowly added up to the brim to form an upper meniscus to allow flotation of parasite eggs or cysts that may be trapped in the soil samples. A coverslip was carefully placed on the mouth of the tube to collect the upper meniscus of the sucrose solution. This was then placed on a glass slide and observed at 100×, 200× or 400× magnification using a compound light microscope (Nikon, Japan) for identification of the presence of parasite eggs, cysts or larvae. Photos and size of specimens were taken using a digital microscope camera (OptixCam, China) and parasite elements were measured using a Toupview software (ToupTek Photonics, China).

Water collection and processing

Water sources in the farm include tap water from the municipal district office, spring water and groundwater. A triplicate of a ten-litre water sample was collected from the identified water sources present in each farm using sterile plastic water bottles. The samples were transported to the laboratory and processed in accordance with Method 1623.1 designated by the United States Emergency Protected Areas (USEPA, 2001). Each sample was filtered using Whatman Nucleopore Track-Etch Membrane with a defined pore size of 1 μm. After filtration, the materials on the filter were eluted with distilled water and the elute was then concentrated by centrifugation under 1500 rpm for 10 min. The resulting supernatant was then decanted, and the remaining residue was pipetted and stored into sterilized Eppendorf tubes.

The water samples were subjected to immunofluorescence assay test (IFA) for the determination of the parasite count. Crypto/Giardia-cel IF test, an *in vitro* direct immunofluorescence test kit, was used to allow simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts (Cellabs Pty. Ltd., Sydney/Australia). The water samples were processed according to the manufacturer's directives.

Survey on knowledge, attitude and farming practices of farmers and farm owners

During the collection of the samples, survey interviews of the farmer and farm owners were conducted to document some of the management practices in the farms. The survey interviews were done to determine the possible association of knowledge, attitudes and practices (KAPs) in farming practices with the occurrence of parasite contamination in the farms. Farm owners and farm managers were asked to be interviewed with informed consent.

Data analysis

Parasite prevalence was calculated as the number of positive samples divided by the total number of samples multiplied by 100. Mean density was calculated as the number of parasites eggs/cyst/oocyst/larvae divided by the total number of positive samples. The comparison of the parasite prevalence (%) and mean density of the parasites observed among the various environmental samples from the farms was analysed by Chi – square test statistics

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press

Table 1. Prevalence and density of parasites recovered from leafy and fruit vegetables from selected farms (F) in Laguna Province, Philippines

	Vegetable ($N = 168$)										
	Prevalence (%)						Mean density (egg/larvae/cyst g^{-1})				
Vegetable type	F1	F2	F3	F4	RF	Total	F1	F2	F3	F4	RF
Leafy vegetables											
Cabbage	0	–	0	0	0	0	0	–	0	0	0
Chinese cabbage	–	0	0	–	–	0	–	0	0	–	–
Lettuce romaine	0	100	16.7	–	66.7	50.0	0	2.0	1.0	–	1.0
Lettuce ice berg	–	100	33.3	16.7	–	66.7	–	2.0	2.0	1.0	–
Lettuce red ruby	83.3	–	–	–	–	83.3	6.0	–	–	–	–
Other lettuce varieties	0	–	0		–	0	0	–	0		–
Fruit vegetables											
Cucumber	–	12.5	0	0	0	12.5	–	1.0	0	0	0
Tomato	0	0	0	0	0	0	0	–	0	0	0
P value*		0.002				17.3%			1.000		

*$P < 0.05$ – significant; RF –reference farm (a certified organic farm located outside the sampling district area).

while the comparison of mean densities of parasites among different vegetable types and among different water sources was analysed by Kruskal Wallis H Test using Quantitative Parasitology 3.0 software and IBM SPSS software (IBM® SPSS® Statistics 2016, Version 24.0). Moreover, an association of the farming practices with a prevalence of contamination and mean density of parasites were analysed using point-biserial correlation. The normality of the data was ascertained using Shapiro–Wilk test. All the data were revealed to be not normally distributed, thus non-parametric tests were employed. All statistical analyses were set at 95% level of confidence and *P* values less than 0.05 was considered significant.

Results

Parasite contamination of vegetables

A total of 29 out of 168 (17.3%) vegetable samples collected from the four selected farms and the reference farm were found contaminated with parasites. The parasites detected in the samples were nematode parasites such as strongylid/hookworm egg or larvae, ascarid and *Toxocara* sp. eggs and protozoan *Balantidium coli* cysts.

Vegetable samples were categorized into leafy vegetables (cabbage and lettuce) and fruit vegetables (tomato and cucumber). Lettuce varieties were found contaminated with red ruby lettuce variety having the highest prevalence of parasite contamination (83.3%) followed by an iceberg (66.7%) and romaine (50.0%) varieties; while cucumber showed the lowest at 12.5% (Table 1). Other fresh produce was not contaminated such as cabbage and tomatoes. Statistical analysis showed significant differences in the prevalence between leafy vegetables and fruit vegetables ($P = 0.002$); while parasite mean densities showed otherwise ($P = 1.000$) in farms positive with parasites.

Comparison of the prevalence of parasite contamination and mean density of parasites recovered from vegetables is shown in Table 2. It revealed strongylids/hookworms with the highest prevalence (13.1%), followed by *Toxocara* sp. (4.8%), *Ascaris* sp. (3.0%) and *B. coli* (1.8%). The highest mean density was also observed in strongylids/hookworms at 6 egg/larvaeg^{-1}. Statistical analysis showed a significant difference among the prevalence of these parasites ($P = 0.0001$).

Parasite contamination of farm soils

A total of 26/55 (47.3%) soil samples were contaminated with at least one parasite. Parasites observed include stronglylid/hookworm larvae (38.2%), *Trichostrongylus* sp. egg (5.5%), *Toxocara* sp. egg (3.6%), *Trichuris* sp. egg (3.6%) and *Taenia pisiformis* egg (3.6%), *B. coli* cyst (2.9%) and *Ascaris* sp. egg (1.8%) (Table 2). Only strongylid/hookworm larvae were observed in the reference farm. Overall, the strongylids/hookworms showed the highest mean density (3 egg/larvae g^{-1}). Interestingly, there was no significant difference observed between the prevalence of parasites ($P = 0.123$) and mean density ($P = 0.850$) in farm soils between the study sites/vegetable farms and the reference farm (Table 3).

Parasite contamination of water samples

The prevalence of parasites and mean density among farms are shown in Table 4. For the four selected farms, it revealed contamination with *Giardia* spp. (66.7%) and *Cryptosporidium* spp. (46.7%); the prevalence of contamination among the different farms was significant at $P = 0.002$, while the mean densities were not significant at $P = 0.272$. It is interesting to note that these protozoan parasites were not observed in the reference farm. Also, all water samples did not show contamination with soil-transmitted helminth (STH) eggs.

Moreover, a survey revealed that each farm had a single type of water source. The identified water sources include pumped groundwater, district water supply and spring water sources. Among the types of water sources, spring water source showed the highest prevalence for *Cryptosporidium* sp. (77.8%) and *Giardia* (100%) (difference not significant at $P = 0.085$). The pumped groundwater samples were found contaminated at 33.33%, but only *Giardia* sp. were observed with a mean density of 1.0×10^5. While the district water supply was found to be free of these parasites. Moreover, STH parasite eggs or larvae were not observed in all water samples.

Knowledge, attitude and practices (KAP)

Almost all of the farms included in the study were organic farms, except Farm 4 which was conventional. However, all farms

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press

Table 2. Parasites recovered from vegetables and environmental samples of selected farms in Laguna Province, Philippines

Parasites	Prevalence (%)			Mean density (egg/larvae/cysts g^{-1}) (cyst/oocyst/L^{-1})		
	Soil	Vegetables*	Water	Soil	Vegetables	Water
Helminths						
Strongylids/Hookworm	38.2	13.1	0	1.5	2.0	0
Toxocara sp.	3.6	0	0	1.0	0	0
Ascaris sp.	1.8	3.0	0	1.0	1.0	0
Trichostrongylus sp.	5.5	4.8	0	2.0	1.0	0
Trichiuris sp.	3.6	0	0	1.0	0	0
Taenia pisiformis	3.6	0	0	1.0	0	0
Protozoan						
Balantidium coli	2.9	1.8	0	1.0	1.0	0
Cryptosporidium sp.	–	–	46.7	–	–	8.0×10^4
Giardia sp.	–	–	66.7	–	–	2.0×10^5

(–) – Immunofluorescence assay (IFA) not performed.
*$P < 0.05$.

revealed the use of animal manure as an additional nutrient supplement for crops. Moreover, common among these farms were the presence of roaming animals (stray dogs and cats, rodents, livestock). Some farms also accept visitors for local tourists to experience a harvest of fresh produce.

A survey was conducted to document some farming practices and associate these with the prevalence of parasite contamination in the farms (Table 5). Factors such as the use of fertilizer, water source and farm sanitation and hygiene practices were documented. Statistical analysis showed a significant association between soil contamination and workers' hygiene and sanitation practices ($P = 0.039$). Moreover, the use of animal manure as fertilizer was associated with water contamination ($P = 0.004$). It was also revealed that farmers do not de-worm their pets and livestock.

Discussion

Several studies have been conducted on the epidemiology of FBD and revealed leafy vegetables as one of the leading food types associated with foodborne illnesses. Several studies have also been done in the Philippines reporting vegetable contamination with parasites (De Leon *et al.*, 1992; Su *et al.*, 2012; Ordoňez *et al.*, 2016; Vizon *et al.*, 2019). However, there is a dearth of studies describing the dynamics of parasite contamination and transmission at the farm level in rural farms. In this study, four vegetable farms and one reference farm were examined for the prevalence of parasite contamination. Results revealed that parasites were found contaminating soils, water and vegetables from the selected farms. Most of these were common parasites of humans, domestic animals, including rodents such as *Ascaris*, strongylids, hookworms, *Toxocara*, *Trichuris*, *Balantidium*, *Cryptosporidium* and *Giardia*.

It is interesting to note that leafy vegetables, particularly lettuce, were found more contaminated with parasites. Other studies also reported lettuce contamination (Adamu *et al.*, 2012; Adanir and Tasci, 2013; Eraky *et al.*, 2014; Ordoňez *et al.*, 2016; Ferreira *et al.*, 2018; Vizon *et al.*, 2019). Parasite contamination of lettuce can be attributed to its broad and large surface area which allows more contact with contaminated soil and water. The uneven surfaces could also allow parasites' eggs, cysts and oocyst to easily trap on the surface as compared to the smooth-surfaced vegetables such as tomatoes and cucumber. Leafy vegetables are also grown in closer proximity to the ground which could lead to a higher risk of contamination due to frequent contact with soil and runoffs. However, no parasites were detected from cabbage, tomatoes and Chinese cabbage (*pechay*). It was observed that outer leaves of cabbage and Chinese cabbage were removed during harvest; while tomatoes have smooth surface reducing the chance of attachment by parasite forms. Some cucumbers were also found contaminated which could be due to possible unhygienic handling of farmworkers as revealed in the KAP analysis.

Several studies also reported contaminated lettuce with parasites that could come from faeces of livestock or animal manure fertilizers (Gupta *et al.*, 2009; Adamu *et al.*, 2012; Adanir and Tasci, 2013; Maikai *et al.*, 2013; Eraky *et al.*, 2014; Adenusi *et al.*, 2015; Luz *et al.*, 2017; Woldetsadik *et al.*, 2017). Variation in the prevalence of parasite contamination in vegetables can be attributed to the state of the vegetables before the examination (pre-washed or unwashed) and/or post-harvest practices. It could also be due to the sensitivity of parasitological techniques employed.

Soil samples from the farms were also collected and examined for the possible presence of parasites. The result of the study revealed a 47.3% prevalence of parasite contamination in soil samples. This is relatively higher compared to other studies at 31–36.4% contamination in house yards and public parks (Paller and De Chavez, 2014; Zibaei *et al.*, 2017). However, a study by Horiuchi *et al.* (2013) showed higher contamination in soils in public and residential areas in a rural village in Laguna, the Philippines at 71%. The presence of parasites contaminating soils in the study sites is indicative of faecal contamination from animals as the farms practised integrated farming. However, the limitation of this study was that no molecular analysis was performed to confirm the specific species of parasites detected to identify the point source. Thus, a molecular analysis will be considered in future studies.

Parasite contamination was also compared with a reference farm located outside the sampling areas of Laguna Province. The reference farm is a certified organic farm and a macro enterprise as opposed to smallholder farms selected in the current study with lower standards of farming practices. Surprisingly,

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press

Table 3. Parasite contamination of vegetables and environmental samples from selected farms (F) in Laguna Province, Philippines

Farm samples	Prevalence (%)					Overall prevalence (%)	P value	Parasite mean density (eggs/larvae/cyst g^{-1})					P value
	F1	F2	F3	F4	RF			F1	F2	F3	F4	RF	
Vegetables ($n = 168$)	13.9	50	4.5	0	33.3	17.3	0.0001*	6	2	1.5	0	1	1.000
Soil ($n = 55$)	60	7	20	50	50	47.3	0.123	2	2	1	1	1	0.850
Water ($n = 15$)[a]	100	33.3	100	100	0	66.7	0.002*	5.0×10^5	1.0×10^4	1.0×10^5	2.0×10^5	0	0.272

*$P < 0.05$ – significant.
[a]Mean density: oocyst/cyst/ L^{-1}; RF – Reference farm.

the prevalence and mean density of parasites in the reference farm (50.0%; 2 egg/larvae g^{-1} soil) were not statistically different ($P = 0.85$) from the study sites (46.7%; 3 eggs/larvae g^{-1} soil). ($P = 0.85$). The results suggest that contamination with parasites in farm soils may be attributed to the presence of animals among all the farms surveyed. The presence of roaming animals such as dogs, cats and rats increases the chance of parasite contamination due to open defecation. Moreover, the study revealed that some farming practices such as the use of animal manure as fertilizer contribute to parasite contamination. Animal manure is a good form of fertilizer, but if not treated or composted properly could pose a health hazard to consumers. Manure from poultry, cattle and pigs are commonly used organic fertilizers in the study sites.

Among the parasites recovered strongyle/hookworm eggs/larvae were the most commonly detected in vegetable and soil samples. Strongylid and hookworm eggs and larvae are difficult to differentiate microscopically. The ova are usually thin-shelled and the larvae have pointed ends. The abundance of strongyle/hookworm in the environment could be due to faecal contamination from animals. There are several species of Strongylids and hookworms that parasitize humans and other animals such as those from rats, pigs and other livestock. A study by Castillo and Paller (2018) observed 100% occurrence of *Angiostrongylus cantonensis* in rodents from Nueva Ecija, Philippines. The intrusion of rodents and other wildlife in farms could also be a source of contamination (Lo *et al.*, 2021). Moreover, cattle, pigs and goats are well-known reservoir hosts of *Trichostrongylus* sp. which was observed in the farms. This parasite may contaminate vegetables through contaminated soils or the use of contaminated water and manure fertilizers. This parasite is now considered as zoonotic.

Another parasite contaminating soils in the farms was *Toxocara* sp. This parasite is a prevalent parasite of dogs (*T. canis*) and cats (*T. cati*). Several studies reported *Toxocara* soil samples in schools, house yards and playgrounds (Paller and De Chavez, 2014; Tudor, 2015; Baneth *et al.*, 2016). The presence of stray cats and dogs that roam around and open defecate in farm areas account for the presence of *Toxocara* sp. Also, the presence of taenid eggs in soils may be due to the presence of rats in the farms. *Trichuris* sp. or whipworms were also found contaminating soils in the farms. It may be transmitted by roaming animals such as dogs (*T. vulpis*) and by using pig manure (*T. suis*); these two species are also infective to humans. *Trichuris trichiura* is also one of the leading STH infections in the Philippines along with hookworm infection (Soares Magalhães *et al.*, 2015; Owada *et al.*, 2018). A study by Paller and Babia-Abion (2019) revealed farm soil contamination with *Trichuris* sp. in Northern Luzon of the Philippines. The presence of *Trichuris* eggs in the farms could be due to the use of manure from pigs or due to open defecation of roaming animals such as dogs near the vegetable farming areas.

Water samples used for watering fresh produce were also examined for possible parasite contamination. No helminth parasites were observed in all water samples. However, *Cryptosporidium* and *Giardia* parasites were detected in spring water samples at high prevalence. Springwater may be contaminated with animal faeces from agricultural runoffs. Meanwhile, water samples from pumped district groundwater sources were also positive for *Giardia*, albeit at lower prevalence. Also, substandard and leaky sewage pipelines and the absence of effective wastewater filter systems could serve as focal polluters of groundwater and surface water (Ćirković *et al.*, 2017). However, the district tap water supply of the reference farm was found to be free of waterborne protozoan parasites implying intact and clean water pipelines in the area.

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press

Table 4. Prevalence and density of *Cryptosporidium* spp. oocyst and *Giardia* spp. cyst in water samples from selected farms (F) in Laguna Province, Philippines

	Water ($N = 15$)										
	Prevalence (%)*						Mean density (oocyst-cyst L^{-1})				
Parasites	F1	F2	F3	F4	RF	Total	F1	F2	F3	F4	RF
Cryptosporidium sp.	100	0	66.7	66.7	0	46.7	2.0×10^5	0	1.0×10^5	8.0×10^4	0
Giardia sp.	100	33.3	100	100	0	66.7	3.0×10^5	1.0×10^5	5.0×10^4	5.0×10^5	0
Total	100	33.3	100	100	0	66.7	5.0×10^5	1.0×10^4	1.0×10^5	2.0×10^5	0

*$P < 0.05$; RF – Reference Farm.

Table 5. Association of farming practices with prevalence and mean density of parasites contaminating vegetables, soil and water samples in selected vegetable farms

	P values					
	Vegetable	Soil	Water	Vegetable	Soil	Water
Farming practices	Prevalence			Mean density		
Fertilizers used						
Processed manure	0.297	0.692	0.327	0.539	0.503	0.004*
Pure manure						
Water source						
Ground water	0.827	0.692	0.400	0.258	0.090	0.993
Surface water						
Municipal tap water						
Toilet facilities						
Absent	0.338	1.000	0.573	0.387	0.935	0.215
Present						
Hygiene						
Use of protective gears during planting, harvesting and soil treatment						
Yes	0.488	0.039*	0.573	0.747	0.878	0.709
No						
Presence of animals in the farms						
Pets						
Present	0.338	1.000	0.573	0.387	0.935	0.215
Absent						
Livestock						
Present	0.783	1.000	0.400	0.320	0.583	0.843
Absent						
Stray animals						
Present	▲	▲	▲	▲	▲	▲
Absent						
Deworming of animals						
Pets						
Yes	0.783	1.000	0.400	0.320	0.583	0.843
No						
Livestock						
Yes	■	■	■	■	■	■
No						

*$P < 0.05$ – significant.
▲■ Cannot be computed because at least one variable is constant – stray animals present in all farms; no deworming of livestock animals in all farms.

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press

The quality of agricultural water could be a cause of soil contamination and the subsequent contamination of vegetables in the farms. The direct use of water from the mountain spring may contaminate vegetables and soils with pathogens from animals (Maffei *et al.*, 2016; Ordoňez *et al.*, 2016; Amoah *et al.*, 2018; Ferreira *et al.*, 2018; Ybañez *et al.*, 2018). However, other sources suggest that vegetable contamination is more related to soil contact rather than the contact with water (Blaszkowska *et al.*, 2011; Gyawali *et al.*, 2013; Adekeye *et al.*, 2016; Hassan *et al.*, 2017). Regardless, the use of clean agricultural water is also an important factor to prevent contamination of vegetables.

Both organic and conventional farms in the present study reported that they use manure as organic fertilizers. According to interviews, the animal manures were either bought from the market or from another farm or formulated in their respective farms. Some farms revealed the use of treated composted animal manure such as the addition of effective microorganisms (EM) and *Lactobacillus* (LABS), while some use sun-dried composted manure before soil application. It was also observed that some farms use untreated manure as fertilizers. The use of improperly processed manure can potentially contaminate soil, water and even vegetable produce, thus posing a health risk to farmers and consumers.

Furthermore, the survey interview revealed poor sanitation and hygiene practices in some farms such as the lack of personal protective equipment during planting and harvest and washing of hands before and after handling crops. Ordoňez *et al.* (2016) reported a significant association between parasite contamination and some farming practices such as the absence of toilet facilities, deworming practices and the presence of animals in the farm. Although the current study showed high awareness of farmers on biological pathogens, there is little awareness of the technological know-how for control and prevention as well as the understanding of the dynamics of pathogen contamination in the farms.

Conclusion

Results of the present study revealed health risks to farmers and consumers due to poor farm practices in some rural communities. There is a need to review food safety guidelines, that is, the Philippine Republic Act (RA) 10 611 on Food Safety should include a 'Produce Safety Rule' to set science-based standards to help ensure that water, soil amendments (e.g. fertilizer or compost), food contact surfaces and other materials that touch produce during growing, harvesting, packing and holding do not contribute to produce contamination. The Produce Safety Rule should also include the promotion of good agriculture practices (GAP) such as addressing animal intrusion into fields and worker hygiene. Also, there have been many claims that eating organic farming increases exposure to biological contaminants in crops. However, this study has found no evidence to support this claim. It is important to realize that both organic and conventional foods must meet the same quality and safety standards. Moreover, providing education and technical assistance to the agricultural sector and other stakeholders is also important, with greater emphasis on the potential impact of adjacent land uses and continued emphasis on the importance of agricultural water quality. Finally, relevant agencies should be focused on promoting good farming practices, communicating risks and conducting research with a multi-faceted approach to help keep leafy vegetables safe for consumers. To prevent possible adverse consequences to public health, it is highly recommended that proactive steps and measures are implemented.

Acknowledgements. The authors would like to thank: The Laguna Provincial Agricultural Office in the Philippines and some selected local government units (LGUs) for providing permits and assistance during sample collection. Also, the Animal Biology Division of the Institute of Biological Sciences, University of the Philippines Los Baños (UPLB) for the facilities and equipment.

Financial support. This work was supported by the National Research Council of the Philippines-Department of Science and Technology (NRCP-DOST).

Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. Prior to the conduct of the study, permits to conduct the study were secured from the LGUs through the Provincial Agriculture Office and respective Municipal Agriculture Offices. Moreover, ethics clearance to conduct survey interviews was approved by the University of the Philippines Manila Research Ethics Board (UPMREB) with permit number 2018-201-01.

References

Adamu NB, Adamu JY and Mohammed D (2012) Prevalence of helminth parasites found on vegetables sold in Maiduguri, Northeastern Nigeria. *Food Control* **25**, 23–26.

Adanir R and Tasci F (2013) Prevalence of helminth eggs in raw vegetables consumed in Burdur, Turkey. *Food Control* **31**, 482–484.

Adekeye TA, Thompson E and Awobode HO (2016) Environmental contamination and public health risk of soil parasites in Ibadan South East Local Government Area, Nigeria. *Zoology and Ecology* **26**, 150–157.

Adenusi AA, Abimbola WA and Adewoga TOS (2015) Human intestinal helminth contamination in pre-washed, fresh vegetables for sale in major markets in Ogun State, Southwest Nigeria. *Food Control* **50**, 843–849.

Amoah ID, Adegoke AA and Stenström TA (2018) Soil-transmitted helminth infections associated with wastewater and sludge reuse: a review of current evidence. *Tropical Medicine and International Health* **23**, 692–703.

Baneth G, Thamsborg SM, Otranto D, Guillot J, Blaga R, Deplazes P and Solano-Gallego L (2016) Major parasitic zoonoses associated with dogs and cats in Europe. *Journal of Comparative Pathology* **155**, 54–74.

Betson M, Nejsum P, Bendall RP, Deb RM and Stothard JR (2014) Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of Ascaris in people and pigs. *Journal of Infectious Diseases* **210**, 932–941.

Betson M, Alonte AJI, Ancog RC, Aquino AO, Belizario VY, Bordado AD, Clark JE, Corales MCG, Dacuma MGB, Divina BP, Dixon MA, Gourley SA, Jimenez JD, Jones BP, Manalo SMP, Prada JM, Van Vliet AHM, Whatley KCL and Paller VGV (2020) Zoonotic transmission of intestinal helminths in Southeast Asia: implications for control and elimination. *Advances in Parasitology* **108**, 47–131.

Blaszkowska J, Kurnatowski P and Damiecka P (2011) Contamination of the soil by eggs of geohelminths in rural areas of Lodz district (Poland). *Helminthologia* **2**, 67–76.

Castillo DC and Paller VGV (2018) Occurrence of *Angiostrongylus cantonensis* in rodents from the rice granary of the Philippines and associated risk factors for zoonotic transmission. *Journal of Parasitic Diseases* **42**, 350–356.

Ćirković V, Uzelac A, Klun I and Djurković-Djaković O (2017) Protozoa as contaminants of surface water – methods of detection. *Water Resources Management* **7**, 45–51.

De Leon WU, Monzon RB, Aganon AA, Arceo RE, Ignacio EJ and Santos G (1992) Parasitic contamination of selected vegetables sold in metropolitan Manila, Philippines. *Southeast Asian Journal of Tropical Medicine and Hygiene* **23**, 162–164.

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

Eraky MA, Rashed SM, Nasr ME, El-Hamshary AMS and El-Ghannam AS (2014) Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. *Journal of Parasitology Research* **2014**, 613960.

Ferreira FP, Caldart ET, Freire RI, Mitsuka-Breganó R, De Freitas FM, Miura AC, Mareze M, Martins F, Urbano MR, Seifert AL and Navarro IT (2018) The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. *Brazilian Journal of Veterinary Parasitology* **27**, 327–337.

Gupta N, Khan DK and Santra SC (2009) Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Food Control* **20**, 942–945.

Gyawali P, Khanal S and Soares Magalhaes RJ (2013) Helminth infections in an indigenous community of Nepal: the role of individual and household socio-economic factors. *Global Journal of Medical Research Diseases* **13**, 33–39.

Hassan AA, Oyebamiji DA and Idowu OF (2017) Spatial patterns of soil-transmitted helminths in soil environment around Ibadan, an endemic area in south-west Nigeria. *Nigerian Journal of Parasitology* **38**, 179–184.

Horiuchi S and Uga S (2016) Modified flotation method, an effective technique for recovering helminth eggs in soil. *Parasitology International* **65**, 576–579.

Horiuchi S, Paller VGV and Uga S (2013) Soil contamination by parasite eggs in rural village in the Philippines. *Tropical Biomedicine* **30**, 495–503.

Jourdan PM, Lamberton P, Fenwick A and Addiss DG (2018) Soil-transmitted helminth infections. *Lancet (London, England)* **391**, 252–265.

Lo CLC, Fernandez DAP, de Luna MCT, de Guia APO and Paller VGV (2021) Diet, parasites, and other pathogens of Sunda leopard cats (*Prionailurus javanensis* Desmarest 1816) in Aborlan, Palawan Island, Philippines. *Journal of Parasitic Diseases* **45**, 627–633.

Luz JGG, Barbosa MV, De Carvalho AG, Resende SD, Dias JVL and Martins HR (2017) Contamination by intestinal parasites in vegetables marketed in an area of Jequitinhonha Valley, Minas Gerais, Brazil. *Brazilian Journal of Nutrition (Revista de Nutricao)* **30**, 127–136.

Maffei DF, Batalha EY, Landgraf M, Schaffner DW and Franco BDGM (2016) Microbiology of organic and conventionally grown fresh produce. *Brazilian Journal of Microbiology* **47**, 99–105.

Maikai BV, Baba-Onoja EBT and Elisha IA (2013) Contamination of raw vegetables with cryptosporidium oocysts in markets within Zaria Metropolis, Kaduna State, Nigeria. *Food Control* **31**, 45–48.

Ordoňez K, Paller VGV, Goh XT and Lim Y (2016) Parasite contamination of vegetables from selected organic and conventional farms in Laguna and Benguet Province, Philippines, Pertanika. *Journal of Tropical Agricultural Sciences* **41**, 1–20.

Owada K, Lau CL, Leonardo L, Clements ACA, Yakob L, Nielsen M, Carabin H and Soares Magalhães RJ (2018) Spatial distribution and populations at risk of *A. lumbricoides* and *T. trichiura* co-infections and infection intensity classes: an ecological study. *Parasite Vector* **11**, 1–13.

Paller VGV and Babia-Abion S (2019) Soil-transmitted helminth (STH) eggs contaminating soils in selected organic and conventional farms in the Philippines. *Parasite Epidemiology and Control* **7**, e00119.

Paller VGV and De Chavez ERC (2014) Toxocara (Nematoda: Ascaridida) and other soil-transmitted helminth eggs contaminating soils in selected urban and rural areas in the Philippines. *The Scientific World Journal* **2014**, 386232.

Soares Magalhães RJ, Salamat MS, Leonardo L, Gray DJ, Carabin H, Halton K, McManus DP, Williams GM, Rivera P, Saniel O, Hernandez L, Yakob L, McGarvey ST and Clementis ACA (2015) Mapping the risk of soil-transmitted helminthic infections in the Philippines. *PLOS Neglect Tropical Diseases* **9**, 1–15.

Su GLS, Mariano CMR, Matti NSA and Ramos GB (2012) Assessing parasitic infestation of vegetables in selected markets in Metro Manila, Philippines. *Asian Pacific Journal of Tropical Diseases* **2**, 51–54.

Torgerson PR and Macpherson CN (2011) The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary Parasitology* **182**, 79–95.

Tudor P (2015) Soil contamination with canine intestinal parasites eggs in the parks and shelter dogs from Bucharest area. *Agriculture and Agricultural Science Procedia* **6**, 387–391.

Uga S, Hoa NT, Noda S, Moji K, Cong LD, Aoki Y, Rai SK and Fujimaki Y (2009) Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. *Nepal Medical College Journal* **11**, 75–78.

USEPA (2001) *Cryptosporidium: Drinking Water Health Advisory*. Washington, D.C., USA: United States Environmental Protection Agency, pp. 1–31.

Vizon KCC, Battad ZG and Castillo DSC (2019) Contamination of food-borne parasites from green-leafy vegetables sold in public markets of San Jose City, Nueva Ecija, Philippines. *Journal of Parasitic Diseases* **43**, 651–657.

Woldetsadik D, Drechsel P, Kerait B, Itanna F, Erko B and Gebrekidan H (2017) Microbiological quality of lettuce (*Lactuca sativa*) irrigated with wastewater in Addis Ababa, Ethiopia and effect of green salads washing methods. *International Journal of Food Contamination* **4**, 3.

Ybañez RHD, Resuelo KGJ, Kintanar APM and Ybañez AP (2018) Detection of gastrointestinal parasites in small-scale poultry layer farms in Leyte, Philippines. *Veterinary World* **11**, 1587–1591.

Zibaei M, Bahadory S, Cardillo N and Khatami AR (2017) Soil contamination with eggs of Toxocara species in public parks of Karaj, Iran. *International Journal of Enteric Pathogens* **5**, 45–48.

https://doi.org/10.1017/S0031182021002031 Published online by Cambridge University Press