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# Microbiological and parasitological contamination of vegetables, water and soil in rural communities

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Microbiological and parasitological contamination of vegetables, water and soil in rural communities of a municipality in the state of Bahia, Brazil, was assessed. Samples of *Lactuca sativa*, L., *Coriandrum sativum* and *Solanum lycopersicum*, soil, and irrigation water were analyzed between August and October, 2015. Total coliforms (TC), *Escherichia coli*, molds and yeasts on vegetables, soil and water, heterotrophic bacteria in water and soil were counted. Parasitological analyses were performed by spontaneous sedimentation method and by Rugai technique for vegetables and soil and by direct and Faust exams for water. Physical and chemical analyses included pH, temperature, dissolved oxygen and turbidity. TC counts were higher in lettuce samples (mean 2.8 log CFU g<sup>-1</sup>). *E. coli* counts did not comply with legislation in a lettuce sample with 3.3 log CFU g<sup>-1</sup>. TC had the highest counts, with mean between 3.7 and 4.9 log CFU g<sup>-1</sup> in soil samples. All water samples showed poor conditions and most samples were positive for at least one parasite. Due to high microbial density and several parasite types in most samples, results showed poor sanitary quality of vegetables with health risks for people. It is crucial to invest in educational activities for handlers and farmers so that a better vegetable quality could be offered to the population. More efficient monitoring is required by health authorities, requiring periodic assessments for parasites so that consumers may have a better life quality.

**Keywords:** Agricultural produce, food production, food hygiene, sanitary profiles.

# INTRODUCTION

Eating habits of many Brazilians have been modified throughout the years. There was great interest in *in natura* food, especially vegetables, due to known and reported health benefits (Ministry of Health, 2014). However, according to their characteristics, vegetables are frequently eaten raw or with inadequate cleaning, featuring possible contamination by microorganisms, such as helminths, protozoa, bacteria, fungi and viruses. The former are more common due to their ability to

survive in soil and water with eventual outbreaks of foodborne diseases (FBD), with great risks for humans (Coutinho et al., 2015).

The contamination of vegetables by bacteria, fungi and parasites may occur through several ways, especially by contact with water used in the irrigation of vegetables contaminated with human fecal material, soil with organic manure derived from fecal dejects or by the contamination of food handlers' hands (Fernandes et al.,

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2015). Microorganisms may indicate pathogens and inadequate hygiene conditions in production, transport and storage (Abreu et al., 2010). Due to the interference of several factors in the contamination of vegetables and lack of studies on these factors as sources of contamination, current paper evaluates the microbiological and parasitological contamination of vegetables, water and soil in rural communities in a municipality of the state of Bahia, Brazil.

#### **MATERIALS AND METHODS**

#### Selection of vegetables

This research was characterized as a descriptive and analytical cross-sectional study. The selection of vegetables for current study was foregrounded by data retrieved from Family Budget Research by the Instituto Brasileiro de Geografia and Estatística which evaluated food consumption data of the Brazilian population. FBR data and local producers´ survey were the criteria employed to include food with the greatest consumption rates and which were produced by communities under analysis. Three vegetables were chosen, namely lettuce (*Lactuca sativa*) and tomatoes (*Solanum lycopersicum*), respectively with consumption percentages 4.7 and 2.4%, and parsley (*Coriandrum sativum*). The latter was included because it was greatly used in crude salads, with a 10.6% consumption rate (IBGE, 2011).

#### Farms characteristics

Qualitative half-structured interviews were previously undertaken on topics agreed upon by farm owners or laborers. The questionnaire consisted of 17 questions divided into three topics: I Property identification (7 questions). II Production data (10 questions). Item I provided information about the presence of pets, existence of sanitary facilities, and destination of sewage and place where it performs the physiological needs. Item II provided information on the type and destination of the vegetables produced, the type of fertilizer and / or pesticide used in the production, origin and treatment of the water used for vegetable production and family consumption.

Producers who reported performing organic planting were chosen. Nine producers residing in four rural communities in the micro region of Santo Antonio de Jesus, Bahia, Brazil, participated. The survey was conducted in the communities Sapucaia 13°2'43"S 39°16'34"W, Cunha 12°57'36"S 39°17'11"W, São Bartolomeu 12°58'26.3"S 39°16'56.1"W and Onha 13°00'41.5"S 39°04'06.9"W. The vegetables were sent to different places: proper consumption, restaurants, supermarkets, town fairs, local community and the National School Meal Program (PNAE).

#### Collection of samples

Material was collected between August and October, 2015. Samples of three vegetables, a sample of the soil where the vegetables were cultivated and a sample of the water for irrigation were retrieved from each producer. Soil samples were taken from different points, forming a compound sample and collected superficially (10-20 cm) at the points where the plant species developed. Each soil sample consisted of five subsamples (Silva et al., 2007). We collected 63 samples, seven samples for each of nine producers: three vegetable samples, three soil samples and one water sample. Soil and vegetable leaves samples were performed by hand, in an

aseptic way, in new plastic bags. Further, 1.5 L water samples were collected in new plastic recipients with lid. Samples were identified, refrigerated at 4°C and transported to the Multidisciplinary Complex for Studies and Research in Health (COMEPS) of the Center of Health Sciences (CCS) of the Universidade Federal do Recôncavo da Bahia (UFRB). There was a maximum 24 h between sample collection and start of analysis.

# Microbiological analysis

Microbial populations of vegetables were estimated by Petrifilm<sup>™</sup> rapid count (3 M Company), from 25 g of each sample, with EC plates for TC and *Escherichia coli* and YM plates (AOAC 997.02) for molds and yeasts. Microbiological analysis of soil comprised TC, *E. coli*, molds and yeasts and heterotrophic bacteria counts. Populations of TC and *E. coli* were calculated with Petrifilm<sup>™</sup> CE plates (3 M Company), whilst molds and yeasts were estimated with medium Martin with chloramphenicol (0.05%); heterotrophic bacteria were calculated by medium Trypticase Soy Agar (TSA), from 10 g of each sample, following Santos et al. (2007).

The water's microbiological analysis comprised TC and *E. coli* by Readycult® Coliforms 100 rapid count (Merck KGaA 01), with estimated populations of heterotrophic bacteria by Petrifilm™ AQHC rapid count (3 M Company) and molds and yeasts with Petrifilm™ AQUA YM plates (AOAC 997.02), from 100 ml of each sample. Colonies were counted with colony count model CP600 Plus (Phoenix ®), calculating colony formation units (CFU) and log CFU g⁻¹ of sample (APHA, 2001).

#### Parasitological analyses

Analytic methodologies for parasitological research in food and soil samples comprised spontaneous sedimentation method (Zeibig, 2014) and modified Rugai technique (Neves et al., 2016). The spontaneous sedimentation method comprised washing of vegetables in distilled water in new plastic bags, elimination of deteriorated and wrinkled parts, manual shaking for 15 min. Four grams of sampled soil were retrieved to which were added 200 ml of distilled water. The samples were filtered by a four-fold gauze and left for sedimentation for 24 h in a conic beaker (Zeibig, 2014). Modified Rugai technique consisted of involve a food sample or 4 g of soil in a four-fold gauze, submerged in a conic beaker with distilled water at 45°C for at least two hours. The gauze was then removed and the supernatant discarded. Further, 50 ml of the sediment was transferred into a sterile collector flask for the preparation of laminas for microscopic analysis (Neves et al., 2016).

Water was analyzed by direct and Faust tests (fluctuation in zinc sulfate). In the case of the direct test, the water sample was maintained under spontaneous sedimentation for 24 h at room temperature. Sediment was then collected by Pasteur pipette. Faust's method comprised a 50 ml aliquot of the sediment in a centrifugal tube and centrifuged at 1,500 rpm. The sediment was then re-suspended with 10 ml of water for later centrifugation at 1,500 rpm. The sediment was mixed with 10 mL of zinc sulfate solution (density 1.18), and centrifuged again. The membrane formed at the liquid surface was removed with a double bacteriological spade and transferred to a lamina, stained with lugol, covered with a laminule and observed under a 400x optic microscope (Neves et al., 2016). Laminas were stained with lugol and directly observed under an optic microscope, magnified 100x and 400x, by counting the number of structures through the surface of the junction, lamina x laminule.

# Physical and chemical analysis of water

Analysis for pH, temperature, dissolved oxygen (DO) and electric

conductivity were prepared on the field after water samples by multi-parameter gauge AK88 (AKSO); turbidity and color analyses were performed in the laboratory by multiprocessor bench test turbidity-meter T-1000 (LABOTEC™) (APHA, 2012).

#### **Ethical aspects**

Current study was submitted to the Committee for Ethics in Research with Human Beings of the Universidade Federal do Recôncavo da Bahia (UFRB) (CAAE: 04022312.0.0000.0056 – Authorization 1.167.637), following Resolution 466/12 of the Brazilian National Health Council. Members of participating families were invited to sign a Term of Free Consent to participate and authorize sample collection of vegetable produced, soil and irrigation water in the allotted sites as a criterion for inclusion in current research (Ministry of Health, 2012).

#### Statistical analysis

Data were analyzed by Fischer's exact test, with Statistical Package for the Social Sciences (SPSS) 20 (IBM) at 5% significance level (p<0.05) to evaluate the relationship between variables and prevalence of microbiological and parasitological contamination.

## **RESULTS AND DISCUSSION**

## Microbial and parasitological contamination

Table 1 shows counts of total coliforms in vegetable samples, soil and irrigation water. TC counts in vegetables ranged between 1.0 and 5.0 log CFU g<sup>-1</sup> of lettuce samples, between 1.5 and 5.3 log CFU g<sup>-1</sup> of parsley samples and between <1 and 2.9 log CFU g<sup>-1</sup> of tomato samples. Highest averages among the vegetables were detected in lettuce (2.8 log CFU g<sup>-1</sup>). Highest count occurred in a parsley sample at 5.3 log CFU g<sup>-1</sup> (Producer 9). The producer 7 had better TC counts for parsley (1.5 log CFU g<sup>-1</sup>) and tomato samples (<1 log CFU g<sup>-1</sup>) and soils samples near them (3.3 and 4.1 log CFU g<sup>-1</sup>) and soils samples (4.7 log CFU g<sup>-1</sup>) and parsley (5.0 log CFU g<sup>-1</sup>) and tomato soil.(6.0 log CFU g<sup>-1</sup>) near them.

Different results were reported in a study by Rincón et al. (2010) in Maracaibo, Venezuela, with parsley being the vegetable with the highest average contamination by TC when compared to other vegetables analyzed. Although Brazilian legislation does not establish any limits for TC, the presence of such microorganisms in the food under analysis is relevant and indicates poor hygiene practice during processing (Silva et al., 2016). Soil samples showed mean TC counts, between 3.7 and 4.9 log CFU g<sup>-1</sup>.

Soil samples from tomato plantation had the highest contamination rates by TC, averaging 4.9 log CFU g<sup>-1</sup>. Results could be a consequence of defecating directly in the soil, a common practice by rural producers

analyzed, the free roaming of domestic animals and the use of manure without due processing (Coutinho et al., 2015).

TC was detected in all water samples. In fact, it failed to comply to the Ministry of Health's Resolution 2914/2011 which establishes that one of the items for drinkable water is the absence of TC in 100 ml of the sample (Ministry of Health, 2011). Average E. coli counts in vegetables ranged between <1.0 and 1.1 log CFU g<sup>-1</sup>. Lettuce showed the highest contamination rate, with mean count of 1.1 log CFU g<sup>-1</sup>, and tomato by the lowest contamination of the bacterium, with populations <1.0 log CFU g<sup>-1</sup> (Table 2). Producers 1 and 2 had the worst TC counts. The highest counts from Producer 1 were from soil samples close to parsley (3.6 log CFU g<sup>-1</sup>) and tomato cultivation (3.0 log CFU g<sup>-1</sup>) and the highest counts from Producer 2 were from lettuce (3.3 log CFU g 1) and the soil near the cultivation of this vegetable (3.6)  $\log CFU g^{-1}$ ).

According to parameters by resolution of the collegiate board (RDC) 12/2001 of the Brazilian Agency of Sanitary Care (Anvisa, 2001), one lettuce sample (Producer 2) was contaminated above the limits by *E. coli* (2.0 log CFU g<sup>-1</sup>) and thus not good for consumption (Table 2). Similarly, Silva et al. (2016) evaluated the quality of water used in the irrigation of five vegetable gardens and in food irrigated in a swampy area in the municipality of Caruaru, in the Agreste region of the state of Pernambuco, Brazil. The authors detected high contamination rates by TC and *E. coli*. Lettuce and parsley among the vegetables analyzed had the greatest contamination rates. In samples of irrigation water, they were all positive for TC and *E. coli*.

According to Yang et al. (2012), lettuce contamination by *E. coli* could occur anytime between the farm and the consumer's table, predominantly by contact with fecal material due to the use of manure in the plantation and/or bad hygiene practices at home. In fact, two samples of soil with lettuce (Producers 2 and 3), a sample of soil with parsley and a sample of soil with tomato (Producer 1) had *E. coli* populations above 3.0 log CFU g<sup>-1</sup>. According to Naganandhini et al. (2015), partially decomposed manure, slurry, wastes from abattoirs and human sewerage could be the source of contamination of arable agricultural land since certain species of *E. coli* could survive in manure based on animal excrement for long periods (between 25 to over 365 days). They could infect humans even with low cell load (10-500/q).

In the case of water samples, *E. coli* was detected in 88.9% of samples (Table 2), which was inadequate according to the Ministry of Health's Resolution 2914 of December 12, 2011, which determined the absence of *E. coli* in 100 ml of the samples. According to Araújo et al. (2015) this contamination could be due to domestic sewage.

One should highlight that samples of tomato in current study had low TC and *E. coli* counts, perhaps due to the anatomical characteristics of the tomato. Similarly, Arbos

Table	1.	Counts	of	total	coliforms	in	vegetables,	soil	and	irrigation	water	of	producers	in	rural
commu	ınit	ies.													

	Vegeta	ıbles¹			Motor2			
Producer	Lettuce	Parsley	Tomato	Soil lettuce	Soil parsley	Soil tomato	Water <sup>2</sup>	
1	1.3	2.6	1.5	3.9	4.7	4.7	Р	
2	4.0	2.0	2.7	4.7	4.0	5.0	Р	
3	5.0	2.5	2.9	4.4	3.3	4.8	Р	
4	3.0	1.7	2.1	3.9	3.6	5.2	Р	
5	2.4	2.8	<1	4.0	3.4	4.9	Р	
6	3.8	4.7	<1	4.0	5.0	6.0	Р	
7	1.0	1.5	<1	3.7	3.3	4.1	Р	
8	3.9	1.8	1.6	3.3	4.3	5.2	Р	
9	1.0	5.3	<1	3.0	2.5	4.6	Р	
Means	2.8	2.7	1.6	3.8	3.7	4.9	-	
SD	1.3	1.2	0.7	0.4	0.7	0.3	-	

<sup>&</sup>lt;sup>1</sup> log CFU g-1; <sup>2</sup> Presence (P); <sup>3</sup> Standard Deviation (SD).

Table 2. E. coli counts in vegetables, soil and irrigation water of producers in the rural communities.

		Vegetables	1		Soil <sup>1</sup>		
Producer	Lettuce	Parsley	Tomato	Soil Lettuce	Soil Parsley	Soil Tomato	Water <sup>2</sup>
1	<1	<1	<1	<1	3.6	3.0	Р
2	3.3	<1	<1	3.6	<1	<1	Α
3	<1	1.5	<1	3.0	<1	<1	Р
4	<1	<1	<1	<1	<1	<1	Р
5	<1	1.0	<1	<1	<1	<1	Р
6	<1	<1	<1	<1	<1	<1	Р
7	<1	1.0	<1	<1	<1	<1	Р
8	<1	1.0	<1	<1	<1	<1	Р
9	<1	1.0	<1	<1	<1	<1	Р
Means	1.1	1.0	<1	1.4	1.2	1.1	-
SD	0.7	0.1	0.7	0.9	0.9	0.6	-

<sup>&</sup>lt;sup>1</sup> log CFU g-1; <sup>2</sup> Presence (P) Absence (A); <sup>3</sup> Standard Deviation (SD).

et al. (2010) also reported low contamination in tomato when compared to the other vegetables under analysis. All vegetable samples showed MY with mean counts between 1.6 and 5.0 log CFU/ g<sup>-1</sup>. MY counts in soil samples were high, varying between 4.7 and 4.9 log CFU g<sup>-1</sup>. Only one sample failed to show MY colony growth, whilst count samples ranged between <1.0 and 5.0 log CFU g<sup>-1</sup>. Producer 2 had the worst MY counts for lettuce (5.5 log CFU g<sup>-1</sup>) and parsley samples (5.8 log CFU g<sup>-1</sup>) (Table 3).

Santos et al. (2010) reported that although MY are important indicators of food contamination, maximum counting limits were not predicted for most foods (Santos et al., 2010). Current study also estimated counts of heterotrophic bacteria (HB) in samples of soil of vegetable gardens. Counts ranged between 6.1 and 6.4

log CFU g<sup>-1</sup>. Studies by Borges Filho and Machado (2013) revealed HB between <1.0 and 10 log CFU g<sup>-1</sup> in soils after the addition of organic manure in Belo Horizonte, Brazil, considered high by the authors. Similarly, bacteria counts were high and may be a source of contamination to cultivated vegetables. HB counts were estimated in irrigation water samples. Sample from Producer 7 had the lowest HB count, whereas sample from Producer 5 proved to be the highest, respectively 1.4 and 5.5 log CFU g<sup>-1</sup>. Sample from Producer 7 was retrieved from an artesian well and stored in a reservoir on the top of the house. The sample of Producer 5 was different even though it was retrieved from the public water supply and stored in an open reservoir on the floor. Only two water samples (Producers 7 and 8) had had populations within the maximum limit allowed

Draducar	\	/egetables	s <sup>1</sup>		- Water <sup>1</sup>		
Producer	Lettuce	Parsely	Tomato	Soil Lettuce	Soil Parsely	Soil tomato	water
1	5.4	5.1	4.5	4.7	4.7	5.1	3.0
2	5.5	5.8	2.1	4.7	5.0	4.6	2.0
3	4.6	4.6	4.5	5.1	4.8	4.9	2.0
4	4.4	4.9	5.0	4.0	5.2	4.7	2.0
5	4.6	4.8	4.3	5.0	4.9	5.0	4.0
6	5.1	5.1	4.0	4.6	6.0	4.6	0
7	5.5	5.3	4.0	4.6	4.1	5.0	5.0
8	4.9	5.3	5.2	4.8	5.2	4.7	4.0
9	3.9	3.9	2.8	4.9	4.6	5.7	1.0
Means	4.8	5.0	4.0	4.7	4.9	4.9	2.5
SD	0.5	0.5	0.7	0.2	0.4	4.3	1.4

**Table 3.** Molds and yeast counts in vegetable, soil and irrigation water of producers in the rural communities.

(respectively 1.4 and 1.8 log CFU g<sup>-1</sup>), or rather 2.7 log CFU ml<sup>-1</sup>. Consequently, 78% of samples did not comply with current legislation (Ministry of Health, 2011). According to Araújo et al. (2015), HB counts are dangerous at high concentrations, although counts are employed as a complementary parameter to thermotolerant coliforms such as *E. coli*.

At least one parasite species was extant in most samples analyzed (79.3%). The 2014 RDC Resolution of ANVISA determined the absence of parasites, at any phase of development, in food (Table 4). According to the above legislation, 77.8% of vegetable garden samples were not fit for human consumption (ANVISA, 2014). Lettuces and parsley had the highest number of positive samples. Similarly, lettuces in studies on vegetables had the highest parasite contamination (Constantin et al., 2013; Coutinho et al., 2015; Brauer et al., 2016). Since lettuce and parsley grow close to the soil, they are more prone to contamination by parasites. Protozoa cysts and helminth eggs survive for long periods in the soil and are still viable at the moment of intake of vegetables (Brauer et al., 2016).

Five tomato samples (55%) were contaminated by helminths, contrasting reports by Abreu et al. (2010) who highlighted that the distance of the tomatoes above the soil makes contamination by parasites difficult. Parasites which are most frequently found in vegetable samples were ciliated protozoa cysts (81%), cysts of other protozoa (44.4%), larvae of nematode helminths (18.5%), adults of nematode helminths (14.8%), adult mites (14.8%), cysts of *Endolimax nana* (14,8%), eggs of *Ancylostoma* spp (3,7%) and eggs of trematode helminths (3.7%). Soil samples contained ciliated protozoa cysts (51.8%), cysts of other protozoa (40.7%), larvae of nematode helminths (37%), adults of nematode helminths (22.2%), eggs of nematode helminths (7.4%), eggs of *Ancylostoma* spp (3.7%), eggs of *Trichuris* spp.

(3.7%).

Since no species classification key is extant in the laboratory, it was not possible to identify the species of several parasites in the material under analysis. Although most species were not pathogenic, they nevertheless indicate the poor sanitary conditions in vegetable breeding. It is also highly relevant to detect helminths such as *Ancylostoma* spp, not merely because of their high prevalence but due to the diversity of their clinical manifestations in the hosts (Mesquita et al., 2015). Prevalence of protozoa was reported in all samples, when compared to helminths. Parasites may be vectors in several ways and, due to their great adaptability and resistance, they could coexist in highly uncommon media (Terto et al., 2014).

As a rule, parasite contamination occurred more in vegetable than in soil samples. This result was due to contamination during handling by infected handlers or through contact with fecal dejects vectored by animals such as fowl, flies, mice and insects. Since the latter stay on faeces in the open air and then fly onto food, they were important mechanical vectors of parasites (Bauer et al., 2016). Current legislation has not provided maximum and minimum rates for water temperature for human consumption. However, temperature between 24 and 32.5°C of water samples (Table 5) was prone to develop TC groups that survive temperatures up to 37°C (Silva et al., 2016).

Further, pH is linked to bacterial multiplication since most the best pH for most bacteria oscillates between 6.5 and 7.5. In other words, pH past neutrality is related to the introduction of organic matter and home effluents. Current study reveals that four samples were inadequate, featuring pH rates below that recommended (6.0- 9.5) (Ministry of Health, 2011).

In the case of turbidity, the sample of Producer 1 only was not satisfactory due to non-compliance with standard

<sup>&</sup>lt;sup>1</sup> CFUv; <sup>3</sup> Standard Deviation (SD).

Table 4. Parasites in samples of vegetables, soil and irrigation water in rural communities.

Camples	Helminths		Protozoa		Bi-pa	Bi-parasitism		Absence	
Samples	n	%	n	%	n	%	n	%	
				Vegetal	bles				
Lettuce	-	-	6	67	3	33	-	-	
Parsley	-	-	6	67	3	33	-	-	
Tomato	5	55	1	11	1	11	2	22	
				Soil					
Lettuce	-	-	2	22	6	67	1	11	
Parsley	1	11	2	22	4	44	2	22	
Tomato	2	22	3	33	2	22	2	22	
Water	-	-	2	22	1	11	6	67	

<sup>&</sup>lt;sup>1</sup> number (n).

**Table 5.** Physical and chemical parameters of irrigation water used in the production of vegetables of producers in rural communities.

	Physical and chemical parameters						
Producer	Temperature. (Cº)	рН	DO (mg L-1)	Turbidity. (NTU)			
1	25.2	5.8	7.0	7.28			
2	25.5	4.95	5.0	0.19			
3	24.2	4.7	4.5	1.25			
4	27.0	5.94	6.0	0.02			
5	24.0	6.5	6.6	4.43			
6	32.5	6.41	4.0	3.86			
7	27.9	6.46	8.7	2.14			
8	25.9	6.5	9.9	3.13			
9	30.5	6.88	7.2	1.34			

<sup>&</sup>lt;sup>1</sup>Dissolved Oxygen (DO); <sup>2</sup> Nephelometric Turbidity Unit (NTU).

of up to 5.0 NTU (Ministry of Health, 2011). So that the water's microbiological quality may be guaranteed, the turbidity pattern should be monitored. In fact, several studies have shown that pathogens, such as *Cryptosporidium* spp., has been associated with turbidity. In fact, the greater the water's turbidity rate, the greater is the possibility of detecting parasites (Daneluz; Tessaro, 2015).

Oxygen is essential for aerobic organisms, DO amounts assess water conditions, during the aerobic equilibrium of the water's organic matter, decomposition-causing bacteria use oxygen in their respiratory processes and may decrease it in the medium. The greater the charge of organic matter, the greater is the number of decomposition-causing microorganisms and, consequently, greater oxygen consumption (Araújo et al., 2015). In current study, DO rates ranged between 4.0 and 9.9 mg/dL, even though Resolution 2914/2011 have not established DO rates in water for human consumption.

Allende et al. (2015) revealed that irrigation water is the

main source of contamination in vegetables. Water contamination by human faeces originates from public or home sewage discharges and from direct release of fecal material on the water surface by domestic or wild animals. Since many people failed to treat water adequately, a possible transmission chain was established. Health risks exist when water is taken or food washed by contaminated water is ingested (Fernandes et al., 2015).

Fischer's test was employed to assess the relationship between contamination of vegetables, soil and water irrigation. No statistical relationship between the variables was detected. Although a statistical co-relationship between variables was not established, the results of the analysis were highly relevant due to the great importance that contamination of vegetables represented for public health. Good Agricultural Practices (GAP) during the cultivation of vegetables is basic to prevent contamination and protect consumers' health.

However, proper washing of vegetables by the

population prior to consumption is mandatory for healthy food, with a reduction in microbial counts. The Brazilian Agency for Sanitary Care (ANVISA) recommends the use of sodium hypochlorite 200 ppm for 10 min as a sanitizing method so that vegetables may be safely consumed (ANVISA, 2004).

#### Farms characteristics

Nine producers were interviewed, of which six were between 30 and 55 years old and three over 55 years old. The survey showed that seven were males and only two were females. Also, seven of the producers had incomplete elementary school and two did not have any formal education. Avendaño-Reyes et al. (2019) in a study conducted with dairy farmers in Mexico found that most farmers were also males (84 and 20%) had no formal education, but 40% had studied at elementary school and the average age of farmers was over 56 years, unlike our own findings.

All interviewed people cultivated planted the vegetables in open fields and called themselves producers of organic vegetables. They informed that they used cattle or goat manure and food wastes, although none had an organic producer certificate issued by the Ministry of Agriculture, Stockbreeding and Supply (MAPA). According to MAPA producers selling organic products should have a certificate issued by MAPA or they should organize in groups for direct commercialization without any certificate. In this case, the producer cannot sell to third parties but only in town fairs (or directly to consumers) and in purchases by the government (Brazilian Program for School Food) and for the National Supply Company (CONAB). Producer 4, for instance, was not authorized to sell organic vegetables but they were sold to restaurants and supermarkets (Mapa, 2003).

When the hygiene and sanitary conditions of the vegetable garden area with regard to domestic animal breeding were evaluated, it may be seen that all producers had domestic animals, such as dogs, cats and fowls, which freely roamed around the vegetable gardens. Only 66.6% of homes had any sanitary installations and used septic cesspits for sewage. The other producers with no sanitary installations excreted directly in the soil. Other residents (55.5%), even with sanitary devices in their homes, preferred to do the same.

According to Jesus et al. (2013), bad sanitary conditions contributed towards the dissemination of intestine parasitosis in rural and urban areas. Constantin et al. (2013) insisted that performing physiological needs directly in the soil was a common habit by rural inhabitants, implying inadequate disposal of human dejects. It increased contamination risks in the field. Further, the roaming of animals within or close to the vegetable gardens could contaminate vegetables by pathogens in their feces.

Eight producers (88.9%) used animal manure and only

one (11.1%) reported the use of vegetable wastes and ashes and the producers did not practice composting. According to Sediyama (2014), animal manure should be processed for composting to decrease microbial load. In fact, when organic manure was treated or stored inadequately, it could contain pathogens and contaminate the vegetables. In fact, it favored the survival of helminth eggs and protozoa cysts in the medium up to the intake of the vegetables. The cistern was the means of water supply for irrigation by 55.5% of participants. Only Producer 5 employed water from the water supply system, even though the water was inadequately stored within an open tank. Unhealthy conditions of irrigation water were the rule in 88.9% of the vegetable gardens.

#### Conclusion

Due to high microbial density and the detection of parasites in most samples, one may say that health quality in the production of vegetables is not satisfactory possibly due to improper use of partially decomposed fertilizers, failure to treat water used for irrigation, contact of vegetables with feces waste, improper handling and bad home hygiene habits and is a risk to human health. It is crucial to invest in educational activities for handlers and farmers so that a better vegetable quality could be offered to the population. More efficient monitoring is required by health authorities, requiring periodic assessments for parasites so that consumers may have a better life quality.

# **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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