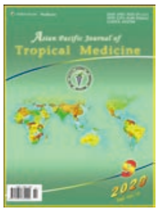

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Soil-transmitted helminth egg contamination from soil of indigenous communities in selected barangays in Tigaon, Camarines Sur, Philippines

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ABSTRACT

Objective: To provide baseline data on the prevalence and intensity of soil-transmitted helminthiasis egg contamination in the soil among indigenous communities.

Methods: A total of 317 soil samples from three barangays of indigenous communities communities in Tigaon, Camarines Sur, Philippines were examined for soil-transmitted helminthiasis egg contamination using optimized sugar flotation method.

Results: Of the soil samples examined, 141 (44.48%) were contaminated by *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp. with cumulative prevalence varying across the study sites (P<0.01). *Ascaris* spp. was predominant in all study sites, followed by *Toxocara* spp. and *Trichuris* spp. with a prevalence of 41.96%, 7.57%, and 5.36%, respectively. Interestingly, *Toxocara* pp. has the highest intensity of contamination, followed by *Ascaris* spp. and *Trichuris* spp. in term of geometric mean soil-transmitted helminthiasis eggs recovered per one gram soil sample (34.25, 21.45, and 11.85 respectively). Each study site harbors significant amount of soil-transmitted helminthiasis eggs and zoonotic *Toxocara* eggs, which present high risk of soil-transmitted helminthiasis infection, particularly among children observed to play and cohabitate with animals known to be hosts of these parasites.

Conclusions: The alarming rate of soil-transmitted helminthiasis and *Toxocara* egg contamination reported in this study suggests that additional measures should be undertaken to control soil-transmitted helminthiasis and zoonotic intestinal infections in the country.

KEYWORDS: Indigenous peoples; Soil-transmitted helminths; *Ascaris*; *Toxocara*; *Trichuris*; Public health

1. Introduction

Soil-transmitted helminthiasis (STH) is one of the neglected tropical diseases (NTD) which is predominant in tropical and subtropical countries affecting more than one billion people particularly in places where populations live in poverty, without adequate sanitation, and in close contact with infectious vectors, domestic animals, and livestock[1]. This STH is a group of intestinal parasites that infect humans when fertilized eggs are ingested by *Ascaris (A.) lumbricoides* and *Trichuris (T.) trichiura* or when infective larvae penetrate exposed skin [hookworms, particularly *Ancylostoma (A.) duodenale* and *Necator (N.) americanus*][2]. Along with other zoonotic parasites such as *Toxocara* spp. and animal hookworms that are recently reported to crossover in humans[3,4], part of their life cycle always involves a transmissive stage in the environment, and once an infected individual defecates or if the feces of an infected person are used as fertilizer, eggs are deposited on soil which further increases the risk of infection[5, 6].

Indigenous peoples (IPs) are considered more prone to diseases such as STH infections, malaria, dengue, malnutrition, and other

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skin diseases[6]. In the Philippines, IPs constitute approximately 9% or 8.1 million of the country's total population and in Bicol Region, the IP group that predominantly thrive in the area are locally called the "Agtas of Mt. Isarog." Compared to the general population, they are marginalized, left behind, and are commonly found clustering in remote areas where basic health and social services are seldom delivered, thus raising the need to provide health data for this particular group[7]. Staggering cases of STH infection accounts to 97% prevalence among Aeta children[8]. Consistent with these findings, STH prevalence observed among IP children is significantly higher than in non-IP children[9-11].

Due to limited access to water and lack of toilet or latrine, it is estimated that 15% of the world's population practice open defecation, which consequently increases the risk of STH transmission and reinfection in the community[12]. Open defecation remains the predominant norm and remains a risk factor in the spread of diarrheal diseases causing morbidity and mortality among children and even adults[13]. To respond to these challenges, the government is reinforcing its efforts in raising awareness through implementation of projects and programs and in turn move towards achieving the Sustainable Development Goals (SDG).

Since STH eggs are predominantly transmitted through open defecation in the soil, analysis for soil contamination of STH eggs offer a much higher chance of recovery and detection compared to examination of water, food contaminated samples, and other environmental matrices. Also, soil examination offers a noninvasive way of estimating the risk of STH infections in community level and reinforce data from human fecal examination to represent STH infection prevalence in a wider scale to collectively provide a deeper understanding of STH occurrence, transport, survival, and risk of transmission to potential hosts[14,15].

This study was conducted to report the presence of parasite egg contamination in the soil of the selected Indigenous Community in Tigaon, Camarines Sur, Philippines.

2. Materials and methods

2.1. Description of study site and population

The Municipality of Tigaon (13°630'981''N, 123°477'066''E) is located in the eastern part of Camarines Sur, Bicol Region in Southern Luzon, Philippines. It has a total land area of 125.75 km^2 that lies between Mt. Isarog and the Lagonoy Gulf, 80% of which is flat and mostly irrigated farmlands. There are 6 out of 23 barangays in the Municipality of Tigaon with identified cluster of Agtas of Isarog (IP) namely; Brgy. Gubat, Brgy. Tinawagan, Brgy. Cabalinadan, Brgy. Coyaoyao, Brgy. Libod and Brgy. Consocep. The Local Government Unit (LGU) of Tigaon was informed and verbal approval from IP leaders concerned were obtained. IP household number, sanitary toilet coverage data were acquired from the LGU office.

2.2. Collection of samples

A total of 317 soil samples (approximately 250 g each) were collected once in October 2018 from the three barangays, specifically from areas: (1) where people or children converge such as front yard, loiter area and playground area, and (2) identified open defecation site, particularly near isolated bushes or trees, and beside unused or unimproved latrines. Sample size (n) was computed based on the prevalence rate (p) of 71% for STH eggs in soil[15] with 5% margin of error (e) and at 5% significance level (z = 1.96).

$$n = \frac{p\,(1-p)\,Z_{\alpha}^{2}}{e^2} = \frac{0.71(1-0.71)1.96^2}{0.05^2} \approx 317$$

Also, to avoid over representation, a 3-meter distance interval was followed in collecting soil samples per site. A shovel was used to collect soil samples three meters apart with a depth of 10 cm, placed in properly labelled airtight ziplock bags and transported into the laboratory for drying. Soil samples were air dried for 24 h and sieved using 150 μm mesh to filter and remove small twigs, debris, and other larger particles that can interfere with STH recovery and detection. Collected samples were brought to De La Salle University Science and Technology Research Center for laboratory analysis.

2.3. Detection and recovery of helminth eggs in soil

Sucrose centrifugal flotation method developed by Horiuchi *et al.*[15] and Uga *et al.*[16] in STH egg detection in soil in rural areas of the Philippines was utilized in this study. The choice of this method is based on its replicability and cost-effectiveness for use in low resource settings where the impact of STH is highest. Two grams of dried, sieved soil sample were suspended in 15 mL centrifuge tube filled with 10 mL of Tween-80 solution and subsequently centrifuged at 500 × *g* for 10 min. Afterwards, supernatant from each sample was discarded and sediment was filled with 10 mL of sucrose solution with specific gravity of 1.200. This was then homogenized using a vortex mixer and centrifuged again at 1 400 × *g*; 5 min and 500 × *g*; 10 min time-speed variations. Aliquot from the surface of the supernatant was transferred to two replicate slides for microscopic observation. Recovered STH eggs were identified using pictorial morphological guide from the World Health Organization Bench Aids for the Diagnosis of Intestinal Parasites[17].

2.4. Quality control and biosafety procedure

To ensure the accuracy and reliability of the parasitic assessment, quality measures employed include proper collection of samples, utilization of fresh reagents, and execution of appropriate laboratory techniques. The counting and identification of the STH eggs were accompanied by cross examination of a parasitologist. Data are double encoded in Microsoft Excel software to reduce errors and ensure accuracy of collected data.

As part of the biosafety procedure, all personnel who helped in the collection of soil specimen have attended an orientation on the conduct of the field and laboratory procedures and were equipped with precautionary biosafety equipment. Also, all soil samples and specimens were autoclaved after the experiment for waste disposal in accordance with the Laboratory Biosafety Manual, 3rd edition[18].

2.5. Data analysis

The prevalence of STH egg in soil per site was calculated by getting the number of samples that tested positive with STH egg, then dividing the number with the total number of soils collected. The STH egg density was defined as STH eggs recovered per one gram (EPG), was computed by the following equation[19] based on two aliquot slides (0.3 mL sample per aliquot) examined per sample with 2 g in 10 mL flotation solution . Thus, to get the EPG, the actual egg count of the replicate was combined and multiplied by 10.

$$\text{EPG}=\frac{\text{Weight of soil sample}+\text{Volume of flotation solution}}{\text{Weight of soil sample}\times\text{Measurement volume used}}=\frac{2+10}{2\times 0.3\times 2}\approx 10$$

The overall egg density was expressed as geometric mean egg counts of the eggs per gram (GMEC EPG) soil. The GMEC EPG values (mean ± standard deviation) of the three study sites were compared using one-way analysis of variance. The level of significance was set to 5%. Statistical analyses were performed using STATA software (STATA version 14.0, Stata Corp, College Station, TX).

2.6. Ethical considerations

This research was reviewed and approved following the existing institutional ethical guidelines of De La Salle University (ethics approval no. ST.001.2018-2019.T1.COS), and was coordinated with the Local Government Unit of Tigaon and had secured the approval of each IP chieftain in three barangays.

3. Results

Out of the 317 collected samples, 141 were tested positive for at least one helminth egg contamination, giving a cumulative prevalence of 44.48%. Prevalence of STH egg contamination was highest in Tinawagan (70.59%, 60/85), followed by Consocep (53.33%, 40/75), and Gubat (26.11%, 41/157). Prevalence varied significantly across study sites (P=0.000, χ^2=855.739).

Helminth eggs isolated from the soil samples resemble eggs of *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp. The occurrence of these parasites was common in all study sites. *Ascaris* was found to be the most abundant and varied significantly across study sites, with prevalence accounting 94.33% (133/141) of the total positive cases (P=0.000, χ^2=207.769) (Table 1). Prevalence of *Ascaris* spp. was highest in Tinawagan (44.36%, 59/133), followed by Gubat at (28.57%, 38/133), and Consocep (27.07%, 36/133). *Toxocara* spp. contamination was recorded second highest with 17.02% (24/141) of the total positive cases and varied significantly across study sites (P=0.000, χ^2=229.685). *Toxocara* spp. contamination was the highest in Tinawagan (41.67% 10/24) followed by Consocep (37.50%, 9/24) and lowest in Gubat (20.83%, 5/24).

Trichuris spp. shown to be the least prevalent (12.05%, 17/141) and does not vary significantly across study sites (P=0.707, χ^2=0.108). Prevalence was highest in Gubat (52.94%, 9/17), followed by Consocep and Tinawagan with the same prevalence (23.53%, 4/17). Intensity of each STH parasite contamination per site was also examined. *Toxocara* spp. has the highest intensity of contamination, followed by *Ascaris* spp. and *Trichuris* spp. in terms of GMEC EPG (34.25, 21.45, and 11.85 respectively). The overall GMEC EPG was 25.07 EPG. Intensity of STH egg contamination was highest in Tinawagan with 40.51 EPG, followed by Consocep with 18.64 EPG, and lastly in Gubat with 16.10 EPG. The GMEC EPG varied significantly across study sites (P=0.000, χ^2=85573.935).

Table 1. Cumulative prevalence and geometric mean egg counts per gram soil of soil-transmitted helminthiasis egg contamination in each study site.

STH egg contamination	Overall (n=317		Consocep (n=75)		Gubat (n=157)		Tinawagan (n=85)	
	Prevalence [n(%)]	GMEC EPG	Prevalence [n(%)]	GMEC EPG	Prevalence [n(%)]	GMEC EPG	Prevalence [n(%)]	GMEC EPG
Cumulative	141(44.48)	25.07±7.00	40 (53.33)	18.64±4.86	41(26.11)	16.10±4.56	60 (70.59)	40.51±7.27
Ascaris spp.	133 (94.33)	21.45±5.55	36 (27.07)	17.38±4.66	38 (28.57)	14.06±4.16	59 (44.36)	32.02±6.78
Toxocara spp.	24 (17.02)	34.25±10.51	9 (37.50)	12.60±3.87	5 (20.83)	10.00±3.53	10 (41.67)	155.91±16.22
Trichuris spp.	17 (12.05)	11.85±4.03	4 (23.53)	10.00±3.65	9 (52.94)	12.77±4.61	4 (23.53)	11.89±4.08

4. Discussion

The present study provided a baseline data on the contamination of STH eggs in the soil of IP communities in Camarines Sur. All three sites tested positive for three STH genera, namely: *Ascaris* spp., *Toxocara* spp. and *Trichuris* spp. based on microscopic examination of egg morphology. *Ascaris* spp. and *Trichuris* spp. are the common intestinal parasites that infect humans while *Toxocara* spp. is a zoonotic species that has the potential to cross-infect humans. Given that almost half of the total soil samples were contaminated with parasite eggs, the level of contamination agrees with the fecal examination survey on intensity of STH infections in Bicol Region, wherein Camarines Sur is listed as one of the high-risk areas with cumulative prevalence of more than 70% and/or an overall proportion of heavy intensity infection exceeding 10%[20]. Similarly, studies conducted in Bangladesh and Kenya[21], Iran[22], Croatia[23], and Nigeria[24] have also reported high cases of STH egg contamination in the soil. However, compared to a similar survey in the Philippines on STH egg contamination in soil which reported 71% prevalence[15], the result of the study is relatively lower.

The distribution of STH eggs across study sites show a significant difference in terms of overall prevalence. Soil contamination was highest in Tinawagan followed by Consocep and Gubat. *Ascaris* was the most prevalent STH found in all study sites, however, in terms of intensity *Toxocara* was the highest. In general, these findings suggest that IPs in these barangays face an extremely high risk of infection by any of these parasitic helminths mainly because of observed unsanitary lifestyle such as practice of open defecation and close contact with domesticated animals and free roaming swine and local environmental condition (geographical features related to moisture content and soil type; and soil contaminated with dog or cat feces). Although the GMEC EPG of each STH egg differ significantly among identified species, single and multiple contamination with *Ascaris* spp., *Toxocara* spp. and *Trichuris* spp. across study sites remain a notable risk considering that these species follow a hand to mouth infection route and are easily dispersed in communities with poor sanitary conditions. Cultural practices such as walking barefoot and eating without proper washing of hands remain an issue that may further increase the risk of harboring *Ascaris* along with other STH infection among IPs. On the subject of rampant open defecation of children, it was observed that not all who defecate in the open lack latrine in their household. In fact, some parents admitted that it is more convenient for them to let children defecate in the backyard or on play areas when they feel the urge to do so, while in some cases the children are afraid to defecate in the latrines for fear of suctioned by it that is why they do not use them. This anecdotal information collectively supports that the lack of Water, Sanitation, Hygiene (particularly, the widespread practice of open defecation) and proper education can lead to the persist of widespread STH egg contamination in the soil. Furthermore, based on the anecdotal reports of IP chieftains, water supply lines were cut-off on areas that are affected by political conflicts leading to the scarcity of water in the area.

Prevalence of STH infections among indigenous groups in the Philippines has reached an alarming point. STH infection among IP children was reported to be significantly higher than non-IP children. Consistent with other results, such findings signify that these group of people are at higher risk of morbidity to STH infection and needs more attention[10,25]. Results of this study showed high level of single and multiple STH egg contamination in all study sites, which support findings on Aeta children with reported 97.4% STH prevalence[8], where each child having at least one STH infection, *T. trichiura* being the most prevalent followed by A. lumbricoides and hookworms.

High density of STH egg contaminating the soil increases the risk of further reinfection. Given the DOH programs for helminth control and elimination, such as the mass drug administration (MDA) of school children ages 1-12, there are still reports of STH infection among post dewormed children. In fact, coinfection of *A. lumbricoides* and *E. vermicularis*, and single infection of *T. trichiura*, *A. lumbricoides*, and *E. vermicularis* were still reported[26]. It is highly probable that the reinfection is possible when children spend more time in contact with the soil which harbors great number of these ubiquitous STH eggs. In fact, 74% cumulative prevalence of STH infection, with single and mixed infections are strongly associated with children having close contact with pets and livestock in the community[27].

In this study, *Ascaris* being the most prominent parasite confirms with several epidemiological surveys conducted on indigenous communities[8-10]. High intensity and widespread distribution of *Ascaris* eggs could be attributed to its adept morphology and resilience to adverse environmental conditions which ensures viability for a longer period. Since these eggs are microscopic and sticky, they easily adhere to almost anything without being noticed. Even raw fruits and vegetables can be contaminated when washed with water from unknown source or fertilized with contaminated night soil[28]. Therefore, soil contamination with STH eggs is a major risk factor in the spread of STH infection.

Trichuris egg contamination was considerably lower than that of *Ascaris* and *Toxocara* egg contamination. This could be explained by the vulnerability of these *Trichuris* eggs to the same ecological conditions given that their morphology (wall thickness) may not provide the same protection compared to other STH. Also, lower rate of soil contamination with *Trichuris* eggs observed might be due to their minimal dispersion as a single female *Trichuris* lay relatively less numbers eggs (2 000 egg per day)[5]. Thus, a few eggs would mean less chance of survival and delicate walls make eggs more vulnerable to inactivation and desiccation.

There is paucity of published reports on zoonotic parasitic infections in the Philippines, particularly among Indigenous Peoples Communities. The presence of *Toxocara* eggs with highest intensity in all study sites calls for an urgent attention to Local Government public health providers in the area. Approximately 35 *Toxocara* eggs are found per gram of soil samples collected. This could be associated to the cohabitation of IPs with their dogs and cats in addition to free-roaming swine raised in backyards which may harbor other parasites such as *Ascaris* suum. Toxocariasis is one of the STH infections considered as an important disease in humans which involves symptomatic conditions resulting from larva migrans. *Toxocara canis* and *T. cati* which are commonly found in dogs and cats, respectively, are parasites known to cause this disease. In general, children are reported to have higher chances of contacting wide range of zoonotic STH infection when left to play in areas where cats and dogs defecate[29]. The staggering rate of *Toxocara* egg contamination reported in this study suggest additional measures to control zoonotic infections in the country in addition to current STH infection control programs by the DOH.

The Integrated Helminth Control Program implemented by the DOH to address STH infection in the Philippines is still a work in progress. Part of the prevention and elimination campaign is the certification of each barangay to have a Zero Open Defecation status. Through coordination with each Local Government Unit, using Community-Led Total Sanitation approach members of each barangays are motivated to participate when an ocular inspection is conducted. The certification for each barangay for ZOD status follows criteria set by the DOH. Although it is a decent step towards prevention of STH infection, the certification procedure lacks scientific basis to support the claim of ZOD free status in each barangay. Since high prevalence of STH egg contamination in the soil is highly associated with deliberate practice of open defecation, it is advisable that soil examination for STH egg detection should be integrated in the certification process. The consideration of environmental aspect in monitoring and assessment of the efficiency of DOH programs in these areas would offer a better chance of addressing the problem on high prevalence of STH infection especially among IPs. Whereas soil examination for STH egg presence clarify the risk of infection among residents more directly and offers a less invasive approach compared to fecal examination, this method supports other approaches used in epidemiological studies to have wider perspective on the extent of contamination and strategically approach the problem with high STH infection in the country.

This study provided a baseline data on the presence of STH eggs contaminating the soil of three IP communities in Tigaon, Camarines Sur. Geared towards reinforcing the effort of the DOH in their campaign for helminth infection prevention and elimination, this study show high level of STH contamination in all study sites with almost half of the samples collected positive for at least one STH egg. The hand-to-mouth route of infection poses high risk of reinfection among IPs in all study sites particularly in children who were observed to play and live in close contact with animals. Since the prevalence of STH infection remains a major health concern among IPs, it is recommended that health services in the IP communities should be improved and ensured. Thus, proper allocation of resources for sanitary improvement should be focused on areas that are reported to harbor high cases of STH egg contamination in the soil. Provision of adequate water supply and sanitary toilet in the area should be considered. Also, more emphasis should be placed on information dissemination about the diseases to raise awareness and proper hygiene and sanitation in the IP communities. Along with monitoring of STH prevalence among IPs and their domesticated animals, the use of this technique to report the environmental contamination of parasites will complete the data that could be used in predictive modeling, risk analysis and mapping of STH contamination in vulnerable communities.

Conflict of interest statement

The authors declare no conflict of interest in this study.

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Authors' contributions

JCD and MCF conceived and designed the study. JCD and MCF were responsible for literature search and screening. JCD and MCF were responsible for data collection. DES, JCD and MCF were responsible for data analyses. JCD, MCF, DPS, JIJ, and VYB contributed to data interpretation. JCD and MCF drafted the manuscript and JCD, MCF, DES, JIJ, and VYB critically revised the manuscript.

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