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Development of a rodent model for late stage rhodesian sleeping sickness

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ABSTRACT

A mouse model of *Trypanosoma brucei rhodesiense* that can be used in pathogenesis and drug studies for Human African Trypanosomosis (HAT) has so far not been developed. In an attempt to develop such a model, a study was undertaken to determine the clinical and pathological changes in mice infected with stock of *T. b. rhodesiense* (KETRI 2357) and its clone (KETRI 3741). The infections resulted in a mean prepatent period of 3 (range 3-4) days for KETRI 2537 and 4 (range 3-5) days for KETRI 3741. The first wave of parasitaemia for both isolates reached peak (antilog 8.7) between 6-8 DPI and was followed by secondary latency. Thereafter, the parasitaemia increased and remained high, with slight fluctuations. The mean survival period was 36 (range: 7-45) and 50 (18-82) days for the mice infected with KETRI 2537 and KETRI 3741 respectively. At post mortem, the spleens were enlarged and there was generalized congestion in most organs. Histopathological changes were more severe in the parent stock compared to its clone. Meningoencephalitis was observed in mice infected with KETRI 3741 that survived up to 77 days. These studies demonstrate the development of late stage disease in mice infected with *T. b. rhodesiense*, with potential for use as a model of sleeping sickness.

Key words: human African trypanosomosis; mice model; virulence; pathology; meningoencephalitis

INTRODUCTION

Sleeping sickness or human African trypanosomosis (HAT) is caused by two species of protozoan parasite *Trypanosoma*, caused by *T. b. gambiense* and *T. b. rhodesiense*. The disease runs a complex course leading to death if treatment is not carried out (Dumas and Bisser, 1999). The pathology of the disease comprises of two distinct stages. During the early haemolymphatic stage trypanosomes are present in the lymph, blood, and extra vascular spaces of most organs, but not in central nervous system (CNS). In the late or meningoencephalitic stage, parasites are found in the CNS where they are protected from drugs that are incapable of crossing the blood brain barrier (BBB) (Penreath, 1989).

A critical area of research in HAT is the development of cheap and reliable experimental models, that can be used increase our understanding of the disease and to evaluate drug regimens that can cure the late stages (Schmidt and Sayer, 1982; Kennedy, 2007). Development of such a model has been difficult because most *T. brucei* organisms tend to cause acute rather than a chronic disease in experimental animals. At present, efficacy trials of drugs against late stage trypanosomosis are performed in both mice and vervet monkeys (*Chlorocebus aethiops*) (Jennings, 1991; Ndung'u *et al*., 1994). The vervet monkey model of Rhodesian sleeping sickness has been extensively used in drug evaluation and pathogenesis studies. The model, which uses a *T. b. rhodesiense* stabilate KETRI 2537, consists of an early, terminal and late stage models. The late stage disease is induced by sub-curative treatment with diminazene aceturate at days 33-35 post infection. Relapses occur 70 to 80 days later and are characterized by clinical signs of late stage disease and histological meningoencephalitis (Schmidt, 1983). On the other hand, the mouse model is based on the

assumption that by 21 days post infection, a considerable number of trypanosomes will have invaded the CNS, and successful cure would depend on the ability of trypanocidals to penetrate the blood brain barrier (Jennings *et al.*, 1977). However, this model was developed using the non-human infective *T. b. brucei* that may have different pathogenicity and drug susceptibilities compared to *T. b. rhodesiense* (Kennedy, 2007). Some studies have also shown that at day 21-post infection only slight meningitis is found in mice in this model (Jennings *et al.*, 1993). However, a proper late stage model presupposes the occurrence of meningoencephalitis.

The objective of this study was therefore to determine the pathogenesis of human infective *T. b. rhodesiense* KETRI 2537 and its clone KETRI 3741 in Swiss white mice, with the aim of developing a murine model of late stage HAT.

MATERIAL AND METHODS

Ethics

All protocols and procedures used in the current study were reviewed and approved by the KARI-TRC Institutional Animal Care and Use Committee.

Trypanosomes

The original isolate of *T. b. rhodesiense* KETRI 2537 was referred to as EATRO 1989 (Fink and Schmidt, 1980). It was isolated from a human patient in Busoga, Uganda in 1972, passaged serially in a monkey, then latter in irradiated mouse before being cryopreserved as *T.b. rhodesiense* KETRI 2537. KETRI 3741 was cloned from KETRI 2537 stabilate.

Experimental Animals

Seventy female inbred Swiss white mice were obtained from International Livestock Research Institute's (ILRI) breeding colony. The mice were kept in Macrolone cages with the following dimensions: 14 cm width, 30 cm length and 15 cm depth. The mice weighed 25-30 g and were fed on mice pellets (Mice pencils®, Unga feeds Kenya) and water *ad libitum*. They were maintained at an ambient temperature of between 20 – 25 ^{o}C. and wood shavings (Tim sales, Nairobi, Kenya) were used as beddings.

Experimental Design

The experiment was divided into a pathogenicity and virulence study.

Pathogenicity study: Fourty mice were infected by intraperitoneal injection with 10^4 *T. b. rhodesiense* KETRI 2537 (20 mice) and KETRI 3741 (20 mice). They were monitored daily for parasitaemia using wet blood smears made from blood collected by tail snip. Parasitaemia levels were estimated using the rapid matching method described by Herbert and Lumsden (1976). Giemsa-stained thin blood films were also made and subsequently examined for parasite morphology. The different forms of the parasites were identified and counted using the criteria described by Stephen (1986). The shape of parasite body, size of free flagellum and presence or absence of distinct undulating membrane were determined. The mice were sacrificed in groups of five at 7, 14, 21 and 28 days post infection. A post mortem examination was carried out and samples from all major organs processed for histopathology.

Virulence study

Two groups of mice, each consisting 10 animals were infected with 10^4 *T. b. rhodesiense* KETRI 2537 and 3741. They were monitored without treatment to determine the comparative virulence of the two parasites. The survival rate of the mice infected with the two isolates was calculated by dividing the number of mice dead at a given point in time with the total number infected. A post mortem examination was carried out whenever a mouse died and samples from all major organs processed for histopathology.

RESULTS

Parasitaemia Pattern

Mice infected with *T. b. rhodesiense* KETRI 2537 and 3741 developed parasitaemia within 3 days (range: 3-4) and 4 (range: 3-5) days respectively. The parasitaemia levels and patterns were similar in the two groups. The first wave of parasitaemia for both isolates reached peak (antilog 8.7) between 6-8 DPI. This was followed by a fall in parasite numbers to undetectable levels between 8 and 10 days after infection. Thereafter, the parasitaemia increased and remained elevated, with fluctuations, throughout the course of the disease (Fig. 1).

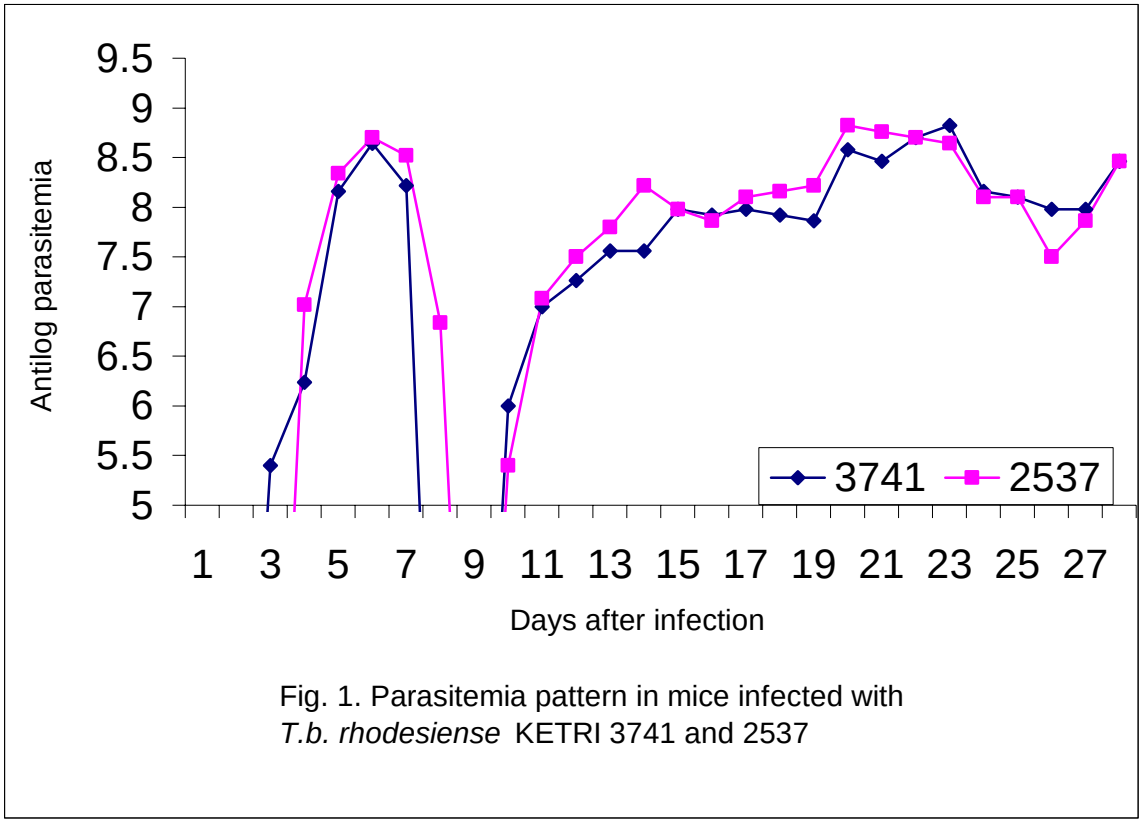


Fig. 1. Parasitemia pattern in mice infected with *T.b. rhodesiense* KETRI 3741 and 2537

Parasite Morphology

Examination of the giemsa stained blood smears showed that both KETRI 3741 and KETRI 2537 had pleomorphism where all the forms i.e., long slender (LS), intermediate (I) and short stumpy (SS) were observed during the course of infection. There was no significant difference ($P>0.05$) between the two parasites in terms of changes in morphology. By day 4 after infection, there was a predominance of long slender forms, with were very few short stumpy ones. However, as the disease progressed the number of short stumpy forms increased while the long slender forms declined. At day 8 after infection the percentage of short stumpy forms were very high as compared to the long slender. Thereafter, the percentage of long slender increased, but remained lower than both the intermediate and short stumpy.

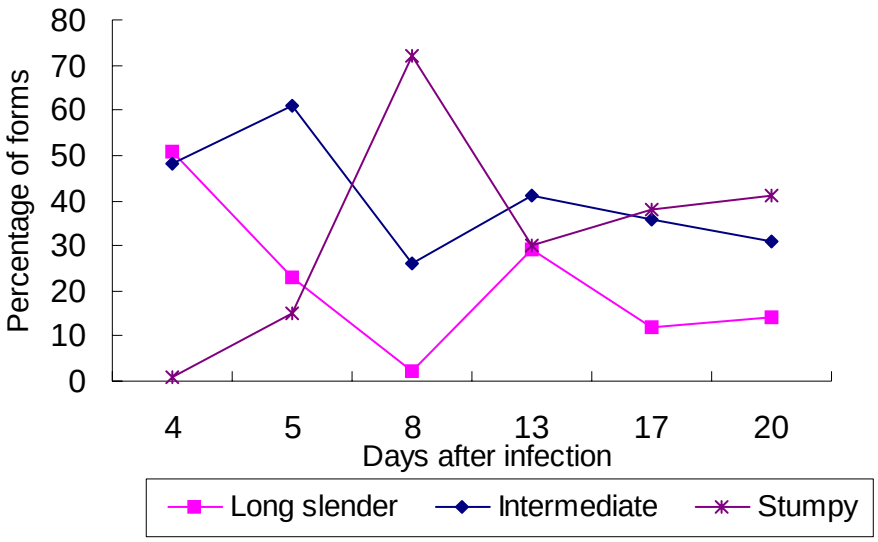


Fig 2. Morphology of trypanosomes in mice infected with *T. b. rhodesiense* KETRI 3741

Survival Period

The mean survival period for the mice infected with *T. b. rhodesiense* KETRI 2537 and KETRI 3741 was 36 (range: 7-45) and 50 (range: 18-82) days respectively (Fig. 2). All the mice infected with *T. b. rhodesiense* KETRI 2537 had died by day 45 compared to only 63% of the mice infected by *T. b. rhodesiense* KETRI 3741 that had died by the same day. The longest surviving mouse was infected with KETRI 3741 and died at 82 days after infection (Fig. 2).

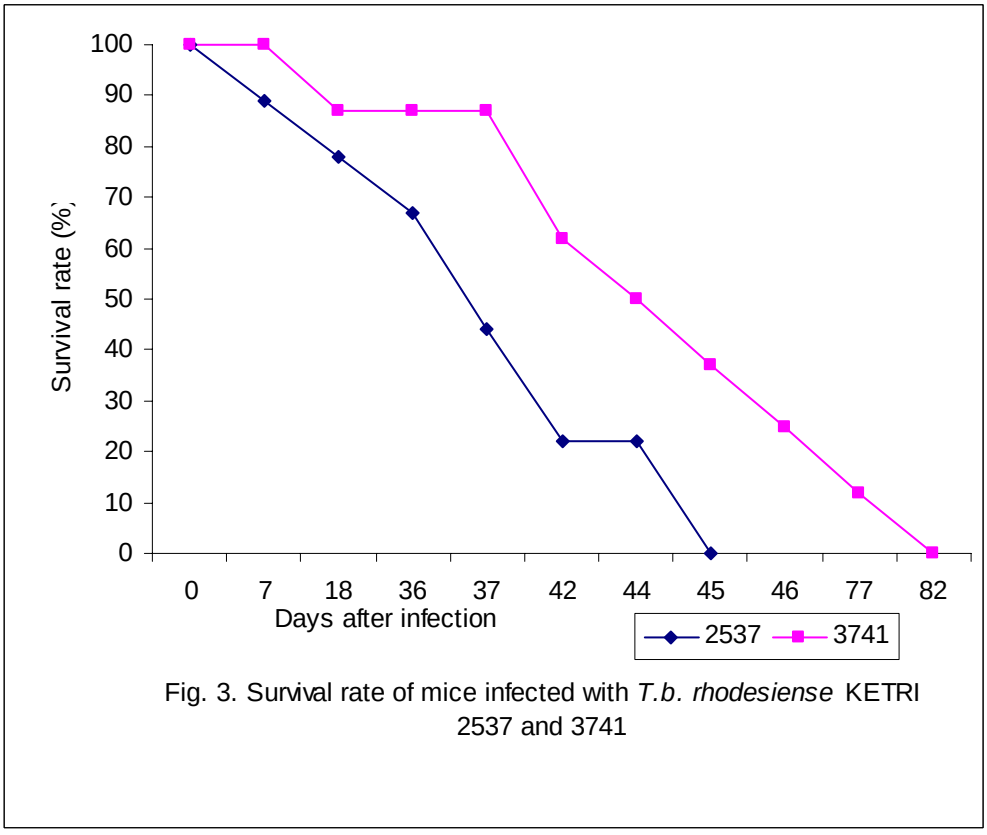


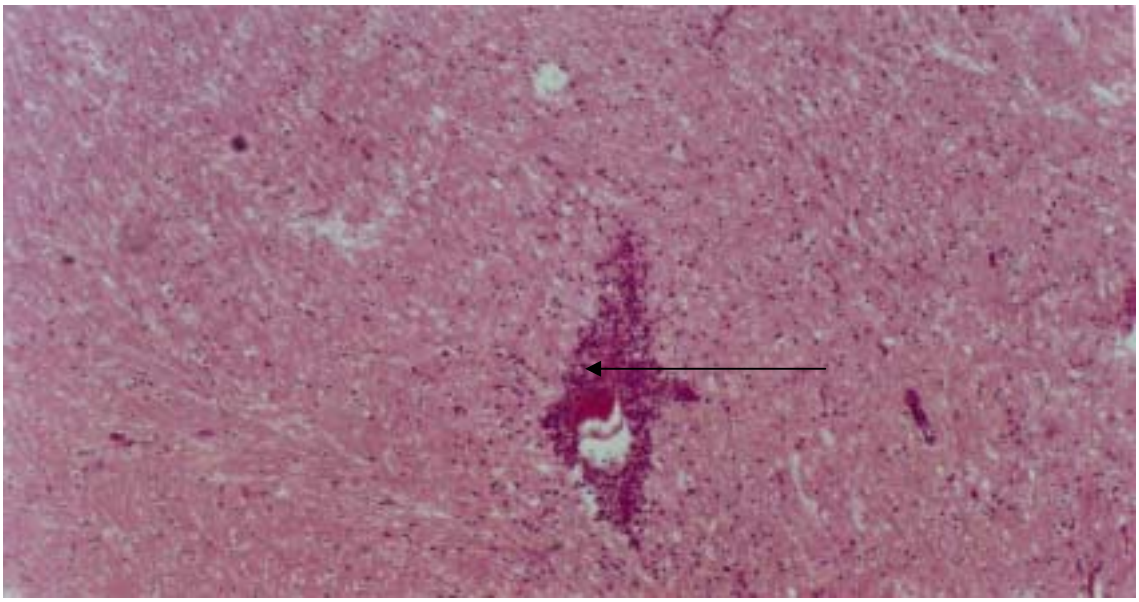
Fig. 3. Survival rate of mice infected with *T.b. rhodesiense* KETRI 2537 and 3741

Post mortem Findings

Grossly, the prominent features were an enlarged spleen and congestion in most organs. The histopathological changes are as detailed below.

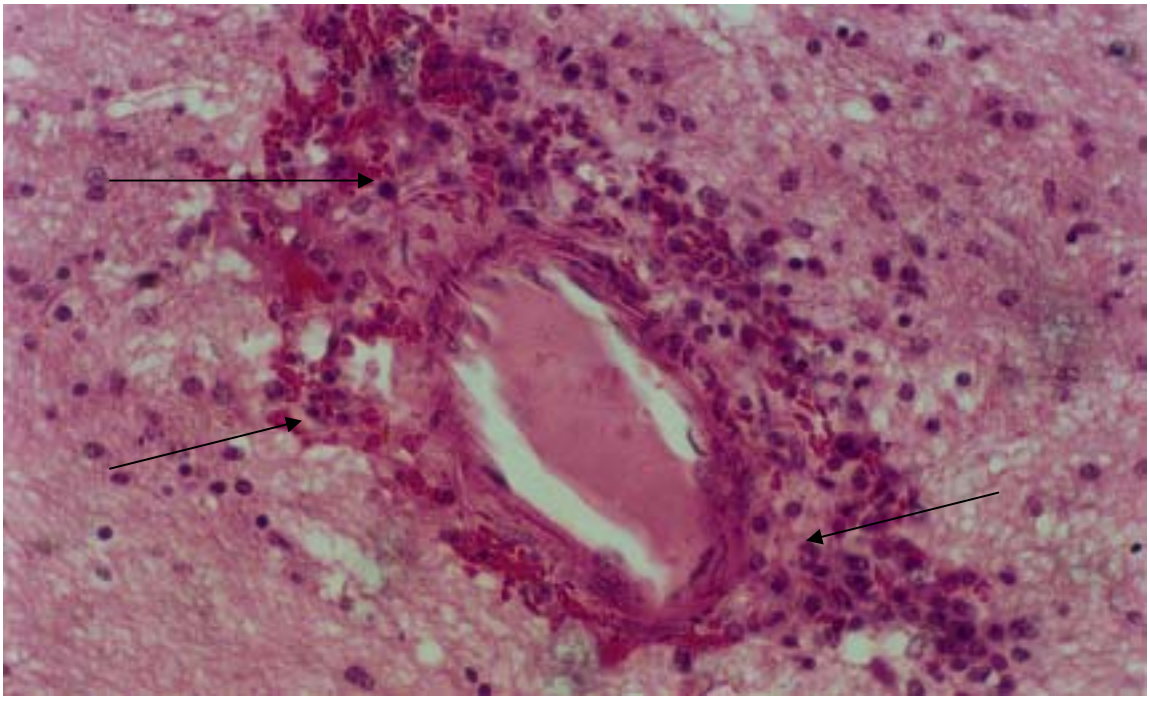
Brain

The brain tissues did not have any significant pathological features up to 14 DPI. Thereafter, the main features observed included discrete inflammation, foci of meningitis characterized by progressive monocellular cell infiltration, perivascular cuffing and congestion of meningeal blood vessels. This progressed with time but was less pronounced in mice infected with KETRI 3741 compared to those infected with KETRI 2537. Infiltration of the brain parenchyma with mononuclear cells was rare and only began to be prominent in mice dying at between 77 and 82 days post infection. In the latter, the brains were characterized by widespread congestion of the blood vessels. Some of these blood vessels were surrounded by lymphocytes, plasma cells and red blood cells (Figs. 4 A and B). The choroids plexus was prominent and contained inflammatory exudates, mainly of mononuclear cells.



Arrow = Mononuclear cells surrounding a blood vessel

Fig. 4 (A). Perivascular cuffing in a brain of a mouse infected with *T. b. rhodesiense* KETRI 3741 for 82 days (Hematoxylin and Eosin, x 250)



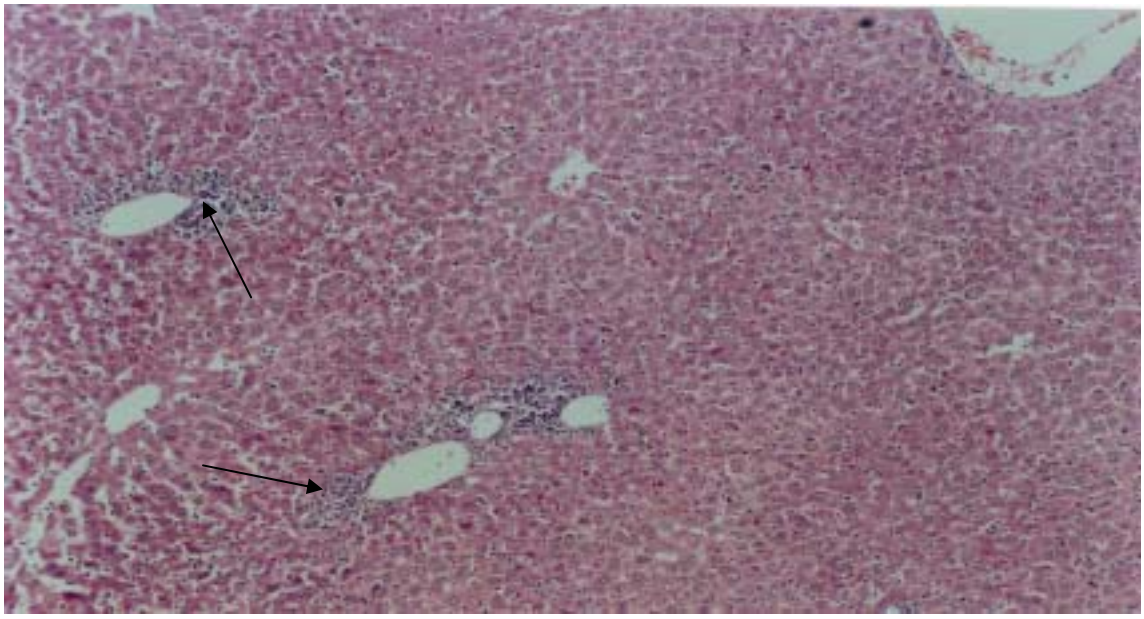
Arrows = Mononuclear cells and hemorrhages surrounding a blood vessel

Fig. 4 (B). Perivascular cuffing in a brain of a mouse infected with *T. b. rhodesiense* KETRI 3741 for 82 days (Hematoxylin and Eosin, x 1,000)

Other organs

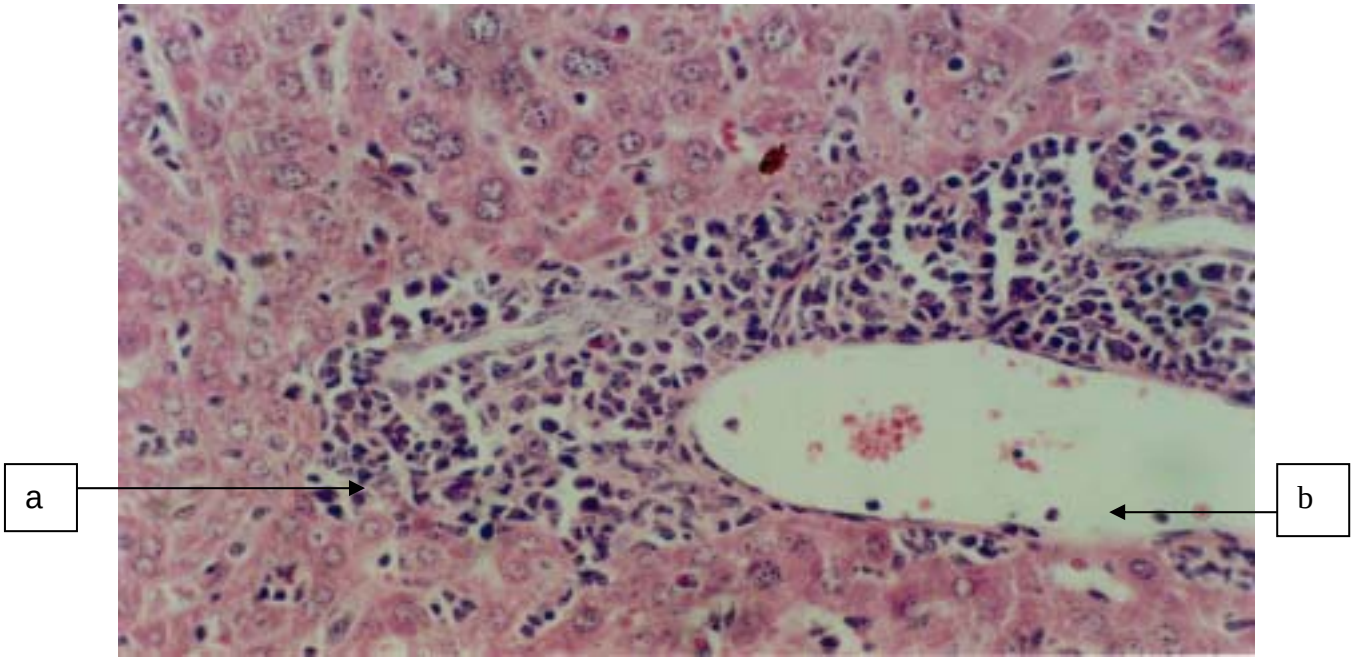
For both group of animals, most of the organs were characterized by mononuclear cellular infiltration into the parenchyma and around the blood vessels (Figs. 5 A and B). The most affected organs were the liver, spleen, heart, and lungs. Apart from tissues obtained on days 7 and 14-post infection when the inflammation was more prominent in mice infected with KETRI 2537, there were no marked differences between the two groups of mice. The germinal centers of the spleens were active with many immature lymphocytes and plasma cells especially on day 14 and 21 PI. However, on day 28 PI these cells were reduced in number.

The lung alveoli and interseptal spaces were infiltrated with inflammatory exudates and cells including lymphocytes, macrophages, and a few neutrophils. This was more prominent in mice over 21 DPI. The liver had extensive lymphocytic infiltration and necrosis of hepatocytes especially in mice sacrificed beyond 38 DPI (Figs. 5 A and B).



Arrows = Mononuclear cells infiltrations

Fig. 5 (A) Infiltration of mononuclear cells in the portal triad of a liver of mouse infected with *T. b. rhodesiense* KETRI 3741 for 77 days (x 250)



a = Mononuclear cells infiltrations, b = Blood vessel

Fig. 5 (B) Infiltration of mononuclear cells in the portal triad of a liver of mouse infected with *T. b. rhodesiense* KETRI 3741 for 77 days (Hematoxylin and eosin x 1,000)

DISCUSSION

The survival time of experimental animal and the pathological changes demonstrates that both KETRI 2537 and 3741 were able to induce a sub-acute to chronic infection in the Swiss-white mice. This contrasts with other *T. brucei* subgroup organisms that produce hyper acute to acute diseases which rarely cause any form of brain pathology. The death of most mice at 35-45 DPI might be due to the intense multiplication of parasites leading to the pathology and organ failure. The incubation periods as well as the

parasitaemia pattern are similar to what was observed earlier in mice infected with EATRO 1989 (Fink and Schmidt, 1979), which is the parental clone of KETRI 2537. Thus, in respect to these two phenotypes the parasite has not changed during the storage.

The results of our study show that at any point of parasitaemia (for both parasites), there were several morphological types of parasites ranging from short stumpy to the long slender forms. At peak parasitaemia (day 8-10) the short stumpy forms constituted the predominant population. This feature has been reported by other workers (Vickerman, 1985; Mathews and Gull, 1994). The slender forms are the multiplicative parasites and appear early in each wave of parasitaemia. However, as the infection progresses these slender forms transforms to a predominance of short stumpy forms, which do not divide and arise as a pre-adaptation for transmission to the tsetse fly (Mathews and Gull, 1994). Thus, due to the presence the pleomorphism, KETRI 3741 can be used in studies involving cyclical transmission.

The pathological changes observed in most organs are characteristic of *T. brucei* infections. Unlike *T. congolense* and *T. vivax*, *T. brucei* is tissue invasive causing cellular infiltration in parenchyma of most organs (Stephen, 1986). The cellular reaction observed in most organs shows that a lymphoid immune reaction is critical in pathogenesis of *T. b. rhodesiense* infection. In the brain the inflammatory reaction starts as meningitis and progresses into Virchows robin spaces before spreading to the parenchyma. In their study, Fink and Schmidt (1979) noted that meningitis in mice occurred as early as 7 DPI, whereas in our study those reactions were observed starting at 21 DPI. Similarly, Fink and Schmidt (1979) observed severe meningoencephalitis in mice as early as day 28 post infection, whereas in the current study the feature only consistently observed in mice which died at between 77 and 82 days after infection. These differences could be attributed to the strain of mice used, whereby the current study utilized Swiss white mice while Fink and Schmidt (1979) used the NMRI mice.

Current drug efficacy trials against late stage trypanosomosis in mice rely on the assumption that trypanosomes have invaded the brain parenchyma by 21 DPI. However, in our study only mild meningitis was reported by this day and parenchymal infiltration started occurring in mice dying 28 days after infection. It is noteworthy that the existing mouse model was developed using the non-human infective *T. brucei* (Jennings *et al.,* 1993), which could have different disease pattern when compared to *T. b. rhodesiense*. Our results show that the *T. b. rhodesiense* KETRI 3741 is suitable for development of a model of a sub-acute to chronic disease in mice. Another method of producing an ideal late stage involves sub-curative treatment with either diminazene aceturate or suramin at 21 days after infection (Jennings and Gray, 1983). Relapses that are accompanied by severe meningoencephalitis are observed within a few weeks. However, the trypanocidals are known to induce brain pathology; and thus it is difficult to interpret the histopathology results. The prior use of a drug in sub-curative doses may also lead to emergence of drug resistance, which may complicate interpretation of efficacy data in case the drug being tested has a similar mode of action.

In conclusion, the study provides evidence of occurrence of a sub acute to chronic trypanosomosis infection in mouse model of HAT. The occurrence of meningoencephalitis in some mice infected with *T. b. rhodesiense* KETRI 3741 indicates that with further improvements, the model may be used in late stage trypanosomosis studies.

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