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

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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

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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