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Short communication

Risk factors with the occurrence of parasites and coliform in vegetables from an agroindustry

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ABSTRACT

In this study, we evaluated the risk factors associated with the occurrence of total coliforms, thermotolerant coliforms, and intestinal parasite eggs in vegetables from an agroindustry in the city of Ibiúna – SP, Brazil. A questionnaire with 17 questions was applied to 24 producers to carry out a diagnosis of the risk factors and their posterior association or not with total coliforms, thermotolerant coliforms, and intestinal parasite eggs. The questions addressed mainly the production system, fertilizer used, source of the water used for irrigation, sanitization of the vegetables, and form of transport. For the investigation of total coliforms, thermotolerant coliforms, and intestinal parasite eggs, the samples were processed at the microbiology and parasitology laboratory of the Federal Institute of São Paulo, São Roque Campus. The results revealed that total coliforms were found in 100% of the investigated samples and thermotolerant coliforms were detected in 95.83% of the samples. There was a significant statistical association for the risk factor use of reservoir water, where there was release of wastewater and absence of sanitization of the vegetables at the moment of harvest in relation to the presence of thermotolerant coliforms. Intestinal parasite eggs were found in 33.33% of the samples.

Key words: good production practices; producers; contaminated water; sanitization.

RESUMO

Fatores de risco e a ocorrência de parasitas e coliformes em hortaliças de uma agroindústria

Avaliou-se neste trabalho os fatores de risco associados com a ocorrência de coliformes totais (CT), coliformes fecais (CF) e ovos de parasitas intestinais (OPI) em hortaliças que chegavam a uma agroindústria do município de Ibiúna- SP. Foi aplicado um questionário com 17 questões para 24 produtores a fim de realizar o diagnóstico dos fatores de risco e a sua posterior associação com a presença ou não de CT, CF e OPI. As questões versavam principalmente sobre o sistema de produção, adubo empregado, origem da água utilizada para irrigação, higienização das hortaliças e forma de transporte. Para a pesquisa de CT, CF e OPI, as amostras foram processadas no laboratório de microbiologia e parasitologia do Instituto Federal de São Paulo, campus São Roque. Os resultados revelaram que em 100% das amostras investigadas foram encontrados CT, e em 95,83%, detectados CF. Houve associação estatística significativa para o fator de risco utilização de água de açude onde havia lançamento de águas residuais e ausência de higienização de hortaliça no ato da colheita em relação a presença de CF. Ovos de parasitas intestinais foram encontrados em 33,33% das amostras.

Palavras chave: boas práticas de produção; produtores; água contaminada; higienização.

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INTRODUCTION

Vegetables are an essential part of the world's population diet (Costa & Oliveira, 2011). In Brazil, the average consumption is of 50 kg per habitant/year (Teixeira *et al.*, 2006).

Currently, there has been an increase in the concern with the sanitary quality of vegetables consumed by the population, as well the practices used to produce them, and the use of technologies aiming at the environment preservation (Oliveira *et al.*, 2014).

It can be said that there are two large groups of vegetable producers: those who will be able to adapt and meet the new sanitary requirements and will be competitive and those who will be marginalized in the process for not following the sanitary and environmental norms (Costa *et al.*, 2011).

Among the set requirements, the maintenance of good production practices, from the cultivation of the vegetables to its commercialization to the final consumer aiming to reduce or eliminate the presence of total coliforms (TC), thermotolerant coliforms (TtC), and intestinal parasites eggs (IPE) in vegetables, mainly those which are consumed raw, takes on economical and public health importance (Silva *et al.*, 2014).

The water contaminated by fecal matter of human or animal origin used in the irrigation of the vegetable gardens, the presence of infected handlers, inadequate sanitization, and transport practices are factors which have been pointed out as causes of contamination by TC, TtC, and IPE in vegetables (Carvalho *et al.*, 2003; Beraldo & Farache Filho, 2012). The investigation of TC, TtC, and IPE in vegetables is important in the public health area, especially considering the production, storing, transport, handling, and commercialization stages of these products (Ferreira *et al.*, 2013). The prevalence and the food-borne infections by TC, TtC, and IPE caused by the ingestion of raw vegetables have been frequently reported (Silva, *et al.*, 2011; França *et al.*, 2013).

This work aimed to evaluate the risk factors associated with the occurrence of TC, TtC, and IPE in vegetables from an agroindustry in the city of Ibiúna – SP, Brazil.

MATERIAL AND METHODS

The work was carried out in a vegetable processing agroindustry located in the in Ibiúna - SP, in the period from 25th August to 7th December 2014.

To perform the diagnosis of the risk factors and their posterior association with the presence or not of TC, TtC, and IPE, a questionnaire with 17 closed questions was initially applied to 24 vegetable producers who deliver their products to the agroindustry. The questions addressed the following areas: production system, type of fertilizer used, origin of the water used for irrigation, sanitization of

the vegetables, and the form of transport used to the agroindustry.

For the investigation of TC, TtC, and IPE, a collection was carried out at the moment each producer was delivering the vegetables, without any processing at the agroindustry. To compose the analysis sample, between 15 and 20 vegetable leaves were collected randomly in aseptic conditions, from various boxes delivered by the producer. The samples were stored in aseptic polyethylene packages and transported the same day under refrigeration. Posteriorly, they were processed in the microbiology and parasitology laboratory of the Federal Institute of Education, Science and Technology of São Paulo, São Roque campus. The analysis of TC and TtC were carried out using the technique of Vanderzant & Splittstoesser (1992) and Silva *et al.* (2007). The samples were diluted from the dilution of 10^{-1} , 10 mL of the samples were plated in three tubes containing 10 mL of Lauryl broth with double concentration with inverted Durhan tubes, 1.0 mL of the sample in three tubes with 10 mL of Lauryl broth with simple concentration, and 0.1 mL in tubes with 10 mL of Lauryl with simple concentration.

The tubes were incubated at 35 ± 1 °C for 24-48 h. After this period, Durhan tubes which presented gas in their interior were considered as positive and compared to the combinations present in the Most Probable Number (MPN) table for TC. The investigation and counting of TtC with the use of inoculation loop aliquots were taken from the positive tubes and reprocessed to a tube containing 10 mL of EC broth also containing inverted Durhan tubes in a kiln at 45 ± 2 °C/24-48 h. After this period, the presence of TtC was confirmed by the formation of gas in the Durhan tube and compared to the MPN table for TtC.

For the verification of the presence or not of IPE in the vegetables, the spontaneous sedimentation technique was used (Hoffman *et al.*, 1934). Two hundred and fifty milliliters of distilled water were added to the samples, being these posteriorly filtered in four-fold gauze and left to rest for 24 h. The following day, the supernatant liquid was discarded and about 30 mL of remaining were transferred to a 50-mL sedimentation calix.

From the sediment obtained from the final part of the tube, three drops were collected with the aid of a plastic pipette and then placed between glass slides. When necessary, to differentiate protozoan cysts, lugol was added to the analyzed sample. Each sample was analyzed in triplicate, in an optical microscope, at 10x magnification, and a scanning was performed to find and identify the parasites. The confirmation of the parasitic structures was carried out using a 40x magnification. The parasitic forms were identified by using the parasitological classification key (OMS, 1991).

For the risk factor analysis, an univariable analysis was performed by using nonparametric tests to compare

proportions (Chi-square or Fisher's exact tests), with a 5% significance level (Zar, 1999). The analysis was carried out with the software SPSS for Windows, version 20.0.

RESULTS AND DISCUSSION

Of the 24 producers investigated, 16 (66.66%) practiced conventional agriculture; four (16.66%), hydroponic agriculture; three (12.5%), conventional and hydroponic agriculture; and one (4.16%), organic agriculture

Table 1 presents the results regarding the investigated risk factors for the presence of TC, TtC, and IPE in vegetables from 24 producers in the municipality of Ibiúna - SP.

Tables 2, 3, and 4 present the results regarding the association analysis for the risk factors and the respective P-values for total coliforms, TtC, and IPE.

For the risk use of non-treated organic fertilizer, more than half of the investigated producers (54.2%) had this practice (Table 1). Silva *et al.* (2011), also evaluating the use of organic fertilizer and the sanitary quality of vegetables produced in Catu and Alagoinhas, in the State of Bahia, detected that, respectively, 100 and 80% of the producers used this type of fertilization in their vegetable gardens. These types of non-treated organic fertilizers used by the horticulturists may be characterized as means of contamination for the vegetables. However, Machado *et al.* (2006) highlighted that the fertilizer is not a determining factor for a possible contamination, also confirmed in this present work, in which there was no statistical association with the use of non-treated organic fertilizer and the occurrence of TC, TtC, and IPE (Tables 2, 3 and 4).

Table 1: Number of producers (NP) and the respective obtained percentage (%) which presented risk factor associated to the presence of thermotolerant and total and intestinal parasite eggs in the vegetables from a total of 24 samples investigated

Risk factor	NP/%
Use of non-treated organic fertilizer	13/54.2
Use of reservoir or river water for irrigation with a close septic tank	05/20.8
Use of reservoir or river water for irrigation where there is release of wastewater	08/33.3
Absence of sanitization in the harvesting of vegetables	11/45.8
Absence of individual sanitization of vegetable with chlorinated water	24/100
Transport in non-sanitized wooden box	02/8.33
Transport in non-sanitized plastic box	19/79.16
Transport in a truck with a wooden truck body	10/41.66

Table 2: Association analysis for the risk factors and the respective P-values for total coliforms (TC)

	Category	Number of producers	TC	
			> 8 (%)	p
Use of non-treated organic fertilizer	No	16	62.5	0.352
	Yes	08	87.5	
Use of reservoir or river water for irrigation with a close septic tank	No	19	63.2	0.272
	Yes	05	100	
Use of reservoir or river water for irrigation where there is release of wastewater	No	16	68.8	1.000
	Yes	08	75	
Absence of sanitization in the harvesting of vegetables	No	13	53.8	0.078
	Yes	11	90.9	
Absence of individual sanitization of vegetable with chlorinated water	No	24	73.7	0.608
	Yes	00	60	
Transport in non-sanitized wooden box	No	22	72.7	0.507
	Yes	02	50	
Transport in non-sanitized plastic box	No	05	80	1.000
	Yes	19	68.4	
Transport in a truck with a wooden truck body	No	14	71.4	1.000
	Yes	10	70	

Water for irrigation with a septic tank and with the release of wastewater represented 20.8 and 33.3%, respectively, of the total evaluated (Table 1). In relation to the sanitization of the vegetables at the moment of harvesting, 54.2% of producers performed it; however, 100%

of them did not carry out the individual sanitization with chlorinated water, evidenced by the absence of high impact measures in the reduction of TC, TtC, and IPE in the vegetables (Jesus *et al.*, 2015). Only 8.33% of the producers still transported the vegetables in non-sanitized wooden

Table 3: Association analysis for the investigated risk factors in the 24 producers and the respective P-values for thermotolerant coliforms (TtC)

	Category	Number of producers	TtC	
			> 0.3 (%)	p
Use of non-treated organic fertilizer	No	16	25	0.099
	Yes	08	62.5	
Use of reservoir or river water for irrigation with a close septic tank	No	19	36.8	1.000
	Yes	05	40	
Use of reservoir or river water for irrigation where there is release of wastewater	No	16	56.3	0.009
	Yes	08	0	
Absence of sanitization in the harvesting of vegetables	No	13	15.4	0.033
	Yes	11	63.6	
Absence of individual sanitization of vegetable with chlorinated water	No	24	36.8	1.000
	Yes	00	40	
Transport in non-sanitized wooden box	No	22	31.8	0.130
	Yes	02	100	
Transport in non-sanitized plastic box	No	05	60	0.326
	Yes	19	31.6	
Transport in a truck with a wooden truck body	No	14	50	0.210
	Yes	10	20	

Table 4: Association analysis for the investigated risk factors in the 24 producers and the respective P-values for the intestinal parasite eggs (IPE)

	Category	Number of producers	IPE	
			> 0 (%)	p
Use of non-treated organic fertilizer	No	11	31.3	1.000
	Yes	13	37.5	
Use of reservoir or river water for irrigation with a close septic tank	No	19	26.3	0.289
	Yes	05	60	
Use of reservoir or river water for irrigation where there is release of wastewater	No	16	37.5	0.667
	Yes	08	25	
Absence of sanitization in the harvesting of vegetables	No	13	54.5	0.082
	Yes	11	63.6	
Absence of individual sanitization of vegetable with chlorinated water	No	24	42.1	0.130
	Yes	00	40	
Transport in non-sanitized wooden box	No	22	31.8	1.000
	Yes	02	50	
Transport in non-sanitized plastic box	No	05	35	1.000
	Yes	19	25	
Transport in a truck with a wooden truck body	No	14	28.6	0.673
	Yes	10	40	

boxes, while 16% already made use of plastic boxes for transport. Despite the progress in substituting wooden boxes for plastic ones, only five producers (or 20.84%) sanitized the plastic boxes. In relation to the type of truck body used to transport the vegetables, the majority (58.34%) used the box model, which reduces the risk of contamination and loss of vegetable leaves by bacteria of the coliform group (Lourenço & Katz, 2010).

In relation to the presence of TC in the vegetables, they were detected in 24 (100%) of the samples, values which oscillated from 0.3 to 240 CFU/mL. As for the investigation of TtC, they were detected in 23 samples (95.83%), with values oscillating from 0.3 to 46 CFU/mL. Despite the broad numerical variation of the TtC found in the samples, this result is of utmost importance in public health due to the potential of these foods as sources of infections and foodborne infections (Martins *et al.*, 2010).

In the correlation analysis with the presence of TtC, there was association for the risk factor use of reservoir water where there was release of wastewater and absence of sanitization of the vegetables at the moment of harvesting (Table 3). The results obtained in this work corroborate those obtained by Santos *et al.* (2010), who studied the hygienic and sanitary quality of vegetables and irrigation water in Salvador – BA, Brazil, and detected unsatisfactory conditions of the investigated areas and the elevated number of vegetables and the water used for their irrigation, which presented contamination by TC and TtC. Such a result was also confirmed by Mattos *et al.* (2009), who affirmed that the microbiological contamination in vegetables is due to the use of contaminated water for irrigation.

For the investigation of IPE, these were found in eight samples (33.33%), with values which oscillated from one to 100 eggs; however, a significant statistical association with the investigated risk factors was not detected (Table 4). Nevertheless, Ferreira *et al.* (2013), who evaluated the quality of water for irrigation in Teresópolis - RJ, Brazil, found the presence of IPE in 23.82% of the investigated samples. Nascimento & Alencar (2014), who investigated 24 vegetable samples in Natal - RN, Brazil, detected parasitic forms in 41.7% of the samples. More elevated frequencies of IPE in vegetables (60%) were detected by Oliveira & Perez (2014) both in those with or without sanitization.

CONCLUSIONS

In the conditions under which this work was carried out, it was possible to conclude that:

Total coliforms were found in 100% of the investigated vegetable samples and thermotolerant coliforms were detected in 95.83%.

Intestinal parasite eggs were found in 33.33% of the samples, with values that oscillated from 01 to 100 eggs,

but there was no significant statistical association for the investigated risk factors.

There was a significant statistical association for the risk factor use of reservoir water, in which there is the release of wastewater and absence of sanitization of the vegetables at the moment of harvesting in relation to the presence of thermotolerant coliforms.

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