Molecular detection of *Giardia intestinalis* in fresh vegetables and watercourses of Giza, Egypt

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**ABSTRACT**

*Giardia* sp. is one of the most important intestinal Protozoa causing food and water-borne diseases to humans and animals. Vegetables are critical means of transmitting infections. In the present study, 68 and 50 fresh field and market vegetables, respectively, were seasonally collected from 3 regions; two from Nahia and one from Saft Al-Laban, Giza Governorate, Egypt. Besides, 12 irrigation water samples (8 ground and 4 surface fresh water) were collected from the same agriculture fields. The samples were separately processed and examined by the amplification of 18S rRNA genes, using the appropriate primers of *Giardia* sp. for determining the prevalence of the protozoan parasite. The 18S rRNA gene of parasite was effectively amplified by using the primers Gi F & Gi R, producing the characteristic diagnostic pattern of *Giardia* with bands at 350 bp. The PCR results showed that 7 (10.2%) out of 68 field vegetables, 5 out of 50 (10%) market vegetables and 1 out of 12 (8.3%) irrigation water samples were positive for *Giardia* cysts. Vegetables collected from Nahia 1 recorded the most prevalence of the parasite (33.3%), followed by Nahia 2 area 5.7% and Saft Al-Laban 4.8%. Dill and tomatoes collected from the field and the market, respectively (42.9% and 30%), were the most contaminated vegetables. One irrigation water sample (surface fresh water), collected from Nahia 1 area, was positive to the protozoan parasite. The seasonal variations in the prevalence of *Giardia* cysts reach the highest value in autumn (21.4% and 25% for field and market vegetables, respectively).

**INTRODUCTION**

*Giardia lamblia* is a food-borne protozoan parasite, responsible for the considerable outspread of human gastrointestinal infections (*Ortega & Adam, 1997*). The clinical presentation varies from asymptomatic to severe diarrhea, abdominal pain, nausea, fatigue and vomiting (the most widespread symptoms) (*Ortega & Kvac, 2013*).

*Giardia* is a highly active flagellated organism with two forms: the trophozoite (9–21µm long and 5–15µm wide) and oval infective thick-walled cyst (7-14µm long) which is excreted in feces of the infected host. *Giardia* has a relatively simple life cycle, which is completed within a single host (*Smith et al., 1993*). *Giardia* cyst is resistant to environmental and water treatment stresses (*Grit et al., 2012, Alum et al., 2014*). It can
survive for several months in the cold surface water and in soil (damp environments) (Ortega & Kváč, 2013), lasting for more than 77 days at less than 10°C (WHO, 2015). While, it can endure for only 4 hours in water at 37°C (Xiao & Ryan, 2008). The infectious dose of Giardia is 10 cysts; up to 300 million cysts can be eliminated per ml of feces from the infected person (Inpankaew et al., 2007). In addition, cyst has the potential to transmit from non-human to human hosts (zoonoses) and vice-versa (Karanis et al., 2007).

Basically, Giardia cyst is detected in the environmental samples using a direct microscopical examination (Nyirenda et al., 2021). This technique requires concentration and separation steps which practically are not efficient, besides the presence of debris and other suspended matter in water and wastewater samples. The recovery efficiency of Giardia cysts by these methods ranges from 20 to 80% (US EPA, 1999). Many studies applied molecular methods for the detection of Giardia cysts in wastewater samples, including PCR and real-time PCR (Guy et al., 2003; Miller & Sterling, 2007; Sripanompong et al., 2021).

The most suitable PCR technique for Giardia detection include the semi-nested one (Jangra et al., 2020). Additionally, the nested PCR technique combined with RFLP analyses (Helmy et al., 2009) or direct sequencing, RT-PCR targeting 18S rRNA, β-giardin (Maloney et al., 2020) are among the most appropriate methods.

Egypt is one of the endemic countries regarding Giardia infections. Thus, in this study, Giardia infection risk associated with using contaminated irrigation water side by side with the consumption of contaminated raw vegetables was assessed. Both field and market vegetables are involved in this study. In addition, the current investigation aimed to determine the extent to which hygienic practices are applied, starting from farm passing through collecting, processing, storage and selling throughout the Egyptian geographical area (Nahia & Saft Al-Laban). Giardia DNA, extracted from the processed samples (irrigation water & vegetables), is tracked using the PCR amplification of a specific target gene (18S rRNA) with appropriate primers.

**MATERIALS AND METHODS**

**Study area**

This study investigated two different areas, depending on the irrigation water type; namely, Nahia (30º 02ʹ 39ʺ N, 31º 09ʹ 53ʺ E) and Saft Al-Laban areas (30º 01ʹ 39ʺ N, 31º 09ʹ 57ʺ E) (Giza Governorate, Egypt). Nahia area has two types of agriculture fields irrigated by two types of irrigation water: Nahia (1) with surface freshwater (Maryotia canal) and Nahia (2) with ground water. Saft agriculture fields are irrigated only by the ground water. Fresh vegetables were collected from Nahia and Saft agriculture fields and from public markets in Giza, Egypt as well. Irrigation water samples were collected from the same fields of Nahia and Saft Al-Laban areas. All samples were collected from June 2020 to June 2021.

**Collection of samples**

A total of 68 and 50 fresh vegetable samples were collected from agriculture fields and public markets, respectively. The seasonally collected fresh vegetables included tomatoes (Solanum lycopersicum), dill (Anethum graveolens), cucumber (Cucumis sativus), parsley (Petroselinum crispum), radish (Raphanus sativus var Longipinnatus),
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lettuce (*Lactuca sativa*), carrot (*Daucus carota*), leek (*Allium porrum*), coriander (*Coriandrum sativum*) and watercress (*Nasturtium officinale*). All vegetable samples were collected in clean nylon bags (500 g each).

Water samples (surface and ground water) were separately collected in 20L sterile polypropylene containers from the neighboring areas around the agriculture fields under study. Samples were transferred on the same day of collection to the Environmental Parasitology Laboratory, National Research Centre, Dokki, Giza, Egypt.

**Processing of collected samples**

**Water samples**

Each collected water sample was filtered through a stainless-steel pressure filter holder (Sartorius) with nitrocellulose membrane (0.45µm pore size and 142mm diameter) (*Brandonisio et al.*, 2000). The membrane filter was washed thrice with sterile physiological saline (100ml each time). The collected washing solution was centrifuged at 2000rpm for 5min (*Kwakye-nuako et al.*, 2007). The supernatants were discarded, and the debris were separately collected in an Eppendorf tube, and each was kept at -20ºC for PCR detection techniques.

**Vegetable samples**

The vegetable sample was washed twice with 2L distilled water, followed by 2L detergent solution consisting of 10ml of Tween 80 diluted in two liters of physiological saline solution (0.85% NaCl) (*Luz et al.*, 2017). The solution was subjected to a vigorous shaking for 15min (*Al-Shawa & Mwafy, 2007*). The washing water of each vegetable sample was separately managed, and the same technique was applied on the irrigation water samples.

**Molecular examination by PCR**

Each processed sample (kept at -20ºC) was separately subjected to DNA extraction using QIAamp DNA stool mini kit (QIAGEN Inc) according to the manufacturer instructions. The extracted DNAs were used as templates in the PCR reaction. The selected forward primer was: Gi F (5'AGCCGGACACCGCTGGCAACC 3') and the reverse one was: Gi R (5' CGGCTGCTGGCACCAGACCTT 3') (*Rai et al.*, 2008). The primers used in this study were checked for their specificity by conducting the National Council on Biotechnology Information (BLAST) searches on the GenBank DNA sequence database (http://www.ncbi.nlm.nih.gov). Primers showed 100% specificity for the target gene with reference accession numbers GLU09492, AF006677, DQ098931, U20351 (rDNA sequences). The target PCR product for *Giardia* was 350 bp.

PCR amplifications were carried out using BIOER Little Genius thermal cycler apparatus, USA. PCR amplification was performed in a total volume of 100µL containing 1µl of DNA, 1X green buffer (Promega), 2.5mM MgCl₂ (Promega), 2.5mM dNTPs (Promega), 1U of Go Taq Hot start polymerase (Promega) and 20pmol of each primer.

PCR cycling conditions began with initial denaturation at 94°C for 5min, followed by 30 cycles of 94°C for 1 min, 67°C for 1min and 72°C for 1min. A final extension of 72°C for 5min (*Rai et al.*, 2008). The PCR product was analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide and photographed under UV
transilluminator, using the Gel Doc 1000 image analysis system (Bio-Rad, Hercules, CA, USA).

**RESULTS**

The 18S rRNA gene of parasite was effectively amplified by using the primers Gi F & Gi R producing the characteristic diagnostic pattern of *Giardia* with bands at 350 bp. (Fig.1)

![Fig. 1. Agarose gel electrophoresis for PCR products of *Giardia intestinalis*](image)

In this study, out of 68 and 50 vegetable samples collected from agriculture fields and markets respectively, 7 (10.2%) and 5 (10%) were positive for *Giardia* sp., respectively. Out of 12 irrigation water samples from the same agriculture fields, one sample only (8.3%) from Nahia (1) was positive for target Protozoa. (Table 1).

**Table 1. Prevalence of *Giardia intestinalis* on some vegetable and irrigation water samples by PCR**

<table>
<thead>
<tr>
<th>Sample source</th>
<th>Total samples</th>
<th>Positive samples Number by PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field vegetables</td>
<td>68</td>
<td>7 (10.3%)</td>
</tr>
<tr>
<td>Market vegetables</td>
<td>50</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Irrigation water</td>
<td>12</td>
<td>1 (8.3%)</td>
</tr>
</tbody>
</table>

For the field vegetable samples, Nahia (1) area (depending on surface water in irrigation) was the most contaminated with the present parasite (33.3%), followed by Nahia (2) (depending on groundwater) 5.7% and Saft Al-Laban areas (depending on groundwater) (4.8%) (Table 2 & Fig. 2).

Concerning the irrigation water, one sample only collected from Nahia (1) area was contaminated with *Giardia* sp., no contamination was detected in ground irrigation water of Saft Al-Laban area and Nahia (2) area (Table 2 & Fig. 2).
Table 2. Prevalence of *Giardia intestinalis* on vegetables and irrigation water samples from different field areas by PCR

<table>
<thead>
<tr>
<th>Samples sites</th>
<th>Nahia(^1)</th>
<th>Nahia(^2)</th>
<th>Saft</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>35</td>
<td>21</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>33.3</td>
<td>5.7</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Irrigation water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1= Field irrigated with surface water
2= Field irrigated with ground water

The most contaminated field and market vegetables were dill and tomatoes (42.9% and 30%), respectively, while radish and watercress were the lowest contaminated field and market vegetables (8.3% and 16.6%), respectively. No parasitic contamination was detected by PCR in field vegetables: water cress, coriander, leek, tomatoes, carrot, cucumber and in market vegetables: lettuce, parsley, dill, coriander, radish, leek and cucumber (Table 3 & Fig. 3).

Table 3. Prevalence of *Giardia* on different types of vegetables by PCR

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Field vegetables</th>
<th>Market vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>11</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Parsley</td>
<td>10</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Water cress</td>
<td>4</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dill</td>
<td>7</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Coriander</td>
<td>8</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Radish</td>
<td>12</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Leek</td>
<td>8</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>4</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Carrot</td>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cucumber</td>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>68</strong></td>
<td><strong>7 (10.2%)</strong></td>
</tr>
</tbody>
</table>
**Fig. 2:** Prevalence of *Giardia intestinalis* in vegetables and irrigation water samples from three different fields by PCR.

**Fig. 3:** Prevalence of *Giardia intestinalis* on different types of field and market vegetables by PCR.

**Fig. 4:** Seasonal prevalence of *Giardia intestinalis* on examined samples by PCR.
Concerning seasonal variation, *Giardia* sp. prevalence rate was the highest in field and market vegetables during Autumn (21.4%, 25%, respectively), while in irrigation water samples, one contaminated water sample only was detected in spring (Table 4 & Fig. 4).

**Table 4.** Seasonal prevalence of *Giardia intestinalis* on examined samples by PCR

<table>
<thead>
<tr>
<th>Sample types</th>
<th>Season</th>
<th>Total examined samples</th>
<th>+ve samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Field vegetables</td>
<td>Autumn</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Market vegetables</td>
<td>Autumn</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Irrigation water</td>
<td>Autumn</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Cysts of *Giardia* are highly prevalent in fresh and waste waters in the global world. Most studies belonging to testing of raw wastewater samples, showed that the concentrations were ranged from 23-100,000 cyst/L (Nasser et al., 2012).

Water-associated outbreaks might be caused by human and animal contaminations of water systems (Navin et al. 1985; Daly et al. 2010). Generally, the zoonotic transmission of giardiasis is not believed to have a major effect in human disease (Hunter and Thompson 2005).

Although that 60% of all *Giardia* infections are acquired through contaminated water (Bennett et al., 1987 and Karanis, et al.2007), still cyst-contaminated food is considerable source of infection. Various publications have shown that irrigation water may be a source of contamination of crops (Chaidez et al., 2005; Thurston-Enríquez et al., 2002).

Earlier studies have shown the presence of *Giardia* cysts in vegetables and fruits in many countries, with the average prevalence is estimated as 4.8% and 35.8% respectively. (Li et al. 2020, Nyirenda et al., 2021). Rai et al. (2008) estimated *Giardia* sp. contamination in a number of Indian environmental samples, including goat meat, milk and market vegetables, he found 12 contaminated vegetable samples, namely; radish, cabbage, spinach, mint leaves, carrot, lettuce, chili fruits and amaranth roots.

Rafael et al. (2017) analyzed 130 samples of vegetables (crisp lettuce, regular lettuce, kale, chicory and rocket) from street markets and 130 ones from community gardens (Brazil). They found 10 out of 130 (7.9%) and 9 out of 130 (6.9%) street market and gardens samples, respectively infected by *Giardia* sp. with a total prevalence of 7.3% (19/260). In India, Utaaker et al. (2017), examined 284 vegetable samples (turnip,
cabbage, carrot, chili, coriander, cucumber, mint leaves, fenugreek leaves, radishes, lettuce, and tomatoes), they stated that 5% of samples were contaminated with *Giardia* cysts. In the advanced countries, the contamination of vegetables by *Giardia* was relatively low, as in study of **Robertson & Gjerde (2001)** reach 2%.

In the present study, higher corresponding results have obtained, with prevalence of infections 10.2% and 10% in fresh field vegetables and market ones, respectively. More higher prevalence of contamination was recorded in some studies, Ferreira et al., 2018 examined 42 Brazilian vegetable samples (Lettuce; Arugula; Chard; Chicory; Chive) with a prevalence of infection 30.9 %. Likewise, Monge et al., 1996, in Costa Rica, a total of 640 samples from eight different vegetables, all were positive for *Giardia* sp. contamination (100%) either collected in dry or wet seasons. Tiyo et al., 2016, examined Brazilian fresh Leafy Vegetables which were collected directly from producers, the *Giardia* contamination reached (12.5%).

The variation in *Giardia* contamination rates of agricultural crops from one locality to another or from one country to another is attributed to the source of irrigation water (treated human sewage, surface fresh water or ground water) or those agricultural fields which receive livestock waste for manuring instead of chemical fertilizers.

In the present study, no remarkable variation between *Giardia* contamination rates between the field and market vegetables, 10.2% corresponding to 10% respectively, this finding was similar to Brazilian study of **Rafael et al., 2017**, 6.9% corresponding to 7.9%.

Nahia1 was the most area which exhibited higher contamination in field vegetables (33.3%), this finding is attributed by that this area used surface freshwater as an irrigation source while other areas under this study (Nahia2 & Saft) used the groundwater, besides, in this study, one water sample only is positive in contamination and related to Nahia1. Generally, surface water may be contaminated due to human or animal faeces or sewage. On contrary, groundwater is usually relatively kept away from contamination and may be considered less likely to be the source of *Giardia* cysts.

The correlation of the parasitic contamination rate of field vegetables with the contamination percentages in the surrounding irrigation water sources may not be clearly established, in some cases, as in the study of **Tiyo et al., 2016**, by Immunofluorescence and PCR examinations, he found no *Giardia* contamination in 14 collected water samples (from rural area) although that contamination rate of surrounding field vegetables, by *Giardia* sp., reach (12.5%). This finding may be explained by presence of other additional sources of parasitic contamination, like extensive using the natural manure in some agricultural areas.

In the present study, the highest prevalence rate for *Giardia* sp. in field vegetables was in dill plant (42.9%). Other Egyptian study, (Gad et al., 2020) indicated higher contamination rates of the same plant (collected from field and markets) by other parasitic infections (*Cryptosporidium* sp, *Entamoeba histolytica* and *microsporidia*), with a total contamination rate reach 69.2%. On contrast, other Egyptian study (Hassan et al., 2012) showed no contamination detected in Dill collected from supermarkets of Alexandria city. This result can be explained through the application of hygiene and health care transactions in these supermarkets. Globally, there are studies that have also proven high *Giardia* contamination values in the Dill plant, **Robertson & Gjerde (2001)**
(28.5%). Generally, the dill plant has a high external surface area, many flexures and highly branched parts that make it a suitable background for adhesion of parasitic stages like cysts. Based on the previous information, lettuce is one of the plants that fall into the challenge of this reason, in the present study, the *Giardia* contamination rate of lettuce was relatively low (18.2%), this is more or less matched with other studies, Robertson & Gjerde (2001), Avazpour et al. (2015); with corresponding contamination values; 30%, 7.5% and 61.5%, respectively. Exceptionally, Amoros et al. (2010) stated a high *Giardia* contamination rate in lettuce reach 61.5%, from a preventive point of view, when washing, leaves of lettuce must have been separated and washed well with anionic detergent.

Concerning to market vegetables, under this study, Tomatoes have the highest contamination rate of *Giardia* (30%). In Egypt, Hassan et al. 2012 reported that market Tomatoes (Alexandria) with a less corresponding contamination rate reach 11%. Al-Nahhas and Abou-alchamat (2020), stated that *Giardia duodenalis* was the most prevalent parasite detected in markets samples (13.2%), abundantly found in lettuce isolates (25%) and then in Parsley (18.7%). In Zambia, the highest parasitic contamination rate was found in Chinese cabbage with 7.4%, followed by rape with 6.3%. (Nyirenda et al., 2021)

Supermarkets, which are generally considered more modern and therefore safer, show variable levels in contamination rates belonging to the displayed vegetables, which varies from one store to another. This fact is controlled by specific factors, including frequent hand-handling between consumers, washing of vegetables in a single large bowl at the same time before offering them to the consumer or the variety of producers from whom vegetables are purchased.

**CONCLUSION**

This work aimed to evaluate the contamination rates of vegetables by *Giardia* sp. and hygienic-sanitary conditions of their production in agricultural fields until exporting to Markets. This study indicates the high contamination rates of field vegetables with the *Giardia* cysts, especially those irrigated with surface water of canals and drains. Opening the door to expand in use of groundwater as an important alternative in agriculture. Besides, there is an urgent need to develop the processes of transferring agricultural crops in a healthy and safe manner, working to increase public awareness of the importance of safety standards and raising the efficiency of quality control, in addition to monitoring irrigation water and ensuring its cleanliness.

**REFERENCES**


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الملخص العربي

الكشف الجزيئي عن الجيارديا المعوية في الخضروات الطازجة والمجاري المائية في الجيزة، مصر

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الملخص

الكشف الجسيم عن الجبارد المعوية في الخضروات الطازجة والمجاري المائية في الجيزة، مصر

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