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ANÁLISE PARASITOLÓGICA DE AMOSTRAS DE AGRIÃO *(Nasturtium officinale, Barbarea verna e Lepidium sativum)* COMERCIALIZADAS EM CURITIBA E REGIÃO METROPOLITANA, PARANÁ, BRASIL

(Parasitological analysis of cress (Nasturtium officinale, Barbarea verna e Lepidium sativum) samples from Curitiba area, Brazil)

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RESUMO: Doenças transmitidas por alimentos têm grande importância na veiculação de vários microrganismos, podendo apresentar risco à população humana e animal. O presente estudo teve como objetivo analisar a contaminação parasitológica em agriões (*Nasturtium officinale, Barbarea verna* e *Lepidium sativum*) comercializados em diversos estabelecimentos na cidade de Curitiba e Região Metropolitana, Paraná. Foram analisadas 50 amostras, submetidas à técnica de lavagem e centrifugação e o método de sedimentação. Dentre as amostras analisadas, 6 apresentaram parasitos sendo eles trofozoítos de *Balantidium coli*, cisto de *Entamoeba coli* e parasitos não determinados da Família Strongylidae e Trichuridae; e 14 amostras possuíam contaminantes como larvas, lesmas e caracóis. Os fatores relacionados a essa contaminação parasitológica podem ser decorrentes a falhas na qualidade da água de plantio e irrigação, e armazenagem e distribuição dos vegetais na região. Visto ser um produto consumido *in natura*, é recomendada a inclusão de procedimentos seguros ao longo de todo o processo para proteger a saúde dos consumidores, já que os vegetais podem ser um veículo transmissor de zoonoses.

Palavras-chave: agrião; segurança alimentar; contaminação ambiental; parasitos; zoonose

ABSTRACT: Food-borne parasite infections affect communities worldwide by transmitting microorganisms, imposing a serious risk to human and animal health. The aim of this study was to analyze the parasitological contamination in cress (*Nasturtium officinale*, *Barbarea verna* e *Lepidium sativum*) from various establishments in the metropolitan area of Curitiba, State of Parana. We analyzed 50 cress samples using the wash and centrifugation technique and the sedimentation method. Among the samples, 6 presented trophozoites of *Balantidium coli*, cyst of *Entamoeba coli* and parasites of Stronylidae and Trichuridae Family and 14 samples were contaminated with maggots, slugs and snails. Factors related to this parasitological contamination may be the failure in the quality of water used for irrigation, and the planting, storage and the distribution of the plants. As these vegetables are mainly eaten in natura, we recommend the inclusion of safety procedures along the entire process to protect the health of consumers, since vegetables can be a zoonosis transmitter vehicle.
Key Words: cress; food safety; environmental contamination; parasites; zoonosis

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INTRODUCTION

Vegetables are widely consumed by the population, being very important in the maintenance and development of humans and animals' health. However, they should be in sanitary conditions suitable for consumption (Cavalcante and Correa, 2010; Neres *et al.*, 2011). Vegetables are also seen as ecological niches that sustain a dynamic and variable microbiota (Rosa and Carvalho, 2000). The microflora of plants can vary considerably depending on the type of environmental and seasonal factors and the source of microorganisms can be from soil, water, insects and animals. These factors are directly influenced by man using safe planting technology, transport and storage conditions (APPCC, 1997; Pacheco *et al.*, 2002; Rosa and Carvalho, 2000).

Most of the plants used for food can be a parasite transmission vehicle, containing protozoan cysts, eggs and larvae of helminths and the lack of minimum health conditions, such as the water contamination by human feces used for irrigation may impose a real threat to consumers (Simoes *et al.*, 2001; Norberg *et al.*, 2008; Silva *et al.*, 2005). A third of the world's population lives in environmental conditions that facilitate the spread of parasite infections. Brazil, as a tropical country, has the climate and socioeconomic conditions that favor the occurrence of parasitic diseases, both in rural and urban areas (Soares *et al.*, 2005).

The lack of personal hygiene when processing and handling fresh food is an important factor for intestinal parasites transmission, so individuals with asymptomatic intestinal parasites that manipulate food represent a major source of parasite contamination and dissemination (Montanher *et al.*, 2007). Transmission of parasites from contaminated soil, air, water, flies, hands and food participate in the life cycle of important helminths (*Ascaris lumbricoides, Fasciola hepatica*) and protozoa (*Entamoeba histolytica* and *Giardia* sp.) (Silva *et al.*, 2014).

Other factors that contribute to the contamination of vegetables are inadequate practices in the field during harvesting and the processing by inefficient cleaning, insect infestation, transport, distribution and improper food display. This contamination is difficult to remove as the vegetables are eaten raw (Machado *et al.*, 2009). The plant structure also facilitates contamination and in the case of watercress, it presents multiple and separate leaves with large contact area, allowing a greater fixation of free-living parasite stages (Silva, 2005).

Watercress is widely distributed mainly in the Southern and Southeastern regions of Brazil (Siqueira *et al.*, 1997). In addition, watercress consumption tends to grow at a rate of at least 10% per year (Agron-team, 2015). Recent studies have shown a high number of lettuce contaminations by intestinal parasites such as helminth and protozoan (Bonilha *et al.*, 1994; Freitas *et al.*, 2004; Garcia *et al.*, 2004; Montanher *et al.*, 2007; Oliveira *et al.*, 2012; Santarém *et al.*, 2012; Silva *et al.*, 2005). Montanher *et al.* (2007) conducted a study on lettuce samples commercialized in restaurants in Curitiba, revealing the presence of *Iodamoeba butschilii*, *F. hepatica*, *Trichocephalus trichiurus* and *E. histolytica*.

The helminth and protozoa infections are among the most common diseases in the world, and may affect the nutritional balance, causing significant complications such as intestinal obstruction and rectal prolapse in humans (Costa-macedo, 1999).

Vegetables, especially watercress and lettuce, can be important transmission vehicles to zoonotic diseases evidenced by the visualization of *Giardia lamblia* that indicates fecal contamination (Alves, 2013; Silva, 2014; Vieira, 2013). *Toxocara* spp. and *Ascaris* spp. eggs were also found, indicating the contamination of these vegetables by faeces of dogs. The ingestion of *Toxocara canis* eggs causes visceral larva migrans and the ingestion of *Ascaris lumbricoides* eggs causes ascariasis in humans (Maciel, 2014; Vieira, 2013).

Laboratory diagnosis of parasites present in vegetables is of great importance to public health as it may provide data on the hygienic conditions involved in the production, storage, transport and handling of these products, as well as the risks of contamination to consumers (Silva *et al.*, 2005). Few national and international studies report the vegetable contamination by intestinal parasites (Garcia *et al.*, 2004). The objective of this work was to determine the presence of parasites and contaminants in cress marketed in the metropolitan region of Curitiba.

MATERIAL AND METHODS

Fifty cress samples commercialized in Curitiba region (local markets, fruit-and-vegetable stores, outdoors and popular fairs) were collected and analyzed. The samples were obtained the day before the analysis and placed in plastic bags at 4ºC until processing. The leaves were considered a sample unit. The analyses were performed at the Laboratory of Parasitic Diseases of the Federal University of Parana, Curitiba.

Initially, one macroscopic analysis was performed removing and discarding the dry/dead parts, and to verify the presence of contaminants, such as mollusks and insects. The analysis consisted of selecting 50 grams of leaves that were washed with 450 mL of sodium chloride (NaCl) at 0,85% with the aid of a spray and a brush. Before the complete wash, the leaves were left to soak 5 min for the product to act. In sequence, the leaves were detached and the solution was filtered through to a series of sieves and subsequently transferred to a 50 mL Falcon tubes. This solution was centrifuged at 9 G for 30 minutes (Monge & Arias, 1996). The remaining fluid was deposited in a sedimentation glass reaching a final volume of 250 mL and allowed to sediment for 24 h at room temperature (Oliveira and Germano, 1992).

For the final analysis, the supernatant was discarded and 5 mL of the resulting sediment was suspended, aspirated and deposited in a glass slide covered with a cover slip. For the sedimentation glass solution, the supernatant was discarded and a 20 mL solution was ressuspended, aspired and deposited in a glass slide covered with a cover slip. Both glass slides were evaluated for parasitic structures in duplicates. The parasite identification was based in morphological keys. We performed a descriptive statistical analysis.

RESULTS

The vegetable samples were: watercress (*Nasturtium officinale*), earth-cress (*Barbarea verna*) and cress-the-dry (*Lepidium sativum*) from which 12% showed some parasite contamination (Table 1). The parasite species found were *Balantidium coli* trophozoites, *Entamoeba coli* cyst, and parasites of Strongylidae and Trichuridae Family. Others organisms were also found (insect larvae, mite, caterpillar, slugs, snails, protozoa and free living nematode larvae and Aphelencoides). The findings were considered not relevant, as they do not represent risk for human consumption.

Table 1 - Frequency of parasites and contaminants in cress samples commercialized in the metropolitan region of Curitiba, Parana, Brazil.

Parasites and Contaminants	Infected Samples	%
Balantidium coli	3	6
Entamoeba coli	1	2
Strongylidae Family	1	2
Trichuridae Family	1	2
Slug suborder Stylommatophora	9	18
Snail Stylommatophora	2	4
Insect larvae	2	4
Caterpillar	1	2

The vegetables where acquired from; outdoor fairs (4%), fruits and vegetables stores (44%), local markets (28%) and fruits and vegetables retail outlets (24%) (Table 2). Although we collected only two samples, the commercialized vegetables from outdoor fairs was by far the most contaminated source. The local market had no positive samples and could be considered secure for safety standards.

Table 2 - Origin of commercialization and comparative positivity of cress samples acquired in the metropolitan region of Curitiba, Parana, Brazil.

Local	Samples	%	Positive Samples	%
Outdoor fairs	2	4	1	50
Fruits and vegetables stores	22	44	3	6,8
Local markets	14	28	0	0
Retail outlets	12	24	2	8,3
Total	50	100	6	12

DISCUSSION

We found an average of 12% of parasite contamination compared to 66% from Silva *et al.* (1995) from Rio de Janeiro, studying cress. Oliveira and Germano (1992) the cress also had a high contamination (66,0%), however the analysis was done during the dry season, time that the irrigation of gardens is done with high intensity, increasing contamination. Moreover, the authors showed that many helminth eggs could survive long periods in wet areas, since the cress were grown in a very moist soil.

Soares and Cantos (2006) studying cress, rocket and lettuce consumed uncooked in the city of Florianopolis, state of Santa Catarina, found that cress had a higher (70,4%) percentage of enteroparasites when compared to lettuce (60%) and rocket (56%). The authors also suggested that the cress structure interferes with the level of contamination, because of the multiple and separated leaves, allowing a greater adherence of parasites.

For this type of study there is the difficulty of identifying protozoa and helminths based on the morphology, because besides the great similarity between eggs and larvae of several species of parasites of man, animals and even plants, vegetables also accumulate pollen grains, vegetables particles and contaminants from the soil, making it difficult for the visualization of parasites (Oliveira and Germano, 1992).

Although the findings, there is no data origin of the samples, as well as the forms of production and producer hygiene issues. There are also no influences of the establishments in the category storage and exposure of cress on the shelves. Despite the high contamination in free markets (50%), it noted that only two samples were analyzed.

According to our results, it is suggested that the cress marketed in Curitiba and region, have a higher quality hygiene during the planting, processing and distribution of vegetables, compared to other studies with high level of *G. lamblia*, *E. coli*, *Endolimax nana*, and *F. hepatica*

(Maciel *et al.*, 2014; Oliveira and Germano, 1992; Soares *et al.*, 2005; Vieira *et al.*, 2010).

The most common species of cress are the earth-cress (*Barbarea verna*) and the hydroponics *L. sativum*. This technique may have improved the quality of the conditions of hygiene and irrigation, resulting in the reduction of contamination. The *B. coli* trophozoites and *E. coli* cyst are considered indicators of fecal contamination, and the *B. coli* is pathogenic to humans, causing balantidiasis.

It is important to note that contamination of vegetables may occur in the cultivation, handling or transport (Falavigna *et al.*, 2005).

In these circumstances, it is necessary that the sanitary inspection act guiding the producer, handlers and consumers about the hygienic and sanitary conditions of vegetables. The washing procedures and hygiene of the vegetables before consumption, recommended by the National Health Surveillance Agency (ANVISA) (Brasil, 2004), should be advertise to reduce the risk of infection by intestinal parasites (Maciel *et al.*, 2014).

CONCLUSION

The results of the study show that on average, 12% of the cress samples commercialized in outdoors fairs, markets and fruits-and-vegetables retail outlets from the region of Curitiba were contaminated with parasites. Therefore, improvement is needed in sanitary conditions from planting to the distribution of vegetables in order to reduce the chances of contamination and the risks to food safety to humans.

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