STUDIES ON THE SUSCEPTIBILITY OF THE ORMA AND GALANA BORAN CATTLE TO TRYPANOSOME INFECTION

By

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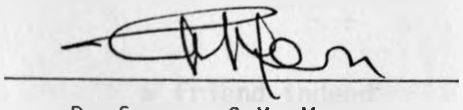
DECLARATION

(a) This Thesis is my original work and has not been presented for a degree in any other University.

Ahmed A. Ismail

(b) This Thesis has been submitted for examination with my approval as University supervisor.



Professor G.M. Mugera

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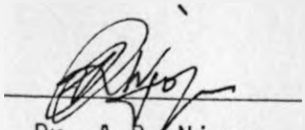

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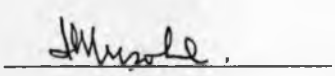

Dr. A.J. Musoke

DEDICATION

This Thesis is dedicated to the late

Mohammad AL-Mahdi Saad,

a friend indeed

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SUMMARY

STUDIES ON THE SUSCEPTIBILITY OF THE ORMA AND GALANA BORAN CATTLE TO TRYPANOSOME INFECTION

Genetic resistance to trypanosomiasis in cattle is a trait generally ascribed only to the Bos taurus breeds indigenous to West Africa, namely, the N'Dama and West African shorthorn. Differences in susceptibility to trypanosomiasis in Bos indicus breeds have not been critically evaluated. However, reports have indicated variations in their susceptibility. For instance a type of Zebu in Sudan and another around the shores of Lake Victoria are believed to have reduced susceptibility to trypanosomiasis. Field studies at a ranch in the coastal region of Kenya (Galana ranch) have suggested that the Orma Boran (Zebu) cattle from the Tana River area appear to be more resistant to trypanosomiasis than the Galana Boran.

In order to verify the latter observation, studies were conducted under laboratory conditions. Groups of Galana and Orma Boran cattle were purchased from the ranch and kept in a fly-free accommodation at Kenya Trypanosomiasis Research Institute (KETRI). The susceptibility of the two Boran types to trypanosomiasis was compared on the basis of the severity of clinical responses, intensity of parasitaemia, severity of anaemia, ability to gain weight and trypanocidal drug requirements.

Four groups of animals were used in the study. The first group (Experiment I) consisting of 12 Galana and 12 Orma steers, was inoculated intravenously with $1x10^6$ organisms of T. congolense (IL 1180). The second group (Experiment II) comprising ten Galana and ten steers of each type was fed upon by tsetse infected with the same stock of T. congolense. The third group (Experiment III) consisting of 12 Galana and ten Orma was injected with $1x10^6$ parasites of T. vivax (stock K2388). The fourth group (Experiment

IV) comprising ten steers of each type was fed upon by tsetse infected with T. vivax (stock K2589).

The results showed that while most Galana animals experienced febrile episodes, elevated heart rates and increased respiratory rates, few Orma experienced these clinical signs. Galana steers lost weight, became weak and were generally in poor condition. In contrast, the Orma steers gained weight throughout the study period. There was no significant difference between the Orma and the Galana steers in the prepatent periods or time to the first peak of parasitaemia following needle infection or fly transmission. The Orma steers, however, were able to reduce and control subsequent parasitaemia. The onset and severity of anaemia was significantly earlier and greater in the Galana as compared to the Orma. In the later stages of infection, 60% of the T. congolense infected, and 80% of the T. vivax infected, Orma animals exhibited "self-cure" - as evidenced by the absence of parasites from the peripheral blood and the steady recovery of the PCV, which was not observed in the Galana groups.

Red blood cell counts and the haemoglobin content also indicated significant difference between the two groups, with the Orma steers having higher values than the Galana steers. The Galana animals experienced a macrocytic hypochromic type of anaemia while the Orma exhibited a normocytic, with tendency to macrocytic normochromic, type of anaemia.

In both types there was a significant panleukopenia during the first 4-5 weeks of infection. The granulocytes and the lymphocytes were affected most. In T. congolense infection, the Orma generally recovered their normal WBC count values within 10-12 weeks of infection, while the Galana remained leukopenic throughout the infection period. In T. vivax infection, severe leukopenia

occurred in the first four weeks, thereafter both groups developed a leukocytosis.

Thrombocytopenia occurred between weeks three and eight of infection in both groups following T. congolense infection, while with T. vivax infection, thrombocytopenia was severe between weeks three and five of infection. The drop in platelet counts in T. vivax infection coincided with positive protamine paracoagulation tests suggesting a disturbance in the coagulation system. However, the fibrinogen values were significantly raised in the Galana as compared to Orma steers infected with T. vivax. There was no increase in the groups infected with T. congolense. In both groups, the osmotic fragility of the erythrocytes was significantly increased during this time (4-8 weeks of infection) although the Orma group was less affected. A slight rise in plasma protein concentration occurred during T. vivax infection, while a slight decrease was observed during T. congolense infection, with no significant differences between the groups.

In these studies animals which developed a PCV value of 15% were treated. Using this value as an indicator of death, a total of 60% of the Galana would have died compared to only 17% in the Orma group.

Thus, in both T. congolense and T. vivax infections initiated either through syring inoculation or tsetse bite, the Orma Boran was consistently less severely affected than the Galana Boran. It was therefore concluded that the Orma Boran, an indigenous Bos indicus breed in East Africa, possesses some degree of resistance to trypanosomiasis. The basis of the resistance was not investigated but it could be genetic.

CHAPTER 1

INTRODUCTION AND OBJECTIVE

Trypanosomes are flagellate haemoprotozoan parasites belonging to the genus Trypanosoma of the family Trypanosomatidae, order Kinetoplastida, class Zoomastigophora and phylum Protozoa (Hoare, 1970). All mammalian trypanosomes occuring in Africa except T. evansi and T. equiperdum are tsetse borne and their distribution is mainly confined to the distribution of the tsetse flies (Glossina spp). They are transmitted cyclically by tsetse and mechanically by other blood sucking flies, the most important being from the genus Tabanus.

Trypanosomes cause a disease known as trypanosomiasis in man and livestock. In man, the disease is called "sleeping sickness" and is caused by Trypanosoma (Trypanozoon) brucei rhodesiense and T.(T.) brucei gambiense. The disease can exist in an acute or a chronic form. The acute form is caused by T.(T.) brucei rhodesiense and classically occurs in East and Central Africa, while the chronic form is caused by T.(T.) brucei gambiense and is generally endemic in West Africa.

The trypanosomes that cause disease in livestock, mainly in cattle, sheep and goats, include T. vivax, T. congolense and T.b. brucei, where the disease is called "nagana". In camels the main species is T. evansi causing a disease generally referred to as "surra". In horses another species, T. equiperdum, causes "dourine" while in pigs, T. simiae and T. suis are the main species. However, T. evansi and T. equiperdum are not cyclically transmitted. In Africa, trypanosomes and their vectors are found naturally in over ten million square kilometers which lie in the most fertile and productive lands and consequently, they have severely hindered livestock production and agricultural exploitation of these areas. As a result, the disease is considered to contribute directly to

human malnutrition in Africa.

In view of these problems, major efforts have been and are being made to control the disease by the eradication of wild animals, bush clearance and the use of traps and insecticides. Recently a new approach of odour-baited targets impregnated with insecticides was introduced and has generated promising results. Vaccination against the disease has proved to be difficult at present because of the problems of antigenic variation. The use of trypanocidal drugs for treatment, or prophylaxis, is faced with the danger of drug resistance. Also no new trypanocidal drugs have been introduced in the market for the last 20 years. Owing to all these constraints an alternative approach to the control of trypanosomiasis is sought for.

THE OBJECTIVE OF THE STUDY

The cost and difficulties of vector control, the absence of a protective vaccine and the limitation of the treatment and/or chemoprophylactic strategies, have stimulated research to identify an additional approach to trypanosomiasis control to allow for efficient utilization of the vast areas occupied by tsetse flies. Considerable attention is now being given to the possibility of the exploitation of trypanotolerant breeds of domestic livestock (Bos taurus - indigenous to West Africa) in tsetse infested areas (reviewed by Murray, Morrison and Whitelaw, 1982; Murray, Trail and Grootenhuis, 1984; Murray and Trail, 1984; Roelants, 1986).

Little or no attention has been given to the possibility that trypanoresistant varieties exist amongst East African cattle. There is, however, epidemiological evidence that Zebu cattle around the shores of Lake Victoria survive and reproduce despite being continuously exposed to tsetse challenge (Cunningham, 1966). Further studies conducted by the Kenya Trypanosomiasis Research Institute (KETRI) on the Galana Ranch - at the Coast Province of Kenya - over a period of 4 years (Wilson, Njogu, Gatuta, Mgutu and

Alushula, 1981; Njogu, Dolan, Sayer, Wilson and Alushula, 1985a; Njogu, Dolan, Wilson and Sayer, 1985b; Dolan, Njogu, Sayer, Wilson and Sayer, 1985) have also suggested that the Orma Boran cattle were more resistant to trypanosomiasis than the Galana Boran cattle. It is not clear as yet, whether this resistance is innate or acquired.

This study was therefore initiated to investigate in detail the resistance exhibited by the Orma Boran cattle to trypanosomiasis and compare them with the Galana Boran cattle under laboratory conditions. Their resistance to trypanosomiasis was evaluated by examining (a) clinical responses, (b) the severity of anaemia, (c) the levels of parasitaemia, (d) the ability to gain weight during infection and (e) the number of trypanocidal drug requirements.

It was hoped that this study would provide evidence under cotrolled laboratory conditions whether or not, the Orma Boran is a trypanotolerant variety of cattle as suggested by field observations.

CHAPTER 2

LITERATURE REVIEW: TRYPANOSOMIASIS CONTROL

Tsetse flies occupy an area of about ten million square kilometers in the most fertile and productive lands of Africa (FAO/WHO/OIE, 1983). Since man came into contact with tsetse flies (Glossina spp) and realized that they are the main transmitters of trypanosomiasis, efforts have continued to control the vector and the disease in both man and animals.

In this chapter a review of the current methods used for the control of trypanosomiasis is presented.

Vector Distribution: There are 22 species and subspecies of the genus Glossina all of which are capable of transmitting trypanosomes (Hoare, 1970). They are adapted to a wide range of habitats, thereby contributing to the widespread nature of the disease. The tsetse fly belt lies between latitudes 15^{0}N and 21^{0}S, on the African continent and occupies potentially fertile land, which if cleared, could support an additional 120 million head of cattle (FAO/WHO/OIE, 1983).

Life Cycle: Trypanosomes are digenetic, the mammals being the final host and the tsetse, the vector host (Hoare, 1970). In the vertebrate host, the metatrypanosomes (metacyclic forms) introduced by the vector, gain access to the blood stream and multiply by binary fission. In the invertebrate host, after ingestion of the infected blood meal, the development is confined to the alimentary tract except T. vivax which develops in the proboscis. Trypanosoma congolense migrates first into the oesophagus, the pharynx and then into the proboscis. Trypanosoma brucei (all subspecies) migrates through the hypopharynx and localize in the salivary glands.

These three main species are pathogenic for domestic livestock, infecting a wide range of domestic and wild animal hosts.

In view of the serious impact of the disease in man and his

livestock, numerous control measures have been attempted. There are essentially two main approaches:

- Vector Control
- Parasite Control

2.1: VECTOR CONTROL

Glossina species have been the targets for a variety of control operations since they were first identified as the vectors for human and animal trypanosomiasis (reviewed by Molyneux, 1982). Each species has its own climatic and vegetational requirements, host preference and behavioral characteristics. Therefore an essential prerequisite for successful control is accurate data on ecology and tsetse population distribution.

Several vector control methods have been tried with varying degrees of success:-

(i) Bush clearance to change the habitat in order to deprive the tsetse of shelter and breeding sites was reported to be successful in a number of areas (reviewed by Ford, Nash and Welch, 1970). Destruction of habitat can be mechanical or by the use of chemicals that defoliate and kill the vegetation.

(ii) Elimination of game animals, and thus reducing the food source for the tsetse, has been practiced in many countries such as Zimbabwe, Zambia, Mozambique, Botswana and Uganda (reviewed by Ford, 1970). From 1946 to 1966, G.m. submorsitans and G. pallidipes were eliminated from more than 20,000 square kilometers following the massive hunting campaigns in Uganda (Wooff, 1968). However, with the increasing world concern for wildlife preservation, the method is no longer advocated.

(iii) Perhaps the early observation that tsetse flies are attracted to man led to the attempts at using hand catching as a method for tsetse control. Direct hand catching was first tried on a larger scale in 1913 against G. palpalis in the Portuguese island

Principe, and against G. fuscipes on Riamigasire island on Lake Victoria (reviewed by Glasgow and Potts, 1970). However, application of this method to the savannah species such as G. pallidipes, was found very difficult because the tsetse did not confine themselves to linear habitats but to a wide range of extensive woodlands (reviewed by Vale, Bursell and Hargrove, 1985). The females of this species are not attracted to humans, thus precluding an efficient hand catching operation (Vale, 1974; Jordan, 1986).

(iv) The successful use of traps was first reported by Harris in Zululand in 1938 where he eradicated G. pallidipes (cited by Ford, 1970). The results were so encouraging that traps were introduced in Uganda and Tanzania. In early 1970s, Challier and Laveissiere developed the biconical trap, designed to offer the flies an attractive combination of blue, white and black surfaces. This trap has been used in many parts of Africa and has proved effective for a wide range of species (reviewed by Allsopp, Hall and Jones, 1985; Vale et al., 1985c; Jordan, 1986). The efficiency of the traps has been increased by treating their surfaces with insecticides so that flies can be killed even if they did not enter the trap (Vale and Hargrove, 1979).

(v) The use of screens impregnated with insecticides and baited with attractants has of late been introduced with varying degrees of success (Vale and Hall, 1985a&b; reviewed by Jordan, 1986). Biconical traps impregnated with insecticides have been successful in Nigeria and in Upper Volta in suppressing the fly population, before the release of sterile males (see below) (reviewed by Allsopp, 1984; FAO/IAEA, 1983). Vale and his colleagues (1985a&b), using odour-baited, insecticide impregnated targets completely eradicated G.m. morsitans and G. pallidipes from an island of 4.5 km^2 in Lake Kariba. Following this successful eradication, target screens were deployed over 600 km^2 of woodland known as the Rifa

Triangle on the Zimbabwe side of the Zambezi river (Vale, Flint and Hall, 1986). G.m. morsitans and G. pallidipes populations declined to apparent extinction within one year.

(vi) Insecticide application, whether ground or aerial, is the most effective method currently in use and is widely used in many national tsetse control programmes. Aerial or ground spraying of insecticides has been the subject of extensive reviews (Burnett, 1970; Molyneux, 1982; Allsopp, 1984; Allsopp et al., 1985). For ground spraying, the insecticide the maximum pupation period, to act on the newly hatched flies (reviewed by Burnett, 1970). Ground spraying is effective, is of relatively low cost compared to aerial spraying, and results in less environmental contamination. However, the method requires thorough knowledge of tsetse ecology in the area to be sprayed. Molyneux (1982) reported satisfactory results with this method in Northern Nigeria where approximately 125,000 km^2 had been cleared.

Aerial spraying is applied when large areas need to be cleared or when conditions are unsuitable for ground spraying. It was first tried in Tanzania in 1948 and then in South Africa where it eliminated G. pallidipes from Zululand region at a very high cost (Burnett, 1970; Allsopp et al., 1985). This method uses non-residual insecticides and thus treatment has to be repeated at precise intervals until all the pupae have emerged. The results from various operations using non-persistent insecticides have indicated that total elimination of tsetse flies is possible provided that the treated area is suitably isolated from other contaminated regions (Molyneux, Baldry, Raadt and Haman, 1978). In Zambia, aerial spraying has led to the eradication of G. morsitans from 15,000 square kilometers at lower cost than ground spraying (Molyneux, 1982). However, the method has several disadvantages. Firstly, it requires total coverage of the tsetse infested zone. Secondly, it leads to more environmental pollution partly because of

the fine insecticide droplets which may be carried outside the target area. Finally it requires several applications (Molyneux, 1982; Jordan, 1986).

(vii) Biological control of tsetse flies is still in an experimental stage. One method has been identified, namely the use of organisms that are predatory or pathogenic to tsetse flies (reviewed by Nash, 1970). This method has not been shown to cause any environmental pollution. In a preliminary study, the release of large numbers of predators or parasitic insects such as Syntomosphyrum glossinae, caused no noticeable reduction in the tsetse population (FAO/IAEA, 1983). Pathogenic organisms such as fungi, protozoa and bacteria, have been identified, but their effectiveness on tsetse population reduction has yet to be evaluated (FAO/IAEA, 1983). Both fly predators and pathogenic organisms are now receiving close attention in several research laboratories (Jordan, 1986).

(viii) Genetic control of tsetse flies through the use of sterile males, involves inducing dominant lethal mutations in the male gametes, thus rendering the male sterile (reviewed by Dame, 1981). After sterilization, the males retain their inseminating ability and are able to compete with their wild counterparts. The method requires large numbers of competing sterile males, which is now possible through the recent improvements in laboratory breeding techniques (Vale et al., 1985c). Before application, the population density of the target species should be reduced as the method is only effective against low density populations, thus requiring integration with other control methods. The integrated approach of biconical traps, insecticide-impregnated screens and the sterile male technique, eradicated G.p. palpalis from an area of 1,500 square kilometers in Central Nigeria (Takken, Oladunmade, Dengwat, Feldmann, Onah, Tenabe and Hamann, 1986).

Vector control approach has contributed substantially over the

past years to reducing the incidence of trypanosomiasis and can be expected to contribute more in controlling trypanosomiasis in tropical Africa, if inter-African collaboration is possible.

2.2: PARASITE CONTROL

Efforts aimed at controlling the parasite are:

1) Vaccination.
2) Chemoprophylaxis and chemotherapy.

2.2.1: VACCINATION

The ideal method for the control of trypanosomiasis would have been by vaccination. But all efforts directed towards vaccine production have been unsuccessful because of the ability of the parasite to undergo antigenic variation. Antigenic variation is the process by which the trypanosomes vary their specific surface coat and thereby evade destruction by the host's immune response. Other members of the order Kinetoplastida (e.g. Trypanosoma cruzi, Leishmania species) escape the host's immune defense by "hiding" in the host's cells.

The biology of antigenic variation has been reviewed in depth by several authors (Gray and Luckins, 1976; Doyle, 1977; Cross, 1977 and Vickerman, 1978). During infection, whether initiated by cyclical or mechanical transmission, antibodies capable of lysing (Crowe, Barry, Luckins, Ross and Vickerman, 1983) or agglutinating (Gray, 1965a&b, 1966) the trypanosome are detectable in the serum. These antibodies destroy those trypanosome populations bearing the surface coat to which antibodies were induced. However, prior to the appearance of these antibodies, a small number of trypanosomes, about one in every 10^4 - 10^5 dividing organisms (Miller, Allan and Turner, 1984), have already varied the antigen type of their surface coat and thus will survive antibody mediated killing and continue multiplying. As early as 1916, Ritz (cited by Turner, 1982) demonstrated that infections initiated with a single trypanosome can

produce multiple variant antigen types (VATs), thus establishing that antigen variation is a property of an individual parasite rather than a population.

Later, in a series of studies, Gray (1962, 1965a&b, 1966) observed that certain VATs always tended to appear early in the course of infection and there seemed to be an ordered sequence of appearance of these variants (Gray 1965a). This phenomenon was observed following syringe passage or fly transmission (Gray, 1965a; Van Meirvenne, Janssen, Magnus, Lumsdan and Herbert, 1975). Gray (1966) termed these regularly occurring variants as "predominant". Interruption of the variant sequence at any point, or infection of new animals is thought to lead to "resetting" the whole sequence once again.

At the molecular level, the phenomenon of antigenic variation has been extensively reviewed (Turner, 1982; Englund, Hajduk and Marini, 1982; Borst, 1983; Turner, 1984; Boothroyd, 1985, Borst, 1986; Steinert and Pays, 1986). Early studies showed that the surface coat of a trypanosome is formed of a single glycoprotein that covers the entire body and the flagellum (Cross, 1975). This is known as the variable surface glycoprotein of the trypanosome (VSG) because it is this molecule which is changed. The coat is electron dense, about 12-15 nm thick (Vickerman, 1969), and represents about 7-10% of the total cell protein.

Each VSG molecule is attached to the plasma membrane of the cell via the C-terminal end, while exposing the N-terminal end at the surface. VSGs from different VATs of T. brucei have molecular weights ranging between 55-65 kD, but differ radically in isoelectric points, amino acid composition, amino acid sequence at the N-terminal region, carbohydrate content (7-17%, w/w) and in the position where the carbohydrate moiety is attached (Cross, 1975; Cross and Johnson, 1976; Johnson and Cross 1979; reviewed by Holder, 1985).

The understanding of antigenic variation at the gene level was made possible in recent years by the use of complementary DNA (cDNA) as a probe in hybridization experiments. Using this technique it has been revealed that the genes for all the VSGs in the VAT repertoire are present within the trypanosome chromosomes but only one gene is expressed at a time. Thus antigenic variation involves the switching off of one gene and the expression of another (reviewed by Englund et al., 1982; Boothroyd, 1985 and Borst, 1986).

This switching from one VSG to another occurs at a low frequency - approximately 10^{-6} - during chronic infection in laboratory animals (Lamont, Tucker and Cross, 1986). It is known that antibody does not induce the switching mechanism since antigenic variation occurs during the early stages of infection before any immune responses have developed (Van Meirvenne et al., 1975); it also occurs in immunosuppressed animals (Hajduk and Vickerman, 1981); and in vitro (Doyle, Hirumi, Hirumi, Lupton and Cross, 1980).

The exact extent of the antigenic repertoire is as yet undetermined, but serological evidence has shown that more than one hundred serologically distinct VATs can be expressed by a cloned population of T. equiperdum (Capbern, Giroud, Baltz and Mattern, 1977), while analysis of the VSG genes in T. brucei have shown that there may be greater than 1000 VSG genes within the genome of a trypanosome (Van der Ploeg, Valerio, De Lange, Bernards, Borst and Grosveld, 1982). Antigenic variation has thus frustrated all efforts for direct vaccination against trypanosomiasis as an approach to control.

The prospects of trypanosomal antigens which have been studied, or are still under study, with regard to their protective potentials, are of interest (Barry, 1986; Nantulya, 1986). When T. brucei bloodstream parasites are ingested by a tsetse fly, they undergo a series of changes and lose their surface coat, developing

into procyclic forms in the midgut. The coat reappears again during maturation of the parasite into metacyclic forms, the mammalian infective forms. Thus two types of antigens are exposed: the common antigens in the procyclics and the VSG in the metacyclics. Both types of antigens are considered possible candidates for vaccination. Mauldin, Turner, Dukes and Miller, (1984) fed different groups of G. morsitans infected with different stocks of T. congolense on rabbit serum immunized against the procyclics of one of these stocks. Fly dissection revealed a reduction in trypanosome maturation and infection rates. It was therefore suggested that vaccination against procyclic antigens lowered the infection rate. Similar studies were carried out by Murray, Hirumi and Moloo, (1985). G.m. centralis flies, fed on goats infected with T. congolense, T. vivax or T.b. brucei, then maintained on goats that had been immunized with freeze-dried, in vitro propagated procyclics were shown to have reduced infection rates. However, the implementation of such approach under field conditions would be difficult.

The observation of the regularly recurring (or predominant) VATs, has stimulated research for a vaccine based on metacyclic antigens because it will eliminate the trypanosomes introduced by the vector and consequently prevent infection and subsequent antigenic variation (Nantulya, Doyle and Jenni, 1980; Emery, Akol, Murray, Morrison and Moloo, 1980; Esser, Schoenbechler, Gingrich and Diggs, 1981; Crowe et al., 1983). Studies in mice (Nantulya et al., 1980) and in cattle and goats (Emery et al., 1980) have shown that immunization with metacyclic antigens by infection followed by treatment, lead to complete protection against homologous but not heterologous fly transmitted challenge in T. congolense or T. brucei infections. The early investigations on the metacyclic populations used polyspecific antisera (Gray 1962, 1965a&b, 1966; Jenni, 1977) which seemed to demonstrate a single antigen type and suggested

predictable metacyclic population patterns. However, the development of monospecific antisera and monoclonal antibodies, and the application of these antisera directly to the metacyclics extruded by the fly, revealed that metacyclics are heterogeneous populations with regard to antigenic type (Miller and Turner, 1981; Hajduk, Cameron, Barry and Vickerman, 1981; Hajduk and Vickerman, 1981). In one study, Crowe et al., (1983) using monoclonal antibodies, were able to demonstrate 12 VATs in metacyclic populations of T. congolense, derived either in vitro, or from the fly. A recent study suggested that there may be more than 16 metacyclic VATs in one stock of T.b. rhodesiense (Esser and Schoenbechler, 1985). Furthermore, the overall composition of the metacyclic VATs of a clone is not stable. Barry, Crowe and Vickerman, (1983) found that the repertoire of a known T.b. rhodesiense had changed over a period of time.

Consequently, there are several problems in an approach to immunization using the metacyclic antigens. The heterogeneity of metacyclic VATs for each serodeme, the numbers of serodemes present in each species of trypanosome and the reported instability of the metacyclic VATs over a period of time, all contribute to the difficulties in implementing vaccination strategies using metacyclic antigens under field situations.

Current biochemical studies of the VSG are aimed at preventing or altering antigenic variation by perhaps interfering with the VSG gene expression (Majiwa, personal communication). The biosynthesis of compounds which either prevent the synthesis and thus the expression of VSG or displace the VSG from the plasma membrane, thereby exposing the trypanosome common antigens, or interfere with the mechanisms of antigenic variation, are anticipated (Turner, 1984; Borst, 1986). However, the changes in the trypanosome genome that are responsible for the appearance of a particular VSG are not yet fully understood.

Other non-variant molecules reported to be located on the surface of the living T. brucei have also been investigated (Stanley, Honigberg and Cunningham, 1978; Beat, Stanley, Choromanski, MacDonald and Honigberg, 1984; Burgess and Jerrells, 1985). Using immunofluorescence, Stanley et al., (1978) showed that there were certain molecular determinants shared by the bloodstream forms of T. brucei irrespective of their VATs. Beat et al., (1984) confirmed that these non-variant molecules were limited only to the bloodstream forms, and have a low molecular weight (22 kD). They appeared not to be associated with the VSG molecule and were destroyed by trypsin digestion, suggesting that they are protein in nature. Binding studies employing live trypanosomes suggested that these molecules were accessible to antibody (Burgess and Jerrells, 1985). Thus it is possible that these molecules are immunogenic, accessible to antibody on the trypanosome surface and also may be present as circulating invariant antigens in trypanosomiasis patients, since antisera raised against these determinants reacted with sera from trypanosomiasis patients but not with sera from malaria or leishmaniasis patients (Burgess and Jerrells, 1985).

However, further investigations of these molecules are needed. This could include methods of isolation, better characterization and possible ways of presentation to the host's immune system.

In conclusion, the parasite has evolved sophisticated mechanisms of evading the host's immune destruction and to date it has resisted any immunological manipulations that may lead to control.

2.2.2: CHEMOTHERAPY AND CHEMOPROPHYLAXIS

In the absence of a vaccine to protect against infection, chemotherapy and chemoprophylaxis have and will continue to play an important role in trypanosomiasis control strategies. This approach has been widely used with considerable success particularly in areas at the periphery of the tsetse fly belt. However, in high fly

challenge areas the frequency of treatment may be economically unacceptable and the emergence of drug resistant strains may cause problems (reviewed by Murray and Urquhart, 1977; Holmes, 1980; Leach and Roberts, 1981).

Treatment of infection depends on detection of the parasite. Diagnosis of trypanosomiasis is generally divided into parasitological and serological methods. Parasitological methods are those that demonstrate the presence of the parasite in the blood or tissues of the host. The reliability of these methods in the field where parasitaemia is often low and sporadic is questionable. Thus treatments based on detection of parasites will miss many cases but treatment of all animals when a certain percentage is found postive will reduce the risk.

The serodiagnostic tests can be grouped into chemical and immunological. The chemical tests are those that make use of compounds that precipitate the globulin fraction of the serum such as the mercuric chloride test and the immunological methods are those which employ antigen - antibody interactions such as agglutination, complement fixation, immunofluorescent antibody, enzyme assays etc., were more reliable but cannot confirm an active infection.

Unfortunately, there are only very few trypanocidal drugs available for treatment of animal trypanosomiasis and no new drugs have been manufactured in the last 20 years (reviewed by Williamson, 1970; Leach and Roberts, 1981; Meshnick, 1982). The drugs that are used in the treatment or prophylaxis of animal trypanosomiasis fall into the following groups:-

1. The Quinoline group which include quinapyramine; (Antrycide sulphate - I.C.I.), curative for T. evansi in camels and T. equiperdum in horses, and (Antrycide Prosalt -I.C.I.), prophylactic against T. congolense and T. vivax in cattle.
2. The Phenanthridine group which include homidium bromide

(Ethidium - Boots) or homidium chloride (Novidium - May & Baker), both curative for T. congolense and T. vivax and isometamidium chloride (Samorin - May & Baker), curative and prophylactic against T. congolense and T. vivax in cattle.

3. The Diamidine group which include diminazene aceturate (Berenil - Hoechst), curative for T. congolense and T. vivax in cattle.
4. Naphthalidine which includes suramin sodium, marketed as (Antrypol -I.C.I.) or (Naganol - Bayer), curative for T. evansi in camels and T. brucei in equines.
5. The Phenanthridine-Pyrimidine group which includes pyrithidium (Prothidium -Boots), prophylactic for T. congolense and T. vivax in cattle.

Chemotherapy and/or chemoprophylaxis have been practiced in the field as a means of trypanosomiasis control for many years. It has been observed on many occasions that animals in the field or laboratory, exposed to an infection - treatment regime, are not only cured of infection, but also develop a substantial degree of acquired immunity (Bevan, 1928; Soltys, 1955; Whiteside, 1960,1962; Wilson, Paris and Dar, 1975a; Wilson, Le Roux, Paris, Davison and Gray, 1975b; Wilson, Paris, Luckins, Dar and Gray, 1976). Bevan (1928) was perhaps the first worker to notice this type of immunity and called it "tolerance".

An elaborate field study to investigate the development of immunity after treatment was conducted by Soltys (1955). After a prophylactic treatment period of 28 months, animals were able to survive for 18 months without apparent infection. Laboratory studies to confirm these observations (Smith, 1958) indicated that repeated dosage of Antrycide (Pro-salt) may leave drug residues in the tissues sufficient to confer prophylaxis for several months afterwards.

Field studies conducted by Whiteside (1960, 1962) greatly contributed to the understanding of parasite-host-drug interactions.

When individual animals were treated with Berenil at the development of parasitaemia, the interval required between subsequent treatments was increased. This increase in time to treatment was due to the development of immunity. In more recent studies, Wilson and his colleagues (1975a, b, 1976) conducted a series of field experiments in an attempt to find the best strategic use of trypanocides and also to investigate the development of immunity conferred by trypanocidal drug treatment. Breeding animals, treated with Berenil in an area of high tsetse challenge, did not develop immunity (Wilson et al., 1975a), but the number of abortions and the calf mortality were noticeably decreased. When groups of beef cattle were introduced into a medium challenge area under three different drug regimes (Wilson et al., 1976), the group that was treated prophylactically with samorin showed the best economic returns because it required fewer treatments and the animals had the highest growth rate and thus they considered this as evidence for the development of immunity. However, when drug cover was removed, the incidence of infection became more frequent, indicating that immunity was short lived, contrary to the findings of Soltys (1955).

Trypanocidal drug treatment or prophylaxis has contributed to the efforts aimed at controlling trypanosomiasis. However, the eventual emergence of drug resistant strains is inevitable. Resistance against the existing trypanocidal drugs can easily develop both in laboratory or field situations (Whiteside, 1960, 1962; reviewed by Leach and Roberts, 1981). In the field, the development of drug resistance can be due to by several factors, such as under-dosing due to incorrect estimation of body weight. This is likely when treatment of large numbers of animals has to be carried out in a limited time. Continued administration of trypanocides to animals kept in high fly challenge areas, irregular dosing with prophylactic drugs, or stopping treatment when the animals are still under trypanosomiasis risk will contribute to the

development of resistance.

The problem of drug resistance exhibited by various species of trypanosomes will be increased if the choice of drug is made without regard to the species present in that locality. For instance, it is known that Ethidium bromide at the normal curative dose is more effective against T. vivax than T. congolense. Thus in an area where T. congolense is the dominant species, higher numbers of relapses will occur if Ethidium bromide is used, as happened in Nigeria (Killick-Kindrick and Godfrey, 1963). Scott and Pegrum (1974) made similar observations in Ethiopia, following Ethidium bromide treatment.

The development of cross-resistance by trypanosomes to many of the drugs can be expected because of their close chemical relationships (reviewed by Williamson, 1979 and Leach and Roberts,1981). Cross resistance to Homidium (Ethidium or Novidium), Prothidium and Samorin has been noted. Berenil does not develop cross-resistance with any of the other existing trypanocidal drugs. To avoid the problem of development of drug resistance and cross resistance, two approaches have been proposed. Firstly Whiteside (1960, 1962) proposed the concept of "sanative" pairs of drugs, that is pairs of drugs that do not induce cross-resistance to each other. These drugs are alternated if resistance is developed to either of them. The best sanative pairs are: Homidium/Berenil and Samorin/Berenil. The second approach is to alternate the trypanocidal drugs available. Ford and Blaser (1971) published general observations on the productivity of cattle in a ranch in which trypanosomiasis was controlled by alternating trypanocidal drugs such as Antrycide-Berenil-Samorin-Berenil etc. This system did not develop local trypanosome resistance to any of the drugs used. Bourn and Scott (1978), following a similar regime were able to protect work oxen in Ethiopia in an area of high fly challenge without signs of drug resistance.

As chemotherapy and chemoprophylaxis continue to play a major role in approaches aimed at trypanosomiasis control, efforts are now being directed towards new compounds with trypanocidal activities. Experimental studies have shown that Samorin complexed with the polyanion dextran sulphate, extended the prophylactic activity of the drug and reduced the skin reaction (Aliu and Sanusi, 1979). In a previous study, James (1978) found that dextran complexed with either Berenil, Samorin or Prothidium, had prolonged protection periods and were markedly less toxic when given to rats or mice. However, laboratory results should be treated with caution when considering field situations. Chemicals which interfere with known trypanosome metabolic pathways have also been under investigation, such as difluoromethylornithine (DFMO) and salicyl hydroxamic acid (reviewed by Croft, 1986).

In conclusion, with careful management, good veterinary supervision and programmed use of trypanocidal drugs - adequate dosage and alternation of the available drugs - an acceptable level of trypanosomiasis control may be achieved and the development of drug resistance can be greatly minimised, at least in medium to low challenge areas.

2.3: AN ALTERNATIVE APPROACH

The genus Glossina occurs over some 11 million km^2. Its northern limit extends across the content from Senegal in the west to southern Somalia in the east (about 14^oN). The southern limit is less defined. In the south-west varies between 10^o and 20^oS corresponding closely to the northern edges of Kalahari & Namibia deserts, whereas in the south-east it is generally about 20^oS but extends as far as 29^oS along the east Africa littoral (Jordan, 1986). This is paralleled by the steady expansion in human population in Africa. There is therefore an urgent need to utilize areas that are currently infested with tsetse flies for pasture

and/or agriculture to meet the needs of the expanding population. It has been estimated that about 30% of the 147 million head of cattle are exposed to the disease (FAO/WHO/OIE, 1983). As a result of trypanosomiasis and other diseases in the tropic, africa produces 70% times less animal protein than Europe (reviewed by Allsopp et al., 1985). However, if trypanosomiasis and other animal production constraints were removed from 7 million km^2 of tsetse infested land, this will result in an additional 120 head of livestock, which would result in the production of an extra 1.5 million tonnes of meat every year (FAO/WHO/OIE 1983; Jordan, 1986), and will contribute significantly to the improvement in other forms of agriculture such as the use of draught oxen for ploughing (Finelle, 1980). As a consequence, the use of trypanotolerant breeds of domestic livestock which might live and be productive in the tsetse infested areas of Africa have been examined as an alternative approach to trypanosomiasis control.

CHAPTER 3

LITERATURE REVIEW: GENETIC RESISTANCE TO TRYPANOSOMIASIS - TRYPANOTOLERANCE

3.1: DEFINITION

David Livingstone (1849-1866), during his travels in Africa, was one of the first observers to link the distribution of trypanosomiasis to the tsetse flies and the game animals on which they feed. Since then it has been observed that game animals, although are present in tsetse endemic areas, do not succumb to the disease as easily as do domestic animals.

McCallum and Anderson (1984) postulated that the resistance of a host to parasitic infection is the product of close association between the host and the parasite over a very long period of time. Thus it is possible that due to the continuous contact between tsetse flies and wild animals over millions of years, wild animals have been gradually selected for resistance to trypanosomiasis and thus they are less susceptible to the ill-effects of the disease and consequently are able to survive and produce in tsetse endemic areas.

There is no clear definition of trypanotolerance. Pagot (cited by Murray et al., 1982) defined it as a racial aptitude (of cattle) allowing them to maintain themselves in good condition and reproduce while harbouring trypanosomes without showing clinical signs of the disease. Murray et al., (1982), based on their field and laboratory observations, defined tolerance as the ability of certain breeds of cattle, sheep and goats as well as some species of wild animals to survive and reproduce in endemic fly infested areas without the aid of chemotherapy, while other breeds cannot. However, while this seems to be the most appropriate definition, all trypanotolerant cattle can and will be affected if they are exposed to high challenge (Stephen, 1966).

3.2: THE ORIGIN OF TRYPANOTOLERANT ANIMALS

3.2.1: THE HAMITIC HUMPLESS LONGHORN

Early paintings and rock drawings of domestic animals showed that the Hamitic humpless Longhorn were the first breed of cattle to be domesticated (Epstein, 1971). Humpless longhorn cattle came to Egypt from Palestine around 5000-3700 B.C. (Epstein, 1971; Reed, 1984). From Egypt they spread along the Mediterranean coast and the Western desert to finally reach West Africa and the Iberian peninsula. From Spain they spread to the rest of Europe and South and North America (Epstein, 1971). Rock engravings in the Nile Valley and Ethiopia have also presented some evidence for the presence of these animals in Eastern Africa (Reed, 1984). Mount Elgon on the Kenya-Uganda border appears to be the southernmost point of their occurrence (Epstein, 1971). This group of longhorns probably reached Ethiopia from Arabia via the Horn of Africa because cattle with long horns were found in rock engravings in central Arabia between 3000-2000 B.C. (Epstein and Mason, 1984).

3.2.2: THE HAMITIC HUMPLESS SHORTHORN

Excavations have revealed that Africa received humpless shorthorn cattle from Asia Minor. Shorthorn cattle began to appear in drawings in Egypt around 3000 B.C. and started to gradually replace the longhorn around 2900 B.C. (Reed, 1984; Epstein and Mason, 1984). Between 1700-1580 B.C. shorthorn cattle spread to West Africa (Epstein, 1971).

3.2.3: THE HUMPED CATTLE (ZEBU)

Zebu cattle probably originated in Eastern Iran but there is no evidence for the domestication of wild humped Zebu cattle (Reed, 1984). They were probably introduced to Egypt from the coast of Somalia which they had reached by sea from the Persian Gulf around 2000 B.C. (Epstein and Mason, 1984). In Somalia and Ethiopia, Zebu

occurred alongside camels and horses before the fourth century A.D. (Reed, 1984). However, Zebu did not become established in large numbers in Africa until the Arab invasion of Africa, around 700 A.D. (Epstein, 1971).

3.2.4: THE SANGA

Typical Sanga are the results of cross-breeding between the Hamitic longhorn and Zebu. They evolved in Ethiopia or Central Eastern Africa or in both, around the first millennium B.C. (Reed, 1984). At present, in most areas, the Sanga have been replaced by Zebu (Epstein, 1971) because the Zebu type appears to be more resistant to rinderpest - which was very frequent in East Africa - and because they have a higher milk yield than the Sanga type (Epstein and Mason, 1984).

The Hamitic humpless longhorn and shorthorn are classified as ancentral Bos taurus type, while the Zebu is a Bos indicus type. From the Hamitic longhorn, the N'Dama cattle descended and from the shorthorn, the West African shorthorn descended. Both breeds are now present in West Africa. It is these two breeds that are considered as trypanotolerant. It is possible that due to the long association between these animals and tsetse flies, they have been selected for their resistance to trypanosomiasis.

3.2.5: THE ORMA BORAN CATTLE

The Orma or Galla tribe is the remnant of the Ormo people who once dominated the southern parts of Ethiopia and Somalia (Kenya Official Handbook, 1983). According to Fedders and Salvadori (1984) the name Galla means wandering - going and coming - thus indicating the nomadic life of these people. As for the Orma, these authors suggest it came from the Arabic name for the Omer people who came from Central Arabia, across the Red Sea, to settle in Northern Somalia with their animals around 2000 years B.C.

They later moved southwards with their animals and spread along

Improved Boran

Galana Boran

Orma Boran

Muturu

N'Dama

the banks of the Tana river from Garissa to Garsen over several centuries (Amin and Moll, 1980; Fedders and Salvadori, 1984).

Amin and Moll (1980) referred to the Orma cattle as playing an important part in the socio-economic life of the tribe. Meat and milk constitute the main diet (Fedders and Salvadori, 1984), and the ownership of over one thousand head of cattle is granted specific recognition denoted by wearing a goat skin bracelet and an enhanced status within the tribe.

The Orma cattle, predominantly white in colour, have longer horns and are larger in size than other Zebu. Whether this would indicate any degree of crossbreeding with the Hamitic longhorns during their evolution is not known. However, the Sanga are a crossbreed between Hamitic longhorn and Zebu and they are not noticeably trypanotolerant (Epstein, 1971). Tana River basin is known to be a tsetse endemic area but, unfortunately, the degree of challenge has not been quantified. It is certain, however, that the Orma cattle have been in contact with tsetse flies over many centuries and consequently there is a great possibility that if natural selection had occurred, it would favour resistance to trypanosomiasis.

Figure 1 shows the features of the Hamitic humpless longhorn (N'Dama), the Hamitic humpless shorthorn (Muturu), the Orma Boran, Galana Boran and the improved Kenya Boran (for meat production).

3.3: EVIDENCE FOR GENETIC RESISTANCE TO TRYPANOSOMIASIS IN CATTLE

Almost all breeds of cattle succumb to trypanosomiasis with varying clinical signs and survival times, depending on the degree of challenge and the virulence of the stock in the area.

Perhaps one of the earliest reports on the resistance of cattle to trypanosomiasis came from the observations made by Pierre in 1906 (cited by Murray *et al.*, 1982), who recorded the ability of certain West African shorthorn cattle to survive and reproduce in tsetse infested areas. Subsequent studies consistently confirmed Pierre's

observations and the resistance of these animals to trypanosomiasis has been increasingly recognized (Stewart, 1937, 1951; Chandler, 1952, 1958). Stewart (1951) described his 20 years of experience with West African shorthorn cattle in Ghana. His overall conclusion was that these animals possess a very high degree of resistance to trypanosomiasis and pointed out that although these animals are rather small for beef or work and have low milk yield, they have the basic value that they survive and reproduce in areas where Zebu and exotic breeds die of trypanosomiasis.

3.3.1: THE N'DAMA

Most studies on trypanotolerance have been carried out on N'Dama. According to Epstein (1971), N'Dama spread in West Africa from Fouta Djallon plateau in Guinea. Now it is the most common trypanotolerant breed in West Africa (Murray et al., 1984) and it is the largest in size and the most productive (ILCA, 1979). Some of the earliest experiments on the tolerance of N'Dama were carried out by Chandler (1952,1958). He confirmed their relative resistance by comparing them with Zebu cattle on weight loss, degree of anaemia and survival. In a series of studies, groups of N'Dama were exposed to natural challenge in an area of G. palpalis and G. morsitans (Chandler, 1952) or challenged under laboratory conditions with G. palpalis infected with T. vivax (Chandler, 1958, Desowitz, 1959) or syringe inoculated with T. congolense (Chandler, 1958). The results of these studies have emphasized the tolerance of the N'Dama. Unfortunately, in these experiments neither the history of the experimental animals nor the characteristics of the infecting strains were precisely known, and only small numbers of animals were involved in the studies.

As a consequence, Stephen (1966), and Roberts and Gray (1973b), have re-evaluated the resistance to trypanosomiasis of N'Dama, Muturu and Zebu which had not previously been exposed to challenge.

Animals were challenged under laboratory conditions with wild-caught G.m. submorsitans infected with T. vivax, T. congolense or T. brucei. The results based on weight loss, degree of anaemia and survival, also confirmed the superior resistance of N'Dama over the other two (Roberts and Gray, 1973b), and presented further evidence that trypanotolerance was an innate characteristic since the experimental animals had not been previously exposed to trypanosomiasis.

In more recent studies, Murray, Murray, Morrison and McIntyre, (1979a&b) conducted a series of large scale experiments in the Gambia to evaluate the extent of the tolerance of the N'Dama as compared to Zebu. At first the animals were syringe inoculated with T. brucei or T. congolense and monitored for five months. The survivors were further exposed to natural challenge for nine months. N'Dama were found to be less susceptible as judged by the levels of parasitaemia, the degrees of anaemia, productivity and mortality. Nine of the 40 Zebu died while none of the N'Dama or their calves died. The Zebu produced no live calves.

A further confirmation of the resistance of N'Dama came from the work of Murray, Clifford, Gettinby, Snow and McIntyre (1981a) and Saror, Ilemobade and Nuru (1981). Murray and his colleagues used N'Dama and Zebu cattle that had not been previously exposed to trypanosomiasis. The animals were subjected to natural fly challenge. All Zebu died of trypanosomiasis within eight months compared to only three deaths out of ten N'Dama animals. The three deaths occurred between 11-14 months after exposure. The prevalence, levels and duration of parasitaemia were significantly less in N'Dama than Zebu. There were greater differences in parasitaemia and anaemia when T. vivax was considered than with T. brucei or T. congolense infections (Murray et al., 1981a).

3.3.2: THE WEST AFRICAN SHORTHORN

The West African Shorthorn are smaller in size than N'Dama. They comprise a large number of breeds (types) in West Africa.

There have been very few studies with regard to the susceptibility of these animals as compared to N'Dama.

One of the earliest accounts of these animals was given by Stewart (1937, 1951). He examined the susceptibility of these animals to syringe inoculation with T. vivax or T. congolense (including a proven pathogenic T. congolense strain from a different geographical area - Tanzania). He found that despite becoming infected, the animals were able to control parasitaemia and did not develop severe clinical symptoms. He reported similar results when these animals were exposed to natural fly challenge in an area of G. palpalis, G. morsitans and G. longipalpis. The resistance, however, broke down when the animals were exposed to a very high challenge or if they were under stress caused by lack of nutrition or repeated bleeding. In a further series of cross breeding (with Zebu) experiments, Stewart (1951) was able to produce much larger animals than West African Shorthorns, that were still able to retain a significant degree of resistance.

The resistance of Muturu cattle to trypanosomiasis was further emphasized by Ferguson (1967). However, it was not until the studies of Roberts and Gray (1973a&b), that critical attempts were made to evaluate the extent of trypanotolerance of West African Shorthorns (Muturu) as compared to N'Dama and Zebu. The studies included animals that were born to dams previously exposed and to dams that had never been exposed to fly challenge. The resistance of West African shorthorns was found to be between N'Dama and Zebu. Similar observations were made earlier by Van Hoeve (1972).

The innate resistance of Muturu to trypanosomiasis was further emphasized by Esuruoso (1977) who used second generation animals of dams that had been reared in a tsetse-free area. They were found to be more resistant to syringe infection with T. vivax as compared to Zebu.

In a series of recent studies, Roelants, Tamboura, Sidiki,

Bassing and Pinder, (1983) and Pinder, Libean, Hirsch, Tamboura, Bauer and Roelants, (1984) used different types of West African shorthorns such as Muturu, Baoule, Simmental, Baoule x N'Dama (F1), Simmental x N'Dama (F1), pure N'Dama and Zebu. It was found that the N'Dama and its F1 generation were most resistant followed by the West African shorthorn. The Zebu were found to be the most susceptible.

3.4: EVIDENCE FOR GENETIC RESISTANCE TO TRYPANOSOMIASIS IN OTHER ANIMALS

3.4.1: WILD ANIMALS

The susceptibility of indigenous livestock to trypanosomiasis is generally considered to be intermediate between susceptible exotic breeds (imported breeds which have had no previous exposure to trypanosomiasis) and resistant wildlife (reviewed by Murray, Grootenhuis, Akol, Emery, Shapiro, Moloo, Dar, Borell and Paris, 1981b). Wild animals are in most cases considered to be refractory to trypanosome infection. This observation was based on the fact that these animals were able to survive and reproduce in areas heavily infested with tsetse flies.

Ashcroft (1959) reviewed the results of several parasitological surveys in which over 1242 wild animals were examined at various times in different locations in East Africa. The results revealed an infection rate of 19.5%. Of these, 4.2% were due to *T. brucei*, 6.3% due to *T. vivax* and 10.4% due to *T. congolense*. In these surveys it was found that the water buck had the highest incidence rate (52%) and the zebra the lowest (6%). Trypanosomes were not found in Sable antelope, rhinoceros, Thomson's gazelle or wildebeest. However, with improvement of the diagnostic methods, parasites have been demonstrated in Thomson's Gazelle and the Wildebeest (Baker, Sacks and Laufer, 1967).

Thus, while there are only very few studies on wild animals, it

would appear that they are seldom severely affected by the disease.

3.4.2: SHEEP AND GOATS

In contrast to cattle, the susceptibility of sheep and goats to trypanosomiasis has received little attention. In West and Central Africa the trypanotolerant sheep are known as Fouta Djallon sheep (reviewed by Murray et al., 1984).

In East Africa studies on sheep and goats are even more poorly documented. However, Griffin and Allonby (1979a,b) found that indigenous breeds of goats, such as Galla, and sheep, such as the Red Masai were more resistant than exotic breeds. However, recently Whitelaw, Kaaya, Moulton, Moloo and Murray, (1985) exposed different breeds of goats to fly challenge or syringe infection with T. congolense. The results showed no significant difference in all parameters investigated between the different breeds.

The epidemiology of trypanosomiasis in sheep and goats is similar to cattle: the dwarf breeds are more resistant than larger breeds. On several occasions these small ruminants have been implicated as reservoirs for human and animal trypanosomiasis. This has been emphasized by Robson and Rickman (1973) and Mahmoud and Elmalik (1977). Goats experimentally infected with T. congolense, remained infected but symptomless for more than two years (Mahmoud and Elmalik, 1977). In an earlier survey, Karib (1961) reported that many sheep and goats although apparently healthy animals, were found to be infected with T. vivax.

It has been shown that sheep and goats in East Africa are more severely affected by T. congolense than T. vivax whereas T. vivax is the predominant cause of severe trypanosomiasis in West Africa (Murray et al., 1984). Reasons for these differences are possibly due to the differences in the pathogenecity between East and West African stocks of T. vivax (reviewed by Gardiner and Wilson, 1987).

3.4.3: MICE

Many studies have been conducted in inbred strains of mice in order to study the mechanisms and inheritance of trypanotolerance (Morrison, Roelants, Mayer-Withey and Murray, 1978; Jennings, Whitelaw, Holmes and Urquhart, 1978; Morrison and Murray, 1979; Whitelaw, MacAskill, Holmes, Jennings and Urquhart 1980). It was found that different strains of mice differ markedly in their susceptibility. Comparing eight strains of mice infected with T. congolense, Morrison et al., (1978) found C57BL/6 to be the least susceptible, while A/J is the most susceptible. Strains such as C3H/HeJ were found to be intermediate. The degree of resistance seems to be related to the capacity of mice to control and reduce parasitaemia (Whitelaw, et al., 1980) and to the ability to influence the rate of parasite differentiation (reviewed by Black, Sendashonga, O'Brien, Borowy, Naessens, Webster and Murray, 1985). Morrison et al., (1978) found no difference in the prepatent periods between susceptible and resistant mice, thus indicating there was no difference in the replication rate at least in the initial phase of infection.

3.5: EVIDENCE FOR GENETIC RESISTANCE TO TRYPANOSOMIASIS IN EAST AFRICAN CATTLE

The possibility that genetic resistance to trypanosomiasis might exist in East African breeds of cattle has largely been ignored. The first indication that there may be trypanotolerant types among the East African Zebu was provided by Archibald (1927). He described a type of Zebu in Sudan that was able to survive and reproduce in an area of tsetse challenge. Later, Cunningham (1966) also pointed out that thousands of Zebu survive around the shores of Lake Victoria without the aid of trypanocidal drugs, despite being under continuous fly challenge. He reported a 30% trypanosomiasis prevalence and was able to demonstrate the presence of neutralizing antibodies in 90% of the animals examined. However, it has not been

established whether this is an innate resistance or an acquired resistance to the serodemes in that area. Variations in susceptibility to trypanosomiasis between different types of Boran cattle have also been demonstrated in Kenya following both natural and experimental challenge (reviewed by Murray and Trail, 1984) and between different Zebu types in Upper Volta following syringe infection with bloodstream forms (Pinder et al., 1984).

More recently, field studies conducted by KETRI on the Galana ranch over a period of four years have provided evidence that the Orma Boran cattle originally from South Eastern Kenya (Tana River basin), were more resistant to trypanosomiasis than Galana Boran cattle originally from the North of Kenya, (Wilson et al., 1981; Njogu et al., 1985a&b; Dolan et al., 1985). It was found that the Orma cattle have a decreased incidence rate of trypanosomiasis and longer time to parasite detection (prepatent period), thus requiring fewer trypanocidal drug treatments.

3.6: MECHANISMS OF TRYPANOTOLERANCE

There is increased evidence that resistance to trypanosomiasis depends on an inherent capacity to limit parasitaemia (reviewed by Black et al., 1985; Roelants, 1986). The mechanisms responsible for this may be operating during the initial host-parasite-vector interactions in the skin or in the circulation once the infection is established. At both sites physiological and/or immunological factors might act against the trypanosomes. Thus the possible mechanisms which may be operational in trypanotolerance are: (a) local skin reaction (b) immune response (c) immunodepression (d) capacity to stimulate pleomorphism (e) capacity to control anaemia and (f) the contribution of physiological factors.

3.6.1: Local skin reaction (chancre)

Following the bite of a trypanosome infected tsetse, a raised indurated lesion (chancre) usually develops in the skin at the site

of the bite (Emery et al., 1980). This is where trypanosomes become established and start multiplying prior to dissemination into the bloodstream. In resistant animals such as eland or buffaloes the chancre is less frequent and is smaller in size compared to susceptible cattle and goats (Dwinger, Grootenhuis, Murray, Moloo and Gettinby, 1986). However, the prepatent periods and the time to the first peak of parasitaemia were similar in susceptible and resistant animals. Thus the reasons for the development of less severe chancres in resistant animals remain to be determined.

3.6.2: The immune response in cattle

Since trypanotolerant animals were able to control the later peaks of parasitaemia, it was thought that their resistance may be due to their ability to mount a better immune response. Desowitz (1959) found that N'Dama cattle with previous trypanosomiasis exposure were able, following rechallenge, to eliminate trypanosomes more rapidly and efficiently than Zebu. This was later confirmed by Roberts and Gray (1973b) who found that the greater differences in susceptibility were only obtained after the second challenge.

Later studies (Shapiro and Murray, 1982) suggested that the ability to control T. brucei infection in experimental N'Dama and Boran cattle was correlated with the ability to recognise at least one of three common trypanosome antigens of molecular weights 110, 150 or 300 kD. All the N'Dama recognised the three antigens, while the five Zebu which died of infection, did not recognise any of these antigens. In contrast, N'Dama and Zebu syringe infected with T. brucei revealed no significant differences in antibody responses (Murray et al., 1979a&b). This was also confirmed by Pinder et al., (1984) who found no difference in antibody response in Zebu, Simmental X N'Dama (F1), Baoule or N'Dama as judged by neutralization, agglutination and complement fixation tests. In more recent experiments, however, Kamanga-Sollo and his colleagues

at ILRAD (Pers. Comm.), have shown that although there appears to be no differences in the kinetics of antibody response between N'Dama and Boran, clear differences exist in the ability of the N'Dama to clear parasites from circulation as compared to Boran cattle.

3.6.3: The immune response in mice

A marked increase in the level of IgM (4-8 fold) was observed in relatively resistant C57BL mice during the first peak of parasitaemia compared to a smaller increase in IgG_1 (2.5 fold) and IgG_2 (1.5 fold) while susceptible A/J mice did not show any increase in IgM, but had a massive increase in IgG, especially IgG_2 (ten fold) (Morrison and Murray, 1979). Later studies by MacAskill, Holmes, Whitelaw, Jennings and Urquhart, (1983) indicated that resistant mice cleared ^{75}S-methionine labelled trypanosomes faster than susceptible mice and that the major antibody involved was IgM. Recently, De Gee, Sonnenfeld and Mansfield, (1985) found that interferon appeared at two stages in resistant mice. The first coincided with the onset of parasitaemia and comprised interferon-a/ß. The second appearance coincided with the high titres of antibody and remission of parasitaemia and was predominantly interferon-g. In contrast, susceptible mice did not produce detectable interferon throughout the infection period.

3.6.4: Immunodepression

A further possible explanation for trypanotolerance is that resistant animals are less susceptible to immunodepression (reviewed by Murray et al., 1982). In laboratory animals, trypanosomes induce depression of T- and B- cell functions by a mechanism that involves T-suppresser cells and macrophages (reviewed by Roelants and Williams, 1982). Mice infected with trypanosomes showed delayed skin graft rejection (Pearson, Roelants, Lundin and Mayor-Withey, 1978). Evidence for a relationship between immunodepression and susceptibility of mice to T. brucei was reported by Selkirk and

Sacks (1980). It was found that strains of mice of different susceptibilities showed different responses to heterologous antigens, the resistant mice being able to mount an early IgM response to DNP-Ficoll, a T-independent antigen. They concluded that the primary difference between resistant and susceptible strains of mice resides in the capacity to produce a T-independent IgM in response to small amounts of immunogen early enough to control the rising parasitaemia. In contrast, the response of susceptible animals is triggered by a larger amount of immunogen and the response only appears when the parasitaemia is already overwhelming and antigenic variation is extensive.

The genes controlling differences in resistance in mice, however, have not been mapped, but they are known to be outside the H-2 complex (Pinder, Fumoux and Roelants, 1985). In T. musculi infection it has been shown that the resistance or susceptibility of mice to infection was controlled by a single, major, incompletely-dominant gene (reviewed by Roelants, 1986). Whether this is the case with other trypanosome species in cattle is not yet known.

3.6.5: Capacity to stimulate pleomorphism

Studies in mice with T. brucei have shown that the rate of differentiation of T. brucei from the rapidly dividing slender forms to non-dividing stumpy forms, together with the production of protective antibodies, is determined by the capacity of the host to stimulate parasite differentiation and to control and reduce parasitaemia (Sendashonga and Black, 1982). The rate of parasite differentiation influences the kinetics of antibody production because antibody response is stimulated by the stumpy but not the slender forms (reviewed by Black et al., 1985). It has been suggested that a similar mechanism may be operative in resistance to T. congolense and T. vivax. Such phenomena have not yet been documented in cattle.

3.6.6: Capacity to control anaemia

One of the major features of infected trypanotolerant animals is the development of less severe anaemia than that shown by infected susceptible animals. A series of erythrokinetic and ferrokinetic studies, in N'Dama and Zebu cattle infected with T. congolense or T. brucei showed that anaemia and its underlying processes are broadly reflected by the number of parasites in the blood (Dargie, Murray, Murray, Grimshaw and McIntyre, 1979 and Dargie, 1980). Thus the differences in anaemia between N'Dama and Zebu were thought to be due to their capacity to control parasitaemia and not due to differences in innate erythropoietic responses (reviewed by Dargie, 1980).

3.6.7: Physiological factors

Murray, Trail and Grootenhuis (1984) related trypanotolerance to reduced susceptibility to the effects of the infection, and thus proposed a number of physiological factors possessed by trypanotolerant breeds which aid survival. Factors such as superior ability to utilize food, to tolerate heat and to conserve water may be responsible.

However, as argued by Frisch (quoted by Murray and Trail, 1984), where food is marginal, breeds with inherently high maintenance requirements will suffer most - as demonstrated by weight loss or reduced weight gain. Tizard (1982) found that a group of Holstein animals infected with T. congolense and kept under a high protein diet and good management, showed spontaneous "self cure".

Little is known about water conservation and heat tolerance in trypanotolerant cattle. N'Dama were found not only to tolerate higher levels of humidity than Zebu, but also have a considerable range of rectal temperature. In some individuals, it varies between 34.4^{o}C and 41.1^{o}C (Greig and McIntyre, 1979). It was found that the water requirements of indigenous East African Zebu are about half

those of exotic breeds and are similar to those of several wild bovids (Murray and Trail, 1984). While no data is available on trypanotolerant breeds, it is possible that they are adapted to an even greater extent. In one study, Dargie (1980) showed that the N'Dama turn over less water than Zebu when expressed in terms of ml per kg body weight. It is quite possible that these physiological factors act separately or together resulting in the observed reduced clinical signs in trypanotolerant animals.

CHAPTER 4

EXPERIMENTAL INFECTIONS OF CATTLE AND RESULTS

4.1: MATERIAL AND METHODS

4.1.1: CATTLE

The animals used in these studies were Galana and Orma Boran steers aged between 1.5 - 2 years, with an average weight between 198 to 210 kg. All animals were purchased from Galana ranch in the Coast Province of Kenya, transported to KETRI and were housed in a fly-free facility.

They were sprayed twice with acaricide at an interval of seven days and then treated with the trypanocidal drug, diminazene aceturate (Berenil - Hoechst, West Germany) at a dose of 7 mg/kg body weight. The animals were also treated with antihelminthic (Nilzan).

They were allowed access to hay, water and mineral salts ad libitum. Feeding was supplemented with ranch cubes (Unga Ltd., Nairobi) as an additional source of protein at a rate of 0.5 kg. per animal per day throughout the study period.

4.1.2: PRE-INFECTION SCREENING

All animals were bled and screened for the presence of trypanosomes by the buffy coat examination method and by mouse inoculation. No trypanosomes were detected.

Fifty to sixty days after Berenil treatment all animals were bled for sera. The sera were tested for the presence of trypanolytic and/or neutralizing antibodies against T. congolense or T. vivax to be used for infection. The presence of anti-trypanosome antibodies was also tested by the indirect immunofluorescence test using acetone-fixed T. congolense or T. vivax. No antibody activity was detected to the infecting parasites.

4.1.3: TRYPANOSOMES

In these studies only T. congolense and T. vivax were used. These two trypanosome species are the most pathogenic species to cattle in East Africa.

4.1.3.1: Trypanosoma congolense

A T. congolense clone, ILRAD 1180 (ILNat 3.1) was used. Details concerning the derivation of this clone from the stock, and its virulence, were reported by Nantulya, Musoke, Rurangirwa and Moloo, (1984). It was cloned from a T. congolense stock originally isolated from Serengeti National Park - Tanzania. In the first experiment animals were infected with bloodstream forms, while in the second experiment tsetse flies infected with the clone were used.

4.1.3.2: Trypanosoma vivax stocks K2388 and K2589

The stocks of T. vivax (K2388 and K2589) were isolated from cows showing the haemorrahagic syndrome on the same farm at Likoni, Mombasa within an interval of one month (E. Opiyo - KETRI, pers. comm.). Stock K2388 was shown to cause haemorrhages following intravenous inoculation into exotic (non-indigenous) cattle, (Mwongela, Kovatch and Fazil, 1981), while Stock K2589 has been proved to induce petechial and ecchymotic haemorrhages after intravenous inoculation into Boran cattle (Wellde, Chumo, Adoyo, Kovatch, Mwongela and Opiyo, 1983).

In the present study stock K2388 was used to infect groups of Galana and Orma steers via the intravenous route, while stock K2589 was used to infect another group of Galana and Orma steers via the bite of infected flies.

4.1.4: PARASITOLOGY - ESTIMATION OF PARASITAEMIA

The improved estimation method of Paris, Murray and McOdimba, (1982) was originally used to detect and estimate the levels of parasitaemia. However, this method uses a very wide range of

parasitaemia score. For example, the presence of one to ten parasites in a whole preparation of a 22x22mm cover slip was scored as 2+ and has an equivalent level of parasitaemia of $1x10^2$ - $1x10^4$ parasites/ml. The presence of one to ten parasites in each microscopic field was scored as 4+ and has a level of parasitaemia equivalent to $1x10^4$ - $5x10^5$ parasites/ml. Thus this estimation system groups together wide ranges of parasitaemia. During chronic infections in cattle, it is noticeable that some animals will always harbour very low parasite numbers such as two to three parasites per whole wet preparation while others consistently have a slightly higher level, such as six to eight parasites per whole preparation. According to this scoring system, both animals will be scored as 2+ while in fact they do not have the same level of parasitaemia. Similarly, an animal that shows 2-3 parasites in each microscopic field should not be grouped with the one that shows 9-10 parasites per field.

Therefore an attempt was made to reduce this wide range by re-evaluating the levels of parasitaemia as indicated by the number of parasites per whole preparation or per microscopic field. For this purpose experiments were done using both infected mouse or bovine blood. Infected blood was diluted serially with normal blood of the respective species. Haemocytometer and buffy coat counts were made from each dilution. The results were compared and adjusted to give an estimate of the total number of parasites per ml of blood (not the absolute scores). Field magnification was the x10 eye-piece and x25 objective lense.

As a consequence, the following table was constructed and used to estimate the levels of parasitaemia throughout the studies reported in this thesis:

a) In the whole preparation (22x22mm coverslip) the detection of:-

1	parasite(s) =	1.0×10^2
2	" =	2.5×10^2
3-4	" =	5.0×10^2
5-6	" =	1.0×10^3
7-8	" =	5.0×10^3
9-10	" =	7.5×10^3

b) The detection of 1 parasite in every 2-3 microscopic fields = 1.0×10^4

c) In each microscopic field (x10 eyepiece and x25 objective lens) the detection of:-

1	parasite(s) =	1.0×10^4
2	" =	2.5×10^4
3-4	" =	5.0×10^4
5-6	" =	7.5×10^4
7-8	" =	1.0×10^5
9	" =	2.5×10^5
10	" =	5.0×10^5

d) >12 per field, a haemocytometer was used for an accurate count of the number of parasites.

For parasitaemia, blood was taken from the jugular vein into bottles containing disodium ethylenediamino-tetraacetic acid (EDTA).

Following infection, the animals were examined daily for parasites for 30-35 days. This was to determine the prepatent period and the height of the first peak of parasitaemia. For the remainder of the observation period, the animals were examined for parasitaemia twice a week.

4.1.5: HAEMATOLOGY

Blood was taken from the jugular vein into Bijou bottles containing EDTA.

4.1.5.1: Blood cell counts

The packed red cell volume (PCV%) was determined by the haematocrit centrifugation technique, using a haemotocrit centrifuge (Hawkins LTD., U.K.).

The total red blood cell counts (RBC) and the total white blood cell counts (WBC) were determined using an electronic particle counter (Coulter Counter Model ZB1, Coulter Electronic, Inc. Hialeah, Florida, U.S.A.).

For the WBC counts a dilution of 1:500 was made. 20 ul of blood sample were mixed with 10 mls of buffered isotonic saline ("Isoton"). Six drops of ZAP-Globin (a lysing reagent - Coulter Electronics, Nairobi, Kenya) were added to lyse the red cells.

For the RBC counts a dilution of 1:50000 was made. 100 ul of the sample used for WBC counts were mixed with 10 mls of the Isoton.

The haemoglobin concentration (Hb) was measured by a haemoglobinometer which was attached to the Coulter Counter. The sample was prepared as described above for measurement of WBC count. ZAP-Globin contains a cyanide reagent which reacts with Hb to form a stable cyanmethaemoglobin, which is measured by the haemoglobinometer.

The differential cell counts were determined on 200 leukocytes per Giemsa-stained blood smear.

All these parameters were measured daily for ten days prior to infection to serve as base line data. Following infection, these parameters were measured daily for the first 30 to 35 days. Thereafter, they were carried out twice weekly until the end of the study period.

4.1.5.2: Calculation of erythrocyte indices

The mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated according to the method of Schalm, Jain, Carrol, (1975).

4.1.5.3: Erythrocyte osmotic fragility test (EOFT):

The osmotic fragility test for the erythrocytes was carried out according to the method described by Schalm et al., (1975) with some modifications. The full description of the method is given in Appendix 3.

4.1.5.4: Thrombocyte counts (Platelets)

Manual counts using a haemocytometer were found to be more reliable and consistent for this blood cell type than using a Coulter Counter. Samples were counted in duplicate. Blood collected in Bijou bottles containing EDTA was diluted 1:20 with 1% ammonium oxalate in distilled water. Both chambers of the haemocytometer were filled and then incubated in a humidified container for 20 min. at $4^{o}C$ in order to allow the thrombocytes to settle. Counting was done by phase contrast microscope, using x40 objective lens and x10 eye-pieces.

4.1.5.5: Total plasma protein concentration (PPC)

The PPC was determined as described by Schalm et al., (1975) using the Goldberg refractometer (American Optical Company - Buffalo, New York).

4.1.5.6: Plasma fibrinogen concentration (PFC)

The PFC was determined by the method of Schalm et al., (1975) using the capillary tube method.

4.1.5.7: Protamine sulphate paracoagulation test (PST):

PST for the presence of fibrin monomer and fibrin split products was performed according to the method described by Seaman

(1970) with some modifications. Full description of the method and the scoring system used in this study are given in Appendix 2. The test was performed twice before infection. After tsetse infection, it was carried out daily for 35 days and twice weekly thereafter.

4.1.6: CLINICAL ASSESSMENT

4.1.6.1: Temperature

The thermometer was held in contact with the rectal mucous membrane for one minute before the value of the temperature was recorded. A temperature of 39.5^{o}C and above was regarded as a febrile response.

4.1.6.2: Heart rates

The number of heart beats per minute was recorded using a stethoscope.

4.1.6.3: Respiration

The number of inspirations per minute was counted using a stethoscope.

The rectal temperature, the heartbeats and respiration were recorded daily for ten days prior to infection. Following infection, these parameters were recorded daily for 30-35 days.

4.1.7: NEUTRALIZATION OF INFECTIVITY TEST (NIT) AGAINST METACYCLIC FORMS OF T. CONGOLENSE (IL 1180)

Serum samples from Galana and Orma animals were assayed for neutralizing antibodies against the metacyclic forms of T. congolense (IL 1180) following the method described by Nantulya, Musoke, Barbet and Roelants, (1979). The metacyclics were obtained from cultures initiated from infected mouse blood and maintained at 28^{o}C in HEPES (25mM) - buffered Eagle's MEM medium supplemented with 20% (V/V) heat-inactivated foetal bovine serum (Hirumi, Hirumi and Moloo, 1982).

The metacyclics were collected by passing the culture medium

which contained mixed population of epimastigotes and metacyclics through Diethylaminoethyl Cellulose (DE52) column which retained the epimastigotes. The recovered metacyclics were concentrated by centrifugation, counted in a haemocytometer and adjusted to $1x10^5$/ml.

A suspension of $1x10^4$ metacyclics (in 0.1 ml volume) in phosphate buffered saline (PSG), pH 8.0 was added to each of five tubes containing 0.1 ml of the test serum and incubated on ice for 45 minutes. The trypanosome suspensions from all the tubes were then separately inoculated intraperitoneally into five Swiss mice. Wet preparations from the tail blood were examined daily for 30 days. Complete neutralization occurred when none of the five mice became positive. The appearance of trypanosome in one or more out of the five mice rendered the result for that group negative.

4.1.8: BODY WEIGHT CHANGES

All animals were weighed by a weighing scale on the day of infection and at monthly intervals thereafter.

4.1.9: TREATMENTS

Any animal that developed a PCV $\leq$ 15% was treated with the trypanocidal drug, Berenil at a dose of 7 mg/kg body weight and withdrawn from the experiment. Under field conditions, animals with this degree of anaemia would be unlikely to survive. Thereafter the time of occurrence of this PCV value was taken as an indication of "life expectancy" in these studies.

4.1.10: METHOD OF DATA ANALYSIS

Analysis of the sequential time data from different groups was carried out using a two factor repeated measures analysis of variance design (Winer, 1971). This design was employed in the analysis of parasitaemia, PCV, WBC, RBC and Hb (see appendix 4). For the purpose of this test, any animal that was treated and

withdrawn from the study, the last value in any variable was used for the analysis. However, all the graphs presented in this study were drawn from the means of the surviving animals only.

Differences between the two groups in the prepatent periods and time to treatment were analysed by the student "t" test for unequal variances (Snedecor and Cochran, 1982) as follows:

Firstly, the t value was calculated using the following formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

Secondly, using a 5% significance level, the ta value was calculated using the following formula:

$$ta = \frac{t(V_1)\frac{SD_1^2}{n_1} + t(V_2)\frac{SD_2^2}{n_2}}{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}$$

The difference between the two samples will be significant at a 5% level if t-value falls below -ta or beyond +ta and non-significant if it falls within the -ta and +ta values.

4.2: EXPERIMENTAL DESIGNS AND PROCEDURES

4.2.1: EXPERIMENT I

THE SUSCEPTIBILITY OF ORMA AND GALANA STEERS TO SYRINGE INFECTION WITH BLOODSTREAM FORMS OF T. CONGOLENSE (IL 1180)

4.2.1.1: Introduction

In the following experiment, groups of Galana and Orma steers were syringe inoculated with a clone of T. congolense known to be virulent to cattle. The susceptibility of these animals was assessed on the basis of clinical findings, severity of anaemia and levels of parasitaemia. Uninfected controls were always included in the experiments, but in this thesis data has been compared exclusively for experimental animals before and after the infection.

4.2.1.2: Experimental design:

Groups of 12 Galana steers and 12 Orma steers were used in this experiment. Three additional animals for each group were left as uninfected controls. Trypanosoma congolense (IL 1180) parasites were grown in leathally irradiated mice. Infected mouse blood was collected five days later, parasites were counted and blood was diluted with PSG pH 8.0 and adjusted to $1x10^6$ parasites/ml. Each animal was then inoculated intravenously with $1x10^6$ organisms of T. congolense IL 1180. One hundred days after infection, the experiment was terminated and the animals remaining in the experiment were treated (see treatments above) with Berenil at 7mg/kg body weight.

4.2.1.3: Experimental procedures:

Clinical assessment, parasitaemia scoring, haemotological parameters, treatments and body weight changes were all carried out as described above.

4.2.2: EXPERIMENT II

THE SUSCEPTIBILITY OF ORMA AND GALANA STEERS TO INFECTION INDUCED BY G.M. MORSITANS INFECTED WITH T. CONGOLENSE (1180)

4.2.2.1: Introduction

The results obtained from groups of animals syringe-infected with T. congolense indicated that the Orma cattle were more resistant to infection than Galana cattle. Significant differences emerged between the two groups in all parameters investigated.

In the light of these results, experiments were designed to examine the susceptibility of these animals to infections induced by tsetse infected with T. congolense.

4.2.2.2: Tsetse flies

Teneral G.m. morsitans of both sexes were obtained from the Walter Reed and the KETRI colonies.

4.2.2.3: Experimental design

New groups of ten Galana and ten Orma steers were used in this study. Each steer was bitten by three infected tsetse flies at different sites on shaved areas of the flank to facilitate subsequent skin measurements. Two additional control animals for each group were bitten with three non-infected flies. Details of the transmission of T. congolense by G.m. morsitans are given in appendix 1A.

4.2.2.4: Experimental procedures

The scoring of parasitaemia, clinical assessment, haematological parameters, PPC, PFC, PST, EOFT, NIT, treatment and body weight changes were all carried out as described above.

4.2.2.5: Skin thickness (chancre)

Skin thickness at the sites of bite was measured with vernier calipers daily for 30 days.

4.2.3: EXPERIMENT III

THE SUSCEPTIBILITY OF ORMA AND GALANA STEERS TO SYRINGE INFECTION WITH BLOODSTREAM FORMS OF T. VIVAX (STOCK K2388)

4.2.3.1: Introduction

The results obtained from previous infections of groups of Galana and Orma steers indicated that the Orma were more resistant to the effects of T. congolense infections compared with the Galana. It was then proposed to examine the susceptibility of these animals to syringe infection with blood stream forms of T. vivax stock K2388 which is known to be virulent to cattle and to produce a haemorrhagic syndrome.

4.2.3.2 Experimental design:

The animals, previously syringe infected with T. congolense and treated with Berenil after 100 days, were syringe infected with T. vivax stock K2388, 90 days later. Groups of 12 Galana steers and ten Orma steers were used. Three additional animals from each group remained as controls. The donor animal was intravenously inoculated with the stabilated stock. Parasitaemia was examined daily and when it was $>1x10^7$, the infected blood was diluted with PSG, pH 8.0 and adjusted to $1x10^6$ parasites/ml. Each animal was inoculated intravenously with $1x10^6$ organisms of T. vivax stock K2388. One hundred and three days after infection, the experiment was terminated and the remaining animals were treated with Berenil at a dose of 7mg/kg body weight.

4.2.3.3: Experimental procedures:

The estimation of parasitaemia, clinical assessments, haemotological parameters, thrombocyte counts, PPC, body weight changes and treatment procedures were carried out as described earlier.

4.2.4: EXPERIMENT IV

THE SUSCEPTIBILITY OF ORMA AND GALANA STEERS TO INFECTION INDUCED BY G.M. MORSITANS INFECTED WITH T. VIVAX (STOCK K2589)

4.2.4.1: Introduction

All stocks of trypanosomes used to infect different groups of Galana and Orma animals whether using bloodstream forms or infected tsetse flies had clearly demonstrated the resistance of the Orma Boran cattle compared with the Galana. In the previous syringe infection with T. vivax stock K2388, the disease appeared to be acute but the haemorrhagic syndrome as reported by Mwongela et al., (1981), was not observed clinically or by testing for blood in faeces. Thus, in this experiment groups of Galana and Orma animals were bitten by G.m. morsitans infected with T. vivax stock K2589 which was reported to be virulent for cattle but produce a less severe haemorrhagic disease than stock K2388 (Wellde et al., 1983).

4.2.4.2: Tsetse flies

Teneral G.m. morsitans of both sexes, obtained from the Walter Reed and the KETRI colonies, were used.

4.2.4.3: Experimental design

New groups of ten Galana and ten Orma steers were used in this study. Each animal was bitten by four infected tsetse flies (see appendix 1B) at different sites on shaved areas of the flank in order to facilitate subsequent skin measurements. Two additional control animals for each group were bitten with three non-infected flies to serve as controls. Details of the transmission of T. vivax by G.m. morsitans are given in appendix 1B.

4.2.4.4: Experimental procedures

Skin thickness was measured as described in experiment two. The detection and estimation of parasitaemia, haemotological parameters, clinical assessments, PPC, PFC, PST, EOFT, body weight changes and treatments were assessed as described previously.

4.3: R E S U L T S

4.3.1: INFECTIONS WITH T. CONGOLENSE

4.3.1.1: CLINICAL ASSESSMENT

(i): Prepatent period:

Following infection whether initiated with bloodstream forms or induced by G.m. morsitans infected with T. congolense (IL 1180), all animals became parasitaemic after varying prepatent periods. In infection initiated with bloodstream forms, Galana animals became parasitaemic between day four and six, while the Orma animals became parasitaemic between day four and nine (Table 1.A). Statistical analysis using "t" test at a 5% significance level indicated no significant differences between the two groups in the prepatent periods.

In infection initiated by infected tsetse, two of the ten Galana animals never became infected as judged by normal PCV (>34%), temperature, heart and respiration rates. However, after 40 days of infection, one of these two animals was intravenously inoculated with approximately $1x10^5$ parasites of T. congolense (IL 1180). This animal became parasitaemic after four days. The other animal was similarly infected at the end of the experiment (day 104) and was parasitaemic five days after infection. Thus it was concluded that these two animals were susceptible although did not get infected initially and therefore were not utilized in the analysis of the data. The eight remaining Galana animals became parasitaemic between days 10 and 14 (Table 1.B). The Orma became patent between days 12 and 14 of infection (Table 1.B). Statistical analysis using "t" test indicated no significant difference between the two groups in the prepatent periods.

(ii): Temperature:

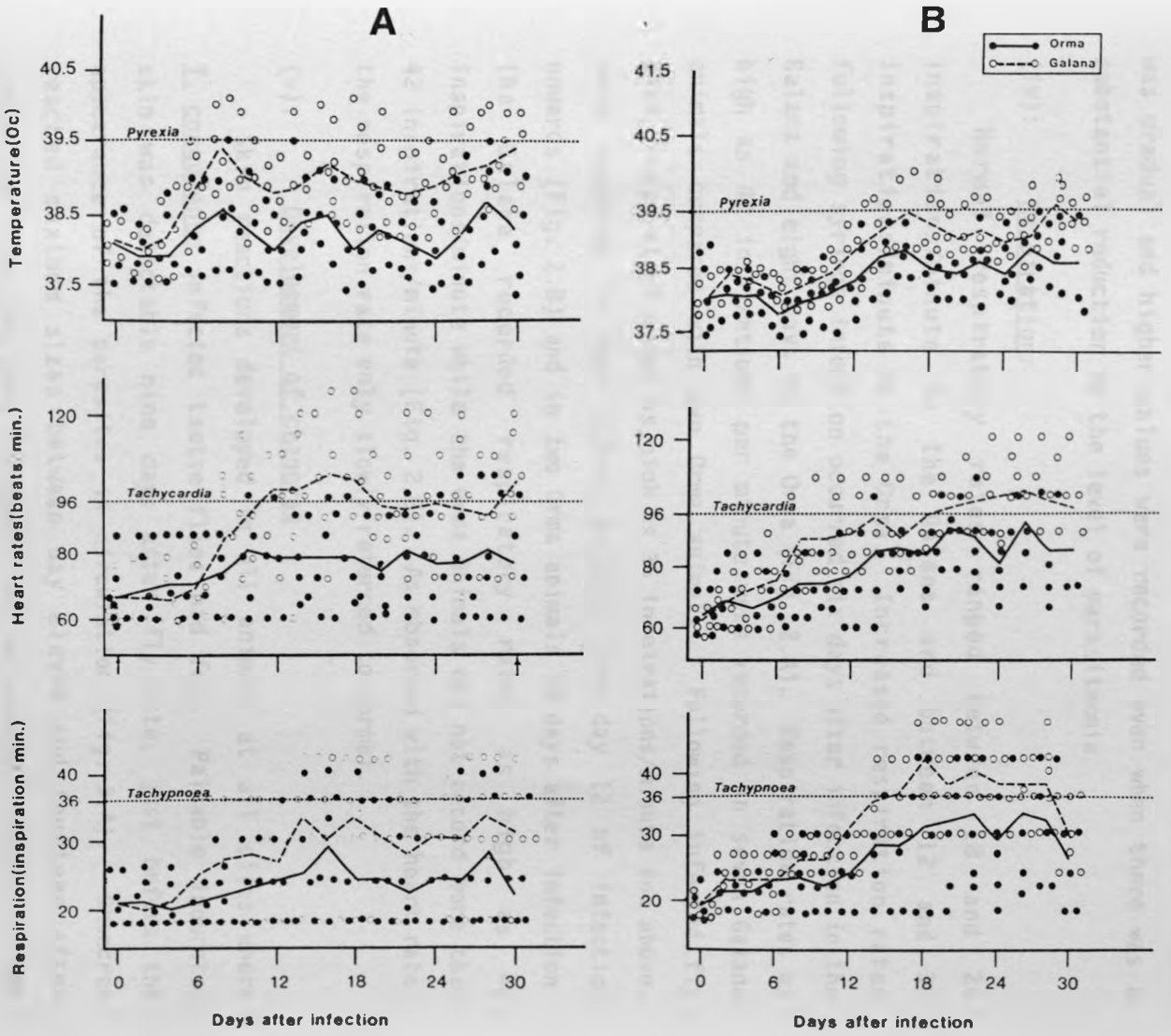
There was a wide range of rectal temperatures but no significant differences were observed between the two groups prior

to infection in either of the experiments. Normal temperatures in the Galana ranged between 37.0°C and 38.8°C while in the Orma, between 36.0°C and 39.1°C. Any rise in temperature beyond 39.5°C was considered as an indication of a febrile response. Seven days after syringe infection, 50% of Galana animals showed temperatures higher than 39.5°C as compared to only two Orma animals. Rise in temperature paralleled the increase in the levels of parasitaemia. In Galana, temperatures as high as 40.3°C were recorded while in Orma the highest temperature recorded was 39.8°C (Fig. 2.A). However, after the first 30 days of infection, the temperatures returned to normal ranges in all animals.

However, in infections induced by tsetse, rise in temperature was observed within 12 days of infection in the Galana and 13 days in the Orma. By day 15 of infection, 75% of the Galana were febrile while only two Orma animals had temperatures as high as 39.5°C. A rise in temperature was observed in both groups from day 15 of infection onwards (Fig. 2.B). However, after day 35 of infection, the temperatures in both groups returned to normal ranges.

(iii): Heart rate:

The mean pre-infection heart rates were not significantly different between the two groups in both infections. Normal heart rates in the Galana ranged between 58 and 78 beats/minute, while in the Orma they ranged between 58 and 84 beats/minute. Increase in heart rate was observed eight and ten days after infection in Galana and Orma respectively following syringe infection. With the increase in parasitaemia, heart rates also increased. Twenty five percent of the Galana recorded 126 beats per minute while the maximum beats recorded in Orma was 102 beats per minute (Fig. 2.A). Following infection induced by tsetse, accelerated heart rates (90 beats/minute and above) were observed eight days after infection in Galana, and 12 days in the Orma (Fig. 2.B). Tachycardia (96


A
B
Orma
Galana
Temperature(Oc)
Heart rates(beats/min.)
Respiration(inapiration/min.)
Pyrexia
Tachycardia
Tachypnoea
40.5
39.5
38.5
37.5
41.5
120
96
80
60
40
36
30
20
0
6
12
18
24
30
Days after infection
Days after infection

beats/minute and above) was observed in both groups from day 22 of infection onwards. The return of the heart rates to normal ranges was gradual and higher values were recorded even when there was a substantial reduction in the level of parasitaemia.

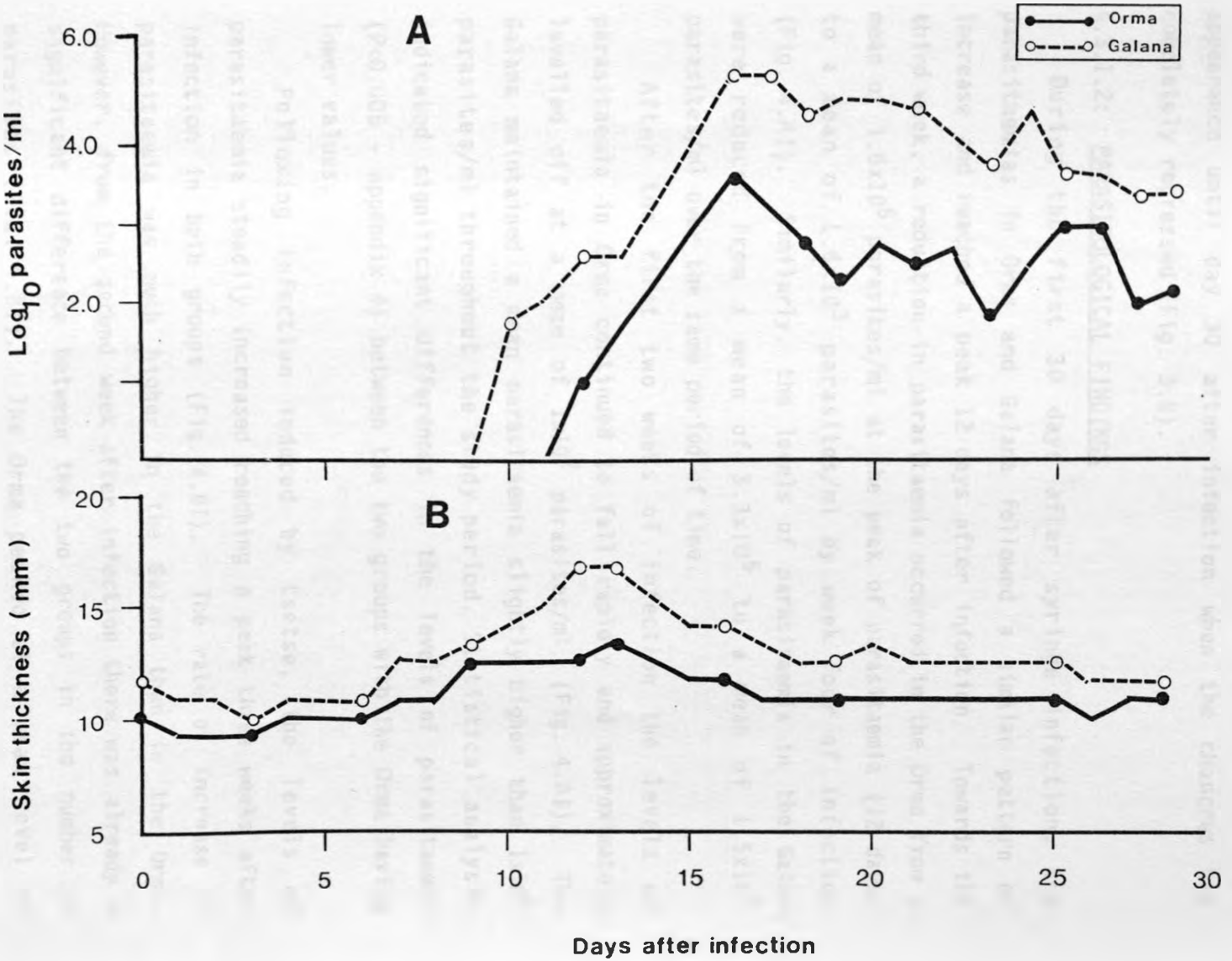
(iv): Respiration:

Normal respiratory rates ranged between 18 and 24 inspirations/minute in the Galana and between 12 and 30 inspirations/minute in the Orma. Increased respiration rates following syringe infection occurred six days after infection in the Galana and eight days in the Orma (Fig. 2.A). Respiration rates as high as 42 inspirations per minute were recorded in seven Galana animals compared with two Orma animals. Following infected fly bite, respiratory rates as high as 36 inspirations/minute and above, were recorded in four Galana animals from day 12 of infection onwards (Fig. 2.B) and in two Orma animals 14 days after infection. The Galana recorded respiratory rates as high as 48 inspirations/minute while the Orma animals did not record more than 42 inspirations/minute (Fig. 2.B). As observed with the heart rate, the respiration rate only slowly returned to normal.

(v): Development of chancres

Skin reactions developed in all animals at all sites where T. congolense infected tsetse flies had fed. Palpable indurated skin was detectable nine days after fly bite, just before the appearance of the parasites in circulation (Fig. 3.A). Chancres reached maximum sizes between day eleven and fourteen after infection, when the skin thickness had an average increase of 60% (day 12 and 13) in the Galana animals and 40% (day 13) in the Orma (Fig. 3.B). Using "t" test at a 5% significance level, the percentage increase in skin thickness was significantly greater in Galana than Orma.

The skin reactions started to regress from day 15 of infection


A
Orma
Galana
6.0
4.0
2.0
Log10 parasites/ml
B
20
15
10
5
Skin thickness (mm)
0
5
10
15
20
25
30
Days after infection

onwards. The areas were painful to touch from the time of appearance until day 30 after infection when the chancres had completely regressed (Fig. 3.B).

4.3.1.2: PARASITOLOGICAL FINDINGS

During the first 30 days after syringe infection, the parasitaemias in Orma and Galana followed a similar pattern of increase and reached a peak 12 days after infection. Towards the third week, a reduction in parasitaemia occurred in the Orma from a mean of $1.6x10^5$ parasites/ml at the peak of parasitaemia (12 days) to a mean of $1.6x10^3$ parasites/ml by week four of infection (Fig. 4.AI). Similarly, the levels of parasitaemia in the Galana were reduced from a mean of $3.3x10^5$ to a mean of $1.5x10^4$ parasites/ml over the same period of time.

After the first two weeks of infection the levels of parasitaemia in Orma continued to fall rapidly and approximately levelled off at a mean of $1x10^2$ parasites/ml. (Fig. 4.AI). The Galana maintained a mean parasitaemia slightly higher than $1x10^4$ parasites/ml throughout the study period. Statistical analysis indicated significant differences in the levels of parasitaemia ($P<0.005$ - appendix 4) between the two groups with the Orma having lower values.

Following infection induced by tsetse, the levels of parasitaemia steadily increased reaching a peak three weeks after infection in both groups (Fig. 4.BI). The rate of increase in parasitaemia was much higher in the Galana than in the Orma. However, from the second week after infection there was already a significant difference between the two groups in the number of parasites (Fig. 4.BI). The Orma peaked at a mean level of parasitaemia of $5x10^3$ parasites/ml, while the Galana peaked at $5.7x10^5$ parasites/ml. From week four onwards both groups progressively decreased their levels of parasitaemia.

Analysis of parasitaemia over the 15 weeks of observation

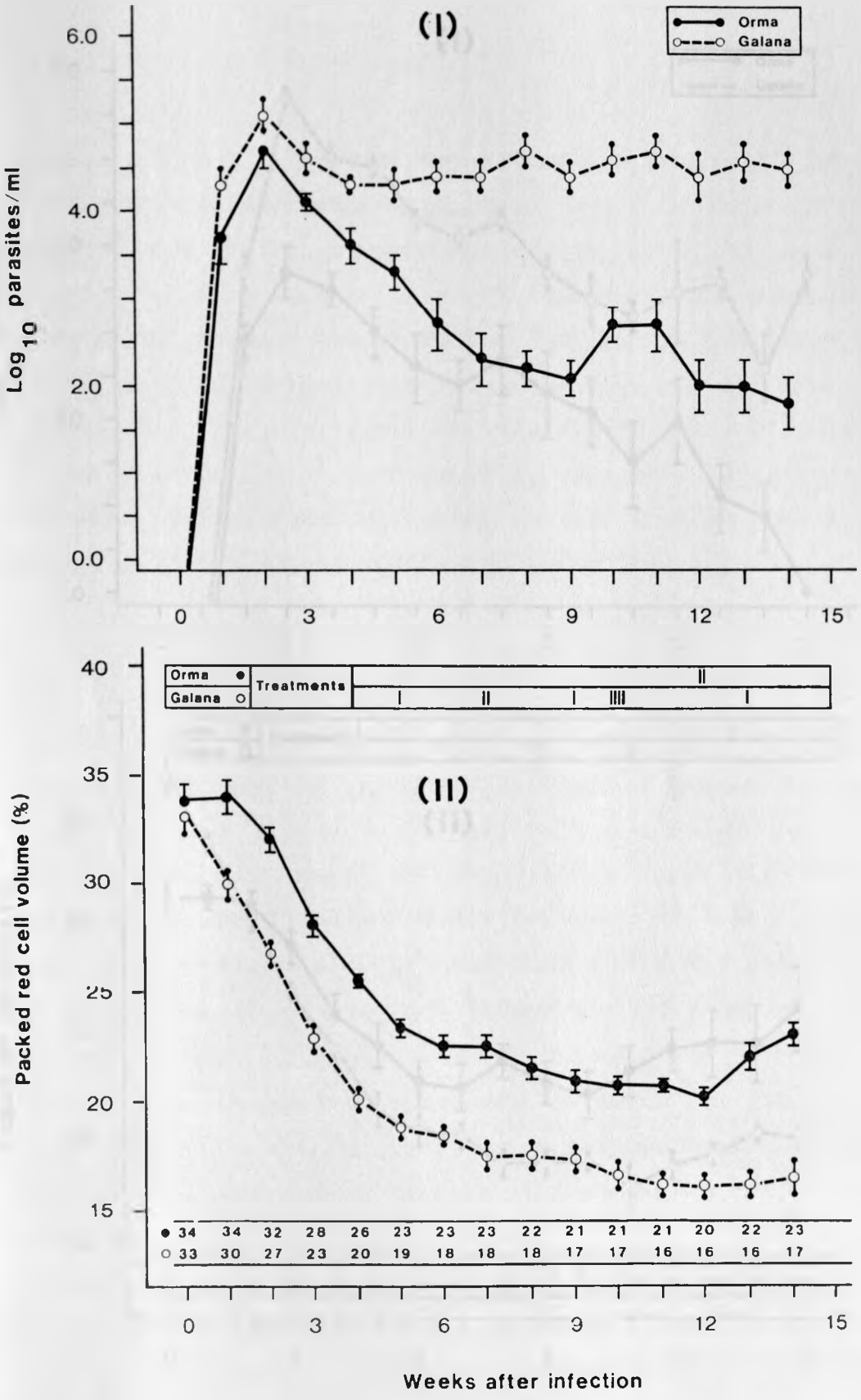


FIGURE 4.A : Mean ± SE weekly parasitaemia (I) and packed red cell volume (II) in Orma and Galana steers following syringe inoculation with *T. congolense* (IL 1180). 53a.

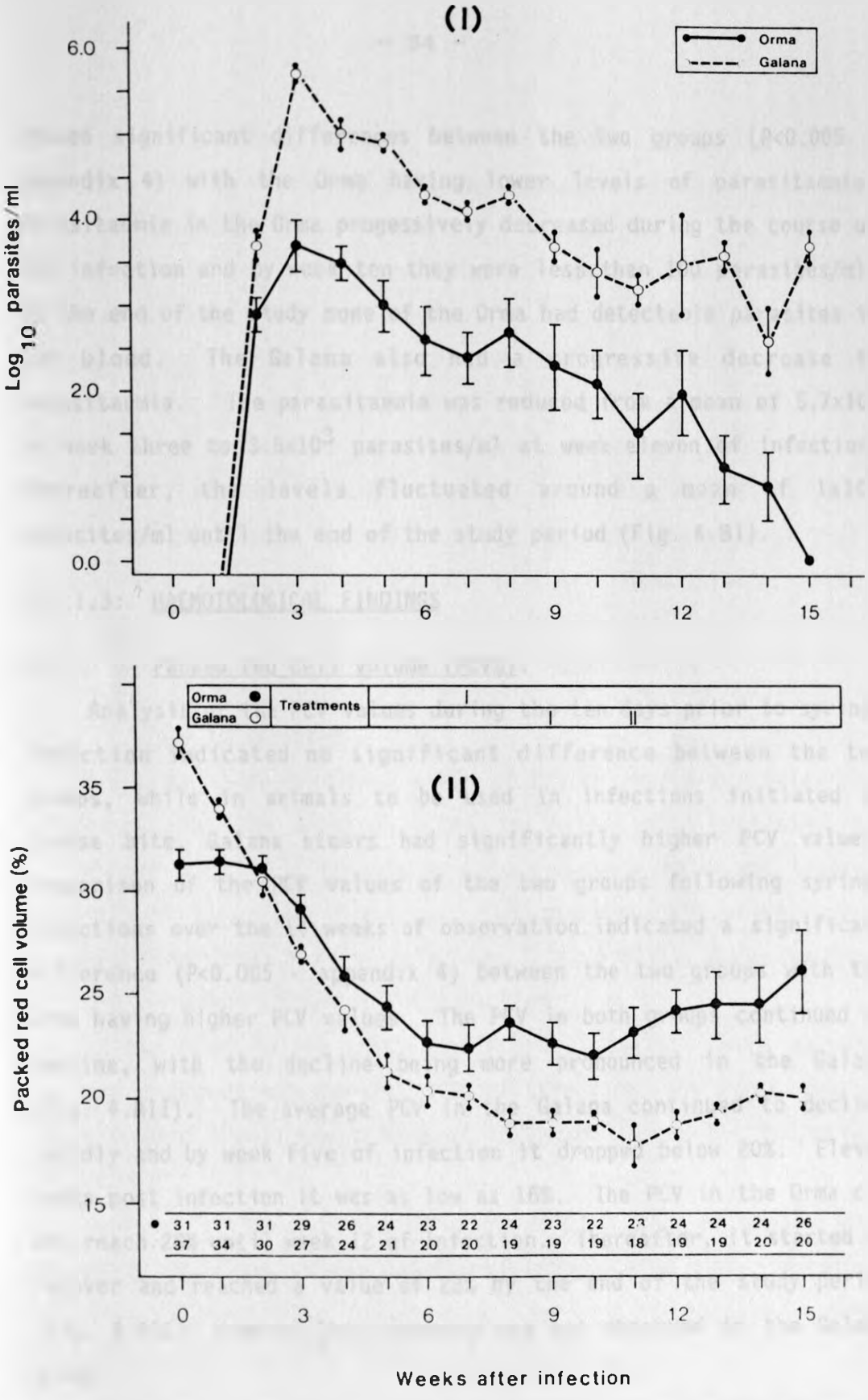


FIGURE 4.B : Mean ± SE weekly parasitaemia (I) and packed red cell volume (II) in Orma and Galana steers after infection with *T. congolense* (IL 1180) induced by tsetse. 53b.

showed significant differences between the two groups ($P<0.005$ - appendix 4) with the Orma having lower levels of parasitaemia. Parasitaemia in the Orma progessively decreased during the course of the infection and by week ten they were less than 100 parasites/ml. By the end of the study none of the Orma had detectable parasites in the blood. The Galana also had a progressive decrease in parasitaemia. The parasitaemia was reduced from a mean of 5.7×10^5 at week three to 3.5×10^3 parasites/ml at week eleven of infection. Thereafter, the levels fluctuated around a mean of 1×10^3 parasites/ml until the end of the study period (Fig. 4.BI).

4.3.1.3: HAEMOTOLOGICAL FINDINGS

(i): Packed red cell volume (PCV%):

Analysis of the PCV values during the ten days prior to syringe infection indicated no significant difference between the two groups, while in animals to be used in infections initiated by tsetse bite, Galana steers had significantly higher PCV values. Comparison of the PCV values of the two groups following syringe infections over the 14 weeks of observation indicated a significant difference ($P<0.005$ - appendix 4) between the two groups with the Orma having higher PCV values. The PCV in both groups continued to decline, with the decline being more pronounced in the Galana (Fig. 4.AII). The average PCV in the Galana continued to decline rapidly and by week five of infection it dropped below 20%. Eleven weeks post infection it was as low as 16%. The PCV in the Orma did not reach 20% until week 12 of infection. Thereafter, it started to recover and reached a value of 23% by the end of the study period (Fig. 4.AII). However this recovery was not observed in the Galana group.

Following infection induced by tsetse, both groups showed a significant fall in PCV values with the decline being more severe in the Galana than in the Orma. Five weeks after infection there was a

significant difference between the two groups with the Galana developing more severe anaemia although they started with higher PCV values than the Orma (Fig. 4.BII).

Analysis of PCV values over the 15 weeks following fly bite indicated a significant difference ($P<0.005$ - appendix 4) between the two groups. The pattern of drop was different in the two groups. A drop between 25-30% of the initial PCV from week six to week ten of infection occurred in the Orma (Fig. 4.BII). Thereafter, there was a gradual recovery until the end of the study period. In the Galana, the PCV dropped severely and by week eight of infection, it had dropped 50% of its initial pre-infection value (Fig. 4.B). Thereafter it fluctuated around an average of 50% drop of its initial value until the end of the study period.

(ii): Time to treatment

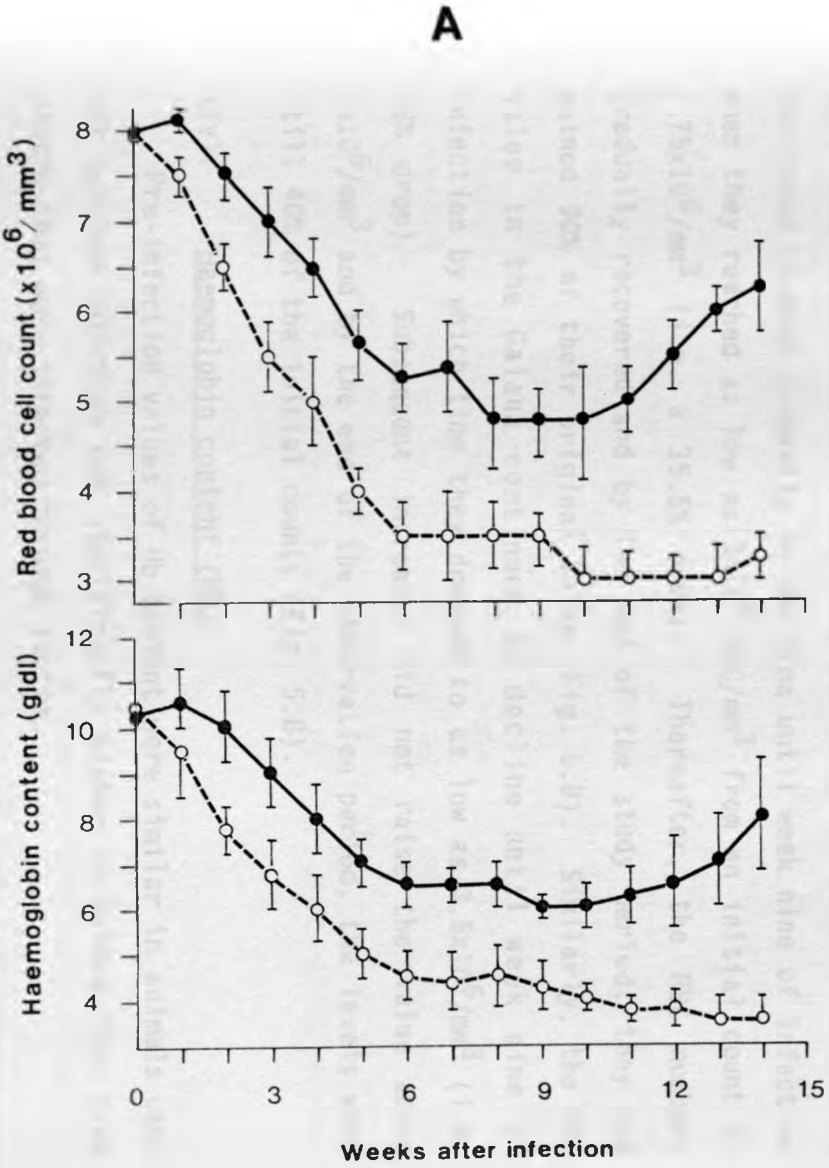
Any animal that developed a PCV of 15% or less was treated and withdrawn from the study. Thus in infections initiated by bloodstream forms of *T. congolense* three Galana animals were treated between week 4-7 after infection. The remaining six animals were treated between week 9-13 of infection (Fig. 4.AII). Only two Orma required treatment at week 12 of infection.

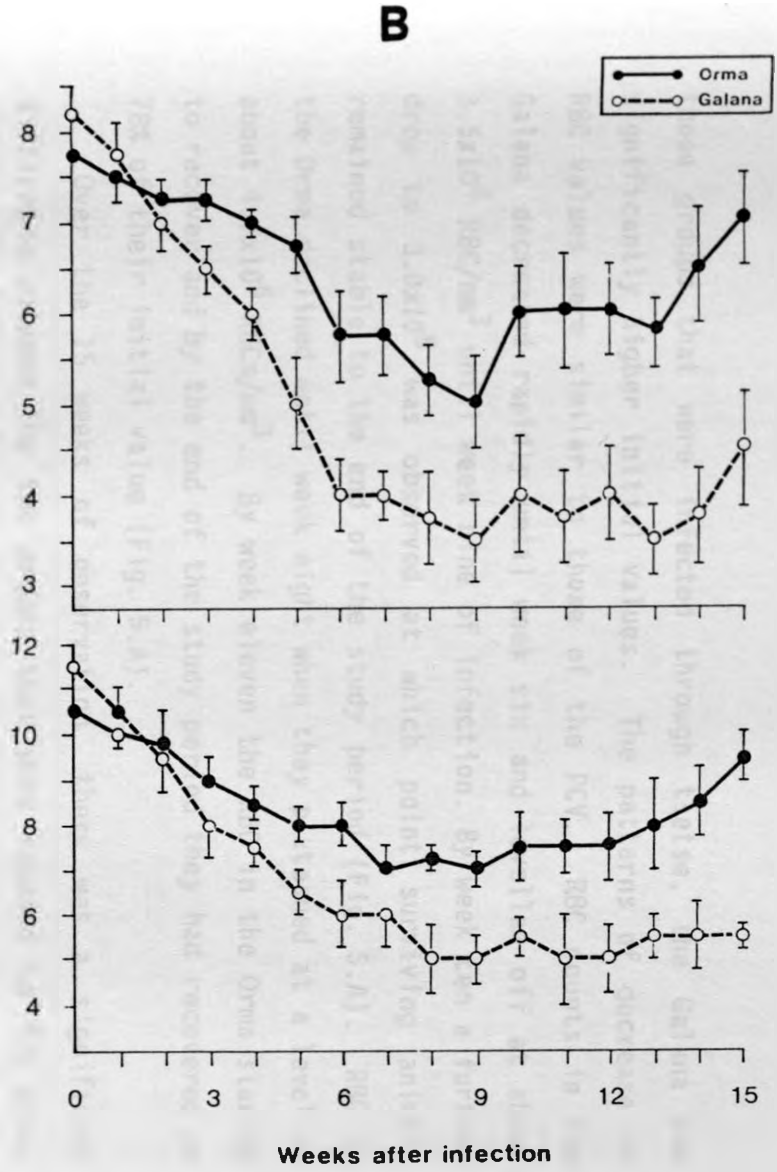
Of the eight Galana animals infected by tsetse, three animals required treatment between week 9-11 of infection, while only one of the ten Orma required treatment at week seven of infection (Fig. 4.BII).

However, if treatment is taken as an indication of death, then 75% and 37.5% of the Galana animals would have died with infections initiated by bloodstream forms and by fly bite respectively. This compares with 17% and 10% in the Orma respectively.

(iii): Red blood cell count (RBC):

There was no significant difference in the pre-infection RBC values between the two groups used in syringe infections, while in


A
Red blood cell count (x10^6/mm^3)
8
7
6
5
4
3
Haemoglobin content (g/dl)
12
10
8
6
4
0
3
6
9
12
15
Weeks after infection


B
Orma
Galana
8
7
6
5
4
3
12
10
8
6
4
0
3
6
9
12
15
Weeks after infection

those groups that were infected through tsetse, the Galana had significantly higher initial values. The patterns of decrease in RBC values were similar to those of the PCV. RBC counts in the Galana decreased rapidly until week six and levelled off at about $3.5x10^6$ RBC/mm^3 until week nine of infection. By week ten a further drop to $3.0x10^6$ was observed at which point surviving animals remained stable to the end of the study period (Fig. 5.A). RBC in the Orma declined until week eight when they plateaued at a level of about $4.7x10^6$ RBCs/mm^3. By week eleven the RBC in the Orma started to recover and by the end of the study period they had recovered to 78% of their initial value (Fig. 5.A).

Over the 15 weeks of observation, there was a significant difference between the two groups that were exposed to fly bite, with Orma being less affected than Galana (Fig. 5.B). RBC counts continued to drop gradually in the Orma until week nine of infection when they reached as low as $5x10^6$ RBC/mm^3 from an initial count of $7.75x10^6/mm^3$ (i.e. a 35.5% drop). Thereafter, the RBC numbers gradually recovered and by the end of the study period, they had gained 90% of their original value (Fig. 5.B). Similarly, the RBC value in the Galana continued to decline until week nine of infection by which time they dropped to as low as $3.5x10^6/mm^3$ (i.e. 58% drop). Subsequent increases did not raise the value above $4x10^6/mm^3$ and by the end of the observation period, the levels were still 40% of the initial counts (Fig. 5.B).

(iv): Haemoglobin content (Hb):

Pre-infection values of Hb content were similar in animals used for syringe infection but significantly higher in Galana than Orma steers that were infected through tsetse.

Analysis of the Hb content over the 14 weeks of observation after syringe infection indicated a significant difference ($P<0.005$) between the two groups with the Orma having a higher Hb content

(Fig. 5.A). By week six of infection Galana lost 52% of their initial Hb content (that is, from 11.5 to 5.5 g/dl of blood), compared to a loss of 34% (from 11.0 to 7.0 g/dl) in the Orma over the same period. Thereafter, the Hb in the Galana continued to drop up to the end of the experiment. By week 14, the Hb in the Galana dropped to as low as 4.5g/100ml of blood, equivalent to a loss of 61%, while in the Orma the Hb started to recover from week ten of infection (Fig. 5.A) and by the end of the study period, it reached a value of 9.0g/100ml of blood (i.e. a loss of only 20% of the initial value).

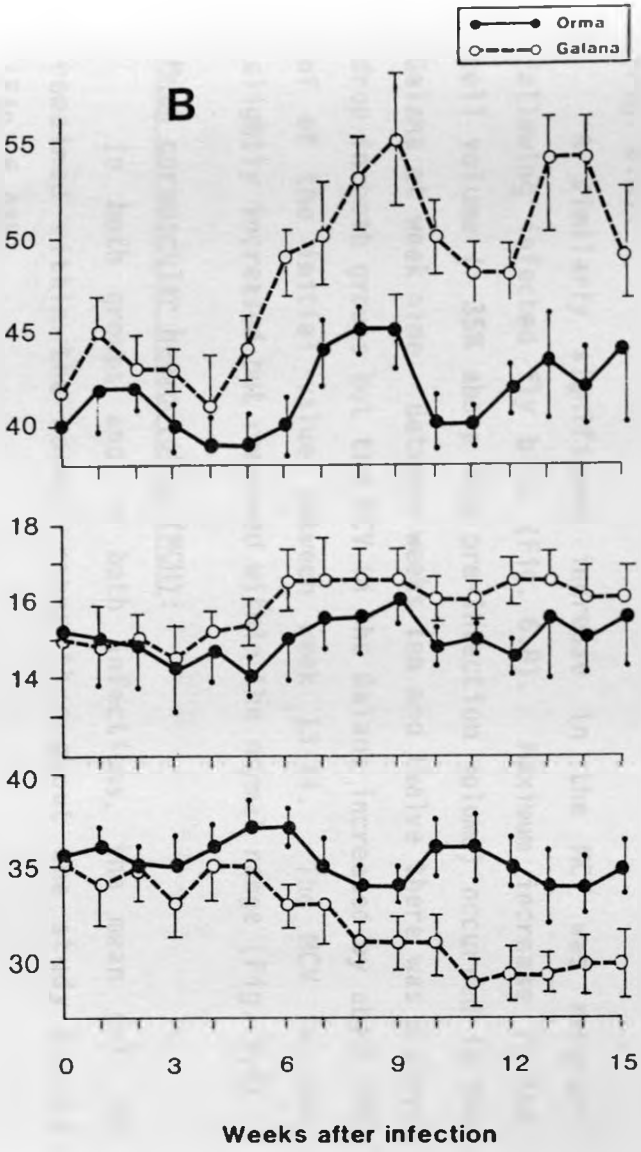
Analysis of the Hb content over the 15 weeks of the study following infected fly bite indicated a significant difference ($P<0.025$) between the two groups with the Galana having lower Hb values than the Orma. By week seven of infection the Hb content in the Orma had dropped from an average of 11.5g/dl to an average of 8.0g/dl (i.e. a drop of 30%). The Hb content in the Galana also dropped from an average of 12.5 g/dl to an average of 6.0g/dl by week eight of infection (i.e. a drop of 50%) (Fig. 5.B). From week nine onwards the amount of Hb in the Orma started to increase and by the end of week 15 the Hb value had attained more than 90% of the pre-infection value (Fig. 5.B). From week nine after infection, unlike the situation in the Orma, the Hb in the Galana did not recover, instead it fluctuated between a value of 6.0 to 6.5g/dl (i.e. a drop of more than 50%) throughout the observation period (Fig. 5.B).

(v): Erythrocyte indeces

Mean corpuscular volume (MCV):

The mean cell volume (MCV) in Galana was significantly increased (Fig. 6.A) compared to the MCV in the Orma group following infection with bloodstream forms of *T. congolense*. By week six of infection, the Galana MCV increased 25% above the pre-infection volume. They continued to increase upto the end of the study period

A
MCV (μm3)
55
50
45
40
MCH (pg)
18
16
14
MCHC (%)
40
35
30
0
3
6
9
12
15
Weeks after infection


Orma
Galana
B
55
50
45
40
18
16
14
40
35
30
0
3
6
9
12
15
Weeks after infection

by which time they had increased 30% more than the initial volume (Fig. 6.A).

A similarly significant increase in the MCV was recorded following infected fly bite (Fig. 6.B). Maximum increase in the cell volume (> 35% above the pre-infection volume) occurred in the Galana at week nine. Between weeks ten and twelve there was a sharp drop in both groups but the MCV in the Galana increased by about 35% of of the initial value between week 13-14. The MCV in Orma slightly increased but remained within the normal range (Fig. 6.B).

Mean corpuscular haemoglobin (MCH):

In both groups and in both infections, the mean cell Hb remained within the normal range throughout the study period (Fig. 6.A&B).

Mean corpuscular haemoglobin concentration (MCHC):

The percent cell Hb concentration (MCHC) in Orma remained within the normal range throughout the infection initiated by bloodstream forms, although there was a slight drop during the second week of infection (Fig. 6.A). However, the Galana experienced a progressive decrease in MCHC (Fig. 6.A). It reached the critical value for hypochromic anaemia (MCHC=30%) by week six of infection which coincided with the maximum increase in the MCV. The Galana MCHC continued to decrease until the end of the study period, by which time it decreased below 28%. However, in infections induced by tsetse, the MCHC was greatly reduced in the Galana, while in the Orma it fluctuated within the normal range throughout the study period. In Galana, the decrease in the MCHC became more pronounced from week seven after infection onwards where it was less than 30% (Fig. 6.B). Thereafter it remained at this level until the end of the study period.

Thus the Galana animals expressed a macrocytic hypochromic type of anaemia in both infections, while the Orma expressed a

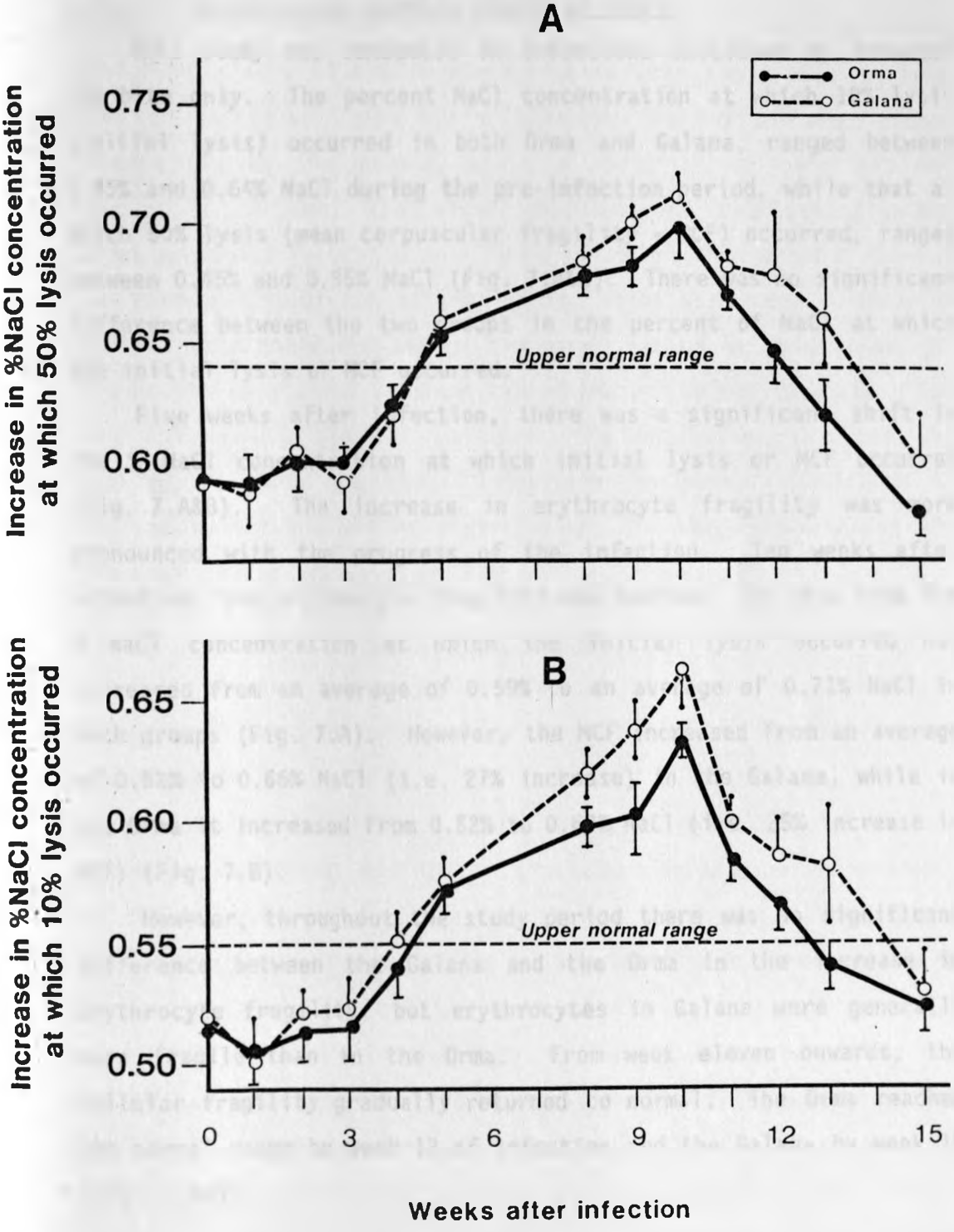


FIGURE 7 : Mean ± SE weekly increase in % NaCl concentration at which (A) initial lysis and (A) MCF occurred in Galana and Orma steers after infection with T. congolense (IL 1180) induced by tsetse. 58a.

normocytic normochromic anaemia.

(vi): Erythrocytes osmotic fragility (EOFT)

This study was conducted in infections initiated by infected fly bite only. The percent NaCl concentration at which 10% lysis (initial lysis) occurred in both Orma and Galana, ranged between 0.55% and 0.64% NaCl during the pre-infection period, while that at which 50% lysis (mean corpuscular fragility = MCF) occurred, ranged between 0.45% and 0.55% NaCl (Fig. 7.A&B). There was no significant difference between the two groups in the percent of NaCl at which the initial lysis or MCF occurred.

Five weeks after infection, there was a significant shift in the % NaCl concentration at which initial lysis or MCF occurred (Fig. 7.A&B). The increase in erythrocyte fragility was more pronounced with the progress of the infection. Ten weeks after infection, the erythrocyte fragility was maximum. By this time the % NaCl concentration at which the initial lysis occurred had increased from an average of 0.59% to an average of 0.71% NaCl in both groups (Fig. 7.A). However, the MCF increased from an average of 0.52% to 0.66% NaCl (i.e. 27% increase) in the Galana, while in the Orma it increased from 0.52% to 0.63% NaCl (i.e. 25% increase in MCF) (Fig. 7.B).

However, throughout the study period there was no significant difference between the Galana and the Orma in the increase in erythrocyte fragility, but erythrocytes in Galana were generally more fragile than in the Orma. From week eleven onwards, the cellular fragility gradually returned to normal. The Orma reached the normal range by week 13 of infection and the Galana by week 15 (Fig. 7.A&B).

(vii): Thrombocyte counts (Platelets):

Pre-infection data indicated no significant difference between the two groups in the number of thrombocytes.

Analysis of the thrombocyte counts over the 15 weeks of observation following infection induced by tsetse indicated significant differences between the two groups with the Galana developing a more severe thrombocytopenia than the Orma. By week three of infection there was a reduction of 45% of the initial thrombocytes in the Galana, while a reduction of only 12% was observed in the Orma. Thereafter, the number of thrombocytes in the Galana gradually recovered until week seven but then dropped in week nine when a loss of 55% of the initial count was noted. Recovery started again until the end of the study period by which time the thrombocyte levels had attained 70% of the pre-infection value. The Orma followed a similar pattern. By week nine the loss in thrombocyte counts was 28%. Thereafter the numbers continued to rise and by the end of the study period, the thrombocytes were 20% above the initial value (Fig. 8.A).

(viii): Plasma fibrinogen concentration (PFC):

Over the ten days prior to infection, there was no significant difference between the Galana and the Orma in fibrinogen concentration.

Following infection, there was an initial drop in the fibrinogen concentration (PFC) of the Galana during the second week, but the PFC returned to normal during the third week. Thereafter, the PFC continued to drop in the Galana until week nine when there was a loss of 58% of the pre-infection value (Fig. 8.B). Subsequently, the PFC of the Galana gradually recovered and by the end of the study period it had attained 65% of the pre-infection level. In the Orma, the PFC increased during the third week of infection (17%) but by week nine it had fallen by 21% of the initial level. Subsequently it recovered more rapidly than that in the Galana and was normal by the end of the study period (Fig. 8.B).

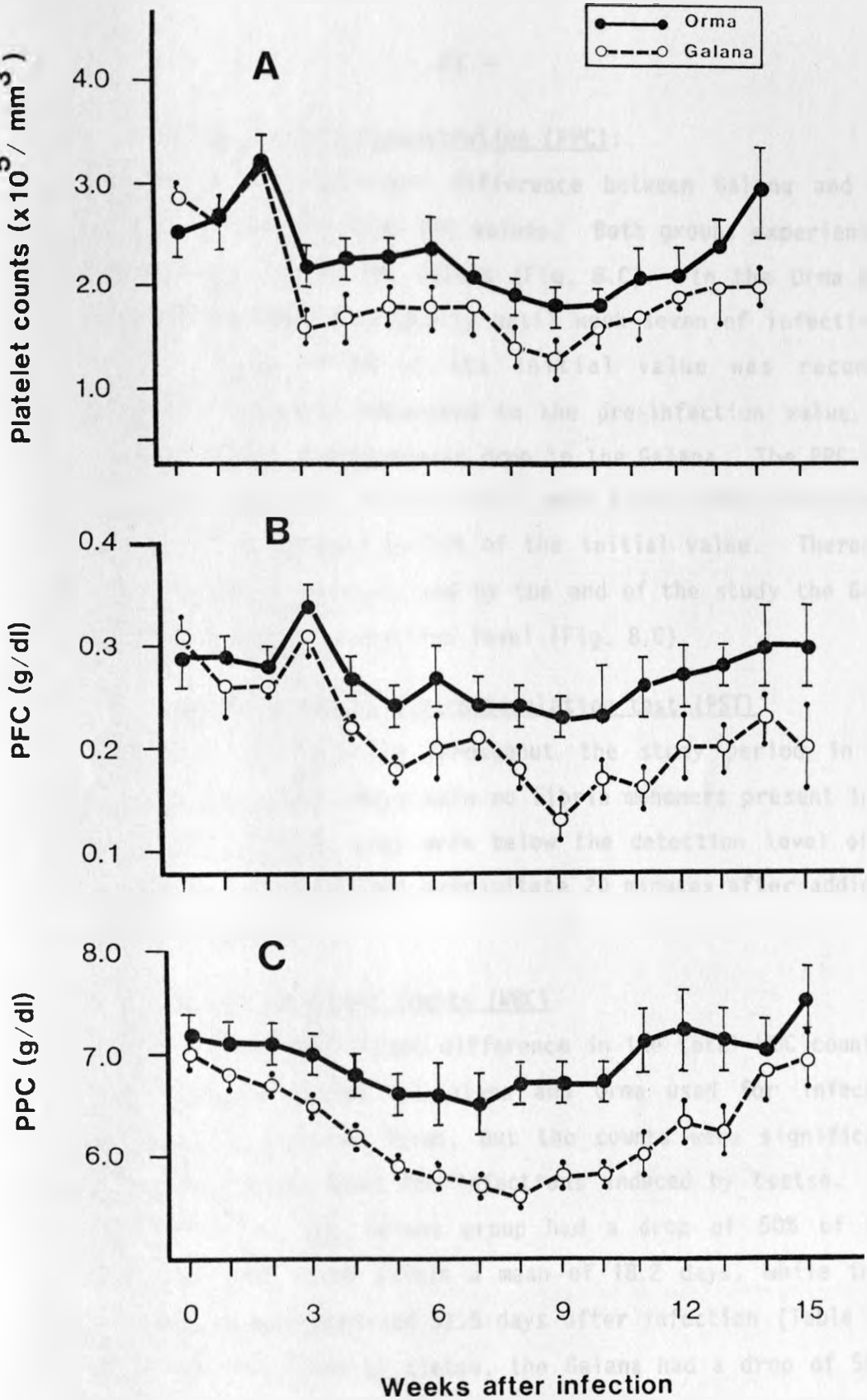


FIGURE 8 : Mean ± SE weekly (A) thrombocyte counts, (A) PFC and (c) PPC in Orma and Galana steers after infection with T. congolense (IL 1180) induced by tsetse. 60a.

(ix): Plasma protein concentration (PPC):

There was no significant difference between Galana and Orma animals in the pre-infection PPC values. Both groups experienced a gradual decrease in the PPC values (Fig. 8.C). In the Orma group the PPC level decreased gradually until week seven of infection at which a decrease of 8% of its initial value was recorded. Thereafter it gradually recovered to the pre-infection value. In contrast, there was a progressive drop in the Galana. The PPC level in the Galana continued to drop until week eight after infection by which time it had dropped by 20% of the initial value. Thereafter there was a gradual recovery and by the end of the study the Galana PPC was 99% of the pre-infection level (Fig. 8.C).

(x): Protamine sulphate paracoagulation test (PST)

The test was negative throughout the study period in both groups indicating that there were no fibrin monomers present in the plasma, or if present, they were below the detection level of the test (presence of flocculent precipitate 20 minutes after adding 1% protamine sulphate).

(xi): White blood cell counts (WBC)

There was no significant difference in the total WBC counts in the pre-infection period in Galana and Orma used for infections initiated by bloodstream forms, but the counts were significantly higher in the Galana used for infections induced by tsetse. With syringe infection, the Galana group had a drop of 50% of their initial total WBC value within a mean of 18.2 days, while in the Orma a similar drop occurred 51.5 days after infection (Table 1.A). In infections initiated by tsetse, the Galana had a drop of 50% of their initial WBC value within a mean of 16.8 days, while in the Orma, this drop did not occur until 78.6 days after infection (Table 1.B).

Analysis of the total WBC counts over the 14 weeks following

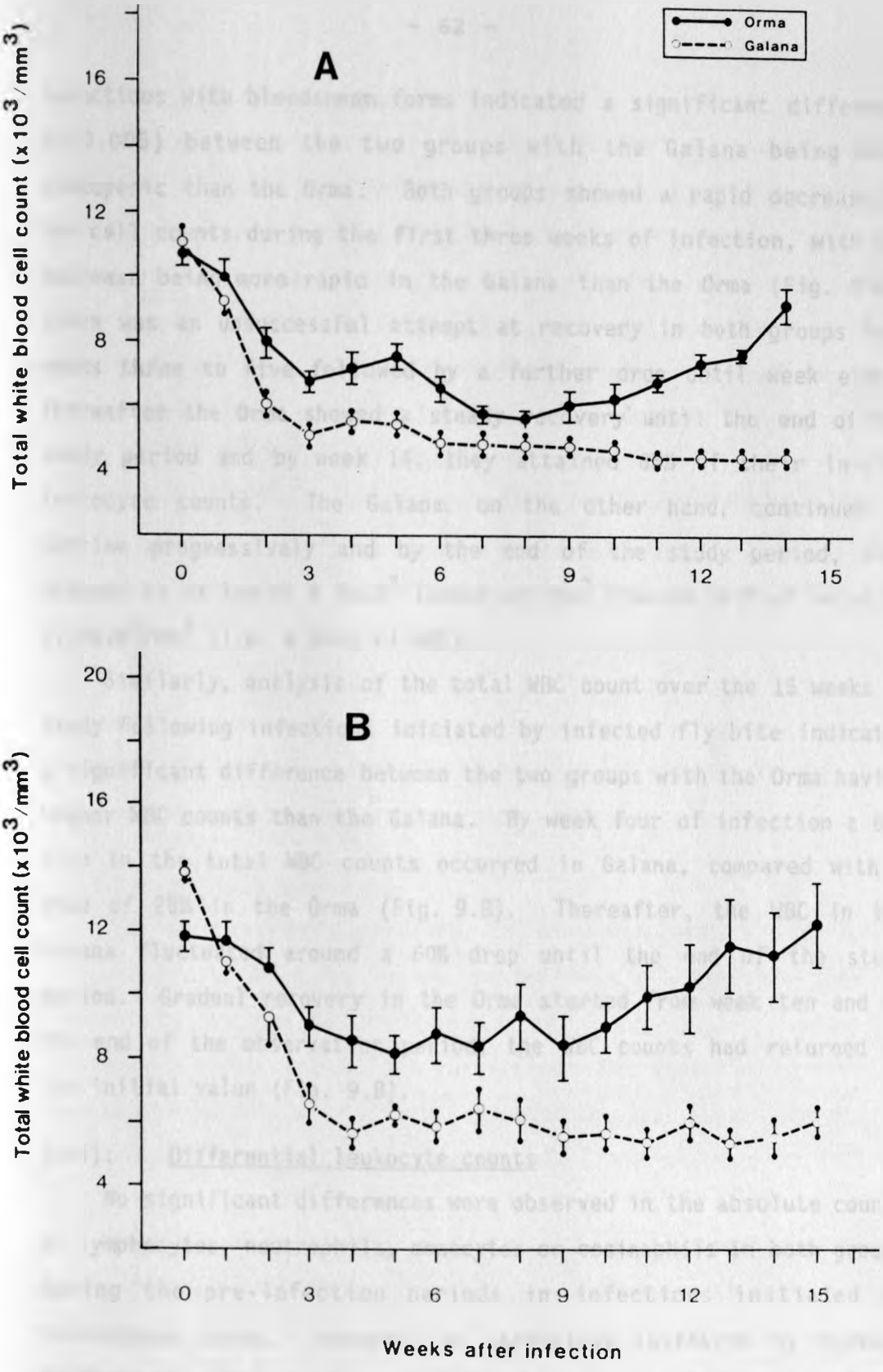


FIGURE 9 : Mean ± SE total WBC counts in Orma and Galana steers after infection with T. congolense (IL 1180) by (A) syringe and (A) tsetse. 61a.

infections with bloodsream forms indicated a significant difference ($P<0.005$) between the two groups with the Galana being more leukopenic than the Orma. Both groups showed a rapid decrease in the cell counts during the first three weeks of infection, with the decrease being more rapid in the Galana than the Orma (Fig. 9.A). There was an unsuccessful attempt at recovery in both groups from weeks three to five followed by a further drop until week eight. Thereafter the Orma showed a steady recovery until the end of the study period and by week 14, they attained 85% of their initial leukocyte counts. The Galana, on the other hand, continued to decline progressively and by the end of the study period, they dropped to as low as $4.5x10^3$ leukocytes/mm^3 from an initial value of $11.0x10^3/mm^3$ (i.e. a loss of 60%).

Similarly, analysis of the total WBC count over the 15 weeks of study following infections initiated by infected fly bite indicated a significant difference between the two groups with the Orma having higher WBC counts than the Galana. By week four of infection a 60% drop in the total WBC counts occurred in Galana, compared with a drop of 25% in the Orma (Fig. 9.B). Thereafter, the WBC in the Galana fluctuated around a 60% drop until the end of the study period. Gradual recovery in the Orma started from week ten and by the end of the observation period, the WBC counts had returned to the initial value (Fig. 9.B).

(xii): Differential leukocyte counts

No significant differences were observed in the absolute counts of lymphocytes, neutrophils, monocytes or eosinophils in both groups during the pre-infection periods in infections initiated by bloodstream forms. However, in infections initiated by tsetse, differences were observed in absolute counts of these cellular components during the pre-infection period, with Galana having higher lymphocyte, neutrophil and monocyte counts than the Orma.

Absolute lymphocyte counts:

Analysis of the absolute lymphocyte counts over the 14 weeks of study following syringe infection indicated a significant difference between the two groups with the Galana having lower lymphocyte counts. The Galana lymphocyte counts remained low throughout the study period, while the Orma lymphocytes continued to drop until week eight of infection and thereafter, showed a gradual recovery. By the end of the study period they had reached the pre-infection value of $7.5x10^3/mm^3$ (Fig. 10.A).

Over the 15 weeks of the observation following infected fly the Orma had higher lymphocyte numbers than the Galana. By week four of infection there was a drop of 57% in the Galana lymphocyte counts (Fig. 10.B). Thereafter the counts fluctuated around this value until the end of the study period. In the Orma, lymphocyte counts dropped by 30% by week four. Thereafter, a gradual recovery occurred and by the end of the study period, a gain of 25% above the pre-infection value was recorded (Fig. 10.B).

Absolute monocyte counts:

Following syringe infection, the monocyte counts in the Galana reduced drastically and by the second week of infection they had dropped to as low as 10 monocytes/mm^3 (Fig. 10.A). During the subsequent weeks, the Galana animals had low monocyte counts that fluctuated around 10 cells/mm^3 throughout the study period. A similar drop was observed following infections mediated by infected fly bite.

Analysis of the monocytes over the observation period indicated a significant difference between the two groups with Galana being more affected by the infection than the Orma. Following infection, the decrease in monocyte counts in Orma was more gradual than in the Galana (Fig. 10.A). The monocytes of the Orma continued to decrease until week ten of infection where they dropped to as low as 20 cells/mm^3, thereafter, they recovered gradually and, by the end of

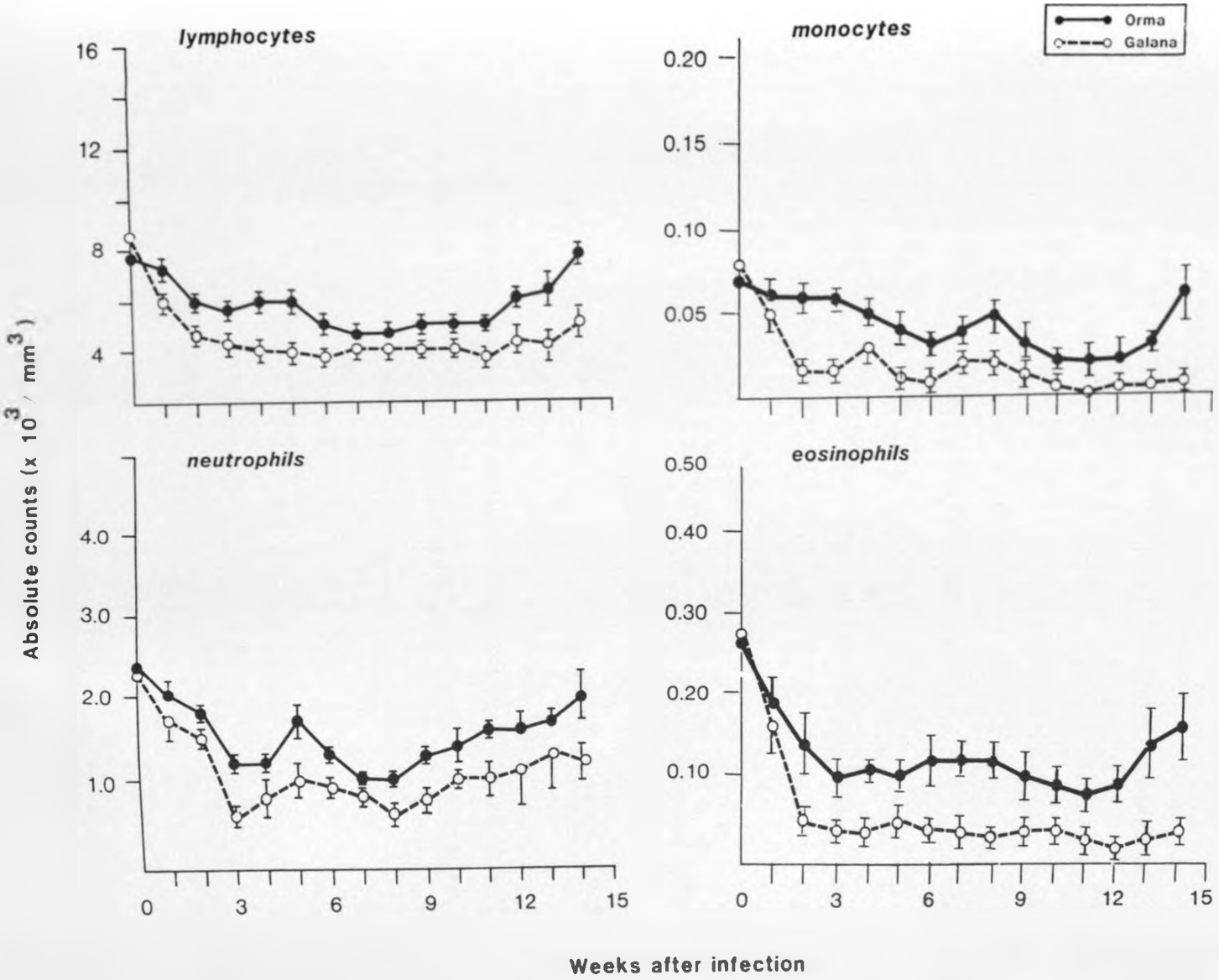
the study period, they had attained 85% of their initial value. Monocyte counts dropped progressively in the Galana and by the end of the study period, they had dropped by more than 90% of their initial count.

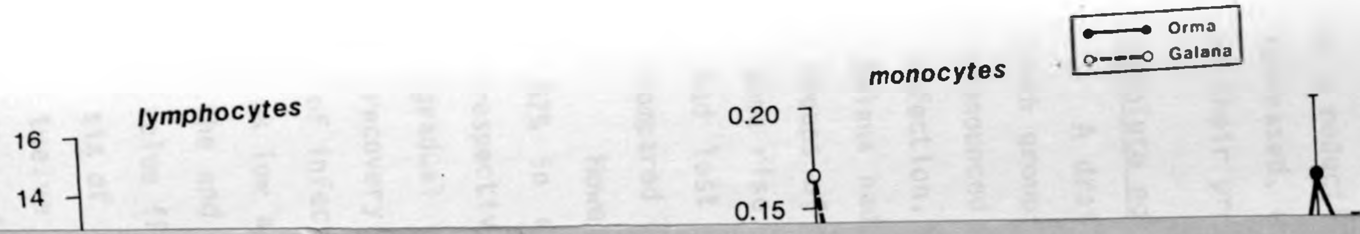
However, in infections initiated by tsetse fly bite, a loss of 95% in the monocyte counts was recorded in the Galana while a loss of 75% occurred in the Orma two weeks after infection (Fig. 10.B). The monocyte numbers in the Galana started recovering from week three to week eight of infection. Thereafter, the counts fluctuated within the low range and monocytes were not detectable on weeks 11 and 15 (Fig. 10.B). The monocytes in the Orma gradually increased from week three to week nine of infection when they reached 67% of the pre-infection value. Inspite of fluctuations, the monocyte counts had, by the end of the study period, attained 83% of the pre-infection value (Fig. 10.B).

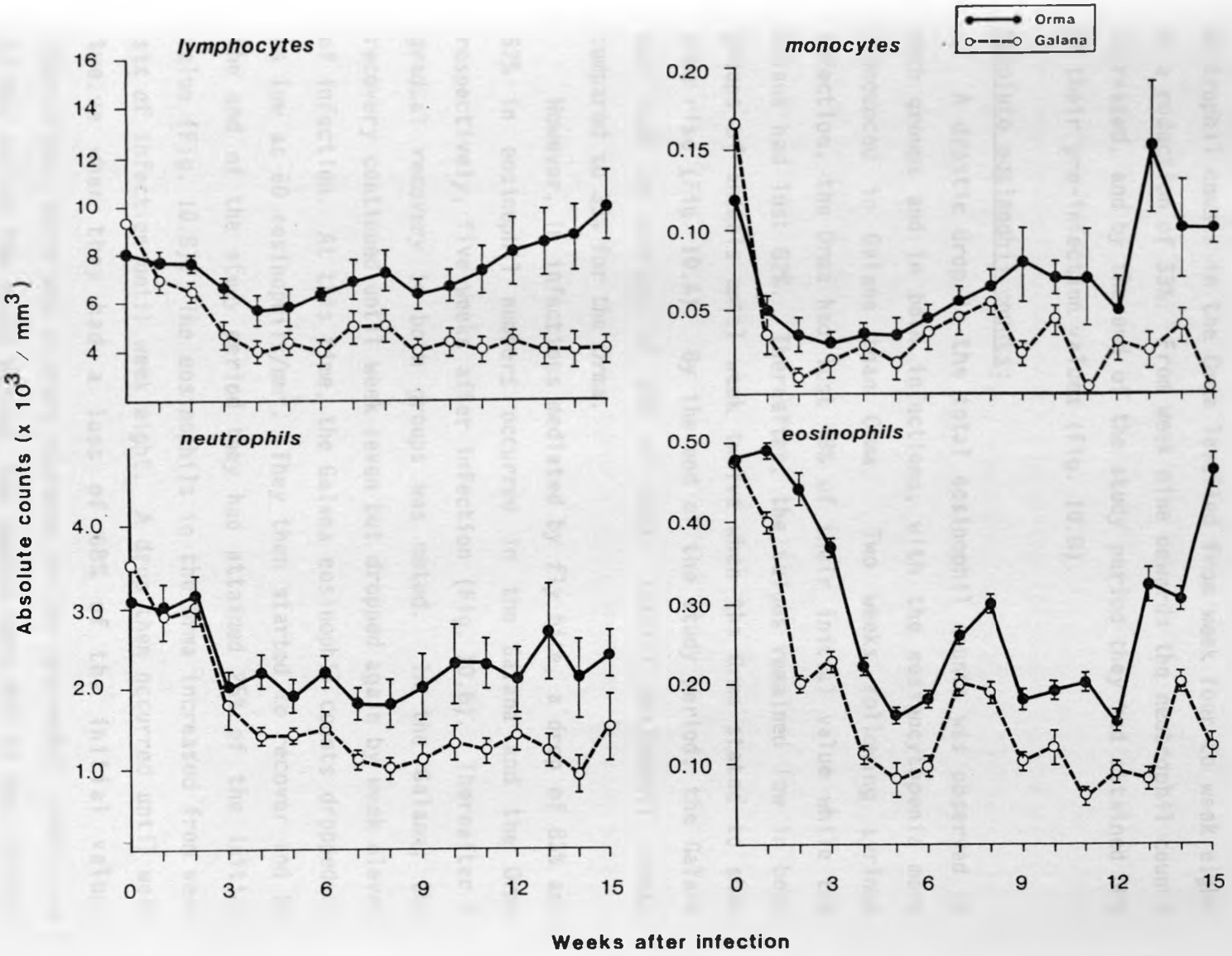
Absolute neutrophil counts:

Following syringe infection, the neutrophils in both groups steadily decreased until week three of infection. By this time, the Galana had lost 73% of their initial value while the Orma had lost 50%. Thereafter both groups fluctuated below the pre-infection values. By the end of the study period the Galana animals attained 50% of their initial value, compared with 83% in the Orma (Fig. 10.A).

Following infection induced by tsetse, the neutrophil counts dropped in both groups. Until week three of infection there was no significant difference between the two groups (Fig. 10.B). From week four onwards there was a significant difference between the two groups with Orma exhibiting higher counts than Galana. The neutrophil counts in the Galana animals continued to drop until week eight of infection when they were 75% of their initial values (Fig. 10.B). Thereafter, they fluctuated around a value 65% lower


lymphocytes
monocytes
neutrophils
eosinophils
Orma
Galana
Absolute counts (x 10^3 / mm^3)
Weeks after infection
16
12
8
4
0.20
0.15
0.10
0.05
4.0
3.0
2.0
1.0
0.50
0.40
0.30
0.20
0.10
0
3
6
9
12
15




lymphocytes
monocytes
neutrophils
eosinophils
Orma
Galana
Absolute counts (x 10³ / mm³)
Weeks after infection
16
14
12
10
8
6
4
0.20
0.15
0.10
0.05
4.0
3.0
2.0
1.0
0.50
0.40
0.30
0.20
0.10
0
3
6
9
12
15

than the initial value to the end of the study period. The neutrophil counts in the Orma levelled from week four to week eight at a reduction of 33%. From week nine onwards the neutrophil counts increased, and by the end of the study period they had attained 80% of their pre-infection values (Fig. 10.B).

Absolute eosinophil counts:

A drastic drop in the total eosinophil counts was observed in both groups and in both infections, with the eosinocytopenia more pronounced in Galana than Orma. Two weeks following syringe infection, the Orma had lost 48% of their initial value while the Galana had lost 82%. Thereafter, the values remained low in both groups of animals until week twelve when the Orma stated to show some rise (Fig. 10.A). By the end of the study period, the Galana had lost an average of 90% of their initial eosinophil counts compared to 35% for the Orma.

However, in infections mediated by fly bite, a drop of 83% and 67% in eosinophil numbers occurred in the Galana and the Orma respectively, five weeks after infection (Fig. 10.B). Thereafter a gradual recovery in both groups was noted. In the Galana, the recovery continued until week seven but dropped again by week eleven of infection. At this time, the Galana eosinophil counts dropped to as low as 60 eosinophils/mm^3. They then started to recover and by the end of the study period they had attained 25% of the initial value (Fig. 10.B). The eosinophils in the Orma increased from week six of infection until week eight. A drop then occurred until week twelve when they had a loss of 68% of the initial value. Thereafter, there was a sharp increase in the eosinophil counts and by the end of the study period, the counts were 96% of the initial values (Fig. 10.B).

4.3.1.4: NEUTRALIZING ANTIBODIES AGAINST *T. CONGOLENSE* (IL 1180) METACYCLICS:

The results of the neutralizing antibody activity against IL 1180 metacyclics during the first four weeks following infection induced by tsetse are shown in Table 3. All the Galana had neutralizing antibody activity during the third and fourth weeks of infection while in the Orma, neutralizing antibody activity was detected in seven out of ten animals in the second week of infection and in all animals as from the third week (Table 3).

4.3.1.5: BODY WEIGHT CHANGES

At the end of the study period in infections initiated by syringe inoculation, the Galana had a mean weight loss of 5.8±3.4 kg compared with a mean gain of 9.7±2.4 kg in the Orma. In infections initiated by tsetse, the Galana had an overall mean loss of 11.4kg compared with a mean gain of 7.9kg in the Orma over the same period.

Table 1A Time to patent parasitaemia in Galana and Orma steers after syringe infection with T. congolense (IL 1180)

Group	Days after infection											mean ± SE
Galana	4	4	4	4	4	4	4	4	4	4	4	4.7 ± 0.2
Orma	4	4	4	4	5	5	5	5	5	6	9	5.1 ± 0.4

Table 1B. Time to patent parasitaemia in Galana and Orma steers after being bitten by tsetse infected with T. congolense (IL 1180)

Group	Days after infection										mean ± SE
Galana	10	11	12	13	13	13	13	14	-	-	12.4 ± 0.5
Orma	12	12	12	13	13	13	14	14	14	14	13.1 ± 0.3

Table 2A Time to 50% drop in WBC in Galana and Orma Steers after syringe infection with T. congolense (IL 1180)

Group	Days after infection												mean ± SE
Galana	6	6	8	9	9	11	12	12	14	40	44	47	18.2± 4.5
Orma	17	18	18	19	19	44	47	54	82	100	100	100	51.5±10.2

Table 2B. Time to 50% drop in WBC in Galana and Orma steers after being bitten by tsetse infected with T. congolense (IL 1180)

Group	Days after infection										mean ± SE
Galana	13	15	15	16	17	18	19	21	-	-	16.8 ± 0.9
Orma	19	19	20	104	104	104	104	104	104	104	78.6 ±12.9

TABLE 3. Neutralizing antibody activity to *T. congolense* (clone 1180) *in vitro* propagated metacyclics in Galana and Orma steers following infected fly bite.

Animal No.	Type	No of mice protected by the Neutralizing activity of antibody Sera taken at week (after infection): 0	1	2	3	4
957		0/5	0/5	0/5	5/5	5/5
958		0/5	0/5	0/5	5/5	5/5
959		0/5	0/5	0/5	5/5	5/5
961	GALANA	0/5	0/5	0/5	5/5	5/5
965		0/5	0/5	0/5	5/5	5/5
966		0/5	0/5	0/5	5/5	5/5
967		0/5	0/5	0/5	5/5	5/5
969		0/5	0/5	0/5	5/5	5/5
970		0/5	0/5	5/5	5/5	5/5
971		0/5	0/5	0/5	5/5	5/5
973		0/5	0/5	0/5	5/5	5/5
974		0/5	0/5	5/5	5/5	5/5
975		0/5	0/5	5/5	5/5	5/5
977	ORMA	0/5	0/5	0/5	5/5	5/5
978		0/5	0/5	5/5	5/5	5/5
980		0/5	0/5	5/5	5/5	5/5
981		0/5	0/5	5/5	5/5	5/5
982		0/5	0/5	5/5	5/5	5/5

4.3.2: INFECTIONS WITH T. VIVAX

4.3.2.1: CLINICAL ASSESSMENT

(i): Prepatent period

Following syringe infection and infection induced by tsetse infected with T. vivax stock K2388 and stock K2589 respectively, all animals became parasitaemic after varying prepatent periods. All Galana animals became parasitaemic following syringe infection between days 9 and 23 of infection, while the Orma were patent between days 16 and 30 (Table 4.A).

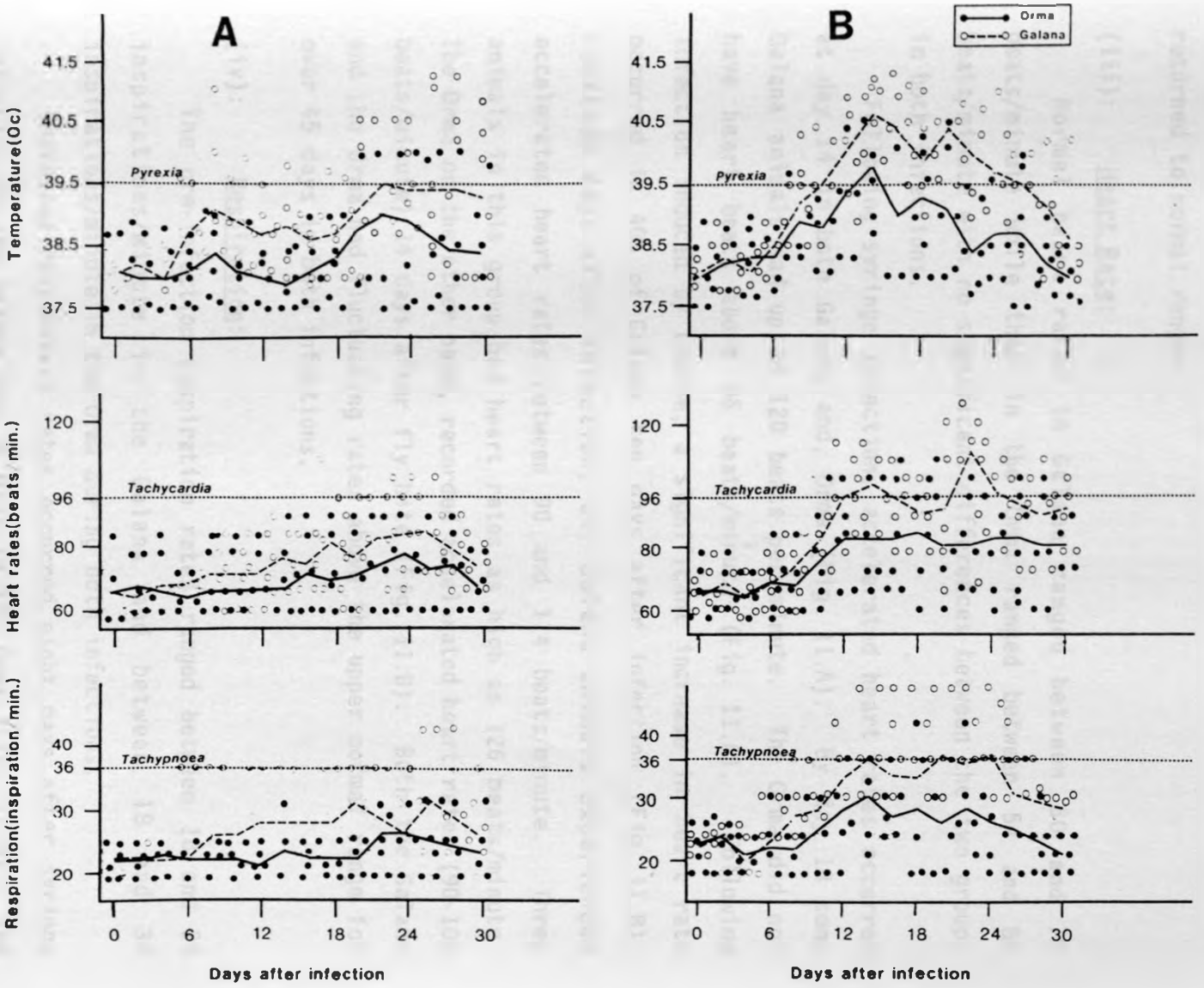
Following infection induced by tsetse, all the Galana became patent between nine and eleven days, while the Orma became parasitaemic between days ten and eleven (Table 4.B).

Statistical analysis using "t" test at a 5% significance level, indicated no significant differences in the prepatent periods in the Galana and Orma.

(ii): Temperature:

Variations in rectal temperatures were observed in all animals during the pre-infection periods in both infections but were not significantly different. Normal temperatures in the Galana ranged between 37.2^{o}C and 38.8^{o}C while that in the Orma ranged between 36.0^{o}C and 39.3^{o}C. By day eight after syringe infection, five Galana animals experienced temperatures above 39.5^{o}C, while only two Orma animals had temperatures of 39.5^{o}C (Fig. 11.A). However, both groups showed elevation of temperatures between days 21 and day 30.

Febrile reactions ($\geq$ 39.5^{o}C) were observed in two Galana, eight days after fly bite and in one Orma animal ten days after fly bite. All of the Galana group experienced fever with temperatures ranging between 39.5^{o}C and 41.5^{o}C. The Orma did not experience temperatures higher than 40.5^{o}C (Fig. 11.B). A significant rise in temperature occurred between day 10 and 22 after infection. This rise corresponded with the first peak of parasitaemia in both infections.


A
B
Orma
Galana
Temperature(Oc)
Heart rates(beats min.)
Respiration(inspiration min.)
Pyrexia
Tachycardia
Tachypnoea
41.5
40.5
39.5
38.5
37.5
120
96
80
60
40
36
30
20
0
6
12
18
24
30
Days after infection
Days after infection

Thereafter temperatures in the two groups in both infections returned to normal ranges.

(iii): Heart Rate:

Normal heart rates in Galana ranged between 60 and 78 beats/minute while that in the Orma ranged between 54 and 84 beats/minute with no significant differences between the two groups in both infections.

Following syringe infection, accelerated heart rates occurred at day 14 in both Galana and, Orma (Fig. 11.A). By day 18 some Galana animals had up to 120 beats per minute. The Orma did not have heart beats above 96 beats/minute (Fig. 11.A). Following infection induced by tsetse, a significant increase in heart rate occurred in 40% of Galana ten days after infection (Fig. 11.B). Fourteen days after infection, all Galana animals experienced accelerated heart rates between 90 and 114 beats/minute. Three animals in this group had heart rates as high as 126 beats/minute. The Orma on the other hand, recorded accelerated heart rates (90-102 beats/minute) 14 days after fly bite (Fig. 11.B). Both the Galana and the Orma had fluctuating rates above the upper normal range for over 45 days in both infections.

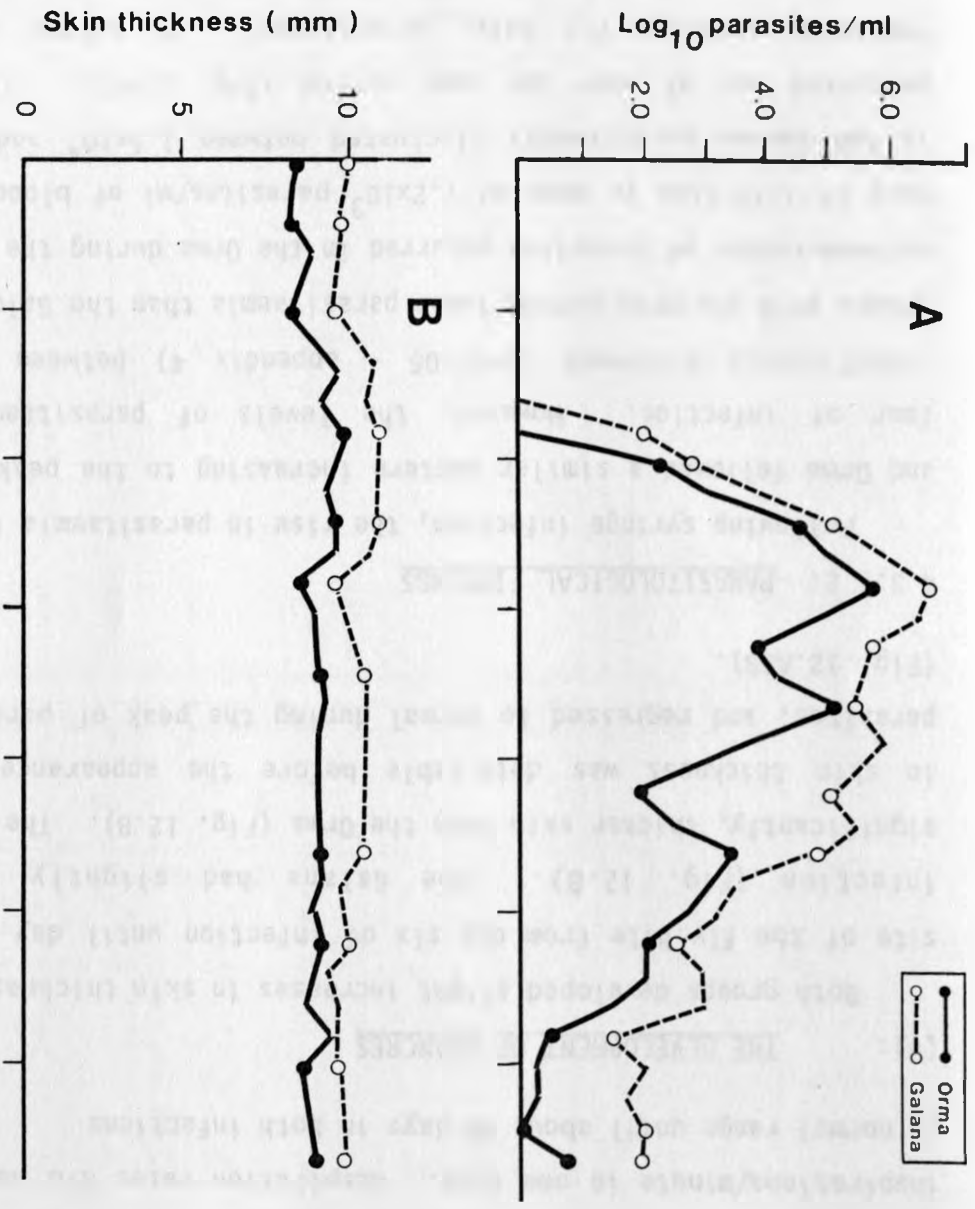
(iv): Respiration:

The pre-infection respiration rates ranged between 18 and 24 inspirations/minute in the Galana and between 18 and 30 inspirations/minute in the Orma during both infections.

Increased respiratory rates occurred eight days after syringe infection in the Galana and 14 days in the Orma (Fig. 11.A). The Galana had respiratory rates as high as 42 inspirations per minute but Orma did not exceed 36 inspirations per minute. Increased respiratory rates were more evident during the first peak of parasitaemia (i.e. 22-35 days post-infection). Increased respiratory rates between 36 and 42 inspirations/minute occurred in

FIGURE 12 :

Mean daily (A) parasitaemia and (A) skin thickness (chancre) in Orma and Galana steers after infection with *T. vivax* (stock 2589) induced by tsetse. 70b.


Log10 parasites/ml
2.0
4.0
6.0
A
Orma
Galana
Skin thickness (mm)
0
5
10
B
0
5
10
15
20
25
30
Days after infection

five Galana animals, twelve days after fly bite (Fig. 11.B). However, 36 inspirations/minute, were recorded in two Orma animals 14 days following infection. Four Galana animals had respiratory rates as high as 48 inspirations/minute while the maximum was 42 inspirations/minute in one Orma. Respiration rates did not return to normal range until about 40 days in both infections.

(v): THE DEVELOPMENT OF CHANCRES

Both groups developed slight increases in skin thickness at the site of the fly bite from day six of infection until day 13 after infection (Fig. 12.B). The Galana had slightly but not significantly, thicker skin than the Orma (Fig. 12.B). The increase in skin thickness was detectable before the appearance of the parasites, and regressed to normal during the peak of parasitaemia (Fig. 12.A&B).

4.3.2.2: PARASITOLOGICAL FINDINGS

Following syringe infection, the rise in parasitaemia in Galana and Orma followed a similar pattern increasing to the peak at week four of infection. However, the levels of parasitaemia were significantly different ($P<0.005$ - appendix 4) between the two groups with the Orma having lower parasitaemia than the Galana. The maximum number of parasites occurred in the Orma during the first 36 days of infection (a mean of 1.2×10^3 parasites/ml of blood), while in the Galana parasitaemia fluctuated between 1.5×10^4 and 1.3×10^5 parasites per ml over the same period (Fig. 13.AI). Similarly, following infected fly bite, parasitaemia in Galana and Orma increased progressively, reaching a peak two weeks after infection in the Orma and three weeks in Galana. The Galana peaked at a mean of 1×10^6 parasites/ml, while Orma peaked at 1.3×10^4 parasites/ml (Fig. 13.AI). The levels of parasitaemia were significantly different ($P< 0.005$ - appendix 4) between the two groups with Orma having lower parasitaemia.

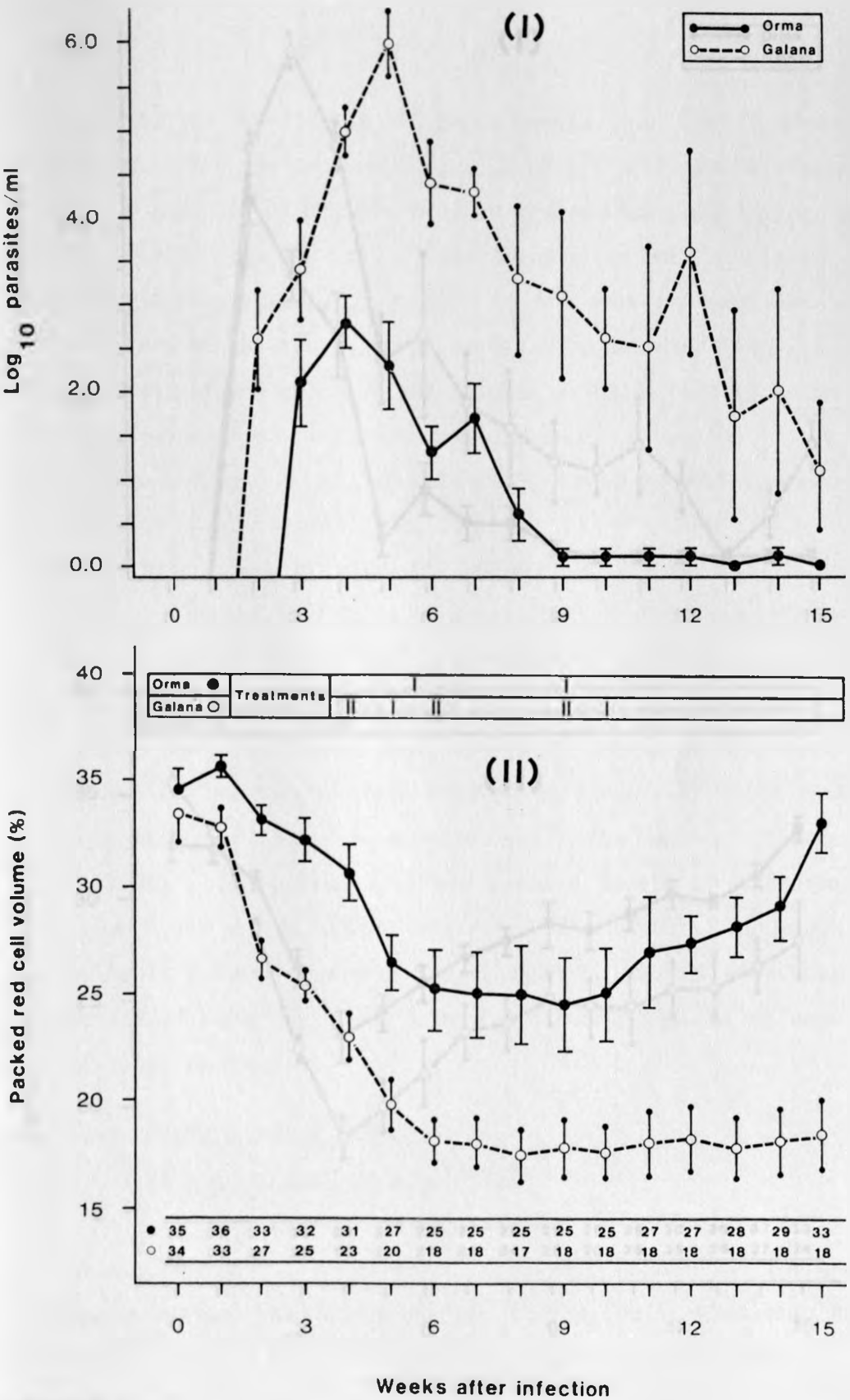


FIGURE 13.A :

Mean ± SE weekly parasitaemia (I) and packed red cell volume (II) in Orma and Galana steers following syringe inoculation with *T. vivax* (stock 2388). 71a.

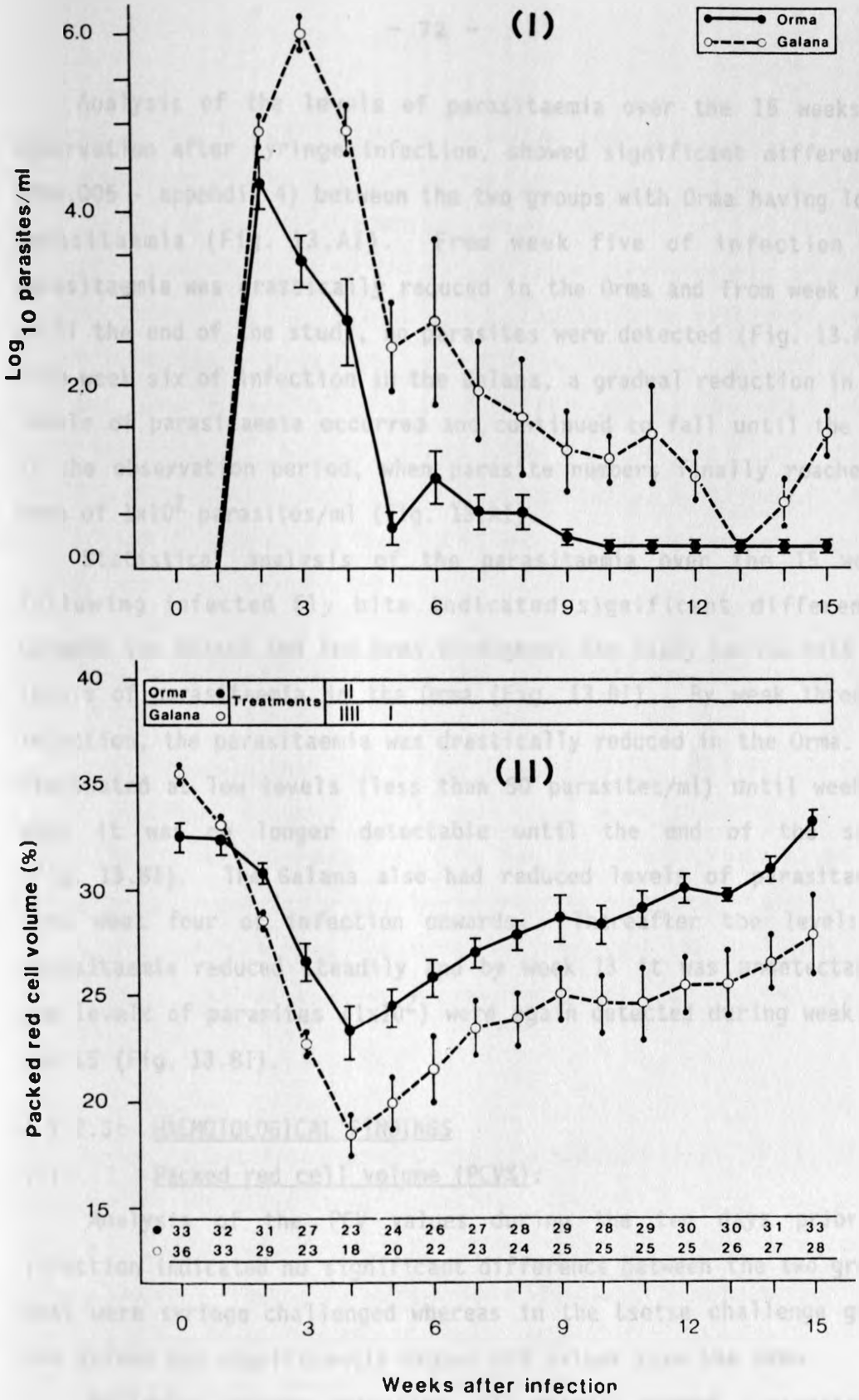


FIGURE 13.B :

Mean ± SE weekly parasitaemia (I) and packed red cell volume (II) in Orma and Galana steers after infection with T. vivax (stock 2589) induced by tsetse.

Analysis of the levels of parasitaemia over the 15 weeks of observation after syringe infection, showed significant differences ($P<0.005$ - appendix 4) between the two groups with Orma having lower parasitaemia (Fig. 13.AI). From week five of infection the parasitaemia was drastically reduced in the Orma and from week nine until the end of the study, no parasites were detected (Fig. 13.AI). From week six of infection in the Galana, a gradual reduction in the levels of parasitaemia occurred and continued to fall until the end of the observation period, when parasite numbers finally reached a mean of $1x10^2$ parasites/ml (Fig. 13.AI).

Statistical analysis of the parasitaemia over the 15 weeks following infected fly bite indicated significant differences between the Galana and the Orma throughout the study period with low levels of parasitaemia in the Orma (Fig. 13.BI). By week three of infection, the parasitaemia was drastically reduced in the Orma. It fluctuated at low levels (less than 50 parasites/ml) until week 10 when it was no longer detectable until the end of the study (Fig. 13.BI). The Galana also had reduced levels of parasitaemia from week four of infection onwards. Thereafter the levels of parasitaemia reduced steadily and by week 13 it was undetectable. Low levels of parasites ($1x10^2$) were again detected during weeks 14 and 15 (Fig. 13.BI).

4.3.2.3: HAEMOTOLOGICAL FINDINGS

(i): Packed red cell volume (PCV%):

Analysis of the PCV values during the ten days prior to infection indicated no significant difference between the two groups that were syringe challenged whereas in the tsetse challenge group the Galana had significantly higher PCV values than the Orma.

Following syringe infection, all animals showed a significant fall in PCV, with the fall being steeper in Galana than in Orma. By week three of infection there was already a significant difference between the two groups in the PCV values. A similar drop in PCV was

observed in both groups following infection induced by tsetse.

Comparison of the PCV of the two groups over the 15 weeks of study following syringe infection showed significant differences ($P<0.005$ - appendix 4) with the Galana being more severely affected than the Orma. The PCV in both groups continued to fall until week six of infection. Thereafter, the PCV in the Orma levelled until week ten, when it gradually started to rise and by the end of the study period, it had attained 94% of the initial value (Fig. 13.AII). In contrast, the PCV in the Galana levelled off at a mean of 18% from week six until the end of the experiment without showing any signs of recovery.

Analysis of the PCV values over the 15 weeks of observation following infected fly bite indicated a significant difference ($P< 0.005$ - appendix 4) between the two groups with the Orma developing less severe anaemia than the Galana (Fig. 13.BII). The PCV in both groups continued to drop until week four when there was a 50% reduction in the Galana and 30% in the Orma (Fig. 13.BII). Subsequently, there was a steady recovery in both groups and by the end of the study period the Orma had regained the pre-infection value while the Galana had gained 80% of the initial value (Fig. 13.BII).

(ii): Time to treatment

During infections mediated by bloodstream forms of T. vivax eight out of 12 Galana animals required trypanocidal drug treatment. This is compared to only two Orma that required treatment (Fig. 13.AII). Following exposure to tsetse fly infections, six Galana and two Orma animals required treatment (Fig. 13.BII).

If treatment is taken as an indication of death, then 67% of the Galana animals used in syringe infection and 60% of those used in fly infection would have died of trypanosomiasis, compared to a death of 20% in the Orma group in either of the infections.

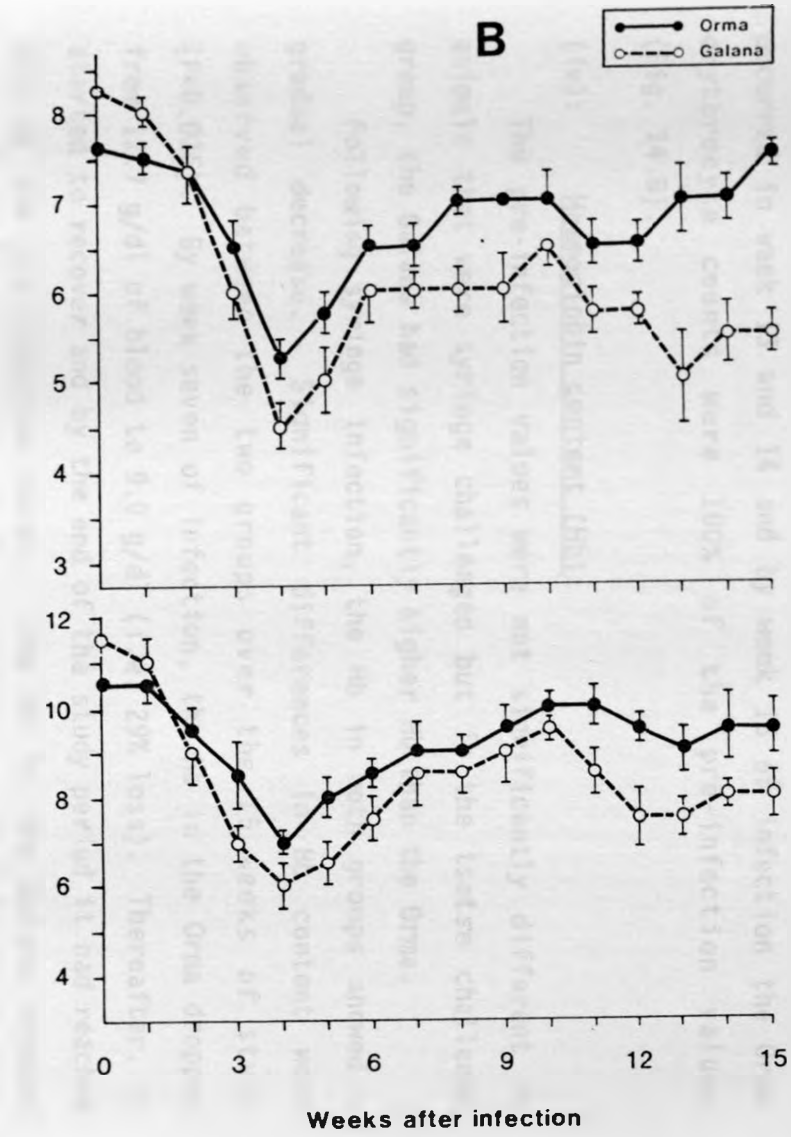
(iii): Red blood cell counts (RBC):

No significant difference was found prior to infection in those groups used for syringe infection, but in those infected with tsetse the Galana had significantly higher RBC counts than the Orma before infection.

Significant differences ($P<0.025$) between the two groups were observed during the 15 weeks of observation after syringe infection. The pattern of loss was gradual in the Orma and rapid in Galana (Fig. 14.A). RBCs in both groups continued to drop until week eight of infection, during which time the RBC counts in the Orma dropped to a minimum of $5.3x10^6/mm^3$ from an initial value of $8.1x10^6/mm^3$, while that in the Galana dropped to $3.5x10^6/mm^3$ from an initial value of $8.1x10^6/mm^3$ (Fig. 14.A). From week nine onwards, the Orma had a steady increase in the total RBC counts and at the end of the study, the RBC counts reached their pre-infection value. The RBCs in the Galana, also started to recover slightly from week nine onwards. By the end of the observation period they had increased from $3.5x10^6/mm^3$ to $4.5x10^6/mm^3$ (Fig. 14.A).

Following infected fly bite, both groups experienced a sharp drop in the total RBC counts which reached their minimum values at week four of infection. Statistical analysis of RBC counts over the 15 weeks of study indicated significant differences between the two groups ($P< 0.025$), with higher counts occurring in the Orma (Fig. 14.B). By this time, there was a loss of 46% of the initial RBC counts in the Galana, compared with a loss of 30% in the Orma. Thereafter, a gradual recovery in the numbers of RBC was observed in both groups. In the Galana the RBC counts gradually increased until week ten dropped until week 13 and levelled out at a cell count of $5.5x10^6/mm^3$ (a loss of 68% of the pre-infection value) until the end of the study period (Fig. 14.B). The RBC counts in the Orma continued to recover as from week five to week ten. They then dropped during weeks eleven and twelve. An increase in the count

A
Red blood cell count (x10^6/ mm^3)
8
7
6
5
4
3
Haemoglobin content (g/dl)
12
10
8
6
4
0
3
6
9
12
15
Weeks after infection


B
Orma
Galana
8
7
6
5
4
3
12
10
8
6
4
0
3
6
9
12
15
Weeks after infection

occurred in week 13 and 14 and by week 15 of infection the Orma erythrocyte counts were 100% of the pre-infection values (Fig. 14.B).

(iv): Haemoglobin content (Hb):

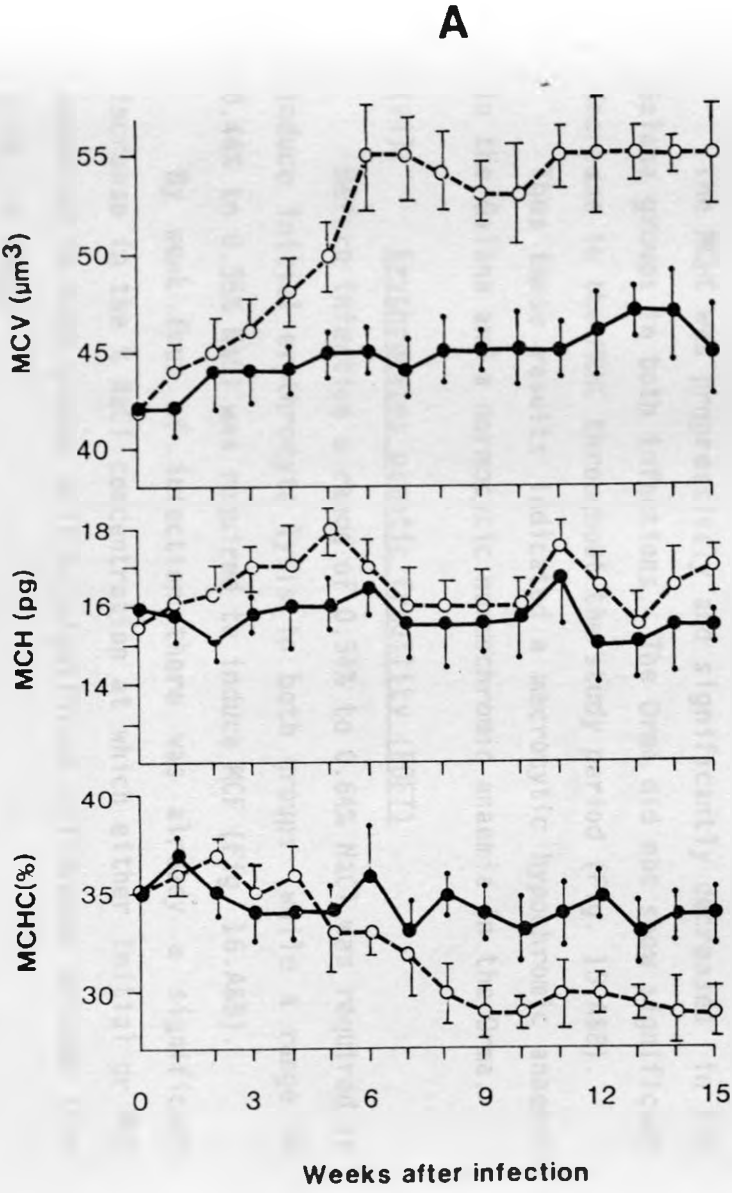
The pre-infection values were not significantly different in animals that were syringe challenged but in the tsetse challenge group, the Galana had significantly higher Hb than the Orma.

Following syringe infection, the Hb in both groups showed a gradual decrease. Significant differences in Hb content were observed between the two groups over the 15 weeks of study ($P<0.025$). By week seven of infection, the Hb in the Orma dropped from 12.7 g/dl of blood to 9.0 g/dl (i.e. 29% loss). Thereafter, it started to recover and by the end of the study period it had reached 91% of the pre-infection value. The Hb in the Galana dropped maximally at week from initial 12.5 g/dl to 7.0 g/dl (45% loss) (Fig. 14.A). By week ten of infection, the Hb in the Galana increased to 8.0 g/dl, and fluctuated around this level until the end of the study (Fig. 14.A).

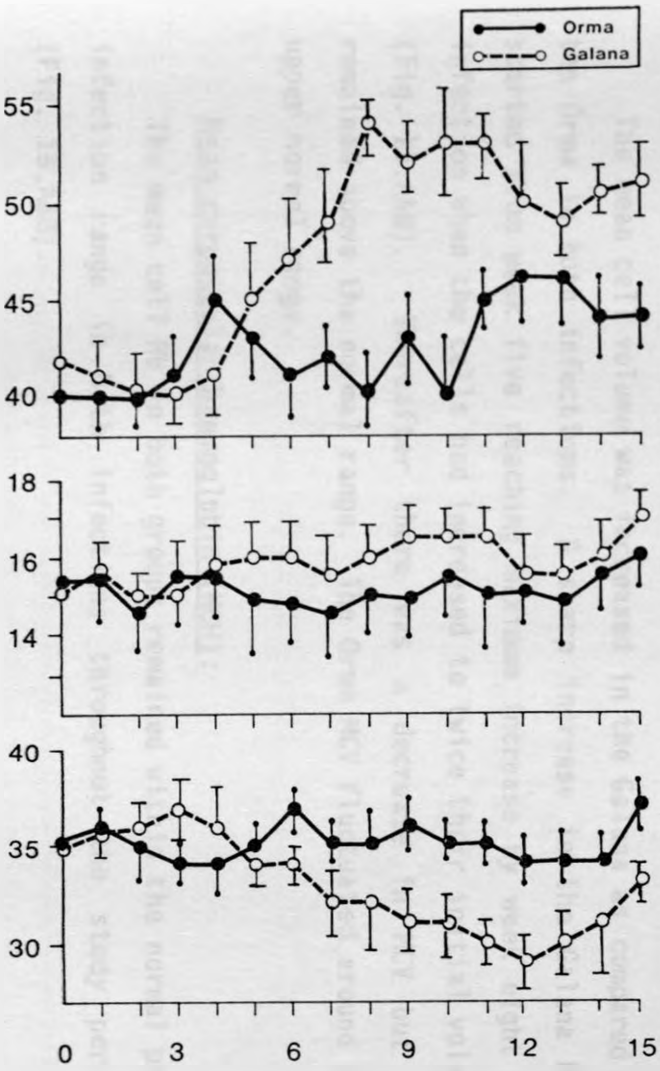
Following infection initiated by infected fly bite, the Hb content declined in both groups with maximum depression occurring at week four when the Galana and the Orma had a reduction of 45% and 30% respectively of their initial values. Statistical analysis of the Hb content over the 15 weeks of observation indicated significant differences between the two groups with the Orma having a higher Hb content than the Galana. A gradual recovery in the Hb content occurred in both groups from week five (Fig. 14.B). The Hb content in the Orma continued to recover and by the end of the study period it regained 90% of the pre-infection value. The Hb content in the Galana followed a similar pattern of gradual recovery to week ten of infection. Thereafter, it dropped again until the end of the observation period when it had not recovered more than 30% of its initial value (Fig. 14.B).

FIGURE 15 :

Mean ± SE weekly MCV, MCH and MCHC in Orma and Galana steers after infection with *T. vivax* by (A) syringe (stock 2388) and (A) tsetse (stock 2589). 75a.


A
MCV (µm3)
55
50
45
40
MCH (pg)
18
16
14
MCHC(%)
40
35
30
0
3
6
9
12
15
Weeks after infection

B


Orma
Galana
55
50
45
40
18
16
14
40
35
30
0
3
6
9
12
15
Weeks after infection

(v): Erythrocyte indices

Mean corpuscular volume (MCV):

The mean cell volume was increased in the Galana as compared to the Orma in both infections. A sharp increase in the Galana MCV started from week five reaching maximum increase by week eight of infection when the cells had increased to twice their initial volume (Fig. 15.A&B). Thereafter there was a decrease in MCV but it remained above the normal range. The Orma MCV fluctuated around the upper normal range.

Mean corpuscular haemoglobin (MCH):

The mean cell Hb in both groups remained within the normal pre-infection range in both infections throughout the study period (Fig. 15.A&B).

Mean corpuscular haemoglobin concentration (MCHC):

The MCHC was progressively and significantly decreased in the Galana groups in both infections. The Orma did not show significant decrease in the MCHC throughout the study period (Fig. 15.A&B).

Thus these results indicated a macrocytic hypochromic anaemia in the Galana and a normocytic normochromic anaemia in the Orma.

(vi): Erythrocytes osmotic fragility (EOFT)

Before infection a range of 0.54% to 0.64% NaCl was required to induce initial erythrocyte lysis in both groups while a range of 0.44% to 0.55% NaCl was required to induce MCF (Fig. 16.A&B).

By week four of infection there was already a significant increase in the % NaCl concentration at which either initial or MCF occurred in both groups with no significant difference between them (Fig. 16.A&B).

However, maximum fragility (both initial lysis and MCF)occurred five weeks after infection and continued at that high level to week nine. Thereafter, the erythrocytes gradually regained their normal osmotic resistance (Fig. 16.A&B).

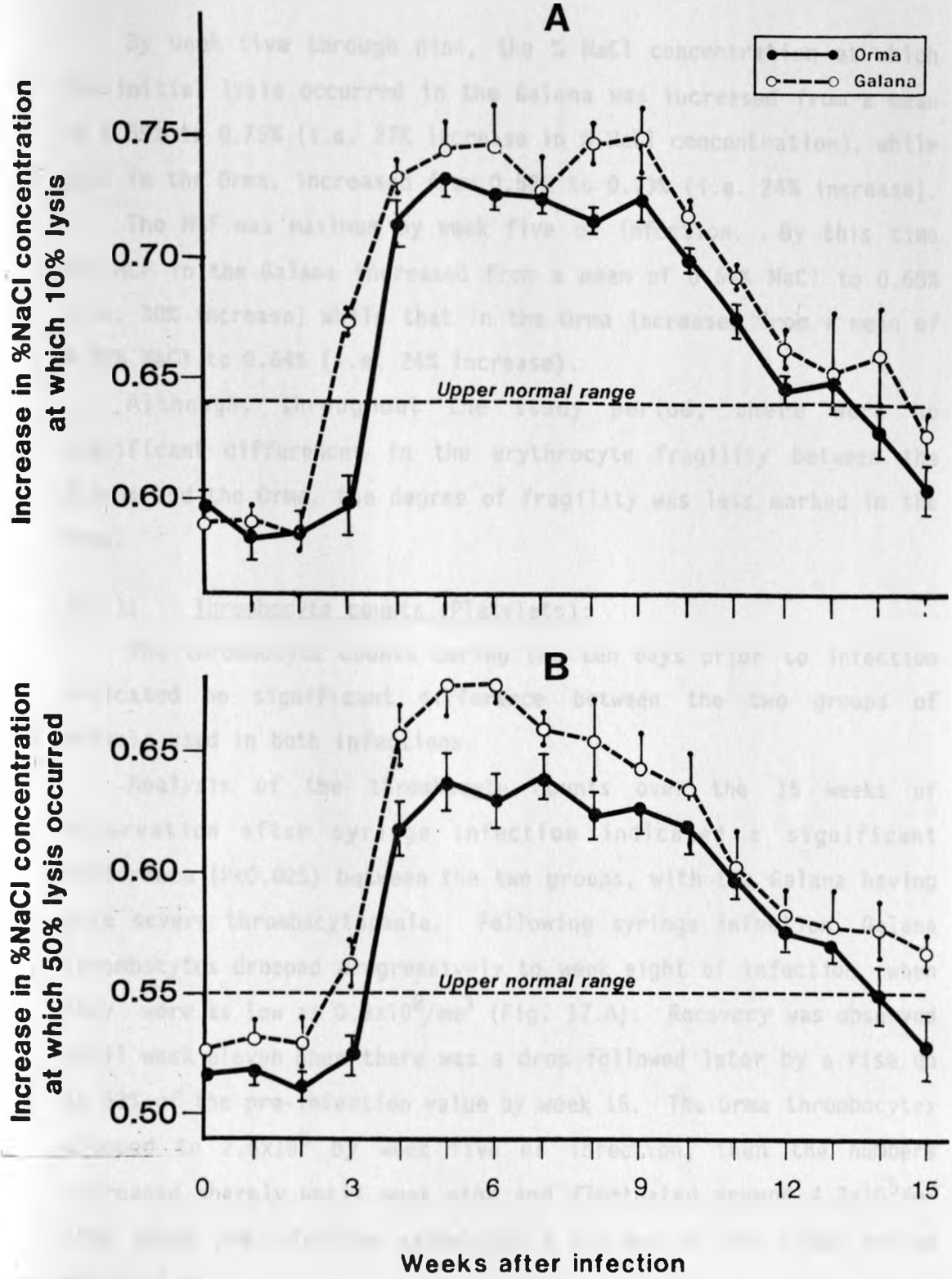


FIGURE 16 : Mean ± SE weekly increase in % NaCl concentration at which (A) initial lysis and (A) MCF occurred in Galana and Orma steers after infection with T. vivax (stock 2589) induced by tsetse. 76a.

By week five through nine, the % NaCl concentration at which the initial lysis occurred in the Galana was increased from a mean of 0.59% to 0.75% (i.e. 27% increase in % NaCl concentration), while that in the Orma, increased from 0.59% to 0.73% (i.e. 24% increase).

The MCF was maximum by week five of infection. By this time the MCF in the Galana increased from a mean of 0.54% NaCl to 0.68% (i.e. 30% increase) while that in the Orma increased from a mean of 0.52% NaCl to 0.64% (i.e. 24% increase).

Although, throughout the study period, there were no significant differences in the erythrocyte fragility between the Galana and the Orma, the degree of fragility was less marked in the Orma.

(vii): Thrombocyte counts (Platelets):

The thrombocyte counts during the ten days prior to infection indicated no significant difference between the two groups of animals used in both infections.

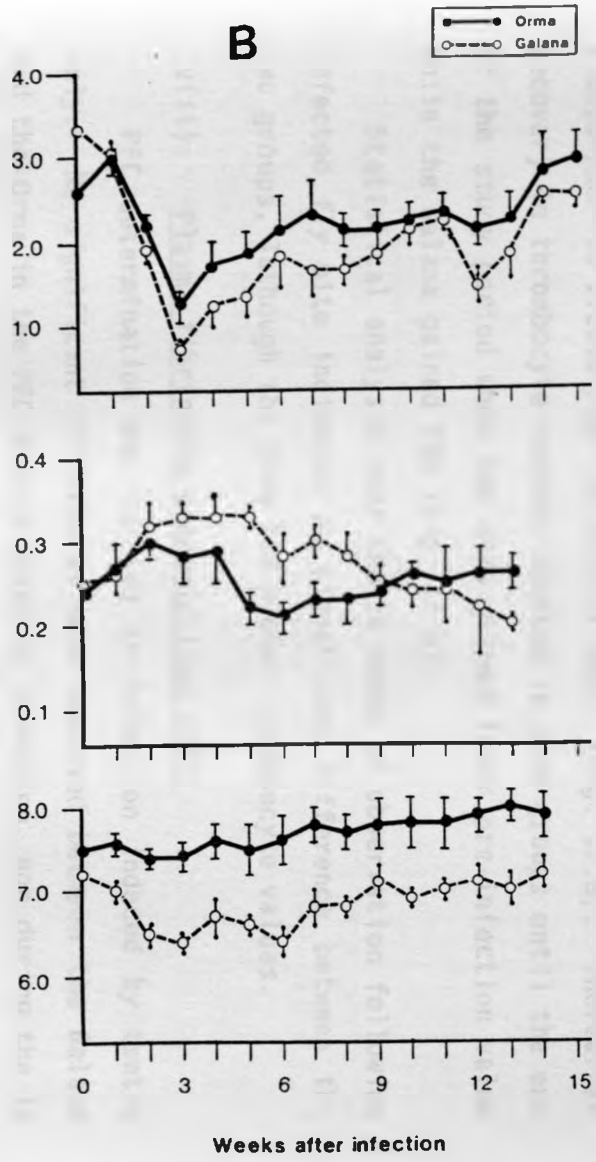
Analysis of the thrombocyte counts over the 15 weeks of observation after syringe infection indicated a significant difference ($P<0.025$) between the two groups, with the Galana having more severe thrombocytopenia. Following syringe infection, Galana thrombocytes dropped progressively to week eight of infection when they were as low as $0.8 \times 10^8/mm^3$ (Fig. 17.A). Recovery was observed until week eleven when there was a drop followed later by a rise up to 53% of the pre-infection value by week 15. The Orma thrombocytes dropped to 2.6×10^8 by week five of infection, then the numbers increased sharply until week nine and fluctuated around $4.2 \times 10^5/mm$ (20% above pre-infection value) until the end of the study period (Fig. 17.A).

Following infection induced by tsetse, both groups experienced severe thrombocytopenia most marked in the third week (Fig. 17.A). By this time, the Galana thrombocyte counts had decreased from

FIGURE 17 :

Mean ± SE weekly thrombocyte counts, PFC and PPC in Orma and Galana steers after infection with *T. vivax* by (A) syringe (stock 2388) and (B) tsetse (stock 2589). 77a.

A
Platelet counts (x10^5/ mm^3)
4.0
3.0
2.0
1.0
PPC (g/dl)
8.0
7.0
6.0
0
3
6
9
12
15
Weeks after infection
Platelet counts (x10^5/ mm^3)
PFC (g/dl)
PPC (g/dl)


B
Orma
Galana
4.0
3.0
2.0
1.0
0.4
0.3
0.2
0.1
8.0
7.0
6.0
0
3
6
9
12
15
Weeks after infection

$3.3x10^5/mm^3$ to $0.07x10^5/mm^3$ (or a loss of 98% of the initial thrombocyte counts), while counts in the Orma were reduced from $2.6x10^5/mm^3$ to $1.2x10^5/mm^3$ (a loss of 54%) (Fig. 17.B). Thereafter recovery in thrombocyte numbers started in both groups until the end of the study period when the Orma gained their pre-infection value while the Galana gained 75% (Fig. 17.B).

Statistical analysis over the 15 weeks of observation following infected fly bite indicated no significant difference between the two groups, lathough the Orma had higher thrombocyte values.

(viii): Plasma fibrinogen concentration (PFC)

PFC determination was carried in infection induced by tsetse only. No significant differences were observed between the Galana and the Orma in the PFC values prior to infection, nor during the 15 weeks study period.

Following infection, both groups had an increase in PFC until week four. By week two of infection, the PFC in the Galana increased from a concentration of 350mg/100ml of blood to 430mg/100ml (23% increase) and maintained that level until week five. Thereafter, PFC gradually returned to the pre-infection concentration (Fig. 17.B). Similarly, the PFC in the Orma increased from 340mg/100ml to 400mg/100ml (12% increase), until week four of infection. Thereafter, it fluctuated within the normal range until the end of the study (Fig. 17.B).

(ix): Plasma protein concentration (PPC)

Measurements of the total plasma protein concentration following infection with bloodstream forms indicated no significant increase from the pre-infection value. Both groups experienced a slight increase from the pre-infection value but they fluctuated within the normal range (Fig. 17.A). The Orma had higher PPC values than the Galana throughout the study period.

Similarly the PPC values before infections initiated by fly

bite were not significantly different between Galana and Orma animals. Following infection, the PPC in the Galana dropped in week three of infection when it was 10% less than the initial levels (Fig. 17.B). Statistical analysis indicated a significantly higher difference in Orma than Galana over the 14 weeks of study.

(x): Plasma protamine paracoagulation test (PST)

The results of PST carried out in this study are shown in Table 5. No fibrin monomers (FM) were detected before infection. There was a direct relationship between the intensity of the test and the levels of parasitaemia. Plasma samples from week two to week five of infection indicated the presence of FM in both groups. Samples collected between week three and four in both groups showed the highest FM levels which coincided with the maximum number of parasites detected (Table 5).

With the decrease in parasitaemia, the number of positives and the intensity of the test were also decreased and by week ten of infection onwards all the Orma were negative for PST which also coincided with the apparent disappearance of parasites from the blood. The test was negative in the Galana from week eleven onwards.

(xi): White blood cell counts (WBC)

There was no significant difference in the total WBC counts during the pre-infection period in animals that were syringe infected with bloodstream forms of *T. vivax*. In animals infected through fly bite, the Galana had higher total WBC counts before infection. The onset of leukopenia was rapid in both groups during the first four weeks of infection. Following syringe infection, a 50% drop in the Galana WBC counts occurred within 23.8 days while in the Orma it occurred after 54.7 days (Table 6.A). A similar 50% drop in WBC counts occurred ten days in Galana and 32 days in the Orma after infected fly bite (Table 6.B). By week four after

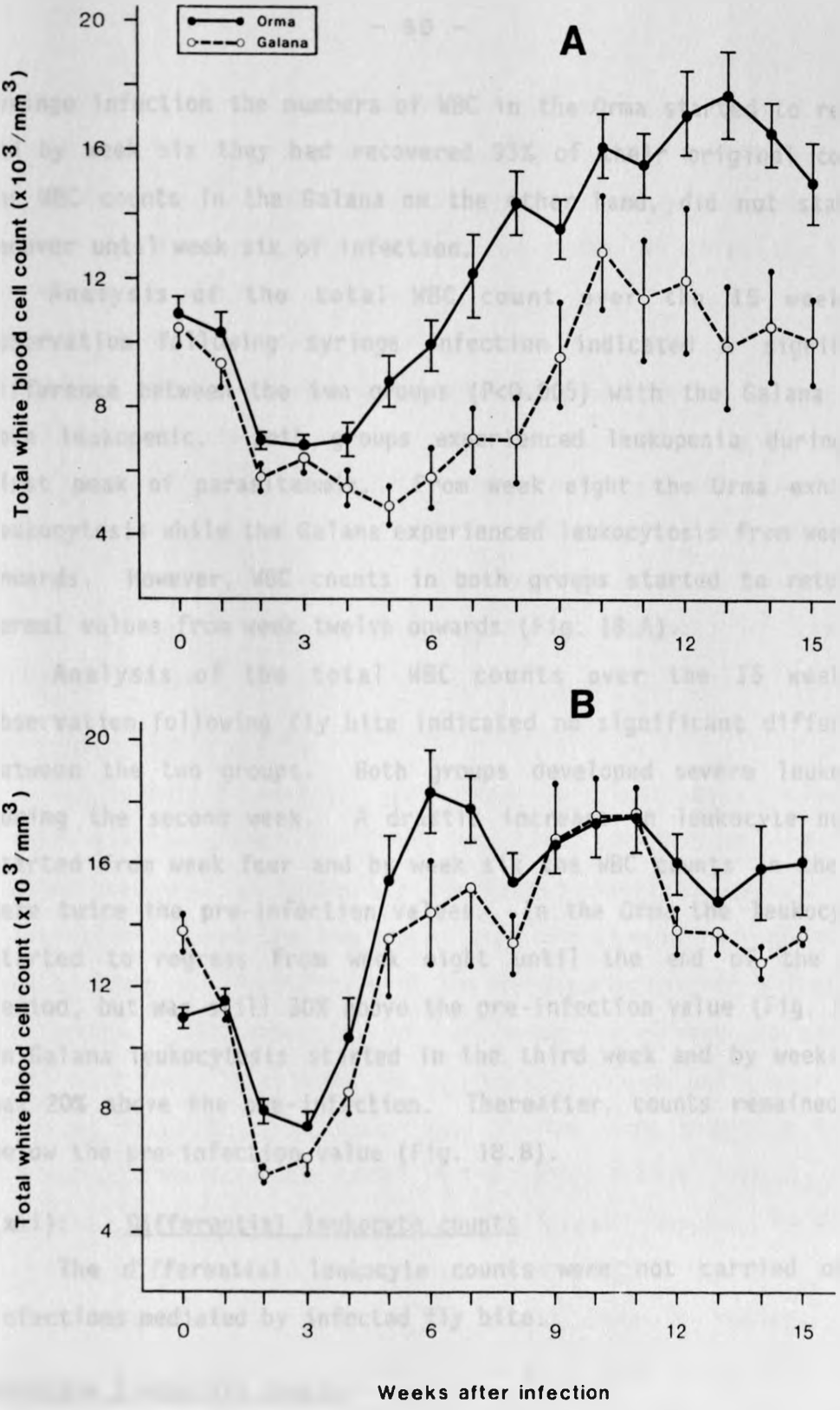


FIGURE 18 : Mean ± SE total WBC counts in Orma and Galana steers after infection with *T. vivax* by (A) syringe (stock 2388) and (A) tsetse (stock 2589). 79a.

syringe infection the numbers of WBC in the Orma started to recover and by week six they had recovered 93% of their original counts. The WBC counts in the Galana on the other hand, did not start to recover until week six of infection.

Analysis of the total WBC count over the 15 weeks of observation following syringe infection indicated a significant difference between the two groups ($P<0.005$) with the Galana being more leukopenic. Both groups experienced leukopenia during the first peak of parasitaemia. From week eight the Orma exhibited leukocytosis while the Galana experienced leukocytosis from week ten onwards. However, WBC counts in both groups started to return to normal values from week twelve onwards (Fig. 18.A).

Analysis of the total WBC counts over the 15 weeks of observation following fly bite indicated no significant differences between the two groups. Both groups developed severe leukopenia during the second week. A drastic increase in leukocyte numbers started from week four and by week six the WBC counts in the Orma were twice the pre-infection values. In the Orma the leukocytosis started to regress from week eight until the end of the study period, but was still 30% above the pre-infection value (Fig. 18.B). In Galana leukocytosis started in the third week and by weeks 9-11 was 20% above the pre-infection. Thereafter, counts remained just below the pre-infection value (Fig. 18.B).

(xii): Differential leukocyte counts

The differential leukocyte counts were not carried out in infections mediated by infected fly bite.

Absolute lymphocyte counts:

There was no significant difference between the two groups in the absolute values of lymphocytes prior to infection. The lymphocyte counts followed a pattern similar to that of the total WBC counts.

Over the 15 weeks of observation, there was a significant difference ($P<0.005$) between the two groups in their lymphocyte counts with the Orma having higher counts. Both groups experienced lymphocytopenia during the first three weeks of infection. The Lymphocytes in the Orma decreased by 28% during the first two weeks following infection compared to a decrease of 43% in the Galana. Thereafter, the Orma lymphocytes remained unchanged until week four and then started to increase, exceeding pre-infection level by week 13. Thereafter, they started to return to the pre-infection value. The number of lymphocytes in the Galana continued to drop until week five of infection. Recovery, began at week six, exceeding pre-infection level by week ten, and gradually returning to normal as from week twelve (Fig. 19.A).

Absolute monocyte counts:

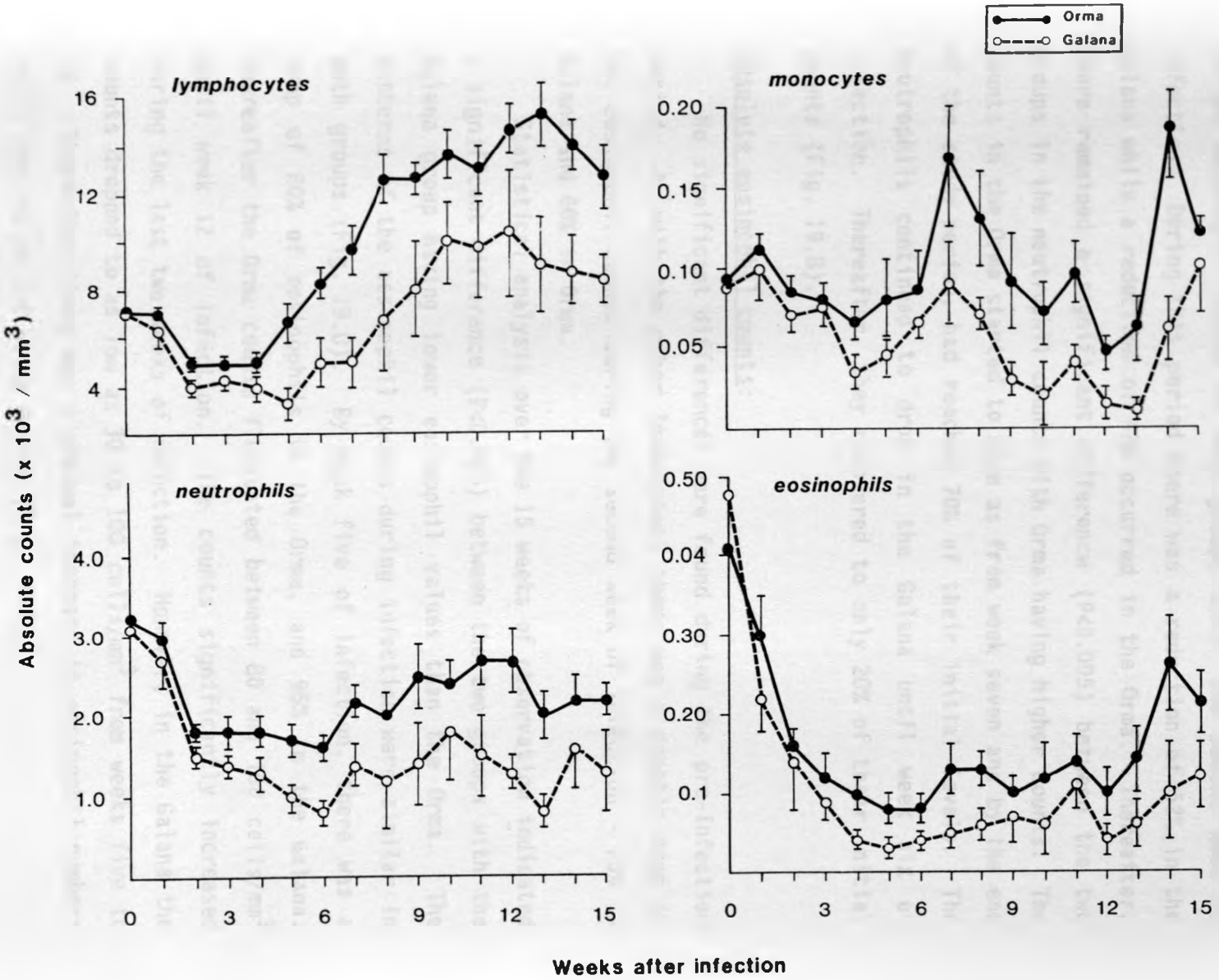
Pre-infection analysis indicated no significant difference between the two groups. Following infection, both groups experienced similar patterns of gradually decreasing monocyte counts. The differences between the two groups over the 15 weeks of observation were significant ($P<0.025$), with the Orma having higher values than the Galana. The monocytes in both groups continued to decrease until week four, then there was a gradual increase until week seven with the increase being more rapid in the Orma than the Galana. Thereafter, the monocyte number in the Galana dropped sharply and by week ten they were not detectable in the blood. However, by week 14 the counts had reached the pre-infection value. The Orma values, though reduced, were consistently higher than the Galana. The lowest value reached was 0.6×10^2 from an initial value of 0.9×10^2. Normal values were reached by the end of the study period (Fig. 19.C).

Absolute neutrophil counts:

The pre-infection values were not significantly different

FIGURE 19 :

Mean ± SE weekly absolute counts of lymphocytes, monocytes, neutrophils and eosinophils in Orma and Galana steers following syringe infection with *T. vivax* (stock 2388). 81a.


Orma
Galana
lymphocytes
monocytes
neutrophils
eosinophils
Absolute counts (x 10³ / mm³)
Weeks after infection
16
12
8
4
0.20
0.15
0.10
0.05
4.0
3.0
2.0
1.0
0.50
0.04
0.30
0.20
0.1
0
3
6
9
12
15

between the two groups. Following infection, there was a sharp drop in the neutrophil counts in both groups until the second week of infection. During this period there was a reduction of 52% in the Galana while a reduction of 42% occurred in the Orma. Thereafter, there remained a significant difference ($P<0.005$) between the two groups in the neutrophil counts with Orma having higher counts. The counts in the Orma started to rise as from week seven and by the end of the study period, had reached 70% of their initial levels. The neutrophils continued to drop in the Galana until week six of infection. Thereafter, they recovered to only 20% of their initial counts (Fig. 19.B).

Absolute eosinophil counts:

No significant differences were found during the pre-infection period. As with the other leukocytes, there was a drastic drop in the eosinophil counts during the second week of infection - 75% in Galana and 60% in Orma.

Statistical analysis over the 15 weeks of observation indicated a significant difference ($P<0.025$) between the two groups with the Galana group having lower eosinophil values than the Orma. The patterns of the eosinophil counts during infection were similar in both groups (Fig. 19.D). By week five of infection, there was a drop of 80% of eosinophils in the Orma, and 95% in the Galana. Thereafter the Orma counts fluctuated between 80 and 160 cells/mm^3 until week 12 of infection. The counts significantly increased during the last two weeks of infection. However, in the Galana the counts dropped to as low as 30 to 100 cells/mm^3 from weeks five to 12. Thereafter there was a gradual increase in eosinophil numbers until the end of the study period (Fig. 19.D).

4.3.2.4: BODY WEIGHT CHANGES

Throughout the study period the Galana lost weight in both infections, while the Orma gained weight. At the end of the study

period, in infections initiated by bloodstream forms, the Galana had a mean loss of 13.6±3.6 kg, while the Orma had a mean gain of 3.7±7.1 kg. However, in infections initiated by infected fly bite, the Galana had a mean loss of 9.2±5.6 kg, while the Orma had a mean gain of 8.9±6.8 kg.

Table 4.A Time to patent parasitaemia in Galana and Orma steers after syringe infection with T. vivax (Stock K2388)

Group	Days after infection	mean ± SE
Galana	9 9 10 16 17 17 20 20 21 22 23 23	17.3 ± 1.5
Orma	16 18 19 19 20 21 22 22 25 30 - -	21.2 ± 1.3

Table 4.B Time to patent parasitaemia in Galana and Orma steers after being bitten by tsetse infected with T. vivax (stock K2589)

Group	Days after infection	mean ± SE
Galana	9 9 10 10 10 10 10 10 10 11	9.9 ±0.18
Orma	10 10 10 10 10 10 10 11 11 11	10.3 ±0.15

Table 5 Results of Protamine Sulphate Paracoagulation Test (PST) in Galana and Orma steers following infection with T. vivax (stock 2589) by tsetse.

		WEEKS POST CHALLENGE															
Breed	Grade	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
GALANA	1	0	0	1	7	6	2	0	0	2	2	0	0	0	0	0	0
	2	0	0	0	6	3	1	0	0	0	0	1	0	0	0	0	0
	3	0	0	1	3	1	0	0	0	1	0	0	0	0	0	0	0
	4	0	0	2	4	2	2	0	0	1	1	0	0	0	0	0	0
	5	0	0	0	13	8	1	0	0	1	2	0	0	0	0	0	0
	TOTAL	0	0	4	33	20	6	0	0	5	5	1	0	0	0	0	0
ORMA	1	0	0	1	4	7	3	0	0	0	2	0	0	0	0	0	0
	2	0	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0
	3	0	0	2	2	1	1	0	0	0	0	0	0	0	0	0	0
	4	0	0	2	2	6	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	4	4	1	0	0	1	1	0	0	0	0	0	0
	TOTAL	0	0	5	14	19	5	0	0	1	4	0	0	0	0	0	0

Table 6.A Time to 50% drop in total WBC in Galana and Orma steers after syringe infection with T. vivax (Stock K2388)

Group	Days after infection												mean ± SE
Galana	8	8	9	14	17	20	21	21	24	27	35	82	23.8± 5.8
Orma	20	29	30	32	32	35	54	103	103	103	-	-	54.1±10.9

Table 6.B Time to 50% drop in total WBC in Galana and Orma steers after being bitten by tsetse infected with T. vivax (stock K2589)

Group	Days after infection										mean ± SE
Galana	8	9	10	10	10	10	10	11	12	12	10.2 ± 0.4
Orma	11	12	14	14	14	15	15	16	103	103	31.7 ±11.9

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1: EXPERIMENTAL INFECTIONS WITH *T. CONGOLENSE*

The pre-infection data showed that the Orma have wider normal ranges of temperature, heart rate and respiration than the Galana. The physiological significance of this is not clear, but similar observations for N'Dama cattle were noted by Grieg and McIntyre (1979). However, Murray, *et al.* (1984) related tolerance to partly reduced physiological susceptibility to the "effects of infection", and proposed a number of factors such as the ability to utilize food, tolerate heat and conserve water, which might aid the survival of these animals.

Following exposure to *T. congolense* infection, whether initiated by bloodstream forms or metacyclics, there were no significant differences between the two groups of animals in the prepatent periods. N'Dama and Zebu cattle exposed to syringe infection (Murray, *et al.*, 1979b) or to natural tsetse infection (Murray, *et al.*, 1981a), also showed no significant differences in the prepatent periods and the time taken to reach the first peak of parasitaemia.

A rise in temperature (39.5^{o}C and above) was observed one to two days before parasite detection. This is similar to the findings of Valli, Forsberg and Robinson, (1978) who recorded fever in calves infected with *T. congolense*, one to two days before parasite detection. Although not statistically significant, more Galana animals experienced febrile episodes, tachycardia and tachypnoea as compared with the Orma. The heart rates and respirations in both groups of animals were significantly elevated during the acute phase of the disease (first parasitaemic peak). The increased heart rates and respiration were maintained above normal values for long periods even when parasites were low in numbers. Although heart beats and

respirations were elevated, they remained rhythmic.

Pyrexia, tachycardia and tachypnoea were found to be the main clinical manifestations during the first peak of parasitaemia. Fever persisted during the high levels of parasitaemia which also coincided with maximum destruction of blood cells. It has been shown that, following phagocytosis of parasites by neutrophils, monocytes or cells of the reticuloendothelial system (RES), some endogenous pyrogens are released (Dinarello, 1979). Sufficient concentrations of these pyrogens alter the concentration of amines in the hypothalamus and thus stimulate the thermo-regulatory centres and lead to elevation of body temperature. In trypanosomiasis the reasons for elevated temperature have not been investigated. A similar phenomenon may be operating since high fever is only detected during high parasitaemias.

The persistence of elevated respiration and heart rates when parasitaemia was significantly low, is not immediately explainable. Because of the decrease in red cell numbers, there is a decrease in oxygen carrying capacity and thus a probable stimulation of respiration and heart rates. The concentration of intra-erythrocytic organic phosphates such as 2,3 diphosphoglycerate (2,3 DPG), was were found to influence the oxyhaemoglobin dissociation (Tenney, 1977). An increase in DPG would retard the unloading of oxygen in the tissues and thus create some sort of tissue hypoxia. This stimulates, via the central nervous system (CNS), a demand for oxygen which will be manifested by increased respirations and heart rates. Whether these disturbances participate in the observed persistence of elevated respiration and heart rates is not known.

Following infection induced by tsetse, chancres developed five days later and reached maximum size 12 to 14 days later. All infected animals experienced such chancres with varying degrees of intensity and size. The average maximum increase in skin thickness

in Galana was 60% above normal, while that of the Orma was 40%. Parasites were detected in the peripheral blood during the rising phase of the chancre. These results were inconsistent with the findings of Akol and Murray (1982) who only detected parasites in the peripheral blood when the chancres had started to regress.

Chancre development following infected fly bite has been observed in a number of experimental situations (Akol and Murray, 1982; Luckins, Rae and Gray, 1983; Dwinger, et al., 1986). It has been demonstrated that resistant animals develop less severe and smaller chancres than susceptible animals. The finding that, eland and buffalo failed to develop chancres after 19 bites of infected tsetse while cattle with fewer bites developed chancre, led to the suggestion that trypanotolerant animals developed less severe and smaller chancres than susceptible animals (Dwinger et al., 1986). The chancre was therefore looked upon as a quick way to identify resistant animals. It appears from the results presented here that chancre development is not a reliable indicator of resistance. Furthermore the chancre does not seem to play any role in limiting the development and the subsequent establishment of parasitaemia.

In the present study significant differences in the levels of parasitaemia emerged between Galana and Orma. Once parasitaemia was established in the Galana, it persisted until treatment. In contrast, the Orma significantly reduced and controlled parasitaemia. Thus, towards the end of the study period, 50% of the Orma infected by syringe inoculation and 90% of those exposed to fly infection, "self cured" as judged by the disappearance of the parasites from the peripheral blood and the steady recovery of the PCV. "Self cure" was not observed in the Galana animals. The findings for the Orma are similar to those reported for N'Dama cattle (Chandler, 1952, 1958; Murray, et al., 1979a&b; Murray, et al., 1981a; Paling, Moloo and Scott, 1987 - submitted). In all these studies, the prevalence, levels and duration of parasitaemia

were significantly lower in N'Dama than Zebu. Comparative studies carried out on West African shorthorn also gave similar results (Stewart, 1951; Roberts and Gray, 1973b; Roelants, et al., 1983; Pinder et al., 1984). In the present study, the Orma was significantly less susceptible to T. congolense infections than the Galana. However, Murray et al. (1981a) presented some evidence that N'Dama cattle were more resistant than Zebu to infections with T. vivax and T. brucei but there were no differences in susceptibility to infection with T. congolense. Recent studies showed that naive N'Dama calves infected with T. congolense were significantly more resistant than age matched Boran calves (Paling, et al., 1987, submitted).

The onset and the degree of anaemia, as indicated by the decrease in PCV, were earlier and more severe in the Galana than the Orma. As a consequence of low PCV, 75% of the Galana in the first experiment, and 38% in the second experiment were treated. This is compared with 17% and 10% respectively in the Orma group.

The Galana animals which had higher levels of parasitaemia developed more severe anaemia, supporting previous observations made by Murray, et al. (1984), Jenkins and Facer (1985) and Holmes (1987), that the degree of anaemia is closely associated with the development and levels of parasitaemia. That the degree of anaemia was directly attributed to the higher and more persistent levels of parasitaemia was also confirmed by Dargie, et al. (1979) in their erythrokinetic and ferrokinetic studies. No difference was found between N'Dama and Zebu in the erythropoietic response.

Ideally resistant animals should have the capacity to control and reduce parasitaemia, to show control of anaemia, to be able to gain weight while infected and require fewer trypanocidal drug treatments. In the present study, loss in body weight, due to trypanosomiasis was more obvious in the Galana than the Orma. Thus, during the present study, the Galana had a net loss in body weight,

while the Orma had a net gain. Studies in West Africa have similarly indicated that trypanotolerant animals exposed to either syringe infection or infection following natural fly exposure, have the capacity to reduce parasitaemia, control anaemia and gain weight (reviewed by Murray and Trail, 1984).

A significant fall in red cell numbers and haemoglobin content was observed in both groups of cattle, and the fall followed similar patterns to the drop observed in the PCV. The drop in the RBC numbers was more rapid and significantly greater in the Galana than the Orma. Recovery of the red cell numbers was observed in the Orma (week 10 of infection) but not in the Galana. Radioisotope studies have revealed that the principal factor causing anaemia was an accelerated red cell loss from the circulation (reviewed by Dargie, 1980; Holmes, MacAskill, Jennings and Urquhart 1982). The spleen was found to be the major site for red cell sequestration and destruction. There is increase in the transit time of RBC in the enlarged spleen during infection, thus exposing them to deleterious factors such as low pH, low glucose and cholesterol levels. These may interfere with Na^+ pump activity, induce spherocytosis and increase RBC fragility and their premature phagocytosis (Prof. V. Anosa, Pers. Comm.; Valli, Forsberg and McSherry, 1978, Anosa and Isoun, 1980; Facer, Crosskey, Clarkson and Jenkins, 1982).

In the present study, the Galana had a macrocytic hypochromic anaemia, while the Orma had a macrocytic normochromic anaemia with a tendency towards normocytic. There have been conflicting reports in the morphologic classification of anaemia caused by cattle trypanosomiasis. It has been classified morphologically as macrocytic normochromic by Naylor (1971b) and as normocytic normochromic by others (Wellde, Lotzsch, Deindl, Sadun, Williams and Warui, 1974; Saror, 1979).

Both Jenkins, McGrorie, Forsberg and Brown, (1980) and Facer, et al. (1982) observed that the increase in MCV correlated

significantly with the increase in reticulocytes, as evidenced by the increased levels of pyruvate kinase, an enzyme which is present in young red cells, which would indicate the recruitment of young cells, and they described anaemia as regenerative in nature. This is supported by the peripheral blood RBC morphological features such as anisocytosis, polychromasia and basophilic stippling, and bone marrow erythroid hyperplasia (Saror, 1979; Valli, Forsberg and Lumsden, 1979; Valli and Mills, 1980), which are indicative of early recruitment of young cells and thus anaemia in trypanosomiasis is described as regenerative, at least in the early stages.

A significant increase in osmotic fragility of red cells was observed in the present studies. The erythrocyte fragility developed during the declining phase of the first peak of parasitaemia (week five) and was maximum during the chronic stage (week ten), which coincided with the maximum increase in MCV. This suggests that the cells increased in volume, becoming more spherical and therefore more fragile. No significant differences were observed between Galana and Orma although the Orma were less severely affected. Measurements of the initial red cell lysis (10% lysis) and the median corpuscular fragility (50% lysis) were shown to follow similar patterns, indicating a uniform effect on the red cells. Increased fragility during trypanosomiasis infection was also reported by Naylor (1971a&b) and Valli and Mills (1980) in cattle infected with T. congolense and in mice infected with T. congolense or T. brucei (Ikede, Lule and Terry, 1977).

In the case of intracellular parasites, the causes of increased osmotic fragility are readily understood. Kreier, Seed, Mohan and Pfister, (1972) suggested that the large Plasmodium gallinaceum and P. berghei mechanically distort the erythrocyte membrane, thus causing permanent structural damage to the cell which renders it more fragile. Similar membrane alterations were observed by Wright (1973) and Dolan (1974) in Babesia infections in cattle. However,

the reasons for increased fragility during trypanosomiasis infections are not known, but the possibility of pharmacological substances such as proteases, hydrolases, lipases and free fatty acids (Tizard, Nielsen, Seed and Hall, 1978; Tizard, Mellors and Nielsen, 1980), being liberated from the trypanosomes and damaging the red cell membrane cannot be overlooked.

The reasons for the observed gradual recovery in red cell osmotic resistance remains unclear, especially in the Orma group which had very low levels of parasitaemia ($< 10^2$) by week 10 of infection and showed a significant recovery of red cell numbers. Cattle erythrocytes were shown to have a life span of 120-150 days (Schalm, et al., 1975), and to have a slow response to demand (Valli, et al., 1978). Thus, if the trypanosomes have any direct or indirect effect on the red cells, it is possible that the observed slowness in overcoming the fragility is because of the deformed population of cells which were only slowly removed from the circulation.

Significant thrombocytopenia occurred during the first peak of parasitaemia in both groups, and thrombocyte counts were low until week ten of infection. This result agrees with the findings of Wellde, Kovatch, Chumo and Wykoff, (1978) and Maxie, Losos and Tabel, (1979), although the thrombocytopenia observed in this study was less severe than that reported by Wellde, et al. (1978) in T. congolense infected cattle.

A protamine sulphate paracoagulation test (PST) was employed in the present study to detect the presence of fibrin monomers (FM) in the plasma, but it gave negative results throughout the study period. These results were contrary to those obtained by Wellde, et al. (1978), who found a positive protamine test, extended partial thromboplastin times and lowered fibrinogen level in cattle infected with T. congolense. The reasons for the discrepancy in results are not clear, but may be related to the mode of infection since Wellde

et al. (1978) infected their animals by syringe. PST has not been tested in any syringe infected animal. The need for the test was not realized until long after the syringe infection experiments had been carried out.

Fibrinogen levels were only increased during the first peak of parasitaemia in both groups, which coincided with the significant thrombocytopenia. However, little is known about the changes of fibrinogen during trypanosomiasis infection in cattle. In man, elevated levels were reported during T. gambiense infection (Greenwood and Whittle, 1976). Thus no conclusions could be drawn from the present observation as the evidence for its degradation into FDP/FM complexes is not available, as the PST was negative throughout the study period.

Significant leukopenia was observed during the first peak of parasitaemia which occurred concurrently with the development of anaemia. The total WBC count in the Orma group dropped to a maximum of 30% of the pre-infection values while that of the Galana dropped to 60% of the pre-infection value. Such concurrent reduction in the total WBC counts and the onset of anaemia has been documented by many authors (Fiennes, Jones and Lewis, 1946; Naylor, 1971b; Wellde, et al., 1974; Valli, et al., 1978 & 1979, Valli and Mills, 1980). Leukopenia in the Orma group persisted until week eight of infection. Thereafter, the leukocyte numbers recovered steadily until the end of the study period. These results were similar to those reported by Anosa and Isoun (1980) who found that the maximum drop in the total WBC counts of sheep and goats infected with T. vivax took place during the acute phase of the disease. In contrast, the Galana remained leukopenic throughout the study period in both experiments. However, the WBC recovery in the Orma coincided with a reduction in the levels of parasitaemia.

Panleukopenia occurred in both groups of animals during the first three weeks of infection. Contrary to the findings of Valli

and Mills (1980), the eosinophils were the most depressed followed by the lymphocytes and monocytes. The neutrophils were significantly affected during the first peak of parasitaemia (week 2-4) but they were within the normal range during the chronic phase of the disease (week 12-15). Using monoclonal antibodies with specificities for bovine leukocyte subsets, Ellis, Scott, MacHugh, Gettinby and Davis, (1987) were also able to show that leukopenia occurred in N'Dama and Boran cattle two weeks following infections initiated by fly bite. They also showed that lymphocytopenia was due to depressed numbers of T-cells, B-cells and Null cells. It was also observed that there were variations, but no significant differences in the overall cell numbers of the monocytes, neutrophils or eosinophils. Leukocytic response in N'Dama was found to be mainly due to the lymphocyte population. In the Orma the leukocytic response occurred in all cell types but the eosinophils took a much longer time to recover compared to other cell types.

It has been observed during trypanosomiasis that the granulocytes were the first cellular components to be noticeably affected (Valli, et al., 1979) and that calves infected with T. congolense showed reduced ability to mount inflammatory responses during the onset of anaemia (Valli, et al., 1979). This may be a contributing factor in the susceptibility of infected animals to intercurrent infections, since granulocytic cells usually comprise the first line of defense against pathogens (Thomson, 1978). Reasons for the preferential depletion of granulocytic cells, apart from being consumed in the defense mechanisms, are not clear. However, Valli and Mills (1980), postulated that since granulocyte depletion occurs at a time when there is a concurrent anaemia, thrombocytopenia and a demand for lymphocytes, it is possible that "the rate limiting step in haemopoiesis is stem cell differentiation, and thus competition between stem cells of various cell lines may occur, with differentiation to granulocytes being

most affected". The depressive action of trypanosome infection upon granulocytes was indicated by the findings that serum from cattle infected with T. congolense or T. vivax depressed myeloid colony formation in vitro (Kaaya, Tizard, Maxie and Valli, 1980).

Sera collected from animals infected by tsetse fly when used to neutralize in vitro propagated metacyclics of the same parasite, showed neutralizing activity by two weeks after infection in the Orma and three weeks in the Galana. These observations were similar to those made by Kamanga-Sollo, Musoke, Nantulya and Masake, (in preparation) in N'Dama and Boran cattle infected with the same parasite stock. However, further immunological studies would have required the use of an antigenically stable population. Unfortunately the clone used in the syringe infections had varied from the original clone type and consequently further immunological studies were not possible.

5.2: EXPERIMENTAL INFECTIONS WITH *T. VIVAX*

As in *T. congolense* infections, the Orma exhibited a wide range of normal temperature, heart rate and respiration. The possible physiological significance of this has been discussed above.

In this study, the animals showed higher temperatures (41.5^{o}C), heart rates (126 beats/minute) and respiration rates (48 inspirations/minute), than in *T. congolense* infections. More Galana animals experienced febrile episodes, tachycardia and tachypnoea as compared to Orma. It was also observed that, unlike *T. congolense* infections, a significant rise in temperature ($\geq$ 39.5^{o}C) was observed two days before parasite detection. This suggests that the *T. vivax* stocks used in the present study caused more acute infection than *T. congolense*. Like in *T. congolense* infections, there were no significant differences between Galana and Orma in the prepatent periods in infections initiated with either bloodstream forms or metacyclics.

Following fly bite, no significant increase was observed in skin thickness. Similarly, Dwinger *et al.* (1986) using stocks of *T. vivax* isolated from the same area, found no chancres after fly bite. However, when they used stocks from West Africa, there was a significant development of chancres. Similar development of chancres following infections with the West African stocks of *T. vivax* was reported by Emery *et al.* (1980) and Emery and Moloo (1981). The chancres were much smaller when compared with those induced by *T. brucei* or *T. congolense* infections (Emery and Moloo, 1981). Chancres produced by *T. vivax* have the main cellular infiltrates composed of lymphocytes and macrophages with a very small contribution of the polymorphonuclear leukocytes (PMN), while those produced by *T. brucei* or *T. congolense*, showed a substantial influx of PMN followed by lymphocytes and macrophages (Emery and Moloo, 1981). However, tsetse flies are known to secrete very few metacyclic of *T. vivax* (Otieno and Darji, 1979) and it was shown

that these few metacyclics disappear from the skin within a few days (Emery et al., 1980). This could be the reason why T. vivax (East African stocks) do not produce chancres or if produced (West African stocks), it is of a smaller size compared to T. brucei or T. congolense.

Significant differences in the levels of parasitaemia were observed between the two groups. Following establishment of the parasites in the peripheral blood, parasitaemia progressively increased. At the height of the first peak, the Orma had a significantly lower number of parasites than the Galana. Infection with either of the two stocks (K2388 or K2589) appeared to produce an acute disease, especially in the Galana, and thus more than 60% were treated within 20-40 days of infection. In the Orma group, although they had a sharp rise in parasitaemia over the same period, they seemed to limit the early parasite growth better than the Galana. Acute infections in cattle caused by T. vivax stocks in East Africa have been previously reported by Hudson (1944), Lewis (1949), Losos and Ikede (1972) and Mwongela et al. (1981).

In the present study, 80% of the Orma survived infection with either of the two stocks. From week nine, the animals were judged to have "self cured" following the disappearance of the parasites and the recovery of the PCV. This "self cure" was not observed in the surviving Galana animals. However, unlike the infections produced by T. congolense where Galana had a sustained parasitaemia until the end of the study period, the surviving Galana significantly reduced their parasitaemia and those infected with stock K2589 also showed a steady recovery in PCV. These findings are similar to those reported by Murray et al. (1981a) who found that N'Dama had greater capacity to control T. vivax infections than Zebu, and with those of Dwinger et al. (1986) in buffaloes and eland.

As in infections with T. congolense, the onset and degree of anaemia was earlier and more severe in the Galana than the Orma. The development of anaemia followed different patterns in infections caused by the two stocks. Stock K2388 produced a sharp drop in the PCV of the Galana which levelled off at a value of 18% until the end of the study period, while the PCV in the Orma remained above 25%. Stock K2589 produced a more acute disease, with a sharp initial PCV reduction in both groups which coincided with the peak of parasitaemia. Thus the T. vivax stocks employed caused a more acute disease than the infections caused by the stock of T. congolense. Studies with West African stocks of T. vivax in goats, sheep and Zebu indicated that animals were anaemic within two weeks of infection (Clarkson, 1968, Anosa and Isoun, 1976, Saror et al., 1981). The possible factors involved in the causes of anaemia have been discussed under the section dealing with T. congolense and are not thought to be fundamentally different in T. vivax infections.

Furthermore, significant reductions in the red cell numbers were observed in the two groups with the Galana more severely affected. In infection with stock K2388, recovery of the red cell numbers was observed in Orma only, while in infection with stock K2589 recovery was observed in both groups with the rate of recovery of red cell numbers being faster in the Orma.

The anaemia caused by infection with T. vivax, whether initiated by syringe inoculation or by infected fly bite, showed morphological types similar to those which resulted from T. congolense infections. The increase in MCV in the Galana correlated with a maximum drop in PCV. Thus infections with T. vivax in the Galana resulted in a macrocytic hypochromic type of anaemia, while that in the Orma was normocytic normochromic.

A significant increase in the red cell fragility occurred much earlier and was of higher magnitude than that induced by T. congolense infections. By week 4 of infection, when the Galana

RBC had become significantly more fragile, the animals had a 50% reduction in the PCV with a similar reduction in the absolute red cell numbers. Furthermore, the extent and duration of the increased red cell fragility was greater in infections caused by T. vivax than in those caused by T. congolense.

Both stocks of T. vivax used in the present study produced a slight, non-significant increase in total plasma protein concentration. Studies with West African stocks (Clarkson, 1968; Anosa and Isoun, 1976) and with an East African stock (Tabel, Losos and Maxie, 1980) have indicated an increase in PPC. It is interesting to note that in the present study, the Orma had a consistently higher normal PPC values than the Galana. The significance of this remains to be investigated.

A profound decrease in thrombocyte numbers inversely correlated with an increase in the levels of parasitaemia. These results are consistent with those of Wellde et al. (1983) who used the same stock of the parasite (stock K2589). It was observed in the present study, that the thrombocyte numbers steadily recovered as the levels of parasitaemia decreased. This was observed clearly in the Orma group, where the thrombocytes attained normal values immediately following the disappearance of the parasites from the blood. However, Wellde et al. (1974) and Davis, Robbins, Weller and Braude, (1974) both found normal numbers of megakaryocytes in bone marrow aspirates during infection and, consequently, Wellde et al. (1974) hypothesized that the suppression of thrombocyte numbers is possibly due to a slow down in the budding or release of thrombocytes from the megakaryocytes.

Thrombocytopenia in trypanosomiasis is brought about by many factors, one of which is thrombocyte pooling, sequestration and destruction in the spleen as evidenced by ^{51}Cr-labelling studies (Preston, Kovatch and Wellde, 1982; reviewed by Meyers, 1985). Davis et al. (1974) showed that thrombocyte aggregation and

destruction occurred when trypanosomes or suppernatents from lysed trypanosomes were added.

In T. vivax infections, unlike T. congolense, positive results of the protamine sulphate paracoagulation test (PST) were obtained. The presence of fibrin monomers (FM) was detected maximally in weeks 3-4 of infection. This coincided with the maximum levels of parasitaemia, maximum increase in plasma fibrinogen concentration (PFC) and most severe thrombocytopenia. It is proposed that procoagulation stimulus of thrombocyte aggregation accompanying high parasitaemia could promote intravascular coagulation. This would result in the formation of FDPs/FM complexes and hence a positive PST (Boreham and Facer, 1974). Impairment of haemostasis, as evidenced by prolonged bleeding times, elevated levels of fibrinogen and FDPs early during haemorrhagic T. vivax infections was reported by Wellde et al. (1983) and Olubayo and Mugera (1985).

In the present study, animals infected with T. vivax developed severe thrombocytopenia, elevated FM levels (PST positive) and increased levels of PFC during the acute phase of the disease. These findings are suggestive of the presence of disseminated intravascular coagulation (DIC). DIC has been reported in several trypanosome infections based on alterations in fibrinogen levels, thrombocytopenia and an increase in FDPs (Barret-Conner, Ugoretz and Braude, 1973; Van den Ingh, Zwart, Van Miert and Schotman, 1976; Forsberg, Valli, Gentry and Donworth, 1979; Anosa and Isoun, (1983). The triggering events of DIC in trypanosomiasis are yet undefined but its presence during the acute phase, when parasitaemia is high, is suggestive of the trypanosomes, or their products, acting directly as initiating factors. The major consequences of DIC include fibrin deposition in blood vessels, which may lead to vascular occlusion. Haemostatic failure (as in the haemorrhagic syndrome accompanying some T. vivax infections) can occur because of the combined effects of excessive consumption of coagulation factors

and an interference with the clotting mechanisms by FDPs which inhibit thrombocyte function and interfere with fibrin polymerization.

Severe leukopenia was observed in both Galana and Orma, either syringe infected with stock K2388, or bitten by flies infected with stock K2589. Unlike the infections with *T. congolense* which produced leukopenia throughout the study period, infections with *T. vivax* produced severe leukopenia lasting for 3-4 weeks. This was followed by leukocytosis in the animals that survived the infection. These findings are in agreement with those of Anosa and Isoun (1980).

In the present study, pancytopenia was observed during the first four weeks of infection. As observed in the *T. congolense* infections, granulocytes were the most severely affected leukocytes. However, the observed leukocytosis in this study is possibly due to lymphocytosis as they are the only cellular population that increased to more than twice the original value. Similar results were reported by Valli and Mills (1980) who demonstrated that lymphocytosis was responsible for the rise in the total leukocytes during the chronic phase of the disease and that the infected calves had marked reductions in the granulocytes.

5.3: CONCLUSIONS

1 - *Trypanosoma vivax* seem to be less virulent to the Orma than *T. congolense*. 80% of the animals attained "self cure" in *T. vivax* infections compared with 60% in *T. congolense*. However, none of the Galana animals attained "self cure" in either of the infections.

2 - In spite of becoming infected the Orma had a net gain of body weight in all experiments compared with a net loss in the Galana.

3 - Although the drop in PCV was more pronounced in the Orma than

that reported for N'Dama following trypanosome infections, PCV levels in the Orma remained higher than those reported in other Zebu types. Only 23% of all the Orma used in this study reached a critical PCV of 20% or less.

4 - Taking treatment as an indication of death, the present study revealed an overall average of 60% ± 7.9 deaths due to trypanosomiasis in the Galana compared to 17% ± 2.4 in the Orma over the 15 week period.

5 - Based on the levels of parasitaemia, haematological parameters and ability to gain weight while infected, the Orma Boran cattle appeared to be more resistant to trypanosomiasis than the Galana Boran cattle. Field observations support this conclusion (Wilson et al., 1981; Njogu et al., 1985a&b; Dolan et al., 1985).

6 - More studies are still required on the Orma. Groups of animals should be moved to other localities such as Trans Mara and Lake Victoria and their resistance should be compared with the local Zebu types resident in these areas. Studies on bone marrow cellular changes, blood biochemical changes and proliferative cellular populations during the acute and chronic phases of infection are still needed. Studies on cellular clearance mechanisms indicated that the N'Dama animals had a greater capacity to clear trypanosomes compared with Boran cattle (Kamanga-Sollo et al., in prepation). Similar studies are also required to be carried out in Orma cattle.

7 - Although the animals used in the present investigation may have had previous exposure to trypanosomiasis, immunological screening indicated no previous experience with the parasite stocks used for infection. It is therefore possible that these results reflect an innate difference in susceptibility to trypanosomiasis rather than an acquired immunity.

Thus, in both T. congolense and T. vivax infections initiated either through syringe inoculation or tsetse bite, the Orma Boran was consistently less severly affected than the Galana Boran. It was therefore concluded that the Orma Boran, an indigenous Bos indicus breed in East Africa, possesses some degree of resistance to trypanosomiasis. The basis of the resistance was not investigated but it could be genetic.

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APPENDIX 1A

The Transmission of *T. congolense* (IL 1180) by *Glossina morsitans morsitans*

Sources of tsetse flies:

Teneral tsetse flies (*G.m. morsitans*) of both sexes were obtained from the Walter Reed colony at Kabete and from the KETRI colony at Muguga.

Trypanosomes:

A donor calf was intravenously infected with a clone of *T. congolense* (IL 1180). Details of this clone are given in chapter 4.

Fly infection with trypanosomes and transmission of infection to steers:

Teneral tsetse flies were allowed to feed on the parasitaemic donor calf. The flies were thereafter maintained on the same calf. To enable a large number of flies to become infected, a new batch of tenerals was added to the group every day for a period of ten days. When the donor calf became too weak from the infection, the "infected" flies were transferred onto a new parasitaemic donor calf until the time that they were used to infect experimental animals. Figure 20 shows the cyclical transmission of infection.

After a period of 25 days from the time the last batch of teneral flies were fed, the flies were separated into individual tubes and were starved for one day before being probed in an attempt to identify the infected individuals. However, due to the type of tubes used in this experiment, probing was found to be difficult. It was therefore decided that individual flies be fed on the steers at predetermined sites (Fig. 20) and then dissected immediately to determine whether they had been infected or not. On demonstrating an infected fly, no further flies were fed on that site. Following this method, each steer was bitten with three infected flies at three different sites.

Results:

A total of 497 flies were dissected of which 65 flies were found positive, thus giving an infection rate of 13.1%.

The number of flies dissected each day is given below.

DATE	No. DISSECTED	No. INFECTED
16/1/86	208	27
17/1/86	228	26
18/1/86	61	12
T O T A L	497	65

Eight Galana steers and ten Orma steers were each bitten by three infected flies. One additional Galana steer was bitten by five infected flies and another Galana steer by one infected fly. These two animals were used to determine the effect of different numbers of infected fly bites on prepatent period and the severity of infection thereafter. The animal that was bitten by five infected flies became positive eleven days later and the one infected with one fly became positive at day 14 after infection. These were within the prepatent period of the other experimental animals. Once the parasitaemia became established, the course of infection followed a pattern similar to that of the other experimental animals. Thus, it was concluded that (in the present study), for a small number of flies, the actual number of infected flies used to infect experimental animals does not influence the course or the severity of the disease.

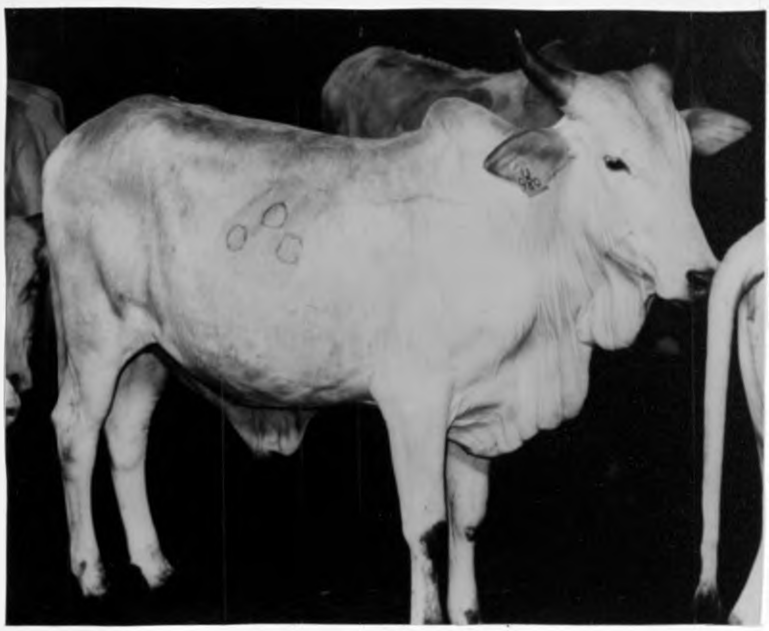
In addition, two Galana and two Orma steers were each bitten by three uninfected teneral flies. These four animals served as uninfected controls.

FIGURE 20 :

Cyclical transmission by G.m. morsitans. 128a.

Feeding tsetse flies on donor animal

Prepared sites for feeding flies



Feeding of individual flies

APPENDIX 1B

The Transmission of *T. vivax* (Stock K2589) by *Glossina morsitans morsitans*

Sources of tsetse flies:

Teneral *G.m. morsitans* of both sexes were obtained from the Walter Reed colony and from the KETRI colony.

Trypanosomes:

Trypanosoma vivax (stock K2589) was raised in a donor calf by intravenous injection of the stabilated blood. When the calf demonstrated parasites in its blood it was used to infect the flies. Details of this *T. vivax* stock are given in chapter 4.

Fly infection with trypanosomes and transmission of infection to steers:

When the donor calf was parasitaemic, teneral flies of both sexes were fed and maintained on the same calf until the time the infection was expected to be mature (day 20 from the first feeding). Each day a new batch of flies was added to feed on the infected calf. A total of 800 flies were fed within a period of four days.

After the last batch of flies had been fed for a period of 16 days on the infected donor, flies were separated and kept singly in tubes, starved for one day and then individually fed on predetermined sites on the steers to be used in the experiment. Any fly that fed (as shown by engorgement) was immediately dissected and the proboscis was examined for the presence of trypanosomes. Any fly found positive was noted and no further flies were allowed to feed on the site. Four infected flies were fed on each steer.

Results:

A total of 98 flies were thus dissected of which 81 were found positive giving an infection rate of 82.7%.
Detailed breakdown of the results is as follows:

DATE	No. DISSECTED	No. INFECTED	% INFECTED
4/5/86	98	81	82.65

Ten Galana and nine Orma steers each had four infected fly bites while one Orma steer got five bites.

In addition two Galana and two Orma steers were each bitten with four uninfected tenerals and were used as controls.

APPENDIX 2

Protamine Sulphate Paracoagulation Test (PST)

1. Introduction:

Disseminated intravascular coagulation (DIC) is attributed to the coexistance of a haemorrhagic diathesis and a thrombosing tendency. It is manifested by thrombocytopenia, prolonged prothrombin time and partial thromboplastin time and the appearance of fibrinolytic activity (Schalm et al., 1975). DIC is rarely a primary disease but is associated with some bacterial, viral or protozoal infection, leukaemia and other malignancies. In these conditions, activation of the clotting process is triggered leading to consumption of clotting factors which may proceed to a severe defibrination syndrome and a resultant haemorrhagic state (Thomson, 1978).

The protamine sulphate paracoagulation test (PST) is used to detect the presence of fibrin monomer (FM) formed in the plasma following triggering of the clotting cascade. Fibrin monomers and fibrinogen degradation products (FDP) are normally present in a complex in plasma. Thus, polymerization of FM in plasma is prevented by the presence of FDP which hold FM in soluble complexes. The addition of protamine sulphate to plasma containing FDP/FM complexes, permits the release of FM molecules from the complex and allows the FM to polymerize and appear as visible insoluble fibrin fibres.

2. Materials:

1 - 3.1% Trisodium citrate in distilled water.

2 - 1% Protamine Sulphate (Grade X - Sigma Chemicals - USA) in distilled water. This solution is always freshly prepared.

3 - Plain 5 ml volume Vacutainer.

4 - 5 ml glass test tubes for harvesting the plasma, washed in dichromic acid and dried with hot air ($100^{o}C$).

5 - glass test tubes 6x100 mm also washed in dichromic acid and hot air dried.

6 - 37^{o}C water bath.

3. Plasma Prepation:

0.5 ml of 3.1% trisodium citrate was drawn into the plain vacutainer and kept on ice. The animals were bled from the jugular into the vacutainer (1 volume citrate to 9 volumes of blood), the contents were mixed thoroughly and put back onto the ice until centrifugation. Using a refrigerated centrifuge (MSE, London), the blood was centrifuged at 2500 RPM for ten minutes. The plasma was transfered to a pre-cooled, clean, dry 5 ml test tubes.

The Test:

The test was carried out as described by Seaman (1970) with some modifications. It was carried out not more than 1.5 hours after bleeding.

1.0 ml of plasma was put into a 6x100 mm glass tube and then put in a 37^{o}C water bath for 15 minutes to raise the temperature of the plasma before the addition of the protamine sulphate.

To the plasma, 100 ul of 1% protamine sulphate was added. The top of the tube was covered and the contents were mixed gently by tilting the tube 90^{0} once and then it was returned to the water bath and immediately the results were read as described below

Reading the results:

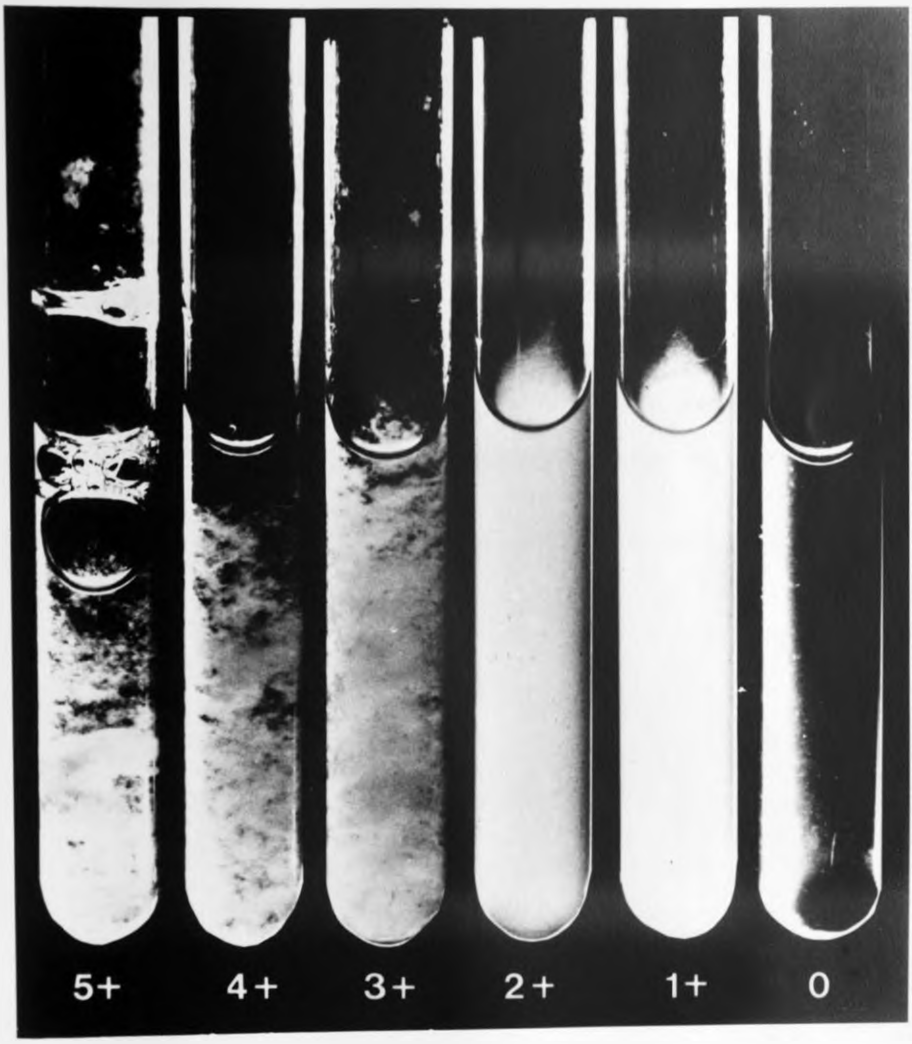
The test tube was removed from the water bath, wiped with tissue paper and held against a flourescent light to read the results. Figure 21 shows the intensity of flocculation and grading. The results were graded according to the amount of visible precipitate and the time taken to achieve this as follows-:

5+ = fibrin clot or obvious flocculent precipitate formed soon after adding the protamine sulphate.

FIGURE 21 :

Intensity and grading of Plasma Protamine Paracoagulation Test (PST). 132a.

Protamine Sulphate Paracoagulation Test (PST)



4+ = fibrin clot or obvious flocculent precipitate formed 30 seconds after the addition of protamine sulphate.

3+ = fibrin strands or obvious flocculent precipitate (usually less in consistency than that for 4+ or 5+) formed within two minutes of adding protamine sulphate.

2+ = Small amount of flocculent precipitate (smaller than that for 3+) formed two minutes after the addition of protamine sulphate.

1+ = Flocculent precipitate present 20 minutes after adding protamine sulphate.

0 = (Negative) No visible precipitate 20 minutes after adding protamine sulphate. Any opalescence as described by Seaman (1970) was also regarded as a negative result.

APPENDIX 3

ERYTHROCYTE OSMOTIC FRAGILITY TEST (EOFT)

INTRODUCTION:

The red cell is composed of 55-65% water, 30-36% haemoglobin and about 5% organic and inorganic matter. The red cell survival time is significantly reduced in anaemias or in nutritional deficiencies of factors such as vitamin B_{12}, folic acid or iron (Shattil and Cooper, 1972).

The red cell membrane is flexible but not essentially elastic. It has been demonstrated that the loss of cholesterol from the red cell membrane causes shrinkage in its surface area, causing it to be more fragile. Increased cholesterol extends the surface area and increases the resistance to osmotic lysis (Cooper, 1970). The membrane could be significantly affected by the relationship between intracellular ATP, Ca^{++} and Mg^{++}. It could also be affected by the pH and the oxygen tension in the local region of the microcirculation (Schalm et al., 1975).

The electrolyte concentration of the intracellular compartments of the red cell is controlled by an ATPase - dependent active transport of Na^{+} and K^{+} (Schalm et al., 1975). Thus any derangement in the permeability of the red cell to electrolytes (Na^{+} and K^{+}) will lead to osmotic swelling and lysis. In hypotonic solutions, the red cell swells to the maximum and becomes spherical before any lysis occurs. Any further increase stretches the red cell membrane and leads to formation of holes large enough to permit the leakage of haemoglobin.

Increase in erythrocyte fragility was observed in bovine Anaplasmosis (Wallace, 1967), in dogs with autoimmune haemolytic anaemia (Schalm et al., 1975) and in calves with Babesia infection (Dolan, 1974) and in mice infected with T. congolense or T. brucei (Ikede, et al., 1977). The test has not been carried out in trypanosomiasis.

The following procedure for carrying out E.O.F.T is modified from Schalm et al., (1975).

Materials

A = Stock solution

10% buffered sodium chloride

NaCl = 90 g

Na_2HPO_4 = 13.655 g

NaH_2PO_4 = 1.87 g

Make up to one litre with distilled water (D.W.).

B = Working solution

1% buffered sodium chloride

Dilute stock solution 1:10

Adjust the pH to 7.44

The Test

1. Tubes were arranged as described in the Working Sheet below:-

Working Sheet

Working Solution					Complementary Sol.			
tube No.	ml.1% NaCl	mls D.W.	% NaCl	Final Vol.	mls of 10% NaCl	mls of D.W.	% NaCl	final Vol.
1	4.25	0.75	0.85	5 mls	0.475	4.525	0.95	5 mls
2	4.00	1.00	0.80	"	0.500	4.500	1.00	"
3	3.75	1.25	0.75	"	0.525	4.475	1.05	"
4	3.50	1.50	0.70	"	0.550	4.450	1.10	"
5	3.25	1.75	0.65	"	0.575	4.425	1.15	"
6	3.00	2.00	0.60	"	0.600	4.400	1.20	"
7	2.75	2.25	0.55	"	0.625	4.375	1.25	"
8	2.50	2.50	0.50	"	0.650	4.350	1.30	"
9	2.25	2.75	0.45	"	0.675	4.325	1.35	"
10	2.00	3.00	0.40	"	0.700	4.300	1.40	"
11	1.75	3.25	0.35	"	0.725	4.275	1.45	"
12	1.50	3.50	0.30	"	0.750	4.250	1.50	"
13	1.25	3.75	0.25	"	0.775	4.225	1.55	"
14	1.00	4.00	0.20	"	0.800	4.200	1.60	"
15	0.50	4.50	0.10	"	0.850	4.150	1.70	"
16	0.00	5.00	0.00	"	0.900	4.100	1.80	"

* A 50 mls volume was made from each and keep at $4^{o}C$ (for both working and complementary solutions).

* Before use, the solutions were brought to room temperature. 5 mls of the working solution was pipetted into each of the respective tubes.
To each tube, 25 ul of heparinized blood was added, mixed well and allowed to stand at room temperature for 30 minutes.
The contents were then mixed again and equal volume of the Complementary solution was added to stop any further reaction.
The tubes were centrifuged at 2500 RPM for 5 minutes.
3.5 mls of the supernatant were transfered into empty clean tubes and the optical density was read at 540 nm using tube 1 as a blank.

The haemolysis in tube 16 is regarded as 100% lysis.

The optical density was converted into percent lysis as follows:-

$$\frac{\text{Optical Density of Unknown}}{\text{Optical Density of tube 16}} \times 100 = \% \text{ lysis}$$

The % lysis was plotted against the % NaCl concentration. A sigmoid curve will be obtained.

* From this curve, the value for the NaCl concentration which produces 10% lysis was found and recorded as initial lysis. Similarly, the value for 50% lysis was found and recorded as the median corpuscular fragility (MCF).
For each individual animal the test was performed three times at seven day intervals to determine the % NaCl concentration required to induce 10% lysis and the 50% lysis. Following infections induced by tsetse, the test was carried out once a week for each animal. A graph was plotted from which the 10% and 50% lysis were determined and recorded.

APPENDIX 4

TABLES OF ANALYSIS OF VARIANCE FOR PARASITAEMIA AND PCV

T.C. syringe infection-parasitaemia, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	421.8457	1	421.8457	92.67754
ERROR	100.1387	22	4.551758	(P< 0.005)
TIME	178.2363	27	6.601346	7.00849
BREED*TIME	119.5039	27	4.426071	4.699049
ERROR	460.5928	489	.9419077	(P< 0.005)

NO OF MISSING DATA 105

T.C. syringe infection-PCV, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	3087.906	1	3087.906	44.104
ERROR	1540.313	22	70.01421	(P< 0.005)
TIME	12530.88	28	447.5313	156.76
BREED*TIME	86.46875	28	3.08817	1.08
ERROR	1459.156	511	2.855	(N.S.)

NO OF MISSING DATA 105

T.C. fly infection-parasitaemia, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	840.5371	1	840.5371	73.58618
ERROR	182.7598	16	11.42249	(P< 0.005)
TIME	876.4434	43	20.38243	22.27919
BREED*TIME	157.4316	43	3.661201	4.001912
ERROR	629.42587	688	.9148631	(P< 0.005)

NO OF MISSING DATA 60

T.C. fly infection-PCV, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	1274.875	1	1274.875	5.798387
ERROR	3517.875	16	219.8672	(P< 0.005)
TIME	19399.31	52	373.0637	73.96048
BREED*TIME	1595.375	52	30.68029	6.082416
ERROR	4196.688	832	5.044096	(P< 0.005)

NO OF MISSING DATA 58

T.V. syringe infection-parasitaemia, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	1944.493	1	1944.493	31.64199
ERROR	1229.059	20	61.45293	(P< 0.005)
TIME	1791.291	47	38.11258	20.3327
BREED*TIME	477.6299	47	10.16234	5.421512
ERROR	1761.981	940	1.874447	(P< 0.005)

NO OF MISSING DATA 218

T.V. syringe infection-PCV, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	13717.94	1	13717.94	20.56229
ERROR	13342.81	20	667.1406	(P< 0.005)
TIME	24702.31	55	449.133	37.56845
BREED*TIME	1015.875	55	11.47046	1.544991
ERROR	13150.56	1100	11.95506	(P< 0.005)

NO OF MISSING DATA 186

T.V. fly infection-parasitaemia, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	1860.509	1	1860.509	34.38898
ERROR	973.834	18	54.10189	(P< 0.005)
TIME	1413.632	44	32.128	22.96911
BREED*TIME	247.959	44	5.635432	4.028911
ERROR	1107.809	792	1.398748	(P< 0.005)

NO OF MISSING DATA 213

T.V. fly infection-PCV, twice weekly

ANOVA : GALANA - ORMA BREED OVER TIME

SOURCE VAR	SS	DF	MS	F
BREED	5687.188	1	5687.188	11.80484
ERROR	8671.812	18	481.7674	(P< 0.005)
TIME	19883.38	51	389.8701	40.1566
BREED*TIME	2235.875	51	43.84069	4.515589
ERROR	8912.625	918	9.708742	(P< 0.005)

NO OF MISSING DATA 214