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## **PREVALENCE OF PARASITIC CONTAMINATION OF LEAFY GREEN VEGETABLES IN MISURATA, LIBYA**

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### **Abstract**

This study was undertaken to determine the prevalence of parasitic contamination in leafy green (lettuce and rocket) vegetables, samples of which were taken up from different regions of Misurata, Libya. A total of 112 raw vegetable samples randomly selected from farms and markets were subsequently examined by a concentration method and then assayed by light microscopy. It was found that 56.3% of the green vegetables were contaminated with different intestinal parasites, the parasites included cysts of *Giardia* spp., *Entameaba histolytica*, *Entameaba coli*, *Coccidia* spp. oocysts, *Balantidium coli* and eggs of *Hymenolepis nana*., *Ascaris lumbricoides*., *Toxocara* spp., *Strongyloides* spp., *Trichius trichura* and *Trichostrongylus* spp. The highest rate of contamination was detected in rocket (64.3%) while contamination was lower in green lettuce (48.2%).

*Toxocara* spp. eggs were the highest prevalent parasite detected in green vegetables (27%) with the highest score density found in the rocket. *Toxocara* was followed by *Entameaba coli* cysts (24%), *Coccidia* spp. Oocysts (22%), *Entameaba histolytica* cysts (19%), *Giardia* spp. cysts (10%), and *Hymenolepis nana* eggs (8%). There were lesser rates of contamination from the parasites *Strongyloides* spp., *Trichius trichura* and *Trichostrongylus* spp. There was no significant difference between single and mixed contamination of rocket and lettuce  $P>0.05$ . However, there was a statistical difference between protozoa and helminths contamination of rocket and lettuce ( $P\leq 0.01$ ). We conclude these findings may have important implications for global food safety and confirm that green

vegetables are a point of transmission of intestinal parasites to humans and so are a threat to public health in Misurata, Libya.

**Keywords:** Intestinal parasites, parasitic contamination, lettuce and rocket vegetables.

### Introduction

Vegetables play an important part in maintaining good health, providing proteins, fibres, minerals and vitamins. But they also play a part in the transmission of helminth eggs and protozoan cysts. Vegetables require a moist environment for their growth and these conditions favour the growth of awkward usage forms of endoparasites (Abougrain *et al.*, 2010; Simoes *et al.*, 2001). This contamination may be caused by several factors, including the use of untreated wastewater (Amoah *et al.*, 2006), or contact with sewage and raw dung used as fertilizer (Simoes *et al.*, 2001).

It follows that the consumption of the raw vegetables plays an important role in the transmission of parasites to humans and animals, especially raw vegetables consumed in salads. (Daryani *et al.*, 2008; Al-Binali *et al.*, 2006). Failure to adhere to health standards in the kitchens of schools, hospitals, and restaurants also can result in the transmission of intestinal parasites such as *Ascaris lumbricoides*, *Taenia* spp., *Hymenolopsis nana*, *Echinococcus* spp., *Toxocara* spp., and the species of protozoa *Cryptosporidium* spp and *Giardia intestinalis* (Abougrain *et al.*, 2010; Uga *et al.*, 2009 and Coelho *et al.*, 2001).

A number of studies in different parts of the world have shown parasitic contamination in fresh vegetables, especially if parasitic infections are endemic in these areas. This is notably the case in developing countries where water used to irrigate the vegetables may be contaminated with human and animal faeces. Research there records the highest rates of contamination with parasite stages (Ulukanligil *et al.*, 2001).

At this time in Libya there are few studies of the parasitic contamination of raw vegetables and the existing scientific literature records only one from Tripoli by Abougrain *et al.*, 2010. The aim of this study is to detect and determine the prevalence of parasitic contamination in raw green vegetables from regions in Misurata, Libya.

### Materials and Methods

**Study area.** This study was carried out in Misurata, northwestern Libya, between April and November 2016. Misurata is located on the Mediterranean coast, at a position of latitude 32°22'39.12"N and longitude 15°05'31.26"E. It has a population of about 500,000, based on the 2012 census. The common climate is a local steppe climate, (Gatehouse, 2012).

#### 2-2 Sample collection

Two types of green vegetables were selected for this study: lettuce (*Lactuca sativa*) and rocket (*Eruca sativa*). A total of 112 samples (56 of each type of vegetables) were randomly selected from the farms and markets, at a total weight of one hundred grams.

#### Sample processing

The green vegetable samples were collected in sterile plastic bags and then carried into the laboratory, where these were immersed overnight in 500 ml 0.9% normal saline at room temperature. The supernatant was discarded and 50 ml 0.9% normal saline was added to the sediment, which was then centrifuged at 3000rpm for 10 minutes. The supernatant was then discarded and the remaining pellet was collected and examined in simple and Lugol iodine stained smears (three for each from each sample) using light microscopy, (Hajjami *et al.*, 2012). Simple and iodine smears were detected for parasitic cysts, eggs and larvae.

#### Statistical analysis

Statistical analyses were carried out using the t-test of the SPSS software to compare the contamination rate among the green vegetables. The differences were investigated as significant at  $p < 0.05$ .

## Results

In the present study, a total 112 samples of leafy vegetables (56 of lettuce and 56 of rocket) were examined for the presence of parasites. The findings of this study show that cysts, eggs and larvae were detected in 56.3% (63 samples) of the total leafy vegetables examined, and that the highest rate of contamination was found in the rocket samples (64.3%. 36 samples) while in the lettuce the prevalence was 48.2% (27). Furthermore, the rate of contamination by a single parasite (77.8%) was higher than the rate of contamination by mixed parasites (22.2%), (Table 1). There was no significant difference between single and mixed contamination between samples of rocket greens and lettuce  $P > 0.05$ .

Table 1

Prevalence of parasites in examined leafy vegetables

Vegetable type	No. examined leafy vegetables	Non-infected	Total parasite Contamination	Single contamination	Mixed contamination
Rocket	56	20	36 (64.3%)	26 (72.2%)	10 (27.8%)
Lettuce	56	29	27 (48.2%)	23 (85.2%)	4 (14.8%)
Total	112	49	63 (56.3%)	49 (77.8%)	14 (22.2%)

Fifty-six of the leafy vegetable samples were contaminated with parasites, whereas 50 (79.4%) infected with protozoan cysts and 30 (47.6%) were infected with helminth eggs -- 6 samples (9.5%) with *Giardia* spp.; 12 samples (19.1%) with Cysts *Enatmeaba histolytica*; 15 samples (23.8%) with *Entameaba coli* cysts; 14 samples (22.2%) with *Coccidia* spp. Oocysts; 1 sample (1.6%) with *Balantidium coli* and 2 samples (3.2%) with unknown cysts. Helminth eggs were also found: 5 samples (7.9%) with *Hymenolepis nana* eggs; 2 samples (3.2%) with *Ascaris lumbricoides* eggs, 17 samples (26.9%) with *Toxocara* spp. eggs, 3 samples (4.8%) with *Strongyloides* spp. eggs, 1 sample (1.6%) with each of *Trichius trichura* and *Trichostrongylus* spp. eggs (Tables 2, 3, 4). There was a weak correlation between protozoa and helminths contamination of the same vegetable (whether rocket or lettuce)  $r = 0.126$ ,  $r = -0.240$  respectively, while there was a strong correlation of the same type of parasite (protozoa or helminths) between rocket and lettuce  $r = 0.760$ ,  $r = 0.914$  respectively. Fig (1) illustrates the statistical difference between protozoa and helminths contamination of rocket and lettuce ( $P \leq 0.01$ ).



Lettuce	27	2 (7.41%)	0	4 (14.8%)	0	0	0
Total	63	5 (7.91%)	2 (3.2%)	17 (27%)	3 (4.8%)	1 (1.6%)	1 (1.6%)

From table (3) it is apparent that the highest contamination in lettuce and rocket samples came from the *Entameaba coli* (29.6%; 19.4% respectively), followed by *Enatmeaba histolytica* cysts and *Coccidia* spp. Oocysts presented in lettuce (25.9% / each), and in rocket (13.4%; 19.4%) respectively. Furthermore, table (4) illustrates that the highest contamination was from *Toxocara* spp, found in both rocket and lettuce (35.1%; 14.8%) respectively, whereas contamination from *Hymenolepis nana* was shown in 8.3% of rocket samples and in 7.4% of lettuce. The density score of the parasites in the contaminated rocket and lettuce samples ranged from high (coccidian oocysts) to rare (helminth eggs).

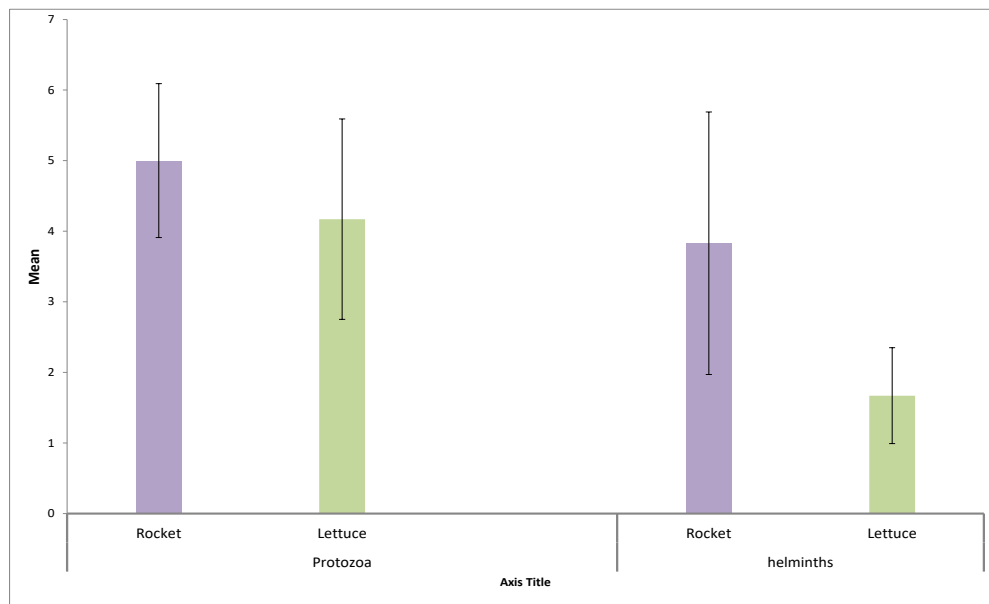


Fig 1. Parasitic contamination (Mean  $\pm$ SE) in leafy green vegetables.

### Discussion

Leafy green vegetables, the consumption of which is so important for human health, can become contaminated by different stages of intestinal parasites through many factors, including irrigation, soil and the type of fertilizer used. Fresh leafy vegetables are the basic ingredient for the traditional salads in many countries including Libya, and so they become an important route of transmission of intestinal parasites, (Slifko *et al.*, 2000; Beuchat, 2002 and Pires *et al.*, 2012). From previous studies in developing countries, the contamination rates of green vegetables was found to range between 16% and 58%. Robertson and Gjerde (2001) in Norway and Adanir and Tasci (2013) in Turkey noted the contamination rates were lower in developed countries compared with developing countries (Daryani *et al.*, 2008; Abougrain *et al.*, 2010; Fallah *et al.*, 2012; Hassan *et al.*, 2012; Ali and Ameen, 2013).

In this study, it was found that 56.3% of the 112 leafy green vegetables examined were contaminated with different parasites. This result is similar to Abougrain *et al.*, 2010 from Tripoli, Libya (58%). Other studies from developing countries such as Iran (56.7%) and Iraq (49.8%) correspond with this study (Daryani *et al.*, 2008 and Ali and Ameen, 2013). The results show a higher rate of contamination compared with results from studies in Egypt (19.4%) and Saudi Arabia (27.2%) by Hassan *et al.*, 2012 and Al-Binali *et al.*, 2006 respectively. Their findings record the highest rate of contamination in the green rocket (64.3%), followed by lettuce (48.2%), and these results were similar to the findings of Abougrain *et al.*, 2010 in Tripoli, Libya. The Tripoli results found 100% and 96% contamination rates in rocket and lettuce respectively. These results agree with Said (2012) in Egypt. These are significant differences and there were found to be strong correlation between rocket and lettuce green vegetables in contamination rates  $P \leq 0.01$ . Many factors contribute to such differences, including the type and number of samples examined, the methods used for detection of the intestinal parasites, and the type of water used for irrigation.

In this study, the *Toxocara* spp eggs were reported to be the most dominant parasite contaminating leafy green vegetables (27%), followed by *Entameaba coli* cysts and coccidian oocysts (23.8% and 22%) respectively. Abougrain *et al.*, 2010, a study from Tripoli, Libya recorded higher contamination rates by *Toxocara* spp eggs in rocket and lettuce and agrees with our results. Moreover, in Egypt Said (2012) found *Toxocara* spp eggs in 31.7% of lettuce samples. In Tripoli, Libya, Abougrain *et al.*, 2010 reported 68% of examined vegetables were contaminated by *Ascaris lumbricoides* eggs. While in this study, just 6% of rocket samples were contaminated by *Ascaris* eggs. The contrast may be due to the fact that the leafy vegetables in this study were washed with tap water before they were embedded in normal saline, as the *Ascaris* eggs are sticky and may adhere to the leaves. Our findings correspond with the 5.8% found by Behrouz *et al.*, 2013 from Iran. It may be deduced from these findings that the wide variation in parasitic contaminants of vegetables were not only related to environmental contamination, but correlate with several other factors, such as type and number of samples and the method of diagnosis. In conclusion, the results in this work indicate that leafy green vegetables in Misurata, Libya are contaminated with intestinal parasites and may pose a health risk to consumers of green vegetables. It is recommended that greater attention be paid to the treatment of sewage water and that the use of untreated waste water for irrigation of plants intended for human consumption be banned. The study points to the continued urgency of proactive and practical education programs that teach the hazards of poor hygienic practices in connection with leafy green vegetable cultivation and subsequent human and animal consumption.

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