HAEMATOLOGICAL AND BLOOD BIOCHEMICAL CHANGES IN THE PATHOPHYSIOLOGY OF *TRYPANOSOMA CONGOLENSE* INFECTION IN INDIGENOUS ZAMBIAN GOATS

BY

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DECLARATION

I, WILLIAM HAROLD WITOLA, DO HEREBY DECLARE THAT THE CONTENTS OF THIS DISSERTATION ARE MY WORK AND THAT THEY HAVE NOT PREVIOUSLY BEEN SUBMITTED TO ANY UNIVERSITY FOR THE AWARD OF A DEGREE OR ANY OTHER QUALIFICATION.

23rd February, 1998

SIGNATURE

DATE

DEDICATION

This dissertation is dedicated to my late Mum and Dad who earnestly supported and encouraged me to soldier-on in my academic work.

APPROVAL

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ABSTRACT

Trypanosomosis is an economically important parasitic disease of almost all domesticated animals in Zambia. However, very little work has been done to establish the pathophysiology of the disease in animals in Zambia. The changes in haematological and blood biochemical parameters of infected animals are important in disease prognosis, treatment rationale and in determining the organs which have undergone pathological changes. Goats are important livestock in tsetse-infested areas of Zambia probably due to their exhibitance of partial trypanotolerance.

The course of experimental *Trypanosoma congolense* (IL3000) infection in indigenous Zambian goats was followed during which the parasitaemia, clinical parameters, haematological parameters and serum proteins profiles were determined and erythrophagocytosis was investigated as a mechanism of anaemia. Parasitaemia was determined using the buffy coat method, while the packed cell volume (PCV) was measured by the haematocrit method. The red blood cell (RBC) counts, white blood cell (WBC) counts and haemoglobin (Hb) were determined using an electronic cell counter. The RBC indices were derived from the measured PCV, RBC counts and Hb. Erythrophagocytosis was investigated using radioisotope assays and microscopic methods. Serum total protein and albumin were determined by chemical methods (Biuret and Bromocresol green dye), the serum globulins concentration calculated by subtraction of globulin concentration from the serum total protein concentration and the A:G ratio determined.

The strain of *Trypanosoma congolense* used was pathogenic, it produced disease in the goats characterised by rapid progressive anaemia, early phase leucopenia, high parasitaemia and classical clinical signs of trypanosomosis. Statistically significant ($P < 0.05$) mean reductions in values of PCV, haemoglobin and RBC counts were observed between the infected and the control goats starting from as early as 17 days post-infection and lasting up to 56 days post-infection. Erythrocyte indices were stable throughout the infection period resulting in normocytic-normochromic anaemia. Significant ($P < 0.05$) evidence of erythrophagocytosis was observed using both radioisotopic and microscopic techniques. The mean serum total protein and globulin levels increased significantly ($P < 0.05$) within 3 weeks of infection and remained elevated until the end of the experiment. It was evident the mean albumin levels did not show any significant ($P > 0.05$) variations while the A:G ratio significantly ($P < 0.05$) dropped in the fifth week and remained consistently low.

This study showed that *T. congolense* infection in indigenous Zambian goats causes anaemia and that erythrophagocytosis is one of the mechanisms of this anaemia. Moreover, marked changes in serum protein profiles develop which are associated with the pathophysiology of the disease.

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CHAPTER ONE

LITERATURE REVIEW

1.1. Livestock distribution in Zambia

Zambia is about 7.5 million km^2 in area and has a population of about 8 million people (Central Statistical Office, Census 1990). The country is comprised of nine Provinces namely, Southern, Northern, Eastern, Western, North-Western, Central, Luapula, Lusaka and Copperbelt Provinces. Each Province has its own inherent kind of occupational activities.

The Southern Province has long been outstanding in terms of livestock and crop production at both traditional and commercial levels. Out of the total of about 3.2 million livestock (cattle, goats, sheep and pigs) present in Zambia, the Southern Province accounts for about 37.5% (Table 1.1). The traditional sector boasts of about 80% of the total livestock in the Province while only about 20% is under the commercial sector. The cattle population in the Province is around 70% while goats and sheep account for 23% and 1.1%, respectively, with the remaining 6% being pigs of the total livestock population (MAFF, 1994).

The Eastern Province ranks second with a population of about 16.7% of the total livestock in the nation, and almost all the livestock in the Province are under the traditional sector. About 44% of the livestock in the Province are cattle while sheep and goats account for 3% and 31% respectively. The pig population in the Province, amount to 22% of the total livestock in the Province (MAFF, 1994).

The Central Province has about 37% of its livestock in the commercial sector with the rest under the traditional sector. The Province has about 13.6% of the total livestock in the country, and of this, 80% are cattle, 12% are goats, 3% are sheep and 5% are pigs (MAFF, 1994).

The Copperbelt Province whose inherent occupational-activities have been mining and industry, is now improving in terms of livestock production with about 3.4% of the total livestock in the nation and out of this, 58% are under the commercial sector. The cattle population is about 57% of the total livestock in the Province. The pig population is around 25% while goats and sheep number 15% and 3%, respectively (MAFF, 1994).

The Northern Province, has about 2.7% of the total livestock in the nation. About 91% of the livestock in the province is under the traditional sector. Cattle account for 54% whilst goats and sheep number 32% and 9%, respectively, with pigs being about 5% of the total livestock in the Province (MAFF, 1994).

The North-Western Province has about 3.2% of the total livestock in the nation and almost all the livestock are under the traditional sector. About 56% of the livestock in the Province are cattle, 29% are goats, 10% are pigs and about 5% are sheep.

The Luapula Province of late, has shown a steady increase in the livestock population. The Province accounts for 2.5% of the total livestock in the country and 97% of this is under the traditional sector (MAFF, 1994).

Lusaka province accounts for about 4.5% of the nation`s total livestock population. The Province has much of its livestock under the commercial sector.

Table 1.1: Livestock distribution in Zambia (MAFF, 1994)

Province	Total Livestock	National %	Traditional sector %	Commercial sector %	Goat %	Cattle %	Sheep %	Pig %
Southern	1,200,353	37.5	80	20	23	70	1.1	6
Western	519,955	16.2	100	0	2	97	0	1
Eastern	533,791	16.7	99	1	31	44	3	22
Central	434,298	13.6	63	37	12	80	3	5
Northern	85,702	2.7	91	9	32	54	9	4
Copperbelt	109,149	3.4	42	58	15	57	3	25
N/Western	101,220	3.2	99	1	29	56	5	10
Luapula	80,317	2.5	97	3	3	16	11	14
Lusaka	144,000	4.5	-	-	-	-	-	-

Starting from the late 1980s, Zambia has recorded gradual decreases in the population of livestock particularly cattle, due to drought and economically important tick-borne diseases (i.e. theileriosis, babesiosis, anaplasmosis and heartwater), trypanosomosis, anthrax, haemorrhagic septicaemia and blackleg. This has been more pronounced in the Southern Province where most peasant farmers have been left with virtually no livestock. From 1990 to about 1993, the country recorded a 12% decline in the total cattle population. This decline was attributed to mortalities due to diseases. The mortality rate due to theileriosis was between 30 - 40%, while that due to trypanosomosis was 24 - 35% and other tickborne diseases was 15 - 20% (MAFF, 1996).

Other infectious diseases (anthrax, haemorrhagic septicaemia, blackleg and cutaneous dermatophilosis) in cattle showed a mortality rate of 15% (MAFF, 1996). However, there has been an increase in the population of small ruminants (goats and sheep), an indication that the integration of improved and serious goat farming in Zambia with other farming activities could be important in finding an alternative activity which can survive the general economic problems caused by drought, high cattle diseases and the general high cost of cattle rearing (Lovelace *et al.*, 1989).

1.2. The indigenous Zambian goat.

Tropical Africa contains about one-third of the world's total goat population. Small ruminants in Africa have been estimated to contribute about 1.15 million tonnes of meat

(16% of the world output), 1.99 million tonnes of milk (14% of the world output) and 21,100 tonnes of skins which is about 15% of the world production (Wilson, 1988).

There is very little work which has been done to establish any biological information or to systematically study health and disease of the small ruminants in Zambia (Lovelace *et al.*, 1989). There over 500,000 goats in Zambia with almost 99% of this in the traditional sector (Lena, 1990; MAFF, 1994). The stocking density of small ruminants in Zambia is less than 7 goats per km^2 with a ratio of less than 0.7 goats to one person. Small ruminants in Zambia contribute less than 8% of the total domestic ruminant biomass (Wilson, 1988).

Africa's human population is growing at a rate of about 3% per annum which is faster than that of any other region of the world. Sub-Saharan Africa alone, with a present population of 500 million people, will have an estimated population of about 1,300 million people by the year 2025 if the present trend of population growth continues (Bulatao *et al.,* 1990). The rate of population growth is posing severe problems for African agriculture (F.A.O., 1986). To meet the escalating population demands, Africa will require an annual increase of 4% in the supplies of livestock products which will mean meat and milk production would have to reach 43 million and 19 million tonnes, respectively, by the year 2025. This will be sufficient to feed the growing population, improve nutrition and eliminate food imports (Plaizier, 1993).

In Zambia, like most countries in Africa, farmers regard cattle as being more important than goats because of the multi-purpose uses of cattle versus goats (Shumba, 1993). However, in Africa there are now brighter opportunities for goat production than cattle production. The consecutive droughts which have continuously occured in the southern part of Africa coupled with the high prevalence of cattle disease (particularly in Zambia) have devastated the cattle population. As a result, most peasant farmers in Zambia have suffered severe losses in cattle populations. However, the goats have stood the test of the hard times and this is attributed to several biological advantages which the goats have over most other livestock (Shumba, 1993).

Goats are versatile animals which are able to adapt to various ecological environments. The semi-arid areas which are prevalent in the parts of Zambia where livestock production is prominent, provide a variety of browse plants suitable for goats` feeding habits. Goats can survive under adverse nutritional conditions and have high resistance to disease and dehydration. The integration of goats with cattle results in efficient resource utilisation (Shumba, 1993). Cattle prefer to graze resulting in under utilisation of the browse component, whereas goats have been found to spend about 60% of their feeding time browsing. Hence, the use of goats and cattle in the production system could increase efficiency of utilisation of the vegetation and prevent bush encroachment (Mukungurutse, 1993).

Moreover, goats have several socio-economic advantages. Goats require low investment, and have low risk of loss by individual deaths compared to cattle. The high productivity and fast turnover rate due to earlier maturity and shorter generation interval is yet another advantage resulting in faster returns on investment. Goats can supply meat in suitable quantities for rural families and the surplus animals can be sold easily unlike cattle which are regarded as capital. Socially, goat meat is acceptable to many people and religions. Goats are also easy to manage with only family labour (Lovelace *et al.*, 1989; Shumba, 1993).

1.2.1. Size and productivity of the indigenous Zambian goat

The indigenous Zambian goat is considered as part of the widespread population of goats called 'Small East African goat' referring to a non-uniform geographical population (Mason and Maule, 1960; Nalubamba, K.S., personal communication). They are variously coloured with a combination of colours (black/white/brown/grey) with a short, smooth coat and short ears. Tassels are common and animals of both sexes are horned. Although small they are not anatomically dwarfs (Mason and Maule, 1960). The fully grown goats have an average weight range of 20 - 27 kg. The animal has a relatively poor growth rate and is particularly adapted to heat stress, high radiation and dry conditions (Lovelace *et al.*, 1993).

The environment in which the Zambian goat thrives is characterised by a short summer rainy season of approximately 5 months duration (November to March-April) and a long dry season. The animals are kept chiefly for meat production. The Zambian goats are efficient in the utilisation of nitrogen and have low requirements of protein for maintenance (Mason and Maule, 1960).

1.2.2. Management

Almost all the indigenous Zambian goats are under traditional management systems where four simple management procedures are used:

a.) Tethering: Here animals are tied to a tree using lose long ropes to allow the animals to move around, graze and browse the nearby grass and shrubs. This system protects animals from predators and theft and further protects crops in fields from being destroyed by the goats. However, the system has some disadvantages. The grazing and browsing area is limited and the animals are denied exercise which can culminate in poor health.

b.) Semi-extensive system: This is the most common kind of management system where animals are housed at night but left to graze freely during the day time. This system gives the goats a wide selection of grazing areas and allow them ample exercise. Often there is somebody assigned to watch after the animals during the day and guide them to the watering spot twice per day.

c.) Extensive system: Under this system animals are treated the same way as under the semi-extensive system except that they are not housed at night but left to congregate together usually under a tree. This system predisposes animals to predators and theft.

Moreover, the survival of the kids is reduced because they are always exposed to adverse weather conditions such as cold spells and rainfall.

d.) Intensive system: This system is only found in commercial settings where animals are confined at night and released to paddocks during the day (Lovelace *et al.*, 1989).

1.2.3. Diet selection

Goats are free to choose their own diet from the available feeds. From the end of the grain harvest in March-April through to an annual burn of residues in September-October, the goats have access to stovers, mainly maize, but also sorghum and millet. In some areas cotton leaves are now becoming an important feed from May to July. A wide range of pods, fruits, fallen leaves, tree and shrub leaves within reach, and weeds of fallow lands form important feed components (Mason and Maule, 1960). Goats in the traditional sector are wholly dependent on rangelands nutrition, with no supplementary feeds during the dry season (Sibanda, 1993). Goats have a good choice of leguminous plants in their grazing habits.

1.2.4. Goat reproduction

Generally, goats (both male and female) attain puberty at the age of 4 - 5 months (Dunn P., 1987). They are polyoestrous animals and breed all year round with a promiscuous pattern of mating. The duration of oestrous (heat) is 12 - 48 hours and this comes once in an oestrous cycle of 21 days. The gestation period is about 150 days (Dunn P., 1987). Studies on the reproductive patterns of indigenous Zambian goats have shown a kidding percentage of 110 %, a weaning percentage (at 6 months) of 87 % and birth weight difference of 20 % between single and twin born kids (Mwenya, 1978). In Zambia, two kidding peaks have been observed to be between March and July, while neonatal mortality as observed in the Gwembe valley, is 21 % (Quartermain and Broadbent, 1974). The twinning rate is 13 % of all births while the average kidding interval is 223 days (Mason and Maule, 1960). A study carried out in the Gwembe valley showed great variation in the herd sizes ranging from about 30 - 300 animals with a buck:doe ratio of 1:18 (Lovelace *et al.*, 1993). Gwembe valley has the highest density of goats in Zambia, while Luangwa (Eastern Zambia) ranks second (Lovelace, personal communication). Quite low values of energy and protein are required for maintainance of in goats in pregnancy (Aregheore *et al.*, 1992). However, some researchers have highlighted major constraints in the reproduction of traditional goats in many parts of Southern Africa. These include high pre-weaning mortality rates, poor kid growth and low fertility rates (Sibanda, 1992). Low kidding rates and low fertility rates have been attributed to several factors one of them being trypanosomosis as evidenced in the study in the Luangwa valley, Eastern Zambia (Wilson, 1988; Bealby et al., 1993).

1.2.5. Diseases of goats in Zambia

The problems of disease have been better researched in cattle than in goats and sheep. Diseases are likely to be the most important problem limiting small ruminant production, and failure to reach maximum reproductive potential due to disease-related wastage is an important constraint in small ruminant production in Zambia (Wilson, 1988). Moreover, health care, if any, for small ruminants is mainly based on traditional ethno-veterinary medicines (Sibanda, 1993). A number of diseases have been identified to be of economic importance in goats in Zambia (Stafford, K.J.S., personal communication). One of the most important haemoparasitic diseases of goats is trypanosomosis, caused by protozoa of the genus *Trypanosoma*. In goats the major species are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosma brucei brucei*. This is a tsetse transmitted disease causing debilitation, loss of production and death. Heartwater, another haemoparasitic disease is a rickettsial disease affecting mostly young goats. Skin diseases of particular importance in goats include mange (caused by *Scabie* species), senkobo (a dermatophilosis) and ringworm. Bacterial footrot is particularly common in the rainy season when grazing areas are muddy. Bacterial pneumonia has been found to be a common problem among young goats during wet humid seasons. Common worm infestations of goats include trichostrongyliosis and fascioliasis. Clinical effects of tick bite lead to lameness in goats and tick fever.

1.3. Tsetse fly distribution and trypanosomosis in Zambia

1.3.1. Tsetse fly distribution in Zambia

Transmission of trypanosomes from one host to another is chiefly by the blood sucking tsetse flies and this is called cyclical transmission. Outside the tsetse areas, other biting flies, particularly of the *Tabanus* species have been seen to be the primary mechanical vectors (The Merck Veterinary Manual, 1991).

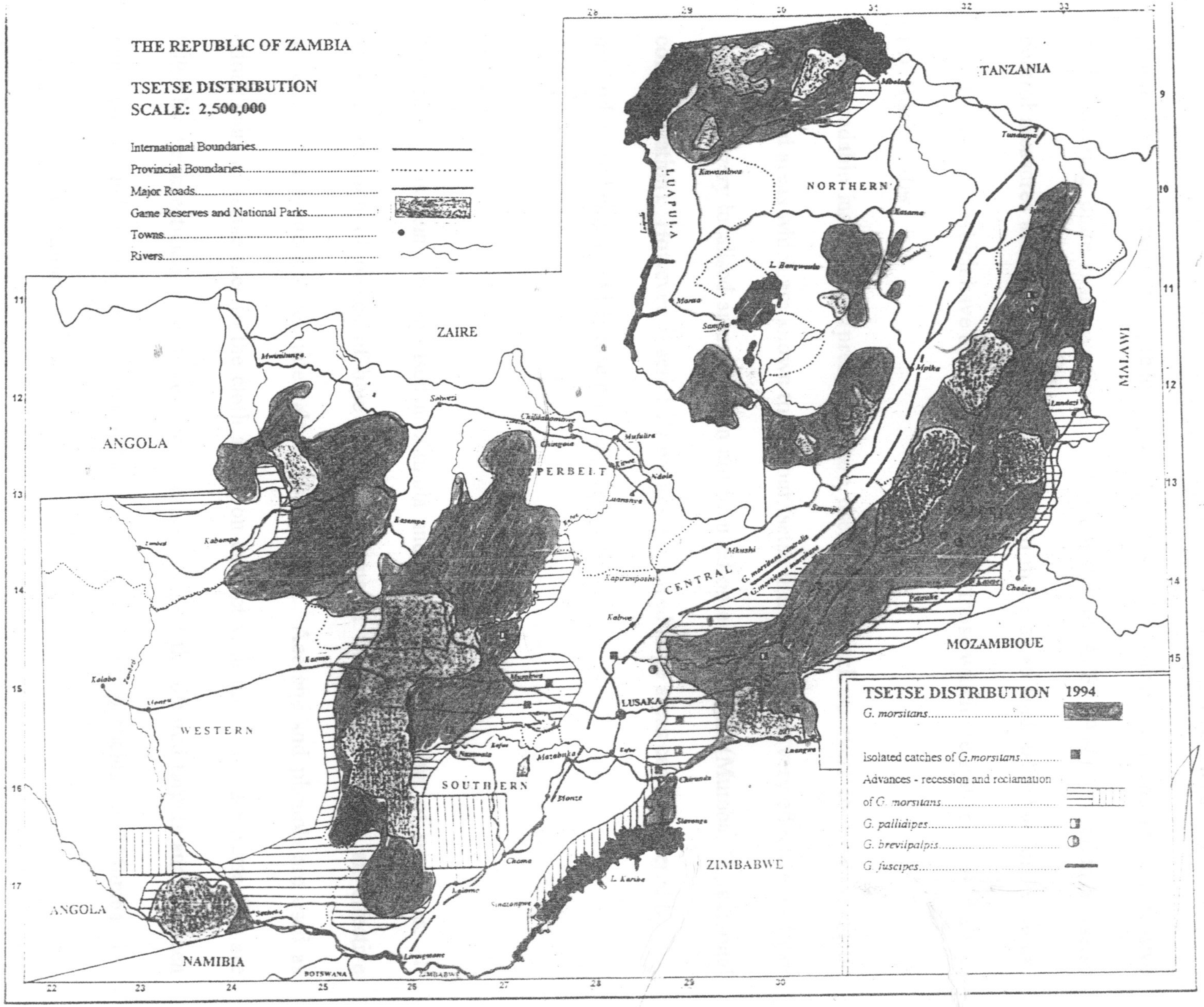
There are over 22 species of the tsetse fly in Africa and the genus *Glossina* is of paramount importance in the transmission of trypanosomosis. The fly is distributed over an area of about 10 million km^2 within latitudes 14^0 North and 30^0 South, about 37% of arable land in Africa (Lorne, 1986; Ngulube, E.M., personal communication). In Zambia much of the arable land is tsetse infested and seven fly-belts based on the ecological conditions have been identified. These include the Western Province extending up to the Upper Zambezi plain of the North-Western Province, the Kafue Basin extending from Kalomo to Namwala, Mumbwa up to Solwezi, the lower Zambezi Basin from Batoka to Chirundu and Luangwa North, the Lunsemfwa South Basin and the River Luapula. This claims a huge proportion of arable land suitable for agriculture (Figure 1.1). Between the extremes of abundant tsetse, presenting what may subjectively be called a 'heavy' trypanosomosis challenge to domestic livestock, and tsetse-free and hence disease-free localities, there is a variety of different patterns of the fly and disease distribution. These patterns are not static and hence the dynamic nature of the epidemiology of animal trypanosomosis is primarily determined by the movements and changing densities of the tsetse fly. Out of the 22 species of tsetse flies found in Africa, 4 species have been recorded in Zambia, namely *Glossina morsitans; Glossina pallidipes; Glossina brevipalpis and Glossina fuscipes* (Mumba, D., personal communication). These tsetse fly species occur in different ecological localities.

Glossina morsitans form the basis of African trypanosomosis because they occupy the savanna woodlands of Africa (often suitable locations for domestic livestock) and feed readily on cattle, sheep and goats (The Merck Veterinary Manual, 1991).

Glossina fuscipes are habitants of either tropical forest or forest outliers in the savanna and, as such, rarely come in contact with domestic animals. With the clearance of the forests for farming, *Glossina fuscipes* are being driven away from most areas. This species of tsetse fly can be heavily infected with trypanosomes and contribute to the maintenance of the reservoir of infection in wild animals (Mumba, D., personal communication).

Glossina pallidipes occupy habitats ranging from humid forests, through dense riparian vegetation in the humid savannas, to sparse riparian vegetation in semi-arid savannas. They occupy a variety of man-made habitats, but they are not as effective vectors of trypanosomes as are *Glossina morsitans* (The Merck Veterinary Manual, 1991).

Figure 1.1: Map of the Republic of Zambia showing the distribution of tsetse flies. Note that where there are tsetse flies, trypanosomosis is prevalent (Adapted from the Regional Tsetse and Trypanosomosis Control Programme, Progress Report for 1997, Lusaka, Zambia).


THE REPUBLIC OF ZAMBIA

TSETSE DISTRIBUTION
SCALE: 2,500,000

1.3.2. Tsetse fly control methods in Zambia

With the growing population in Zambia, more land is being claimed for livestock production. However, the spread of the tsetse fly is becoming more and more extensive now, covering more than two-thirds of the country (MAFF, 1994). Therefore, tsetse control measures are now being used in order to make more land habitable for livestock. Control methods which have been used in Zambia and those which are still being used are:

(a) Insecticides

Biodegradable insecticides (prethroids) are used in knapsack sprayers to spray thickets, bushes and areas which are known to harbour tsetse flies. This is not very effective as it can cover only very localised areas and can not be used on a large scale (Mumba, D., personal communication). However, if aerial spraying is employed large areas can be covered but the method is not being used because it is very expensive.

(b) Targets

This method is being widely used in Zambia and has scored a number of successes. It involves erection of pieces of blue cloth (impregnated with deltamethrine, an effective insecticide) at predetermined intervals of 100 m apart. Acetone and phenols are used as odour baits of tsetse flies to the cloth and upon contact with the cloth, the tsetse flies are killed by the insecticide. The system involves servicing of the planted targets and has been modeled in such a way as to incorporate local community participation (Mumba, D., personal communication).

c.) Use of acaricides

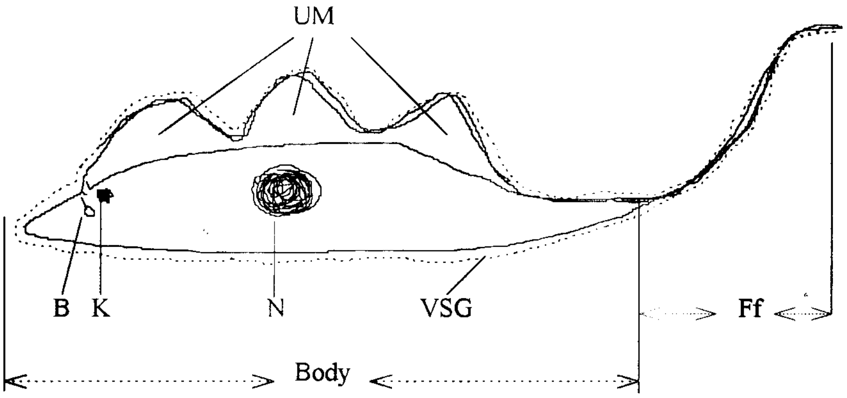
Certain acaricides used for the control of ticks (i.e. pyrethroids) are also effective in the control of tsetse flies. However, this is one method which is not being used widely unless there is a tick control programme in a particular area (Mimba, D.C., personal communication).

1.3.3. Morphology of trypanosomes

Trypanosomosis is a disease caused by the protozoa of the genus *Trypanosoma* affecting all mammalian species with varying degrees of pathogenicity (Lorne, 1986). Trypanosomes are extra-erythrocytic haemoprotozoan parasites of mammals and amphibians. They are flagellated protozoa of the family *Trypanosomatidae* with several genera and species in existence. All species are parasitic in habit and and most of the species require a vector for transmission. They live in the blood of the host, but some do also invade other body fluids (cerebrospinal fluid) and body tissues (Adam *et al.,* 1971; Lorne, 1986).

Morphologically, trypanosomes are elongated cells with one nucleus near the centre and have a flagellum which appears to arise from the kinetoplast (organelle with structural similarities to mitochondria which contains DNA). They do possess an undulating plasma membrane which is externally covered by a variable surface glycoprotein (Figure 1.2).

Figure 1.2: Morphology of blood stream trypanosomes (Chitambo, H., personal communication)



Key to abbreviations:

B - blephaloplast

K - kinetoplast

N - nucleus

UM - undulating membrane

VSG - variable surface glycoprotein (pellicle)

Ff - free flagellum

NB: only blood stream forms (trypomastigotes and metacyclic forms) have a pellicle with variable antigenic types which is a major obstacle towards the development of a potent vaccine.

Trypanosomes of particular economic importance in Subsaharan region are *Trypanosoma brucei brucei (T.brucei); Trypanosoma congolense (T. congolense); Trypanosoma vivax (T. vivax)* and *Trypanosoma simiae (T. simiae).* These trypanosomes not only differ in their pathogenicity in different hosts (Table 1.2.), but they also have different morphological features which are used as a tool in their identification. Visually, *T. vivax* has a short free flagellum and a poorly developed undulating membrane. Most of its cytoplasm is posterior to the nucleus and the kinetoplast is large and subterminal. The posterior end is broad and bulbous. *T. congolense* is a small trypanosome without a free flagellum. The undulating membrane is poorly to moderately developed while the kinetoplast is marginal and away from the blunt posterior end. *T. brucei* exists in 3 forms; the short form, the intermediate form and the long slender form. The short form is very stout with a well developed undulating membrane but no free flagellum. The kinetoplast is small and subterminal to the broad posterior end. The intermediate form is moderately long with a free flagellum and a well developed undulating membrane. The kinetoplast is small and subterminal to the blunt posterior end. The long form has a slender body with a long free flagellum and well developed undulating membrane. The kinetoplast is small and subterminal to the posterior pointed end. *T. simiae* has a body structure like *T. congolense* but does exist in polymorphic forms (Chitambo, H., personal communication).

Table 1.2: Pathogenicity of trypanosomes in various hosts (Lorne, 1986)

Trypanosome	**Horse**	**Cattle**	**Sheep**	**Goats**	**Camels**	**Pigs**	**Dogs**
T. brucei	+++	+	+	++	+++	±	+++
T. congolense	++	+++	++	++	++	±	+++
T. simiae	R	-	+	++	+++	+++	R
T. vivax	++	+++	++	++	+	R	R

+++, ++, +, ± = level of pathogenicity; R = resistant.

1.3.4. Life cycle of important trypanosomes

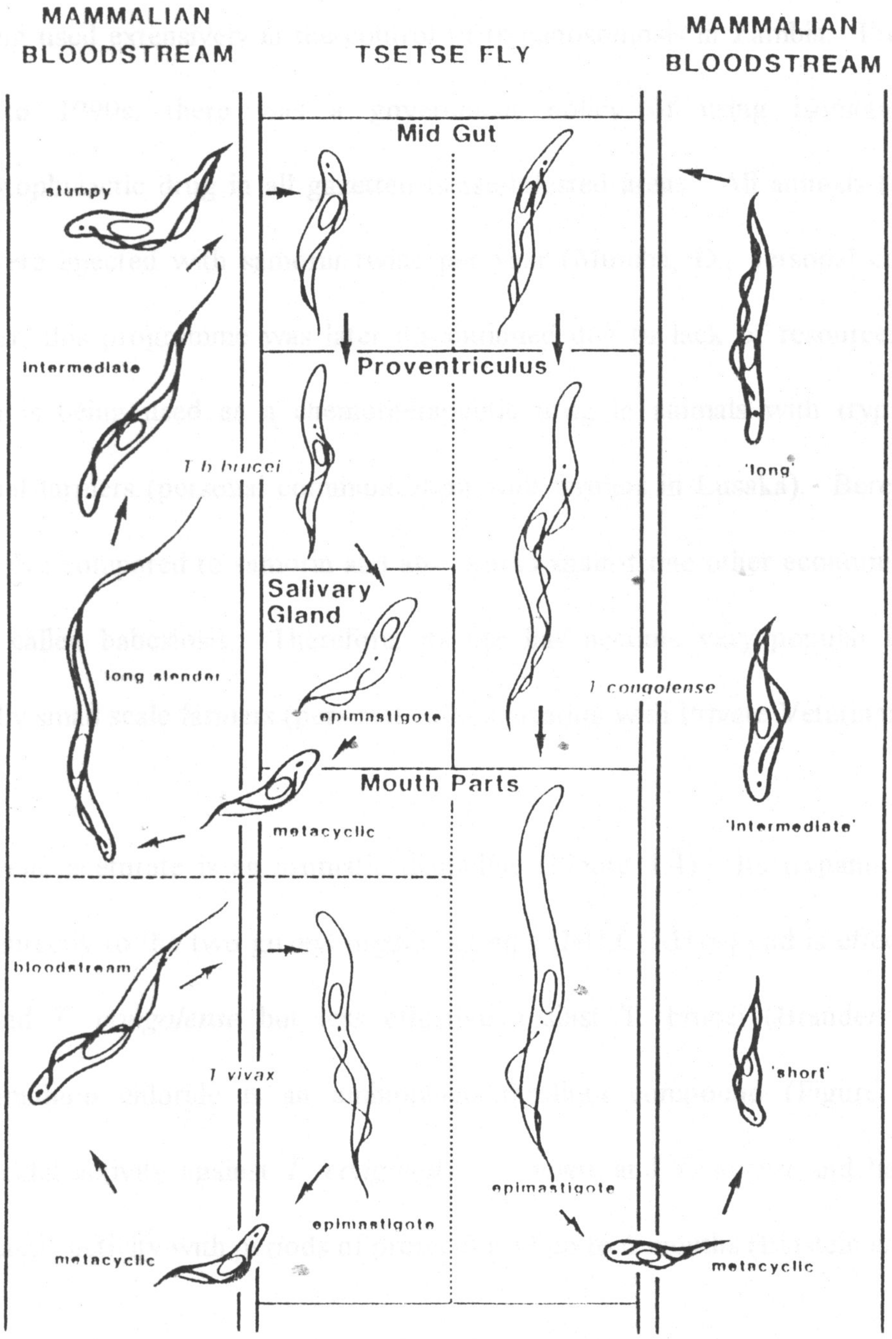
Different species of trypanosomes of the salivarian type found in the sub-Saharan regions exhibit different life cycles (Figure 1.3).

T.brucei has the tsetse fly as the vector in which it develops in stages. The first stage of development occurs in the mid-gut of the tsetse fly after picking the parasites (in the trypomastigote form) when the tsetse fly takes its first meal. The parasites then move to the proventriculus where the second stage of development takes place. The final developmental stage into the infective metacyclic form takes place in the tsetse fly salivary glands. This form possesses variant surface coat glycoproteins (Adam, *et al.,* 1971; Lorne, 1986).

T.congolense parasites develop over a 2 - 4 weeks period in the tsetse fly mid-gut, proventriculus and finally in the mouth-parts (hypopharynx) where the infective metacyclic forms are produced. The tsetse flies are usually infected when they take their first meal (with the trypomastigote form) after emerging from the puparium (Adam *et al.,* 1971; Lorne, 1986). *T.vivax* parasites develop entirely in the mouth-parts. Tsetse flies of any age can become infected but teneral flies are more easily infected.

During the course of infection in the mammalian host, *T. brucei* undergoes morphological changes to forms which are infective to the tsetse fly. *T. congolense* and *T. vivax* are said to undergo differentiation in the mammalian host, which is not accompanied by obvious morphological changes.

Figure 1.3: Life cycle of important trypanosomes namely *Trypanosoma brucei, Trypanosoma congolense* and *Trypanosoma vivax.* Heavy outlines indicate parasite forms with surface coats containing variable glycoprotein antigens. Light outlines indicate uncoated forms which are not infective to mammals (Adapted from International Laboratory for Research on Animal Diseases, ILRAD, 1983, Annual report p27, Nairobi, Kenya).


MAMMALIAN BLOODSTREAM
TSETSE FLY
MAMMALIAN BLOODSTREAM
Mid Gut
Proventriculus
Salivary Gland
Mouth Parts
stumpy
intermediate
T b brucei
long slender
epimastigote
metacyclic
bloodstream
T vivax
epimastigote
metacyclic
'long'
T congolense
'intermediate'
'short'
epimastigote
metacyclic

1.3.5. Trypanosomosis control using drugs

Isometamedium chloride (samorin) and diminazene aceturate (berenil) have been and are still being used extensively in the control of trypanosomosis in Zambia. From about early 1980s to 1990s, there was a government policy of using isometamedium as a chemoprophylactic drug in all gazetted tsetse-infested areas. All animals (cattle) in these areas were injected with samorin twice per year (Mumba, D., personal communication). However, this programme was later discontinued due to lack of resources. At present, samorin is being used as a chemotherapeutic drug in animals with trypanosomosis by individual farmers (personal communication with farmers in Lusaka). Berenil is relatively inexpensive compared to samorin and also works against one other economically important disease called babesiosis. Therefore, its use has become very popular among farmers especially small scale farmers (personal communication with Private Veterinarians).

Diminazene aceturate is an aromatic diamidine (Figure 1.4). Its trypanocidal activity is related directly to the two guanyl organic groups [NH:C(NH_2)-] and is effective against *T. vivax* and *T. congolense* but less effective against T. brucei (Brander *et al.*, 1991). Isometamidium chloride is an aminophenanthridium compound (Figure 1.5). It has trypanocidal activity against *T. congolense, T. vivax* and *T. evensi* and has considerable prophylactic activity with periods of protection of up to 6 months (Einstein *et al.*, 1994).

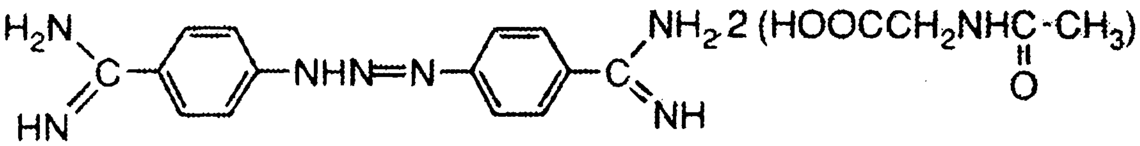


Figure 1.4: Formula of diminazene aceturate (Brander *et al.*, 1991).

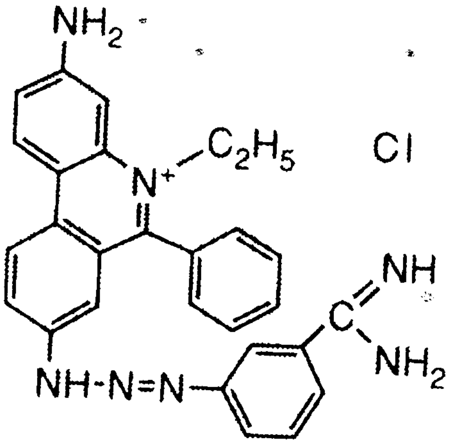


Figure 1.5: Formula of isometamidium chloride (Einstein *et al.*, 1994).

1.4. Characteristics of goat blood

Blood is a tissue consisting of fluid and cellular constituents. It is always circulating through vessels supplying the substances for the survival of all body cells as well as carrying metabolic waste products to excretion organs. As its major function, blood carries supplies of oxygen, essential nutrients, hormones, water, electrolytes and buffering substances to the tissue cells. The supply of these substances is received either by direct contact of blood with other tissues or by diffusion of the substances through membrane barriers. Broadly, blood can be grouped into four distinct components namely erythrocytes, platelets, leucocytes and plasma (Coles, 1986).

1.4.1. Erythrocytes

Goat erythrocytes are the smallest among all the domestic animals. The average diameter of a goat erythrocyte is 4 µm (Swenson, 1977). Their shape can be fairly discocytic and slightly biconcave or bluntly triangular and they lack a central pallor nor do they exhibit rouleaux formation (Jain, 1986). In goat kids variations in size (anisocytosis) of erythrocytes is a common feature but this is very infrequent in adult animals. Variation in shape (poikilocytosis) is prominent in kids and in some mature goats of certain breeds (Jain, 1986). Age-related variations in the total number of erythrocytes occur, with relatively low levels at birth (7 to 8 million/µl) which reach highest levels at about 8 months of age and then gradually fall to stabilize at about 8 to 18 million/µl by 1 year of age (Coles, 1986; The Merck Veterinary Manual, 1991).

Erythrocytes contain haemoglobin which give them their main inherent function of transporting oxygen. Erythrocyte indices namely, the Mean Corpuscular Haemoglobin (MCH), and the Mean Corpuscular Haemoglobin Concentration (MCHC) are used to express the haemoglobin content in an erythrocyte. The MCH expresses the amount of haemoglobin (in picograms) in an average erythrocyte of a population of cells, while the MCHC expresses the ratio of weight of the haemoglobin to the volume of the erythrocyte in grams per decilitre (g/dl). The published goat normal values for MCH are in the range of 5.2 - 8 pg, while for MCHC are in the range of 30 - 36 g/dl (The Merck Veterinary Manual, 1991). The Mean Corpuscular Volume (MCV) is the red cell index which expresses the average volume of a population of erythrocytes in femtolitres (fl) and the published normal values are in the range of 16 - 25 fl. These indices are important in determining the state of erythrocytes. Variations from the normal range of the indices would indicate a problem with the integrity of erythrocytes which can be caused by a number of pathological factors (Jain, 1986; Dacie and Lewis, 1994). The Packed Cell Volume (PCV) expresses the percentage of blood composed of erythrocytes and in goats the published normal range is 22 - 38 % (The Merck Veterinary Manual, 1991). The PCV is an important haematological parameter in determining pathological aberrations of the blood system. Erythrocytes are primarily made in the bone marrow and the spleen where they also undergo a series of maturation stages before they are released into circulation.

1.4.2. Platelets

These are structures with various sizes and shapes but are mostly small, round and densely packed with functional prominent azurophilic granules. They are numerous in circulating blood and function in blood clotting or haemostasis. When blood vessel walls are damaged, collagen is exposed and circulating platelets attach and undergo a change in shape with the accompanying release of Adenosine diphosphate (ADP). This ADP stimulates local platelet aggregation with the formation of the primary plug which is consolidated by the action of platelet contractile proteins (Jain, 1986; The Merck Veterinary Manual, 1991). Platelets are also important in the inflammatory response by releasing various mediators and are a source of phospholipids needed for normal coagulation. Platelets are made in the bone marrow from megakaryocytes.

1.4.3. Leucocytes

Leucocytes are what are termed as the white blood cells and when a haemogram is developed they form a distinct greyish-white layer at the interphase of the plasma and the red cell mass. Leucocytes are less numerous in the blood stream than the erythrocytes are. The total number of leucocytes in the blood stream is very dynamic because of their functional diversity in immune mechanisms (Coles, 1986). Leucocytes are subdivided into several functional units namely neutrophils, eosinophils, basophils, lymphocytes, monocytes and macrophages.

a.) Neutrophils: These cells form the first line of cellular defense against microbial infection. Neutrophils are polymorphonuclear (cells with several morphologies of the nucleus) leucocytes which develop from the finely granular myelocytes of the bone marrow. The nucleus is segmented with undulated nuclear membrane and clamped chromatin, while the cytoplasm is clear and contains numerous granules that are typically neutral in reaction (Allan, 1979; Jain, 1986). Neutrophils are the most active cells in acute inflammation where they migrate through the endothelial cells (cells lining the inner membrane of the blood vessels) junctions into the inflammatory exudate in large numbers and ingest (phagocytose) the intruding microorganisms and later die to form pus. Extensive infection leads to a temporary increase in the numbers of circulating neutrophils causing leucocytosis (increase in number of leucocytes). Neutrophils account for about half the total number of circulating leucocytes (Dacie and Lewis, 1994).

b.) Eosinophils: These cells are a little larger than neutrophils. When stained with Romanowsky dyes, eosinophils have bright pinkish-red, uniformly stained cytoplasmic granules and a polymorphic nucleus that is smoother and less segmented than that in mature neutrophils (Allan, 1979; Jain, 1986; Dacie and Lewis, 1994). The nucleus of the mature eosinophil in goat may be of band form or may present 2 or 3 lobes. The granules are small, round, numerous and strongly acidophilic and nearly fill the cytoplasm which stains pale blue (Jain, 1986). Eosinophils are important in controlling helminth infections and in regulating allergic and inflammatory reactions (Allan, 1979; Jain, 1986).

c.) Basophils: These cells are the rarest of the circulating leucocytes (Dacie and Lewis, 1994). Goat basophils contain numerous densely packed, round granules which stain (with Romanowsky dyes) purple with a reddish halo that gives the cytoplasm an overall reddish tinge. The nucleus is often in eccentric position and stains diffusely purple (Jain, 1986). Basophils play an important role in allergic and inflammatory processes.

d.) Lymphocytes: These cells are morphologically and functionally heterogeneous and are generally classified as small, medium and large. Functionally, they are grouped on the basis of their involvement in the immune response. T-cells (thymic-derived) are lymphocytes concerned with the cell-mediated immunity and immuno-regulatory functions while B-cells (bone marrow-derived) are concerned with the formation of humoral antibodies (Jain, 1986). The goat lymphocytes have a round to oval nucleus with an occasional kidney-bean indentation or are binucleated cells. With Romanowsky dyes, the cytoplasm stains pale blue with a few small to large, reddish-purple azurophilic granules. Lymphocytes provide a specific acquired immune response upon stimulation by antigenic components of the infecting microorganisms (Allan, 1979).

e.) Monocytes and macrophages: Blood monocytes and macrophages are components of the mononuclear phagocyte system (MPS). Other components of the MPS include histiocytes in the connective tissue; fixed and free macrophages in the lymph nodes, spleen and bone marrow; pleural and peritoneal macrophages; kupffer cells of the liver; alveolar macrophages; osteoclasts; and macroglial cells in the nervous system (Jain, 1986).

Generally, while circulating in the blood stream, macrophages are called monocytes (Allan, 1979). The goat monocyte is a large cell with distinctly blue cytoplasm having a ground-glass appearance. Several vacuoles, usually clustered to one side of the cell, are common. The nucleus of the goat monocyte can be ovoid, bandform or three-pronged with a stringy, diffuse chromatin. Macrophages have an abundant cytoplasm with many coarse azurophilic granules or vacuoles of varying sizes, mitochondria, bundles of microfilaments and microtubules, scattered rough endoplasmic reticulum and a well developed golgi complex. The nucleus is oval, indented or elongated with spongy chromatin, several prominent nucleoli, and a distinct nuclear membrane (Allan, 1979; Jain, 1986). Monocytes and macrophages function in phagocytosing infective agents (or their antigenic components) and also prepare the antigenic components of the infective agents in their cytoplasm and convey the infective agent to lymphoid tissue where the antigenic components are presented to the immunocytes to initiate an immune response (Allan, 1979).

1.4.4. Plasma

Plasma is the liquid medium in which the solid elements (erythrocytes, leucocytes and platelets) are suspended and it consists of water, electrolytes, metabolites, nutrients, proteins and hormones. When blood is coagulated the liquid media is termed serum which is devoid of coagulation factors (fibrinogen, prothrombin, calcium) which are present in plasma.

1.4.4.1. Plasma proteins

A major component of plasma is protein which is a very complex mixture including simple proteins and conjugated proteins such as glycoproteins and various types of lipoproteins. There are over 200 plasma proteins described and quantified in domestic animals. With the use of various methods such as fractionation, electrophoretic and chromatographic techniques, plasma proteins can be separated into fibrinogen, albumin and globulins (Kaneko,1980; Martin *et al.,* 1981).

a.) Albumin: Constitutes about 40 - 60% of the total plasma proteins, but it has the lowest molecular weight among the major protein molecules found in plasma. Its tertiary structure is globoid or ellipsoid (Kaneko,1980; Martin *et al.,* 1981; Coles, 1986). Albumin is synthesized by the liver as a chain of 610 amino acids and is catabolised by all active tissues. Its rate of metabolism varies among species. Albumin functions as a storage reservoir of amino acids and as transporter of amino acids for tissue proteins. Albumin can act as a carrier molecule for fatty acids, trace elements and many drugs. This assists in preventing rapid excretion of drugs and in detoxification and inactivation of materials that may be toxic to the animal body. Due to its abundance, albumin is the most osmotically active plasma protein and hence the largest contributor to the intravascular colloid osmotic pressure (Kaneko, 1980). Concentration of albumin in plasma can be used to assess an animal's protein intake, degree of hydration and liver function. Albumin concentration is also important in evaluation of other plasma constituents especially calcium, which is 50% albumin-bound in plasma (Bain, 1986).

b.) Fibrinogen: This is the precursor of fibrin that forms the blood clots and is synthesised in the liver where it is produced by the microsomes of the hepatocytes. Of all the plasma proteins, fibrinogen has the most rapid turnover and it is necessary to supply new fibrinogen to protect the vascular endothelium (Coles, 1986).

c.) Globulins: Globulins are protein molecules that are insoluble in plain water but soluble in salt water. The serum globulins are a heterogeneous complex mixture of protein molecules identified as alpha- (α-), beta- (β-) and gamma- (γ-) globulins according to their mobility in an electrical field. In the goat, each of these globulins has further been sub-divided.

Alpha globulins: This category of globulins consists of high density lipoproteins (HDL) i.e. α_1 and very low density lipoproteins (VDL) i.e. α_2. They are synthesized by the liver and are important positive markers of acute inflammatory diseases. The α_2 component is, for instance, markedly increased in both bacterial and viral infections (Kaneko, 1980; Coles, 1986). Alpha globulins are the most rapidly migrating globulins in serum electrophoretograms in the goat. In normal goat serum electrophoretograms, the α_2 fraction is not visualised.

Beta globulins: These are the second fastest migrating globulins in goats. They are subdivided into β_1 (fast) and β_2 (slow) fractions. Some important proteins in this group are transferrin, ferritin, complement (C3, C4), hemopexin and C-reactive protein. β-globulins are positive markers of acute inflammatory disease (Kaneko, 1980).

Gamma globulins (immunoglobulins): These globulins are a complex mixture of antibodies and are synthesized in B-lymphocytes or their derivative plasma cells. They are glycoproteins whose basic structure is a monomer composed of two heavy (H) and two light (L) chains joined by disulphide bonds. The H and L chains have variable regions which lead to further subdivision of the immunoglobulins (Igs) into subtypes and subclasses. This further leads to a variation in function of each subdivision. Igs are thus subdivided into IgG, IgM, IgE, and IgA. Increases in immunoglobulin levels occur in infections due to viruses, bacteria and parasites. Elevation in Igs can either be monoclonal (increase in one specific Ig associated with neoplasia) or polyclonal (overall increase in Igs). Polyclonal Ig increases occur in a variety of diseases which include trypanosomosis, chronic bacterial infections and liver disease (Tizard, 1987).

1.5. Trypanosomosis in ruminants

Little attention has been paid to trypanosomosis in sheep and goats compared to cattle in Africa because of the general view that goats and sheep are more tolerant to trypanosomosis than cattle (d`Ieteran and Trail, 1987). However, some observations in tsetse infested areas of Zambia (Bealby *et al.,* 1993), Zaire (Makumyaviri *et al.,* 1989), Kenya (Griffins and Allonby, 1979a) and Nigeria (Adah *et al.,* 1993) indicate that the disease is of considerable importance in goats and sheep (Luckins, 1992). Reports from East Africa further show that *T. congolense* infections in sheep and goats have been associated with severe economic losses (Kanyari *et al.,* 1983), however, the general view is that goats are more tolerant to trypanosomosis than cattle. Goats have been reported to be particularly susceptible to *T. congolense* and *T. vivax* infections (Ugochukwu, 1983) and in certain cases goats can even be more susceptible exhibiting higher parasitaemias than cattle (Masake, 1980). Small ruminants may also be important reservoirs (probably because of being highly trypanotelerant) of trypanosomes which may later be passed on to other livestock (Mahmoud and Elmalik, 1977).

Trypanosomes are inoculated into the skin of the animal in their metacyclic form where they grow causing a localised swelling known as the chancre. Trypanosomes then enter the local lymph nodes near the point of inoculation where they undergo further multiplication and then enter the blood stream via the lymph drain. In cattle with *T. congolense* infection, the chancre has been reported to be the first clinical sign following a bite from an infected tsetse fly (Akol and Murray, 1986) and this has been reported to always precede the detection of parasitaemia by several days (Akol and Murray, 1982). Akol and Murray, (1986), observed that in cattle with *T. congolense* infection, the chancre was detectable at around 5 days post-infection and was often followed within 1 or 2 days by marked enlargement of the regional lymph nodes (this has been proposed to be the induction of the immune response).

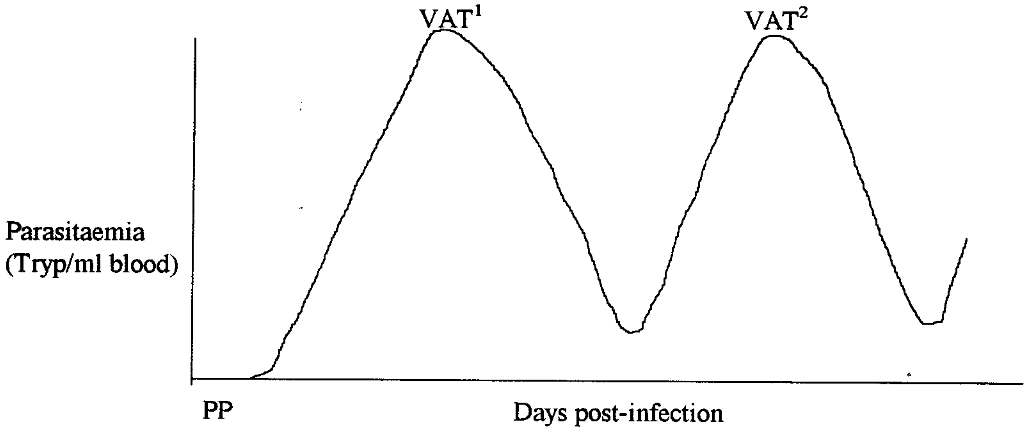
Prior to development of parasitaemia, trypanosomes have been reported to be detectable in lymphatic vessels draining superficial lymph nodes close to the site of the infected tsetse fly bite (Gray and Luckins, 1980; Akol and Murray, 1986).

In the bloodstream, trypanosomes divide rapidly by binary fission. In *T. congolense* infections, the trypanosomes then attach to the endothelial cells and localise in the capillaries and small blood vessels. *T. brucei* and *T. vivax* invade tissues causing tissue damage in several organs (Bungener and Mehlitz, 1977; Murray *et al.*, 1980; Emery and Moloo, 1981). Waves of trypanosome multiplication (Figure 1.6) are detectable in the host's blood, the peak and remission of each wave being characterised by an increasing percentage of non-dividing short stumpy forms of trypanosomes (Vickerman and Tetley, 1978; The Merck Veterinary Manual, 1991). The incubation period is usually 1 - 4 weeks.

The host's immune response to infection is vigorous, forming immune complexes (antigen-antibody complexes) which are thought to be involved in the pathogenesis of the disease. The antigens are components of lysed trypanosomes or are exposed variable antigens of the surface coat of the trypanosome. The surface coat is a mono-layer of glycoproteins and trypanosomes have multiple genes that code for different surface coat glycoproteins that are not vulnerable to the prevailing host immune response (Cross, 1978; Barbet *et al.*, 1978). These genes become keep changing to renew the surface coat with different glycoproteins. This leads to antigenic variation which results in trypanosomes eluding the host's immune response and hence persistence of the trypanosomes in the host. The antigen complexes cause inflammation which results in signs of the disease.

The vigorous locomotory activity of the trypanosomes inflict mechanical damage on their host by penetrating through or between cells. Trypanosomes have been reported to consume plasma proteins particularly albumin by pinocytosis through their flagella pocket (Langreth and Balber, 1975). Moreover, trypanosomes have been observed to release destructive lysosomal enzymes which are thought to be lytic to the host tissue cell (Vickerman and Tetley, 1978).

Figure 1.6: Repetition of the variable antigenic type population of trypanosomes



PP - Prepatent period is the time lapse between infection and the appearance of the trypanosomes in blood stream and varies from a few days to a few weeks.

VAT - Variable Antigenic Type is a given repertoir or trypanosome population which corresponds to specific immune response. Each VAT gives rise to the development of a different antibody response. The number of VATs in a given infection is not yet well defined (Chitambo, H., personal communication).

1.5.1. Effect of trypanosomosis on the productivity of ruminants

Trypanosomosis as a disease entity affects both the production of goats and sheep in a number of ways. Animals are wasted, lethargic, have a dull and starring coat (standing hairs) and show a 'hunched-up' appearance. The superficial lymph nodes are visibly enlarged and animals may show a sero-mucoid eye and nasal discharge and mucous membranes are pale. Goats have been noted to continue eating even when severely ill with *T. congolense* (Kaaya *et al.,* 1977). Anorexia, facial and submandibular oedema with intermittent temperature have been observed (Ikede, 1986; Ogunsunmi,*et al.,* 1994a), and terminally ill animals are extremely weak and are unable to rise. As the disease progress animals are tachycardic (show increased heart rate), show pulsating jugular vein and a progressively weaker pulse as bradycardia sets in. Terminally, they are bradycardic i.e. have low heart rate (Murray *et al.,* 1983). The reproductive performance of the goats is compromised with very low fertility rates (Bealby *et al.,* 1993). Trypanosomosis affects the general performance and production of the animals and is associated with quite high mortalities (MAFF, 1994). Animals with trypanosomosis tend to have a compromised immune system and are, therefore, more susceptible to secondary infections which would otherwise not affect them (Rurangirwa *et al.,* 1978).

Trypanosomosis acts as an environmental constraint, reducing livestock productivity or even making large rangeland areas unsuitable for livestock production (Jahnke *et al.,* 1987). However, small populations of cattle, sheep, and goat breeds have been found to be trypanotolerant (tolerant to trypanosomosis) in West and Central Africa (d'Ieteran and

Trail, 1987). These animals can survive and thrive in areas where the tsetse challenge is substantial although very high tsetse challenge leads to lower productivity of even trypanotolerant animals. In West Africa, certain breeds of cattle, the N'Dama and Muturu are well known to be trypanotolerant (Ayoade, 1977).

In explaining why the indigenous West African cattle, N'Dama and Muturu, are more resistant to trypanosomosis than the West African Zebu, Weitz, (1979), suggested that this is dependent upon immunologic differences (innate tolerance). Fiennes, (1970), noted that trypanotolerant breeds' ability to withstand the disease while adult depends on infection during calf-hood and constant exposure thereafter, implying that the tolerance is acquired and not innate.

Other factors observed to contribute to trypanotolerance are genetics, the transfer of maternal antibodies from the dam to the calf, low incidence of intercurrent disease and good management (Stephen, 1966; Roberts and Gray, 1973). Griffin and Allonby, (1979b), showed that indigenous breeds of goats and sheep in Kenya were able to withstand infections of *T. congolense* and concurrent parasitaemia, lasting several weeks, better than the exotic breeds. This upheld the concepts that constant exposure to the disease and innate tolerance lead to enhanced trypanotolerance.

1.5.2. Trypanosomosis effects on the blood parameters of ruminants

1.5.2.1. Haematological effects

Haematological parameters have been reported to be fundamentally dynamic during the pathogenesis of many haemoparasitic infections in domestic animals. In the assessment of the health status of an animal and in the diagnosis, treatment and prognosis of many tropical diseases, a recourse to the examination of the animal's blood is of paramount importance (Aba-Adulugba *et al.,* 1990a). As far back as 1861, David Livingstone had described the progress of trypanosomosis as depicting reduced blood volumes, severe anaemia, tissue destruction and presence of toxic substances in the blood.

Haematological and other changes in trypanosome infections are affected by several factors including the virulence of the parasite, the susceptibility of the host and the period of the infection during which samples are taken (Anosa, 1988). The development of anaemia is the most reliable indicator of the progress of a trypanosome infection. However, certain animals, particularly of the trypanotolerant breeds, have been reported to be parasitaemic but not to show evidence of anaemia (Murray, *et al.,* 1983). Observations made in goats with *T. vivax* (Emery *et al.*, 1980) and *T. brucei* infections (Barry and Emery, 1984), have shown that anaemia starts developing even before parasitaemia develops. This early stage anaemia could result from the reported presence of trypanosomes in lymphatic vessels prior to parasitaemia (Gray and Luckins, 1980; Akol and Murray, 1986). Adah *et al.*, (1993) reported that the course of *T. congolense* infection in goats showed progressive anaemia i.e. continually falling PCV, haemoglobin concentration and red blood cell counts. This has also been observed by earlier workers in sheep and goats with *T. vivax* and in mice with *T. congolense* (Ikede *et al.*, 1977; Ugochukwu, 1983).

Anaemia is therefore, no doubt, the most prominent sign of trypanosomosis in domestic animals. The changes in the blood picture pointing to anaemia are noted to begin during the prepatent period. The rapid decline in haemoglobin concentration, erythrocyte numbers and haematocrit values plus clear clinical signs of pallor of the mucous membranes in infected animals indicate that anaemia is important in the pathogenesis of trypanosomosis. Ikede *et al.*, (1977), Ugochukwu (1983), Ikede, (1986), and Igbokwe and Anosa (1989), observed that anaemia in ruminants with *T. vivax* and *T. congolense* infection was characterised by depressed erythrocyte values (PCV, haemoglobin concentration and red blood cell counts). The type of anaemia which occurs during trypanosomosis has been reported to be predominantly haemolytic in nature and associated with decreased life span of the erythrocytes (Oyewale, 1987).

The haematological effects during the pathogenesis of trypanosomosis are influenced by a range of variables, many of which are still unknown and others that are only poorly understood (Katunguka *et al.*, 1992a). It was established that anaemia in sheep infected with *T.congolense* was seen in 2 phases. In phase one, anaemia was seen to develop rapidly with increase in parasitaemia and reached a peak at 14 days post infection. In phase two, there was a persistent low packed cell volume (PCV) with or without presence of parasites and the anaemia seen was typed as macrocytic and normochromic (anaemia with red blood cells having larger size than normal but with a normal haemoglobin content). In both cases the anaemia seen was attributed to haemolysis as a result of immunological factors, trypanosome products, dyshaemopoiesis and haemodilution. The immunological factors are said to contribute to anaemia by production of antibodies against autologous erythrocytes and this has also been reported to occur in cattle infected with *T. vivax* (Katunguka *et al.*, 1992a).

The haemolysis observed above raises a lot of queries in that significant decreases in haematological values occurred within a few days of the animal having been infected even in the prepatent phase when trypanosomes are not seen in the peripheral blood. This leads to speculations on the production of haemolysin (erythrocyte damaging factor) by the parasite in whatever form it occurs in the body (Lorne, 1986). It has been reported that *T. congolense* and *T. vivax* are capable of producing haemolytic factor or factors (Murray *et al.,* 1980).

A number of aetiological factors for the anaemia observed in trypanosomosis have been proposed and the severity of the anaemia has been said to be dependent upon the species of the trypanosome, host and the course of the infection i.e. acute, subacute or chronic (Jennings *et al.,* 1974; Cox, 1979; Wery *et al.,* 1982). Haemolysis is one such mechanism and has been categorised into intravascular and extravascular haemolysis (Kobayashi *et al.*, 1976; Valli and Mills, 1980; Amole *et al.,* 1982). This has been particularly established in calves and mice infected with *T. congolense* and *T. brucei,* respectively. Dargie *et al.*, (1979), also cited extravascular and intravascular haemolysis to be an important aetiology of anaemia in *T. congolense* and *T. vivax* infections in cattle but whose mechanism was not clearly established.

The osmotic and mechanical fragility of erythrocytes have been observed to increase in *T. congolense* infection in cattle (Huan *et al.,* 1975). This leads to reduced lifespan of erythrocytes and hence decreased red cell mass as has been noted in ruminants with *T. vivax* and *T. congolense* infection (Anosa *et al.*, 1979; Igbokwe and Anosa, 1989) and this can be categorised as intravascular haemolysis. Disseminated intravascular coagulation of erythrocytes, a notable feature in trypanosomosis (Rickman and Cox, 1980) can be a mechanical aetiology of intravascular haemolysis. However, disseminated intravascular haemolysis has not been identified in goats (Jain, 1986).

1.5.2.1.1. Erythrophagocytosis as a cause of anaemia

Erythrophagocytosis (monocyte and macrophage phagocytosis of erythrocytes) in trypanosomosis has been reported to contribute significantly to anaemia through an immunological mechanism (Dargie *et al.*, 1979). The antibody-antigen (Ab-Ag) complexes deposited on the surface of the erythrocytes of infected animals as a result of trypanolysis and release of antigens (Ags), have been reported to convert the erythrocytes into foreign proteins which are then engulfed by macrophages derived from monocytes (Facer *et al.*, 1982; Lorne, 1986;; Kimeto, 1989). Increased erythrophagocytosis in cattle with trypanosomosis has been reported to have a multifactorial aetiology that may include haemolytic factors produced by trypanosomes, immunologic mechanisms, fever, disseminated intravascular coagulation and an active mononuclear phagocytic system (Karhe, 1974; Huan *et al.*, 1975; Murray, 1978).

Erythrophagocytosis is reported to be immunoglobulin-mediated through the macrophage Fragment crystallizable (Fc) receptors (receptors on surface of macrophages) for the IgG. This occurs due to altered antigenicity of the host animal's erythrocytes (Weiss and Klausner, 1988) during trypanosomosis. Trypanosomes contain many potentially destructive molecules i.e. destructive lysosomal enzymes, phospholipase haemolysins and the residual bodies of autophagosomes. Murray *et al.*, (1977), reported the presence of haemolytic factors in the sera of trypanosome infected cattle. The haemolytic material from *T. congolense* has been found to consist of a mixture of free fatty acids and phospholipase-A, whereas a haemolytic factor from *T. brucei* has been observed to be a small molecular weight protein (Amole *et al.*, 1982). These are discharged through the flagellar pocket and initiate erythrocyte cell surface change i.e. exposure of a cryptic antigen on the erythrocyte membrane, specific cleavage of erythrocytes and clustering of erythrocytes (Vickerman and Tetley, 1978).

Phospholipases released from trypanosomes have been suspected to cause endothelial damage of blood vessels resulting in disseminated intravascular coagulation. These phospholipases are also thought to act on the host phospholipids leading to liberation of potentially haemolytic non-esterified fatty acids (NEFA). Phospholipases together with

NEFA may cause damage to erythrocytes leading to early removal of the erythrocytes from the circulation by an activated mononuclear phagocytic system (Huan *et al.*, 1977; Gardiner *et al.*, 1988; Assoku and Gardiner, 1988).

The lysates of dying trypanosomes are reported to coat erythrocytes surface membranes thereby imposing on them a coat recognised as foreign (Murray, 1978). Some of the affected erythrocytes are directly lysed by these effects, but those which remain viable induce host immunological responses against self-erythrocytes.

The host animal produces IgG and IgM which bind the modified erythrocytes through an antigen-antibody complex formation using complement C-3 (Kay and Douglas, 1977; Facer *et al.*, 1982). Immunoglobulins have been observed to adsorb onto erythrocytes of calves with *T. congolense* infection (Kobayashi *et al.*, 1976). Amole *et al.*, (1982), noted that IgM was the particular Ig involved in the immunological mechanism of anaemia in mice with *T. brucei* infection. The increase in the concentration of IgM in the sera appears in the early phase of the disease especially in acute cases and diminishes as the disease progresses to the chronic state. Increased IgM has also been reported in sheep with *T. brucei* infection (Ogunsanmi *et al.*, 1994b). Hence, the correlation of the rise in the concentration of IgM in serum with the haemolytic crisis lead Amole *et al.*, (1982), to deduce that IgM was the Ig involved in coating the erythrocytes. Some researchers have established that preformed antigen-antibody complexes are adsorbed onto erythrocytes and then fix complement on the erythrocyte surface resulting in intravascular haemolysis and/or erythrophagocytosis

(Woodruff *et al.*, 1973; Woodruff, 1973). Amole *et al.*, (1982), further reported that only the binding of antigen-antibody complexes onto erythrocytes lead to complement fixation and erythrophagocytosis and not the binding of either antigen or antibody alone.

Complement is an intricate multimolecular enzyme system found in the blood and other body fluids. Complement has two functions namely, activation and/or cellular destruction and plays an important role in the animal's natural defense mechanism. In trypanosomosis (*T. congolense* infection in calves), the total haemolytic complement levels and complement component C1, $C1_q$ and C3 have been reported to decrease within three weeks of infection (Nielsen *et al.*, 1978; Olaho-Mukani *et al.*, 1996) This can be an indication that the complement and complement components are used up in erythrophagocytosis.

Assoku and Gardiner, (1988), demonstrated a regular generation of IgM and IgG during the course of *T. vivax* infection in cattle following the first peak of parasitaemia and observed that the peak antibody activities occurred at about 1 month post-infection. They noted that these Igs were against self-RBCs and were commonly detected between 10 and 40 days post-infection. The erythrocytes coated with antigen-antibody complexes activate the mononuclear phagocyte cells (monocytes and macrophages) which then phagocytose the erythrocytes (Grosskinsky, *et al.*, 1983).

Trypanosome-mediated B-cells (cells involved in antibody production) stimulus is polyclonal and it has been observed that this enhances the autoimmune humoral responses against host tissues (for instance erythrocytes) and can thus induce erythrophagocytosis (Mackenzie and Boreham, 1974). Genuine autoantibodies which do not cross-react with the infecting trypanosome antigen have also been reported to increase in *T. vivax* infection (Assoku and Gardiner, 1988). These autoantibodies have been reported to include immunocoglutin, cold-active haemagglutinin and antibody against fibrinogen (Rickman and Cox, 1979). Recently, Uche *et al.*, (1993) and Olaho-Mukani *et al.*,(1996), observed that in *T. evansi* infection in goats, there was a strong antibody response characterised by elevated IgG and IgM class-specific antibodies with a progressive fall in the haemolytic complement, and upon treatment, the IgM and complement levels recovered rapidly while IgG levels remained high for sometime. Olaho-Mukani *et al.,* (1996), therefore, postulated that IgM has strong complement fixing properties (hence utilising much complement) than IgG which accounted for the rise in IgM and fall in complement levels and vice versa.

In trypanosomosis, the activity of erythrophagocytosis has been reported to be highly pronounced in the spleen, liver, bone marrow and haemal lymph nodes (Anosa *et al*, 1977; Rickman *et al*, 1979; Anosa and Kaneko, 1983). However, erythrophagocytosis has also been reported to occur in peripheral blood (involving monocytes and macrophages in circulation) and in the myocardium of cattle with *T. vivax* infection (Kimeto, 1989). In the liver of cattle with *T. congolense* infection, macrophages have been observed to be abundant with an active appearance of vacuolated cytoplasm packed with erythrocytes and chromatin-like particles (Anosa and Kaneko, 1983). Splenomegaly (enlargement of the spleen) has been reported in trypanosomosis and attributed to the work of the spleen in clearing macrophages (which have phagocytosed erythrocytes) from the circulation and also due to erythrophagocytosis occurring within the spleen (Rickman and Cox, 1979).

In trypanosomosis, the activation of macrophages leads to blood coagulation abnormalities (disseminated intravascular coagulation, increased blood clotting time, thrombocytopenia, decreased coagulation factors in plasma, increased fibrin degradation products and increased blood viscosity) attributed to the possible role of the macrophages as the causative agents of the disorders (Robins-Browne *et al.*, 1975). Therefore, the observation of coagulation disorders during trypanosomosis can be an indicator of activated macrophages and probable sign of enhanced erythrophagocytosis.

In vitro experiments have shown that IgG-laden erythrocytes can readily be phagocytosed even by peritoneal macrophages (Weiss and Klausner, 1988). Makumyaviri, A., (personal communication) observed using *in vitro* experiments that monocytes and macrophages derived from peripheral blood readily phagocytosed erythrocytes presumably already coated with immunoglobulin. In normal (uninfected) animals, a naturally occurring antibody in plasma binds to an antigen present on the surface of senescent (aged) but not young erythrocytes leading to physiological erythrophagocytosis (Weiss and Klausner, 1988; Makumyaviri, A., personal communication).

Erythrophagocytosis can, therefore, be cited as a mechanism contributing to intravascular haemolysis by peripheral blood mononuclear cells (monocytes and macrophages).

Erythrophagocytosis may also be a significant factor of extravascular haemolysis using resident macrophages in organs like the liver, spleen, bone marrow, kidneys, haemal lymph nodes, heart muscle and lungs.

1.5.2.1.2. Changes in leucocyte counts

A marked lymphoid proliferative response and an increase in constituent cells of the mononuclear phagocyte system have been reported to be an indication of the activated immune system during *T. vivax* infection in goats and cattle and work to determine whether the immune system could be involved in causing anaemia has been advocated (Masake, *1980). Katunguka, (1992a), has reported marked increases in WBC count of sheep with T.* *congolense* infection. This leucocytosis was mainly due to lymphocytosis and was associated with good immunological response to limit the parasitaemia.

Recently, Ogunsanmi *et al.*, (1994a), reported that severe anaemia in ewes with *T. brucei* was associated with leucocytosis. This overall leucocytosis is important in keeping the parasitaemia low and is a common feature in trypanotolerant animals. Valli *et al.*, (1980), observed neutropenia in cattle infected with *T. congolense*. Lymphopenia had earlier been noted in sheep with *T. vivax* infection (Anosa and Isoun, 1976). Further, Saror, (1975), had earlier reported leucopenia in cattle infected with *T. vivax* and *T. congolense* and in sheep and goats with *T. vivax*. This leucopenia had been said to be consistent in most trypanosome infections of sheep and goats (Anosa and Isoun, 1980).

Adah *et al.*, (1993), observed that there were no reports of leucocytosis in *T. congolense* infected goats. This was in line with the findings of Kaaya *et al.*, (1977), that there was no significant change in leucocyte counts of goats with *T. congolense* infection. The leucocytosis observed in trypanotolerant animals is important in boosting the immune system and hence in keeping the parasitaemia very low and eventual elimination of the parasites.

1.5.2.1.3. Immunosuppression

Due to the reports of leucopenia in highly susceptible animals with trypanosome infection, trypanosomosis is considered to have possible immunosuppressive effects. In view of the increasing use of vaccines in domestic animals throughout Africa, trypanosome infections may be important in the efficacy of the vaccines (Holmes *et al.*, 1974; Scott *et al.*, 1977; Griffin *et al.*, 1980). The suggested mechanism by which trypanosomes suppress the host's immune system is by inhibition of the reactive B-lymphocytes from responding fully to antigen challenge and hence having a reduced antibody production. The degree of immunosuppression caused by trypanosomosis has been observed to be dependent on the parasitaemia. For instance, in two experiments, *T. congolense* which had much lower parasitaemia caused less suppression than *T. brucei* which gave higher parasitaemia (Freeman *et al.*, 1974; Lesley *et al.*, 1980). *T. congolense* infection in cattle and goat was observed to cause mild immunosuppression (Scott *et al.*, 1977).

T. brucei infections in mice have shown elevated levels of nitric oxide synthesis associated with activated macrophages (Sternberg and McGuigan, 1992; Sternberg, 1996). The continued release of the nitric oxide and other activated macrophage products such as prostaglandin-E_2, have been noted to contribute to immunosuppression in the spleen and lymph nodes, through interference with lymphocyte proliferative responses (Schleifer and Mansfield, 1993; Mabbott *et al.*, 1995). It was further reported that release of nitric oxide by bone marrow macrophages contributed to the anaemia associated with trypanosomosis through inhibition of erythropoiesis and consequently, inhibition of nitric oxide synthesis *in vivo* in the mouse lead to improved control of *T. brucei* parasitaemia (Sternberg *et al.*, 1994).

1.5.2.1.4. Changes in blood volume

Haemodilution has been reported to contribute to anaemia in sheep and goats with *T. vivax* infection (Anosa and Isoun, 1976) and in cattle with *T. congolense* infection (Naylor, 1971; Van Den Ingh *et al.*, 1976). These reports were supported by increased plasma volume, decreased albumin concentration and increased globulin concentration causing an osmotic effect. However, haemodilution has been observed to be absent in acute trypanosomosis or in early stages of the disease (Dargie *et al.*, 1979).

Suggestions put forward to explain the haemodilution seen in anaemia are that it may be a response to a decrease in erythrocytes count as an attempt to prevent circulatory collapse and that it might be due to marked increases in gamma-globulins (common in trypanosomosis) leading to increased plasma colloid osmotic pressure (Anosa *et al.,* 1976). In addition, changes in aldosterone levels, release of amines or metabolic products from trypanosomes have been suggested to contribute (Katunguka *et al.*, 1992b). Holmes, (1976) and Valli and Forsberg, (1979) observed haemodilution in calves with *T. congolense* infection and attributed the haemodilution to microvascular damage and hence intravasation of body fluids.

1.5.2.1.5. Dyshaemopoiesis

In most haemoparasitic diseases (including trypanosomosis) which cause anaemia, the erythropoietic activities are usually altered. Low erythropoietic (decreased erythrocyte production) potential or failure of erythropoiesis altogether, has widely been suggested to be an occurrence of bone marrow depression in trypanosomosis in ruminants. Igbokwe and Anosa, (1989), noted depressed erythropoiesis in *T. vivax* infection in sheep and in *T. congolense* infection in cattle. Earlier, it had, however, been established that there was no total failure of erythropoiesis in *T. vivax* and *T. congolense* infections because of the common occurrence of bone marrow erythroid hyperplasia (Mackenzie and Cruickshank, 1973), increased iron uptake (Dargie *et al.,* 1979) and increase in young erythrocytes indicated by macrocytosis (increased production of erythrocytes with size larger than normal) (Wellde *et al.*, 1974). Naylor, (1971), and Saror, (1975), both reported erythroid hyperplasia of the bone marrow in cattle with *T. congolense*. However, Murray *et al.*, (1977), noted bone marrow inactivity in chronic infections.

Ogunsanmi *et al.*, (1994a), showed that anaemia in trypanosomosis in ruminants in the acute phase of the disease, was normocytic (normal erythrocyte size) and normochromic (normal haemoglobin content in erythrocytes), and in the chronic phase of the disease, the anaemia was macrocytic (erythrocyte size larger than normal). Normocytic-normochromic anaemia in the acute phase of the disease was deduced to indicate inadequate erythropoiesis while macrocytic anaemia in the chronic phase indicated erythropoietic response. This was in contrast with earlier workers (Fiennes, 1954; Valli *et al.*, 1978; Igbokwe and Anosa, 1989) who had observed macrocytic anaemia in the early acute phase and normocytic or microcytic changes in the chronic phase.

Wellde *et al.*, (1989), observed macrocytic-hyperchromic anaemia in the early stages (first ten weeks) which then gradually reverted to normocytic-normochromic anaemia by the 21st week post-infection in cattle with *T. congolense* infection. They, however, noted no changes in the MCHC. In line with these observations, Wellde *et al.*, (1989), reported elevated serum iron levels, increased total iron binding capacity and plasma iron turnover rate and elevated plasma iron clearance rates in the first 8 weeks post-infection which later decreased to control levels. The early changes indicated an erythroid response. Kaaya *et al.*, (1977) noted that normocytic-normochromic anaemia was a common finding in cases of depressed erythropoiesis (a usual finding in cattle with *T. congolense*). Naylor, (1971), showed that trypanosomosis in goats resulted in anaemia due to haemolysis and bone marrow inhibition.

1.5.2.1.6. Serum proteins changes

Serum albumin and globulin concentration evaluation is of aid to Veterinary clinicians in diagnosis of several important diseases which are generally accompanied by high levels of total serum globulins. Relatively little precise data exists on Caprine albumin and total serum globulin concentration in tropical Africa (Aba-Adulugba and Joshua, 1990b). Increased gammaglobulin concentrations and decreased albumin concentrations have been noted in animals with trypanosomosis (Anosa and Isoun, 1976). In goats with trypanosomosis, the total plasma protein concentration has been reported to be decreased, unaltered (Kalu *et al.*, 1989) or increased (Igbokwe and Mohammed, 1992).

Anosa, (1988), observed that in animals with little resistance to trypanosome infection, the total plasma protein concentration did not increase while it did increase in animals with high resistance to the disease. This can be an indication that trypanosomosis suppresses the immune system in highly susceptible animals, while the immune system in trypanotolerant animals is responsive to infection and this keeps the parasitaemia low.

The increase in total serum protein has been noted to be due to increased concentrations of the globulins fraction and suggestions put forward to explain this increase are that there could be a non-specific polyclonal stimulation of B-lymphocytes in infected animals (Manfield, 1978) which leads to the production of greater amounts of immunoglobulins (Igbokwe and Mohammed, 1992). The immunoglobulins which have been noted to undergo changes in concentration in animals with trypanosomosis are IgG, IgG1, IgG2, IgM and heterophile antibodies. In N'Dama and Zebu cattle with natural *T. congolense* infection, IgG concentration was observed to increase two-folds, while IgG1 underwent a transient decrease to about 70% of the control values. IgG2 serum levels increased by about 1.5 times at 7 weeks post-infection. IgM and heterophile antibodies showed variations with some animals periodically having elevated IgM levels whereas others showed marked low levels Tabel (1978).

In highly susceptible animals, low plasma albumin concentrations have been reported in several trypanosome infections and attributed to plasma dilution (haemodilution), proteinuria (loss of protein through urine) or hepatocellular damage leading to compromised albumin synthesis in the liver (Van Den Ingh *et al.*, 1976; Saror, 1980). Tabel, (1988), also observed significantly lowered plasma albumin concentrations in cattle with trypanosomosis and he attributed this to plasma dilution, decreased rate of albumin synthesis (due to hepatocellular damage) and increased rate of albumin catabolism.

Complement is an important fraction of the total serum proteins and can be considered to contribute to the total serum protein concentration levels. Decomplementation (hypocomplementaemia), has been reported to be a common occurrence in cattle infected with either *T. congolense* or *T. vivax*. Low complement levels in serum have been observed to first occur during the decline of first wave of parasitaemia. Complement component C3 has been found to be the one which shows particularly low levels and has been identified as the haemolytic complement. The decrease in complement concentration comes about because complement is used up in immune complexes in combating repeated parasitaemias and also in erythrophagocytosis (Tabel, 1978). Hence decomplementation should lead to a decrease in total serum proteins concentration.

A pneumococcal carbohydrate-reactive protein (CxRP), an acute phase plasma protein, has been reported to increase in the plasma of rabbits infected with *T. congolense* during the early acute phase of the disease and later decline as the disease becomes severe with more pronounced pathological changes (Cook, 1979). CxRP has been observed to be a protein associated with early inflammatory lesions of trypanosomosis and can, therefore, contribute to increase in total serum protein concentration during the early acute phase of trypanosomosis.

The albumin:globulin (A:G) ratio is an important indicator of the level of either serum albumin or serum globulin. In sheep infected with *T. brucei,* (Ogunsanmi *et al.*, 1994b) and *T. congolense* (Rees and Clarkson, 1967), the A:G ratio was reported to decrease and this was attributed to a decrease in albumin concentration and an increase in globulin concentration.

1.6. Objectives of the study

a) To determine changes in blood parameters during the course of experimental *T. congolense* infection in indigenous Zambian goats.

b) To establish serum proteins profiles during the course of experimental *T. congolense* infection in indigenous Zambian goats.

c) To investigate erythrophagocytosis as a mechanism of anaemia during the course of trypanosomosis in indigenous Zambian goats.

CHAPTER TWO

MATERIALS AND METHODOLOGY

2. Experimental animals

The first set of 8 goats obtained were used for preliminary experiments in order to perfect the methods to be used in the final experiments. The first set of goats was also used for investigation of erythrophagocytosis using thin smears as explained in section 4.0.8. over a period of 3 months. The second set of goats obtained was used for the final experiments and for the investigation of erythrophagocytosis using radioisotopes. Both sets of goats were prepared for the experiments in the same way explained below.

Male indigenous Zambian goats aged between 10 and 18 months were purchased from a cross-section of apparently tsetse-free (Nalubamba, K., personal communication) villages in Lusaka-West, Shibuyunji area. The ages of the goats were determined at purchase by asking the farmers as well by examination of the teeth (The Merck Veterinary Manual, 1991).

2.1. Preparation of the goats

Upon arrival at the Veterinary School, the animals were kept in one pen overnight. The next day, the animals were ear-tagged. All the goats were injected intramuscularly with reconstituted diminazene aceturate (Berenil, Hoechst, Ireland) at a dose rate of 3 - 5 mg per kilogramme body weight to clear any unforeseen infections due to trypanosomes and

Babesia. Oxytetracycline, 20% (Dopharma, Holland) was administered intramuscularly at 20 mg/kg body weight to control any infections due to bacteria, rickettsia and *Anaplasma.* The goats were also dewormed with albendazole, 1.9% M/V (Valbazen, Smithkline, Beecham, Zimbabwe) at 5 ml/10 kg body weight orally to control infestations of roundworms, liverflukes and lungworms. The animals were then dressed with tick grease (Tick dressings, Milborrow and company) by applying the grease thinly and evenly by hand under the tail, over the perineal area, on the scrotum, inside thighs, along the ventral aspect of the abdomen, thorax and neck, in the axilla, in and around the ears and in-between the hooves to control ticks. The animals were then left in the same pen for a day before being moved to the quarantine pen. At the quarantine, two identical fly-proof pens (3 x 6 m size) were thoroughly cleaned and disinfected with Carbolic acid, coefficient 8 - 10, (Kynol, Roussel Uclaf) and left to dry for three days before occupation. On the day of occupation, the floor of the pens was covered with a bedding of hay (Plate 1). The goats were randomly divided into 2 groups of 5 goats each by lining them up and drawing a card for each goat from a box containing 10 cards with 5 of the cards marked 'Group A' and the other 5 cards marked 'Group B'. Group A goats were put in pen-A while group B goats were put in pen-B.

In the first three days, the animals were provided with grass hay and water *ad libitum.* On the fourth day, fresh lucerne was introduced at 50 g per goat. Number-3 maize meal (National Milling limited) containing 5% coarse salt (National milling limited) and 5% dicalcium phosphate (Kynoch, South Africa) was also introduced at 50 g per goat

(Siulapwa, N.J., personal communication). The quantities of both lucerne and number-3 maize meal were increased daily by 50 g per goat until 250 g per goat was attained and maintained for both fresh lucerne and number-3 maize meal. Previous work with Zambian goats (Lovelace, C.E.A., personal communication) had shown that goats from free range feeding did not take easily to confinement and being fed concentrate and they needed to adapt. The animals were left to acclimatize for a period of two months before commencing the experiments on them. The pens were cleaned twice per week by washing and disinfecting the floor while enclosing the animals to one corner using a wire mesh. During the acclimatization period, the animals were screened weekly for blood parasites, worm infestations and thoroughly examined clinically.

2.1.1. Screening for blood parasites

2.1.1.1. Trypanosomes

The dark ground/phase contrast buffy coat method of Murray *et al.*, (1983) was used to screen for trypanosomes. Two ml of blood was collected from each goat into a heparinised test tube by jugular puncture using sterile vacutainer needles (21 gauge). The blood was mixed thoroughly with heparin by inverting 3 times. In the laboratory, the test tubes were put on a mixer (Spiramix model A-253, Denley, England) to keep the blood thoroughly mixed. From each test tube, blood was aspirated into a plain microcapillary tube (75 mm long, Funakoshi company, Japan) to three-quartres of the tube's length and the end of the tube sealed with wax (Kristaseal). The microcapillary tubes (prepared in duplicate) were then packed into a haematcrit centrifuge (Kubota, model KH-1200S, Japan) in a balanced arrangement and centrifuged at 12000 rpm for 5 minutes. The tubes were cut 1 mm below the buffy coat layer to include the top layer of red blood cells and 1 cm above to include the plasma. The contents of the cut piece were expressed onto a clean slide, mixed with the edge of a cover slip and covered with the same cover slip of 22 x 22 mm size. The slide was examined using a light microscope under the X40 objective with the diaphragm almost shut to achieve critical illumination. 50 fields of each slide were scanned for trypanosomes.

2.1.1.2. Other blood parasites (*Babesia, Anaplasma, Theileria* and *Ehlrichia*)

Screening for other blood parasites was done using the procedures described by the Technical Centre for Agricultural and Rural Co-operation, (1989). New glass slides were immersed in 98% ethanol and wiped clean and dry using a soft clean cloth. The tip of the ear of each goat was pricked with a sterile 18 gauge needle and pressure applied on the ear lobe to express out blood from the pricked point. A drop of blood was collected onto a glass slide. While holding the slide flat in the left hand, the edge of another slide was brought in contact with the first slide at the point of the blood drop at about 45° angle and the drop allowed to spread along the entire edge, then dragged gently, but quickly towards one end of the glass slide. The blood smear was then air-dried by waving the slide and fixed in 98% methanol for one minute and further air-dried. The slides were then stained with 10% Giemsa solution.

a.) Preparation of 10% Giemsa solution

Phosphate buffer solution (PBS) pH 7.2 for diluting concentrated stock solution was prepared by weighing:

Hydrated disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) = 1.77 g

Hydrated sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) = 0.70 g

Sodium azide (NaN_3) = 1.00 g

and dissolving in about 900 ml of distilled water in a conical flask. The pH of the solution was adjusted to pH 7.2 with 1 M sodium hydroxide (NaOH) solution and the whole contents transferred to a 1000 ml volumetric flask and made to mark with distilled water. The buffer solution was kept in a tightly closed polyethylene bottle. To prepare 10% Giemsa solution in PBS at pH 7.2, 5 ml of the Giemsa stock solution was dissolved in 45 ml of the buffer (Makumyaviri, A., personal communication).

b.) Staining of the fixed blood smears

The glass slides were placed flat on the rack (with the smeared side facing up) and flooded with the diluted Giemsa for 30 minutes. The slides were thereafter rinsed with running tap water and left to dry while standing in the upright position.

c.) Scanning for blood parasites

A dry slide was placed on the microscope and grossly scanned under the X40 objective lens with a X10 eye piece for blood parasites. The slide was then scanned in detail under a X100 objective lens with immersion oil added to the slide. About 50 fields were scanned.

2.1.2. Screening for helminths

This procedure was done using the McMaster method. Fresh faecal samples were collected from each goat by inserting a forefinger into the rectum and scooping out faeces into a test tube, then corking the tube to avoid hatching of the worm eggs. About 2 g of the collected faecal sample were weighed and 28 ml of saturated sodium chloride solution added and thoroughly mixed with a glass rod. The solution was strained into a small beaker and the strainer washed with 30 ml saturated salt solution. The 2 chambers of the McMaster slide were filled with the solution using a pasteur pipette held horizontally and the slide stood for 1 minute to allow eggs to float up in the two chambers.

Under the microscope, the grids inscribed on the underside of the top of the chamber, where the eggs were floating was brought into focus. The number of eggs in both chambers were counted. For 1 slide, 1 helminth egg = 100 eggs per gram (e.p.g.). The average e.p.g. for a mixed infestation of *Haemonchus, Ostertagia* and *Trichostrongylus* was found to be around 2000 at 10 days after deworming with valbazen. This was an indication that the efficacy of the valbazen was very low and, therefore, ivermectin (1.0% W/V solution) was used in the second week at dose rate of 0.5 ml/25 kg body weight by subcutaneous injection. The e.p.g. was rechecked a week later and found to be an average of 50 of a mixed infestation of *Haemonchus, Ostertagia* and *Trichostrongylus.* The goats were dewormed again with ivermectin 3 weeks before commencing the experiments.

2.2. Passaging of trypanosomes in mice

2.2.1. Source of the mice and parasites

Five adult male albino mice were obtained from the School of Veterinary Medicine quarantine facility for laboratory animals. Stabilates of *Trypanosoma congolense IL3000* (Plate 5) obtained from the International Livestock Research Institute, Kenya which had been frozen at -190°C in liquid nitrogen were thawed out at room temperature. About 0.5 ml of the stabilate was aspirated using a 2.5 ml syringe and a 20 gauge needle, and a drop put on a clean glass slide. A cover slide was added and the slide observed under the light microscope using a X40 objective lens (with a X10 eye piece) with the diaphragm shut to achieve critical illumination in order to determine the viability of the trypanosomes by observing their mobility. The trypanosomes were observed to be considerably motile i.e. swimming vigorously and therefore, apparently, viable.

2.2.2. Inoculation of mice

More of the stabilate (about 1 ml) was aspirated into the syringe. Mice were restrained by holding the tail with the forefinger and the thumb, and using the other hand, the scruff was grabbed with the forefinger and thumb, the mouse was turned upside down and the tail held with the same hand between the palm and the index finger. Holding the syringe with the right hand, 0.2 ml of the stabilate was injected intraperitoneally at mid-point of the abdomen while inclining the needle at 45° angle to the abdominal surface and inserting only 3 mm of the needle (Murray *et al.*, 1983). The inoculated mice were placed in a separate cage to which an identification card was attached and the cage kept in the quarantine.

Two days post-inoculation, the parasitaemia development was checked as follows:

Each mouse was held by the tail and the tip of the tail nipped with a sharp pair of scissors. Then pressure was applied on the tail to express a drop of blood onto a clean glass slide. A cover slide (22 x 22 mm) was added and the parasitaemia checked using a light microscope under a X40 objective lens with critical illumination. The parasitaemia was checked daily until the fifth day when the parasitaemia reached the peak with more than 100 trypanosomes being observed per field.

2.2.3. Harvest of trypanosomes and further passage in mice

Sodium citrate with a 0.1 molarity was used as an anticoagulant and was prepared by weighing 2.94 g of sodium citrate, dissolving in about 90 ml distilled water and making-up the solution to 100 ml with distilled water in a 100 ml volumetric flask.

2.2.3.1. Bleeding the infected mice

The infected mice were anaesthetised by placing them one at a time in a large covered beaker (in a hood) containing cotton wool on which chloroform had been poured and covering the top. When the mouse became unconscious, it was removed and pinned upside-down on a dissection board. The thoracic cavity was accessed by cutting into the abdominal cavity and through the diaphragm using a pair of scissors. A 2.5 ml syringe containing 0.25 ml of the 0.1 M sodium citrate and a 21 gauge needle was inserted into the throbbing heart and slowly blood was drawn. The blood from all the 4 mice was pooled in a 10 ml test tube. The viability of trypanosomes was checked and found to be very viable (swimming vigorously). Another 5 male adult mice were obtained from the same source and inoculated with the infected blood in the same way as was done to the first group. The parasitaemia was checked on a daily basis. One mouse died on the third day post-inoculation, while the rest were very weak with staring coats and looking emaciated by the third day. The mice were bled and the blood pooled as was done for the first group.

2.2.3.2. Determining the trypanosome concentration in pooled blood

The method described by Murray *et al.*, (1983), was used. Sodium chloride solution (0.85%) was prepared by weighing 0.85 g of sodium chloride and dissolving in about 90 ml of distilled water and making up the volume to 100 ml in a volumetric flask. Exactly 200 µl of the pooled mouse blood was measured using a 200 µl micropipette and added to 1.98 ml of 0.85% sodium chloride solution (ten times dilution) and the number of trypanosomes in the diluted blood counted using a haemocytometer chamber (Improved Neubauer deep, 1/10 mm, Erma, Japan).

A bit of saliva was smeared on the edges of the haemocytometer chamber and a cover slip (22 x 22 mm) mounted by pushing it into position along the edges until Newton's rings developed along the covered edges of the chamber. A small amount of the diluted blood was collected in a capillary tube and the tube touched onto the edge of the cover slip to allow movement of the blood into the counting chamber and left to settle for 120 seconds.

The chamber was later mounted on a light microscope and the number of trypanosomes in one 1mm^2 corner counted using a X10 objective lens (with a X10 eye piece) with critical illumination. The trypanosomes/ml of the pooled blood was calculated as follows:

A x 10 x dilution factor x 1000 = trypanosomes/ml of pooled blood.

Where: A = number of trypanosomes counted in one 1 mm^2 corner

dilution factor = 10.

A was found to be equal to 1000, therefore, the concentration of the trypanosomes in the pooled blood was = 1000 x 10 x 10 x 1000

= 1×10^8 trypanosomes/ml.

The required concentration of trypanosomes/ml for inoculation was 3×10^6 trypanosomes/ml.

Therefore,

$$C_1 \times V_1 = C_2 \times V_2$$

Where:

$C_1 = 10^8$ trypanosomes/ml (actual concentration of trypanosomes in pooled blood)

$C_2 = 3 \times 10^6$ trypanosomes/ml.

V_1 = required volume of the pooled blood

V_2 = 50 ml (desired final volume of the diluted pooled blood)

Therefore,

$$V_1 = \frac{\underline{3 \times 10^6 \text{ tryps/ml} \times 50 \text{ ml}}}{10^8}$$

$$V_1 = \underline{1.50 \text{ ml}}$$

Hence, 1.50 ml of the pooled blood was transferred to a clean conical flask containing 48.5 ml of the 0.85% sodium chloride solution, to make a trypanosome concentration of 3 x 10^6 trypanosomes/ml. The diluted trypanosomes suspension was ready for inoculation into the goats.

2.3. Inoculation of goats with trypanosomes

The inoculation was done immediately after bleeding the mice and preparing the right dilutions. The conical flask containing the diluted blood was corked and the contents thoroughly mixed by inverting 3 times. Using a new 10 ml syringe and an 18 gauge needle, 2 ml of 0.85% sodium chloride solution was aspirated, and then 1 ml of the thoroughly mixed trypanosomes suspension aspirated into the same syringe to make a total of 3 ml mixture containing 3 x 10^6 trypanosomes (Holmes, P.H., personal communication).

The inoculation was done into the jugular vein by restraining a goat in a standing position, raising the jugular vein by applying digital pressure mid-way along the jugular vein and when the vein was bulging, a sterile 18 gauge needle was inserted into the vein at about 15° angle until blood started dripping out. Then the syringe containing the trypanosome suspension was connected to the needle, some blood aspirated from the vein (to make sure the needle was still in the vein) and then the whole content in the syringe injected into the vein slowly and gently, the needle withdrawn and the site of inoculation massaged to stop any bleeding (Holmes, P.H., personal communication). The infected goats beared tag numbers: **1, 2, 3, 4** and **5.** The control goats beared tag numbers: **6, 7, 8, 9** and **10.**

2.4. Establishing baseline parameters

The previous day before the day of inoculation of trypanosomes into the goats, the baseline data for all the goats was established for the following parameters:

- Body weight
- Rectal temperature
- Packed cell volume (PCV)
- Red blood cell (RBC) count
- White blood cell (WBC) count
- Haemoglobin (Hb) concentration
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Haemoglobin (MCH)
- Mean Corpuscular Haemoglobin Concentration (MCHC)
- Total serum protein concentration (TSP)
- Serum albumin concentration
- Serum globulins concentration
- Albumin:globulin (A:G) ratio

The methodologies used for each parameter were as described in the proceeding methods.

2.5. Clinical examination

All the goats were clinically examined a day before inoculation (baseline data) and then examined every 2 days after inoculation. The general appearance, activity, eating and drinking habits, colour and consistency of the stool and urine were noted. The state of the mucous membranes (of the eyes and mouth), the size and texture of superficial lymph nodes (parotid and prescapular) were determined. The heart rate and respiratory rate were assessed by auscultation.

2.5.1. Body weight

The animals were weighed on a weekly basis (starting from the day before inoculation) on an FHK 30-3 platform scale for animals. Both the hind and forelegs of the animal were tied together, the animal placed on the scale platform and the scale reading noted.

2.5.2. Rectal temperature

Temperature readings were done every 2 days. The rectal temperatures of the goats were taken while restraining the animal in a standing position. A short blunt bulb clinical thermometer (Premier, UK), was shaken vigorously, lubricated with water and inserted into the rectum through the anal opening and held for 90 seconds. Upon withdrawal, the temperature reading was recorded in degrees Celsius (°C).

2.6. Haematology

Haematological parameters were determined thrice per week during the first 5 weeks post-inoculation and thereafter (after the commencement of the erythrophagocytosis experiments) twice per week.

2.6.1. Collection of blood samples for haematology

The blood sampling procedure illustrated by Jain, (1986) was followed. Blood samples for haematology were collected on the morning of the day of determining haematological parameters. Blood was collected into 2.5 ml heparinized vacutainer tubes using 21 gauge vacutainer needles. The jugular vein was raised by applying pressure midway along the jugular groove and when the vein was bulging, the vacutainer needle was inserted into the vein at about 30° angle (Plate 2). When the blood was dripping from the needle, a vacutainer tube was connected to the needle and blood allowed to collect into the tube until the vacuum was exhausted. The tube was then inverted three times to thoroughly mix the blood with the anticoagulant (heparin). In the laboratory, the blood was put on a roller mixer (Denley Spiramix, model A253, Sussex, England) to continue mixing the blood before measuring of any haematological parameters.

2.6.2. The electronic cell counter

The Baker Instruments electronic haematology cell counter (series 130, for veterinary use), was used to determine the RBC counts, WBC counts and the haemoglobin concentration (Plate 3).

2.6.2.1. Calibration of the cell counter

The following reagents specific for use on the Baker Instruments electronic haematology cell counter series 130, were obtained from IFCI Clone systems (an agent of Baker Instruments, USA):

a.) Haem-QC Plus Normal and Abnormal: This consisted of vials of quality control standards accompanied by a chart of the predetermined haematological values meant for calibrating the cell counter.

b.) Haema-lyse 100: This was a reagent for lysing RBCs in a WBC dilution in order to release haemoglobin and eliminate RBCs. The haema-lyse also acted as a lubricant for the instrument lining.

c.) Haema-line 2: This reagent was used for diluting blood samples in preparation for cell counting and was used for flushing the system.

d.) Haema-clean 100: This was used as a detergent for washing clean the system lining.

e.) Haema-standby: This was used to flush the system into standby state if it was to be left idling for more than 10 minutes. It was also used for flushing the system after a day's use.

An electronic autodilutor (Nichiryo) was used for diluting the blood samples. The assembled cell counter was checked to make sure that all the connections were in place as illustrated in the operation manual. The autodilutor was also checked and the inlet tubing connected to the reservoir of haema-line 2. The dilutor was flushed five times with haema-line 2 and set to dispense 10 ml of the haema-line 2 and to aspirate 40 μl of a blood sample. The cell counter was switched on and left to warm-up for 5 minutes.

About 20 ml of haema-clean 100 was dispensed into a clean and dry haema-vial. The vial was placed under the snorkel of the cell counter and the snorkel dipped into the haema-clean 100 so that the tip was close to the bottom of the vial. A flush cycle was initiated by pressing the 'Standby Flush' (STBY/Flush) button. The flush cycle was repeated two more times and then the counter left to warm-up for 10 minutes. Then, 20 ml of haema-line 2 was dispensed into a clean, dry haema-vial using the dilutor and 6 drops of haema-lyse 100 added and mixed by inverting the capped vial 2 times. This mixture was used to flush the cell counter 3 times to rinse off the haema-clean. Another 10 ml of haema-line 2 was dispensed into a clean, dry vial, 3 drops of haema-lyse added and the vial placed under the snorkel. Using the Threshold potentiometers, the RBC threshold was adjusted to 0.65 while that of the WBC was adjusted to 0.90. The 'Self Test' button was placed to run a self test cycle in order to determine whether the system was clean or not. When the system was clean, the panel gave the background count in the range of 0.00 - 0.04 for a brief time and then gave 'Ready 000' reading.

The electronic check for WBC, RBC, Hb lamp and the Hb cell was done by pressing the scan button and adjusted to:

WBC = 0.90

RBC = 0.65

Hb = 2.00

Hb cell = 4.20

The adjustment was done by turning the potentiometer screws for each parameter (while the particular parameter was on display) until the right voltage was attained.

2.6.2.2. Quality control

A new vial of Haem-QC Plus Normal was brought to room temperature and mixed thoroughly using the Denley Spiramix. Then 40 µl of the standard was aspirated using the autodilutor and dispensed into a clean haema-vial along with 10 ml of haema-clean. Three drops of haema-lyse were added to the vial and mixed by inverting the vial 3 times. This made a 1:250 WBC dilution for priming the cell counter. Another 40 µl of the standard was aspirated and dispensed into a clean vial along with 10 ml of the haema-line, the vial capped and contents mixed by inverting two times to get a 1:250 WBC dilution. Then 40 µl was aspirated from the 1:250 WBC dilution, the tip of the dilutor wiped clean with a fluffless tissue (Kimwipes) and dispensed into another clean vial along with 10 ml of haema-line. The vial was capped and the contents mixed by inverting gently three times to make a 1:62,500 RBC dilution. Three such sets of WBC and RBC dilutions were made.

Three drops of haema-lyse were added to each of the WBC dilution and mixed gently by inverting. At least 60 seconds were allowed to elapse after adding the haema-lyse before the WBC dilution could be run on the cell counter. The priming dilution was placed under the snorkel and the snorkel dipped into the dilution close to the bottom of the vial. The WBC/Hb button was placed to initiate a priming cycle. The results were disregarded. The first WBC dilution sample was then run by pressing the WBC/Hb and the WBC counts and Hb concentration recorded in x $10^3/\mu l$ and g/dl units, respectively. The first RBC dilution was run by pressing the 'RBC' button when the snorkel (wiped clean with fluffless tissue after every run) was dipped in the dilution. The results were recorded in x $10^6/\mu l$ units. The remaining samples were run one after another, alternating between WBC sample and RBC sample to ensure the system was well lubricated by the haema-lyse in the WBC dilution. The recorded results were used to calculate the calibration factors as follows:

Table 2.1: Calculation of the electronic cell counter calibration factors

Set Number	**WBC (x $10^3/\mu l$)**	**Hb (g/dl)**	**RBC (x $10^6/\mu l$)**
1	8.00	13.8	4.83
2	7.90	13.5	5.19
3	8.10	13.6	5.54
Average	8.00	13.6	5.18
Reference Mean	9.40	13.7	4.53

The calibration factors were calculated using the formulae:

$$\frac{(R - A) \times 100}{A}$$

Where: R = Reference value

A = Actual average value.

<u>WBC calibration factor:</u>

$$= \frac{(R - A) \times 100}{A}$$

$$= \frac{(9.4 - 8.00) \times 100}{8.00}$$

$$= \underline{17.5.}$$

Hb calibration factor:

$$= \frac{(R - A) \times 100}{A}$$

$$= \frac{(13.70 - 13.63) \times 100}{13.63}$$

$$= \underline{0.5}$$

RBC calibration factor:

$$= \frac{(R - A) \times 100}{A}$$

$$= \frac{(4.53 - 5.18) \times 100}{5.18}$$

$$= \underline{-12.5.}$$

The calibration factors were then entered into the cell counter by pressing the 'CAL' button until the particular parameter was displayed and the factor punched in at the numeric key board and the 'Enter' button pressed.

2.6.2.3. Calibration check

Another series of 3 sets of haem-QC Plus Normal WBC and RBC dilutions were prepared and run. The values were counter-checked with the reference ranges to make sure they were within the reference range before proceeding. Three sets of WBC and RBC dilutions were prepared from a vial of Haem-QC Plus Abnormal standard, and run. The values were counter-checked with the reference values to make sure they were within range before proceeding.

2.6.2.4. RBC and WBC Threshold determination for the research goats

Blood samples were collected from 3 uninfected research goats and WBC and RBC dilutions prepared. At 1:62,500 RBC dilution, there was 'Count overflow' display, indicating the cell concentration was very high for the counter and hence a further dilution was done. The RBC dilution was further diluted three times by aspirating and discarding 5 ml with a 500 µl micropipette from the diluted RBC sample. A further 10 ml of haema-line were added to the remaining 5 ml of the RBC dilution in the vial and thoroughly mixed by inverting the capped vial twice. This meant that the RBC readings displayed by the cell counter were to be multiplied by a factor of 3 to get the actual value. The counts were done at different potentiometer settings as follows:

Table 2.2: Potentiometer settings for WBC threshold determination

WBC (x 10^3/µl) counts			**Potentiometer setting**
Goat-1	**Goat-7**	**Goat-8**	
24.8	25.2	25.0	0.30
19.3	20.1	19.7	0.40
19.0	18.7	21.4	0.50
18.1	17.8	20.2	0.60
17.4	17.6	18.3	0.63
17.2	17.3	17.4	0.67
17.2	17.2	17.4	0.70
17.1	17.3	17.1	0.72
15.7	16.0	16.3	0.80
13.7	14.0	14.1	0.90
10.7	11.0	11.8	1.00

Table 2.3: Potentiometer settings for RBC threshold determination

RBC (3 x $10^6/\mu l$) counts			**Potentiometer Setting**
Goat-1	**Goat-7**	**Goat-8**	
5.41	5.40	5.60	0.20
4.41	4.50	4.80	0.22
4.44	4.48	4.77	0.25
2.72	3.00	3.20	0.30
1.73	2.00	2.20	0.35
1.39	1.60	1.90	0.40
1.09	1.40	1.70	0.45
0.88	1.00	1.20	0.50

The average WBC count at each threshold level was plotted against the threshold and the threshold level of the portion of the curve that exhibited small variations in count was taken as the threshold that would separate instrument background noise from the cell population being counted (Figures 2.1 and 2.2). This was 0.70, taken as the WBC threshold. Likewise, the RBC threshold was found to be 0.22. These threshold levels were entered by pressing the 'SCAN' button and when 'RBC' was displayed, the RBC potentiometer screw was turned to attain the correct threshold level (0.22). And when the 'WBC' was displayed, the WBC potentiometer screw was turned to the required threshold level (0.70).

Figure 2.1: WBC threshold plot

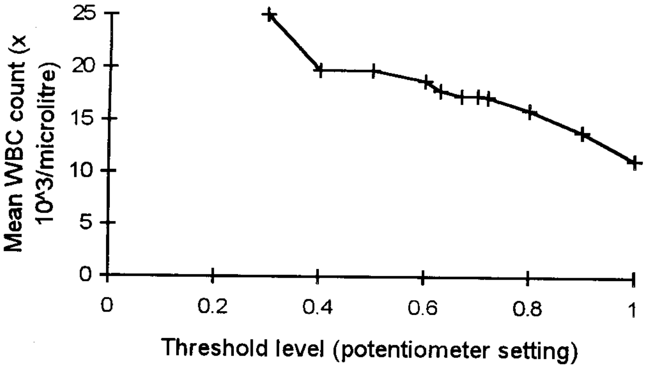
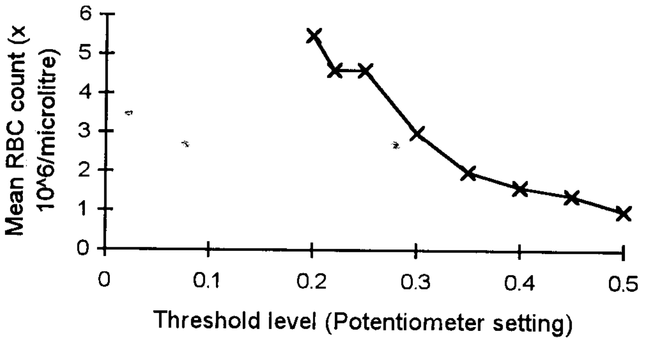


Figure 2.2: RBC threshold plot



2.6.2.5. Counting blood cells of experimental goats

The waste container stopper was reset every time before switching on the cell counter. The power switch was turned on and when the operation code (OP1) was displayed, a vial containing haema-clean was placed under the snorkel and the flush cycle initiated by pressing the 'STBY/FLUSH' button. The flush cycle was repeated two more times and then the counter left to warm-up for about 15 minutes. Exactly 20 ml of haema-line was dispensed into a clean vial, 6 drops of haema-lyse added and the capped vial inverted 2 times to mix. This preparation was used to further flush the system 3 times. The thresholds were then set to 0.90 and 0.65 for WBC and RBC counts, respectively. A further 10 ml of haema-line were dispensed into a clean vial, 3 drops of haema-lyse added and mixed by inverting the capped vial 2 times. This preparation was placed under the cleaned snorkel and the 'self-test' cycle run. When the 'Ready 000' reading was displayed, the thresholds were set to 0.70 and 0.22 for the goat WBC and RBC thresholds, respectively.

A vacutainer tube containing a blood sample which had been rolling on the Spiramix was opened and the cleaned tip of the dilutor inserted into the blood sample. The aspiration cycle was initiated by pressing the 'Aspirate/dispense' button and 40 µl of the blood sample collected. The dilutor tip was wiped clean with a fluff-free tissue in a downward motion. The aspirated blood was dispensed along with 10 ml of haema-line into a clean vial while holding the vial at an angle with the dilutor tip placed against the inner side of the vial making sure it remained above the fluid level. The vial was capped and contents mixed by inverting 2 times, giving a 1:250 WBC dilution. For the RBC dilution, a further 40 µl were aspirated from the WBC dilution and dispensed into another vial along with 10 ml of haema-line to make a 1:62,500 RBC dilution. The vial was capped and the contents mixed by inverting twice.

The RBC dilution was further diluted by aspirating and discarding 5.00 ml from the 10 ml in the vial using a 5000 µl micropipette. Then a further 10 ml of haema-line was dispensed to the remaining 5 ml in the vial to dilute it 3 more times. This meant that the RBC readings displayed by the cell counter after running a RBC sample were to be multiplied by a factor of 3 to get the actual count. Finally a further WBC dilution was prepared from one control goat blood sample to be used as a priming sample.

To each of the WBC dilution, 3 drops of haema-lyse were added and mixed by inverting twice. The haema-lyse was allowed to act for at least 1 minute before running the WBC sample. The WBC priming sample was run first by pressing the 'WBC/Hb' button and the result disregarded. This was followed by running an actual WBC dilution sample and the WBC count and Hb concentration readings recorded. The snorkel was wiped clean and a RBC dilution sample run and the value recorded. Upon completion of the cell counting, the snorkel was dipped in a vial containing haema-clean and the flush cycle run 3 times by pressing the 'STBY/Flush' button. The system was further flushed twice with haema-standby and finally the 'SHND/Clean' button was pressed to initiate the 'Shutdown' cycle. The counter was then switched off and the waste container emptied. A vial full of haema-standby was left under the snorkel and the stopper on the waste container was reset.

2.6.2.6. Determination of red blood cell indices

The MCV, MCH and MCHC were calculated from the PCV, RBC count, and Hb concentration using the formulae of Dacie and Lewis (1994), as follows:

$$\text{MCV (fl)} = \frac{\text{PCV } (10^{-2}) \times 1000}{\text{RBC } (\times 10^{12}/\text{L})}$$

$$\text{MCH (pg)} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC } (\times 10^{12}/\text{L})}$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{PCV } (\times 10^{-2})}$$

2.6.2.7. Determination of packed cell volume (PCV)

The PCV was determined by the Microhaematocrit Centrifuge Technique using the same samples collected for RBC, WBC and Hb determination. A blood sample which had been rolling on the Spiramix was opened and blood aspirated up a 75 x 1.5 mm plain capillary tube for three quarters of its length and the outside wiped clean with a tissue. The bottom end of the capillary tube was sealed with cristaseal and the tubes placed in the microhaematocrit centrifuge (Kubota, model KH 12005, Japan) with the sealed end outermost (Plate 3). The capillary tubes were loaded symmetrically to ensure good balance, the rotary cover screwed on and the lid closed. The centrifuge was switched on, the speed set to 12,000 rpm and the time set to 5 minutes. After centrifugation, the PCV was read as a percentage using the Hawsby microhaematocrit reader.

2.7. Parasitaemia (trypanosomes/ml blood) determination

The number of trypanosomes per millilitre of blood was determined using the Buffy coat method (as described in section 2.1.1.1.) with the buffy coat from the capillary tube samples used for reading the PCV and the average number of trypanosomes per field determined by counting the trypanosomes physically. The number of the counted trypanosomes was then expressed in trypanosomes/ml of blood using the chart in table below (Murray *et al*, 1983):

Table 2.4: Parasitaemia estimation

Number of trypanosomes	**Score**	**Parasitaemia (tryps/ml)**
Swarming > 100/field	6^+	$> 5 \times 10^6$
> 10/field	5^+	$> 5 \times 10^5$
1 - 10/field	4^+	10^4 to 5×10^4
1/2 fields to 1/10 fields	3^+	5×10^3 to 5×10^4
1 - 10/preparation	2^+	10^3 to 10^4
1/preparation	1^+	10^2 to 10^3

2.8. Serum proteins estimation

The total serum protein concentration, serum albumin concentration, serum globulins concentration and the albumin:globulin (A:G) ratio were estimated. These parameters were determined thrice per week during the first 5 weeks post-infection and thereafter (after the commencement of erythrophagocytosis experiments) twice per week.

2.8.1. Collection of blood samples

Blood samples for serum protein estimation were collected on the morning of the day of estimation. Blood was collected into 5 ml plain vacutainer tubes using sterile 21 gauge vacutainer needles as described in section 2.6.1. The vacutainer tubes containing blood were placed on the test tube rack in an upside down position (with the corked end sitting down).

2.8.2. Extraction of serum from whole blood

The vacutainer tubes containing blood were left in an upside position for two and half hours at room temperature to allow the blood to clot. Each tube was turned upright to let the serum collect at the bottom leaving the clot adhered to the rubber cork. The rubber cork was then pulled out slowly and gently along with the blood clot. The tubes, now containing sera were placed in a bench centrifuge (Hitachi, model 05p-21, Japan) and spun for 10 minutes at 2000 rpm in order to allow all the particles to sediment leaving only clear serum. The sera was transferred to clean labelled 5 ml test tubes and was ready for use.

2.8.3. Estimation of serum total protein concentration

The serum total protein concentration was estimated using the Biuret method. The reaction between the copper ions in an alkaline copper sulphate solution (Biuret reagent) and the peptide nitrogen atoms in serum proteins resulted in a violet-blue complex. This reaction was specific such that other non-protein nitrogen compounds did not react (Ministry of Agriculture, Fisheries and Food, 1984).

2.8.3.1. Preparation of Biuret solution

a.) Reagents:

- Potassium sodium tartrate tetrahydrate = $C_4H_4KNaO_6.4H_2O$ (Wako Pure Chemical Industries Limited, Japan).
- Copper sulphate pentahydrate = $CuSO_4.5H_2O$ (Wako Pure Chemical Industries Limited, Japan)
- Potassium Iodide = KI (Cica, Kanto Chemical Company, Japan)
- Sodium hydroxide = NaOH (Cica, Kanto Chemical Company, Japan)

b.) Method:

The following chemicals were weighed using a Libror scale, (model EB-3200H, Shimadzu, Japan) with a capacity of 3120.00 g:

- Potassium sodium tartrate tetrahydrate = 90.00 g
- Copper sulphate pentahydrate = 30.00 g
- Potassium iodide = 10.00 g
- Sodium hydroxide = 16.00 g

The sodium hydroxide was immediately (because it is highly hygroscopic) added to about 900 ml of distilled water in a 1000 ml conical flask and dissolved. The solution was transferred to a 2000 ml volumetric flask and made up to the mark with distilled water to make a 0.2 M sodium hydroxide solution.

About 500 ml of the sodium hydroxide solution was put in a 1000 ml conical flask and 90.00 g of potassium sodium tartrate, 30.00g copper sulphate and 10.00g potassium iodide dissolved in it. The solution was transferred to a 2000 ml volumetric flask and the volume made up to the mark with the 0.2 M sodium hydroxide solution. This was now Biuret reagent working solution and was stored in a polyethylene container at room temperature.

2.8.3.2. Preparation of the blank diluent reagent

This reagent was prepared to be used in case there was haemolysed, icteric or turbid sera. In such a case a blank sample in the same way as the sample with biuret solution was to be prepared except that instead of using the biuret reagent, the blank diluent was to be used. Then the blank sample`s absorbance was to be subtracted from the absorbance of the sample in biuret reagent.

To about 500 ml of 0.2 M sodium hydroxide solution in a 1000 ml conical flask, 9.00 g of potassium sodium tartrate and 5.00 g potassium iodide were dissolved and the volume made to 1000 ml with 0.2 M sodium hydroxide. This made the blank diluent reagent which was stored in a polyethylene bottle at room temperature.

2.8.3.3. Standard protein solution

A set of vials of standard Bovine serum albumin solution (BSA) with concentrations of 2, 4, 6, 8 and 10 g/dl was obtained from Sigma Aldrich (U.K). From these, a set of 10 vials, each containing 2 ml of the BSA at dilutions of 1 - 10 g/dl was prepared by directly pipetting 2 ml of each standard at the particular concentration into a sterile vial with the particular concentration label. Concentrations at 1, 3, 5, 7 and 9 g/dl were worked out (Table 2.4) using the formula:

$$V_1C_1 = V_2C_2$$

Where, V_1 = Volume of the original standard BSA concentration

C_1 = Concentration of the original standard BSA

V_2 = 2 ml (Final volume to which V_1 was to be made with 0.85% sodium chloride solution)

C_2 = BSA desired concentration

Table 2.5: Dilution of stock BSA to working BSA concentrations

Working BSA (g/dl)	**Stock BSA (g/dl)**	**Stock BSA (ml)**	**Sodium chloride (ml)**
1	2	1.00	1.00
3	4	1.50	0.50
5	6	1.70	0.30
7	10	1.40	0.60
9	10	1.80	0.20

The solutions were pipetted into clean, sterile glass test tubes and mixed thoroughly. The vials containing the standards were corked and kept in a refrigerator at 2°C.

2.8.3.4. Calibration curve for total serum protein concentration

Six 15 ml test tubes were labelled with concentrations of 1, 2, 4, 6 and 8 g/dl and 'Blank' in duplicate. 5.00 ml of the biuret reagent was pipetted into each tube and 5.00 ml of 0.85% sodium chloride solution added using a 10.00 ml glass pipette. The tubes were stoppered with clean paraffin film and the contents mixed by gently inverting several times. Using a 200 µl micropipette, 200 µl of the standard BSA at each particular concentration was pipetted into the tube with that particular concentration label. After aspirating the 200 µl from the reservoir, the outside of the micropipette tip was wiped dry with fluffless tissue before dispensing. After dispensing, the tip was rinsed with the reagent in the test tube by aspirating and squirting back into the same test tube. To the tube labelled blank, 200 µl of the 0.85% sodium chloride solution was added and all the tubes covered with the paraffin film and the contents mixed by inverting gently several times.

The tubes were then incubated in a water bath maintained at 37°C for 15 minutes to allow the reaction to take place between the BSA and the reagent. Immediately after incubation, the absorbances of the samples were read using a spectrophotometer (Hitachi, model 100-20, Japan) set at 540 nm wavelength.

The absorbances (A_{540}) of the samples were read successively with another cuvette which was being cleaned with distilled water and drained dry with blotting paper before the next sample was added. The sample to be run was mixed thoroughly by inverting the tube twice and about 2 ml of the sample was poured into the cuvette which was then positioned in the light path in the holding rack of the spectrophotometer, the sample compartment closed and the digital meter reading recorded (Table 2.6)

Table 2.6: Absorbances (A_{540}) at different BSA concentrations

BSA (g/dl)	1	2	4	6	8
A_{540}	0.059	0.083	0.176	0.270	0.369

A curve of A_{540} against BSA concentration was plotted on a graph paper and the best fitting line was a linear curve. The curve was used to determine the goats' total serum protein concentration for that set of reagents. When a new set of reagents was prepared a new calibration curve was created.

2.8.3.5. Determination of serum total protein

Samples of goat serum were taken in duplicate and treated in an identical manner as the standard BSA. 200 µl of each serum sample was used and the mean of the duplicate A_{540} readings taken for determination of the protein concentration from the standard curve.

2.8.4. Estimation of serum albumin concentration

The capability of albumin to bind Bromocresol green dye (BCG) made it possible to determine serum albumin concentration. When albumin bound to the dye, a change in peak absorbance wavelength occurred and this was measured at 640 nm using the spectrophotometer (Ministry of Agriculture, Fisheries and Food, 1984).

2.8.4.1. Preparation of Bromocresol green dye solution

a.) Reagents:

- Bromocresol green = $C_2H_{14}Br_4O_5S$ (Cica Kanto Chemical Company, Japan)
- Sodium azide = NaN_3 (Wako Pure Chemical Industries, Japan)
- Succinic acid = $HOOCCH_2CH_2COOH$ (Cica Kanto, Japan)
- Sodium hydroxide (Cica Kanto, Japan)
- 'Brij-35', Polyoxythylene dodecyl ether = $CH_3(CH_2)_{11}O(CH_2CH_2O)_nH$, (Cica Kanto).
-

b.) Method:

The following were weighed on a Libror scale:

- Succinic acid = 17.70 g
- Bromocresol green dye = 0.22 g
- Sodium azide = 0.20 g
- 'Brij-35' = 7.50 g
- Sodium hydroxide = 20.00 g

The weighed sodium hydroxide was quickly dissolved in about 300 ml distilled water in a 500 ml beaker. The solution was transferred to a 500 ml volumetric flask and made up to the mark with distilled water to make 1 M sodium hydroxide solution (for pH adjustment).

The weighed 'Brij-35' was put in about 10 ml distilled water in a 50 ml graduated beaker. The beaker was covered with a glass cover and heated slowly until the 'Brij-35' had dissolved, then the solution was made to the 25 ml mark with warm distilled water, the beaker covered and heated again to further dissolve the 'Brij-35' and kept warm.

Meanwhile, the weighed succinic acid, Bromocresol green and sodium azide were dissolved *in about 1500 ml distilled water in a conical flask by stirring with a magnetic stirrer. Using a* 10 ml pipette, 8.00 ml of the warm 'Brij-35' was measured and very quickly squirted into the solution while stirring. When all the particles in the solution had dissolved, the pH of the solution was checked using an electronic pH meter (Horiba, model M-8AD, Japan) and the pH adjusted to pH 4.2 with sodium hydroxide solution. The solution was transferred to a 2000 ml volumetric flask and made to mark with distilled water. The solution was stored in a polyethylene container in the dark at room temperature.

2.8.4.2. Calibration curve for serum albumin concentration

Clean, 5 ml test tubes were labelled with concentrations of 1 - 5 g/dl and 7 g/dl and 'blank' in duplicate. 4.00 ml of Bromocresol green dye solution was pipetted into each tube. Then, 20.0 µl of the standard was aspirated with a 50 µl micropipette, the outside of the tip wiped dry with a blotting paper and the sample added to the tube by squirting into the dye solution, then aspirating and squirting it back 2 more times. The tubes were covered with paraffin film and the contents mixed by inverting 3 times very gently. The samples were incubated for 15 minutes in a water bath set at 37°C.

Immediately after incubation of the samples, the A_{640} of the samples were read successively using the same cuvette which was being rinsed clean with distilled water and drained dry by pressing it upside down on blotting paper for about 30 seconds before running the next sample and the A_{640} recorded (Table 2.7).

Table 2.7: Absorbances (A_{640}) at different BSA concentrations

BSA (g/dl)	1	2	3	5	7
A_{640}	0.232	0.308	0.438	0.692	0.880

A curve of A_{640} against concentration was plotted on a graph paper and the best fitting line was a linear curve. The curve was used to determine the goats' serum albumin concentration using the same Bromocresol green dye solution used for creating the curve. When a new set of reagents was prepared, a new curve was created.

2.8.4.3. Determination of serum albumin concentration

Samples of goat serum were taken in duplicate and treated in an identical manner as the standard BSA. 20.0 µl of each goat serum sample was used and the mean of the duplicate A_{640} readings taken for determination of the serum albumin concentration from the standard curve.

2.8.5. Estimation of serum globulin concentration

Upon determination of the serum total protein and the albumin concentrations, the globulin concentration was arrived at by subtracting the albumin concentration from the serum total protein concentration and expressed in g/dl.

2.8.6. Calculation of the albumin:globulin (A:G) ratio

The A:G ratio was calculated by dividing the albumin concentration by the globulin concentration.

2.9. Investigation of erythrophagocytosis

2.9.1. Preparation of solutions

2.9.1.1. Reconstitution of 1million IU penicillin and 1 g streptomycin

Penicillin was obtained as an ampule containing 1 million IU penicillin powder while streptomycin was obtained as an ampule containing 1 g streptomycin powder. The 1 million IU penicillin powder was reconstituted to a 500 IU penicillin solution.

Distilled water was sterilised by autoclaving at 120°C for 30 minutes. Two clean vials in which the reconstituted drugs were to be kept were also autoclaved. The reconstitution was done in a sterile hood. 5.0 ml sterile water was drawn with a sterile syringe and needle and squirted into the 1 million IU penicillin and the vial thoroughly shaken to dissolve the powder. The resulting penicillin solution was 200,000 IU/ml

The final 500 IU/ml penicillin was worked out using formula:

$$V_1C_1 = V_2C_2$$

Where, V_1 = volume to be drawn from the 200,000 IU/ml penicillin solution.

V_2 = 50 ml; the volume of the final preparation required.

C_1 = 200,000 IU/ml

C_2 = 500 IU/ml.

0.13 ml of the 200,000 IU/ml penicillin solution was diluted with sterile water to a solution of 500 IU/ml penicillin.

The 1.0 g streptomycin, was dissolved in 5.0 ml sterile water to get a 200 mg/ml solution. The final 500 µg/ml streptomycin concentration was worked out using formula:

$$V_1C_1 = V_2C_2$$

Where, V_1 = volume to be drawn from the 200 mg/ml streptomycin solution.

C_1 = 200 mg/ml streptomycin concentration

C_2 = 500 µg/ml streptomycin concentration

V_2 = 50 ml; desired final volume of the final preparation.

0.125 ml of the 200 mg/ml streptomycin was diluted with sterile water to give a solution of 500 µg/ml. The solutions were kept in a fridge at 2 - 4°C.

2.9.1.2. Preparation of phosphate buffered solution (PBS) at pH 7.2

The following were weighed on the Libror scale:

- Anhydrous disodium hydrogen phosphate (Na_2HPO_4, Cica, Japan) = 13.48 g
- Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$, Cica Japan) = 4.26 g
- Sodium chloride (NaCl, Cica, Japan) = 4.26 g

The weighed quantities were dissolved in about 800 ml of distilled water and the volume made up to the mark in a 1000 ml volumetric flask. The PBS was prepared fresh for use on each day of the experiment.

2.9.1.3. Preparation of Rochester Park Memorial Institute (RPMI)-1640 media

RPMI-1640 media powder was obtained from Sigma Aldrich, England and prepared as per manufacturer's instructions. 10.39 g of the RPMI-1640 powder was weighed on the Libror scale and quickly (it is hygroscopic) transferred to a conical flask containing about 900 ml of distilled water while stirring until dissolved. The pH of the solution was checked (by pouring a bit of the solution into a beaker and checking its pH with the electronic pH meter) but found to be lower than pH 7.2. The pH was raised by adding drops of 1 M sodium hydroxide solution to the full volume of the prepared media solution and checking the pH after stirring until pH 7.2 was attained. Sodium bicarbonate (0.20 g) was added to the solution and stirred until dissolved. The solution was finally transferred to a 1000 ml volumetric flask and the volume made to the mark with distilled water.

The prepared media was sterilised by filtration using a filter membrane with a porosity of 0.22 µm (Millipore, Ireland). The filtration apparatus i.e. a 1000 ml flask, funnel, a membrane supporter as well as the membrane were sterilised by autoclaving at 120°C and 2.5 kg/cm^2 pressure for 30 minutes using a neoclave (model AS-2401, Sakura, Japan). Five, 120 ml storage bottles and stoppers were also autoclaved. The apparatus and the media were placed in a sterile hood. A vacuum pump (Millipore, Ireland), was connected to the vacuum line of the hood. The operator's hands were swabbed with cotton wool soaked in 98% ethanol before touching anything in the hood. The filtration apparatus was assembled in the hood and connected to the vacuum line. The suction pump was switched on, and the media poured into the funnel of the filtration apparatus while the filtrate was collected in the flask. The filtrate was transferred to the storage bottles and stored in the fridge at 2°C.

On the morning of the experiment, one bottle containing 100 ml of the media was brought to room temperature and made to 'complete' RPMI-1640 by addition of the following:

- 10 ml sterile fetal bovine serum (Flow Laboratories, Australia)
- 0.1 ml 2-mercaptol ethanol (Wako Pure Chemical industries, Japan)
- 0.5 ml of the 500 IU/ml penicillin solution
- 0.3 ml of the 500 µg/ml streptomycin solution

and mixed by gentle inversion several times. The media was at this point 'complete' with full nutrients for cells (Makumyaviri, A., personal communication).

2.9.2. Separation of blood cells

2.9.2.1. Collection of blood samples

Blood sample collection for investigation of erythrophagocytosis in the preliminary experiments started at day 0 while in the final experiment it started at 37 days post-infection. The samples were collected on a weekly basis on the day when no other experiment was being done. About 5 ml of blood was collected from each animal into vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA). The collection procedure was done as described in the procedure for collecting blood samples for blood cell counting (section 2.6.1). Six goats were sampled for the preliminary experiment.

2.9.2.2. Isolation of RBCs and mononuclear cells (MNC) from whole blood

The procedure of Makumyaviri, A., (personal communication) was followed. The vacutainer tubes containing blood were placed on the Spiramix and left to mix for about 5 minutes. Meanwhile, 15 ml centrifugation conical glass tubes were labelled with sample numbers and about 5 ml histopaque-1077 solution (Sigma, England) which had been brought to room temperature was poured into each tube. Holding the centrifugation tube at about 30° angle, a blood sample was slowly and gently poured onto the top of the histopaque. The samples were placed in a bench centrifuge and spun at 2500 rpm for 30 minutes. This resulted in the separation of the sample into 4 distinct layers i.e. the upper clear plasma layer, then immediately below was the opaque whitish layer of mononuclear cells. Below the mononuclear cells layer was the transparent histopaque layer and at the bottom was the RBCs mass.

A set of six 15 ml centrifugation tubes were labelled with sample numbers plus a 'W' mark. Using a clean pasteur pipette for each sample, the plasma layer was aspirated and discarded. The MNC layer was aspirated and put in a labelled tube. The histopaque layer was aspirated and discarded. To the MNC samples, about 2 ml of the red cell lysing buffer (8.3 g/l ammonium chloride in 0.01 M Tris-HCl buffer, pH 7.5 ± 0.2) obtained from Sigma, England, was added, mixed by shaking gently then incubated for 10 minutes to lyse any contaminating RBCs. After incubation of the MNC suspension, they were centrifuged at 1500 rpm for 5 minutes and the supernatant discarded. About 5 ml of the 'complete' RPMI-1640 media was added and mixed with the cell mass by aspirating and squirting back with a pasteur pipette.

To the RBC mass remaining in the original centrifuge tubes, about 5 ml of PBS at pH 7.2 was added and mixed with a pasteur pipette by aspirating and squirting back the cell suspension into the test tube. The RBC suspension was then centrifuged at 1500 rpm for 10 minutes and the supernatant discarded. This washing procedure was repeated two more times and finally the RBCs mass was resuspended in a volume of PBS (pH 7.2) approximately equal to the volume of the cell suspension by pouring the PBS gently onto the cell mass and mixing by aspirating and squirting back with a pasteur pipette.

2.9.2.3. Establishing the cell concentration in the RBC and MNC suspensions

The counting of cells in the RBC suspension and MNC suspension was done using the electronic cell counter (Makumyaviri, A., personal communication). The cell counter was switched on and prepared for use as explained in section 2.6.2.5. To prepare the RBC dilution, a RBC suspension sample which had been rolling on the Spiramix was obtained and 40 µl of the sample aspirated with the autodilutor. The sample was dispensed along with 10 ml of haema-line into a clean dry vial while holding the vial at about 45° angle with the dilutor tip placed against the inner-side of the vial and making sure it remained above the fluid level. The vial was capped and contents mixed by inverting twice to give a 1:250 dilution. A further 40 µl was aspirated from the dilution and dispensed into another vial along with 10 ml of haema-line to make a 1:62,500 RBC dilution. The vial, was capped and contents mixed by inverting twice.

The RBC dilution was further diluted by aspirating and discarding 5 ml from the 10 ml of the RBC dilution. A further 10 ml of haema-line was dispensed into the vial to dilute the remaining 5 ml RBC suspension. This meant that the RBC readings displayed by the cell counter after running the RBC sample, were to be multiplied by a factor of 3 to get the actual count.

A MNC suspension was diluted as follows: a MNC suspension sample which had been rolling on the Spiramix was obtained and 40 µl aspirated with an autodilutor, then dispensed along with 10 ml haema-line into a clean vial and the contents mixed by inverting twice to make a 1:250 MNC dilution. Three drops of haema-lyse were added to the MNC dilution and incubated for at least 60 seconds before running the samples. The MNCs and RBCs samples were run alternatively. The MNC suspension samples' concentrations were compared and the sample with the least concentration noted. Then all the other samples were diluted (with 'complete' RPMI-1640 media) down to this concentration.

The MNC cell suspensions were for the time being kept in the fridge at 4°C. Likewise the RBC suspension cell concentrations for each sample were compared and the sample with the least concentration noted. Then all the other samples were diluted (with PBS, pH 7.2) down to this concentration. This was in order to have uniform cell concentration in all the suspensions.

2.9.3. Microscopic counting method

2.9.3.1. Dispensing of MNC and RBC suspensions to incubation tubes

The MNCs and RBCs were incubated at a ratio of 1:20 i.e. 1 MNC to 20 RBCs (Makumyaviri, A., personal communication). For convenience, the total volume of the mixture was kept in the range of 1.0 - 1.5 ml for each sample. The following formulae were used to work out the volumes for the MNC and RBC suspension:

a.) $\times$ RBCs = 20 x Actual MNC count

Where, $\times$ gave the number of RBCs needed to make a 1:20 ratio with MNCs counted in 1 ml of the suspension (Actual MNC count).

b.) $Z \text{ ml} = \frac{\times}{Y}$

Where: Z ml = volume of the RBC suspension which will contain $\times$ RBCs

Y = Actual RBCs counted in 1 ml RBC suspension.

The MNC and RBC suspensions were left on a mixer for 10 minutes before dispensing. The worked out volume of MNC suspension was dispensed in duplicate to labelled 5 ml glass sterile test tubes using a 5000 μl micropipette. Then the worked out volume of RBC suspension was dispensed to the same tube (using a 200 μl micropippette) and mixed by aspirating and squirting back 3 times. This was done for each sample and the test tubes corked and incubated at 37°C in an incubator for 3 hours.

2.9.3.2. Counting phagocytosed RBCs

When the incubation period was over, the tubes were centrifuged at 1000 rpm for 5 minutes, the sediment aspirated using a pasteur pipette, a drop added to a clean glass slide and spread with the edge of another slide to form a thin smear. The smear was air-dried by waving and fixed in 98% methanol for 1 minute and further air-dried by waving. A 1:10 dilution of Giemsa stain was prepared and the slides stained as explained in section 2.10.3. When dry, the slides were scanned on a light microscope using the X100 objective and a X10 eye piece under immersion oil. Twenty monocytes and/or macrophages were randomly picked and the number of erythrocytes in each counted and summed-up (Makumyaviri, A., personal communication). This was done in duplicate and the mean tabulated.

2.9.4. Radioactive chromium uptake method

2.9.4.1. Dilution of ^{51}Cr stock solution

A stock solution of ^{51}Cr was obtained from Amersham (South Africa) as 1.0 ml sodium chromate in aqueous solution at a concentration of 23.8 μg Cr/ml (i.e. 185 MBq/ml or 5 mCi/ml) lot number 225CA. The dilution was done 10 days after the manufacture date of the solution. To dilute the 185 MBq/ml stock solution to 36 MBq/ml, 0.195 ml of the 185 MBq/ml solution was pipetted in to 0.805 ml PBS (pH 7.2) pre-pipetted to a clean sterilised 10 ml test tube and mixed thoroughly by aspirating and squirting the solution back into the tube 3 times. This was ready for use in the experiment.

On every morning of the experiment day, 0.195 ml of the stock solution was diluted similarly and used for that particular day. All the procedures involving the handling of the samples containing radioisotopes were done with the operator wearing disposable surgical gloves, laboratory goggles and a radiation coat with a radiation monitor.

2.9.4.2. Labelling processed RBCs with ^{51}Cr

The labelling rate of RBCs was 10^9 cells in PBS (pH 7.2) incubated with 6 Mbq (University of Glasgow, 1996). The RBC concentration on each particular day of the experiment was used to calculate how many MBq were to be used per sample. This was done by the formula below:

$$Y \text{ MBq} = X\,\frac{(6 \text{ MBq})}{10^9 \text{ RBCs}}$$

Where, X = the day's uniform RBC concentration in PBS

Y = the MBq to be worked out.

The diluted sodium chromate solution containing a concentration of 36 MBq /ml was used. After calculating the required MBq (Y), the volume (Z ml) to contain Y MBq from the solution with a concentration of 36 MBq/ml was worked out as follows:

$$Z \text{ ml} = \frac{Y \text{ MBq} \times 1 \text{ ml}}{36 \text{ MBq}}$$

Hence, Z ml of the sodium chromate solution were pipetted with a 200 μl micropipette and added to the X RBC concentration in a 5 ml test tube and mixed by aspirating and squirting back the contents 3 times with the same tip. The pipette tips used for this purpose were kept for disposal in a lead shielded reservoir (special reservoir). The test tubes were covered with paraffin film, placed in a lead can and incubated at 37°C in an oven for 1 hour. After incubation, the meniscus level of the volume of each sample was marked and the samples were placed in a bench centrifuge and spun at 1000 rpm for 5 minutes. The supernatant was discarded, then about 2 ml of PBS (pH 7.2) added and mixed with the cells by aspirating and squirting back with a pasteur pipette. This was spun at 1000 rpm for 10 minutes, the supernatant discarded and the washing procedure repeated 2 more times. Finally, PBS (pH 7.2) was added to the cell sediment up to the mark made and the cells resuspended by aspirating and squirting back with a pasteur pipette.

2.9.4.3. Dispensing of MNCs and labelled RBCs to incubation tubes

The samples from this stage were prepared in duplicate. For each sample, incubation and counting tubes (5 ml glass test tubes) were coded as follows:

- **Mp_o**: tube which contained MNCs plus labelled RBCs and from which the supernatant for measuring the supernatant radioactivity was taken. The pellet radioactivity was read from this tube after lysing and washing off unphagocytosed labelled RBCs. The pellet radioactivity measured the amount of ^{51}Cr emitted by labelled RBCs engulfed by MNCs.

- **Mt$_o$**: tube which contained MNCs plus labelled RBCs and from which the total input radioactivity was read. The radioactivity got from here was a measure of the amount of ^{51}Cr added and was used to compare with that got from tube Rt$_o$ so as to monitor whether the addition of MNCs impaired the detection of radiation.
- **Rs$_o$**: tube which contained labelled RBCs only and from which the supernatant for measuring the spontaneous radioactivity was taken. Spontaneous radioactivity was a measure of the amount of ^{51}Cr being liberated from labelled RBCs and was used to monitor whether there was a loss of ^{51}Cr from intact labelled RBCs.
- **Rt$_o$**: tube which contained labelled RBCs only and from which the total input radioactivity was read.
- **Msp**: tube which contained the supernatant taken from Mp$_o$ and from which the supernatant radioactivity was read. Supernatant value was a measure of the amount of ^{51}Cr liberated due to intracellular lysis of phagocytosed RBCs (by MNCs) and also extracellular lysis of ^{51}Cr-labelled RBCs by MNCs.
- **Rs**: tube which contained the supernatant taken from tube Rs$_o$ and from which the spontaneous radioactivity was read.

The MNCs and RBCs were incubated at 1:100 ratio i.e. 1 MNC to 100 RBCs (Makumyaviri, A., personal communication). The total volume of the mixture was maintained within the range of 1.0 - 1.4 ml for each sample. For instance, in one experiment, the cell concentration in each ^{51}Cr-labelled RBC suspension was 3.0 x 10^9/ml and that of MNCs was 3.0 x 10^6/ml. Hence, 0.1 ml of the labelled RBCs suspension was incubated with 1 ml of the MNC suspension to give a ratio of 1 MNC to 100 RBCs and 1.1 ml total volume of the mixture.

The MNC suspension which had been kept at 4°C in the fridge was retrieved and put on the Spiramix roller for 10 minutes. Then the worked out appropriate volume which would give the 1:100 ratio was dispensed to tubes Mp_o and Mt_o using a 200 µl micropipette The same volume, but of 'complete' RPMI-1640 media was added to tubes Rs_o and Rt_o. Then the worked-out appropriate volume of ^{51}Cr-labelled RBCs was added to tubes Mp_o, Mt_o, Rs_o, and Rt_o using a 200 µl micropipette and the contents mixed by gently aspirating and squirting back 3 times.

The tubes were covered with paraffin film, placed in a lead shielded container and incubated at 37°C in an incubator for 3 hours. After incubation, tubes Mp_o and Rs_o were spun at 1000 rpm for 10 minutes and a supernatant volume equivalent to one-quarter of the total volume of the tube contents taken from tubes Mp_o and Rs_o and placed in tubes Msp and Rs, respectively. Red cell lysing buffer was added to Mp_o (one-quarter of the total volume of tube Mp_o), mixed with a pasteur pipette and incubated for 10 minutes to lyse all unphagocytosed labelled RBCs. Then the tubes were spun at 1000 rpm for 10 minutes and the supernatant discarded into a special reservoir. The pellet was washed three times with 2 ml of 'complete' RPMI-1640 media by adding the media to the pellet, mixing with a pasteur pipette (aspirating and squirting back), centrifuging at 1000 rpm for 10 minutes and discarding the supernatant into a special reservoir. Finally, 1.0 M sodium hydroxide solution (a volume equivalent to half the original volume in Mp_o) was added to the pellet, the tube corked and contents mixed by shaking thoroughly to break-up the pellet and lyse the MNCs. This was then kept for measuring pellet radioactivity. Tube Rs_o plus its remaining contents were discarded into the special reservoir.

To tubes Mt_o and Rt_o, 1.0 M sodium hydroxide solution (a volume equivalent to half the volume of the contents in each tube) was added, the tubes corked and thoroughly shaken to break-up all the cells contained in the tubes. Tubes Mt_o and Rt_o were then kept for measuring total input radioactivity (for the mixture of RBCs and MNCs) and total input radioactivity (for RBCs only), respectively. The tubes were orderly packed in lead shielded containers ready for transportation to the radioactivity counting laboratory.

2.9.4.4. Reading radioactivity

The radioactivity was counted using a Gamma Scintillation Counter (Sourcerer Radioimmunoassay System, Oakfield Instruments Limited, Oxford, U.K) with a counterpoint system 9 software (Plate 4). The scintillation counter was based at the University Teaching Hospital (School of Medicine) and its operation was done with the help of the Chief Technician in-charge of the Radioisotope laboratory.

The machine was switched on and set to 'Other Isotopes' menu (it had a choice of iodine isotopes, cobalt, selenium and 'Other isotopes'). The time for counting was set (60 seconds for the first and second experiment and 90 seconds for the proceeding experiments) and the reference tray (of ^{159}I) was inserted into the wells and the radioactivity counted to check the machine's calibration factors. Then the background count was done for the tray to be used for counting the radioactivity of the samples. The machine had a capacity to carry 8 samples at a time and so, 8 tubes were packed in an orderly manner (noting the well number and the number of the sample) and the machine set to count radioactivity of the samples. The counts were automatically processed and printed by the computer with the background count being subtracted automatically for each counting well. The count of each sample was identified by the number of the well in which it was placed.

The mean of duplicate values was obtained. The supernatant count and the spontaneous count were multiplied by 2 to get the actual value since the volume of the supernatant used was just half of the actual supernatant volume. To calculate the ^{51}Cr-incorporation% and the ^{51}Cr-release% the following formulae were used:

$$^{51}\text{Cr-Incorporation\%} = \frac{\text{Pellet radioactivity x 100}}{\text{Total input - Spontaneous release}}$$

Where:

^{51}Cr-incorporation = the measure which determined the amount of ^{51}Cr-labelled RBCs phagocytosed by MNCs after a 3 hours incubation period. The measure was used to show the magnitude of erythrophagocytosis.

Pellet radioactivity = radioactivity read from tube Mp_o after lysing and washing off unphagocytosed labelled RBCs. The radioactivity read here was being emitted from phagocytosed RBCs.

Spontaneous release = measure of the amount of ^{51}Cr which was being liberated from labelled RBCs and was read from tube Rs.

Total input = radioactivity read from tube Rt_o and measured the total amount of radioactivity possessed by labelled RBCs.

$$^{51}\text{Cr-Release\%} = \frac{\text{Supernatant radioactivity - Spontaneous release x 100}}{\text{Total input - Spontaneous radioactivity}}$$

Where:

^{51}Cr-release% = a measure of the amount of radioactivity released from labelled RBCs which were extracellularly lysed by MNCs as well as that due to digestion of the engulfed RBCs and excretion of the ^{51}Cr by the MNCs.

Supernatant radioactivity = measure of the amount of ^{51}Cr liberated due to intracellular lysis of phagocytosed RBCs and also extracellular lysis of of the labelled RBCs. This was read from tube Ms_p.

All the experiment residues and glassware were packed in the special reservoir and delivered to the National Council for Scientific Research Radiation Unit for disposal.

2.10. Pathological examination of some of the experimental goats

2.10.1. Sacrifice of one control and two infected goats for pathology

One control and 2 infected animals were sacrificed at 85 days post-infection and post-mortem done. Euthanasia was achieved by intravenous injection (into the jugular vein) of excessive dose thiopentone sodium (5% solution). The gross post-mortem was conducted in the post-mortem room of the School of Veterinary Medicine.

2.10.2. Making impression smears

To make impression smears of the liver, kidney, superficial lymph nodes and lungs, a slicing incision was made in each organ with a sharp knife and the organ gaped open by applying digital pressure. Then a clean glass slide surface was dabbed on the cut surface and waved around to air-dry (Bancroft and Cook, 1995). After drying, the slides were dipped in 98% methanol for 1 minute to fix and then retrieved and waved to air-dry.

To make impression smears of the bone marrow, a long bone (femur) was broken in the middle and a drop of the bone marrow was applied on a clean glass slide and spread with the edge of another slide (Makumyaviri, A., personal communication). The slide was air-dried by waving and dipped in 98% methanol for 1 minute to fix and later air-dried.

2.10.3. Staining impression smears with Giemsa solution

2.10.3.1. Dilution of stock Giemsa solution

The stock Giemsa solution which had been kept at 4°C was brought to room temperature by standing it on the bench for 30 minutes. Then 10 ml of the Giemsa solution was measured in a 100 ml measuring cylinder and 0.01 M PBS (pH 7.2) added to the mark of 100 ml and mixed thoroughly to make a 1:10 dilution of the stock Giemsa solution.

2.10.3.2. Staining the smears

The fixed and air-dried impression smears were placed on a rack in a horizontal position with the smeared surface facing upwards. Using a pasteur pipette, the diluted Giemsa solution was applied to flood the surfaces of the slides and left to stain for 30 minutes. After staining the excess stain was washed off the slides with running tap water by holding each slide under a running tap in a horizontal manner for about 5 seconds and excess water shaken off the slide. The slides were placed in a vertical position and left to dry.

2.10.4. Scanning of the stained smears for evidence of erythrophagocytosis

The stained impression smears of different organs were scanned for evidence of erythrophagocytosis using a light microscope (Olympus, model BH-2, Japan). Firstly, the scanning of each slide was done grossly under the X40 objective with a X10 eye piece, and when a satisfactory field was picked, immersion oil was applied on the slide and the objective changed to X100 to pick details. The scanning was basically done to look for monocytes and/or macrophages and check for evidence of engulfed erythrocytes and establish the organs which showed more erythrophagocytosis activity.

2.11. Statistical analysis

C-stat for Windows version 1.0 computer statistical package (Holman and Kennedy, 1993) was used. The Student t-test method was employed to determine whether there was any statistical difference ($P < 0.05$) between the control and the infected group means for the parameters measured.

CHAPTER THREE

RESULTS

The results reported in this chapter from the first set of 8 goats are only for the erythrophagocytosis experiment using the microscopic method. Results for the other parameters were not recorded consistently because they were merely trial results and as such they were not included. Results reported for all the other parameters were obtained using the second set of 10 goats.

3.1. Parasitological observations

In all the 5 infected goats, the parasitaemia was first evident on the third day post-infection (PI) and continued rising to reach the first peak at 4 days PI with goat-1 showing the highest peak (5000 trypanosomes/ml) (figure 4.6a) while goat-2, 3, 4 and 5 (figures 4.6b - 4.8) had lower similar peaks (1000 trypanosomes/ml) (Table 3.0). Thereafter, the parasitaemia started dropping, with the disappearance of the first parasitaemic wave being recorded at 7 days PI in goat-2, 3 and 5, while goat-4 showed a minimal parasitaemia of 100 trypanosomes/ml (not complete disappearance). Goat-1 showed the end of the first wave of parasitaemia at 9 days PI.

The second parasitaemic wave appeared at 11 days PI in goats-2, 3, 4, and 5 with goat-4 showing a peak (5000 trypanosomes/ml) higher than that of the first wave. Goat-1 showed the reappearance of parasitaemia at 14 days PI and died 2 days thereafter. The peak of the second wave of parasitaemia was observed at 14 days PI in goat-2, 3 and 5 with goat-3 scoring the highest peak (10,000 trypanosomes/ml), about 10 times higher than that of goat-2 and 5. Goat-4 showed the next highest peak (5000 trypanosomes/ml) during the second wave of parasitaemia.

A decline in the second parasitaemic wave was observed to start at 17 days PI in all the infected goats with goat-4 having a complete disappearance of parasitaemia which later resurfaced at 19 days PI indicating the start of the third parasitaemic wave (barely 2 days after the end of the second wave). Goat-2 and 5 had a prolonged (6 days duration) second wave of parasitaemia which finally disappeared at 17 days PI. Goat-3 was exceptional in that its second wave never disappeared completely but was maintained at a relatively high level (1000 trypanosomes/ml) for 17 days (up to day 28 PI) when the third peak (10,000 trypanosomes/ml) was recorded and, thereafter, the parasitaemia kept on fluctuating until day 53 PI when it disappeared completely and never reappeared for the remaining 3 weeks of the experiment.

Goat-5 showed a remission of parasitaemia (third wave) 24 days PI (1 week after disappearance of the second wave) with the lowest peak (100 trypanosomes/ml) being recorded, and thereafter, the parasitaemia became very scanty and finally completely disappeared at 46 days PI for the rest of the remaining 4 weeks of the experiment.

The start of the third wave of parasitaemia in goat-2 was observed at 24 days PI (5 days after the end of the second wave) and lasted for a prolonged period (18 days) with a relatively very high peak (5000 trypanosomes/ml) being noted at 35 days PI. A very short fourth parasitaemic wave, but with the highest peak (7000 trypanosomes/ml) ever observed for goat-3, was noted at 53 days PI (1 week after the disappearance of the third wave). This was followed by a prolonged (11 days) absence of parasitaemia with the fifth wave appearing at 67 days PI with a relatively high peak (5000 trypanosomes/ml), but later disappeared 4 days before the end of the experiment.

Goat-4 showed rather consistent parasitaemic waves lasting about 1 week per wave with a 3 days interval between the waves. However, after the disappearance of the fifth parasitaemic wave, there was a prolonged period (17 days) of complete absence of parasitaemia which later resurfaced at 60 days PI with the highest peak (15,000 trypanosomes/ml) recorded among all the infected goats, but which lasted only a short while. The final wave was observed at 71 days PI and at the last day of the experiment (74 days PI) no parasitaemia was observed.

The mean parasitaemia of the infected goats depicted a fluctuating wavy pattern throughout the experimental period (Figure 3.0). Eight mean parasitaemic waves were observed with each wave lasting an average of about 6 - 7 days. After the end of the second mean parasitaemic wave, there was a prolonged period (7 days) of relatively very low mean parasitaemia. However, the intervals between the proceeding mean parasitaemias were characteristically very short. The highest mean parasitaemic peak (12,500 trypanosomes/ml) was observed during the seventh mean parasitaemic wave, while the lowest peak (1180 trypanosomes/ml) was noted during the fifth mean parasitaemic wave. At the last day of the experiment, there was no observed mean parasitaemia.

3.2. Rectal temperatures

All the infected goats depicted highly variable temperatures which corresponded to parasitaemic waves (Table 3.1). The day before infection all the experimental animals (both the infected and the control goats) had rectal temperatures in the range of 38.4 - 39.4°C which was almost within the normal published range of 39.1 ± 0.5 (The Merck Veterinary Manual, 1991). The control group goats maintained rectal temperatures within this range though the temperatures were variable at different days until the end of the experiment (Table 3.1).

In all the infected goats, the temperatures rose steadily to reach the first peak over a period of about 3 - 4 days (which corresponded to the prepatent phase). Goat-1 showed only one peak of temperature (40.5°C) which corresponded to its first parasitaemic peak and thereafter, gradually declined over a period of 10 days to reach the original temperature (38.7°C). The second parasitaemic wave didn't result in temperature rise until the animal died. The observed first temperature peak (41°C) of goat-2 (figure 4.9) was the highest among the infected goats. This temperature rapidly declined to near original level with the disappearance of parasitaemia, but rapidly rose again reaching a second peak of 40.3°C at

11 days PI when the second wave of parasitaemia resurfaced. With the disappearance of this second wave of parasitaemia, goat-2`s temperature fell slightly below the original level (at 20 days PI) and then started rising rapidly to give a third peak at the start of the third parasitaemic wave. With the prolonged third parasitaemic wave, relatively high rectal temperature was maintained with a peak of 41.3°C being recorded at 50 days PI which coincided with goat-2`s highest parasitaemic peak. The temperature then declined rapidly and fluctuated until the end of the experiment.

Goat-3 showed a pattern of rectal temperatures (Table 3.1) similar to that of goat-2 which closely followed parasitaemic waves. The first rise in temperature was noted at 3 days PI when the first parasitaemic wave started. A rapid decline was observed with the disappearance of the first wave at about 8 days PI and thereafter (10 days PI) there was a steep rise in temperature reaching another peak (40.5°C) at 11 days PI (with the start of the second parasitaemic wave). The temperature went on fluctuating but maintained a relatively high level until day 53 PI when it dropped to its original level (of about 38.5°C) with the complete disappearance of parasitaemia and maintained this low level up to the end of the experiment.

Goat-4 also showed a fluctuating pattern (Table 3.1) of temperature (rise and fall) corresponding to the parasitaemic patterns (i.e. high parasitaemia corresponded to high temperature and vice versa), with the highest rectal temperature being recorded when the animal had the highest parasitaemic score. Goat-5 which maintained relatively low parasitaemia showed its highest rectal temperature (Table 3.1) at the start of the second wave of parasitaemia and thereafter maintained low temperature.

The mean rectal temperature of the infected goats showed a very steep rise to reach a peak of 40.3°C (which was the highest mean temperature recorded throughout the experimental period) at 4 days PI (Figure 3.1). Thereafter infected goats maintained fluctuating mean rectal temperatures which were relatively higher than the mean rectal temperature of the control goats throughout the experimental period. However, at about 19 days PI, the mean rectal temperature of the infected goats dropped to normal. This was the time when the mean parasitaemia was observed to be very low for a prolonged period

Also, at 67 days PI the mean rectal temperature of the infected goats was noted to drop to below the mean rectal temperature of the control goats. This period was associated with correspondingly low mean parasitaemia. At the last day of the experiment, the mean rectal temperature of the infected goats was noted to drop to the normal level.

The control goats depicted a fluctuating pattern of the mean rectal temperatures which maintained the published normal range of 39.1 ± 0.5 °C (figure 4.9).

Statistically, the infected goats' mean rectal temperatures were found to be very significantly ($P < 0.01$) higher than the control goats' means at 11, 14, 17 and 21 days PI. At 35 days PI, the two means were observed to be significantly ($P < 0.05$) different (Figure 3.1).

3.3. Other clinical signs

Clinical examinations done prior to infection showed that both the infected and the control goats were bright, alert, very active and drinking and eating all the feeds which were offered. The observed mucous membranes of the eyes and mouth were pinkish-red and moist (normal appearance). The coat was smooth with lustre. The faecal samples were pelleted smooth and solid. The readily palpable superficial lymph nodes (parotid and prescapular) were an average of about 0.5 cm in diameter with a smooth firm texture to touch. The heart beats were steady, strong and fell within a range of 70 - 90 beats/minute which was within the normal published range of 70 - 135 beats/minute (The Merck Veterinary Manual, 1991). The respiratory rates were rhythmic, deep and steady and were in the range of 25 - 30 breath/minute.

On the third day post-infection, the prescapular lymph nodes nearest to the site of trypanosomes inoculation in infected goats were highly inflamed, warm and oedematous. Other superficial lymph nodes were slightly reactive. With the first rise in rectal temperatures the infected goats looked a bit lethargic with a staring coat but were eating and drinking effectively. With the start of the second parasitaemic wave at 11 days PI, the infected goats were more lethargic, tended to sit rather than stand and had a seromucoid discharge from the eyes and this was more pronounced in goat-4. All palpable superficial lymph nodes were enlarged. Goat-1 was very inactive, had stopped eating and could hardly move. Its heart rate was sluggish with shallow laboured breaths. It was found dead in the morning 3 days after the start of the second parasitaemic wave.

Goat-4 was particularly sick during the second parasitaemic wave, with very sluggish heart beats and classically shallow laboured breaths and was producing a seromucoid nasal discharge. It was, however, able to eat and drink but had a tendency of eating more maize meal than lucerne or hay. Goat-3, which had prolonged parasitaemia, was notably lethargic during the third parasitaemic wave and also tended to eat much maize meal but was indifferent to other food stuffs. Goat-2 had developed a typically staring coat during the third parasitaemic wave. Goat-5 did not show very notable features of the disease during the second parasitaemic wave apart from enlarged prescapular and parotid lymph nodes.

During the third parasitaemic wave all the infected goats did develop pale observable mucous membranes of the eyes and mouth coinciding with drastic drop in PCV and RBC counts. Goat-2 and 4's mucous membranes progressively became pale until after the fourth parasitaemic wave when they started recovering. Goat-3 and 5's mucous membranes were slightly pale and came back to the normal state after the complete disappearance of parasitaemia at 46 days PI and 49 days PI for goat-3 and 5, respectively. These 2 goats then became very active, could eat feeds offered without discriminating and developed a smooth coat. Their superficial lymph nodes were still enlarged though they were diminishing in size. The heart rate and respiratory rates recovered. Goat-2 and 4 still had staring coats though they were now able to eat all the feeds offered equally well.

The body weight for the infected goat-4 showed a slight gradual decline and the drop was particularly notable during the fourth wave of parasitaemia (Table 3.2). The rest of the infected goats and the control goats showed rather stable body weights (Table 3.2). There was no observable statistical significance between the mean body weights of the infected goats and that of the control goats (Figure 3.2). The control group goats showed stable normal clinical parameters throughout the experimental period.

3.4. Haematological observations

3.4.1. Packed cell volume (PCV)

The day before infection both the control group and the infected group goats had PCV (%) within the range of 28 - 40%. This was within the normal published range of 22 - 38% (The Merck Veterinary Manual, 1991) except for goat-5 which had 40% which was slightly above the upper normal limit. In the infected goats, the PCV dropped steeply by an average 5.5% even before the appearance of parasitaemia, and thereafter a slight recovery was observed at the end of the first parasitaemic wave (Figure 3.3). With the start of the second parasitaemic wave the PCV in the infected goats declined further during which goat-1 died with its lowest PCV being 26.5%. Goat-2 showed a continuous drop in PCV with slight recoveries between parasitaemic waves recording its lowest PCV (17.0%) at 53 days PI which was half its original PCV and then the PCV showed a tremendous recovery reaching 25.0% at 67 days PI (Table 3.3). With the onset of another parasitaemic wave at 67 days PI, the PCV dropped rapidly until the end of the experiment.

A trend of fluctuating PCV following the parasitaemic wave patterns was also observed in goat-3 (Figure 3.27) which reached its lowest PCV (19.0%), almost half the starting PCV, at 48 days PI. With the complete disappearance of parasitaemia at 53 days PI, goat-3 showed a progressively rapid recovery of PCV getting to 30%, 3 days before the end of the experiment. Goat-4 showed a remarkable drop in PCV from 28% to the lowest point of 14.0% at 46 days PI and maintained very low PCV throughout the experimental period, with only a slight recovery being observed during the last 10 days of the experiment (Table 3.3).

Goat-5 showed a more gradual decline in PCV (Table 3.3). After the end of the second parasitaemic wave there was a slight recovery in PCV and then it maintained a low, but relatively steady PCV until about 45 days PI when the fifth parasitaemic wave started and there was a rapid drop in PCV reaching its lowest level (20.0%) at 48 days PI. With the complete disappearance of parasitaemia at about 46 days PI, a gradual recovery in PCV started which continued until the end of the experiment.

All control goats showed stable PCVs, however, goats-7, 9 and 10 showed a notable drop in PCV during the first 10 days which later recovered (Table 3.3). The drop was , however, not significant since the PCVs were still within the normal published range and the PCV later recovered and maintained stable levels. The initial drop in PCV should have been due to blood sampling for experimental purposes, and when the erythropoietic centres became responsive to the blood sampling, the PCV recovered.

Statistically, the mean PCV of the infected goats was first observed to be significantly ($P < 0.05$) lower than that of control goats at 17 days PI and remained at this level until 24 days PI when the significance level increased ($P < 0.01$) which lasted up to day 56 PI (Figure 3.3). As from day 60 PI, there was no observable significant ($P > 0.05$) difference between the mean PCV of the infected group and the control group until the end of the experiment.

3.4.2. Red blood cell (RBC) counts

One day before infection both the control group goats and infected group goats had RBC counts in the range of 11.88 - 17.89 x $10^6/\mu l$. This was within the normal published range of 8 - 18 x $10^6/\mu l$ (The Merck Veterinary Manual, 1991). Infected goat-1 showed a gradual decline in the RBC count starting during the prepatent phase from 17.19 x $10^6/\mu l$ to reach its lowest count (12.60 x $10^6/\mu l$) 7 days PI and thereafter, a slight recovery was observed shortly before the animal died (Table 3.4).

Goat-2 (infected) showed RBC count pattern similar to its PCV pattern starting with a rapid decline during the prepatent phase and showing a considerable recovery after the end of the first parasitaemic wave at 9 days PI. With the onset of the second parasitaemic wave, a steep drop in the RBC count was noticed and there was no appreciable recovery observed thereafter but the counts kept on dropping (with only negligible recovery at the end of each parasitaemic wave) to reach its lowest count (8.07 x $10^6/\mu l$) at 53 days PI (Table 3.4). With the end of the fifth parasitaemic wave goat-2 showed a considerable recovery in RBC count getting to about 11.88 x $10^6/\mu l$ at 67 days PI.

Goat-3 and 5 (infected) depicted very gradual declines in RBC count during the prepatent phase, but with the onset of the first parasitaemic wave in goat-3, there was a steep drop in RBC count unlike goat-5 which only had a notable steep decline in RBC count during its second parasitaemic wave (Table 3.4). The prolonged second parasitaemic wave in goat-3 led to a continuous gradual decline in counts reaching the lowest count (10.01 x $10^6/\mu l$) at 53 days PI and thereafter starting to show an increase in counts reaching 14.52 x $10^6/\mu l$, 3 days before the end of the experiment. Similarly, goat-5 (infected) maintained low counts with no appreciable recovery in between the third, fourth and fifth parasitaemic waves and depicted its lowest count (10.15 x $10^6/\mu l$) at 60 days PI. Thereafter with the complete disappearance of parasitaemia, there was remarkable continuous recovery reaching 13.80 x $10^6/\mu l$, 3 days before the end of the experiment.

Goat-4 (infected) depicted a gradual drop in RBC count (Table 3.4) during the prepatent phase. The drop became rapid with the onset of the first parasitaemic wave to reach 7.11 x $10^6/\mu l$ at 17 days PI. Thereafter a low level of counts was maintained with no notable recoveries between the parasitaemic waves, reaching the lowest count (6.84 x $10^6/\mu l$) at 46 days PI and then showed a slight recovery but only temporarily. A further decline was observed during the last 2 days of the experiment. Generally in all the infected goats , a decline in RBC count corresponded to a decline in PCV of the same magnitude and vice versa. This indicated that the anaemia was normocytic.

All the control goats showed steady counts which were maintained within the published normal range (Table 3.4). Statistically, the mean RBC count of the infected goats was observed to be significantly ($P < 0.05$) lower than that of the control goats at 24 days PI and at 39 - 60 days PI (Figure 3.4). During the last 2 weeks of the experiment, there was a considerable recovery in the mean RBC counts of the infected goats reaching 11.81 x $10^6/\mu l$ at 71 days PI (Figure 3.4). Infected experimental goat-2 depicted a particularly classical wavy pattern of parataemia and hence its red blood cell counts plot at different days post-infection is shown in figure 5.0a.

3.4.3. Haemoglobin (Hb) concentration

The starting Hb concentration in both the infected group and control group goats was in the range of 9.4 - 12.8 g/dl and this was found to be almost within the normal published range of 8 - 12 g/dl (Jain, 1986). The Hb patterns of the infected goats closely followed the RBC count patterns i.e. a drop in RBC count showed a corresponding drop in Hb of the similar magnitude and vice versa. This was an indication of normochromic anaemia.

Goat-9 and 10 showed a slight decline in the Hb concentration during the first 2 weeks of the experiment (though the Hb concentration was still within the normal range) and thereafter stabilised (Table 3.5). This slight decline could be attributed to blood sampling. There was also a notable very gradual decline in the Hb concentration of control goat-6 and 7 throughout the experiment.

Statistically, the mean Hb concentration of the infected goats was observed to be significantly ($P < 0.05$) lower than that of the control goats starting from 17 days PI until 60 days PI and only showed no significance ($P > 0.05$) at 32 days PI (Figure 3.5). At 35 days PI, the mean Hb of the infected goats was noted to be lower than that of the control goats at a higher significance level ($P < 0.01$).

3.4.4. Mean corpuscular volume (MCV)

At the starting of the experiment all goats (both the infected and the control) had their MCV (Table 3.6) in the range of 20.0 - 25.1 g/dl which was within the normal range of 15 - 30 g/dl (Jain, 1986). With the progress of infection, there was no particular notable pattern which correlated with the parasitaemia or the RBC count and the PCV, but the MCV continued to show very minimal fluctuations and the values were maintained within the normal range throughout the experimental period.

Overall, during the first 2 weeks PI, there was an observable decline in the mean MCV of the infected goats which later recovered during the third week PI (Figure 3.6). Another notable decline in the mean MCV of the infected goats was noted in the seventh week PI but was followed by a steady recovery. Statistically, there was no observable significant ($P > 0.05$) difference between the infected goats' mean MCV and the control goats' mean MCV throughout the experiment (Figure 3.6).

3.4.5. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

The starting MCH values for both the infected and the control group goats were in the range of 6.8 - 8.0 pg (Table 3.8) which fell within the published normal range of 5.2 - 8.0 pg (The Merck Veterinary Manual, 1991), while the starting MCHC values ranged from 31.2 - 36.7 g/dl (Table 3.8) almost within the normal published range of 30 - 36 g/dl (The Merck Veterinary Manual, 1991). With the progress of infection, there was only insignificant fluctuations in both MCH and MCHC in all the infected goats up to day 45. However, an appreciable increase in MCHC was observed between 45 and 60 days PI (but was short-lived) particularly in goat-2 and 3 (Table 3.8). MCH values in infected goats also showed only minimal fluctuations but with a very gradual increase being observed in goats-2, 3 and 5 between 46 days PI and 60 days PI, which was followed by a gradual decline to normal values (Table 3.7).

Overall, the mean MCH and mean MCHC values of the infected goats followed relatively stable patterns with only insignificant fluctuations (Figure 3.7). However, there was an appreciable increase in the mean MCHC values between the seventh and eighth week PI.

Control goats had very stable MCH values and MCHC values (Table 3.7 and 3.8) throughout the experiment. There was no notable significant ($P > 0.05$) difference between the infected and control goats' mean MCH and MCHC (Tables 3.7 and 3.8). These MCH and MCHC values plus the MCV values in infected goats indicated normocytic/normochromic anaemia.

3.4.6. White blood cell (WBC) counts

The starting WBC counts for both the infected and control goats ranged from 12.2 - 23.2 x 10^3/µl (Table 3.9). Some of the counts were, however, lying out of the normal published range of 4 - 13 x 10^3/µl (The Merck Veterinary Manual, 1991). With progression of infection (in infected goats), there was a general decline in WBC counts until a few days after the first parasitaemic wave had ended. Thereafter, all the infected goats depicted a general increase in the counts with goat-2, 3 and 4 having rapid rise in the counts while goat-1 and 5 showed very gradual increases (Table 3.9). During the first decline in WBC counts, goat-3 and 4 depicted their lowest WBC counts of 7.3 x 10^3/µl and 6.9 x 10^3/µl, respectively, at 14 days PI and this was followed by a very rapid increase in both goats, reaching their highest counts of 32.1 x 10^3/µl (at 24 days PI for goat-9) and 18.3 x 10^3/µl (at 19 days PI for goat-11). Goat-2 had a similar rapid increase in counts, depicting 16.5 x 10^3/µl at 28 days PI, a count which was almost its starting count. Goat-5 showed a rather rapid increase in WBC counts reaching its highest count (20.4 x 10^3/µl) at 35 days PI. After attaining the peak, all the infected goats thereafter, followed a pattern showing WBC count increase with increase in parasitaemia and vice versa, but generally, there was a gradual decline in WBC counts with progression of the infection.

Overall, the mean WBC count of the infected goats showed typical leucopenia, recording about 41% drop in the mean WBC count by the fourteenth day PI (Figure 3.9). Thereafter, there was a rapid increase in the mean counts getting to about the starting level within a week. During the following weeks (weeks 5 - 10) there was a steady decline in the mean counts until the last day when a sharp increase was observed (Figure 3.9).

All the control goats showed rather stable patterns in WBC counts, though minimal fluctuations were depicted but they were not of the same big magnitude as compared to the infected goats (Table 3.9). Statistically, the mean WBC counts for the infected goats were found to be significantly ($P < 0.01$) lower than the mean WBC counts for the control goats at 4, 11 and 14 days PI (Figure 3.9). However, at 7, 9 and 17 days PI the significance level was at $P < 0.05$. On the last day of the experiment the mean WBC count for the infected goats was observed to be significantly ($P < 0.01$) higher than that of the control goats.

3.5. Serum biochemical observations

3.5.1. Total serum protein concentration (TSP)

At the start of the experiment all the goats in both the control and infected groups had their TSP in the range of 5.77 - 6.47 g/dl which was almost within the normal published range of 6.1 - 7.4 g/dl (The Merck Veterinary Manual, 1991). Goat-1 and 2 had an initial gradual decline in TSP during the first week (Table 4.0) which continued in the second week for goat-1 until it died. Goat-2 depicted an increase in TSP at the start of the second week PI corresponding to the start of the second parasitaemic wave, but there was a further drop to the lowest concentration (5.12 g/dl) towards the end of the second parasitaemic wave. With the onset of the third prolonged parasitaemic wave, there was a notable, gradual rather prolonged increase in TSP reaching 6.51 g/dl at 32 days PI. Thereafter, there was a fluctuating pattern and the highest concentration of 6.66 g/dl was attained at 63 days PI. Goat-3 depicted a stable pattern during the first 1 week, but there was a notable increase in TSP, which later declined after the peak of the second parasitaemic wave. A gradual increase in the TSP of goat-3 was observed was maintained with the second parasitaemic wave reaching the highest concentration (8.19 g/dl) at 42 days PI shortly after the complete disappearance of parasitaemia. Thereafter, a sudden slight drop in TSP was observed followed by an increase to attain a plateau higher than normal until the end of the experiment.

Goat-4 had a steady pattern in the first week (Table 4.0) but with the start of the first parasitaemic wave, a rise in TSP was observed followed by a drop to below the starting concentration (6.08 g/dl) at 14 days PI. With the onset of the second parasitaemic wave, there was a steady increase in the TSP to reach the highest concentration of 7.75 g/dl at 42 days PI and this was followed by a fairly high plateau of TSP until the end of the experiment. Goat-5 showed a slight drop, followed by a rise in TSP at the start of the first parasitaemic wave, with a later decline in TSP at the end of the wave. With the start of the second parasitaemic wave, a gradual rise in TSP was observed reaching the highest concentration of 7.43 g/dl at 42 days PI and thereafter maintaining a fairly high plateau of TSP until the end of the experiment.

The mean TSP concentration of the infected goats showed a fluctuating pattern during the first 2 weeks PI (Figure 4.0). Thereafter, a gradual increase in the mean TSP followed, characterised by intermittent sharp rises matching mean parasitaemic patterns. A relatively high level of mean TSP was maintained until the end of the experiment.

All the control goats had reasonably stable TSP (Table 4.0) throughout the experiment with only minimal fluctuations. The mean TSP concentration of the control goats showed an equally stable pattern (Figure 4.0). Statistically, the mean TSP for the infected goats was observed to be significantly ($P < 0.01$) higher than that of the control goats at 28 days PI until 32 days (Figure 4.0). At 35 days PI the significance level in the difference of the 2 means observed was at $P < 0.05$ and this difference was also seen from day 46 to day 60 PI. At day 63 PI, the level of significance in the difference again increased to $P < 0.01$, and thereafter dropped to $P < 0.05$ at day 67 PI and this level was maintained until the end of the experiment.

3.5.2. Serum globulin concentration (SGC)

The SGC for the experimental goats at day 0 PI ranged from 3.02 - 4.10 g/dl which fell within the normal range of 2.7 - 4.4 g/dl (The Merck Veterinary Manual, 1991). SGC in all the infected goats depicted patterns (Table 4.1) similar to those depicted by total serum protein concentration (TSP) with disease progression, an indication that it was actually SGC which determined the magnitude of increase in TSP. Goat-1 died while the SGC was making a gradual rise. Goat-2 had its lowest SGC (2.77 g/dl) at 14 days PI (at the peak of its second parasitaemic wave) and thereafter a gradual fluctuating increase in the SGC until it attained its highest concentration (4.00 g/dl) at 63 days PI. This was followed by a slight decline just before the end of the experiment. Goat-3 showed a pattern of SGC typically corresponding to the TSP with the highest concentration (5.52 g/dl) being attained 42 days PI. This was followed by a steep decline in concentration to reach the lowest concentration (3.72 g/dl) at 46 days PI and thereafter a gradual increase started to reach a plateau of high concentration which was maintained until the end of the experiment.

Goat-4 also had an SGC pattern similar to its TSP pattern with the lowest concentration (3.78 g/dl) being observed at 14 days PI at the end of the second parasitaemic wave. This was followed by a gradual increase in concentration, attaining the highest concentration (5.25 g/dl) at 42 days PI and then a plateau of relatively high SGC was maintained until the end of the experiment. Goat-5's SGC varied in correspondence to the TSP with the lowest concentration (2.50 g/dl) being noted at 17 days PI (i.e. at the end of the second parasitaemic wave). This was followed by a steady increase until the highest concentration (4.79 g/dl) was attained at 42 days PI (at the end of the fourth parasitaemic wave). Thereafter, a slight decline followed and then a fairly high plateau of SGC was maintained until the end of the experiment. The mean SGC pattern of the infected goats (Figure 4.1) closely followed the pattern depicted by the mean TSP of the infected goats.

Control goats showed stable SGC (Table 4.1) with minimal fluctuations throughout the experiment. Statistical analysis indicated that the mean SGC for the infected goats was significantly ($P < 0.05$) higher than the mean SGC of the control goats at 28 and 32 days PI (Figure 4.1). This significant difference was also observed at 39, 49 and 53 days PI. At 42 and 63 days PI, the mean SGC for the infected goats was found to be significantly ($P < 0.01$) higher than that of the control goats. At 67 and 71 days PI, the significance level in the difference reverted back to $P < 0.05$.

3.5.3. Serum albumin concentration (SAC)

At day 0 PI, all the experimental animals (both the infected and the controls) had SAC ranging from 2.38 - 2.93 g/dl (Table 4.2) which was within the normal published range of 2.3 - 3.6 g/dl (The Merck Veterinary Manual, 1991). With the progress of infection, goat-1 had a fairly rapid decline in the SAC with about a 50% reduction being observed at 14 days PI, 3 days before the animal died. The rest of the infected goats had fairly stable SAC throughout the experiment with only minimal fluctuations being observed (Table 4.2).

The mean SAC of the infected goats showed an appreciable decrease during the first 2 weeks PI but, thereafter, the mean SAC levels recovered to starting levels and stabilised with only minimal fluctuations being shown for the rest of the experimental period (Figure 4.2).

Control goats showed steady SAC (Table 4.2) throughout the experiment. There was no notable statistically significant ($P < 0.05$) difference between the SAC of the infected goats and the means of the control goats throughout the experimental period (Figure 4.2).

3.5.4. Albumin:globulin (A:G) ratio

At day 0 PI, the range of the A:G ratio in all the experimental animals was from 0.64 - 0.95 (Table 4.3) which fell within the normal published range of 0.6 - 1.1 (The Merck Veterinary Manual, 1991). With progression of infection, the A:G ratio was dictated by the SGC. Goat-1 had a rapid decline in the A:G ratio with 0.38 (about 50% reduction) being recorded at 14 days PI, 3 days before the animal died.

Goat-2 showed a rapid decline during the first 7 days PI (coinciding with the increase in the SGC and the development of the first parasitaemic wave), but showed a recovery in the next week (Table 4.3). A further decline in the A:G ratio ensued with the development of the second parasitaemic wave reaching 0.72 at 17 days PI, a level which was maintained until about day 30 (towards the peak of the third parasitaemic wave) when a further drop was evidenced to reach its lowest ratio (0.66) at 42 days PI. Thereafter, a fluctuating trend followed but with a maintained relatively low ratio until the end of the experiment. Goat-3 developed a rapid drop in the A:G ratio in the first 10 days PI with only a slight recovery being noticed at the end of the parasitaemic wave, but this was followed by a further gradual decline to reach its lowest ratio (0.46) at 39 days PI (Table 4.3). Towards the end of the last parasitaemic wave, goat-3 showed a tremendous increase in the A:G ratio to 0.73 (the starting ratio) and thereafter, a rapid decline followed by a gradual increase until the end of the experiment.

Goat-4 had a rapid decrease in the A:G ratio during the first parasitaemic wave with a very steep decline being observed towards the peak of the second parasitaemic wave leading to its lowest A:G ratio (0.43) at 17 days PI (Table 4.3). This was followed by a steady temporary rise in the ratio and then a relatively low ratio was maintained over a 30 days period with a gradual increase being noticed between day 50 and day 60 PI.

Goat-5 depicted a gradual and steady decline in the A:G ratio from day 0 reaching its lowest ratio (0.55) at 42 days PI (Table 4.3). This was followed by a steady gradual increase in the ratio reaching 0.74 at 63 days PI and thereafter a decline was observed until the end of the experiment.

The mean A:G ratio of the infected goats showed a very rapid decline within the first week of infection with about 23% reduction being recorded by day 9 PI (Figure 4.3). Thereafter, a rather gradual decline followed over the next 5 weeks until the lowest mean A:G ratio (0.54) was observed at day 42 PI. This was followed by a recovery in the mean ratio.

Control goats-6 - 10 had fairly stable ratios with only minimal fluctuations being observed throughout the experimental period (Table 4.3). Statistically, the mean A:G ratio for the infected group was significantly ($P < 0.05$) lower than the mean A:G ratio for the control group at 19 days PI. This difference was later observed also at days 28, 42, 49 and 63 PI (Figure 4.3).

3.6. Microscopic erythrophagocytosis observations

3.6.1. Number of RBCs/20 mononuclear cells (MNC)

A preliminary experiment was carried out with the first set of goats. At day 0 PI, all the goats in both the control and infected groups had the number of phagocytosed erythrocytes/20 MNC in the range of 1 - 3 (Table 4.4). At day 5 PI, there was an appreciable increase in the number of phagocytosed erythrocytes/20 MNCs of all the infected goats while the control goats maintained relatively very low numbers. The increase in the infected goats continued, reaching an average of 27 RBC/20MNC at 20 days PI (Figure 4.4). Thereafter, a slight decline was observed at day 28 PI but this was followed by another sharp increase reaching an average peak, 29 days PI. A notable decrease in the mean number of phagocytosed RBCs was observed at 42 days PI (at the end of the experiment).

Statistically, at 5 days PI the mean number of phagocytosed RBC/20 MNC for the infected goats was found to be significantly ($P< 0.05$) higher than that of the control goats (Figure 4.4). From day 12 PI until the end of the experiment, the observed significance level for the difference in the means was at $P < 0.01$.

3.7. Isotopic assays observations of erythrophagocytosis

3.7.1. ^{51}Cr-incorporation% (measure of the magnitude of erythrophagocytosis)

Results in this section were obtained from the final set of experimental goats.

Erythrophagocytosis experiments in the final batch of goats started at 37 days PI due to logistical delays in the arrival of isotopes from the manufacturer. Infected goats started with higher ^{51}Cr-incorporation% (Table 4.5) than the control goats. Infected goat-2's ^{51}Cr-incorporation was 34% at day 37 and this was its highest percentage recorded throughout the experiment. This highest percentage occurred during the prolonged third parasitaemic wave and when the SGC was highly elevated. Moreover, goat-2 experienced a tremendous drop in RBC counts shortly thereafter. At day 44 PI, goat-2 showed a slight decline in the percentage to 28.8% (Table 4.6) which occurred shortly after the disappearance of the third parasitaemic wave, and with the drop in SGC. However, the animal still maintained relatively low RBC counts for the next 1 week. A further drop in ^{51}Cr-incorporation% to 23.8% was observed at 51 days PI (Table 4.7) with the continued absence of parasitaemia. This was followed by a further decline to 18.5% at 58 days PI (Table 4.8) after which an appreciable increase in RBC counts was observed with no parasitaemia. At day 65 PI, goat-2 showed neither a decline nor increase in the ^{51}Cr-incorporation% (Table 4.9).

Goat-4 (infected) started with a ^{51}Cr-incorporation% (36.8%) a bit higher than that of goat-2 and at 44 days PI only a slight decline to 33.3% was observed. The first higher percentage was associated with the period when SGC was increasing rapidly with very low RBC counts and short intervals between parasitaemic waves. The maintained relatively high percentage at 44 days PI had correspondingly maintained low RBC counts and a prolonged parasitaemic wave. At day 51 PI, goat-4 showed an appreciable decline in the percentage to 24%, which was associated with a prolonged absence of parasitaemia but with relatively high SGC. For the next 2 weeks a gradual decline in the percentage ensued with 19.4% being recorded at 65 days PI associated with rather erratic parasitaemia but with maintained low RBC counts.

Goat-5 (infected) started with a very high ^{51}Cr-incorporation (38.3%) at 37 days PI which was shortly thereafter followed by low RBC counts. This high percentage occurred when the SGC was high and during the fourth parasitaemic wave. Thereafter, a rapid decline in the ^{51}Cr-incorporation occurred with 26% being recorded at 51 days PI and 17.1% at 58 days PI after which a fairly stable percentage was maintained with 17.7% being observed at 65 days PI. The rapid decline in the percentage was associated with the complete disappearance of parasitaemia and with a recovery in RBC counts with relatively high SGC.

The mean ^{51}Cr-incorporation% of the infected goats was observed to be exceedingly higher than that of the control goats at 37 days PI (Figure 4.5). During the following weeks (i.e. 8th - 10th week PI), the mean ^{51}Cr-incorporation% of the infected goats showed a rapid decline and stabilised at very low levels (almost getting to the mean control levels) in the last 2 weeks of the experiment.

All control goats (Goat-6, 8 and 9) had comparatively lower ^{51}Cr-incorporation% (Table 4.5) at 37 days PI with goat-6 having 19.1%, while goat-8 had 17.9% and goat-9 had 18.1%. Stable percentages were observed throughout the experimental period with only very minimal fluctuations (Table 4.6 - 4.9). Statistically, the mean ^{51}Cr-incorporation% for the infected goats was found to be significantly ($P < 0.01$) higher than that of the control goats at 37 days PI (Figure 4.5). The significance level in the difference reduced to $P < 0.05$ at 44, 51, 58 and 65 days PI.

3.7.2. ^{51}Cr-release%

This was the ^{51}Cr released after the intracellular breakdown of the phagocytosed erythrocytes (by MNCs) as well as extracellular lysis of erythrocytes and was detectable in the supernatant. However, ^{51}Cr-release% was only detectable at 44 days PI (Table 4.6) and at 58 days PI (Table 4.8) and only a negligible amount was observed. At 44 days PI, the mean ^{51}Cr-release from the infected goats was found to be significantly ($P < 0.05$) greater than the mean of the control goats. At 58 days PI, the mean ^{51}Cr-release for the infected goats was, however, not significantly ($P > 0.05$) higher than the mean of the control goats.

3.7.3. Comparison of total input radioactivity from tube Rt_o to that of tube Mt_o

There was no significant ($P > 0.05$) observable difference between the mean total input radioactivity from tube Rt_o and that from tube Mt_o throughout the experiment (Tables 4.5 - 4.9).

3.7.4. Spontaneous radioactivity observations

The obtained spontaneous radioactivity values for the experimental goats were very low (almost negligible) compared to the total input radioactivity values (Tables 4.5 - 4.9).

3.8. Post-mortem findings

3.8.1. Gross pathological findings

Goat-2 and goat-4 (infected goats) and goat-6 (control) were sacrificed. Notable pathological changes in both infected goats were:

- Accumulation of light, straw-coloured serous fluid in the pericardial cavity causing extended pericardial sac. Similar fluid accumulation (but only very small amounts) in the thoracic cavity and abdominal cavity.
- Lymph nodes were enlarged and oedematous
- Blood had a watery consistence, (more so in goat-2 than goat-4) and was bright red.
- The spleen and the liver were slightly enlarged in both goats.
- Goat-4 had oedematous testicles

The general carcass appearance was pale in both goats.

The control goat-6 showed no pathological changes from normal.

3.8.2. Impression smears observations

Microscopic scanning of Giemsa-stained impression smears of the liver, spleen, bone marrow, lymph nodes, heart muscle and lungs revealed the following:

Plenty of macrophages with their cytoplasms heavily laden with erythrocytes were observed in the impression smears of the liver (Plate 8) and the bone marrow of both infected goats indicating enhanced erythrophagocytosis in the liver and the bone marrow of the infected animals. The spleen impression smears of the infected goats also showed a good number of macrophages laden with erythrocytes, but not as much as in the liver and bone marrow. The lung and the lymph nodes showed very few macrophages with erythrocytes. Impression smears of organs (liver, bone marrow, spleen, lungs and lymph nodes) from the control goat-6 did not show any presence of phagocytosed erythrocytes in their cytoplasms. Infact, it was more difficult to locate macrophages on the smears from the control goat than it was on smears from the infected goats. Moreover, the control goat smears possessed mainly small monocytes (Plate 7) unlike the infected goat smears which had mostly macrophages (Plate 8).

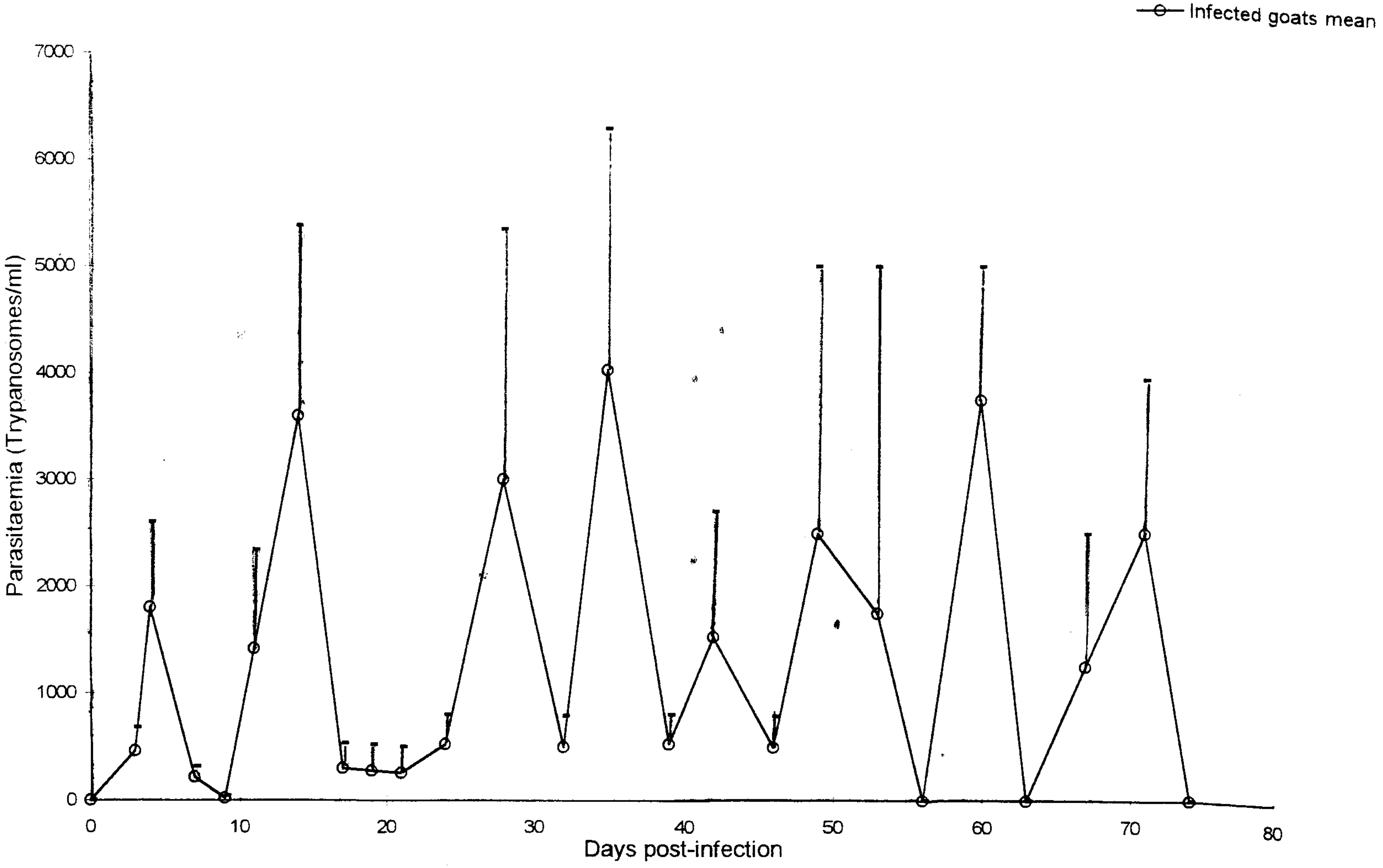


Figure 3.0: Variations in mean parasitaemia (trypanosomes/ml blood) of the infected experimental goats at different days post-infection. Vertical bars indicate standard error of the means.

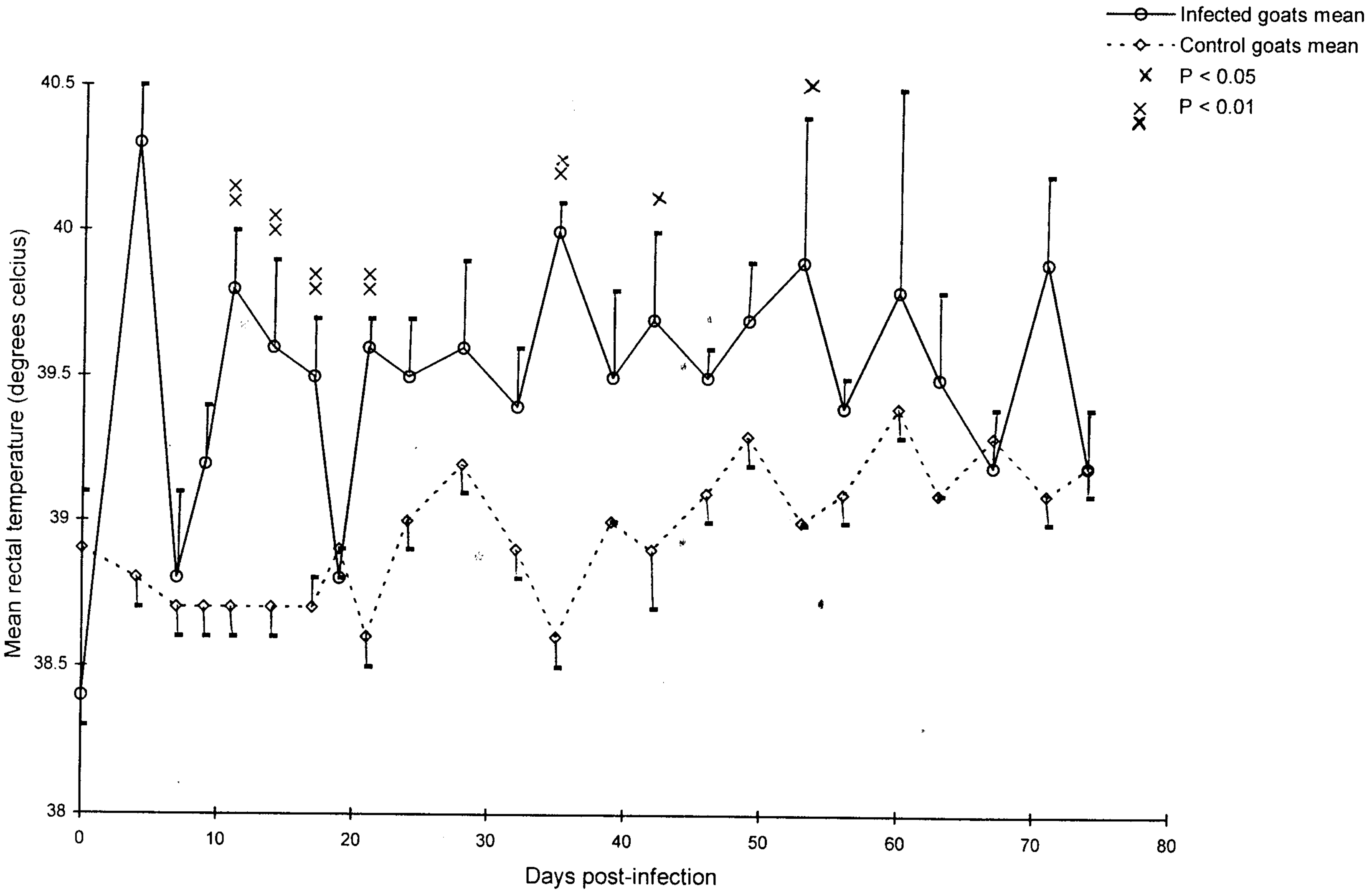


Figure 3.1: Variations in mean rectal temperatures (^{o}C) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

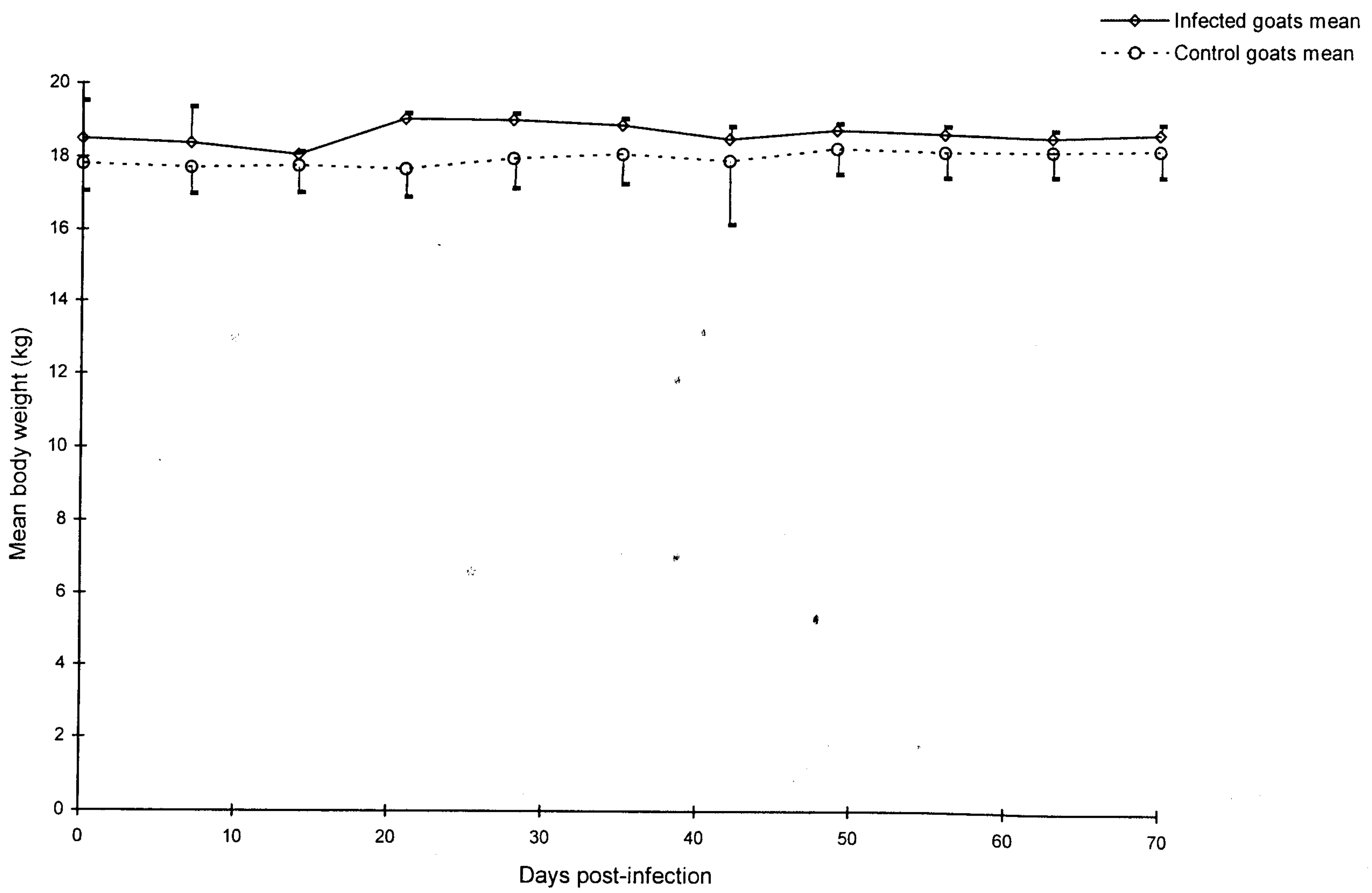


Figure 3.2: Variations in mean body weights (kg) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

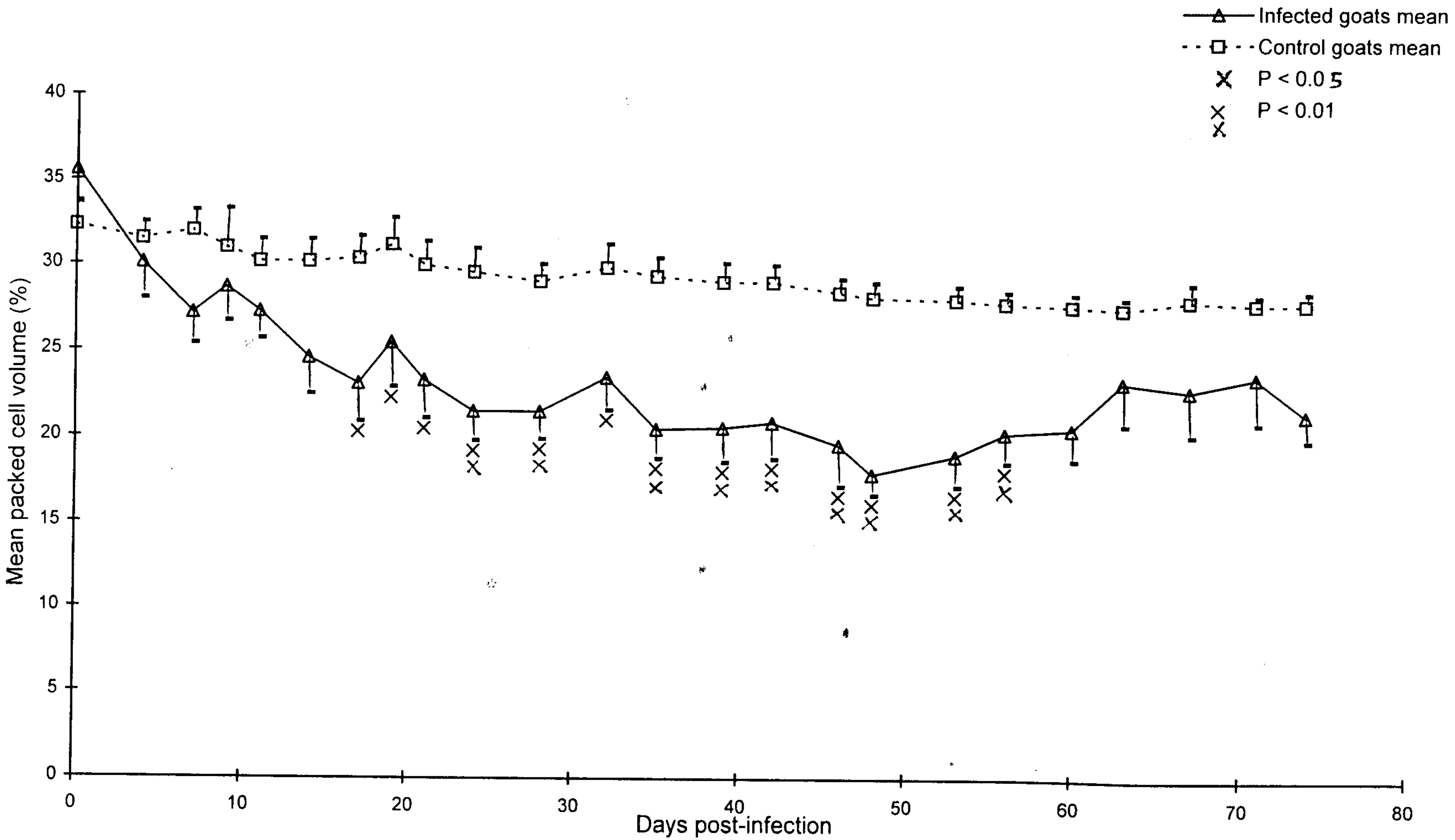


Figure 3.3: Variations in mean packed cell volume (%) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

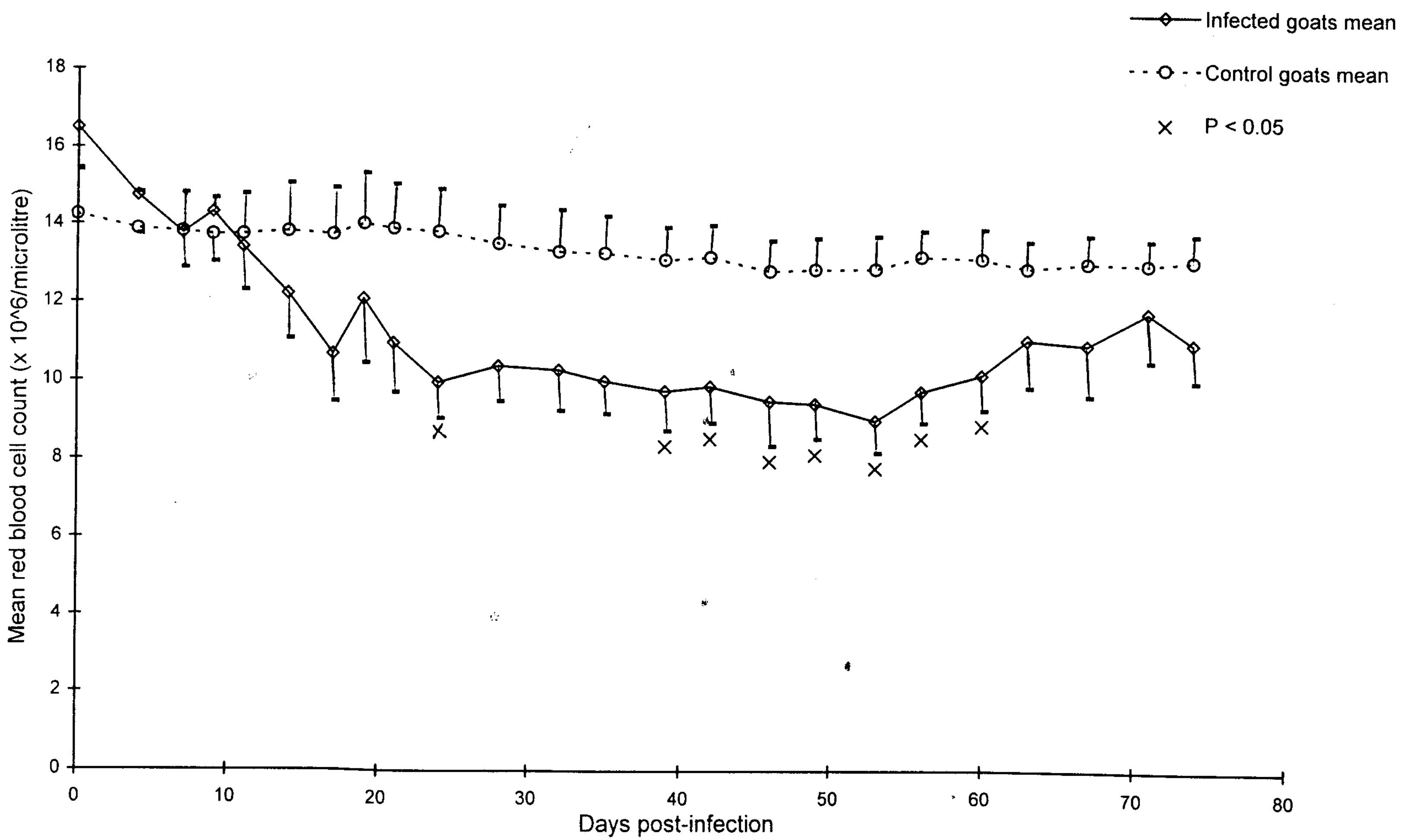


Figure 3.4: Variations in mean red blood cell counts (x $10^6/\mu l$) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

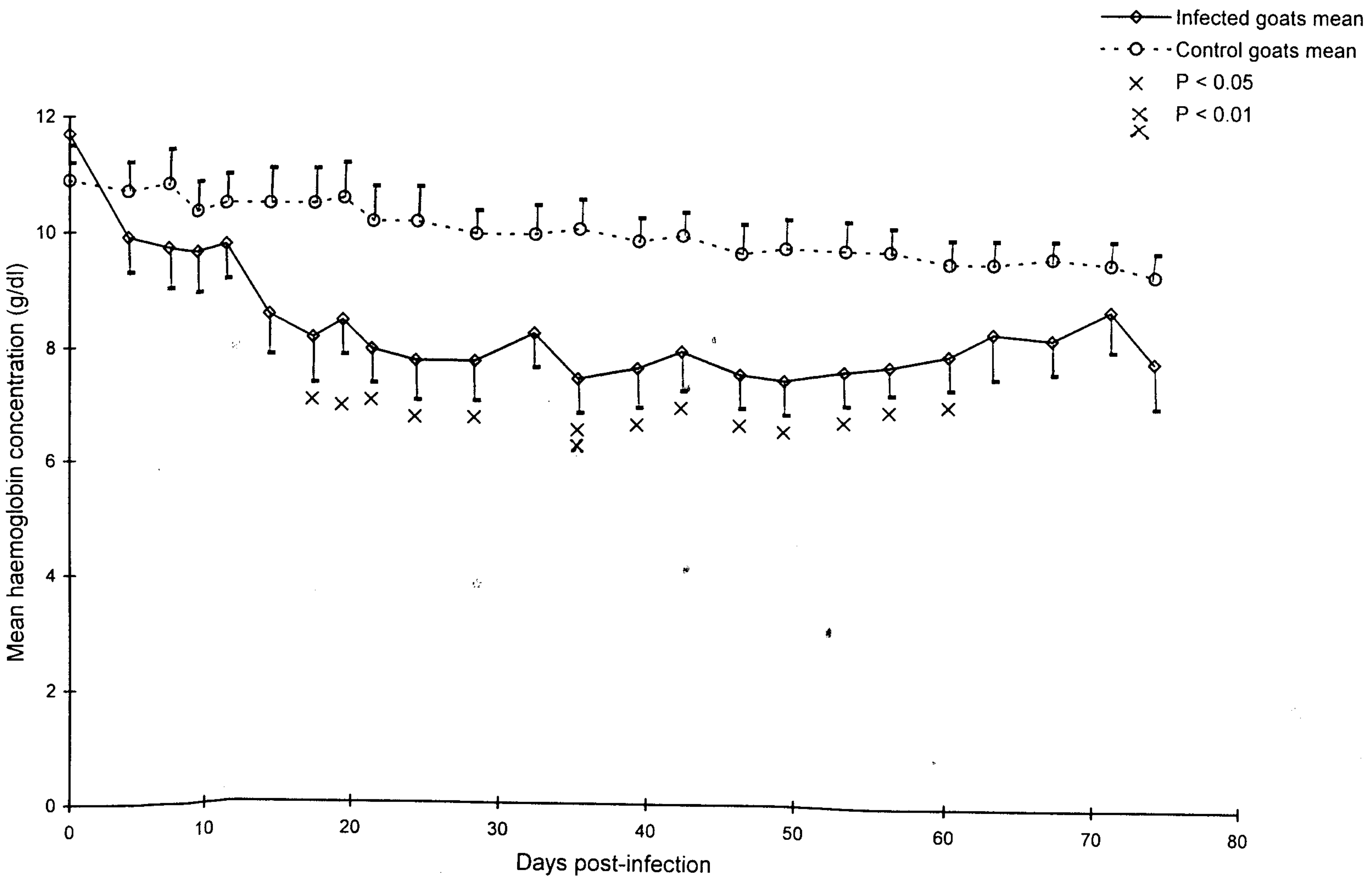


Figure 3.5: Variations in mean haemoglobin concentrations (g/dl) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

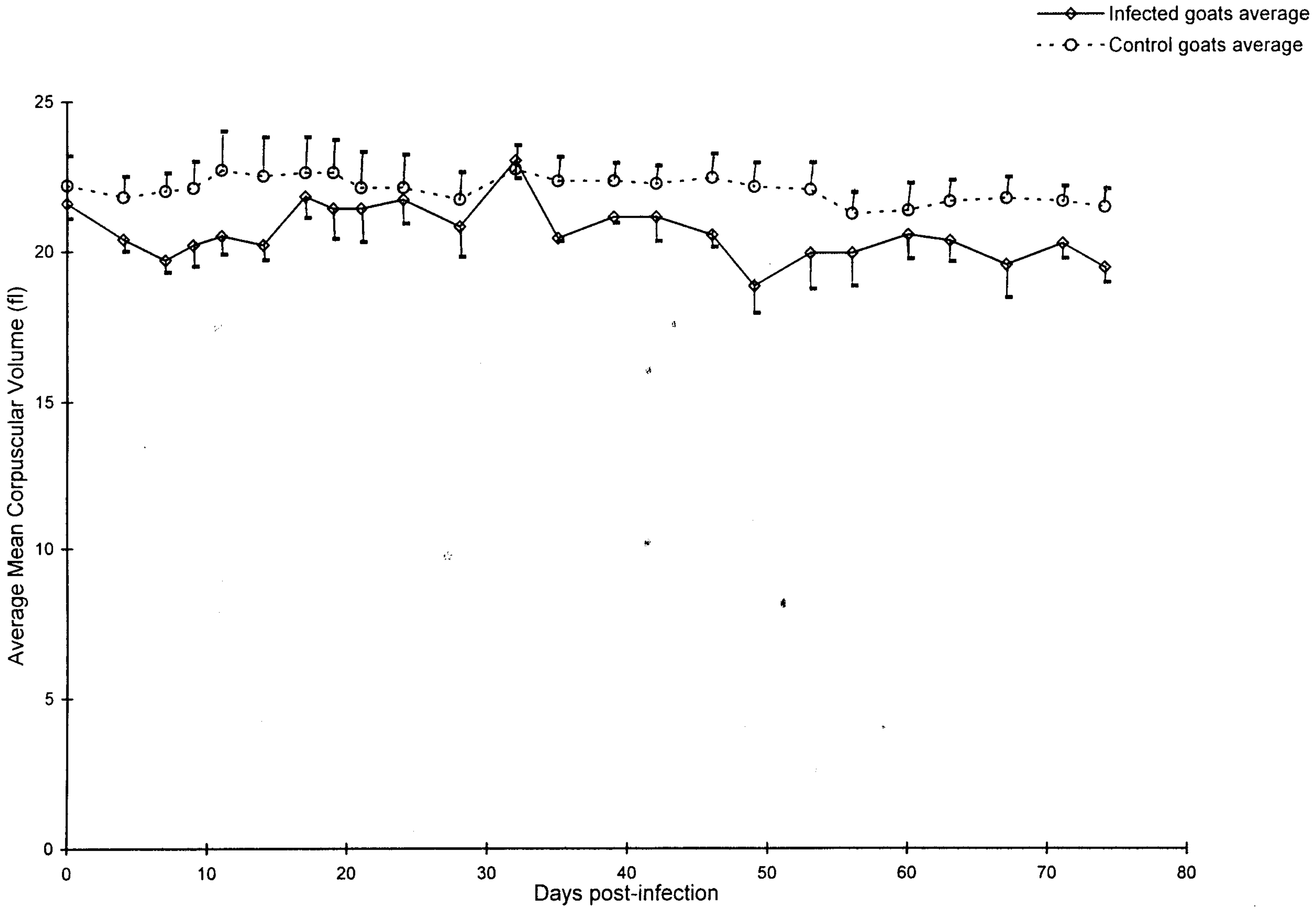
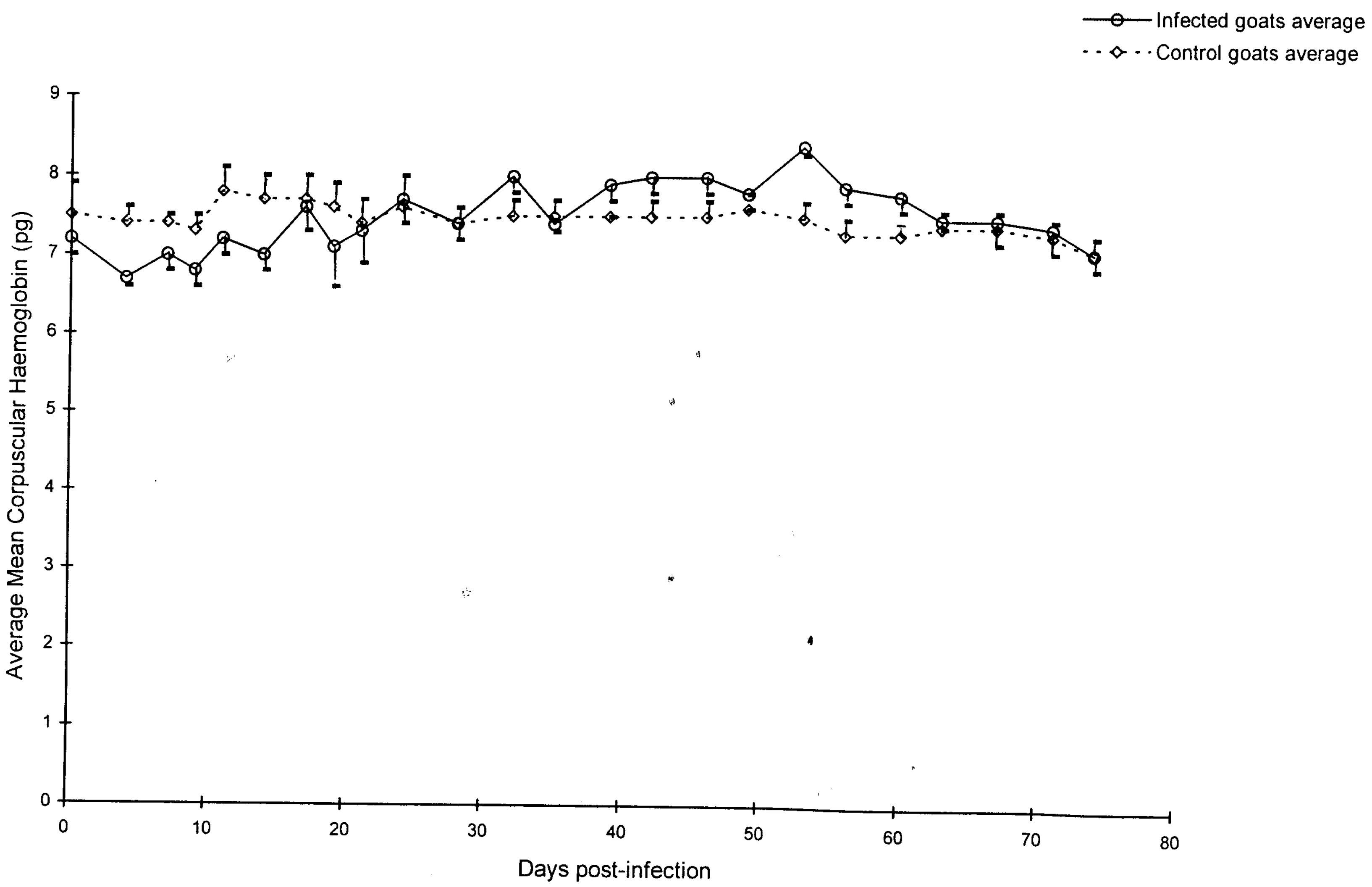


Figure 3.6: Variations in average mean corpuscular volume (fl) of the experimental goats at different days post-infection. Vertical lines indicate the standard error of the means.



Figrue 3.7: Variations in average mean corpuscular haemoglobin (pg) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means

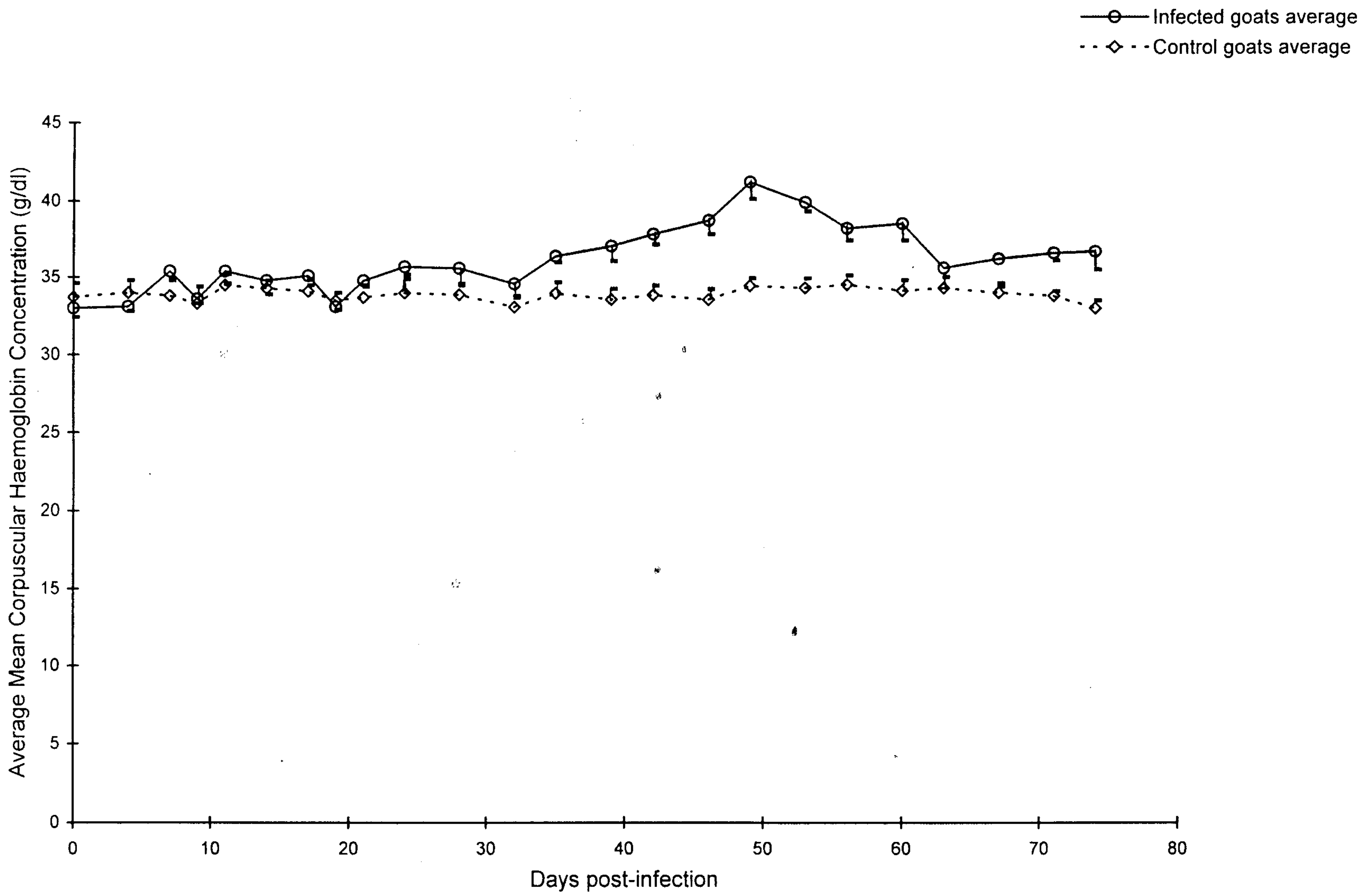


Figure 3.8: Variations in average mean corpuscular haemoglobin concentrations (g/dl) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

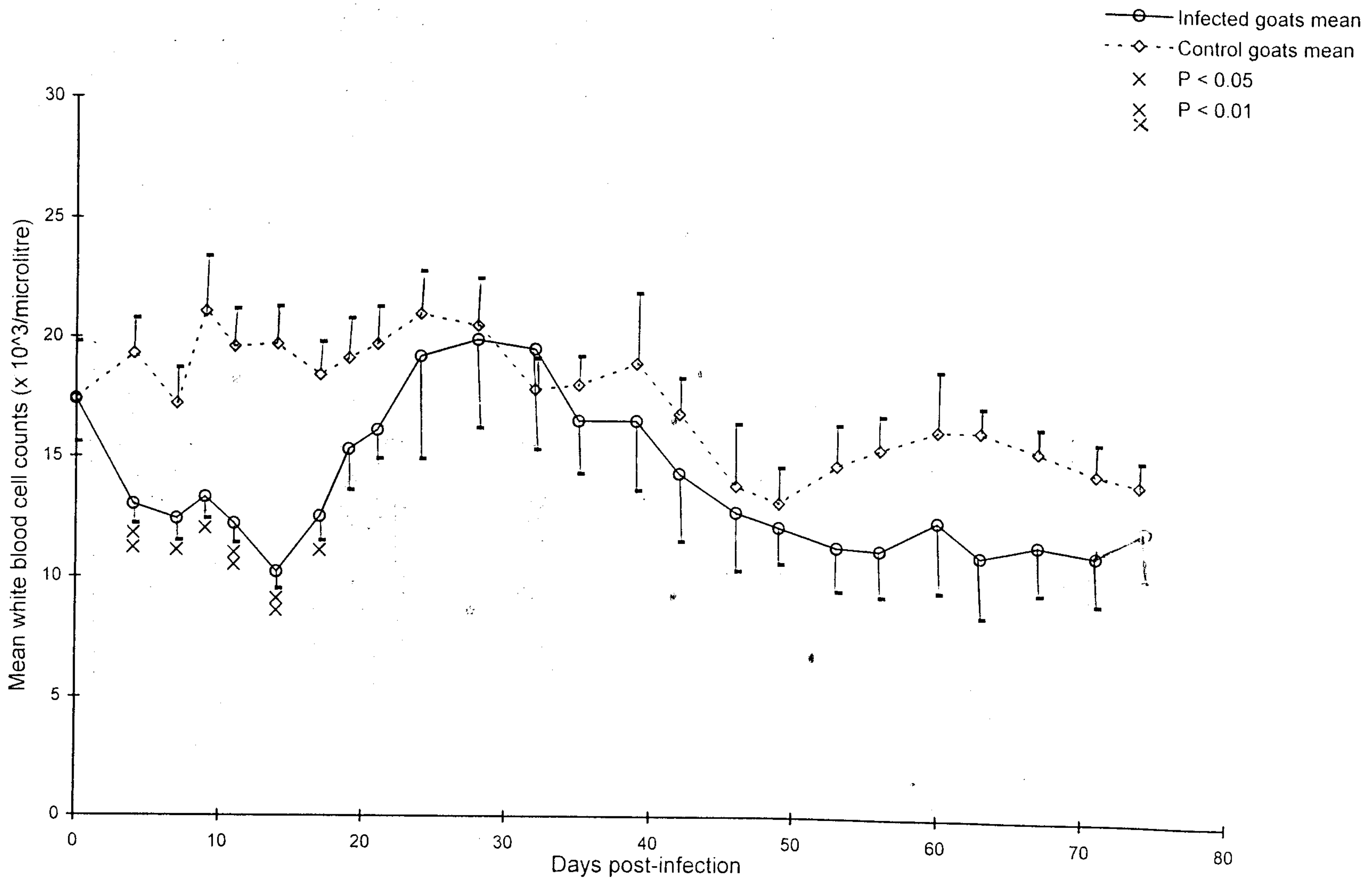


Figure 3.9: Variations in mean white blood cell counts (x $10^3/\mu l$) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

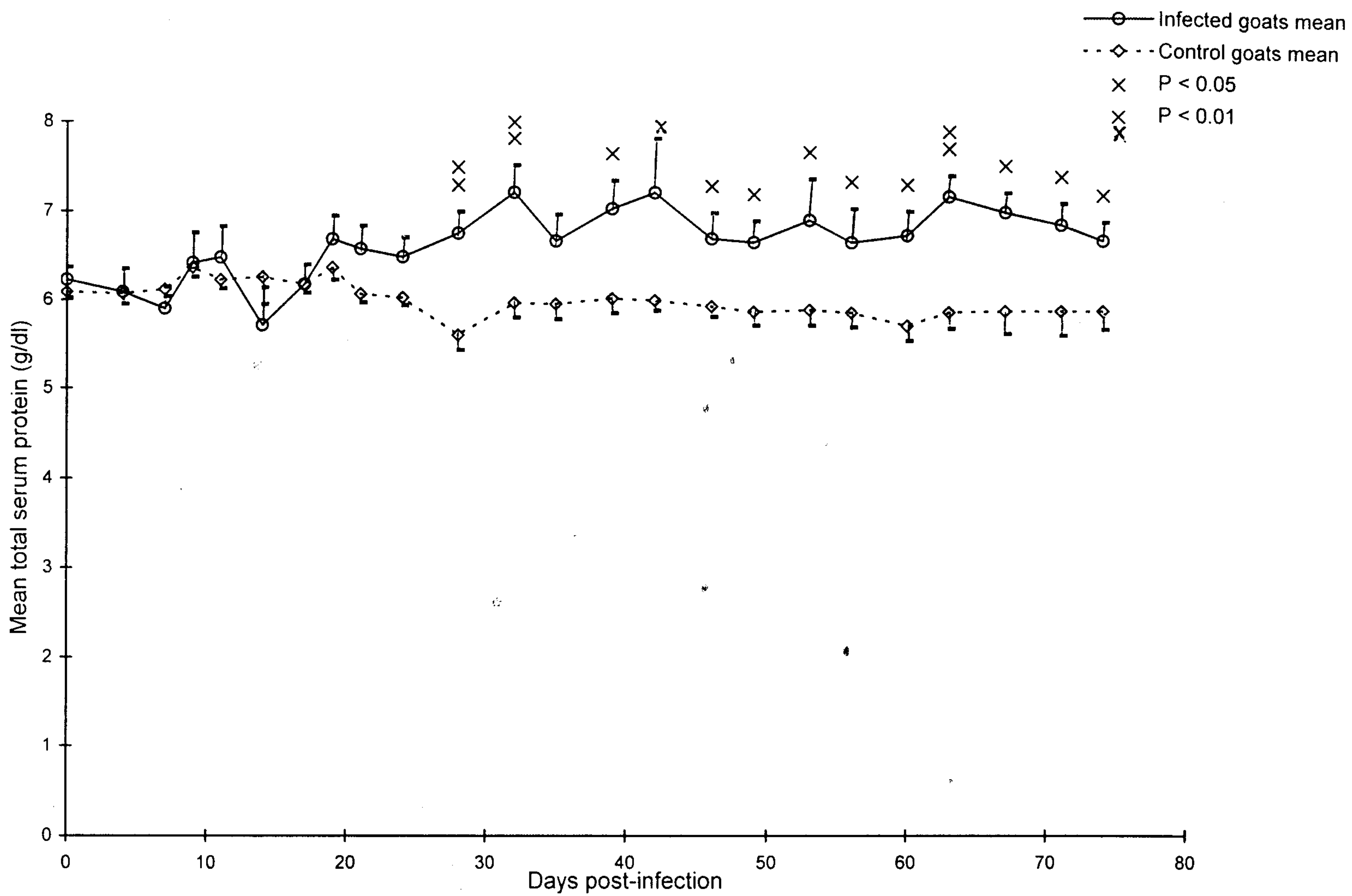


Figure 4.0: Variations in mean total serum protein concentrations (g/dl) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

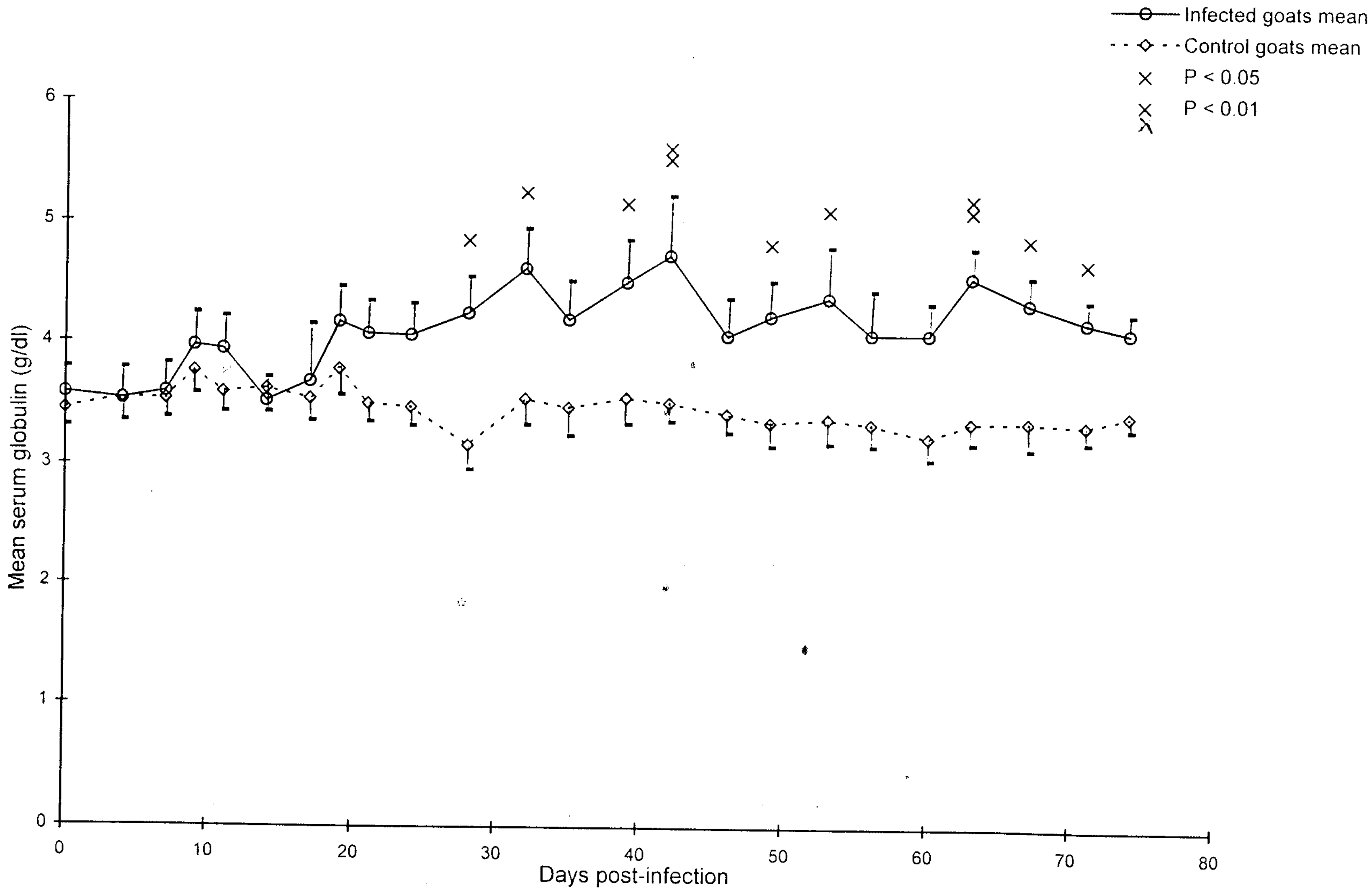


Figure 4.1: Variations in mean serum globulin concentrations (g/dl) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

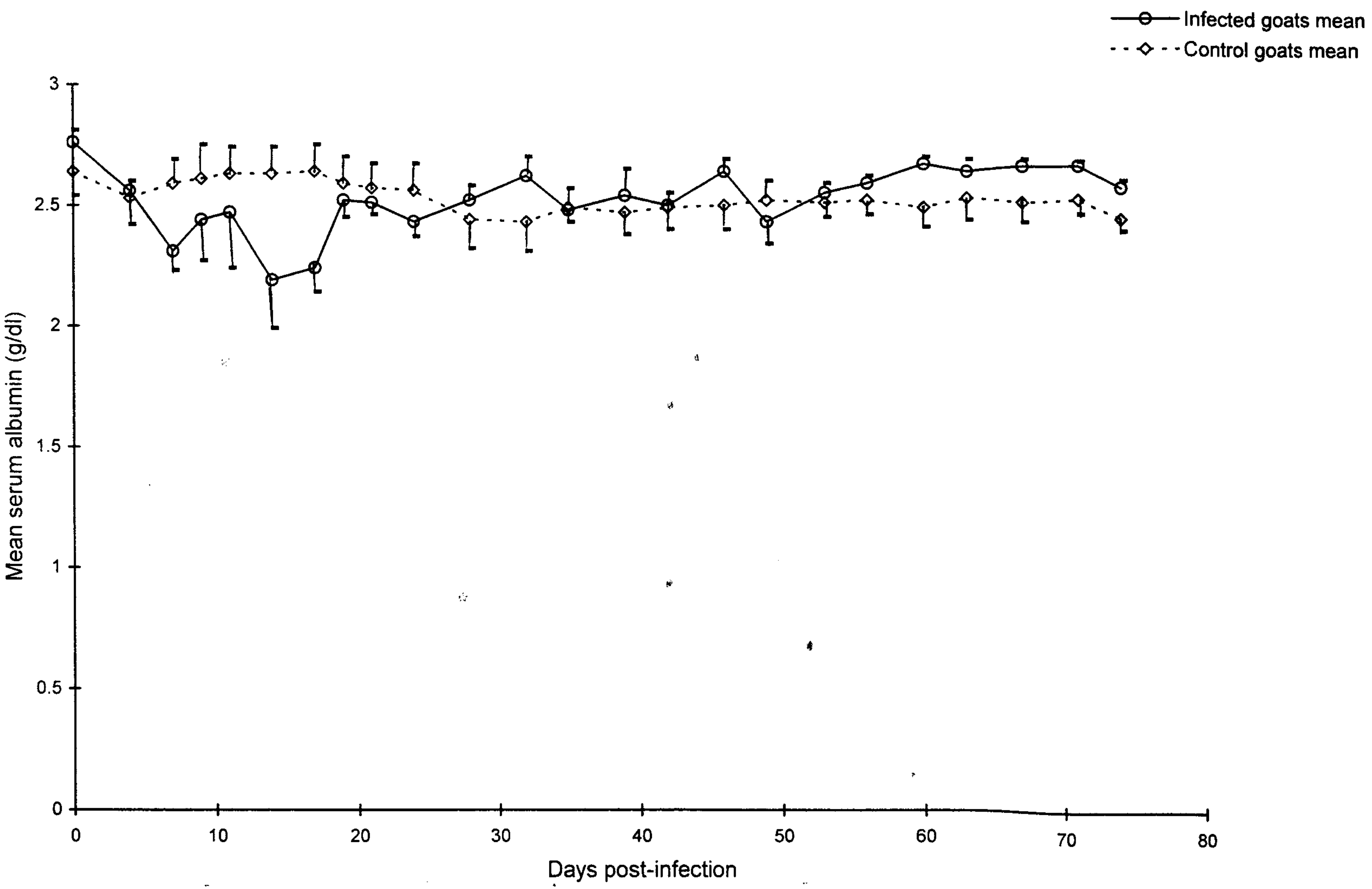


Figure 4.2: Variations in mean serum albumin concentrations (g/dl) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

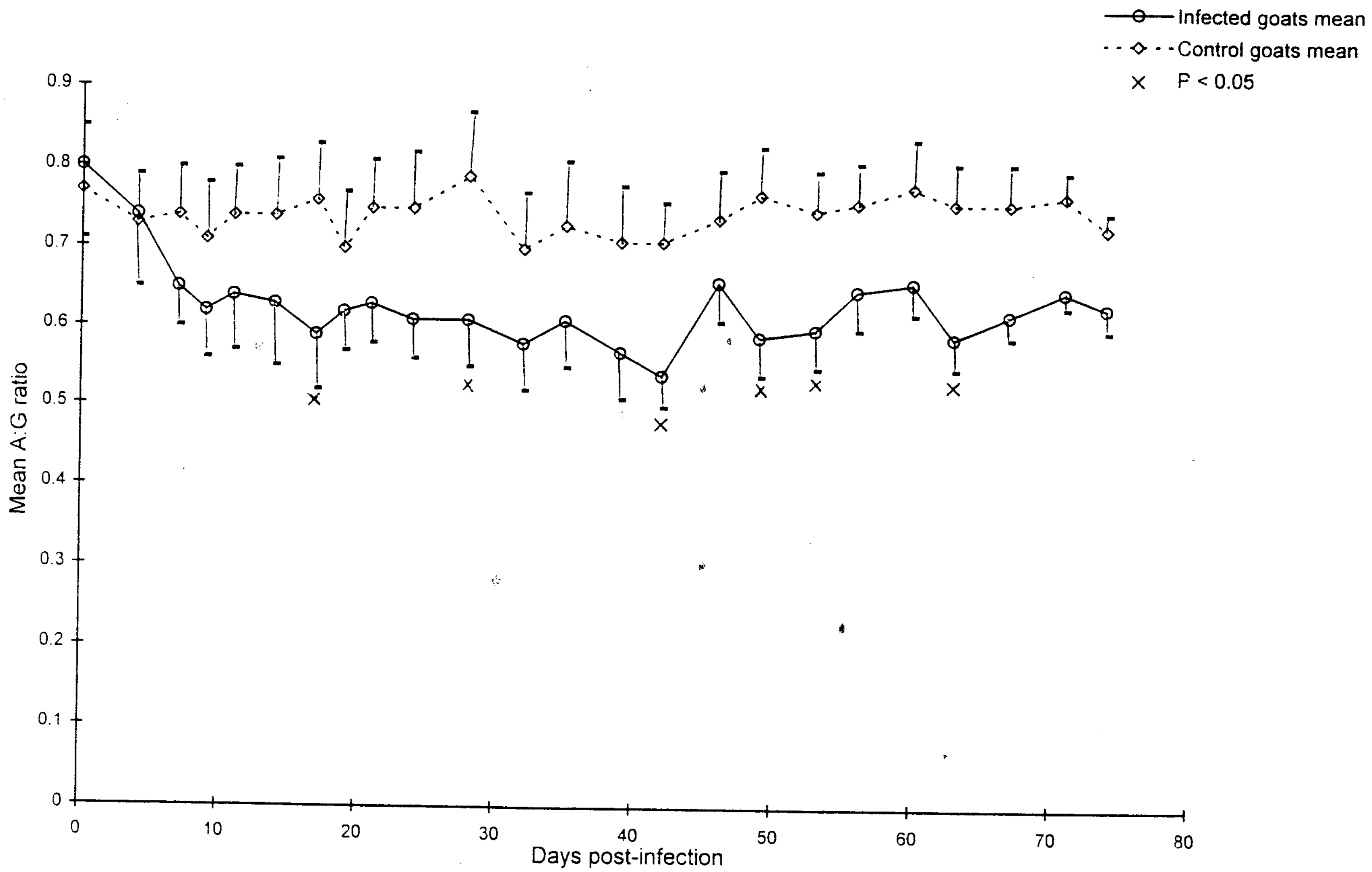


Figure 4.3: Variations in mean albumin:globulin (A:G) ratio of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

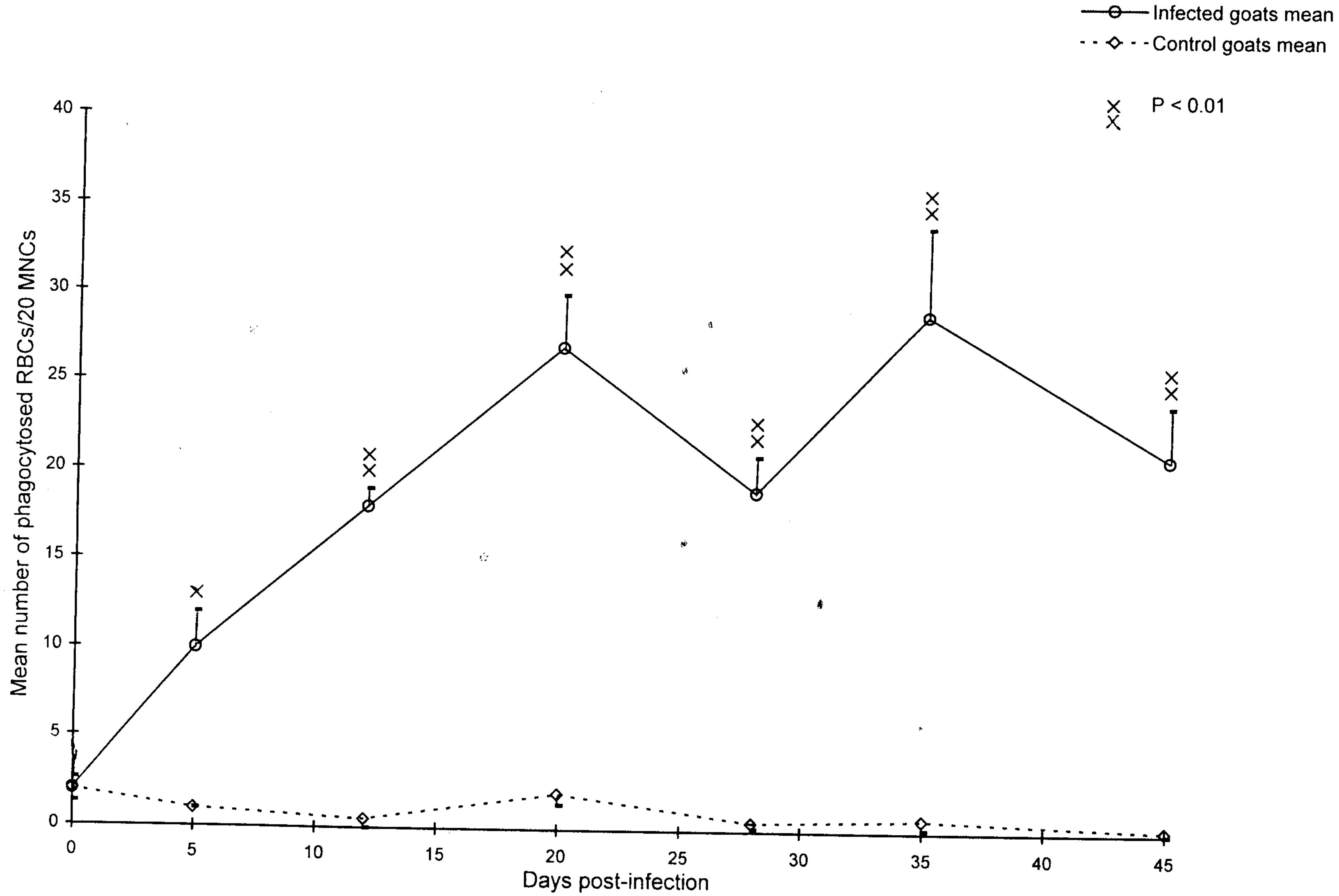


Figure 4.4: Variations in mean number of phagocytosed erythrocytes/20 mononuclear cells (MNCs) of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

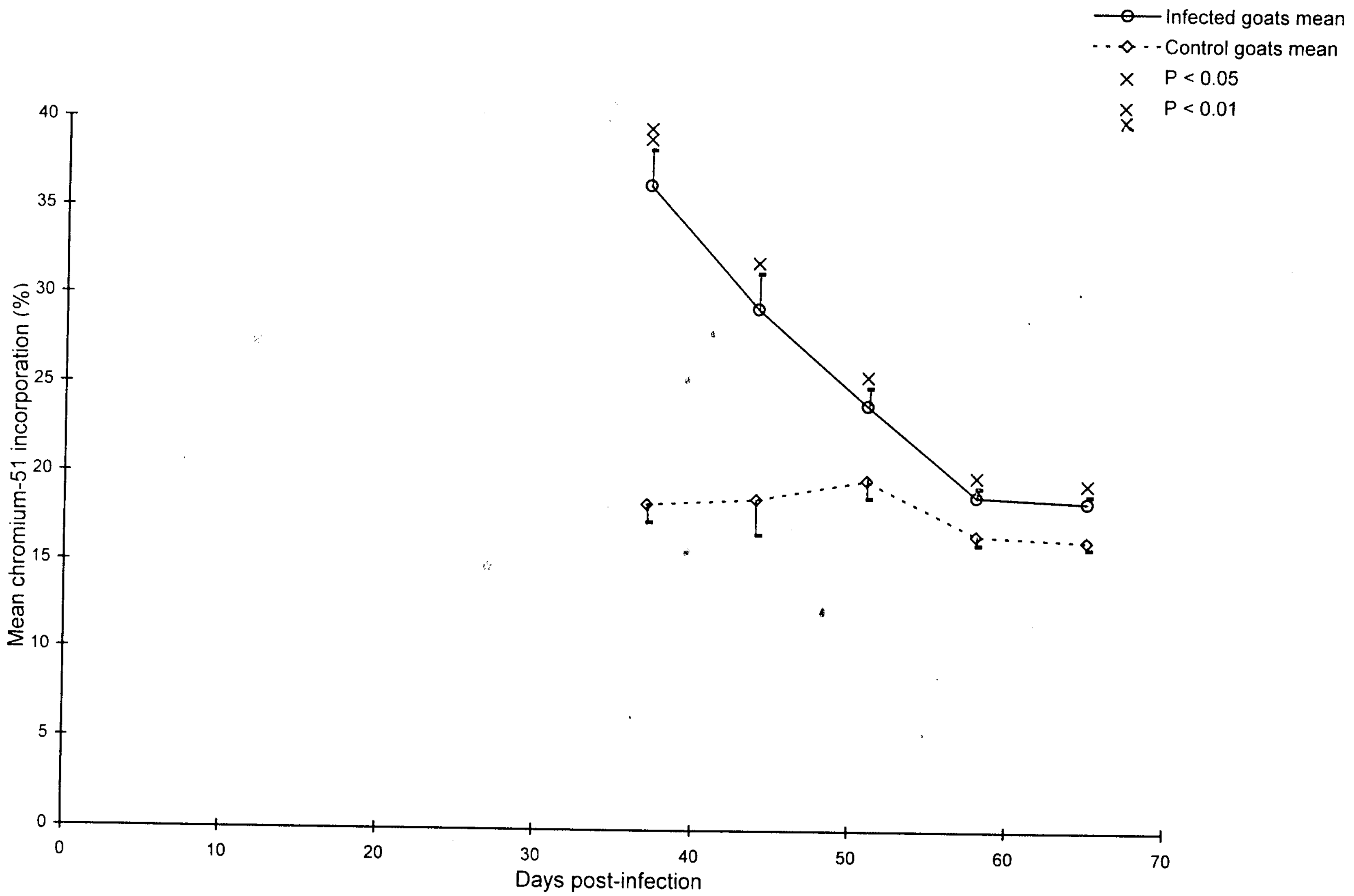


Figure 4.5: Variations in the mean chromium-51 (^{51}Cr) incorporation percentage of the experimental goats at different days post-infection. Vertical lines indicate standard error of the means.

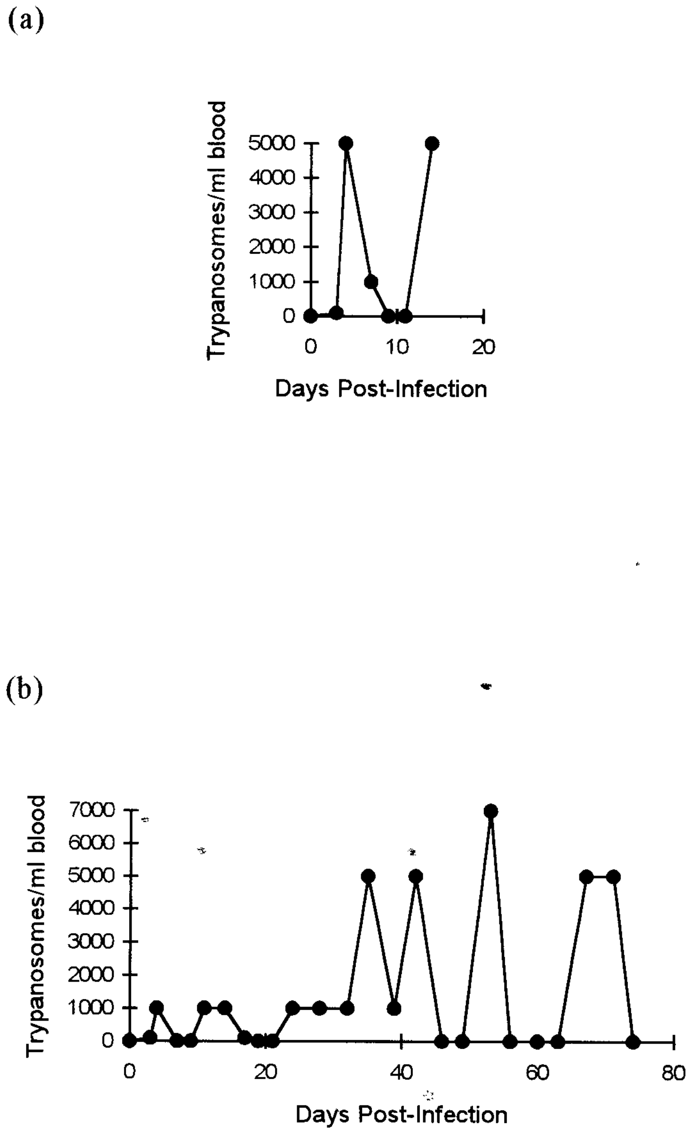


Figure 4.6: Variations in parasitaemia (trypanosomes/ml blood) of experimental infected goat-1 (a) and goat-2 (b) at different days post-infection.

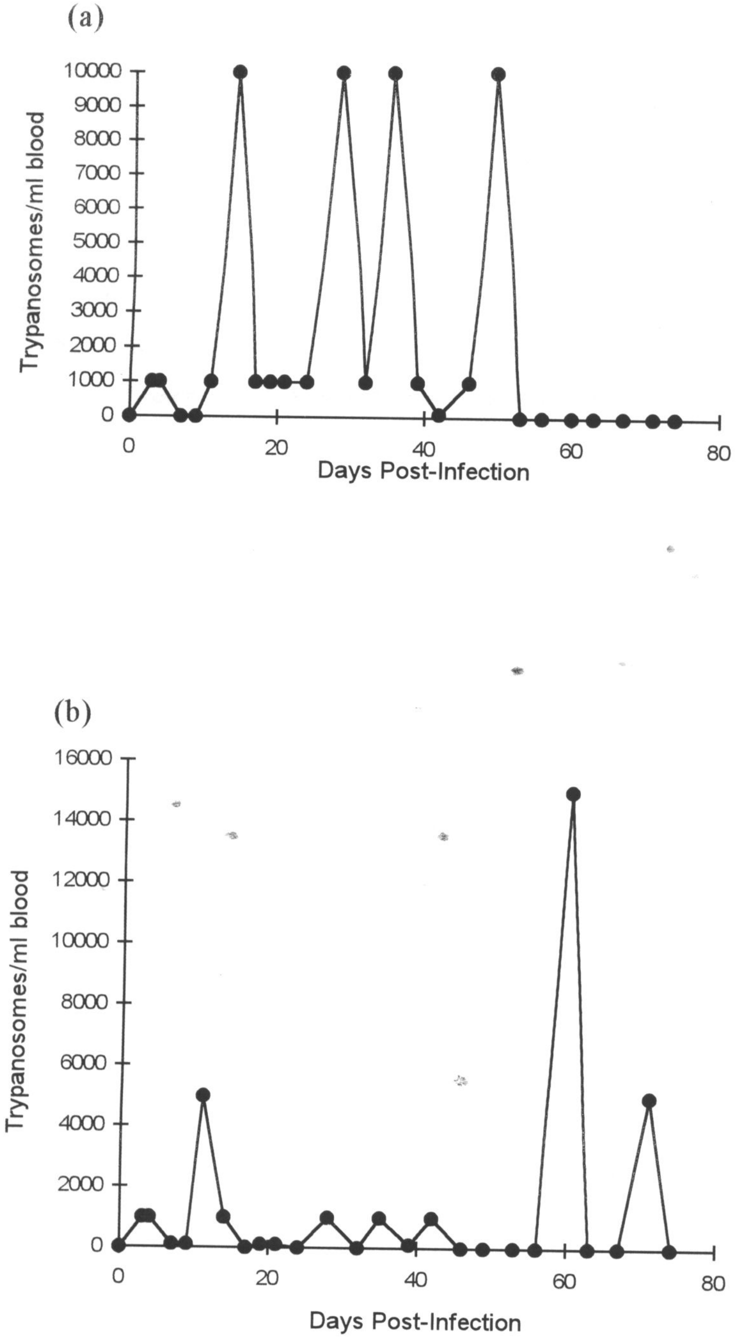


Figure 4.7: Variations in parasitaemia (trypanosomes/ml blood) of the experimental infected goat-3 (a) and goat-4 (b) at different days post-infection.

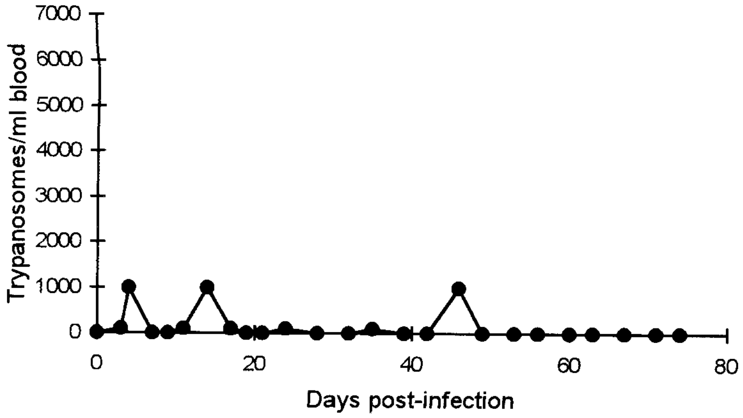


Figure 4.8: Variations in parasitaemia (trypanosomes/ml blood) of experimental infected goat-5 at different days post-infection.

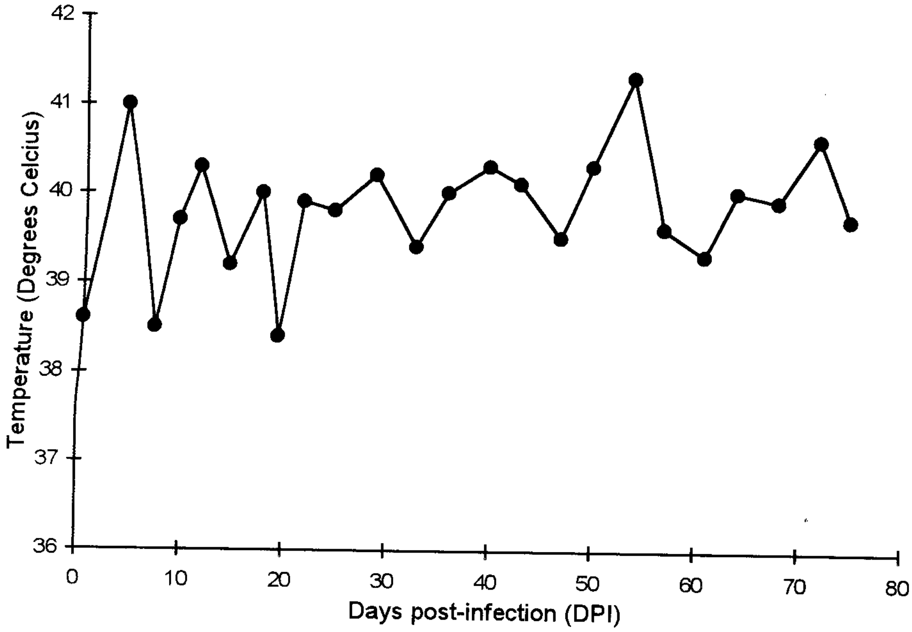
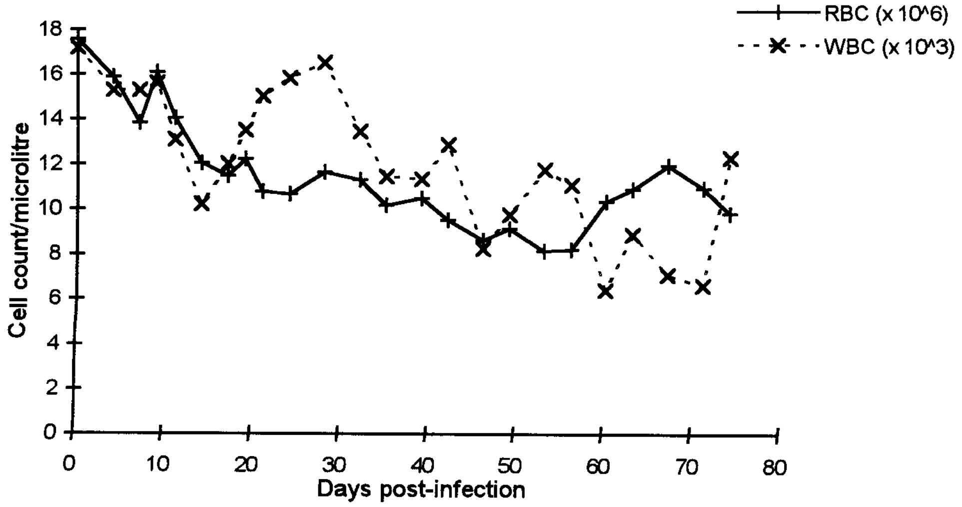


Figure 4.9: Variations in rectal temperatures (^{0}C) of experimental infected goat-2 at different days post-infection

(a)



(b)

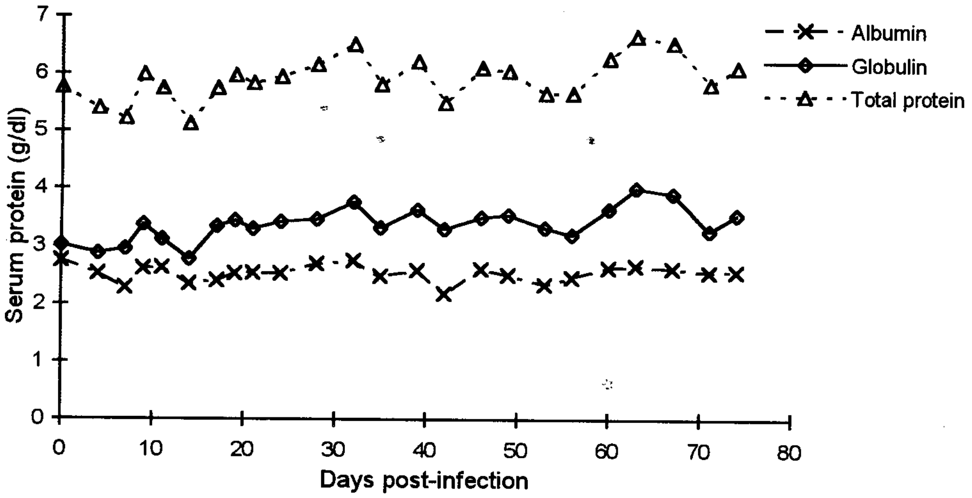


Figure 5.0: Variations in red and white blood cell counts (a) and serum proteins concentrations (b) of infected goat-2 at different days post-infection.

Table 3.0. Parasitaemia (Trypanosomes/ml blood) for the experimental goats at different days

DAYS PI	INFECTED GOATS NUMBERS						
	1	2	3	4	5	MEAN	SEM
00	0	0	0	0	0	0	0
03	100	100	1000	1000	100	460	220
04	5000	1000	1000	1000	1000	1800	800
07	1000	0	0	100	0	220	196
09	0	0	0	100	0	20	20
11	0	1000	1000	5000	100	1420	920
14	5000	1000	10000	1000	1000	3600	1778
17	Died!	100	1000	0	100	300	235
19		0	1000	100	0	275	243
21		0	1000	100	0	257	243
24		1000	1000	0	100	525	275
28		1000	10000	1000	0	3000	2345
32		1000	1000	0	0	500	289
35		5000	10000	1000	100	4025	2258
39		1000	1000	100	0	525	275
42		5000	100	1000	0	1525	1180
46		0	1000	0	1000	500	289
49		0	10000	0	0	2500	2500
53		7000	0	0	0	1750	2500
56		0	0	0	0	0	0
60		0	0	15000	0	3750	1250
63		0	0	0	0	0	0
67		5000	0	0	0	1250	1250
71		5000	0	5000	0	2500	1443
74		0	0	0	0	0	0

PI Post-infection; SEM Standard error of the mean.

Table 3.1: Rectal temperatures (°C) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	38.7	38.6	38.3	38.3	38.3	38.4	0.1	39.4	39.0	38.4	38.8	38.7	38.9	0.2
04	40.4	41.0	39.9	40.6	39.6	40.3	0.2	38.7	39.1	38.6	39.1	38.5	38.8	0.1
07	39.8	38.5	38.7	38.3	38.6	38.8	0.3	38.6	39.0	38.7	38.8	38.6	38.7	0.1
09	39.4	39.7	39.0	38.5	39.2	39.2	0.2	39.1	38.6	38.8	38.4	38.7	38.7	0.1
11	39.4	40.3	39.8	40.1	39.3	39.8	0.2	38.7	38.8	38.6	38.7	38.6	38.7	0.1
14	39.0	39.2	40.4	39.0	40.4	39.6	0.3	38.4	38.6	38.6	39.4	38.4	38.7	0.1
17	Died!	40.0	39.1	39.5	39.2	39.5	0.2	38.8	38.8	38.5	38.8	38.5	38.7	0.1
19		38.4	38.7	39.0	39.0	38.8	0.1	39.3	39.2	38.6	38.8	38.6	38.9	0.1
21		39.9	39.4	39.5	39.5	39.6	0.1	38.6	38.6	38.5	38.9	38.4	38.6	0.1
24		39.8	38.8	39.8	39.7	39.5	0.2	39.0	39.2	39.0	38.9	39.0	39.0	0.1
28		40.2	39.2	38.9	39.9	39.6	0.3	39.0	39.4	39.1	39.1	39.3	39.2	0.1
32		39.4	39.9	38.9	39.4	39.4	0.2	38.9	38.8	39.1	39.2	38.6	38.9	0.1
35		40.0	40.1	40.0	39.8	40.0	0.1	38.6	38.6	38.6	38.8	38.5	38.6	0.1
39		40.3	39.0	39.3	39.4	39.5	0.3	39.0	39.0	39.0	39.1	39.0	39.0	0.01
42		40.1	40.1	39.4	39.1	39.7	0.3	38.6	38.8	39.7	38.8	38.6	38.9	0.2
46		39.5	39.5	39.4	39.7	39.5	0.1	39.1	39.1	39.3	39.0	39.0	39.1	0.1
49		40.3	39.9	39.2	39.5	39.7	0.2	39.3	39.4	39.5	39.3	39.2	39.3	0.1
53		41.3	39.9	39.2	39.3	39.9	0.5	38.8	38.9	39.0	39.1	39.0	39.0	0.1
56		39.6	39.3	39.3	39.4	39.4	0.1	39.2	38.8	39.2	39.2	38.9	39.1	0.1
60		39.3	38.9	42.0	39.2	39.8	0.7	39.2	39.8	39.4	39.4	39.4	39.4	0.1
63		40.0	39.1	39.0	39.9	39.5	0.3	39.1	39.2	39.0	39.1	39.1	39.1	0.01
67		39.9	39.1	39.1	38.8	39.2	0.2	39.3	39.31	39.2	39.3	39.5	39.3	0.1
71		40.6	39.5	40.2	39.2	39.9	0.3	39.2	39.3	39.1	39.2	38.9	39.1	0.1
74		39.7	39.2	38.8	39.1	39.2	0.2	39.3	39.0	39.2	39.3	39.1	39.2	0.1

PI = Post-infection; SEM = Standard error of the mean.

Table 3.2: Body weight (kg) of the experimental goats at different days post-infection.

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	14.50	18.80	20.00	19.50	19.70	18.50	1.02	19.00	15.40	17.00	12.60	18.10	17.82	0.75
07	14.60	18.60	19.80	19.50	19.40	18.38	0.97	18.70	15.30	17.10	12.60	17.90	17.72	0.73
14	13.80	18.50	19.70	19.00	19.30	18.06	1.08	18.80	15.40	17.00	12.70	17.90	17.76	0.74
21	Died!	18.70	19.30	18.90	19.20	19.03	0.14	18.85	15.20	17.00	12.60	17.70	17.67	0.76
28		18.60	19.10	18.90	19.40	19.00	0.17	19.60	15.30	17.30	12.80	17.80	17.96	0.82
35		18.70	19.00	18.45	19.30	18.86	0.18	19.70	15.50	17.40	12.85	17.90	18.07	0.80
42		18.40	19.0	17.50	19.00	18.48	0.35	19.30	15.60	17.45	12.70	17.45	17.90	0.74
49		18.50	18.70	18.50	19.20	18.73	0.17	19.50	15.70	18.20	12.50	18.20	18.22	0.69
56		18.70	18.70	18.00	19.10	18.63	0.23	19.35	15.75	18.00	12.65	18.00	18.15	0.69
63		18.85	18.65	18.00	18.80	18.58	0.20	19.30	15.80	18.10	12.70	18.10	18.20	0.68
70		18.65	18.60	18.00	19.40	18.66	0.29	19.40	15.75	18.30	12.70	17.95	18.22	0.70

PI = Post-infection; SEM = Standard error of the mean

Table 3.3: Packed cell volume (PCV) % of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	37.5	35.5	37.0	28.0	40.0	35.6	2.0	35.0	33.0	28.0	35.5	30.0	32.3	1.4
04	30.0	31.0	30.5	23.0	36.0	30.1	2.1	33.4	32.0	28.0	33.7	30.5	31.5	1.0
07	26.0	28.5	28.5	21.0	32.0	27.2	1.8	34.5	32.5	28.0	34.0	31.0	32.0	1.2
09	26.5	30.5	31.0	22.0	33.5	28.7	2.0	35.0	30.0	27.5	33.0	29.5	31.0	1.3
11	28.0	28.0	29.0	21.0	30.5	27.3	1.6	35.0	30.5	27.5	30.0	28.0	30.2	1.3
14	26.5	26.0	22.0	18.0	30.5	24.6	2.1	35.0	31.0	28.0	29.0	28.0	30.2	1.3
17	Died!	24.5	25.0	16.5	26.5	23.1	2.2	34.7	32.0	28.0	29.5	28.0	30.4	1.3
19		26.0	28.0	18.0	30.0	25.5	2.6	36.5	33.5	28.0	29.0	29.0	31.2	1.6
21		22.5	24.0	18.0	28.5	23.3	2.2	35.0	30.5	27.5	29.5	27.5	30.0	1.4
24		22.0	21.5	17.0	25.5	21.5	1.7	34.5	30.0	27.0	29.5	27.0	29.6	1.4
28		22.0	21.0	17.5	25.5	21.5	1.6	33.0	29.0	27.0	29.0	27.5	29.1	1.0
32		25.0	24.5	18.0	26.5	23.5	1.9	35.0	31.0	27.0	29.0	27.5	29.9	1.4
35		21.0	21.0	16.0	24.0	20.5	1.7	33.0	30.0	27.0	29.5	27.5	29.4	1.1
39		22.5	21.0	15.0	24.0	20.6	2.0	32.5	30.0	26.5	29.5	27.0	29.1	1.1
42		18.0	22.5	17.0	26.0	20.9	2.1	32.5	30.0	27.0	29.0	27.0	29.1	1.0
46		17.5	24.0	14.0	23.0	19.6	2.4	31.0	29.0	27.0	29.0	26.5	28.5	0.8
48		18.0	19.0	14.5	20.0	17.9	1.2	31.5	28.5	26.5	28.0	26.5	28.2	0.9
53		17.0	21.0	15.0	23.0	19.0	1.8	31.0	28.5	26.5	28.0	26.5	28.1	0.8
56		17.0	23.0	17.5	23.5	203	1.7	30.5	28.0	26.5	27.5	27.0	27.9	0.7
60		19.5	24.0	16.0	23.0	20.6	1.8	30.5	28.0	27.0	27.0	26.5	27.8	0.7
63		22.0	27.0	17.0	27.5	23.4	2.5	30.0	28.0	26.5	27.5	26.5	27.7	0.6
67		25.0	26.5	15.0	25.0	22.9	2.6	32.0	28.5	27.0	27.0	26.5	28.2	1.0
71		21.0	30.0	17.0	27.0	23.7	2.7	31.0	28.0	27.0	27.5	26.5	28.0	0.5
74		18.0	27.0	15.0	26.0	21.5	1.7	30.0	28.0	27.0	28.0	27.0	28.0	0.7

PI = Post-Infection; SEM = Standard error of the mean.

Table 3.4: Red blood cell (RBC) counts (x $10^6/\mu l$) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	17.19	17.58	17.58	12.27	17.89	16.50	1.06	17.25	16.47	11.88	16.14	11.50	14.25	1.16
04	14.31	15.90	15.66	11.07	16.83	14.75	1.01	15.99	15.82	11.79	15.77	11.60	13.88	0.95
07	12.60	13.83	15.48	11.04	15.90	13.77	0.90	16.00	16.00	11.90	15.90	11.20	13.82	1.00
09	12.87	16.08	16.65	9.87	16.20	14.33	1.30	16.32	15.03	11.87	15.83	11.30	13.74	0.95
11	13.35	14.04	14.26	9.33	16.11	13.42	1.12	16.40	15.80	11.41	13.72	11.40	13.75	1.05
14	13.35	12.04	11.75	8.44	15.50	12.22	1.15	17.55	15.85	11.44	13.09	11.20	13.83	1.25
17	Died!	11.48	12.41	7.11	11.71	10.68	1.21	16.91	16.13	11.22	13.51	10.98	13.75	1.22
19		12.20	14.34	7.46	14.45	12.11	1.64	17.49	16.83	11.62	13.20	11.11	14.05	1.32
21		10.75	12.81	7.50	12.89	10.99	1.26	17.44	15.71	11.87	13.32	11.34	13.94	1.16
24		10.63	10.81	7.27	11.22	9.98	0.91	17.46	15.08	11.73	13.45	11.51	13.85	1.11
28		11.61	10.80	7.75	11.46	10.40	0.90	16.74	14.73	11.83	13.08	11.34	13.54	0.99
32		11.28	10.41	7.35	12.15	10.30	1.04	16.88	14.89	11.40	12.00	11.51	13.34	1.09
35		10.14	10.29	7.80	11.85	10.02	0.83	16.31	14.78	11.60	12.45	11.43	13.31	0.96
39		10.44	10.02	6.93	11.70	9.77	1.01	15.55	14.64	11.48	12.63	11.40	13.14	0.84
42		9.48	10.02	7.77	12.30	9.89	0.93	15.61	14.69	11.62	12.61	11.50	13.21	0.83
46		8.55	12.03	6.84	10.62	9.51	1.14	14.37	14.57	10.97	13.32	11.02	12.85	0.79
49		9.07	10.81	7.06	10.82	9.44	0.89	15.09	14.03	10.88	13.26	11.17	12.89	0.81
53		8.07	10.01	7.26	10.71	9.01	0.81	14.97	14.21	10.71	13.43	11.20	12.90	0.84
56		8.13	11.40	8.64	10.82	9.75	0.80	15.03	13.98	11.70	13.70	11.67	13.22	0.66
60		10.26	12.24	8.04	10.15	10.17	0.86	15.33	13.63	12.00	13.86	10.97	13.16	0.76
63		10.80	12.42	7.80	13.29	11.08	1.21	15.17	13.51	11.86	12.95	11.10	12.92	0.70
67		11.88	12.33	7.32	12.33	10.96	1.30	15.36	14.48	11.92	12.54	11.03	13.07	0.72
71		10.83	14.52	8.10	13.80	11.81	1.25	15.21	14.02	11.90	12.63	11.50	13.05	0.63
74		9.72	13.65	7.53	13.23	11.03	0.98	15.00	13.87	11.82	13.25	11.85	13.16	0.68

PI = Post-Infection; SEM = Standard error of the mean.

Table 3.5: Haemoglobin (Hb) concentration (g/dl) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	11.7	11.9	12.3	9.8	12.8	11.7	0.5	12.0	12.1	9.4	11.4	9.6	10.9	0.6
04	9.6	10.5	10.1	7.7	11.8	9.9	0.6	11.2	11.8	9.5	11.4	9.7	10.7	0.5
07	8.8	10.1	10.3	7.3	11.8	9.7	0.7	11.9	12.0	9.3	11.3	9.6	10.8	0.6
09	8.9	10.6	10.1	7.4	11.2	9.6	0.7	11.4	11.2	9.2	10.9	8.9	10.3	0.5
11	9.7	9.6	10.0	7.3	11.8	9.7	0.6	12.0	11.1	9.4	10.7	9.0	10.4	0.5
14	9.0	9.0	8.4	6.2	10.0	8.5	0.7	12.2	11.0	9.3	10.1	9.3	10.4	0.6
17	Died!	8.2	8.9	6.0	9.2	8.1	0.8	12.1	11.7	9.4	9.8	9.0	10.4	0.6
19		8.1	9.9	6.3	9.3	8.4	0.6	12.1	11.8	9.4	9.6	9.4	10.5	0.6
21		7.8	8.6	6.3	9.0	7.9	0.6	12.1	11.0	9.1	9.3	9.1	10.1	0.6
24		7.6	7.9	6.1	9.1	7.7	0.7	12.2	11.0	9.0	9.3	9.0	10.1	0.6
28		8.0	8.0	5.8	8.9	7.7	0.7	11.4	10.4	9.3	9.4	8.9	9.9	0.4
32		8.2	9.0	6.1	9.3	8.2	0.6	11.2	10.8	9.1	9.2	9.1	9.9	0.5
35		7.7	7.8	5.8	8.5	7.4	0.6	11.4	10.8	9.2	9.4	9.2	10.0	0.5
39		7.7	8.1	5.8	8.9	7.6	0.7	11.0	10.7	8.9	9.3	9.0	9.8	0.4
42		7.2	8.4	6.3	9.7	7.9	0.7	11.1	10.8	9.0	9.3	9.2	9.9	0.4
46		7.0	8.8	5.7	8.7	7.5	0.6	10.6	10.4	8.9	9.2	8.8	9.6	0.5
49		7.2	8.5	5.8	8.1	7.4	0.6	11.4	9.8	8.9	9.7	8.9	9.7	0.5
53		7.0	8.5	6.0	8.8	7.6	0.6	11.3	10.0	8.8	9.6	8.8	9.7	0.5
56		6.9	8.7	6.6	8.7	7.7	0.5	11.3	9.6	8.9	9.5	9.1	9.7	0.4
60		7.8	9.0	6.5	8.2	7.9	0.6	11.3	9.2	9.1	9.3	8.8	9.5	0.4
63		8.2	9.5	6.1	9.5	8.3	0.8	11.2	9.3	9.0	9.2	9.0	9.5	0.4
67		8.7	8.9	6.1	9.0	8.2	0.6	11.3	9.9	9.1	8.9	9.0	9.6	0.3
71		7.9	10.8	6.4	9.6	8.7	0.7	11.0	9.7	9.0	9.0	8.9	9.5	0.4
74		7.3	9.4	5.6	9.0	7.8	0.8	10.6	9.1	8.8	9.3	8.6	9.3	0.4

PI = Post-Infection; SEM = Standard error of the mean

Table 3.6: Mean corpuscular volume (fl) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	21.8	20.2	21.0	22.8	22.4	21.6	0.5	20.3	20.0	23.6	22.0	25.1	22.2	1.0
04	21.0	19.5	19.5	20.8	21.4	20.4	0.4	20.9	20.2	23.7	21.4	23.1	21.8	0.7
07	20.6	20.6	18.4	19.0	20.1	19.7	0.4	21.6	20.3	23.5	21.4	23.3	22.0	0.6
09	20.6	19.0	18.6	22.3	20.7	20.2	0.7	21.4	20.0	23.2	20.8	25.0	22.1	0.9
11	21.0	19.9	20.3	22.5	18.9	20.5	0.6	21.3	19.3	24.1	21.9	26.7	22.7	1.3
14	19.8	21.6	18.7	21.3	19.7	20.2	0.5	19.9	19.6	24.5	22.1	26.3	22.5	1.3
17	Died!	21.3	20.1	23.2	22.6	21.8	0.7	20.5	19.8	24.9	21.8	26.1	22.6	1.2
19		21.3	19.5	24.1	20.8	21.4	1.0	20.9	19.9	24.0	22.0	26.1	22.6	1.1
21		20.9	18.7	24.0	22.1	21.4	1.1	20.1	19.4	23.2	22.1	25.9	22.1	1.2
24		20.7	19.9	23.4	22.7	21.7	0.8	19.8	19.9	23.0	21.9	25.8	22.1	1.1
28		18.9	19.4	22.6	22.3	20.8	1.0	19.7	19.7	22.8	22.2	24.3	21.7	0.9
32		22.2	23.5	24.5	21.8	23.0	0.6	20.7	20.8	23.7	24.2	23.9	22.7	0.8
35		20.7	20.4	20.5	20.2	20.4	0.1	20.2	20.3	23.3	23.7	24.1	22.3	0.8
39		21.5	21.0	21.6	20.5	21.1	0.2	20.9	20.5	23.1	23.3	23.7	22.3	0.6
42		19.0	22.5	21.9	21.1	21.1	0.8	20.8	20.4	23.2	23.0	23.5	22.2	0.6
46		20.5	19.4	20.5	21.6	20.5	0.4	21.6	19.9	24.6	21.8	24.0	22.4	0.8
49		19.8	16.3	20.5	18.5	18.8	0.9	20.9	20.3	24.4	21.1	23.7	22.1	0.8
53		21.1	16.3	20.7	21.5	19.9	1.2	20.7	20.1	24.7	20.8	23.7	22.0	0.9
56		20.9	16.7	20.3	21.7	19.9	1.1	20.3	20.0	22.6	20.1	23.1	21.2	0.7
60		19.0	20.4	19.9	22.7	20.5	0.8	19.9	20.5	22.5	19.5	24.2	21.3	0.9
63		20.4	18.4	21.8	20.7	20.3	0.7	19.8	20.7	22.3	21.2	23.9	21.6	0.7
67		21.0	16.3	20.5	20.3	19.5	1.1	20.8	19.7	22.6	21.5	24.0	21.7	0.7
71		19.4	20.7	21.0	19.6	20.2	0.5	20.4	20.0	22.7	21.8	23.0	21.6	0.5
74		18.5	19.8	19.9	19.6	19.4	0.5	20.0	20.2	22.8	21.1	22.8	21.4	0.6

PI = Post-infection; SEM = Standard error of the mean

Table 3.7: Mean corpuscular haemoglobin (pg) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	6.8	6.8	7.0	8.0	7.2	7.2	0.2	7.0	7.3	7.9	7.1	8.0	7.5	0.2
04	6.7	6.6	6.4	7.0	7.0	6.7	0.1	7.0	7.5	8.1	7.2	7.3	7.4	0.2
07	7.0	7.3	6.7	6.6	7.4	7.0	0.2	7.4	7.5	7.8	7.1	7.2	7.4	0.1
09	6.9	6.6	6.1	7.5	6.9	6.8	0.2	7.0	7.5	7.8	6.9	7.5	7.3	0.2
11	7.3	6.8	7.0	7.8	7.3	7.2	0.2	7.3	7.0	8.2	7.8	8.6	7.8	0.3
14	6.7	7.5	7.1	7.3	6.5	7.0	0.2	7.0	6.9	8.1	7.7	8.7	7.7	0.3
17	Died!	7.1	7.2	8.4	7.9	7.6	0.3	7.2	7.3	8.4	7.3	8.4	7.7	0.3
19		6.6	6.9	8.4	6.4	7.1	0.5	6.9	7.0	8.1	7.3	8.5	7.6	0.3
21		7.3	6.7	8.4	7.0	7.3	0.4	6.9	7.0	7.7	7.0	8.6	7.4	0.3
24		7.1	7.3	8.4	8.1	7.7	0.3	7.0	7.3	8.4	6.9	8.6	7.6	0.4
28		6.9	7.4	7.5	7.8	7.4	0.2	6.8	7.1	7.9	7.2	7.8	7.4	0.2
32		7.3	8.6	8.3	7.7	8.0	0.3	6.6	7.3	8.0	7.7	7.9	7.5	0.3
35		7.6	7.6	7.4	7.2	7.4	0.1	7.0	7.3	7.9	7.5	8.0	7.5	0.2
39		7.4	8.1	8.4	7.6	7.9	0.2	7.1	7.3	7.8	7.4	7.9	7.5	0.2
42		7.6	8.4	8.1	7.9	8.0	0.2	7.1	7.3	7.7	7.4	8.0	7.5	0.2
46		8.2	7.3	8.3	8.2	8.0	0.2	7.4	7.1	8.1	6.9	8.0	7.5	0.2
49		7.9	7.9	8.2	7.4	7.8	0.2	7.5	7.0	8.2	7.3	8.0	7.6	0.2
53		8.7	8.5	8.3	8.2	8.4	0.1	7.5	7.0	8.2	7.1	7.8	7.5	0.2
56		8.5	7.6	7.6	8.0	7.9	0.2	7.5	6.9	7.6	6.9	7.8	7.3	0.2
60		7.6	7.4	8.1	8.1	7.8	0.2	7.4	6.7	7.6	6.7	8.0	7.3	0.3
63		7.6	7.6	7.8	7.1	7.5	0.1	7.4	6.9	7.6	7.1	8.1	7.4	0.2
67		7.3	7.2	8.3	7.3	7.5	0.3	7.4	6.8	7.6	7.1	8.2	7.4	0.2
71		7.3	7.4	7.9	7.0	7.4	0.3	7.2	6.9	7.6	7.1	7.7	7.3	0.2
74		7.5	6.9	7.4	6.8	7.1	0.2	7.1	6.6	7.4	7.0	7.3	7.1	0.2

PI = Post-infection; SEM = Standard error of the mean

Table 3.8: Mean corpuscular haemoglobin concentration (g/dl) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	31.2	33.5	33.2	35.0	32.0	33.0	0.6	34.3	36.7	33.6	32.1	32.0	33.7	0.9
04	32.0	33.9	33.1	33.5	32.8	33.1	0.3	33.5	36.9	33.9	33.8	31.8	34.0	0.8
07	33.8	35.4	36.1	34.8	36.9	35.4	0.5	34.5	36.9	33.2	33.2	31.0	33.8	1.0
09	33.6	34.6	32.6	33.6	33.4	33.6	0.3	32.6	37.3	33.5	33.0	30.2	33.3	1.1
11	34.6	34.3	34.5	34.8	38.7	35.4	0.8	34.3	36.4	34.2	35.7	32.1	34.5	0.7
14	34.0	34.6	38.2	34.4	32.8	34.8	0.9	34.9	35.5	33.2	34.8	33.2	34.3	0.5
17	Died!	33.5	35.6	36.4	34.7	35.1	0.6	34.9	36.6	33.6	33.2	32.1	34.1	0.8
19		31.1	35.4	35.0	31.0	33.1	1.2	33.2	35.2	33.6	33.1	32.4	33.5	0.5
21		34.7	35.8	35.0	33.7	34.8	0.4	34.6	36.1	33.1	31.5	33.1	33.7	0.8
24		34.5	36.7	35.9	35.7	35.7	0.5	35.4	36.7	33.3	31.5	33.3	34.0	0.9
28		36.4	38.1	33.1	34.9	35.6	1.1	34.5	35.9	34.4	32.4	32.4	33.9	0.7
32		32.8	36.7	33.9	35.1	34.6	0.8	32.0	34.8	33.7	31.7	33.1	33.1	0.6
35		36.7	37.1	36.2	35.4	36.4	0.4	34.5	36.0	34.1	31.9	33.4	34.0	0.7
39		34.2	38.6	38.7	37.1	37.1	1.0	33.8	35.7	33.6	31.5	33.3	33.6	0.7
42		40.0	37.3	37.1	37.3	37.9	0.7	34.1	36.0	33.3	32.1	34.1	33.9	0.6
46		40.0	36.7	40.7	37.8	38.8	0.9	34.2	35.9	33.0	31.7	33.2	33.6	0.7
49		40.0	44.7	40.0	40.5	41.3	1.1	36.2	34.4	33.6	34.6	33.6	34.5	0.5
53		41.2	40.5	40.0	38.3	40.0	0.6	36.4	35.1	33.2	34.3	33.2	34.4	0.6
56		40.6	37.8	37.7	37.0	38.3	0.8	37.0	34.3	33.6	34.5	33.7	34.6	0.6
60		40.0	37.5	40.6	35.7	38.6	1.1	37.0	32.9	33.7	34.4	33.2	34.2	0.7
63		37.3	35.2	35.9	34.5	35.7	0.6	37.3	33.2	34.0	33.4	34.0	34.4	0.7
67		34.8	33.6	40.7	36.0	36.3	1.6	35.3	34.7	33.7	33.0	34.0	34.1	0.4
71		37.6	36.0	37.6	35.5	36.7	0.5	35.5	34.6	33.3	32.7	33.6	33.9	0.3
74		40.5	34.8	37.3	34.6	36.8	1.2	35.3	32.5	32.6	33.2	31.8	33.1	0.5

PI = Post-infection; SEM = Standard error of the mean

Table 3.9: White blood cell counts (x $10^3/\mu l$) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	20.5	17.2	22.1	13.0	14.0	17.4	1.8	12.9	23.2	23.2	16.7	12.2	17.4	2.4
04	14.2	15.3	12.4	10.3	12.9	13.0	0.8	18.5	22.2	21.6	20.1	13.9	19.3	1.5
07	10.9	15.3	13.8	10.7	11.1	12.4	0.9	16.8	19.7	19.8	17.2	12.3	17.2	1.5
09	13.6	15.6	13.7	13.2	10.2	13.3	0.9	20.0	23.6	23.2	26.1	12.7	21.1	2.3
11	13.8	13.1	10.8	13.4	9.8	12.2	0.8	19.4	22.9	21.4	20.7	13.6	19.6	1.6
14	16.6	10.2	7.3	6.9	10.2	10.2	1.7	19.2	23.6	20.7	21.4	13.8	19.7	1.6
17	Died!	12.0	15.4	12.3	10.3	12.5	1.0	18.3	21.7	20.1	18.9	13.1	18.4	1.4
19		13.5	18.1	18.3	11.3	15.3	1.7	22.0	22.1	18.4	20.2	13.0	19.1	1.7
21		15.0	22.1	15.6	11.7	16.1	2.2	23.1	22.0	18.1	21.2	14.2	19.7	1.6
24		15.8	32.1	13.9	14.9	19.2	4.3	25.4	22.1	18.8	23.4	15.4	21.0	1.8
28		16.5	31.1	17.0	15.2	19.9	3.7	21.3	23.0	23.5	22.2	12.5	20.5	2.0
32		13.4	31.8	14.7	18.2	19.5	4.2	17.4	19.6	18.4	20.7	13.1	17.8	1.3
35		11.4	19.9	14.2	20.4	16.5	2.2	17.3	20.0	19.2	19.6	13.7	18.0	1.2
39		11.3	24.9	15.0	15.0	16.5	2.9	12.2	24.8	15.6	27.4	14.5	18.9	3.0
42		12.8	22.5	10.3	11.7	14.3	2.8	15.3	20.1	16.1	20.2	12.3	16.8	1.5
46		8.2	19.4	10.1	13.1	12.7	2.4	11.9	22.5	15.8	12.0	6.7	13.8	2.6
49		9.7	16.3	12.4	10.1	12.1	1.5	10.9	18.1	11.5	15.2	9.7	13.1	1.5
53		11.7	16.3	8.4	8.9	11.3	1.8	13.2	19.8	12.9	17.7	10.1	14.7	1.7
56		11.0	16.7	9.3	7.9	11.2	1.9	14.6	20.1	12.1	16.9	13.1	15.4	1.4
60		6.3	20.4	11.5	11.4	12.4	2.9	13.3	25.7	11.8	17.2	12.9	16.2	2.5
63		8.8	18.5	8.5	8.4	11.0	2.5	14.7	18.0	15.7	19.1	13.3	16.2	1.0
67		7.0	16.3	9.9	13.2	11.5	2.0	17.5	17.7	13.7	15.2	12.8	15.4	1.0
71		6.5	13.0	10.2	14.6	11.1	2.0	13.6	18.3	14.2	14.8	11.5	14.5	1.3
74		12.2	16.6	8.4	12.6	12.4	2.3	10.4	19.1	12.8	14.2	13.9	14.1	1.0

PI = Post-infection; SEM = Standard error of the mean

Table 4.0: Total serum protein concentration (g/dl) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	6.45	5.77	6.47	6.40	6.08	6.23	0.14	6.32	5.97	6.19	5.99	5.99	6.09	0.07
04	5.88	5.40	6.66	6.73	5.77	6.09	0.26	6.45	5.71	6.10	5.99	6.12	6.07	0.12
07	5.55	5.23	6.49	6.47	5.75	5.90	0.25	6.38	5.86	6.16	6.10	6.10	6.12	0.08
09	5.51	5.99	7.21	7.21	6.16	6.42	0.34	6.42	6.12	6.60	6.60	6.10	6.37	0.11
11	5.60	5.75	7.17	7.25	6.64	6.48	0.35	6.36	6.10	6.56	6.08	6.03	6.23	0.10
14	5.12	5.12	6.14	6.08	6.10	5.71	0.24	6.32	6.05	6.69	6.14	6.08	6.26	0.12
17	Died!	5.75	6.62	6.49	5.84	6.18	0.22	6.38	5.94	6.47	6.05	6.05	6.18	0.10
19		5.97	7.01	7.10	6.69	6.69	0.26	6.71	6.01	6.69	6.23	6.21	6.37	0.14
21		5.84	6.69	7.08	6.69	6.58	0.26	6.29	5.88	6.25	6.14	5.81	6.07	0.10
24		5.95	7.01	6.47	6.53	6.49	0.22	6.32	5.84	6.14	6.01	5.86	6.03	0.09
28		6.16	7.21	7.06	6.62	6.76	0.24	5.88	5.55	6.08	5.36	5.14	5.60	0.17
32		6.51	7.93	7.38	7.08	7.22	0.30	6.29	5.84	6.45	5.68	5.60	5.97	0.17
35		5.81	7.12	7.06	6.69	6.67	0.30	6.21	5.53	6.42	6.08	5.55	5.96	0.18
39		6.21	7.73	7.19	7.03	7.04	0.31	6.23	5.75	6.47	6.12	5.53	6.02	0.17
42		5.49	8.19	7.75	7.43	7.22	0.60	6.18	5.88	6.27	6.05	5.62	6.00	0.12
46		6.10	6.42	7.43	6.84	6.70	0.29	6.14	5.94	6.18	5.88	5.49	5.93	0.12
49		6.05	7.00	7.08	6.49	6.66	0.24	6.25	5.66	6.21	5.79	5.44	5.87	0.16
53		5.66	7.84	7.21	6.95	6.91	0.46	6.32	5.64	6.32	5.68	5.47	5.89	0.18
56		5.66	7.41	7.08	6.49	6.66	0.38	6.27	5.60	6.25	5.66	5.51	5.86	0.17
60		6.27	7.47	6.82	6.40	6.74	0.27	5.99	5.49	6.21	5.60	5.27	5.71	0.17
63		6.66	7.73	7.36	6.97	7.18	0.23	6.32	5.86	6.25	5.55	5.38	5.87	0.19
67		6.53	7.34	7.41	6.71	7.00	0.22	6.45	5.90	6.42	5.51	5.10	5.88	0.26
71		5.81	7.25	7.45	6.93	6.86	0.24	6.25	5.77	6.29	5.64	5.44	5.88	0.28
74		6.10	6.99	6.95	6.71	6.68	0.21	6.14	5.88	6.23	5.66	5.49	5.88	0.21

PI = Post-Infection; SEM = Standard error of the mean

Table 4.1: Serum globulin concentrations (g/dl) of experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	3.85	3.02	3.77	4.10	3.15	3.58	0.21	3.64	3.06	3.77	3.18	3.61	3.45	0.14
04	3.48	2.87	4.09	4.11	3.11	3.53	0.25	3.81	2.85	3.84	3.39	3.83	3.54	0.19
07	3.54	2.95	4.11	4.08	3.26	3.59	0.23	3.70	2.99	3.75	3.44	3.77	3.53	0.15
09	3.74	3.37	4.63	4.62	3.51	3.97	0.27	3.81	3.12	4.24	3.77	3.87	3.76	0.18
11	3.68	3.12	4.56	4.51	3.82	3.94	0.27	3.68	3.09	4.08	3.42	3.69	3.59	0.16
14	3.70	2.77	3.78	3.78	3.59	3.52	0.19	3.63	3.04	4.22	3.51	3.72	3.62	0.19
17	Died!	3.34	4.34	4.55	2.50	3.68	0.47	3.71	2.94	3.99	3.39	3.67	3.54	0.18
19		3.44	4.62	4.63	3.99	4.17	0.29	4.06	3.07	4.31	3.57	3.87	3.78	0.21
21		3.30	4.34	4.55	4.09	4.07	0.27	3.69	2.96	3.84	3.51	3.50	3.50	0.15
24		3.42	4.67	4.15	4.00	4.06	0.26	3.71	2.91	3.76	3.39	3.58	3.47	0.15
28		3.46	4.78	4.64	4.10	4.24	0.30	3.36	2.73	3.85	2.87	2.98	3.16	0.20
32		3.76	5.27	4.99	4.40	4.61	0.33	3.80	3.03	4.21	3.19	3.46	3.54	0.21
35		3.32	4.65	4.70	4.08	4.19	0.32	3.73	2.74	4.07	3.59	3.22	3.47	0.23
39		3.63	5.28	4.76	4.34	4.50	0.35	3.80	2.94	4.13	3.65	3.21	3.55	0.21
42		3.30	5.52	5.25	4.79	4.72	0.50	3.62	3.08	3.96	3.57	3.34	3.51	0.15
46		3.49	3.72	4.92	4.11	4.06	0.31	3.68	3.06	3.84	3.40	3.13	3.42	0.15
49		3.54	4.66	4.73	3.97	4.22	0.29	3.74	2.84	3.83	3.26	3.08	3.35	0.19
53		3.32	5.28	4.69	4.18	4.37	0.42	3.78	2.95	3.92	3.14	3.10	3.38	0.20
56		3.20	4.78	4.50	3.80	4.07	0.36	3.71	2.88	3.83	3.14	3.15	3.34	0.18
60		3.64	4.73	4.24	3.67	4.07	0.26	3.48	2.79	3.78	3.02	3.06	3.23	0.18
63		4.00	5.06	4.81	4.28	4.54	0.24	3.78	3.11	3.73	2.95	3.16	3.35	0.17
67		3.91	4.64	4.79	3.99	4.33	0.22	3.89	3.22	3.88	2.93	2.87	3.36	0.22
71		3.26	4.50	4.75	4.20	4.18	0.18	3.72	3.03	3.78	3.09	3.09	3.34	0.14
74		3.54	4.37	4.44	4.04	4.10	0.15	3.67	3.15	3.78	3.23	3.25	3.42	0.11

PI = Post-infection; SEM = Standard error of the mean

Table 4.2: Serum albumin concentrations (g/dl) of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	2.60	2.75	2.70	2.80	2.93	2.76	0.05	2.68	2.91	2.42	2.81	2.38	2.64	0.10
04	2.40	2.53	2.57	2.62	2.66	2.56	0.04	2.64	2.86	2.26	2.60	2.29	2.53	0.11
07	2.01	2.28	2.38	2.39	2.49	2.31	0.08	2.68	2.87	2.41	2.66	2.33	2.59	0.10
09	1.77	2.62	2.58	2.59	2.65	2.44	0.17	2.61	3.00	2.36	2.83	2.23	2.61	0.14
11	1.55	2.63	2.61	2.74	2.82	2.47	0.23	2.68	3.01	2.48	2.66	2.34	2.63	0.11
14	1.42	2.35	2.36	2.30	2.51	2.19	0.20	2.69	3.01	2.47	2.63	2.36	2.63	0.11
17	Died!	2.41	2.28	1.94	2.34	2.24	0.10	2.67	3.00	2.48	2.66	2.38	2.64	0.11
19		2.53	2.39	2.47	2.70	2.52	0.07	2.65	2.94	2.38	2.66	2.34	2.59	0.11
21		2.54	2.35	2.53	2.60	2.51	0.05	2.60	2.92	2.41	2.63	2.31	2.57	0.10
24		2.53	2.34	2.32	2.53	2.43	0.06	2.61	2.93	2.38	2.62	2.28	2.56	0.11
28		2.70	2.43	2.42	2.52	2.52	0.06	2.52	2.82	2.23	2.49	2.16	2.44	0.12
32		2.75	2.66	2.39	2.68	2.62	0.08	2.49	2.81	2.24	2.49	2.14	2.43	0.12
35		2.49	2.47	2.36	2.61	2.48	0.05	2.48	2.79	2.35	2.49	2.33	2.49	0.08
39		2.58	2.45	2.43	2.69	2.54	0.11	2.43	2.81	2.34	2.47	2.32	2.47	0.09
42		2.19	2.67	2.50	2.64	2.50	0.05	2.56	2.80	2.31	2.48	2.28	2.49	0.09
46		2.61	2.70	2.51	2.73	2.64	0.05	2.46	2.88	2.34	2.48	2.36	2.50	0.10
49		2.51	2.34	2.35	2.52	2.43	0.09	2.51	2.82	2.38	2.53	2.36	2.52	0.08
53		2.34	2.56	2.52	2.77	2.55	0.04	2.54	2.69	2.40	2.54	2.37	2.51	0.06
56		2.46	2.63	2.58	2.69	2.59	0.03	2.56	2.72	2.42	2.52	2.36	2.52	0.06
60		2.63	2.74	2.58	2.73	2.67	0.03	2.51	2.70	2.43	2.58	2.21	2.49	0.08
63		2.66	2.67	2.55	2.69	2.64	0.05	2.54	2.75	2.52	2.60	2.22	2.53	0.09
67		2.62	2.71	2.62	2.72	2.67	0.03	2.56	2.68	2.54	2.58	2.23	2.52	0.08
71		2.55	2.75	2.70	2.73	2.68	0.02	2.53	2.74	2.51	2.55	2.35	2.54	0.06
74		2.56	2.62	2.51	2.67	2.59	0.03	2.47	2.73	2.45	2.43	2.24	2.46	0.05

PI = post-infection; SEM = Standard error of the mean

Table 4.3: Albumin:globulin (A:G) ratio of the experimental goats at different days post-infection

DAYS PI	INFECTED GOATS NUMBERS							CONTROL GOATS NUMBERS						
	1	2	3	4	5	Mean	SEM	6	7	8	9	10	Mean	SEM
00	0.68	0.91	0.72	0.78	0.93	0.80	0.05	0.74	0.95	0.64	0.88	0.66	0.77	0.06
04	0.69	0.88	0.63	0.64	0.86	0.74	0.05	0.69	1.00	0.59	0.77	0.60	0.73	0.08
07	0.57	0.77	0.58	0.59	0.76	0.65	0.05	0.72	0.96	0.64	0.77	0.62	0.74	0.06
09	0.47	0.78	0.56	0.56	0.75	0.62	0.06	0.69	0.96	0.56	0.75	0.58	0.71	0.07
11	0.42	0.84	0.57	0.61	0.74	0.64	0.07	0.73	0.97	0.61	0.78	0.63	0.74	0.06
14	0.38	0.85	0.62	0.61	0.70	0.63	0.08	0.74	0.99	0.59	0.75	0.63	0.74	0.07
17	Died!	0.72	0.52	0.43	0.67	0.59	0.07	0.72	1.02	0.62	0.78	0.65	0.76	0.07
19		0.74	0.52	0.53	0.68	0.62	0.05	0.65	0.96	0.55	0.75	0.60	0.70	0.07
21		0.77	0.54	0.56	0.64	0.63	0.05	0.70	0.99	0.63	0.75	0.66	0.75	0.06
24		0.74	0.50	0.56	0.63	0.61	0.05	0.70	1.01	0.63	0.77	0.64	0.75	0.07
28		0.78	0.51	0.52	0.61	0.61	0.06	0.75	1.03	0.58	0.87	0.72	0.79	0.08
32		0.73	0.50	0.48	0.61	0.58	0.06	0.66	0.93	0.53	0.78	0.62	0.70	0.07
35		0.75	0.53	0.50	0.64	0.61	0.06	0.66	1.02	0.58	0.69	0.72	0.73	0.08
39		0.71	0.46	0.51	0.62	0.57	0.06	0.64	0.96	0.57	0.68	0.72	0.71	0.07
42		0.66	0.48	0.48	0.55	0.54	0.04	0.71	0.91	0.58	0.69	0.68	0.71	0.05
46		0.75	0.73	0.51	0.66	0.66	0.05	0.67	0.94	0.61	0.73	0.75	0.74	0.06
49		0.71	0.50	0.50	0.63	0.59	0.05	0.67	0.99	0.62	0.78	0.77	0.77	0.06
53		0.70	0.48	0.54	0.66	0.60	0.05	0.67	0.91	0.61	0.81	0.76	0.75	0.05
56		0.77	0.55	0.57	0.71	0.65	0.05	0.69	0.94	0.63	0.80	0.75	0.76	0.05
60		0.72	0.58	0.61	0.74	0.66	0.04	0.72	0.97	0.64	0.85	0.72	0.78	0.06
63		0.67	0.53	0.53	0.63	0.59	0.04	0.67	0.88	0.68	0.88	0.70	0.76	0.05
67		0.67	0.58	0.55	0.68	0.62	0.03	0.66	0.83	0.65	0.88	0.78	0.76	0.05
71		0.78	0.61	0.57	0.65	0.65	0.02	0.68	0.90	0.66	0.83	0.76	0.77	0.03
74		0.72	0.60	0.56	0.66	0.63	0.03	0.67	0.87	0.65	0.75	0.69	0.73	0.02

PI = Post-Infection; SEM = Standard error of the mean

Table 4.4: Number of phagocytosed erythrocytes/20 mononuclear cells of the preliminary experimental goats at different days post-infection

DAYS	**INFECTED GOATS NUMBERS**						**CONTROL GOATS NUMBERS**					
PI	**1**	**2**	**3**	**4**	**Mean**	**SEM**	**5**	**6**	**7**	**8**	**Mean**	**SEM**
0	3	2	0	1	2	0.6	0	3	1	3	2	0.7
5	16	10	8	6	10	2	1	1	1	1	1	0
12	20	19	14	18	18	1	0	2	0	0	0.5	0.5
20	35	29	22	20	27	3	3	2	0	2	2	.06
28	30	20	27	24	19	2	1	0	0	1	0.5	0.3
35	33	43	21	20	29	5	0	0	2	1	0.7	0.5
45	20	30	15	18	21	3	0	1	0	0	0.2	0.3

PI = Post-Infection; SEM = Standard error of the mean

Table 4.5: Isotope assay values of the experimental goats at 37 days post-infection

INFECTED GOATS NUMBERS						CONTROL GOATS NUMBERS				
Radioactivity Value	**2**	**4**	**5**	**Mean**	**SEM**	**6**	**8**	**9**	**Mean**	**SEM**
Pellet	13045	17021	18331	16132	614	11017	8931	9248	9732	1869
Supernatant	1105	1885	1432	1474	226	1736	1386	1279	1467	138
Spontaneous	1280	1940	1586	1602	191	1798	1440	1303	1514	148
Tot Input mix	39202	54167	52051	48474	4676	56278	49702	43145	49708	3791
Tot input RBC	39680	48174	49444	45766	3065	59554	50917	52993	54488	2603
Incorporation (%)	34	36.8	38.3	36.4	2	19.1	18.1	17.9	18.4	1
Cr-Release (%)	-	-	-	-	-	-	-	-	-	-

Table 4.6: Isotope assay values of the experimental goats at 44 days post-infection

INFECTED GOATS NUMBERS						CONTROL GOATS NUMBERS				
Radioactivity Value	**2**	**4**	**5**	**Mean**	**SEM**	**6**	**8**	**9**	**Mean**	**SEM**
Pellet	6879	7768	6531	7059	368	2976	5037	5784	4599	840
Supernatant	927	1260	1399	1195	140	652	732	479	621	75
Spontaneous	297	437	359	364	41	285	260	338	294	23
Tot Input mix	23099	24077	26873	24683	1131	19256	25444	25422	23374	2059
Tot input RBC	24192	2377	25800	24591	7554	20798	25081	26905	24261	1810
Incorporation (%)	28.8	33.3	26	29.4	2	14	20.3	21.8	18.7	2
Cr-Release (%)	2.6	3.5	3.3	3.1	0.2	1.8	1.9	0.5	1.4	0.4

Table 4.7: Isotope assay values of the experimental goats at 51 days post-infection

INFECTED GOATS NUMBERS						CONTROL GOATS NUMBERS				
Radioactivity Value	**2**	**4**	**5**	**Mean**	**SEM**	**6**	**8**	**9**	**Mean**	**SEM**
Pellet	14170	13985	14574	14243	174	11954	12630	10426	11670	652
Supernatant	720	770	557	682	64	273	866	469	536	174
Spontaneous	1125	887	828	947	91	386	919	703	669	155
Tot Input mix	60565	59219	61125	60303	566	61486	58856	59030	59791	849
Tot input RBC	60604	55339	63549	59831	2401	63400	64397	58954	62250	1673
Incorporation (%)	23.8	24.0	24.2	24.0	0.1	19.6	21.8	17.9	19.8	1
Cr-Release (%)	-	-	-	-	-	-	-	-	-	-

Tot Input mix = Total Input Radioactivity value from the whole mixture content of RBCs and monocytes.
Tot Input RBC = Total Input Radiactivity value from the whole content of RBCs only.
SEM = Standard error of the mean

Table 4.8: Isotope assay values of the experimental goats at 58 days post-infection

INFECTED GOATS NUMBERS						CONTROL GOATS NUMBERS				
Radioactivity Value	**2**	**4**	**5**	**Mean**	**SEM**	**6**	**8**	**9**	**Mean**	**SEM**
Pellet	6816	7098	6143	6686	283	6077	5656	6730	6154	312
Supernatant	2315	1115	1096	1509	403	668	625	399	564	83
Spontaneous	1012	506	1029	846	172	562	614	242	473	116
Tot Input mix	37769	33992	37035	36265	1156	36283	36610	38652	37182	741
Tot input RBC	37143	34996	37012	36384	695	33152	34509	39456	35706	1916
Incorporation (%)	18.5	21.2	17.1	18.9	1	17.0	15.7	17.5	16.7	0.5
Cr-Release (%)	3.6	1.8	0.2	1.9	1	0.3	0.0	0.4	0.2	01

Table 4.9: Isotope assay values of the experimental goats at 65 days post-infection

INFECTED GOATS NUMBERS						CONTROL GOATS NUMBERS				
Radioactivity Value	**2**	**4**	**5**	**Mean**	**SEM**	**6**	**8**	**9**	**Mean**	**SEM**
Pellet	5919	5722	4787	5476	349	4696	4661	4991	4783	105
Supernatant	422	629	438	496	66	518	367	356	414	52
Spontaneous	580	572	438	530	46	705	890	588	728	88
Tot Input mix	32255	30011	27404	29890	1402	29836	30335	29644	29938	206
Tot input RBC	31006	30773	28374	30051	841	31604	28234	30154	29997	976
Incorporation (%)	18.7	19.4	17.7	18.6	0.5	16.1	15.8	17.2	16.4	0.4
Cr-Release (%)	-	-	-	-	-	-	-	-	-	-

Tot Input mix = Total Input Radioactivity value from the whole mixture content of RBCs and monocytes.
Tot Input RBC = Total Input Radiactivity value from the whole content of RBCs only.
SEM = Standard error of the mean

Plate 1: Experimental goats under confinement in a fly-proof pen.

Plate 2: Collection of blood samples for haematology and serum proteins determinat from an experimental goat under restraint.

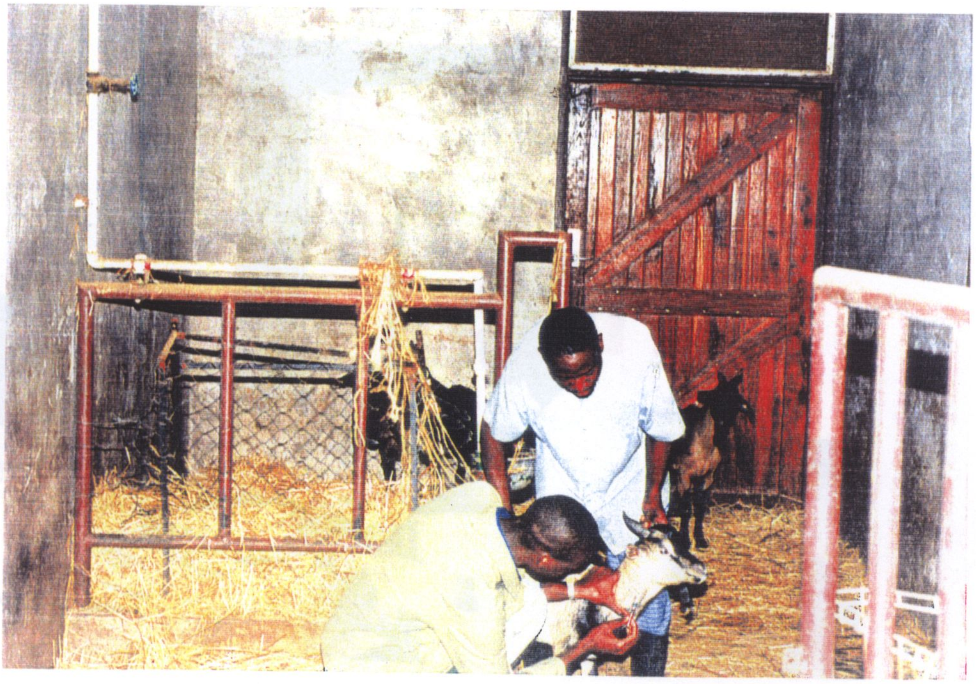


Plate 3: Haematology equipment used in the study. Note: (a) Electronic cell counter; (b) Haematocrit centrifuge.

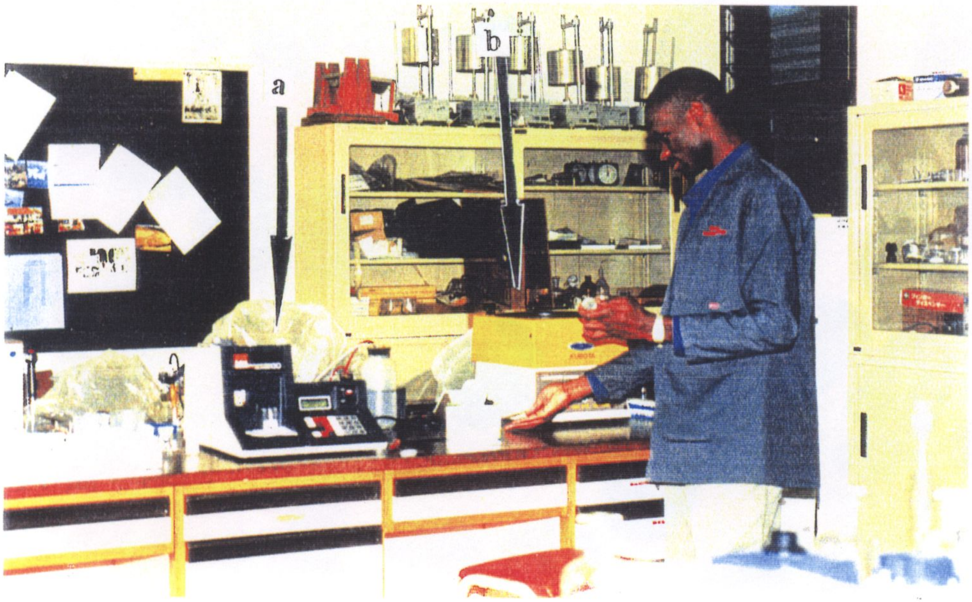


Plate 4: Reading radioactivity using a Gamma Scintillation Counter machine (a), connected to a counterpoint system-9 software (b).

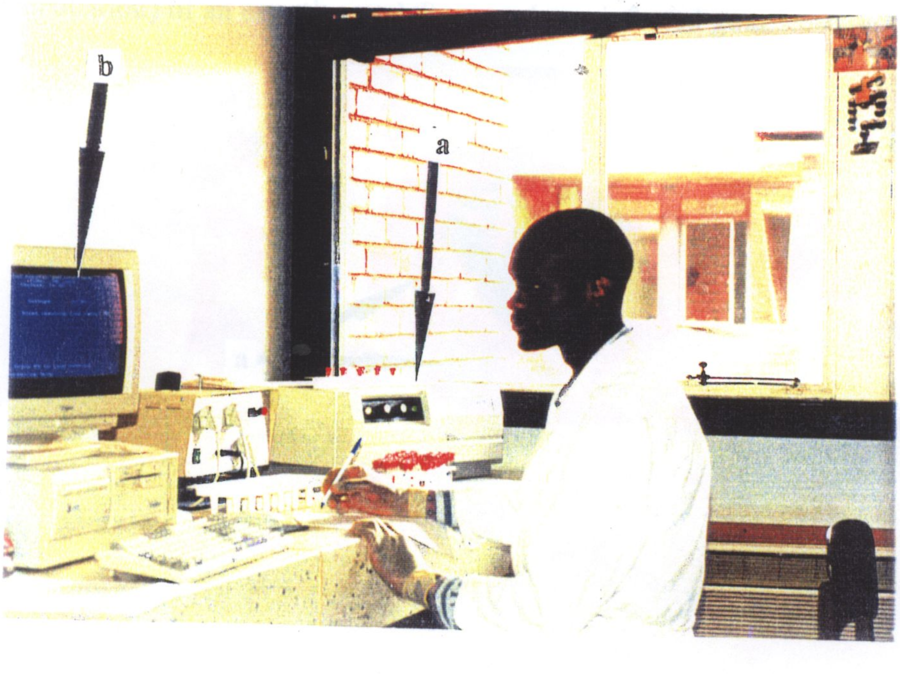


Plate 5: Three trypanosomes (*Trypanosoma congolense* IL3000) on a Giemsa-stained t
blood smear of a mouse used for passaging the trypanosomes.

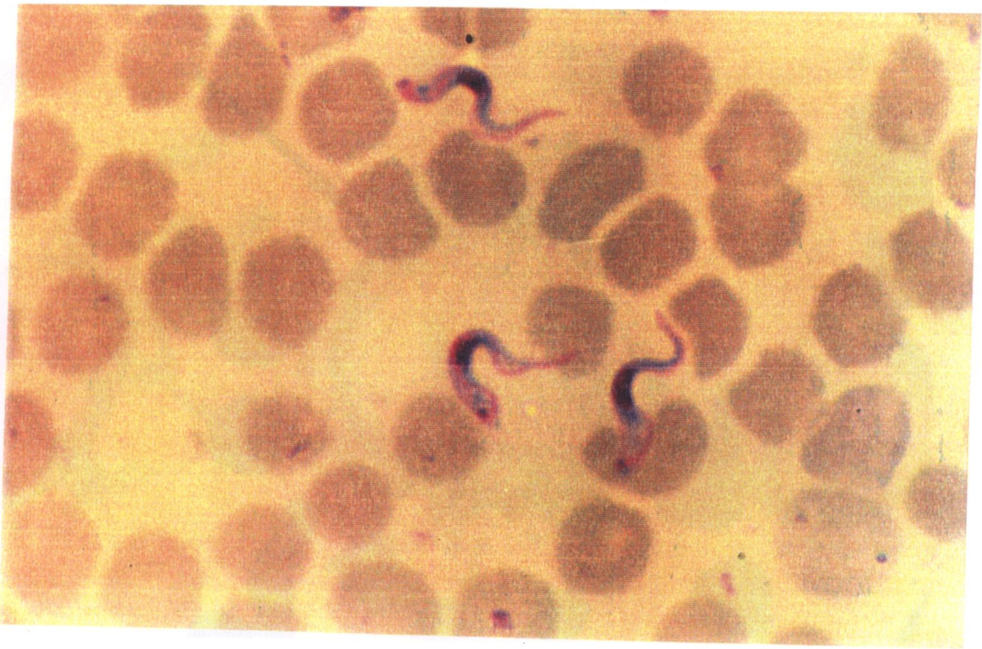


Plate 6: A monocyte with phagocytosed erythrocytes (a) in its cytoplasm. The Giemsa-stained smear was made from the cell sediment of a mixture of MNCs and RBCs (after 3 hours incubation) of an experimental *T. congolense* infected goat.

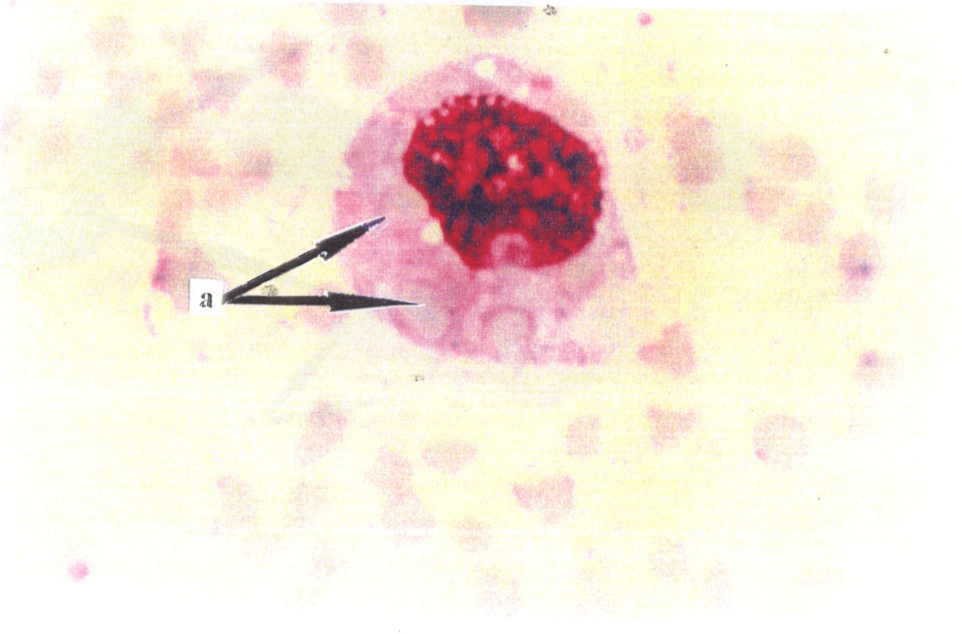


Plate 7: A Giemsa-stained liver impression smear of an experimental control goat showing a monocyte (a). Note: no phagocytosed erythrocytes are present in the cytoplasm.

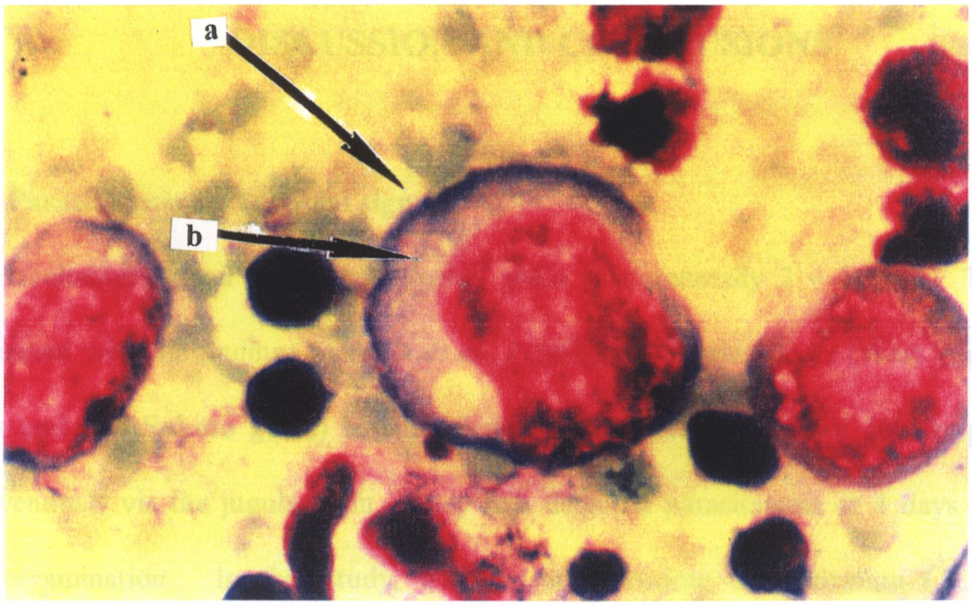
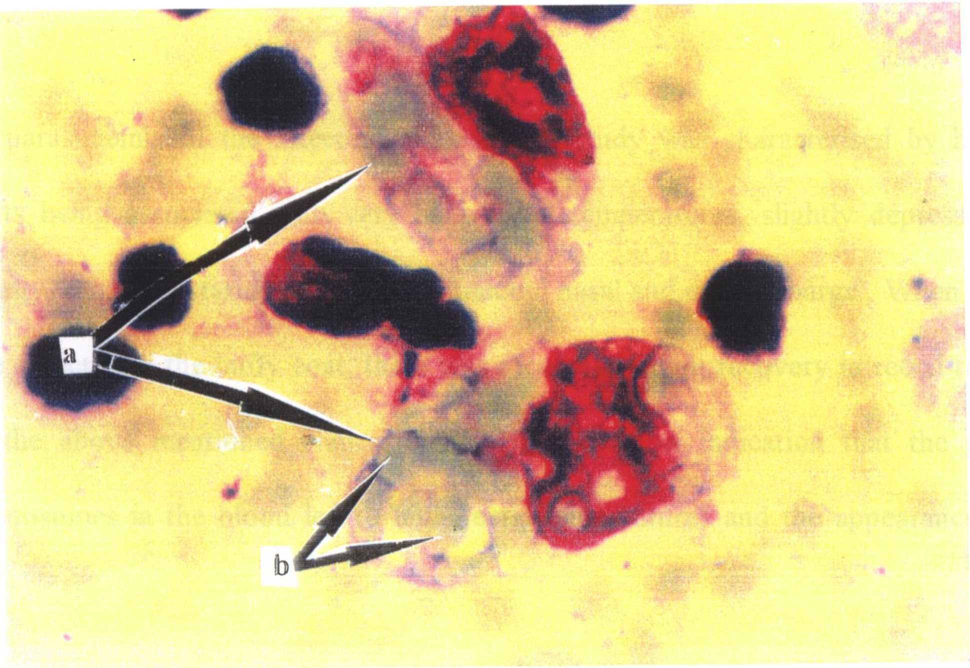


Plate 8: A Giemsa-stained liver impression smear of a *T. congolense* infected experimental goat showing macrophages (a) containing phagocytosed erythrocytes (b) in their cytoplasm.



CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

4.0. Parasitaemia and clinical signs

The incubation period in all the infected goats was generally 3 - 4 days. This was in agreement with the findings of Adah *et al.*, (1993), in Nigerian goats (West African Dwarf goats and Red Sokoto goats) inoculated with *T. congolense* (1 ml x $10^{7.8}$ trypanosomes) intravenously via the jugular vein. They first detected parasitaemia at 4 days PI by buffy coat examination. In this study, a lower trypanosome concentration (3 ml x 10^{6} trypanosomes) than that used by Adah *et al.*, (1993), was used and the prepatent period found was similar. The ability of a host animal to delay development and/or limit parasitaemia is a good determinant of its ability to control the development of clinical trypanosomosis (Adah *et al.*, 1993).

The parasitaemia of the infected goats in this study was characterised by high parasite counts being associated with very high rectal temperatures, slightly depressed appetite, lethargy, enhanced staring coat and seromucoid nasal and eye discharge. When no parasites were detected in the buffy coat, there was a corresponding recovery in rectal temperatures and the above mentioned clinical signs. This was an indication that the presence of trypanosomes in the blood led to high rectal temperatures and the appearance of clinical signs.

The high parasite counts observed were in conformity with the findings of Masake, (1980), and Ugochukwu, (1983), who observed that goats infected with *T. congolense* and *T. vivax* were in some cases very susceptible with very high parasitaemias. The association of rectal temperature peaks with high parasitaemia of infected goats in this study was contrary to the reports of Kaaya *et al.*, (1977), who observed rectal temperature peaks not being necessarily associated with high parasitaemia of goats with *T. congolense* infection.

The parasitaemia showed wavy patterns of presence and absence of parasites in the blood in line with the observations of Vickerman and Tetley, (1978), and the Merck Veterinary Manual, (1991). With the presence of parasitaemia, the host animal's immune system builds up an immunological response directed against the antigenicity of the parasites and this leads to clearance of the parasitaemia. The antigenicity of the trypanosomes is expressed by the surface coat glycoproteins (Cross, 1978). Trypanosomes have multiple genes that code for different surface coat glycoproteins that express different antigenicity. Hence, the observed wavy patterns of parasitaemia in the infected goats demonstrated that once the immune system became responsive to the antigens being expressed by the trypanosomes, the parasitaemia was cleared.

It has been reported that the trypanosomes comprising a parasitaemic peak population are usually a mixture of antigenic types in various proportions (Lorne, 1986). The host animal's immune system is said to eliminate the major type(s) at each parasitaemic remission, leaving a few heterotypes to form the parent population of the next parasitaemic wave (Barbet *et* *al.*, 1978; Lorne, 1986). This could have been the reason of the occurrence of wavy patterns of absence and presence of trypanosomes in the infected experimental goats in this study.

Generally, the first parasitaemic wave in all the infected goats was very short, a probable indication that the host's immune response to trypanosome infection was vigorous. The terminal complete disappearance of parasitaemia in goat-3 and goat-5 which was associated with the complete disappearance of all the clinical signs of the disease was a manifestation of self-cure. However, the persistence of high parasitaemia in goat-2 and goat-4 throughout the experimental period, even with the diminishing clinical signs of trypanosomosis, correlates to the observations that small ruminants may be important reservoirs of the disease (Mahmoud and Elmalik, 1977).

Some classical clinical signs of trypanosomosis were observed as reported by earlier workers. These included visibly enlarged superficial lymph nodes, seromucoid eye and nasal discharge, pale mucous membranes, lethargy, dull staring coat and 'hunched-up' appearance as observed by Kaaya *et al.*, (1977), in East African goats with *T. congolense* infection. However, these workers also observed progressive emaciation and oedema of the face and submandibular lymph nodes which were not observed in this study. This could imply that the indigenous Zambia goats were to some extent more tolerant to the *T. congolense* strain used.

The animals continued to eat even when they were sick, but they tended to eat more of number-3 maize meal (which had the highest carbohydrate content) than any other feed offered. This complemented the observations of Katunguka *et al.*, (1992a), that sheep infected with *T. congolense* still maintained their normal good appetite for carbohydrate-based feed stuffs even when notably ill. This ability of goats to continue taking nourishment may help the goats to withstand trypanosomosis infection better than other domestic ruminants.

During the period that goats-1 and 4 were observed to be very sick with high parasitaemia, the sluggish heart beat and laboured breathing observed were an indication of a decompensated heart. These clinical signs pointed to worsening anaemia and can be used to show that in these two infected goats, the anaemia was severe. The blood transport system delivers oxygen from lungs to the body cells and also transports carbon dioxide to outside the body. Blood with insufficient oxygen-carrying capacity would, therefore, lead the body system to crave for oxygen through laboured breathing (The Merck Veterinary Manual, 1991). Insufficient PCV would result in decompensation of the heart manifested by sluggish heart beats (The Merck Veterinary Manual, 1991). Shortly before death, goat-1 was bradycardic. These observations were in line with the findings of Murray *et al.*, (1983).

The progression of infection in goat-1 was acute, terminating in death during the peak of the second parasitaemic wave. However, the rest of the infected goats survived the acute stage of the disease which later progressed as a chronic disease. This observation meant that, in the early phase of *T. congolense* infection before the immune system fully responded to the infection, the disease had very detrimental effects, even leading to death. However, in animals whose immune response was vigorous, the acute disease progressed to a chronic state which was tolerable. There was no appreciable decrease in weight of the infected animals which differed from earlier reports that trypanosomosis causes wasting in goats (Ikede, 1986; Ogunsanmi *et al.*, 1994a). This could be evidence of tolerance to *T. congolense* infection in Zambian goats.

4.1. Haematological parameters

The decrease in PCV, RBC count and Hb concentration was noted to start during the prepatent phase of the infection. This was in conformity with the observations of Barry and Emery, (1984), in goats with *T. brucei* infection and the observations of Emery *et al.*, (1980), in goats with *T. vivax* infection that anaemia starts developing during the prepatent phase. Akol and Murray, (1986), reported presence of trypanosomes in the lymphatic vessels before development of parasitaemia. These trypanosomes present in the lymphatic vessels could have been producing haemolytic factors which were then being transported to the blood stream to cause anaemia. Therefore, the speculation that trypanosomes (regardless of whatever form they occur in the host's body) produce haemolysins (Lorne, 1986) is supported by the observations in this study.

With the development of parasitaemia, the drops in PCV%, RBC counts and Hb concentration were more pronounced during parasitaemic peaks but tended to be more gradual when there was no parasitaemia during the first three parasitaemic waves. For instance, in goat-4, with the peak of the first parasitaemic wave, there was a drastic drop in PCV from 28.0 to 21.0%, with a corresponding drop in RBC count (from 12.27 to 9.87 x $10^6/\mu l$) and Hb (from 9.8 to 7.3 g/dl). These low levels of PCV, Hb and low RBC count were maintained until the appearance of the peak of the second parasitaemic wave when steep drops were observed in PCV (from 21.0% to 16.5%), RBC count (from 9.87 to 7.11 x $10^6/\mu l$) and Hb (from 7.3 to 6.0 g/dl).

This trend continued into the third parasitaemic wave. This meant that the presence of trypanosomes in the blood stream led to production of haemolytic factors in larger quantities than when there was no parasitaemia. However, during the later parasitaemic waves, the PCV, RBC count and the Hb in all the infected animals remained consistently low regardless of whether there was parasitaemia or not. This could have implied that as the disease progressed, not only the presence of parasites caused decreases in levels of PCV, RBC count and Hb, but also that the disease caused depression of erythropoietic centres. This disease pattern was also observed by Katunguka *et al.*, (1992a), in sheep with *T. congolense* infection. Therefore, the report that dyshaemopoiesis is one contributing factor to anaemia in trypanosomosis (Murray *et al.*, 1980; Lorne, 1986) was also evident in this study.

The observed appreciable recovery in the PCV, RBC count and Hb concentration in goats-3 and 5 with the prolonged absence of parasitaemia during the last one-third of the experimental period was an indication of self-cure with complete disappearance of trypanosomes from the blood stream depicting a certain degree of tolerance to *T. congolense* infection in indigenous Zambian goats.

The RBC indices (MCV, MCH and MCHC) did not show any appreciable changes compared to the control group. The MCV particularly was very stable throughout the infection though the PCV and RBC counts decreased significantly. This was an indication of normocytic anaemia.

In cases of anaemia, an increase in MCV indicates increased production of erythrocytes resulting in the presence of immature erythrocytes in circulation (macrocytosis). This situation is associated with responsive haemopoiesis (Jain, 1986). If the MCV decreases, it means that erythrocytes with a smaller cell volume than normal are more numerous in circulation than the erythrocytes with normal cell volume. This indicates microcytosis. This situation is associated with dyshaemopoiesis (Jain, 1986). On the other hand, if there is anaemia but the MCV remains normal, then the anaemia is typed as normocytic and this is attributed to non-responsive erythropoietic centres (Jain, 1986).

The fairly stable levels of MCH and MCHC throughout the experiment was evidence of normochromic anaemia. When these indices are found to be lower than normal, the erythrocytes are said to be hypochromic, and when the indices are higher than normal, the erythrocytes are hyperchromic. If the indices are within normal range, then the erythrocytes are normochromic (Jain, 1986). Therefore, the type of anaemia observed in this study was normocytic-normochromic because the MCV remained within the normal range while the MCH and MCHC showed only insignificant fluctuations throughout the experimental period. This was in agreement with the findings of Kaaya *et al.*, (1977) and Losos *et al.*, (1977), who observed normocytic-normochromic anaemia to be a common feature in cattle with *T. congolense* infection.

The findings of Ogunsanmi *et al.*, (1994a), that acute trypanosomosis in ruminants depicted normocytic-normochromic anaemia were also evidenced in this study. However, their findings that in the chronic phase of the disease, the anaemia became macrocytic were contradictory to the findings in this study. The observations of Ogunsanmi *et al.*, (1994a), suggested that in the early acute phase of trypanosomosis in ruminants, there was no stimulation of the erythropoietic centres. However, as the disease got into the chronic phase probably the erythropoietic centres became responsive leading to the enhanced production of erythrocytes. This resulted in the presence of immature erythrocytes in circulation, increasing the MCV.

The anaemia observed in this study maintained normocytic-normochromic form indicating inadequate erythropoiesis throughout the course of the disease which was in line with the observations of Naylor, (1971), that trypanosomosis in goats resulted in anaemia due to haemolysis and bone marrow inhibition. The above observed haematological changes were evidence of the development of anaemia in indigenous Zambian goats as the major consequence of the disease and this was in agreement with observations of Losos and Ikede, (1972), in domestic animals with *T. congolense* infection and the reports of Igbokwe and Anosa, (1989), in sheep with *T. vivax* infection.

The fluctuations in the total WBC counts during the infection followed a similar pattern in all the infected goats. The observed sharp decrease in the total WBC counts (by 47% in goats-1 and 4, 40% in goat-2, 63% in goat-3 and by 30% in goat-5) during the early acute phase of the disease was probable evidence of immuno-suppression by trypanosomosis. However, with the development of the chronic disease state, there was a notable increase (by 45% in goat-3, 7% in goat-4 and 46% in goat-5) in the total WBC counts getting to levels higher than those obtained at 0 day PI in infected goat-5 while goat-2 counts only rose to the starting level. The counts then declined to maintain stable levels for the rest of the experimental period.

The initial sharp leucopenia was probably brought about, as reported in highly susceptible animals with trypanosomosis (Holmes *et al.*, 1974; Scott *et al.*, 1977; Griffin *et al.*, 1980), by inhibition of the reactive B-lymphocytes from responding fully to antigen challenge and hence resulting in reduced production of lymphocytes. This idea was supported by the fact that during the same early phase of the infection, there was a correspondingly notable gradual decline in the serum total protein and the globulins concentration since B-lymphocytes are responsible for the production of antibodies which constitute serum globulins.

With the end of the second parasitaemic wave in all the infected goats, there was a sharp rise in the total WBC counts indicating the start of the responsiveness of the host's immune system to infection and this had a corresponding gradual increase in the serum levels of the total protein and globulins which meant that there was now enhanced antibody production. This complemented the observations of leucocytosis in sheep with *T. congolense* infection by Katunguka *et al.*, (1992a), and also in ewes with *T. brucei* infection (Ogunsanmi *et al.*, 1994a) but was contrary to the reports of Adah *et al.*, (1993), that leucocytosis was not evident in goats with *T. congolense*.

Leucocytosis is a common feature in trypanotolerant animals (Katunguka *et al.*, 1992a) and helps in keeping the parasitaemia low and/or clearing the parasitaemia altogether. This should explain the complete disappearance of parasitaemia and recovery of other haematological parameters (PCV, Hb and RBC counts) in goats-3 and 5. The leucocytosis observed should have been due to lymphocytosis (enhanced production of lymphocytes) as evidenced by the increase in serum globulins and total protein levels. This idea can be supported by the findings of Katunguka *et al.*, (1992a), in sheep with *T. congolense* infection that lymphocytosis was the cause of the leucocytosis observed.

4.2. Serum proteins parameters

Goat-1 which recorded a 50% drop in albumin concentration shortly before it died was an indication that a drop in albumin concentration may be used to determine the severity of the disease. The concentration of albumin in the serum is used to assess an animal's protein intake levels, degree of hydration and liver and kidney function. When the concentration is low, it is a reflection of compromised liver function (reduced albumin production), increased loss through kidneys (due to kidney pathological changes), decreased protein intake or blood dilution. Albumin has the lowest molecular weight among the major protein molecules in plasma (Martin *et al.*, 1981), therefore, it is more likely to be easily lost from plasma through the kidneys when there is kidney pathological change. Albumin is synthesized by the liver and a compromise in the functioning of the liver would lead to impaired albumin production rate. Therefore, goat-1 should have developed very acute trypanosomosis which compromised either liver and kidney functions or both leading to decreased albumin concentrations resulting in death. This has been reported to be a common finding in highly susceptible animals with trypanosomosis (Van Den Ingh *et al.*, 1976; Saror, 1980).

Moreover, on postmortem, goat-1 showed extensive accumulation of straw-coloured fluid in the thoracic and abdominal cavity which was a sign of oedema. Oedema can result from hypoproteinaemia (low plasma protein levels) which causes loss of intravascular colloid osmotic pressure and hence fluid extravasation into body cavities. Hypoproteinaemia also pointed to compromised liver function since the liver is the major organ where plasma proteins are synthesized. Albumin has vital functions which include maintenance of intravascular colloid osmotic pressure, acting as a carrier molecule for fatty acids and other substances and assisting in inactivation of toxic substances (Tabel, 1987). Hence the drastic reduction of albumin concentration in the plasma of goat-1 meant that the functions of albumin were impaired, upsetting the intravascular colloid osmotic pressure. This should have led to accumulation of the straw-coloured fluid in the thoracic and abdominal cavities.

The other 4 infected goats had stable albumin levels throughout the experimental period indicating that the disease did not induce hepatocellular or kidney damage. This showed that these goats were not highly susceptible as could be evidenced by the disease progression in these animals.

The observed significant increases in total serum protein levels of infected goats-2, 3, 4 and 5 obviously resulted from an increase in the globulin concentration during infection. This could be attributed to hypergammaglobulinaemia, which is a prominent feature of trypanosomosis in sheep and goats, primarily due to increase in IgM levels (Anosa and Isoun, 1976; Igbokwe and Mohammed, 1992; Ogunsanmi *et al.*, 1994b). The increased globulin levels during infection was in conformity with the reports of Manfield, (1978), who attributed this increase to a non-specific polyclonal stimulation of B-lymphocytes in the infected animals leading to enhanced production of antibodies (immunoglobulins). The simultaneous increase in total WBC counts and globulin levels in this study can be evidence of non-specific polyclonal stimulation of B-lymphocytes. Moreover, Katunguka *et al.*, (1992a), had attributed the leucocytosis observed in trypanosomosis to be due to lymphocytosis. Increase in globulins is an indication of good immunological response in infected animals and is important in keeping parasitaemias low and/or clearing parasitaemia resulting in self-cure. This has been found to be a common feature of trypanotolerant animals (Anosa, 1988).

Reports that increases in globulins and total serum protein levels in sheep and goats with *T. vivax* infection lead to increased blood and plasma volumes due to an osmotic effect (Anosa and Isoun, 1976) are incompatible with the findings in this study. This is because increase in blood and plasma volumes would lead to blood dilution and hence lowered albumin levels (Clarkson, 1968) which was not observed in this study, since albumin levels were stable throughout the course of the disease except for goat-1.

The observed significant decrease in A:G ratio in this study was certainly the result of increased globulins levels. This coincided with the report of Ogunsanmi *et al.*, (1994b), concerning West African Dwarf sheep with *T. brucei* infection and also with the findings of Rees and Clarkson, (1967). Decreases in A:G ratio are a good measure of the immunological response in infected animals.

4.3. Investigation of erythrophagocytosis

Preliminary erythrophagocytosis observations depicted evidence of relatively low numbers of engulfed erythrocytes before infection in both the control and infected goats. The low numbers were observed throughout the experiment in the control group, while the infected group showed a progressive increase in the numbers with progression of infection. The observed erythrophagocytosis in the control goats was basically physiological erythrophagocytosis as reported by Weiss and Klausner, (1988); a mechanism meant to get rid of senescent erythrocytes from circulation (Makumyaviri, A., personal communication). The average lifespan of a normal goat erythrocyte in circulation is about 125 days (Jain, 1986). However, erythrocyte survival time is said to be reduced in haemolytic anaemias caused either by an intracorpuscular or extracorpuscular defect or both. Goats are among domestic animals with the longest erythrocyte lifespan i.e. more than 100 days. These animals are said not to normally have reticulocytes (immature erythrocytes) in circulation (Jain, 1986). Immature erythrocytes have a shorter lifespan than normal erythrocytes. Removal of senescent erythrocytes from circulation may occur by changes in membrane permeability leading to osmotic swelling and lysis. Erythrophagocytosis of senescent and pathological erythrocytes by the mononuclear phagocyte system cells is also said to be important in removal of senescent cells from circulation (Coles, 1986). Erythrocyte fragmentation because of extrinsic damage, or intrinsic cellular abnormality due to partial phagocytosis has also been cited to an important mechanism of erythrocyte removal from circulation, (Jain, 1986).

The observed enhancement of erythrophagocytosis with progression of infection in infected goats (Plate 6) was a positive indication that erythrophagocytosis is prominent in goats with *T. congolense* infection and should contribute to the low RBC counts as reported in cattle with *T. congolense* infection (Dargie *et al.*, 1979) and with *T. vivax* infection (Kimeto, 1989). Erythrophagocytosis mainly takes place in the mononuclear phagocyte system. However, phagocytosis of whole erythrocytes has been said to occur in peripheral blood during the course of haemoparasitic diseases such as trypanosomosis in ruminants (Kimeto, 1989), eperythrozoonosis in sheep and haemobartonellosis in cats (Jain, 1986).

The results obtained in the second experiment involving use of radioisotopes (^{51}Cr), largely complemented the preliminary observations of visually counting phagocytosed erythrocytes. The fact that the starting ^{51}Cr-incorporation percentages at 37 days PI were the highest percentages observed in infected animals coincided with the observation of the highest counts of phagocytosed RBCs at about 29 days PI in the preliminary experiments and was an indication that the peak erythrophagocytosis activity occurred at about 1 month PI in infected goats. This was in conformity with the observations in cattle with *T. vivax* infection (Assoku and Gardiner, 1988) that the peak production of antibodies against self-erythrocytes was commonly detectable at about 1 month post-infection. Grosskinsky *et al.*, (1983), had earlier shown that the presence of these antibodies activated erythrophagocytosis.

The occurrence of parasitaemia and elevated levels of serum globulins concomitantly with high percentages of ^{51}Cr-incorporation portrayed an interplay between parasitaemia and increased serum globulin levels with enhanced erythrophagocytosis. Moreover, the observation of lowered RBC counts a few days after the occurrence of high ^{51}Cr-incorporation percentage meant that considerable amounts of RBCs were being removed from circulation through the process of erythrophagocytosis and this justified the involvement of erythrophagocytosis in causing anaemia. Therefore, the postulated idea that erythrophagocytosis is immunoglobulin-mediated (Weiss and Klausner, 1988) was in this study supported by the occurrence of enhanced erythrophagocytosis with corresponding elevated serum globulins levels.

During *T. evansi* infection in goats, IgM and IgG have been reported to be particularly elevated leading to overall elevated serum globulins levels (Uche *et al.*, 1993; Olaho-Mukani *et al.*, 1996). It has further been postulated that enhanced generation of IgM and IgG comes about as a result of parasitaemia in cattle with *T. vivax* infection (Assoku and Gardiner, 1988). Therefore, suggestions that IgM and IgG are involved in the production of Ag-Ab complexes which later adsorb to host erythrocytes and fix complement (Kay and Douglas, 1977; Facer *et al.*, 1992) leading to phagocytosis of the modified erythrocytes by mononuclear cells can also be deduced in this study.

The complete disappearance of parasitaemia in two of the infected goats coincided with appreciable recovery in RBC counts and with very low ^{51}Cr-incorporation percentages (almost equal to control values) even though serum globulins were still notably high. This could imply that the presence of parasitaemia was necessary for enhanced erythrophagocytosis. The trypanosomes in this case were a source of antigens which were used by immunoglobulins to form Ag-Ab complexes which then coated host erythrocytes and fixed complement. The modified erythrocytes should have then been phagocytosed by activated mononuclear cells. The fixing of complement on erythrocytes coated with Ag-Ab complexes has been said to cause activation of the cells of the mononuclear phagocyte system (Grosskinsky, *et al.*, 1983). The activity of activated mononuclear cells has been reported to be directed towards phagocytosing the modified erythrocytes (Jenkins, *et al.*, 1980).

The fact that there was no difference between the total input radioactivity obtained from a mixture of labelled RBCs plus MNCs and the total input radioactivity obtained from labelled RBCs only goes to show that the addition of MNCs did not compromise the emission of radiation. It was thought that the addition of MNCs to the ^{51}Cr-labelled erythrocyte suspension would have resulted in a reduction in the amount of radiation emitted from the labelled erythrocytes. If this had happened, then it would have impaired the detection of pellet radioactivity (radioactivity from phagocytosed erythrocytes).

The observed negligible values of spontaneous radioactivity meant that there was no significant amount of ^{51}Cr which was being liberated and which would otherwise have labelled the MNCs. When labelling RBCs the ^{51}Cr was in the anionic hexavalent chromate form. It penetrated the RBCs and was reduced to cationic trivalent chromium and became bound to the globin moiety and the haemoglobin. The unbound ^{51}Cr was washed off and hence there was no free ^{51}Cr which could have labelled the MNCs (University of Glasgow, 1996).

The intracellular breakdown of phagocytosed erythrocytes by MNCs should have led to the excretion of ^{51}Cr from MNCs detectable in the supernatant as ^{51}Cr-release percentage.

However, this was detectable on 2 occasions only and in negligible amounts, indicating that probably the incubation period was not long enough to allow for the intracellular digestion of engulfed erythrocytes and consequent excretion of the ^{51}Cr. Another probable reason was that perhaps a more highly sensitive scintillation counter machine was necessary to allow for the consistent detection of ^{51}Cr-release. Nevertheless, it appeared that the three hours incubation period was optimum to prevent excretion of the ^{51}Cr which otherwise would have resulted in lower final ^{51}Cr-incorporation percentage values rendering it difficult to tell whether there was a difference between control goat values and the infected goat values. Macrophages are said to destroy ingested erthrocytes through proteolytic and lipolytic enzymes. In the process, haemoglobin is degraded with release of iron, globin and bilirubin (Jain, 1986).

4.4. Post-mortem findings

The fluid accumulation observed in the pericardial cavity, thoracic cavity and the abdominal cavity of the infected goats was a sign of oedema most often associated with hypoproteinaemia. Hypoproteinaemia upsets the intravascular colloid osmotic pressure and this leads to movement of blood fluids from blood vessels into body cavities (Coles, 1986). When this happens, the functioning of the cardiovascular system is compromised i.e. decreased blood volume resulting in low blood pressure and impairment of the whole range of blood functions. However, looking at the obtained protein profiles, the albumin levels were within the acceptable normal range (except for infected goat-1 in which albumin levels decreased) while the serum total protein levels were considerably elevated. So the probable cause of the fluid accumulation (in infected goats-2 and 4) could be associated with the pathogenesis of the disease whereby *T. congolense* parasites tend to localise in the endothelium (The Merck Veterinary Manual, 1991) leading to endothelial damage of a vast number of capillaries. Consequently, this results in plasma fluid loss from the blood system into the body cavities. The endothelium is a layer of cells (endothelial cells) lining the inner side of blood vessels and is involved in maintaining the integrity of blood vessels. It can thus be postulated that not only decreases in plasma albumin levels but also blood vessel endothelial damage cause plasma fluid loss from the blood system into body cavities.

The watery consistency of the blood was a sign of anaemia because of the lowered cell:fluid ratio in the blood due to increased RBC loss. The overall pale carcass appearance of the infected goats was also a sign of anaemia. Paleness of a carcass is usually a prominent sign of blood loss either through bleeding or haemolytic factors. However, in this case, the goats were not bled at sacrifice and there was no blood loss through bleeding. Therefore, the paleness of the carcass of the infected goats should have been as a result of the haemolytic anaemia during the course of the *T. congolense* infection.

The observed slight enlargement of the spleen and liver of the infected goats was due to the activated mononuclear phagocyte system cells in these organs leading to enhanced erythrophagocytosis as evidenced from impression smears of these organs. The presence of oedema of the testicles also points at endothelial cells damage leading to blood fluid extravasation into interstitial spaces of the testicles (since the testicles are heavily vascularised). This could be a probable reason of infertility in Zambian goats with trypanosomosis.

The observation that macrophages and monocytes on impression smears of the infected goats (unlike the control goat organ smears) had plenty of phagocytosed erythrocytes was visible evidence of enhanced erythrophagocytosis in goats infected with *T. congolense.* Moreover, the fact that infected goat smears had more macrophages than the smears from the control goat could have been an indication that *T. congolense* infection led to activation of macrophage proliferation. This could also have resulted in increased numbers of macrophages and monocytes in circulation and hence occurrence of considerable erythrophagocytosis in circulation as could be evidenced from the results of erythrophagocytosis investigation by isotopic assays. Erythrophagocytosis is otherwise a very uncommon feature in healthy animals (Jain, 1986).

The observed enhanced erythrophagocytosis in the bone marrow of infected goats could be one of the factors contributing to dyshaemopoiesis. The phagocytosed RBCs have to be catabolised by the phagocytes and the catabolites excreted within the bone marrow. Some of these catabolites could have negative effects on the functioning of the bone marrow. Also the enhanced erythrophagocytosis in the spleen could have led to an overload of liver functions through increased amounts of excreted end products of breakdown of phagocytosed erythrocytes.

The lack of development of icterus throughout the infection period would be an indication that erythrophagocytosis is an important mechanism of the development of anaemia. The mononuclear phagocyte system cells (i.e. macrophages and monocytes) play a vital role in the catabolism of haemoglobin and are a major storage site of iron in the body for reutilisation (Jain, 1986). Since icterus will come about as a result of the presence of bilirubin pigments (end products of haemoglobin catabolism) in the blood, it is, therefore, logical to postulate that if much of the observed haemolytic crisis in trypanosomosis is due to erythrophagocytosis, then no icterus would be observed because the haemoglobin is trapped in the mononuclear phagocyte system. The products of haemoglobin resulting from the intracellular breakdown of phagocytosed erythrocytes by macrophages, are reutilised (Lorne, 1986). Moreover, the absence of pathological changes involving the liver in trypanosomosis in goats entails that the liver's capability to catabolise bilirubin remains intact and, therefore, there is no accumulation of bilirubin in plasma which would otherwise lead to icterus.

Erythrophagocytosis has also been observed to be a cause of anaemia in anaplasmosis and it is said to be immune-mediated. Erythrocytes containing the *Anaplasama* parasites are extracellularly phagocytosed in the spleen and the bone marrow (Jain, 1986). However, in anaplasmosis unlike in trypanosomosis, the liver undergoes pathological changes which compromises its functions. Hence catabolism of the haemoglobin products is impaired leading to accumulation of the products in plasma and hence icterus. Icterus was not, however, evident in this study and has not been reported to occur in goats with trypanosomosis (Holmes, P.H., personal communication)

4.5. Conclusions

This study showed that *T. congolense* IL3000 infection in indigenous Zambian goats leads to development of acute trypanosomosis in the early phase of the disease. However, with progress of infection the disease became chronic, though with continuing high parasitaemia. The study also depicted eventual self-cure, a classical feature of trypanotolerant animals. Therefore, indigenous Zambian goats can be said to be exhibiting higher degree of trypanotolerance.

This study has also shown that *T. congolense* infection in indigenous Zambian goats leads to development of anaemia. The onset of the anaemia is seen during the prepatent phase and is characterised by significant decreases in PCV, RBC counts and Hb concentration. However, no significant changes in the RBC indices are observed and this qualified the anaemia seen in indigenous Zambian goats with *T. congolense* infection to be typed as normocytic-normochromic anaemia. The total WBC counts are noted to fall during the early phase of the disease but later recover and fluctuate throughout the infection. It is, therefore, concluded that during *T. congolense* infection in Zambian goats, there is leucopenia in the early phase of the disease which later reverts to normal WBC counts.

Trypansome infection also leads to significant increases in the concentration of total serum proteins and serum globulins while the albumin levels are unaltered. However, goats which fail to tolerate the acute phase of the disease show significant reductions in albumin levels. The albumin:globulin ratio is found to significantly drop due to the elevated globulins levels. It would therefore be necessary to determine whether differences in haematological parameters and serum proteins occur in infections with other trypanosome species. It is, also concluded that erythrophagocytosis is an important feature of the aetiology of the anaemia associated with trypanosomosis in indigenous Zambian goats. However, further work on this aspect is required to elucidate the mechanism.

The integration of indigenous Zambian goat rearing with other livestock, particularly in tsetse-infested areas would be highly beneficial in the provision of food security because of the trypanotolerant nature of the goats. However, it should be born in mind that goats can be reservoirs of trypanosomes which can be passed onto other animals such as cattle and cause devastating effects on the productivity of these animals. Therefore, in tsetse-infested areas where no tsetse fly control methods are in use, goats can be prime livestock for peasant farmers who cannot afford the high cost of trypanosomosis therapy.

If goats are to be reared on a commercial scale in tsetse-infested areas, tsetse fly and trypanosomosis control measures should be embarked on in order to maintain optimum productivity. This is because even though indigenous Zambian goats are considered trypanotolerant, their productivity is actually decreased by trypanosomosis due to the negative effect which trypanosomosis has on their reproduction (Bealby, *et al.*, 1993).

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