

BRIEF COMMUNICATION

SURVIVAL OF *Trypanosoma cruzi* IN SUGAR CANE USED TO PREPARE JUICE

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SUMMARY

Chagas disease can be transmitted to man by many different means, including contact with infected triatomine feces, blood transfusion, laboratory accidents, organ transplants, and congenital or oral routes. The latter mode has received considerable attention recently.

In this assay, we evaluate the survival of *Trypanosoma cruzi* contaminating sugar cane used to prepare juice, as well as the viability and capacity for infection by the parasite after recovery. Thirty triatomines were contaminated with *T. cruzi* Y strain and 45 days later pieces of sugar cane were contaminated with the intestinal contents of the insects. The pieces were ground at different intervals after contamination (time = 0, 1, 4, 6, 12 and 24 hours) and the juice extracted and analyzed. Different methods were used to show *T. cruzi* in the juice: direct analysis, hematocrit tube centrifugation and QBC, and experimental inoculation in 47 female BALB/c mice (five control mice and seven mice for each interval examined (five inoculated orally and two intraperitoneally). Positive results were found using the direct analysis and QBC methods for juice prepared up to 12 hours after initial contamination. However, by the centrifugation technique, positivity was found only up to four hours after contamination of the sugar cane. Inoculated animals showed parasitemia during a 14 day observation period, demonstrating the high survival rate of *T. cruzi* in sugar cane.

KEYWORDS: *Trypanosoma cruzi*; Experimental infection; Sugar cane juice.

American trypanosomiasis is found in the Americas from the southern United States to Argentina and Chile, with a high incidence in Brazil. The main vectors are triatomine insects, particularly the species *Triatoma infestans*, *Rhodnius prolixus* and *Panstrongylus megistus*, popularly known as the kissing bug. Transmission of parasitosis habitually depends on the participation of infected triatomines, but may also take place through alternative mechanisms such as congenital or oral routes, maternal milk, blood transfusion, laboratory accidents and organ transplants². The oral mode of infection is suspected in several recent events.

There are several reports on the outbreak of human Chagas disease in Brazil, probably by oral contamination^{8,9,11}. Only a few experimental studies have attempted to demonstrate the likelihood of this route of contamination^{7,10}.

The objective of the present work was to evaluate the survival of *Trypanosoma cruzi* present in contaminated sugar cane used in the preparation of fresh cane juice, as well as the viability and infective capacity of the parasite in the juice.

Thirty nymphs of *T. infestans* were infected with *T. cruzi* Y strain, and maintained at the Parasitology laboratory of the Instituto de Medicina Tropical de São Paulo, Brazil. The insects were dissected 45 days later and, after confirming the presence of metacyclic trypomastigotes, their intestinal contents were mixed with physiological saline (approximately 2.0×10^5 parasites/mL) which was then used to contaminate six pieces of sugar cane (≈ 5 mL of solution/piece, applied with dropper). The cane pieces were then processed in a mechanical grinder to extract the juice at different intervals after contamination (time = 0 and 1, 4, 6, 12 and 24 hours after contamination). The juice was analyzed for *T. cruzi* using a direct method, and by techniques similar to the hematocrit tube centrifugation test and acridine orange Quantitative Buffy Coat (QBC).

Forty two, male Balb/c mice, in groups of seven animals each, corresponding to each juice extraction interval, were infected using one of two different routes: five mice were given 20 μ L of contaminated juice orally (≈ 1000 parasites); and two mice were injected intraperitoneally with 0.5 mL of contaminated juice. A further five animals were given 20 μ L of uncontaminated sugar cane juice orally, and served as the control group.

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Table 1
Infection of mice with juice prepared from sugar cane contaminated with *Trypanosoma cruzi* after various time intervals

Time to extraction of juice after cane contamination (hours)	Method used to show <i>T.cruzi</i> in juice			Inoculation route			
	Direct analysis	Hematocrit centrifugation	QBC	Oral No.	Positivity * (%)	Intraperitoneal No.	Positivity * (%)
0	Positive	Positive	Positive	5	100	2	(-)
1	Positive	Positive	Positive	5	100	2	50
4	Positive	Positive	Positive	5	100	2	50
6	Positive	Negative	Positive	5	100	2	(-)
12	Positive	Negative	Positive	5	100	2	50
24	Negative	Negative	Negative	5	100	2	(-)

* Infection 14 days after inoculation; (-) mortality during experiment.

Fresh blood, obtained from tailsnips of each mouse, was examined for circulating parasites from the third day after infection. Parasitemia was accompanied up to day 14 after infection. The results are given in Table 1.

Mortality of animals was also accompanied up to four months after beginning the experiments. All mice inoculated orally using juice prepared at the different intervals after initial sugar cane contamination, survived.

In 1990, an investigation was performed to evaluate the viability of *T. cruzi* present in sugar cane juice. This study demonstrated that the parasite was viable for up to four hours in the sugar cane juice kept at room temperature⁷. However, in our experiment, in which the unprocessed sugar cane stayed contaminated for different intervals until the juice was extracted, the parasite survived up to 24 hours after initial contamination.

In 1997, in Goiânia, Brazil, oral transmission of the disease was demonstrated using inoculates consisting of blood from mice infected previously with *T. cruzi* Y strain. The animals died within 20 days after infection¹⁰. In our investigation the inoculum consisted of juice from sugar cane contaminated with *T. cruzi* Y strain. The animals thus inoculated survived for at least four months after infection.

We chose to infect mice orally with *T. cruzi* contaminated sugar cane juice in an attempt to reproduce recent events in Santa Catarina State, Brazil, where several individuals were infected by Chagas disease in the acute phase. We also chose this route based on previous reports showing that in mice the oral cavity is more susceptible to infection than the esophagus^{1,3,5}.

Sugar cane juice is not the only beverage capable of transmitting the parasite: assai palm fruit juice was implicated in cases of infection by *T. cruzi* via the digestive route in an outbreak in Pará State, Brazil, in 2001 and in 2006, 26 cases were reported in Amapá State. Many family episodes also are reported from the Brazilian Amazon^{4,6,11}.

Our findings suggest that oral *T. cruzi* transmission should be considered likely since this mode of infection may be responsible for the appearance of new cases in areas where natural transmission by vectors is well controlled. We also demonstrate a high degree of survival by the trypanosomatid in the sugar cane itself, up to 24 hours, suggesting

that inadequate storage of the cut cane in areas endemic for Chagas disease can represent a risk to health.

RESUMO

Avaliação da sobrevivência de *Trypanosoma cruzi* em cana de açúcar utilizada no preparo do caldo

A doença de Chagas pode ser transmitida ao homem através de vários mecanismos: fezes de triatomíneo infectado; transfusão sanguínea; acidente em laboratório; transplante de órgão; vias congênita ou oral convindo salientar que esta última tem motivado ocorrências recentemente.

Neste estudo procuramos avaliar a sobrevivência de *Trypanosoma cruzi* presente em cana de açúcar contaminada com o parasita, utilizada no preparo do caldo e, também, a viabilidade e a capacidade de infecção do parasita depois de ser recuperado. Trinta triatomíneos foram contaminados com a cepa Y de *T. cruzi*; após 45 dias realizamos a contaminação de pedaços de cana de açúcar com o conteúdo intestinal dos insetos. Estes pedaços foram moídos em diferentes tempos: no início (tempo 0) e após 1, 4, 6, 12 e 24 horas da contaminação e o caldo extraído foi analisado por diferentes métodos: direto, centrifugação em tubo de hematócrito, QBC. Este caldo contaminado foi inoculado em 47 camundongos machos BALB/c, sendo cinco controles (com caldo de cana limpo) e sete para cada tempo estudado (cinco inoculados pela via oral e dois pela intraperitoneal). Na análise direta e no QBC obtivemos resultados positivos até 12 horas e, na centrifugação, ocorreu positividade somente até as quatro horas. As parasitemias dos animais inoculados foram todas positivas em um período de 14 dias de observação, demonstrando alto grau de sobrevivência do *T. cruzi* na cana de açúcar.

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