STUDIES ON THE BIOLOGY OF *TRYPANOSOMA* (*NANNOMONAS*) *SIMIAE*

BY

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1984

DECLARATION

I, Elizabeth Auma Opiyo, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

Signature .....E.A.Opiyo.....

I, Professor George K. Kinoti, hereby declare that this thesis has been submitted for examination with my approval as University Supervisor.

Signature .....

I, Doctor Leonard H. Otieno, hereby declare that this thesis has been submitted for examination with my approval as Supervisor.

Signature .....

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STUDIES ON THE BIOLOGY OF *TRYPANOSOMA* (*NANNOMONAS*) *SIMIAE*

ABSTRACT

This thesis is primarily a study of factors which influence the virulence of *T.* (*N*). *simiae* for pigs. The factors investigated include the species of tsetse vectors, size of inocula of trypanosomes injected into pigs and the method of maintenance of the trypanosomes. The rate of development of *T.* (*N*). *simiae* in different species of tsetse and its relationship to the resulting infection in pigs was also investigated. In addition, an attempt was made to adapt *T.* (*N*). *simiae* to laboratory rodents.

In this study three stocks of *T.* (*N*). *simiae* were cyclically transmitted through tsetse. Transmitting the isolates through *Glossina morsitans* and *G. pallidipes* resulted in a disease less severe than did syringe inoculations. Pigs infected through tsetse bites survived much longer than had previously been reported, with some pigs exhibiting self-cure after running infection for varying lengths of time. The response of pigs to experimental infection with *T.* (*N*). *simiae* varied from one animal to another and pigs appear to fall into three categories. Pigs in the first group were the very susceptible and died during the first peak of parasitaemia. The parasitaemia built up fast and the pigs died soon after the first appearance of trypanosomes in the peripheral blood circulation.

The second group developed parasitaemia, and survived more than one parasitaemia peak but eventually succumbed to the infection. The third group became parasitaemic, apparently controlled the parasitaemia for some weeks at very low levels and eventually threw off the infection.

Grouping the pigs according to their place of origin shows that those which experienced chronic, self-limiting infections were from the same farm. The less susceptible pigs came from this farm. This suggest that the course of infection observed might have been determined by the ability of the individual pig to control the infection rather than by the vector. No clear evidence was obtained to show whether or not the species of the vector influenced the virulence of T. (N). simiae.

Teneral tsetse flies became infected with T. (N). simiae when they were fed on pigs carrying predominantly stumpy trypanosomes in their blood, but the number of trypanosomes circulating in blood did not appear to influence the infection rate in tsetse.

At 25°C G. morsitans developed mature T. (N). simiae infections in 19 days and G. pallidipes in 23 days. G. morsitans was more frequently infected than G. pallidipes. In pigs neither the prepatent period nor the period of patency appeared not to be related to the rate of development or the infection rate in the tsetse fly.

VII

One isolate of T. (N). simiae (EATRO 1786) has been successfully adapted to rats. The parasite has been maintained in rats through 20 passages without pig serum and 14 passages with pig serum. The infection first became lethal to rats in the seventh passage. Abnormal trypanosomes were observed during the initial stages of adaptation. The rat adapted T. (N). simiae were shorter than trypanosomes from the original strain. Trypanosomes from the original isolate had a mean length of range 13.5 µm to 17.43 µm. In rats the trypanosomes measured a mean of 12.59 µm (range 8.8 and 14.4 µm). In pigs the rat adapted T. (N). simiae measured a mean of 12.35 um (range 9.9 - 14.38 µm). The rat adapted T. (N). simiae strain has remained infective to tsetse and to pigs. It infects mice readily.

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C H A P T E R I

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Flagellate protozoans belonging to the genus Trypanosoma include some of the most important parasites of man and his livestock. African trypanosomiasis is largely a disease of man and livestock which occurs in practically all the countries of tropical Africa. African human trypanosomiasis, or sleeping sickness, results from infection with Trypanosoma gambiense and T. rhodesiense. The tsetse borne trypanosome species which infect livestock are Trypanosoma vivax, T. congolense, T. simiae, T. brucei and T. suis. All the above species of trypanosomes are transmitted by tsetse flies which belong to the genus Glossina. Two other species of trypanosomes affect livestock in Africa but are not transmitted by tsetse flies. These are Trypanosoma evansi and T. equiperdum. The infective forms of the Trypanosoma species occur in the mouthparts of the vector, and are transmitted to the definitive host when the fly feeds. In the South American trypanosomiasis, caused by Trypanosoma cruzi, the infective forms occur in the faeces of the vectors, reduviid bugs. T. cruzi is confined to the South American continent.

African human trypanosomiasis occurs in epidemics. The greatest of these occurred at the end of the 19th Century in

Busoga and Buvuma Island in Uganda during which an estimated 250,000 people died. This led to the evacuation of people from the Lake Victoria shores as a control measure (Ford 1972). In between epidemics, the infection maintains a low level of prevalence in different countries. The level of prevalence in these countries is a reflection of the control measures being practised by the countries.

Trypanosomiasis in livestock causes significant economic losses and constitutes a major contraint to livestock development in vast areas of tropical Africa. It therefore deprives man of much needed protein.

Tsetse fly is found throughout the tropical regions of Africa. It occurs in over 10 million km^2 of potential pastoral land stretching across the middle of Africa and lying between latitudes $12^{o}N$ and $20^{o}S$. Almost the whole of this area is rendered unsuitable for cattle production because of the threat of trypanosomiasis (Moeller, 1976). The infection results in poor milk and meat production and the eventual death of the affected animals. Trypanosomes infect a large number of mammalian species. The severity of the disease they cause varies a great deal from one mammalian species to another, from one trypanosome species to another and from one parasite strain to another.

From among trypanosome species, Trypanosoma simiae is a very important parasite of pigs in which it often causes a fatal disease.

It is not infective to laboratory rodents. Sheep and goats are sometimes susceptible to infections with T. simiae (Hoare, 1972). Very little is known about the immunology of T. simiae and so clinical diagnosis is only confirmed by the finding of trypanosomes in the blood of the affected animal. When this blood is subinoculated into a naive pig and the pig develops the disease and dies soon afterwards, then the diagnosis is confirmed (Hoare, 1972). There is no immunodiagnostic method available and the prevalence of T. simiae in domestic stock is unknown.

After a pig has become infected, the course of the infection is usually very fast, an animal dying soon after parasites have been detected in its blood (Stephen, 1966). Treatment is limited to very few trypanocidal drugs. Dimidium chloride (Wilson, 1948) and metamedium chloride (Stephen, 1966) were shown only to suppress parasitaemia and prolong the lives of the affected pigs. Wilson (1949) cured T. simiae in pigs using antrycide dimethyl sulphate in Uganda. Unsworth (1952) failed to cure infected pigs in Nigeria using the same drug. Relapses in pigs after treatment using the same drug was observed by Watson and Williamson (1958).

It was observed that T. simiae rapidly developed resistance to antrycide chloride (Wilson, 1949; Watson and Wilson, 1958). Berenil was found ineffective but suramin was found effective (Watson and Williamson, 1958).

Commercially available trypanocidal drugs and experimental diamidines failed to cure pigs once the disease had been

established (Mahaga and Rottcher, 1983). However, Joshua et. al. (1983) successfully cured a sheep infected with T. simiae using berenil administered at 7.00 mg/kg. Aliu (1983) recommended isometamedium given intramuscularly at between 12.5 and 35.5 mg/kg or a combination of quinapyramine sulphate at 7.5 mg/kg and diminazine at 5 mg/kg given subcutaneously for the treatment of T. simiae infection. The dosages used or recommended above are much higher than the normal curative doses given during T. vivax and T. congolense infections in other livestock and are not economical. The recommended dosage for diminazine is 3.5 mg/kg and for isometamedium 0.5 mg/kg (Williamson, 1970).

Ilemobade and Balogun (1981) found that pigs infected with T. simiae performed poorly, failed to grow and had a high and uneconomical feed cost per unit weight gain compared to pigs infected with T. brucei or T. congolense and uninfected pigs.

Little is known about the physiology and biochemistry of this parasite. Studies can only be conducted in the pig because laboratory animals are either refractory or poorly susceptible to infection with the parasite. Recent observations at the Kenya Coast that infections in pigs with T. simiae gave rise to deseases which varied between acute and chronic forms (Steel; 1983) and a later correlation of their fluctuation and the transmitting species of tsetse (Janssen and Wijers, 1974) prompted this study. This study aimed at determining factors which influence the virulence of T. simiae in pigs. An attempt is also made to adapt T. simiae

to laboratory rodents so that investigations into aspects such as immunology, chemotherapy and host - parasite relationships could be undertaken more conveniently on this parasite.

1.2 LITERATURE REVIEW

1.2.1 Classification

The genus Trypanosoma belongs to the phylum Protozoa, the order Kinetoplastida and the family Trypanosomatidae. Hoare (1972) divided the genus into two main sections: the stercoraria and the salivaria. The stercorarian trypanosomes have a free flagellum, a large kinetoplast and a pointed posterior end. In these trypanosomes reproduction in the mammalian host is discontinous, taking place in the amastigote or epimastigote stages. Development in the vector is completed in the posterior station and transmission is by contamination. With T. rangeli, however, development takes place in the anterior station and transmission is by inoculation. Trypanosomes belonging to this section are typically non-pathogenic with the exception of T. cruzi. T. cruzi is pathogenic to both man and animals.

The salivarian trypanosomes may or may not have a free flagellum; the kinetoplast is either terminal or subterminal and the posterior end of the body is usually blunt. Multiplication in the mammalian host is continous and takes place in the trypomastigote stage. Development of the trypanosomes in the vector is completed in the anterior station and transmission

is inoculative. The only exception is T. equiperdum which is transmitted by contact during copulation. In Africa all trypanosomes of medical and veterinary importance belong to the salivaria.

According to Hoare (1972) salivarian trypanosomes belong to four subgenera. The subgenus Dutonnella consists of a single species, T. vivax which is an important parasite of cattle, sheep and horses. T. vivax is widely distributed throughout tropical Africa wherever tsetse flies are found. This species has been introduced through human agency to countries outside Africa, such as Mauritius, West Indies, Central and South American, where it is maintained by mechanical transmission (Hoare 1970).

The subgenus Nannomonas consists of two species: T. congolense and T. simiae. This subgenus is considered more fully below.

The subgenus Trypanozoon consists of a number of species which are morphologically indistinguishable and differ only in certain biological features. The subgenus is made up of five species. Three of the species, T. brucei, T. rhodesiense, T. gambiense are transmitted by tsetse flies where they develop in the midgut and salivary glands. The other two species, T. evansi and T. equiperdum, are transmitted directly (Hoare, 1970). T. evansi is transmitted by mechanical vectors such as tabanid flies and T. equiperdum is sexually transmitted.

The subgenus Pcynomonas is represented by a single species, T. suis, a parasite of pigs. T. suis infections cause a mild disease in adult pigs but are very pathogenic to young animals (Hoare 1972). Not much is known about this species.

1.2.2 Subgenus Nannomonas

The subgenus Nannomonas is made up of relatively small trypanosomes measuring between 8µm and 24µm in length (Hoare, 1970). They are typically without a free flagellum and have a medium sized kinetoplast usually situated in a marginal position, and an incospicuous undulating membrane. Peculiar to this subgenus is the siting of the flagellum. The intracytoplasmic portion of the flagellum typically arises somewhat behind the kinetoplast before emerging on the opposite side of the body. The posterior end of the body is usually rounded in the smaller forms or pointed in the longer forms. The nucleus is situated in the middle of the body. The number of species belonging to this subgenus is still not known but two distinct species are usually recognised, namely T. congolense and T. simiae. T. congolense is monomorphic while T. simiae is polymorphic. The trypanosomes of this subgenus develop in the midgut and proboscis of tsetse flies (Hoare, 1972).

1.2.3 Trypanosoma (Nannomonas) congolense Broden 1904.

Trypanosoma (N.) congolense is responsible for the most important form of trypanosomiasis of domestic mammals. It occurs

throughout the tropical regions of Africa wherever tsetse flies occur. The name was given by Broden in 1904 (Hoare, 1972) to a small trypanosome discovered by him in the blood of sheep and a donkey from Congo. Later a dimorphic trypanosome was named T. dimorphon and a similar but smaller trypanosome was named T. nanum. These names were eventually regarded as synonyms of T. congolense. Studies by Godfrey (1961) suggested that in Nigeria there are strains of T. congolense which are separable by their mean lengths. The strains differ in their pathogenicity in cattle, sheep, dogs and rats. Godfrey (1961) classified the strains into three groups: the congolense type, the intermediate type and the dimorphon type. The congolense type trypanosomes are characterised by their small size, measuring between 11.2 and 13.8μm in length. They are not very infective to mammalian hosts and are not virulent, and the host develops a low parasitaemia. The dimorphon type trypanosomes are long, measuring between 13.8 and 15.7μm in length. These trypanosomes were shown to be highly infective and pathogenic, and the host developed a high parasitaemia. The intermediate type trypanosomes measuring between 11μm and 15μm however were shown to be intermediate in infectivity and virulence but giving rise to high parasitaemia.

T. congolense can be transmitted by ten species of tsetse, namely: G. palpalis, G. fuscipes, G. tachinoides, G. morsitans, G. austeni, G. swynertoni, G. pallidipes, G. longipalpis,

G. brevipalpis and G. vanhoofi. Infection rates in tsetse flies are intermediate between those with T. vivax which can be as high as 20% and those with T. brucei infection which under natural conditions are less than 1% (Nash, 1969). In tsetse, development of T. congolense commences in the endoperitrophic space of the midgut, mainly in the posterior third where the trypomastigotes ingested with the blood continue to multiply for some days (Hoare, 1970). The elongated trypomastigotes then escape through the open end of the membrane into the ectoperitrophic space reaching the cardia by the 20th day or earlier (Hoare, 1970). Kaddu (1978) observed T. congolense in the proboscis of G. pallidipes by day 10 post infection. In the cardia, they become longer and penetrate into the endoperitrophic space of the cardia from where they migrate through the oesophagus to the proboscis. In the proboscis, the trypomastigotes develop into metacyclic trypanosomes. The complete cycle of development takes from 19 to 53 days depending on the conditions in which the flies are kept (Hoare, 1972). Mechanical transmission by other blood sucking Diptera, especially tabanid flies is thought to occur (Hoare, 1972).

Almost all species of domestic mammals are susceptible to infection with T. congolense. The course of the infection varies with the strain of the parasite and with the species of host. The infection may produce hyperacute, acute or chronic disease syndromes in cattle, sheep, goats, horses and camels or mild disease in pigs (Stephen, 1966).

1.2.4 Trypanosoma (Nannomonas) simiae Bruce et. al. 1912

A trypanosome which had the characteristics of T. simiae was first seen in 1909 by Montgomery and Kinghorn (1909) in Kambole (Zambia), where a pig which suddenly developed severe symptoms of disease, with trypanosomes swarming in the blood, died 6 hours later. The authors called the trypanosome Trypanosoma nanum. Three years later, two papers described a new species of trypanosome with characters similar to those of the parasites described by Montgomery and Kinghorn (1909). In both instances the trypanosomes were isolated from monkeys on which wild G. morsitans had been allowed to feed. The first paper was published by Bruce, Harvey, Hamerton, Davey and Bruce (1912) who had isolated the trypanosomes in Nyasaland (Malawi). The second paper was by Kinghorn and York (1912) who had isolated the trypanosomes in Northern Rhodesia (Zambia). Bruce et. al. called their parasite Trypanosoma simiae and Kinghorn and York called their parasite Trypanosoma ignotum. It was later realised that the two parasites were identical and since Bruce et. al. (1912) were the first to report the discovery, the new trypanosome was named T. simiae. Later Bruce et. al. (1913) demonstrated that T. simiae was highly pathogenic to domestic pigs and that it occurred naturally in warthogs.

T. simiae is a common parasite affecting pigs and some other domestic mammals in the tropical zone of Africa (Hoare, 1972). Its distribution stretches across the continent of Africa from

Portuguese Guinea (Guinea Bissau) in the West to Somalia in the east (hoare, 1972). On the eastern side of the continent, the southrn most point from which T. simiae infection has been reported in Zululand in South Africa (Neitz quoted by Hoare, 1972). In total, T. simiae has been reported from 19 countries of Africa. These are Tanzania, Zaire, Malawi, Zambia, Zimbabwe, Guinea Bissau, Sierra Leone, Mali, Ghana, Dahomey, Nigeria, Burundi, Uganda, Kenya, Mozambique, Somalia, Zululand in South Africa, Rwanda and Liberia (Hoare, 1972).

Initially there was much controversy regarding the morphology of T. simiae. Hoare (1972) states that the general morphology of T. simiae conforms to the pattern of T. congolense. The common features are a medium sized kinetoplast which typically occupies a marginal position near the posterior end of the body and the absence of a free flagellum in the great majority of morphological forms. The posterior end of the body can be pointed or rounded. T. simiae is polymorphic, exhititing three types. The simiae type measure between 14 and 24 μm (mean 18 μm) and constitutes 90% of the population. The simiae type trypanosomes have a well developed undulating membrane. The rodhaini type constitutes 7% of the population and measure between 11 and 20 μm (mean 15.8 μm) while the shortest forms are the congolense type. The congolense type form a very small proportion of the trypanosomes and they measure between 9 and 17 μm in length.

Bruce et. al. (1912) however, described T. simiae as monomorphic trypanosomes measuring between 14 and 24µm. They noticed that the body was elongated and markedly undulating and that it was difficult to say whether the species had a free flagellum or not. Different workers reported that T. simiae was either monomorphic or polymorphic. Hoare (1972), while accepting the variation in the mean length of T. simiae reported by different authors, is of the opinion that the differences reported by different workers are related to the time when the stained blood preparations were made during the course of infection. He notes that the separation of the forms was based on the extreme forms of each type. Stephen (1966) concluded that since it was a matter of individual judgement as to where the intermediate forms of T. simiae fall using the measural technique, the technique should not be recommended as a diagnostic aid. Generally it is accepted that the average length of T. simiae trypanosomes varies during the course of the infection as follows:- on the first day of patent infection, they measure on average 16.5µm, on the second day 17.57µm; on the third day 19.54 µm and on the fourth day 21.28µm (Stephen, 1966). The short congolense type is therefore found in the early part of the infection and the simiae type in the terminal stages. Stephen (1966) therefore inferred that this could help explain the variation in average length reported by different authors. He also noted that short forms sometimes predominate in camels and sheep but not in pigs.

Stephen (1966) observed that the rate of drying of blood films affected the morphology of T. simiae in stained

preparations. In slowly dried blood films, the trypanosomes are broader than in quickly dried films. Wenyon (1927) observed that the appearance of trypanosomes differed from pig to pig due to the fact that dividing forms were more common in some pigs than in others. Hoare, (1972) noted that the occurrence of flagellµm was puzzling. This is because it is difficult to determine where the undulating membrane ends in relation to the flagellum. Some preparations appeared to have a membrane which runs to the tip of the flagellum while in others a short free flagellum appeared to be present.

All in all, therefore, there is wide variation in the lengths of trypanosomes of T. simiae species and this makes it very difficult to use length to distinguish this species from related species. With T. congolense a relationship has been established between the type of infection seen in cattle and the length of trypanosomes (Godfrey, 1961), but no such relationship has been established in T. simiae infection in the pig or any other host.

The domestic pig is very susceptible to infection with T. simiae. In contrast, guinea pigs, rats, mice and dogs are refractory to infection and attempts to infect them by injection of infected blood or by fly challenge have failed (Stephen, 1966). Rabbits are sometimes susceptible. Desowitz and Watson (1953) showed that when inoculated with 3ml. of infected pig blood some rabbits became infected and died from massive infection while others were refractory to infection. These authors did not

indicate the number of parasites present in the 3ml. of blood injected in the rabbits or the sex and age of the rabbits they used. These are some of the factors which might influence susceptibility. Splenectomy rendered rabbits susceptible to infection. Splenectomised rabbits developed an intese parasitaemia and died from the infection. However splenectomized rats, mice and guinea pigs remained refractory to infection with T. simiae.

Sheep and goats show varying degrees of susceptibility to infection with T. simiae (Bruce et. al. 1912). The authors infected 15 goats by feeding infected G. morsitans on them. Thirteen of the goats died on an average within 46 days of infection while two of them recovered from infection. When blood from the infected goats was inoculated into clean goats, the goats became parasitaemic after a prepatent period of 8 days and the patency of infection ranged from 29 to 107 days. Bruce et. al. (1912) observed that when goats were infected cyclically through tsetse most of them died, but that the mortality rate fell when the goats were inoculated with parasites which had been maintained by syringe passage in goats, monkeys or warthogs. When Joshua and Kayit (1984) cyclically infected sheep with T. simiae using G. tachinoides or G. morsitans, the sheep developed a low parasitaemia and survived for 74 days. Inoculation of a pig with the parasite from the sheep resulted in death within 9 days. Syringe inoculation of the parasite from pigs into goats caused a less virulent disease than in pigs. Mahaga and Sabwa (1982)

observed that a strain of T. simiae which was readily infective to pigs in which it caused acute fatal disease, caused in goats a disease which ended fatally in some but not all. In sheep it resulted in parasitaemia which was detectable on wet film for only about 10 days. Subinoculation of whole blood from these animals into pigs caused acute disease.

T. simiae was first isolated from a monkey - hence the name. Only Cercopithecus, however, has been shown to be susceptible (Hoare, 1970). In Cercopithecus monkeys infection with T. simiae is highly pathogenic, killing the animals in a few days (Hoare, 1972). Monkeys of the genera Colubus, Cercocebus, Callitriclus and Macaca as well as the chimpanzee are refractory to infection (Hoare, 1972).

Culwick and Fairbairn (1947) isolated a polymorphic trypanosome from a horse which they claimed could have been T. simiae. This is the first mention of an infection of a horse by T. simiae. Blood from this horse was passaged into sheep and then cyclically through G. morsitans to Cercopithecus monkeys. All their attempts to transmit the infection cyclically to goats failed. Since these workers failed to infect a pig it is difficult to be sure whether the parasite isolated from the horse was T. simiae. Cattle can be infected with T. simiae (Willet, 1970) and camels have been shown to be susceptible (Pellegrini, 1948).

Circumstantial evidence suggests that the warthog and bush pig are the reservoir hosts of T. simiae (Stephen, 1966). In all the instances where T. simiae has been isolated by feeding wild caught G. morsitans on a monkey, pig or goat, warthogs and bush pigs were observed to be present in the areas from where tsetse were caught. Bruce et. al. (1912) examined 180 animals of different species and only the warthog was proved to harbour T. simiae. The dog, monkey and cattle have also been suggested as possible reservoirs of the infection (Bruce et. al. 1912; Kinghorn and York, 1912; Culwick and Fairbairn, 1947).

Eleven species of tsetse from different countries of Africa have been incriminated as vectors of T. simiae. They are G. morsitans, G. longipalpis, G. pallidipes, G. palpalis, G. tachinoides, G. austeni, G. fusca, G. tabaniformis, G. brevipalpis, G. fuscipleuris and G. vanhoofi. It has been suggested that T. simiae is usually introduced into a herd of pigs when an occasional tsetse bites the pigs. Once this infection has been introduced into the herd, other blood sucking insects spread the infection by mechanical transmission through the herd (Unsworth, 1952). Development of T. simiae in the tsetse vector was shown to be like that of T. congolense. The development begins in the midgut (Bruce, Harvey, Hamerton, Davey and Bruce (1912 . The trypanosomes then migrate to the proboscis where they assume the epimastigote form and attach themselves to the walls of the food canal. The flagellates then invade the hypopharynx where they develop into metacyclic trypanosomes. The metacyclic

trypanosomes measure 10 - 12µm in length (Culwick and Fairbairn, 1947). Like T. congolense, T. simiae is without a free flagellum in all stages of its development in the insect vector.

Bruce et. al. (1912) found that at 28.3°C, the complete cycle of development took about 20 days in G. morsitans. The infection rate may vary from 7.1% under field conditions (Gray, 1961) to 9.4% (Stephen and Gray, 1966). Stephen and Gray (1960) have obtained an infection rate of 12.7% after feeding laboratory reared G. morsitans submorsitans on a large white pig infected with T. simiae.

In pigs infection with T. simiae runs a fast course. The pig survives on average 3.6 days from the time trypanosomes are first detected in the blood. In pigs experimentally infected, the prepatent period is about 3 days after inoculation with infected blood and 4 to 5 days after challenge with infected tsetse flies (Stephen, 1966).

Bruce et. al. (1912) observed a rapid change in the virulence of T. simiae. When a cage containing wild G. morsitans was placed on a monkey and then on a goat, both animals became infected. But the monkey developed such an acute disease that the average survival was only a few days. When the authors inoculated blood from a warthog into goats, none of the goats became infected. When the infection was transmitted by G. morsitans, the mortality in the goats was from 86% to 90%. This observation

suggests that when T. simiae is transmitted by G. morsitans the infection is more virulent than when it is inoculated with blood. It would appear that the virulence of T. simiae is considerably enhanced by passage through G. morsitans. However, there was no confirmation that the warthog from which blood had been used to infect goats was infected with T. simiae and not some other species. It was assumed that wild caught G. morsitans picked infection from warthog infected with T. simiae.

In pigs infections due to T. simiae do not respond to treatment with most trypanocidal drugs in use. The drugs that can be used effectively are antrycide suramin complex (Watson and Williamson, 1958) and antrycide chloride (Stephen and Gray, 1960).

Little is known about the physiology, biochemistry and immunology of this parasite. This is because of the rapidity with which the infection in the pig develops (Hoare, 1972). For physiological and immunolgical studies of any parasite, it is necessary that the parasite remains in the host long enough. Stephen (1960) noticed that macrophages phagocytosed trypanosomes during infection with T. simiae.

Observations at the Kenya Coast suggested that infections in pigs due to T. simiae gave rise to a disease which ranged between acute and chronic forms. Steel (1966) came across a strain of trypanosomes which in morphology conformed to the classical description of T. simiae. However, this strain did not give

rise to the fulminating infection characteristic T. simiae. When the natural infections were left to run the normal course, the pigs died during the third or fourth week of parasitaemia and not within 7 days. Previous workers had observed that when pigs were infected with T. simiae, they died in an average of 3.6 days (range 24 hours to eight days) from the time when trypanosomes were seen in the blood (Stephen 1966).

Janssen and Wijers (1974), working in the same area as Steel (1966), studied the course of T. simiae infection in pigs to determine whether chronic strains existed and how they differed from the virulent ones. This they did by isolating T. simiae from naturally infected tsetse flies, G. brevipalpis, G. austeni and G. pallidipes, and transmitting the isolates through tsetse to pigs. Their observations suggested that there was a correlation between the virulence of T. simiae to pigs and the transmitting species of tsetse. Thirteen strains were isolated in pigs from G. brevipalpis. The infection in the thirteen pigs ranged between hyperacute and subacute disease. Four of the pigs had hyperacute infection, with the pigs surviving less than three days. Six pigs had acute infection and survived from 4 to 8 days while the other three pigs had subacute infection, with the pigs surviving from 8 to 16 days. Two strains were isolated from G. austeni. One strain gave rise to subacute infection, with the pig surviving thirteen days of parasitaemia and the other had a chronic infection lasting 58 days. Only one isolate was

obtained from G. pallidipes. This strain gave rise to chronic infection which lasted 109 days. No trypanosomes were seen in the blood of the pig after this period. When the authors transmitted the G. pallidipes isolate through G. brevipalpis, the pig experienced a hyperacute disease and transmission of the G. brevipalpis isolate through G. pallidipes gave rise to chronic disease in the pig. For cyclical transmission the authors had used both teneral wild flies and flies reared from pupae. The authors were aware that such wild flies could have picked infections from a wild suid in addition to the experimentally induced infection from the pigs.

A chronic T. simiae was isolated by Agu (1982) from wild caught flies. When a pig infected with the chronic strain, and a clean pig were challenged with a known virulent strain the "clean" pig died after 8 days but the other pig, after showing three waves of heavy parasitaemia, later reverted to the chronic state and finally parasites could not be detected in the blood. This is an additional report on the presence of chronic T. simiae strains.

1.3 OBJECTIVES OF THIS STUDY

In Africa, pig farming is practised on a very small scale and is limited to areas free of tsetse. With an ever increasing demand for animal protein, pig farming can be expected to assume greater importance.

Pig trypanosomiasis is a major constraint to pig farming in an area of over 10 million square kilometers. Fuller knowledge of the infection may help in devising suitable control methods. Such methods may consequently be used to reclaim vast areas for pig husbandry. This study has two main objectives.

1. Perhaps the most striking features of T. simiae is its virulence to pigs. It is clear from the literature review that little is known about the factors which influence the virulence of the parasite. This study aims at finding out factors which influence the virulence of T. (N.) simiae for pigs. The factors investigated include the species of tsetse vectors, the size of trypanosome inocula injected into pigs and the method of maintenance of the trypanosomes. The rate of development of T. simiae in different species of tsetse and its relationship to the resulting infection in pigs was also investigated.

2. Lack of a convenient experimental host has greately restricted physiological, immunological and other related studies of T. simiae. This study also aims at adapting T. simiae to rats and mice.

CHAPTER 2

MATERIALS AND METHODS

This chapter describes the materials and methods used during this study.

2.1 Pigs

All the pigs that were used in this study were of the Large White breed and were bought from farms in the Muguga area, which is tsetse free. Only male pigs aged between two and six months were used.

Pigs were housed in fly-proof pens, infected and uninfected groups being housed separately. Infected pigs which lost the infection spontaneously and those treated for the infection were kept in separate pens in order to observe possible relapses.

The animals were given a commercially available feed ("finishing mash") and water *ad lib* with the food and water being replenished at the end of each day. "Finishing mash" consists of grains, bone meal, blood meal, minerals and vitamins. The animals slept on hay spread on the floor of the pens. Hay was changed when it became wet.

Before pigs were used for experiments, they were kept in pens for a minimum period of two weeks. During this time a visual check was made on their condition. It was possible to detect helminth infections in the stool of the pigs during this

time or to detect any skin infections or presence of ectoparasites. When mites were detected, the pens were washed with lysol. When worms were detected in the stool, pigs were treated with Panacur at 5mg/kg. body weight and were not used for the study for the next two weeks. Treatment of pigs resulted in a lot of ascarids being voided with the stool. It was found necessary to treat only pigs which had been acquired from outside the Veterinary Research Depatment, Muguga. This is because animals from the department are routinely vaccinated and treated against helminthic infections, a practice which many small scale farmers cannot always afford. None of pigs from the department were found infected with worms.

2.2 Rats and Mice

The albino rats used for this study originated from National Public Health Laboratories at the Kenyatta National Hospital, Nairobi. The rats were brought to the Veterinary Research Department Muguga in 1960. They are probably Wistar.

Swiss mice from the Veterinary Research Department originated from Carworth Farm, New York. This strain of mice was taken to Rio de Jenairio Laboratories of the Rockefeller Foundation from where they were taken to Lagos, Nigeria. From Lagos they were brought to Entebbe in 1936. In 1956, they were brought to the then East African Veterinary Research Organisation, now called the Veterinary Research Department, Kenya Agricultural Research Institute, Muguga.

Swiss mice from the Kenya Trypanosomiasis Research Institute originated from London. These mice were brought into Kenya through the Walter Reed Army Medical Research Project based at the Veterinary Research Laboratories, Kabete. Swiss mice were also acquired from the Dutch Medical Research Institute at Kenyatta National Hospital animal house.

Mice were used for experiments at 8 weeks and rats at 12 weeks after birth. Only male rats and mice were used. They were housed in cages in the experimental groups and were fed on pellets and water ad lib.

2.3 Tsetse Flies

All the tsetse flies used for the transmission experiments were maintained at the Kenya Trypanosomiasis Research Institute. Only teneral flies were used.

2.3.1 Glossina pallidipes Austen

The flies used in the present study originated from Kibwezi forest, Kenya. Wild caught flies were brought to Muguga and were used to initiate a colony. Details on the initiation and the performance of this colony are given elsewhere (Opiyo, 1979; Opiyo, Mgutu, Okumu and Amakobe, 1980; Opiyo and Okumu, 1981). The insectary in which the flies were kept was maintained at 25^{o}C ± 1^{o}C and a relative humidity of between 70% and 75%.

2.3.2 G. morsitans Newstead

Glossina morsitans of both sexes were initially obtained from a colony maintained at the International Centre for Insect Physiology and Ecology (ICIPE). The ICIPE colony originated from flies supplied by the Tsetse Laboratory in Langford, Bristol, U.K. The flies from ICIPE were used to start the colony at the Kenya Trypanosomiasis Research Institute (KETRI) and all flies used for the studies came from the KETRI colony. This species of tsetse was kept in the same insectary and under the same condition as G. pallidipes described under 2.3.1 above.

2.3.3 G. brevipalpis Newstead

This species of tsetse originated from Kibwezi forest, Kenya and was brought in at the same time as G. pallidipes. Flies of this species were used when they were available since the colony expanded very slowly indeed. The maintenance procedures were the same as for the other two species.

2.4 Trypanosomes

2.4.1 Trypanosoma simiae

Four stabilates of T. simiae were used in this study. The four stabilates were isolated from Muhaka Forest, Kwale District Kenya during 1970. Three of the isolates were made by Dr. A. J. Wilson during the month of October, 1970. These isolates were designated EATRO 1806, 1861 and 1880. Stabilate

EATRO 1806 was isolated from the infected proboscis of a G. brevipalpis fly which was put in a capillary tube, sealed and stored in liquid nitrogen. Later the capillary tube was cut open and the proboscis was macerated under the dissecting microscope in phosphate buffered saline (PBS) pH 8.0. The suspension was then withdrawn into a syringe and inoculated into a pig. The pig became parasitaemic after a prepatent period of 6 days and the stabilate was made 10 days after inoculation and stored in liquid nitrogen. Stabilate EATRO 1861 was also made from an infected proboscis of G. brevipalpis fly. The fresh proboscis was macerated in PBS and the suspension was inoculated into a pig. The pig became parasitaemic after a prepatent period of 9 days and the stabilate was made 29 days later. Stabilate EATRO 1880 was also isolated from G. brevipalpis. The infected proboscis of the fly was macerated in PBS and the suspension was stored in liquid nitrogen for 29 days after which it was inoculated into a pig. The pig became parasitaemic after a prepatent period of 29 days and the stabilate was made 58 days after inoculation.

The fourth stabilate EATRO 1786 was isolated by Dr. Jan Janssens in September 1970, from Muhaka Forest. He fed male G. austeni on a pig. The pig became parasitaemic after a prepatent period of 5 days and the stabilate was made 20 days after the flies had fed on the pig. On dissection, two out of nineteen flies were found infected.

2.5 Infection of pigs with T. simiae

Trypanosomes in capillary tubes were taken from the trypanosome bank where they had been kept in liquid nitrogen and allowed to thaw at room temperature. After thawing, the capillary tubes were cut open and the trypanosomes were suspended in ESG (EDTA - Saline - Glucose; pH 7.4) and a drop of the suspension was put on a microscope slide on which a cover slip was placed and then trypanosomes were microscopically enumerated using a x 40 objective. The number of trypanosomes in one millilitre of the suspension was determined and each pig was inoculated with 10^5 trypanosomes subcutaneously. When a subpassage was made from donor pigs, infected blood from an ear vein was suspended in ESG, the number of trypanosomes in the suspension was determined microscopically and then the required number of trypanosomes were inoculated into a uninfected pig.

For cyclical transmission of T. simiae to pigs, flies which had been previously fed on infected pigs or rats were fed on clean pigs for one week or until the pig became positive with trypanosomes on microscopical examination of tail blood. These flies were kept for three weeks from the time of the infective feed before being used to transmit the infection to pigs.

2.6 Infection of mice and rats with T. simiae

Stabilates of different strains were removed from the trypanosome bank where they had been preserved in liquid nitrogen

and suspended in ESG and inoculated into mice and rats intraperitoneally. Mice received 10^5 and rats 5×10^5 trypanosomes each. When mice and rats were inoculated with trypanosomes from an existing infection in rats or pigs, infected blood was suspended in ESG and inoculated as previously described.

Starting from day 2 after inoculation, rats and mice were examined daily for the presence of trypanosomes in tail blood. For this purpose a wet preparation was made by putting a drop of tail blood on a microscope slide and placing a cover slip over it. Trypanosomes were counted and recorded using the method of Walker (1968), see Appendix 1. Thin blood smears were made and stained using Giemsa's stain for morphological study.

2.7. Infection of Tsetse Flies with *T. simiae*

Teneral flies were fed on parasitaemic pigs. The number of teneral flies fed on a pig on any day depended on the number of flies that emerged on that day. This exercise continued daily for as long as the pig was alive or for one week where infected pigs remained alive longer than a week.

After feeding on an infected pig, teneral flies were taken back to the insectary where they were routinely fed on rabbits. The maintenance conditions were the same as for the main insectary with a temperature of $25^{\circ}C \pm 1^{\circ}C$ and a relative humidity of 70 - 75%. Any fly dying during the period of maintenance was dissected and

examined for the presence of trypanosomes in the gut and proboscis. After three weeks, flies were fed on pigs in an attempt to transmit the infection. When the infection had been successfully transmitted or after the infected flies had fed on an uninfected pig for one week, the flies were dissected in a 0.01 molar phospate buffered saline at pH 8.0. The gut and the proboscis were then examined for the presence of trypanosomes. When a proboscis was found to be infected the exact location of trypanosomes was noted, that is whether they were in the labrum, labium or hypopharynx. When trypanosomes were seen in the labrum, labium and hypopharynx, the infections were considered mature but those in which trypanosomes were only found in the labrum were considered immature. Trypanosome infection rates in the flies were estimated from the proportion of flies with mature infections out of the total number of flies dissected.

Attempts were made to identify infected flies by the examination of Giemsa stained saliva smears, but none of the 100 saliva smears examined were found to contain trypanosomes. The method was therefore not used in this study.

2.8 Infectivity Titration of Trypanosomes

Samples of trypanosomes to be titrated were made by suspending infected blood from pigs, rats or mice in ESG and serially diluting them ten fold using the same buffer. When the infected blood to be titrated was from mice or rats, groups of six mice were inoculated at each of five consecutive dilutions.

The dilution containing the highest number of trypanosomes was adjusted to contain 10^6 trypanosomes per ml. Mice in the first groups received 10^4, 10^3, 10^2 and 10^1 trypanosomes respectively. Starting from day 3 after inoculation, mice were examined for the presence of trypanosomes using tail blood. This examination was carried out daily and any mouse found parasitaemic was killed. Examination for trypanosomes was carried out for 21 days after which the mice were killed.

The number of mice which become infected in each group at each of the five dilutions was recorded. Using this number the infectivity of the appropriate suspension was determined according to Lumsden, Herbert and McNeillage (1973). See Appendix 11. The infectivity of the suspension was expressed as the ID_{63}, which is defined as the number of trypanosomes required to infect 63 per cent of the animals inoculated. This is the volume of suspension of trypanosome that on average contains one infective organism, assuming that any given organism in the suspension may not be infective and that a single organism will infect.

C H A P T E R 3

FACTORS AFFECTING THE VIRULENCE OF *TRYPANOSOMA SIMIAE* IN PIGS

3.1 INTRODUCTION

Many factors influence the virulence of trypanosomes in their vertebrate hosts. Walker (1970) observed that when *T. brucei* was passaged frequently through mice, the virulence of the strain increased. This he said was because only the fast growing trypanosomes are selected for by harvesting and inoculating trypanosomes during the rising phase of parasitaemia. Like syringe passage, mechanical transmission of trypanosomes is suspected to enhance the virulence of trypanosomes in the same way. Stephen (1966) suggested that during an epidemic of *T. simiae* in pigs, tsetse flies play only a secondary role in the transmission. He suggested that the infection may be introduced into a herd from the wild by a few tsetse flies and then maintained by horse flies which transmit it mechanically. This mode of transmission is believed to enhance the virulence of *T. simiae* to the extent that a whole herd could be decimated within a few days of the initial introduction of the disease from the wild.

The virulence of trypanosomes may also be related to the morphology of the trypanosomes. Fairbairn (1953) suggested that the mean lengths of strains of *T. vivax* from West Africa were

generally shorter than those of the parasites from East Africa and that the shorter forms of trypanosomes were responsible for the acute, virulent infections in cattle which occur mainly in West Africa. However for T. congolense trypanosomes the long forms give rise to virulent infections with heavy parasitaemia (Godfrey, 1961).

There is evidence that tsetse may influence virulence of trypanosomes in mammalian hosts. Willet (1970) observed that there was a marked difference in the risk of domestic stock contracting infections with T. congolense depending on whether the animals were in contact with tsetse of the palpalis or of the morsitans group. He observed that sometimes cattle could be kept safely in an area infested with G. fuscipes but not in one infested with G. pallidipes. He concluded that the constrast between the two species in the transmission of T. congolense might be the result of the feeding habits of the flies. G. pallidipes tended to feed on bovids which may harbour the infection whereas G. fuscipes tended to feed on man and reptiles. The history of the epidemics of human sleeping sickness which have occurred in Eastern Africa seems to relate the disease form to the transmitting species of tsetse. It was recognised that the gambian type of infection was transmitted by G. fuscipes while the rhodesian type was transmitted by G. pallidipes (Willet, 1965; Ford, 1972). Scott (1970) noted that variation in the virulence of sleeping sickness occurred naturally and that there was a constant

association of the more virulent strains with transmission by the G. morsitans group and of less virulent strains by the palpalis group. Lewis (1949) observed that there were two forms of T. vivax infections in cattle in Kenya. The chronic infection he associated with G. palpalis and the virulent form with G. brevipalpis and G. fuscipleuris.

Observations at the Kenya Coast indicated that Trypanosoma simiae infections in pigs gave rise to diseases which fluctuated between acute and chronic forms (Steel, 1966). It was later shown that there was a correlation between virulence and the transmitting species of tsetse (Janssen and Wijers, 1974). T. simiae was isolated from naturally infected tsetse flies (Glossina brevipalpis, G. austeni and G. pallidipes) and cyclically transmitted to pigs. All the isolates from G. brevipalpis were very virulent, with no pig surviving longer than 16 days of patent infection. The one isolate from G. pallidipes caused a chronic infection, while G. austeni isolates were intermediate in virulence between the G. brevipalpis and G. pallidipes isolates.

Janssen and Wijers (1974) felt that there was need to further investigate the effect of the vector species on the virulence of T. simiae in pigs. They had used in addition to laboratory reared flies, wild caught teneral G. brevipalpis and were therefore not sure of the source of the infecting trypanosomes. Flies with teneral flies could have taken a partial blood meal before being caught.

When transmitting the infection to pigs, flies fed for a period of one week, and during this period an unknown number of trypanosomes could have been inoculated into the pig. They did not assess the number of trypanosomes being inoculated into the pig by the flies.

This study endeavours to determine factors which may influence the virulence of T. (N). simiae for pigs. The factors investigated were the species of tsetse, the number of trypanosomes inoculated into the pig and the syringe passage of the trypanosomes.

3.1 MATERIALS AND METHODS

3.2.1 Pigs

All the pigs used for this study were of the Large White breed and were bought from farms in the Muguga area. Only male pigs aged between two and six months were used. Details about the maintenance of pigs before and during the study are given in Chapter Two.

3.2.2 Tsetse flies

Three tsetse species were used for this study. They are G. pallidipes, G. morsitans and G. brevipalpis. The history and rearing conditions of these flies is given in Chapter Two.

3.2.3 Trypanosomes

Three stabilates of T. simiae were used, namely EATRO 1806, 1861 and 1786. Detailed information on the history of isolation

and maintenance of these stabilates is given in Chapter Two.

3.2.4 Infection of pigs and tsetse flies with T. (N). simiae

The methods used for infecting both pigs and tsetse flies are described in Chapter Two.

Transmission of the infection by tsetse flies was either from pigs which had been inoculated with a stabilate direct from the bank or from a pig which had been cyclically infected. The flow chart for cyclical transmission was as follows:

(a) bank → pig → G. morsitans → pig

(b) bank → pig → G. morsitans → pig → G. morsitans → pig

(c) bank → pig → G. pallidipes → pig

(d) bank → pig → G. pallidipes → pig → G. pallidipes → pig

(e) bank → pig → G. pallidipes → pig → G. morsitans → pig

(f) bank → pig → G. morsitans → pig → G. pallidipes → pig

3.2.5 Examination of pigs after infection

Starting from the day when pigs were inoculated with trypanosomes or when infected flies were first put to feed on clean pigs, the rectal temperatures of pigs were taken twice daily, in the morning and afternoon. Blood from the tail vein was at the same time collected into two heparinised capillary tubes for the Haematocrit Centrifugation Technique (HCT) using a Hawksley haematocrit centrifuge. After spinning blood in the capillary tubes, the packed cell volume for each pig was estimated by

reading off the scale from the reader and then the tube was cut at the buffy coat interface. The buffy coat was the expressed on to a slide and examined for trypanosomes. As soon as trypanosomes were detected in the buffy coat the wet method was used for the enumeration of trypanosomes (Walker, 1969). When a pig became parasitaemic the prepatent period in days was recorded as was the patent period and the number of days when trypanosomes were observed in the blood circulation. The patent period was either the period from the day when trypanosomes were first detected to the day the pig died or, if a self-limiting infection occurred, the period from the day when trypanosomes were first detected to the last day on which they were seen. The survival of infected pigs was taken as the main indicator of the virulence of an infection.

Giemsa stained, thin blood smears were made as often as possible from the infected animals for examining the morphological forms of trypanosomes present. The stained smears were examined under oil immersion and the lengths of the trypanosomes were measured using a pre-calibrated ocular micrometer. This procedure was also followed for T. simiae infections in mice and rats. The positions of the kinetoplast and nucleus were noted and the presence or absence of a free flagellum was checked.

Some very sick pigs were treated with Antrycide chloride (at 50 mg/kg) and these were examined as often as possible for trypanosomes for six months and thereafter only occasionally in

order to detect any relapses that might occur.

Whenever an isolate was being used for the first time, tail blood from the parasitaemic pig was mixed with ESG and inoculated (ip) into a group of four albino mice as a quick check that the isolate was T. simiae since the parasite is not usually infective to mice.

3.2.6 Serial syringe maintenance of T. simiae in pigs

Two stabilates of T. simiae (EATRO 1806 and EATRO 1786) were used for this study. Starting with an infected pig, blood from an ear vein was suspended in ESG to give a dilution of 10^5 trypanosomes per millilitre of the suspension. A clean pig was then inoculated and was thereafter examined as previously described. When infection became established a further subpassage was made into a clean pig. For EATRO 1806, the trypanosomes were serially passaged to three pigs and for EATRO 1786, two times on each of two occasions.

For each pig the prepatent period, the patency period and the pattern of parasitaemia were recorded.

3.3 RESULTS

3.3.1 The effect of vector species on virulence

a) Glossina morsitans

The behaviour of three isolates was studied in a series of experiments with a view to determining the influence of G. morsitans

order to detect any relapses that might occur.

Whenever an isolate was being used for the first time, tail blood from the parasitaemic pig was mixed with ESG and inoculated (ip) into a group of four albino mice as a quick check that the isolate was T. simiae since the parasite is not usually infective to mice.

3.2.6 Serial syringe maintenance of T. simiae in pigs

Two stabilates of T. simiae (EATRO 1806 and EATRO 1786) were used for this study. Starting with an infected pig, blood from an ear vein was suspended in ESG to give a dilution of 10^5 trypanosomes per millilitre of the suspension. A clean pig was then inoculated and was thereafter examined as previously described. When infection became established a further subpassage was made into a clean pig. For EATRO 1806, the trypanosomes were serially passaged to three pigs and for EATRO 1786, two times on each of two occasions.

For each pig the prepatent period, the patency period and the pattern of parasitaemia were recorded.

3.3 RESULTS

3.3.1 The effect of vector species on virulence

a) Glossina morsitans

The behaviour of three isolates was studied in a series of experiments with a view to determining the influence of G. morsitans

on the virulence of T. simiae in pigs.

Three pigs on separate occasions were given i.p. 10^5 trypanosomes from the isolate 1861, each time the trypanosomes being taken directly from the trypanosome bank. When the infections became patent, teneral G. morsitans flies were fed on the pigs and used in the next part of the experiment.

The results are shown in Tables 1 and 11. The prepatent period for the pigs directly inoculated from the bank varied from 2 to four days with a mean of 3.33 ± 0.66 days. The parasitaemia in these pigs rose sharply after trypanosomes were first detected in the blood of the pigs. The rise in parasitaemia was preceeded by a rise in rectal temperature. Pig No. 3 (Fig. 1) in which the infection was allowed to run the full course died with a heavy fulminating parasitaemia after 3 days. The parasitaemia by then was very high, with more than 120 trypanosomes in a (x40 objective) microscope field in a wet preparation. The PCV of this pig had dropped from 45% to 35% the time of death.

This isolate was transmitted by G. morsitans to five pigs on various occasions as follows:-

i) Transmission by G. morsitans from pigs which had been inoculated from trypanosomes from the bank was to pig Nos. 9 and 4 (Table II).

b) Pig No. 9 experienced a chronic disease and survived 28 days of patent parasitaemia. Pig No. 4 died of acute disease after

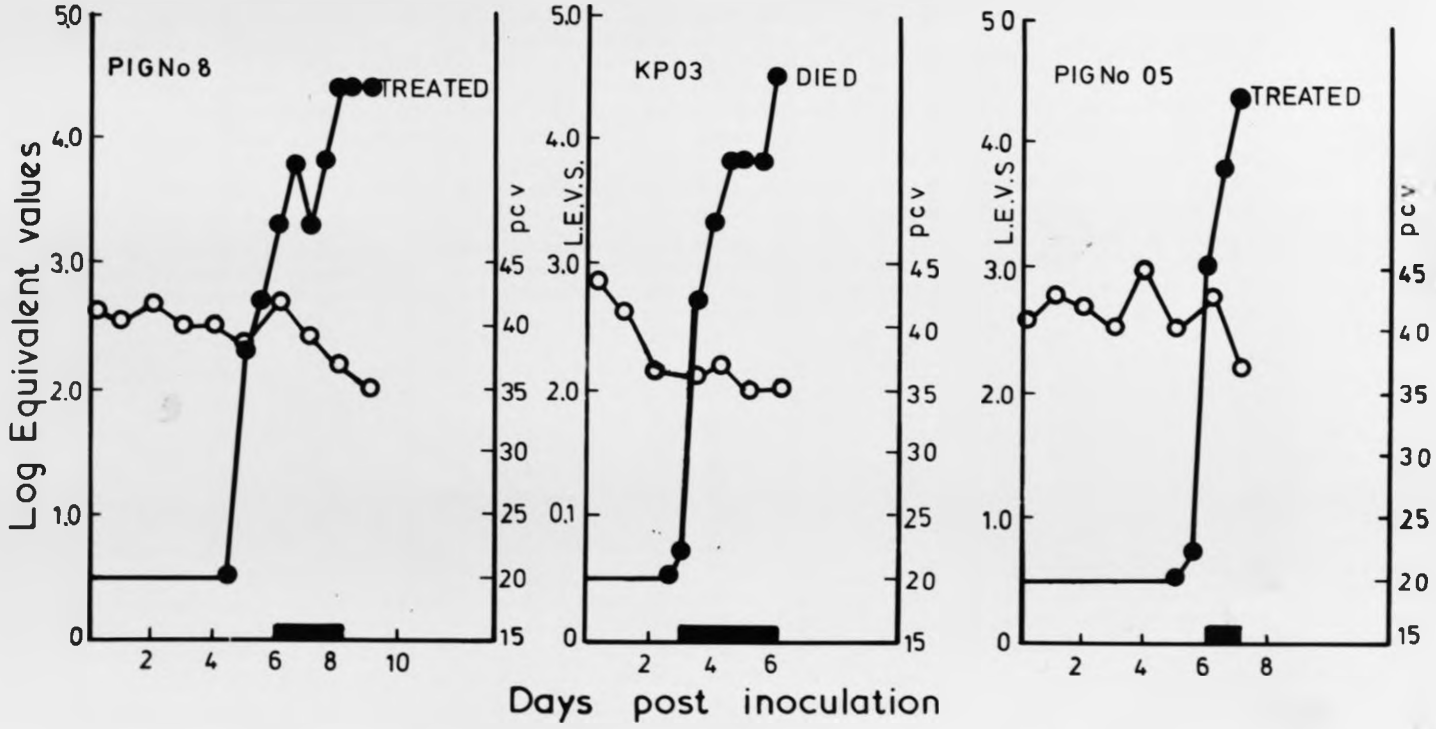


FIG.1. The course of infection in three pigs directly inoculated with EATRO 1861. The graphs show parasitaemia (●—●), PCV (o—o) and days when temperature rose above 40.0°C (▬)

3 days of patent parasitaemia. The prepatent periods for these pigs were 23 and 13 days, respectively.

ii) On another occasion G. morsitans transmitted infection from a pig which had been cyclically infected by G. morsitans to another pig. This was to pig No. 18 (Table II (c). This pig developed parasitaemia after six days and died of a heavy fulminating parasitaemia 7 days later.

iii) G. morsitans was also used to transmit this isolate from a pig which had been cyclically infected by G. pallidipes (Table II (f) to two pigs (Nos. 14 and 16). Pig No. 14 had a prepatent period of 14 days and died after 11 days of patent parasitaemia. During the period of infection, the parasitaemia was generally low rising only at the time of death. The PCV of the pig fluctuated steadily and dropped just prior to death. For pig No. 16, the prepatent period was 18 days and the pig had a self - limiting infection which disappeared after 115 days. The pig had low and intermittent parasitaemia separated by period when parasites were not detectable in the peripheral blood of the pig. After the 115 days no trypanosomes were observed in the blood of this pig. Examination of the pig continued for six months from the day when trypanosomes were last seen in blood. The PCV for pig No. 16 fluctuated steadily during the period of infection and did not drop below 30%. Parasitaemia reading ranged between one and ten trypanosomes per microscope field.

Table 1: Mean prepatent periods and mean patent periods recorded for pigs infected directly from the Bank and for pigs infected by transmission through different species of tsetse.

Stock No. EATRO No.	Mode of Transmission	Mean Prepatent Period	Mean Patent Period
1861	Direct inoculation (3)*	3.33 ± 0.66	8.66 ± 5.66
	G. pallidipes (6)	8.33 ± 1.63	5.66 ± 0.92
	G. morsitans (5)	14.8 ± 2.82	32.8 ± 20.99
1786	Direct inoculation (7)	3.86 ± 0.6	10.43 ± 5.18
	G. pallidipes (4)	8.0 ± 1.78	40.5 ± 24.09
	G. morsitans (7)	9.57 ± 2.15	7.71 ± 0.02
1806	Direct inoculation (3)	3.33 ± 0.33	2.0 ± 0.58
	G. brevipalpis (3)	5.67 ± 1.86	12.33 ± 8.41
	G. morsitans (4)	5.75 ± 0.95	11.0 ± 6.10

*Numbers in brackets are numbers of animals in each group.

The prepatent period for the five pigs to which G. morsitans transmitted the infection ranged from 6 to 23 days giving a mean of 14.8± 2.82 days (Table 1). The patency of infection ranged from a hyperacute disease with the pig surviving only 3 days to a very chronic, self- limiting infection which persisted for 115 days. The mean patency of the infection was 32.8 ± 20.99 days.

For the isolate EATRO 1806, three pigs (Nos. 1, 23, 71) were inoculated directly from the bank. The prepatent period for these three pigs ranged from 3 to 4 days giving a mean of 3.33 ± 0.33 days. The pigs died soon after trypanosomes were detected in their peripheral blood. The survival of the pigs ranged between 1 to 3 days with a mean of 2.0 ± 0.58 days. All the pigs died of heavy fulminating parasitaemia (Table 1 and III (a)). This isolate was transmitted by G. morsitans as follows:-

(i) G. morsitans transmitted the infection from pigs directly inoculated from the bank to clean pig Nos. 80 and 70. Pig No. 80 became parasitaemic after a prepatent period of 6 days and died 4 days later. Pig No. 70 had a prepatent period of 7 days and survived 29 days of patent infection (Table III (b)).

(ii) In another experiment G. morsitans flies transmitted infection from a pig (No. 80) which had been cyclically infected by G. morsitans to pig No. 90 (Table III (c)).

Table II: Details of experiments with EATRO 1861.

(a) Direct inoculation from the bank.

Bank	Pig No.	Prepatent Period	Patent Period	Remarks
	3	2	3	Died
	5	5	4	Treated
	8	4	20	Treated

(b) Transmission by G. morsitans

Bank	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Remarks
	5		9	23	28	Died
	3		4	13	3	Died

(c) Transmission by G. morsitans from a G. morsitans initiated infection

Bank	Pig No.	G. morsitans	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Remarks
	8		9		18	6	7	Died

(d) Transmission by G. pallidipes

Bank	Pig No.	G. pallidipes	Pig No.	Prepatent Period	Patent Period	Remarks
	3		10	11	5	Died
	5		17	6	4	Died
	8		11	8	6	Died

(e) Transmission by G. pallidipes from a G. pallidipes initiated infection.

Bank	Pig No.	G. pallidipes	Pig No.	G. pallidipes	Pig No.	Prepatent Period	Patent Period	Remarks
	8		10		13	5	10	Died
	8		10		15	5	4	Died

(f) Transmission by G. morsitans from a G. pallidipes initiated infection

Bank	Pig No.	G. pallidipes	Pig No.	G. morsitans	Pig No.	PPP	PP	Remarks
	10		15		16	18	15	Self cured
	8		10		14	14	11	Died

(g) Transmission by G. pallidipes from a G. morsitans initiated infection.

Bank	Pig No.	G. morsitans	Pig No.	G. pallidipes	Pig No.	PPP	PP	Remarks
	8		9		19	15	5	Died

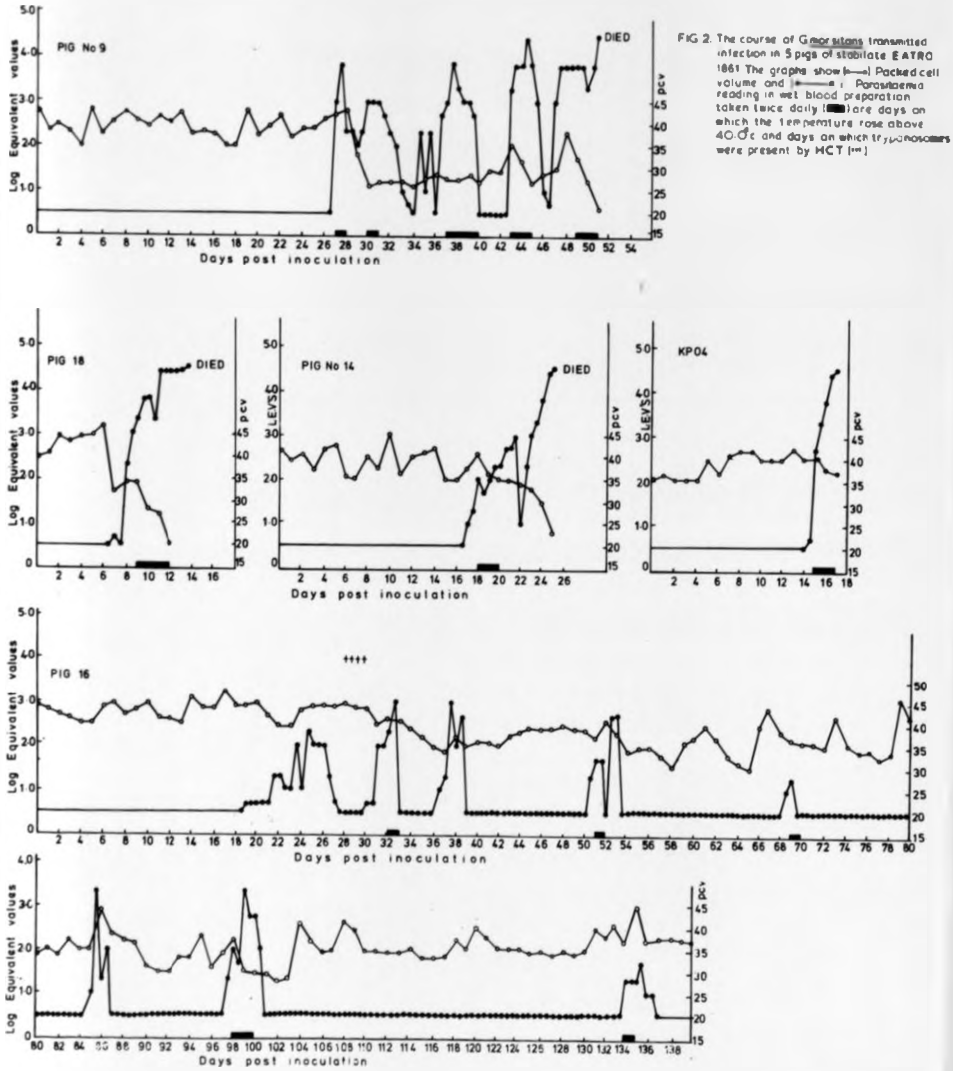


FIG 2. The course of G.morsitans transmitted infection in 5 pigs of stabilate EATRO 1861 The graphs show |—| Packed cell volume and |•——•| Parasitaemia reading in wet blood preparation taken twice daily [■■] are days on which the temperature rose above 40.0°c and days on which trypanosomes were present by HCT [▪▪]

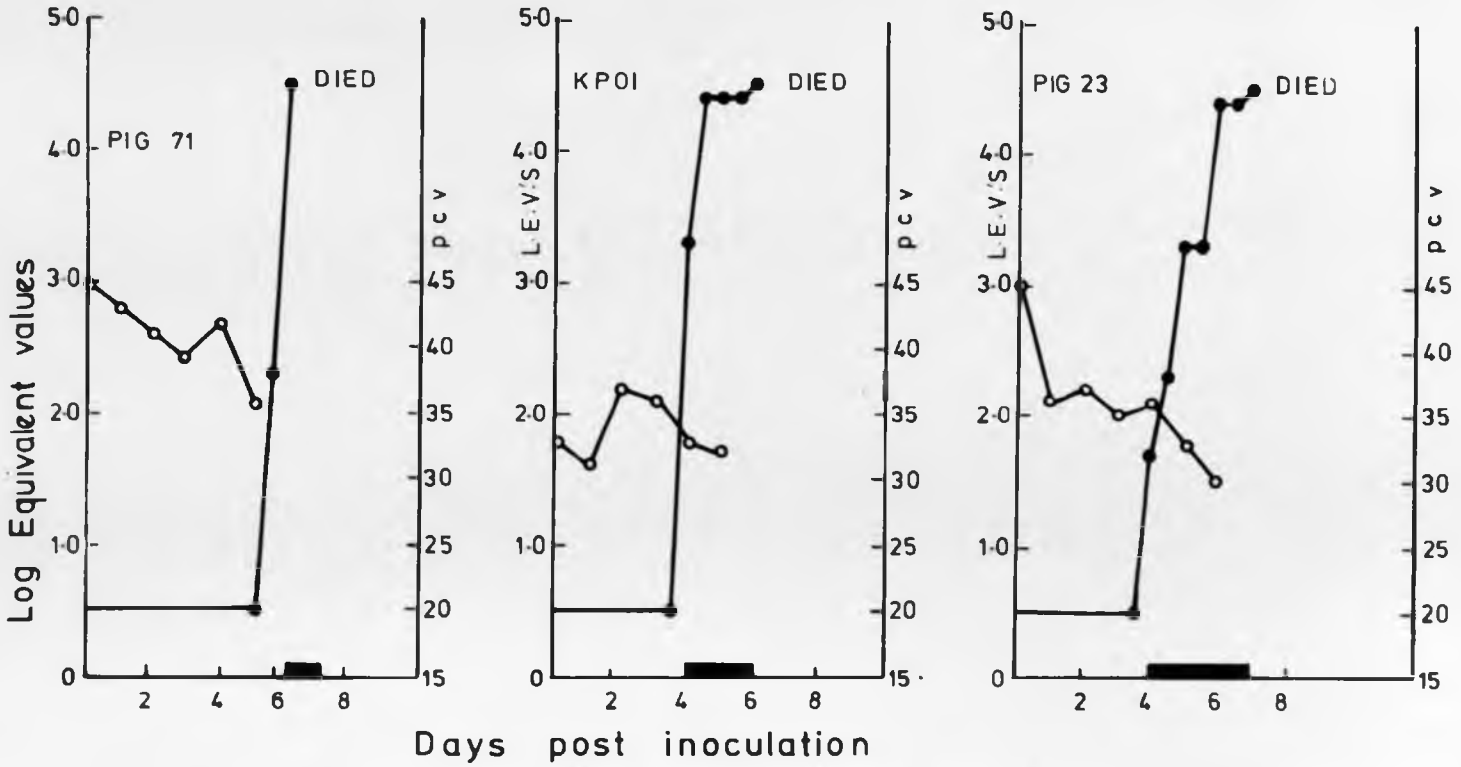


FIG.3. The course of infection in three pigs directly inoculated with EATRO 1806. The graphs show the Pcv (o—o), parasitaemia (•—•), and(▬) days when the temperature rose above 40.0°c.

Table III: Details of experiments with EATRO 1806

(a) Direct inoculation from the bank

Bank	Pig No.	Prepatent Period	Patent Period	Remarks
	71	4	1	Died
	01	3	2	Died
	23	3	3	Died

(b) Transmission by G. morsitans

Bank	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Remarks
	04		80	6	4	Died
	23		70	7	29	Died

(c) Transmission by G. morsitans from a G. morsitans initiated infection.

Bank	Pig No.	G. morsitans	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Rema
			80		90	7	8	Died

(d) Transmission by G. brevipalpis

Bank	Pig No.	G. brevipalpis	Pig No.	Prepatent Period	Patent Period	Remarks
	23		68	7	29	Died
			67	2	6	Died
			66	8	2	Died

(e) Transmission by G. morsitans from a G. brevipalpis transmitted infection.

Bank	Pig No.	G. brevipalpis	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Remarks
	23		68		72	3	3	Died

(f) Transmission by G. pallidipes from a G. brevipalpis transmitted infection.

Bank	Pig No.	G. brevipalpis	Pig No.	G. pallidipes	Pig No.	Prepatent	Patent	Remar
	23		68		73	5	2	Treated

Table IV: Details of experiments with 1786.

(a) Direct inoculation from bank.

Bank	Pig No.	Prepatent Period	Patent Period	Remarks
	78	2	1	Died
	02	5	3	Treated
	5	3	5	Died
	79	5	7	Died
	27	2	14	Treated
	07	4	40	Treated
	01	6	3	Died

(b) Transmission by G. morsitans

Bank	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Remarks
	79		08	10	9	Died
	1		4	6	9	Died
	5		6	20	12	Died

(c) Transmission by G. morsitans from G. morsitans initiated.

infection

Bank	Pig No.	G. morsitans	Pig No.	G. morsitans	Pig No.	Prepatent Period	Patent Period	Rema
	79		08		87	14	5	Died

(d) Transmission by G. pallidipes

Bank	Pig No.	G. pallidipes	Pig No.	Prepatent Period	Patent Period	Remarks
	5		2	8	110	Self cured
	27		12	13	7	Died
	79		9	6	36	Self Cured

(e) Transmission by G. pallidipes from G. pallidipes initiated infection.

Bank	Pig No.	G. pallidipes	Pig No.	G. pallidipes	Pig No.	Prepatent Period	Patent Period	Remarks
	79		9		85	5	9	Died

(f) Transmission by G. morsitans from G. pallidipes initiated infection.

Bank	Pig No.	G. pallidipes	Pig No.	G. pallidipes	Pig No.	Prepatent Period	Patent Period	Remarks
	79		9		86	6	7	Died

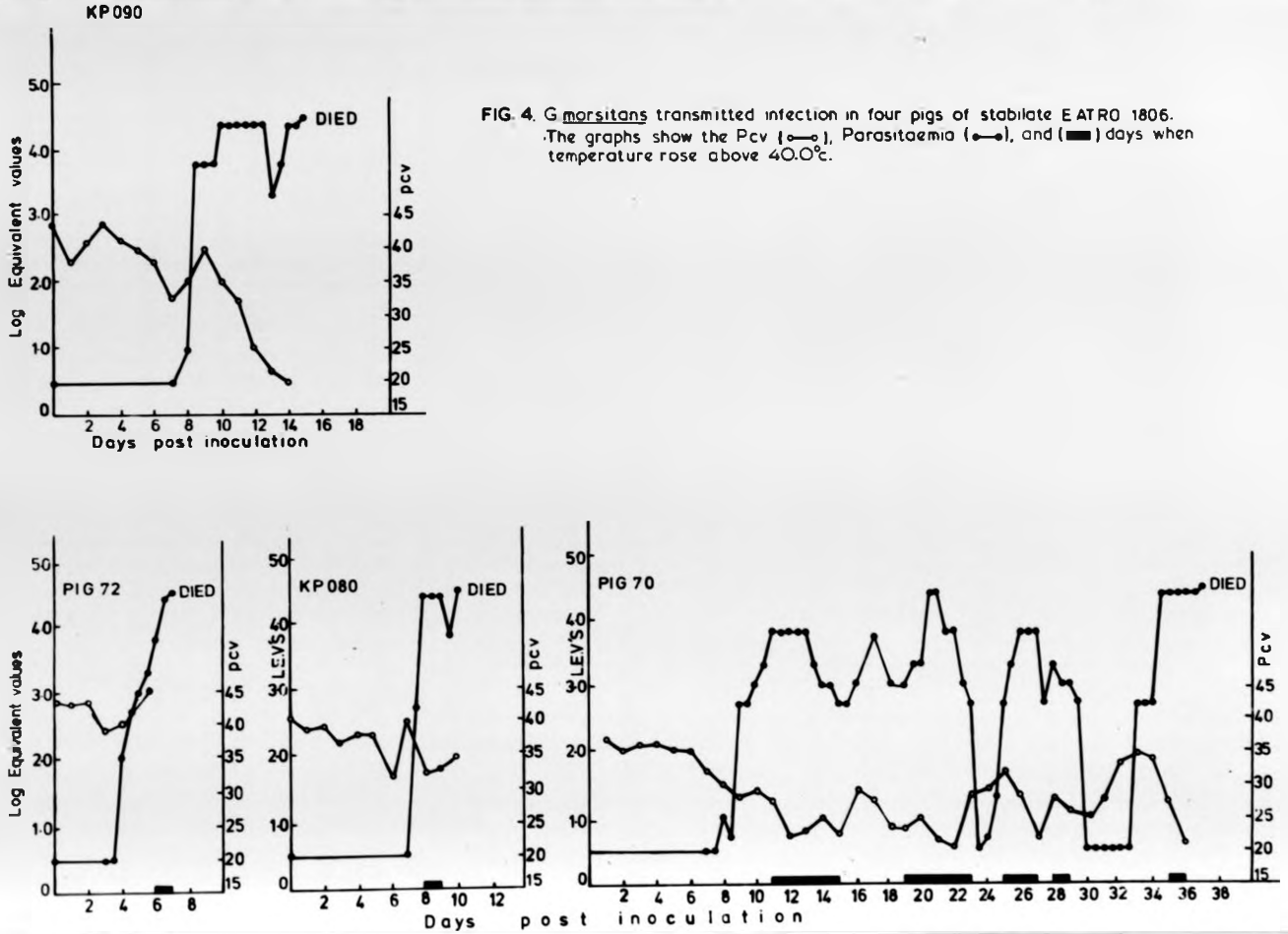
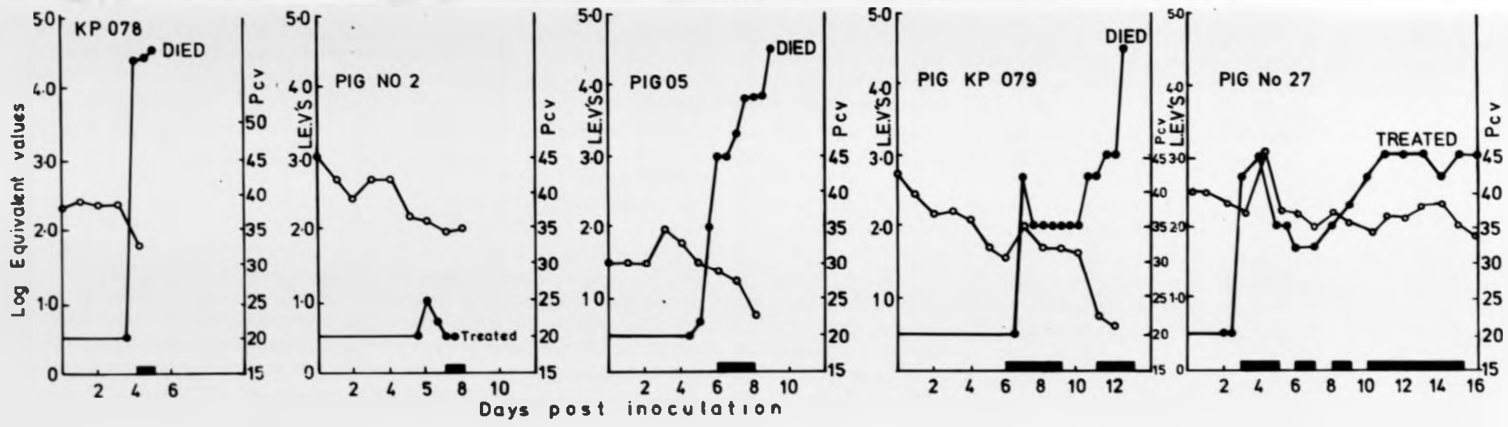


FIG. 4. G morsitans transmitted infection in four pigs of stabilate EATRO 1806. The graphs show the Pcv (o—o), Parasitaemia (•—•), and (▬) days when temperature rose above 40.0°C.



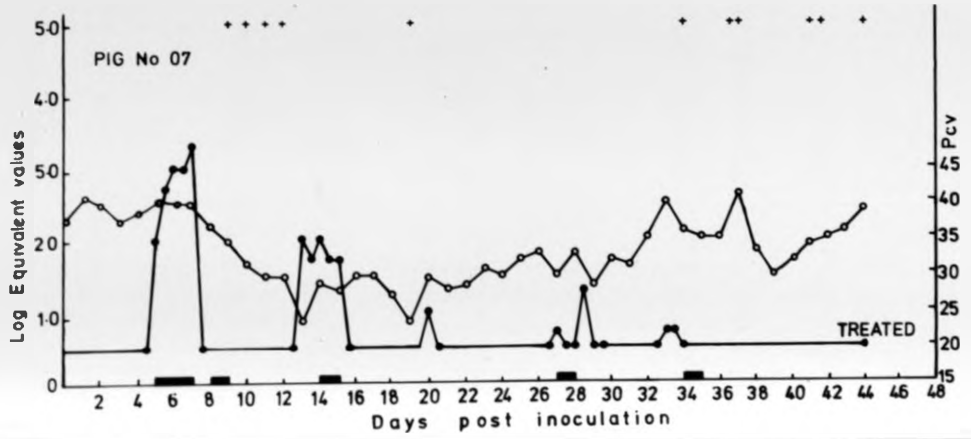


FIG. 5. The course of infection in six pigs directly inoculated with stabilate EATRO 1786 The graphs show the parasitaemia (●—●), Pcv (○—○), days when temperature rose above 40.0°c (▬) and days when trypanosomes were detected by HCT and not in the wet blood preparation (+++).

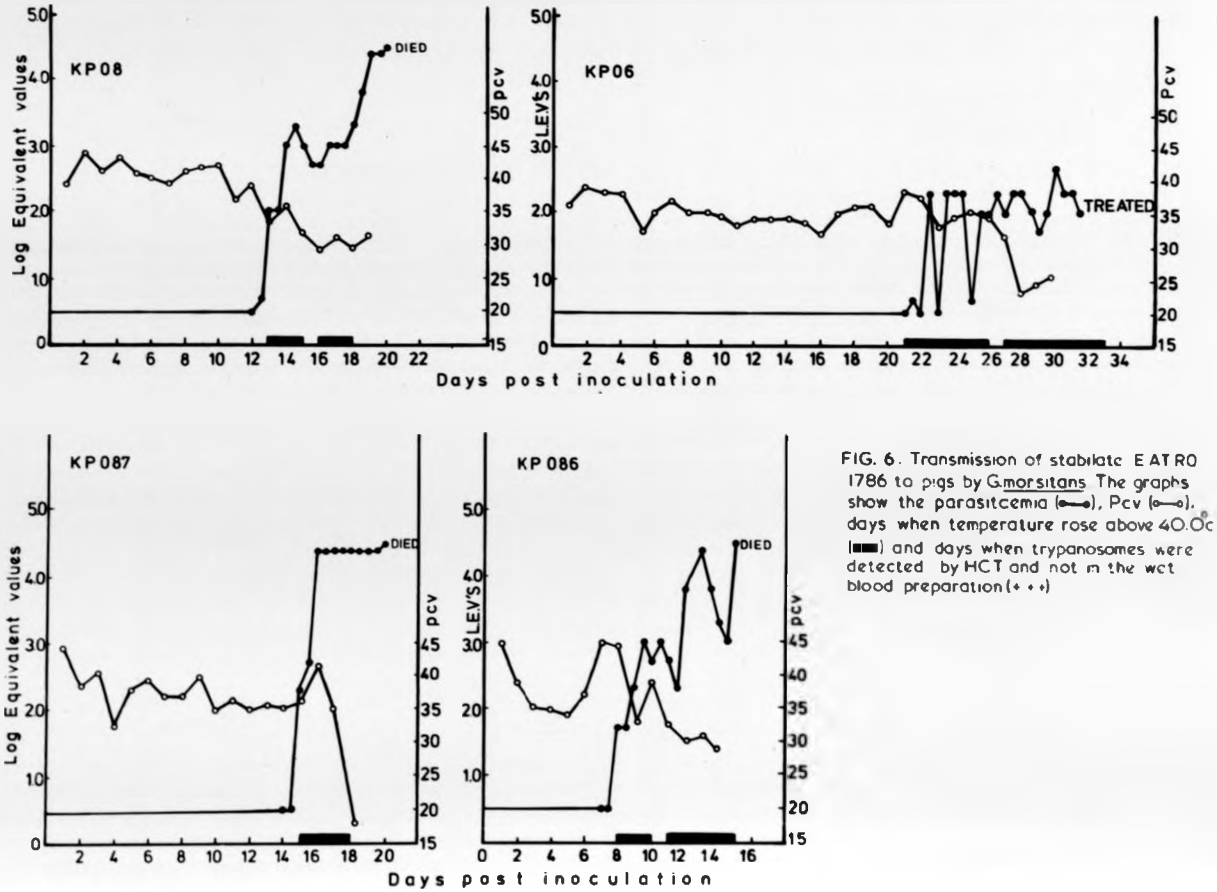


FIG. 6. Transmission of stabilate E AT RO 1786 to pigs by G.morsitans. The graphs show the parasitcemia (o—o), Pcv (o—o), days when temperature rose above 40.0°c (■■) and days when trypanosomes were detected by HCT and not in the wet blood preparation (+ +)

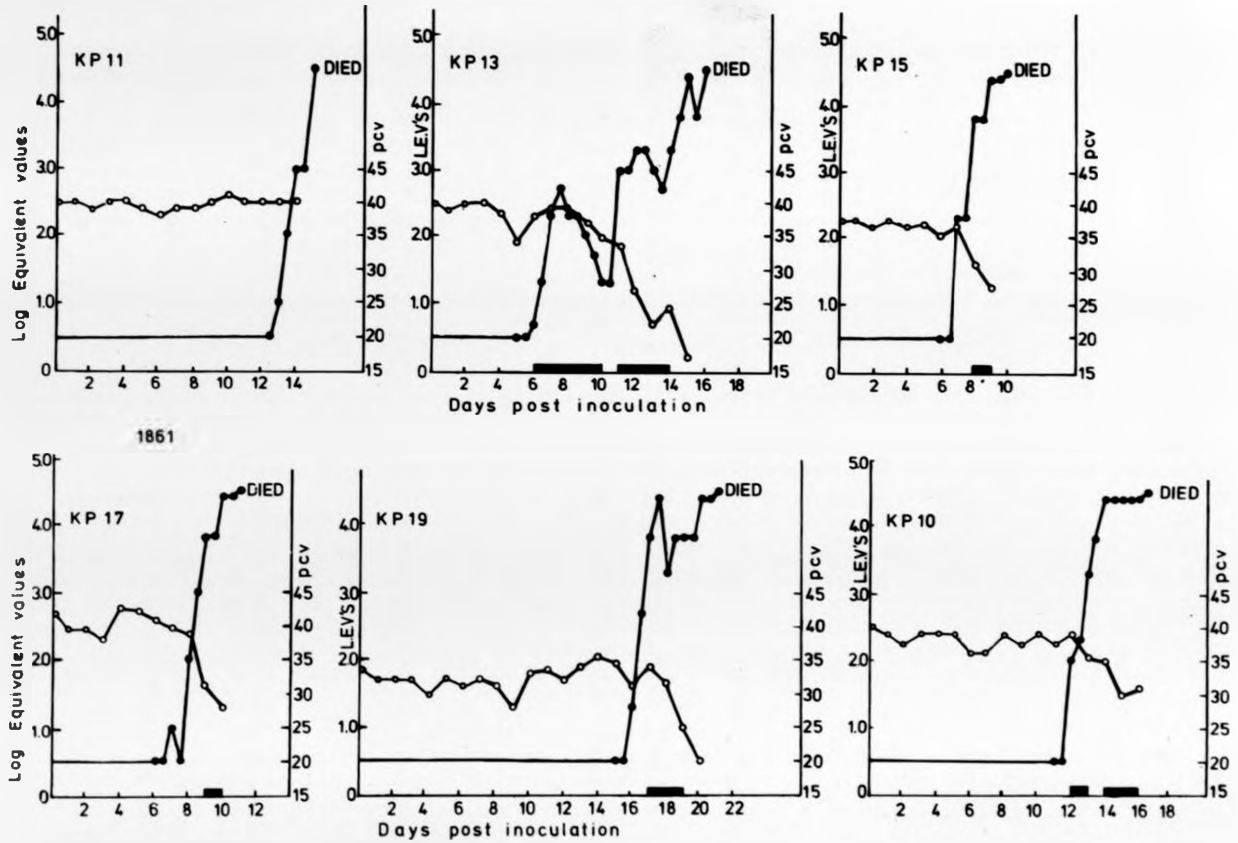


FIG.7. Transmission of stabilate EATRO 1861 by G.palliipes The graphs show parasitaemia (●–●), Pcv (○–○) and days when temperature rose above 40.0°c (▬).

(iii) G. morsitans also transmitted a G. pallidipes initiated infection to pig No. 86. This pig became parasitaemic after 6 days and had a self - limiting infection which disappeared after 36 days (Table IV (f)).
The mean prepatent period for pigs to which infection was transmitted by G. morsitans was 9.57 ± 2.15 days and the patency of infection was 7.71 ± 0.92 days (Table 1).

When observations in all the pigs to which the infection was transmitted by G. morsitans are grouped together, the mean prepatent period is 12.31 ± 2.26 days and the mean patent period 20.6 ± 7.86 days (Table V).

(b) Glossina pallidipes

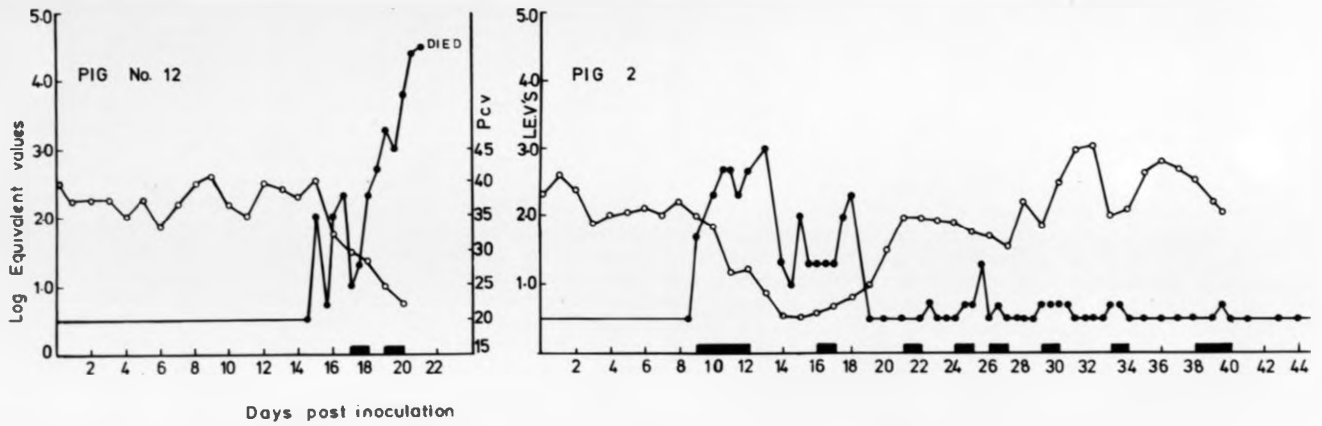
The behaviour of the three isolates was also studied to determine the influence of G. pallidipes on the virulence of T. simiae in pigs.

The procedure followed for the study was the same as that used for transmission by G. morsitans. Isolate EATRO 1861 was inoculated directly into 3 pigs (Nos. 3, 5, 8) on various occasions and G. pallidipes was used to transmit infection from them to other pigs as follows:-

(i) G. pallidipes transmitted the infection to three other pigs. These were pig Nos. 10, 17 and 11. Pig No. 10 developed parasitaemia after a prepatent period of 11 days and died five days later. Pig Nos. 17 and 11 had prepatent periods of 6

Table V: Mean prepatent periods and mean patent periods for all the pigs infected variously with the three stabilates of *T. simiae.*

Mode of transmission	Mean prepatent Period (Days)	Mean Patent Period (Days)
Direct Inoculation (13)	3.6 ± 0.34	8.07 ± 3.07
G. morsitans (16)	12.31 ± 2.26	20.6 ± 7.84
G. pallidipes (10)	8.2 ± 1.14	19.6 ± 10.49



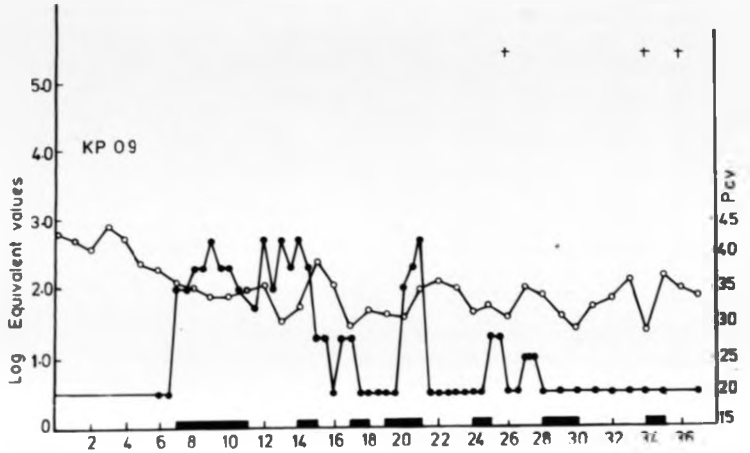


FIG. 8. Transmission of stabilate EATRO 1786 by G pallidip
graphs show the parasitaemia (●—●), Pcv (o–o) days wher
rose above 40.0°c (▬) and days when trypanosome:
detected by HCT and not in the wet blood prepar

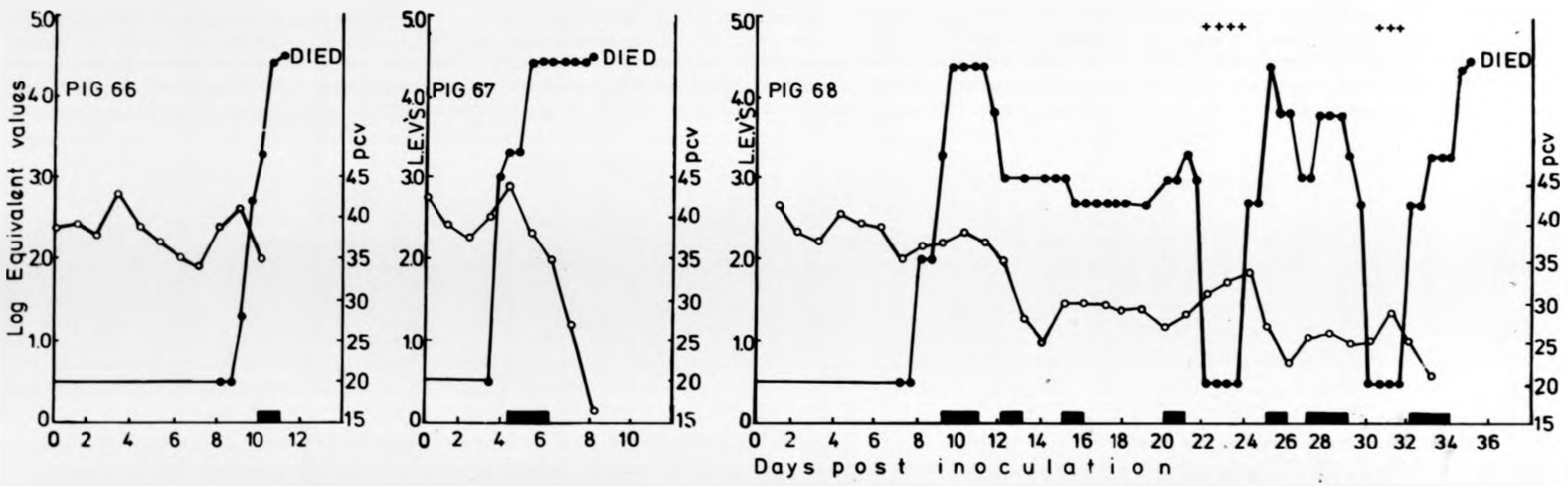


FIG. 9. Transmission of stabilate EATRO 1806 to three pigs by G brevipalpis The graphs show the Pcv (o—o), parasitaemia (•—•), days when temperature rose above 40.0°c (▬) and days when trypanosomes were detected by HCT and not in the wet blood preparation (+++)

(c) Glossina brevipalpis

There are a few G. brevipalpis kept in the KETRI insectary. As described previously, these flies were brough in from the wild at the time when the G. pallidipes colony was started. The few flies that emerged were therefore used for breeding and not for experiments. At one time seven teneral G. brevipalpis fed on pig No. 23 which had been inoculated directly from the bank with EATRO 1806. These flies then transmitted the infection to pig Nos. 66, 67 and 68. Pig No. 66 became parasitaemic after a prepatent period of 8 days and died 2 days later. Pig No. 67 which was infected by the same batch of flies became parasitaemic after 2 days and died after 6 days. One fly was found infected in the batch of 7 flies giving an infection rate of 14.3%. When the proboscis of the one fly was macerated and inoculated into pig No. 68, it became parasitaemic after 7 days and died 29 days from the infection (Table III (d)). The parasitaemias, PCVs and temperatures of these pigs are shown in Fig. 9. The mean prepatent period for these pigs was 5.67 ± 1.86 days and the mean patency was 12.33 ± 8.41 days.

When all the observations are grouped for both prepatent period and patency according to the mode of transmission, it is found that pigs which were inoculated directly from the bank developed parasitaemia after a mean prepatent period of 3.6 ± 0.34 days and had patent infections for a mean period of 8.07 ± 3.07 days. These observations made on thirteen

pigs. Infections were established in 16 pigs after transmission by G. morsitans. The prepatent period in these pigs ranged between 3 and 38 days, with a mean of 12.31 ± 2.26 days. Infections in this group of pigs were patent for a mean period of 20.6 ± 7.84 days. The patency ranged between 3 and 115 days. One pig from this group had a self-limiting infection which disappeared after 115 days. G. pallidipes transmitted infection to 10 pigs from stabilates EATRO 1861 and EATRO 1786. The prepatent period in the ten pigs was 8.2 ± 1.14 days and the pigs survived the infection for a mean of 19.6 ± 10.49 days. Two pigs from this group had self-limiting infections which lasted 36 and 110 days (Table IX).

The results of observations on transmission were compared for each stabilate using Students 't' test. For stabilate EATRO 1861, there were no significant differences among the different groups of pigs in either the prepatent period or the patency. The same type of result was obtained for stabilate EATRO 1806. A comparison of the prepatent periods of infections with EATRO 1786, showed that the pigs inoculated directly developed parasitaemia much faster than those infected by G. morsitans The differences in prepatent period were statistically significant at between the 1% level and the 2% level. The difference in prepatent periods for G. pallidipes transmitted infection was also significant at the 5% level. The patency of infection for pigs infected by this stabilate was not

statistically significant.

3.3.2 The effect of serial syringe passage on virulence of T. simiae

Two stabilates, EATRO 1786 and 1806, were serially maintained in pigs.

Trypanosomes from donor pig No. 68 infected with EATRO 1806 were subpassaged into pig No. 71 and subsequently to pig Nos. 74 and 65. The donor pig (No. 68) died after a patent infection of 29 days during which it had experienced several parasitaemic waves. When trypanosomes from this pig were subpassaged serially through three pigs, two of the pigs died after a day of infection and the third pig was treated *in extremis* (Table VI)

EATRO 1786 was serially passaged through pigs on two occasions. The first time, the donor pig (No. 9) self-cured after 36 days of patent parasitaemia. When Pig No. 089 was inoculated with trypanosomes from pig No. 9, it also experienced a self-limiting infection which lasted 64 days. A further subpassage from pig No. 089 to pig No. 91 resulted in death of the pig after 15 days. The parasitaemia was low and rose at the time of death (Table VI).

On the second occasion, pig No. 4, which had been infected by G. morsitans, served as the donor. A subpassage from it to pig No. 6 resulted in death of the pig after 5 days. A passage from pig No. 6 to pig No. 3 resulted in the death of the pig after 11 days (Table VI).

3.3.3 The effect of the trypanosome number inoculated into the pig on virulence of T. simiae infection.

Two stabilates, EATRO 1806 and 1861, were used for this study. With EATRO 1806, pigs which were inoculated with 10^6, 10^5 or 10^4 trypanosomes all became infected after a prepatent period of 3 days and had to be treated within 2 days. Although the three pigs showed trypanosomes in their blood on the same day, the pig that received the largest number of organisms had the highest parasitaemia (Table VII). Pigs that were inoculated with 10^3 and 10^2 trypanosomes did not become infected.

When EATRO 1861 was serially diluted and then inoculated into pigs, only the pig that received 10^2 trypanosomes did not become infected. For pigs which received 10^6 and 10^5 trypanosomes, the infection became established after a prepatent period of 2 days while the pig that received 10^4 trypanosomes became parasitaemic after a prepatent period of 3 days (Table VIII). The pig (No. 13) that was inoculated with 10^3 trypanosomes became parasitaemic after a prepatent period of 45 days and died after

Table VI: Virulence of T. (N). simiae during serial syringe passage

STRAIN	PIG NO.	Prepatent period (days)	Patent period (days)	Remarks
EATRO 1806	68 (Donor)	7	29	Died
	71	4	1	Died
	74	2	1	Died
	65	3	1	Treated in extremis
EATRO 1786	9 (Donor)	6	36	Self-cured
	089	5	64	Self-cured
	91	5	15	Died. Low parasitaemi rising at the time of death
EATRO 1786	4 (Donor)	5	9	Died
	6	4	5	Died
	3	2	11	Died

Table VII: Effect of parasite numbers on the course of infection in pigs infected with T. simiae EATRO 1806.

No. of trypanosomes inoculated into pig	Pig No.	Prepatent Period (Days)	Patent Period (Days)	Remarks
Donor	70	7	30	Infected by G. morsitans
10^6	65	3	1	Treated
10^5	01	3	2	Treated
10^4	03	3	2	Treated
10^3	02	Did not become parasitaemic		
10^2	04			

Table VIII: Effect of parasite numbers on the course of infection in pigs infected with T. simiae EATRO 1861.

No. of trypanosomes inoculated into pig	Pig No.	Prepatent period (days)	Patent period (days)	Remarks
Donor	10	4	5	
10^6	9	2	2	* 10/1
10^5	11	2	6	* 1/1
10^4	12	3	6	
10^3	13	45	13	
10^2	14	Did not become parasitamic		

* Parasitaemia readings on day 3 post inoculation for pigs No. 9 and 11 which showed parasitamia on the same day after inoculation.

10/1 - Ten trypanosomes per microscope fied in wet preparation.

1/1 - One trypanosomes per microscope field.

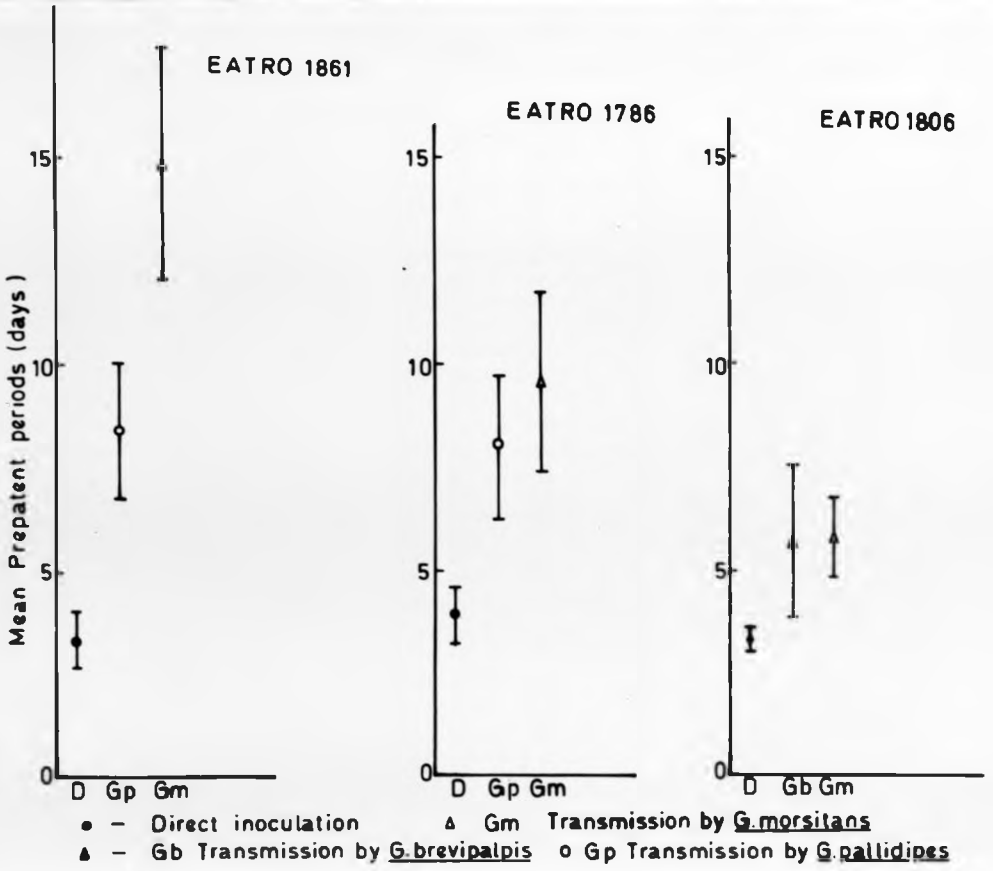


FIG. IO. The mean prepatent period in days of groups of pigs infected directly and Cyclically for the three stabilates.

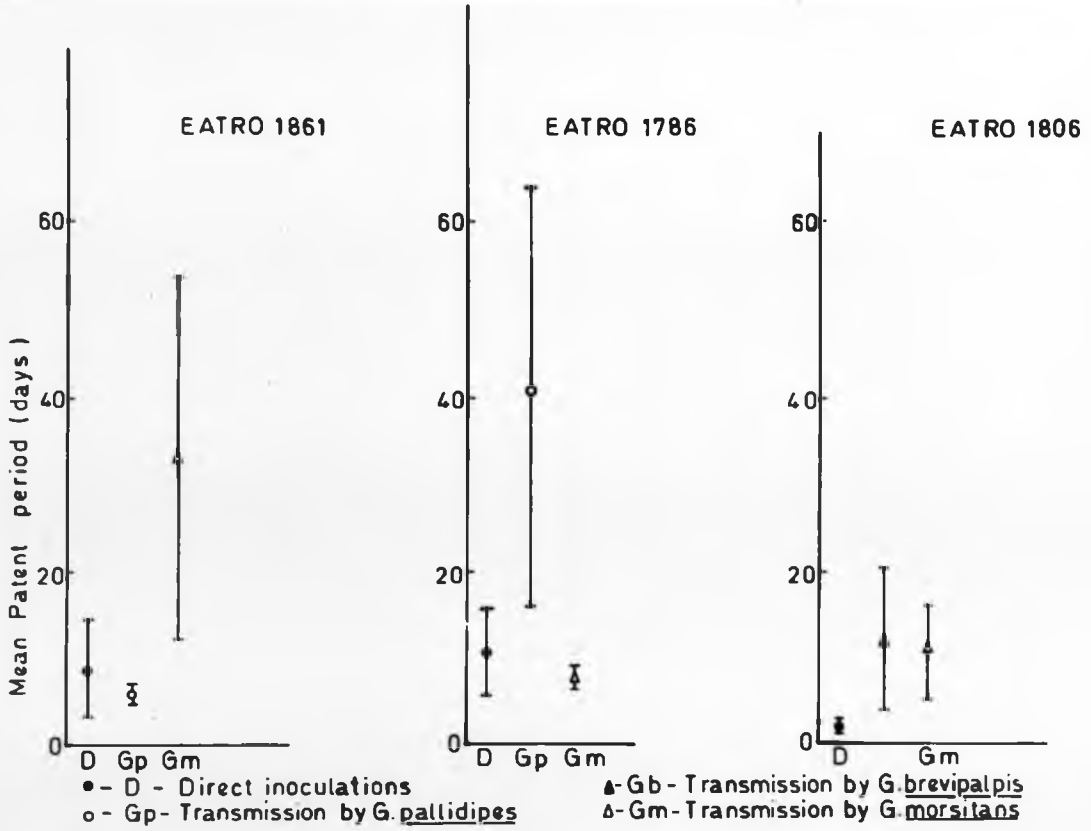


FIG. 11. The mean patent period in days of groups of pigs infected directly and Cyclically for the three stabilates.

3 days. The pig (No. 14) that was inoculated with 10^2 trypanosomes did not become parasitaemic.

3.4 DISCUSSION

3.4.1 Transmission of *T. simiae* by tsetse

It has been suggested that the virulence of trypanosome infections may be related to the transmitting species of tsetse (Bruce *et. al.*, 1912; Van Hoof, 1927; Lewis, 1949; Willet, 1960; Scott, 1970; Ford, 1972; Janssen and Wijers, 1974). Except for Lewis (1949) and Janssen and Wijers (1974), the suggestions were based on circumstantial evidence indicating that the disease form depended on species of tsetse present in an area. Lewis (1949), after noticing that virulent strains of *T. vivax* originated from the Emali area of Kenya in areas infested with *G. pallidipes* and that chronic strains was commonly found in *G. palpalis* area, experimentally transmitted the Emali strain through 6 species of tsetse, including *G. pallidipes* and *G. palpalis* (*G. fuscipes*) to cattle. His results indicated that *G. pallidipes* transmitted acute disease to cattle while *G. palpalis* transmitted a chronic one. Recently Janssen and Wijers (1974) suggested that *G. pallidipes* transmitted chronic *T. simiae* infections to pigs while *G. brevipalpis* was responsible for acute, fatal infections. They found that the infection transmitted by *G. austeni* resulted in disease forms intermediate in virulence between those caused by the *G. brevipalpis* and *G. pallidipes* strains.

In this study three isolates of T. simiae were cyclically transmitted to pigs. Two of the isolates (EATRO 1861 and 1806) were G. brevipalpis isolates and one was a G. austeni isolate (EATRO 1786). Passage of the three isolates through G. morsitans and G. pallidipes resulted in a disease which was less virulent than that produced by syringe passaged organisms. The mean survival period of syringe inoculated pigs was 8.07±3.07 days, compared to 20.6 ± 7.84 days of the G. morsitans transmitted infections. For G. pallidipes infections the survival time was a mean of 19.6 ± 10.49 days.

For the syringe passaged infections, the course of infection was generally of short duration, with the trypanosomes multiplying fast and the infection rapidly overwhelming the host. The disease in this group of pigs ranged from hyperacute to chronic. Out of thirteen pigs only three survived for more than 10 days. These findings closely resemble those reported for natural T. simiae infections in pigs. Stephen (1966) found that the duration of infection averaged 3.6 days (range 24 hours to 8 days). In this study three pigs survived longer than the 8 days. The prepatent period for the syringe inoculated infection did not vary a great deal. This could be the result of starting with blood stream trypanosomes.

Infections transmitted by G. morsitans had longer prepatent periods than those induced by syringe. The long prepatent period for fly transmitted infections may be related to the rate at which the metacyclic trypanosomes

adjust to life in the mammalian blood stream. The mammalian blood stream , unlike the tsetse proboscis, has abundant supply of oxygen and glucose. The adjustment may involve changes in the mitochondrial configuration, from an active phase to a passive phase (Vickerman, 1970). After this initial adjustment the metacyclic trypanosomes give rise to blood stream forms.

The number of trypanosomes inoculated from the bank into pigs was standardised while the number of organisms inoculated from infected flies could not be controlled and this could have contributed to the wide range of prepatent periods observed in fly transmitted infections. It is also known that infected flies do not always extrude trypanosomes each time they feed (Harley and Wilson, 1972; Roberts, 1981).

In their response to infection, whether syringe inoculated or cyclically transmitted, pigs appeared to fall into three groups. In the first group, trypanosomes got established and continued to multiply unchecked by the host. There was a continuous rise in temperature and the pig died with a fulminating infection with parasites swarming in the blood. In this group death occurred during the first peak of parasitaemia which set in within the first day or two from the time parasites were detected in the blood. Death followed very fast and there was no detectable change in PCV (Fig. 3). This was particulary true of syringe inoculated pigs and pigs infected with stabilate EATRO 1861 by G. pallidipes.

In the second group, parasitaemia got established and rose slowly but steadily to reach a peak at the time of death. Some pigs experienced more than one parasitaemic wave but they all eventually succumbed to the infection. The survival time of those pigs was much longer than that of the pigs in the first group. The change in PCV depended on the duration of infection.

In the third group infection became established but the parasitaemia remained low throughout the duration of infection. Peaks of parasitaemia alternated with periods when no parasites could be detected in the peripheral blood. The parasitaemic interval increased in duration until no parasites were seen in the blood. In this group the parasitaemia never rose above 10 trypanosomes in one microscope field.

Janssen and Wijers (1974) observed that the strain they had isolated from G. pallidipes gave rise to chronic infection in pigs. When they transmitted an apparently virulent isolate from G. brevipalpis through G. pallidipes to pigs, it gave rise to chronic infection. In the present study two G. brevipalpis isolates (EATRO 1806 and 1861) were transmitted in several experiments to pigs. Isolate 1861 was transmitted to pigs through G. pallidipes on six occasions. The pigs experienced diseases which ranged between acute and subacute. None of them survived more than 10 days of patent infection.

The second isolate when transmitted by G. pallidipes gave rise to hyperacute infection. The third isolate used in

this study was from G. austeni (EATRO 1786). In their study Janssen and Wijers (1974) described it as a moderately virulent strain. In the present study when this strain was transmitted by G. pallidipes it gave rise to infections ranging from subacute to very chronic. Two pigs had self-limiting infection. When the same strain was transmitted through G. pallidipes the disease was initially acute. After the first transmission, the pig had self-limiting infection after a patent period of 36 days. When this chronic strain was transmitted to another pig, the pig had a subacute disease. The difference in response could probably be a result of variation in the susceptibility of the pigs used. The observations made in this study do not support the suggestion that transmission of a virulent strain of T. simiae through G. pallidipes gives rise to a chronic type infection.

If transmission by G. brevipalpis enhances the virulence of T. simiae (Janssen and Wijers, 1974) then transmission of stabilate EATRO 1806 (originally a G. brevipalpis isolate), through G. brevipalpis should have given rise to hyperacute disease. Transmission of this stabilate by this species of tsetse to three pigs gave rise to infections of 2, 6 and 29 days duration.

Using G. morsitans submorsitans, Gray (1961) and Stephen (1966) observed that the survival period of pigs cyclically infected with T. simiae ranged from two to four days. In this

study the sixteen pigs infected by G. morsitans survived from 3 to 78 days. One pig had a self-limiting infection lasting 115 days. For all the three stabilates the disease form ranged between the acute and the chronic form. The self-limiting infection in the pig was in a group infected with EATRO 1861 (original G. brevipalpis isolate). There was however no pattern displayed in either the parasitaemia or survival of pigs to which the flies transmitted the infection. Until the report of Janssen and Wijers (1974) there had been no report of pigs surviving T. simiae infection. They found that some survived T. simiae infections transmitted by G. pallidipes and G. austeni. In the present study G. morsitans transmitted chronic infections to pigs from an originally virulent strain.

There was much variation in behaviour of the different isolates. The ability of the individual pig to contain the infection seems to be an important factor determining the outcome of an infection. The pigs which survived longest always had a low parasitaemia, indicating that a factor or factors in the pig controlled the replication of the trypanosomes. Some pigs were able to control parasitaemia and spontaneously recovered from it. It is interesting that all the pigs with self-limiting infection came from one farm.

3.4.2 Serial syringe passage of T. simiae in pigs

Janssen and Wijers (1974) suggested that syringe passage of a chronic strain of T. simiae did not affect its virulence

for pigs. With EATRO 1806, a virulent T. simiae isolate, the subpassages had a shorter prepatent period than the original infection (Table VI). This was as expected because blood stream trypanosomes were being taken from one host to another. However the patency of the infection was drastically reduced from 29 days in the donor pig to 1 day in the recipient. This reduction was too sudden to have been the effect of the subpassage. It could have been the effect of the extreme susceptibility of the recipient, whereby the trypanosome overwhelmed the pig within 24 hours while the donor was able to suppress the infection for 29 days prior to succumbing to it. The reduced prepatent period could as well therefore be a reflection of the enhanced ability of the trypanosomes to establish themselves in a new host as a result of serial syringe passage.

Pig No. 9 a donor for the subpassages using EATRO 1786 was infected by G. pallidipes which had taken the infective feed from a pig inoculated with the original strain from the bank. The prepatent period for pig No. 9 was six days and the pig had a self-limiting infection after 36 days. The recipient of this subpassage pig No. 089 developed the parasitaemia after a prepatent period of five days and although the infection was self-limiting it lasted a longer period of 64 days. A subpassage from this infection was not self-limiting, however. The pig No. 91 was positive with trypanosomes on day

6 post inoculation and died 15 days later with low parasitaemia rising at the time of death. One feature common to these three infections was that the parasitaemia readings were low throughout except for pig 91 in the second passage when there was a rise in parasitaemia at the time of death. There was no change in the prepatent periods. However, the infection which initially was self-limiting after 36 days only lasted a little longer after a subpassage although the infection was also self-limiting but after 64 days. A further subpassage resulted in the death of the pig.

On the second occasion when EATRO 1786 was cyclically transmitted by G. morsitans there was reduction in prepatent period during the subpassages to 4 and 2 days respectively. The patency of the infection however did not show any trend. The parasites therefore appeared to have been able to establish themselves faster with the subpassages (Table VI).

It could therefore be inferred that serial syringe passage can enhance the virulence of T. simiae by enabling the parasites to establish themselves faster with each subpassage but once established the infection is left to the mercy of the immune capability of the pig. Either the pig can control the parasitaemia, keeping it at a low level and eventually throwing off the infection, or the parasites may multiply very fast and the pig succumbs to the infection in a few days.

3.4.3 Inoculation of pigs with varying number of trypanosomes

Walker (1964) working with a virulent, mouse-adapted strain of T. brucei (NIMR2) found that mice inoculated with 10^6 parasites would die in three to four days but the same strain would kill in seven days if only one parasite was inoculated in mice. The course of infection with this strain was influenced by varying the inoculum size.

The ten fold dilutions of trypanosomes which were inoculated into pigs did not appear to influence the course of the disease in pigs. All the pigs became parasitaemic on the same day (Table VII) and yet they had received 10^6, 10^5 or 10^4 parasites in the inoculum. Pigs which received 10^3 or 10^2 parasites however did not become parasitaemic. It is difficult to say for sure the effect of the parasite burden on the course of the disease because only one pig was used at each inoculum level.

On the second occasion pigs that were inoculated with 10^6 or 10^5 trypanosomes became positive on the same day, i.e 2 days after inoculation (Table VIII). However there was a tenfold difference in the parasitaemia reading. The pig inoculated with 10^4 trypanosomes was positive on day three. Inoculation of a pig with 10^3 trypanosomes resulted in an infection after prepatent period of 45 days and the pig died 13 days later. A pig inoculated

with 10^2 trypanosomes did not become infected. There was no dose response relationship portrayed in these pigs for either the prepatent period or the patency of the infection. It would have been expected that the larger the inoculum the shorter the prepatent period and therefore the shorter the survival of the host with the infection. In this simple system the resulting infection in the pig appeared not to be related to the inoculum size. However, it would appear that inoculation of a pig with 10^2 trypanosomes would not result in an infection.

3.5 CONCLUSION

Transmission of the three stocks of T. (N). simiae through the different species of tsetse did not always affect the virulence of the trypanosome stocks for pigs. For EATRO 1861, transmission through G. pallidipes did not alter its virulence for pigs and only on one occasion transmission of this strain through G. morsitans gave rise to a chronic self-limiting infection. Regarding the virulence of EATRO 1806, transmitting it through the different species of tsetse also did not alter the virulence of the stock significantly. Unlike the two stocks above which were G. brevipalpis isolates, EATRO 1786 was a G. austeni isolate and transmitting it through G. pallidipes gave rise to chronic self-limiting infection in two pigs while its transmission through G. morsitans did not significantly affect its virulence for pigs. What determines the course of the infection once established appears not to be the species of tsetse. However,

in all the chronic infections the parasitaemia was characteristically low rising only towards the time of death or disappearing completely from the peripheral blood after a period giving rise to self-cure.

The number of trypanosomes inoculated into the pig did not affect the virulence of the disease in the pig. The trypanosomes will appear after a prepatent period but once they are seen they continue to multiply and the course of the disease will get influenced by the ability of the individual animal to control the parasitaemia.

The pigs used in this study appear to vary so much in their susceptibility to the parasite and only a limited number of animals were used for each observation. It would therefore be necessary to have the observations repeated in a larger number of animals from defined breeds of pigs before a definite conclusion could be reached.

C H A P T E R 4

DEVELOPMENT OF *T. SIMIAE* IN TSETSE FLIES

4.1 INTRODUCTION

Many factors seem to affect the ability of tsetse flies to transmit trypanosomes from one vertebrate host to another. They are related to the trypanosome itself, the vertebrate host and the vector. It has been demonstrated by various workers that some species of tsetse are refractory to infection by trypanosomes. Godfrey (1966) failed to infect *G. palpalis* with *T. congolense* in the laboratory after several attempts. Harley and Wilson (1967) demonstrated that *G. morsitans* and *G. pallidipes* were better vectors of *T. rhodesiense* in the laboratory than *G. fuscipes* and this, they said, was due to the inability of infected *G. fuscipes* flies to extrude infective trypanosomes when they fed. Jordan (1974) points out that for *Trypanosoma congolense* and *T. vivax*, *G. morsitans* and *G. austeni* are several times more frequently infected than *G. pallidipes* and *G. brevipalpis*. Trypanosome infection rates also vary between localities. With *T. brucei* not all strains are transmissible by tsetse in nature (Hoare 1972). The reasons for non-transmissibility are not fully understood but they are probably related to a reduced ability of the trypanosomes to complete their development in tsetse. The strains which are not transmissible cyclically are maintained by mechanical vectors such as tabanids.

The species of the mammal from which flies take the infective feed also seems to affect the development of the trypanosomes in the fly. It is difficult to infect G. palpalis from a person with a chronic T. gambiense strain. Strains with low virulence are readily capable of producing infection in the fly. Jordan (1974) states that a tsetse fly that is not innately refractory to infection is most likely to be infected if it occurs in an area of high mean temperature, but not so high as to preclude a long adult life and feeds early in life on heavily infected species of Bovidae.

With T. brucei trypanosomes it has been demonstrated that the short, stumpy forms are the infective stage for tsetse. Vickerman (1970) demonstrated a correlation between morphological changes and changes in the mitochondrial structure and suggested that structural changes prepared the trypanosome for life in the tsetse gut which has low glucose and oxygen supplies. He showed that the short stumpy forms have well developed mitochondria while the long slender forms have rudimentary mitochondria. The long slender trypanosomes when ingested by tsetse flies in a blood meal are subjected to an environment for which they are not equipped. They lack the Krebs cycle enzymes and cytochrome chain components and are unable to oxidise stagnant blood in the tsetse gut and can not live in the environment. The absence of short stumpy trypanosomes in a blood meal would therefore result in the inability of the infection to establish

itself in tsetse flies. T. evansi, a Trypanozoon trypanosome, does not develop to maturity in tsetse and Vickerman (1970) demonstrated that T. evansi trypanosomes resemble monomorphic T. brucei trypanosomes, which are not infective to tsetse, in having inactive mitochondria.

In the present study the rate at which T. simiae develops in G. morsitans and G. pallidipes was investigated.

4.2 MATERIALS AND METHODS

4.2.1 Trypanosomes

Two stabilates of T. (N). simiae, EATRO 1786 and 1806, were used for these studies.

4.2.2 Pigs

Pigs used for these studies were as described in Chapter Two.

4.2.3 Tsetse Flies

Tsetse flies used in this study were described in Chapter Two.

4.2.4 Development of T. simiae in Tsetse

To determine the rate at which T. simiae develops in tsetse, teneral flies of G. pallidipes or G. morsitans were fed on a

parasitaemic pig and dissected at different time intervals. In an experiment involving both species, a few flies were dissected in PBS and examined for trypanosomes one hour and six hours after feeding, and then daily for thirty days. For the first four days after the feed, only the midguts were examined. From day five onwards, both the gut and the proboscis of the flies were examined for trypanosomes.

In another experiment G. morsitans flies were dissected 5, 10, 15, 20, 25, 30, 35 and 40 days after the infective feed.

4.2.5 Relationship between trypanosome numbers and morphology in the blood of the pig and the infection rate in tsetse

The effect of trypanosome numbers present in the blood of the pig at the time when the flies take the infective feed on the infection rates in tsetse was determined as follows. Teneral G. morsitans flies were fed on an infected pig daily for nine days in the first experiment and 4 days in the second experiment, a new batch each day. After feeding on the pig, the flies were maintained on a rabbit for 3 weeks after which they were dissected and the infection rate determined. During this period when flies were being fed on a pig thin blood smears were made each day and stained for morphological study.

4.3 RESULTS

4.3.1 Development of T. simiae in Tsetse

Two experiments were carried out. In the first experiment,

Table X : Development of T. simiae (Stock EATRO 1806) in G. morsitans and G. pallidipes

Day after infection	No. Flies dissected	No. G. morsitans with infection in			No. Flies dissected	No. G. pallidipes with infection in		
		Midgut	Labrum	Hypopharynx		Midgut	Labrum	Hypopharynx
1 Hour	4	4	0	0	2	2	0	0
6 Hours	3	3	0	0	2	2	0	0
1 Day	3	3	0	0	1	1	0	0
2	5	5	0	0	1	1	0	0
3	7	2	0	0	4	4	0	0
4	7	3	0	0	4	2	0	0
5	4	4	0	0	4	0	0	0
6	4	0	0	0	4	0	0	0
7	7	1	0	0	4	0	0	0
8	7	0	0	0	6	0	0	0
9	10	0	0	0	6	0	0	0
10	10	2	1	0	6	1	0	0
11	10	4	0	0				
12	7	0	0	0	11	2	0	0
13	6	0	0	0				
14	20	0	0	0	5	0	0	0
15	19	1	0	0	5	0	0	0
16	35	4	1	0	5	0	0	0
17	3	0	0	0				
18	15	0	0	0	6	0	0	0
19	10	1	1	1	4	0	0	0
20	12	1	1	0	5	0	0	0
21	10	0	0	0	5	0	0	0
22	8	1	1	1	5	0	0	0
23	8	0	0	0	5	1	1	1
24	15	0	0	0	10	0	0	0
25	12	0	0	0	7	0	0	0
26	9	1	0	0	12	0	0	0
30	17	0	0	0	9	1	1	1

Table XI: Development of T. simiae (Stock EATRO 1806) in G. morsitans and G. pallidipes

Time post-infection	No. Flies dissected	No. G. morsitans with infection in			No. Flies dissected	No. G. pallidipes with infection in		
		Midgut	Labrum	Hypopharynx		Midgut	Labrum	Hypopharynx
1 Hour - 5 Days	29	24	0	0	18	12	0	0
6 - 10 Days	38	3	1	0	26	1	0	0
11 - 15 Days	62	5	0	0	21	2	0	0
16 - 20 Days	75	6	3	1	20	0	0	0
21 - 25 Days	53	1	1	1	32	1	1	1
25 - 30 Days	26	1	0	0	21	1	1	1

teneral G. morsitans and G. pallidipes flies were fed on a pig infected with trypanosomes from stock EATRO 1806. A total of 289 G. morsitans and 138 G. pallidipes flies were dissected over a period of 30 days. Mature infections were found in 2 G. morsitans and 2 G. pallidipes flies.

Trypanosomes were first detected in the proboscis of G. morsitans on day 10 after feeding on an infected pig. The trypanosomes were present in the labrum. Trypanosomes were found in the hypopharynx on days 19 and 22 after the infective feed (Table X . With G. pallidipes, trypanosomes were found in the hypopharynx on days 23 and 30 (Table X). When the dissections are grouped at five-day intervals (Table XI), it is found that most of the flies dissected up to day 5 had trypanosomes in the midgut. This is true for both species.

In the second experiment, 67 G. morsitans flies were fed on a pig infected with trypanosomes from stock EATRO 1786 and dissected at five day intervals for a period of 40 days. Trypanosomes were first detected in the proboscis of the flies on day 30 after infection (Table XII). A total of five tsetse flies had mature infections, two, one and two infections being found on day 30, 35 and 40 respectively.

4.3.2 Relationship between trypanosome numbers and morphology and infection rate in tsetse

Two experiments were carried out. In the first experiment teneral flies were fed on pig No. 1 (which was infected with

Table XII: Development of T. simiae (Stock EATRO 1786) in G. morsitans

Day after infection	No. Flies dissected	No. Flies with infection in		
		Midgut	Labrum	Hypopharynx
5	9	2	0	0
10	10	9	0	0
15	10	10	0	0
20	9	8	0	0
25	8	8	0	0
30	5	2	2	2
35	6	2	1	1
40	10	4	2	2

Table XIII: Trypanosome infection rate in tsetse, parasitaemia and morphology of trypanosomes in pig blood.

Day after inoculation of pig	Parasitaemia in pig	Morphology of Trypanosomes in pig blood as % of count		Trypanosome infection rate of G. morsitans
		Long	Short	
6	0/20	-	-	-
7	2/1	45.0	55.0	-
8	+	48.7	51.3	12.5
9	+	75.0	25.0	0

Table XIV: Trypansome infection rate in tsetse, parasitaemia and morphology of trypanosomes in pig blood.

Day after inoculation	Parasitaemia in pig	Morphology of Trypanosomes in pig blood as % of count		Trypanosome infection rate of G. morsitans %
		Long	Short	
6	1/20	*		0
7	1/5	*		12.5
8	5/1	*		0
9	10/1	62.7	37.3	0
10	10/1	56.5	43.5	5.0
11	10/1	54.5	45.5	0
12	10/1	63.0	37.0	0
13	5/1	60.8	39.2	0
14	+	78.3	21.7	0

*Very few trypanosomes were present in the stained smears and counts were not made.

Table XIV: Trypansome infection rate in tsetse, parasitaemia and morphology of trypanosomes in pig blood.

Day after inoculation	Parasitaemia in pig	Morphology of Trypanosomes in pig blood as % of count		Trypanosome infection rate of G. morsitans %
		Long	Short	
6	1/20	*		0
7	1/5	*		12.5
8	5/1	*		0
9	10/1	62.7	37.3	0
10	10/1	56.5	43.5	5.0
11	10/1	54.5	45.5	0
12	10/1	63.0	37.0	0
13	5/1	60.8	39.2	0
14	+	78.3	21.7	0

*Very few trypanosomes were present in the stained smears and counts were not made.

Table XV: Summary of trypanosome infection rates in some tsetse flies.

Stabilate No.	Pig No.	Trypanosome infection rate in	
		G. morsitans	G. pallidipes
		%	%
1861	10	0	4.0
	12	1.81	0
	16	4.1	2.2
	03	30	10.0
	5	0	1.9
	18	1.4	0
	9	0.6	0
1806	65	0.4	0
	66	1.4	0
	70	2.7	0
	23	1.5	0
	73	0	8.3
	68	2.5	2.9
1786	07	0	1.8
	09	2.8	2.0
	79	0	2.6

stock EATRO 1786) over a period of 3 days. The donor pig had become parasitaemic on day 7 after inoculation. Thirty two G. morsitans flies fed on it on the second parasitaemia day. When dissected one month later six of the flies had midgut infections. Four of the six flies also had infections in the proboscis, giving an infection rate of 12.5%. The labrum and hypopharynx were heavily infected with trypanosomes. The parasitaemia when the flies fed on the pig was approximately 60 parasites per microscope field (Walker 1968) in a wet blood preparation. Twenty two flies were fed on the pig on the ninth parasitaemic day, when parasiteamia was 60 trypanosomes had mature infections on dissection. Two of the flies had gut infections, which are regarded as immature. The overall infection rate for the flies that fed on pig No. 1 was 7.4%. Examination of Giemsa stained, thin blood smears showed that on day 7 the majority of the trypanosomes were of the short forms, measuring less than 14 µm (Table XIII).

By day 9 after inoculation only 25% of the trypanosomes were of the short form. The pig died on day 10 after inoculation.

In the second experiment, teneral flies took infection blood from pig No. 4 (Table XIV) on the first parasitaemia day, which was day 6 after inoculation. This pig remained alive with parasitaemia for 9 days. Only flies which fed on days 7 and 10 after taking the infective feed became infected (Table XIV). It was observed that on days 6 and 7 after inoculation the few trypanosomes present were predominantly

of the short forms. Flies that fed on the pig on day 7 after inoculation, gave an infection rate of 12.5% on dissection. On day 10 the infection rate was 5%. The overall infection rate for the batch of 288 flies dissected was 2.1%.

4.4 DISCUSSION

4.4.1 Development of T. simiae in Tsetse

Many factors are known to influence the establishment of trypanosomes in tsetse flies among which are the age of the fly at the time of the infective feed (Harmsen, 1973), the sex of the fly (Fairbairn and Culwick 1950), the genetic differences between species of flies (Harley and Wilson 1968), source of blood meal (Jordan, 1974) and strain of parasite (Vickerman 1970). For some trypanosomes the development is completed in the proboscis. Once the infection has been established in tsetse, the trypanosomes are expelled with the saliva when the fly feeds. During this study identification of infected flies by the probing technique was not possible because out of 100 stained saliva smears examined no trypanosomes were seen. This finding supports the observation made by Roberts (1981) during which he was unable to identify G. morsitans infected with T. congolense by examination of stained saliva.

During the first five days after the infective feed, trypanosomes were found in the gut of the majority of G. morsitans flies. This was followed by a reduction in the number of flies found with trypanosomes in the gut. The pattern was observed for G. pallidipes infected with EATRO 1806. However for stabilate EATRO 1786, the number of flies found with gut infection was relatively higher throughout the period of observation (Tables X and XI). The infection in the host used at the two times had been low for EATRO 1786 in pig KPO 2 while for EATRO 1806, the initial parasitaemia had built up fast and the pig was treated in extremis. The parasites used for infecting flies was from a relapsed infection.

It is generally accepted that only the short forms of trypanosomes are capable of developing in tsetse. With relapsing infections, during which the long forms of trypanosomes give rise to short forms which are destroyed by antibodies during a crisis, chances of tsetse flies picking up infection are higher than with an infection that develops fast and overwhelms the host before the action of antibodies set in to kill the trypanosomes. EATRO 1786 normally gave a less virulent relapsing infection in pigs with two pigs ending up self-cured and parasitaemia was generally lower.

Once the right morphological form of trypanosome has been ingested by tsetse it has to adapt itself to the new environment to enable development to continue. Several trypanosomes are destroyed at this stage and the maximum period for these 'wrong' morphological forms of trypanosomes to survive in the gut of tsetse depends on a number of factors and varies among species of trypanosomes. Hoare (1972) demonstrated experimentally that T. evansi is incapable of developing in Glossina for the trypanosomes ingested by the fly are killed in the gut within six hours and digested together with the blood. The rapid reduction in the nubmer of flies found with gut infection after the first five days during this study could have been due to the trypanosomes dying in the gut of the flies because they were not destined to develop further in the tsetse gut.

The temperature at which the trypanosomes were developing was the same for both species of tsetse and for the second experiment (Table X) trypanosomes were detected in the labrum of G. morsitans by day 10 post infection. The mature infection in which the hypopharynx was involved was demonstrated by day 19 post infection, while for G. pallidipes was by day 23 (Table X). In the other group of flies (Table XI) mature infection was first detected in the flies in the period between 26 - 30 days. In this study, development of mature infection in a limited number of tsetse flies with T. simiae was shown to take a minimum of 19 days as had been shown for the congolense group trypanosomes but

the labrum could be invaded as early as day 10 post infection. This is in agreement with observations made by Bruce et. al. (1912), Peel and Chardrome (1954), Kaddu, (1978).

Among the various species of tsetse flies there is variation with regard to susceptibility to trypanosome infection. Duke (1936) found that the overall infection rate of G. morsitans significantly exceeded that of G. fuscipes fed on the same animals. Harley (1971) showed that G. pallidipes and G. fuscipes were quite susceptible to infection with T. rhodesiense, followed by G. morsitans. G. brevipalpis was not susceptible.

When the infection rates for flies of both species which had all along been dissected to determine the infection rate were compared, G. morsitans was at times more frequently infected than G. pallidipes. The overall infection rate for flies fed on pigs infected with EATRO 1861 was 2.6% for G. morsitans and 1.9% for G. pallidipes. With EATRO 1806 the infection rates recorded for G. morsitans and G. pallidipes was 1.7% and 1.04% respectively. During these investigations (Results on Table XI and XII) the infection rates were 13.1% for G. morsitans and 2.7% for G. pallidipes. G. morsitans appeared to be more frequently infected than G. pallidipes especially with strain EATRO 1806 (Table XV).

Unfortunately it was not possible to repeat this aspect of investigation using the two species of tsetse due to lack of G. pallidipes flies. An attempt was made to relate the infecting species of tsetse, the infection rate in the batches of flies and the course of the resulting disease in pigs. The observations made appear to suggest that there was no relationship among the three factors. Parasitaemia generally appeared earlier in syringe initiated infections than in cyclically transmitted infections. The prepatent period for cyclically initiated infection varied between 5 days and three weeks (Chapter 3). The response of pigs to infection suggest individual variation in susceptibility.

4.4.2 Relationship between trypanosome numbers, trypanosome morphology and infection rate in tsetse

The infection rates in G. morsitans flies which fed on the two pigs (Nos. 1 and 4) reveal that the infection rate in tsetse fly does not depend on the number of circulating trypanosomes during the course of infection in the pig. Only teneral tsetse flies less than 24 hours old were used for this aspect of investigation to try and overcome the problems of the pertrophic membrane hindering the establishment of the infection in tsetse. When the infection rate of trypanosomes in these flies were estimated day by day, there were days when no fly became infected despite the numerous parasites which could have been ingested in the blood meal. However, the

morphology of trypanosomes present during the whole course of the infection, is taken into account, flies appear to become infected after ingesting trypanosomes in a blood meal when there are many short forms of trypanosomes circulating in the blood of the pig. In table XIV and XV for example, on day 8 post inoculation, 12.5% of flies which fed on the pig became infected, on day 7, 12.5% while for those that fed on day 10 was 5%. The percentage of short form trypanosomes circulating were 51.3% for pig No. 1 on day 8 and 43.5% on day 10. No estimation of the morphological forms was done for days 6, 7 and 8 because there were very few trypanosomes in the stained blood smear for pig No. 4. When the trypanosomes were predominantly of short forms. Similar observation was made with pig No. 1.

Recently isolated trypanosome strains are usually pleomorphic, long slender forms predominating in the ascending parasitaemia, the shorter and probably infective forms, appearing as the parasitaemia goes into remission (Hoare, 1970).

Infecting tsetse with T. simiae on these two occasions follow the observations made for the other trypanosome species, that is flies will become infected when stumpy trypanosomes predominate but the infectivity of T. simiae for tsetse appear to be specifically defined in that a population which give rise to a fairly high infection rate in tsetse will fail to infect any fly when flies are fed to the same host twenty four hours later.

4.5 CONCLUSION

Glossina morsitans developed mature infections with *T. simaie* in 19 days and *G. pallidipes* in 23 days. *G. morsitans* was found more frequently infected than *G. pallidipes* on dissection. The rate of development of mature infection and the higher infection rate appeared not to bear any relationship to the course of the resulting disease in pigs both in the prepatent periods and patency recorded.

Teneral tsetse flies became infected with *T. simiae* when fed on a host carrying trypanosomes but only on some days during the infection and the number of trypanosomes circulating in the pig did not appear to affect the trypanosome infection rate in tsetse.

CHAPTER 5

ADAPTATION OF TRYPANOSOMA (NANNOMONAS) SIMIAE TO LABORATORY RODENTS

5.1 INTRODUCTION

Among the Nannomonas trypanosomes, T. congolense infects a wide range of mammalian species while T. simiae is apparently infective to a very few. Stephen (1966) in his review of pig trypanosomiasis summarised the attempts of various workers to transmit T. simiae to laboratory and other animals by blood inoculation and by fly challenge. Guineapigs were found to be refractory by 4 of the reports while one author reported transient parasitaemia. Rabbits were reported to be sometimes susceptible. Desowitz and Watson (1953) reported extreme individual variation in the susceptibility of rabbits to T. simiae. Some animals were completely refractory to infection while others died after exhibiting a massive infection for a number of days. After splenectomy, every rabbit in which the infection was allowed to run its full course died and in every case an intense parasitaemia was produced. Attempts to infect rats and dogs have never been successful. There is one report of mice becoming infected, but Stephen (1966) regards the report as suspect.

Splenectomy of rats, mice and guinea pigs failed to render these animals susceptible to infection with T. simiae (Desowitz and Watson, 1953). Sheep, goats and monkeys also were

variably susceptible to infection by this species of trypanosomes (Bruce et. al., 1913). Warthogs and wild pigs have been incriminated as reservoirs of T. simiae infection (Hoare, 1972). The effect of T. simiae in cattle and horses is unknown. Janssen and Wijers (1974), working with T. simiae at the Kenya Coast, demonstrated the variable susceptibility of goats and rabbits to infection with the parasite. Pigs on which wild Glossina brevipalpis were fed developed a disease which ranged between hyperacute and acute. When two goats were inoculated with blood from pigs with hyperacute infections they developed subacute to chronic infections from which they eventually died. A goat which was inoculated with blood from a pig which had an acute infection developed a chronic, self-limiting disease. When blood from a pig which had been infected by G. pallidipes was inoculated into a goat, the goat developed a very mild infection. When eight rabbits were inoculated with blood from pigs infected by wild G. brevipalpis, they developed short-lived, self-limiting infections. In all rabbits, the parasitaemia remained very low, not exceeding eight parasites per field of a thick film. Rabbits inoculated with the G. pallidipes isolate never showed any parasitaemia.

The susceptibility of mammalian hosts to infection with trypanosomes, as with most other infective organisms, varies from species to species and from one breed or strain to another. Desowitz (1959) working in West Africa, demonstrated that infection with T. vivax differed between breeds of cattle. Zebu

cattle suffered fatal infections particularly in areas of heavy challenge. N'dama cattle could live in tsetse infested areas without succumbing to the infection. This variable susceptibility was later confirmed under experimental conditions in the laboratory by Morrison et. al. (1977) who demonstrated the factors governing the susceptibility to African trypanosomiasis in strains of inbred mice. It was observed that there were large differences in survival time between the different strains of mice and it was thought that the differences were related to the ability of individual animals to control the parasitaemia.

Like T. simiae, T. vivax is not infective to laboratory rodents under normal conditions and investigations with T. vivax could not be conducted using mice and rats until Desowitz and Watson (1959) adapted a strain to albino rats. Unsworth and Nesbitt (1952) reported that it was possible to maintain T. vivax by serial syringe passage in rabbits provided that at each passage, the injection of infected blood was followed within twenty four hours by a supplementary inoculation of uninfected sheep or bovine blood. Desowitz and Watson (1959) used a similar procedure was used to passage a strain of T. vivax in albino rats. This strain was maintained by serial syringe passage through rats, at first with supplementary serum but later without it. It adapted well to rats, as well as to mice, and it is now widely used in various laboratories both in Africa and oversease without

supplementary sheep's serum. At passage 71 the dependence on the supplementation was lost and at the same time a 25% mortality was recorded in rats. In later work involving the rat-adapted strain it was shown that death occurred with both high and low parasitaemia throughout the period of infection.

The work reported in this chapter was an attempt to adapt T. simiae to laboratory rats and mice. Availability of a rodent-adapted strain would greatly facilitate experimental studies of this parasite which are now difficult because of the expense and inconvenience of working with pigs.

5.2 MATERIALS AND METHODS

5.2.1 Trypanosomes

Two stabilates of T. simiae were used for this study. They were EATRO 1786 and 1880.

5.2.2 Rats

Rats were used after weaning and they were from the Small Animal House, the Kenya Trypanosomiasis Research Institute. Only male rats were used for the investigation.

5.2.3 Irradiated Rats

Irradiated rats were obtained from the International Laboratory for Research on Animal Diseases (ILRAD), Kabete. The rats were irradiated at 900 rads per minute for seven minutes and 40 seconds from a Cesium Source.

5.2.4 Cyclophosphamide treated Rats

Adult rats were each given a total of 200 mg/kg body weight of cyclophasphamide in 4 doses over 4 consecutive days. Treatment was by the intraperitoneal route.

5.2.5 Young Rats

Ten day old rats were removed from the breeding colony but were kept with their mother during the experiments.

5.2.6 Mice

Mice in KETRI colony were brought in from the Walter Reed Project. They had originally been brought from London. Only male mice aged 8 weeks were used.

5.2.7 Rabbits

Adult male rabbits of the New Zealand White variety, which were used for maintaining tsetse flies, were obtained from the Veterinary Research Department, Muguga.

6.2.8 Pigs

All the pigs that were used in the experiments were of the Large White breed and were bought from the Muguga area. They ranged in age between 2 and 6 months at the start of the experiments.

5.2.9 Tsetse Flies

All the tsetse flies which were used for the transmission experiments were bred at the Kenya Trypanosomiasis

Research Institute. Only teneral flies were used.

5.2.10 Infecting pigs, rats and mice with *Trypanosoma (N). simiae*

Trypanosomes in capillary tubes were withdrawn from the trypanosome bank, where they are kept in liquid nitrogen, and were allowed to thaw. After thawing, the capillary tubes were cut open and the trypanosomes were suspended in ESG (EDTA - Saline - Glucose, pH 7.4) and microscopically enumerated using a x 40 objective in a wet preparation. Pigs were inoculated with 10^5 trypanosomes subcutaneously behind the ear. When subpassage was made from a donor pig, trypanosomes in infected blood were suspended in ESG and then inoculated as described above. Inoculation of rats either directly from the trypanosome bank or from a donor pig followed the same procedure. Adult rats received 5×10^5 trypanosomes while the young rats received 10^5 trypanosomes. Rats were inoculated intraperitoneally.

Mice were inoculated ip with 10^5 trypanosomes or as specified.

Initially when infecting normal adult rats with *T. (N). simiae* a group of five rats was inoculated and then injected with 0.5ml of normal pig serum 24 hours after inoculation. Another group of five rats was at the same time inoculated with the parasite but not treated with pig serum.

5.2.11 Examination of pigs, rats and mice after inoculation

Starting from the day when pigs were inoculated with trypanosomes, rectal temperatures of the pigs were taken daily in the morning and afternoon. Blood from the tail vein was collected at the same time in two heparinished capillary tubes for the Haematocrit Centrifugation Technique (HCT). The blood in the capillary tubes was then span using a Hawksley haematocrit centrifuge and the packed cell volume (PCV) for each pig was estimated by reading off the scale from the reader. The tube was then cut just at the buffy coat interface, the buffy coat expressed onto a microscope slide and the slide then examined as for the wet blood preparation. When taking blood for estimation of PCV and examination of the buffy coat, wet blood preparations were also made for direct estimation of trypanosomes in the blood of the pig using the method of Walker (1968). After trypanosomes had been detected in the blood of the pig by the wet preparation method, buffy coat examination was discontinued and the HCT was used only for estimating the PCV. Thin blood smears were made from the blood of the pig to check the morphology of the trypanosomes. After drying the smears they were fixed in redistilled methanol, stained with Giemsa's stain and examined microscopically under oil immersion.

Rats and mice were examined for trypanosomes by the wet preparation method using fresh tail blood. Trypanosomes were

then counted and recorded as for pigs. Examination of mice and rats began two days after inoculation. When mice or rats were found parasitaemic, thin blood smears were made and stained using Giemsa's stain before examining under the microscope. Blood from a parasitaemic rat was used to infect other rats. The prepatent periods for rats and mice were recorded as was the patency of an infection, patency being the period when an animal was parasitaemic. The end point of the patent period was either the day when the host died or the last day on which parasites were seen in the blood of the host.

5.2.12 Infection of tsetse flies with T. simiae from pigs and Transmission of infection to pigs and rats

Teneral G. morsitans of both sexes were fed on parasitaemic pigs or rats after which they were maintained on rabbits as described in Chapter Two.

5.2.13 Infectivity of rat-adapted T. simiae for mice

Samples of trypanosomes to be titrated were made by suspending infected blood from rats in ESG and serially diluting them tenfold. A group of six mice were inoculated at each of six consecutive dilutions. Details of procedure is given in Chapter Two. Stabilate EATRO 1786 was used and titrations were carried out from rats in passage Nos. 13 and 20 (Table XXI and XXII).

5.3 RESULTS

5.3.1 Ten-day old rats

Eight 10-day old rats were inoculated with 10^5 trypanosomes of EATRO 1786 from the trypanosome bank. One out of the eight rats became parasitaemic after a prepatent period of 14 days and was parasitaemic for 6 days after which no trypanosomes were detected in the blood of this rat even by buffy coat examination.

A blood sample was obtained from the infected rat on parasitaemia day 3, suspended in ESG and used to inoculate a further nine 10 - day old rats. All the nine rats became parasitaemic after a prepatent period of 1 or 2 days and remained parasitaemic for periods ranging from 3 days to 50 days (Table XVI)

Trypanosomes from one of the rats were inoculated into five adult rats. The infection was 4 days old at the time the passage was made and it persisted for another 6 days. No rats became infected.

In another attempt trypanosomes from an infected pig (No. 1) were inoculated into ten 10 - day old rats. None of the rats became parasitaemic. (Table XVII).

5.3.2 Irradiated rats

Trypanosomes used for inoculating the rats were from pig No. 1 which had been inoculated with stabilate EATRO 1786. Five irradiated rats were inoculated with 10^5 trypanosomes. None

Table XVI: Infection of 10 day old rats with T. (N). simiae.

Trial No.	No. of rats infected/ No. inoculated	** Max. No. Trypanosomes per dield	* Duration of Parasitaemia (Days)
I	1/8	20	6
II	1/9	20	3
	1/9	+	7
	2/9	+	10
	1/9	+	17
	1/9	+	39
	1/9	++	50
III	0/10	NIL	NIL

** Maximum number of trypanosomes seen in one microscope field in wet blood preparation.

\+ Approximately sixty trypanosomes per field

++ Approximately 120 trypanosomes per field

* All the infections were self-limiting

of the rats became parasitaemic.

On the second occasion another group of five irradiated rats were inoculated with trypanosomes from the same source (pig No. 1). None of the rats became parasitaemic.

5.3.3 Cyclophosphamide treated rats

Trypanosomes inoculated into these rats were also from pig No. 1. Five cyclophosphamide treated rats were inoculated with 5×10^6 trypanosomes on day 0 and none of the rats became parasitaemic.

Another group of five cyclophosphamide treated rats was inoculated with trypanosomes from the same pig and again no infection was established. In both experiments all the rats died by day 16 post inoculation, probably from drug toxicity or as a result of immunosuppression caused by cyclophosphamide.

5.3.4 Normal adult rats

Five adult rats were inoculated with 5×10^6 trypanosomes from the same pig (No. 1) which was inoculated with stabilate EATRO 1786. Three out of five rats became parasitaemic after a prepatent period of 18 days and were parasitaemic for 3 days (Table XVII). From one of the parasitaemic rats five more rats were inoculated. All the five rats became parasitaemic. Four rats became parasitaemic after a prepatent period of two days and remained so for 3 days. One rat became parasitaemic after

Table XVII Adaptation of T. (N). simiae to rats without pig serum

Passage No.	Mean Prepatent Period (Days)	Mean Patent Period (Days)	No. Infected/ No. Inoculated
1	18.0 ± 0.00	3.0 ± 0.00	3/5 (3)*
2	2.2 ± 0.20	34.4 ± 31.39	5/5 (5)*
3	8.0 ± 0.00	6.5 ± 0.69	4/5 (4)*
4	21.3 ± 11.00	15.3 ± 5.33	3/5 (3)*
5	13.0 ± 4.90	32.5 ± 12.50	2/5 (2)*
6	12.0 ± 0.00	31.0 ± 0.50	1/5 (1)*
7	11.6 ± 1.17	20.4 ± 8.20	5/5 D
8	4.2 ± 0.37	39.2 ± 1.45	5/5
9	2.0 ± 0.00	31.8 ± 7.38	5/5
10	7.4 ± 0.40	40.6 ± 2.23	5/5
11	4.2 ± 0.24	31.6 ± 6.85	5/5
12	3.0 ± 0.32	45.0 ± 2.43	5/5
13	3.8 ± 0.49	30.4 ± 9.53	5/5
14	4.2 ± 0.20	42.4 ± 3.53	5/5
15	5.4 ± 0.40	36.6 ± 2.23	5/5
16	5.0 ± 0.00	21.2 ± 2.24	5/5
17	3.0 ± 0.58	26.0 ± 7.36	5/5
18	8.0 ± 0.58	32.0 ± 7.56	5/5
19	4.2 ± 0.20	31.4 ± 2.27	5/5
20	5.2 ± 0.20	33.1 ± 2.59	5/5

* The number of rats in which infection was transient is in brackets.

3 days and remained infected for 160 days when it was accidentally killed. From passage No. 2, serial syringe passage was continued using blood from one infected rat to infect a group of five rats. The results are summarised in Table 14.

From passage No. 1 to passage No. 7, rats were parasitaemic for varying lengths of time and none died of the infection. Death from infections was first observed in passage No. 7 and thereafter became the normal outcome. From this time onwards all rats had persistent parasitaemias.

In another experiment, samples of infected blood from pig No. 24 were inoculated into groups of 2 rats over a period of 20 days. The results are summarised in Table XVIII. Only one out of two rats became parasitaemic after a prepatent period of 3 days and trypanosomes were observed in the blood of the rat for only two days. None of five rats inoculated with blood from this rat became infected.

5.3.5 Inoculation of adult rats with *T. simiae* followed by injection of normal pig serum

Five rats were each given 5×10^5 trypanosomes from stabilate EATRO 1786 and twenty four hours later they were injected with 0.5 ml of normal pig serum. None of the rats became infected. The experiment was repeated six times, with the same result. An examination of the effect *in vitro* of the pig serum on trypanosomes revealed that trypanosomes suspended

Table XVII: Results of inoculation of rats with blood from a pig infected with T. simiae on 12 consecutive days.

Age of infection in donor pig* (Day)	No. trypanosomes/ No. fields	No. rats infected/ No. inoculated
0	0/20	
8	0/20	0/2
9	2/1	0/2
10	5/1	0/2
11	5/1	0/2
12	10/1	0/2
13	20/1	0/2
14	20/1	1/2
15	20/1	0/2
16	20/1	0/2
17	5/1	0/2
18	5/1	0/2
19	++	0/2

* Pig No. 24

++ - Approximately 120 trypanosome per microscope field.

in the serum clumped together and many of them had very sluggish movements. It was concluded that the serum was killing the trypanosomes and another batch which had no effect on the trypanosomes was used.

In order to see if pig serum would enhance infectivity, five rats were each given 5 x 10^5 trypanosomes from a parasitaemic rat (Passage No. 6 Table XVII) followed by pig serum 24 hours later. All the five rats became parasitaemic and remained infected until they died. Trypanosomes from one of these rats were passaged into another five rats, all of which became infected. A total of 14 passages were done and the results are summarised in Table XIX). The mean prepatent period tended to decrease with time.

In a second experiment two normal rats were inoculated with infected pig blood daily, followed by pig serum 24 hours after inoculation, until the donor died. As can be seen from Table none of the rats became infected.

5.3.6 Infectivity of rat - adapted *T. simiae* for mice

The infectivity for mice of the rat - adapted strain was tested using different numbers of trypanosomes from passage numbers 13 and 20 (Table XXI). Trypanosomes from all the four passage 13 rats were infective for mice (Table XXI). The smallest infective dose was 10^3 trypanosomes. The ID_{63} values (infective dose required

Table XIX: Adaptation of T. (N). simiae to rats using pig serum

Passage No.	Mean prepatent Period (Days)	Mean patent Period (Days)	No. rats infected/ No. Inoculated
1*	3.4 ± 0.75	38.4 ± 7.9	5/5
2	1.8 ± 0.37	32.4 ± 5.7	5/5
3	5.6 ± 0.24	36.4 ± 1.96	5/5
4	8.2 ± 0.80	40.2 ± 3.84	5/5
5	5.6 ± 1.21	33.0 ± 7.69	5/5
6.	3.0 ± 0.55	35.2 ± 8.22	5/5
7	5.4 ± 0.24	28.4 ± 10.86	5/5
8	5.8 ± 1.20	30.6 ± 5.50	5/5
9	6.0 ± 0.00	13.0 ± 4.71	5/5
10	3.8 ± 0.49	28.0 ± 8.89	5/5
11	3.0 ± 0.00	12.8 ± 2.82	5/5
12	3.2 ± 0.20	36.4 ± 2.56	5/5
13	3.2 ± 0.20	16.0 ± 4.64	5/5
14	3.0 ± 0.00	11.6 ± 6.38	5/5

* The donor was a rat which had not received pig serum (passage No. 6, Table XVII).

Table XX: Results of inoculation of rats with blood from a pig infected with T. simiae plus normal pig serum.

Age of infection in donor pig* (Day)	Parasitaemia in pig **	No. rats infected/ No. inoculated
0	0/20	-
8	0/20	0/2
9	2/1	0/2
10	5/1	0/2
11	5/1	0/2
12	10/1	2/2
13	20/1	0/2
14	20/1	0/2
15	20/1	0/2
16	20/1	0/2
17	5/1	0/2
18	5/1	0/2
19	++	0/2

* Pig No. 24

** No. trypanosomes/No. of fields

++ Approximately 120 trypanosomes per microscope field.

Table XXI: Infectivity of rat-adapted *T. simiae** for mice

Donor rat No.	Parasiraemia in donor rat	Parasitaemia day of donor rat.	No. trypanosomes inoculated into mice	No. of mice infected/ No. inoculated	ID_{63}
Rat No. 8	10**	4	10^6	6/6	
			10^5	4/6	
			10^4	3/6	2.5 ± 0.5
			10^3	6/6	
			10^2	0/6	
Rat No. 8	20**	5	10^6	6/6	
			10^5	0/6	
			10^4	0/6	1.4 ± 0.5
			10^3	0/6	
			10^2	0/6	
Rat No. 7	+	5	10^6	6/6	
			10^5	4/6	
			10^4	0/6	2.0 ± 0.3
			10^3	0/6	
			10^2	0/6	
Rat No. 7	+	9	10^6	6/6	
			10^5	6/6	
			10^4	0/6	1.4 ± 0.5
			10^3	0/6	
			10^2	0/6	

* From passage No. 13. (Table XVII).

** Number of trypanosomes per microscope field.

\+ Approximately 60 trypanosomes per microscope field.

ID_{63} Dose required to infect 63% of the group of rats inoculated.

Table XXII: Infectivity of rat - adapted T. simiae* for mice

Donor rat No.	Parasitaemia in donor rat	Parasitaemia day of donor	No. Trypanosomes inoculated into mice	No. of mice infected/ No. Inoculated	ID_{63}
Rat No. 4	10**	2	10^6	2/6	
			10^5	0/6	
			10^4	0/6	1.6 ± 0.5
			10^3	0/6	
			10^2	0/6	
Rat No. 1	+	12	10^6	6/6	
			10^5	6/6	
			10^4	2/6	2.6 ± 0.3
			10^3	0/6	
			10^2	0/6	
Rat No. 1	+	13	10^6	6/6	
			10^5	6/6	
			10^4	2/6	2.6± 0.3
			10^3	0/6	
			10^2	0/6	
Rat No. 1	20/1	14	10^6	6/6	
			10^5	6/6	
			10^4	0/6	
			10^3	0/6	2.4 ± 0.5
			10^2	0/6	

* From passage No. 20 (Table XVII).

** Number of trypanosomes per microscope field.

\+ Approximately 60 trypanosomes per microscope field.

ID_{63} Dose required to infect 63% of the group of rats inoculated.

to infect 63 per cent of animals inoculated) indicate that the infectivity of the trypanosome population in a rat varied from day to day (Table XXI). The results with passage 20 trypanosomes were similar to those with passage 13 trypanosomes except that the smallest infective dose was 10^4 trypanosomes. They are summarised in Table XXI and XXII).

5.3.7 Morphology of trypanosomes

The infections in rats that were becoming infected were generally transient during the first four passages and the trypanosomes were morphologically different from what was being observed in the pig. This observation was made during the first three passages. Some of the trypanosomes were very long and sluggish in movement. In Giemsa stained smears, trypanosomes were observed to reach length up to 36.3 µm. The trypanosomes had a mean length of 28.99 ± 0.45 µm, with a range of 24.2 to 36.3 µm. The kinetoplast was a mean of 6.01 ± µm from the anterior end with a range of 3.3 to 15.47 µm. The nucleus was a mean of 18.11± 0.46 µm (range 14.4 to 27.5 µm) from the anterior end. In one rat which became infected in passage No. 2, the unusually long trypanosomes were present but at the same time a few shorter forms were present and after several attempts, a successful subpassage was made and trypanosomes from this rat is what gave rise to the line which was serially passaged in rats with and without pig serum. The mean length of T. simiae trypanosomes from pigs inoculated with stock EATRO

e: XXIII: Mean length * of T. simiae (Stock EATRO 1786) trypanosomes from three pigs.

Parasitaemia Day	Pig Nos.		
	2	4	5
1			
2	13.5 ± 1.06	15.06 ± 0.30	15.05 ± 0.29
3		14.92 ± 0.46	15.53 ± 0.33
4	15.5 ± 0.41	15.4 ± 0.26	16.12 ± 0.22
5	16.7 ± 0.46	15.38 ± 0.31	15.80 ± 0.25
6	16.06 ± 0.34	15.09 ± 0.34	
7	15.31 ± 0.44	15.23 ± 0.37	
8	16.13 ± 0.38	15.43 ± 0.28	

* Mean lengths in micrometres.

n micrometres of T. simiae (Stock EATRO
somes from three rats.

Passage No./Rat No.		
6/8	11/6	16/5
.59 ± 0.28	12.30 ± 0.27	10.13±0.26
.43 ± 0.29	13.5 ± 0.26	12.15±0.32
.39 ± 0.33	13.9 ± 0.27	11.88±0.36
.59 ± 0.34		
.74 ± 0.24		
.89 ± 0.25		

ble: XXIV: Mean lengths in micrometres of T. simiae (Stock EATRO 1786) trypanosomes from three rats.

Parasitaemia Day	Passage No./Rat No.		
	6/8	11/6	16/5
1	12.59 ± 0.28	12.30 ± 0.27	10.13±0.26
2	12.43 ± 0.29	13.5 ± 0.26	12.15±0.32
3	12.39 ± 0.33	13.9 ± 0.27	11.88±0.36
4	12.59 ± 0.34		
5	12.74 ± 0.24		
6	12.89 ± 0.25		

1786 ranged between 13.5 and 10.12 µm. The mean for trypanosomes from the rat adapted strain was 12.59 µm in rat blood and 12.35µm in pig blood (Table XXIII and XXIV).

5.3.8 Transmission of T. simiae from rats to pigs

Two hundred and nine teneral G. morsitans flies were fed on rats infected with T. simiae, maintained on a rabbit for 3 weeks and then fed on a normal pig (No. 19). They successfully transmitted the infection to the pig. The pig became parasitaemic after a prepatent period of 17 days and died on day 67 post infection. The infection was subpassaged from this pig (No. 19) into rats and into another pig (No. 22). Dissection of the flies which had transmitted the infection to pig No. 19 revealed mature infections in 10 out of 209 flies, giving an infection rate of 23.2%.

Trypanosomes from the rats were also successfully transmitted to pigs by syringe. One pig (No. 16) was inoculated subcutaneously with trypanosomes from a rat (passage No. 11, Table XVII). It became parasitaemic after a prepatent period of 16 days and remained so for 244 days after which it apparently self-cured. Another pig (No. 17) received trypanosomes from the same rat via the intravenous route. Parasitaemia was first detected 6 days after inoculation and persisted for 186 days. A subinoculation from pig No. 17 to another pig (No. 21) also gave rise to a low parasitaemia. All the three pigs had a consistently low

parasitaemia which terminated by self cure.

5.3.9 Attempts to transmit T. simiae from pig to mice

A group of five mice were inoculated with trypanosomes from pig No. 1 which had not been passaged through rats. None of the mice became infected.

5.4 DISCUSSION

A stock of T. simiae (EATRO 1786) has successfully infected both ten - day old and adult rats. One line of the infection has been successfully maintained in rats without pig serum through 20 syringe passages and 14 passages with pig serum. Initially the infection in rats was transient, in some animals trypanosomes being present for 1 or 2 days only. In some cases there would be no increase in parasitaemia. With other transient infections, the trypanosomes would multiply and a heavy parasitaemia would get established but the trypanosomes would then suddenly disappear from the perpheral blood and the rat would thereafter remain free from parasitaemia. Desowitz and Watson (1959), when adapting T. vivax to rats, observed similar behaviour by the trypanosomes. They noted that trypanosomes would appear in the peripheral blood of a rat 1 or 2 days after inoculation, increase in number over a period of 3 to 7 days and then decline more or less rapidly, usually over a period of 1 or 3 days. Thereafter no trypanosomes could be detected in the majority of the rats examined. They observed some non-fatal

relapses but none were seen in the present study.

During the first 7 passages in which no pig serum was used infections were transient, except for one rat which run a chronic infection for 160 days until it was accidentally killed on day 164 post inoculation. Desowitz and Watson (1959) noted that although T. vivax caused definite indisposition in many of the infected rats, no fatal cases occurred. In the present study rats started to die from infection from passage 7 (Table XVII). They also noticed that T. vivax in the rat appeared to retain its normal morphological and other characters. Observations with T. simiae showed that trypanosomes present in the blood of rat during the transient infections included unusually long trypanosomes. These long trypanosomes appeared to have free flagella and in some cases a very long snout. This only happened in adult rats. In the rat which was chronically infected in passage No. 2 (Table XVII) the long trypanosomes were also present and these gave way to shorter and smaller trypanosomes. This is the infection from which the present line of rat adapted T. simiae arose.

The disappearance of trypanosomes from peripheral rat blood after a period of multiplication appears to resemble the response of rats to infections with T. lewisi. After inoculation into the rat, the T. lewisi trypanosomes multiply and a mature population of parasites gets established. Meanwhile the rat raises antibodies against the mature trypanosomes which then

kill the entire population and the rat remains free from infection thereafter (Lumsden, 1972). With salivarian trypanosomes generally, the infections are periodic with waves of parasitaemia following each other at intervals of a few days. Each wave of parasitaemia represents a new antigenic trype which is destroyed in its descending phase by host antibody. The continuation of the infection is a result of trypanosomes evading the immune response of the host by repeatedly cnaging their antigenic character (lumsden, 1972). The host will continue to live until overwhelmed by the infection.

The first batch of pig serum used was observed to be lethal to trypanosomes. Subsequently the new batch of serum had no effect on trypanosomes and it did not enhance the adaptability of trypanosomes to rats and was probably unnecessary.

One must consider the possibility that the stabilate (EATRO 1786) from which the rat-adapted strain originated might have contained one or trypanosome species, in addition to T. simiae, which could have multiplied in the first rat and become better adapted with subsequent passages. Failure to infect mice with the stabilate argues against this possibility and confirms that the original stabilate contained only T. simiae. In addition, isoenzyme electrophoresis comparing this stock (EATRO 1786) with stocks of T. congolense and T. brucei showed that this stock of T. simiae had distructive isoenzyme profiles (Gashumba, Gibson and Opiyo, 1986). This is the stock

that became adapted to rats and mice.

G. morsitans flies successfully transmitted the rat-adapted strain of T. simiae to a pig and rats. It is interesting that syringe passage of the strain in rats for 8 months had not affected its ability to develop in the vector. The infection rate in the flies was much higher (23.2%) than the rates in flies which fed on infected pigs, which were generally less than 10% (see chapter 4).

The three pigs to which the rat adapted strain of T. simiae was transmitted, whether cyclically or by syringe, developed chronic, self-limiting infections. This result could be attributed either to the rat adapted strain being less virulent in pigs than the normal strains or, since as shown in chapter 3 individual pigs differ markedly in their susceptibility to infection, to the three pigs being resistant to infection.

5.5 SUMMARY

It has been possible to adapt T. (N). simiae to rats and mice without pig serum through 20 passages.

Infection in rats in the initial stages of the adaptation was transient, with rats throwing off the infection after running a parasitaemia for a period ranging from 1 day to 50 days.

T. simiae progressivley more infective to rats, becoming lethal from passage No. 7. Passages progressed rats died faster. In rats which received pig serum 24 hours post inoculation both

the prepatent period and the patency of infection was less variable than in rats which did not receive pig serum post inoculation, but pig serum was not necessary for T. simiae to adapt to rats.

The rat adapted T. (N). simiae has remained infective to tsetse. However it is less virulent for pigs than the original strain. This is true whether it is transmitted by syringe inoculation or through tsetse.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.1 DISCUSSION

In pigs disease due to Trypanosoma simiae develops after an average prepatent period of six days after challenge by tsetse (Stephen and Gray, 1960) and 3.3 days after injection of infected blood (Bruce et. al., 1913). At the end of the prepatent period, the parasitaemia developed to a high level very fast and the survival of the pigs after fly challenge averaged 3.6 days (Stephen, 1966) or 3.4 days (Gray, 1961). Survial of syringe inoculated pigs was 1.3 days (Bruce et. al. 1913). Steel (1966) observed that some pigs could survive 3 weeks of patent infection with T. simiae. Later Janssen and Wijers (1974) observed chronic syndromes in pigs cyclically infected. They related the disease syndromes to the transmitting species of tsetse.

In the present study the trypanosome isolates that were used originated from the same area studied by Janssen and Wijers (1974). The mean prepatent period of the syringe inoculated infections was in agreement with what has been documented. The infection was established after a mean period of 3.6 ± 0.34 days and the pigs survived a mean of 8.07 ± 3.07 days which was more than twice what had been previously reported. Bruce et. al. (1912) noticed an enhacement of the virulence of T. simiae after

transmission by G. morsitans. During this study, transmission by G. morsitans gave rise to infections which ranged between hyperacute and chronic forms. The mean patent period was 12.31 ± 2.26 days and the mean patency was 20.6 ± 7.84 days. In addition to the lowering of virulence of T. simiae by G. morsitans, one pig exhibited self-cure after a period of 115 days. These observations are not in agreement with what Bruce et. al. (1912) observed. In another experiment G. morsitans serially transmitted the infection two times to pigs and as such was given two chances for enhancing the virulence of the stabilate. The infection in the second pig was more chronic than after the first transmission. Janssen and Wijers (1974) suggested that transmission of T. simiae by G. pallidipes gave rise to chronic infection in the pig. When the three stabilates were transmitted by G. pallidipes to pigs, the disease was less virulent than for the original stock. None of the ten pigs developed hyperacute infection. Self-limiting infection was observed in two pigs. This observation appear to be in agrement with observations made by Janssen and Wijers (1974). When G. pallidipes serially transmitted the infection twice to pigs, a very chronic disease syndrome was not obtained which weakens the obove argument. It was concluded that another species of tsetse, G. brevipalpis, was the cause of the virulent T. simiae infections at the Kenya Coastal area (Janssen and Wijers, 1974). In this study when G. brevipalpis transmitted a G. brevipalpis isolate of T. simiae to pigs a hyperacute disease was expected, but instead

a chronic infection was observed in one of the three pigs. The present findings appear to suggest that transmission by G. morsitans and G. pallidipes to pigs may give rise to chronic infections. However, the frequency with which these chronic syndromes appear does not conform to any predictable pattern. In each case where chronic conditions were observed, there were individual animals with very virulent disease syndromes included in the same group. In every case, chronic infections were characterised by low parasitaemia and not too frequent rise in temperature above 40.0^{o}C. Parasitaemia readings for T. simiae infections are known to rise very fast and pigs die within a few days with fulminating parasitaemia. In this study parasitaemia, in the would be chronic cases, developed very slowly and remained at a low level. The occurrence of the chronic infections in pigs appeared not to be related to the number of trypanosomes inoculated into the pig. For T. brucei infections in mice Walker (1964) was able to relate the survival of the mice to the number of trypanosomes inoculated.

The varying response of pigs to cyclically transmitted infection further suggest that the population of the large white variety of pigs used in this study could have been heterogenous. There were individuals that were highly susceptible that exhitited the classical T. simiae infection. There were those that were susceptible but were able to suppress parasitaemia and to go through more than one parasitaemic wave, but which eventually

succumbed to the infection. Then there was a group that became parasitaemic but controlled the parasitaemia effeciently and later threw off the infection. The wide variation in the response of pigs to infection appear to support the suggestion of Ormerod (1970) that normally an infectious disease changes its natural history as parasite and host establish a genetic balance. He noted that where reservoir hosts and transmitting agents got involved, the tendency to vary greatly increased. These variations in response among individuals were present in one group of animals and not in the other. The large white variety of pigs was introduced into Kenya around 1906 (Sande, 1981) and it appears that among them there are individuals that can withstand infection with T. simiae. This type of pigs could have been in the group studied by Steel (1966) and Janssen and Wijers (1974). When the development of T. simiae was compared in the two species of tsetse, infection was first detected in the labrum of G. morsitans by day 10 and in the hypopharynx by day 19 and for G. pallidipes by day 23. This observation is in agreement with the developmental cycle recorded by Bruce et. al. (1913). Harley and Wilson (1968) noticed T. congolense trypanosomes in the hypopharynx of tsetse by day 14 and Kaddu (1978) by day 10.

As with other trypanosomes, infection of T. simiae in tsetse appeared to be dependent on the right morphological forms being present in the blood meal (Vickerman, 1970).

Attempts were made to adapt two isolates of T. simiae to rats. The isolate which was very virulent to pigs failed. The less virulent strain was adapted to rats both in the presence of normal pig serum and without pig serum. Desowitz and Watson (1951) were able to adapt T. vivax in the presence of sheep serum but the strain could not be maintained in the absence of the serum for quite a period of time.

In this study the lethal effect of normal pig serum which was observed at the initial stages of the adaptation made it difficult to assess what would have been the fate of this experiment. However, a repeat experiment further pointed out that daily inoculation of rats with T. simiae from an infected pig over a period may not give rise to infection of T. simiae in rats. The failure to infect the cyclophosphamide treated rats and irradiated rats could have been due to rats in these groups dying too early before the infection was established or perhaps they were not given equal chance (daily inoculation) as for the normal rats.

This is the first time that T. simiae has been adapted to go into rats and mice. The rat adapted strain has remained infective to pigs and is transmissible by tsetse to rats and pigs.

6.2 CONCLUSION

This study provides some new information about T. simiae infection in pigs. There appears to exist individual pigs in the Large White breed that can withstand infection with T. simiae. In these pigs the infection is characterised by low parasitaemia and spontaneous recovery. Cyclical transmission of T. simiae by G. morsitans and G. pallidipes may give rise to chronic disease syndromes in pigs, but in an unpredictable way. Virulence of T. simiae therefore may be influenced in a variety of ways including the response of the pig to the infection. A rat adapted T. simiae strain has been developed which should facilitate futher work on the biology of T. simiae.

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Appendix I

From Walker (1968)

LOG EQUIVALENT VALUES (L.E.V.'s) FOR GIVEN OBSERVED NUMBERS OF TRYPANOSOMES PER MICROSCOPE FIELD

Observed No. of tryps. per field.	L.E.V.	
0/20	0.5	A practical Value for the 'veil line' (see text)
1/20	0.7	
1/10	1.0	
1/5	1.3	
1/2	1.7	
1/1	2.0	
2/1	2.3	
5/1	2.7	
10/1	3.0	
20/1	3.3	
+	3.8	Approx. 60 tryps. per field but uncountable
++	4.4	Approx. 120 tryps. per field but uncountable
Dead	4.5	A practical value

The term 'veil line is used in the sense that Preston (1948 Ecology, 29: 264 - 283) intended; namely that when making counts of rare events there comes a degree of rarity when the

counts are unreliable by virtue of the limited size of the habitat sampled. For convenience, in this case the value is about 1/30 or an L.E.V. of 0.5. It will be noticed that the log values have been raised by a value +2 which is equivalent to multiplying the original observations by x100.

Appendix 2

From Lumsden et. al. (1973)

TABLES FOR ESTIMATION OF ID_{63} VALUES AND THEIR STANDARD ERRORS

These tables (from Lumsden et. al., 1963 are computed for tenfold dilution intervals and for groups of six susceptible reciptients at each dilution, these being convenient and commonly used arrangements and as described in this book. The tables are applied to results as follows:

(a) Results for each recipient group are expressed as fractional ratios, numerators being numbers of recipients infected, over a denominator of 6, the number of recipients inoculated; e.g. 1/6 means 1 out of 6 was infected.

(b) 0/6 and 6/6 are regarded as 'weightless' data attention confined to 'useful' results, 1/6, 2/6, 3/6, 4/6 or 5/6.

(c) In any given dilution series there may occur 0, 1, 2, or 3 useful results; the tables cover all these contingencies. Any titration series in which four 'useful' result occur, or in which there are reversals (e.g. 6/6-3/6-4/6-0/6) should be regarded as a suspect experiment and repeated.

(d) Proceeding from the lower to the higher dilutions (e.g. from say $\bar{4}$ towards 8) the first useful result encountered in designated x, x being the characteristic of the log dilution of the result designated, with the sign reversed (e.g. for a first useful result at $\bar{5}$ (-5) log dilution, x=5).

(e) Depending, then, on the number os subsequent useful points, the appropriate table is entered, the particular pattern occurring located, and the ID_{63} value and its standard error read off. See below for action in case there are no 'useful' results.

(f) It is important to remember that this ID_{63} value will refer to the volume of inoculum used and adjustment is required for relation to other volumes; e.g. if the ID_{63} found for an inoculum volume of 0.1 ml. per recipient is antilog 5.6, the Id_{63} per ml will be 10 times higher, antilog 6.6.

Series with ONE useful result

Ratio	ID_{63}	S.E.
5/6	x.1	0.3
4/6	x.0	0.3
3/6	(x - 1).8	0.3
2/6	(x - 1).6	0.3
1/6	(x - 1).4	0.5

Series with TWO useful results

Ratio	ID_{63}	S.E
5/6 4/6	x.8	0.4
5/6 3/6	x.7	0.3
5/6 2/6	x.4	0.3
5/6 1/6	x.3	0.3
4/6 4/6	x.5	0.6
4/6 3/6	x.5	0.3
4/6 2/6	x.3	0.2
4/6 1/6	x.1	0.3
3/6 3/6	x.3	0.5
3/6 2/6	x.2	0.4
3/6 1/6	(x - 1).9	0.5
2/6 2/6	x.1	0.6
2/6 1/6	(x - 1).8	0.5

Series with THREE useful results

Where one point is 5/6 or 1/6, it is ignored and the remaining centrally placed points (4/6, 3/6 or 2/6) are used

and 0.1 is added to the S.E. obtained for the two points. Where two points are 5/6 or 1/6 and are consecutive, the extreme result is ignored and 0.2 is added to the S.E. given to the last two points. Where two points are 5/6 and 1/6 (on each side of 4/6, 3/6 or 2/6) they are ignored, the value for the single central point is used, and 0.2 is added to the S.E. otherwise.

Ratio	ID_{63}	S.E.
4/6 4/6 3/6	(x + 1).2	0.6
4/6 4/6 2/6	(x + 1).0	0.5
4/6 3/6 3/6	(x + 1).2	0.6
4/6 3/6 2/6	x.8	0.4

Series with No useful results

In these the last 6/6 is treated as x; then the ID_{63}= x.4 with S.E. 0.5.

Figure 30 shows a representative titration result, and the process of obtaining ID_{63} values.

In reducing S.E.'s to one decimal point, adjustment is always to the larger figure so that a significant 't' test result is certainly significant statistically. For practical

purposes a difference between ID_{63} estimates may be taken as significant (p 0.05) if it equals or exceeds 1.2 ($S.E._1 + S.E._2$).