Veterinary Parasitology: Regional Studies and Reports 13 (2018) 205–211

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

Infectivity and virulence of *Trypanosoma evansi* and *Trypanosoma equiperdum* Venezuelan strains from three different host species

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ARTICLE INFO

Keywords:
Parasitemia
T. evansi
T. equiperdum
Virulence
Pathogenicity
Anemia

ABSTRACT

The infectivity and virulence of seven *Trypanosoma evansi* and *Trypanosoma equiperdum* Venezuelan strains isolated from horses, donkeys and capybaras were compared in a mouse model up to 41 days, for parasitemia, animal weight, survival rates, packed cell volume, haemoglobin and erythrocyte count. Two *T. equiperdum* strains and three of the *T. evansi* strains resulted in 100% mice mortality, while the two *T. evansi* donkey strains exhibited lower infectivity and mortality. *T. equiperdum* strains had shorter pre-patent periods (4 days) than the *T. evansi* strains (4–12 days). In terms of pathogenicity, only the *T. evansi* horse strain and the two capybara strains produced a significant decrease of the packed cell volume, in haemoglobin concentration and in red blood cell count. In contrast, the *T. evansi* donkey strains did not show any changes in the hematological parameters. From the seven variables studied, only pre-patent period, day of maximum parasitemia, day of first parasitemia peak and number of parasitemia peaks were statistically significant. Weight decrease was only observed in mice infected with the *T. evansi* horse strain. *T. equiperdum* strains showed the highest mice lethality (7% survival by day 8 post-infection) with no change in the hematological parameters. The three *T. evansi* horse and capybara strains showed 80%, 87% and 97% survival rates, respectively by day 12 post-infection. However, by day 20 post-inoculation all the mice infected with the *T. evansi* horse strain died, while 53% and 27% capybara strains infected survived. Whereas by day 40 post-infection, 53 and 73% of the mice infected with the *T. evansi* donkey strains had survived. These results demonstrate striking infectivity and virulence differences between Venezuelan *T. evansi* and *T. equiperdum* strains in NMRI mice and open new possibilities to characterize inter and intra-species variations that may contribute to the pathogenicity of these two species.

1. Introduction

Trypanosoma evansi can infect a wide range of animal species, including horses, donkeys, capybaras, camels, buffaloes, goats, sheep, dogs and others and is spread by mechanical transmission by *Tabanus* and *Stomoxys* spp. biting flies (Lun and Desser, 1995; Sumba et al., 1998; Reid, 2002). This parasite has a wide geographical distribution, mainly in tropical and subtropical regions of Northern Africa, Southeast Asia, Central and South America. The trypanosomosis caused by *T. evansi*, is a multispecies, polymorphic disease (Desquesnes et al., 2013a) and is responsible for outbreaks with high mortality, morbidity and great economic impact. The disease is known as “Derrengadera” or “mal de cadeiras” (Hoare, 1972; Levine, 1983; Luckins and Dwinger, 2004; Rodrigues et al., 2005; Desquesnes et al., 2013b). It is currently thought that *T. evansi* evolved from a *T. brucei* ancestor on several occasions, through the loss of mitochondrial DNA minicircle heterogeneity and the complete loss of the of kinetoplastic mitochondrial DNA maxicircles (Lai et al., 2008; Wei et al., 2011; Silva-Iturriza et al., 2013; Carnes et al., 2015). In Venezuela, *T. evansi* was first described (Guárico State) during a disease outbreak (Rangel, 1905). It was initially named *T. venezuelense* (Mesnil, 1910), but later recognized as *T. evansi* (Lavier, 1929; Hoare, 1956). However, according to Llamozas (1856) in (Diaz Ungría, 1960), the disease (equine trypanosomosis) was unknown until 1826 when it was recognized in Apure State, without any evidence of

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https://doi.org/10.1016/j.vprsr.2018.07.004
Received 4 March 2018; Received in revised form 30 May 2018; Accepted 6 July 2018
Available online 09 July 2018

blood parasites. Horses and donkeys are considered highly susceptible to *T. evansi* infections in the Venezuelan plains, while capybaras are considered asymptomatic hosts or reservoirs (Clarkson, 1976; Reverón, 1992; García et al., 2000).

T. equiperdum is the causative agent of Dourine or "Mal du Coit", a sexually transmitted disease that affects equids, characterized by white patches in the genitalia (OIE Terrestrial Manual, 2013; Claes et al., 2005; CFSPH and IICAB, 2009; Scacchia et al., 2011; Wei et al., 2011). Dourine is endemic in many regions of Asia and Africa, outbreaks have been reported in the Middle East and in Europe (Zablotskij et al., 2003; Claes et al., 2005; Gari et al., 2010; Hagos et al., 2010; Scacchia et al., 2011; Calistri et al., 2013; Vulpiani et al., 2013). There have been sporadic reports in America, including Canada (1921), USA (1934) and Mexico (1973) (OIE World Organisation for Animal Health). In Venezuela, the presence of Dourine was first documented in a domestic horse with dourine-plaques, however, no parasites were detected (Rivas Larralde, 1939), and the presence of *T. equiperdum* was not confirmed in Venezuela, and Latin America until 2015 when Sanchez et al. reported Venezuelan *T. equiperdum* strains (TeAp-N/D1 and TeGu-N/D1) based on genotyping by Coinertia analysis of microsatellite data and Procyclin PE repeats and the phylogenetic analysis of maxicircle sequences (Sánchez et al., 2015a, 2015b), which are not present in *T. evansi*.

Genomic and phylogenetic analyses has resulted in the classification of *T. evansi* and *T. equiperdum* strains in four different groups corresponding to independent origins from genetically distinct *T. brucei* strains (Carnes et al., 2015). The existence of *T. equiperdum* as a separate distinct species has been questioned for over a decade (Claes et al., 2005; Lai et al., 2008). Support for the reclassification of *T. evansi* and *T. equiperdum* as *T. brucei* subspecies derives from the comparative genomic and phylogenetic analysis of the akinetoplastic *T. evansi* STIB805 strain (Carnes et al., 2015). The first-draft genome sequence of the *T. equiperdum* OVI strain (South-Africa) has also been reported (Hébert et al., 2017). These genomic analyses constitute valuable tools for the classification of the *Trypanozoon* subgenus.

Hematological parameters and parasitemia levels have been used to describe the degree of virulence and pathogenicity of *Trypanosoma* spp. isolates and strains in natural or experimental infections (Mekata et al., 2013; Motloang et al., 2014; Gitonga et al., 2017). In the literature, human and animal trypanosome infections are classified as hyperacute, acute, subacute and chronic (Anosa, 1988; Spickler et al., 2010). Hematocrit or packed cell volume, haemoglobin and erythrocyte count are considered important parameters of infectivity and virulence of trypanosome strains that correlate with the physiological condition of the infected host (Hoare, 1972). The aim of the present study was to compare the infectivity and virulence patterns of seven Venezuelan trypanosoma strains (heterogeneous populations) – five *T. evansi* and two *T. equiperdum* – isolated from naturally infected horses, donkeys and capybaras, using a murine model. A complementary study of infectivity and virulence was performed using clones (homogeneous populations) derived from three strains used in the present study. (Perrone et al., 2018). This is an essential step to further typify these Venezuelan strains and to unravel the complex mechanisms underlying infectivity and virulence in *T. evansi* and *T. equiperdum*.

2. Materials and methods

2.1. Trypanosoma strains and experimental infections

Five *T. evansi* and two *T. equiperdum* Venezuelan strains were obtained from naturally infected hosts (Perrone et al., 2009; Sánchez et al., 2015a, 2015b), expanded in Sprague–Dawley rats and cryopreserved in liquid nitrogen using phosphate buffer saline with 1% glucose (PBSG) and 5% dimethyl sulphoxide (DMSO). The TeAp-Cedral12 and TeAp-ElFrío01 strains were isolated from capybaras (*Hydrochoerus hydrochaeris*), TeAp-Mantecal01, TeAp-N/D1 and TeGu-N/D1 were isolated from horses (*Equus caballus*), while TeGu-Terecay01 and TeGu-Terecay323 were obtained from donkeys (*Equus asinus*). The cryopreserved *T. evansi* and *T. equiperdum* strains were reactivated by intra peritoneal (IP) inoculation into individual NMRI (Naval Medical Research Institute) mice. When the parasitemia reached approximately 1×10^5 trypanosomes/mL, the mice were sacrificed and 500 µL of blood were taken in the presence of 0.14% EDTA and diluted in PBSG to a final concentration of 200 parasites/mL.

Mice were inoculated intra-dermally at the base of the skull with 1–2 parasites/g of body weight, suspended in 50 µL of PBSG. Thirty mice were inoculated with each strain and divided into three cages with 10 mice. Mice in the control group were injected identically with hemoflagellates-free PBSG (Perrone et al., 2006). All strains had been maintained in the laboratory for more than 10 years with multiple passages.

2.2. Variables studied

Prior to inoculation (day 0) and then on every other day, 3 of the 10 mice in each cage, were randomly selected, for a total of 9 mice inoculated with each strain at each time point. Parasitemia, packed cell volume (PCV), haemoglobin (Hb), erythrocyte count (RBC) and animal weight were determined. These parameters were followed for 41 days or until the animals succumbed to the infection. Animal weight was measured on a Sartorius® balance and survival rates (%) were calculated as the percentage of mice that remained alive in the infected groups. Parasitemia was calculated (Brenner, 1962) and PCV determined by the microhematocrit method (Woo, 1969). The number of RBC (erythrocytes/mm^3) was counted-using a Neubauer chamber. The cyanmethaemoglobin technique (Van Kampen and Zjlstra, 1965) was used to determine the Hb concentration expressed as g/dL.

2.3. Statistical analysis

The statistical analysis was performed between mice inoculated with the same strain (no difference between cages were considered). The normality and homogeneity of variance of the hematological data were assessed for the experimental infections and the control group. The parametric, one-way Analysis of Variance (ANOVA) and Newman-Keuls Multiple Comparison post-test were used to analyze and compare the arithmetic means of the hematological data in the interval between the first peak of parasitemia and the end of the assay. Multivariate Analysis of Variance (MANOVA) was performed to evaluate differences among three groups of parasite strains: *T. evansi* (TeGu-Terecay01 and TeGu-Terecay323), *T. evansi* (TeApElFrío01, TeAp-Cedral12 and TeAp-Mantecal01) and *T. equiperdum* (TeAp-N/D1 and TeGu-N/D1) and to determine the significance of the variables that contribute to the behavior of the pathogenic strains. The mean and the standard deviation of hematological and parasitological variables were standardized individually to ensure normal distribution and to make residual variables comparable using the following equation:

$$X_T = \frac{(X_0 - \overline{X_i})}{S_i^2} \quad (1)$$

where X_T is the value of the transformed variable, X_0 is the value of the initial variable, X_i is the mean of the variable i values and s_i^2 is the standard deviation of variable i.

A Canonical Analysis of Populations (CAP) was performed to determine the relationships between significant scaled-variables and pathogen groups, to understand differences between them and determine how the variables correlate to a particular group more than to another. CAP is a methodology based on the Mahalanobis distance, which uses a linear combination of predictor variables to identify new canonical components that maximize the variability between groups, relative to the variability within groups, maximizing the following function.