

## **Variation in the Susceptibility of 6 Strains of Mouse to Infection with *Trypanosoma evansi*.**

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### **ABSTRACT**

The mouse inoculation test (MI) is generally considered the most sensitive method for the detection of *T. evansi* in blood from infected animals. However, the effect that strain of mouse used may have on the sensitivity of the test is not known. It was investigated in this study.

Forty mice from each of 4 inbred strains (BALB/c, DPJ, CBA/CaH and C57BL/6J) and 2 outbred strains (ARC and Quackenbush) were inoculated in groups of ten, by intraperitoneal injection, with 0.3ml of bovine blood containing 1,000, 125, 25 or 0 *T. evansi* per ml. Mortality was monitored twice daily and parasitaemia score was recorded every 2-3 days for 21 days. Totals of 96%, 100% and 95% of the mice from all strains developed detectable parasitaemia when inoculated with blood containing 1,000, 125 or 25 *T. evansi* per ml of blood respectively. CBA/CaH mice survived significantly longer (11.6 days) than Quackenbush, BALB/c and C57BL/6J mice (8.8, 8.2 and 8.8 days respectively) inoculated with blood containing 125 *T. evansi* per ml ( $p < 0.05$ ). However, there was no measurable difference in duration of survival of mice from different strains when inoculated with blood containing 1,000 or 25 *T. evansi* per ml.

It was concluded that the choice of mouse strain for use in the MI test is unimportant and that practical considerations such as colour and robustness are more important in deciding on an appropriate strain. Of the six strains evaluated, the BALB/c strain was considered the strain of choice as it was the most robust, is readily available in laboratory colonies and, being white, identifying marks made on the coat with hair dye were easy to visualise.

### **INTRODUCTION**

The mouse inoculation test (MI) is generally regarded as the most sensitive method for the diagnosis of infection with *T. evansi* (Monzon et al. 1990). However, there is little published information on conditions which standardise or optimise sensitivity of the test and false negative results have been recorded using MI where parasites have been detected using other methods (Monzon 1990). The reasons for this are not clear but may be due to a loss of infectivity of the trypanosomes for mice as peaks of new antigenic variants arise (Wilson and Cunningham 1972) or because the strain of mouse used was resistant to infection. The latter possibility has not been demonstrated for *T. evansi* but was investigated in this study as Herbert and Lumsden (1968) and Black et al. (1983) demonstrated marked differences in the susceptibility of two strains of laboratory mouse to infection with *T. brucei*.

### **MATERIALS AND METHODS**

Forty mice from each of 2 outbred (ARC, Quackenbush) and 4 inbred strains (Balb/C, CBA, DPJ and C57 BL/C6) were allocated at random into 4 groups of 10. Mice in each

group were inoculated, by intraperitoneal injection, with 0.3ml of bovine blood to which *T. evansi* was added to give parasite levels of 1000, 125, 25 or 0 per ml of inoculum. The mice were checked visually twice daily and every 2-3 days for 21 days by wet smear examination of tail-tip blood for the presence of *T. evansi*. Differences in susceptibility were made by comparison of the prepatent period and the duration of survival of mice of each strain. The significance of differences in duration of survival of mice between strains and infecting doses was calculated using Analysis of Variance and Tukey test for significant difference.

## RESULTS

Totals of 96%, 100% and 95% of the mice from all strains developed a detectable parasitaemia after inoculation with blood containing 1,000, 125 or 25 *T. evansi* per ml of blood respectively. CBA/CaH mice survived longer (11.6 days) post inoculation than Quackenbush, BALB/c and C57BL/6J mice (8.8, 8.2 and 8.8 days post inoculation respectively) when inoculated with blood containing 125 *T. evansi* per ml ( $p < 0.05$ ) (Fig.1). There was no difference in the duration of survival of mice from different strains when inoculated with blood containing 1,000 or 25 *T. evansi* per ml. *Trypanosoma evansi* was found in the blood of CBA/CaH mice, inoculated with blood containing 1,000 *T. evansi* per ml 3-6 days post inoculation, earlier ( $p < 0.05$ ) than in Quackenbush, ARC and DPJ mice, in which *T. evansi* was found 6 days post inoculation; however, there was no difference in prepatent period between strains after inoculation with 125 or 25 *T. evansi* per ml.

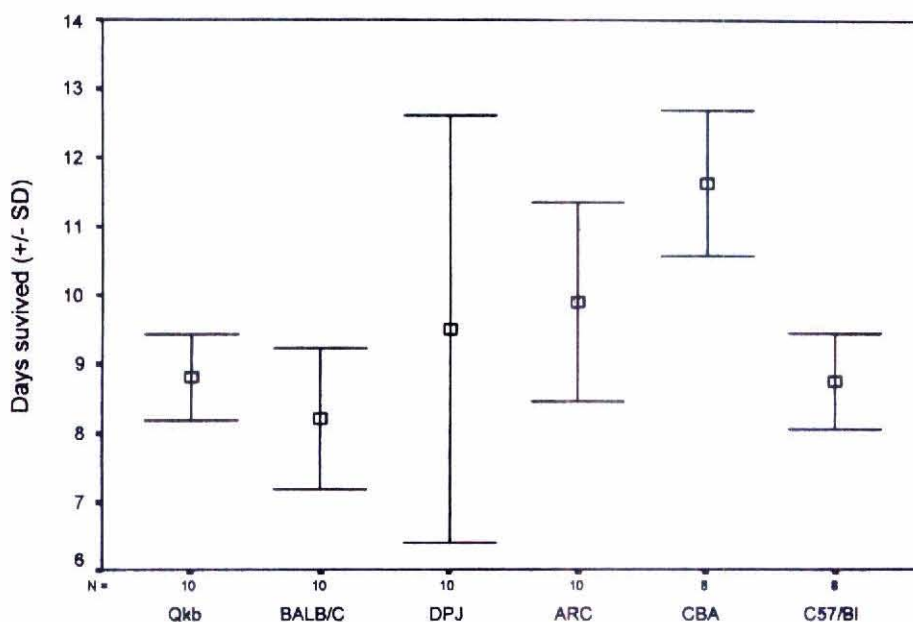


Figure 1. Length of survival of 6 strains of mice infected with 125 *T. evansi* per ml of bovine blood.

## DISCUSSION

The high susceptibility of mice to infection with *T. evansi* was confirmed in this study. Furthermore, the observation that susceptibility was unaffected by strain of mouse suggests that choice of strain for use in the MI test is unimportant. However, extrapolation of results from the six strains used in this study to encompass all strains is not justified. This is because of the large number of different mouse strains available and the possibility that one



or more will be resistant in keeping with the documented differences in susceptibility between selected strains of mice to *T. brucei* (Herbert and Lumsden 1968; Black et al. 1986). Thus, while all six strains used were equally susceptible to infection with *T. evansi*, prudence dictates that prior to selection for use in the MI test susceptibility to *T. evansi* should be confirmed.

While high susceptibility to infection is a necessary criterion for selection of strain of mouse for the MI test, there are also other more practical considerations. Of the six strains evaluated, the BALB/c strain was considered the strain of choice as it was the most robust, is commonly available in laboratory colonies and, being white, identifying marks on the coat with hair dye were easy to visualise.

The lower limit of the infecting dose of *T. evansi* for mice has not been defined. This study demonstrated that mice of all strains inoculated with about eight organisms (0.3 ml of 25 *T. evansi* per ml) reliably developed parasitaemia. Furthermore, within strains, prepatent period and duration of survival were independent of the infecting dose, indicating that mice are likely to be infected by fewer than eight organisms.

There was an indication that CBA/CaH mice were more resilient to infection and also had a shorter prepatent period than mice of other strains. While this did not affect sensitivity of the MI test, examination of the underlying mechanisms may provide further understanding of the pathogenesis of infection with *T. evansi*.

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## REFERENCES

- Black, S.J., Sendashonga, C.N., Lalor, P.A., Whitelaw, D.D., Jack, R.M., Morrison, W.I. & Murray, M. 1983. Regulation of the growth and differentiation of *Trypanosoma (Trypanozoon) brucei brucei* in resistant (C57Bl/6) and susceptible (C3H/He) mice. *Parasite Immunol.* 5: 465-478.
- Black S.J., Sendashonga, C.N., Webster P., Koch, G.L.E. & Shapiro, S.Z. 1986. Regulation of parasite-specific antibody responses in resistant (C57Bl/6) and susceptible (C3H/HE) mice infected with *Trypanosoma (Trypanozoon) brucei brucei*. *Parasite Immunology* 8: 425-442.
- Herbert W.J. and Lumsden W.H.R. 1968. Infectivity and virulence of *Trypanosoma (Trypanozoon) brucei* for mice. I. Comparison of two mouse strains. *J. Comp. Pathol.* 78: 341-344.
- Monzón, C.M., Mancebo, O.A. & Roux, J.P. 1990. Comparison between six parasitological methods for diagnosis of *Trypanosoma evansi* in the subtropical areas of Argentina. *Vet. Parasitol.* 36: 141-146.
- Wilson, A.J. & Cunningham, M.P. 1972. Immunological aspects of bovine trypanosomiasis. I. Immune Response of cattle to infection with *Trypanosoma congolense* and the antigenic variation of the infecting organism. *Exptl. Parasitol.* 32: 165-173.