

Susceptibility of different mouse strains to experimental infection with a Venezuelan isolate of *Trypanosoma evansi*

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ABSTRACT

The susceptibility of BALB/c, C57Bl/6, DBA/2, NMRI, NIH and CD1 mouse strains to experimental infection with a Venezuelan equine isolate of *Trypanosoma evansi* was examined through hemoglobin concentration, packed red cell volume, number of erythrocytes per blood μ l, parasitemia, body weight, and mice survival time. The said variables seem to be suitable markers to contrast the mouse susceptibility to *T. evansi* experimental infections. Among the evaluated mouse strains, NMRI was the more susceptible and DBA/2 the less vulnerable to the local *T. evansi* isolate used in the study. The resistant mouse strains could help in the identification of factors conferring natural resistance against *T. evansi*, while the susceptible could be used to investigate the heterogeneity of virulence observed in this parasite.

Key words: *Trypanosoma evansi*; mouse susceptibility; experimental infection

INTRODUCTION

Trypanosoma evansi is a hemoflagellate with a broad geographic distribution in the tropics, mainly in countries of North Africa, Southeast Asia, as well as Central and South America (Luckins, 1998). Under natural condition *T. evansi* is competent to parasitize mammals including carnivore, bat, deer, edentate, rodent, and different herd (bovine, camel, caprine, equine, ovine, and porcine) (Hoare, 1972; Shaw, 1977; Luckins; 1988; Brun, 1998; Herrera *et al.*, 2004). *T. evansi* trypanosomosis is an endemic disease causing potentially lethal outbreaks in horses. Ample South American areas, including Colombian and Venezuelan plains, Brazilian Pantanal, and Northern Argentina are used for horse-dependent extensive bovine farming; consequently, horse health acquires a significant economic meaning (Clarkson 1976; Dávila *et al.* 1996; Monzón and Russo, 1996; Rivera 1996; García *et al.* 2000; Queiroz *et al.* 2000). In the region, *T. evansi* also infects the capybara (*Hydrochaeris hydrochaeris*), which represents an important reservoir host sharing habitat with bovine and equine herds (Reverón, 1992, Franke *et al.*, 1994, García *et al.*, 2000).

Even though *T. evansi* is a pathogen for most domestic and wild hosts, its effect upon the mammal varies according to the virulence of the parasite, the host species, unspecific factors as simultaneous infections and general stress, among others, as well as epizootiological conditions (Hoare, 1972). In addition, the species, age, and physiological status of the host specifies the susceptibility to the infection and the clinical manifestations of the disease. Furthermore, the degree and type of infection (acute or chronic) just as the level of parasitemia affect the transmission through insect vectors (Luckins, 1998). Certain bovine, caprine and ovine breeds show a significant resistance to *T. congolense* trypanosomosis, since they are able to control the levels of parasitemia, as well as the severe parasite induced anemia, and consequently, tolerate

the presence of the trypanosomes (Kemp and Teale 1998).

This susceptibility variation also occurs in mice. Indeed, Herbert and Lumsden (1968) established marked dissimilarities in the vulnerability of C57Bl/6 and C3H/HE mice to experimental injection with *T. brucei*; Morrison and Murray (1978) demonstrated differences in the resistance of B57BL/6, BALB/c and A/J strains of laboratory mouse to infection with *T. congolense*; and Reid and Husein (1998) tested the defenselessness of ARC, Quakenbush, BALB/c, DPJ, CBA/CaH and C57BL/6J mouse strains to inoculation with *T. evansi*. Kemp and Teale (1998) indicated that the ability to control the disease is genetically established. In view of the fact that the search of host differences in the degree of susceptibility represent the first step to facilitate the identification of factors associated to the trypanotolerance, in this paper we studied the susceptibility of six mouse strains to the infection with an indigenous *T. evansi* isolate.

MATERIALS AND METHODS

Parasites

The utilized trypanosomes derived from a naturally infected horse suffering “derrengadera” (vernacular designation for Surra) symptoms at Mantecal (7° 33’ N – 69° 6’ W; 62 m above sea level; Apure State, Venezuela). Flagellates were expanded in cyclophosphamide immunosuppressed BALB/c mice (300 mg/Kg) (Aisenberg, 1970). Axenic infected blood came from bleed BALB/C mice was diluted in PBSG (Saline buffer phosphate 0.02 M + 1% glucose; pH 7.0) to a final 100 trypomastigote/ml concentration, and stored in liquid N₂ until use.

Animals

Eight weeks old female mice of the BALB/c, C57Bl/6, DBA/2, NMRI (provided by The Venezuelan Institute of Scientific Investigations, Caracas), NIH and CD1 (provided by The National Institute of Hygiene, Caracas) strains were used in the tests. The experimentation consisted in 40 animals of each strain (240 in total) randomly placed in four metal boxes with food and water provided *ad libitum*; three boxes made the experimental group and the fourth one the control group.

Experimental infection

The rodents of the experimental groups were intra-dermal inoculated in the base of the skull with 1 trypomastigote/g of body weight, suspended in 50 µl of PBSG. Control groups were identically injected with hemoflagellates-free PBSG. One day after inoculation, and every other day until host death, 100 µl of caudal vein blood was collected with the aid of a heparinized microhematocrit tube from 1 randomly selected mice from each box (n=18). Control mice were identically chosen and bled (n=6); their blood was also processed.

Blood samples were distributed in the following aliquots: 10 µl to determine hemoglobin concentration (Hb) according to Van Kampen and Zijlstra (1965), 40 µl to establish packed red cell volume by standard microhematocrit method (PCV), 5 µl to ascertain erythrocytes per blood µl (EC), and 5 µl to quantify parasitemia (PAR) according to Brener (1962). Mice body weight (MBW) and survival time were also registered. Experiments were repeated three times.

Statistics

Once corroborated the data normal distribution, independence and variance homogeneity a one-way Analysis of Variance (ANOVA), a mean contrasting parametric test, was done. Analyses detecting significant differences were evaluated with Duncan *post hoc* test. Differences were considered to be statistically significant with ANOVA values of $p < 0.05$ and Duncan *post hoc* $p < 0.05$.

This investigation complies with the norms set out in the Guide for Care and Use of Laboratory Animals, published by the U.S. National Institute of Health (NIH publication N° 85-23, revised 1985).

RESULTS

Development of parasitemia infected mice

The initial inoculum produced patent PAR in most rodents (98%) independently of the strain. Even though the PAR increased progressively after the prepatent period (6 days) in the 96.4% of the mice, 1.6% was able to control it, and 2% of the rodent population never showed patent PAR. Table 1 shows the infectivity characteristics of local *T. evansi* in the mouse strains under investigation. Mouse strains bearing highest mean PAR were NMRI (3.4×10^8 trypomastigotes/blood ml), BALB/c (3.3×10^8 trypomastigotes/blood ml), and NIH (2.2×10^8 trypomastigotes/blood ml). The higher PAR peaks correspond to NMRI (9.6×10^9 trypomastigotes/blood ml), BALB/c (9.3×10^9 trypomastigotes/blood ml) and NIH (5.6×10^9 trypomastigotes/blood ml) strains. The shorter time to appear the peak of PAR goes with C57Bl/6 and NIH (13 days), while the longer fits with NMRI, CD1 and DBA/2 (19 days). However, the extent of the patent period was identical in all strains (6 days).

Strain	Prepatent Period (days)	Parasitemia Mean	Parasitemia Peak	Day appearing the peak
NMRI	6	3.4×10^8	9.6×10^9	19
BALB/c	6	3.3×10^8	9.3×10^9	15
C57Bl/6	6	1.4×10^8	1.07×10^9	13
NIH	6	2.2×10^8	5.6×10^9	13
CD1	6	1.2×10^8	1.9×10^9	19
DBA/2	6	6.7×10^7	9.7×10^8	19

Table 1. Main infectivity indicators in the mouse strains under study. Parasitemia mean and Parasitemia peak expressed in trypomastigotes/blood ml.

Clinical changes of infected mice

The PCV values show a decrease in all experimental groups with respect to controls; NMRI and NIH strains are evidence for higher variations; decreasing PCV values in DBA/2 strain was not significant ($p > 0.05$) (Figure 1). Figure 2 shows Hb the arithmetic mean and standard error values throughout the experiments underlining the negative correlation between Hb and PAR. In other words, the way PAR increases the way Hb diminishes. Nevertheless, Hb variations were statistical significant for the NMRI strain only, reaching 11g/dl, approximately; smaller values were observed in the C57Bl/6 strain. The EC means are presented in Figure 3. The results are consistent with the anemic condition already pointed, since EC decrease consistently through the infection. Such diminution is statistical significant for the NMRI strain only. The MBW decrease was generalized to every mouse isolate nevertheless NMRI mice exhibited the greater diminution (figure 4). Variations in MBW were statistically significant for NMRI and BALB/c, all other strain modifications lack of statistical significance.

Mouse susceptibility to *Trypanosoma evansi*.

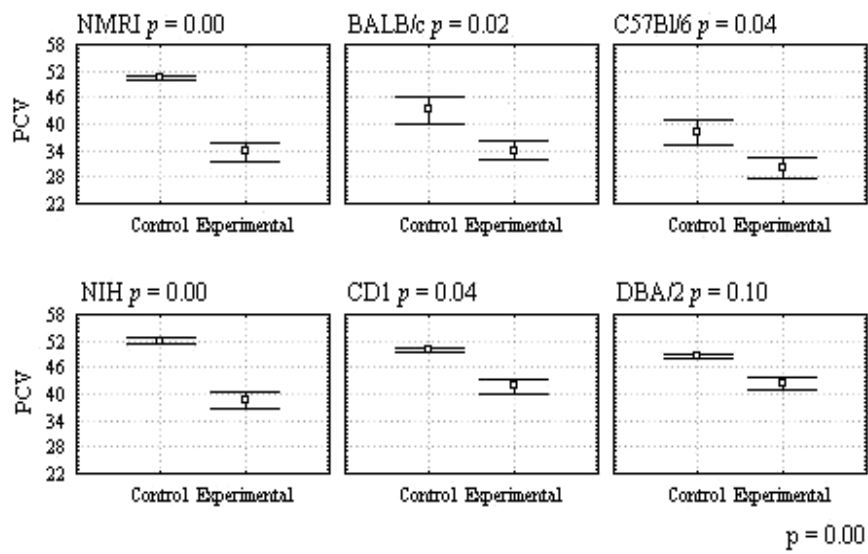


Figure 1. Arithmetic means and standard errors of the PCV. ANOVA $p=0.00$; p , Duncan *post hoc* probability.

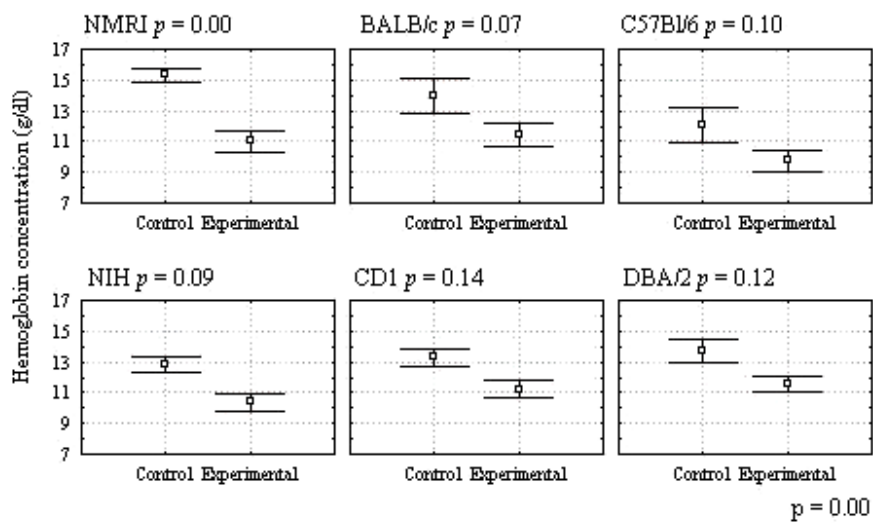


Figure 2. Arithmetic means and standard errors of the hemoglobin concentration. ANOVA $p=0.00$; p , Duncan *post hoc* probability.

Mouse susceptibility to *Trypanosoma evansi*.

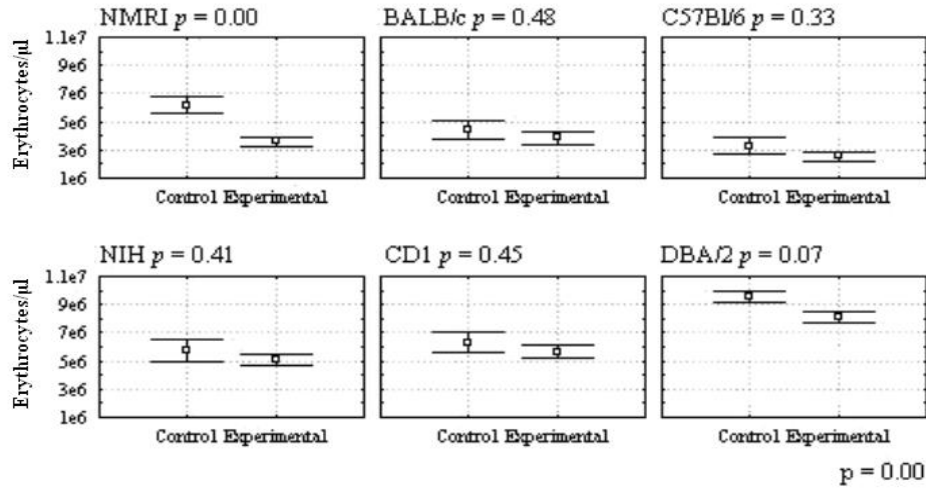


Figure 3. Arithmetic means and standard errors of the erythrocyte number per blood ml. ANOVA $p=0.00$; p , Duncan *post hoc* probability.

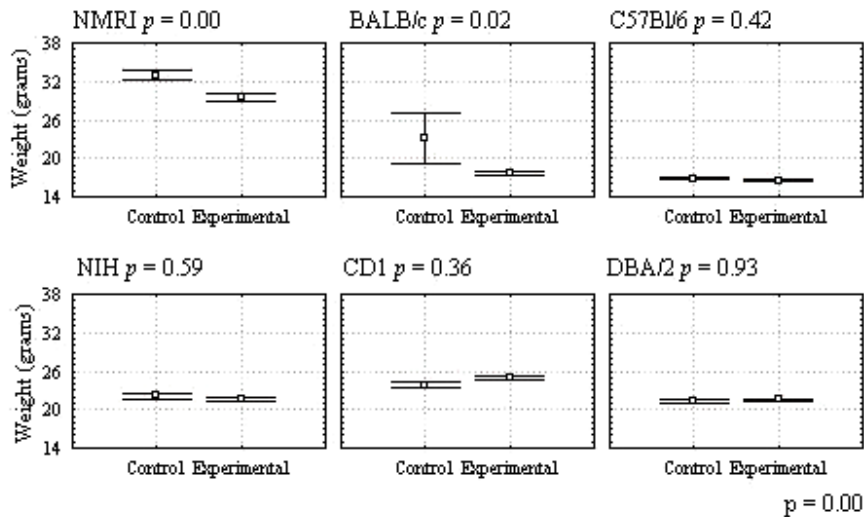


Figure 4. Arithmetic means and standard errors of the mouse body weight. ANOVA $p=0.00$; p , Duncan *post hoc* probability.

DISCUSSION

The results here presented showed clearly the variable mouse susceptibility to the infection with a Venezuelan isolate of *T. evansi* derived from a naturally infected native horse. Mouse strains under investigation exhibited statistical significant differences in hematology and infection characteristics. In every case the infection passed progressively until the host death in a relatively short period of time, which could mean that mouse is not a proper model to study the typical parasitemic waves described in the horse. The mouse susceptibility reported in this research does not parallel the typical *T. evansi* behavior in natural rodent hosts as capybara, since the latter deploys high parasitemias developing neither anemia nor clinical symptoms (Franke *et. al.*, 1994; Herrera *et. al.*, 2004; Menezes *et. al.*, 2004). Among the six evaluated mouse strains, NMRI was the more susceptible as ANOVA analyses proved to be the unique strain in which all registered variables diminished significantly ($p < 0.05$) with respect to their respective controls. On the other hand, DBA/2 strain ought to be considered the less susceptible because no significance ($p > 0.05$) was detected between the experimental groups and its particular controls. Although, anemia was not the

predominant feature of the DBA/2 infection, as it was in NMRI, all mice died meaning that the pathogenesis expression could be different in diverse mouse strains. All prepatent periods were identical (6 days) with a host survival ranging 15-25 days, which suggest that the initial trypanosome replication is a strain independent process. Our results are partially in concordance with Menezes *et al.* (2004), who infected four mouse strains with six *T. evansi* isolates, obtaining prepatent periods varying 2-4 days and survival times stretching 12-24 days.

Araque (1985) investigated the susceptibility of four mouse strains to *T. evansi*. The author found two distinct infection patterns, one characterized by great susceptibility and the other typified by evident resistance. The BALB/c strains led the first group, since all animals died in the acute phase and never exceeded 10 days of patent parasitemia. On the other hand, DBA/2 and C57Bl/6 strains were able to overcome the acute phase with survival times up to 60 days. Even though we obtained similar prepatent times, our results contrast with it, since no animal was capable to survive for more than 25 days. Reid and Husein (1998) considered the variation in the susceptibility of six mouse strains to infection with *T. evansi*. They found high percentages (95-100%) of the mice from all six strains developing a detectable parasitemia after inoculation. The authors found no statistical difference in the duration of survival of infected mice. Our results underline the heterogeneity of the infection, also indicating, the complexity and multifactorial condition of the pathologic process, which include the genetic characteristics of the host just as the virulence of the parasite. Both factors could determine the pathogenesis of the disease in a murine model. Kemp and Teale (1998), found an analogous phenomenon with African trypanosomes infecting bovine, caprine and/or ovine herds. Morrison and Murray (1978) put in evidence the relative resistance of C57Bl/6 mice to *T. congolense* infection, since they stayed alive for 110 days, while BALB/c and A/J mice survived for 50 and 16 days, respectively. Morrison and Murray (1978) and DeGee *et al.* (1988) hybridized susceptible and unsusceptible mouse strains, concluding that the immune system ability to control the parasitemias is inherited as a recessive character, while the susceptibility is transmitted as a dominant feature.

We were not able to relate mouse susceptibility to H-2 haplotype, however and in spite of the parasitologic differences among the considered mouse strains, survival time ranged over 15 and 25 days. Even though all strains (with the DBA/2 strains exception) showed diminution in every recorded variable, NMRI strain exhibited higher statistical differences with respect to the controls. Levine and Mansfield (1981) studied the locus H-2 role in the murine resistance to *T. rhodesiense* infection. The authors found that mouse strains with identical H-2 haplotype exhibited significant differences in the mean outlive times. In contrast, congenital mice expressing different H-2 haplotypes, but identical genetic content unrelated to the H-2 haplotype, showed similar results. Such findings suggest that genes associated to the Major Histocompatibility Complex do not fulfill a fundamental function in the control of the parasitemia, mean survival time, and general resistance of *Mus musculus* to *T. rhodesiense* infection. Kemp and Teale (1998) paper speculates about the participation of particular Major Histocompatibility Complex haplotypes in the bovine trypanotolerance; the tolerance-susceptibility model is well documented in two African bovine species, *Bos taurus* and *B. indicus*. *B. taurus* is able to control the parasitemia, hematocrit and body weight better than *B. indicus*; this natural resistance made easy the identification of chromosomal zones potentially involved in the trypanosomosis tolerance named Quantitative Trait Loci regions (QTL). Hanotte *et al.* (2003) suggested that the presence of several QTL, localized in different chromosomes, contribute to handle the trypanotolerance in terms of anemia, hematocrit decrease, emaciation and parasitemia. Kemp and Teale (1998), Nakamura *et al.* (2003), and Graefe *et al.* (2003) reported differences two or three genes associated to chromosomes 5 and 17, which probably are implicated in the resistance-susceptibility phenomena to

trypanosomes, named Tir1, Tir2, and Tir3. The identification of genes, as well as proteins associated to trypanotolerance or trypanoresistance could facilitate the understanding of evasion mechanisms allowing the host survive to or control such infections.

The study of the susceptibility of different mouse strains to *T. evansi* set up a first step to identify factors and/or mechanisms related to trypanosusceptibility and trypanoresistance. The hematological variable fluctuations could be a pathology reference point to the mouse *T. evansi* disease; so, hematocrit, hemoglobin concentration, erythrocyte count and parasitemia would represent appropriate indicators of the physiological state of the host, reason why they have been profusely used to compare the susceptibility of different mouse strains, and diverse bovine breed to *T. evansi* (Kemp and Taele, 1998). The resistant mouse strains could help in the identification of factors conferring natural resistance against *T. evansi*, while the susceptible ones could be used to investigate the heterogeneity of virulence observed among *T. evansi* isolates.

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