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CASE REPORT

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Trypanosoma cruzi DTU II coinfection with bacteria producing prolonged cutaneous lesion in a healthy young male

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ABSTRACT

Chagas disease (CD) is a neglected tropical disease caused by *Trypanosoma cruzi* and is genetically classified in six discrete typing units (DTUs). The isolates reported in Mexico are generally associated with DTU I. We presented a case of a prolonged cutaneous lesion in a Mexican man, caused by DTU II in coinfection with *Bacillus velezensis* and *Corynebacterium* sp. The patient assessment included a complete clinical history, physical exam, laboratory tests, and a skin biopsy. In the facial tissues, intracellular parasites were revealed. The PCR tests were positive for *T. cruzi* in tissue and blood samples. DNA satellite sequencing was correlated with the DTU II. The initial serological tests reported negative results. However, four months later, two serological tests reported positive results. These exams were performed in different health centers. Mexico is considered an endemic area for CD; nevertheless, this is just the second cutaneous case associated with a DTU different from DTU-I noted in this country. From an ecological point of view, this fact suggests a geographical expansion of DTU II and an association with atypical skin manifestations. Further studies should be conducted to understand this exciting association between DTU-II and prolonged cutaneous expression in humans.

KEYWORDS: *Trypanosoma cruzi.* Chagas disease. DTU II. Cutaneous infection.

INTRODUCTION

Chagas disease (CD) is a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi,* and is primarily transmitted by triatomine insects. Lately, *T. cruzi* was genetically classified by genetic background in six discrete typing units (DTUs)[1]. In 2012, Ramos-Ligonio *et al*.[2] reported for the first time the presence of TcII, TcIII, TcIV, and TcV genotypes in *Triatoma dimidiata*, from Veracruz state, Mexico (19°26'05"N 96°22'59"O). After that, other DTUs were also identified in Mexican wildlife. For example, *Canis lupus familiaris*, *Suis domesticus*, *Sigmodon hispidus*, *Artibeus lituratus,* and *Ateles geoffroyi* were reported to be infected with *T. cruzi* belonging to DTU-II, whereas DTU-III, DTU-V, and DTU-VI have been identified in *Alouatta palliata*, *Alouatta pigra,* and *Ateles geoffroyi*[2]. However, studies on the genetic classification of DTUs in humans are scarce, reporting only DTU-I[3]. Nevertheless, in 2019, our group reported the first case of infection by DTU-II in Mexico, which presented an atypical cutaneous disseminated disease in the acute phase without apparent immunosuppression[4]. In addition, we found a second case of cutaneous infection by DTU-II in a male Mexican patient, who was coinfected with *Bacillus velezensis* and *Corynebacterium* sp.

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CASE REPORT

A 27-year-old male sought the dermatology care service for a skin lesion located on the right mandible, characterized by increased volume, erythema, and fistulous tracts. The dermatosis comprised an area of 3x4 cm and exuded a slightly seropurulent secretion (Figure 1A). He claimed it had been evolving for more than five months and mentioned having undergone previous treatment with different topical and oral antibiotics, without improvement. He claimed to be a permanent resident in Mexico City (19°25'10"N 99°08'44"O), but he had made frequent business trips to Veracruz state (19°26'05"N 96°22'59"O) in the last year for periods of one to two weeks. The suspected clinical diagnoses were actinomycosis, cutaneous tuberculosis, or sporotrichosis. Thus, a skin biopsy, histopathological analysis, and culture from a tissue sample were performed. Also, a blood count and blood chemistry were requested.

Regarding histopathology, the hematoxylin and eosin stain exhibited an epidermal hyperplasia and ulceration. Tissue slices (Figure 1B) exhibited an inflammatory infiltrate composed of histiocytes, plasma cells, lymphocytes, and polymorphonuclear located in the dermis and subcutaneous tissue (Figure 1C). There was also vascular damage.

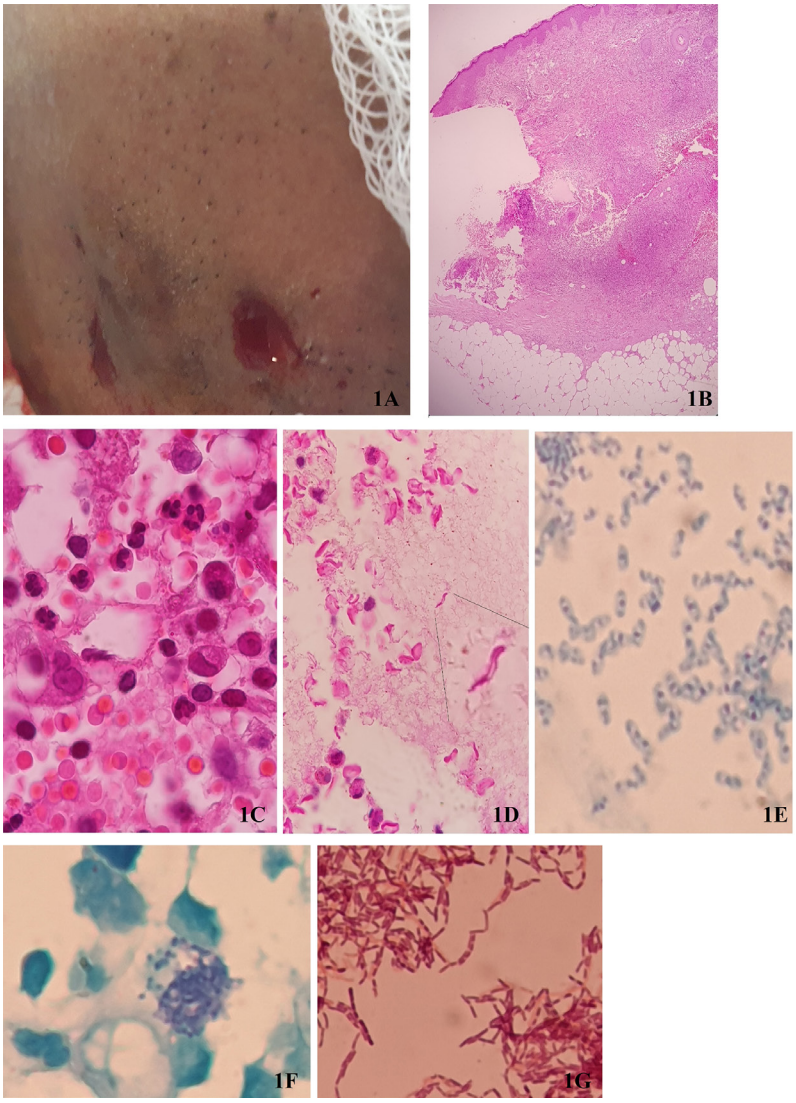

Figure 1 - 1A) Clinical picture. The right mandible showed edema, hyperpigmentation, ulcer, and seropurulent secretion; 1B) Histopathology [5X, hematoxylin-eosin (H&E) stain]. Lower magnification exhibits epidermal hyperplasia, fistulous tract, cutaneous ulcer, vascular damage, and inflammatory infiltrate dermis; 1C) (40X, PAS stain) High magnification showed inflammatory infiltrate composed of histiocytes, plasma cells, lymphocytes, and polymorphonuclear infiltrate with extravasated erythrocytes in dermis; 1D) (40X plus close-up H&E stain) Exhibits a trypomastigote in the deep dermis; 1E) [100X, Ziehl-Neelsen (ZN) stain] In dermis, observe multiple amastigotes that display nuclei and kinetoplasts; 1F) (100X, ZN stain) In fat tissue, observe intracellular bacteria clusters, ZN negative; 1G) (100x, Gram stain) A direct examination from the MacConkey culture obtained from tissue sample reveals gram-negative bacilli.

No fungal structure or mycobacteria was observed in special stains such as Gram, Ziehl-Neelsen, PAS [periodic acid-Schiff stain], and Giemsa. No fungal growth was observed in the culture media (Sabouraud Dextrose Agar with Cycloheximide and Chloramphenicol [Mycobiotic Agar], potato dextrose agar, and brain heart infusion). Surprisingly, parasites (amastigotes and trypomastigotes), as well as intracellular bacteria, were presented in the tissue samples (Figures 1D, 1E, and 1F). Furthermore, the bacterial culture analyses showed evidence for the growth of two different isolates. The former corresponded to a Gram-negative bacillus, Ziehl-Neelsen negative, and catalase-negative, identified by PCR and sequencing as *Bacillus velenzensis* (Figure 1G), and the second was identified as *Corynebacterium* sp. The antibiogram reported a *Bacillus* sp., sensitive to cephalothin, ciprofloxacin, fosfomycin, gentamicin, tetracycline and oxycline, resistant to nitrofurantoin and trimethoprim/sulfamethoxazole, and intermedium resistant to clindamycin, erythromycin, and penicillin. Moreover, culture media for fungi were evaluated weekly and did not present any growth during five weeks. Consequently, serological diagnoses were requested to confirm the diagnosis of Chagas disease or *Leishmania* sp. The initial tests, executed in 3 independent laboratories (blood bank laboratory [chemiluminescence architect Chagas kit (Abbott)], a research laboratory specialized in parasitic infections in our institution [ELISA home with the Ninoa strain as antigen][3,4], as well as an external private laboratory [chemiluminescence technique]), were reported as negative.

PCR analysis was done to identify *Leishmania* sp. and specific *Leishmania* species with DNA isolated from the tissue biopsies and blood; these tests showed negative results. However, a PCR established with the DNA satellite showed the presence of *Trypanosoma cruzi* DNA[4]. The sample sequence showed an identity of 99% with the *T. cruzi* ClBE07 clone (GenBank Nº AY520036). Furthermore, Bayesian phylogenetic analysis (Figure 2) supported the grouping of the sequence obtained inside *T. cruzi* DTUs and showed high posterior probability values (1.00) for DTU II. On the other hand, PCR-sequencing analysis to identify bacterial *16S* revealed the presence of *Bacillus amyloliquefaciens,* also known as *velezensis,* in the tissue samples and culture (GenBank ascension Nº ON737906).

Laboratory tests were requested, including blood count and chemistry, reporting neutrophilia and eosinophilia. Other parameters were within the average reference values.

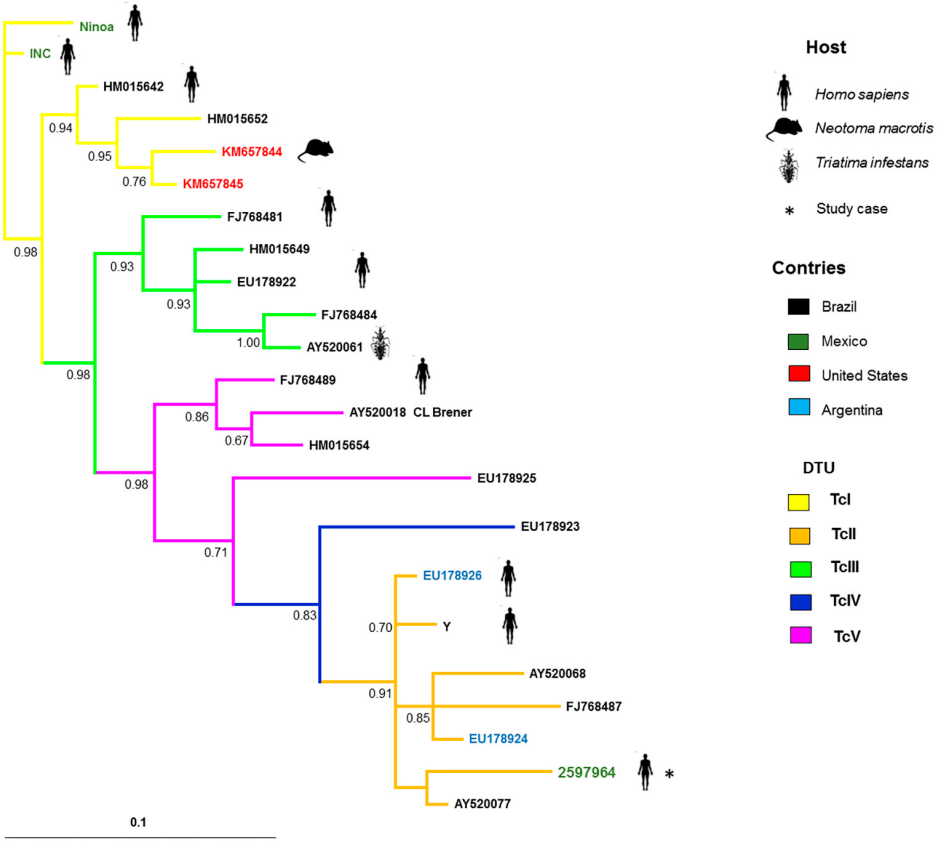

Figure 2 - The phylogenetic tree showed reference sequences from DTU I to DTU V, and the sequence from the present case belongs to DTU II group, and is located near to the sequence obtained from South America (Brazil and Argentina).

Also, a complete cardiac assessment was performed, including thorax radiography, an electrocardiogram, and an echocardiogram. All tests were reported within the standard values. In addition, two independent laboratories (public and private) repeated ELISA and Western blot during the patient follow-up, and the results were positive four months after the original assessment.

The initial reports established a mixed cutaneous infection; thus, according to the antibiogram report, the patient received standard doses of cefalexin and ciprofloxacin with clinical improvement in three weeks. However, according to PCR and histology results that established Chagas disease (CD), the patient was referred to the Tropical Medicine Center, an authorized center for the diagnosis and management of Chagas disease in Mexico[5], because the antiparasitic treatment for CD is not commercially available in Mexico. In addition, the CD is a mandatory report disease, because only special health authorities are able to provide the treatment (Secretaria de Salud). Sadly, current Mexican health care system lineages for Chagas treatment do not allow established treatment if the serology is negative, so for our patient, the treatment was delayed for several months, despite the parasite being visualized in tissue slices and the infection being confirmed with a PCR test[6]. Thus, the opportunity to treat the patient in the acute phase, when the benefit of treatment is more significant, was lost. Four months after the diagnosis, when the serology reported a positive result, treatment with nifurtimox was started, and doses of 240 mg at 8:00, and 180 mg at 15:00 and 22:00, were administered for 60 days. Afterwards, serology tests performed in August 2021 and January 2022 reported negative results. The last follow-up performed in July 2022 included a PCR and ELISA, which were also negative.

DISCUSSION

The DTU I is Mexico's most abundant and widely dispersed *T. cruzi* group[3]. It has been associated with sylvatic, peridomestic, and domestic cycles[7]. However, other DTUs, such as TcII/TcV/TcVI, have been less frequently identified in *Triatoma dimidiata* from the Veracruz state. Moreover, TcI, TcII, TcIII, TcV, and TcVI were isolates in different primates in Veracruz (19°26'05"N 96°22'59"O), Tabasco (17°58'20"N 92°35'20"O), and Campeche (18°50'11"N 90°24'12"O) states, whereas TcII was identified in domestic mammals and didelphis from Veracruz and Yucatan (20°50'00"N 89°00'00"O)[7,8]. López-Cancino *et al.*[9] mentioned DTU II isolates that were detected in two human cases of a study that mainly included wildlife, small mammals, and some domestic animals in the Yucatan Peninsula, but they do not mention the epidemiologic or clinical data of these patients. While in Baja California state, a 50-year-old female with disseminated cutaneous infection was diagnosed with DTU II *T. cruzi* infection[4].

In conclusion, in Mexico, the identification of DTUs is scarce, especially among infected humans (Table 1). Nevertheless, the data available support the existence of DTU II from Baja California (24°08'55"N 110°18'24"O, far north) to the Yucatan Peninsula (19°33'04"N 89°17'47"O, far south of the country), revealing the presence of other DTUs in vectors, animal hosts and humans from Mexico[4,9].

In the present case, the strain was identified as TcII. Thus, this patient represented the second case of human infection manifested as a prolonged cutaneous infection in Mexican subjects associated with a DTU different from DTU I. In the previous patient, ages, medications, and associated diseases could favor the multiple and prolonged cutaneous lesions[4]. On the contrary, in this case, the patient is a young man without a previously known condition, who denied having used any medication. Thus, we considered that possible factors for this rare clinical presentation could be the type of *T. cruzi* strain belonging to the TcII group, as well as the tissue presence of other microbes. In addition, *B. velezensis,* previously identified as plant-associated bacillus, has been reported in reptile cutaneous lesions[10-12]. According to our knowledge, this is the first time that *B. velezensis* has been related to human skin grazes.

As for treatments, there is no consensus in Mexico on the methods of diagnosis for acute and chronic Chagas diseases in maternity wards and blood banks, and the therapy with trypanocide is not administered to chronic patients[13]. Moreover, neither benznidazole nor nifurtimox (the two WHO-approved treatments for Chagas disease) are marketed in Mexico. They are not included in the national formulary and the institutional formularies, so the access to treatment is highly restricted[14].

Nowadays, only nifurtimox is occasionally available in Mexico[13]. Consequently, we considered that the concepts and systems that allow for the starting of treatment for CD must be reviewed and updated. Actual parameters do not refer to new diagnostic tools such as PCR or MALDI-TOF-mass spectrometry, which are highly sensitive and specific and could quickly establish a diagnosis in the acute phase[15,16]. In cases like this, the diagnosis was performed in the acute phase, but the nifurtimox treatment was delayed for several months. The antitrypanosomal therapy with benznidazole or nifurtimox prevents or delays the progression of chronic Chagas disease and increases quality-adjusted life expectancy, especially if the treatment is administered in the acute phase of the infection, which

Table 1 - Summary of *T. cruzi* DTUs, niche, host, vectors, and geographic distribution of Chagas diseases reported in Mexico and southern USA (Texas).

Article	DTU genotype	Niche	Host	Sylvatic vectors	Geographic distribution	Chagas diseases
This work	TcII	Domestic	Human	NR	Veracruz, Mexico	Localized Cutaneous lesion
Rangel-Gamboa *et al*.[4]	TcII	Domestic	Human	NR	Baja California, Mexico	Disseminated Cutaneous lesion
Ballinas-Verdugo *et al.*[3]	TcI	Domestic	Human	NR	Guerrero, Mexico	Chronic chagasic heart disease
Ruíz-Sánchez *et al*.[7]	TcI	Domestic	Human	NR	Veracruz, Mexico Nayarit, Mexico	Ventricular tachycardia, heart failure and cardiomegaly
Ramos-Ligonio *et al*.[2]	TcI, TcII, TcIII, TcIV, TcV	Domestic	*Triatoma dimidiata*	Triatomine	Veracruz, Mexico	NR
Murillo-Solano *et al*.[8]	TcI, TcII, TcV, TcVI	Domestic and peridomestic	*Homo sapiens, Canis lupus, Ovis aries, Rattus spp., Mus muscullus, Sciurus colliaei, Didelphis spp., Procyon lotor, Peromyscus spp., Bufo marinus, Columba livia, Gallus gallus*	Triatomine	Veracruz, Mexico	NR
López-Cancino *et al*.[9]	TcI, TcII , TcVI	Peridomestic and sylvatic	*Artibeus jamaicensis, Artibeus lituratus, Sturnira lilium, Sturnira ludovici, Dermanura phaeotis, Carollia brevicauda, Myotis keaysi, Heteromys gaumeri, Sigmodon hispidus.*	Triatomine	Yucatan Peninsula	NR
López-Cancino *et al.*[9]	TcII	Domestic and peridomestic	Human	Triatomine	Yucatan Peninsula	NR
Monteón *et al*.[19]	TcI	Domestic, Peridomestic and sylvatic	*Triatoma dimidiata*	Triatomine	Yucatan Peninsula	
Monteón *et al*.[19]	TcI	Domestic	Human	NR	Oaxaca, Mexico Nayarit, Mexico	Chagas disease
Garcia *et al*.[20]	TcI, TcII, TcV, TcVI	Domestic	Human	NR	Texas, USA	Chagas disease

NR = Not reported

may restrict infection in target organs of Chagas disease[17,18]. In addition, the treatment in the acute phase could reduce the adverse effects of treatment, consequently reducing the dropout rate[17].

CONCLUSIONS

The patient presented an acute Chagas disease, known as inoculation chagoma. This report supports the presence of DTU II in humans from Mexico, suggesting an expansion of DTU II from its original territory in South America. Changes in DTU distribution could be related to human and mammal migrations, climate changes, habitat destruction, and urbanization. Thus, understanding the population structure of *Trypanosoma cruzi* is critical to establishing the links to transmission cycles and disease. Therefore, we considered that this work emphasizes the importance of determining the infecting strain in humans to establish possible associations with clinical behavior and ecological distribution. It also provides evidence of the relevance of new diagnosis tools in establishing rapid and correct diagnoses.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

Written informed consent was obtained from the patient. All procedures followed the Regulations of the General Health Law in the Field of Health Research in Mexico: NOM-012-SSA3-2012, Title II, Chapter II, General Dispositions.

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