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ORIGINAL ARTICLE

PARASITOLOGICAL EVALUATION OF LETTUCE SERVED IN SCHOOL MEALS AT A FEDERAL STATE SCHOOL IN RIO DE JANEIRO, BRAZIL

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ABSTRACT

The Brazilian "National School Lunch Program" (PNAE) must provide healthy food for students in public primary education. Thus, it is necessary to ensure both nutritional and parasitological quality, reducing health risks. Vegetables must be clean, presenting no parasites and larvae, according to Brazilian legislation. Lettuce (*Lactuca sativa* L.) is one of the favorite vegetables among students, and is eaten raw in salads. This paper aims to evaluate the parasitological quality of lettuce served in school lunches. 72 samples of leaf lettuce, 36 from conventional agriculture (CA) and 36 from family agriculture (FA), were analyzed. Half the samples in each group were sanitized by immersion in a sodium hypochlorite solution, with 2% active stabilized chlorine for 10 minutes (n=18/each) and half were not sanitized (n=18/each). Parasite load evaluation was performed by two methods in each subgroup: spontaneous sedimentation (SS) and sedimentation by centrifugation (SC). The parasite frequencies found were evaluated by the chi-squared test. Medically relevant parasites identified were helminths (*Strongyloides stercoralis, Ascaris lumbricoides* and Hookworms) as well as protozoa (*Balantidium coli* and *Entamoeba coli*). 44.6% of the FA samples presented some form of parasite by SS evaluation and 33.4% by SC evaluation; 66.7% of the CA samples presented parasites by SS evaluation, and 44.5% by SC evaluation. No significant differences were noted between the FA and CA groups in either technique. No parasites were found in any of the sanitized lettuces, regardless of the subgroup or technique applied. These results evidence the importance of adequate training and guidance for vegetable growers, food handlers as well as the general population regarding proper hygiene of lettuce leaves prior to consumption.

KEY WORDS: Parasites; lettuce; school meals; National School Lunch Program (PNAE); family agriculture.

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INTRODUCTION

School lunches are a right guaranteed to schoolchildren in public primary education by the Brazilian legislation in article 208 of the Federal Constitution (Brazil, 1988).

To safeguard this right, the National School Lunch Program (PNAE) was devised and is managed by the National Fund for Educational Development (FNDE), the government agency in charge of educational policies in the Ministry of Education (MEC). This program has been through numerous modifications and, in 2009, it began including high school and young adult students in the public education system. Another milestone for the program was the compulsory purchase of at least 30% of the food from family agriculture (Brazil, 2009).

This is of utmost importance to Family Agriculture, as it stimulates the local economy, the permanence of families in the countryside, as well as fostering sustainable development (Brazil, 2016).

The acquisition of food from family agriculture, by managers and nutritionists, ensures better nutritional quality of student lunches while respecting regional eating habits (Saraiva et al., 2013). According to Triches and Schneider (2010), family agriculture is characterized by agro-ecologically-based production preserving the biological diversity of local ecosystems; the healthy use of soil, water and air and the recycling of organic waste, reducing to a minimum the use of non-renewable resources, as well as the diversification of crops and reduction in the use of chemical products.

Foodstuffs from family agriculture are generally products of rural farming, in which management and labor come from family cores, where planting is mostly done manually and managed more sustainably (Moreira, 2013). This kind of farming can often favor the presence of parasites in foodstuffs, especially leafy vegetables such as lettuce, produced in soil contaminated with eggs and cysts, particularly when irrigated with contaminated water (Peña et al., 2013).

Other forms of inadequate handling which can lead to contamination are: the use of organic fertilizers with excrements of fecal matter; presence of animals in cultivation areas and inadequate forms of vegetable storage and transportation during product distribution (Souto et al., 2011). Raw lettuce not correctly sanitized may contain helminth eggs and larvae, as well as protozoa cysts, presenting an important transmission route for intestinal parasites (Perez, 2014; Silva, et al., 2017; Vidigal & Landivar, 2018).

Parasitological studies highlight that enteroparasite transmission occurs by soil contaminated with helminth eggs due to domestic animal feces (Gallina et al., 2011) or orally, through the ingestion of contaminated water and/or food (Coelho et al., 2001), caused by poor hygiene and sanitary conditions as well as inadequate handling of vegetables (Severo et al, 2012).

The purpose of this study is, therefore, to evaluate the parasitological state of the lettuce (*Lactuca sativa* L.) offered in school meals at a Federal School in Rio de Janeiro, within the PNAE context.

MATERIAL AND METHODS

72 samples of leaf lettuce were analyzed, 36 from conventional crops, obtained in the Supply Center of Rio de Janeiro State (CEASA/RJ) and 36 originated from family agriculture from the town of Nova Friburgo, through a Rural Cooperative, which made weekly lettuce deliveries directly to the school.

On each day of the study, the conventional agriculture (CA) samples were obtained randomly from a local market, and family agriculture (FA) samples were obtained directly from the school kitchen, establishing as a sample unit a single, entire head of lettuce, regardless of weight and size. Four units (heads) were collected from each group, in the morning, totaling eight samples analyzed per day. These collections were performed from August 2018 to December 2018. Neither lettuces from FA or CA presented roots, however, FA specimens appeared to have more dirt in their leaves. The lettuce samples were placed in clean, previously identified disposable plastic bags.

Half the sample units from both sources (CA and FA – n=18 each) were sanitized, according to the Standard Operating Procedure (SOP) recommended by the National Sanitary Vigilance Agency (ANVISA-RDC, resolution number 216 (Brazil, 2004), from September 15, 2004 and RDC number 275 (Brazil, 2002), from October 21, 2002), carried out by an outsourced company, responsible for the school meals, while the other half (CA and FA – n=18 each) was analyzed without sanitation.

For the sanitized lettuce samples, the following procedure was adopted: removal of deteriorated units, parts or leaves; washing of leafy vegetables in running water (leaf by leaf); immersion in sodium hypochlorite solution, with 2% active stabilized chlorine (200 ppm for 10 minutes) followed by rinsing in running water.

Subsequently, sanitized as well as non-sanitized samples were sent to the Laboratory of Professional Education in Laboratorial Health Techniques (LATEC) at the Joaquim Venâncio Polytechnic School of Health/FIOCRUZ for parasitological evaluation. The non-sanitized samples were processed individually in the laboratory, utilizing procedure gloves for manual shedding and disposal of deteriorated leaves and stems.

The samples from each origin, family agriculture (FA) and conventional agriculture (CA) were subdivided into four other subgroups: sanitized family agriculture (SFA – n=18); non-sanitized family agriculture (NSFA – n=18); sanitized conventional agriculture (SCA – n=18) and non-sanitized conventional agriculture (NSCA – n=18).

The sediments were analyzed by the spontaneous sedimentation method or Hoffman, Pons and Janer method (HPJ) (Hoffman et al., 1934) and by the sedimentation by centrifugation method (Faust et al., 1938). Grouping occurred in the following fashion: SFA by spontaneous sedimentation; NSFA by centrifugation; SCA by spontaneous sedimentation and NSCA by centrifugation. For each method sample analysis was conducted in triplicates. It is noteworthy that the washed samples from each sampling subgroup were then submitted to both methods of sedimentation.

Each lettuce head (sample unit) was put in a polyethylene box and washed in 300mL of distilled water. With the assistance of a silicone brush, each lettuce leaf was carefully brushed, to remove possible parasites. To avoid any cross contamination, a different silicon brush was used on each lettuce sample. Between different experimental days, all silicon brushes were carefully cleaned in running water with neutral detergent, immersed in 70% ethylic alcohol and dried with paper towels. The suspension volume obtained was sufficient for the application of both techniques. The resulting suspension was filtered using a funnel and twice folded gauze.

An aliquot of 250mL was deposited in a sedimentation cup and left resting (spontaneous sedimentation) for five hours, according to the HPJ method; another 15mL aliquot was homogenized and transferred to a tapered head tube and centrifuged (Excelsa 206 B) at 630g for five minutes.

The resulting supernatant from both methods was discarded, utilizing a Pasteur pipette. The sediment was placed on a microscope slide with the assistance of a pipette, stained with a drop of Lugol solution, homogenized and covered with a cover slip.

Next, the sediments were analyzed by bright field microscopy, under an Axiohome microscope with an Axiocam Hrc5 camera, using a 10x magnification ocular lens. The Zeiss Zen Black software was used to obtain all images, as well as to set up all calibration bars. The reading was performed through a scan for identification of parasites or free-living larvae. Three slides from each subgroup were prepared and analyzed.

The statistical analysis of the data was conducted utilizing version 20.0 of the SPSS program for Mac. Parasite and helminth frequencies were analyzed by the chi-squared test, comparing each method (spontaneous sedimentation x centrifugation) among the different lettuces analyzed. Differences were considered meaningful when p-value <0.05.

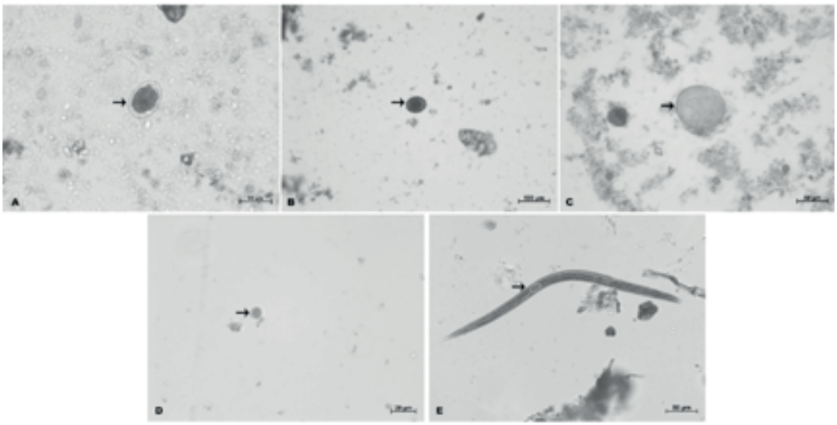
RESULTS

No parasites were found in the SFA group in any of the sedimentation methods applied (0 out of 18). In the NSFA group, subjected to the spontaneous sedimentation method, parasites were noted in 38.9% (7 out of 18) of the samples. When NSFA samples were centrifuged, 33.30% (6 out of 18) presented positive results to some sort of parasite.

No parasites were detected in the SCA group in both sedimentation methods (0 out of 18). In the NSCA group, 66.7% (12 out of 18) of the samples analyzed through spontaneous sedimentation showed positive results for some type of parasite, while under centrifugation, 44.50% (8 out of 18) of the samples analyzed presented parasites.

Among the parasites found in these samples, helminths and protozoa of medical relevance were detected, as shown in figure. Other nematodes (eggs, larvae), free-living organisms, non-identified arthropods (insects and mites), as well as mushroom spores, were also detected.

Figure. Photomicrographs of parasites found in lettuce.



A: egg of *Ancylostoma* sp; B: egg of *Ascaris lumbricoides*; C: cyst of *Balantidium coli*; D: cyst of *Entamoeba coli*; E: larvae of *Strongyloides stercoralis*.

Parasite frequencies were evaluated by the chi-squared test and showed no differences in the frequencies of parasites found in FA and CA groups, by either centrifugation method ($\chi2=2.933$; df=2; $p=0.231$) or spontaneous sedimentation method ($\chi2=1.265$; df=2; $p=0.531$).

Table shows the parasite species of medical relevance found on the lettuces, by both techniques. In the FA group, nearly 44% of the samples were positive for parasites by SS analyses, and 34% were positive by SC technique; in the CA group, 66% of the samples were positive for parasites by SS analyses, and 44% were positive by SC technique. Among helminths, *Strongyloides stercoralis* larvae and *Ascaris lumbricoides* and hookworm eggs were detected. Among protozoa, *Balantidium coli* cysts and *Entamoeba coli* cysts were noted.

Table. Parasitological evaluation of lettuces from Family Agriculture and Conventional Agriculture

	Family Agriculture n=18				Conventional Agriculture n=18			
	Spontaneous Sedimentation		Sedimentation by Centrifugation		Spontaneous Sedimentation		Sedimentation by Centrifugation	
Forms found:	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Helminth								
Strongyloides stercoralis	3	16.7%	2	11.1%	6	33.3%	3	16.7%
Ascaris lumbricoides	0	0.0%	1	5.6%	0	0.0%	1	5.6%
Ancylostoma sp	1	5.6%	0	0.0%	1	5.6%	0	0.0%
Total	4	22.3%	3	16.7%	7	38.9%	4	22.3%
Protozoa								
Balantidium coli	2	11.1%	1	5.6%	5	27.8%	2	11.1%
Entamoeba coli	1	5.6%	2	11.1%	0	0.0%	2	11.1%
Total	3	22.3.%	3	16.7%	5	27.8%	4	22.3%
Total Parasitized Samples	*7/18	38.9%	*6/18	33.4%	*12/18	66.7%	*8/18	44.5%

*Relative frequency

DISCUSSION

Raw vegetable intake is an important pathway for parasite contamination when sanitization is not performed effectively, even leading to the development of diseases (Barcelo et al., 2017). In this study, helminths and protozoa, free-living as well as those of medical relevance, were found in samples from FA and CA groups. RDC 14/2014 – Anvisa (Brazil, 2014) determines, in its seventh article, that producers must apply procedures to reduce contamination of foodstuffs to its lower level as frequently as possible. The data in this study show that this goal has not been achieved, as several parasites of medical relevance were found in non-sanitized samples. The same RDC, in its eighth article, establishes that the final product, in its commercialized form (ready for consumption), must meet hygienic-sanitary criteria to avoid health risks.

Lettuces for human consumption must undergo a sanitization cycle, according to ANVISA resolutions RDC number 216 (Brazil, 2004) and RDC number 275 (Brazil, 2002) which recommend sanitizing raw vegetables by applying a sodium hypochlorite solution, with 2% active stabilized chlorine (200 ppm for 10 minutes).Thus, all sanitized samples in this study would be considered appropriate for consumption. However, in the literature, several authors have reported contamination in vegetable samples ready for consumption in self-service restaurants, with protozoan cysts, eggs and/or helminth parasite larvae. Barcelo et al. (2017) examined parasites in leafy vegetable samples (lettuce, rocket salad leaves, cabbage and chard) ready for consumption, i.e., which had already undergone sanitization, being served in a self-service restaurant in the city of Ji-Paraná/RO. The authors found a high rate of parasitic contamination by helminths and protozoa in all samples analyzed (*Balantidium coli, Entamoeba coli, Endolimax nana, Giardia* sp., *Ascaris lumbricoides, Ancylostomidae*, *Hymenolepis diminuta, Strongyloides stercoralis*), which were, therefore, not up to standard according to the ANVISA resolutions and as such, not appropriate for consumption. It is important to note that the methodology used by Barcelo et al., 2017 is similar to that used in this study, in which parasites are detected in water and through shaking or friction.

Oliveira and Germano, 1992 proposed a methodology to study parasites in leafy vegetables, in which they used neutral detergent (extran) followed by a zinc sulfate solution, prior to centrifugation as these chemicals could increase the method`s sensitivity. This methodology has been used frequently since then in spite of being different from the ANVISA recommendation, since no neutral detergent is required. For example, Macena et al. (2018), conducted a study in the city of Teixeira de Freitas/BA, and they found parasitic forms in 96.67% of the lettuce served in self-service restaurants. Gonçalves et al. (2013) collected samples of lettuce served in self-service restaurants in the city of Porto Alegre/RS, and in spite of finding parasites in the leafy vegetables ready for consumption, none were pathogenic to man. Several studies that found parasites in lettuces have suggested that this contamination is due to the lack of hygienic-sanitary training and the precariousness in hygiene care when handling vegetables, aside from insufficient inspection by sanitary surveillance agencies in food establishments (Silva et al., 2017; Macena et al., 2018; Vidigal e Landivar, 2018). This study corroborates those studies, as it demonstrates that leafy vegetable sanitization, when correctly performed by qualified professionals, reduces the parasite load to zero, and is, therefore, an efficient and vital measure to avoid food-borne parasitic diseases.

In this study, contamination by helminths and protozoa was detected among non-sanitized samples from FA as well as CA. Both methods of sedimentation utilized showed efficiency in retrieving parasites and there was no significant difference between them. The helminthes found were: *Strongyloides stercoralis, Ascaris lumbricoides* and hookworms. The protozoa found were: *Balantidium coli* and *Entamoeba coli*. These results support previous studies that identified the parasitic forms of helminths and protozoa in leafy vegetables from street markets and supermarkets, i.e., before any sanitization cycles (Silva et al., 1995; Mesquita et al., 1999; Falavigna et al., 2005; Soares & Cantos, 2005; Santana et al., 2006; Neres et al., 2011; Alves et al., 2013; Pinto et al., 2018).

The results obtained in the current study lead to the conclusion that the evaluated lettuce samples, from groups NSFA and NSCA, demonstrated contamination by enteroparasites, protozoa or helminth alike evidencing the risk that the population is exposed to when ingesting raw leaf vegetables without correct sanitization. This study verified that the presence of parasites in the lettuce samples analyzed, may be the result of poor hygienic-sanitary quality, as noted by Barcelo et al. (2017); Silva et al. (2017) and Melo (2011), linked to a number of factors, namely lack of hygiene, cultivation with organic manure containing fecal waste material, vegetable irrigation with contaminated water, food handlers who are either parasitic carriers themselves or who are unqualified and poorly trained, as well as the presence of animals in the cultivation area to mention only a few. Although not tested for parasites in this study, the FA lettuces were irrigated with water from a stream alongside the properties.

The study conducted by Melo et al. (2011), verified the prevalence of enteroparasites in food handlers and the correlation with enteroparasitic contamination of leafy vegetables in the city of Parnaíba, Piauí, indicating parasitic contamination in 62.5% of the samples analyzed. Among the identified parasites were *Entamoeba coli* and *Strongyloides* spp. The presence of *Entamoeba coli* indicates vegetable contamination by human feces, as it is a non-pathogenic protozoan that inhabits human intestines, which can be caused by lack of proper sanitation or incorrect food handling. Contamination by *Strongyloides* spp. may be caused by organic fertilization, as producers reported using cattle manure as fertilizer. In another study by Pinto et al. (2018), which analyzed the level of contamination by parasitic structures in lettuce samples commercialized in street markets in the city of Jardim, Ceará, 80% of the samples were reportedly contaminated by helminth parasitic structures.

In the current study, besides helminths and protozoa of medical relevance, other nematodes (eggs, larvae), free living organisms, unidentified arthropods (insects and mites) and mushroom spores unimportant to public health were also detected, all occurring in the non-sanitized samples from both groups (data not shown). These results indicate that the lettuce samples from family agriculture as well as those from conventional agriculture present parasitological quality indicating contamination at some point in the productive chain, either in the field, in the transportation process or in the food preparation facilities. This situation may indicate that the water used in lettuce production could be inadequate. Research results from Neres et al., 2011; Corrêa et al., 2012; Alves et al., 2013 corroborate the results here shown, highlighting a potential health risk and attesting the importance of evaluating sanitary conditions when regarding parasitological aspects.

The consumption of leafy vegetables contaminated by parasites may be a pathway for the propagation of intestinal parasites. Pathogenic parasites have been found in samples of non-sanitized lettuce; however, when the sanitization is done correctly, the parasitological load is reduced to zero, making the leafy vegetables adequate for human consumption. This demonstrates that sanitization processes ensure food safety. According to the resolution from ANVISA-RDC number 216 (Brazil, 2004), training of food handlers must be periodic and supported by a formal food handling program in all units. It is noteworthy that food handlers from outsourced companies which supply meals to schools also go through periodic training programs. The results emphasize that high standards of hygienic-sanitary quality in lettuces in salads in school meals are possible through food handler training. The results from this study reveal the importance of enforcing the Sanitary Surveillance system and the need to train/guide leafy vegetable producers, food handlers, and the public on the importance of correctly sanitizing lettuce leaves before consumption. Further research, among agricultural families is necessary to evaluate production in the field and to devise better agricultural practices, improving the sanitary quality of leafy vegetables

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