DOI: 10.5433/1679-0359.2017v38n2p801

Parasitological analysis of green leaf lettuce cultivated in different production systems

Análise parasitológica em alfaces cultivadas em diferentes sistemas de produção

Juliana Santiago Santos[1]; Cristina Atsumi Kuba[1]; Francislaine Anelize Garcia Santos[2]; Aline da Silveira Batista[3]; Stênio Clemente Paião Sitolino[4]; Ana Caroliny Carrion Pereira[5]; Rogério Giuffrida[6]; Vamilton Alvares Santarém[7*]

Abstract

This study analysed parasite contamination in green leaf lettuce (*Lactuca sativa*), grown in different cultivation systems (conventional, organic, and hydroponic), from a family farmer cooperative in the municipality of Presidente Prudente, São Paulo, Brazil. Samples were collected at weekly intervals during five months, totalling 180 vegetable samples (60 samples of leaf lettuce from each cultivation system). Lettuce leaves were washed with 0.5% Extran MA 02, and the resulting fluid subjected to sedimentation and centrifugal flotation for recovery of parasite structures. Overall, 71 samples (39.4%) were contaminated with at least one parasite structure, 34 (47.9%) from lettuce grown in the hydroponic system, 20 (28.2%) from the organic system, and 17 (23.9%) from the conventional system. *Entamoeba* spp. cysts were the most common parasite structures found in the leafy vegetables, with the highest cyst counts found in the hydroponic system (p = 0.003). It is concluded that, regardless of the cultivation system (conventional, organic, or hydroponic), there is a possibility of green leaf lettuce contamination by intestinal parasites. Measures that improve sanitary conditions during production, as well as proper hygiene during the preparation of raw leafy vegetables, may be important to reduce contamination and consequent transmission of parasite diseases from raw leafy vegetable consumption.
Key words: Enteroparasites. Cultivation systems. Leafy vegetables. Zoonoses.

Resumo

O presente estudo analisou a contaminação parasitológica em alfaces crespas (*Lactuca sativa*) cultivadas em diferentes sistemas de produção (convencional, orgânico e hidropônico), fornecidas por uma cooperativa de produtores da agricultura familiar, no município de Presidente Prudente, São Paulo. As coletas foram repetidas em intervalos semanais, durante um período cinco meses, totalizando 180

[1] Nutricionistas. Discentes, Curso de Mestrado do Programa de Pós-Graduação em Ciência Animal, Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, São Paulo, Brasil. E-mail: nutri.julianasantiago@gmail.com; cris_kuba@hotmail.com

[2] Bióloga. Discente, Curso de Mestrado do Programa de Pós-Graduação em Ciência Animal, UNOESTE, Presidente Prudente, São Paulo, Brasil. fran02garcia@gmail.com

[3] Médica Veterinária. Discente, Curso de Mestrado do Programa de Pós-Graduação em Ciência Animal, UNOESTE, Presidente Prudente, São Paulo, Brasil. li_sbatista@hotmail.com

[4] Discente, Curso de Graduação em Medicina Veterinária, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: agropaiao@hotmail.com

[5] Discente, Curso de Graduação em Ciências Biológicas, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: carolcarrion@hotmail.com

[6] Prof., Programa de Pós-Graduação em Ciência Animal. Curso de Medicina Veterinária, Departamento de Medicina Veterinária Preventiva, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: rgiuffrida@unoeste.br

[7] Prof., Programa de Pós-Graduação em Ciência Animal, Curso de Medicina Veterinária, Departamento de Parasitologia, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: rgiuffrida@unoeste.br

[*] Author for correspondence

Received: Apr.20, 2016 – Approved: Oct. 208, 2016

amostras de hortaliças (60 amostras de alface por tipo de produção). As folhas das alfaces foram lavadas com Extran MA 02 a 0.5% e o fluído resultante submetido às técnicas de sedimentação e de centrífugo-flutuação, para recuperação das estruturas parasitárias. Observou-se que do total de 180 amostras, 71 (39,4%) estavam contaminadas por pelo menos uma estrutura parasitária, sendo 34 (47,9%) das alfaces cultivadas no sistema hidropônico, 20 (28,2%) no orgânico e 17 (23,9%) no sistema convencional. Cistos de *Entamoeba* spp. foram as estruturas parasitárias mais frequentes nas hortaliças, com maior contagem de cistos naquelas produzidas no sistema hidropônico (p=0,003). Conclui-se que independentemente do sistema de cultivo (convencional, orgânico e hidropônico), existe a possibilidade de contaminação de alfaces por enteroparasitos. Medidas que propiciem a melhoria na qualidade higiênico-sanitária na produção, assim como a adequada higienização das verduras antes do consumo "in natura" podem ser importantes para a redução da contaminação e consequente transmissão de doenças parasitárias pelo consumo "in natura" de hortaliças.

Palavras-chave: Enteroparasitos. Sistemas de cultivo. Verduras. Zoonoses.

Introduction

In recent years, increased consumption of leafy vegetables has been encouraged, as they have a key role in promoting health and longevity, by providing nutrients of plant origin, such as vitamins, minerals, antioxidants, and fibre (SLAVIN; LLOYD, 2012; WANG et al., 2014), that may contribute to a lower incidence of cardiovascular disease, cancer, and total mortality (WANG et al., 2014; BRADBURY et al., 2014).

Leafy vegetables may be cultivated in conventional, organic, or hydroponic systems. The conventional system consists of the cultivation of leafy vegetables in soil, using chemical fertilizers and pesticides. The organic system uses soil cultivation but precludes the use of pesticides, synthetic fertilizers, growth regulators, and other chemicals. In the hydroponic system, leafy vegetables are grown in plastic tubes, containing a solution of nutrients and chemical fertilizers (GOMES NETO et al., 2012).

Consumption of raw food has increased as the population aspires to healthier eating (GREGÓRIO et al., 2012). However, the quality of leafy vegetables, in terms of microbiological hygiene, is a concern, because the intake of raw leafy vegetables can be an important means of transmission of parasite diseases, including some with zoonotic importance (RAMOS et al., 2014). In the United States, 22% of foodborne illnesses are caused by leafy vegetable consumption (PAINTER et al., 2013).

Contamination can occur at various stages along the chain of production, from cultivation, through the use of organic fertilizer and contaminated water and/or soil, to consumption (GREGÓRIO et al., 2012). The use of animal manure increases the risk of microbiological and parasite contamination, making the food unsuitable to be consumed raw without proper sanitization (SANTANA et al., 2006; ARBOS et al., 2010). In the hydroponic system, contamination results from, among other factors, the sanitary conditions of the water, as, in this system, the plant nutrients are pre-dissolved in water (SANTANA et al., 2006).

Although several studies in Brazil have assessed the presence of intestinal parasites in leafy vegetables (NERES et al., 2011; GREGÓRIO et al., 2012; PACIFICO et al., 2013), little research has been done comparing leafy vegetables grown in different cultivation systems, thus justifying this study, which aimed to analyse parasite contamination in green leaf lettuce (*Lactuca sativa*) grown in different production systems (conventional, organic, and hydroponic).

Materials and Methods

Sample collection

From August to December 2015, 180 samples of green leaf lettuce (*L. sativa*) supplied by a cooperative of family farmers in the municipality of Presidente Prudente, São Paulo, Brazil, were analysed. The cooperative has 45 members, of whom 20 are vegetable producers (15 using the conventional system, two the organic system, and three the hydroponic system).

Collections were performed weekly, with 9-18 samples selected per collection in order to allow analyses to be carried out on equal proportions of samples from the three production systems, as the production of leafy vegetables in the organic system could be lower than in other systems. Overall, 60 samples from each lettuce production system were analysed.

The samples were obtained at the distribution stage, with proper identification, and then transported in cool boxes to Oeste Paulista University Preventive Veterinary Medicine Laboratory II in Presidente Prudente, Sao Paulo, Brazil, for parasitological analysis.

Parasitological analysis

Recovery of helminth eggs and larvae, and protozoan cysts or oocysts, from leafy vegetables followed the procedure described by Arbos et al. (2010), with some modifications. Dead leaves were removed from each plant and discarded. Five intact leaves were collected from the internal and external portions of each sample, placed in fresh, suitably identified plastic bags, and then 200 mL of 0.5% Extran MA 02 detergent solution was added for washing. After homogenization, the material in the plastic bag was transferred directly, without filtering, to a sedimentation cup, to which was added sufficient distilled water to make a final volume of 200 mL. The material was allowed to stand for 12 to 24 hours under refrigeration (7 °C). Afterwards, 10 mL of the sediment was transferred to graduated centrifuge tubes. The tubes were centrifuged at 2500 rpm (873 *g*) for 5 min. After centrifugation, the pellets were aspirated and placed onto glass slides for microscopic assessment of parasite structures.

To assess the presence of *Giardia* spp. cysts, the centrifugal-flotation technique described by Faust et al. was also carried out, with minor modifications (HOFFMANN, 1987). From each sedimentation cup prepared in the previous step, 1.0 mL of sediment was transferred to graduated centrifuge tubes containing zinc sulphate solution (density 1.18 g cm^{-3}). The tubes were centrifuged at 2500 rpm (873 *g*) for 5 min, and, after standing for 5 min, 50 μL of supernatant was removed with a pipette for optical microscopy evaluation.

Identification of parasite structures was performed in duplicate, stained with Lugol and analysed using an optical microscope at 100× and 400× magnification.

Parasite structures were identified at the level of genus or family, according to the criteria described by Bowman (2009) and Carli (2011). *Strongyloides* spp. eggs were identified by their small size (less than 60 μm), thin-layer and presence of larvae inside, while trichostrongylid eggs were characterized as measuring more than 60 μm, having a double layer of skin, and by the presence of blastomeres inside (CARLI, 2011).

Microscopic evaluation was performed "blind", to prevent influencing the analyst during microscopic examination.

Statistical analysis

Prior to statistical analysis, data normality assumption was assessed with the Kolmogorov-Smirnov test, which determined that the data had nonparametric distribution. To determine whether the parasite structure count was different among the vegetable growing systems, the nonparametric Kruskal-Wallis test was used, with contrasts assessed by the Student-Newman-Keuls test; 5% significance level was used for all comparisons. The frequencies of parasite structures between different systems were compared with Pearson's chi-square test, and contrasts with the bisection method, adjusting the level of significance using the Bonferroni method ($p = 0.05/3 = 0.016$). All analyses were performed with R software (R DEVELOPMENT CORE TEAM, 2013).

Results and Discussion

In this study, 71 of 180 samples analysed (39.4%) were contaminated with at least one parasite structure, of which 34 (47.9%) were from lettuces grown in the hydroponic system, 20 (28.2%) from the organic system, and 17 (23.9%) from the conventional system.

Neres et al. (2011) also observed that vegetable samples from hydroponic systems had a high level of contamination by infective and non-infective forms of intestinal parasites. Data in this study also support the results by Santana et al. (2006) and Gomes Neto et al. (2012), evaluating parasite contamination of green leaf and iceberg lettuces, respectively, in the three cropping systems, and studies analysing lettuces grown in conventional and hydroponic systems (NERES et al., 2011; GREGÓRIO et al., 2012; PACIFICO et al., 2013). Similarly, Santarém et al. (2012), Alves et al. (2013), Vieira et al. (2013), and Ramos et al. (2014) found contamination in conventional farming system leafy vegetables, and Arbos et al. (2010) in an organic production system.

Cysts of *Entamoeba* spp. were the most frequently recovered parasite structures (Table 1), in line with other studies conducted in Brazil assessing lettuce contamination (NERES et al., 2011; GOMES NETO et al., 2012; GREGÓRIO et al., 2012). In this study, the frequency (Table 1) and number (Table 2) of *Entamoeba* spp. cysts was significantly higher in lettuce grown in the hydroponic system, contrasting with the results of Gomes Neto et al. (2012), who found more cysts in lettuce produced in organic and conventional systems, and of Santana et al. (2006), who found contamination only in the samples of lettuce grown in the organic system. Pacifico et al. (2013) observed no significant difference in the frequency of contamination between hydroponic and conventional system-grown lettuces.

Table 1. Frequency (%) of positive results for parasite structures present in green leaf lettuces grown in different production systems (n = 180; 60 samples per production system). Presidente Prudente, São Paulo, Brazil. 2016.

Parasite structure	Production system			p
	Conventional % (n = 60)	Hydroponic % (n = 60)	Organic % (n = 60)	
Protozoa				
Entamoeba spp. Cysts	21.7 (13)	51.7 (31)	26.7 (16)	<0.001*
Oocysts	0 (0)	5.0 (3)	1.7 (1)	0.167
Nematodes				
Eggs	5.0 (3)	1.7 (1)	5.0 (3)	0.552
Trichostrongylid	3.3 (2)	1.7 (1)	3.3 (2)	
Strongyloides spp.	1.7 (1)	0 (0)	1.7 (1)	
Larvae	3.3 (2)	6.7 (4)	1.7 (1)	0.353
Trichostrongylid	3.3 (2)	5.0 (3)	1.7 (1)	
Strongyloides spp.	0 (0)	1.7 (1)	0 (0)	

p = significance level for comparison between different systems; * statistically significant differences between conventional and hydroponic system (p = 0.001) and between organic and hydroponic system (p = 0.008).

Table 2. Counts of parasite (mean ± standard deviation) structures present in green leaf lettuces grown in different production systems (n = 180). Presidente Prudente, São Paulo, Brazil. 2016.

Parasite structure	Production system			p
	Conventional (n = 60)	Hydroponic (n = 60)	Organic (n = 60)	
Protozoa				
Entamoeba	0.58 ± 1.44	2.25 ± 4.45	0.49 ± 0.95	0.003*
Oocysts	0 ± 0	0.1 ± 0.54	0.36 ± 2.84	0.892
Nematodes				
Eggs	0.05 ± 0.22	0.05 ± 0.39	0.066 ± 0.31	0.939
Larvae	0.033 ± 0.181	0.1 ± 0.44	0.05 ± 0.38	0.892

p = significance level for comparison between different systems; * Hydroponic system counts were statistically significantly different from the conventional (p = 0.002) and organic (p = 0.006) systems.

Other parasite structures, such as trichostrongylid eggs, were recovered at low frequency in this study (Table 1). Trichostrongylid eggs or larvae were detected in samples from all three systems, whereas *Strongyloides* spp. eggs were recovered in samples from conventional and organic systems, and one larva from the hydroponic system. Santana et al. (2006), when assessing vegetable contamination, reported *Strongyloides* spp. and trichostrongylids, with greater recovery of trichostrongylid eggs, in lettuce samples from organic and traditional system crops.

In the present study, no cysts of *Giardia* spp. were recovered. This protozoan structure was observed by Gomes Neto et al. (2012), in organic lettuce samples. Other studies, evaluating lettuces from conventional and hydroponic systems, identified *Giardia lamblia* cysts (NERES et al., 2011; GREGÓRIO et al., 2012).

Several factors may contribute to the contamination of leafy vegetables by intestinal parasites at different stages in the production chain, such as climatic conditions, hygiene and sanitation, water quality, environmental contamination, and handling during transport or sale (SANTANA et al., 2006; MELO et al., 2011; GOMES NETO et al., 2012; ALVES et al., 2013). Guilherme et al. (1999) and Melo et al. (2011) analysed faeces samples from handlers, observing intestinal parasites in 26.4% and 50% of samples, respectively. Guilherme et al. (1999) also observed the presence of *Entamoeba* cysts in handlers' nail beds, indicating that they may contribute to contamination of leafy vegetables.

In most studies on contamination of leafy vegetables, samples were acquired from commercial establishments such as markets, grocery stores, and street markets (SANTANA et al., 2006; NERES et al., 2011; GOMES NETO et al., 2012; SANTARÉM et al., 2012; PACIFICO et al., 2013; VIEIRA et al., 2013; RAMOS et al., 2014). The leafy vegetables analysed in this study were collected at a distribution point, from samples obtained directly from producers, reducing the chances of contamination during transport or sale. Thus, the presence of *Entamoeba* spp. cysts, protozoan oocysts, and nematode eggs or larvae indicate a likely contamination of the leafy vegetables analysed by organic waste, either of animal or of human origin (GUILHERME et al.,1999; SANTARÉM et al., 2012), during fertilization, as postulated by Santana et al. (2006).

Studies have shown that lettuces grown in a hydroponic system have lower contamination with intestinal parasites (SANTANA et al., 2006; GOMES NETO et al., 2012). In the present study, however, the highest frequency of parasite structures was observed in in hydroponic-grown lettuce, supporting results from Machado et al. (2015) in

Santa Maria, Rio Grande do Sul, Brazil, in which a higher frequency of parasites was observed in green leaf lettuce grown by hydroponics (68.75%), than in the conventional system (50.0%). According to these authors, inappropriate sanitary conditions of the irrigation water used in farming are the main cause of contamination of lettuce in this production system. This hypothesis may be confirmed by results presented by Takayanagui et al. (2007), who found faecal coliform contamination and intestinal parasites in leafy vegetables' irrigation water.

Contamination of leafy vegetables may occur in several stages of the production chain, including planting, harvesting, processing, distribution and final preparation, but the critical stage for contamination occurs during planting, especially in fertilization and irrigation, often carried out with untreated water and manure (GREGÓRIO et al., 2012). However, as postulated by Machado et al. (2015), in this study, the presence of parasite structures in lettuces produced in the three production systems, especially in hydroponics, may be associated with the quality of water used for irrigation.

Another limitation of our study was that only green leaf lettuce samples were analysed. However, this variety of leafy vegetables was chosen for being the most widely consumed worldwide, and for not suffering seasonal interference.

Raw leafy vegetables are an important means of disseminating parasite structures (COELHO et al., 2001; PAULA et al., 2003). The presence of parasite structures in different crop systems demonstrates their relevance to public health. Although not causing, in most cases, high lethality, intestinal parasites may affect nutritional balance, trigger intestinal bleeding, interfere with the absorption of nutrients, and reduce food intake (WHO, 1981). The results in our study support this assumption and show that leafy vegetable contamination appears to be independent of the crop system.

Thus, in addition to proper hygiene treatment of leafy vegetables before consuming them raw, (PACIFICO et al., 2013), adopting educational measures to promote the improvement of sanitary conditions in the supply chain can minimize or eliminate the risk of parasite infection in leafy vegetables.

Conclusions

The present study shows that, regardless of the cultivation system (conventional, organic or hydroponics), lettuces may be contaminated by intestinal parasites. Measures conducive to improving sanitary conditions in the production of leafy vegetables may be important for reducing contamination and consequent transmission of parasite diseases during raw leafy vegetable consumption.

Acknowledgements

The authors wish to thank the Family Farming Cooperative (COAF) "Melhor da Roça", Presidente Prudente, São Paulo, Brazil, for supplying the leafy vegetables.

References

ALVES, A. S.; CUNHA-NETO, A.; ROSSIGNOLI, P. A. Parasitos em alface-crespa (*Lactuca sativa* L.), de plantio convencional, comercializada em supermercados de Cuiabá, Mato Grosso, Brasil. *Revista de Patologia Tropical,* Goiânia, v. 42, n. 2, p. 217-229, abr./jun. 2013.

ARBOS, K. A.; FREITAS, R. J. S.; STERTZ, S. C.; CARVALHO, L. A. Segurança alimentar de hortaliças orgânicas: aspectos sanitários e nutricionais. *Ciência e Tecnologia de Alimentos,* Campinas, v. 30, n. 1, p. 215-220, maio 2010.

BOWMAN, D. D. Diagnostic parasitology. In: ______. (Ed.). *Georgi's parasitology for veterinarians*. 9th ed. St. Louis: Saunders-Elsevier, 2009. p. 295-371.

BRADBURY, K. E.; APPLEBY, P. N.; KEY, T. J. Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *The American Journal of Clinical Nutrition,* Bethesda, v. 100, p. 394S-398S, jul. 2014. Suplemento 1.

CARLI, G. A. de. *Parasitologia clínica:* seleção de métodos e técnicas de laboratório para o diagnóstico das parasitoses humanas. 2. ed. São Paulo: Atheneu, 2011. 906 p.

COELHO, L. M. P. S.; OLIVEIRA, S. M.; MILMAN, M. H. S. A.; KARASAWA, K. A.; SANTOS, R. P. Detecção de formas transmissíveis de enteroparasitas na água e nas hortaliças consumidas em comunidades escolares de Sorocaba, São Paulo, Brasil. *Revista da Sociedade Brasileira de Medicina Tropical,* Uberaba, v. 34, n. 5, p. 479-482, set./out. 2001.

GOMES NETO, N. J.; PESSOA, R. M. L.; QUEIROGA, I. M. B. N.; MAGNANI, M.; FREITAS, F. I. S.; SOUZA, E. L.; MACIEL, J. F. Bacterial counts and the occurrence of parasites in lettuce (*Lactuca sativa*) from different cropping systems in Brazil. *Food Control,* Amsterdã, v. 28, n. 1, p. 47-51, nov. 2012.

GREGÓRIO, D. S.; MORAES, G. F. A.; NASSIF, J. M.; ALVES, M. R. M.; CARMO, N. E.; JARROUGE, M. G.; BOUÇAS, R. I.; SANTOS, A. C. C.; BOUÇAS, T. R. J. Estudo da contaminação por parasitas em hortaliças da região leste de São Paulo. *Science in Health,* São Paulo, v. 3, n. 2, p. 96-103, maio/ago. 2012.

GUILHERME, A. L. F.; ARAÚJO, S. M.; FALAVIGNA, D. L. M.; PUPULIM, A. R. T.; DIAS, M. L. G. G.; OLIVEIRA, H. R.; MAROCO, E.; FUKUSHIGUE Y. Prevalência de entroparasitas em horticultores e hortaliças da Feira do produtor de Maringá, Paraná. *Revista da Sociedade Brasileira de Medicina Tropical*, Uberaba, v. 32, n. 4, p. 405-411, jul./ago. 1999.

HOFFMANN, R. P. *Diagnóstico de parasitismo veterinário.* Porto Alegre: Sulina, 1987. 155 p.

MACHADO, V. S.; MONTEIRO, D. U.; WEIBLEN, C.; RIBEIRO, T. C.; LORENSETTI, D. M.; EMMANOUILIDIS, J.; LA RUE, M. L. de; SANGIONI, L. A.; PEREIRA, D. I. B.; BOTTON, S. de A. Ocorrência de estruturas parasitárias em alface – (*Lactuca sativa*) de cultivo hidropônico e convencional comercializadas em Santa Maria, RS. *Revista Analytica*, São Paulo, ago./set. 2015. Available at: <http://http://corpoemformasuplementos.com/revista_digital/78/artigo-2.pdf>. Accessed at: 3 oct. 2016.

MELO, A. C. F. L.; FURTADO, L. F. V.; FERRO, T. C.; BEZERRA, K. C.; COSTA, D. C. A.; COSTA, L. A.; SILVA, L. R. Contaminação parasitária de alfaces e sua relação com enteroparasitoses em manipuladores de alimentos. *Revista Trópica: Ciências Agrárias e Biológicas,* Chapadinha, v. 5, n. 3, p. 47-52, abr. 2011.

NERES, A. C.; NASCIMENTO, A. H.; LEMOS, K. R. M.; RIBEIRO, E. L.; LEITÃO, V. O.; PACHECO, J. B. P.; DINIZ, D. O.; AVERSI-FERREIRA, R. A. G. M.; AVERSI-FERREIRA, T. A. Enteroparasitos em amostras de alface (*Lactuca sativa*, var. crispa), no município de Anápolis, Goiás, Brasil. *Bioscience Journal,* Uberlândia, v. 27, n. 2, p. 336-341, mar./abr. 2011.

PACIFICO, B. B.; BASTOS, O. M. P.; UCHÔA, C. M. A. Contaminação parasitária em alfaces crespas (*Lactuca sativa* var. crispa), de cultivos tradicional e hidropônico, comercializadas em feiras livres do Rio de Janeiro (RJ). *Revista do Instituto Adolfo Lutz,* São Paulo, v. 72, n. 3, p. 219-225, ago. 2013.

PAINTER, J. A.; HOEKSTRA, R. M.; AYERS, T.; TAUXE, R. V.; BRADEN, C. R.; ANGULO, F. J.; GRIFFIN, P. M. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerging Infectious Diseases*, Atlanta, v. 19, n. 3, p. 407-415, 2013.

PAULA, P.; RODRIGUES, P. S. S.; TORTORA, J. C. O.; UCHÔA, C. M. A.; FARAGE, S. Contaminação microbiológica e parasitológica em alfaces (*Lactuca sativa*) de restaurantes self-service de Niterói, RJ. *Revista da Sociedade Brasileira de Medicina Tropical,* Uberaba, v. 36, n. 4, p. 535-537, jul./ago. 2003.

R CORE TEAM. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2013. Available at: <http://www.R-project.org/.> Accessed at: 3 jul. 2016.

RAMOS, M. O.; BEGOTTI, I. Z.; ROSA, G.; VIEIRA, G. F. P.; MESSA, V.; MERLINI, L. S. Avaliação parasitológica de alfaces (*Lactuca sativa*) comercializadas no município de Umuarama, Paraná. *Revista Brasileira de Higiene e Sanidade Animal,* Fortaleza, v. 8, n. 3, p. 1-12, jul./set. 2014.

SANTANA, L. R. R.; CARVALHO, R. D. S.; LEITE, C. C.; ALCANTARA, L. M.; OLIVEIRA, T. W. S.; RODRIGUES, B. M. Qualidade física, microbiológica e parasitológica de alfaces (*Lactuca sativa*) de diferentes sistemas de cultivo. *Ciência e Tecnologia de Alimentos*, Campinas, v. 26, n. 2, p. 264-269, abr./jun. 2006.

SANTARÉM, V. A.; GIUFFRIDA, R.; CHESINE, P. A. F. Contaminação de hortaliças por enteroparasitas e *Salmonella* spp. em Presidente Prudente, São Paulo, Brasil. *Colloquium Agrariae*, Presidente Prudente, v. 8, n. 1, p. 18-25, jan./jun. 2012.

SLAVIN, J. L.; LLOYD, B. Health benefits of fruits and vegetables. *Advances in Nutrition: An International Review Journal,* Bethesda, v. 3, n. 4, p. 506-516, jul. 2012.

TAKAYANAGUI, O. M.; CAPUANO, C. A. D.; OLIVEIRA, A. M.; BERGAMINI, M. H. T.; OKINO, A. A. M. C.; CASTRO E SILVA, M. A. O.; RIBEIRO, E. G. A.; TAKAYANAGUI, A. M. M. Avaliação da contaminação de hortas produtoras de verduras após a implantação do sistema de fiscalização em Ribeirão Preto, SP. *Revista da Sociedade Brasileira de Medicina Tropical,* Uberaba, v. 40, n. 2, p. 239- 241, mar./abr. 2007.

VIEIRA, J. N.; PEREIRA, C. P.; BASTOS, C. G. G.; NAGEL, A. S.; ANTUNES, L.; VILLELA, M. M. Parasitos em hortaliças comercializadas no sul do Rio Grande do Sul, Brasil. *Revista de Ciências Médicas e Biológicas*, Salvador, v. 12, n. 1, p. 45-49, jan./abr. 2013.

WANG, X.; OUYANG, Y.; LIU, J.; ZHU, M.; ZHAO, G.; BAO, W.; HU, F. B. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *British Medical Journal,* Londres, v. 349, p. g4490-4503g, jul. 2014.

WORLD HEALTH ORGANIZATION – WHO. Intestinal protozoa and helminthic infections: report of a WHO scientific group. Geneva: World Health Organization, out./nov. 1981. Available at: <http://apps.who.int/iris/bitstream/10665/41519/1/WHO_TRS_666.pdf>. Accessed at: 15 dec. 2015.