IMMUNITY ENGENDERED THROUGH SELF-CURE IN ZEBU CALVES EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA VIVAX*

BY

NDUBISI RAPHAEL UZOIGWE

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THESIS APPROVAL

This thesis by Ndubisi Raphael Uzoigwe meets the regulations governing the degree of Masters of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

External Examiner

B.O. Ikede,
Professor,
Department of Veterinary
Pathology,
University of Ibadan,
Ibadan.

Internal Examiners

A.A. Ilemobade
Professor & Former Head,
Department of Veterinary
Parasitology & Entomology,
Ahmadu Bello University,
Zaria.

A. Sannusi,
Senior Lecturer,
Department of Veterinary
Parasitology & Entomology,
Ahmadu Bello University,
Zaria.

DIGITIZED

D.S. Adegboye,
Reader,
Department of Veterinary
Pathology & Microbiology,
Ahmadu Bello University,
Zaria.

D E D I C A T I O N

Dedicated to my late father Wilfred Orjiemezie Uzoigwe

(Died March 23rd 1980)

DECLARATION

I hereby declare that the work reported in this thesis was carried out by myself in the Department of Veterinary Parasitology and Entomology, under the supervision of Professor A.A. Ilemobade, Dr. A. Sannusi and Dr. D.S. Adegboye.

No part of this thesis has been accepted in any previous application for a higher degree. All sources of information are specifically acknowledged by means of references.

Ndubisi Raphael Uzoigwe

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ABSTRACT

The response of White Fulani Zebu calves to primary *Trypanosoma vivax* infection was observed over a period of 24 weeks. This was followed by a study of the effects of challenge infection with a homologous strain on the calves. The calves were between the ages of six and 12 months and were purchased from Jibiya, a tsetse-free area of Nigeria. A stock of T. *vivax* isolated from Ilorin in Kwara State of Nigeria was used for the primary inoculation and later for the homologous challenge. Each of the eight calves used as the experimental group was inoculated through the jugular vein with 1.6×10^8 trypanosomes. Three other calves served as the control. Temperature, parasitemia, PCV, Hb concentration, leukocyte changes and weight gain/loss were monitored. All the eight calves became infected after prepatent periods ranging between two and five days. Three (38%) out of the eight infected calves died. All calves that survived after the third week of infection were self-cured. High parasitemia and anemia were observed in the dead calves. The surviving calves showed early signs of recovery from the anaemia and exhibited ability to control the parasitemia. None of the four self-cured calves became infected following challenge with the homologous strain while the three control calves became infected.

In both the primary and challenge infections the white blood cell picture did not seem to indicate adverse effect on the lymphoid system of the infected calves. It is concluded

from this study that immunization of calves with live trypanosomes may have potentials for the control of trypanosomiasis in the field.

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CHAPTER ONE

GENERAL INTRODUCTION

In Africa south of the Sahara the tsetse-borne human trypanosomes pose a great threat to the health of man while the tsetse-borne animal trypanosomes cause great economic drain on his livestock resources.

The effects of trypanosomiasis on the livestock industry include low productivity arising from high mortality, abortions and poor growth. Trypanosomiasis is caused by protozoan parasites (flagellates) which are transmitted biologically in tropical Africa by the tsetse (Glossina species).

The geoecological distribution of tsetse flies (Glossina sp.) and the trypanosome parasites they transmit is over a large part of pasture producing land areas (Buxton, 1955; Glover, 1961; Ford, 1971; and Davis, 1977). Nigeria has only about a fifth of the total land area of 920,000 sq. km free of tsetse flies (Davis, 1977). This situation has led to the problem of over-stocking and over grazing with attendant soil erosion in the restricted tsetse free areas of the north.

At present, the available practical methods for the control of trypanosomiasis, viz, control of tsetse flies, chemotherapy and chemoprophylaxis, do not seem to be completely effective. These methods have some attendant

disadvantages. For instance, the reclamation of tsetse-infested land adversely affects the terrestial ecosystem while the use of insecticides for the eradication of tsetse incurs huge expenditure. Furthermore, run-offs of such insecticide constitute a source of pollution to the environment and are also hazardous to other non-target life in the environment. In a similar manner, chemotherapy and chemoprophylaxis are expensive and drug resistant trypanosomes have emerged. Because of these limitations it is clear that if our livestock industries must be improved to meet the national demand for animal protein, more efficient methods for the control of trypanosomiasis should be developed.

It has been observed by some workers that calves often show some degree of resistance or tolerance to trypanosome infections. Increased resistance to trypanosome infections either through self-cure or chemotherapy have been reported (Soltys 1955, Smith 1958, Fiennes 1970, Wilson et al., 1976 and Wellde et al., 1978). From these reports, there seems to be some hope that a well planned procedure using calf models could lead to the application of this protective mechanism as a possible control method.

This study was therefore designed with the following objectives:

(a) To investigate if protective immunity through self-cure in calves can be induced against an

economically important species of trypanosome viz, T. vivax.

(b) To study the degree of protection conferred on such infected calves against artificial challenge with homologous trypanosome strain.

CHAPTER TWO

LITERATURE REVIEW

Introduction

African trypanosomiasis of both man and livestock is caused by a group of extracellular protozoan haemoflagellates; the trypanosomes. The trypanosome parasites belong to the genus Trypanosoma of which there are three subgenera, namely, the Trypanozoon (represented by T. brucei, T. gambiense and T. rhodesiense); the Nannomonas (represented by T. congolense and T. simiae); and the Duttonella (represented by T. vivax and T. uniforme).

The life cycle of the members of the three subgenera is digenic and involves development both in the arthropod vector-the Glossina, and the vertebrate hosts. The details of the morphological characteristics, life cycles and range of hosts of the subgenera are described by Hoare (1972).

Infections in humans are caused by the members of the Trypanozoon subgenus, viz., T. gambiense and T. rhodesiense, while animal trypanosomiasis is commonly caused by members of the other two subgenera including T. brucei. The parasites are cyclically transmitted from one host to another through the bite of the tsetse flies. Sometimes mechanical transmission occurs through other biting flies. In the laboratory, transmission is achieved both cyclically and through syringe or needle passage.

This review discusses mainly the infections caused in livestock (and to some extent laboratory rodents) by the trypanosomes and the factors affecting them. It also deals with aspects of host resistance against infection and self-cure phenomenon in animal trypanosomiasis, and finally outlines some practical attempts so far made to protect animals against trypanosomiasis through immunization.

Trypanosomiasis in susceptible hosts

Nature of parasitemia

The appearance of the trypanosomes in the blood stream of a susceptible host following cyclical or needle transmission is usually preceded by a prepatent period which may last from few days to weeks. The prepatent period in cattle with *T. congolense* has been reported to have an inverse relationship with the dose of trypanosome given (Wellde *et al.*, 1981). Following patency, the level of parasitemia increases progressively until it attains a peak. This is then followed by a fall in parasitemia. Thereafter, periodic waves of parasitemia succeed each other at few days intervals (Edwards *et al.*, 1956; Godfrey, 1961; Assoku, 1975; Maxie *et al.*, 1976; Esievo, 1982). The ascending phase of each wave represents the multiplication of a new antigenic population while the descending phase represents the destruction of some members of the population by the hosts immune responses (Lumsden, 1972).

Temperature

A rise in body temperature usually marks the onset of parasitemia. In cattle infected with T. congolense, a rise in temperature to about 103°F has been reported (Stephen, 1970). The rise coincides with the appearance of trypanosomes in the peripheral blood of the animals. Valli et al. (1978) reported that in calves infected with T. congolense, the first temperature peak occurred five to six days after infection and corresponded with the appearance of trypanosomes in the peripheral blood. Similarly, rises in temperature have been reported in young sheep and goats infected with T. congolense (Griffin and Allonby, 1981). These observations indicate that pyrexia is one of the clinical signs of early trypanosomiasis.

Haematological changes

Anaemia

Anaemia is a cardinal sign in trypanosomiasis. The anaemia is manifested as decrease in red blood cell count (RBC), packed cell volume (PCV) and haemoglobin concentration (Hb). It has been observed that the onset of anaemia corresponds with decline in the first peak of parasitemia (Fiennes, 1970; Roberts and Gray, 1973; Ige and Amodu, 1975) and rise in serum trypanosomal antibody level in mice (Assoku, 1975). In T. congolense-infected Holstein calves and Holstein x Hereford calves, Valli et al. (1978) reported

that the onset of anaemia occurred after the first peak of parasitemia, while in T. congolense-infected Ndama and Zebu cattle, Dargie et al. (1979b) observed that the onset of anaemia correlated closely with the appearance of parasites in the blood. The severity of the anaemia has been shown to depend on the level and duration of parasitemia in T. brucei and T. congolense infected Ndama and Zebu breeds of cattle (Dargie et al., 1979a,b), and in T. vivax infected Zebu cows (Saror, 1979). The same relationship was also reported in sheep and goats infected with T. vivax (Anosa and Isoun, 1980). Saror (1980) however did not observe a similar relationship in Red Sokoto goats infected with the Y58 strain of T. vivax.

Leukocyte responses

Fiennes et al. (1946) reported that in adult cattle and calves infected with T. congolense, marked leukopenia occurred in the adults while leukocytosis was observed in the calves. They noted that the leukopenia developed gradually and was concurrent with neutropenia, while the leukocytosis was a result of lymphocytosis and attained a peak during anaemic crisis. Naylor (1971) and Losos et al. (1973) reported that leukopenia in bovine T. congolense infection was a result of neutropenia, lymphopenia and eosinopenia. In calves infected with T. congolense, Wellde et al. (1981) reported that thrombocytopenia and leukopenia were prominent manifestations of the disease. In other studies, Maxie et al. (1976, 1979) observed that transient leukopenia and

thrombocytopenia with concomitant lymphopenia and neutropenia occurred earlier among T. vivax infected cows than those infected with T. congolense. Saror et al. (1979) reported that in T. vivax infections in Ndama and Zebu breeds of cattle, there was an early transient leukopenia which was followed later by leukocytosis which occurred as a result of lymphocytosis. It is possible that the initial leukopenia during the early stages of infection may correspond to the period of activation of the haemopoietic system to produce more leukocytes.

Body weight

Changes in body weight is another feature of trypanosomiasis. Stephen (1966) observed that Ndama and Zebu cattle undergoing chronic trypanosome infection suffer severe retardation in body growth. It has also been reported (Fiennes, 1970) that during the course of trypanosomiasis in cattle, marked weight loss occur in the adults while calves do not gain weight as quickly as the unifected ones. Ikede and Losos (1975) also observed that in sheep carrying T. brucei infection. loss of body weight and other clinical signs (dullness, weakness, peripherial lymph node enlargement, odema and progressive anorexia) developed gradually and were noticeable from the 6th week of infection onwards. In T. simiae infection in pigs, it is reported that marked loss in body weight occurred during the course of the infection (Ilemobade and Balogun, 1981).

Factors affecting the course and outcome of infections

The course and outcome of trypanosome infection is influenced by a number of factors involving both the parasites and the host.

Challenge dose

The number of infective organisms introduced into a host determines whether an infection would occur in the host or not (Stephen, 1970). Willet (1956) reported that 20,000 living blood forms of human trypanosomes were unable to initiate an infection but when 80,000 trypanosomes were used for the challenge, infection occurred. Failure of an inoculum containing a large number of infective organisms to initiate an infection could occur in hosts which have very strong immunity (Weitz, 1970). It thus seems that there has to be a minimum infective dose of trypanosomes before an infection can occur.

Parasite species/strain and infectivity

Infection of different hosts by the different trypanosome species, result in different types of disease syndrome (Stephen, 1970). Ilemobade and Balogun (1981) infected pigs with T. brucei, T. congolense and T. simiae. They observed that both the T. brucei and T. congolense produce mild or symptomless infection while the T. simiae produce a severe infection. Stephen (1966a and 1970) observed that T. simiae is non-infective to cattle and sheep, and that T. vivax which

produce severe infection in cattle remain non infective to pigs. Furthermore, the West African strain of T. vivax has been found more virulent than the East African strain (Fairbarin, 1953). It has been noticed that generally in Nigeria compared with East Africa, the T. congolense infection is characterised by low infectivity, pathogenicity and parasitemia, and that the dimorphon type is very virulent in our livestock (Godfrey, 1961).

Desowitz and Watson (1951) suggested that the infectivity of T. vivax in rats deponded on the stage of infection in the donor animal. Infections initiated in rats with trypanosomes taken at the initial peak of infection in cattle, were of greater intensity and longer duration than those resulting from inoculation of trypanosomes collected during relapse infection (Desowitz and Watson, 1952). Ilemobade et al. (1975) related the difference in infectivity observed in T. vivax infection in cattle, sheep and goats to the stage of infection in the donor animal. Also Leeflang et al. (1976a) described the infectivity of natural bovine isolates of the T. vivax to mice and suggested that only early parasites from the early stages of natural infection are readily capable of infecting mice. Leeflang et al. (1976b) had also demonstrated in another study using cyclically infected ox, sheep and goat as donor animals and mice and rats as receipients that trypanosomes isolated from cyclical infections of less than 4 weeks were infective to mice and rats.

Susceptibility of hosts

There are differences in the susceptibility of different animals to infection with the various trypanosome species. Murray and Morrison (1978) observed that while some animals are readily susceptible to infection with some trypanosome species, some are completely refractory to such infections. These workers also related susceptibility to the capacity of the host to limit the level of parasitemia and the ability to control or even eliminate the trypanosomes. A similar observation was also reported by Dargie et al. (1979b) who stated that superior resistance of the Ndama cattle over the Zebu breed lies in their ability to control parasitemia rather than their capacity to mount a higher erythropoietic response. It is known that T. congolense produces severe disease in cattle (Stephen, 1966b), while in pigs it gives rise to a mild or symptomless infection (Stephen, 1966a; Ilemobade and Balogun, 1981).

In Trypanosoma vivax infections of goats and sheep (Anosa and Isoun, 1976; 1980) it was observed that goats suffer more severely than sheep. Parallel observations show that T. brucei causes severe disease in dogs and horses while cattle showed only the mild disease (Ikede and Losos 1972, 1975, and Ikede et al., 1977).

The three main breeds of cattle in Nigeria, viz, Muturu, Ndama and Zebu, show differences in their degrees of susceptibility to infection. This difference is believed to have influenced their distribution in the country (Leeflang, 1974). Both the Ndama and the Muturu are trypanotolerant and capable of surviving in the tsetse endemic southern part of the country while the Zebu are susceptible to trypanosome infections and live in the more northern part (Chandler, 1952; Desowitz, 1959; Maclenann, 1970; Weitz, 1970 and Roberts and Gray, 1973). Cross-breeds of Ndama and Zebu, and Muturu and Zebu occupy an intermediate position in susceptibility to trypanosome infections and are found in the derived savannah zone.

Age

Ashoroft, (1959) demonstrated that young rats are more susceptible than the adults to trypanosome infection. Similarly, in sheep and goats it has also been reported that young sheep and goats aged between two days to eight weeks are more susceptible to T. congolense infection than the adult (Griffin and Allonby, 1979). In the bovine however, Fiennes et al. (1946) observed that young cattle would normally survive prolonged T. congolense infection while the adults die. Also, recently Wellde et al. (1981) demonstrated that Hereford cattle not more than one year old are more resistant than those of two years and above. These reports thus suggest that age resistance to trypanosomiasis is not a common factor in all animal species.

Nutrition and husbandry

The level of nutrition in animals determines the effect of trypanosomiasis on the animals. Hill (1956) demonstrated that cattle under good husbandry were able to tolerate clinical infections more than those under poor standard of husbandry. Some workers including Chandler (1952) and Stephen (1966b) have reported that nutritional deprivation and deviation from a high standard of nutrition to a low one could result in fatal infection even among the trypanotolerant breeds. Maclenann (1970) from his own studies concluded that animals in low nutritional states (as in time of drought become less able to withstand the effects of infection. Thus, adequate feeding and good husbandry are factors that help animals to ameliorate the severity of the disease.

Secondary/Intercurrent infection

The presence of intercurrent disease and other stress conditions reduce the ability of infected animals to resist infections (Chandler, 1952; Stephen, 1966a; Maxie et al., 1979). In both T. vivax and T. congolense infections in cattle, Maxie et al. (1979) observed that both groups of infected animals became susceptible to secondary bacterial infection which consequently caused the intensification of the primary trypanosomiasis. Other observations on the aggravation of the effects of trypanosomiasis due to secondary infection have also been reported by Losos and Ikede (1972). Immunosupression which occurs during trypanosomiasis

(Allit et al., 1971; Greenwood et al., 1973; Terry, 1976; and Pearson et al., 1981) may enhance concurrent infections since the humoral response of the host to other antigens is suppressed (Goodwin et al., 1972; Murray, 1974; Hudson et al., 1976).

Immunity to trypanosome infection

Humoral immune response

Trypanosomal infections like other infections stimulate the production of antibodies by the host. In both human and animal trypanosomiasis, it is reported that increases in the level of IgM antibody usually occur first before that of IgG (Lumsden, 1965; Houba and Allison, 1966; Freeman et al., 1970; Luckins, 1972; Greenwood, 1974; Luckins, 1976; Luckins and Mehlitz, 1976; Mackenzie et al., 1979). More increases in IgG usually occur during infections. Two functional types of antibody are directed against the parasite (Lumsden, 1970; 1972; de Raadt, 1974; Gray and Luckins, 1976; Terry, 1976). These are the specific antibody against the variant antigens and antibody against the common antigens.

Cell-mediated immune response

Immune responses indicative of cellular immunity have been observed in some human and animal trypanosomiasis. Koten and de Raadt (1969) reported perivascular infiltration of the brain and heart by mononuclear cells in human sleeping sickness. This reaction is suggestive of cell-mediated immune

response. Also it has been observed that lymphoid hyperplasia along with parasite proliferation occurred in the spleen of rabbits infected with T. brucei (Esuruoso, 1967, 1970). Follwoing the initial hyperplasia, a severe lymphoid depletion was observed in the organ during the course of the infection. Tizard and Soltys (1971) successfully demonstrated skin hypersensitivity reaction in T. brucei infected rabbits. In another study, Mansfield and Kreier (1972) however failed to demonstrate the same feature in rabbits infected with T. congolense. It is therefore likely that cell-mediated immune response is also involved in protection against trypanosomiasis.

Innate resistance

Human beings are naturally resistant to infection by T. brucei but are infected by their morphologically identical subspecies T. gambiense and T. rhodesiense. This innate resistance of humans against T. brucei is attributed to the susceptibility of T. brucei to human serum and the insusceptibility to T. gambiense and T. rhodesiense (Desowitz, 1970). Earlier Laveran (1902) and Laveran and Mensil (1902) cited by Terry (1976) demonstrated that injection of normal human serum into mice depressed and sometimes completely inhibited T. brucei infection in the mice. They therefore concluded that man's insusceptibility to T. brucei was due to some property of normal human serum. Yorke et al. (1930) also showed that normal human serum possesses powerful trypanocidal effect in vitro against a laboratory strain of T. rhodesiense.

Aaronovitch and Terry (1972) showed by gel filtration and ultracentrifugation technique that the trypanocidal factor was a high molecular weight globulin. They further observed that the globulin was reduced when reacted with a rabbit antiserum which is specific for IgM. The authors therefore concluded that the globulin is probably IgM. The conclusion was however disputed a year later by Hawkings (1973) who by chromatographic studies revealed that although the factor is a macroglobulin, it is an alpha-2-macroglobulin rather than IgM.

Terry (1957) observed that the cotton seed rat (Sigmodon hispidus) was innately resistant to a rodent adapted T. vivax. He found that the rats remained refractory when inoculated with as many as 200 million live trypanosomes and that normal cotton rat serum would agglutinate the T. vivax suspension and bring about their lysis in vitro. He therefore concluded that the factor responsible for the resistance was a natural antibody produced in the rats in the absence of antigenic stimulus.

Acquired immunity

The exposure of animals to natural infections in the field accompanied with drug treatments confers some degree of immunity on such animals. Soltys (1955) reported that Zebu cattle which received prophylactic treatment of antrycide pro-salt for two and half years at two months intervals before being released in an enzootic tsetse area for 18 months,

became resistant to both local and imported strains of T. congolense. Such resistance also occurred among cattle that were similarly treated but were initially left in a tsetse and trypanosome free area for ten months before they were returned to the enzootic area. Smith (1958) confirmed and further extended these observations. He injected a group of Zebu cattle with the prophylactic doses of quinapyramine. Then, one week later he inoculated the same animals with T. congolense. To a second group, he injected only the prophylactic dose of the drug. A third group was left as control. Each treatment regime was carried out on seven different occasions at two months intervals. Six months after the last treatment the animals were challenged with T. congolense. It was observed that all the six that received the dual treatment and four of those that received only the prophylactic treatment resisted the challenge while the control group came down with infection. Wilson et al. (1975a), observed that in beef cattle maintained in an area of medium tsetse challenge, group treatment with Berenil or Samorin enhanced the growth of the animals better than treatment on individual basis. In another study, Wilson et al. (1975b) claimed that no significant resistance developed through periodic treatment with Berenil in a herd of breeding cattle kept in a high challenge area. Wilson et al. (1976) observed that cattle maintained in a tsetse area and treated with Berenil upon the development of clinical disease developed partial immunity to trypanosomiasis after two years of treatment while treatment of animals as a group following the development of patent parasitemia did not confer any immunity.

Some other earlier observations have also shown that some degree of immunity could be acquired through birth. Whiteside (1962) observed that calves born of immune dams when infected within the first two weeks of birth developed greater resistance to infection than those born of non immune dams. It was therefore suggested that immunity was as a result of an inherited immune factor from the dams.

Antigenic variation

During the course of infection changes in surface antigens (surface coat) of trypanosomes occur through the process of antigenic variation. The phenomenon of antigenic variation represents one major device by which trypanosomes establish chronic infections in spite of the protective immune responses mounted by the host (Vickerman, 1974; Doyle, 1977; Cross, 1978; de Gee, 1979; Person et al., 1981). The entire outer surface of trypanosomes is known to be covered by glycoproteins (Cross, 1975; Rovis et al., 1978). These glycoproteins which constitute the surface antigens of the trypanosomes are unstable (de Raadt, 1974; Vickerman, 1974). The changes in surface coats of the trypanosomes are thought to occur as a result of the antibodies produced and directed against the trypanosomes by the host. It has been suggested that the survival of the trypanosomes is favoured on one hand by the fact that the surface coats or antigens undergo variation during the course of infection (Shapiro and Murray, 1982) and, on the other hand by the fact that it constitutes

a barrier between the trypanosomes and its immediate surrounding (Pearson et al., 1981).

There has been speculations over the decades on whether the phenomenon is antibody induced or selective. In vitro cultivation and cloning of animal infective forms of the trypanosomes has made it possible for the controversy to be investigated. Recently, Doyle et al. (1980) reported that variable antigen types developed from clones of T. brucei in vitro 18-46 days following initiation of the clone. They also found that new variable antigen types are generally first detected 10-12 days after initiation of the infection in vivo whereas new variable antigen types were first detected after 18-46 days in vitro. These reports show therefore that the host factor is not important in the induction but in the maintenance of antigenic variation.

Self-cure in animal trypanosomiasis

Evidences based on both field observations and experimentations show that some young animals are capable of resisting trypanosomiasis. A number of early workers including Schilling (1935 and 1936), Bevan (1936) and Hornby (1941) recognised that calves possess greater resistance to trypanosome infection than did adult cattle and they suggested the exploitation of this ability of the calves to develop cattle with reasonable degree of tolerance to the disease. In an experimental study, Fiennes et al. (1946) showed that

calves of dams infected with T. congolense recovered after an acute disease while the adults with similar infection died. Also, Fiennes (1950) found that calves infected within the first two weeks of their birth made spontaneous recovery from the infection. In a recent study, Wellde et al. (1978, 1981) reported that calves between the ages of four months to one year self-cured primary T. congolense infection while their older counterparts died or needed treatment to survive. Griffin and Allonby (1981) however showed that age resistance does not appear to be important in the small ruminants. They observed that most young sheep and goats infected with T. congolense died of the infection. The younger animals suffered more severely and showed higher mortality than the older animals.

Immunization of animals against trypanosomiasis

Murray and Urquhart (1977) reviewed the attempts by many workers to immunize animals against trypanosomiasis by using different trypanosome materials which include; exoantigens, dead trypanosomes, attenuated trypanosomes, X-irradiated living trypanosomes and adjuvants. None of these materials, however, conferred absolute protection.

Antigens

Exoantigens

Weitz (1960) showed that the serum from T. brucei infected mice and rats, gave protection against challenge with homologous variant or strain, when the serum was used

to immunize the animals. The result indicated that soluble trypanosome antigen or exoantigens are present in the serum of the infected animals. It was suggested that the exoantigens may be plasmanemes (filopodia) of the T. brucei which are readily detached from the organisms into the serum (Macadam and Herbert, 1970a and 1970b). It was also found that infected sera containing filopodia gave better protection than sera free from filopodia in terms of survival (Herbert and Macadam, 1971). Exoantigens of T. rhodesiense from sera of infected rats have also been used in achieving considerable levels of protection in mice, rats and monkeys (Seed and Weinman, 1963; Seed, 1963).

Killed trypanosomes

Kligler and Berman (1935) reported that rats immunized with dead T. gambiense suspensions developed immunity against its homologous strain. Also T. brucei killed by formalinization, freeze-thawing and heating at 56^{o}C have been used to vaccinate rats, rabbits and sheep (Soltys, 1964). It was found that trypanosomes which were thermally killed for 30 minutes did not confer any protection while all the animals immunized with the formalinized and freeze-thawed trypanosomes protected against infection.

Trypanosome extracts

Seed (1972) obtained about 80% protection by immunizing mice with a trypanosome extract prepared by DEAE-sepharose

fractionation. The assessment of protection was based on survival at day 17. The infected unimmunized controls lived only for five days. Powell and Mathaba (1978) used T. rhodesiense homogenate and the kinetoplast fraction (fraction-3-extract) to immunize sheep against T. vivax. They observed that all the sheep which were vaccinated with the "kinetoplast-fraction" developed very low parasitemia and became apatent 25 days post challenge and remained alive for extra 75 days. The group that was immunized with the homogenate were less protected and this was evidenced by increased parasitemia and death of the animals within 34 days after challenge. Apart from the indication that the fraction 3 extract could provide a good immunization preparation against heterologous strains, the duration of its effect as an immunogen was long enough since the experimental sheep were still protected 57 days after the last immunizing injection.

Attenuated trypanosomes

James et al. (1973) found that immunization of rats with T. brucei attenuated by X-irradiation failed to protect the rats against challenge with a homologous strain. Out of 13 rats that were vaccinated, only two were completely protected. The others had their survival time extended. Duxbury et al. (1972) obtained no protection whatsoever when they vaccinated cattle with four to seven inoculations of irradiated T. congolense. They found that the only effect of the vaccination was an extension of the prepatent period by three days. On

the contrary, Wellde et al. (1973) induced strong and lasting resistance against a homologous strain of T. rhodesiense when they immunize cattle with irradiated T. rhodesiense. The immunity lasted eight months before it began to wane. Discussing their failure to obtain any effect in an earlier attempt Duxbury et al. (1972) opinied that both their immunizing and challenge inocula might have undergone antigenic variation. Takayanagi and Nakatake (1976) reported that thymic cells transferred from T. gambiense immune mice into lethally irradiated thymectomized mice gave protection to mice against experimental infection with T. gambiense. James (1976) briefly exposed T. brucei and T. congolense to low concentration of Samorin and Berenil before using them for vaccination in laboratory rodents. Inocula which contained 10^6 to 10^7 treated parasite were given to each recipient. All the recipients became protected when challenged with an inoculum containing 10^4 untreated homologous parasites. Although a high degree of protection may be achieved, the risk of drug resistant parasites emerging by the use of this method exists.

Adjuvants

Many workers including Aiyedun (1977), Osaki et al. (1979), Ryu and Shaey (1980) and Johnson and Chi (1981) have experimented on the use of adjuvants to induce immunity in animals.

Freund's complete adjuvants administrered to rats one week before they were infected with T. gambiense and T. brucei, and later treated with Berenil, induced high levels of active immunity in the rats against three subsequent infections with homologous strains (Aiyedun, 1977). The incomplete adjuvant was unable to confer such level of protection. Osaki et al. (1979) reported that immunization of mice with a combination of T. gambiense infected mouse blood plus Freund's complete adjuvant confered protection in the mice when challenged two weeks later with T. gambiense. Attempts have also been made to vaccinate with a mixture of dead trypanosomes suspension and eolite powder (Ryu and Shaey, 1980). It was observed that sub-cutaneous inoculation of the mixture in mice gave 85% protection against infection with 100 live T. gambiense. Also recently, Johnson and Chi (1981) demonstrated that suspending T. brucei in N-Nitroso-guaminidine (NTG) for 15 minutes before using them to vaccinate mice at seven to 14 days intervals, confered absolute immunity in the surviving mice when rechallenged with normal homologous trypanosomes 35 and 88 days later.

Immunostimulants

The use of bacterial species as immunostimulants to boost immunity against trypanosome infection was attempted by Murray and Morrison (1979). They found that the use of Corynebacterium paruum, Bacillus calmette Guerin (BCG) and Bordetella pertussis

as immunostimulants prior to challenge with T. congolense or at the same time, did not confer any protection in mice. It was observed however, that the immunostimulants only caused an extension of the prepatent period of infection and the survival time of the mice. Other prospective methods which could lead to field applications of immunization against trypanosomiasis has been reviewed (Murray et al., 1979, 1980).

CHAPTER THREE

STUDIES ON THE RESPONSE OF ZEBU CALVES TO PRIMARY AND CHALLENGE INOCULATION WITH *TRYPANOSOMA VIVAX*

INTRODUCTION

Trypanosoma vivax is one of the most economically important species of trypanosomes which infect cattle in Nigeria. As early as 1927, *T. vivax* was recognised as the most common trypanosome in Nigerian cattle (Glover, 1961). High incidence of *T. vivax* infection in cattle has equally been reported in northern Nigeria (Ilemobade, 1971).

In view of its importance, a study was designed to assess the ability of susceptible Zebu calves to self-cure following primary infection with a wild isolate of *T. vivax*, and then the ability of self-cure calves to resist challenge infection with a homologous strain of *T. vivax*.

MATERIALS AND METHODS

Experimental animals

Twelve White Fulani Zebu calves between the ages of three and nine months were purchased for the study from Jibiya, a known tsetse-free area within the Sudan savannah zone of Nigeria. The ages of the animals were determined based on the anterior teeth eruption (Akerejola and Ojo, 1979).

On arrival at the Ahmadu Bello University, Samaru, Zaria, an area free of tsetse, the animals were preconditioned for three months before use. During the period, they were housed in pens. All the calves received routine anthelmintic treatment with therapeutic dose of Thiabendazole; those that had clinical anaplasmosis were treated with Biocycline (UpJohn). All animals were vaccinated against anthrax, contagious bovine pleuropneumonia and rinderpest. Tick control was carried out on weekly basis by dipping or spraying with Pfizona (Supona: Shell), and acaracide. The animals were grazed on pasture but during the period of pasture scarcity they were fed on grass hay supplemented with concentrate (cotton-seed cake and maize). Salt licks and water were supplied ad lib.

Prior to the commencement of the experiment, the animals were found to be free of trypanosomes based on wet and thin blood films, haematocrit centrifuge test (Woo, 1969) and mice inoculation of blood from the calves.

Trypanosome stock

A stock of Trypanosoma vivax isolated in 1980 from Zebu cattle exposed to natural infection at Ilorin was used for the primary inoculation of the animals. Stabilates were made from the isolate and these were cryopreserved at -196^{o}C in liquid nitrogen until used.

Experimental procedure

One donor calf was inoculated with the stabilate. On the third day of detectable parasitemia, blood from the donor calf was then subinoculated intravenously into each of eight experimental calves. Each inoculum contained approximately 1.6 x 10^8 trypanosomes in 3 ml of donor blood. The trypanosomes were enumerated in a haemocytometer using the trypanosome counting fluid described by Sannusi (1977). The three uninoculated calves served as controls.

Six weeks after their last patent parasitemia (based on absence of detectable trypanosomes by wet blood film, thin blood smear and haematocrit centrifuge test) all four calves which survived the primary infection, and the three previously uninoculated control calves were challenged with the cryopreserved homologous T. vivax (Ilorin strain). Each calf was injected through the intravenous route with approximately 2.2 x 10^8 trypanosomes in 4 ml blood of the donor calf previously cryopreserved.

Clinical examinations

Following the inoculations, rectal temperatures and blood samples were initially taken daily for the first seven days until the prepatent period of infection was determined. Thereafter rectal temperatures were taken and blood samples collected from the jugular vein three times a week (Mondays, Wednesdays and Fridays). About 1.0 to 1.5 ml of blood were

collected in an anticoagulant (disodium salt of ethylene diamine tetra-acetic acid, EDTA).

The packed cell volume (PCV) was determined by the microhaematocrit centrifuge technique and values were read using Hawksley microhaematocrit reader (Gelman Hawksley Ltd, England). The haemoglobin (Hb) concentration was estimated as cyanmethaemoglobin using an electronic haemoglobinometer (Coulter Electronics, Herts England). Total white blood cell (WBC) counts were made using the haemocytometer. Thin blood smears stained in Giemsa solution were prepared for the differential leukocyte counts, and for identification of any blood parasites present.

Parasites were estimated using the Haematocrit centrifuge technique (Woo, 1969) and depending on the degree of parasites obtained this was then rated as follows:

+	=	Less than 10 trypanosomes seen
++	=	10 to 15 trypanosomes seen
+++	=	16 to 20 trypanosomes seen
++++	=	more than 20 trypanosomes seen.

Changes in weight of the calves were determined once each week with an Avery Scale (Avery Burmingham England).

RESULTS

Table 1 summerizes the pertinent results obtained as they relate to age of animals, trypanosome dose, prepatent period, parasite patency and survival.

Table 1: Effects of a primary infection of eight Zebu calves with an Ilorin strain of T. vivax

Animal No	Age at Infection (Months)	Parasite Dose	Prepatent period (Days)	Patent period (Days)	Survival period (Days)	Remarks
*981	9	1.6×10^8	3	105	120	Died
*982	6	"	2		20	Died
*984	9	"	3		11	Died
986	6	"	2	69	Beyond 24 weeks	Self-cured
+987	6	"	4	91	126	Self-cured but died of other cause
988	6	"	4	91	Beyond 24 weeks	Self-cured
990	12	"	4	91	"	Self-cured
992	12	"	2	101	"	Self-cured

*Death due to trypanosomiasis.

+Death due to organophosphate poisoning.

Parasitemia

All the eight inoculated calves became infected after a prepatent period raning between two and four days. The parasitemia was highest in all the calves during the second week of infection and resulted in the death of a calf. Another calf died in week 3, while a third calf died in week 17 although it was apatent 10 days before death. After the 4th week all the surviving calves with the exception of the one that died in week 17, showed very low parasitemia. This persisted for 10 weeks (Fig. 1), leading to a fluctuating parasitemia up to week 19 after which time no parasites were detected.

Packed cell volume (PCV)

The mean PCV in all the surviving infected calves dropped from a preinfection value of 27.5% (± 5.5) to 21.4% (± 4.5) in the second week. This sharp drop coincided with the parasitemic peak observed in week 2 (Fig. 1A). The first calf which died in the same week had a PCV of 19.5% prior to its death in week 3. The third calf which died in week 17 also had a PCV of 13.0% before death. Decreases in PCV in the surviving calves continued till week 5 when it reached the lowest mean of 19.0% (± 3.8). Gradual increase was observed between the 6th and 11th weeks after which it started to drop. By week 13 the PCV value had dropped to 19.3% (± 4.0). Increases occurred thereafter and the value rose once more to preinfection level in week 20. The sharp rise in week 20 occurred as a result of the effects of

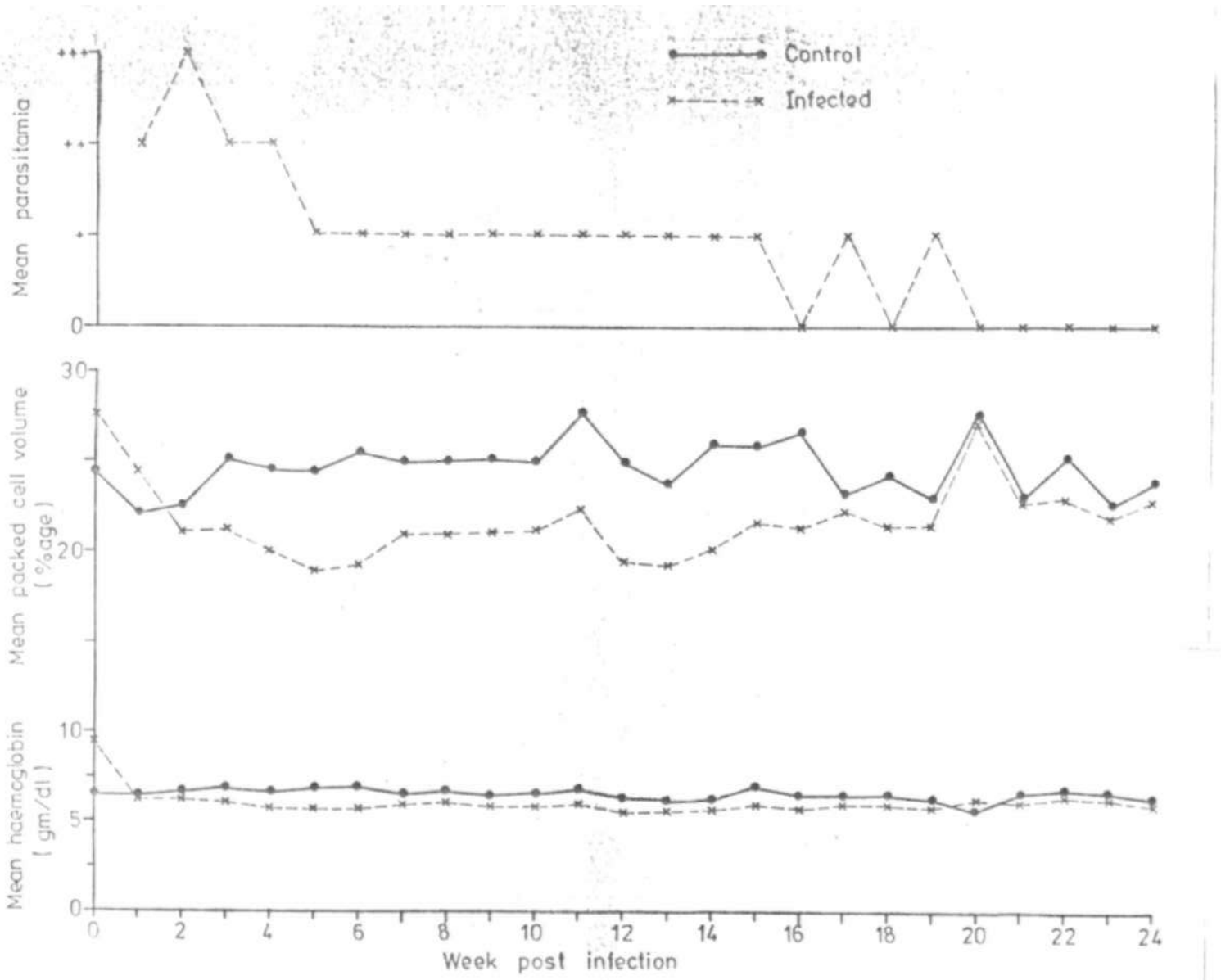


FIG.1. MEAN PARASITEMIA, PACKED CELL VOLUME AND HAEMOGLOBIN CONCENTRATION OF INFECTED AND CONTROL ZEBU CALVES DURING PRIMARY *T. VIVAX* INFECTION.

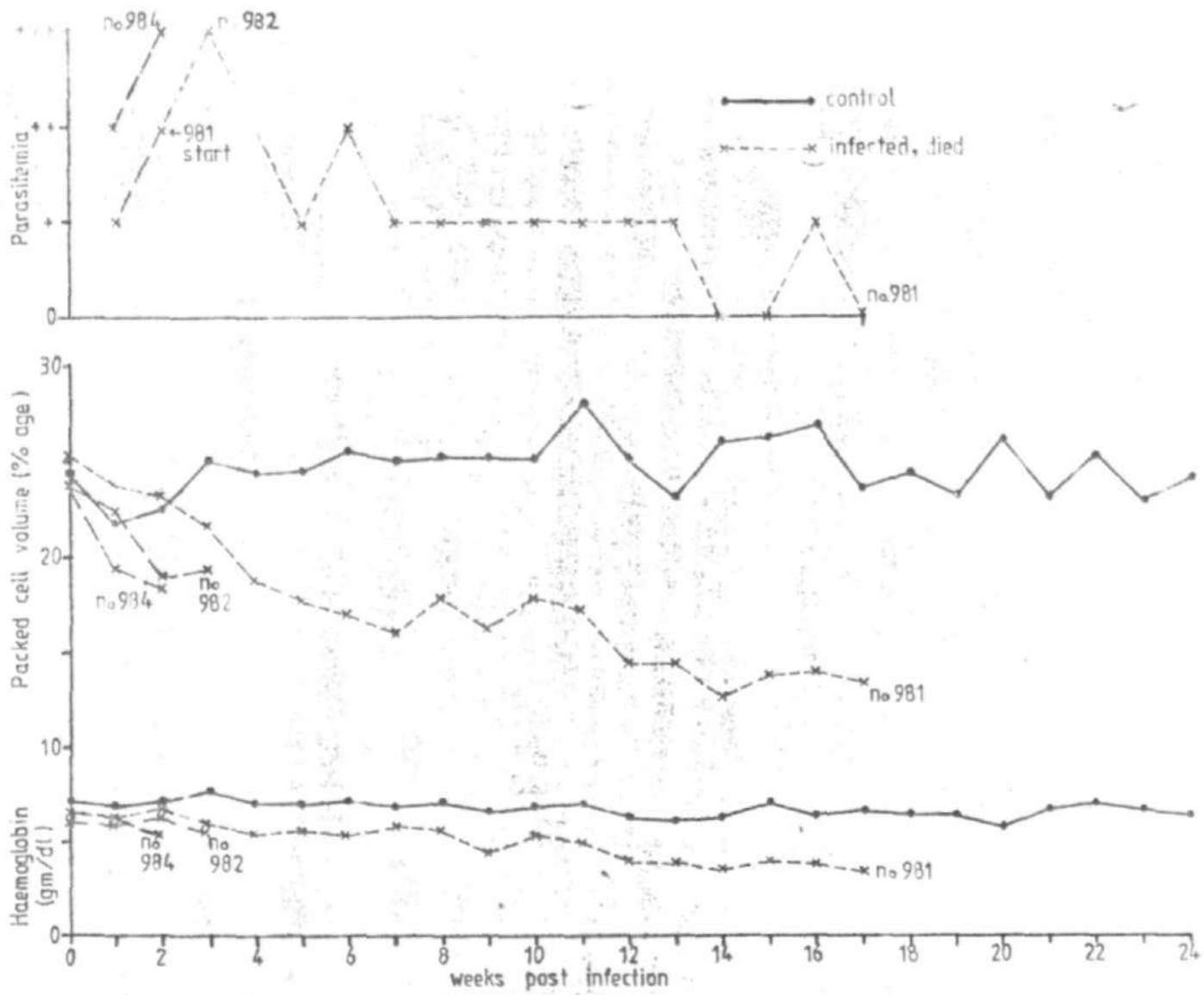


FIG.2. PARASITEMIA, PACKED CELL VOLUME AND HAEMOGLOBIN LEVELS IN THREE CALVES THAT DIED DURING PRIMARY T. VIVAX INFECTION. (CONTROL AS IN FIG.1.)

organophosphate poisoning during one of the routine sprayings with Pfizona (acaracide) which led to the death of one calf. The control calves maintained higher PCV values than the infected calves during the course of infection. The parasitemia, packed cell volume and haemoglobin levels in the three infected calves that died during the primary T. vivax infection is shown in Fig. 2. The PCV was markedly lowered in these calves compared with the controls especially at the point of death.

Haemoglobin (Hb) concentration

There were slight differences in the haemoglobin levels of the dead calves and the control. With the exception of the decrease from a preinfection value of 9.02 (± 1.4) to 7.54 (± 1.2) noticed in week 1 in the infected group, the haemoglobin levels among the infected as well as the control calves did not show significant variations during the course of infection (Fig. 1).

White blood cell (WBC) counts

The WBC counts in both the infected and control calves did not show any marked changes during the course of the infection. However, there were fluctuations in the total WBC counts in both the infected and control calves ((Fig. 3). No data was obtained in week 20 due to organophosphate poisoning which affected all the calves.

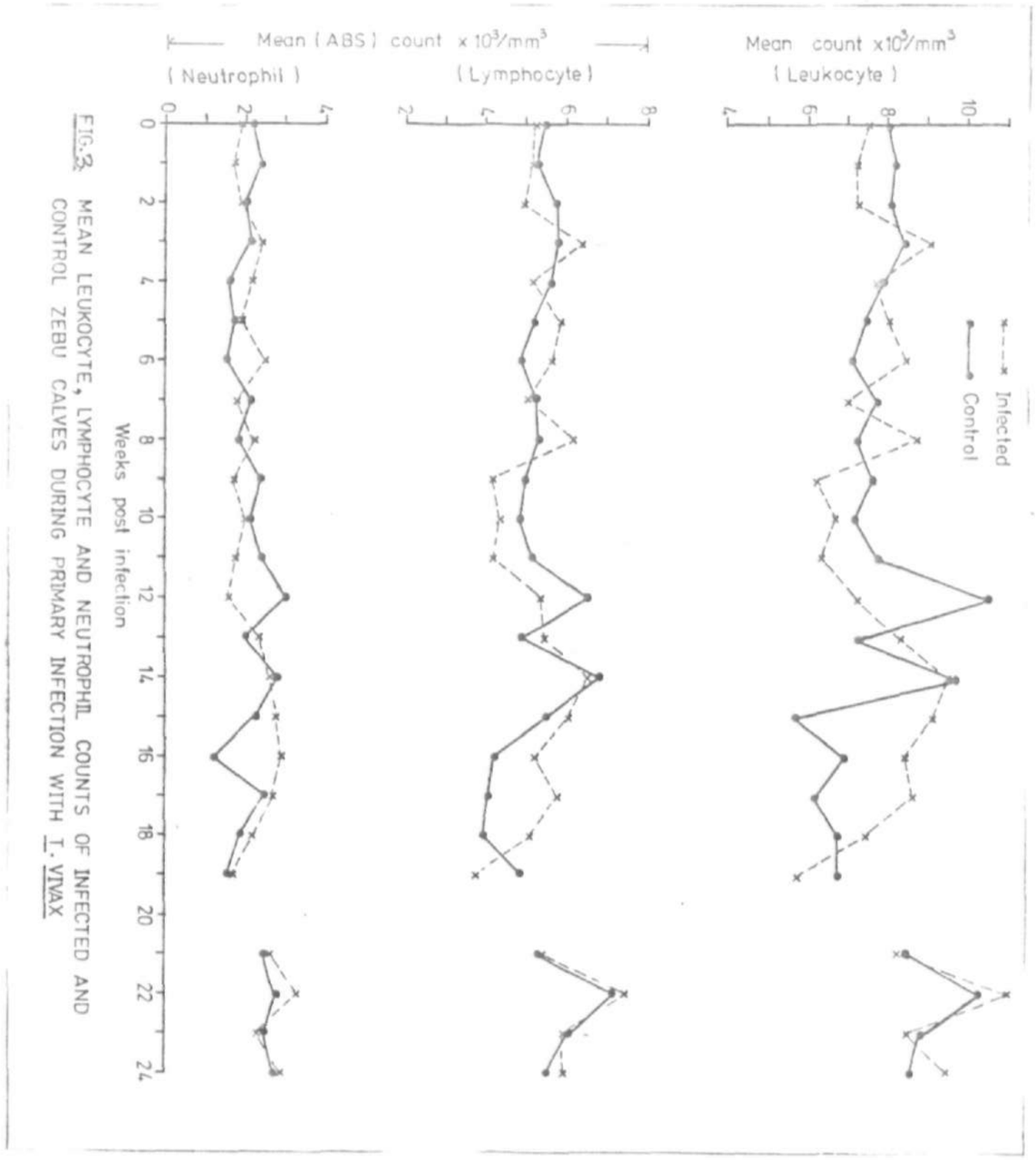


FIG.2. MEAN LEUKOCYTE, LYMPHOCYTE AND NEUTROPHIL COUNTS OF INFECTED AND CONTROL ZEBU CALVES DURING PRIMARY INFECTION WITH T. VIVAX

The lymphocyte and neutrophil counts in both infected and control calves also did not show marked changes. The pattern of variation in these counts corresponded with those of the total WBC counts (Fig. 3).

Changes in body weight

Loss in body weight became evident among the infected group two weeks post infection. Thereafter intermittent weight gains and loss occurred in both groups of calves.

Response of self-cured calves to challenge with a homologous stock of Trypanosoma vivax

The observations made on the self-cured calves when challenged with a homologous stock of T. vivax are summarized on Table II.

Parasitemia

For the seven weeks of this phase of the study no parasites were detected in the blood of any of the self-cured calves. The three control calves became infected after prepatent periods which varied between five to seven days. The parasitemia reached a peak in week 3 (Fig. 4) and persisted into the following week after which the level dropped. All the three infected control calves became non-patent by the seventh week after they had been treated with Berenil (7 mg/kg body weight) in the fifth week.

Table II: Summary of observations following challenge with a homologous strain of T. vivax on self-cured calves and previously uninfected control calves.

Animal No	Age at time of challenge (Months)	Approximate parasite Dose	Prepatent period (Days)	Drug treatment	Remarks
SELF-CURED CALVES					
986	12	2.1×10^8	-	Untreated	Refractory
988	12	"	-	"	"
990	18	"	-	"	"
992	18	"	-	"	"
PREVIOUSLY UNINFECTED					
983	12	"	5	Treated	Susceptible
985	18	"	5	"	"
991	15	"	7	"	"

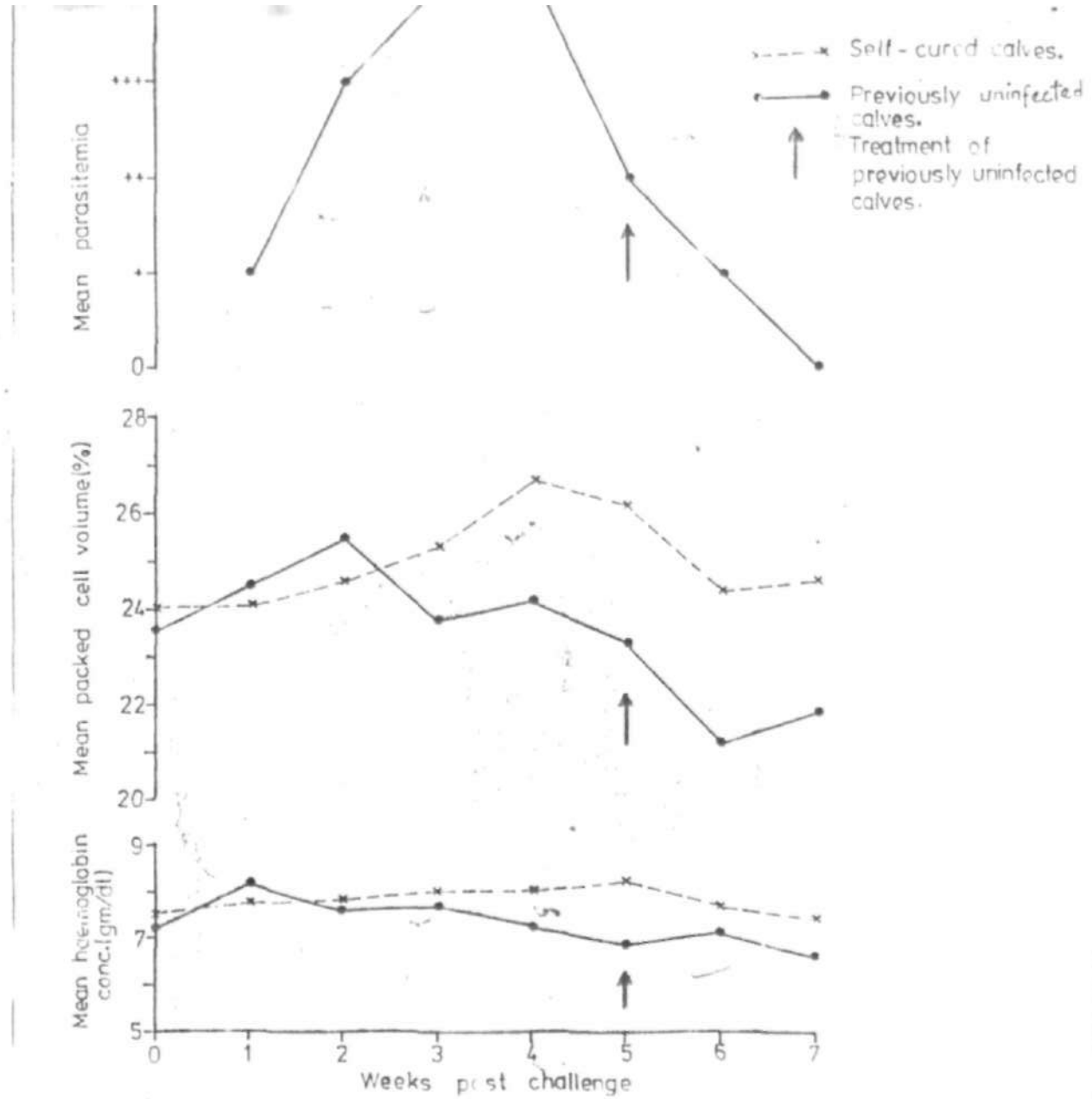


FIG.4. MEAN PARASITEMIA, PACKED CELL VOLUME AND HAEMOGLOBIN CONCENTRATION OF 4 SELF-CURED AND 3 INFECTED CONTROL CALVES.

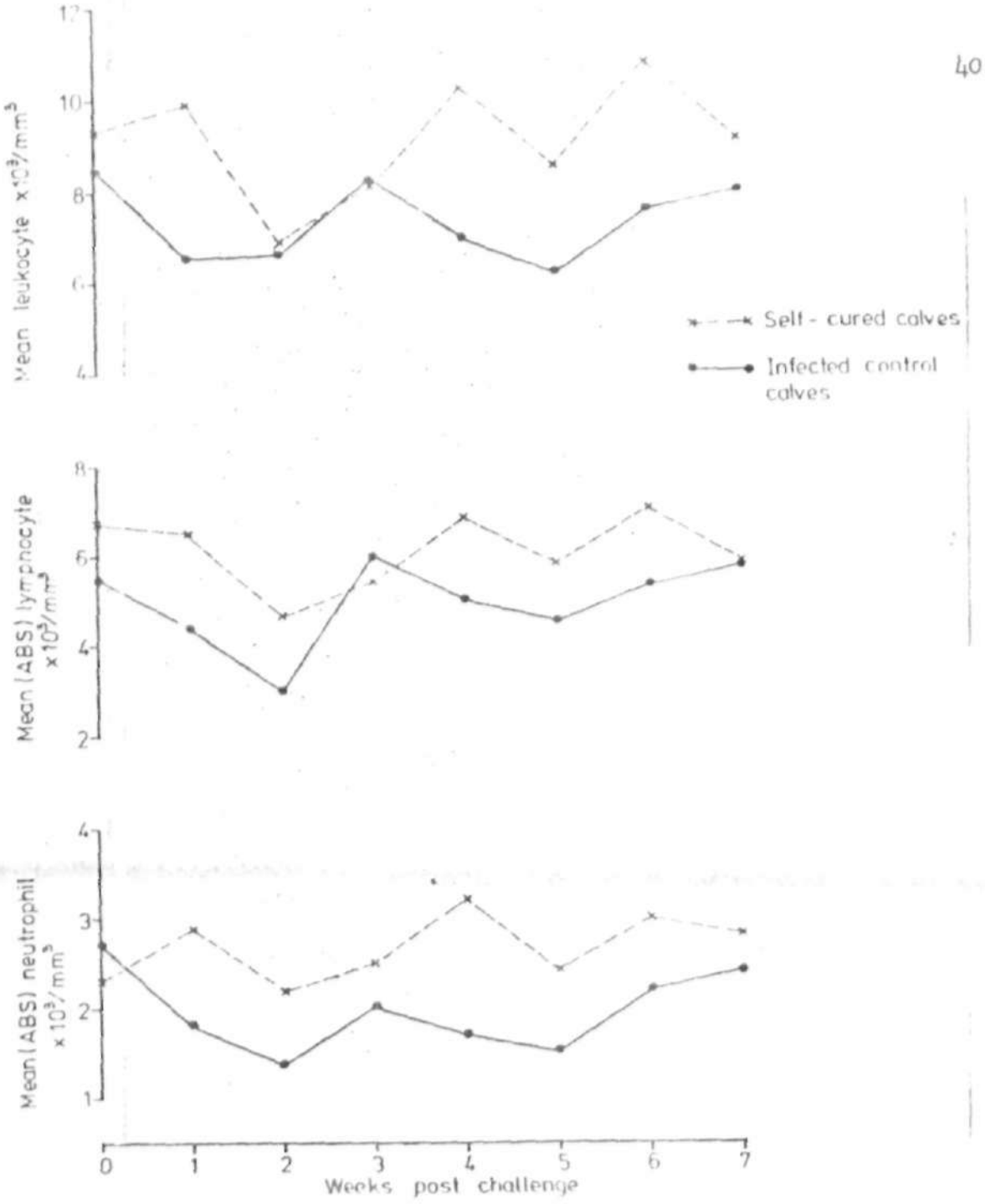


FIG. 5. MEAN LEUKOCYTE, LYMPHOCYTE AND NEUTROPHIL COUNT OF SELF-CURED AND INFECTED CONTROL CALVES

DISCUSSION

The results of this study have shown that White Fulani Zebu calves between the ages of six and 12 months possess the ability to self-cure primary T. vivax infection. Although evidence of clinical trypanosomiasis was obtained, the disease process in the surviving calves was not as severe as reported in cases of trypanosomiasis (Stephen, 1970). This is evident from the mild temperature reaction, level of parasitemia and mild nature of anemia. Most of the deaths (two out of three) occurred at the early stage of the infection within the first three weeks and was characterised by rapid decline in PCV and persistent high parasitemia. In contrast all the calves that were able to survive maintained low parasitemia and were able to control it until they self-cured.

Dargie et al. (1979a, 1979b) compared the responses of Ndama and Zebu cattle experimental infected with both T. brucei and T. congolense. From the studies, they concluded that the superior resistance of the Ndama over the Zebu was due mainly to their ability to control parasitemia rather than their capacity to mount a more efficient erythropoietic response. This observation has also been made for T. congolense infection in the same breeds (Murray and Morrison, 1978). In this study, the surviving calves have demonstrated similar response as indicated by the short period of high parasitemia and the protracted period of low parasitemia and apatency prior to complete self-cure. Unlike the dead calves all the self-cured

calves showed initial signs of recovery from the anaemia and improvement in PCV five weeks post infection. This early recovery contrasts with reported observations on anaemia during animal trypanosomiasis (Dargie et al., 1979b; Wellde et al., 1981). Therefore, it could be inferred that the responses of some White Fulani Zebu calves used in this study is similar to the response of the Ndama breed during exposure to infection and that their ability to survive and self-cure could be dependent as in the Ndama on their capability to limit and control parasitemia and also on the ability of their bone marrow to regenerate new red blood cells for the replacement of damaged ones. This is indicated by the recovery from anaemia reflected in the rise in PCV by the fifth week.

In the present study, three out of eight calves (38%) died of the infection. The difference in mortality in this study and that of Wellde et al. (1981) in which one out of eleven calves died could be due to differences in the dose and species of trypanosome used. In the present experiment the challenge dose was approximately 1.6×10^8 T. vivax organisms against 3.0×10^8 T. congolense organisms per 277 kg body weight used by Wellde and his colleagues. Apart from the higher dose, the West African T. vivax is known to cause more severe disease in cattle than either the East African T. vivax or T. congolense. Another factor which could be associated with these deaths is virulence of the strain. The strain used in this study had already been known to be virulent since almost all adult animals that

were exposed to it either naturally or experimentally died of trypanosomiasis (Ilemobade unpublished; TTD drug sensitivity trials). Considering the parasitemic profile, the parasitemia was high or fairly high at the early stage of infection. It has been shown that trypanosome variants with high degree of virulence are usually expressed at the early period of infection (Van Meirvenne et al., 1975) and that animals which were unable to survive this period become liable to death while those that survive beyond this period in most cases eventually recover from the infection. This observation is in accord with the results of this experiment in which two of the three calves that died did so at the early stage of infection while all but one of those that survived beyond this period self-cured the infection. Death in these calves was believed to be due to anaemia as evident from the low PCV values at the point of death.

Although this study did not include observation on the immune responses of the calves during the course of infection, the need for such a study in future investigation is suggested for better understanding of the actual mechanism(s) involved in self-cure against trypanosome infections which has been demonstrated in the Zebu calves.

The lack of infection in the self-cure calves following challenge with homologous T. vivax strain indicates that self-cure from primary infection is accompanied with a high degree of protection against future challenge with same strain.

Wellde et al. (1981) reported in their own studies similar acquisition of immunity by calves against homologous T. congolense challenge either through syringe or cyclical passage. The immunity acquired by the calves used in this study was solid for at least a period of six months (period of observation). This was based upon non-detectability of parasitemia, good blood picture and absence of other clinical signs indicative of animal trypanosomiasis. It must also be stated that the refractory response of the self-cured calves was not due to loss of infectivity of the cryopreserved stabilate since the controls became infected with the same stabilate materials. A trial aimed at finding out the response of these self-cured calves to a heterologous challenge with a mice infective strain (T. vivax Y58) suggested that the acquired immunity did not prevent reinfection since all the calves became infected within two to four days.

These observations are in consonance with earlier findings by other workers, notably Bevan (1936), Hornby (1941) and Fiennes et al. (1946) on trypanosome infections in young cattle. They are also in agreement with the findings of Wellde et al. (1981) on T. congolense infection in Hereford calves. Thus, even though Weitz (1970) could not demonstrate acquired protection in cattle that had recovered from trypanosomiasis, these previous reports support the current findings.

SUMMARY AND CONCLUSION

This study has shown that a proportion of White Fulani Zebu calves possess some ability to limit, control and self-cure primary infection with Trypanosoma vivax. This ability of the calves compares with that of trypanotolerant Ndama breed, but contrasts with reports on adult Zebu cattle. The factors responsible for this include the ability to limit and control parasitemia early during the course of infection and the ability of the marrow to maintain a relatively normal blood picture.

Early deaths occurred in calves that were unable to withstand primary infection but most of the calves which survived the first three weeks of infection were self-cured. Calves that self-cured the primary infection resisted challenge with the homologous stock, which was, in this study, the "Ilorin" strain of T. vivax.

It is inferred that live trypanosomes could be used for immunization of cattle under 12 months of age. The potentials of the Zebu calves as shown in this study make them a favourable model for any such immunization programme.

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APPENDIX ONE

1A

PRIMARY INFECTION OF CALVES WITH T. VIVAX
PACKED CELL VOLUME OF INFECTED AND CONTROL CALVES

WEEKS	INFECTED*		CONTROL**	
	MEAN	± SD	MEAN	± SD
0	27.5	5.5	23.8	2.5
1	24.0	3.9	22.3	2.4
2	21.4	4.5	22.6	2.5
3	21.7	3.2	25.2	0.3
4	20.1	3.4	24.6	0.7
5	19.0	3.8	24.5	1.9
6	19.5	3.4	25.5	2.3
7	21.3	3.6	25.2	2.0
8	21.1	2.9	25.3	2.6
9	21.1	3.5	25.2	1.3
10	21.4	3.6	25.1	0.2
11	22.5	5.2	27.8	2.2
12	19.6	4.2	25.1	0.8
13	19.3	4.0	23.0	1.0
14	20.3	4.3	26.1	1.4
15	21.8	4.3	26.0	1.6
16	21.6	4.8	26.8	1.8
17	22.4	4.7	23.5	4.1
18	21.6	5.7	24.5	2.2
19	21.5	4.8	23.2	1.7
20	27.3	8.2	26.2	1.8
21	22.8	2.6	23.0	1.4
22	22.9	3.9	25.4	0.9
23	22.2	3.2	22.7	1.5
24	21.7	2.1	24.1	0.8

*Number of calves was 5 from wk 0 to 18, and 4 from 19 to 24

**Number of calves = 3.

1B

PRIMARY INFECTION OF CALVES WITH T. VIVAX
HAEMOGLOBIN CONCENTRATION OF INFECTED
AND CONTROL CALVES

WEEKS	INFECTION*		CONTROL**	
	MEAN	± SD	MEAN	± SD
0	9.02	1.4	7.8	0.7
1	7.54	1.2	7.0	1.2
2	7.46	1.7	8.3	0.3
3	7.38	1.2	8.6	0.2
4	6.30	1.2	8.3	0.4
5	6.32	1.3	8.5	0.4
6	6.44	1.4	8.9	0.7
7	6.88	1.0	8.0	0.5
8	7.14	1.0	8.4	0.8
9	6.82	1.4	7.9	0.7
10	6.70	1.0	8.3	0.2
11	6.90	1.6	8.5	0.3
12	6.34	1.3	7.9	0.6
13	6.24	1.1	7.8	0.7
14	6.66	1.1	7.8	0.5
15	7.16	1.3	9.0	0.6
16	6.62	1.3	8.3	0.4
17	7.38	1.3	8.2	1.6
18	7.20	1.8	8.1	2.2
19	6.68	1.2	7.5	0.3
20	7.53	2.2	7.4	0.9
21	7.50	1.3	8.1	1.2
22	8.00	1.1	8.0	0.6
23	7.75	1.0	8.0	0.4
24	7.16	0.6	7.4	0.3

*Number of calves was 5 from wk. 0 to 18, and 4 from 19 to 24.

**Number of calves = 3.

1C

PRIMARY INFECTION OF CALVES WITH T. VIVAX
TOTAL WBC COUNTS OF INFECTED AND CONTROL VALVES

WEEKS	INFECTED*		CONTROL**	
	MEAN	± SD	MEAN	± SD
0	7500.0	959.0	8033.3	202.1
1	7260.0	1588.7	8250.0	1096.5
2	7265.0	1538.6	8183.3	1068.0
3	9020.0	2610.6	8416.6	1501.9
4	7750.0	863.8	7883.3	2232.8
5	8096.0	693.4	7497.6	2397.0
6	8450.0	1467.9	7116.6	2635.0
7	7050.0	1903.3	7750.0	2422.3
8	8770.0	1566.9	7216.6	2010.8
9	6220.0	1305.5	7566.6	1109.4
10	6700.0	2094.3	7166.6	2426.0
11	6310.0	1995.1	7850.0	1702.2
12	7250.0	1169.4	10516.6	2382.4
13	8350.0	2773.8	7250.0	1178.9
14	9500.0	2359.8	9616.6	2155.4
15	9100.0	3246.5	5616.6	1200.3
16	8450.0	2659.4	6950.0	626.5
17	8630.0	2865.4	6280.0	1075.0
18	7490.0	2049.2	6283.3	1078.6
19	5712.5	1049.9	6716.6	2490.6
20	-	-	-	-
21	8275.0	2088.6	8416.6	768.6
22	10937.5	1521.7	10333.3	1118.4
23	8492.5	1988.5	8883.3	987.8
24	9462.5	1443.0	8566.6	1059.8

*Number of calves was 5 from wk. 0 to 18, and 4 from 19 to 24.

**Number of calves = 3.

1D

PRIMARY INFECTION OF CALVES WITH T. VIVAX
ABSOLUTE LYMPHOCYTE COUNT OF INFECTED AND CONTROL CALVES

WEEKS	INFECTED*		CONTROL**	
	MEAN	± SD	MEAN	± SD
0	5256.4	1023.5	5558.2	706.1
1	5204.0	1020.4	5271.3	1262.4
2	5054.6	961.2	5706.7	1406.3
3	6407.8	2462.3	5806.7	482.1
4	5234.4	853.2	5696.0	1491.2
5	5829.2	614.8	5201.3	1561.0
6	5755.0	991.5	4935.0	1820.1
7	5105.4	1849.2	5299.3	1237.0
8	6212.2	2104.1	5359.0	2120.2
9	4269.8	650.4	5033.3	1012.5
10	4435.0	1597.8	4912.4	1870.1
11	4277.0	1645.2	5227.0	583.5
12	5476.0	782.7	6505.0	1865.2
13	5522.0	1407.7	4913.3	1329.9
14	6566.8	1793.6	6997.6	2394.4
15	6031.6	2187.8	5520.0	683.5
16	5261.0	1178.9	4252.0	918.4
17	5870.4	2015.7	4173.3	750.2
18	5125.4	1538.1	4050.3	869.7
19	3844.5	87.1	4911.0	1949.1
20	-	-	-	-
21	5476.0	1480.4	5484.0	1211.5
22	7452.3	1443.8	7137.3	906.5
23	5989.0	1543.1	6035.0	878.2
24	5981.3	675.6	5538.3	1094.3

*Number of calves was 5 from wk. 0 to 18, and 4 from 19 to 24.

**Number of calves = 3.

1E

PRIMARY INFECTION OF CALVES WITH T. VIVAX
ABSOLUTE NEUTROPHIL COUNT OF INFECTED AND CONTROL CALVES

WEEKS	INFECTED*		CONTROL**	
	MEAN	± SD	MEAN	± SD
0	1908.0	888.5	2264.6	955.8
1	1775.4	678.8	2406.6	543.0
2	1980.6	867.2	1986.6	202.3
3	2407.4	889.5	2145.0	654.8
4	2285.6	547.6	1672.0	518.2
5	1963.8	651.8	1786.3	735.8
6	2597.0	946.0	1569.0	674.9
7	1884.2	750.5	2186.3	1280.7
8	2252.8	681.9	1835.6	414.2
9	1707.2	888.6	2416.6	853.3
10	2029.0	999.3	2014.3	1361.4
11	1867.4	744.2	2495.0	1290.3
12	1610.4	780.5	3031.6	691.7
13	2343.6	1413.9	2041.3	481.9
14	2619.6	1225.0	2701.3	609.8
15	2894.0	1283.8	2373.3	280.9
16	2960.2	1595.7	1223.3	493.9
17	2744.0	1127.6	2568.6	1065.2
18	2210.6	882.6	1962.3	836.8
19	1715.0	918.9	1685.3	747.8
20	-	-	-	-
21	2670.3	595.9	2641.3	702.3
22	3374.0	454.7	2857.0	749.2
23	2317.3	876.5	2588.0	974.4
24	2984.0	774.1	2917.3	1125.4

*Number of calves was 5 from wk. 0 to 18, and 4 from 19 to 24.

**Number of calves = 3.

1F

PRIMARY INFECTION OF CALVES WITH T. VIVAX
WEIGHT GAIN/LOSS

WEEKS	INFECTED*		CONTROL**	
	MEAN	± SD	MEAN	± SD
1	0.0	3.3	1.3	2.3
2	0.8	5.1	5.7	1.2
3	-1.2	1.2	1.0	1.7
4	0.2	2.9	3.3	1.5
5	-0.8	1.9	-1.7	1.2
6	5.0	5.5	9.7	6.4
7	-1.2	6.0	-1.7	6.4
8	5.2	4.3	-1.0	5.2
9	-5.8	3.4	4.3	3.2
10	4.8	5.8	2.0	4.0
11	5.0	6.1	-4.3	2.9
12	10.2	6.0	11.0	2.6
13	-0.6	3.3	-3.7	2.3
14	1.0	4.4	1.3	2.1
15	-5.0	4.1	-5.3	1.2
16	-5.2	2.3	-4.3	2.5
17	5.0	4.8	3.7	0.6
18	-10.6	2.7	-6.7	4.9
19	-	-	-	-
20	-6.5	4.4	-5.3	3.1
21	7.5	4.2	4.7	1.5
22	-.75	3.6	-1.3	5.5
23	0.0	2.9	1.7	2.1
24	2.25	1.4	2.7	1.5

*Number of calves was 5 from wk. 0 to 18, and 4 from 19 to 24.

**Number of calves = 3.

APPENDIX TWO

2A

PACKED CELL VOLUME (%)
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
0	24.0	0.8	25.0	2.0
1	24.1	1.3	24.5	1.0
2	24.6	0.6	25.5	0.3
3	25.3	0.7	23.8	1.3
4	26.7	1.3	24.2	0.3
5	26.2	1.4	23.3	1.6
6	24.4	0.3	21.2	0.8
7	24.6	1.8	21.9	1.1

*Number of calves = 4.

**Number of calves = 3.

2B

HAEMOGLOBIN CONC. (GM/DL)
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
0	7.5	0.2	7.2	0.2
1	7.8	0.7	8.2	0.3
2	7.8	0.8	7.6	0.1
3	8.0	0.5	7.6	0.1
4	8.0	0.4	7.2	1.0
5	8.2	1.0	6.9	1.5
6	7.6	1.3	7.1	0.6
7	7.4	0.9	6.0	0.5

*Number of calves = 4.

**Number of calves = 3.

2C

TOTAL WHITE BLOOD CELL COUNT
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
0	9462.5	1443.0	8566.6	1059.8
1	9862.5	3260.7	6616.7	246.6
2	6950.0	1203.0	5003.3	1470.3
3	8187.5	495.6	8266.7	596.5
4	10250.0	2212.5	7083.3	1037.2
5	8600.0	1155.4	6283.3	175.6
6	10712.5	809.7	7666.7	351.2
7	9150.0	543.1	8050.0	150.0

*Number of calves = 4.

**Number of calves = 3.

2D

ABSOLUTE LYMPHOCYTE COUNT
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
0	6765.6	124.0	5534.0	103.8
1	6588.1	283.6	4479.5	9.8
2	4587.0	54.1	3097.6	155.2
3	5485.6	59.9	6034.	17.8
4	6947	287.6	5007.8	60.1
5	5891	128.2	4561.6	16.5
6	7155.9	62.3	5389.6	25.9
7	5947.5	60.2	5820.1	5.7

*Number of calves = 4.

**Number of calves = 3.

2E

ABSOLUTE NEUTROPHIS COUNT
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
o	2337.2	13.7	2792.7	116.5
1	2939.0	303.2	1832.8	19.7
2	2244.8	39.7	1403.4	47.0
3	2521.7	58.9	2091.4	25.0
4	3228.7	261.0	1792.0	95.4
5	2433.8	125.9	1507.9	9.1
6	3031.6	72.0	2246.3	23.9
7	2863.9	47.7	2439.1	8.5

*Number of calves = 4.

**Number of calves = 3.

2F

WEIGHT GAIN/LOSS (KG)
DURING HOMOLOGOUS CHALLENGE WITH T. VIVAX

WEEKS	SELF-CURED*		INFECTED CONTROL**	
	MEAN	± SD	MEAN	± SD
1	4.3	1.0	3.0	1.0
2	4.8	1.7	3.7	2.1
3	0.3	2.6	0	0
4	7.5	3.0	0	0
5	4.8	6.3	-1.3	4.7
6	3.3	2.5	6.3	3.8
7	2.5	1.3	-1.3	1.2

*Number of calves = 4.

**Number of calves = 3.