



CASE REPORT

Histopathological Damage in the Myocardium of Balb/C Mice of a *Trypanosoma cruzi* Isolate from an Endemic Area of Puebla, Mexico

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Abstract

Purpose: Chagasic cardiomyopathy is the most important clinical manifestation of the Chagas disease due the irreversible damage and the outcome it causes. The objective of this study is to report the behavior of a *T. cruzi* isolate from *Meccus pallidipennis* collected in Huatlatlauca, a municipality in the state of Puebla with a seroprevalence of 14.2%.

Methods: A total of 40 Balb/c mice were used, of which 20 formed the control group and 20 were inoculated with metacyclic trypomastigotes isolated from 20 adult specimens of *M. pallidipennis*, a parasitemia curve was made and the sacrifice of the animals of the experiment when a concentration of 1x10⁷ bloodstream trypomastigotes (BT) per field was found, histological sections of the excised hearts were obtained and analyzed with conventional microscopy.

Results: After 37 post infection days, the highest concentration of parasitemia was reported with an average of 10.57x10⁶ BT per field; 100% of the infected hearts presented diffuse histological lesions with a predominance of amastigote nests and inflammatory cells. The mortality rate was 35% in the models inoculated with *T. cruzi*, while the control group presented a parasitemia curve of zero and normal histological findings.

Conclusion: The carried out analysis concludes the possible circulation of TcI strains, given the characteristics of the histological studies that show a coincidence with the aspects described for this, which require definitive molecular studies.

Keywords: *Trypanosoma cruzi*; Chagas Disease; Chagasic Cardiomyopathy; Balb/c Mice; Huatlatlauca

Abbreviations: DTUs: Discrete Typing Units; MT: Metacyclic Trypomastigotes; BT: Bloodstream Trypomastigotes; LIT: Liver Infusion Tryptose; PB: Phosphate Buffer; H & E: Hematoxylin-Eosin; Pf: Per Field

Introduction

In México, it is estimated that between 1 to 2 million people are infected with *Trypanosoma cruzi*, and more than 6 million are at risk of contracting the American trypanosomiasis or Chagas disease [1]. Cardiovascular and digestive damage are the main manifestations of the disease, which occurs between 43% and 21% of the cases respectively, with chagasic cardiomyopathy being the most clinically important due the irreversible alterations it generates in the heart, where a dilated cardiomyopathy develops and can end with a sudden death or with a heart transplant [2-4]. Histopathological studies in the myocardium indicate that during the acute phase we can observe the presence of intracellular amastigote nests, which in most cases is followed by an acute inflammatory process; in the chronic phase it is characterized by the remodeling of the myocardium that depends on the severity of the infection, which is also related to the concentration of the inflammatory infiltrate that contributes to the destruction of cardiomyocytes and fibrosis, however, in this stage the visualization of amastigotes is less [3,4]. The virulence of the *T. cruzi* strains depends on different factors: the vector associated with the transmission, the dynamics of the parasite, the immunological state of the host, as well as the environmental conditions where it develops, which determine the severity of the disease [5,6].

T. cruzi is a species that presents a great genetic and phenotypic diversity, it is classified into 7 discrete typing units (DTUs), TcI-TcVI and one associated to TcBat bats, said diversity is believed to be a determining factor in its geographical distribution, transmission, tissue tropism, clinical manifestations and severity, response to treatment and prognosis [2,3,6]. TcI infections predominate in Mexico, while TcIII, TcV and TcVI are less frequent [7-10]. Vector-borne transmission is the main route of

infection in Puebla and in the rest of the states of the Mexican Republic, where more than 70% of the rural cases correspond to this route [11]. The *Meccus phyllosomus pallidipennis* (Stål 1872) and the *Triatoma dimidiata* (Latreille 1802) are the species with the greatest epidemiological importance, however, there are a few studies on the virulence and expression of the *T. cruzi* strains that they transmit [11-13]. The objective of this study is to report the behavior of the isolated strain from *M. pallidipennis* collected in an area with seropositive cases and a seroprevalence of 14.2% [1], other studies in Puebla have reported that TcI circulates in the state [14], an aspect that could be verified with the histopathological damage in the murine models.

Materials and Methods

Animals

a) **Insects:** we used 20 adult specimens of *M. pallidipennis* collected in the peridomicile area of Huatlatlauca, Puebla. They were fed bi-weekly with rabbit blood, according to the disposition and use of the insects. All were kept in jars for their breeding.

b) **Mice:** we used 40 Balb/c mice that were male, 8 to 10 weeks old, weighing between 20 and 26 grams, which were provided by the "Claude Bernard" bioterium of BUAP.). All were kept under the same light / dark conditions, temperature and relative humidity. Cyclophosphamide (1g injectable solution) was used for the immunosuppression of the 40 Balb/c Mice, administering 2 doses of 150 mg/kg of weight intraperitoneally on days 1 and 4 of the study [15].

The mice were divided into an experimental group consisting of 8 groups of 5 mice, 20 for infected cases (4 groups) and 20 for the control groups of each repetition (4 groups).

Experimental Design

It is a descriptive experimental study carried out from January to July 2020 in the facilities of the research laboratories of the Faculty of Biological Science of the Benemérita Universidad Autónoma de Puebla (BUAP) (Puebla, México), for which an analysis is performed of the histopathological damage in Balb/c mice with isolates of metacyclic trypomastigotes (MT) of the *Trypanosoma cruzi* obtained from triatomines. The droppings were obtained after each blood intake, they were checked between covers and slides in the search of MT, the positive droppings were immediately inoculated into 20 immunosuppressed Balb/c mice. To monitor the parasitemia, measuring curves were made on the development of the infection (Parasitemia Curves) for each case and it was decided to sacrifice the mice with previous cardiac puncture after identifying a concentration greater than 1×10^7 bloodstream trypomastigotes (BT) per field or when reaching the day 41 of the study, with an overdose of barbiturate (pentobarbital sodium 120-210 mg / kg / dose). The blood obtained was preserved in a LIT (Liver Infusion Trypouse).

Parasitemia Curve

The parasitemia curve was drawn up in the 8 groups by means of blood samples obtained after a small cut in the distal region of the tail of the mice, samples were collected on days 1, 5, 9, 13, 17, 21, 25, 29, 33, 37 and 41 post infection. In 1.5 mL microtubes, with a 100 μ L micropipette, 30 μ L of anticoagulant (EDTA 200 nM pH 7.4) and 100 μ L of blood was placed per mouse, then, with a 2 mL capacity Pasteur pipette, a sample was taken of the previous dilution and then placed a drop in the center of a 7.5 cm x 2.5 cm glass microscope slide, later, 12 μ L of PB 1x (phosphate buffer) (pH 7.4) is added with a 100 μ L micropipette at the same time as mixing, the sample is covered with a glass coverslip to be observed in a light microscope at 40x in search of BT. After identifying the parasite, it was counted in a Neubauer chamber with dilutions of 50 μ L of blood plus 450 μ L of PB 1x (pH 7.4) to obtain a 1:10 dilution.

Histopathological Study

A necropsy of the models was performed to obtain the heart which was fixed with 10% formalin for 24 hours. The already fixed samples were introduced into an automatic tissue processor and subsequently the hearts of the infected mice were embedded in paraffin, a wash was performed with H₂O, dehydration with different degrees of alcohol (70°, 90°, 96° y 100°) and clarification with xylol, then the tissues are placed in cassettes for biopsies and the inclusion in liquid paraffin is made to obtain the block, once it solidifies, it is proceeded to cut them through a manual rotary microtome to obtain 4 micron thick sections, then placed in warm water for its spreading and subsequent placement on glass microscope slides. Once they have dried, they are deparaffinized with xylol and later stained with H&E (hematoxylin-eosin). The observation of the sections was carried out with an optical microscope at 40x and 100x (immersion).

Bioethics

All the procedures of use in the study models were carried out in accordance with the ethical code for the handling of animals for experimentation (CICUAL) endorsed by the BUAP management of research and postgraduate studies.

Results

Parasitaemia Curve

The presence of bloodstream trypomastigotes (BT) in peripheral blood was detected starting from day 9 of post infection in the four groups of infected experimental animals, with variations from 3.7×10^5 BT per field (pf) to 5×10^5 BT pf, reaching the highest point of concentration on day 37 post infection, with levels between 10.1×10^6 BT pf and 11.2×10^6 BT pf. No parasites were observed in the control group. Figure 1 represents the growth pattern of the curve and average obtained from the BT count of both groups.

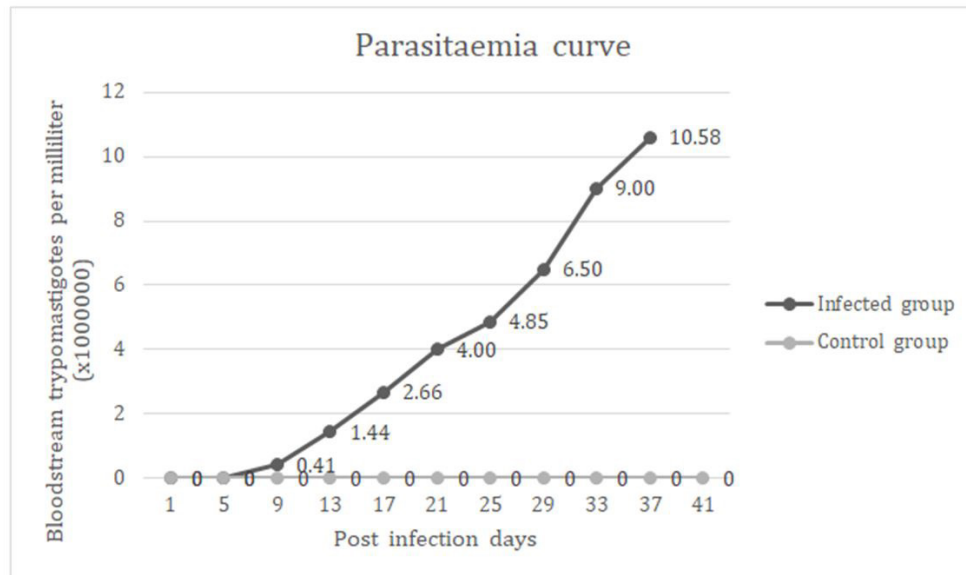


Figure 1: The Parasitaemia curve in the infected group of mice and in the control group

In the infected mice with *T. cruzi* there was a mortality rate of 35% that corresponds to 7 deaths between days 5 and 17 of post infection. In the animals in the control groups, no deaths were recorded.

Image Examination

Figure 2

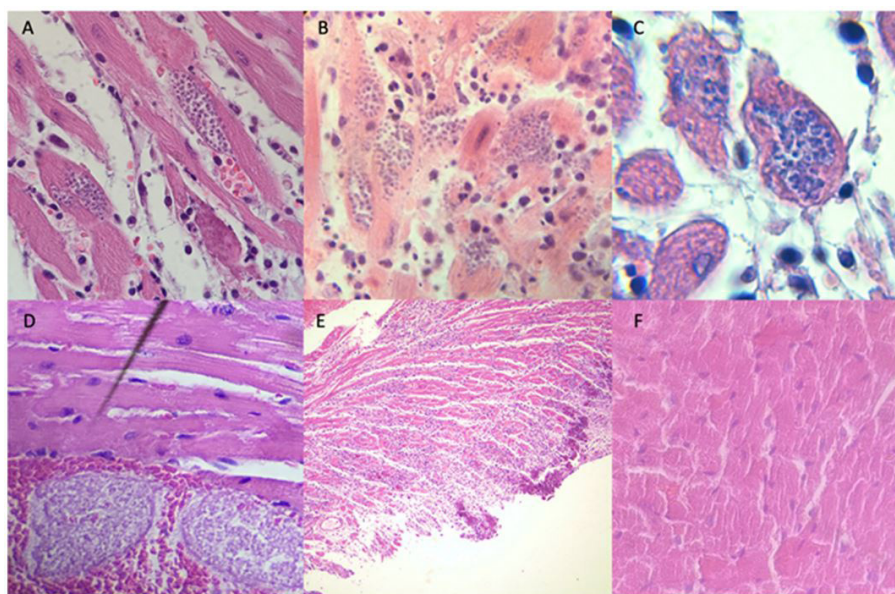


Figure 2: The 6 pictures correspond to histological sections obtained from the study: A, B and C correspond the obtained hearts after the sacrifice of the infected mice, while D and E correspond to the post mortem retrieved heart. (*) amastigote nests (+) mural thrombi. A, B and F are visualized at 40x, C and D at 100x (immersion) and E at 10x. H&E Staining

Histopathological Study

40 histological sections were made with the following findings:

After 37 days post infection, the hearts of the infected mice presented similar lesions in all repetitions, finding a diffuse histological damage, being more evident in the ventricles than in the atrium. In 100% of the cases, intracellular lesions with a pseudocystic appearance were observed, which correspond to amastigote nests. An average of 8 nests were quantified that presented a great variety of sizes and that in turn determined the conservation or loss of the integrity of the cardiac fibers, an inflammatory infiltrate was also identified with a predominance of mononuclear cells, showing a higher concentration of these around the lesions, other findings observed were the presence of hemorrhagic areas, vascular congestion and edema. In the hearts of the killed mice, the presence of amastigote nests, mural thrombi, abundant areas of necrosis and a higher concentration of inflammatory cells were also identified. In the control group, no lesions suggestive of infection by *T. cruzi* and histology with normal appearance were observed.

Discussion

Some authors recognize the forms of TcI in the acute phase by the serological values of the confirmed reported cases and with evidence of parasites in the blood [14,16]. In this case, when using the murine model, a rapid and abrupt growth of the strain that circulates in Huatlatlauca is detected, where, according to the published data, a high seroprevalence (14.2%) is observed even in the child population [1]. This area of the state corresponds geographically to Palmar de Bravo, where serological studies carried out in an open population provided a lower prevalence figure [17] and the same rates of infestation and infection were not found despite the existence of intra domiciliar triatomines such as the *Triatoma barberi* and the *M. pallidipennis*, species that have been considered important in the transmission of the parasite in Mexico. The mortality rate of mice in a relatively short period of time could be reporting the characteristics of the infection in the acute phase, an important reference to draw up strategies for serological studies in the susceptible child population and to complete those already started in the municipality [1]. The studies carried out on the histopathological damage in the murine model with the strains of *T. cruzi* can, together with the molecular studies, provide information on the clinical behavior in confirmed cases. In these cases, patients with arrhythmias and other pathologies and other pathologies suggestive of the disease were reported [18].

With the histological findings presented, we could suppose that myocardial damage is due the circulation of TcI strains, which are known to have tropism in cardiac cells [4,6], and that are also supported thanks to the publications made with Mexican strains that report predominance of this lineage in the state of Puebla and in Mexico [10,16,19]. The myocardial damage observed in this study is similar to the one obtained in a study carried out in Molcaxac, where the alterations described here could be seen in the model [16], however, the severity of the findings is much more evident in this study, the same could explain the difference in the percentage of mortality. Other aspects that may condition the differences are: the species of the vector collected and the state of immunosuppression induced in the experimental animals, since this condition favors the replication of the parasite as well as the natural evolution of the infection. Study carried out with Mexican strains of TcI inoculated orally in mice, reports low levels of parasitaemia, histological damage and mortality compared to our results, emphasizing the importance of the inoculation route, the vector and the strain in the development and manifestation of the disease [19].

Conclusion

The analysis carried out concludes the possible circulation of TcI strains, given the characteristics of the histological studies that show a coincidence with the aspects described for this, which require definitive molecular studies.

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