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Review

The revised *Trypanosoma cruzi* subspecific nomenclature: Rationale, epidemiological relevance and research applications

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ABSTRACT

The protozoan *Trypanosoma cruzi*, its mammalian reservoirs, and vectors have existed in nature for millions of years. The human infection, named Chagas disease, is a major public health problem for Latin America. *T. cruzi* is genetically highly diverse and the understanding of the population structure of this parasite is critical because of the links to transmission cycles and disease. At present, *T. cruzi* is partitioned into six discrete typing units (DTUs), TcI–TcVI. Here we focus on the current status of taxonomy-related areas such as population structure, phylogeographical and eco-epidemiological features, and the correlation of DTU with natural and experimental infection. We also summarize methods for DTU genotyping, available for widespread use in endemic areas. For the immediate future multilocus sequence typing is likely to be the gold standard for population studies. We conclude that greater advances in our knowledge on pathogenic and epidemiological features of these parasites are expected in the coming decade through the comparative analysis of the genomes from isolates of various DTUs.

Contents

Abbreviations: ITS1 rDNA, internal transcribed spacer 1 of rDNA; MLEE, multilocus enzyme electrophoresis; MLST, multilocus sequence typing; NTS, non-transcribed spacer; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNPs, single-nucleotide polymorphisms; SL, spliced leader; SL-IR, spliced leader intergenic sequence.

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1. Introduction

Infection with *Trypanosoma cruzi* is a complex zoonosis, transmitted by many hematophagous triatomine species and sustained by over 70 genera of mammalian reservoir hosts. *T. cruzi* has a broad endemic range that extends from the Southern United States to Argentinean Patagonia. The human infection, named Chagas disease in recognition of Carlos Chagas who first discovered American trypanosomiasis in 1909, is found mostly in South and Central America, primarily affects poor rural populations, and is considered to be the most important parasitic infection in Latin America with serious consequences for public health and national economies.

The spectrum of pathological outcomes associated with acute and chronic Chagas disease ranges from subclinical infection through the cardiac and digestive syndromes to death. Specific outcomes may be determined by a variety of non-exclusive factors including parasite genetics, host genetics, mixed infections, and cultural and geographical factors (Macedo et al., 2002, 2004; Buscaglia and Di Noia, 2003; Campbell et al., 2004).

The diversity of the *T. cruzi* genome and multiplicity of its genotypes and phenotypes is well recognized (Dvorak et al., 1982; Barnabé et al., 2000; Brisse et al., 2000; Devera et al., 2003; Lewis et al., 2009a). Designation of ecologically and epidemiologically relevant groups for *T. cruzi* has oscillated between a few discrete groups (Miles and Cibulskis, 1986; Souto and Zingales, 1993; Souto et al., 1996; Zingales et al., 1999) and many (Tibayrenc and Ayala, 1988). Currently, six discrete typing units (DTUs) are assigned (Brisse et al., 2000). In 2009, these DTUs were renamed by consensus as TcI–TcVI (Zingales et al., 2009). Several reviews already describe how these DTUs correspond with former nomenclatures and with prospective biological and host associations (Campbell et al., 2004; Miles et al., 2009; Sturm and Campbell, 2010; Zingales et al., 2009).

The aim of this review is to explain further the rationale for naming TcI–TcVI, with reference to their known molecular genetics, eco-epidemiological features and pathogenicity. We also summarize methods for DTU genotyping, and discuss a possible seventh *T. cruzi* branch, provisionally named Tcbat. An understanding of the *T. cruzi* DTUs and their epidemiological implications will provide new insights to guide research and future interventions against this devastating infectious disease.

2. The concept of discrete typing unit

Since the late 1970s, *T. cruzi* has become one of the models for molecular epidemiologists and population geneticists, and consequently this protozoan parasite is a pathogenic agent for which evolution and population structure are among the best studied, although not necessarily the best understood. The emerging picture is that of a typical pattern of reticulate evolution, similar to that of many plant species (Avise, 2004).

The concepts of DTUs and clonal evolution have been designed within the framework of evolutionary research on *T. cruzi*. Tibayrenc and co-workers devised descriptive concepts and terminology to make such research and its implications accessible to non-specialists, including medical professionals and epidemiologists, and to bypass certain demands of classical evolutionary biology definitions. Classical cladistic and population genetics approaches imperfectly depict the biological realities of the evolution of pathogenic microorganisms.

2.1. The clonal model of evolution in T. cruzi

In the framework of this model, a "clonal species" refers to all cases where descendant multilocus genotypes are virtually identical to the founding genotype. The main parameter focused on in this scenario is the inhibition of genetic recombination. The term "clone" in this context refers to the population structure of the species under study, not to its precise mating system. Different methods of propagation can generate genetic clones, including classical cell division, several cases of parthenogenesis and gynogenesis. Following this definition (Tibayrenc et al., 1990), selfing and extreme inbreeding are not alternative hypotheses to clonality (Rougeron et al., 2009), but rather a particular case of it. Selfing refers to mating between identical genotypes, which can be issued from the same clone (Tibayrenc et al., 2010). Extreme inbreeding refers to mating between extremely similar genotypes. The result is a lack or extreme limitation of genetic recombination, hence genetic clonality.

Stating that *T. cruzi* is a basically clonal species means neither that recombination is totally absent in the parasite's natural populations, nor that it does not have an impact on the evolutionary scale, but rather that it is too rare to break the prevalent pattern of clonality. The potential for genetic exchange is still present (Gaunt et al., 2003). Moreover, some localized transmission cycles suggest that genetic recombination does occur within DTUs of *T. cruzi* (Carranza et al., 2009; Ocaña-Mayorga et al., 2010). The possibility of limited genetic exchange between DTUs is also under debate (Lewis et al., 2011). However, the species *T. cruzi* considered as a whole shows all the signs for a typical clonal population structure: departures from panmictic expectations, strong linkage disequilibrium (non-random association of genotypes at different loci) within and especially between DTUs, and division into discrete genetic clusters (see DTUs, below).

2.2. Discrete typing units and clonets

Often the genetic subdivisions identified by evolutionary studies in pathogen species do not fulfill the criteria demanded by rigorous cladistic analysis. The main reason is that even in predominantly clonal species such as *T. cruzi* there is a certain amount of genetic recombination that clouds the distinction of