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Prevalence of soil-transmitted helminth in soil irrigated with wastewater in Sharkia Governorate, Egypt with a reference to the seasonal variation

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ABSTRACT

The objective of this study was to assess the extent of parasitological contamination of soil irrigated with wastewater. Samples were randomly collected including soil, and water samples from which soils were watered. Samples were taken from different locations in Sharkia governorate, in the east of Nile Delta, Egypt. Such locations were irrigated from water canals, namely El-Ganabia, Sherwida, El-mahmodyia, El-Senety, and Saft Zerek. The samples were collected monthly during September 2021 to September 2022. More than half of the collected samples (60.7%) tested significantly positive for parasite contamination. The largest proportion of pollution was found in areas near the El-Senety Canal with a rate of infection (80%), whereas soil samples near the Moias Canal area had significantly the lowest (40%). 40.8% of positive samples had one parasite species, whereas 37.9% and 11.5% of positive samples were infected with parasites.

Introduction

All levels of human beings rely on vegetables as essential sources of energy or nutrients (Simon-Oke *et al*., 2014). As abundant providers of water, vitamin C, carotene, iron, and vitamins including thiamine (vitamin B12), niacin, and riboflavin, they significantly increase the quality of food (Nazemi *et al*., 2012; Sunil *et al*., 2014). According to Etewa *et al*. (2017) and Traoré *et al*. (2021), a healthy diet should include fresh vegetables. However, soil might pass some parasites to vegetables, particularly when soil irrigated with wastewater. Subsequently, if vegetables were eaten uncooked, they might act as carriers of intestinal parasites.

A wide variety of eukaryotic creatures called protozoan and metazoan parasites can infect both humans and animals through a number of different transmission pathways, such as soil, water, and food (Ortega and Sterling, 2018). According to Van Pelt *et al*. (2018), certain of these parasites are more likely to persist in the environment. Life cannot exist without water, but it is a limited, vulnerable resource with both qualitative and quantitative constraints. By 2025, 60% of the world's population could experience water scarcity, according to predictions.

In Sharkia Governorate, irrigation with sewage water undoubtedly becomes a harsh reality, primarily because of water scarcity and inefficient sewage disposal (Henin, 2021). Consuming raw veggies, especially when they are still fresh, puts the population at tremendous risk by isolating several types of parasites that have a pathological effect on living organisms (human and animal). For instance, Jassim (2022) shows that contamination of fresh vegetables with parasites, bacteria, and fungus is a public health risk. Additionally, the freshwater of the Euphrates River in Anbar Governorate is contaminated with pathogenic and nonpathogenic parasites (Asfaw *et al*., 2023). The consumption of raw vegetables without proper washing is an important route in the transmission of parasitic diseases (Slifko *et al*., 2000).

According to Muamar *et al*. (2014) and Li *et al*. (2017), the use of untreated wastewater for crop irrigation can also result in soil hardening and shallow groundwater contamination. Potentially toxic elements (PTEs) are primarily exposed to humans through food crops, which could result in a variety of health problems in people if the PTE concentration exceeded the safe limit (Xiong *et al*., 2016; Mombo *et al*., 2016). In addition to these pathogens, wastewater may also contain viruses, bacteria, nematodes, and protozoa (Uyttendaele *et al*., 2015). Intestinal parasites rank among the most significant global public health issues, particularly in tropical and subtropical nations (Wakid *et al*., 2009).

Fresh vegetable consumption has been associated with an increase in reported cases of foodborne disease in recent years. The most significant intestinal parasites that are spread through consumption of contaminated, uncooked, or improperly washed fruits and vegetables are intestinal parasites (Al-Megrm *et al*., 2010), which play a significant epidemiological role in the transmission of parasitic foodborne diseases (Kozan *et al*., 2005). An estimated 1.5 billion people are infected with parasites that are transmitted through soil, the majority of which are annelids such as Ascaris and Strongyloides stercoralis. These causes also include whipworms and hookworms (Stolk *et al*., 2020).

According to Amoah *et al*. (2017), the majority of these contaminations appear to take place in tropical and subtropical areas with unfavorable health, economic, and social conditions. Vegetables can become contaminated by various stages of parasites. Three main paths exist for the contamination of vegetables with various parasite stages: the contamination of raw vegetables through contaminated food handles, the contamination of irrigation or washing water, and the farm during harvest (Ishaku *et al*., 2013). According to recent reports, eating fresh veggies is primarily to blame for an increase in occurrences of food-borne diseases

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(Alhabbal, 2015).

The objective of the current study was to investigate the prevalence of helminths, of medical importance for humans and animals, in soil and wastewater used for irrigation in Sharkia Governorate, Egypt.

Materials and methods

Study area

The study was carried out in Sharkia Governorate, Egypt which is east of the Nile Delta. From the El-Ganabia, Sherwida, El-mahmodyia, El-Senety, and Saft Zerek canals, water samples were collected. The adjacent farms and agricultural fields in the various agricultural zones of Sharkia are irrigated by these canals. In order to assess the physicochemical properties and parasite contamination of these irrigation water sources over the course of a year, water samples were taken each month.

Collection of water and soil samples

Sixty surface water samples were taken, 12 from each irrigation canal at various locations throughout its length. In previously cleaned and labelled polystyrene bottles, 2 to 3 liters were gathered. Concentrated nitric acid was used to acidify samples for the detection of heavy metals at a pH of 2. On the same day after collection, samples were sent to the labs in a cool icebox (4°C). As soon as the samples arrived at the lab, they were analyzed. In addition, sixty soil samples, 12 samples nearby each water stream were collected.

Soil Sample Processing

For soil samples, a modified flotation technique was applied (Downes and Ito, 2001; Garcia *et al*., 2007; Horiuchi and Uga, 2016). A test tube containing 5 g of sieved soil was placed in it before being washed with 8 ml of distilled water using a vortex mixer. The suspension was then centrifuged at 1800 rpm for 10 minutes. Using a vortex mixer, 8 ml of sucrose solution (specific gravity 1.2) was added to the sediment in the tube after the supernatant was decanted. For 10 minutes, the tube was centrifuged at 1800 rpm. After centrifugation, an upper meniscus was generated by gradually injecting sucrose solution with a 10-ml syringe up to the tube's brim. A cover slip was carefully placed on the meniscus in order to capture the top and the prepared slides were checked for parasites using different stains and examined by light microscopy.

Parasite detection in water samples

According to Brandonisio *et al*. (2000), the water samples were filtered via nitrocellulose membrane filters with 0.45mm pore size and 142mm diameter. Physiological saline (0.85 NaCl) was used to wash the membrane filter three times. After that, the filtrate was centrifuged for 5 minutes at 2000 rpm (Kwakye-nuako *et al*., 2007). Each sample's supernatant and four sediment samples were analyzed. To find lightweight eggs and protozoan cysts, the supernatant was subjected to a zinc sulfate flotation examination (Daryani *et al*., 2008). The sediment was then analyzed using the subsequent techniques: To identify parasites, simple and lugol's iodine-stained smears were made (Bakir *et al*., 2003). Each sample's two smears were air dried, methanol-fixed, and Giemsa-stained (Garcia, 2001). The precipitate was also used to create modified Ziehl-Neelsen stain and Kato-Katz slides. Microscopy was used at various magnifications to identify the types of parasites based on their size and shape (ElShazly *et al*., 2007).

Results

Figs. 1-5 depict the prevalence of parasite infection in the soil near irrigation canals scattered throughout the study area. More than half of the samples that were collected (60.7%) had parasite contamination that was significantly positive. Areas close to the El-Senety Canal had the highest contamination percentage (80%), whilst soil samples close to the Moias Canal area had the lowest (40%). Nearly 40% of positive samples contained one parasite species, compared to 37.9% and 11.5% of positive parasite-infected samples that contained one Helminthes cestode (*Taenia* sp. egg, *Moniezia, Dipylidium* sp.) nematode as (*Ascaris* sp. egg, free-living probably *rhabditidae*, Nematode larvae). Three parasite species (*Ancylostoma* larva, *Fasciola hepatica*, and *Enterobius vermicularis* egg) and two parasitic species (*Strongyloide* egg and, *Ascaris* egg) were also discovered to have positively infected the trematodes (*Fasciola* sp., and *Clonorchis sinensis*). The likelihood of isolating more than three parasite species (*Taenia* sp., *Endolimax* nana, and *Ancylostoma* larva) did not exceed 10% (9.9%) in all positive samples, which was a significant finding.

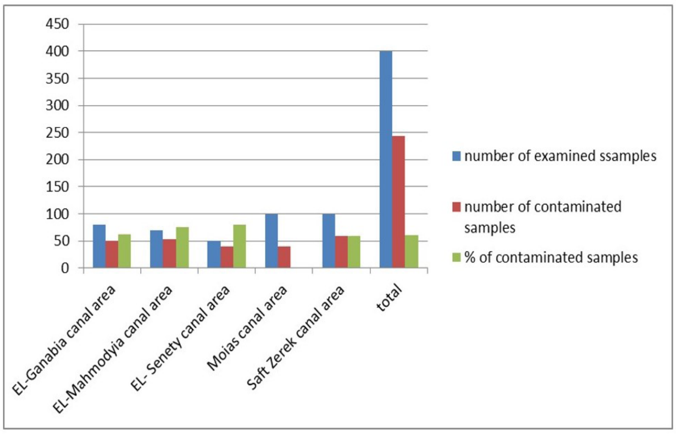

Fig. 1. Number and incidence of positive soil samples contaminated with parasite species.

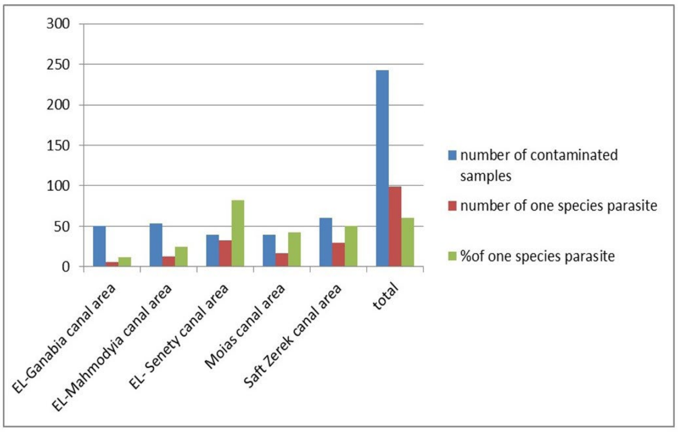

Fig 2. Number of positive soil samples contaminated with parasite species compared with the number and percentage of one species parasite in each area.

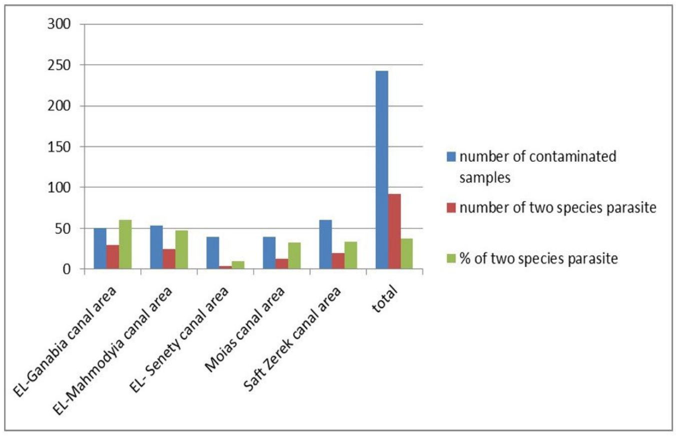

Fig. 3. Number of positive soil samples contaminated with parasite species compared with the number and percentage of two species of parasite in each area.

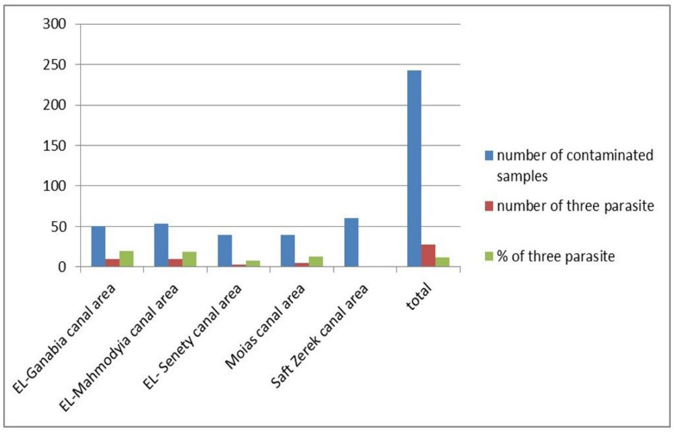

Fig. 4. Number of positive soil samples contaminated with parasite species comparative with the number and percentage of three species of parasite in each area.

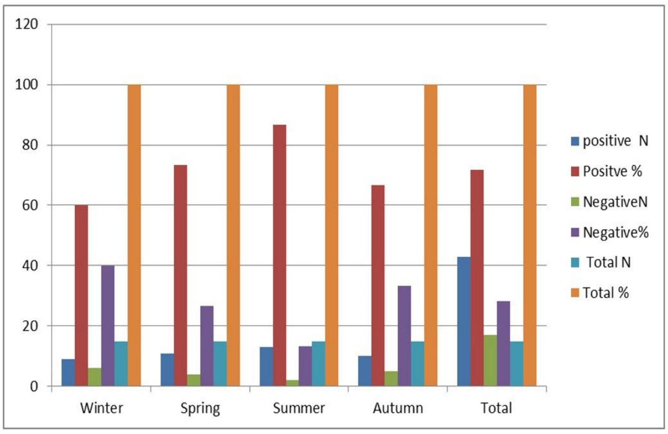

Fig. 5. Seasonal variation of parasitic contamination in studied irrigation canals.

Discussion

Food-borne parasites are particularly important globally among food-borne diseases (WHO, 2015). In Egypt's large agricultural province of Sharkia, a variety of green vegetables are grown and eaten by the locals. The goal of the current investigation was to determine whether some green vegetables that are frequently consumed raw and used in salads on a daily basis might be contaminated with parasites from the farming mud soil of this region. Variable contamination rates with helminths and protozoa of different species were found in the current study's evaluation of vegetables and environmental materials that included soil for parasites using concentration, staining, and light microscopy.

At least one parasite type was significantly present in more than half of the soil samples that were taken. The fact that most parasites can survive for a long time in mud soil, which has adequate moisture and little sunshine, and the tightly adhering particles that stop parasitic larvae from spreading to deeper soil levels, supports this conclusion (Collender *et al.*, 2015). As a result, we were successful in identifying parasitic entities in most samples. Similar investigations into soil in other nations (Paller and Babia-Abion, 2019; Haghparast-Kenari *et al.*, 2021) found that any type of soil, including agricultural lands, was contaminated with numerous parasite larvae, oocysts, and various eggs.

It is challenging to distinguish parasitic entities that might be caught in the soil sample particles. Given that samples with a high concentration of particles, including soil particles, may easily trap or attach to the parasitic structures, resulting in reduced recovery and viewing, it is intriguing that we cannot rule out the presence of parasites in the negative soil samples obtained in this study (Amoah *et al.*, 2017). This may also be related to the random sampling method for soil utilized in this investigation, as some samples may have been taken far from fecal material hot spots that serve as the first sites of diffusion (Collender *et al.*, 2015). The findings of our investigation strengthen the case that soil in the area may be a source of contamination for parasite infections in humans and animals.

Irrigation water is the main component that may cause soil pollution in agricultural areas. This raises serious concerns when using treated wastewater is necessary owing to a lack of freshwater resources or when fecal material contamination of irrigation water is a plausible possibility. According to Abi Saab *et al.* (2022) and Tram *et al.* (2022), the use of treated sewage water may be a significant source of soil contamination with parasite eggs. The same as in other Sub-Saharan African towns, the lack of proper drainage and sanitation infrastructure contaminates water bodies used for irrigation, which contaminates the soil, which becomes a route for human infection (Douglas *et al.*, 2018).

STHs (Ascaris and Trichuris) and Toxocara spp. were found to be the most common parasites in soil samples, according to many studies that have documented soil contamination with various parasite structures. This contamination may have occurred due to accidental and unintended exposure to contaminated water sources or as a result of the intentional human practice of infecting soil with fecal material as part of organic farming practices (Paller and Babia-Abion, 2019). Additionally, having animals around while farming and using their dung as fertilizer both have the potential to contaminate the soil with parasites (Klapec and Borecka, 2012). The use of animal wastes as fertilizer for the farmlands is customary because of the extensive livestock expansion in the studied area. The number of farm animals and the degree of soil contamination by parasitic life forms are intimately correlated.

However, according to Paller and Babia-Abion, (2019), the rate of parasitic structure recovery from soil is unaffected by depths up to 10 cm. Additionally, the kind of soil may have a significant impact on the recovery of parasite life forms from the soil, particularly helminthic eggs. According to Amoah *et al.* (2017), the uniform loose character of the big particles in sandy soils is thought to lead to larger parasite recoveries than in other soil types.

Compared to other crops examined in this study, the majority of leafy greens had parasite species that we could identify. It is claimed that leafy crops like parsley and coriander are frequently grown and collected in close proximity to the soil, subjecting them to contaminated irrigation water, manure, and dirt. Furthermore, these plants' uneven surfaces and dense foliage ensure a high parasitic load in comparison to vining crops like tomatoes, cucumbers, and green peppers, which are typically grown and harvested above ground and have a smooth surface that discourages parasite attachment and is simple to clean of leftover soil (Srisamran *et al.*, 2022).

Globally, there are notable variations in the prevalence of these parasites in plants. These discrepancies may be attributed to variations in environmental and climatic conditions, hygienic conditions, laboratory techniques, and the types of vegetables harvested, all of which play a role in the transmission of parasite diseases. The presence of these parasites in various green vegetables at various points along the production chain, from the fields to the consumer's table, has been reported in numerous research studies published in development (Enogiomwan *et al.*, 2020; Agbalaka *et al.*, 2019; Al Nahhas and Aboualchamat, 2020) and developed (Trelis *et al.*, 2022) countries. This suggests that green vegetables might serve as important vehicles for parasites transmitted by food.

In the current study, the presence of intestinal parasites from humans and animals shows that various sources of contamination have a significant impact on the ongoing contamination of vegetables produced in open fields. This condition is strongly impacted by inadequate human sanitation practices, including wastewater and sewage treatment, as well as soil contamination through the use of irrigation water that has been contaminated with feces. Since the same parasites were found in the study area, it seems that these critical factors had a long-term impact on vegetable safety (Healy *et al.*, 2022).

Particularly when survival time surpasses the growth cycle, as is commonly the case with the researched vegetables, this prolonged life period is sufficient to pose a risk to those who handle or consume fresh produce goods (Amahmid *et al.*, 2023). Ascaris eggs are the most frequently found parasite in vegetables throughout the entire production chain, not just during farming, due to their sticky character from the mamillated eggshell surface (Enogiomwan *et al.*, 2020). It is vital to emphasize that finding D. caninum eggs in vegetable samples is a key sign of zoonotic infections from infected animals, especially dogs and cats.

It is significant to note that non-infectious life forms, including the un-embryonated ova of Ascaris and Trichuris species, have been discovered in this study. These life forms may eventually transform into infectious stages and pose serious health risks. Even though helminth egg load concentrations were not found in this experiment, Cotruvo *et al.* (2004) reported that just one embryonated helminthic egg may be enough to cause infection.

Compared to other research' findings, which have a compaa ratively low rate, Our results were in line with earlier research from Egypt (Eraky *et al.*, 2014) and other nations that show a higher percentage of contaminated samples during the dry seasons (spring and summer) than during the rainy seasons (winter and autumn).In this study, the seasonal change in the rate of parasite infection in vegetable samples was evaluated. The majority of positive samples were found in the spring (29.3%), followed by the summer (27.7%), and the fall (24.5%). The proportion of positive samples was lowest in the winter (20.1%). Due to seasonal changes in temperature and moisture, there are noticeable differences in the incidence of these parasites in vegetables.

According to a different study conducted in Hanoi, Vietnam, more eggs were collected from vegetables during the dry season (78%) compared to the wet season (22%) (Uga *et al*., 2009). This may be because there are more parasites present in soils during dry seasons, particularly Soil-Transmitted Helminths (STH). In addition, a lack of rain makes it harder for eggs to be washed away from the soil and vegetables, necessitating an additional irrigation cycle (Collender *et al*., 2015). In contrast, a study conducted in Turkey (Aydenizoz *et al*., 2017) indicated that rain had a positive impact on the parasite burden of vegetables. It is clear from our observations that the autumn light rain in our area may have a favorable impact on the parasite burden of vegetables found in this study.

One of the biggest dangers that both industrialized and developing nations face is water contamination. Global environmental quality has decreased as a result of significant industrialization and the production of numerous chemical compounds (Chakravarty *et al*., 2010). The pollution of Egypt's River Nile system has increased over the past few years as a result of population development, new irrigation projects, as well as other activities along the Nile. Due to the challenges of agricultural growth and climate change, the quality of irrigation water has become a significant global problem (Zaman *et al*., 2018).

In Egypt, sewage receives just minimal treatment before being dumped into the Nile River, lakes, and oceans. The presence of trees provided shelter for individuals urinating and producing geohelminthic larvae and eggs, and waste and debris were carelessly deposited into the watercourses. These conditions were favourable for the spread of numerous parasites. Elshazly *et al*. (2007). Helminths such Nematodes larvae, Ascaris lombricodes, Taenia ssp. and Toxocara, and fasciola eggs were found in this study's contaminated samples in amounts of 16.22%, 5.41%, 2.7%, 2.7%, and 2.7%, respectively. Similar findings were made by research in Ethiopia and West Africa (Drechsel and Keraita, 2014; Woldetsadik *et al*., 2017).

Similar to the findings of Eraky *et al*. (2014), who discovered a substantial seasonal variation, with the highest contamination of vegetables in summer and the lowest in winter, the prevalence of parasite contamination in the current study was higher in summer and autumn compared to winter and spring prevalence. Jaran (2016) found that the seasons of summer and spring had more positive cases of protozoa in water samples than those of winter and fall. Increased population activity in the summer and autumn increases the risk of irrigation water contamination. However, due to rising pathogen concentrations, irrigation with treated and recycled contaminated water may be detrimental to human health.

Conclusion

This study declared that soil and water used for irrigation are critical determinants for the parasitic contamination of vegetables. Such parasites are significant for both humans and animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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