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Contamination of Raw Herbs with Parasitic Protozoa and Helminths in Shushtar City, Southwestern Iran

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ABSTRACT

Introduction: Intestinal parasites are among the most prevalent foodborne diseases worldwide, and raw vegetables and herbs are among the primary sources of human infection by these parasites. This study aimed to investigate the prevalence of parasitic contamination of fresh herbs in Shushtar, Khuzestan Province, Southwest of Iran. **Methods**: In this study, 129 herb samples from various farms were collected and washed with water. The washing waters were centrifuged, and the resulting sediments were examined by formol-ether concentration and Sheather's sugar flotation procedure, as well as a wet smear and Ziehl-Neelsen staining. **Results**: Among the 129 samples, 73.6% (n=95) showed contamination with at least one parasite, including trophozoite like amoebae (52.6%), followed by *Giardia lamblia* (14.7%), *Cryptosporidium* spp. (2.1%), *Blastocystis* sp. (21%), free-living nematodes larvae (3.1%), Trichostrongilid nematodes (1.05%), *Ascaris lumericoids* eggs (2.1%), *Hymenolepis* spp. (2.1%) and Taeniid eggs (1.05%). **Conclusion**: A high prevalence rate of parasitic contaminations of herbs in Shushtar necessitates proper washing of herbs and vegetables by consumers to prevent parasitic infections.

INTRODUCTION

Parasitic infections, mainly soil-transmitted helminthiases, are among the most severe health problems, particularly in unprivileged, rural, and deprived urban communities of developing economies with poor personal and environmental hygiene and inadequate access to safe drinking water supply [1, 2]. About 3.5 billion people are infected by different parasites worldwide, among which only ~450 million are symptomatic [3, 4]. Amoebiasis is a widespread infection affecting 104 million people of the world's population [5]. About 184 and 64 million people worldwide have an infection with *Giardia lamblia* and *Cryptosporidium* spp., respectively, and 576-740 million are assumed to have hookworm infection. Hookworm, *Ascaris* sp., and whipworm infections are significant worldwide burdens [4].

Various studies indicate intestinal parasitic infections of 18.4%-39% in different areas of Iran [6]. These parasites are mainly transmitted to humans through the fecal-oral route [5-7]. The most critical intestinal parasitic complications are malnutrition and stunted growth, especially in children and pregnant women [4]. In Iran, herbs (sometimes referred to as vegetables) including basil, mint, tarragon, chives, radish, scallion, cilantro, parsley, dill, and watercress constitute an essential part of a healthy diet due to their nutritional value. Simultaneously, contamination of these herbs with various enteric pathogens such as viruses, bacteria, or parasites poses a severe threat [8, 9]. The contamination source may be human or animal feces used as fertilizer or wastewaters used for irrigation [10, 11]. Dietary habits, such as consuming raw or unwashed vegetables, play a pivotal role in transmitting parasitic infections [9]. The most critical parasites transmitted to humans by contaminated vegetables and herbs include *Giardia duodenalis*, *Cryptosporidium*, *Toxoplasma gondii*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Ascaris* spp., *Hymenolepis* spp., *Toxocara* spp., *Trichostrongylus* spp., and *Taenia* spp. [9, 11, 12]. Raw or unwashed vegetables are considered a major source of intestinal parasitic infections, especially in developing and underdeveloped countries [13]. Consumption of raw herbs constitutes a major part of the Iranian dietary regimen; hence, increasing the plausible risk of parasitic infections [14, 15]. Intestinal parasites are abundant in subtropical areas, including the southwestern regions of

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Iran; the intestinal parasitic infection prevalence in this area is about 37.5% [2, 16-18].

Concerning the high prevalence rate of intestinal parasites and their health threats, the present study aimed to investigate the frequency of parasite contamination in fresh herbs of Shushtar in Khuzestan province, southwestern Iran.

MATERIALS AND METHODS

Study area. The study area comprised suburbans around Shushtar city in the north part of Khuzestan Province, southwestern Iran (Fig. 1). The Shushtar is 2436 $km^{2,}$ with a history of several millenniums evident in its historical sites. Shushtar is known as the paradise of Khuzestan and can be the best destination for travelers in winter and spring due to its Mediterranean climate. This city is very suitable for agriculture, especially vegetable, herbs, grain, cotton, and sugarcane. The orchards in the city are full of good pomegranates, vines, and date palms.

Sample preparation and examination. One hundred twenty-nine herb samples, each weighing ~5-10 kg, were purchased from various farms in suburban areas in the north, south, east, and west of Shushtar city (Fig. 1). The samples were immersed in 5 liters buckets of tap water for 15 min, and then the sediments were obtained by centrifugation of wash water at 500 g for 7-10 min. The sediments were examined by formalin-ether and Sheather's sugar flotation techniques described elsewhere [19-21], and prepared smears were examined under 10X and 40X objective lenses. Smears stained with the Ziehl-Neelsen method were examined under the oil immersion objective lens (100x).

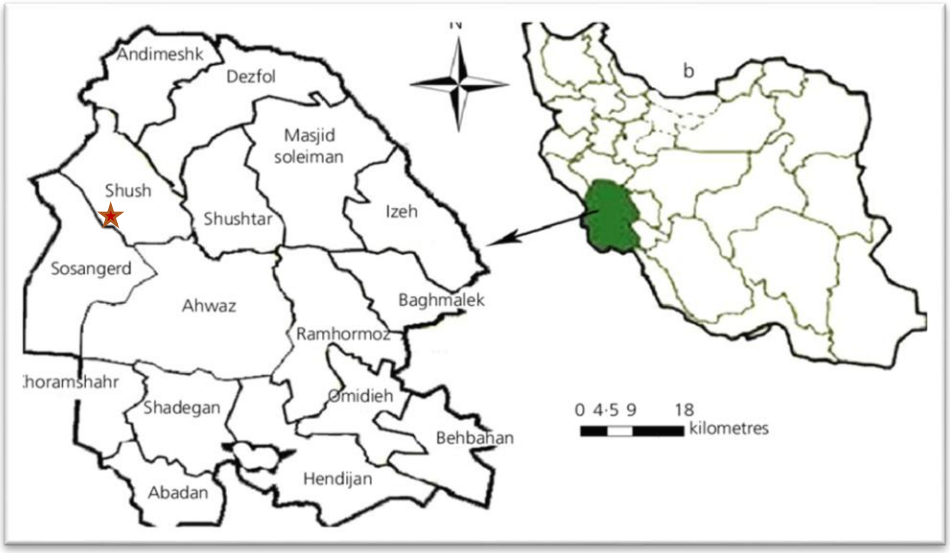

Fig. 1. Map of Iran and Khuzestan Province, the red star indicates the study area, Shushtar city.

The ethical committee of the Shoushtar Research Center of Medical Sciences, Shoushtar, Iran, approved the project (code number: IR.SHOUSHTAR.REC.13970).

RESULTS

Of the 129 herb samples, 73.6% (n=90) showed parasite contamination, including trophozoite like amoebae showing the highest rate of 52.6% (n=50) followed by *Giardia lamblia* 14.7% (n=14), *Cryptosporidium* spp. 2.1% (n=2), *Blastocystis* sp. 21% (n=20). Moreover, free-living nematodes larvae 3.1% (n=3), trichostrongilid nematodes 1.05% (n=1), *Ascaris lumericoids* eggs 2.1% (n=2), Taeniid eggs 1.05% (n=1), *Hymenolepis* spp. 2.1% (n=2), and some flagellates were observed in the samples (Fig. 2 A, B) and (Fig. 3 A, B, C).

DISCUSSION

Raw or unwashed vegetables and herbs have an important role in the transmission of fecal pathogens. Previous studies have shown a relatively high prevalence of intestinal parasite infections (IPIs) in the south of Iran [1, 2, 4, 18]. Our study revealed a high rate of parasite contamination in raw herbs in Shushtar city, Khuzestan province (south of Iran); about 73% of the samples showed contamination with at least one protozoa or a helminth.This rate was higher than those detected in other areas of Iran.

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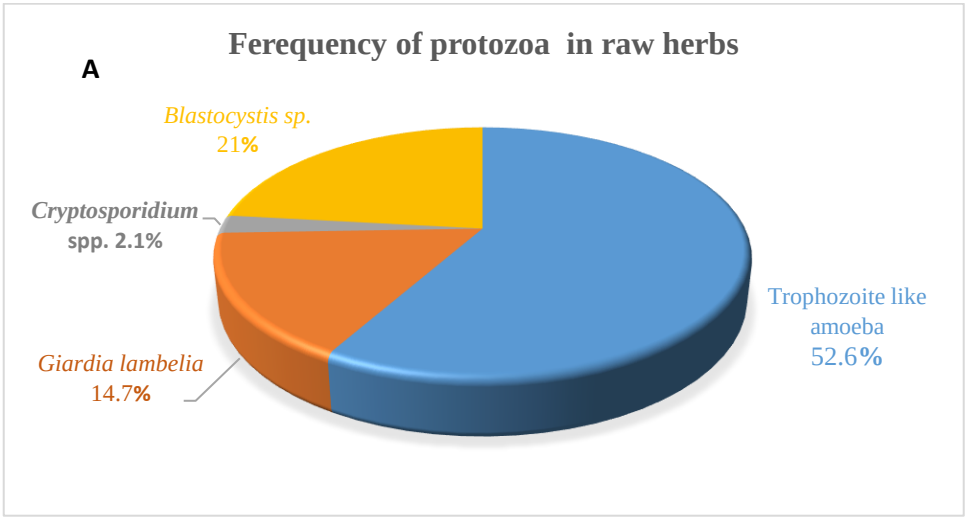

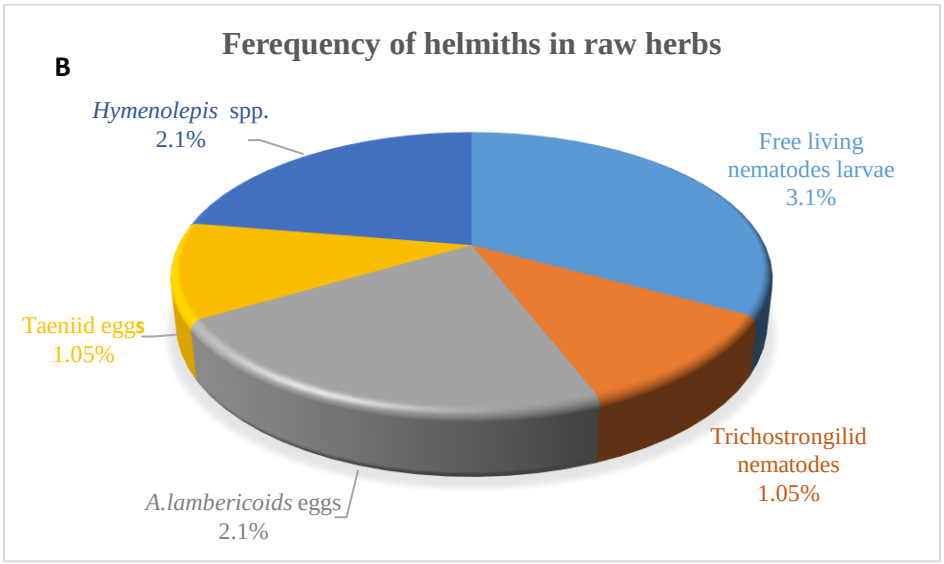

Fig. 2. Frequency of parasite contamination in raw herbs of Shushtar city, Khuzestan province, southwestern Iran; A, protozoa; B, helminths.

The overall frequencies of vegetable parasitic contamination in Tehran were 65% [22], followed by Shahrekord (34.78%) [23], Qom (31.55%) [24], Ahvaz (15.5%) [25] and Hamedan (8.4%) [26]. In other countries, the vegetable contamination rates with parasites were 13.5% in Sudan [27], 50.9% in Brazil [28], 57.8% in Southwest Ethiopia [29], and 15.1% in United Arab Emirates [30]. In our study, the contamination rates with protozoa and helminths were 90.5% and 9.47%, respectively. This finding agrees with previous reports from Iran [14, 18, 22]. Trophozoites like amoeba were the most frequent parasites in our samples (52.6%), followed by *Giardia lamblia* (14.7%), *Cryptosporidium* spp. (2.1%), and *Blastocystis* sp (21%).

Previous studies have shown that the human prevalence rate of *Giardia* was about 1%-60% in Iran [2, 18, 31] and 5.7%-9.5% in Iraq [32]. Blastocystis infection were reported 5.6%-27.5% in Iran [2, 31, 33]. *Blastocystis hominis* is also among the most prevalent IPIs in villages in southwestern Iran [2]. The overall frequency of helminths in our samples was 9.47%, including free-living nematode larvae (3.1%), *Ascaris lumericoids* eggs (2.1%), *Hymenolepis* spp. (2.1%) and Trichostrongylidae and Taeniid eggs (1.05%). The

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vegetable contamination rate can be influenced by various factors, including the water used in irrigating agricultural lands, season, and unprocessed organic fertilizer used in farms and gardens [14]. Recent studies have revealed the impact of unwashed and pre-washed vegetables used in salads on parasite transmission to humans [14].

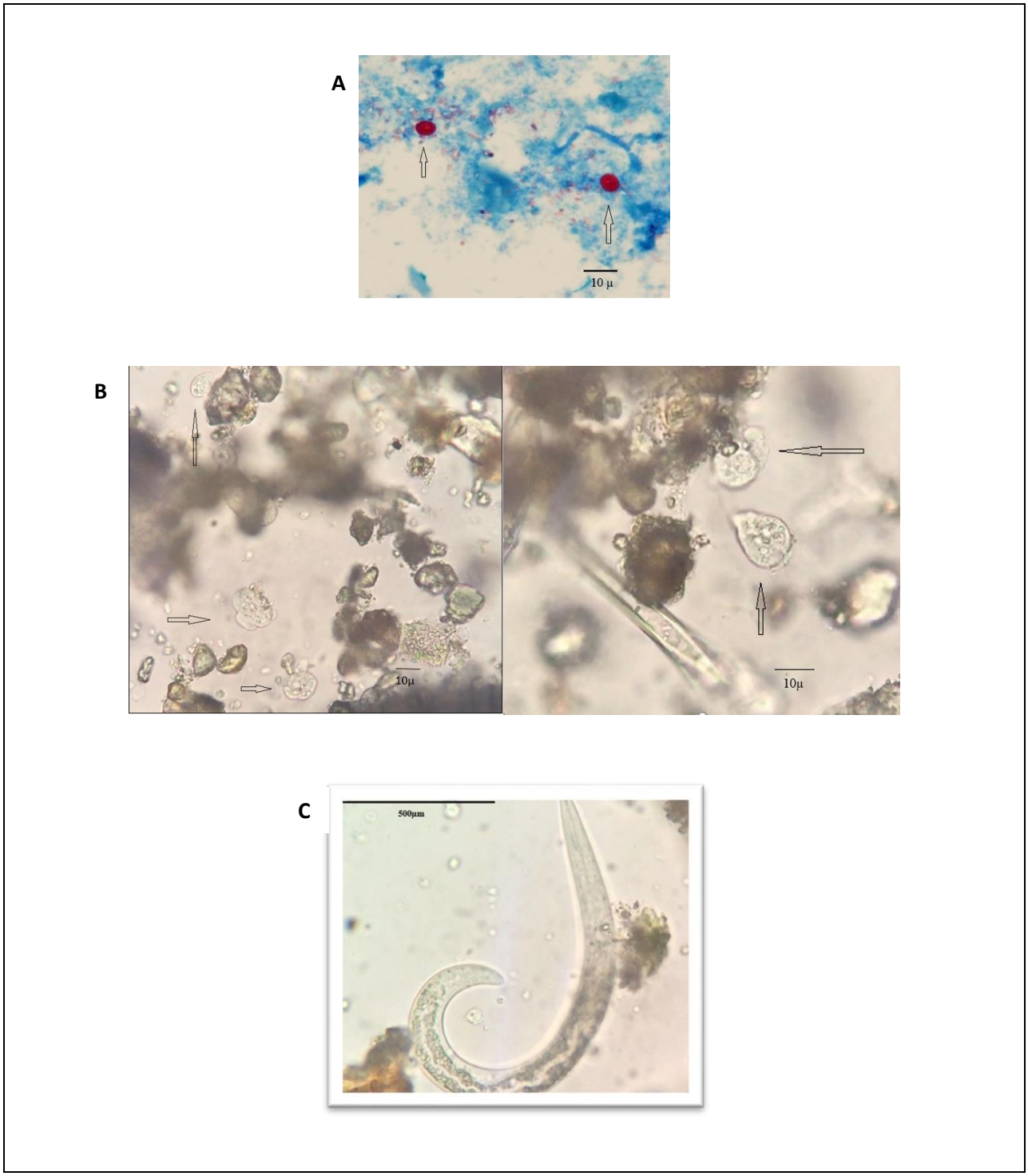

Fig. 3. Microscopical Detection of parasites in sediments from herbs wash waters. A) Ziehl-Neelsen staining shows *Cryptosporidium* oocyst. (Scale bars= 10µ) 100x, B) trophozoite like amoebae, (scale bars= 10 µ) 40x, and C) a free-living nematode larva (scale bars= 500 µm) 10x.

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Contaminated fresh vegetables have also been indicated as a food-borne parasitic infection in unprivileged, rural, and deprived urban communities of developing economies [34, 35]. Iran shows a decline in the prevalence of geohelminthe infections [36], which may be related to improved sanitation and health status.

Educational programs for proper washing of vegetables can prevent parasitic infections. Farmers should be aware of the impact of using organic human and animal fertilizer in spreading parasitic diseases. Regular monitoring of vegetable and herbs parasitic contamination by health care authorities can keep people alert of the risk of Parasitic infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests associated with this manuscript.

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