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Temporal and spatial patterns of *Trichuris trichiura* eggs: a potential threat to human health in Pakistan

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Summary

This study investigates the presence of *Trichuris trichiura* eggs in soil samples collected from urban areas in Lahore, Pakistan. A total of 3600 soil samples were collected over two years from Lahore's urban regions. The detection of helminth eggs in these samples was performed using sodium hypochlorite (NaOCl) as a diagnostic technique. The study reveals an overall prevalence rate of *T. trichiura* at 0.97 % (35 out of 3600) in the contaminated soil samples from Lahore's slum areas. When analyzing the data by geographical areas, the study found the highest prevalence of *T. trichiura* in Allama Iqbal Town (1.83 %, 11 out of 600), followed by Samanabad (1.16 %, 7 out of 600), Wapda Town (1.00 %, 6 out of 600), Gulberg (1.00 %, 6 out of 600), and Cantt (0.50 %, 3 out of 600). Conversely, Valencia Town had the lowest prevalence rate at 0.33 % (2 out of 600). However, these variations in prevalence rates were not statistically significant ($p = 0.117$). Prevalence rates of *T. trichiura*'s eggs varied significantly across different sampling seasons ($p>0.001$). In autumn, a total of 900 soil samples were collected, with 19 samples (2.11 %) testing positive for *T. trichiura*. This rate was notably higher compared to the prevalence rates observed in winter, spring, and summer, which were 0.66 %, 0.22 %, and 0.88 %, respectively. Regarding the sampling months, the study observed a significantly higher prevalence during September (3.33 %, 10 out of 300), followed by October (2.33 %, 7 out of 300), and August (1.33 %, 4 out of 300). Prevalence rates gradually decreased in other months, ranging from 1 % to 0.33 % (3 to 1 out of 300), with no parasite detection in March (0 %, 0 out of 300) ($p < 0.001$). This research underscores soil contamination due to fecal waste and highlights public unawareness of parasite biology, driven by open defecation practices.

Keywords: Trichuriasis; *Trichuris trichiura* eggs; contaminated soil; prevalence; spatio-temporal assessment; Pakistan

Introduction

Soil Transmitted Helminths (STHs) infections afflict a staggering two billion people worldwide (Ortega *et al.*, 2010; Caldrer *et al.*, 2022). These infections persist predominantly in human communities characterized by low Socio-Economic Status (SES), inad-

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equate sanitation, limited access to healthcare services (World Health Organization, 2012; Kelechi *et al.*, 2015), close clustering of family members (Nematian *et al.*, 2009), deficient personal hygiene practices (Mishra *et al.*, 2008; Garn *et al.*, 2022), habits such as pica (World Health Organization, 2006), nail biting, and neglecting hand hygiene before eating and after defecation (Khanum *et al.*, 2010; Garn *et al.*, 2022). These factors collectively foster the prevalence of trichuriasis (Rana & Pokhrel, 2023).

Trichuris trichiura (*T. trichiura*) is a highly prevalent intestinal infection found in humans, as well as in canines and felines on a global scale (Varkey *et al.*, 2007; Fallatah *et al.*, 2010; Alelign *et al.*, 2015). First documented by Linnaeus in 1771, *T. trichiura* is a Soil Transmitted Helminth commonly found in regions characterized by hot and humid climates, particularly endemic in tropical and subtropical areas (World Health Organization, 2012), where inadequate sanitation practices are prevalent (Silva *et al.*, 2011), and socio-economic conditions may be suboptimal (Nkengazong *et al.*, 2010). This human whipworm, *T. trichiura*, is a roundworm that takes up residence in the large intestine of humans, causing the ailment known as trichuriasis.

Trichuriasis exhibits lower incidence rates in urban areas compared to slums, largely attributable to superior infrastructural facilities, particularly in the realms of sanitation and sewage systems (Garn *et al.*, 2022). Infection occurs when the host inadvertently ingests embryonated eggs present in the environment, typically via the fecal-oral route (Maipanich, 2008). These ingested eggs hatch within the small intestine, liberating stichosomal larvae, which subsequently mature into adult worms. These adult worms predominantly migrate to the large bowel, specifically the cecum and the ascending colon (Fallatah *et al.*, 2010). Within this habitat, the female worm is prolific, producing between 2,000 to 10,000 eggs per day, which are then excreted through feces into the soil. Under stable temperature conditions (around 24°C), these eggs reach their infective stage within a span of 54 days; however, in fluctuating temperatures ranging from 6°C to 24°C, the development period extends to 210 days (World Health Organization, 2012).

The diagnosis of intestinal trichuriasis is commonly accomplished by detecting the presence of its eggs in fecal samples (Lee *et al.*, 2016). Approximately 2 – 3 weeks after being deposited in the soil, these eggs undergo the process of embryonation, becoming infective (Damen *et al.*, 2010). These eggs, once deposited in the soil, become a source of contamination, spreading to water sources, adhering to vegetables (Gupta *et al.*, 2009), infiltrating food through airborne means, and clinging to the wings and legs of insects like house flies and cockroaches (Maipanich, 2008). Fecal contamination stands out as the primary risk factor, particularly for the health of children (Veesenmeyer, 2022).

From the initial infection to the maturation of adult worms (prepatent period), it takes approximately 60 days within the host's body (Ichhpujani & Bhatia, 2003). Trichuriasis can manifest in various distressing symptoms including weight loss, rectal prolapse, bloody diarrhea, malnutrition, epigastric and abdominal pain when the worms reside in the cecum. It can also lead to appendicitis when the worms are present in the appendix. Additionally, geophagia (the habit of eating soil) and anemia are common signs among children aged 1 – 12 years old (Silva *et al.*, 2011; Caldrer *et al.*, 2022).

Trichuriasis is particularly prevalent in preschool children, often resulting in severe anemia in infected individuals (Hagel & Giusti, 2010). Children face an elevated risk of infection due to their outdoor play habits, which are most common in the 2 – 7 year age group, where pica, the consumption of non-food items, is also more frequent (Al-Mekhlafi *et al.*, 2008). Erlanger *et al.* (2008) reported cases of malnutrition in 5-year-old children due to *T. trichiura* infection. The prevalence of trichuriasis tends to be higher in preschool children who may neglect hygiene practices due to poor sanitary facilities (Rim *et al.*, 2003; Garn *et al.*, 2022).

Numerous surveys have been conducted across different cities in Pakistan to gauge the prevalence of *T. trichiura* in soil samples contaminated by infected carriers of the parasite, whether human or animal. Previous research efforts have been somewhat limited in scope, with data scattered across various studies. Reported prevalence rates of *T. trichiura* in humans or soil samples have varied, such as 2.4 % in Lahore (Qureshi, 1995), 1.6 % in Islamabad (Jamil *et al.*, 1999), 4.1 % in Islamabad and Larkana (Shaik *et al.*, 2000; Chaudhry *et al.*, 2004), 1.0 % in Muzzafarabad (Kamran *et al.*, 2005), and as high as 19.1 % in Karachi and Swat (Khan *et al.*, 2012).

It is worth noting that the majority of individuals in the secondary phase of parasite intestinal infections are asymptomatic (Ezeamama *et al.*, 2005), and carriers often go undetected, posing a significant threat to potential outbreaks. The lifespan of adult worms within the human intestine typically ranges from 2 to 7 years (World Health Organization, 2012). Colonoscopy has proven to be a valuable diagnostic tool for trichuriasis (Ok *et al.*, 2009; Khurana *et al.*, 2021).

Hence, the present study aims to comprehensively address the spectrum of prevalence of *T. trichiura* in soil samples from Lahore's slum areas, Pakistan, providing valuable insights to researchers and assisting the government in implementing measures to combat parasite infestations and associated diseases, particularly by improving SES in affected regions.

Materials and Methods

Selection of study regions and soil sampling

In accordance with a predetermined flowchart, specific zones and the number of sampling sites and samples per month were carefully chosen for this study. Emphasis was placed on selecting sampling areas that included backyards of houses, public parks, and crop fields if they were in proximity to the designated locality. This choice was guided by the fact that manure, fertilizers, and night soil were commonly used as fertilizers in these areas. Notably, in certain urban regions such as Valencia, Allama Iqbal City, and large sections of Wapda City, significant construction activities were ongoing, with daily laborers working on-site and utilizing temporary toilets, including “gurkies.” Moreover, open areas were often used for defecation. Families resided on these sites, and children played barefoot alongside their pets, including dogs, cats, hens, and more, due to the absence of proper sanitation facilities. Often, a single bucket of water served both as a drinking source for animals and as a means of cooling during hot weather. As a result, these urban areas were selected for inclusion in the study, alongside more densely populated urban areas like Gulberg, Cantt, and Samanabad.

Soil sampling methodology and laboratory processing

The study involved the collection of a total of 3600 soil samples, with 600 samples gathered from each of the six urban areas (Allama Iqbal Town, Wapda Town, Valencia Town, Cantt, Gulberg, and Samanabad) in Lahore, Punjab, Pakistan. Sampling was conducted over the course of two years, from November 2010 to October 2012, with 150 samples collected per month. This timeframe was divided into the four seasons: summer, winter, spring, and autumn. Each sampling site yielded multiple samples, with soil collected from five different depths (2 cm, 4 cm, 6 cm, 8 cm, and 10 cm). This depth variation was necessary to observe the presence of parasite eggs, particularly during rainy periods when overflow water could introduce leachate into the soil. To ensure accurate tracking, the collected soil samples were carefully stored in screw-capped plastic bottles, each clearly labeled with the date and place of collection. All samples were promptly transported to the Parasitology Laboratory at the University of the Punjab, Lahore, Pakistan for further analysis and evaluation.

Detection of helminth eggs

To ascertain the presence of helminth eggs, the collected soil samples underwent examination on the same day, employing the Sodium hypochlorite recovery technique, as outlined by the World Health Organization (1991). In this method, 2 grams of a sifted and laboratory-dried soil sample were combined with 5 milliliters of a 30 % sodium hypochlorite (NaOCl) solution, following the procedure detailed by Soulsby (1982). The mixture was intermittently agitated until a uniform and homogeneous supernatant blend of soil was achieved. Ten microliters of the prepared solution were subsequently positioned on a glass slide, covered with a cover glass, and examined under a microscope at 40x magnification. The identification of helminth eggs was based on their morphological characteristics, in accordance with the descriptions provided by Urquhart *et al.* (2001).

Fig. 1. Photographs showing some sampling sites: (a) Samanabad, (b) Gulberg, (c) Cantt, (d) Allama Iqbal Town.

Statistical analysis

Exact confidence intervals (CI) for prevalence rates at the 95 % level were calculated. Comparison of the prevalence of *T. trichiura* infection among different areas, seasons, and months were performed by using the Epi Info 6.01 software (CDC, Atlanta). Observed differences were considered to be statistically significant at a 0.05 threshold value.

Ethical Approval and Informed Consent

The experimental procedures and protocols in this study were approved by the Ethical Research Committee of Abdul Wali Khan University, Madran, Pakistan, and informed consent was obtained from all participants before collecting soil samples in urban areas.

Results

Presence of T. trichiura's eggs at different depths

No eggs were detected at the shallowest depth of 2 cm, just beneath the surface layer. Similarly, no eggs were found at the subsequent depth of 4 cm. It was only at the fifth depth, located 10 cm below the surface layer, that a slightly softer and humid soil condition conducive to the survival of parasitic eggs was observed. Consequently, the presence of eggs was exclusively noted at this fifth depth of 10 cm.

Overall prevalence rate of T. trichiura's eggs

The overall prevalence rate of *T. trichiura*'s eggs in the soil was calculated to be 0.97 %, which translates to 35 positive samples out of the total 3600 collected from urban areas (Table 1).

Prevalence rates of T. trichiura's eggs by sampling areas

Prevalence rates of *T. trichiura*'s eggs varied by sampling areas. The highest prevalence was observed in Allama Iqbal Town at 1.83 % (11 out of 600 samples), followed by Samanabad at 1.16 % (7 out of 600 samples), Wapda Town at 1.00 % (6 out of 600 samples), Gulberg at 1.00 % (6 out of 600 samples), and Cantt at 0.50 % (3 out of 600 samples). In contrast, the lowest prevalence rate was recorded in Valencia Town at 0.33 % (2 out of 600 samples). However, observed differences in prevalence rates among these areas were statistically insignificant (p = 0.117) (Table 1).

Prevalence rates of T. trichiura's eggs by sampling seasons and months

Prevalence rates of *T. trichiura*'s eggs varied significantly across different sampling seasons. In autumn, a total of 900 soil samples were collected, with 19 samples testing positive for *T. trichiura*, resulting in a prevalence rate of 2.11 %. This rate was notably higher compared to the prevalence rates observed in winter, spring, and summer, which were 0.66 %, 0.22 %, and 0.88 %, respectively. The differences in prevalence rates among these seasons were statistically significant (p < 0.001) (Table 2). Additionally, the prevalence rates of *T. trichiura*'s eggs displayed variations according to the sampling month. The highest cumulative prevalence was observed in September, with 3.33 % (10 out of 300 samples) testing positive for the parasite. This was followed by October at 2.33 % (7 out of 300 samples), and August at 1.33 % (4 out of 300 samples). Subsequent months exhibited lower prevalence rates, ranging from 1 % to 0.33 % (3 out of 300 samples), with a complete absence of the parasite in March, recording 0 % (0 out of 300 samples). The differences observed in prevalence rates among these months were statistically significant (p < 0.001) (Table 2).

Discussion

The transmission of human diseases by helminth parasites through contaminated soil poses a significant public health concern. Soil infested with STHs, such as *T. trichiura*, serves as a potential source of these diseases, often leading to chronic morbidity and impairment. Parasitic infestations and resulting diseases represent a substantial public health challenge in developing countries, especially in regions like Pakistan characterized by low SES, limited parental education, and prevalent unsanitary conditions (Welch *et al.*, 2017). The findings of our study differ from previous observations in Mazaferabad (1 %) (Chaudhry *et al.*, 2004) and Lahore (6.20 %) (Maqbool *et al.*, 2007), both located within Pakistan. These variations can likely be attributed to differences in

Table 1. Prevalence rates of *Trichuris trichiura*'s eggs, in overall and according to sampling urban areas, in soil samples of urban areas of Lahore, Punjab, Pakistan.

Sampling urban areas	Number of soil samples	Infested	Prevalence (%±C.I.[1])	P value
Allama Iqbal Town	600	11	1.83±0.009	0.117
Wapda Town	600	6	1.00±0.007	
Valenchia Town	600	2	0.33±0.003	
Cantt	600	3	0.50±0.005	
Gulberg	600	6	1.00±0.007	
Samanabad	600	7	1.16±0.007	
Total	3600	35	0.97±0.003	

Abbreviations:
[1]: C.I.: 95% confidence interval

Table 2. Prevalence rates of *Trichuris trichiura*'s eggs, in overall and according to sampling seasons and months, in soil samples of urban areas of Lahore, Punjab, Pakistan.

Sampling seasons or months [1]	Number of soil samples	Infested	Prevalence (%±C.I.[2])	P value
Autumn	900	19	2.11±0.009	0.000*
Winter	900	6	0.66±0.005	
Springer	900	2	0.22±0.003	
Summer	900	8	0.88±0.005	
September	300	10	3.33±0.019	0.000*
October	300	7	2.33±0.017	
November	300	2	0.67±0.009	
December	300	3	1.00±0.011	
January	300	2	0.67±0.009	
February	300	1	0.33±0.005	
March	300	0	0.00±0.000	
April	300	1	0.33±0.005	
May	300	1	0.33±0.005	
June	300	2	0.67±0.009	
July	300	2	0.67±0.009	
August	300	4	1.33±0.013	
Total	3600	35	0.97±0.003	

Abbreviations: [1]: Sampling months or seasons for the two sampling years, [2]: C.I.: 95% confidence interval, *: Statistically significant test.

site selection, fluctuations in climatic conditions, and the utilization of distinct techniques for recovering eggs from the soil (Khurana *et al.*, 2021).

In our study, we utilized the conventional microscopic diagnostic technique known as the sodium hypochlorite technique to detect parasite eggs in soil samples. This method is widely acknowledged as one of the simplest and most reliable techniques for this type of diagnosis (Urquhart *et al.*, 2001). It is noteworthy that our prevalence rate differs from that reported in Australia (Speare *et al*., 2006), where a higher rate was estimated (68.4 %). This disparity can likely be attributed to variations in diagnostic techniques employed and differences in geographical distribution. The prevalence rate of eggs in the soil serves as an indicator of fecal pollution, primarily originating from unsanitary defecation practices in slum areas. Inhabitants, particularly in slums, often practice open defecation in fields or in depressions created within their own homes, commonly referred to as "gurkies." This unhygienic behavior significantly contributes to soil contamination (Welch *et al.*, 2017).

In urban areas, particularly in regions such as Allama Iqbal Town, Valencia, and Wapda Town, elevated prevalence rates are associated with ongoing construction activities and the presence of transient populations, including nomads and daily wage laborers, who reside in these areas for livelihood purposes. These transient residents often resort to open defecation practices. Additionally, these communities tend to live in close-knit family setups and may not prioritize personal hygiene. The feces they deposit not only contaminate the soil but also adhere to vegetables such as carrots, spinach, salads, radishes, coriander, mint, and sugar cane, as well as other herbs grown in nearby fields (Else *et al.*, 2020).

Inadequately constructed toilets, often lacking doors, are common in these areas, especially within buildings under construction that lack proper sewage systems. Water supply is typically limited to a single tap or hand pump, and a shared bucket of water serves multiple purposes, including bathing and utensil washing. Due to the lack of proper sanitation facilities, pets have easy access to the toilet areas and often drink from the same buckets of water. To escape the heat, these animals are also drawn to these spots. These observations are consistent with those made by Esfandiari (1995). This unsanitary environment fosters soil infestation and infection in humans, particularly among children who exhibit herding behavior. Farmers who use soil as fertilizer, especially at night, contribute to an increased prevalence of ascariasis infestation in children who play barefoot in these fields. Alarmingly, 90 % of these children engage in geophagia (eating soil), thumb-sucking, and nail-biting behaviors. Insects like house flies and cockroaches, as well as puppies or stray dogs that frequent these contaminated areas, may carry *T. trichiura*'s eggs on their fur. When children come into contact with these animals and then fail to wash their hands before meals or after defecation, they risk ingesting the eggs. Younger children, in particular, are less discerning and may consume dropped food items, such as bread, biscuits, or bananas, from contaminated soil using their unclean hands (Veesenmeyer, 2022). This escalating contamination issue aligns with the observations of Steinmann *et al.* (2010). Disturbingly, even the youngest children were seen with flies covering their nipples and teats when they rested on wooden rope beds (khats) in open areas. These makeshift cribs were frequently used by the children, often without any intervention from their mothers. Parents, in general, seemed unaware of the critical importance of hygiene and lacked knowledge about the biology of parasites. Many held misconceptions, believing that parasites were the natural consequence of residing in their bodies, whether as ectoparasites or endoparasites. These findings echo those of Tengku *et al.* (2014).

Soil contamination levels were notably exacerbated during the rainy season, as water played a significant role in both soil contamination and the transmission of eggs from one location to another. The presence of moisture in the soil provides favorable conditions for the growth of helminths, as essential ions required for egg development are present in the soil, facilitating hatching. Similarly, a depth of 10 cm of moist soil was found to be infested with STHs' eggs, corroborating the observations made by Rai *et al.* (2000). Children were also observed playing in ponds, potentially ingesting infected eggs through the fecal-oral route or by handling contaminated soil with unwashed hands, consistent with the findings of Paller and de Chavez (2014) in the Philippines.

The data reveal a notable pattern in the prevalence rates of *T. trichiura*'s eggs across different seasons. Particularly, autumn, with its characteristic environmental conditions, witnessed the highest and statistically significant prevalence rates, especially during the month of September. The gradual decrease in prevalence rates from autumn to spring, notably in March, corresponds to environmental factors such as decreasing temperature and humidity during this period. These environmental changes likely render the eggs unable to hatch, resulting in their dormancy within the soil. Consequently, the decline in prevalence rates during spring suggests a temporary decrease in the transmission potential of the parasites, as the dormant eggs await favorable conditions for further development and transmission to their final host (Turgeon *et al.*, 2018).

The presence of stray or pet dogs and cats, which often defecate in open areas, significantly contributes to soil contamination. This is supported by the findings of Areekul *et al.* (2010), who observed high rates of parasitic infections, particularly *Trichuris* spp., in dogs. Their study revealed a significant proportion of dog stool samples containing Trichuris eggs, indicating the potential for environmental contamination through fecal matter. Dogs, being common in urban and rural areas, frequently roam freely and deposit feces in various outdoor locations, including parks, streets, and residential areas. Similarly, cats also contribute to soil contamination with their fecal matter. This behavior aligns with the observations of Mizgajska-Wiktor and Jarosz (2007), who highlighted the role of domestic animals, including dogs and cats, in contaminating soil with parasitic eggs. Therefore, considering the high prevalence of parasitic infections in dogs and their habit of defecating in open areas, it is plausible to suggest that dogs, along with cats and rodents (Jones, 2021), serve as significant sources of soil contamination, posing a potential risk of disease transmission to humans.

The differences found in the prevalence of intestinal helminth infestation observed among the six urban localities are statistically non-significant, despite the disparities in SES between regions. This observation contradicts a study conducted in Ethiopia (Alelign *et al.*, 2015). Following counseling sessions and educational outreach involving union council leaders and heads of families in the studied slums, as well as the provision of informative banners and cards, and the establishment of free medical and homeopathic camps, the local residents gradually recognized the value of life and the importance of adopting hygienic practices to safeguard themselves from helminth infections and diseases. These insights echo the findings of Alemu *et al.* (2011).

Conclusion

The present study underscores the pervasive issue of soil contamination with *T. trichiura* resulting from fecal waste disposal practices. Soil contamination is poised to worsen further as a consequence of open defecation and a general lack of awareness regarding the biology of parasites among the population. To reduce the transmission of infections within slum areas, it is imperative for the government to take proactive measures aimed at improving sanitation infrastructure. Local authorities should rigorously enforce ordinances related to deworming programs, with a particular focus on educational institutions. Recognizing that this is a pressing public health concern, the government should also consider incorporating hygiene and parasitology education into academic curricula. Additionally, the development and implementation of control and prevention strategies for parasitic infections are of utmost importance. Furthermore, educators should play a pivotal role in instilling good hygiene practices, particularly emphasizing the importance of handwashing, especially after defecation and before meals, among children. The data collected in this study can serve as a valuable resource for pinpointing specific areas of infection and informing targeted interventions aimed at preventing further loss of human resources to parasitic infections.

Conflicts of interest

There are no conflicts of interests.

Consent for publication

Authors are providing a consent to the publisher to publish their manuscript upon acceptance, along with obtaining written consent from the inhabitants of the studied regions to capture photographs of themselves and their premises.

Data Availability

All data that were generated or analysed during this study are included in this published article.

Author's Contributions

SAA designed this study, collected soil samples, performed experimental work in the laboratory and wrote the manuscript. SAA and MBS carried out statistical analysis. SN, NN, AK, SS, LA-M, WAS, MAZ, JF and MBS edited and finalized the manuscript. All authors approved the final version of the manuscript for submission.

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