Parasitological analysis of sugarcane juice marketed in the Federal District

Análise parasitológica de caldo-de-cana comercializados no Distrito Federal

Análisis parasitológico de jugo de caña de azúcar comercializado en el Distrito Federal

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REVISA

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RESUMO

Objetivo: analisar amostras de caldo-de-cana comercializados no Distrito Federal, com o intuito de investigar a presença de parasitas que possam ser nocivos à saúde, ocasionando o aparecimento de Doenças Transmitidas por Alimentos. **Método:** Foram analisadas 10 amostras de caldo-de-cana provenientes estabelecimentos distribuídos em diferentes regiões no Distrito Federal. Todas as amostras foram submetidas ao método de sedimentação e analisados em triplicata. A análise dos resultados foi realizada com auxílio de microscopia óptica, para investigação da presença de ovos, cistos, oocistos ou demais formas evolutivas parasitárias. **Resultados:** Em todas as amostras foi constatado a presença de algum tipo de contaminação, sendo registrado leveduras em 100% das amostras, em 50% se obteve presença de Entamoeba sp., em 10% Taenia sp., em 10% Giardia sp. e em 10% Ascaris sp. **Conclusão:** É possível observar uma ineficiência nas práticas higiênico-sanitárias dos estabelecimentos responsáveis pelo processamento e moagem do vegetal, viabilizando contaminação na maior parte das amostras. Esta contaminação, pode ocasionar diversos tipos de doença para os seres humanos, particularmente oferecem mais riscos a indivíduos imunocomprometidos, sendo necessário uma monitorização mais eficaz desse tipo de alimento, bem como da sua distribuição.

Descritores: Contaminação Alimentar; Caldo-de-cana; Doenças Parasitárias.

ABSTRACT

Objective: to assess samples of sugarcane juice marketed in the Federal District in order to investigate the presence of parasites that can be harmful to health, causing Foodborne Diseases. **Method:** 10 samples of sugarcane juice from establishments distributed in different regions in the Federal District were analyzed. All samples were submitted to the Sedimentation method and analyzed in triplicate. The analysis was performed with the aid of optical microscopy to investigate the presence of eggs, cysts, oocysts or other parasitic evolutionary forms. **Results:** In all samples, the presence of some type of contamination was found, with yeasts being registered in 100% of the samples, in 50% Entamoeba sp., 10% Taenia sp., 10% Giardia sp. and in 10% Ascaris sp. **Conclusion:** We observed an inefficiency in the hygienic-sanitary practices of the establishments responsible for the processing and grinding of the vegetable, allowing contamination in most of the samples. This contamination can cause different types of disease for humans, particularly they offer more risks to immunocompromised individuals, requiring more effective monitoring of this type of food, as well as its distribution.

Descriptors: Food Contamination; Cane juice; Parasitic diseases.

RESUMEN

Objetivo: Analizar las muestras de jugo de caña de azúcar que se venden en el Distrito Federal para investigar la presencia de parásitos que pueden ser dañinos para la salud y causar la aparición de enfermedades transmitidas por los alimentos. **Método:** Se analizaron diez muestras de jugo de caña de azúcar de establecimientos distribuidos en diferentes regiones del Distrito Federal. Todas las muestras fueron sometidas al método de sedimentación y analizadas por triplicado. El análisis de los resultados se realizó con la ayuda de microscopía óptica, para investigar la presencia de huevos, quistes, ooquistes u otras formas evolutivas parasitarias. **Resultados:** En todas las muestras, se encontró la presencia de algún tipo de contaminación, con levaduras registradas en el 100% de las muestras, en 50% Entamoeba sp., 10% Taenia sp., 10% Giardia sp. y en 10% Ascaris sp. **Conclusión:** Es posible observar una ineficiencia en las prácticas higiénico-sanitarias de los establecimientos responsables del procesamiento y molienda del vegetal, lo que permite la contaminación en la mayoría de las muestras. Esta contaminación puede causar diferentes tipos de enfermedades en los humanos, particularmente ofrecen más riesgos para las personas inmunocomprometidas, lo que requiere un monitoreo más efectivo de este tipo de alimentos, así como su distribución.

Descriptores: Contaminación de alimentos; Jugo de caña; Enfermedades parasitarias.

ORIGINAL

Introduction

Due to the increase in factors and diseases that lead to immunosuppression, parasitic infections have been considered a prominent point in public health worldwide. Cross-contamination by food and water is the main vehicle for the transmission of infectious diseases, notably contributing to the occurrence of food-borne diseases.[1–3] Infection from food intake has increased worldwide[4–6], and is closely associated with the population's growing demand for food on the streets and careless health inspection.[7–11]

According to data from the World Health Organization (WHO), Foodborne Diseases (FD) are currently the biggest public health concern worldwide. FD can be fatal, especially in children under five, the elderly and people with immunodeficiency, causing 420.000 deaths annually. In the American region, diarrheal diseases account for about 95% of DTA.[7,12-13]

Food contaminants can be of diverse microbiological origins, including parasites, bacteria, viruses, as well as toxins.[6,9,14–19] These are associated with serious diseases causing health problems due to food intake.[14] Enteroparasites (helminths and protozoa) are part of a neglected group of tropical diseases [20]. Although enteroparasitosis is a worldwide public health problem.[5,6,16,18,21–23] in more severe cases, infections can generate morbidity and lead to death, as food contamination by these organisms is poorly investigated.

The number of infections from eating contaminated food [4–6] had greater prominence after the expansion of the population's demand for consuming food on the street, where the preparation of these may be under precarious sanitary hygiene inspection [8–11,24]. In tropical and populous regions like Brazil, access to street food is easy. In this context, the consumption of refreshing and nutritious foods such as sugar cane juice, known as sugarcane juice or garapa, is widely consumed throughout the country. Sugarcane belongs to the family of Gramineaes, Saccharum officinarum species, which has been part of the history of Brazil since 1502.[21]

Brazil is the largest sugar cane producer in the world [25]. The harvest in 2019 was 622.3 million tons, having a great notoriety for Brazilian agribusiness and a significant economic contribution in the involvement and production of sugar, fuel alcohol, cachaça, and for the extraction of food, as is the case of sugar cane, sugarcane juice.[21,26]

The cane juice is obtained by grinding, being a refreshing and energetic drink, which in its composition contains sugars such as glucose, fructose and sucrose. Likewise, the broth is rich in nutrients such as iron, calcium, phosphorus, proteins, vitamins A, C, B1 and B6.[22,27-28] Due to its nutritive character, sugarcane juice generates a favorable environment for contamination and growth of microorganisms. Much of the contamination occurs through the handling of sugarcane, grinding and storage of juice. In addition, the sanitary conditions of the establishment are essential for the quality and non-contamination of the product.[29–32] Although cane juice is widely consumed in Brazil, especially during the summer, studies on the possibility of food acting as a vehicle for the transmission of pathogenic microorganisms are scarce.

One way to assess food contamination and poor sanitary hygiene is to investigate the presence of helminth eggs, indicative of the quality of the product from its production to its arrival at the consumer's table. Parasitological research studies indicate a high prevalence of protozoa and helminths of medical interest, found in vegetables sold in supermarkets and public fairs in Brazil.[33-34] With regard to contamination by parasites, several enteroparasitoses can be transmitted by eating vegetables: Cryptosporidium sp., Giardia lamblia, Entamoeba histolytica/dispar, Ascaris lumbricoides, Ancylostomidae sp., Strongyloides stercoralis, Trichuris sp., Taenia sp.[5-6,35], among others. The presence of any of these parasites in the food, suggests contamination by faeces and absence of health care.[35]

To certify ethical practices at all stages of food production, it is essential to prioritize protocols guided by the Hazard Analysis System and Critical Control Points (HASCCP) and the principles of good hygiene practices, including Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP).[4,23]

This study was carried out to analyze the parasitological contamination profiles present in sugarcane juice, sold in public establishments in different regions of the state of Federal District/ Brazil. The data obtained through this study are relevant to public health, making it possible to guarantee to the consumer, through actions of applicability of legislation and inspection, good practices in food handling.

Method

Ten samples of fresh cane juice were collected from different establishments, located in the state of Federal District. The choice of establishments was based on the turnover of people, easy access to food, as well as different consumer profiles. Federal District is a state located in a tropical region[36], where the consumption of sugarcane juice is common and typical. Of the 10 samples collected, 2 samples are from the Samambaia region (A1 and A2), 1 sample from Brasilia Bus Station (A3), 1 sample from Taguatinga – Federal District (A4), 2 samples from Águas Claras (A5 and A6), 2 samples from Riacho Fundo I (A7 and A8), 1 sample from Guará (A9) and 1 sample from Asa Sul - Brasília (A10) as shown in Figure 1. The samples were collected in sterile flasks, identified at the time of collection and packed in thermal bag. The samples were transported to the Clinical Analysis laboratory at Paulista University UNIP – Federal District, immediately after each collection.

Figure 1 - Map registered sample collection points in Brasília - Distrito Federal.
Source: Google Maps.

Parasitological analysis of the samples was carried out using spontaneous sedimentation methods such as Hoffman, Pons and Janer or Lutz's method, which are routinely used for EPF (Stool Examination) exams. These methods allow the visualization of parasites, protozoa, cysts, oocysts, eggs and intestinal helminth larvae.[37] In each sample, 200 ml of cane juice homogenized with 50 ml of distilled water were used, the liquid was subsequently strained through a plastic sieve and filtered through sterile gauze, disposed in graduated glass goblets for decanting. After 2 hours, the sediment was analyzed in triplicate. The slides were prepared with a drop of lugol and visualized with the aid of optical microscopy. For each sample, 3 slides were analyzed, 2 hours after the start of the test and 3 slides after 24 hours of sedimentation, as detailed in Table 1.

Table 1- Methods applied to sugarcane juice samples in the Federal District. Brasilia, DF, Brazil.

Samples	Date of gathering and analysis	Method applied
A1	18/09/2018 and after 24 hours	Hoffman, Pons e Janer
A2	20/09/2018 and after 24 hours	Hoffman, Pons e Janer
A3	24/09/2018 and after 24 hours	Hoffman, Pons e Janer
A4	26/09/2018 and after 24 hours	Hoffman, Pons e Janer
A5, A6, A7, A8 e A9	27/09/2018 and after 24 hours	Hoffman, Pons e Janer

A10	04/10/2018 and after 24 hours	Hoffman, Pons e Janer

Results

Among the 10 samples of sugarcane juice analyzed, it was possible to identify the presence of yeasts in different budding phases, in 100% of the samples, as can be seen in Figure 2 (a-f), where it shows images acquired at the time of analysis.

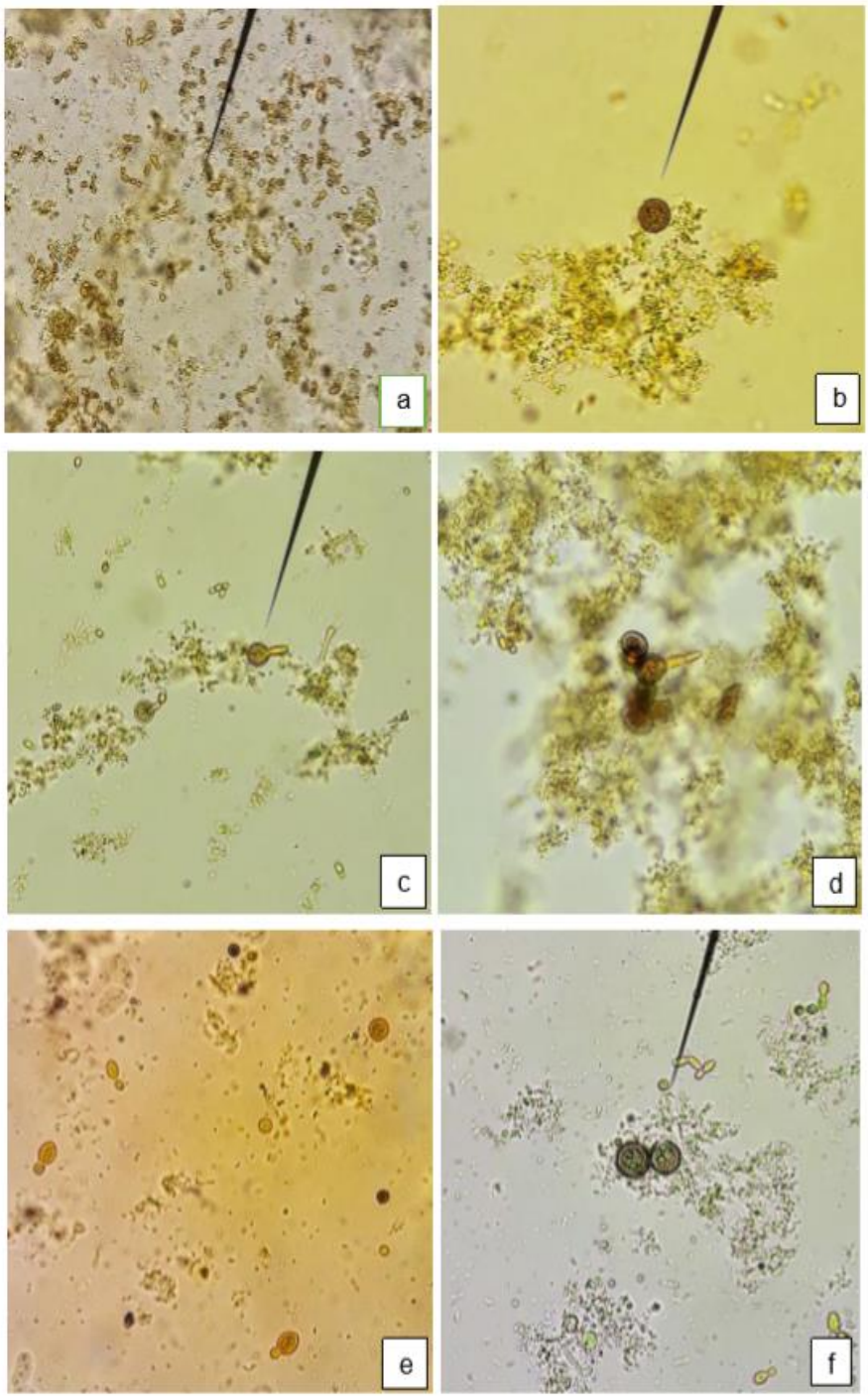

Figure 2 – Presence of budding yeasts and vegetative spore. Brasilia, DF, Brazil.
Note: A. Yeasts present in large quantities in sample A9. B. Germ pore has an A4 sample. C. Yeasts with germ tube (pseudo-hyphae) sample A4. D. Sprouting yeasts with germ tube, (pseudo-hyphae) A4. E. Yeasts in budding by asexual reproduction sample A1. F. germline spores present in sample A3.

The frequency of parasitic contamination was positive in 70% of the samples, as can be seen in Figure 3. In the image, morphological structures compatible with parasitological findings can be seen, found in samples A1, A2, A3, A4, A5, A7 and A8 . Sample A1 was sent to the microbiology sector and after staining using the gram method, it was possible to observe gram-positive bacteria in the form of rods and gram-negative cocci, not being subjected to the other biochemical tests (data not shown). Specimens of protozoan cysts Entamoeba sp. were observed in samples A1, A2, A5, A7 and A8, corresponding to 50% of the analyzed samples. In sample A3, it was possible to observe Taenia sp. and cysts of Giardia sp., corresponding to 10%. In sample A4, it was possible to identify Ascaris sp. Egg, equivalent to 10% of samples.

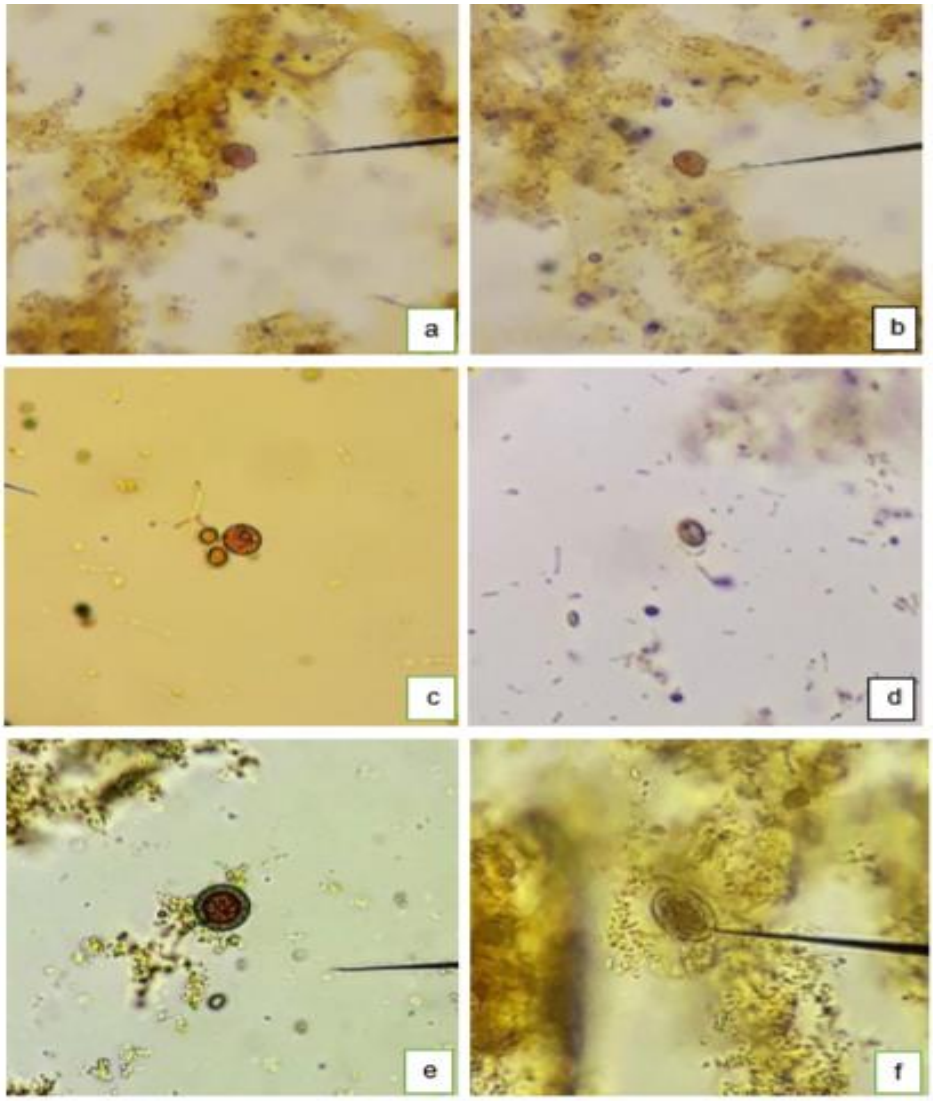

Figure 3 – A. Entamoeba sp. cyst in sample A2. B. Entamoeba sp. cyst in sample

A5. C. Entamoeba sp. cyst in sample A7. D. Giardia sp cyst in sample A3. E. Taenia sp. egg in A3 sample. F. Infertile egg of Ascaris sp. cyst in sample A4.

The global parasitological analysis of this study is detailed in Table 2 below, where "x" indicates presence and "-" indicates the absence of a certain microorganism.

Table 2 – Parasitological results of the analyzed sugarcane juice. Brasilia, DF, Brazil.

Samples	Giardia sp.	Entamoeba sp.	Ascaris sp.	Taenia sp.	Yeasts
Sample 1	-	x	-	-	x
Sample 2	-	x	-	-	x
Sample 3	x	-	-	x	x
Sample 4	-	-	x	-	x
Sample 5	-	x	-	-	x
Sample 6	-	-	-	-	x
Sample 7	-	x	-	-	x
Sample 8	-	x	-	-	x
Sample 9	-	-	-	-	x
Sample 10	-	-	-	-	x

"x" presence.
"-" absence.

Discussion

Food contamination is a serious public health problem.[6,38] Foods with a high nutritional and sugar content, such as sugarcane juice, can easily act as a vehicle for the transmission of foodborne diseases, since it offers conditions conducive to the growth of contaminants. Because it is a favorable medium for the growth of different microorganisms and also due to its high consumption and low level of inspection, sugarcane juice ends up becoming a food potentially harmful to health. The risks arising from microbiological contamination in sugarcane juice have been reported since 1991, with episodes of contamination with T. cruzi, an agent of Chagas Disease.[39]

In the analyzes presented in this study, although it was not the focus of the research, all samples showed the presence of budding yeasts. Some fungi, like Saccharomyces cerevisae are present in sugar cane, and can be commonly found in sugarcane juice.[40-41] S. cerevisae is a yeast, generally non-pathogenic, used for the production of bioethanol, due to its fermentative capacity and high tolerance to alcohol, besides being widely used in beer, wine and yeast production industries for baking.[42-43]

With regard to parasitological analysis, our data indicate the presence of different contaminating parasites in the sugarcane juice samples. Similar data were observed in the study by Maldone and collaborators investigating contamination in vegetables sold in the Federal District, where the following

parasites were found: Trichuris sp., Strongyloides sp., Taenia sp. and Giardia sp. were detected in vegetables sold at fairs, Entamoeba sp., E. coli, Strongyloides sp. Ascaris sp, Enterobius vermicularis and Ancylostomidae were detected in vegetables sold in the markets of the Federal District.[5]

In our study, in 50% of the samples it was possible to observe the presence of Entamoeba sp., A unicellular protozoan that appears in the form of a cyst or trophozoite. The form of transmission is usually oral-fecal through contaminated food and water.[44-45]. Santos and Biondi showed parasitic contamination 8.2% of vegetables obtained from different establishments (restaurants, fairs and markets) in the Federal District.[46]

In one of the samples analyzed, cysts of Giardia sp., A flagellated protozoan, can be found in two characteristic forms, cyst or trophozoite. The form of cyst is responsible for oral-fecal transmission.[35,47] Giardiasis is a highly contagious disease and is involved with gastrointestinal symptomatological processes such as diarrhea, fever, colic, vomiting and consequent weight loss.[48]

One of the samples showed the presence of egg Taenia sp., A hermaphrodite helminth, responsible for causing the diseases of teniasis and cysticercosis.[49] The form of transmission can occur through the ingestion of cysticercus present in the meat of animals, causing teniasis or through the ingestion of Taenia solium eggs, whose transmission is oral-fecal, through eggs eliminated in the host's feces.[49]

In our study, eggs of Ascaris sp. were also found. This nematode is responsible for the disease ascariasis, popularly known as roundworms. They have a wide geographical distribution with high prevalence, being found in all countries of the world.[50] Transmission occurs via the oral-fecal route, through water and food contaminated with eggs containing the parasite's larva. Currently, it is estimated that more than 800 million people are infected with helminth Ascaris sp. this number may reach 1.2 billion people.[50] A study carried out in open markets in the municipality of Vitória da Conquista in Bahia, had a result where the presence of Entamoeba sp. in more than 70% of the samples, with the presence of Ascaris sp. in 38%, cysts of Giardia sp. in 27%, with identification of Isospora belli oocysts in 5.5%.[51]

A relevant point to the data of this study is that during the collection of the samples, it was possible to observe that most establishments did not follow the standards defined by the Technical Regulation of Good Practices for Food Services, and the Technical Regulation of Hygienic-Sanitary Procedures for Handling Food and Drinks Prepared with Vegetables.[52-53] The presence of such parasites can be justified by the absence of adequate hygienic sanitary conditions, which are essential for the preparation of raw foods, by establishments that did not comply with the rules regulated in RDC 216 of 2004 and RDC No 218, which has Technical Regulation of Hygienic-Sanitary Procedures for Handling Food and Beverages Prepared with Vegetables, which came into force in 2005, after cases of contamination with sugarcane juice; and establishes rules for the storage and grinding of sugarcane, as well as the use of disposable gloves and hand washing. Such procedures are necessary to ensure greater control over foods that do not undergo any type of decontamination before ingestion, thus complementing RDC No.12 of 2001, which inspects microbiological standards for

foods by determining the acceptable amount of fecal and thermotolerant coliforms in foods.[28-30]

Although the possibility of contamination through the consumption of sugarcane juice is evident, when not handled and / or processed under good hygienic-sanitary practices, parasitological and microbiological studies of an investigative nature in sugarcane juice are scarce.

Conclusion

Based on the analysis results, we found the presence of contaminating organisms in all samples, showing poor hygienic-sanitary practices in establishments where sugarcane juice is marketed in the Federal District.

Such contamination can be avoided by taking measures such as more effective monitoring of the material, care in maintenance and storage, actions added to the correct training in food handling. The concern with ensuring that food is not contaminated must be continuous to ensure health for the population, thus mitigating the recurrence of infectious cases.

It is worth mentioning the scarcity of studies analyzing the potential of commercial sugarcane juice to offer risks to human health. Thus, this work has a significant impact in terms of public health, in order to warn about the dangers of eating a food, although popular in the national territory, and still poorly evaluated.

Therefore, it is necessary to have a monitoring system that is more comprehensive and that can avoid such occurrences, bringing security to the consumer, especially those who are at risk, such as children, the elderly and the immunosuppressed.

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