BEHAVIOUR OF *BABESIA MICROTI* IN MONGOLIAN GERBILS (*MERIONES UNGUICULATUS*) AND THE USE OF TICKS (*HYALOMMA A. ANATOLICUM* AND *IXODES RICINUS*) AS POTENTIAL VECTORS

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SUMMARY

The behaviour of *Babesia microti* in Mongolian gerbils (Meriones unguiculatus) and the use of *Hyalomma a. anatolicum* and *Ixodes ricinus* as vectors was studied. The Mongolian gerbil (Meriones unguiculatus) was found to be a suitable host for *B. microti* and the used Ixodid ticks. Attempts were made to transmit *B. microti* using larvae of *H. a. anatolicum* and *I. ricinus* as vectors. Larvae were fed on gerbils which were known to be exhibiting a parasitaemia of 20-30% infected red blood cells. After moulting the resulting nymphs were applied to uninfected gerbils and the gerbils monitored for the presence of *B. microti* in thin blood smears and by sub-inoculation of blood into uninfected gerbils.

*B. microti* was not detected either in any of the gerbils on which the nymphs had been fed or in any of the sub-inoculated gerbils, suggesting, that under the conditions of this study, *H. a. anatolicum* and *I. ricinus* larvae are incapable of affecting the transmission of this parasite.

Some evidence was obtained, however, that *B. microti* undertook some form of development following the ingestion of infected red blood cells by immature larval stages of both ticks and nymphs of *H. a. anatolicum*. 
INTRODUCTION

*Babesia microti* is a protozoan parasite capable of infecting a range of indigenous and laboratory rodents, and has been frequently used as a laboratory model for the economically important *Babesia* species. Furthermore, interest in *B. microti* has recently increased due to its potential as a zoonosis (Hickerton, 1980).

*B. microti* has been used as an experimental model in tick transmission experiments by several workers (Young, 1970; Barnett and Croft, 1976; Hussein, 1980 and Walter, 1981).

The knowledge about transmission acquired from studies on tick transmission of rodent *Babesia* has been fragmentary due to the limitations of experimental designs or to restricted character of the given information.

The development of *Babesia* in their vectors is still not fully understood and many aspects of *Babesia* morphology, structure, immunogenicity, histocompatibility, and biochemistry have not yet been studied. Heavily infected tick stages or tissues are needed for further research and to this end detailed data on tick transmission of *Babesia* in the laboratory is also needed (Friedhoff and Smith, 1981).

The object of this work was to study the behaviour of *B. microti* in Mongolian gerbils (*Meriones unguiculatus*) and the use of *H. a. anatolicum* and *I. ricinus* as potential vectors. It was hoped that the results of this study would contribute to the development of a suitable model of transmission for studies on babesiosis.
Hyalomma a. anatolicum and Ixodes ricinus

Hyalomma a. anatolicum (Koch, 1844) is an ixodid tick, distributed throughout Southern Europe, Northern Africa, Middle East and Indian subcontinent (Arthur, 1962). Ixodes ricinus (Koch, 1844) is distributed worldwide and is the most common amongst the British ticks (Arthur, 1962).


The morphological characteristics and the life cycles of these ticks have been described by Arthur (1962). The attachment and feeding mechanisms are described by Balashov (1968).

Ixodid ticks are the vectors of several important protozoan blood parasites of domestic and wild mammals. Hyalomma spp. are the vector for Theileria annulata in Asia and Equine babesiosis in its locus. In Europe I. ricinus plays an important role in the transmission of the protozoan of bovine piroplasmosis (Arthur, 1962).

The life cycle of ixodid ticks is summarised by the well known developmental stages of egg, larva, nymph and adult (Soulsby, 1968).

In field conditions H. a. anatolicum behaves as a two or three host tick, in which the first moult takes place on the host
and the second on the ground. The first host is usually a small animal. *I. ricinus* behaves as a three host tick (Balashov, 1968).

Hadani et al. (1969) using gerbils (*Meriones tristani*) studied the life cycle of several ixodid ticks in laboratory and field conditions. In laboratory conditions the tick *Hyalomma excavatum* behaves like a three host tick and its life cycle was described as follows: larva feed for 4 to 15 days, moulted after 6 to 18 days with a nymphal prefeeding period of 6 to 16 days. Nymphs feed for 6 to 14 days and moult after 15 to 27 days. All stages were kept at 28°C.

In the life cycle of *Ixodes ricinus* the larva feed for 4 to 8 days and moult after 42 days, with a maturation period of 10 to 14 days at 11°C. Nymphs feed for 8 days on rabbits and moult after 30 days at 28°C.

The Life Cycle of Babesia in the Tick

The life cycle of *Babesia bigemina* in the tick vector was first studied by Koch (1906) in *Boophilus (Margaropus) australis, Rhipicephalus eversti* and *Hyalomma aegyptium*. In the gut of the tick Koch observed pear-shaped parasites which became ameboid, these forms were apparently associated in pairs to give rise to organisms having at first two nuclei, which later fused to form a single nucleus. He also recorded multiple division stages. Ultimately "clavate" parasites were observed in the gut contents and in the ova.

Later, Riek (1964, 1966) studied the life cycle of *Babesia bigemina* (Smith and Kilborne, 1893) and *Babesia argentina* (Bovis)
(Lignieres, 1903) in their tick vector Boophilus microplus (Canestrini). Riek (1964) found that most of the parasites in erythrocytes were destroyed and that certain spherical bodies survived and developed. Invasion of the epithelial cells of the gut occurred about 24 hours after repletion and subsequent multiplication of the parasite in these cells was by multiple fission. By about 72 hours "vermicules" were produced and released into the haemolymph. On the fourth day some of the "vermicules" invaded the cells of the malpighian tubules and of the haemolymph, a secondary cycle of multiple fission gave rise to vermicules. Others invaded the ova of the tick, and subsequently undergo a similar cycle in the gut cells of the developing larvae, the final cycle takes place in the salivary glands of the nymphs and the infective forms of the parasite appeared 8 to 10 days after larval attachment.

The development of the parasite in the lumen of the tick gut was uncertain, but Riek (1964) described "spiky-rayed" forms which tended to form clumps, as the most obvious forms. These forms were named by Koch (1906) as "strahlenkörper" and described in relation to the sexual cycle of the parasite.

Re-examining the developmental cycle of *B. bigemina* in *Boophilus microplus*, Friedhoff and Busher (1976) rediscovered Koch’s "strahlenkörper". They also suggested that those forms could give evidence of the sexual reproduction of *B. bigemina*.

The current state of the life cycle of *Babesia* in the tick vector has been revised by Friedhoff (1981) and a hypothetical life cycle has been proposed by the author in which indefinite
merogony occurs in normal erythrocytes followed by gamogony in tick gut contents and epithelium and indefinite sporogony in all active stages in various tick tissues.

Mode of Infection and Transmission

As a rule, the engorging female tick is the only stage that can acquire infection from its mammalian host. Babesia from rodents are an exception to this rule; in Nuttallia type babesias the infection is acquired by engorging larvae and transmitted by nymphs. Babesia merionis (syn Nuttallia danii and N. tadhikistanica) and B. microti are examples of this type of transmission (Friedhoff and Smith, 1981).

Tick transmission of piroplasms has been investigated by several workers: (Joyner, et al., 1963; Anthony and Holbrook, 1967; Fay and Rausch, 1969; Mamatkulov, 1970; Donnelly and Pierce, 1975; Stiller and Frerichs, 1979; Lewis and Young, 1980).

Tick transmission of rodent babesias has been achieved as early as 1952 by Adler and Feldman-Musham. The authors, transmitted a Nuttallia spp. to a gerbil (Meriones tristani) by Rhipicephalus sanguineus nymphs infected as larvae.

Tsur, Hadani and Pipano (1960) transmitted Nuttallia danii to gerbils by Hyalomma excavatum (Hyalomma a. anatolicum; Koch, 1844; Hoogstraal and Kaiser, 1959) nymphs infected as larva from gerbils.

Krilov (1965), transmitted Nuttallia tadhikistanica to gerbils (Meriones erythrorus) by H. anatolicum nymphs infected as larva from gerbils. In 1970 Young transmitted B. microti to mice by I. trianguliceps, but Irvin and Brocklesby (1978) failed in the
transmission attempt of B. microti to mice by nymphs of Rhipicephalus appendiculatus.

Barnett and Croft (1976) transmitted B. microti to gerbils by nymphs of I. trianguliceps. B. microti was transmitted by nymphs of I. scapularis and I. pacificus to gerbils and hamsters (Oliveira and Kreier, 1979); and by I. excapularis nymphs to hamsters (Spielman and Piesman, 1979). B. microti transmission was also achieved by Hussein (1980) and Walter (1981) using nymphs of I. canisuga, I. trianguliceps and I. ricinus.

In all these transmission experiments larvae picked up the infection and transmitted it to nymphs, which were infective for the host.

In the mentioned studies Hyalomma spp and Ixodes spp. were predominantly and successfully used as experimental vectors, which stress their importance as experimental models in transmission experiments.

Babesia microti

Babesia microti is a haemoprotozoan parasite which infects indigenous and laboratory rodents.

França (1912) described a piroplasm of Microtus agrestis, which he called Smithia microtia. The parasite has also been known by the synonyms of Nuttallia microti (Coles, 1914) and Babesia rodhaini (Van der Berghe, Vinche, Chardome and Van der Bulke, 1950).

Shortt and Blackie (1965) in a study on the course of infection in laboratory rodents of strains of B. microti found no
differences in infectivity or morphology.

Levine (1971) considered *Babesia microti* to be the same organism as *Babesia rodhaini*, but Killick-Kendrick (1974) on the basis of morphology considered *B. rodhaini* to be distinct from *B. microti*.

Recently, however *B. microti* has been differentiated from other rodent *Babesia* spp. on the grounds of biochemical and immunological studies (Momen, Chance and Peters, 1979; Hickerton and Jones, 1981).

In the latest classification of the protozoan, Levine (1980) now considers *B. microti* a separate species and classified it as follows: Phylum *Apicomplexa*; Class *Sporozoea*; Subclass *Piroplasmea*; Order *Piroplasmida*; Family *Babesidae*; Genus *Babesia*.

The parasite has been recovered from several small mammals in Europe (França, 1912; Coles, 1914; Jacobs, 1953; Shortt, 1961; Shortt and Blackie, 1965; Backer, Chitty and Phipps, 1963; Nowell, 1969; Young quoted by Cox, 1970) and U.S.A. (Tizeer, 1938; Kirner, Barbehein and Travis, 1958; Van Peneen and Duncan, 1968), but recent interest has focussed on its role as a zoonosis (Cox, 1980) and its importance as such was stressed by the successful isolation of the parasite from 19 cases of human babesiosis (Roebush, 1980).

**Morphology**

The morphology of *B. microti* was first described by França (1912) as being mostly annular and 2 to 3 μm in diameter, large piroplasms up to 6.5 μm x 2 μm were also detected along with oval and pyriform types 1.5 μm long and elongated ellipsoidal types.
The chromatin masses occupied peripheral situations and varied in size, from a small compact mass to an elongated peripheral line.

The morphology and behaviour of *B. microti* has been studied in a number of laboratory rodents: *Talpa europaea*, *Apodemus syvalticus*, *Clethrionomys glareolus britannicus*, *Microtus agrestis* (Shortt and Blackie, 1965); *Mus musculius*, *Nastomia saucha*, *Neotoma fuscipes*, *Peromiscus maniculatus*, *Rattus norvegicus* (Levine, 1971); mice and rats (Young, 1970) and a number of morphological stages have been described as developing during the course of infection in these hosts. Shortt and Blackie (1965), however considered that the parasite demonstrated a common pattern of development and differentiation in these hosts and that the predominant morphological stages were the annular forms as first described by França (1912), results from ultrastructure studies on *B. microti* developing in hamster by Rodzinska (1976), suggest that the parasite is highly pleomorphic with the ability to change its shape continuously. Such changes in shape could account for the large number of morphological stages described for these parasites.

**Mode of Infection and Parasitaemia**

*B. microti* can easily be adapted to rats and less easily to mice under laboratory conditions and produces chronic infections. In mice the parasitaemia reaches a peak on day 10–12 after the inoculation of infected red cells. Thereafter the level of parasitaemia declines and in most cases becomes undetectable by the 30th day (Cox, Young and Nowell, 1969; Young, 1970; Hickerton, 1980). Shortt and Blackie (1965) however, found that in multimamate rats (*Praomys natalensis*) a small number of parasites were
continuously present after recovery and that periodic relapses occurred.

Young (1970) found no significant difference in the course of infection produced by three different strains of \textit{B. microti} in gerbils (\textit{Gerbillus gerbillus} and \textit{Meriones tristani}). He showed that infected \textit{G. gerbillus} reached a peak of 13-18\% parasitaemia 13-18 days post infection. While \textit{M. tristani} reached a peak of 6-11\% parasitaemia 16-20 days post infection.

\textbf{Babesia microti as an Experimental Model}

The piroplasms of rodents have been widely used as models for the infections of cattle, especially in the field of chemotherapy (Beveridge, 1953).

In the past three decades the laboratory model for studies on babesiosis in laboratory rodents was usually \textit{Babesia rodhaini}. Rats recovered from \textit{B. rodhaini} infection but mice died in a few days (Nowell, Cox and Young, 1969); \textit{Babesia microti}, however, develops in a more predictable way and does not kill the mice (Cox and Young, 1969). The parasite may therefore provide a suitable model for biological and immunological studies on babesiosis. Consequently in biological studies particular attention has been given to the interaction between host and parasite by means of fine structure studies (Rudzinska \textit{et al.}, 1976; Rudzinska and Tragger, 1977); mechanisms of entry (Rudzinska, 1976); reproduction (Rudzinska, 1976; Rudzinska \textit{et al.}, 1979) and feeding mechanisms (Friedhoff, 1974).

\textit{Babesia microti} has been used as an experimental model for studies on the immunology of babesiosis and the immunization
against babesiosis by Cox and Young (1969); Cox and Turner (1970); Gravelly and Kreier, Clark and Allison (1974); Clark et al. (1975); Perez et al. (1977); Rosenberg and Evans (1979); Roebush, Hanson and Cox (1980); Roebush et al. (1981).

The Mongolian Gerbil

The Mongolian gerbil (Meriones unguiculatus, Milne-Edwards, 1867) is an indigenous small rodent commonly found in Mongolia and China. Simpson (1945) classified Meriones unguiculatus, as a rodent of the family Cricetidae, sub-family Gerbillinae. The genus Meriones is a member of 12 genera with over 300 forms (Rich, 1968).

The name "Mongolian" gerbils refers particularly to Meriones unguiculatus and has a number of common names, sand rat, antelope rat, desert rat and jirds (Rich, 1968).

All laboratory colonies of Mongolian gerbils are descended from the gerbils captured by Kasuga in 1935 (Marston, 1976).

The United Kingdom's population of Mongolian gerbils was established in 1964 from the colony described by Marston and Chang (1965).

Among the characteristics that makes the Mongolian gerbil an attractive experimental host are, its docility towards man, adaptability to a wide range of laboratory conditions without evidence of stress (Schwentker, 1963) and breeding capacity in ordinary systems of management (Marston and Chang, 1965).

Among the reported undesirable characteristics, is their excitability when confronted with an unexpected stimulus, e.g. they can suffer from epileptiform convulsions or become aggressive.
Such behaviour can be a serious problem when gerbils are mismanaged in the laboratory (Marston, 1976).

The real importance of the Mongolian gerbil lies in its broad spectrum of susceptibility to a number of conditions (Schwentker, 1963; Rich, 1968).

Colonies of the Mongolian gerbil were established in Japan as early as 1935 by Kasuga. They were then recommended for Rickettsia studies (Rich, 1968).

Gerbils were recommended as experimental hosts for studies on a number of etiological agents by Schwentker (1963) and their usefulness in this context later confirmed by Rich (1968) and Marston (1976). Consequently gerbils have been used extensively as laboratory models in behavioural studies (Swanson, 1974; Yahr and Kessler, 1975) in microbiological studies (Schwentker, 1963, 1969; Rich, 1968) and in various problems of cholesterol metabolism (Clarkson, King and Warnock, 1957; Gordon, Stolzenber and Cekleniak, 1959; Gordon et al., 1961; Roscoe and Fahrenback, 1962).

A Laboratory Host for Babesia Species

Various genera and species of gerbils have proved to be useful experimental hosts in studies of infections caused by a limited number of haemoproteozoa; Nuttallia spp. (Adler and Felman-Mushman, 1952); Nuttallia danii (Tsur, Hadani and Pipano, 1960, 1963); Nuttallia tadzhikistanica (Krilov, 1965); Nuttallia meri (Gunders, 1971); Babesia microti (Oliveira, 1979); Babesia divergens (Lewis and Young, 1980; Williams, 1980).

The Mongolian gerbil has proved useful for studies on Babesia hyalomysci (Bafort, Timperman and Molineux, 1970); Babesia microti
(Barnett and Croft, 1970); Babesia rodhaini (Mahmoud, 1973); Babesia divergens (Entrican, et al. 1979) and Babesia divergens (Lewis and Williams, 1979).

Befort, Timperman and Molineux (1970) isolated and described Babesia hyalomysci from the tree rat Hyalomyscus stella. The authors also studied the susceptibility of laboratory animals to the parasite by syringe passage. In this study the Mongolian gerbil was found highly susceptible, showing an acute infection and high parasitaemia.

The success in this transmission experiment, proves that the Mongolian gerbil is a useful experimental host in which susceptibility to rodent babesiosis is an important factor.

During a year, Barnett and Croft (1976) studied the incidence of B. microti in the vole Clethrionomys glareolus. Simultaneously, the accuracy of determining natural Babesia infections of C. glareolus from Giemsa stained blood films was assessed by intraperitoneal inoculation of the blood of a number of wild caught rodents into gerbils. The Mongolian gerbil was used as an experimental host because of its susceptibility to B. microti infection.

The authors found the proposed method highly accurate. The accuracy was determined by the ability of Mongolian gerbils to pick up the infection from animals in which parasites were not seen in their blood films.

Entrican et al. (1979) reported a fatal human case of babesiosis in which the isolated organism was shown to be Babesia divergens.
In the microbiological investigations, 0.15 ml of the patient's heparinized blood showing approximately 50 percent parasitised red blood cells, was inoculated intraperitoneally into pairs of rats, cotton rats, guinea pigs, mice, nude mice, hamsters and gerbils. Giemsa stained blood films from the nude mice showed a few parasitised red cells 24 and 48 hours later, but the parasitaemia did not develop. None of the other animals showed parasitized red blood cells with the exception of the gerbils. In this experimental transmission, susceptibility of the Mongolian gerbil to Babesia divergens was shown. The indications are that this useful animal can provide a sensitive method for the isolation of B. divergens from humans.

In Scotland, Lewis and Williams (1979) studied the possibility of infecting Mongolian gerbils with Babesia divergens infected blood from cattle. Intact and splenectomized gerbils were infected by intraperitoneal injection of 0.7 ml of infected blood from a calf. The strain of B. divergens was successfully transmitted and later maintained by syringe passage in gerbils.

The B. divergens gerbil-adapted strain was later inoculated into splenectomized calves and splenectomized control gerbils by injection of 0.2 ml of infected blood. The infection was successfully transmitted but calves reacted mildly. Subsequently the authors showed that B. divergens normally transmitted between cattle by ixodid ticks could be adapted by passage into gerbils and that the gerbil-passage parasite appears to invoke a mild atypical reaction when inoculated into calves.
These studies have indicated that gerbils were potentially valuable experimental hosts for studies on infections caused by Babesia spp. Furthermore, these studies seem to indicate that the Mongolian gerbil could provide a small animal model for B. divergens and the economically important Babesia spp.

A Laboratory Host for Ixodid Ticks

In the field of acarology, various genera and species of gerbils have proved to be useful laboratory animals. Gerbils have been used for studies of experimental transmission of haemoparasites and for rearing of preimaginal stages of some ixodid ticks.

Adler and Feldman-Mushman (1952, 1958) transmitted a Nuttallia spp. to a gerbil (Meriones tristani) by Rhipicephalus sanguineus and Rhipicephalus secundus. Tsur, Hadani and Pipano (1960) successfully transmitted Nuttallia danii to gerbils (Meriones tristani) by nymphs of Hyalomma excavatum and Krilov (1963, 1965) transmitted Nuttallia tadzhikistanica to gerbils (Meriones erythromus) by nymphs of Hyalomma anatolicum.

Hadani et al. (1969) used red tailed gerbils (Meriones tristani) for feeding and rearing preimaginal stages of some ixodid ticks. These gerbils were also used for studies of behaviour and life cycles of the following ixodid ticks: Rhipicephalus sanguineus, Rhipicephalus secundus, Hyalomma excavatum, Hyalomma dromedarii, Hyalomma aegyptium, Haemaphisalis atophila, Haemaphisalis cratica and Ixodes ricinus.

In Britain Barnett and Croft (1976) in a study of the epidemiology of B. microti in the bank vole (Clethrionomis glareolus)
used the Mongolian gerbil (*Meriones unguiculatus*) as experimental host for feeding unfed larva and nymphs of *Ixodes trianguliceps* and to test ticks for infectivity. Partially fed females of *Ixodes trianguliceps* were also allowed to feed on gerbils. The results of this study have shown that only a few feeds were successful. Partially fed ticks removed from *O. glareolus* were rarely attached and fed on gerbils.

The Mongolian gerbil was used as an experimental host by Barnett and Croft (1976) because it is very susceptible to *B. microti* infection. It has been proved to be a suitable host for other ixodid ticks and it makes little attempt to remove ticks by grooming.
EXPERIMENTAL APPROACH

This study attempted the experimental transmission of Babesia microti by ixodid ticks, to the Mongolian gerbil host.

Two species of ixodid ticks, *Hyalomma a. anatolicum* and *Ixodes ricinus* were used as vectors in this study due to their availability in the C.T.V.M. Entomology Laboratory, the ease with which they could be maintained under laboratory conditions and their importance as vectors of protozoal diseases.

Preliminary experiments were conducted with larval and nymphal stages of these ticks, to establish the optimum conditions for attachment and feeding on the experimental host.

Initial studies were conducted to establish the course of infection of Babesia microti in gerbils so that a reproducible and predictable infection level could be provided as a source of tick infection.

The Mongolian gerbil (*Meriones unguiculatus*) was used in this study as a source of infection for the tick vectors and as a means of monitoring the infectivity of the ticks due to their susceptibility to *B. microti* infection and their suitability as a host for ixodid ticks.

From the results obtained during the initial studies on the behaviour of *B. microti* in gerbils and on the attachment and feeding behaviour of the ticks on gerbils it was decided to attempt the experimental transmission of the haemoparasite, by feeding large numbers of *Hyalomma a. anatolicum* and *Ixodes ricinus* larvae onto infected gerbils. After moulting to the next instar ticks would be
applied to uninfected gerbils which would then be monitored for the presence of *Babesia* infection. Moulting larvae and nymphs would also be monitored for the presence of developmental stages of the parasite.
MATERIALS AND METHODS

The Vectors

The colony of *H. a. anatolicum* was derived from ticks supplied by Professor B.S. Gill from a colony of ticks isolated near Ludhiana, Punjab, India. The identity of the ticks has been corroborated by Hoogstraal (personal communication to A.R. Walker).

The colony of *I. ricinus* was derived from ticks isolated near Lochaline, West Scotland. The original isolated ticks comprised a mixture of all instars totalling 200 individuals. The identity of the ticks was according to Walker (personal communication).

Unfed and uninfected larvae and nymphs of both *Hyalomma a. anatolicum* and *Ixodes ricinus* were obtained from the respective tick colonies of the entomology laboratory of the Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian.

The Parasites

An example of *Babesia microti* (King's strain) was obtained from the low temperature storage bank of the Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian and was originally obtained from Professor D.W. Brocklesby, Institute for Research into Animal Diseases, Compton, (Brocklesby, personal communication).

The Experimental Hosts

Both sexes of random bred albino mice and natural colour and white Mongolian gerbils were obtained from the small animal house of the Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian.
Mice were fed *ad lib* on a balanced commercially available diet. Mongolian gerbils were fed *ad lib* on a similarly obtained diet for rats. Both experimental hosts had constantly available water.

Cages of identical design were used throughout. Mice were caged in groups and gerbils were caged individually and identified by means of cage cards, showing *B. microti* code number (TREU).

**Viability of Mice-adapted Stabilate**

After storage for eight months at -196°C, a mice-adapted cryopreserved stabilate of *Babesia microti* was checked for viability. Two capillary tubes of the stabilate material (TREU 1550, Figure 5) were thawed in a jar of phosphate buffered saline (PBS) pH 7.2 at room temperature and diluted with PBS pH 7.2 to a total of 0.2 ml which was on two occasions injected intraperitoneally into each of two mice using 16-5 hypodermic needles fitted to a 1.0 ml plastic disposable syringe.

**Withdrawal of Blood from Experimental Host**

Mice and gerbils were placed in a glass jar containing cotton wool and anaesthetic ether (May and Baker Ltd.) until adequately anaesthetised. They were then removed from the jar and pinned out on a cork board ventral side uppermost. A small pad of cotton wool soaked in ether was placed over the head to ensure continuing anaesthesia. The rib cage was reflected anteriorly exposing the heart and blood withdrawn from the right ventricle of the heart through a 16-5 needle into 1.0 ml sterile plastic disposable syringe containing ten international units of heparin (Pularin, Duncan, Flockhart
and Company Limited) in 0.1 ml PBS pH 7.2.

**Infection of Mice**

From stablate initiated infection in a mouse (No. 2), 0.1 ml of blood containing $9.6 \times 10^7$ *B. microti* infected red blood cells per ml was injected intraperitoneally into a mouse (No. 7, Figure 5, Table 9) using a 16-5 hypodermic needle fitted to a 1.0 ml plastic disposable syringe.

From the blood of another mouse (No. 1, Figure 4, Table 9) containing $8.1 \times 10^8$ ml *B. microti* infected red blood cells, four serial dilutions were made ($10^7$, $10^6$, $10^5$ and $10^4$ ml) four mice (Nos. 3-6) were each injected intraperitoneally with 0.1 ml of each dilution (Figure 5, Table 9).

**Adaptation of Babesia microti to the Gerbil**

*Babesia microti* was adapted to the Mongolian gerbil (*Meriones unguiculatus*) by intraperitoneal inoculation of 0.1 ml of mouse blood containing $3.7 \times 10^7$ ml infected red blood cells (No. 3, Figure 5, Table 9).

**Monitoring Infections in Mice and Gerbils**

During the observation period *Babesia microti* infections in mice and gerbils were monitored by means of thin blood smears.

On the first day after inoculation, the hair of the gerbil's tail tip was cut. After this, the blood was obtained by tipping the tail end. The tail was gently massaged and the second drop of blood was collected in a slide previously washed for 24 hours in a mixture of methanol/ether 50:50, and the slide identified by means of a diamond pen (Plate 1).
PLATE 1  Method of sampling blood from gerbils
Thin blood smears were prepared daily and processed as follows: the smears were immediately air dried, fixed in methanol for five minutes and stained in ten percent Giemsa stain (Gurr) diluted in buffered water (pH 7.2) in a coplin jar for 20 minutes. The stained slides were differentiated in buffered water at pH 7.2, air dried and examined by light microscopy, under an oil immersion objective lens at x 1000 total magnification for the presence of parasites.

A minimum of ten fields from the tail of the smear containing approximately 2000 red blood cells were examined and the proportion of parasitised red cells expressed as a percentage of the total red cells counted.

Preparation of Working Stabilates

After adaption of the mouse derived strain of *B. microti* to Mongolian gerbils (*Meriones unguiculatus*) working stabilates were made to ensure the supply of similar infective parasites for further experiments.

One gerbil (No. 8, Figure 5, Table 9) was exanguinated into heparin as described previously and the blood was mixed by gentle inversion. The blood from the gerbil was divided into 0.5 ml aliquots and to each of these was added 88 μl of a 50% solution of dimethyl sulphoxide (DMSO) in PBS pH 8.0 to give a final DMSO concentration of 7.5%. The mixture was then allowed to flow into sterile 6.4 cm long glass capillary tubes until they were two-thirds full, the ends heat-sealed and the tubes cooled rapidly to -196°C by placing them in a liquid nitrogen refrigerator. Each stabilate was given a unique identifying number (TREU - Trypanosomiasis
Two examples of stabilates (TREU 1616 and 1624; Figure 5, Table 9) were made on different occasions. The original parasitaemia of the blood used to prepare TREU 1616 was $9.6 \times 10^8$ infected red blood cells/ml, while that of the blood used to prepare TREU 1624 was not determined.

After storage for one month at $-196^\circ C$ the viability of stabilates was checked by the intraperitoneal injection of the contents of two capillaries of each stabilate into a single gerbil. This was repeated on one (TREU 1616) and two further occasions (TREU 1624) (Gerbil Nos. 5, 6, 7, 18, 19; Figure 5, Table 9). The course of infection of the parasite was studied daily by means of thin blood smears stained with Giemsa stain (Gurr).

**Preliminary Infections in Gerbils**

Preliminary tests were done to study the course of infection in animals infected with the gerbil-adapted strain of *Babesia microti*. A group of six gerbils (Nos. 8-13, Figure 5, Table 9), of both sexes, four months old were injected with 0.1 ml of blood containing $1.7 \times 10^7$ infected red blood cells/ml. The animals were inoculated and the infection monitored as described before.

Preliminary tests in mice showed that the prepatent period and the severity of the infection depended on the number of infected red blood cells in the inoculum. Establishing a standard inoculum, therefore, became necessary. This was done by inoculating gerbils with series of dilutions of *B. microti* infected gerbil blood.
Four serial dilutions were prepared from gerbil (Figure 5, Table 9) blood containing 5.1 x 10^9 infected red blood cells per ml. 0.1 ml of blood was diluted in 0.9 ml of sterile PBS and from this four further dilutions were made (10^7/ml, 10^6/ml, 10^5/ml and 10^4/ml). Each one of a group of four gerbils (Nos. 14-17, Figure 5, Table 9) were injected intraperitoneally with 0.1 ml of each blood dilution. The animals were inoculated and the gerbils were monitored for the presence of parasites as described before.

As a result of this experiment an infected red blood cell concentration of 10^7/ml was selected as an inoculum for further studies. Gerbils injected with this concentration of infected red blood cells produced parasitaemia levels of 22-32% (Table 4) in the first 12 days of infection which was judged to be a suitable level of infection for attempts at transmission of the parasite by ticks.

Infection in Gerbils

The original source of Babesia microti infection for Mongolian gerbils was derived from gerbil-adapted stabilates (TREU 1616). Two gerbils (Nos. 25 and 26), three months old were injected with two capillaries diluted in sterile PBS at pH 7.2 to a volume of 0.3 ml. The blood from which stabilates were prepared had contained 9.6 x 10^8/ml infected red blood cells (Table 9).

Due to the apparent unpredictable nature of B. microti infections initiated directly from stabilate material, further experiments were carried out with gerbils infected from freshly collected B. microti infected gerbil blood obtained after one or two passages from stabilate infected gerbils (Nos. 27, 46, 59, 60, 61; Figure 5).
When establishing a standard infected red blood cell concentration, twelve gerbils were infected (Nos. 34-45, Figure 5). Four gerbils of both sexes and three months old were infected with 0.1 ml of the following dilutions: $4.8 \times 10^7$/ml, $4.8 \times 10^6$/ml, $4.8 \times 10^5$/ml. The dilution of $10^7$ was chosen and tested as a reliable source of infection for further experiments. For this purpose twelve gerbils (Nos. 47-68, Figure 5), of both sexes and two to five months old were inoculated with $6.5 \times 10^7$/ml infected red blood cells.

For the study of the course of infection of *B. microti* in the experimental hosts, a group of six female gerbils (Nos. 28-33, Figure 5), four to five months old were injected with $8.0 \times 10^7$/ml infected red blood cells.

**Tick Feeding**

When feeding larvae or nymphs in infected or uninfected gerbils, similar metal mesh cages of 10.5 x 10.5 x 10.5 cm with a base of aluminium foil and covered with hay were used. Gerbils were confined individually for a period of 24 hours to allow ticks to attach. Cages were identified with the gerbil number and placed on plastic dishes. They were then arranged on a metal tray containing a 0.5% solution of "Savlon" (I.C.I.) (Plate 2).

One thousand larvae per gerbil (Nos. 3, 4, 10-13, 47-58, Figure 6) of both *H. a. anatolicum* and *I. ricinus* were spread on the gerbil's body, the remaining ticks in the plastic container were left in the cage for four hours to allow ticks to search for the host. After this period of time the plastic containers and caps were removed to avoid the gerbils eating them.
PLATE 2 Method of caging gerbils for tick application

PLATE 3 Gerbil caging after tick application and method for the prevention of tick scape. The arrow indicates an accumulation of engorged ticks.
Two hundred Ixodes nymphs were fed on gerbils (Nos. 64-73, Figure 6) following the same procedure used for larvae feeding.

Gerbils were fed on a rat commercially balanced diet as previously described but water deprived for the period of tick attachment (12-24 hours). Larvae and nymphs were identified by means of a feeding number.

After the attachment period the gerbils were transferred individually to plastic cages of similar size (31 x 31 x 11 cm) and containing a bed of wooden shavings. Food and water were constantly available. Gerbils were identified by cage cards showing experimental number.

The cages were transferred to a metal tray which was placed on a larger metal tray containing approximately 0.5% solution of "Savlon" (I.C.I.) (Plate 3).

Maintenance of Tick Colonies

Ticks of all instars were maintained by feeding on rabbit ears. The ticks were kept in tissue paper (Kleenex) on plastic containers with metal gauge. Ixodes ticks were kept at a temperature of 18°C, 65% relative humidity and 16 hours daylight period. Hyalomma ticks were kept in the same conditions, except that moulting was carried out at 28°C.

Ticks for use in Behaviour and Transmission Experiments

The batches of ticks used in the experiments were as identical as possible, in that they came from one or more egg batches laid at approximately the same time and which had been reared on the same host subsequently.
Tick Maintenance and the Development of *B. microti* in Ticks

Larvae (1000/animal) and nymphs (100-200/animal) of *Hyalomma* and *Ixodes* ticks were infected by feeding them on *Babesia microti* infected gerbils. Ticks were applied at a time when it was judged that the parasitaemia was at a maximum as described before, but cages were covered with fine nylon mesh bags, either individually in the case of larvae or in groups of three in the case of nymphs (Plate 3). After detachment engorged larvae and nymphs were collected daily from the bags (Plate 4). Engorged larvae were also collected from the cages by means of an electrical pump (Plate 5) and a paint brush (No. 6 sable water colour brush) as described by Hosie and Walker (1979).

While moulting to the next instar, ticks were kept under laboratory conditions described before. After this, a minimum ten day period of maturation at a temperature of 18°C was given to both species of ticks.

The development of the parasite in the tick was monitored daily during the moulting period by means of smeared preparations. Smeared preparations of larval tissues were made by compression of the larva's body in between forceps and smearing the released contents on a clean slide previously washed in a mixture of methanol, ether 50:50. Smeared preparations of nymph tissues were made by smearing the tick contents on a clean slide (as described before) after perforation of tick cuticle over the dorsal anterior area of the tick's body, with a needle and compression of the body with forceps, as described before.
PLATE 4 Close-up of engorged larvae collected in the preventive bag after detachment.

PLATE 5 Method of collecting engorged larvae from cages.
Figure 4 shows the typical course of infection in gerbils infected with different levels of infection.

The course of Babesia microti infection in gerbils

The course of *Babesia microti* initiated with $8.0 \times 10^7$ infected red blood cells was studied in a group of six female gerbils, five months old (gerbils Nos. 28-33, Table 9) to assess the suitability of this inoculum for transmission studies. The pre-patent period for five of the gerbils was 24 hours, and for one gerbil six days. The parasitaemia rose to a peak between 33 and 65.2% infected red blood cells, six days after infection.

Two gerbils died nine to ten days after infection with parasitaemias of 42.4 to 65.2% infected red blood cells. Two of the remaining gerbils showed a second rise in blood parasite levels twelve days after infection which rose to a peak of 6.6 to 9.8 infected red blood cells, before falling again with few parasites present at 26 days post infection. The remaining gerbils showed low levels of parasitaemia (less than one percent) for 16 days. These results are summarised in Table 6.

Feeding performance of immature instars

Experiments were carried out to test the suitability of gerbils as host for the immature instars of *Hyalomma a. anatolicum* and *Ixodes ricinus* and to establish the feeding performance of these ticks.

The feeding performance of the immature instars of *I. ricinus* was studied in six gerbils and the feeding performance of *H. a. anatolicum* was studied in 23 gerbils (Figure 6). The results are summarised in Tables 7 and 8.
Slides were identified by means of a diamond pen and stained with 10% Giemsa stain (Gurr) with added Azur II (Shortt, 1966) and diluted in buffered water pH 7.2.

Transmission Experiments

To attempt experimental transmission of *Babesia microti* by the tick vectors a group of gerbils of both sexes, two to five months old were injected with a standard concentration of *B. microti* infected red blood cells. At the time of maximum parasitaemia 1000–2000 *Hyalomma a. anatolicum* and *Ixodes ricinus* larvae were applied for feeding. Nymphs derived from the above larvae were tested for infectivity by application to uninfected gerbils, which were then monitored daily for evidence of clinical signs of babesiosis and the presence of parasites in thin blood smears. Gerbils that fed *I. ricinus* nymphs were monitored for a 30 day period. Gerbils that fed *H. a. anatolicum* nymphs were monitored for a 20 day period after which blood from two gerbils (Nos. 77 and 79, Figure 6) was injected into a further two gerbils (Nos. 81 and 82, Figure 6), which were monitored for the presence of *B. microti* infection for a ten day period due to the limited time.

Twelve gerbils (Nos. 47–68, Figures 5 and 6) were inoculated intraperitoneally with 0.1 ml of a concentration of 6.5 x 10⁷/ml *B. microti* infected red blood cells and at the appropriate parasitaemia 1000 *H. a. anatolicum* larvae per gerbil were applied for feeding. Following the same procedure, two gerbils (Nos. 12 and 13, Figures 5 and 6) were inoculated with 1.7 x 10⁷/ml *B. microti* infected red blood cells and at the appropriate parasitaemia 2000 *I. ricinus* larvae were applied for feeding.
After feeding, detached larvae of both tick species were collected and allowed to moult and mature as described in a previous section. For the transmission experiments 100 of the resultant *H. a. anatolicum* nymphs per gerbil, were fed on a group of four gerbils (Nos. 77-80, Figure 6) and 200 were fed on a group of three gerbils (Nos. 74-76, Figure 6). The development of the parasite in both instars were monitored daily during the moulting period of ticks as described in a previous section.

Nymphs of both ticks were tested for infectivity by application to susceptible gerbils. After tick feeding the gerbils were then monitored for both evidence of clinical signs and determination of percentage of parasitaemia. Twenty days post application on gerbils *Hyalomma a. anatolicum* and *Ixodes ricinus* nymphs were tested for infectivity by syringe passage of two gerbils' (Nos. 77 and 79) blood on two gerbils (Nos. 81 and 82). Infection in them was monitored for ten days (Figure 6).
RESULTS

PRELIMINARY STUDIES ON BEHAVIOUR OF BABESIA MICROTI

Viability and course of infection of Babesia microti mice-derived stabilate in mice

Mouse-derived stabilate (TREU 1550) was shown to be infective after inoculation into mice (Nos. 1 and 2) with a prepatent period of 7-8 days. The mice showed a rising parasitaemia during the 14-17 day observation period with maximum parasitaemia levels of 12.8 to 30.2% infected red blood cells. These results are shown in Table 1.

The prepatent period of a mouse (No. 7) infected with B. microti stabilate TREU 1616 was four days. Once established, the parasitaemia in this mouse increased rapidly to a peak of 21% infected red blood cells, twelve days after injection before declining to levels less than one percent in 26 days. This level of parasitaemia persisted until the end of the 31 day observation period. These results are shown in Table 1.

Course of infection and mortality in mice injected with different numbers of Babesia microti infected red blood cells

The prepatent period of mice (Nos. 3-6) injected with different numbers of B. microti red blood cells (Table 2) varied from one to eleven days. Maximum levels of infection were reached in 11 to 28 days post infection (24-82% infected red blood cells). Parasites were not detected in the animals after 27 to 45 days after infection. A mouse injected with the highest number of infected red blood cells rapidly developed an infection and died seven days
later. The course of infection of mice injected with different numbers of *B. microti* infected red blood cells is shown in Figure 1.

Viability and course of infection of Babesia microti mouse-derived stabilate in gerbils

Mouse-derived stabilate (TREU 1616) was shown to be infective after inoculation into gerbils (Nos. 6 and 7, Figure 5). The resulting infection had a prepatent period of 15-17 days, after which the parasitaemia rose to a maximum level of 20% 18-23 days post inoculation. Twenty four days after inoculation the parasitaemia decreased to a level less than one percent and the parasitaemia persisted at this level until the end of the 30 days period of observation. The resultant course of infection for these gerbils is shown in Figure 2.

The course of *B. microti* infection derived from mouse stabilate was repeated in gerbils (Nos. 5, 25, 28, Figure 9) with a resultant prepatent period of nine to eleven days and a maximum level of infection of 20%, which was reached 15 to 19 days after inoculation.

Babesia microti adaptation to gerbils

*Babesia microti* infected mouse blood (Mouse No. 3, Table 9) was passaged by intraperitoneal injection in gerbils (Nos. 1 and 2, Table 9).

Gerbils were found to be susceptible and the parasites were first detected four to five days after inoculation.

*B. microti* infected gerbil blood (Gerbil No. 2, Table 9) was passaged into a further gerbil (No. 4, Table 9). Parasites were
TABLE 1  Course of infection of *Babesia microti* mice derived stabilate in mice

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>TREU</th>
<th>Prepatent period (days)</th>
<th>Maximum level of infection (%)</th>
<th>Peak of infection (days)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
</tr>
</thead>
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<tr>
<td>1*</td>
<td>1550</td>
<td>7</td>
<td>12.8</td>
<td>-</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>2*</td>
<td>1550</td>
<td>8</td>
<td>30.2</td>
<td>-</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>1616</td>
<td>4</td>
<td>21.2</td>
<td>12</td>
<td>26</td>
<td>31</td>
</tr>
</tbody>
</table>

*killed

TABLE 2  Course of infection in mice injected with different numbers of *Babesia microti* infected red blood cells

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Inoculum (T. Inf. RBC)</th>
<th>Prepatent period (days)</th>
<th>Maximum level of infection (%)</th>
<th>Peak of infection (days)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3*</td>
<td>$8.1 \times 10^7$</td>
<td>1</td>
<td>82.2</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>$8.1 \times 10^6$</td>
<td>2</td>
<td>24.0</td>
<td>11</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>$8.1 \times 10^5$</td>
<td>3</td>
<td>29.0</td>
<td>13</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>$8.1 \times 10^4$</td>
<td>11</td>
<td>30.0</td>
<td>28</td>
<td>43</td>
<td>45</td>
</tr>
</tbody>
</table>

*died*
Figure 1  The course of infection in mice injected with different numbers of Babesia microti infected red blood cells.
Figure 1
Figure 2 The course of infection in gerbils injected with stablilates of *Babesia microti* (1616).
Figure 2
first detected in this gerbil 24 hours after infection reaching a maximum level of parasitaemia (21.8%) seven days after inoculation. Parasites were detected at various levels in the blood of the gerbil for 24 days after inoculation.

As a means of providing reliable sources of infection for further studies on the behaviour of *B. microti*, gerbil-derived stabilates were prepared (TREW 1624) and tested for infectivity in gerbils (Nos. 18 and 19, Figure 5). One gerbil became infected showing a patent infection 15 days after inoculation, dying of babesiosis 21 days post inoculation with 57% parasitaemia. The other gerbil did not show a patent infection during the 26 day observation period.

Course of infection of *Babesia microti* in gerbils

Preliminary studies on the behaviour of *Babesia microti* were undertaken in a group of four gerbils (Nos. 10-13, Table 9) of both sexes, five months old injected with *B. microti* infected gerbil red blood cells. All the animals were susceptible in that they developed a patent parasitaemia two to three days after inoculation. The maximum levels of 27 to 30% infected red blood cells were reached eight to ten days post inoculation, after which the parasitaemia decreased rapidly to a level of less than one percent 16 days after inoculation. These low levels of parasitaemia persisted for a further six to eight days after which parasites were not seen in the blood smears for several days until the end of the observation period. One gerbil died after 11 days. These results are shown in Table 3.
Figure 3  The course of infection in three gerbils injected with different numbers of Babesia microti infected red blood cells.
Figure 3
### TABLE 3 Course of infection in gerbils with a number of *Babesia microti* infected red blood cells

<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Inoculum (T. Inf. RBC)</th>
<th>Prepatent period (days)</th>
<th>Maximum level of infection (%)</th>
<th>Peak of infection (days)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
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<td></td>
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</tr>
<tr>
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<td>2</td>
<td>27.8</td>
<td>8</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>3</td>
<td>29.0</td>
<td>10</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
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<td></td>
<td>2</td>
<td>28.2</td>
<td>8</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

*died*

### TABLE 4 Course of infection in gerbils with different numbers of *Babesia microti* infected red blood cells

<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Inoculum (T. Inf. RBC)</th>
<th>Prepatent period (days)</th>
<th>Maximum level of infection (%)</th>
<th>Peak of infection (days)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
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</thead>
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<td>4</td>
<td>28.8</td>
<td>9</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>15</td>
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<td>4</td>
<td>22.0</td>
<td>12</td>
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<td>19</td>
</tr>
<tr>
<td>16</td>
<td>$5.1 \times 10^5$</td>
<td>6</td>
<td>31.8</td>
<td>10</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>17</td>
<td>$5.1 \times 10^4$</td>
<td>NPS</td>
<td>NPS</td>
<td>NPS</td>
<td>NPS</td>
<td>19</td>
</tr>
</tbody>
</table>

NPS = no parasites seen
Course of Babesia microti infection and mortality in gerbils injected with different numbers of infected red blood cells

Preliminary studies on the effect of infection initiated with different numbers of infected gerbil red blood cells was undertaken on a group of four gerbils of both sexes, five months old (Gerbils Nos. 14-17, Table 9). The gerbils were injected with $5.1 \times 10^7$ to $5.1 \times 10^4$ B. microti infected red blood cells. The prepatent period for the gerbils injected with $5.1 \times 10^7$ and $5.1 \times 10^6$ infected red blood cells was four days, while the prepatent period for the gerbil injected with $5.1 \times 10^5$ infected red blood cells was six days. The maximum levels of parasitaemia obtained varied from 22 to 31.8% of infected red blood cells and were reached nine to twelve days after inoculation. Parasites were detected in the blood of each animal at varying levels for 16 to 23 days after inoculation.

Fatalities did not occur and one gerbil injected with $5.1 \times 10^4$ infected red blood cells did not develop a patent parasitaemia. These results are summarized in Table 4.

Figure 3 shows the different course of infection for these gerbils.

FURTHER STUDIES ON BEHAVIOUR OF BABESIA MICROTI

Studies on the course of infection of Babesia microti in gerbils infected with different numbers of infected red blood cells

The course of infection of Babesia microti in gerbils injected with different numbers of infected red blood cells was studied on a group of 12 gerbils of both sexes and three months old (Gerbils
The prepatent period of four gerbils (Nos. 34-37) injected with $4.8 \times 10^7$ *B. microti* infected red blood cells was 24 hours. Thereafter the parasitaemia rose to a peak between 29.4 and 38.6% infected red blood cells five to six days after infection, before declining to a level of less than one percent 17 to 19 days later. All the gerbils in this group showed a second rise in blood parasite levels 12 to 14 days after infection which rose to a peak between 2.2 and 15% infected red blood cells, before falling again with few parasites present 23 to 25 days after infection. Fatalities did not occur.

The prepatent period of the four gerbils infected with $4.8 \times 10^6$ *B. microti* infected red blood cells (Gerbils Nos. 38-41, Table 9) was two days. The parasitaemia in this group of gerbils rose to a peak between 22.8 and 27.8% infected red blood cells, eight to ten days after infection before declining to low levels (less than one percent) nine to eleven days later. None of the gerbils showed a second rise of infection and deaths did not occur.

The prepatent period of the four gerbils infected with $4.8 \times 10^5$ *B. microti* infected red blood cells (Gerbils Nos. 42-45, Table 9) was four days. The parasitaemia in this group rose to a peak between 21.6 and 26% infected red blood cells nine to eleven days after infection before declining to low levels (less than one percent) nine days later. Deaths did not occur. None of the gerbils of this group showed a second rise of infection, but one reached the peak parasitaemia eleven days after. These results are summarised in Table 5.
PLATE 6  *Babesia microti* merozoites in gerbil red blood cells (x 500 magnification)
rf  ring form
p  periform
mf  maltese cross form
eb  evidence of budding
ym  young merozoites
<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Inoculum (T. Inf. RBC)</th>
<th>Prepatent period (days)</th>
<th>Maximum level of infection (%)</th>
<th>Peak of infection (days)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td></td>
<td>1</td>
<td>29.4</td>
<td>5</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>35</td>
<td>4.8 x 10^7</td>
<td>1</td>
<td>32.2</td>
<td>6</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>1</td>
<td>38.6</td>
<td>6</td>
<td>10</td>
<td>23</td>
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<td>37</td>
<td></td>
<td>1</td>
<td>37.0</td>
<td>6</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>2</td>
<td>22.8</td>
<td>9</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>39</td>
<td>4.8 x 10^6</td>
<td>2</td>
<td>26.8</td>
<td>9</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>2</td>
<td>27.8</td>
<td>8</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td>2</td>
<td>23.0</td>
<td>10</td>
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<td>23</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>4</td>
<td>26.0</td>
<td>9</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>43</td>
<td>4.8 x 10^5</td>
<td>4</td>
<td>24.0</td>
<td>9</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
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<td></td>
<td>4</td>
<td>22.4</td>
<td>9</td>
<td>13</td>
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<td>45</td>
<td></td>
<td>4</td>
<td>21.6</td>
<td>11</td>
<td>15</td>
<td>24</td>
</tr>
</tbody>
</table>
Figure 4 The course of infection in gerbils injected with different numbers of *Babesia microti* infected red blood cells. Each point is the mean value for 4 animals and the vertical bar in each point represents the standard deviation of the mean.
Figure 4
<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Inoculum (R. Inf. RBG)</th>
<th>Prepatent period (days)</th>
<th>Peak of infection (days)</th>
<th>Maximum level of infection (%)</th>
<th>Duration of infection (days)</th>
<th>Observation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28*</td>
<td>8.0 x 10^7</td>
<td>1</td>
<td>6</td>
<td>42.2</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>6</td>
<td>6</td>
<td>36.4</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>30*</td>
<td></td>
<td>1</td>
<td>6</td>
<td>65.2</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>31</td>
<td></td>
<td>1</td>
<td>6</td>
<td>58.6</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>1</td>
<td>6</td>
<td>33.0</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>1</td>
<td>6</td>
<td>33.4</td>
<td>6</td>
<td>25</td>
</tr>
</tbody>
</table>

* died
### TABLE 7  Feeding performance of immature instars of *Ixodes ricinus*

<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Tick Species</th>
<th>Feeding Code</th>
<th>Instar</th>
<th>Numbers applied (p. Ger)</th>
<th>Numbers recovered (Total)</th>
<th>Pre feeding period (days)</th>
<th>Feeding period (days)</th>
<th>Peak feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td><em>Ixodes ricinus</em></td>
<td>14</td>
<td>Larvae</td>
<td>2000</td>
<td>200</td>
<td>10</td>
<td>2/3</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td><em>Ixodes ricinus</em></td>
<td>15</td>
<td>Larvae</td>
<td>2000</td>
<td>200</td>
<td>10</td>
<td>2/3</td>
<td>6</td>
</tr>
<tr>
<td>74-76</td>
<td><em>Ixodes ricinus</em></td>
<td>19</td>
<td>Nymphs</td>
<td>200</td>
<td>198</td>
<td>33</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

### TABLE 8  Feeding performance of immature instars of *Hyalomma a. anatolicum*

<table>
<thead>
<tr>
<th>Gerbil No.</th>
<th>Tick Species</th>
<th>Feeding Code</th>
<th>Instar</th>
<th>Numbers applied (p. Ger)</th>
<th>Numbers recovered (Total)</th>
<th>Pre feeding period (days)</th>
<th>Feeding period (days)</th>
<th>Peak feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>47-58</td>
<td><em>Hyalomma a. anatolicum</em></td>
<td>241</td>
<td>Larvae</td>
<td>1000</td>
<td>2400</td>
<td>20</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>62-73*</td>
<td><em>Hyalomma a. anatolicum</em></td>
<td>243</td>
<td>Nymphs</td>
<td>200</td>
<td>240</td>
<td>20</td>
<td>3</td>
<td>1-6**</td>
</tr>
<tr>
<td>77-80</td>
<td><em>Hyalomma a. anatolicum</em></td>
<td>245</td>
<td>Nymphs</td>
<td>100</td>
<td>279</td>
<td>69.7</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

* Some gerbils did not become infected (6)

** Some gerbils died (5) in the tick feeding period
In all experiments gerbils of both sexes between two and five months old proved to be suitable in that larvae and nymphs of the ticks readily attached and fed until fully engorged. Very little attempt was made by the gerbils to remove ticks by grooming, but on the day of application they showed some degree of irritation.

The pattern of feeding is described as follows: immature instars of the ticks started to detach in an engorged state three days after application. The peak of detachment was three days later (six days from application). After reaching the peak of detachment, the numbers of detached ticks decreased rapidly until the end of the feeding period which was six to seven days. After detachment ticks left the gerbil's cage and were found in the bags (Plate 4). No fatalities could be ascribed to the ticks alone, but five out of six B. microti infected gerbils (Nos. 64, 65, 70, 72, 73) on which Hyalomma nymphs were fed, died due to a severe anaemia (Figure 6, Table 8) even when the feeding period was extended by several days it was found that most ticks fed at the same time, six days after application (peak detachment). It was observed that the majority of ticks, when applied directly to the gerbil's body attached almost immediately, while nymphs took longer to do so and appeared to have more difficulty in remaining on the host to effect attachment. Plates 7 and 8 show Hyalomma a. anatolicum larvae and nymphs feeding on gerbils.

Developmental stages of Babesia microti in tick tissues

To confirm that larvae of Hyalomma a. anatolicum and Ixodes ricinus were able to pick up the parasites from infected gerbils, tissue smears from 24 larvae were prepared and stained during the
PLATE 7 Close up of engorging larvae. The arrow indicates the location of an attached larva on a gerbil (al)

PLATE 8 Close up of attached nymphs onto gerbil's back
moulting period in an attempt to confirm the presence and define
the developmental stages of Babesia microti in the ticks. To
confirm that nymphs of H. a. anatolicum were able to pick up the
parasite an experiment was designed and tick tissue smears were
made daily. It was not possible to reproduce the experiment for
the nymphs of I. ricinus due to its long moulting period and the
limitations on time.

Even though the results obtained are not consistent in both
species of tick, evidence of a similar pattern of B. microti dev-
elopment was seen. Developmental stages of B. microti were found
in larval tissue smears from the first day after detachment until
the sixth day of a moulting period of eight days. On the first day
merozoites were seen in ingested gerbil red blood cells. Several
small ring forms with well defined nuclei arranged along the peri-
iphery were also seen on this day. Slightly larger ring forms
with a single short spike-like projection were seen approximately
48 hours after detachment. Smaller ring forms some with a well
defined blue cytoplasm were also seen at this time along with
larger ring forms with a single spike. A few small triangular
or elongated bodies having several projections were seen. After
this period the following pattern of development took place:
approximately at 72 hours after detachment small and large ring
forms and spiky rayed forms ("Strahlenkorper"; Friedhoff, 1981)
were seen. Strahlenkorper is the original term used by Koch (1906)
to describe long, thin and rayed forms of some developmental stages
of Babesia bigemina.
Between 96 and 130 hours after detachment larger ring forms with well defined blue cytoplasm, few spiky rayed forms and few clusters of spiky rayed forms were observed.

When isolated spiky rayed forms were detected with a concentrated heavily stained (pink) nucleus and a diffuse, not very well defined bluish cytoplasm were seen. When clusters of spiky rayed forms were detected only the nuclei were seen as a heavily stained dot. In both cases the spike-like projections were lightly stained and therefore difficult to be seen. From 130 to 15½ hours post detachment developmental stages of *B. microti* in the larvae of *H. a. anatolicum* could not be detected.

Developmental stages of *B. microti* were found in *H. a. anatolicum* nymph tissue smears from the first day to the sixth day of the moulting period of 13 days. Approximately 2½ hours post detachment, merozoites were seen to be free of ingested red blood cells and ring forms with well defined blue cytoplasm were also seen. From 48 to 72 hours post detachment several small ring forms were seen but 72 to 96 hours post detachment no developmental stages were detected. At 106 hours post detachment a single large ring form was seen (Plate 9). One hundred and thirty hours post detachment a small ring form with blue cytoplasm and a large ring form were seen, but from 15½ to 290 hours post detachment developmental stages of *B. microti* were not seen. One and two days after moulting tissue smears were prepared from the nymphs, but no developmental stages of *B. microti* could be detected.

Developmental forms of *B. microti* were found in *I. ricinus* larvae tissue smears from 2½ to 72 hours post detachment. After
PLATE 9  Developmental stage of *B. microti* in tick (nymph) tissue smear.

*rf* = ring form
24 hours post detachment Babesia bodies were seen in ingested gerbil red blood cells along with small ring forms. From 48 to 72 hours post detachment only small ring forms were seen and from 96 to 274 hours post detachment no developmental stages of B. microti were detected.

Parasites, when detected in tissue smears were rare and were not present in all smears.

TRANSMISSION EXPERIMENTS

Attempt to transmit Babesia microti with nymphs of Ixodes ricinus

None of the gerbils on which nymphs of Ixodes ricinus which had previously fed on B. microti infected gerbils as larvae developed a patent B. microti infection. Parasites were not seen in any of the stained thin blood smears prepared from the three gerbils which had been monitored daily for 30 days after receiving 100 nymphs each.

Attempts to transmit Babesia microti with nymphs of Hyalomma a. anatolicum

None of the gerbils on which nymphs of Hyalomma a. anatolicum which had previously fed on B. microti infected gerbils as larvae, developed a patent B. microti infection. Parasites were not seen in any of the stained blood smears prepared from the four gerbils which had been monitored daily for ten days after receiving 200 nymphs each. The blood of two of these gerbils was passaged by intraperitoneal injection but failed to infect two further gerbils which had been monitored for 13 days. Tick tissue smears of nymphs made 24 to 72 hours after detachment showed that parasites were present and that they developed to some extent.
DISCUSSION

The aim of this study was to attempt transmission of *Babesia microti* from infected to uninfected gerbils using larval and nymphal stages of *Hyalomma a. anatolicum* and *Ixodes ricinus* as vectors. Before undertaking attempts at transmission however, it was important to confirm the suitability of gerbils as experimental host for *B. microti* and both tick species. Furthermore, it was essential to be able to produce a reproducible high level of parasitaemia early after infection without killing the gerbil host. Such a parasitaemia was thought to be advantageous in effecting the successful transmission of the haemoparasite, in that relatively large numbers of the parasite would be available to the vector before the onset of an immune response (Cox and Turner, 1970; Hussein, 1977, 1979) which could reduce the infectivity of *B. microti* to the vector. Once the condition for the production of the above parasitaemia levels were established, attempts at transmission were undertaken using large numbers of larval ticks (1000 to 2000) as it was again thought that this would maximise the successful transmission of the haemoparasite. Since the ability of the parasite to invade ticks may depend on the parasite rate in the vertebrate host, initial studies on the behaviour of *B. microti* in mice showed that certain parameters describing the course of infection (prepatent, period, time to the peak parasitaemia and parasitaemia at peak) were dependant on the number of *B. microti* infected red blood cells injected into the host. This is similar to the results reported by Nowell (1970) when studying
the relationship between sizes of inoculum dose and the resultant course of infection. Mice injected with different numbers of *B. microti* infected mouse red blood cells produced a progressive reduction in prepatent period, time to the peak parasitaemia and an increase in peak parasitaemia (Young, 1970; Hickerman, 1980). Mice injected directly with stabilate material (TREU 1616) of mouse origin however, showed a relatively longer prepatent period suggesting that the infectivity of this particular stabilate was low which could be due to a variety of factors, such as the length of storage, initial infectivity of the stabilate, poor survival of the haemoparasite during cryopreservation.

Mongolian gerbils were shown to be highly susceptible to *B. microti* infections in that high parasitaemias developed soon after the injection of *B. microti* infected mouse blood. Furthermore there was evidence of increasing virulence (reduction in prepatent period, etc.) following subsequent passage into the gerbils. Results with stabilate material prepared from *B. microti* infected gerbil blood was however disappointing in that the infectivity of such stabilates appeared to be very low and not predictable, probably due to a low number of infected red blood cells when prepared and/or a factor of host resistance. It was initially hoped to use gerbil-derived stabilates to indicate infections due to the reported stable characteristics of this material, but in the present study the parasitaemia produced following the infection of such material did not meet the required criterion described above. Consequently further studies were undertaken with gerbils which had
been injected with *B. microti* infected gerbil red blood cells collected directly from infected gerbils.

Gerbils were inoculated with different numbers of *B. microti* infected red blood cells in order to establish a suitable inoculum which would reliably produce a parasitaemia with the characteristics required for transmission experiments. A similar relation between the numbers of infected red blood cells and the parameters of prepatent period, days to peak and maximum levels of infection (%) was seen in gerbils as in mice i.e. a reduction of prepatent period and days to peak parasitaemia, with increasing numbers of infected red blood cells. From these results it was concluded that an inoculum of approximately $10^7$ infected red blood cells could produce a parasitaemia of 20–30% infected red blood cells in 10–12 days after injection and was considered suitable for use in transmission experiments.

Further studies were conducted in gerbils inoculated with this number of infected red blood cells to confirm the characteristics of the infection produced by this particular inoculum. The course of infection in six gerbils injected with a total of $8.0 \times 10^7$ infected red blood cells was found to be similar in that they exhibit a prepatent period of one day and reached a peak parasitaemia in six days after infection.

Ticks were put to feed on these animals to test the effect of infection and tick challenge. Gerbils became infected developing an acute anaemia that was made worse by the ticks, causing some fatalities.
To confirm that larvae of *Hyalomma a. anatolicum* and *Ixodes ricinus* were able to pick up the parasites from infected gerbils and develop, tick tissue smears were studied. Even though the obtained results are not consistent due to the small number of ticks studied and the difficulties caused arising from the excess of tick material when searching for parasites; evidence of a similar pattern of development was observed for both ticks. During the moulting period the most obvious forms were spherical bodies (ring forms). These forms were earlier described by Koch (1906) and later for *B. bigemina* by Riek (1964) and Friedhoff and Busher (1976). Koch (1906) reported a gap of information between the appearance of these forms and the appearance of kinetes. In this study further developmental forms were not detected probably due to the difficulties in searching for parasites as mentioned before, or because they were not in the ticks. The latter could be explained by the need for a "stimulation" which happens when the tick feeds as a nymphs. From the observation of the life cycle of *B. microti* in this study, a chronological sequence of its developmental stages can be suggested.

Experiments were carried out to study the feeding performance of the immature instars of *Hyalomma a. anatolicum* and *Ixodes ricinus* in Mongolian gerbils.

Due to the laboriousness of the tick counting techniques and the limitations on the time it was not possible to study in detail the feeding performance of these ticks. Furthermore, on the understanding that a suitable parasitaemia and course of infection were needed to ensure the conditions of which transmission
of parasites by ticks could be attempted, the study of the parasite behaviour in gerbils was stressed.

The results obtained were based on estimations of the numbers of recovered ticks. Even though the recovery rate in both *H. a. anatolicum* and *I. ricinus* larvae was sufficient for further transmission experiments most of the applied larvae did not attach to the gerbils, due to their escape from the gerbil's cage, which could be explained by their high activity and the lack of a system of tick containment. The same situation was observed with nymphs. In such conditions, the obtained recovery rate could be explained by the large numbers of ticks applied to the gerbil host. With a better design of cage the recovery rate could be increased.

The feeding performance of *H. a. anatolicum* and *I. ricinus* was characterised by a pattern in which larvae and nymphs of both ticks were found to engorge and detach in small groups at the beginning of the feeding period, these numbers increased rapidly until the peak detachment was reached; thereafter the numbers of detached ticks decreased slowly until the end of the feeding period. Most of the engorged ticks were detached at the peak feeding.

A similar feeding performance is that reported for *I. ricinus* fed on gerbils by Hadani et al (1969). The same authors reported a much longer feeding period for three different *Hyalomma* spp. On the contrary Snow (1969) found a shorter feeding period using *H. a. anatolicum* fed on guinea pigs.

Although the mortality rate of the above ticks was not studied when moulting most of them were found apparently healthy when applied to gerbils as ticks of the next instar.
Throughout the experiments Mongolian gerbils were easy to handle and no adverse reactions in their behaviour were observed as described by Marston (1976), although during the tick application and a few hours later gerbils showed some degree of irritation.

The animals did not remove ticks by grooming and allowed the ticks to feed. This confirms the findings of Barnett and Croft (1976).

_Hyalomma a. anatolicum_ and _Ixodes ricinus_ fed as larvae on parasitic gerbils failed to transmit infection to other gerbils. Blood from gerbils in which _H. a. anatolicum_ nymphs had fed on infected gerbils as larvae, was proved to be uninfecive to other gerbils.

The failure to transmit the parasite could be explained in different manners. The parasite was unable to undergo successful development in the ticks even though taken by them, as mentioned for _Rhipicephalus appendiculatus_ by Irvin and Brocklesby (1972). These authors also discussed the possibility of an incompatibility of ticks and piroplasms system. This could well be the case of _H. a. anatolicum_, even though the transmission of another rodent _Babesia (Nuttallia daniil)_ was successful using laboratory-bred nymphs of _H. excavatum_ and gerbils, but it is unlikely to be the case of _I. ricinus_, since transmission of _B. microti_ by _Ixodes_ spp. was achieved by several authors.

Young (1970) reported the transmission of _B. microti_ (Kings strain) using nymphs of _I. trianguliceps_ that fed as larva on
infected rats and mice that were bred in the laboratory. The author was not able to demonstrate transmission using nymphs of *H. a. anatolicum, H. dromedarii, I. ricinus* and *I. exagonus*.

Oliveira and Kreier (1979) reported the transmission of *B. microti* using nymphs of *I. pacificus* and *I. scapularis* that fed as larvae on infected hamsters and gerbils. In this study *B. microti* was either an isolate from the Gray strain or a fresh isolate from a field mouse. No information was available on the origin of the ticks.

Hussein (1980) reported the transmission of *B. microti* using nymphs of *I. trianguliceps* and *I. canisuga* that fed as larvae on infected field voles. Transmission of the parasite was successful in eight out of ten trials with *I. trianguliceps* and once in 25 trials with *I. canisuga*. In this study ticks were bred in the laboratory.

Walter (1981) reported transmission of *B. microti* using unfed *I. ricinus* nymphs collected from field mice to uninfected hamsters bred in the laboratory.

Regardless of the number of larvae that successfully engorged on gerbils, if few of them were infected, the opportunity to transmit Babesiosis was reduced due to the dilution of the numbers of nymphs that could feed per gerbil. It could also be possible that the inoculum was insufficient to produce a patent infection due to host resistance. The given results offer some evidence of age resistance in gerbils. Similarly, age resistance factors were found in *B. microti* infected mice (Cox and Young, 1969).
Krylov (1965) reported that *R. a. anatolicum* may infect gerbils no sooner than on the fourth day of blood sucking. A shorter feeding period than needed would also decrease the possibility of transmission.

Although none of the gerbils developed a patent parasitaemia parasites were seen in tick tissue smears shortly after detachment and some developmental stages of *B. microti* were later observed.
CONCLUSIONS

The Mongolian gerbil was found to be a suitable experimental animal for studies on *Babesia microti* infections and also to be a suitable host for the immature instars of *Hyalomma a. anatolicum* and *Ixodes ricinus*.

*B. microti* infections in gerbils could be reproduced dependant on the numbers of infected red blood cells injected. Even though gerbils were found highly susceptible a factor of age host resistance was observed.

Transmission of the parasite from infected to uninfected gerbils was not achieved interstadially with larvae and nymphs of *H. a. anatolicum* or *I. ricinus* although, there was evidence of some development of *B. microti*.
ACKNOWLEDGEMENTS

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APPENDIX
<table>
<thead>
<tr>
<th>Experimental Host</th>
<th>No.(s)</th>
<th>Origin</th>
<th>Inoculum</th>
<th>T.Inf</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>1,2</td>
<td>TREU 1550</td>
<td>1 Stabilate + 0.1ml PBS</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>3-6</td>
<td>Mouse 1</td>
<td>0.1ml blood</td>
<td>$8.1 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>7</td>
<td>TREU 1616 (Mouse)</td>
<td>2 Stabilates + 0.1ml PBS</td>
<td>$9.6 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>8,9</td>
<td>Gerbil 2</td>
<td>0.1ml blood</td>
<td>$7.6 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>1,2</td>
<td>Mouse 3</td>
<td>0.1ml blood</td>
<td>$3.7 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>3,4</td>
<td>Gerbil 2</td>
<td>0.1ml blood</td>
<td>$7.6 \times 10^7$</td>
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</tr>
<tr>
<td>Gerbils</td>
<td>5-7</td>
<td>TREU 1616 (Mouse)</td>
<td>1 Stabilate + 0.1ml PBS</td>
<td>$9.6 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>8-10</td>
<td>Gerbil 5</td>
<td>0.1ml blood</td>
<td>$1.7 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbil</td>
<td>11</td>
<td>Gerbil 5</td>
<td>0.1ml blood</td>
<td>$1.7 \times 10^6$</td>
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</tr>
<tr>
<td>Gerbil</td>
<td>12</td>
<td>Gerbil 5</td>
<td>0.1ml blood</td>
<td>$1.7 \times 10^5$</td>
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</tr>
<tr>
<td>Gerbil</td>
<td>13</td>
<td>Gerbil 5</td>
<td>0.1ml blood</td>
<td>$1.7 \times 10^4$</td>
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</tr>
<tr>
<td>Gerbils</td>
<td>14-17</td>
<td>Gerbil 9</td>
<td>0.1ml blood</td>
<td>$5.1 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>18,19</td>
<td>TREU 1624 from gerbil</td>
<td>2 Stabilates + 0.1ml PBS</td>
<td>N.D.</td>
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</tr>
<tr>
<td>Gerbil</td>
<td>24</td>
<td>Gerbil 18</td>
<td>0.5ml blood</td>
<td>$1.1 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>25,26</td>
<td>TREU 1616 Mice</td>
<td>2 Stabilates + 0.2ml PBS</td>
<td>$9.7 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Gerbil</td>
<td>27</td>
<td>Mouse 9</td>
<td>0.2 ml blood</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Gerbils</td>
<td>28-33</td>
<td>Gerbil 26</td>
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<td>$3.9 \times 10^7$</td>
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</tr>
<tr>
<td>Gerbils</td>
<td>34-37</td>
<td>Gerbil 25</td>
<td>0.1 ml blood</td>
<td>$4.8 \times 10^7$</td>
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</tr>
<tr>
<td>Gerbils</td>
<td>38-41</td>
<td>Gerbil 25</td>
<td>0.1 ml blood</td>
<td>$4.8 \times 10^6$</td>
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</tr>
<tr>
<td>Gerbils</td>
<td>42-45</td>
<td>Gerbil 25</td>
<td>0.1 ml blood</td>
<td>$4.8 \times 10^5$</td>
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</tr>
<tr>
<td>Gerbil</td>
<td>46</td>
<td>Gerbil 27</td>
<td>0.1 ml blood</td>
<td>$3.9 \times 10^7$</td>
<td></td>
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<tr>
<td>Gerbils</td>
<td>47-59</td>
<td>Gerbil 46</td>
<td>0.1 ml blood</td>
<td>$6.5 \times 10^7$</td>
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<tr>
<td>Gerbil</td>
<td>60</td>
<td>Gerbil 59</td>
<td>0.1 ml blood</td>
<td>$1.9 \times 10^7$</td>
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<tr>
<td>Gerbils</td>
<td>61-73</td>
<td>Gerbil 60</td>
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<td>$9.3 \times 10^7$</td>
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<tr>
<td>Gerbil</td>
<td>81</td>
<td>Gerbil 77</td>
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<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Gerbil</td>
<td>82</td>
<td>Gerbil 79</td>
<td>0.1 ml blood</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

N.D. = not determined
Fig. 5  Schematic representation of *Babesia microti* infections in mice and gerbils.
Fig. 6 Schematic representations of the tick feeding and transmission experiments with Babesia microti in gerbils.