THE ULTRASTRUCTURE OF A *TRYPANOSOMA VIVAX* ISOLATE ADAPTED TO LABORATORY RODENTS.

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Abstract
The ultrastructural morphology of a rodent-adapted isolate of *Trypanosoma vivax* was carried out. The subcellular analysis shows representative characteristics of the eukaryotic cell. In addition, demonstrates special features peculiar of the trypanosomes. The interaction trypomastigote-macrophage is described, indicating possible autoimmune reactions induced by the parasite. This report is the first done with a murine-adapted isolate of *T. vivax* in Venezuela.
Key words: *Trypanosoma vivax*, ultrastructure.

Resumen
Este trabajo describe la morfología ultraestructural de un aislado de *Trypanosoma vivax* adaptado a roedores. El análisis subcelular muestra características representativas de la célula eucariota. Además, evidencia peculiaridades propias de los tripanosomas. Se describe la interacción macrófago-tripomastigote, cuyas características indicarían reacciones autoinmunes inducidas por el parásito. Este es el primer trabajo realizado en Venezuela con un aislado de *T. vivax* adaptado a murinos.
Palabras calve: *Trypanosoma vivax*, ultraestructura.

Introduction

Trypanosoma vivax is the etiological agent of the bovine trypanosomosis ("cacho hueco" or "huequera" in vernacular language). The disease, widely distributed in the Venezuelan livestock zones, is characterized by anemia, wasting and reproductive function loss. Such clinical picture causes diminution in livestock production, increments in production costs by veterinary assistance and medicaments, as well as animal death. Therefore, detailed studies, including the morphological description of isolates able to infect laboratory hosts [1–3], are imperative and could be useful, as a datum point, in future studies with Venezuelan stocks of this trypanosome. This work describes the ultrastructure of *T. vivax* trypomastigotes in an easy to use experimental murine model.

Material and methods

The infective material, *T. vivax* TVFG12 isolated in French Guyana and adapted to rodents, was i.p. injected in Sprague-Dawley rats to an inoculum of 10^2 trypomastigotes per rat. When the parasitemia reached 10^4-10^5 trypomastigotes/ml, the rats were bled and the trypanosomes purified by anionic exchange through a DEAE-cellulose chromatographic column.

The eluates containing parasites were diluted in glucose-phosphate buffer. The samples were fixed in Karnovsky solution (2.5% glutaraldehyde, 37% formaldehyde and Millonig's phosphate buffer to pH 7.4 and 320 mOsm). Washes were done with Millonig's phosphate buffer and post-fixation with 1% osmium tetroxide. Immediately after, samples were soaked in distilled water and dehydrated with an ethanol series of increasing concentrations [4]. Subsequently, the samples were infiltrated with

propylene oxide and included in epoxy resin. Ultrathin sections (60-90 nm) were contrasted with uranyl acetate [4] and lead citrate [5].

The sections were examined with a Jeol JEM-1011 electron microscope (80kV) and the permanent registers were done in 8.3×10.2 cm negatives.

Results and discussion

The micrographs show the typical ultrastructural organization of the eukaryotic cell. Nucleus, mitochondrial profiles and kinetoplast, rough endoplasmic reticulum, polysomes, electron-dense granules 0.2-0.3 μm in diameter, and autophagic vacuoles were obvious. In addition, typical ultrastructural characteristics of the Trypanosomatidae, as microtubular subpellicular corset, flagellar pocket lacking of microtubular support, and paraxial rod of the flagellum, were also evident (Figure 1).

The absence of microtubular subpellicular corset in the flagellar pocket region agrees with the inter-membranous fusion processes of endo and exocytosis occurring in the zone [6]. By its part, the great amount of autophagic vacuoles suggests high autolytic activity; in other systems such characteristic correspond to Ca^{++} dependant phenomena related to aging [7].

The electron-dense granules could represent acidocalcisomes [8,9]. These are highly specialized organelles involved in cation and polyphosphate storage, as well as in trypanosomes stress adaptative mechanisms [10,11].

T. vivax trypomastigotes interact with macrophages [12–14]; our results show such interaction too (Figures 2A and Figure 2B). Alike relations in our experimental model could indicate an activation of the mononuclear phagocytic system due to increments of the population density of the circulating trypomastigotes [15,16]. On the other hand, macrophage mitochondrial degeneration suggests induced damage by other phagocytes. So, the existence of autoimmune pathologic manifestations due to the parasite, have to be considered. The literature reports different inmmunopathogenic alterations in other trypanosomes [15,17,18], suggesting that accumulation of certain parasite metabolites could alter tissues enough to unleash auto-antigenicity so resulting auto-antibodies could react against modified antigens on the host cells.

In this order of ideas, certain trypanosome antigens (heteropolysaccharides and glycoproteins) could be similar to those present on the infected host cells, inducting anti-trypanosome specific antibodies that could show cross reactivity with the host's cell and tissue autoantigens; this phenomenon is known as molecular mimicry [16].

Finally, we do not rule out a contaminative origin for autoimmunity, since trypanosome cytoadherence would "contaminate" blood cells and other host's tissues. Exogenous antigens could be picked up by host cells being, in consequence, possible targets for the own immune system.

Conclusions

The ultrastructural analysis of the isolate TVFG12 of *T. vivax*, as well as having characteristics of the eukaryotic cell and show the subcellular particularities of the Trypanosomatidae, exhibited autolysis signs and host macrophages degeneration. This report is the first done with a murine-adapted isolate of *T. vivax* in Venezuela.

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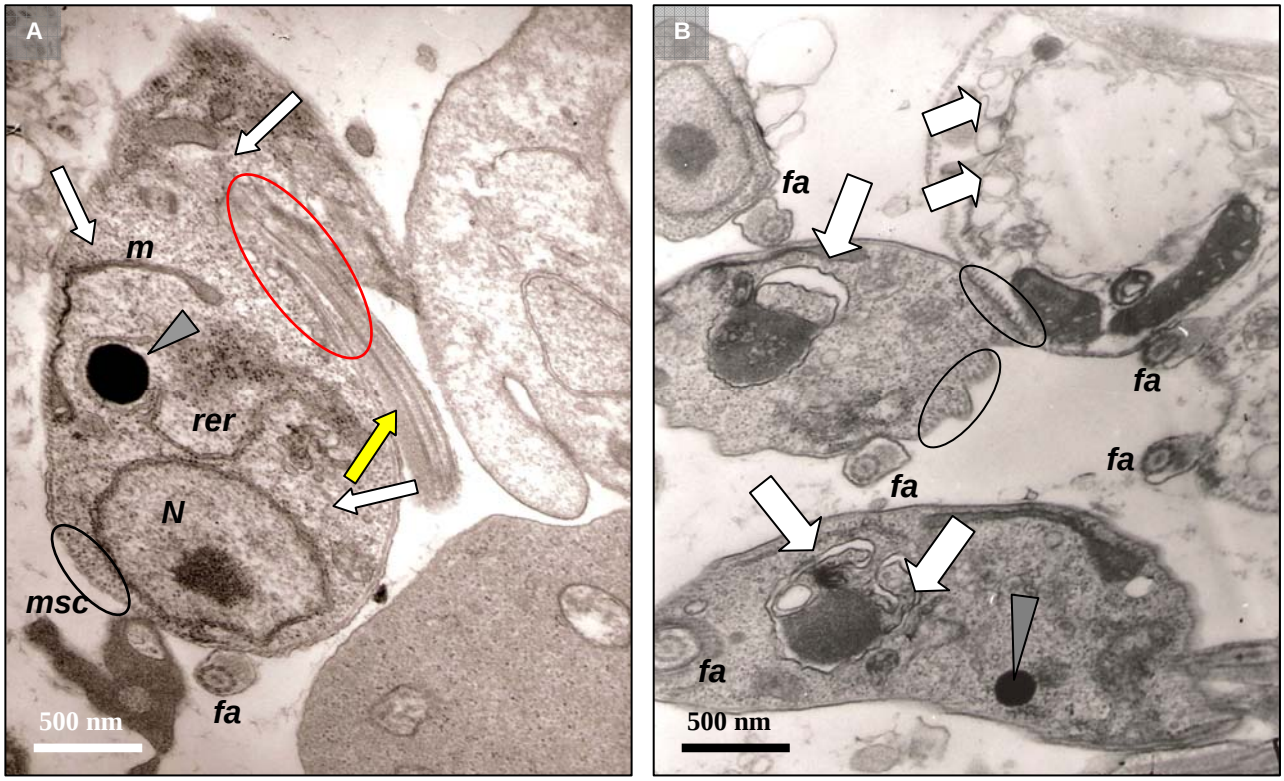

Fig. 1. Ultrastructural details of the trypomastigotes of the *Trypanosoma vivax* TVFG12 isolate. A-B. Microtubular subpellicular corset (msc and black ovals), nucleus (N), sections of the mitochondria (m), flagellar pocket (red oval), flagellar axoneme (fa), flagellum paraxial rod (yellow arrow), rough endoplasmic reticulum (rer), multiple ribosomes (white arrow), electron-dense granules (grey arrowhead), and numerous autophagic vacuoles (wide white arrows).

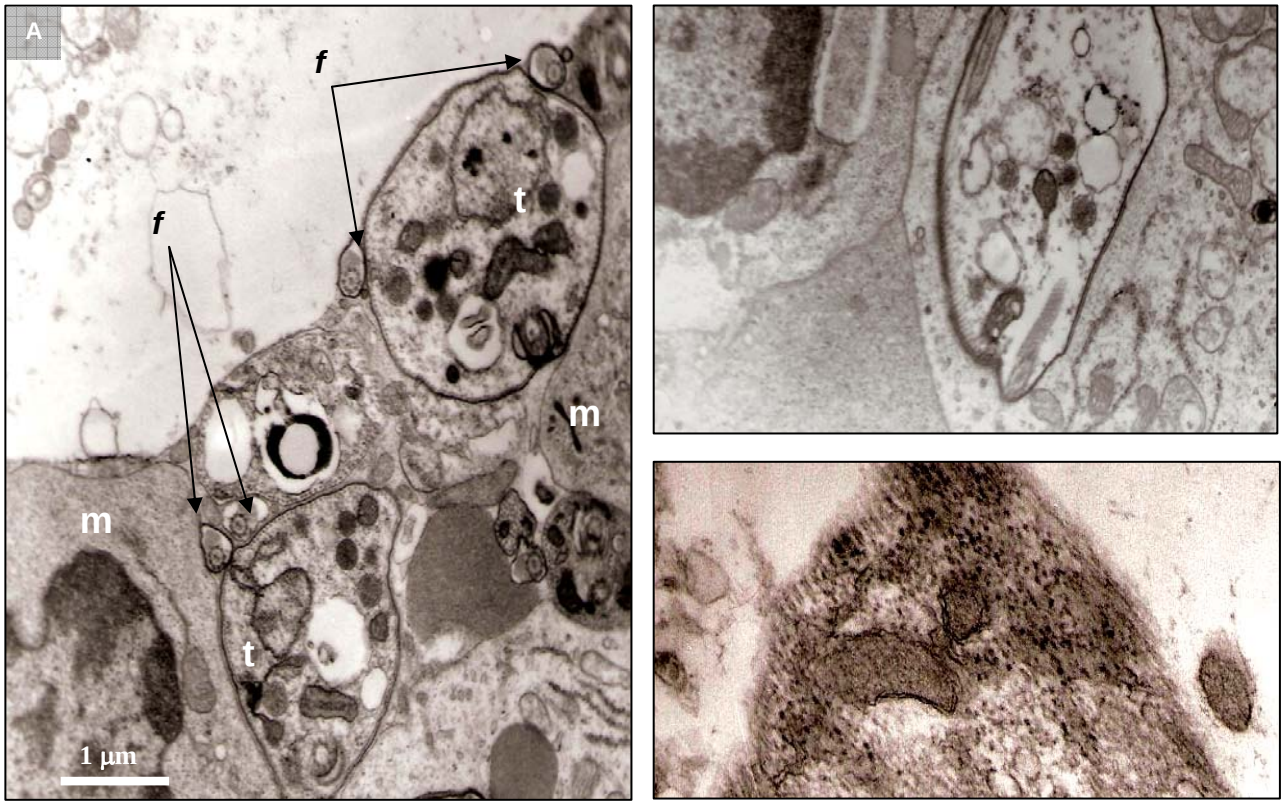

Fig. 2. *Trypanosoma vivax* trypomastigote-macrophage interaction. A. Trypomastigotes (t), macrophages (m), and parasite's flagella (f). B. The trypomastigotes show signs of degeneration (white arrows). The macrophages exhibited ring-like mitochondria (white arrow head). C. Transversal section of the trypomastigote's kinetoplast (red oval).

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