



Prevalence and morphological characters of *Strongyloides stercoralis* contaminating some fresh raw vegetables in Sharkia province, Egypt

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Accepted: 01 January 2019

Published: 26 December 2020

ABSTRACT

Fresh green vegetables are considered an essential source of all nutrients required for a healthy body. However, parasitic contaminations of fresh raw vegetables are common in most developed countries. Therefore, this study explored the prevalence of *Strongyloides stercoralis* on some fresh raw leafy vegetables in Sharkia province markets because there is not enough data. Raw fresh vegetables of leek (*Allium porrum*) ($n=80$) and green onion (*Allium ascalonicum*) ($n=80$) were examined. The current study showed that the prevalence of *S. stercoralis* on leek (*Allium porrum*) was (60/80) 75% and on green onion (*Allium ascalonicum*) was (56/80) 70%. Moreover, morphological characters of *S. stercoralis* were described.

Those results revealed that consumption of raw green vegetables represented a high risk of human and animal's infections with *S. stercoralis* in Sharkia, Egypt. Hence, effective control measures should be applied to minimize parasitic contaminations of vegetables.

Key words: *Strongyloides stercoralis*, Leek, Green onion, Prevalence, Sharkia, Egypt.

INTRODUCTION

Soil-transmitted helminth (STH) infections are major prevalent infectious agents in many countries especially developing countries where environmental sanitation and personal hygiene are lacking (Maikai et al., 2012; Strunz et al., 2014). Human strongyloidiasis is due to a soil-transmitted nematode of threadworm *Strongyloides stercoralis*, represented a global public

health concern as it affects 30 to 100 million people worldwide (Genta, 1989; Nutman, 2017). However, two intestinal nematodes species of *Strongyloides*, *S. stercoralis* and *S. fuelleborni* are mainly responsible for strongyloidiasis which *S. stercoralis* is the most widespread and worldwide distribution of human clinical pathogens, while *S. fuelleborni* is found sporadically in Africa (Siddiqui and Berk, 2001).

S. stercoralis has a complicated life cycle in which the cycle starts when the hosts walk barefooted on contaminated soil and the infective filariform larvae (L3) penetrate the skin. Then, it passed via bloodstream to lungs, move to airways, coughed up and finally reach small intestine, where they developed into adult females worms reproduce asexually (parthenogenic) and lay embryonated eggs into gastrointestinal tract that hatch internally into non-infectious free-living first-stage larvae which are excreted in the stool to external environment. Outside host, unusual to other human nematodes, these larvae either develop into mature free-living non parasitic males and females worms that reproduce sexually or transform directly into third filariform larval stage ready to infect a new host (Greaves et al., 2013). With free-living cycle, human intestinal nematode of *S. stercoralis* has an internal auto-infective life cycle which some of rhabditiform larvae further develop to become infectious filariform (L3) larvae that remain in the human host. These larvae able to cause reinfection of the host through penetration of gut wall and to reenter the circulation and complete cycle (DiGiulio, 2019). A distinctive characteristic of autoinfective cycle of *S. stercoralis* helps

the worms to survive and continue their infections for several years inside human hosts which reached 65 years in a recorded one case (Page et al., 2018). In addition, strongyloidiasis is associated with pulmonary disease, abdominal pain and diarrhea as well as potentially life threatening hyperinfection among immunocompromised patients particularly in untreated cases (Keiser and Nutman, 2004; Becker et al., 2011; Forrer et al., 2019). Soil transmitted helminthes as *S. stercoralis* are transmitted via oral fecal routes and are affected by favorite food among people (Zeehaida et al., 2011). In consequence, leafy vegetables and dark green herbs are an essential component of Egyptian diet as Egyptian societies consumed daily either fresh raw or cooked as it used in many dishes (Hassan, 2004). However, the consumption of raw vegetables without proper washing, contaminated drinking water and uncooked food are considered an essential route of parasitic diseases transmission (Macpherson et al., 2000; Slifko et al., 2000). Furthermore, increasing the number of food borne illness cases are primarily related to consuming fresh vegetables (Mohamed et al., 2016).

Several reports in many countries around the world have suggested parasitic contamination of fresh vegetables included *S. stercoralis* larvae on green *Coriandrum sativum* as recorded by **Muniswamappa et al. (2012)**, while, vegetables and herbs were potentially sources of *S. stercoralis* infection in Malaysia (**Zeehaida et al., 2011**). Nevertheless, the soil transmitted roundworm, *S. stercoralis*, is represented as one of the most neglected infections between neglected tropical diseases (**Schär et al., 2013; Krolewiecki and Nutman, 2019**). Likewise, the majority of data obtained in Egypt have focused on other major soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, and *Ancylostoma duodenale* and *Toxocara spp.* eggs, *Hymenolepis nana* eggs and *Trichuris trichiura* contamination of raw vegetables (**Said, 2012**); however, information on *S. stercoralis* is scarce. Therefore, to our knowledge the current investigation was a first study conducted to assess prevalence of *S. stercoralis* eggs or larvae contaminated raw vegetables (leeks and green onion) which constitute a risk to human in Sharkia province, Egypt. As well, morphological characters of *S. stercoralis* eggs, larval stages and free living adult females were described.

MATERIALS AND METHODS

Sample collection

In the present study, two types of green vegetables were selected Leek (*Allium porrum*) ($n=80$) and green onion (*Allium ascalonicum*) ($n=80$) which were randomly collected from sellers at regional local markets at Sharkia province, Egypt between April, 2018 and November, 2018. Fresh vegetable samples (400g each) were separately collected in clean nylon bags and transferred to Parasitology lab. Fac. Vet. Zagazig Univ., Egypt. for further examinations.

Processing of collected samples

Fresh vegetables samples were chopped into small pieces about (200g), soaked in normal saline and incubated in a plastic container and left overnight at 4°C. Then, normal saline solution was collected, filtrated and transferred into a centrifuge tube, centrifuged at 3000 rpm for 5 min. The supernatant was removed and the sediment from each sample was examined with iodine to detect parasite stages under light microscopy at 10× and 40× objectives (**Al Nahhas and Aboualchamat, 2020**).

Samples culturing and parasites isolation

The samples were processing using fecal culture technique as described by **Khanna et al. (2015)** with some modifications to diagnosis and isolate the parasites in temperatures between 25 - 30°C.

RESULTS

The current study revealed that the overall prevalence of *S. stercoralis* among examined fresh raw vegetables was 72.5%. While the contamination rates of leek and green onion were 75% and 70% respectively (**Table 1**).

In the present study, eggs, first stage larvae (L1), rhabditiform second stage larvae (L2), filariform third stage larvae (L3) and adult free living females of *S. stercoralis* were identified. Hence, using light microscopic examination, eggs were thin shell and ellipsoidal with no significance difference between the size of three egg developmental stages of early deposited fresh eggs, eggs with embryo and egg containing larvae which were measuring about 70 – 95 × 50 – 60 µm (**Fig. 1A, B, C**). In addition, the first stage larvae body length was approximately ranged from 215 - 263 µm and width (15 – 25 µm). The length of esophagus was 65 - 73 µm. While intestine and end of tail length were about (110 – 123 µm) and (45 - 64 µm)

respectively. Also, identified L1 larvae have a short buccal with double bulb esophagus and unclear genital primordium (**Fig. 2A**). On the other hand, the length of rhabditiform larvae was about 228 – 284 µm and the width was 25 - 36 µm. Moreover, the length of esophagus, intestine and tail end were (64 – 82 µm), (132 – 153 µm) and (35 - 52 µm) respectively. Those larvae had double bulbous thickening of esophagus which had a club-shaped anterior portion and a bulb posteriorly with short buccal cavity and a constriction in the middle. While intestine with grain pigments mass in two rows. Moreover, large, prominent genital primordium and pointed tail were observed (**Fig. 2B & C**). However, thin slender third larval stage (L3) was identified which the data analysis revealed that the length was 524 - 636 µm with 35 – 55 µm width. The larvae have non bulbous filariform long cylindrical esophagus measuring about 210 – 230 µm (**Fig. 2D**). Also, the total length of intestine and tail end were (215 - 288 µm) and (95 - 112 µm) respectively. In addition, a notched tail was observed.

The current findings demonstrated that the body of free living females was small and flat (**Fig. 3A**). The length was 850 – 965 µm

and width was 55 – 60 μm . Head with two lateral cephalic lobes with small papilla in subdorsal, subventral and lateral sides. Esophagus was rhabditiform which reached 130 – 135 μm length with a short muscular part which ended distally with incomplete bulb. Vulva was near center of body. The length of gut was 688-700 μm and tail end

length ranged from 85 – 130 μm (**Fig. 3B, C & D**).

The culture results showed that the development of first larvae lasted two days to be noticed, but infective stage larvae were detected within seven days.

Table 1: Prevalence of *Strongyloides stercoralis* on leafy vegetables

Vegetables	No. examined	No. infected	%
Leek	80	60	75
Green onion	80	56	70
Total	160	116	72.5

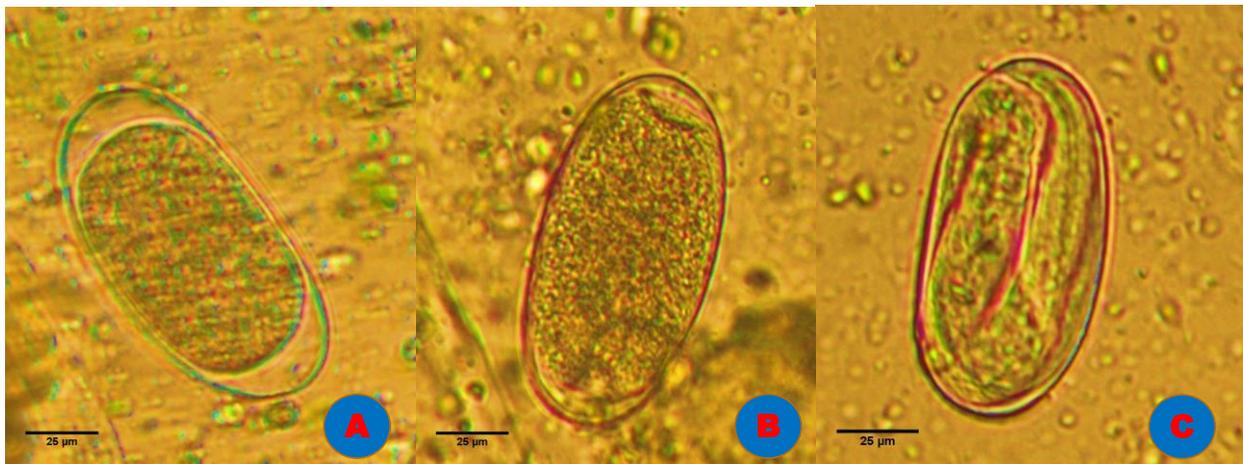


Fig. 1: Morphological characters of *S. stercoralis* eggs.

A. Fresh deposited egg **B.** Egg containing embryo. **C.** Egg containing larva. 400X (Bar=25 μm).

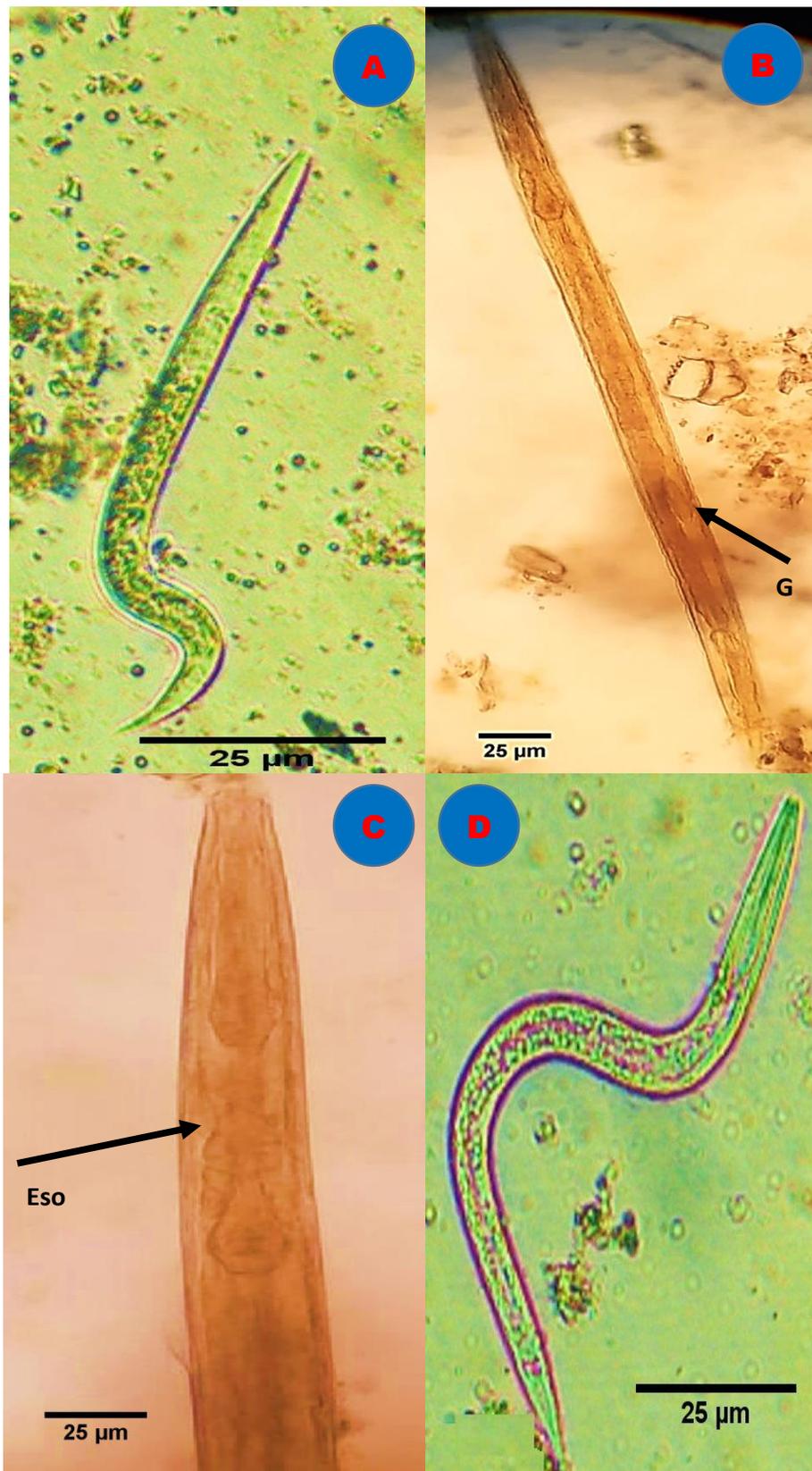


Fig. 2: Larval stages of *S. stercoralis*. **A.** First larval stage (L1). **B.** Second larval stage (L2); (G: Genital primordium) (100X). **C.** Second larval stage (L2); anterior end; (Eso: Rhabditiform esophagus). **D.** Third stage larvae (L3). 400X. (Bar=25 µm)

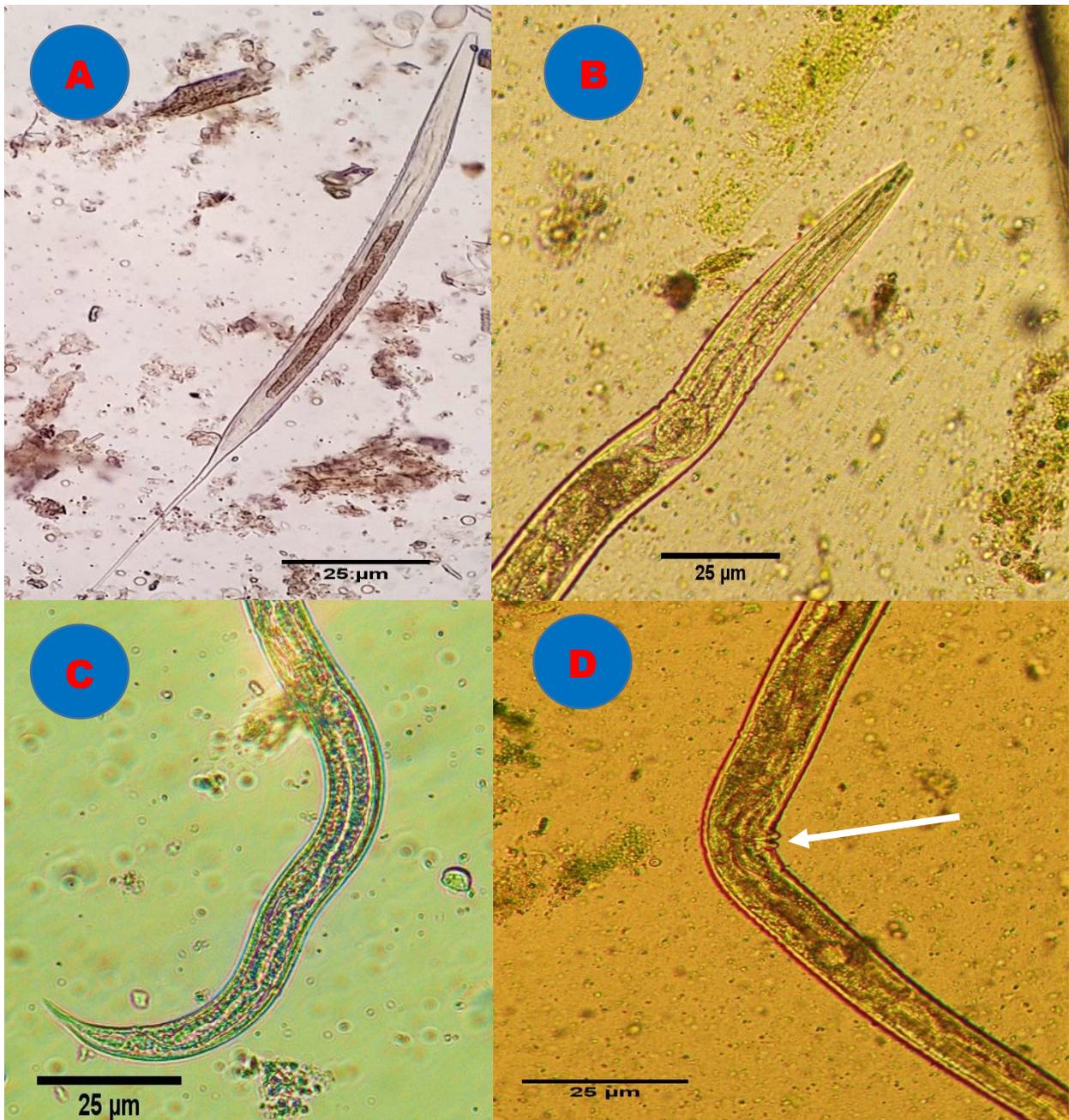


Fig. 3: Adult free living females of *S. stercoralis*. **A.** Adult female (160X). **B.** Female anterior end. **C.** Female posterior end. **D.** Female vulval region (White arrow indicate small vulval slit). 400X. (Bar=25 µm).

DISCUSSION

The threadworm *S. stercoralis* is a soil-transmitted nematode and is distributed worldwide causing Strongyloidiasis as a more critical medical and social neglected problem (Wang et al., 2017). Furthermore, transmission of intestinal parasites is rise due to consumption of raw vegetables and fruits which farmers always use untreated human or animal fertilizer and contaminated or untreated wastewater for irrigation particularly in developing countries (Alemu et al., 2020). To our knowledge, contaminations of vegetables with *S. stercoralis* were based on very limited data as few studies have focused on soil helminthes parasites in Egypt. So far, we believe that the current study was a first report discussed the contamination of vegetables with free living nematodes in Sharkia province as well as morphological criteria with no previous surveys were conducted.

In the present study, we found that the prevalence of *S. stercoralis* was 75 and 70% on leek and green onion respectively at local markets. Our findings were approximately similar to results obtained by Muniswamappa et al. (2012) who detected *S. stercoralis* larvae in 63% of fresh vegetables of *Coriandrum sativum* in India.

However, Ogbolu et al. (2009) found 45.8% of *S. stercoralis* contaminated vegetables from open markets in south western Nigeria. Further, Kudah et al. (2018) recorded *S. stercoralis* contaminations of spring onions, lettuce and tomatoes were 36.4, 31.4 and 41.6% respectively in Ghana.

Other studies showed much lower prevalence as Mohamed et al. (2016) in Sudan who reported *S. stercoralis* (8.6 %) in vegetables samples and 42.9 % in water samples used to irrigate those vegetables which highest numbers of green onion and beet were contaminated. Furthermore, in Nigeria (Adamu et al., 2012) has detected *S. stercoralis* in only 2.0% and 1.3% of cabbage and carrot respectively; and Adenusi et al. (2015) who recorded *S. stercoralis* larvae only in 2.08% of examined cabbage, cucumber and spinach but lettuce was 1.04%. In addition, Ezatpour et al. (2013) recorded 1.8% infection rate of *S. stercoralis* of both leek and green onion in Iran.

In contrast to our findings, Said (2012) and Hassan et al. (2012) didn't detect any contamination with *S. stercoralis* of fresh leafy vegetables in Alexandria, Egypt. Likewise, no *S. stercoralis* contamination were found in consumed raw vegetables in

Iran (Daryani et al., 2008; Asadpour et al., 2016; Rostami et al., 2016).

The discrepancy might be due to variations of collection, number and type of examined samples, different geographical locations, processing, methodology for parasites detection, type of water used for irrigation, fertilizer type and handling procedures of vegetables (Ezatpour et al., 2013; Alemu et al., 2020; Hajipour et al., 2020). Moreover, inadequate sanitation, inappropriate treatment of human swage and using of untreated waste water to irrigate vegetables and crops were significant causes of dissemination such parasites contaminations (Amoah et al., 2007; Youssef and Uga, 2014). As well, we claimed that contamination of vegetables with *S. stercoralis* in examined area in terms of soil properties in which *Strongyloides spp.* were detected in Sharkia province soil samples as described by Etewa et al. (2016). Hence, the high contamination rate with *S. stercoralis* in the present study might due to the conception of the external free-living cycle that doesn't need host to continue (Punsawad et al., 2019). Supporting our assumptions of fresh raw vegetable probably a risk for human infections with *S. stercoralis*, a previous study was done by Atia (1984) in Telega

village Sharkia province, Egypt who found the prevalence of *S. stercoralis* was 13.5 % in stool samples among infected persons.

The current study casts a light on the morphometric of *S. stercoralis* development stages which our results were slightly different than obtained by Little, (1966) and Gugosyan et al. (2019). However, it is unlikely to detect *S. stercoralis* eggs in stool as parasitic females lay eggs in the intestinal epithelium and hatch to larvae before passing in the stool (Puthiyakunnon et al., 2014), therefore it is interesting to detect it in the present research.

As the findings of the current study highlighted that consuming fresh raw vegetables represented a possible risk to human, consequently further surveillance study on symptomatic and asymptomatic patients with *S. stercoralis* infections in that area are needed to be conducted.

In this study, we have used a microscopic examination in agreement with all aforementioned studies as it is a simple, low cost and routinely used to identify different intestinal parasites. However, it is difficult to identify *Strongyloides* larvae and adult by morphological parameters as well as difficult clinical diagnosis (Metwally and Abdel-Rahman, 2014).

Therefore, further studies are required to develop molecular assays to investigate *S. stercoralis* infections.

CONCLUSION

This study revealed that fresh raw vegetables in Sharkia, Egypt were contaminating with *S. stercoralis*. Also, this investigation illustrated the role of commonly consumed vegetables as a route of foodborne parasitic diseases. So, public knowledge and awareness about proper washing of raw vegetables before consuming are needed. In addition, effective management and control programs in terms of irrigation systems and sanitation should be carefully investigated.

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الملخص العربي

دراسات وبائية ووصفية لسترونجيلويدز ستركوراليس التي تلوث بعض الخضروات الطازجة النيئة في محافظة الشرقية بمصر

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تعتبر الخضروات الطازجة مصدرًا أساسيًا لجميع العناصر الغذائية اللازمة لصحة الجسم. ومع ذلك ، فإن تلوث هذه الخضروات الطازجة النيئة بالطفيليات شائع في معظم البلدان النامية. لذلك ، أجريت هذه الدراسة على مدى انتشار الأسطوانيات البرازية (سترونجيلويدز ستركوراليس *Strongyloides stercoralis*) على بعض الخضروات الطازجة المختلفة في أسواق محافظة الشرقية بسبب عدم وجود بيانات كافية عن هذه الطفيليات. حيث تضمنت الدراسة الحالية ١٦٠ عينة خضروات تم جمعها من أسواق محافظة الشرقية. في حين أظهرت الدراسة الحالية أن انتشار سترونجيلويدز ستركوراليس على الكراث (*Allium porrum*) كان ٧٥٪ والبصل الأخضر (*Allium ascalonicum*) كان ٧٠٪. علاوة على ذلك ، تم وصف الخصائص المورفولوجية للسترونجيلويدز ستركوراليس. أظهرت هذه النتائج أن استهلاك الخضروات النيئة قد تمثل مصدرا لعدوى الإنسان بسترونجيلويدز ستركوراليس. ومن ثم ، ينبغي تطبيق التدابير الفعالة للتقليل من تلوث الخضروات بهذه الطفيليات من خلال توعية المجتمع بضرورة تنظيف الخضروات جيدا وطهيها قبل تناولها ، وكذلك الإلتزام بقواعد السلامة عند استخدام المياه المعالجة للرى.