Pathogenicity and Serological response in pigs, sheep and goats infected with Kenyan isolates of *Trypanosoma simiae* and *Trypanosoma congolense*.

By

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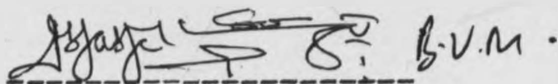

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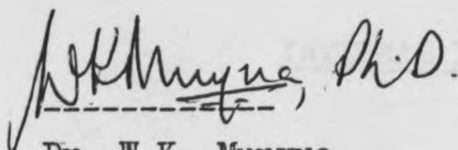

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DEDICATION

This work is dedicated to my father the late Fabianus Mahaga Mudongi who passed away in 1970. May his soul rest in peace.

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SUMMARY

The progress of infection and serological response of T. simiae 'KETRI' and T. congolense 'Transmara' in pigs, sheep and goat was studied.

Inoculation of all the three animal species with the T. simiae strain resulted in infection detectable in wet films. There were significant drops in the packed cell volume and body weight gains in all the T. simiae infected groups of animals as compared to the negative controls ($P<0.05$). Clinical observations revealed changes ranging from acute disease in pigs and goats to a more chronic infection with low parasitaemias in sheep.

In contrast, T. congolense 'Transmara' inoculations in the three animal species showed some variasion. It failed to infect pigs but caused acute infection in goats and resulted in very chronic infections with persistent parasitaemia lasting for more than 100 days in sheep. There was no statistical significance in PCV and body weight gains between the T. congolense 'Transmara' infected sheep and the controls ($P>0.05$). A later trial inoculation of pigs with T. congolense 'Ukunda' and T. congolense 'Matuga' isolates resulted in infections of very low parasitaemias which were only occasionally detectable by Woo's micro-haematocrit

centrifugation technique. These pigs failed to portray any obvious signs of infection.

The serological investigation of infections using the complement fixation test enabled the detection of circulating *Trypanosoma* antibodies even during the early stages of infection. However, the test invariably failed to distinguish between infections with either of the *Trypanosoma* species. Furthermore, incorporation of *T. vivax* and *T. b. brucei* positive control serum samples and antigens, showed that there were cross-reaction with the two *Nannomonas* isolates. The cross-reaction with the *Nannomonas* isolates was significantly lower with *T. vivax* antisera. This study also showed the rate of disappearance of the circulating trypanosomal antibodies in the different groups of infected animals after treatment with appropriate chemotherapeutic compounds.

1. INTRODUCTION

Trypanosomes are flagellated protozoan parasites classified in the genus Trypanosoma (Hoare, 1972). They have been subdivided into various subgenera on the basis of their morphology and mode of development in the *Glossina*.

The subgenera are *Trypanozoon*, *Duttonella*, *Nannomonas* and *Pycnomonas* (Hoare, 1936). The subgenus *Trypanozoon* is composed of the human pathogens *Trypanosoma* (*Trypanozoon*) *rhodesiense* and *T*(T)*gambiense*, and the animal trypanosomes *T*. (T) *equiperdum*. The subgenus *Duttonella* is comprised of *T*. *vivax* and *T*. *uniforme*, both animal parasites. The *Pycnomonas* group contains the porcine parasite *T*. *suis* only.

The subgenus *Nannomonas* is comprised of *T*. *congolense* and *T*. *simiae*. These are typically devoid of a free flagellum and therefore their movement in fresh wet blood film preparations is sluggish without any evidence of progressive forward motion. They have a medium kinetoplast and their development in the vector occurs only inside the midgut and proboscis. The morphological similarity between the two trypanosomes is so close that they are not only being classified in the same subgenus (Hoare, 1936) but are morphologically almost undistinguishable.

The pathogenicity of *T. simiae* in different animal species has been reported by several workers (Bruce *et al.* 1913; Wilson, 1949; Roberts, 1970; Janssen and Wijers, 1974).

Serological diagnosis of trypanosomiasis has been utilised by several workers. Killick-Kendrick (1968) stated that the diagnosis of bovine trypanosomiasis by conventional parasitological technique is often difficult since trypanosomes cannot always be detected in peripheral blood and some species do not readily infect small laboratory rodents. *T. simiae* does not infect small laboratory rodents and in rabbits it has been reported to cause only a transient parasitaemia (Pelligrini, 1948; Watson and Williamson, 1948).

The above facts make the diagnosis of *T. simiae* during low parasitaemia very difficult. On the other hand some pig blood samples known to contain *T. congolense* from direct microscopic examination failed to infect rats (Killick-Kendrick and Godfrey, 1968). This complicates the issue further as regards the differentiation between the two *Trypanosoma* species.

Luckins (1977) stated that although the demonstration of parasites in the infected host is the

only unequivocal means of diagnosing trypanosomiasis, it is sometimes necessary to use immunodiagnostic procedures, even though the possibility of mixed infections with two or even three species of trypanosomes may produce a varied and complex host response.

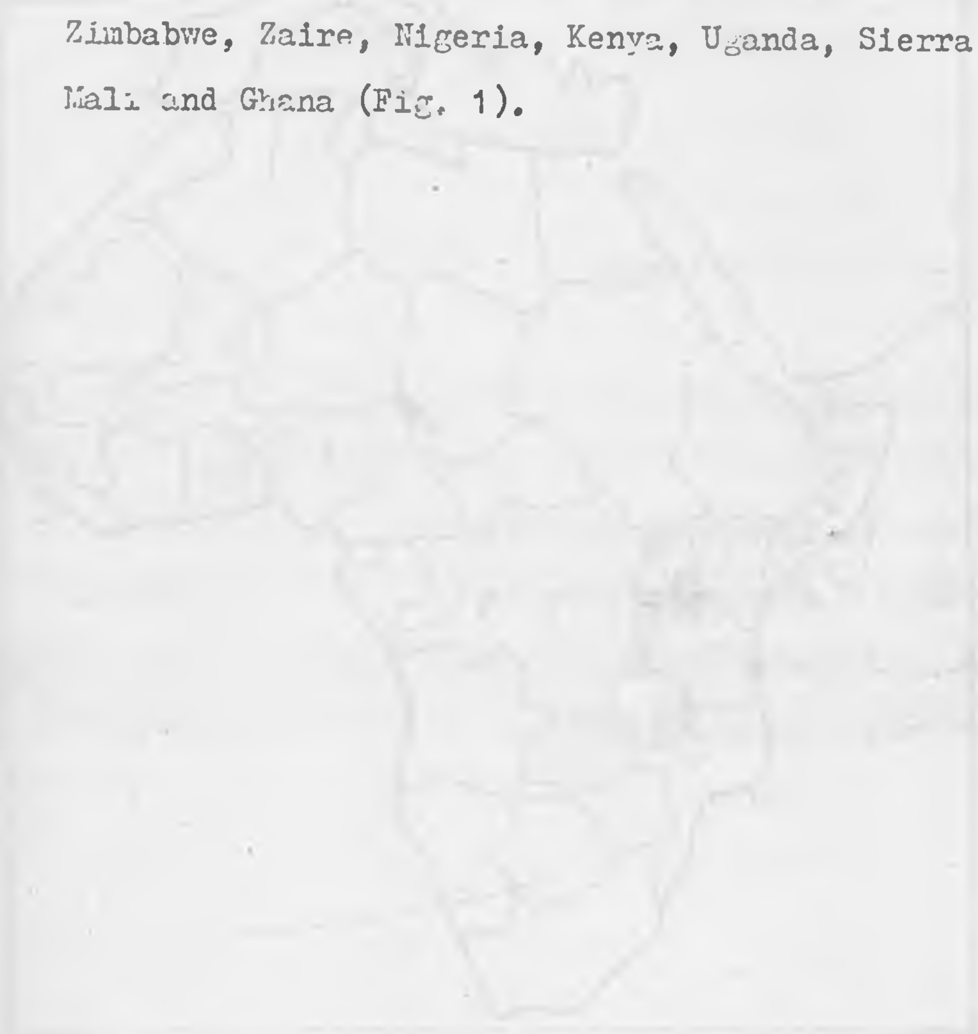
T. simiae was first described by Bruce et al. (1912). It has since been known that it causes extremely virulent and rapidly fatal trypanosomiasis in domestic pigs. In tsetse infested areas this can result in severe losses. The parasite has been classified as follows:

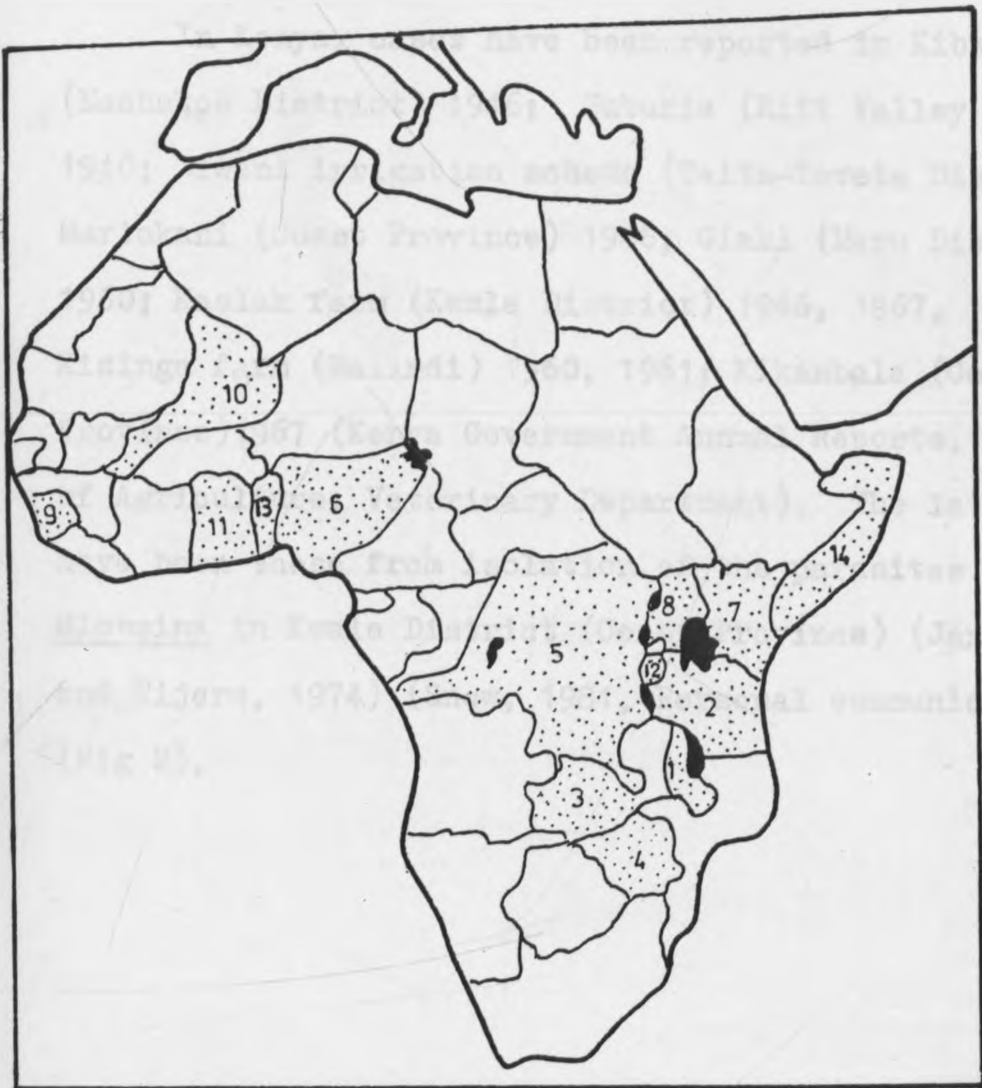
Phylum	-	Protozoa
Subphylum	-	Sarcomastigophora
Super Class	-	Mastigophora
Class	-	Zoomastigophorea
Order	-	Kinetoplastida
Suborder	-	Trypanosomatina
Family	-	Trypanosomatidae
Genus	-	Trypanosoma
Subgenus	-	Nannomonas
Section	-	Salivaria

(from Stephen, 1966)

Cases of pig trypanosomiasis caused by T. simiae have been reported in several tropical

African countries including Malawi, Tanzania, Zambia, Zimbabwe, Zaire, Nigeria, Kenya, Uganda, Sierra Leone, Mali and Ghana (Fig. 1).

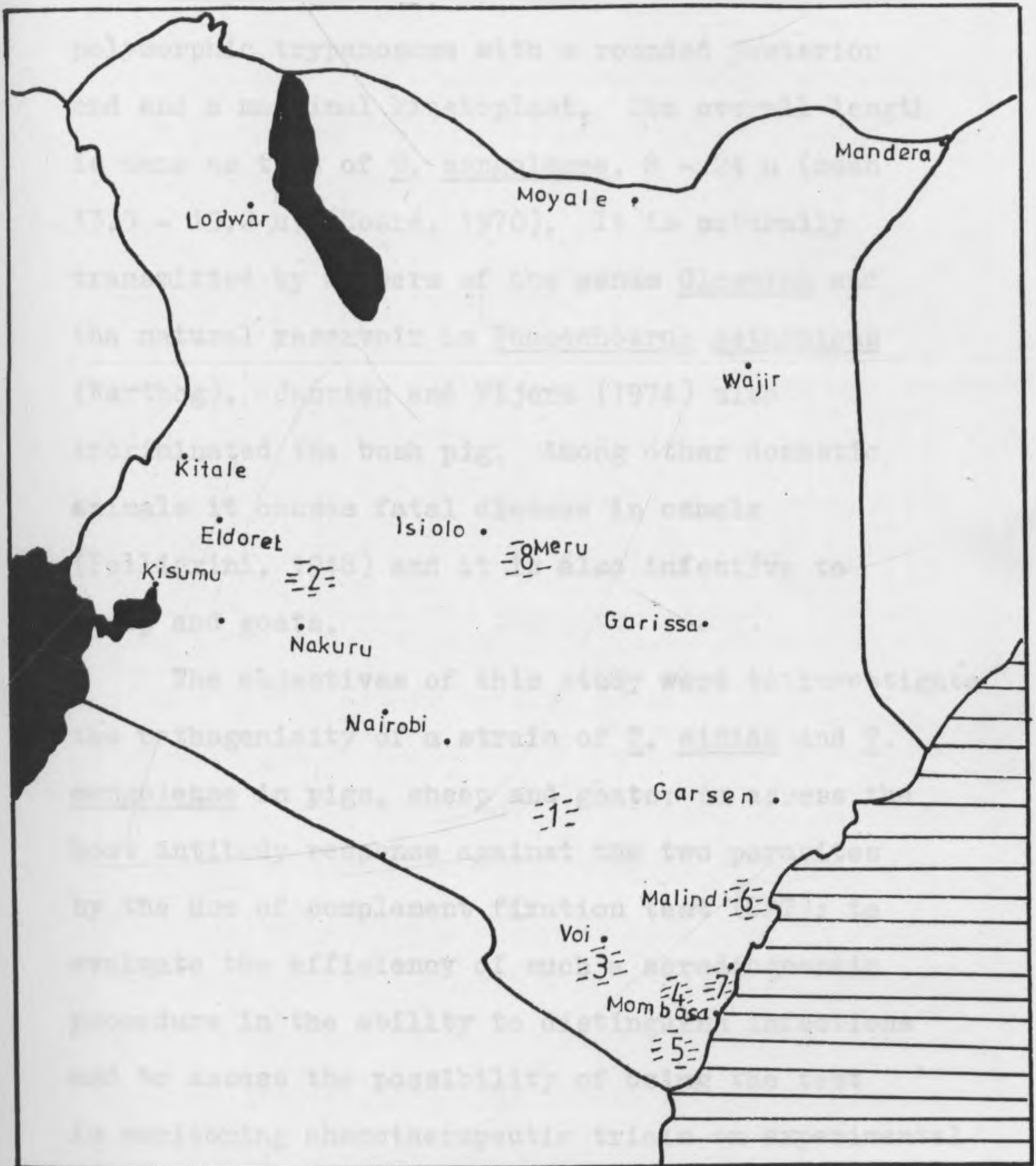




1. Malawi.
2. Tanzania (Mainland).
3. Zambia.
4. Zimbabwe.
5. Zaire.
6. Nigeria.
7. Kenya.
8. Uganda.
9. Sierra Leone.
10. Mali.
11. Ghana.
12. Burundi.
13. Dahomey.
14. Somalia.

Fig 1. Map showing the countries where _T. simiae_ has been reported in Africa. (From Stephen, 1966).

In Kenya, cases have been reported in Kibwezi (Machakos District) 1946; Subukia (Rift Valley Province) 1940; Ziwani irrigation scheme (Taita-Taveta District), Mariakani (Coast Province) 1946; Giaki (Meru District) 1960; Kaslak farm (Kwale District) 1966, 1967, 1968; Kizinga farm (Malindi) 1960, 1961; Kikambala (Coast Province)1967 (Kenya Government Annual Reports, Ministry of Agriculture, Veterinary Department). The latest cases have been those from isolation of the parasites from Glossina in Kwale District (Coast Province) (Janssen and Wijers, 1974) (Snow, 1981, Personal communication) (Fig 2).



1. Kibwezi (Machakos district).
2. Subukia (Rift valley province).
3. Ziwani irrigation scheme (Taita-Taveta district).
4. Mariakani (Coast province).
5. Kaslak farm (Kwale district).
6. Kizinga farm (Kilifi district).
7. Kikambala (Coast province).
8. Giaki (Meru district).

Fig 2 : Map showing the areas where *T. simiae* has been reported in Kenya.

Morphologically, T. simiae is a medium sized polymorphic trypanosome with a rounded posterior end and a marginal kinetoplast. Its overall length is same as that of T. congolense, 8 - 24 µ (mean 13.5 - 19.2 µ) (Hoare. 1970). It is naturally transmitted by members of the genus Glossina and the natural reservoir is Phacochoerus aethopicus (Warthog). Janssen and Wijers (1974) also incriminated the bush pig. Among other domestic animals it causes fatal disease in camels (Pelligrini, 1948) and it is also infective to sheep and goats.

The objectives of this study were to investigate the pathogenicity of a strain of T. simiae and T. congolense in pigs, sheep and goats; to assess the host antibody response against the two parasites by the use of complement fixation test (CFT); to evaluate the efficiency of such a serodiagnostic procedure in the ability to distinguish infections and to assess the possibility of using the test in monitoring chemotherapeutic trials on experimental T. simiae infections in domestic animals.

2. LITERATURE REVIEW

2.1. T. SIMIAE INFECTION IN PIGS, SHEEP AND GOATS.

T. simiae infection in pigs produce as very rapidly fatal disease (Bruce et al., 1912; Wilson, 1946). The incubation period varies from 3 - 10 days under experimental conditions (Stephen, 1962; Roberts, 1970). The clinical manifestation of the disease in pigs can result in death within one or two days after the onset of pyrexia. It was claimed that the characteristic signs do not develop in such cases and the only symptoms encountered are those of an acute septicaemic disease. In cases where the disease lasted longer several clinical signs have been observed by workers. These include pyrexia of up to 41,1°C, dullness, inappetence and sometimes anorexia, decreased activity varying from slight stiffness and disinclination to rise, an unsteady gait, respiratory distress and coldness of extremeties. Other workers have also reported hyperaemia of the skin, salivation, frothing at the mouth, diarrhoea, oedema of the prepuce and cough (Stephen, 1966).

Cases have been reported where pigs lasted with T. simiae infection survived for up to six days after the initial pyrexia (Unsworth, 1952; Roberts, 1970).

Hudson (1944) stated that the swarming of *T. simiae* in the blood of infected pigs was considered unique. Unsworth (1952) observed heavy *T. simiae* infections in pigs during an outbreak in Nigeria. He observed inappetence, pyrexia of varying degree, weakness of the hind limbs, pallor of the skin and mucous membranes and the tendency of pigs to be more easily caught as major clinical signs associated with infection. He also incriminated biting flies as having been responsible for the secondary spread of the infection during that outbreak. Dijk *et al.* (1973) found that respiratory distress was common both in acute and chronic cases of *T. simiae* infection in pigs. They also observed frothing in the mouth and nostrils, nervous symptoms in one form of paddling movements when lying on the ground and anaemia.

Following his chemotherapeutic studies, Stephen (1962) stated that any attempt at raising pigs where *T. simiae* infected tsetse flies occur would be attended by serious losses if it relies solely on treatment of infected animals as they occur. This comment was made because of the

acuteness and rapidly fatal the course of the infection assumes in domestic pigs.

Some T. simiae isolates which cause chronic infections in pigs have been reported. (Mettam, 1940; Janssen and Wijers, 1972; Opiyo, 1981). Cases of self-cure of such infections in pigs, have also been reported (Opiyo, 1981. Unpublished observations).

Sheep are quite readily infected with T. simiae and show a similar resistance to that shown by goats (Stephen, 1966). The infections in sheep and goats have been studied to some extent. The sheep are more readily infected with tsetse resulting in a more chronic disease than in goats (Bruce et al., 1913; Pelligrin, 1948; Wilson, 1949). Stephen (1962) indicated that the course of the infection in sheep was similar to goats. Wilson (1948) observed that syringe infection in sheep was non-pathogenic although trypanosomes were detected in blood in considerable numbers for irregular periods from second to twelfth week post-infection. He found that a T. simiae isolate (T. simiae Mbarara (Uganda), was very pathogenic for pigs. The same isolate when maintained in sheep proved non-pathogenic but sub-inoculation from the sheep to pigs were highly pathogenic for

the latter. However, he cited a case where a sheep died from T. *simiae* infection 1½ months after inoculation with 10 ml of highly positive pig blood. The worker mentioned that T. *congolense* was not very pathogenic but prevented pigs from normal and rapid weight gain.

2.2. T. CONGOLENSE INFECTIONS IN PIGS, SHEEP AND GOATS.

T. *congolense* infections in pigs have been observed and studied by several workers (Macfie and Gallagher, 1914; Parkin, 1935; Peel and Chardome, 1954; Killick-Kendrick and Godfrey, 1963). Most of these workers agree on the low pathogenicity of the trypanosome in pigs. However, Pelligrini (1937) failed to infect pigs with T. *congolense* by sub-inoculation from an infected bovine. Mettam (1938) referred to pigs as being very resistant to T. *congolense* infections. Stephen and Gray (1960) observed that T. *congolense* infections caused only the mildest clinical effects in a pig infected by wild flies.

Parkin (1935) reported that T. *congolense* infection in pigs caused only sporadic and scanty parasitaemia. Stephen (1947) observed that

T. congolense was harboured by many pigs in Zaire.

Stephen (1962) reported pigs showing relapses with *T. congolense* after having been treated for *T. simiae* infections with berenil (R) and prothidium (R). The *T. congolense* in the relapse was resistant for 7.0 mg/kg berenil, double the normal dose and 2.5 mg/kg prothidium.

T. congolense infections in sheep and goats are known not to produce the striking symptoms observed in bovine. The disease assumes an initial acute form which subsequently becomes chronic, (Parkin, 1935; Henning, 1956). Pale mucosae, accelerated pulse and respiration, some loss of condition, persistent striking temperature variations were some of the clinical manifestations that were reported (Parkin, 1935).

2.3. THE USE OF COMPLEMENT FIXATION TEST (CFT) IN TRYPANOSOMIASIS.

The occurrence of trypanocidal and agglutinating antibodies in sera of animals infected with trypanosomes is a well known phenomenon (Kligler, 1931; Russell, 1936; Desowitz and Watson, 1953). Fife (1972) grouped serodiagnostic tests used in the detection of blood parasites into two. Those that employ the intact organisms as antigen

and those that employ soluble antigen. The CFT is an example of the latter group.

Serological techniques are often used to detect circulating antibodies in the serum of the host animals. The methods are used for the epidemiological population studies and in laboratory oriented research (Grunnel, 1981). The CFT has been used in the diagnosis and studies of various viral, bacterial, protozoan and other parasitic infections in domestic animals. It is also useful in the identification and quantification of antibody content and as such is used mainly as a research tool(merchant and Packer, 1971). The test has been identified as one of the serological diagnostic procedures that complement direct parasitological diagnosis of infections (Targett, 1978).

In vitro a substance known as complement, usually added in form of fresh guinea pig serum is used up or 'fixed' during antigen - antibody reactions. A secondary indicator system is one which determines the degree of 'fixation' of the added complement. Lysis of erythrocytes by complement *in vitro* is a well known phenomenon. Thus addition of sheep erythrocytes which have

been sensitized by rabbit anti-sheep red blood cells (haemolysin) comprises the secondary indicator system (Waghela, 1975).

The application of the test to the diagnosis of sleeping sickness has been reported (Weitz, 1970). Generally, the test has been found to be highly sensitive (Weitz, 1970; Fife, 1972) and ultrasonically disintegrated trypanosome antigens have been used in the test (Pautrizel *et al*., 1959; Staak and Lohding, 1979).

Staak and Kelly (1979) utilised the test in the assessment of therapy of bovine trypanosomiasis in parts of Kenya. Staak and Lohding (1979) applied it in assessing the antibody responses in cattle infected with *T. vivax*, *T. congolense* and *T.* (*T*) *brucei*.

Neujean and Evan (1958) using *T. equiperdum* antigen found the CF test a reliable diagnostic aid in the diagnosis of *T. gambiense* infections in human patients, especially in the early cases of infection. Robinson (1926) observed that the close affinity between all species of subgenus *Trypanozoon*. This provided further support in possession of common antigens by *T.* (*T*) *brucei*, *T.* (*T*) *evansi* and *T.* (*T*) *equiperdum*.

The test was found to be a valuable tool for detection of cryptic infections and evaluation of treatments in *T. vivax* infected cattle (Staak, 1976). However, such a serodiagnostic study has never been reported for *T. simiae* in any animal species.

Durations between the appearance of parasites in peripheral blood and the first positive CF reaction in infected individuals have been reported for *T. (T) brucei*, *T. congolense* and *T. vivax* infections in bovines. On the other hand, the periods and pattern of disappearance of CF antibody titres in treated animals has also been studied for *T. (T) brucei*, *T. congolense* and *T. vivax* infections in bovine (Staak and Lohding 1979). In this study similar aspects have been investigated in pigs and small ruminants infected with *T. simiae*.

3. MATERIALS AND METHODS

3.1. ANIMALS: Pigs, sheep, goats, guinea-pigs, rats and mice were utilised in this study.

(i) Pigs. Eighteen pigs belonging to the Large White breed were used. All had been bred on the station (Veterinary Research Laboratories, Kabete). They weighed approximately 21 kg at the beginning of the experiment.

(ii) Goats. Seven cross-bred goats obtained from the Veterinary Farm, Machakos were used.

(iii) Sheep. Six sheep belonging to the Dorper breed were utilised.

The pigs, sheep and goats were all kept in concrete floor pens in fly-proof stables and were dewormed regularly. The pigs were fed on commercial feed which was distributed in adequate quantities twice daily. The sheep and goats were fed on hay with daily supplementation with calf pellets. Drinking water was available to the animals *ad libitum*.

(iv) Rats and mice. The rats and mice were maintained in metal cages in a fly-proof room and were fed on commercial feed.

(v) Guinea pigs. Thirty (30) male guinea pigs were obtained from the animal section of Veterinary Research Laboratories, Kabete (V.R.L.). They were utilized as a source of complement.

3.2. TRYPANOSOMA SPECIES AND STRAINS.

(i) Trypanosoma simiae "Ketri" (T.S.). This stock was obtained from Kenya Trypanosomiasis Research Institute, Muguga. The stock was isolated from a pig at the Kenya coast near the Kenya - Tanzania border.

(ii) Trypanosoma congolense "Transmara" (T.C.T.). This was obtained from trypanosomiasis section (V.R.L.). The stock was originally isolated from bovine in the Transmara area of Kenya.

(iii) Trypanosoma congolense "Ukunda" (T.C.U.). T.C.U. was available from the chemotherapy of trypanosomiasis research project, (Chemotryp), and was isolated from a bovine at Ukunda, Coast Province, Kenya.

(iv) Trypanosoma congolense "Matuga" (T.C.M.). Also isolated by Chemotryp project from a bovine at Matuga, Coast Province, Kenya.

TABLE 1. SUMMARY OF THE GROUPING AND USE OF THE EXPERIMENTAL ANIMALS.

SPECIES	INFECTION				
TRYP. / ANIMAL	T. simiae "Ketri"	T. congolense "Transmara"	"Matuga"	"Ukunda"	Non-infected controls
PIGS	No 102 No 109 No 110	No 106 No 107	No 144	No 122	No 111 No 112
GOATS	No 6 No 549	No D No 521			No C No 27
SHEEP	No 129 No 356	No 146 No 255			No 367 No 378
	OTHERS				
PIGS	No 125. T. simiae subinoculation from sheep Nos 129 and 356 before treatment. No 131. T. simiae subinoculation from sheep Nos 129 and 356 after treatment. Other pigs were involved in inoculum preparation and isolation of T. simiae antigen. No 130. T. simiae sub-inoculation from goats 29 & 449				
GOATS	Nos 29 and 449. T. simiae infection after longer passage in pigs.				
RATS	Isolation of T. congolense for antigen preparation; Isolation of T. brucei for antigen preparation; Preparation of T. congolense "transmara" inoculum.				
MICE	Isolation of T. vivax for antigen preparation; Subinoculations with T. congolense from pig Nos 106, 107, 122 and 144.				
GUINEA PIGS	Isolation of complement.				

3.3. INOCULUM PREPARATION.

The *T. simiae* inoculum for all the test animals was obtained from a pig with high parasitaemia. The pig had earlier been infected with stabilate of *T. simiae* (Ketri). The stabilate had been stored in straws in liquid nitrogen at the Central Artificial Insemination Station (C.A.I.S.).

A 1 x 10^5 trypanosomes inoculum dose per animal was prepared by determination of parasitaemia by aid of Neubear haemocytometer and subsequent dilution with phosphate buffered saline (PBS).

The *T. congolense* "Transmara" inoculum was obtained from highly parasitaemic mouse blood. The preparation and standardization of the inoculum was as for *T. simiae* above.

3.4. INOCULATION METHOD.

The inoculation method in pigs, sheep and goats was by intravenous syringe injection. The ear vein was used in pigs. In sheep and goats the jugular vein was used. Rats and mice were inoculated intraperitoneally.

3.5. PARASITOLOGICAL METHODS.

The status of infection was determined by the following methods.

- Daily wet blood film examination (Killick-Kendrick, 1968).
- Occasional application of the Woo technique (Woo, 1969).
- Subinoculation into pigs and mice.

(i) Wet blood film examination.

This method was employed for the direct examination of infectivity status in this study. The blood was obtained from the tail in pigs. In sheep and goats, ear blood was used. The standard wet blood film preparation and examination was done as specified by Baker (1970). For examination of the blood films a Leitz Laborlux 12 phase contrast microscope with phaco 40/0.65 objective and X 10 eyepiece (X 400 magnification) was used. The wet blood film results were graded as -ve, +ve, ++ve, +++ve and ++++ve.

-ve: This represented the failure to detect any trypanosomes in more than 100 microscopic fields examined at the above magnification.

+ve: One to five trypanosomes detected per field examined at the X 400 magnification.

++ve: Six to ten trypanosomes detected per microscopic field at the above magnification.

+++ve: More than ten trypanosomes detected per field.

++++ve: Numerous trypanosomes observed per microscopic field examined.

(ii) Woo technique.

The Woo capillary (micro-haematocrit) centrifugation method (Woo, 1969) was occasionally employed on animals which had persistently exhibited negative results on wet film examination.

The blood samples were collected in heparinised microhaematocrit tubes from the tail in pigs and ear in sheep and goats. The procedure was carried out as described by Woo, 1969. This involved collection of blood in the haematocrit tubes, the sealing of one end of the tubes with plasticene and then centrifugation. The tubes were examined on a special glass slide prepared as follows: 2 rectangular pieces of glass 25 x 10 x 1.2 mm

were longitudinaly stuck on a glass slide at a distance 1.5 mm apart. This ensured a continuous longitudinal groove of 1.5 mm in which a microhaematocrit tube fitted. The tube to be examined was placed in the groove. It was examined for the presence of motile trypanosomes by checking the junction between the buffy coat and the plasma microscopically using a Leitz Laborlux 12 Phaco microscope. The 25/0.50 objective and X 10 eye-piece were used in this examination.

(iii) Sub-inoculations into piglets and mice.

The inoculation of citrated whole blood from *T. simiae* and *T. congolense* infected animals into pigs or mice respectively was done as an additional method. This was done to determine presence of infections in animals with very low parasitaemia. Citrated whole blood from the pigs, sheep or goats was injected into pigs or mice. Five and 0.5 ml amounts were used in subinoculations into pigs and mice respectively. The infection in the recipients was subsequently monitored by daily wet film examination.

3.6. CLINICAL METHODS.

The following procedures were used for the assessment of the clinical status of the pigs, sheep and goats in the experiment.

- Daily physical examination.
- Daily rectal temperature reading.
- Packed cell volume (PCV) determination.
- Body weight variations.

(i) Daily physical examination.

This was done mainly by visual examination. It also included the checking of environmental conditions around the animals. The health, feeding, watering and sanitary conditions were checked. Animals which appeared sick were given further attention.

(ii) Rectal temperatures.

Daily rectal temperatures of the pigs, sheep and goats were measured.

(iii) Packed cell volume.

Hornby, 1952 stated that the estimation of degree of anaemia is probably the most reliable indication of the progress of disease in trypanosome infected cattle.

The PCV was determined twice weekly in experimental pigs, sheep and goats. Heparinised microhaematocrit tubes were used. The samples were collected in a manner similar to that described for the wet film technique. The processing and reading of the PCV in percentage

percentage were done by using the standard method with a microhaematocrit reader (Hawksley, England).

(iv) Weight determination.

Weekly body weight measurements of every experimental animal was done by weighing the animals in a cage-like portable weighing machine (Animatics, Kenya).

3.7. SEROLOGY.

(i) Serum samples.

Blood samples for serum preparation were obtained by puncture of the jugular vein in case of sheep and goats, and the anterior venacava in case of pigs (Hoerlein *et al*., 1971).

The samples were collected in clean test tubes. The blood was left to stand on the bench for serum to separate. It was then centrifuged and serum was recovered by sunction using Pasteur pipettes. Sheep and goat serum samples were kept in clean bijoux bottles. The pig serum samples were kept in clean Eppendorf tubes.

All the samples were stored in a deep freeze at -20°C until required for serological testing.

(ii) Antigens.

T. simiae, *T. congolense* and *T. vivax* for antigen preparation were isolated from highly parasitaemic pig, rat and mouse blood respectively. The isolation of pure trypanosomes involved initial concentration by the density gradient centrifugation using Percoll^TM^ (Pharmacia Fine Chemicals, Uppsala, Sweden). Percoll^TM^ is a medium based on colloidal silica coated with polyvinyl pyrrolidone (PVP). Thereafter the trypanosome rich resuspended portion was passed through the DEAE-52 cellulose columns.

T. simiae, *T. congolense*, *T. vivax* and *T. brucei* soluble antigens were used to test each serum sample. They were prepared as soluble extracts of pure trypanosomes.

Separation of *T. brucei* for antigen preparation was achieved by passing highly parasitaemic rat blood through a DEAE (Diethylaminoethyl) cellulose (DEAE-52 Whatman) chromatography column (Godfrey and Lanham, 1970).

3.8. DETAILED PROCEDURE FOR T. BRUCEI BRUCEI ANTIGEN PREPARATION.

The pure trypanosomes were obtained by separation from blood of 30 highly parasitaemic

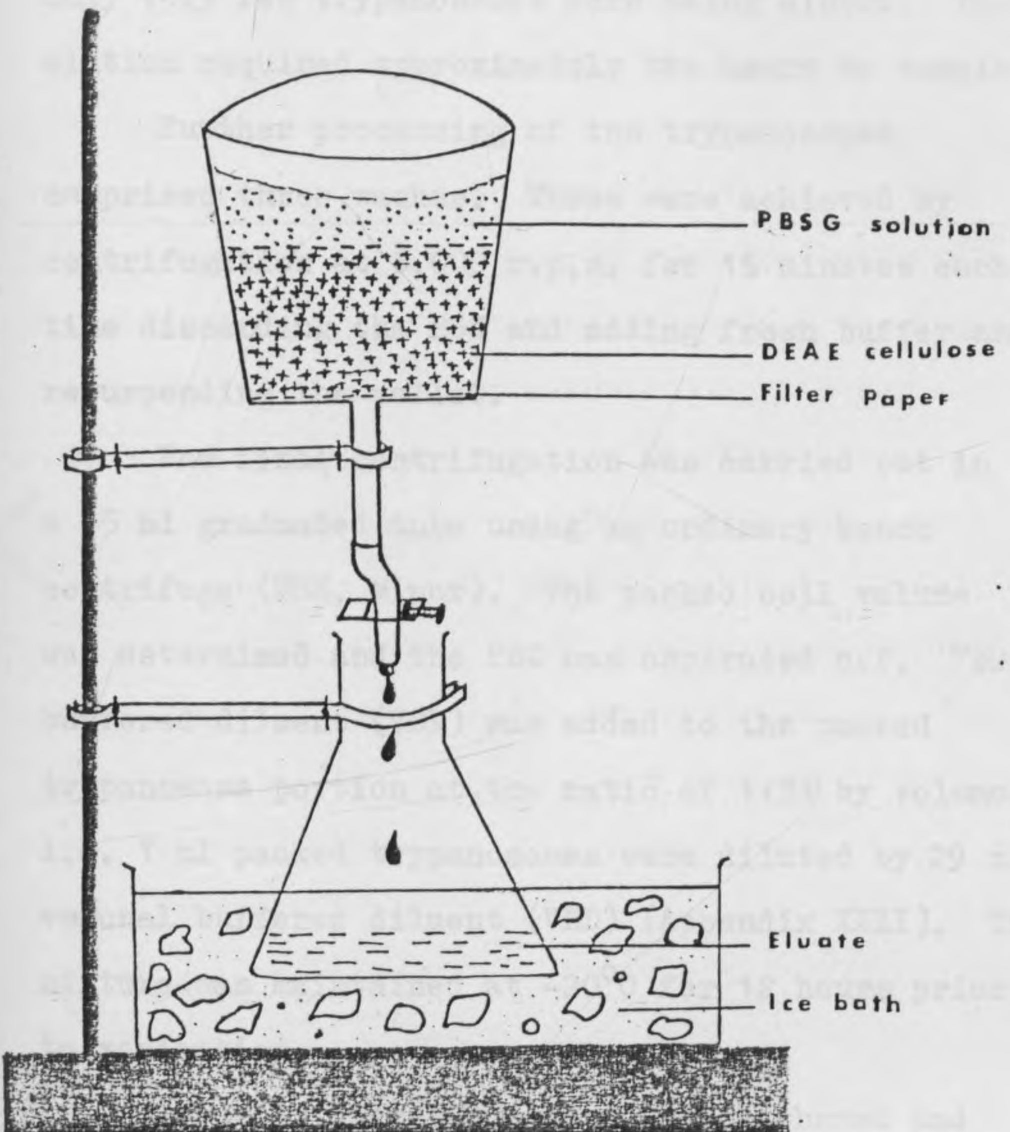
rats, exhibiting a ++++ve parasitaemia. The rats were anaesthetised in a jar containing diethy ether prior to bleeding. They were bled from the heart after exposing it by cutting the rib cage and the sternum area. The blood was collected in a jar containing 3.8% sodium citrate as anticoagulant. The blood was diluted with phosphate buffered saline glucose (PSG) buffer pH 8.0 in the ratio 1 to 2 ratio.

The PSG of 0.217 ionic strength (Lanham and Godfrey, 1970) used in this isolation was prepared from phosphate buffered saline stock solution (Appendix XVI). The PSG was prepared on the day of isolation.

The basis of this technique is the isolation of trypanosomes from infected blood by adsorbing blood components onto DEAE-cellulose and eluting the trypanosomes (Lanham, 1968).

Two hundred grams of Whatman DEAE-52 pre-swollen cellulose was used in isolating trypanosomes from 300 ml of the 1:2 blood: PSG mixture. The cellulose preparation and column packing was as specified by Godfrey and Lanham (1970). A Buchner glass funnel, 11 centimeters in diameter were used in setting up the columns.

Fig 3. Diagram of anion - exchange column set in Buchner flask during the isolation of T. b. brucei.


PBSG solution
DEAE cellulose
Filter Paper
Eluate
Ice bath

Wet films of the eluate were regularly checked for presence of trypanosomes. Collection of the required elute commenced on the first detection of trypanosomes. Wet film examination continued until only very few trypanosomes were being eluted. The elution required approximately two hours to complete.

Further processing of the trypanosomes comprised three washes. These were achieved by centrifugation at 6,000 r.p.m. for 15 minutes each time discarding the PSG and adding fresh buffer and resuspending the pellet.

The final centrifugation was carried out in a 15 ml graduated tube using an ordinary bench centrifuge (MSE, Minor). The packed cell volume was determined and the PSG was aspirated off. Veronal buffered diluent (VBD) was added to the packed trypanosome portion at the ratio of 1:30 by volume i.e. 1 ml packed trypanosomes were diluted by 29 ml veronal buffered diluent (VBD) (Appendix XXII). The mixture was maintained at -20°C for 12 hours prior to sonication.

After 12 hours, the mixture was thawed and sonicated using an ultrasonicator (MSE 100 Watt ultrasonic disintegrator).

The sonicated mixture was centrifuged at 6,000 r.p.m. for one hour. The supernatant was collected and utilised as the actual antigen

in the complement fixation test. The antigen was titrated by checkerboard before it was dispensed into ampoules and stored at -70°C.

3.9. DETAILED PROCEDURE FOR T. SIMIAE, T. VIVAX AND T. CONGOLENSE ANTIGEN PREPARATION.

The separation of the *T. simiae*, *T. vivax* trypanosomes was achieved from pig, mouse and rat blood respectively. The only difference between isolation of these antigens and that of *T. b. brucei* was in the initial concentration of trypanosomes by density gradient centrifugation of whole blood followed by the use of small DEAE-cellulose columns.

Percoll™ (Pharmacia, Fine Chemicals, Uppsala, Sweden) was utilised for the density gradient centrifugation. In this procedure highly parasitaemic blood was collected in 3.8% sodium citrate from the appropriate animal species. The blood was centrifuged on a Percoll™ gradient to concentrate the trypanosomes in one layer on the basis of relative density, (Pertoft et al., 1968; Wolff, 1975).

The final Percoll centrifugation base was prepared by mixing two solutions which were refered to as the 'heavy' and 'light' solution.

(i) <u>'Heavy' solution.</u>

This was prepared by addition of 1 part by volume of Hank's buffered saline solution (BSS) without sodium bicarbonate (Gibco Bio-cult, Glasgow, Scotland) to 9 parts by volume of Percoll™. The two were thoroughly mixed and kept at +4°C. prior to use.

(ii) <u>'Light' solution.</u>

This was prepared by addition of 1 part by volume of Hank's BSS to 9 parts by volume of distilled water. The solution was thoroughly mixed by several inversions and kept at +4°C.

Due to the risk of contamination by bacteria, it was necessary to prepare both solutions on the day of isolation.

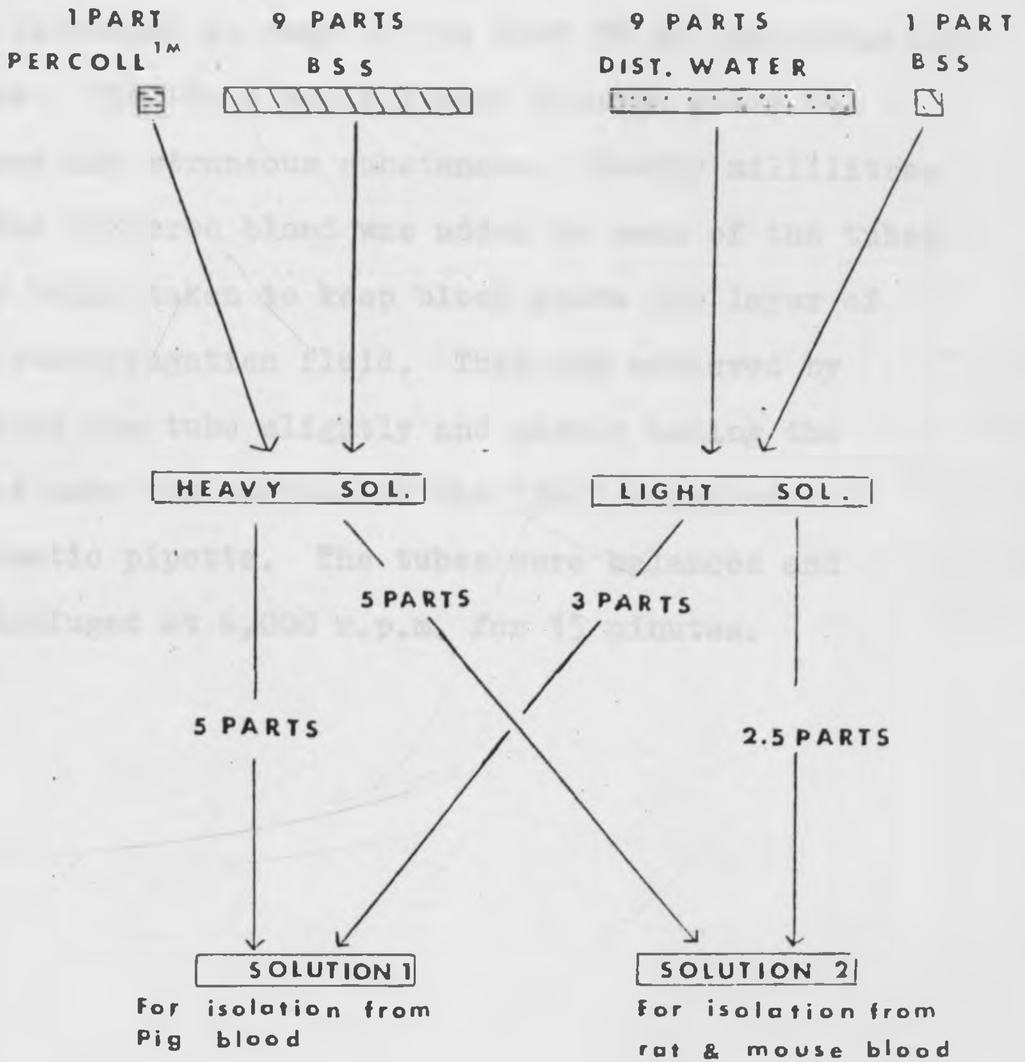
(iii) <u>The final centrifugation solution.</u>

The final centrifugation base was prepared by mixing the 'heavy' and 'light' solutions in different ratios depending on the animal species from which the blood for trypanosome isolation was collected. The ratios were determined by several trials. For concentration of trypanosomes in pig blood five parts by volume of the heavy solution were mixed with three parts of the light solution. The ideal mixture for concentration of trypanosomes

in rat and mice blood was prepared by mixing five parts by volume of the heavy and 2.5 parts of the light solutions.

The final centrifugation mixture was thoroughly mixed by inversion of the tube and maintained at +4°C prior to use.

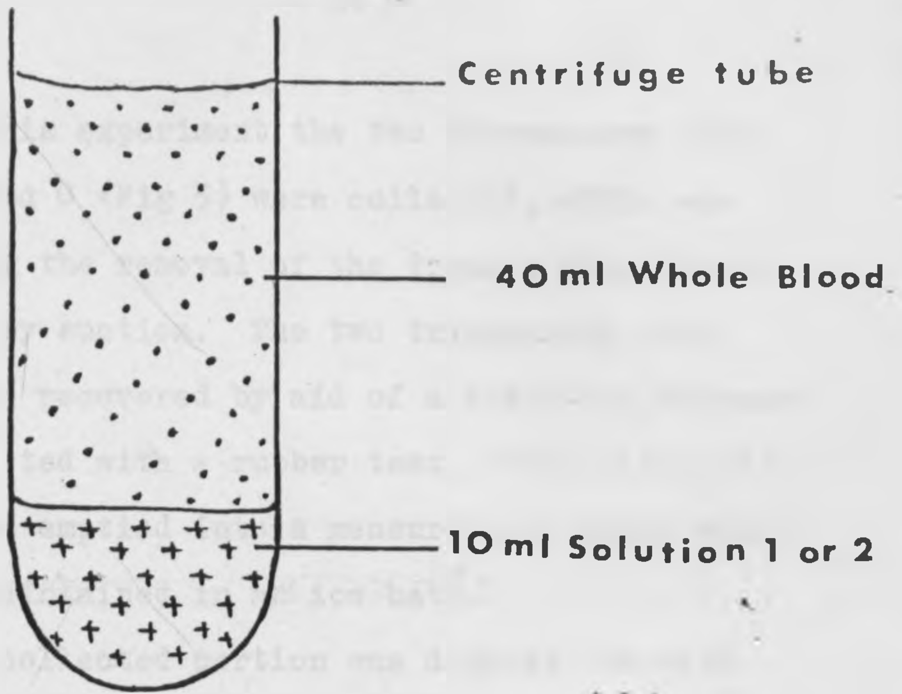
Fig 4. Chart showing the preparation of the density gradient centrifugation solutions.


1 PART
PERCOLL TM
9 PARTS
BSS
9 PARTS
DIST. WATER
1 PART
BSS
HEAVY SOL.
LIGHT SOL.
5 PARTS
3 PARTS
5 PARTS
2.5 PARTS
SOLUTION 1
For isolation from Pig blood
SOLUTION 2
For isolation from rat & mouse blood

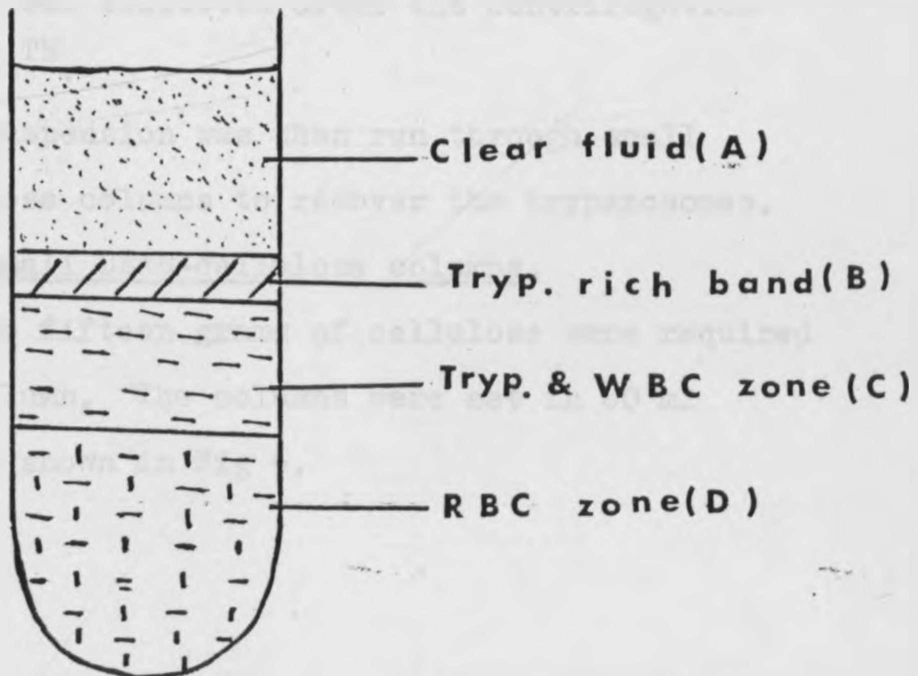
(iv) <u>The centrifugation procedures.</u>

10 ml of the final centrifugation solution was dispensed in each of the four 50 ml centrifugation tubes. The blood was filtered through gauze to remove any straneous substances. Fourty millilitres of the filtered blood was added to each of the tubes, care being taken to keep blood above the layer of the centrifugation fluid. This was achieved by tilting the tube slightly and slowly adding the blood onto the surface of the fluid using an automatic pipette. The tubes were balanced and centrifuged at 6,000 r.p.m. for 15 minutes.

Fig 5. Diagram showing centrifugation tube contents before (I) and after (II) the density gradient centrifugation procedure.



I. BEFORE CENTRIFUGATION



II. AFTER CENTRIFUGATION

In this experiment the two trypanosome rich layers B and C (Fig 5) were collected. This was achieved by the removal of the topmost clear fluid layer (A) by suction. The two trypanosome rich layers were recovered by aid of a wider-tip Pasteur pipette fitted with a rubber teat. The collected portion was emptied into a measuring cylinder which had been maintained in an ice bath.

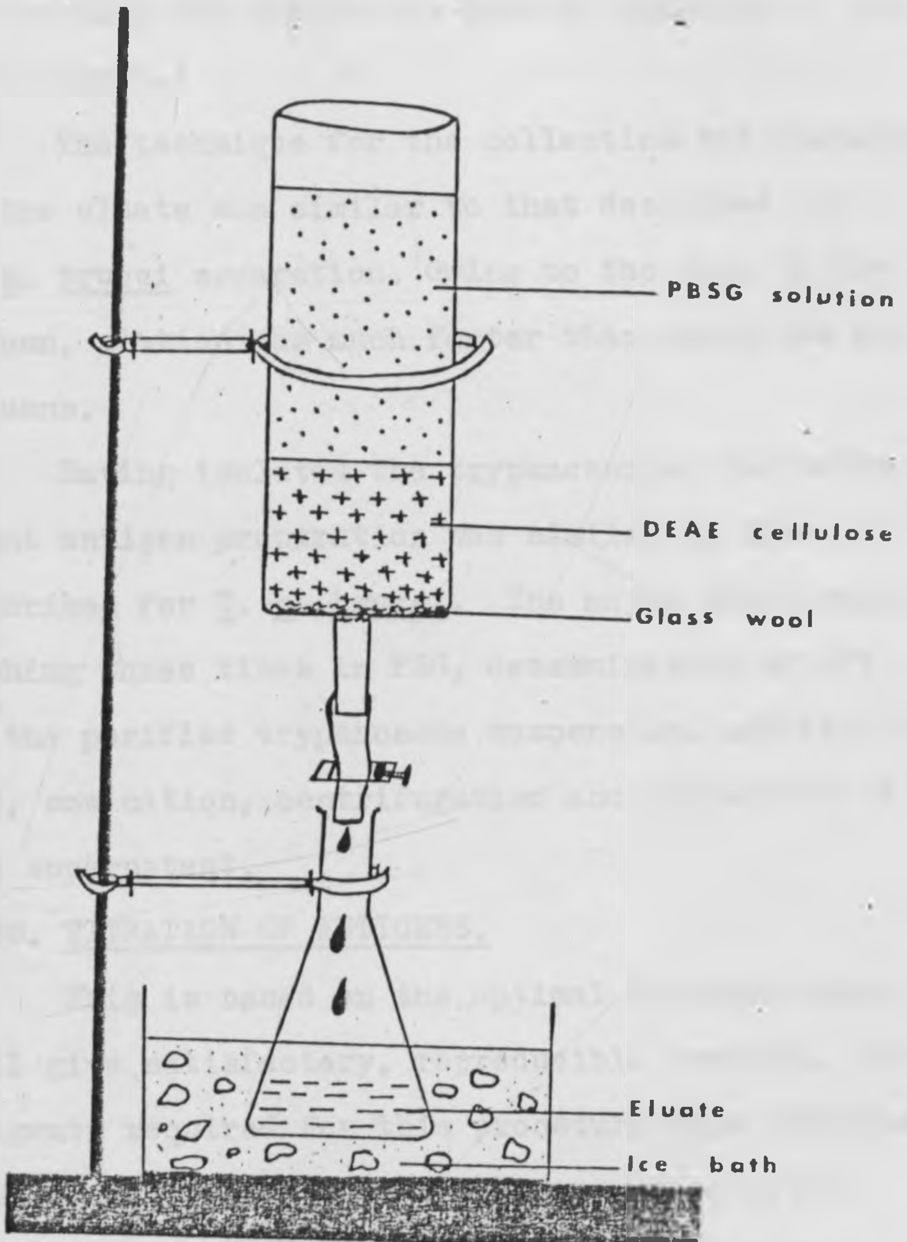
The collected portion was diluted 1:1 with the appropriate PSG. The resultant suspension was dispensed into the centrifugation tubes and centrifuged for a further 15 minutes at 6,000 r.p.m. The supernatant was sucked off and the remaining portion was resuspended in PSG to the original volume that was collected after the centrifugation with Percoll™.

The suspension was then run through small DEAE-cellulose columns to recover the trypanosomes.

(iv) <u>The small DEAE-cellulose columns</u>.

Ten to fifteen grams of cellulose were required for each column. The columns were set in 60 ml syringes as shown in Fig 6.

Fig 6. Diagram of the small DEAE-cellulose anion exchange column set in a 60 ml syringe.


PBSG solution
DEAE cellulose
Glass wool
Eluate
Ice bath

The preparation of the cellulose and the packing of the columns was similar to that described in the isolation of T. b. brucei. The time required for packing the column was shorter compared to the big columns.

The technique for the collection and checking of the eluate was similar to that described for T. b. brucei separation. Owing to the size of the column, elution was much faster than using the big columns.

Having isolated the trypanosomes, the subsequent antigen preparation was similar to that described for T. b. brucei. The major steps being washing three times in PSG, determination of PCV of the purified trypanosome suspension, addition of VED, sonication, centrifugation and collection of the supernatant.

3.10. TITRATION OF ANTIGENS.

This is based on the optimal dilution which will give satisfactory, reproducible results. The reagents required for this procedure were titrated complement, positive control serum, haemolytic system, negative control serum and the antigen to be titrated.

A Microtiter plate method (Anon, 1965) was used in the antigen checker-board titration. Two ml of 1:5 dilution control serum was inactivated by incubation at 60°C for 30 minutes. The inactivated serum was serially diluted in 1 ml amounts up-to tube No. 11. 0.025 ml VBD was placed into every well of a microtitre plate by use of a dropper. 0.025 ml antigen was serially diluted using a 0.025 ml Eppendorf pipette starting from A to G while H remained free. Control serum was added to all wells. Two volumes of 0.05 ml complement was added to every well of the test plate. The plate was fitted with a cover-plate and kept in a refrigarator at +4°C for 18 to 20 hours. The addition of the haemolytic system, sealing and incubation was performed in the standard manner described for the test proper.

Example of an antigen checker-board titration result.

This was the result with *T. vivax* antigen checker-board in this study.

Table 2. The setting of antigen titration.

A: PLATE WITH POSITIVE CONTROL SERUM.

Ag dil.	1.5	10	20	40	80	160	320	640	1280	2560	5120	VBD
1:2	4	4	4	3	1	0	0	0	0	0	0	0
1:4	4	4	4	3	1	0	0	0	0	0	0	0
1:8	4	4	4	3	1	0	0	0	0	0	0	0
1:16	4	4	4	3	1	0	0	0	0	0	0	0
1:32	4	4	3	2	1	0	0	0	0	0	0	0
1:64	4	3	1	1	1	0	0	0	0	0	0	0
1:128	3	2	1	0	0	0	0	0	0	0	0	0
VBD	0	0	0	0	0	0	0	0	0	0	0	0

B: PLATE WITH NEGATIVE CONTROL SERUM.

Ag dil.	1.5	10	20	40	80	160	320	640	1280	2560	5120	VBD
1:2	0	0	0	0	0	0	0	0	0	0	0	0
1:4	0	0	0	0	0	0	0	0	0	0	0	0
1:8	0	0	0	0	0	0	0	0	0	0	0	0
1:16	0	0	0	0	0	0	0	0	0	0	0	0
1:32	0	0	0	0	0	0	0	0	0	0	0	0
1:64	0	0	0	0	0	0	0	0	0	0	0	0
1:128	0	0	0	0	0	0	0	0	0	0	0	0
VBD	0	0	0	0	0	0	0	0	0	0	0	0

KEY:

0= - 100% Lysis
1 - 75% Lysis
2 - 50% Lysis
3 - 25% Lysis
4 - 0% Lysis

The antigen dilution is selected at the dilution showing 50% lysis with the highest serum dilution. This is refered to as the limiting antigen dilution. In this case (above) it was 1:32. To be sure of the working dilution the dilution immediately lower than the limiting antigen dilution was utilised in the tests. In the above example, working antigen dilution of 1:16 was chosen.

The controls using negative control serum samples resulted in 100% lysis in all the wells. This was important because it ruled out the anti-complementary behaviour with the antigen preparations.

All the antigens which were used in this study were tested in a similar manner. The dilution factor guided in the dispensing of the antigens into small aliquots in ampoules so as to avoid wastage.

3.11. TITRATION OF RABBIT ANTI-SHEEP HAEMOLYSIN (AMBOCEPTOR).

Commercial amboceptor (Behringwerk AG, Marburg/Lahn, W. Germany) was used in the study. The titration was done in test tubes.

The emboceptor was prediluted "jumping" dilutions.

1:500 ↓ 1:750 ↑ 1:1,000 ↓ 1:1,500 ↑ 1:2,000 ↓ 1:3,000 ↑ 1:4,000 ↓ 1:6,000 ↑ 1:8,000 ↓ 1:12,000

0.5 ml was transfered from the above tubes into a set of tubes in order of the dilution. Care was taken to start with the highest dilution. The following steps were subsequently followed in this procedure.

- 1 ml of VBD was added to each test tube.
- 0.5 ml of 2% sheep red blood cells (SRBC) was added.
- It was left on the bench for 10 minutes.
- 1 ml of complement at working dilution or 1:40 diluted complement was added.
- Incubation followed in a 37°C waterbath for 30 minutes.

The results were read and recorded as the highest dilution exhibiting complete haemolysis. also refered to as minimal haemolytic dose (MHD). The working dilution of the amboceptor is four times the MHD titre. For example, if MHD is present in dulution of 1:4,000, the working dilution is usually 1:1,000.

3.12. COMPLEMENT.

Pooled sera from thirty healthy male guinea pigs which had been starved for one day

prior to bleeding was the source of complement used throughout the study. It was stored at -20°C and it was titrated on the day when tests were performed. The complement was kept in small ampoules. The portion used in a test had to be from the ampoule that had been titrated the same day before the test.

(i) Titration of complement.

Like in titration of haemolysin it was performed in test tubes. Nine tubes were set up. A 1:40 complement dilution was prepared. Varying volumes of the 1:40 diluted complement were placed in tubes and varying amounts of VBD were added to make uniform final volume (Table 3). The tubes were incubated at 37°C for 1 hour. 0.5 ml of 2% SRBC and 0.5 ml amboceptor at working dilution were added to every tube. The tubes were inverted to ensure proper mixing and they were placed back in 30 minutes. The test was read by recording minimal haemolytic dose of complement (MHD,C) which was represented by the first tube showing complete haemolysis. The next tube is the full unit complement (FHD,C).

The complement dilution to be used in the test proper was calculated by using the formula

$\frac{40}{2}$ Full units. (Staak et al., 1979).

EXAMPLE.

If the MHD,C was present in tube No. 3 the FMD,C was represented by tube No. 4. The calculation was as follows:

$$\frac{40}{2} \times 0.25 = 1{:}80$$

TABLE 3. DILUTION AND SETTING OF COMPLEMENT TITRATION TUBES.

Tube No.	C 1:40 ml	VBD ml	Total Volume ml
1	0.10	1.90	2.0
2	0.15	1.85	2.0
3	0.20	1.80	2.0
4	0.25	1.75	2.0
5	0.30	1.70	2.0
6	0.35	1.65	2.0
7	0.40	1.60	2.0
8	0.45	1.55	2.0
9.	0.50	1.50	2.0

3.12. SHEEP RED BLOOD CELLS (SRBC)

Three (3) Merino sheep were kept for regular bleeding to obtain erythrocytes. Usually 37.5 ml blood was collected into 62.5 ml Alsevers solution (Appendix XVIII) by venipuncture of the jugular vein.

Penicillin was added to the blood which was then stored in sterilised bijou bottles for use within one week.

Prior to the test proper or any of the titrations the blood cells were washed three times with VBD. This was followed by dilution to 2% red cell suspension. The cells were sensitised by the amboceptor at working dilution in 1:1 ratio before being incorporated in the test as the haemolytic system.

3.13. CONTROL SERUM SAMPLES.

Positive and negative control serum samples were available for the tests.

The positive control samples were those obtained from animals with known status of infection with a particular trypanosome species. Several serum samples were tested in order to choose the appropriate ones for use. T. simiae, T. congolense, T. vivax and T. brucei positive control serum samples were obtained.

The negative control serum samples were obtained from animals known to have had no exposure to trypanosomes. The samples had to give negative results with all the antigens. They were available from pig, sheep and goat.

The positive and negative serum samples were included in every test that was performed.

3.14. COMPLEMENT TITRATION TEST.

The technique closely followed the CFT for brucellosis by Alton and Jones, (1967) with minor modifications and was based on a microtitre system (Anon, 1965). Doubling serum dilutions of 1:5 to 1:640 were used. One volume (0.025 ml) of test serum in VBD ph 7.4, one volume (0.025 ml) antigen and two volumes (0.050) complement at working dilution, were utilised. All serum inactivations were done at 60°C for 30 minutes.

The test plates were kept in a refrigerator at +4 °C for 18 to 20 hours before addition of two volumes of hemolytic system. The system was composed of sensitized 2% sheep red blood cells. It was prepared by mixing equal parts by volume of 2% SRBC and haemolysin at working dilution. The mixture was allowed to stand on the bench for at least 15 minutes before it was added to the test.

In all tests, four - fold concentration of minimum hemolytic dose of anti-sheep hemolysin was used.

After the addition of the haemolytic system the plates were sealed with sellotape, inverted several times to mix and then incubated at 37°C

for 30 minutes. Thereafter they were left standing on the bench for one hour before the test was read by aid of a microtiter reader.

Each serum sample was tested against antigens prepared from T. simiae, T. congolense, T. b. brucei and T. vivax. Each test included an anticomplementary control for the serum at ½ dilution i.e. the antigen portion was replaced by an equal volume of VBD. Standard positive and negative serum samples were included in each batch of tests.

The testing of individual serum samples against the four antigens. Was performed on different plates on the same day using some complement and haemolytic system.

3.15. INTERPRETATION OF RESULTS.

The results were recorded according to the degree of fixation at a particular titre. The degree of fixation was rated as follows:-

100%	fixation	was	recorded	as	4
75%	"	"	"	"	3
50%	"	"	"	"	2
25%	"	"	"	"	1
Slight fixation				"	Trace
No fixation				"	Negative (-ve)

In this study, trace reaction was taken as suspicious. Twenty five percent fixation and above were considered positive.

3.16. TRYPANOCIDAL COMPOUNDS.

The suppression of the trypanosome parasitaemia employed the use of the following trypanocidal compounds.

6-amidino-2- (4-amidinophenyl) Indol (DAPI)

This was a test diamidine synthesised by Prof. Dann of University of Erlangen, West Germany. The compound was used at 5 and 10 mg/kg in 5% aqueous solution administered intramuscularly to suppress *T. simiae* parasitaemia in pigs. This was chosen following initial drug trials (Mahaga, 1981, unpublished). It was coded D in the study.

Quinapyramine isothionate (8111):-

This was a quinapyramine isothionate test compound from May and Baker. It was used at 10 mg/kg in 2% solution in attempting to cure *T. simiae* in pigs and sheep. This was also in accordance with earlier work (Maloo, 1982, unpublished). It was coded Q in this study.

Antrycide methyl sulphate (I.C.I.) :-

This was utilised in a manner similar to 8111, although it was only restricted to pigs. This was coded A in this study.

Berenil (Hoechst, Frankfurt, West Germany):-

This drug was employed in treatment of *T. congolense* infection in sheep. It was applied at a dose of 7 mg/kg in 5% solution intramuscularly. The compound was coded B in this study.

3.17. STATISTICAL ANALYSIS

Results of packed cell volume and weight gain variations (expressed as percentages and kilograms respectively) were subjected to statistical analysis. The means computed for the various experimental animal groups were analysed by aid of Student's t-test as applied to paired observations.

4. RESULTS.

4.1. PARASITOLOGICAL AND CLINICAL OBSERVATIONS.

(i) Pigs.

The *T. simiae* strain was very virulent to pigs. The period between inoculation and the first detection of parasites by wet blood film method was 4 to 5 days. Clinical signs of acute septicaemic conditions were observed. Pigs developed pyrexia of up to 41.2°C and the temperature peaks corresponded with periods of parasitaemia (Fig 7). During the initial parasitaemia the disease was more acute with pigs becoming anorexia and isolating themselves in corners as well as showing reluctance in standing up. High parasitaemias of up to ++++ve developed within 48 hours after the initial detection of parasites. Pigs developed anaemia as shown in Fig 8.

One pig (No 110) died of trypanosomiasis relapse on the 28th day post-inoculation despite the suppression of parasitemia with DAPI on the 5th and 19th days post-inoculation. Two other pigs (No 102 and No 109) survived after the initial suppression of parasitemia with DAPI. However, both subsequently relapsed and had to be treated with antrycide methyl sulphate. It was clear from previous experiments that all *T. simiae* infected

pigs would have died within a few days if it were not for the suppression of the parasitaemia using the above compounds.

The pigs lost up to 4 kilograms body weight within the first three weeks of infection. The average packed cell volume values in the *T. simiae* infected pigs dropped as low as 25%. The lowest recorded PCV values for the individual animals were 20% in pig 102, 27% in pig 109 and 14% in pig 110. Pig No 110 died on the 29th day post-inoculation. The PCV values corresponded well with the duration of parasitaemia within the experimental group.

T. congolense "Transmara" (TCT) failed to infect three pigs (Nos 106, 107 and 123). All remained persistently negative by the daily wet film examination, accasional Woo technique and on several subinoculation of their blood into mice. All were healthy throughout the observation period of two months. The temperature and PCV values remained within the normal range throughout the study period. The pattern of their body weight gains was closely similar to that of unifected control group (Fig 9).

The uninfected control group composed of two pigs (Nos 111 and 112) remained healthy throughout the observation period.

Trial infection of a pig (No 122) with a second stock of *T. congolense*, *T. congolense* "Ukunda" (TCU) resulted in infection with a very low parasitaemia. The pig remained negative on wet film examination and parasites were only occasionally detected by the Woo technique. There were no marked changes in the rectal temperatures, PCV and weight gains in the animal within the first sixty days observation period. Subinoculation of citrated whole blood into three mice on the 50th day post-inoculation resulted in positive infection. High parasitaemia of up to ++++ve were observed in mice.

A later trial infection of another pig (No 144) with another congolense isolate, *T. congolense* "Matuga" (TCM) produced similar results to that obtained with the "Ukunda" isolate.

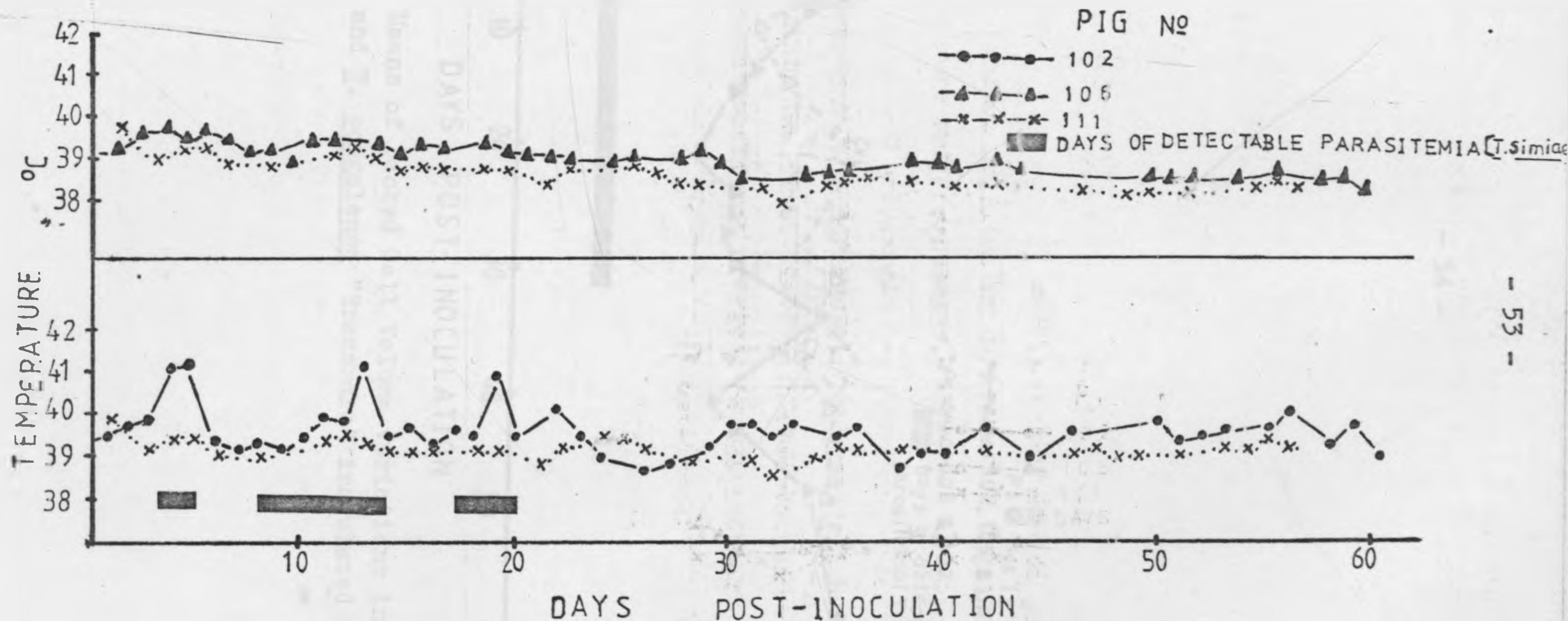


Fig 7. Graphs comparing daily post-inoculation temperature variations in pigs representing T. simiae and T. congolense "Transmara" experimental groups to the negative controls.

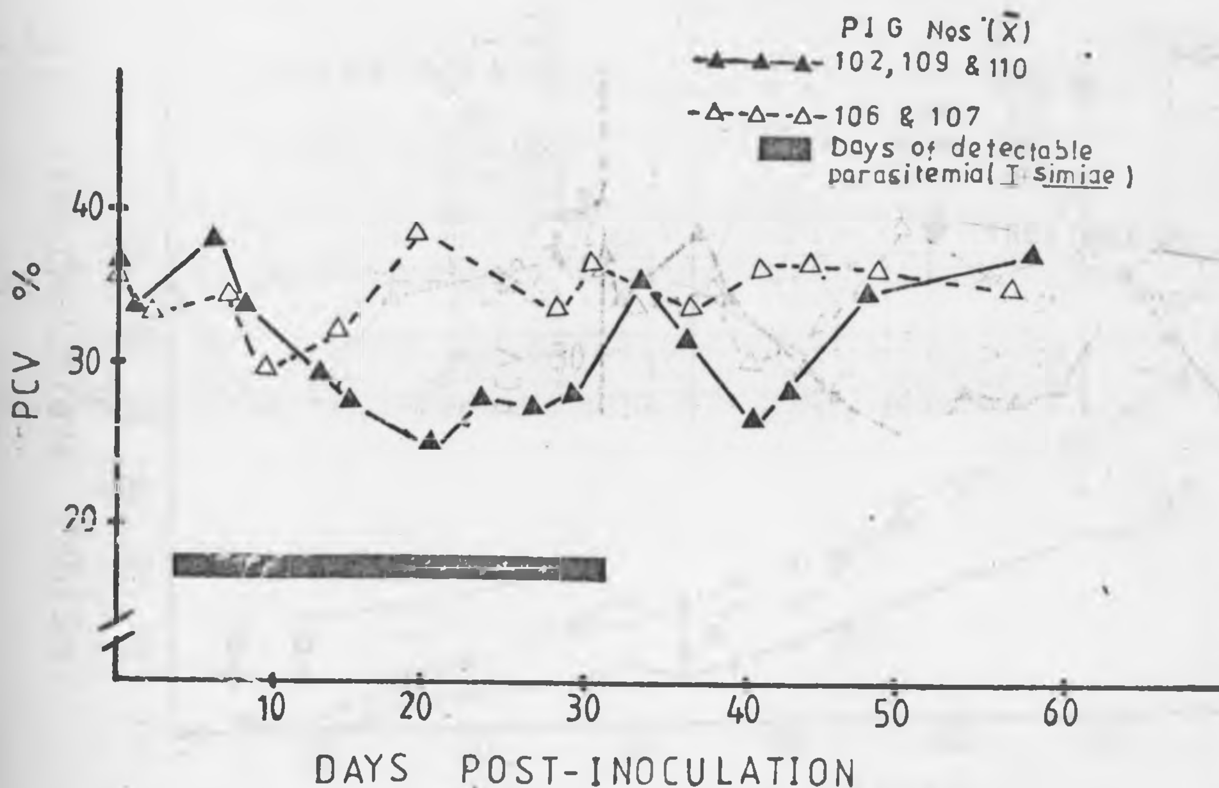


Fig 8. Means of Packed Cell Volume variations in T. simiae and T. congolense "Transmara" inoculated pigs.

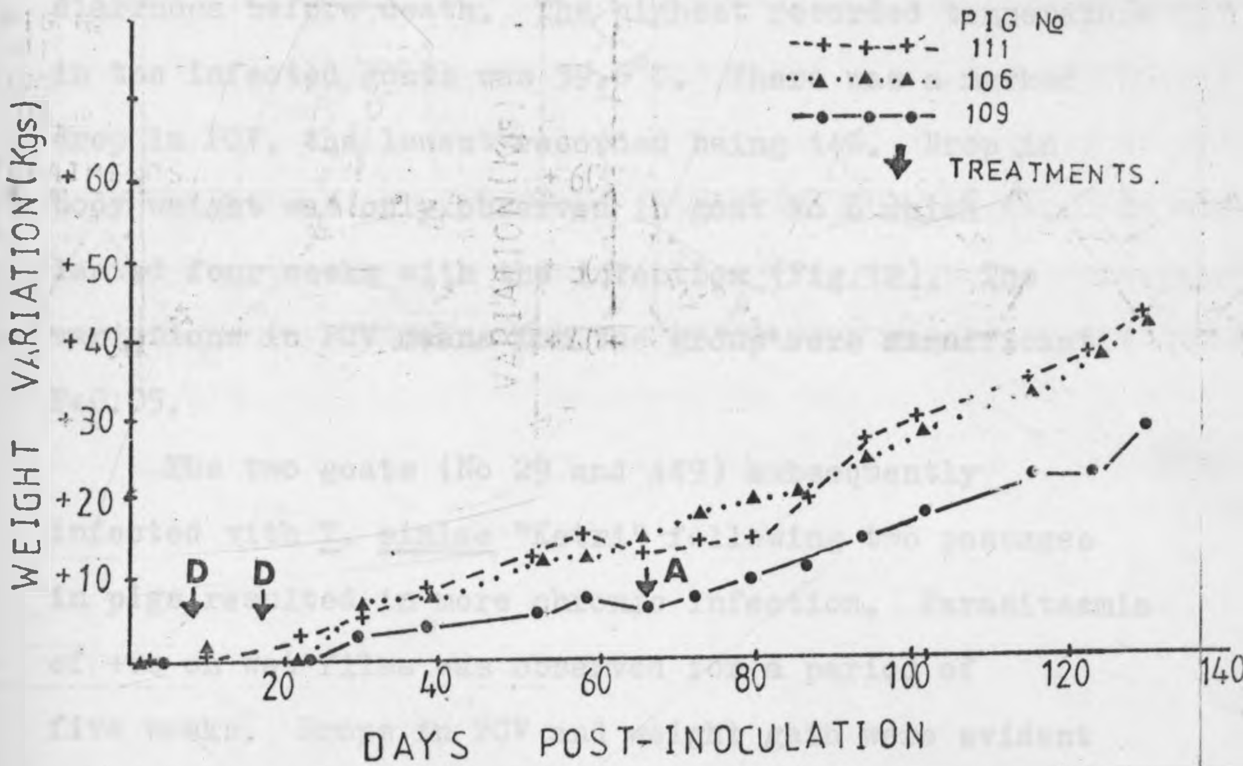


Fig 9. Variations in body weight in groups of pigs inoculated with T. simiae, T. congolense "Transmara" and the negative control.

(ii) Goats.

Both T. simiae infected goats (No 6 and No 549) ran an acute disease that terminated fatally on the 46th and 25th day post-inoculation. The period between inoculation and detection of first parasitaemia was twelve days. The parasitaemia remained lower that in pigs, up to +++ve. The animals remained dull, their mucosae appeared anaemic and they developed slight diarrhoea before death. The highest recorded temperature in the infected goats was 39.6°C. There was a marked drop in PCV, the lowest recorded being 14%. Drop in body weight was only observed in goat No 6 which lasted four weeks with the infection (Fig.12). The variations in PCV means for the group were significant $P<0.05$.

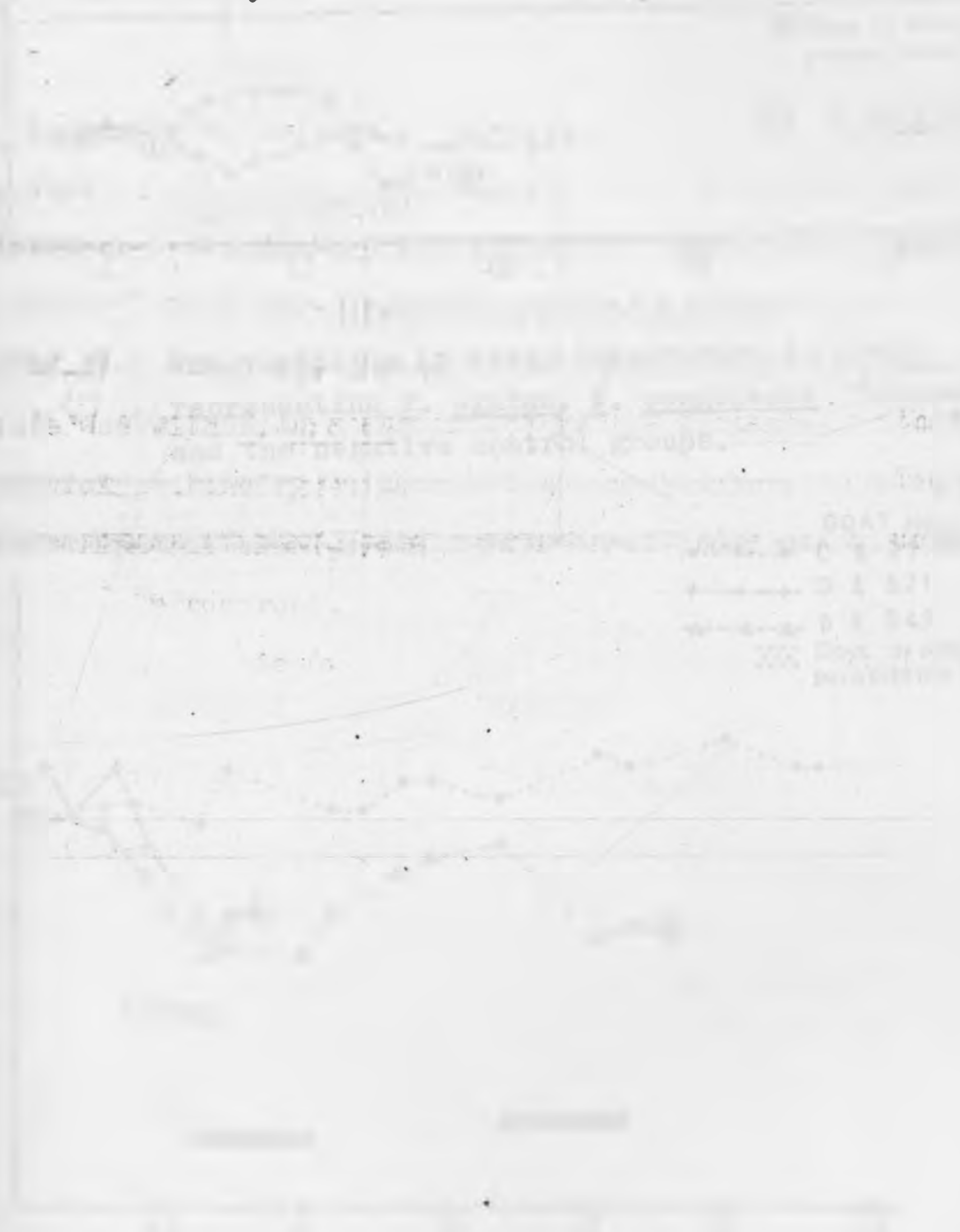
The two goats (No 29 and 449) subsequently infected with T. simiae "Ketri" following two passages in pigs resulted in more chronic infection. Parasitaemia of +ve on wet films was observed for a period of five weeks. Drops in PCV and weight gain were evident and corresponded with the duration of parasitaemia (Fig 120). The lowest recorded PCV readings were 22% and 24% in Nos 29 and 449 respectively. The parasitaemia was undetectable in wet films after the 36th day post-inoculation. Subsequent application of Woo technique failed to detect any parasites in peripheral circulation. During this non-parasitaeimic

period the PCV values were within normal ranges and the animals gained weight (Fig 12a). Pooled whole blood from these two goats was inoculated into pig No 130 on the 103rd day post-inoculation. The inoculation failed to cause any detectable infection in the pig. It was therefore concluded that the goats were self-cured after five weeks of parasitemia.

The T. congolense "Transmara" (TCT) infected goats (D and 521) showed similar acute disease as that observed in goats infected with T. simiae. The period between inoculation and the first detection of parasites in peripheral blood was 8 and 9 days respectively: Maximum recorded temperature was 40°C. High parasitaemia of ++++ve was observed and the PCV dropped significantly to 17% and 19% respectively before death (Fig 1). Other signs observed during the course of infection were anorexia, anaemic mucosae, tachycardia and tachypnea. Death occured on the 6th and 19th day post-inoculation in both goats respectively.

The negative control group of goats Nos C and 27 appeared normal with normal temperature variation; normal PCV ranges and regular gains in body weights during the observation period (Fig 12).

The non-infected control goats were observed for a period of two weeks following the death of those infected with T. simiae "Ketri" and T. congolense "Transmara".



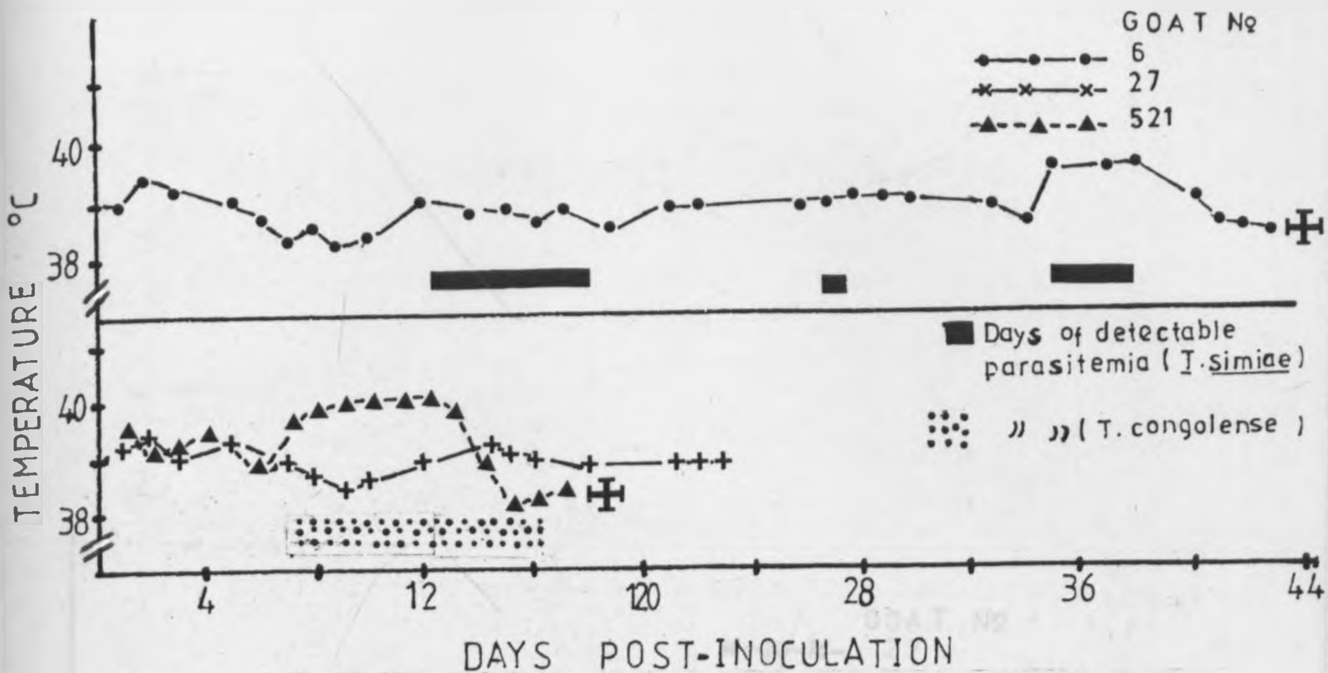


Fig 10. The variation in daily temperature in goats representing T. simiae, T. congolense "Transmara" and the negative control groups.

GOAT Nos ($\bar{X}$)
C & 27
D & 521
6 & 549
Days of detectable parasitemia (T. congolense)

PCV %

DAYS POST-INOCULATION

Fig 11. The variation in means of packed cell volume in groups of goats inoculated with T. simiae T. congolense "Transmara" and the negative controls.

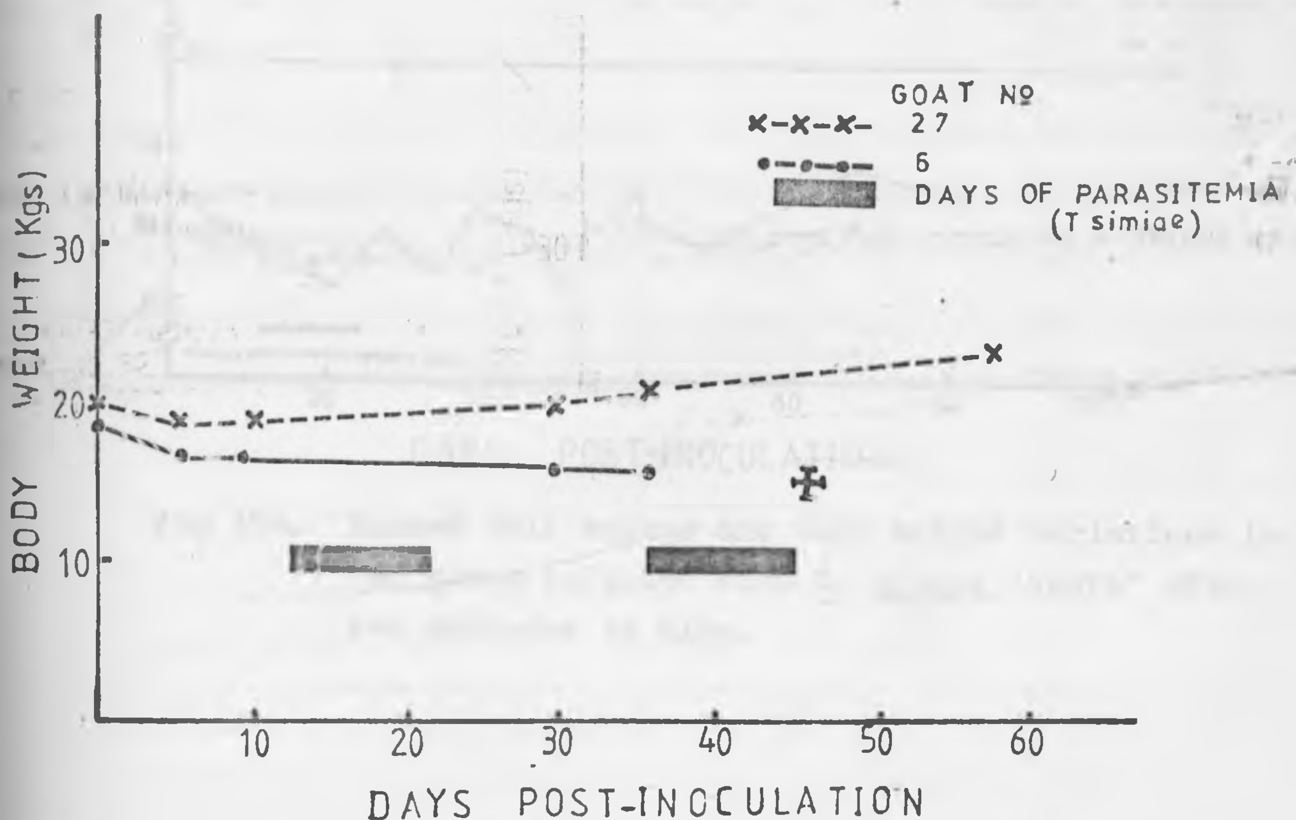


Fig 12. The body weight variation in goats representing the T. simiae infected group and the negative control.

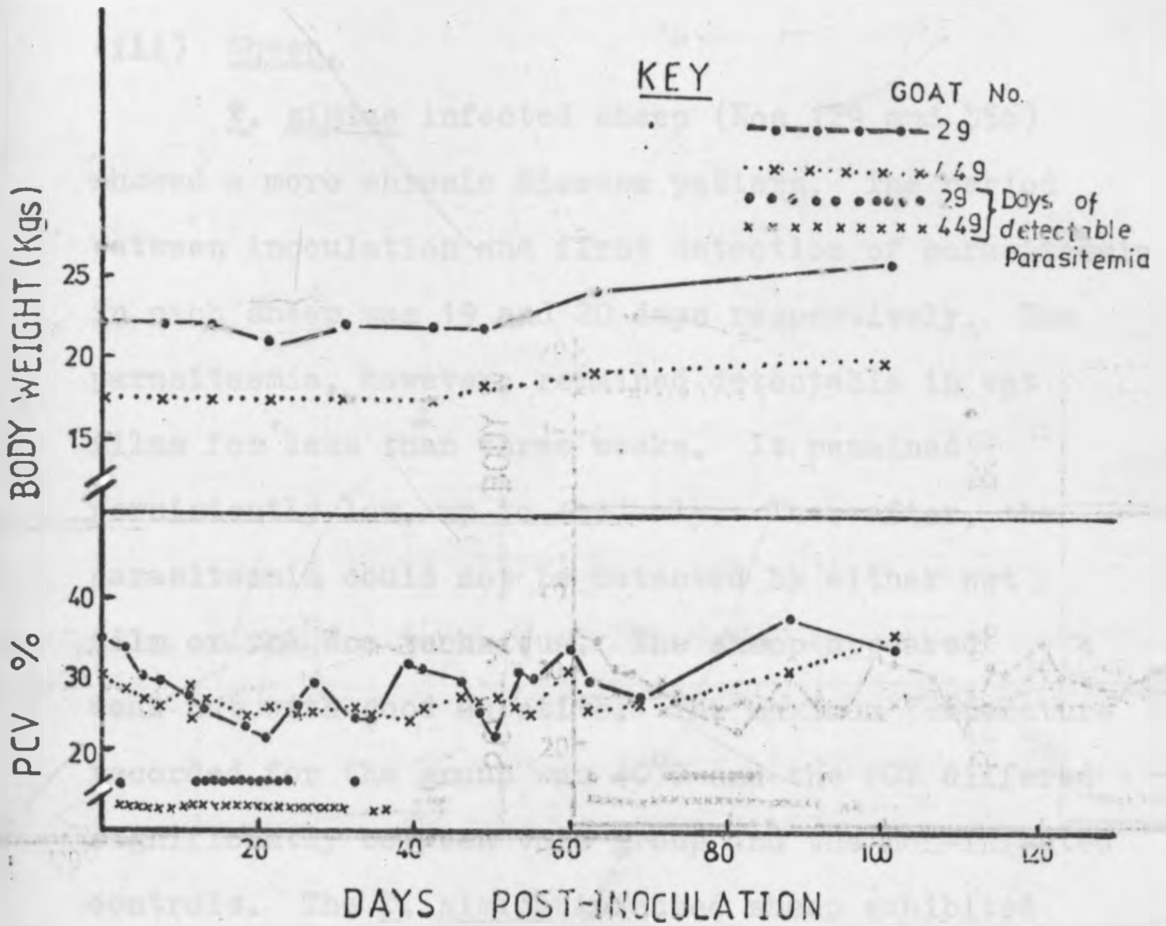


Fig 12a. Packed cell volume and body weight variations in two goats infected with T. simiae 'Ketri' after two passages in pigs.

(iii) Sheep.

T. simiae infected sheep (Nos 129 and 356) showed a more chronic disease pattern. The period between inoculation and first detection of parasitaemia in each sheep was 19 and 20 days respectively. The parasitaemia, however, remained detectable in wet films for less than three weeks. It remained persistently low, up to +ve only. Thereafter, the parasitaemia could not be detected by either wet film or the Woo technique. The sheep appeared weak but with good appetite. The maximum temperature recorded for the group was 40°C and the PCV differed significantly between this group and the non-infected controls. The *T. simiae* infected sheep exhibited very slow weight gains and developed sub-mandibular oedema inspite of persistent negative results from several faecal samples examined for helminth infection during that period. Pig No 125 inoculated with pooled whole blood from sheep Nos 129 and 356 on their 64th day post-inoculation (26th day of inability to detect the parasites in wet films) resulted in positive infection. The inoculated pig became infected and exhibited an acute disease with very high parasitaemia. The prepatent period for the infection in the pig was five days and it was treated with antrycide methyl sulphage (A).

The sheep in the infected group were treated with 8111 on 106th day post-inoculation. After treatment there was a gradual rise in PCV to normal ranges (Fig 13). The sub-mandibular oedema subsided and the group exhibited reasonable body weight gains. There was statistical significance both in the PCV and weight variation in T. simiae infected and the negative control sheep. The significance exceeded the P = 0.05 level for each of the two parameters.

The sheep infected with T. congolense "Transmara" strain (No 146 and No 255) ended up with very chronic type of infection. The prepatent period was 15 and 19 days respectively. The parasitaemia remained persistently low, up to ++ve and they consistently showed positive results on wet film for a period of 100 days until treatment. The clinical condition of the animals remained good and was closely comparable with the non-infected controls. In the first 60 days observation period there were no marked differences in PCV between this group and the negative controls. Thereafter the difference between the two groups became more marked until after treatment with Berenil (B) (Fig 13) on 128th day post-inoculation. The pattern of the weight gains between the two groups was similar to that of PCV. The difference in body weight gains was

minimal between the T.C.T. infected group and the controls during the first 80 days of the experimental period. Thereafter the difference became more marked until treatment of the parasitaemic group (Fig 14). The PCV variation between the T. congolense "Transmara" infected group and the negative control group was not significant ($P>0.05$).

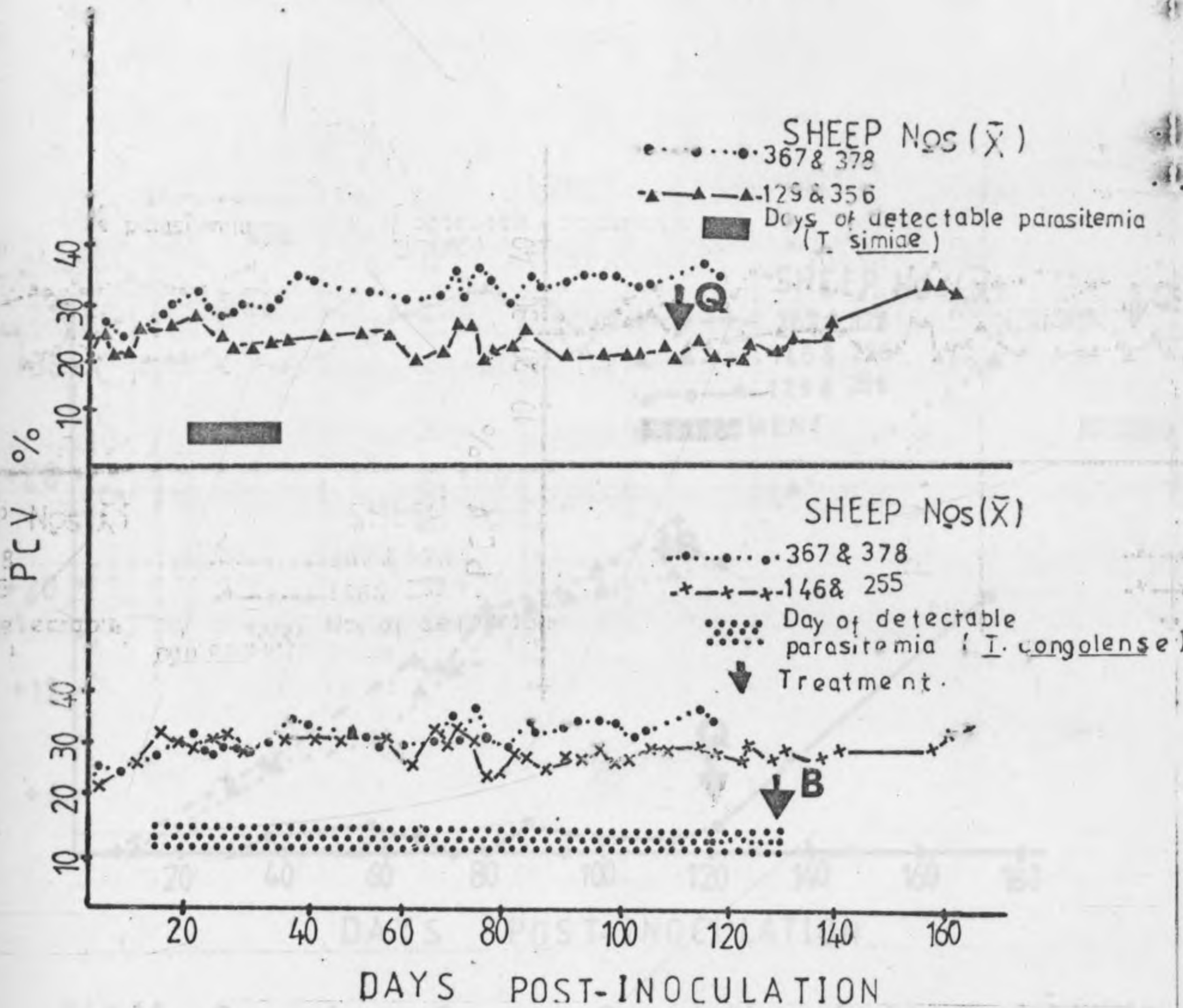


Fig 13. Variations in means of packed cell volume in groups of sheep inoculated with T. simiae and T. congolense "Transmara" to the negative controls.

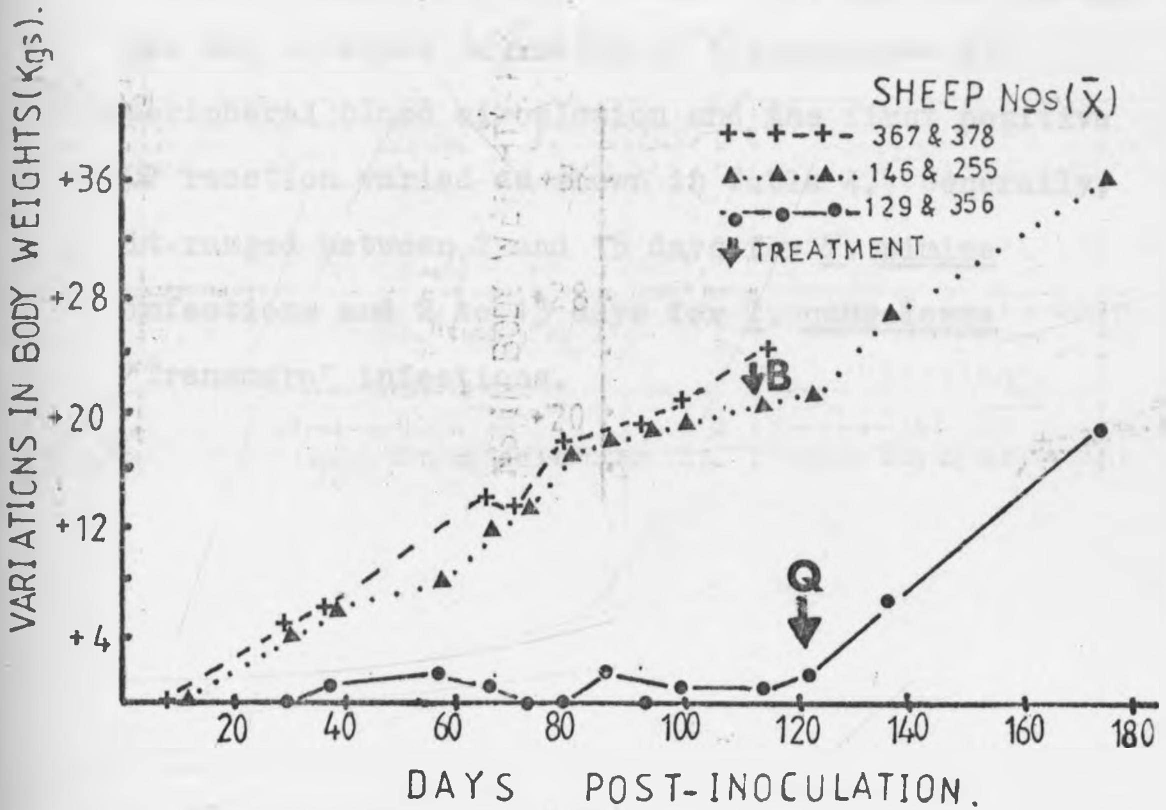


Fig 14. Comparison of means of variations in body weights in sheep representing the negative control, the T. congolense "Transmara" and T. simiae inoculated groups.

4.2. SEROLOGY RESULTS.

All pre-infective serum samples from all the animals which were incorporated in the study showed negative results on CFT. All *T. simiae* and *T. congolense* infected individuals exhibited positive antibody titres on the CF test. The periods between the day of first detection of trypanosomes in peripheral blood circulation and the first positive CF reaction varied as shown in Table 4. Generally, it ranged between 2 and 15 days for *T. simiae* infections and 2 to 13 days for *T. congolense* "Transmara" infections.

TABLE 4. PERIOD IN DAYS BETWEEN THE FIRST DETECTION OF PARASITES AND THE FIRST APPEARANCE OF ANTIBODIES

ANIMAL NO		TRYPANOSOME SPECIES	CFT RESULT OF PRE-INFECTIVE SERUM SAMPLE	DAYS BETWEEN FIRST POSITIVE WET FILM AND FIRST POSITIVE CFT REACTION
PIG	102	*T. simiae*	-ve	2
"	109	"	-ve	2
"	110	"	-ve	5
SHEEP	129	"	-ve	5
"	356	"	-ve	3
GOAT	549	"	-ve	8
"	6	"	-ve	15
PIG	106	*T. congolense* "transmara"	-ve	-ve throughout obseration period
"	107	"		"
SHEEP	146	"	-ve	13
"	255	"	-ve	4
GOAT	521	"	-ve	2
"	D	"	-ve	2
PIG	111	None	-ve	-ve throughout observation period
"	112	"	-ve	"
SHEEP	367	"	-ve	"
SHEEP	378	"	-ve	"
GOAT	27	"	-ve	"
GOAT	C	"	-ve	"

The results of the complement fixation test (serological) for the different experimental animal groups are represented in Table 4.

The highest complement fixation antibody titres in T. simiae infections were detected in pigs (Appendix XI, XII, XIII and XIV). Whereas, sheep and goats infected with the same T. simiae strain exhibited low titres even after long duration of parasitaemia.

The three T. congolense "Transmara" inoculated pigs remained persistently negative on CF test throughout the study period (Appendix XV). The sheep and goats infected with the same T. congolense strain exhibited antibody titres of up to 1:20.

Studies of cross-reactivity using the CF test revealed definite Trypanosoma inter-species cross-reaction. This was more marked between T. simiae, T. congolense and T. b. brucei, but to a much lower degree between antibodies to T. simiae and antigen prepared from T. vivax as shown below.

From the test, 23 (42.59%) of the total of 54 serum samples from positive T. simiae infected pig serum samples exhibited similar antibody titres on testing with either T. simiae and T. congolense antigen. 28 sera (51.85%) exhibited higher titres with the T. congolense antigen than with the T. simiae

one. Only 3 (5.56%) serum exhibited higher titres with *T. simiae* antigen than with the *T. congolense*.

Similar titres were exhibited with 21 (38.39%) of the above total on testing with *T. simiae* or *T. vivax* antigens. Higher antibody titres with *T. simiae* antigen than with the *T. vivax* one were exhibited in 33 (61.11%) of the total samples. Only 16 (29.63%) of the above total revealed equivalent titres using either *T. simiae* or *T. b. brucei* antigen. However, 37 (68.52%) resulted in higher titres with *T. b. brucei* antigen as compared to the *T. simiae* one.

The positive *T. simiae* control sample that was used throughout the study generally exhibited higher antibody titres with the *T. b. brucei* and *T. congolense* antigens than with *T. simiae* (Appendix XX). It was either negative or showed lower titres with the *T. vivax* antigen. On the other hand on the eight different CF test setting (different days), the *T. vivax* positive control serum sample constantly revealed significantly higher antibody titres with *T. vivax* antigen than with those prepared from trypanosomes of sub-genus *Nannomonas* (Appendix XX).

The T. brucei positive control serum persistently exhibited the highest antibody titres with T. brucei antigen than with T. simiae, T. congolense and T. vivax antigens.

All the three pigs which had been inoculated with T. congolense "Transmara" exhibited persistently negative results with test using T. simiae, T. congolense, T. vivax and T. b. brucei antigens. However, pigs which were subsequently inoculated with T. congolense "Ukunda" and T. congolense "Matuga" portrayed positive CF results.

The T. simiae infected sheep and goats revealed positive CF results as represented by sheep No 129 and goat No 6 in Fig 16.

The negative control groups of pigs, sheep and goats remained persistently negative on CFT with all the individual four trypanosome antigens. Sheep No 378 was later inoculated with T. congolense "Transmara" and consequently showed presence of antibodies.

Both the T. simiae and T. congolense infected animals exhibited a decrease in antibody titres after treatment with trypanocidal drugs.
Fig No 109 showed a fall in titre from 1:40 on the day of treatment with antrycide methyl sulphate

to negative 3 months later (Appendix XII). The second pig No 102 showed antibody titre drop from 1:160 on the day of treatment with antrycide methyl sulphate to 1:80 one month later and 1:5 two months after the treatment (Fig 15).

In sheep No 129 there occured a titre drop from 1:20 on the day of treatment with compound 8111 to 1:5 two months later and subsequently negative 3½ months after the treatment (Fig 16).

The fall in antibody titres was also evident in the T. congolence "Transmara" infected sheep after treatment. Sheep No 146 exhibited an antibody titre drop from 1:20 on the day of treatment with Berenil to 1:10 15 days later and was negative 1½ months after treatment. The second one, No 255 likewise exhibited a drop from 1:20 on the day of treatment with Berenil to 1:5 15 days later and subsequently negative one month after treatment (Fig 17).

The negative control serum samples portrayed negative CF results throughout the study period (Appendix XX).

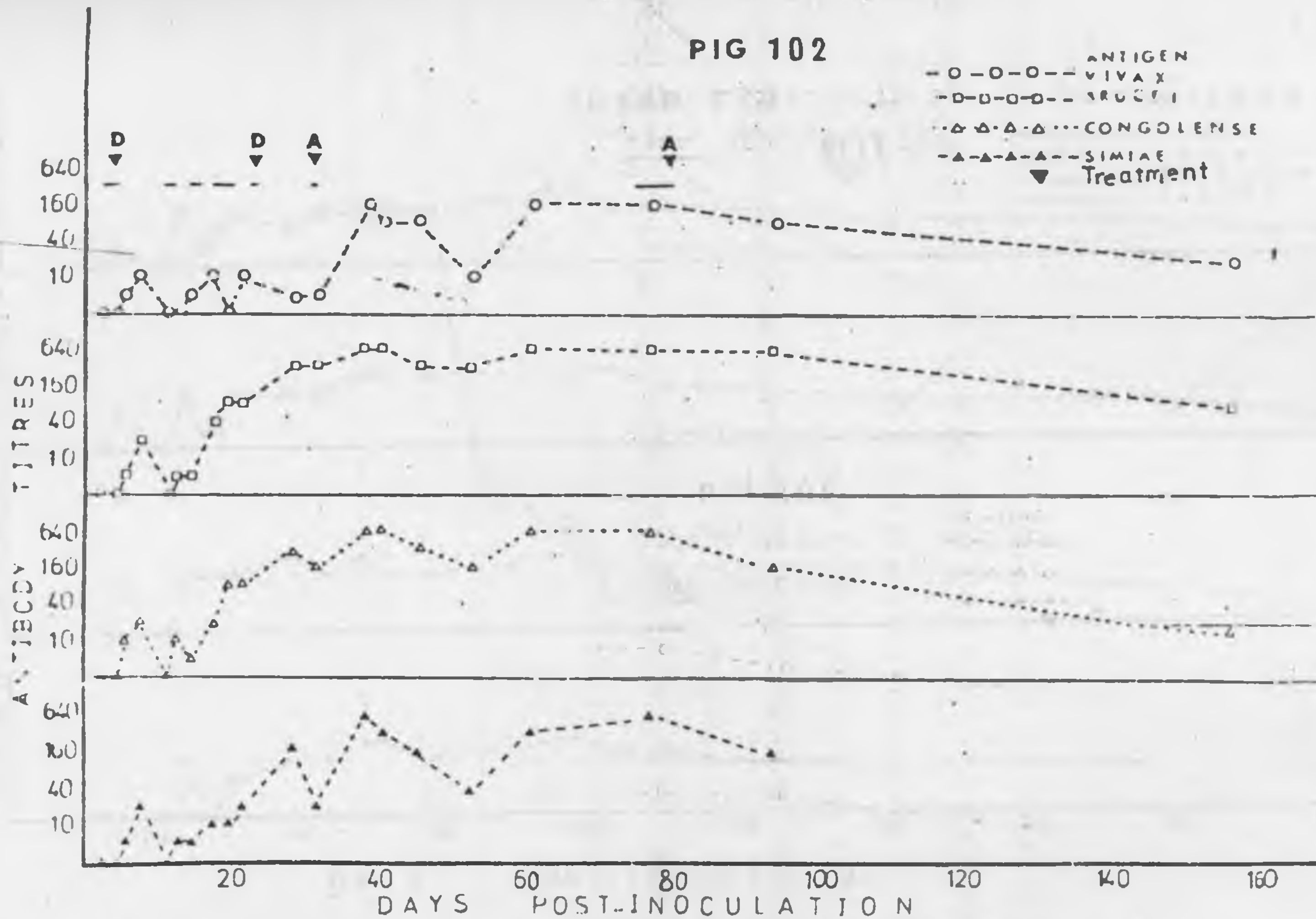


Fig 15. The variation in CF antibody titres in pig No 102 representing the *T. simiae* infected pigs.

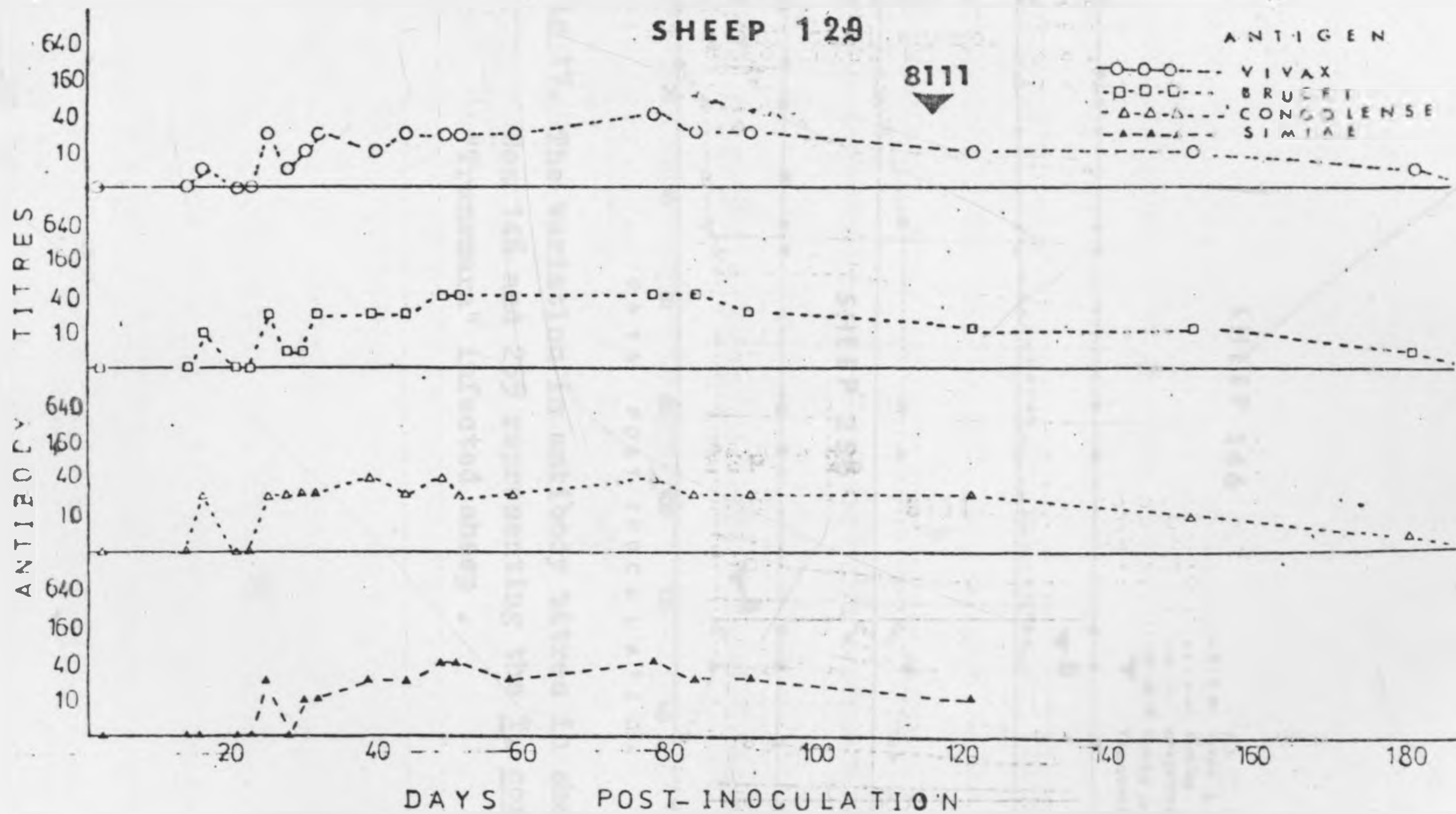


Fig 16. The variation in antibody titres in sheep No 129 representing the *T. simiae* infected sheep.

Fig 17. The variation in antibody titres in sheep Nos 146 and 255 representing the T. congolense "Transmara" infected sheep .

5. DISCUSSION

The virulence of the strain of *T. simiae* to pigs, sheep and goats was confirmed. Though very virulent to pigs, the clinical picture that was observed in this study was less dramatic than that described earlier for certain isolates (Bruce *et al.*, 1913., Peel and Chardome, 1954). The prepatent period following syringe inoculation by intravenous route was longer than the 2.8 days (Peel and Chardome, 1954) and 3.3 days (Bruce *et al.*, 1913). In this experiment, the prepatent period was 4 to 5 days. It is possible that the differences was due to the trypanosome inoculum used. The variation in the prepatent periods between the three animal species that were used in this study is clear.

Stephen (1962) indicated that *T. simiae* infections in sheep and goats were similar. However, results from the present study have indicated certain differences. The prepatent period was much longer in sheep, the parasitaemia was obviously higher in goats and the course of infection was more chronic in sheep. The drop in PCV was more rapid and more marked in caprine than ovine groups. Pelligrini (1948) and Wilson (1949), reported that *T. simiae* infections resulted in more chronic disease in sheep than in goats.

Although these workers used tsetse infection, their findings agree with those from the present work. Bruce *et al.* (1913) reported considerable variation in the pathogenicity of *T. simiae* in goats depending on whether the donor was a monkey or another goat. Peel and Chardome (1954), reported that sub-inoculation of parasitaemic goat blood into another goat resulted in a more acute disease with shorter prepatent period than infection after exposure to *G. brevipalpis*. This is likely to occur due to differences in the initial inoculum. Subsequent infection in goats after two passages in pigs was more chronic and terminated in self-cure. This indicates that the pathogenicity of the strain to goats was reduced by the several passages in pigs. In this study, infections with inoculum prepared after a single passage in pigs resulted in a more acute and fatal disease than did the infections resulting from inoculum prepared after several passages in pigs. This finding agree with those reported by Bruce *et al.* (1913), that the mortality of goats infected with *T. simiae* after being passed for a generation or two through goats, monkeys or warthog is lower than that after direct bite of the fly.

In this study it was more clear that the sheep tolerated T. simiae infection much longer than the goats. However, infections did result in the reduction of PCV and weight gains in sheep but to a lesser degree than that in goats (Fig 11 and 13). Even though the parasitaemia was not detectable in wet films three weeks after the onset, it was shown that it continued to exist at low levels. This was proved by the positive infection which resulted after the sub-inoculation of a clean pig with the sheep blood. The infected pig developed acute infection five days post-inoculation. This agrees with the findings by Wilson (1948), working with the T. simiae "Mbarara" isolate. The author reported acute infections in pigs after inoculation with T. simiae from sheep. This indicated that in tsetse infested areas where sheep and pigs coexist, the former can play a role in the epizootiology of T. simiae infections to the later resulting in heavy losses. The absence of domestic pigs at the Kenyan coast makes this situation non-existent there. However, this may indicate some importance of small ruminants in the perpetuation of T. simiae in the area even in the absence of domestic pigs. The findings from this study agree with those of Wilson (1948). Working with T. simiae "Mbarara" (Uganda) isolate the author reported

similar results. He stated that the strain was non-pathogenic to the sheep. In the present study T. simiae "Ketri" infections in sheep resulted in reduction in PCV and weight gains.

T. congolense "Transmara" failed to infect pigs. The findings with T. congolense "Transmara" are similar to those reported by Pelligrini (1937). However, T. congolense "Ukunda" and T. congolense "Matuga" successfuly infected pigs. The infection of these two isolates in pigs simulate those of Parkin, (1935) and Stephen and Gray (1960). Infections with both isolates were similar with very scanty parasitaemia which remained undetable on wet film examination. This confirms that certain strains of T. congolense are incapable of causing infection in pigs. Unlike T. simiae, the two T. congolense isolates successfully infected mice and rats.

From the results T. congolense "Transmara" was more pathogenic to goats than sheep. Acute infections of shorter prepatent periods, higher parasitaemia, lower PCV which terminated fatally within two weeks of detectable parasitaemia were observed in goats. On the other hand the sheep haboured T. congolense "Transmara" parasitaemia for more than 100 days without any obvious signs of

disease. Parkin, (1935) stated that T. congolense infections in sheep , when concomitant disease were eliminated, were seldom as striking as on bovines. He further stated that the common course was that of a primary acute form followed with a chronic disease. The primary acute form was not observed in this study. Anaemia was less marked in T. congolense infected sheep than in the T. simiae infected ones. These findings showed that the T. simiae strain was more pathogenic to sheep than T. congolense "Transmara".

The results from T. congolense "Ukunda" and T. congolense "Matuga" infection in pigs agree with those reported by Parkin, (1935) using the Rhodesian and the Zululand dog strain of T. congolense. He added that the infection with any of the two T. congolense strains did not interfere with the growth of the pigs. However, he argued that the low parasitaemia might have been due to the good condition in which the pigs were maintained. The findings from the present study may suggest that it may also be due to species tolerance. This is so because the T. simiae infected group of pigs although maintained under the same conditions developed in very acute infections with significant interference in their growth. Earlier trial infections of goats with T. congolense "Ukunda" and T. congolense

"Matuga" isolates resulted in chronic infection of higher parasitemia than that recorded in this study for pigs. Parasitaemia rating ++ve on wet films was detected in the goats (Rottcher, Personal communication).

Hoare, (1936) and Mettam, (1940) also reported T. congolense infections in pigs as being chronic and without impairing the health of pigs. However, Killick-Kendrick and Godrey, (1963) studying infection in pigs Nsukka found it difficult to isolate T. congolense strains with low infectivity to rats. On the other hand, some T. congolense strains with low infectivity to rats have been reported (Godfrey, 1961). T. simiae is not infective to rats (Desowitz and Watson, 1953). Therefore confusion between the T. simiae strains which cause chronic infections in pigs and T. congolense infections in pigs still exists.

Mettam, (1940) working in Agege (West Africa) described T. simiae strains causing chronic infections in pigs and later showed that experimentally infected pigs survived for nine months (Mettam, 1951). However, the basis on which the worker identified the aetiological agent as T. simiae is not clear.

Janssen and Wijers, (1974) reported that there exists at the Kenya coast T. simiae which causes a less acute disease with pigs surviving 3 or 4

weeks even longer. The authors maintained their experimental pigs under fly-proof conditions and infected them by *Glossina* caught from the sorrounding. In that study, they observed that the low virulence of some *T. simiae* strains encountered in the area might have been due to the species of *Glossina* involved in the transmission. In *G. brevipalpis*, the trypanosome becomes more virulent for pigs and in *G. pallidipes* they lose part of the virulence. This however, was not part of this study.

This study confirmed that like any other trypanosome infection in livestock, *T. simiae* evoked immune response in pigs, sheep and goats. This resulted in production of antibodies by the hosts. The antibodies were detectable using the complement fixation test (CFT).

As stated by Weitz, (1970) and Fife, (1972) the CFT was of high sensitivity. It however failed to distinguish between *T. simiae* and *T. congolense* infections in various animals as it was determined in this work. The strong cross-reactivity between the two *Trypanosoma* species indicates presence of antigens which are shared between the two species.

This test cannot therefore be utilised in distinguishing between infections with different species of trypanosomes using field samples. The lowest degree of cross-reaction with *T. simiae*

was found to be with T. vivax. This again confirms the fact that T. vivax is only slightly related to T. simiae. This agrees with the observation by Luckins, (1981) that bovine trypanosomiasis caused by T. brucei, T. congolense and T. vivax presents a complex serodiagnostic problem. He further stated that serological tests often failed to distinguish between infections with the three species because they elicit the formation of cross-reacting antibodies. Similar cross-reaction was found in this study.

The CF test is still of value in experimental infections. This would be important in monitoring the status of infection and in assessing cure in chemotherapeutic experiments. As such, it is of value only where general screening is required or in cases of known experimental infections.

In field cases the test may be of some value in epizootiological studies especially those concerned with determination of Trypanosoma infection rates in areas regardless of the trypanosome species involved.

The results show that following effective treatment, trypanosomal antibody titres dropped from the initial levels on the days of treatment to half or less within a period of one month. It may therefore be useful in assessing the effect of

drugs or potential trypanocidal compounds on infections.

In view of the above serodiagnostic problem, those encountered with morphological similarity and animal infectivity overlap, a precisely specific method of distinguishing between *T. simiae* and *T. congolense* remains unaccomplished.

In this study, the highest antibody titres against *T. simiae* infection were detected in pigs, followed by sheep and finally goats. It was rather inconclusive for goats due to their early death. However, it was rather surprising that even after several weeks of parasitaemia the sheep did not develop equally high antibody titres as were the case in pigs. This may be due to the persistently low parasitaemia that the sheep maintained during the infection with the *T. simiae* strain.

In their studies of *T. congolense* infection in bovine, Staak and Lohding, (1979) found that the period between the first detection of trypanosomes in peripheral blood and the first positive CF reaction was 2 - 19 days. In this study the same period was 4 - 13 days in *T. congolense* "Transmara" infected sheep and goats; and 2 - 15 in *T. simiae* infected pigs, sheep and goats. This agrees with the findings

of the above workers. However, to establish the validity of such figures, one would require larger groups of animals than those incorporated in this study.

Similar to the findings by Robinson, (1926) for the *Trypanozoon* subgenus, this work has confirmed close affinity between the members of the subgenus *Nannomonas*. Consequently it provides further support for their present classification.

6. CONCLUSIONS

The present study confirmed the virulence of *T. simiae* "Ketri" and *T. congolense* "Transmara" in goats, sheep and goats. The virulence of *T. simiae* "Ketri" is greater in goats than in sheep. However, the isolate is less virulent to goats after passages in pigs.

T. congolense "Transmara" is not infective to pigs but results in more virulent infection in goats than in sheep. Unlike the "Transmara" *congolense* isolate, two other isolates (*T. congolense* "Matuga" and *T. congolense* "Ukunda") are infective to pigs, causing chronic infection with very low parasitaemia. The parasitaemia in pigs infected with these two isolates is only detectable by more sensitive parasitological methods than wet film examination. It is therefore conclusive to state that some *T. congolense* isolates are capable of infecting domestic pigs while other are not.

The complement fixation test was found sensitive in the detection of *Trypanosoma* antibodies in the three animal species. However, the test could not distinguish between *T. simiae* and *T. congolense* infections in animals. The test can therefore be of use in cases of known experimental *Trypanosoma* infections. It can therefore be of value

in the assessment of cure in chemotherapeutically based studies.

Comparative inoculation of domestic pigs and small laboratory rodents including mice, rats and guinea pig remains the easiest available method of distinguishing between *T. simiae* and *T. congolense*. This procedure takes a few weeks before results are confirmed and it may be confusing with *T. congolense* strains having low infectivity to the laboratory rodents.

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APPENDIX I.

TABLE SHOWING PCV (%) VARIATIONS AND MEANS IN THREE GROUPS OF PIGS.

	GROUP I				GROUP II			GROUP III		
DAYS POST-INOCULATION	PIG NOS 102	109	110	$\bar{X}$ 102 & 109	106	107	$\bar{X}$	111	112	$\bar{X}$
0	33	37	33	35.0	36	35	35.5	40	37	38.5
1	29	32	37	30.5	36	32	34.0	35	35	35.0
6	40	39	35	39.5	36	34	35.0	40	38	39.0
8	34	35	34	34.5	31	29	30.0	33	35	34.0
13	30	27	29	28.5	30	35	32.5	32	30	31.0
15	24	28	28	26.0	33	34	33.5	28	32	30.0
20	20	30	20	25.0	36	39	37.0	28	30	29.0
23	20	35	30	27.5	34	32	33.0	31	29	30.0
27	30	37	14	33.5	34	34	34.0	32	29	30.5
29	27	28		27.5	37	37	37.0	35	27	30.0
34	35	34		34.5	29	34	31.5	36	28	32.0
36	31	32		31.5	30	37	33.5	27	38	32.5
41	21	30		25.5	39	34	30.5	32	25	28.5
43	26	30		28.0	36	35	35.5	39	35	37.0
48	30	39		34.5	35	36	35.5	37	40	30.5
59	36	36		36.0	30	40	35.0	33	37	35.5
				$P>0.05$			$P>0.05$			

Key. GROUP I = *T. simiae* infected.

GROUP II = *T. congolense* "Transmara" inoculated.

GROUP III= Non-inoculated control.

APPENDIX II.

TABLE SHOWING PCV (%) VARIATIONS AND MEANS IN THREE GROUPS OF SHEEP.

DAYS POST-	GROUP I			GROUP II			GROUP III		
	PIG NOS								
INOCULATION	129	356	$\bar{X}$	146	255	$\bar{X}$	367	378	$\bar{X}$
0	26	19	22.5	20	23	21.5	23	17	20.0
2	23	23	23.0	15	27	21.0	24	16	20.0
7	21	19	20.0	18	23	20.5	26	22	24.0
9	25	24	24.5	21	27	24.0	27	22	24.5
13	25	26	25.5	27	30	28.5	29	27	28.0
15	25	25	25.0	25	36	30.5	32	26	29.0
20	27	26	26.5	24	35	29.5	36	27	28.5
24	25	25	25.0	27	34	30.5	30	26	29.0
27	22	20	21.0	28	32	29.5	32	25	28.5
29	22	19	20.5	29	28	30.5	29	29	29.0
34	22	21	21.5	31	29	30.0	30	27	28.5
36	24	20	22.0	30	31	30.5	33	28	30.5
43	26	22	24.0	32	29	30.5	35	35	35.0
48	25	22	23.5	31	29	30.0	34	29	31.5
50	23	24	23.5	29	29	29.0	35	31	33.0
55	25	21	23.0	31	30	30.5	28	32	30.0
			$P<0.05$			$P>0.05$			

Key. GROUP I = *T. simiae* infected.

GROUP II = *T. congolense* "Transmara" infected.

GROUP III= Non-infected control.

APPENDIX III.

PCV (%) MEANS IN THREE GROUPS OF GOATS

DAYS POST-INOCULATION	GROUP I $\bar{X}$ Nos 6 & 459	GROUP II Nos D & 521	GROUP III Nos C & 27
0	27.0	28.5	27.0
1	27.5	29.5	26.0
3	26.5	30.5	27.0
6	26.5	30.5	28.0
8	23.0	24.5	28.0
13	17.5	19.0	27.0
15	18.5	20.0	31.0
20	17.5		29.0
22	20.5		28.0
24	18.0		28.0
27	24.0		30.0
29	25.0		30.0
34	26.0		29.0
41	19.0		32.0
43	20.0		31.0
	$P < 0.05$		

Key. GROUP I = *T. simiae* infected.

GROUP II = *T. congolense* "Transmara" infected.

GROUP III = Non-infected control.

APPENDIX IV.

TABLE SHOWING BODY WEIGHT (KGS) VARIATIONS AND MEANS IN THREE GROUPS OF PIGS.

DAYS POST-INOCULATION	GROUP I				GROUP II			GROUP III		
	102	109	110	$\bar{X}$ 102 & 109	106	107	$\bar{X}$	111	112	$\bar{X}$
3	58	23	22	40.5	26	27	26.0	21	21	21.0
10	58	25	24	41.5	26	28	27.0	22	23	22.0
23	55	23	21	39.0	26	30	28.0	24	25	24.5
30	58	26		42.0	33	40	36.5	26	30	28.5
38	60	27		43.5	32	42	38.0	30	30	30.0
51	64	28		46.0	38	45	41.5	33	37	35.0
60	70	30		50.0	38	48	43.0	36	37	36.5
68	68	29		48.5	40	47	43.5	33	38	35.5
75	70	30		50.0	42	49	45.5	35	38	36.5
82	75	32		53.5	44	52	48.0	36	40	38.0
89	70	33		51.5	44	54	49.0	40	44	42.0
96	80	37		58.5	48	60	54.0	47	50	48.5
103	78	40		59.0	52	65	58.5	50	55	52.5
118	75	45		60.0	55	68	61.5	55	53	54.0
125	87	45		66.0	60	75	67.5	58	53	55.5

Key. GROUP I = _T. simiae_ infected.

GROUP II = _T. congolense_ "Transmara" infected.

GROUP III = Non-infected control.

APPENDIX V.

TABLE SHOWING BODY WEIGHT (KGS) VARIATIONS AND MEANS IN THREE GROUPS OF SHEEP.

DAYS POST- INOCULATION	GROUP I			GROUP II			GROUP III		
	SHEEP NOS								
	129	356	$\bar{X}$	146	255	$\bar{X}$	367	378	$\bar{X}$
0	19	16	17.5	16	17	16.5	20	15	17.5
5	20	16	18.0	16	17	16.5	20	15	17.5
9	18	16	17.0	16	17	16.5	20	15	17.5
29	18	16	17.0	19	18	18.5	20	20	20.0
32	19	16	17.5	20	19	19.5	23	18	20.5
55	20	17	18.5	24	17	20.5	26	19	22.5
63	20	16	18.0	25	20	22.5	27	21	24.0
70	19	16	17.5	25	21	23.0	27	20	23.5
77	19	16	17.5	27	23	25.0	30	22	26.0
84	20	17	18.5	28	23	25.5	30	23	26.5
91	19	16	17.5	29	23	26.0	31	23	27.0
98	20	16	18.0	29	23	26.0	31	24	27.5
120	21	16	18.5	31	26	27.0			
134	24	18	21.0	34	26	30.0			
171	30	23	26.5	39	30	34.5			
		$P<0.05$				$P>0.05$			

Key. GROUP I = *T. simiae* infected.

GROUP II = *T. congolense* "Transmara" infected.

GROUP III = Non-infected control.

APPENDIX VI

PIG 102 : CFT RESULTS

INFECTION ; T. SIMIAE

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T. SIMIAE	T. CONG	T. VIVAX	T. BRUCEI
0		0	0	0	0
4		0	0	0	0
6		1:5	1:10	1:5	1:5
8		1:20	1:20	1:20	1:20
10		0	0	0	0
11		1:5	1:10	0	1:5
13		1:5	1:5	1:5	1:5
16		1:10	1:20	1:10	1:40
18		1:10	1:80	0	1:80
20		1:20	1:80	1:10	1:80
27		1:160	1:320	1:5	1:320
30		1:20	1:160	1:5	1:320
37		1:640	1:640	1:160	1: 640
39		1:320	1:640	1:80	1: 640
44		1:160	1:320	1:80	1: 640
51		1:40	1:160	1:10	1:320
59		1:320	1:320	1:160	1: 640
75		1:320	1:320	1:160	1: 640
93		1:160	1:320	1:40	1: 640
157		–	1:20	1:20	1:80

APPENDIX VII.

FIG 109 : CFT RESULTS
INFECTION ; T. SIMIAE

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS			
		T. SIMIAE	T. CONG	T. VIVAX	T. BRUCEI
0		0	0	0	0
5		0	1:5	1:5	1:5
6		1:5	1:10	1:5	1:5
8		1:20	1:40	1:20	1:20
10		1:10	1:10	1:5	1:20
13		1:5	1:5	0	1:5
15		1:20	1:40	1:10	1:20
17		1:10	1:40	1:10	1:40
20		1:20	1:80	1:10	1:20
24		1:5	1:20	1:5	1:20
27		1:40	1:80	1:20	1:80
44		1:80	1:160	1:40	1:160
48		1:80	1:160	1:40	1:320
50		1:20	1:40	1:10	1:80
51		1:80	1:80	1:40	1:320
66		1:20	1:20	1:10	1:40
100		1:20	1:20	1:20	1:20
153		-	0	0	0

APPENDIX VIII.

SHEEP 129 : CFT RESULTS

INFECTION : T. SIMIAE

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T. SIMIAE	T. CONG	T. VIVAX	T. BRUCEI
0		0	0	0	0
13		0	0	0	0
16		0	1:20	1:10	1:10
21		0	0	0	0
23		0	0	0	0
25		1:20	1:20	1:20	1:20
28		0	1:20	1:10	1:10
30		1:10	1:20	1:10	1:10
32		1:10	1:20	1:20	1:20
39		1:20	1:40	1:10	1:20
44		1:20	1:20	1:20	1:20
49		1:40	1:40	1:20	1:40
51		1:40	1:20	1:20	1:40
58		1:20	1:20	1:20	1:40
78		1:40	1:40	1:40	1:40
84		1:20	1:20	1:20	1:40
91		1:20	1:20	1:20	1:20

APPENDIX IX.

GOAT 6 : CTF RESULTS

INFECTION : T. SIMIAE

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T.SIMIAE	T.CONG	T.VIVAX	T.BRUCEI
0		0	0	0	0
2		0	0	0	0
8		0	0	0	0
13		0	0	0	0
17		0	C	0	0
21		0	0	0	0
25		0	0	0	0
28		1:20	1:20	1:5	1:40
35		0	0	0	0
37		0	0	0	0
39		1:10	1:10	1:10	1:10
44		1:10	1:10	1:10	1:10

APPENDIX X.

PIG 106 AND 107 CFT RESULTS

INOCULATION : T. CONGOLENSE "TRANSMARA"

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T.SIMIAE	T.CONG	T.VIVAX	T. BRUCEI
0	Negative throughout the study period.	0	0	0	0
2		0	0	0	0
4		0	0	0	0
7		0	0	0	0
9		0	0	0	0
11		0	0	0	0
14		0	0	0	0
16		0	0	0	0
18		0	0	0	0
21		0	0	0	0
25		0	0	0	0
30		0	0	0	0
37		0	0	0	0
39		0	0	0	0

APPENDIX XI.

SHEEP 146 : CFT RESULTS

INFECTION : T. CONGOLENSE "TRANSMARA"

DAYS POST INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T.SIMIAE	T.CONG	T. VIVAX	T. BRUCEI
0		0	0	0	0
7		0	0	0	0
21		0	0	0	0
23		0	0	0	0
28		0	1:5	0	0
39		0	0	0	0
44		0	1:10	0	0
49		0	0	0	0
84		1:5	1:10	0	0
89		0	1:5	0	0
124		1:5	1:10	0	0
131		0	1:20	0	0
140		-	1:10	0	0
146		-	1:10	0	0
149		-	0	0	0

APPENDIX XII.

SHEEP 255 : CFT RESULTS

INFECTION : T. CONGOLENSE "TRANSMARA"

DAYS POST-INOCULATION	DURATION OF PARASITAEMIA	TITRE WITH ANTIGENS T.SIMIAE	T. CONG	T.VIVAX	T.BRUCEI
0		0	0	0	0
14		0	0	0	0
16		0	0	0	0
18		0	1:5	0	0
23		0	1:10	0	0
35		0	1:5	0	0
44		0	1:10	0	0
49		0	1:20	0	0
84		0	1:20	0	0
89		0	1:20	0	0
124		0	1:20	0	0
131		0	1:20	0	0
146		–	1:5	0	0
165		–	0	0	0
179		–	0	0	0

APPENDIX XIII

PIGS 111 AND 112 CFT RESULTS

NEGATIVE NON-INFECTED CONTROLS

DAY	111				112			
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0

APPENDIX XIV.

SHEEP 367 AND 378 CFT RESULTS

NEGATIVE NON-INFECTED CONTROLS (NO 378 WAS LATER INFECTED WITH T. CONGOLENSE "TRANSMARA" EX RATS

DAY	367				378			
	TS	TC	TV	TB	TS	TC	TV	TB
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0
144	0	0	0	0	-	1:5	0	0
150	0	0	0	0	-	0	0	0
169	0	0	0	0	-	1:20	1:15	1:20
183	0	0	0	0	-	1:10	1:5	1:5

<u>APPENDIX XV.</u>

CONTROL SERUM SAMPLES
CFT RESULTS

DATE OF SETTING TESTS	T.SIMIAE POSITIVE CONTROL SERUM				T. CONGOLENSE POSITIVE CONTROL				T. VIVAX +ve CONTROL SERUM				T. BRUCEI +ve CONTROL				NEGATIVE CONTROL SERUM SAMPLE			
	ANTIGENS				ANTIGENS				ANTIGENS				ANTIGENS				ANTIGENS			
	TS	TC	TV	TB	TS	TC	TV	TB	TS	TC	TV	TB	TS	TC	TV	TB	TS	TC	TV	TB
19.3.82	1:640	1:640	1:10	1:320	1:10	1:20	1:10	1:20	1:5	1:5	1:40	1:20	1:5	0	0	1:160	0	0	0	0
22.3.82	180	320	0	180	10	20	10	20	0	5	20	5	40	20	40	40	0	0	0	0
24.2.82	180	320	0	640	5	20	5	20	0	0	20	5	20	40	40	40	0	0	0	0
29.3.82	640	640	40	640	20	80	20	20	10	20	80	20	40	40	80	320	0	0	0	0
31.3.82	180	640	10	640	5	20	10	10	5	5	40	5	40	20	20	80	0	0	0	0
1.4.82	180	640	0	640	0	20	5	10	0	0	20	5	5	20	20	80	0	0	0	0
16.4.82	180	180	20	320	10	20	10	20	0	0	20	5	10	0	20	80	0	0	0	0
21.4.82	180	640	5	640	10	40	10	20	0	5	40	5	20	20	40	160	0	0	0	0
20.5.82	–	640	20	640	–	20	5	20	–	0	40	5	–	–	–	–	–	0	0	0

APPENDIX XVI.

PHOSPHATE BUFFERED SALINE STOCK SOLUTION pH 8.0

Na_2HPO (anhydrous)		13.48 gm
$NaH_2PO_4.H_2O$		0.78 gm
NaCl		4.25 gm
H_2O (distilled)	to	1000 ml

APPENDIX XVII.

VERONAL BUFFERED DILUENT (VBD) pH 7.3-7.4

VBD was prepared as a concentrated solution (stock solution) and diluted 1:5 for use.

VBD STOCK SOLUTION

Sodium chloride	83 gm
Sodium-5.5-diethyl barbiturate	10.19 gm
Distilled water	500 ml
N/1 Hydrochloric acid	34.58 ml
Stock solution of 20.3 gm of $Mgcl_2 6H_2O$ and 4.4 gm of $Cacl_2 2H_2O$ in 100 ml distilled water	5 ml

APPENDIX XVIII.

ALSEVERS SOLUTION pH 6.1

Glucose	20.5 gm
Sodium chloride	4.2 gm
Trisodium acetate	8.0 gm
Citric acid	0.55 gm
Distilled water	1000 ml