THE SUSCEPTIBILITY OF LABORATORY ANIMALS TO TRYPANOSOMA EVANSI INFECTIONS

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INTRODUCTION

Trypanosoma evansi, one of about 200 trypanosomes species encountered in mammals in the world, is the causative agent of an economically important disease among domestic animals called surra. Many studies on *T. evansi* has been made using various experimental animals such as chicks and fowls for kifectivity (Alwar 1962, Manuel & Cajita 1967), mice for influences of temperature (Marthur 1976) and rats for immunological studies (Assoku 1975). Even larger animals such as goats, pigs, horses, cattle and buffaloes have been used by several workers in studying *T. evansi* infections (Chand & Singh 1971, Gill 1971, Srivastava & Ahluwalia 1972).

The present paper deals with the susceptibilities of small laboratory animals to *T. evansi* infections in order to determine which animals are suitable for maintaining *T. Svansi* in the laboratory. All works in this study were done at the Filariasis Research Division of the Institute for Medical Research (IMR), Kuala Lumpur.

MATERIALS AND METHODS

Trypanosoma evansi used in this study was maintained by the Filariads Research Division of the IMR continuously for 2 years in white mice. This *T. evansi* was then reinoculated and maintained in white rats for the study.

White mice *(Mus musculus)* weighing 35 to 55 g, multimammate rats *(Mastomys natalensis)* weighing 48 to 78 g, white rats *(Rattus norvegicus* of the Spraque — Dawley Albino Rat) weighing 130 to 187 g and rabbits *(Oryctolagus caniculus)* weighing 2 to 2.7 kg were used as the experimental animals. These animals, males and females, were obtained from the Animal Resources Division of the IMR.

The blood of white rats showing high parasitaemia examined in direct fresh blood was used for initiating infections in experimental animals. The blood was taken from the tail and sterile citrate-saline solution was used as the diluent for preparing the inocula suspension to a concentration of approximately 50;000 trypanosomes (inoculum A) and 100,000 trypanosomes (inoculum B) per ml. Counting of trypanosomes were made with a haemocutometer, using Turk's fluid as diluting substance (Samaddar & Gill 1962, Dill & Sen 1963). In order to prevent possibilities againts bacterial contamination, 1 drop of penicillin was added to each 10 ml inoculum.

The infections were set up by inoculating intraperitoneally 1 ml inoculum per animal. A group of 10 animals were injected at any one time. Ten white mice, 10 multimammate rats and 10 white rats were inoculated with prepared inoculum A. Another batches of 20 white mice, 20 multimammate rats, 20 white rats and 10 rabbits were inoculated with inoculum B.

Giemsa stained thick blood smears were made daily after infection up to 14 days and thereafter once a week for the remaining live animals. The smears for white mice, multimammate rats- and white rats were prepared from peripheral blood drawn from the tails. For rabbits the blood was drawn from the ears.

RESULTS

The results of experiments which involving animals inoculated with inoculum A and B are summarized in Figures 1 and 2. The results in groups of animals are explained briefly below.

White mice

When inoculum A were inoculated into white mice, the prepatent period was 2 to 5 days (Table 1). Most of the white mice became positive parasitaemia on the 3rd day post-infection. Mortality commenced on the 5th day and by the 9th day all were dead. Eight out of 20 (40%) white mice

Table I. Parasitaemia and mortality in white mice inoculated intraperitonealy with approximately 50,000 *Trypanosoma evansi*

No. Animal	Parasitaemia on day (post-infection) 1	2	3	4	5	Dead on day (post-infection)
1		–	–	–	+	9
2	–	–	+	+	+	6
3	–	+	+	+	+	7
4	–	+	+	+	+	5
5	–	–	+	+	+	6
6	–	–	–	–	+	8
7	–	–	+	+	+	7
8	–	–	+	+	+	7
9	–	+	+	+	+	6
10	–	+	+	+	+	6

died on the 7th day post-infection.

When inoculum B were inoculated into white mice, the prepatent period was 1 to 2 days. The number of parasitaemic white mice both on the 1st and 2nd day post-infection were 50%. Mortality began at the 4th day after infection in 10% of mice and ended on 12 th day. The highest number died (9 out of 20) on the 5th day post-infection.

Multimammate rats

When inoculum A were inoculated into multimammate rats it was found that the prepatent period was 1 to 4 days. Greatest number of parasitaemic rats was on the 4th day post-infection. The first mortality occurred on the 5 th day, the latest pn the 7th day, whilst the highest number of death was on the 5th day post-infection.

When inoculum B were inoculated into multimammate rats, the prepatent period was also 1 to 4 days. Eight out of 20 (40%) of the multimammate rats were parasitaemic on the 1st day post-infection. Death started on the 4th day. Three rats were found still alive 7 days post-infection. Their blood examinations showed a steady, very light parasitaemia. The last observed death among parasitaemic multimammate rats was on the 7th day post-infection. Eight percent multimammate rats were found dead by this time (Figure 2).

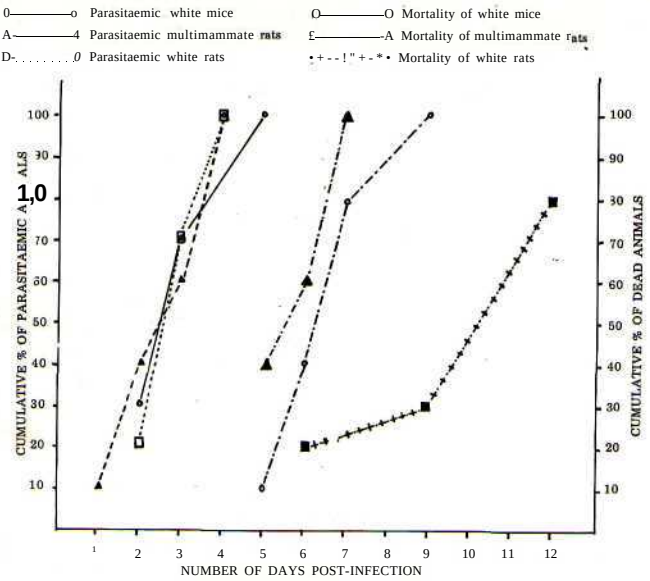

FIG 1. PARASITAEMIA AND MORTALITY AMONG WHITE MICE, MULTIMAMMATE RATS AND WHITE RATS INOCULATED INTRAPERITONEALLY WITH APPROXIMATELY 50.000 *TRYPANOSOMA EVANSI* EACH.

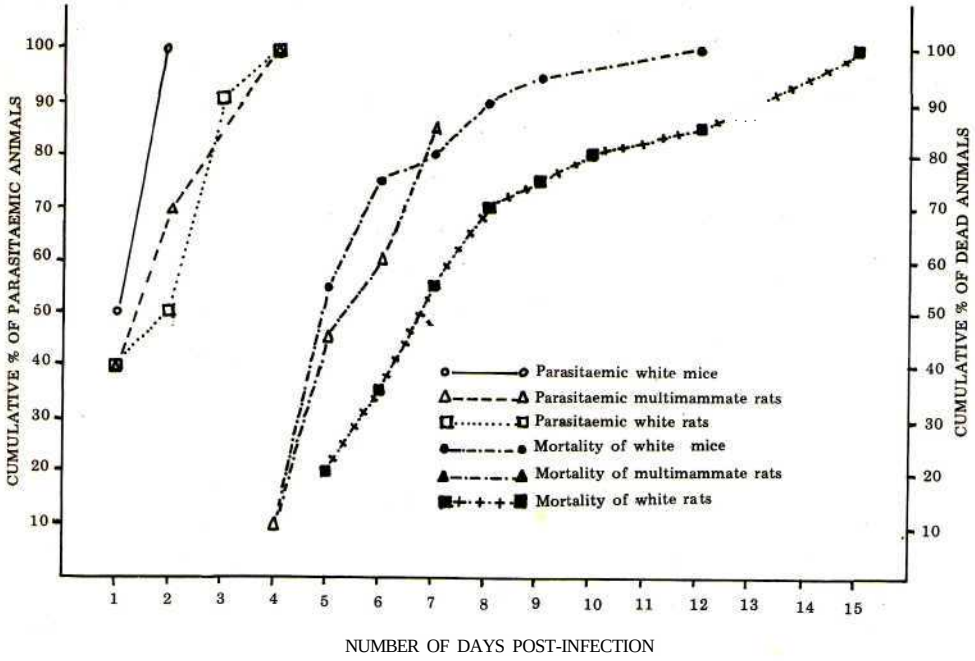

FIG. 2. PARASITAEMIA AND MORTALITY AMONG WHITE MICE, MULTIMAMATE RATS AND WHITE RATS INOCULATED INTRAPERITONEALLY WITH APPROXIMATELY 100,000 *TRYPANOSOMA EVANSI* EACH.

White rats

The prepatent period of white rats inoculated with inoculum A was 2 to 4 days. This was the shortest latent period among the groups of animals inoculated with the same number of trypanosomes. Fourteen out of 20 (70%) of the white rats were parasitaemic by the 3rd day post-infection. Mortality among parasitaemic white rats was first detected on the 6th day. The last observations on mortality stopped on the 12th day post-infection. This was at 80% mortality without reaching 100% of cumulative mortality rate (Figure 1). Two parasitaemic white rats died due to effects of anaesthesia.

When inoculum B were inoculated into white rats, the prepatent period was 1 to 4 days. The highest number to be patent was on'the 1st and 2nd day post-infection. Beginning with 20% of mortality among parasitaemic white rats on the 5th day post-infection, it was found that the last animal died on the 15th day post-infection. There were 2 peaks of mortalities, these being on the 5th and 7th day post-infection.

Rabbits

Rabbits were inoculated with inoculum B only. The earliest detection of parasitaemia was in 3 rabbits on the 4th day after infection (Table 2). This parasitaemia lasted for 2days, but appeared again on the 11th and 14th day in 2 out of those 3 rabbits, respectively. The parasitaemia lasted for 1 day only. The other rabbits showed parasitaemia were on the 5th to 11th day post-infection. On the first day of weekly examination of blood, 2 rabbits were parasitaemic, but negative on the second day. On the third day of weekly examination. 6 out of 10 rabbits showed parasitaemia. One rabbit did not show any parasitaemia at all and no mortalities occurred **among rabbits during** the study.

fabel 2. Parasitaemia in rabbits inoculated intraperitoneally with approximately 100,000 *Trypanosoma evanst*

No. Animal	Parasitaemia on day (post-infection)																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	21»	28*	35*
1	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	–	+
2	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+
3	–	–	–	+	+	–	–	–	–	–	–	–	–	+	+	–	+
4	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+
5	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
8	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+
9	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–

* = Weekly observation.

General appearances of the inoculated animals

Paleness and dullness were the only symptoms observed on white mice, multimammate rats and white rats on 1 to 2 days before death, while their appetites remained normal. Distinct symptoms of illness were shown by 5 rabbits entering the second day of weekly examination of blood. These rabbits had inflammation on their head region, especially around the eyes and ears. Their appetites, however, remained normal.

DISCUSSION

From the data presented in Figures 1 and .2, it is evident that in white mice, multimammate rats and white rats the length of the prepatent period and average duration of survival were inversely proportional to the number of trypanosomes inoculated. Gill & Sen (1963) also found this phenomenon when they worked with mice and rabbits inoculated with *T. evansi*. Figures 1 and 2 also show a distinct early parasitaemia and relatively short survival period in multimammate rats in the group of animals infected with inoculum A. On the other hand, a shorter prepatent period and shorter average survival period is seen in white mice among given inoculum B. Thus, multimammate rats and white mice are the most susceptible experimental animals to *T. evansi* infection with low and high infective doses, respectively.

White mice, multimammate rats and white rats showed parasitaemia a day post-infection and parasitaemia continued until death. This may be due to the rapid growth of parasites in these animals without allowing much time for host defence mechanism to come into play (Gill & Sen 1963). Meanwhile, the paleness of animals was explained by Assoku (1975) as due to increased extravascular destruction of erythrocytes rather than inhibition of haerrtopoitic activity. He examined the histopathology of liver, spleen and bone marrow of rats infected with *T. evansi*. The survival of some multimammate rats in high dose inoculum groups presumably indicated what Yutuc (1963) referred as age resistance of the hosts. As a whole, white rats appeared less susceptible, but could still be infected. They showed the longest survival period, which suggests that white rats may be usefull for maintaining *T. evansi* in the laboratory.

The replasing and short parasitaemia in the peripheral blood of rabbits showed them to be the most resistant to trypanosomeinfection. The scanty appearances of parasitaemia were also noted by Samaddar & Gill (1962) in goats when they used animals in Surra disease transmission studies. The peculiar inflammation of the head region of rabbits was also seen by Goel & Singh (1970). They studied the histopathology of the eyes of rabbits infected with *T. evansi* and reported on conjunctivities and keratitis of the rabbits. It is interesting to note that the symptoms of inflammation were not always parallel with the appearances of trypanosomes in the peripheral blood.

In conclusion, white rats can be considered as the most useful animal for maintaining *T. evansi* in the laboratory due to their long survival period after infection.

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