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Contamination of *Cryptosporidium* spp. Oocysts in Raw Vegetables Produced in Koya City, Iraq

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HIGHLIGHTS

- The average prevalence rate of *Cryptosporidium* was 6.5% in vegetables of Koya, Iraq.
- Contamination rates were not related to vegetable types and also sampling seasons.
- Serious control measures and training must be carried out in the studied area.

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ABSTRACT

Background: *Cryptosporidium* spp. is one of the most important parasitic pathogen which causes acute gastroenteritis in human. This study aimed to determine and compare the contamination rates of *Cryptosporidium* oocysts in some common vegetables that are cultured and consumed in Koya City, Iraq.

Methods: Totally, 400 vegetable samples were collected randomly from vegetable farms in Koya city during spring and summer, 2016. The vegetables, including celery, parsley, leek, radish, and green onion were examined for the presence of *Cryptosporidium* spp. by Ziehl-Neelsen acid-fast staining method. Data were analyzed by SPSS statistical software (version 21).

Results: The prevalence rate of *Cryptosporidium* spp. oocysts in parsley, celery, leek, radish, and green onion were 6.25, 7.50, 6.25, 6.25, and 6.25%, respectively, showing no significant differences (p>0.05). Also, *Cryptosporidium* spp. oocysts were detected in 12 out of 200 (6%) samples from spring and 14 out of 200 (7%) samples from summer, without any significant difference (p>0.05).

Conclusion: Our findings highlighted that if the proper washing as well as disinfecting actions are neglected before consumption of the vegetables, consumption of raw vegetables could be potentially hazardous, inducing *Cryptosporidium* infection in Iraqi people.

Introduction

Consumption of raw vegetables without proper washing is one of the most significant causes of human parasitic diseases (Said, 2012). *Cryptosporidium* spp. is an important zoonotic parasitic agent which its excreted oocyst in the feces can infect the hosts, especially mammalians. Although the oocysts cannot multiply out of the host but they have the ability to tolerate environmental adverse conditions for a several months until their inges-

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tion by a new suitable host. Within the new host, the parasite's life cycle begins again, involving multiplication through utilizing resources of infected host (Chalmers and Katzer, 2013; Checkley et al., 2015; Rossle and Latif, 2013).

The main clinical feature of cryptosporidiosis is watery diarrhea that can differ from relatively mild to quite severe. There is also complained of abdominal pain and mild fever by infected persons. The immunodeficiency persons are more sensitive to infection (Petri et al., 2000). This protozoan parasite can also spread by water and soil. Human get infection by fecal-oral transmission and consumption of raw vegetables contaminated by oocysts. The food handlers in catering or restaurants with poor personal hygiene and unsanitary practice are the major sources of the infection (Chalmers and Katzer, 2013). Irrigation of vegetables using untreated or fecal-contaminated water is the main contamination source of these products prior to harvesting (Al-Binali et al., 2006; Amoah et al., 2007; Pires et al., 2012); however, contamination could be occurred at the time of harvesting, transportation, handling, distribution, or even at home settings (Said, 2012; Tefera et al., 2014).

There are some studies performed previously to assess the fact of intestinal parasite transmission through raw vegetables in several countries such as Egypt (Said, 2012), Libya (Abougrain et al., 2010), Canada (Dixon et al., 2013), Ghana (Duedu et al., 2014), Saudi Arabia (Al-Megrm, 2010), Peru (Ortega et al., 1997), Philippines (Su et al., 2012), Nigeria (Damen et al., 2007), Iran (Rostami et al., 2016), and also Iraq (Hadi, 2011). In our literature study, we did not find any published data about the contamination of *Cryptosporidium* oocyst in produced edible raw vegetables in Koya city, Kurdistan Region-Iraq. Therefore, this study aimed to determine and compare the contamination rates of *Cryptosporidium* spp. oocysts in some common vegetables that are cultured and consumed in this area from Iraq.

Materials and methods

The samples

The Koy Sanjaq or Koya city is located in the Erbil Governorate of Iraqi-Kurdistan, close to the Iranian border. There are 27 agriculture farms which they cultivate and supply the raw vegetables. Totally, 400 vegetable samples were collected randomly from farms in Koya city during spring and summer, 2016. Eighty samples were collected from each of the most common raw consumed types of fresh vegetables, including celery, parsley, leek, radish, and green onion (Figure 1).

Parasitological examination

The vegetable samples were about 400-500 g from each vegetable type. Each sample was soaked in one L of physiological normal saline (0.95% NaCl) and followed by mechanical shaking for 15 min. The top layer was discarded and the remaining wash solution was filtered through a sieve (micron pore size) to remove large debris, and then centrifuged at 2000 rpm for 15 min. The supernatant was decanted, and after that a few drops of the sediment were put on the glass slides and analyzed for parasites using Ziehl-Neelsen acid-fast staining method. For this work, the smear on a slide prepared from the sediment; then, the smears were air dried, and then fixed by ethanol. In the next step, alkaline fuchsine was poured on each slide and heated, but not boiled, for 5 min. Each slide was then washed carefully by water and decolorized using sulfuric acid (2.5%) for about 1 min. Next, each slide was stained with 1% methylene blue for 1 min, rinsed with tap water, and air dried. The detection of *Cryptosporidium* spp. oocysts was carried out by light microscope observation with ×100 oil-immersion objective lenses.

Data analysis

The collected data were analyzed by SPSS statistical software (version 21). The *p* value less than 0.05 were used to declare association.

Results

The average contamination rate of *Cryptosporidium* spp. oocysts in produced edible green vegetables in Koya city, Iraq was 6.5%. The results revealed that the prevalence rate of *Cryptosporidium* oocysts in parsley, celery, leek, radish, and green onion were 5 of 80 (6.25%), 6 of 80 (7.50%), 5 of 80 (6.25%), 5 of 80 (6.25%), and 5 of 80 (6.25%), respectively. There was no statistically significance difference between the vegetable types and the contamination rate of *Cryptosporidium* spp. oocysts ($p>0.05$).

Cryptosporidium spp. oocysts were detected in 12 out of 200 (6%) samples from spring and 14 out of 200 (7%) samples from summer. However, no significant ($p>0.05$) seasonal variation was found regarding contamination rates of *Cryptosporidium* spp. oocysts in the vegetables samples during spring and summer.

Discussion

In different areas of the world, the vegetables may be contaminated during pre-harvesting, harvesting, and post

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Figure 1: The most common local vegetables in Koya City, Iraq sampled in this research

harvesting. Sometimes, untreated wastewater is used to irrigate the vegetables, therefore consumption of raw vegetables induce parasitic infection if no appropriate washing is done (Damen et al., 2007; Gupta et al., 2009; Insulander et al., 2008; Ma et al., 2014; Pires et al., 2012; Said, 2012). In our studied area in Iraq, most vegetable farms are irrigated by wastewater and fertilized by natural fecal sources; these are likely the major causes of *Cryptosporidium* contamination rate of 6.5% found in the samples. Similar findings have been previously indicated by Al-Binali et al. (2006) from Saudi Arabia that reported the prevalence of 8.33 and 2.78% for *Cryptosporidi-*

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um oocysts in watercress and leek samples, respectively. In another research, Dixon et al. (2013) found *Cryptosporidium* spp. in 5.9% of pre-cut salads and leafy greens sold in Ontario, Canada. In agreement with the results of the current survey, Ranjbar-Bahadori et al. (2013) showed that 6.6% of examined vegetable samples of Tehran, Iran were contaminated with *Cryptosporidium* spp. Also, they found green onion as the most contaminated vegetable among all vegetables samples. In addition, Ahmad et al. (2016) determined the occurrence of *Cryptosporidium* spp. oocyst in Egyptian raw vegetable salads, including onion (1.5%), diil and parsley (3.4%), tomato (1.1%), and carrot (2.4%) which in accordance with the present survey. However, much higher incidence rate of this parasite reported by some Egyptian researchers who found *Cryptosporidium* oocyst in 33.3% of parsley (Sherbini et al., 2016) and 19% of green onion samples (Said, 2012). Such high rate of *Cryptosporidium* was also seen in vegetables samples of Ilam, West of Iran reported as 30.7% (Avazpoor et al., 2015). The observed differences might be due to differences in environmental conditions, sanitary practice, and the sample size difference between the studies.

Based on the present research, contamination rates were not significantly related to vegetable type which is similar to the results of Maikai et al. (2013) who studied the prevalence of *Cryptosporidium* spp. in 200 vegetable samples marketed in Zaria metropolis, Nigeria with no difference between contamination rates in lettuce (48%), spinach (40%), and waterleaf (36%). Accordingly, Rahman et al. (2014) have reported that the rates of *Cryptosporidium* oocysts did not differ among the total examined vegetables in Bangladesh.

The contamination rates of *Cryptosporidium* spp. in our vegetable samples had no difference between spring and summer. Rostami et al. (2016) announced that parasitic contamination of Iranian vegetable samples was higher in warm seasons than in cold seasons. Similarly, Fallah et al. (2012) stated that although the rate of parasitic contamination rates in vegetable samples were not significantly different between spring and summer (as warm seasons), but this rates were reduced considerably in autumn and in winter (as cold seasons). Since our vegetable samples just were obtained in warm seasons, we could not achieve comprehensive comparison in this regards, which is limitation of this study.

Conclusion

Our findings highlighted that if the proper washing as well as disinfecting actions are neglected before consumption of the vegetables, the consumption of raw vegetables could be potentially hazardous for inducing *Cryptosporidium* spp. infection in Iraqi people. Since inappropriate hygienic conditions and poor food handling practices are common in Iraq, serious control measures must be carried out by health authorities. Also, training of the farmers, food handlers and consumers is too important to improve their knowledge about risk of this parasitic infection and its preventive actions.

Author contributions

H.S.A. designed the study and wrote the manuscript. G.R.J.K. and S.N.M. conducted the study and supervised the analysis and the manuscript. N.S., M.Y., and M.R. drafted and consulted the analysis. H.S.A. and K.K.S. performed the experiments. All authors revised and approved the final manuscript.

Conflicts of interest

There is no conflict of interest.

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