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First report of a family outbreak of Chagas disease in French Guiana and posttreatment follow-up

Denis Blanchet [a], Simone Frédérique Brenière [b,*], Alejandro G. Schijman [c], Margarita Bisio [c], Stéphane Simon [a], Vincent Véron [a], Claire Mayence [d], Magalie Demar-Pierre [a], Félix Djossou [e], Christine Aznar [a]

[a] Laboratoire Hospitalier et Universitaire de Parasitologie et Mycologie Médicale, Equipe EA 3593, EPaT, UFR de Médecine, Université des Antilles et de la Guyane, Centre Hospitalier Andrée Rosemon (CHAR), Cayenne, French Guiana
[b] IRD, Institut de Recherche pour le Développement, UMR INTERTRYP (IRD-CIRAD), Interactions hôtes-vecteurs-parasites dans les infections par trypanosomatidae, 911 Av. Agropolis, 34394 Montpellier cédex 5, France
[c] Laboratorio de Biología Molecular de la Enfermedad de Chagas, LabMECh, INGEBI-CONICET, Buenos Aires, Argentina
[d] Service d'accueil des urgences, Centre Hospitalier de l'Ouest Guyanais (CHOG), St Laurent du Maroni, French Guiana
[e] Service de maladie infectieuse et tropicale, Centre Hospitalier Andrée Rosemon (CHAR), Cayenne, French Guiana

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ABSTRACT

The outbreak of acute Chagas disease due to oral transmission of the parasite is a well-known phenomenon mainly occurring in the Amazon. Such an event is described here for the first time in French Guiana. Eight patients of the same family, presenting epidemiological and clinical histories compatible with recent *Trypanosoma cruzi* infection of Chagas disease due to the ingestion of palm *Oenocarpus bacaba* juice were, rather late after the putative date of infection, underwent four parasitological and two serological specific tests for confirmation of the diagnosis. Real-time PCR results were positive for all the patients; strains were isolated by hemoculture from four patients, PCR identification of TcI DTU was made for six patients, while parasites were not detected in any of the patients by direct microscopic examination. The results of two serologic tests were positive. All patients were treated with benznidazole, and two patients were additionally given nifurtimox. A 6-year follow-up was possible for six patients. Real-time PCR was negative for these patients after 1 year, while the antibody rates decreased slowly and serology results were negative only after several years (1–5 years). Our findings confirm the occurrence of an outbreak of Chagas infection in members of the same family, with the oral mode of infection being the most likely hypothesis to explain this group of cases. Our results show the successful treatment of patients infected by TcI and the usefulness of real-time PCR for the emergency diagnosis of recent Chagas disease cases and in posttreatment follow-up.

1. Introduction

Chagas disease is a widespread anthropozoonosis in Latin America. Its causative agent, *Trypanosoma cruzi*, is transmitted mainly through the dejections of infected vectors that are hematophagous bugs of the subfamily Triatominae (Hemiptera, Reduviidae) (WHO, 2002). In the Amazonian region, Chagas disease has been recognized since the middle of the twentieth century, with documented human cases in French Guiana and in the Brazilian Amazonian area (Floch and Boulan, 1956; Floch and Tasqué, 1941; Shaw et al., 1969). However, because of the absence of domestic vectors, the region has previously been considered as nonendemic for human Chagas disease (WHO, 1990). Only in the last 15 years, has the transmission of *T. cruzi* to man in the Amazonian region been considered as an emergent problem (Coura et al., 2002). The major epidemiological features have been family microepidemics of acute Chagas disease due to oral contamination (Pinto et al., 2001).

The diagnosis of acute Chagas disease is currently based on clinical and biological criteria among which the detection of *T. cruzi* by microscopic examination of peripheral blood is still considered essential (Ministério da Saúde do Brasil, Secretaria Nacional de Vigilância em Saúde, Sistema de Notificação de Agravos de Notificação (SINAN), 2004) (Von et al., 2007). However, this technique is of low sensitivity, and depends on the time elapsed between the first

* Corresponding author. Tel.: +33 (0)467416447.
E-mail address: Frederique.Breniere@ird.fr (S.F. Brenière).

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clinical manifestations and the blood sampling (Laranja et al., 1956; Miller, 1931). During the acute phase, general clinical signs tend to be fairly nonspecific, such as fever, myalgia, asthenia (WHO, 2002). Conventional (Moser et al., 1989; Sturm et al., 1989) and real-time polymerase chain reaction (PCR) (Britto et al., 1999) have been developed for detection of *T. cruzi*-specific DNA from peripheral blood samples of chronic Chagasic patients, and recently, for diagnosis of acute cases (Nicholls et al., 2007).

In this report, we present the first description in French Guiana of a family infected by *T. cruzi* due to oral transmission after consuming fruit juice of the *Oenocarpus bacaba* palm tree. The diagnosis was supported by parasitological, serological, and PCR tests, by the clinical symptoms, by the patients' answers to epidemiological queries, and by the posttreatment follow-up. Eight members of the same family were affected. Of these, six patients were infected by the *T. cruzi* DTU TcI, and all six can be considered as cured approximately 1, 3, or 5 years after the treatment.

2. Materials and methods

2.1. Study area and patients

The patients were living in a village of Littoral in French Guiana, in the north of Cayenne city (5°28′46.2″N 53°12′14.0″W), the biotope mainly constituted of savannas. Eight cases of Chagas disease were reported, all being members of the same family. Four patients (1-HEN, 2-LAU, 3-VER, and 4-JER) consulted the emergency department of the hospital of Saint Laurent du Maroni (SLMH) in November 2005, where the diagnosis of acute Chagas disease was suspected. One week later, these patients were hospitalized in Cayenne. Two other members of the family were immediately referred for clinical and laboratory examinations to assess possible *T. cruzi* infection (patients 5-JUS and 6-PHI), while the two last members of the family were only tested in February 2006 (patients 7-DOR and 8-JUL). Patient 5-JUS was previously hospitalized in Kourou to treat an ovarian cyst. Initially, patient 6-PHI had a diagnosis of toxoplasmosis and accordingly received specific treatment. Specific ethical approval is not required because the standard procedures were applied for the patients at the Cayenne Hospital in French Guiana.

2.2. Samples

Blood samples were obtained from the Emergency Unit of the SLMH and from the Infectious Disease and Intensive Care Unit of the Cayenne Hospital. Blood was sampled by venipuncture into EDTA tubes for PCR and culture, and into dry tubes for serology. Pericardial fluid sample was taken from patient 1-HEN, who had severe pericardial effusion, and a liver biopsy was also collected from patient 3-VER, who presented with acute cytolytic hepatitis.

Positive control samples were derived as aliquots of 10 ml of whole human blood artificially contaminated by CL-Brener and Guyanese strains of *T. cruzi*, using a mechanical micromanipulator in which the trypanosomes were introduced one by one into parasite-free blood samples to obtain two defined parasite loads: 1 trypanosome per ml and 1 trypanosome per 10 ml of blood. Peripheral blood samples from six persons having never lived in or traveled to endemic countries were used as negative controls.

2.3. Microscopic examination

Peripheral blood, buffy-coat, and pericardial fluid samples were examined by direct microscopic observation by a single operator for approximately 40 min. Blood films, pericardial fluid samples, and liver tissue prints stained with May-Grünwald Giemsa were also microscopically examined for 40 min each.

2.4. Culture

Cultures for *T. cruzi* were performed from 30 ml of peripheral blood in EDTA tubes (Castro et al., 2002). For patients 1-HEN and 3-VER, liver biopsy and pericardial fluid in tubes containing liver-infusion tryptose (LIT) medium were also cultured. Cultures were incubated at 28 °C and microscopically examined each day during the first week and then each week for 6 months (Castro et al., 2002).

2.5. Serology

Serological testing for *T. cruzi* was performed using the Bioelisa Chagas test (Biokit, Barcelona, Spain) and ELISA cruzi (BioMerieux, Rio de Janeiro, Brazil), according to the manufacturer's instructions for both processing and interpretation (antibody rates were expressed as a ratio of optical densities of the sample/cut-off value). In order to establish the antibody kinetic for each patient after treatment, all conserved sera were processed simultaneously. Other analyses were also carried out to address infections by endemic parasites, such as *Plasmodium* sp. and *Toxoplasma gondii*, and viral pathogens such as dengue virus, CMV, and EBV, by current procedures performed at the reference Polyvalent Laboratory of Cayenne Hospital.

2.6. DNA extraction

Samples for PCR were prepared as follows: 10 ml of blood was mixed with guanidine hydrochloride 6 M as previously described (Britto et al., 1993). After boiling for 15 min, the lysate was allowed to cool at room temperature. DNA extraction was immediately carried out from 200 µl of lysate using the DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.7. Real-time PCR

The *T. cruzi* kDNA real-time PCR was performed as described later by Qvarnstrom et al. (2012), except for the TaqMan® probe (71P, 5′ CATCTC(A/C)CCCGTACATT 3′), which in the present study was labeled at 5′ with FAM and at 3′ with a dark quencher dye (MGB). This system was also used (test LbG/1) in the international validation study several years later (Schijman et al., 2011). Real-time PCR reactions were carried out in duplicate in a 50-µl final volume containing 1× TaqMan universal PCR Master with UNG Mix (Applied Biosystems, Foster City, USA), 900 nmol/L of each primer, 250 nmol/L of probe, and 10 µl of template DNA. All reactions were run on a 7300 real-time PCR System (Applied Biosystems, Foster City, USA). The Universal Thermal Cycling Protocol as provided by the manufacturer was used: each sample was initially warmed for 2 min at 50 °C to activate UNG, and then denatured at 95 °C for 10 min and cycled 50 times, each cycle consisting of 95 °C for 15 s and 60 °C for 60 s. *T. cruzi*-positive (1 trypanosome cell per 1 ml and per 10 ml) and -negative samples were included in each PCR run.

The samples were considered positive for *T. cruzi* when the threshold cycle (Ct) for the *T. cruzi* target was inferior to 45. The presence of PCR inhibitors was tested in the negative PCR samples, using the TaqMan Exogenous Internal Positive Control (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions.

The analytical sensitivity of the real-time strategy calculated using serial dilutions of DNA extracted from a starting sample containing one *T. cruzi* cell per milliliter of blood was 10^{-4} trypanosome

Table 1
Schedules of treatments applied to six of the patients.

Patient	Drug	Dosage (mg/kg/days)	Start	End	Approximate duration (days)
1-HEN	Benznidazole	5	12/9/2005	1/6/2006	28
	Nifurtimox	8	3/1/2006	5/1/2006	61
2-LAU	Benznidazole	5	12/9/2005	3/2/2006	83
3-VER	Benznidazole	5	12/9/2005	3/3/2006	84
4-JER	Benznidazole	7	12/9/2005	12/21/2005	12
	Benznidazole	5	12/24/2005	12/29/2005	5
	Nifurtimox	8	3/1/2006	4/30/2006	60
5-JUS	Benznidazole	5	12/9/2005	3/10/2006	91
6-PHI	Benznidazole	5	12/16/2005	3/25/2006	99

equivalents per reaction tube. The other positive control (1 trypanosome cell per 10 ml) gave consistent and reproducible positive signals in each sample.

2.8. T. cruzi molecular typing

Parasite discrete typing units (DTUs) (Zingales et al., 2009) were identified from kDNA-PCR-positive blood samples using a combination of PCR strategies targeted to nuclear genomic markers, such as the intergenic region of the spliced leader genes and the D7 domain of the 24Sα ribosomal DNA genes, which had been optimized to allow the direct identification of DTUs in human peripheral blood (Burgos et al., 2007).

2.9. Treatments

Patients were treated as recommended by the World Health Organization [1]. All chemotherapies were started with benznidazole a few days after the diagnosis was established. For two patients, adverse cutaneous reactions led to stopping benznidazole before the end of the first month and switching to nifurtimox for 2 months (Table 1).

3. Results

3.1. Case reports and biological tests before treatment

Clinical and biological results of the patients are summarized in Table 2. The first four patients who had presented to the emergency department of the hospital (SLMH) in November 23, 2005, had the following clinical signs: fever, myalgia, asthenia, and mild edema of the legs. The diagnosis of malaria was discarded after microscopic examination of blood samples and that of dengue after specific serological test. As *T. cruzi* infection was suspected, blood samples were immediately sent to the Parasitology and Medical Mycology Laboratory of Cayenne Hospital. The second microscopic examinations of the four blood samples were negative for flagellate parasites. The specific *T. cruzi* serology and real-time PCR for *T. cruzi* were positive for the four patients. Ten days later, it was decided to treat the patients with benznidazole. Later, blood cultures were positive for patients 1-HEN, 2-LAU, and 3-VER after 14, 22, and 30 days of culture, respectively. Moreover, the culture from pericardial fluid of patient 1-HEN, who had severe pericardial effusion, was also positive after 41 days. Similarly, patient 3-VER, who showed an acute cytolytic hepatitis, had a positive culture from a liver biopsy sample after 12 days. After discussions with these first four patients, the oral transmission of *T. cruzi* after consuming fresh *O. bacaba* fruit juice was suspected, occurring during the period of active picking of palm fruits and traditional juice preparation in this family. The presumed date for the contamination was established around the last week of October 2005. Consequently, four other members of the family (patients 5–8) who had consumed the juice were also invited to attend the hospital. Patients 7-DOR and 8-JUL had left the department on November 23, 2005, and they consulted only in February 2006. As presented in Table 2, specific *T. cruzi* serologic testing using the two Bioelisa Chagas kit yielded positive results for patients five and six, it was doubtful for patient seven (only one positive test result), and for patient eight one test result was positive and the second was missing. Real-time PCR for *T. cruzi* yielded a positive result for patients 5-JUS to 8-JUL and blood culture was positive only for patient 6 after 16 days. As done previously, the physicians decided to treat the patients.

3.2. Identification of the T. cruzi discrete typing unit

Direct identification of *T. cruzi* DTUs was achieved from blood samples for six of the eight patients. All the samples rendering positive findings by means of lineage-specific PCR procedures were infected by parasite populations belonging to the DTU Tc I.

Table 2
Chagas infection diagnosed by serology, real-time PCR, and blood culture.

Patient code	Date of sample	Age	Sex	Clinical signs[a]	Parasitological diagnosis		Serological diagnosis		PCR	
					Blood microscopy	Blood culture	BioElisa Biokit	BioElisa BioMerieux	Real-time	DTU of *T. cruzi*
1-HEN	11/23/2005	47	M	1, respiratory disturbances	Positive	Positive[b]	Positive	Positive	Positive	TcI
2-LAU	11/23/2005	17	F	1	Negative	Positive	Positive	Positive	Positive	TcI
3-VER	11/23/2005	40	F	1, nausea	Negative[c]	Positive[c]	Positive	Positive	Positive	TcI
4-JER	11/23/2005	10	M	1, nausea	Negative	Negative	Positive	Positive	Positive	TcI
5-JUS	12/4/2005	36	F	1, sore throat, abdominal pain	Negative	Negative	Positive	Positive	Positive	TcI[d]
6-PHI	12/4/2005	32	M	1	Negative	Positive	Positive	Positive	Positive	TcI
7-DOR	2/11/2006	66	F	1	Negative	Negative	Positive	Negative	Positive	ND
8-JUL	2/11/2006	41	M	1	Negative	Negative	Positive		Positive	ND

[a] 1, for the following signs: fever, myalgia, asthenia, lower-limb edema.
[b] Positive in pericardial fluid.
[c] Positive in liver biopsy.
[d] TcI determined by only 24s ribosomal DNA-PCR, the spliced-leader PCR was negative.

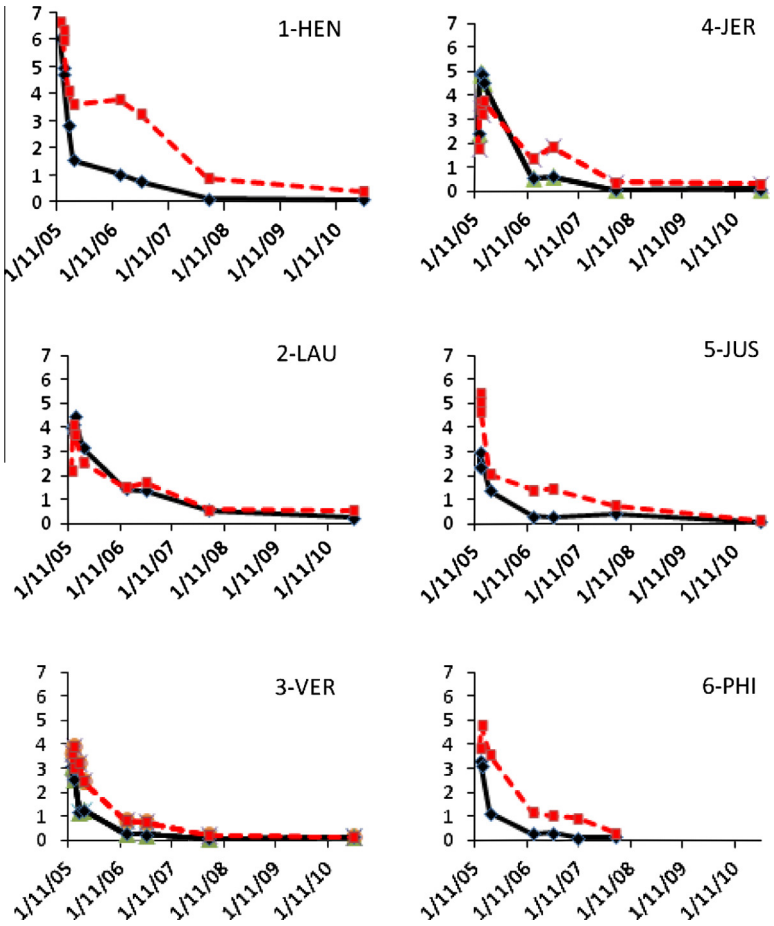

Fig. 1. Kinetics of *T. cruzi* antibody levels before and after treatment with benznidazole and nifurtimox established using two commercial ELISA tests: black solid line (Biokit), red dotted line (BioMerieux). For both tests, the reaction was considered positive for ratio values >1.00, negative for ratio values <0.9 (Biokit) and <0.8 (BioMerieux), and doubtful between positive and negative ratio values; these standards were according to the manufacturer's instructions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Serological and real-time PCR evaluation after treatment

Six of the eight patients received treatment with benznidazole first, which was generally well tolerated except for two patients (1-HEN and 4-JER) who developed a skin reaction such that the treatment was discontinued; a second treatment with nifurtimox was then administered to these two patients (Table 1). Annual serological posttreatment follow-ups were undertaken for the six patients between 2005 and 2011. The rates of *T. cruzi* antibodies measured by the two ELISA tests are presented in Fig. 1. For all patients and for both tests, the antibody levels decreased after treatment, reaching a negative serology for the two tests after 1 year for patient 3-VER, and about 2 years and 8 months for the others, except for patient 1-HEN whose therapy took longer than 5 years. In general, a negative antibody response was seen first with the Biokit test than with the BioMerieux test. The real-time PCR essays were performed for the six patients during their post-treatment from 14 December 2006 and then on the scheduled follow-up dates; all test results were negative except those for patient 1-HEN, whose real-time PCR results were positive on 14 December 2006 but negative on the following dates.

4. Discussion

Outbreaks of Chagas disease due to suspect oral contamination are increasingly reported in the Amazonian basin and in neighboring regions (Coura et al., 2002). Recent outbreaks in Brazil and in Venezuela (Rodriguez-Morales, 2008; Alarcon de Noya et al., 2010; Ianni and Mady, 2005) have incriminated contaminated palm fruit juice, cane juice, and guava juice. These cases of oral contamination are usually in the form of micro-epidemics in a group of individuals who have shared the same food source. Similarly, in the present work, we reported for the first time in French Guiana an outbreak of Chagas disease due to oral contamination identified among the members of a same family. The correct diagnosis was delayed and not easy to make. Indeed, in French Guiana, the medical professionals are not familiar with Chagas disease, and the clinical signs of fever, cephalalgia, and myalgia tend almost exclusively to suggest malaria or dengue fever.

Most of the current patients presented at least the following clinical signs: fever, myalgia, asthenia, and lower-limb edema; these signs in association with other have already been described in acute cases in the Amazon (Pinto et al., 2008). After ruling out other infectious diseases and after evaluating the epidemiological context, and because the clinical signs were suggestive of acute infection by *T. cruzi*, the medical staff examined a battery of biological tests to support the suspected diagnosis. The results of these tests are discussed below.

Microscopic examinations can lead to a strong suspicion of *T. cruzi* infection when detection is made, but *T. cruzi* was not directly detected in any of the eight patients. This result may be due to the delay between the presumed infection date (end of October) and the subsequent blood sampling, which was at least 24 days for patients 1–4 and longer for the others (Laranja et al.,

1956; Miller, 1931). The limit beyond which parasites cannot be readily detected by direct examination of blood has been classically the fourth week after infection (Hoare, 1972). Moreover, acute Chagas cases with negative results after microscopic examination despite typical severe clinical manifestations have already been reported in similar family outbreaks in the Brazilian Amazon (Pinto et al., 2008) and in Rio Grande do Sul (Guimãraes et al., 1968). Standardized real-time PCR-based detection of *T. cruzi* infection was made for all the patients, allowing for rapid decisions to be made regarding therapy. The current PCR system was adapted from a conventional PCR that targets the *T. cruzi* kDNA, but is also known for cross-matching with *T. rangeli* (Vallejo et al., 1999). Our TaqMan system can also detect some strains of *T. rangeli* that can possess minicircles whose sequence matches perfectly with our primers and probes (Recinos et al., 1994). As the real-time current system is promising for the diagnosis of *T. cruzi* infection, other DNA targets, such as mini-satellite or ribosomal DNA sequences, could be used (Piron et al., 2007). Hemoculture is a reference test for diagnosing *T. cruzi* infection, but it does not lead to a rapid diagnosis, making this approach inappropriate for prompt therapeutic decisions. In the present case, this test fully confirmed the parasite infections in four out of the eight cases. Blood culture is undoubtedly useful for confirming the specific diagnosis of *T. cruzi* through the genetic characterization of the strain when other trypanosome infections, such as *T. rangeli*, are possible. Moreover, cryopreservation of the strains can open ways for deeper investigations of the biology of these Amazonian strains.

The serological tests provided confirmation on the *T. cruzi* infection in all the patients and helped in the medical decision making.

The retrospective review of the current diagnostic process shows the importance of examining several biological tests, but also of evaluating the epidemiological context in which the patients are living. These data led clinicians to suspect contamination in the family via consumption of palm juice and to determine the approximate date of the contamination. This allowed the doctors to call the other family members to the hospital. The infection was then also diagnosed in these new patients and they received the appropriate treatment.

In the context of the epidemiological and clinical patterns strongly suggestive of acute Chagas disease due to oral contamination in patients of the same family, real-time PCR was a fundamental biological tool with which to immediately confirm the diagnosis and thereby avoid delay in patient management.

This study highlights the diagnostic weakness in detecting recent *T. cruzi* infection in the epidemiological and clinical contexts in French Guiana. Furthermore, despite many reports of cases of oral transmission in the Amazon and knowledge of Chagas disease in French Guiana since 1939 (Floch and Tasqué, 1941), there is still little clinical experience of the illness and some difficulties in diagnosing acute or chronic Chagas cases in this territory. This situation is not limited to French Guiana, but is likely more widespread in many regions of the Amazon. A hallmark of this study was that recent *T. cruzi* infections can be clinically very severe; the decision to treat the patients and the posttreatment monitoring showed a positive clinical progress of the majority of the patients as well as seroconversion, which is in agreement with a parasitological cure.

The several years of follow-up of the patients after their treatment allowed us to validate the first experience of Chagas treatment in recently infected patients in French Guiana. Seroconversion was observed in all patients, which is expected in acute infections unlike chronic cases, but it generally took more than 2 years (Rumi et al., 2013). Indeed, the treatment was not applied immediately after the infection (~4 weeks later), a delay that would allow the infection to become established and humoral immune response to be well developed. This monitoring also allows us to say that the progressive decrease in antibody levels would be a good sign of recovery.

Based on this report, there is an urgent need (a) to develop better information on epidemiological and clinical patterns of Chagas disease primarily for health staff but also (b) to better understand the factors associated with the risk of the transmission so as to act accordingly. The epidemiological situation is becoming better known in French Guyana. The presence of fourteen species of triatomine is now reported and the two principal species *Panstrongylus geniculatus* and *Rhodnius pictipes* are notorious potential vectors (Bérenger et al., 2009). We have known for several years that TcI circulates in the forest (Lewicka et al., 1995; Dereure et al., 2001) and recently, TcI and also *T. cruzi* belonging to TcIII–TcIV, infecting vectors and mammals, have been identified in various urban areas (Peneau et al., 2014). However, the ecoepidemiology of the principal vectors and the transmission risk situations are not yet known. The state of knowledge of the eco-epidemiological context and the transmission risk situations are clearly insufficient, but valuable to consider Chagas disease in French Guyana as a public health problem.

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