

Parasite Contamination of Freshly Harvested Vegetables from Selected Organic and Conventional Farms in The Philippines

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ABSTRACT

Vegetables are considered as an important part of a healthy diet. However, there have been reports showing contamination of vegetables with parasites. This study aimed to assess parasite contamination of freshly harvested vegetables from selected organic and conventional farms in the Philippines. A total of 252 freshly harvested vegetables were collected from 20 farms through systematic random sampling and were processed by means of sedimentation technique. Positive samples were subjected to molecular analysis for further identification of species. Results showed that 58 out of 252 (23.02%) vegetable samples were contaminated with parasites eggs/cysts/oocysts. The parasites found were *Ancylostoma ceylanicum*, *Toxocara* sp., *Trichuris trichiura*, *Ascaris suum*, *Hymenolepis* sp., unknown trematode egg, *Iso spora*, *Balantidium*, *Giardia intestinalis* and *Cryptosporidium*. *Ascaris suum* had the highest contamination rate in organic and conventional farms at 13.09% and 8.33%, respectively. *Cryptosporidium* (≥ 800 oocysts/kg) and *Giardia intestinalis* (≥ 240 cysts/kg) had the highest mean density in both farms. Also, lettuce showed the highest contamination rate among the sampled vegetables in both types of farms. Furthermore, results revealed that texture of vegetables, distance to the soil substrate, and farming practices could possibly contribute to the parasite contamination of

vegetables in this study. These findings have important implications on public health that may aid regulatory agencies for prevention and control strategies for food safety.

Keywords: Farming practices, food-borne parasites, food safety, Philippines, vegetables

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INTRODUCTION

Philippines is a largely agriculture-based country where 13M hectares are devoted to agriculture, 6.1 M hectares of which are highly suitable for cultivation (Carating, Fernando, Abrina, & Tejada, 2010). There are two types of farming systems that are mainly practiced in the Philippines: organic and conventional farming. Though there have been differences in these farming practices, both farm types aim to produce large quantities of crops, such as vegetables. However, there have been reports showing vegetable contaminated with parasites that maybe associated with farming practices (Abe, Ajah, Ayuba, Mogaji, & Ekpo, 2016; Matini, Shamsi-Ehsan, & Maghsood, 2016; Mohamed, Siddig, Elaagip, Edris, & Nasr, 2016).

The aforementioned gap led the researchers to assess the parasite contamination of vegetables and to associate this with the different farming practices from selected organic and conventional farms in the Philippines.

MATERIALS AND METHODS

Study Sites

The study sites were two provinces in the Northern and Southern Luzon, Philippines, which produced and supply large quantities of fresh produce within the regions. The present study includes 20 farms, ranging from 1 to 2.5 hectares; 14 of which are from Northern Luzon (7 organic and 7 conventional farms) and six are from Southern Luzon (3 organic and 3 conventional farms).

Sampling Method

A total of 252 vegetable samples were freshly harvested from organic and conventional farms of the selected farms in Northern and Southern Luzon Provinces. The collection period in both types of farms was done during dry season (November to March). Systematic random sampling was used in harvesting vegetable samples. One sample unit of vegetables was equivalent to 250g. Samples were placed in individual plastic bags, labeled and placed in a cooler box (4 ° C). The samples were transported to the laboratory for immediate processing.

Recovery and Identification of Parasites from Vegetables

Pooled samples were processed through sedimentation technique as described by Nazemi, Raei, Ameri and Chaman (2011). Approximately 100µl of the sediment from each sample was examined for parasites' eggs/cysts/oocysts under a compound microscope (Olympus, Tokyo, 100x – 400x magnification) (Uga et al., 2009). For positive samples, the number and developmental stage of detected parasite were recorded. Another 100µl from the sediment was also used for immunofluorescence assay in examining *Cryptosporidium* and *Giardia* using *Crypto/Giardia* detection kit (Cellabs Pty. Ltd., Brookvale, New South Wales, Australia), following manufacturer's instructions. Prepared slides were scanned at 400x and 1000x under an epifluorescence microscope.

Molecular Characterization of Parasites

The vegetable samples that were positive with *Ascaris*, *Trichuris*, hookworm, *Giardia*, and *Cryptosporidium* were subjected to molecular identification. Different protocols and gene markers were used for each parasite. For all PCR reactions, positive and negative controls (distilled water) were included. Confirmation of parasites identification at the species level is important to provide information on the source of contamination whether from animal or human feces.

DNA Extraction

The eluate (approximately 200µl) from samples positive for *Ascaris*, *Trichuris*, hookworm, *Giardia*, and *Cryptosporidium* were extracted using Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Inc) following manufacturer's instructions.

Multiplex PCR for the detection of soil-transmitted helminths

The protocol of Phuphisut et al. (2014) were modified to obtain the target genes for *Ascaris*, *Trichuris* and hookworm. The target gene for *Ascaris* is COI (Fw: 5' GGSGGTTTTGGGTCTTTGG 3'; Rw: 5' CCAAACAAGGTAGCCAACCA 3') which amplified 192 bp; 18S (Fw: 5' CTGCGAGGATTGACAGATCA 3'; Rw: 5' GTACAAAGGGCAGGGACGTA) amplified 498 bp for *Trichuris* and; ITS1 (Fw: 5' ATGCTTGGCAAGAGTCGTTT 3'; Rw: 5' TGTGGCGTCCACACATATT 3') amplified 330bp for hookworm. The primary reaction (20 µl) consisted of the following: 10 µl Premix (X-Prime Taq

Premix 2x), 1 µl for each primer, 1µl of dH₂O and 3 µl of DNA template. The PCR thermocycling program was modified having 94°C for 5 minutes, followed by 30 cycles of denaturing for 30 sec at 94°C, annealing for 30 sec at 55°C and extension for 30 at 72°C, followed by a final extension at 72°C for 7 minutes.

Semi-nested PCR for Hookworm Speciation

The semi-nested protocol of Ngui, Lee, Tan, Roslan and Lim (2012) for hookworm speciation was modified to obtain the ITS region. All the positive eluate samples for hookworm in multiplex were subjected to semi-nested PCR for hookworm speciation. The primary PCR reaction used the forward primer, NC1 (5' ACG TCT GGT TCA GGG TTC TT-3') and reverse primer, NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3'), which amplified 310 and 420 bp amplicons. The primary reaction (20 µl) consisted of the following: 10 µl Premix (X-Prime Taq Premix 2x), 2 µl for each primer, 3µl of dH₂O and 3 µl of DNA template. The PCR thermocycling program was as follows: 94°C for 5 minutes, followed by 30 cycles of denaturing for 30 sec at 94°C, annealing for 30 sec at 55°C and extension for 30 sec at 72°C, followed by a final extension at 72°C for 7 minutes.

The samples that produced the 310 and/or 420 bp amplicons were subjected to a second amplification. An amplicon of 250 bp for *Necator* and 130 bp for *Ancylostoma* were amplified using the forward primer NA (5' ATG TGC ACG TTA TTC ACT-3') for *Necator americanus* and AD1 (5'-

CGA CTT TAG AAC GTT TCG GC-3') for *Ancylostoma* sp. NC2 was used as the common reverse primer for both species. The secondary reaction (20 µl) consisted of the following: 10µl of Premix (X-Prime Taq Premix 2x), 2 µl for each primer, 1µl of dH₂O and 3 µl of the first PCR product. The thermocycling program for the secondary PCR were as follows: 94°C for 5 mins, followed by 40 cycles of denaturing for 1 min at 94°C, annealing for 1 min at 55°C and extension for 1 min at 72°C, followed by a final extension at 72°C for 7 minutes.

Nested PCR for the Detection of *Giardia* Species

Detection of *Giardia* was carried out targeting the TPI gene by the protocol used by Sulaiman et al., (2003). The forward and reverse primers used were AL3543 (5'-AAATIATGCCTGCTCGTCG-3') and AL3546 (5'-CAAACCTTITCCGCAAACC3'), which amplified the 605 bp amplicon. The primary reaction (20 µl) consisted of 10 µl of Premix (SolGent™ 2x *h-Taq* PCR Smart Mix), 1 µl for each primer, 5 µl of dH₂O and 3 µl of the DNA template. The PCR thermocycling program were: 94°C for 5 minutes, followed by 40 cycles of denaturing for 45 sec at 94°C, annealing for 45 sec at 50°C and a final extension at 72°C for 10 minutes.

For the secondary reaction, an amplicon of 530 bp was amplified using the forward and reverse primers: AL3544 (5'-CCCTTCATCGGIGGTA ACTT3') and AL3545 (5'-GTGGCCACCACICCCGTGCC3'), respectively. The secondary reaction (20

µl) consisted of 10 µl of Premix (SolGent™ 2x *h-Taq* PCR Smart Mix), 1 µl for each primer, 5 µl of dH₂O and 3 µl of the first PCR product. The secondary PCR thermocycling program used the same modification with the primary PCR reaction.

Nested PCR for the Detection of *Cryptosporidium* Species

The protocol used by Nichols, Campbell and Smith (2003) was modified to obtain the SSU rRNA gene that was the target gene for the nested PCR protocol in detecting *Cryptosporidium*. The forward and reverse primers used were N-DIAGF2 (5'-CAA TTG GAG GGC AAG TCT GGT GCC AGC-3') and N-DIAGR2 (5'-CCT TCC TAT GTC TGG ACC TGG TGA GT-3'), which amplified the 655 to 677 bp amplicons. The primary reaction (20 µl) consisted of 10 µl of Premix (X-Prime Taq Premix 2x), 1 µl for each primer, 5 µl of dH₂O and 3 µl of the DNA template. PCR thermocycling was performed as follows: 95°C for 5 mins, followed by 35 cycles of denaturing for 30 sec at 94°C, annealing for 1 minute at 68°C and extension for 30 sec at 72°C, followed by a final extension at 72°C for 10 minutes.

Meanwhile the protocol of Johnson, Pieniasek, Griffin, Misener and Rose (1995) was modified for the secondary reaction, wherein an amplicon of 435 bp was amplified using the forward and reverse primers: CPB-DIAGF (5'-AAG CTC GTA GTA GTT GGA TTC TG-3') and CPB-DIAGR (5'TAA GGT GCT GAA GGA GTA AGG-3'), respectively. The

secondary reaction (20 µl) consisted of 10 µl of Premix (X-Prime Taq Premix 2x), 1 µl for each primer, 3 µl of dH₂O and 5 µl of the first PCR product. PCR thermocycling was performed as follows: 95°C for 5 minutes, followed by 35 cycles of denaturing for 30 sec at 94°C, annealing for 1 min at 60°C and extension for 30 sec at 72°C, followed by a final extension at 72°C for 10 minutes.

Sequencing and Analysis of Sequence Data

The primary and secondary amplifications were incubated in the MyCycler thermal cycler (Bio-Rad, Hercules, USA). PCR amplicons were then analyzed by 2% agarose gel-electrophoresis at 100V for 50 minutes (multiplex for STH), 90V for 35 minutes (hookworm speciation and *Giardia* species) and 100V for 40 minutes (*Cryptosporidium* species). Positive bands were excised from the gel and purified using QIAquick Gel Extraction Kit (Qiagen, Germany), according to the manufacturer's instructions, and sent to Genomics BioScience and Technology Co., Ltd. Malaysia for bidirectional sequencing. Sequences were aligned and checked manually using BioEdit (version 7.1.11) (Applied Biosystem, UK).

Documentation of Farm Practices and Hygiene

Farm practices were documented through survey interviews of farmers and farm owners during the sample collection of vegetables. The purpose of this documentation was to determine the farming practices that might affect the contamination rate of parasites

on vegetables. Informed consent was taken from the respondents before the survey was conducted.

Statistical Analysis

Contamination rate (%) was calculated as the number of positive samples divided by the number of total vegetables sampled multiplied to 100. Mean density was calculated as the total number of parasites divided by the total number of positive samples. The density of parasite's eggs/cysts/oocysts in contaminated vegetables were converted to per kg. For instance, one parasite egg/cyst/oocyst in 0.1ml (1 drop) of the concentrate would have 20 parasite eggs/cysts/oocysts in 2 ml (from a 250g pooled vegetable sample unit) and 80 parasite eggs/cysts/oocysts per kg vegetable. Independent sample t-test was used to assess the difference between the contamination rates of vegetable samples from organic and conventional farms; Moreover, the association between contamination rates and farming practices were analyzed using Point-Biserial correlation. All statistical analyses were done at 95% level of significance.

RESULTS AND DISCUSSION

Identification of Parasites from Vegetables

Parasites were detected in 58 out of 252 (23.02%) vegetable samples collected from selected organic and conventional farms in Northern and Southern Luzon Provinces. The parasites observed microscopically were hookworm, *Toxocara* sp., *Trichuris* sp., *Ascaris* sp., *Hymenolepis diminuta*,

unknown trematode egg, *Isospora* sp., *Balantidium coli*, *Giardia* sp. and *Cryptosporidium* sp. (Figure 1).

The samples that were positive for parasites were subjected to molecular analyses. Out of 58 vegetable samples that have undergone DNA extraction and PCR amplification, 16 showed the expected bands. BLAST results of the obtained sequences for parasites are summarized in Table 1. Species of parasites confirmed via molecular techniques include *Ancylostoma ceylanicum*, *Trichuris trichiura*, *Ascaris suum* and *Giardia intestinalis*. *Cryptosporidium* was not identified to species level which could be due to the low *Cryptosporidium* DNA concentration from the samples.

Parasite Contamination Rates in Vegetables between Organic and Conventional Farms

Among the parasites identified, *Ascaris suum* had the highest contamination rate in both organic and conventional farms at 13.10% and 8.33%, respectively. On the other hand, *Cryptosporidium* sp. and *Giardia intestinalis* have the highest mean density with ≥ 800 oocysts and ≥ 240 cysts per kg in both farms respectively (Table 2).

Ascaris suum showed the highest contamination rate in organic and conventional farms which could be attributed to the presence of pigs in the farm. According to the farmers, pig manure is commonly used in organic farms while chicken manure is a widely used supplement with chemical fertilizers in conventional farms. Abe et al. (2016), Adamu, Adamu and Mohammed (2012), Elom, Eze, Nworie and

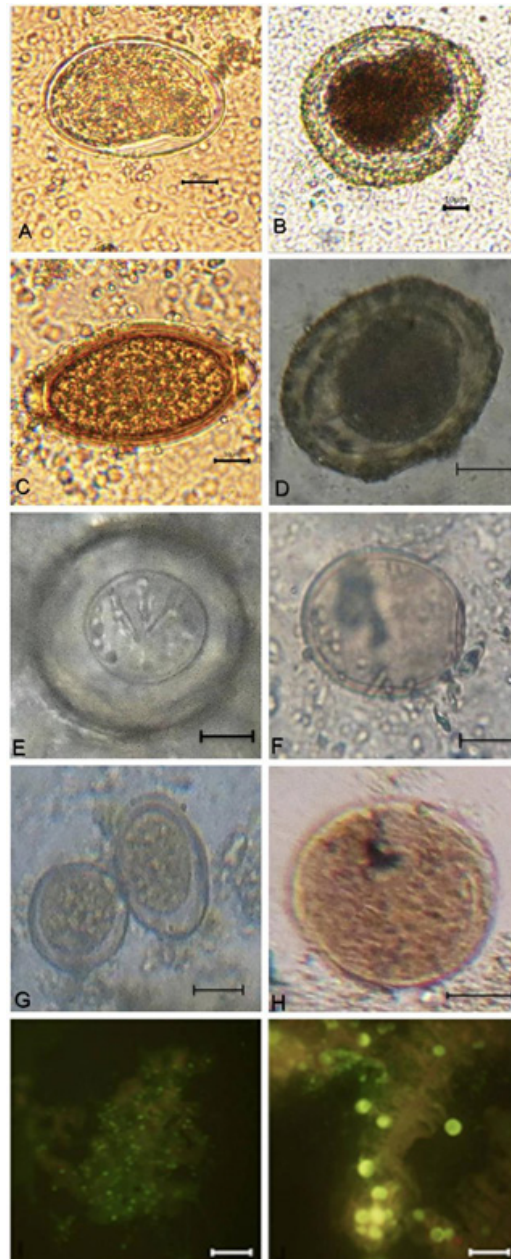


Figure 1. Eggs and cysts recovered from vegetables in organic and conventional farms (A) Hookworm/strongylid (B) *Toxocara* sp. (C) *Trichuris* sp. (D). *Ascaris* sp. (E) *Hymenolepis* sp (F) Fluke (G) *Isospora* (H) *Balantidium* (I) *Cryptosporidium* (J) *Giardia* (scale bar: 10 μ m)

Table 1

BLAST results of sequences of parasites recovered in positive vegetable samples

Vegetable Samples Code	Closest Match		Identity (%)
	Accession No. From Genbank	Species Name	
CGI6	JN120881.1	<i>Ancylostoma ceylanicum</i>	100
LT14-GI3	KF279134.1	<i>Ancylostoma ceylanicum</i>	100
HW	JN120871.1	<i>Ancylostoma ceylanicum</i>	98
LT21-GI2	LC036567.1	<i>Ancylostoma ceylanicum</i>	98
CLR1B	KF279134.1	<i>Ancylostoma ceylanicum</i>	98
LT10-GI3a	HQ452519.1	<i>Ancylostoma ceylanicum</i>	98
CLRIA	KC632568.1	<i>Ancylostoma ceylanicum</i>	98
LT10-GI2a	JQ673421.1	<i>Ancylostoma ceylanicum</i>	97
LT10-GI4	LC036567.1	<i>Ancylostoma ceylanicum</i>	97
LT14-GI4	LC036567.1	<i>Ancylostoma ceylanicum</i>	97
LT1-GI1	KR075936.1	<i>Giardia intestinalis</i>	99
LT10-DR	JQ863257.1	<i>Giardia intestinalis</i>	99
LT10-GI1	KR075936.1	<i>Giardia intestinalis</i>	98
CGI4	X54253.1	<i>Ascaris suum</i>	99
CLR2	AB591802.1	<i>Ascaris suum</i>	99
CGI2	AB699090.1	<i>Trichuris trichiura</i>	99

Akpotomi (2012) and Klapac and Borecka (2012) observed the same results, having *Ascaris* as the most prevalent parasite in their studies. *Ascaris* species is known for its thick shell that allows it to withstand the adverse environmental conditions and thus, can survive longer in the environment.

Meanwhile, *Toxocara* was found to be present only in organic farms while high density of hookworm (*Ancylostoma ceylanicum*) was also recorded in organic farms, where presence of stray dogs and

cats was a common sight. Previous studies showed that *T. canis* and *T. cati* were the most prevalent parasites in stray dogs (69.5%) and cats (24.2%), respectively (Borecka, 2005; Klapac & Borecka 2012). Contamination of vegetables were also linked to the presence of stray dogs and cats in the West of Iran (0.8%) and in Mexico, City (33.3%) (Matini et al., 2016; Vázquez, Martinez-Barbabosa, Tay Zavala, Ruiz Hernandez & Perez Torres, 1997).

Table 2

Contamination rate and mean density of parasites recovered from vegetables in organic and conventional farms according to types of parasites

Parasites	Organic Farms			Conventional Farms		
	Contamination Rate (%)	Mean Density (eggs/cysts/oocysts per kg)	SD	Contamination Rate (%)	Mean Density (eggs/cysts/oocysts per kg)	SD
Protozoans						
<i>Balantidium coli</i> ¹	2.98	80	40	-	-	-
<i>Isospora</i> sp. ¹	3.57	160	0	1.19	400	
<i>Giardia intestinalis</i> ¹	0.59	~240	-	2.38	~240	113.14
<i>Cryptosporidium</i> sp. ²	1.19	~800	56.57	5.95	~800	230.94
Cestodes						
<i>Hymenolepis diminuta</i> ³	0.59	240	-	1.19	80	-
Trematodes ³	0.59	80	-	-	-	-
Nematodes						
<i>Ascaris suum</i> ³	13.10	80	55.21	8.33	160	92.38
<i>Ancylostoma ceylanicum</i> ³	9.52	160	120.44	2.38	80	0
<i>Toxocara</i> sp. ³	2.98	320	0	-	-	-
<i>Trichuris trichiura</i> ³	0.59	160	-	-	-	-

~ estimation (too many to count) ¹cysts ²oocysts ³eggs

- no SD value, as parasites were only found in only one sample

The presence of parasites such as *Giardia intestinalis* and *Cryptosporidium* may be due to the contaminated water source for the crops. Some of the farms were located near sewers which could be an ideal source of parasitic protozoans. The use of contaminated water for watering crops could be a potential source of contamination with these parasites. Hajjami, Ennaji, Fouad, Oubrim and Cohen (2013) reported that untreated and treated wastewater with even just one helminth egg/L must not be used in irrigations for green leafy vegetables because these are commonly eaten raw.

Hymenolepis diminuta (organic farms: 0.59%; conventional farms: 1.19%) and *T. trichiura* (organic farms: 0.59%; conventional farms: 0%) have the lowest contamination rate among the detected parasites. The presence of *H. diminuta* in some farms could be due to the sporadic occurrence of rats, as affirmed by the farmers. While the presence of *Trichuris* in organic farms might be due to the fertilizers from pig's feces that the farmers were using. Humans are the principal host of *T. trichiura* though pigs have also been reported to be infected (Stephenson, Holland & Cooper, 2000).

Meanwhile, among the different types of vegetables, deep red lettuce was found to have the highest contamination rate among the vegetables. The current data concurred with the study in Sudan, Benha, Egypt and Alexandria, Egypt (Eraky, Rashed, Nasr, El-Hamshary & El-Ghannam, 2014; Mohamed et al., 2016; Said, 2012). Texture

and distance of the vegetable in soil may have a great impact on the contamination of vegetables. Rough, highly textured surfaces with deep crevices would be more likely to harbour soil, with the possible consequence of increased numbers of parasitic organisms. Also, vegetables closer to the ground could be more susceptible to contamination with helminth eggs especially during heavy rains and floods (Mohamed et al., 2016; Omoyawe & Falola 2012; Said, 2012).

In addition, this study revealed that organic farms (23.81%) showed higher contamination rate than conventional farms (21.43%), albeit not significantly different ($p > 0.05$) (Table 3). Gharavi, Jahani and Rokni (2002) also reported that in Tehran, there was a relatively low parasitic contamination in farms that uses chemical fertilizers. Conversely, a study in Poland showed that conventional farms had higher contamination rate than organic farms (Klapec & Borecka, 2012). Most of the organic farms in the current study have fences around their vegetable plots to avoid the entrance of stray animals, however, the use of animal manure as fertilizer could be potential source of contamination. On the other hand, most of the conventional farms in this study were smaller in scale than the organic farms, making it easier for the former to manage and control possible contamination.

Table 3

Contamination rate of parasites in organic and conventional farms according to types of vegetables

Vegetables	Organic Farms			Vegetables	Conventional Farms		
	Samples Examined	No. Positive	%		Samples Examined	No. Positive	%
Leafy vegetables							
Broccoli	8	0	0	Broccoli	8	0	0
Cabbage	8	0	0	Cabbage	-	-	-
Camote tops	4	1	25	Camote tops	-	-	-
Kangkong	4	0	0	Kangkong	-	-	-
Lettuce varieties							
Deep Red	12	9	75	Deep Red	8	4	50
Green Ice	24	11	46	Green Ice	12	4	33
Ice Berg	16	6	38	Ice Berg	-	-	-
Romaine	16	7	44	Romaine	12	3	25
Singkang	12	0	0	Singkang	4	0	0
Onion Leaves	8	0	0	Onion Leaves	4	0	0
Parsley	8	1	13	Parsley	-	-	-
Polonchai/Pechay	4	1	25	Polonchai/Pechay	4	0	0
Spinach	4	0	0	Spinach	-	-	-
Taro	4	1	25	Taro	-	-	-
Fruit vegetables							
Eggplant	8	0	0	Eggplant	-	-	-
Luffa	8	2	25	Luffa	8	2	25
Okra	4	0	0	Okra	4	1	25

Table 3 (Continue)

Vegetables	Organic Farms			Vegetables	Conventional Farms		
	Samples Examined	No. Positive	%		Samples Examined	No. Positive	%
Fruit vegetables							
Squash	-	-	0	Squash	4	0	0
Strawberries	4	1	25	Strawberries	4	1	25
String beans	-	-	-	String beans	4	1	25
Tomato	4	0	0	Tomato	4	0	0
Root crop							
Carrot	8	0	0	Carrot	4	2	50
Total	168	40	23.81	Total	84	18	21.43

Association between Farming Practices and Parasite Contamination in Vegetables

Farming practices observed in both organic and conventional farms were associated with the parasite contamination rates of vegetables. Statistical analysis revealed that only the presence of toilet facilities ($p = 0.020$), deworming of farmers and farm animals ($p = 0.026$) and presence of animals in or near the farm ($p = 0.025$) showed significant relationship with the presence of parasites on vegetables (Table 4).

It was observed that some farms have clean toilets with available water, while some farms have toilets but do not have enough access to water for cleaning, some others do not have toilets in the farms. There was also no proper sewage system

observed in most of the sampled farms, increasing the chance of contamination. It was stated that infectious organisms may be present in human or animal by-products and if these were not properly disposed, it might become a source of contamination, especially to crops that are planted near to it (McLaughlin, 2012; Nazemi et al., 2011).

Improper handling of farm produce by farmers could also be a source of contamination. Farmers are commonly using gloves only during application of chemical fertilizers. Also, some farmers were observed washing their hands in water buckets used for watering crops. Meanwhile, deworming of animals also showed significant relationship with vegetable contamination in farms ($p = 0.026$). Since it is not a habitual practice to deworm farm

and companion animals, it is likely that these may increase the risk for parasite contamination in the environment. Several studies also revealed that animal feces was a common cause of contamination of vegetables by intestinal parasites (Idahosa, 2011; McLaughlin, 2002).

Table 4

Association of farming practices with organic and conventional farms in northern and southern Luzon provinces

Farming Practices	Northern (N=14)		Southern (N=6)		<i>p</i> value*
	Organic (n=7)	Conventional (n=7)	Organic (n=3)	Conventional (n=3)	
Fertilizers used					
Processed manure with inoculants	7	7	2	3	0.452
pure manure	0	0	1	0	
Water sources					
district tap water	7	7	3	1	0.441
mountain spring	0	0	0	2	
Hygiene					
Toilet facilities					
Present	3	1	2	1	0.020
Absent	4	6	1	2	
<i>Using gloves during planting, harvesting, treating of soil</i>					
Yes	2	1	2	2	
No	5	6	1	1	0.071

Table 4 (Continue)

Farming Practices	Northern (N=14)		Southern (N=6)		<i>p</i> value*
	Organic (n=7)	Conventional (n=7)	Organic (n=3)	Conventional (n=3)	
Hygiene					
Washing of hands before and after eating, planting, harvesting, treating of soil					
Yes	7	7	1	0	0.410
No	0	0	2	3	
Deworming of farmers and farm animals					
Yes	0	0	1	0	0.026
No	7	7	2	3	
Farm animals in or near the farm					
Present	4	0	3	3	0.025
Absent	3	7	0	0	

* $p > 0.05$ not significant

Raising farm animals inside or near the farm could also contribute to differences in levels of contamination between farms ($p = 0.025$). Animal feces maybe converted as fertilizers or may directly contaminate the soils in farms. Heavy rains and flooding may also aggravate the contamination of vegetables in the farms. Also, the farmers themselves could step on the feces and might bring soiled shoes or slippers into the planting area.

CONCLUSION

This study revealed parasite contamination of freshly harvested vegetables in both organic and conventional farms. Thus, consumers should be conscientious with food preparation, such as thorough washing of freshly harvested vegetables and fruits from farms. Also, the role of various farming practices underscores the dynamics of parasite transmission and contamination in agricultural setting. Hence, measures should be taken to mitigate the impacts of foodborne parasites from the source to the consumers. Measures should also be emphasized regarding health and well-being of all those who work in food supply systems and of the animals and plants destined for human consumption. It is essential that there are standards by which food can be produced in a safe manner. As these guidelines and regulatory documents are developed and revised, food producers should pay attention to the best scientific information that reduces risks for parasites in food.

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