SEROLOGICAL SURVEY OF BOVINE ANAPLASMOSIS ON SHIKA FARM AND TREATMENT OF THE CLINICAL DISEASE

BY

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THESIS APPROVAL

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"A bad beginning makes a good ending...."

D E D I C A T I O N

This thesis is dedicated to my loving Mother, Madam Abisatu Amoke Jagun (nee Akarakiri), through whose efforts, kindness, devotion and care, my leap to this height has been possible. May Almighty Allah continue to preserve her soul in order to eat more fruit of her labour.

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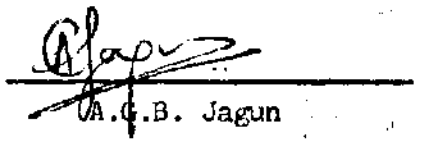
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This is to certify that I carried out the work reported in this thesis in the protozoology laboratory of the Department of Veterinary Parasitology and Entomology, as well as on the farm of the National Animal Production Research Institute (N.A.P.R.I.), under the supervision of Profs. A.A. Ilemobade, I.A. Abdulkadir and D.I. Saror.

The work of other investigators, in this field, is duly acknowledged and referred to appropriately. No part of this thesis has been submitted elsewhere for a degree or diploma.



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CHAPTER I

INTRODUCTION

Bovine anaplasmosis is an infectious, noncontagious disease of cattle caused by rickettsial-like parasites of the genus Anaplasma. More than 19 species of ticks are now known to transmit Anaplasma organisms under natural condition. However, its mode of transmission is complicated by the fact that other biting flies mechanically transmit the organism. Humans have similarly, been incriminated in the mechanical transmission of the disease, through contaminated instruments, while in utero transmission has also been demonstrated (Fowler and Swift, 1975; Swift and Paumer, 1976).

The severity of the disease varies from peracute to protracted chronic course and is characterised chiefly by a progressive anaemia, icterus and pyrexia. The organism is located in the erythrocytes of the host. It is essentially a disease of cattle, affecting all breeds and age groups, but calves are generally more resistant to clinical infection than the adults. Cattle imported from an area free of the disease, however, appear to be more susceptible than the

native population. Animals which recover from the clinical disease become carriers for the rest of their lives (Jones, 1968).

Tetracyclines have been the main chemotherapeutic agent used in the treatment of bovine anaplasmosis, for the past two decades. Two other new drugs, Gloxazone^{R} and Imidocarb^{R}, have recently been introduced; but Gloxazone^{R} was never marketed due to its toxicity, while Imidocarb^{R} has not yet been fully accepted as the tetracyclines. In some parts of the world, especially in the United States of America, vigorous efforts to control the disease, to some extent, have been successful through the use of drugs and various methods of vaccination programmes. In Nigeria, for example, the clinical disease is treated with Oxytetracycline, while further infection is prevented through seasonal control of the ticks and other arthropod vectors, using some broad spectrum acaricides. So far such control measures have been limited to government farms and absentee farmers. In general, the control of the vector is hardly undertaken routinely and cattle are, in most cases, left to develop immunity through constant tick challenge. This leads to great economic losses, through deaths and reduced production.

This thesis, therefore:

(a) reviews pertinent literature on bovine anaplasmosis, especially with particular reference to Nigeria;

(b) investigates the prevalence of the disease among cattle in the National Animal Production Research Institute (NAPRI) Farm at Shika and its environs and in slaughter cattle and

(c) evaluates certain common dosages of Oxytetracycline used in the treatment of bovine anaplasmosis and the duration of such treatment.

CHAPTER II

REVIEW OF LITERATURE

A Review of Bovine Anaplasmosis.

Historical Developments

Anaplasmosis was first observed in conjunction with the study of piroplasmosis of cattle by Smith and Kilborne (1893 a,b). In their investigation they recorded the occurrence of "intra-corpuscular bodies", which were referred to as the "coccus-like bodies or marginal points"; in the blood of affected cattle, but which were considered to be a stage in the life cycle of Piroplasma bigeminum. Kolle (1898) described apparently similar bodies in the erythrocytes of South African Cattle. Occurrence of "marginal point" in erythrocytes of cattle suffering from piroplasmosis has been observed by Dschunkowsky and Luhs (1904). They concluded that the inclusions represented a form of P. annulatum, the "causative agent of tropical piroplasmosis". Similar observations were recorded by Lignières (1914) in Argentine Cattle. Subsequent investigations revealed that the above workers were dealing with a mixed infection of anaplasmosis and piroplasmosis.

In the course of his studies, Theiler (1910 a,b; 1911 a,b; 1912 a,b) concluded that the "chromatic bodies" or so-called "marginal points" present in the erythrocytes of affected animals represented a new genus of protozoan - the Anaplasma - which was responsible for the genuine gall sickness of South African Cattle. He designated the peripheral bodies found in the bovine erythrocytes, A. marginale, the disease it causes, anaplasmosis. By means of cross-immunity experiment, Theiler demonstrated that anaplasmosis was different and distinct disease from piroplasmosis.

Distribution.

Since the announcement by Theiler, anaplasmosis has been reported from most tropical and subtropical countries throughout the World, and during the past three decades it has been identified in many areas of the temperate zones. The gradual spread of the disease into the temperate zones, within the past years has been attributed mainly to the development of modern transportation; because of the ease with which many exposed and carrier animals are moved into previously clean areas (Goss, Mills and Guard, 1941; Moore, Riley and White, 1950; Griffiths and Hadlow, 1951; Thomas, 1958).

Classification of Anaplasma.

Despite the intensive investigations conducted since the discovery of the organism (Theiler, 1909, 1910, 1911, 1912; Lignières, 1914; Boyton, 1917; Leclainche, 1930) opinions were still divided for a long time, as to the nature of the anaplasma agent and its relationship to other blood parasites.

Problems of establishing the genus Anaplasma.

Through the use of phase microscopy and a variety of special techniques, many workers (Boynton, 1932; Boynton and Woods, 1944; Espana and Espana, 1959, 1963; Pilcher, Wu and Muth, 1961; Krier and Ristic, 1963, a,b) reported that Anaplasma have structures resembling tails associated with marginal body. This impression that Anaplasma is a polymorphic organism has greatly increased the controversy over its classification. Some standard texts have even reflected this controversy. For instance, Jubb and Kennedy (1963), Bruner and Gillespie (1966), in their texts, placed Anaplasma among "pathogenic protozoa of undetermined classification".

In 1934, Neitz, Alexander and du Toit suggested that Anaplasma, Eperythrozoon, Bartonella, and Grahamella, being

closely related, should be classed together in the family Anaplasmidae, which is then placed in the order Haemosperidia. Perhaps as a result of the strong criticism levelled against this familial grouping by Weinman (1944), Neitz's suggestion was not generally accepted. In his criticism Weinman (1944) among other things had remarked that it would be improper to place Anaplasma among the sporozoa, since it shows neither nuclear differentiation nor evidence of sexuality. On the other hand, Foote, Geer and Stich (1958), suggested that Anaplasma is a virus, on the basis of the organism's obligate intracellular parastism and its filterability characteristics. This suggestion was later disproved by Ristic (1968), when he contended that Anaplasma is clearly not a virus, because of its cellular organisation, cellular growth and possession of a limiting membrane, both of which were lacking in viruses.

Stainer, Doudoroff and Adelberg (1963), using structural characteristics divided all biological cells into two types; the procaryotic and the eucaryotic (Table I). The former includes the bacteria, mycoplasma, rickettsia, chlamydia, spirochetes, leptospira, and the blue green algae. The eucaryotic type includes green algae and higher plants, protozoa and higher animals, and the fungi.

TABLE I*

Characteristics of Procaryotic and Eucaryotic cells and of Anaplasma

Criteria	Eucaryotic	Procaryotic	Anaplasma
Nucleus:	Demonstrable with conventional stains (e.g. Giemsa)	Demonstrable only by special procedures (e.g.; thin section electron microscopy, staining after hydrolysis of RNA, with phase microscopy)	Demonstrable only by special procedures
Nuclear membrane:	Present except during cell division.	Absent	Absent
Membranous Organelles:	Prominent in cytoplasm e.g. mitochondria, endoplasmic reticulum).	Undeveloped; if present usually clearly associated with plasma membrane.	Undeveloped or absent.

*The Microbial World, 2nd Ed., Stainer et al. (1963)

Using this new classification, Stainer et al. (1963) grouped Anaplasma with the procaryotes; suggesting that Anaplasma is not a protozoan. This suggestion has been accepted in Bergey's Manual, 8th edition 1974; where the organism is placed in the family Anaplasmataceae, in the order Rickettsiales.

Like rickettsia, Anaplasma are obligate intracellular parasites which maintain their limiting membranes during intracellular growth stage. The parasite has thus been described by Ristic (1968) as one which develops inside a limiting membrane in the host cell and produces an inclusion body. Although inclusion bodies were commonly associated with viral infections, Krier and Ristic (1972) had remarked that inclusions may be formed by agents other than viruses. Chlamydia, and Anaplasma were thus given as examples of such agents. On the basis of the foregoing, Anaplasma is considered to be a non-protozoan organism, but rickettsial-like (Bergey's Manual of determinative bacteriology, 8th Ed., 1974).

Family Anaplasmataceae

According to the current edition (8th edition) of the Bergey's Manual of determinative bacteriology (1974), the family Anaplasmataceae has been defined as obligate

parasites found within or on erythrocytes or free in the plasma of various wild and domestic vertebrates. They do not multiply in any other tissue. When blood smears are treated with Giemsa's stain, they appear as red-shaped, spherical, coccoid - or ring-shaped bodies, staining reddish violet and measuring 0.2 - 0.4 micron in diameter. They may also occur in short chains or irregular groups in blood plasma or within erythrocytes. Each organism is enclosed in a membrane, with internal structure resembling that of rickettsiae. Multiplication is by binary fissiion. They are Gram-negative and not acid-fast. Cultivation in tissues outside the host has recently been reported (Marble and Hank, 1972, 1973; Hidalgo 1975). Transmission is mainly by arthropods.

Some general characteristics of the genus Anaplasma.

When stained with Romanowsky - type stains, this organism appears in the erythrocytes as dense, homogeneous, bluish purple round structures, measuring 0.3 - 1.0 micron in diameter. These structures have been revealed by electron microscopy to be inclusions; and are separated from the cytoplasm of the erythrocyte of their host by a limiting membrane. Each of these inclusions contains from one to

eight subunits or 'initial bodies', which are believed to be the actual parasitic form of the organism (De Robertis and Epstein, 1951; Moulton and Christensen, 1955; Espana, 1957; Foote et al, 1958; Ristic and Watrach, 1960a; Ristic, 1960b). These initial bodies are described as dense aggregates of fine granular material embedded in an electron-lucid plasma and enclosed in a double membrane (Ristic and Watrach, 1961). The initial body is reported to enter erythrocyte by causing invagination of cytoplasmic membrane and subsequent formation of a vacuole. In the cacuole the initial body multiplies by binary fusion, to form an inclusion. During acute and convalescent phases of the infection, the formation of the inclusion body is the one mostly encountered. No spores or resistant forms have been reported for the organism. However, Pilcher et al, (1961) described the organism as aerobic, and its production of catalase was reported by Wallace and Dimopoullos (1965). Production of pigments has not been reported, but incorporation of amino acids from plasma in vitro by the organism has been described (Mason and Ristic, 1966). A comprehensive study of the histochemical analysis of the parasites reveals positive reactions for DNA, RNA, protein and organic iron (Moulton and Christensen, 1955).

Species of Bovine Anaplasmosis.

Differentiation of the genus Anaplasma into species has been based entirely on host range, as well as the location of the inclusions in the erythrocytes. On this basis, three different species of Anaplasma have been described (Theiler, 1910, 1911; Lignières, 1914; Lestoquard, 1924; Yakimoff and Belawine, 1927; Neitz, 1957). These species are, Anaplasma marginale, Anaplasma centrale and Anaplasma ovis (Table II). However, current Classification is in support of A. marginale and A. centrale as the two main species of bovine Anaplasmosis. Anaplasma ovis has been described (Lestoquard, 1924) as the causative agent of the disease in Sheep and Goats, but may infect Cattle, without any disease being produced.

The two valid species of bovine anaplasmosis are, therefore, described below:-

Anaplasma marginale.

A. marginale is the most pathogenic of the three species of the genus Anaplasma, and is the main causative agent of bovine anaplasmosis.

In the blood smears stained with Giemsa, it appears as reddish violet spherical, coccoid, dense homogeneous structure, measuring 0.3 - 1.0 micron in deameter.

TABLE II

Differential characteristics of species of genus Anaplasma

Species	Host	Location in erythrocyte	Disease
1. A. marginale	Cow, Deer,	Predominantly marginal.	Severe anaplasmosis.
2. A. centrale	Cow.	Predominantly Central.	Generally mild anaplasmosis.
3. A. ovis	Sheep, Deer, Goats.	Predominantly marginal.	Mild to severe disease.

Other related species of wild ruminants such as antelopes, deer, elk, black wildebeest, duiker, and blesbok, as well as buffalo, have also been considered to be susceptible to natural infection (Mottet and Buck, 1931; Neitz and du Toit, 1932; Boynton and Woods, 1933, 1940; Neitz, 1935; Stiles, 1942; Honess and Winter, 1956; Christensen, Osebold and Rosen, 1958; Howe and Hepworth, 1965; Peterson, Kistner and Davis, 1973; Peterson and Roby, 1975; Vaughn, Renshaw and Frank, 1976).

The virulence of A. marginale for cattle is not decreased by sub-passage in deer (Christensen et al, 1958). Anaplasma isolated from deer was readily passaged, by innculation from deer to calf, from calf to calf, from calf to deer and from deer to deer (Osebold, Christensen, Longhurst and Rosen, 1959). Through these studies deers have been incriminated as an important reservoir of anaplasma infection. The antelope, Antilocapra americana, had also been demonstrated to act as reservoir for A. marginale (Jacobson, Worley and Hawkins, 1977).

Bedell and Dimopoullos (1962, 1965) reported that the infectivity of A. marginale can be destroyed by heating the organism at 60°C for at least 50 minutes or through exposure to sonic oscillation at 35°C for at least 90 minutes.

Infectivity of A. marginale can also be destroyed by exposure to X-ray doses of 100,000 roentgens or higher (Wallace and Dimopoullos, 1965; Dammert, Wallace, Finnerty, Schrader, Rogers, Gough, Cano and Dimopoullos, 1965).

Preservation of A. marginale in bovine blood, to which glycerin has been added and frozen, can be for many months, (Barnett, 1964). A more recent method of preservation by lyophillization (freeze-drying) process was described by Richey, Kliewer, Brock and Jones (1973). These authors believe that this latter method is more advantageous in terms of cost and shelf-life, than ordinary freezing, especially when the material has to be used outside laboratories.

Anaplasma centrale.

After the recognition of A. marginale, as the main causative agent of bovine anaplasmosis, Theiler (1910a) described a second disease entity, which is caused by a centrally located, coccus-like body, closely resembling A. marginale. These bodies were most often seen in the centre of infected erythrocytes. Theiler first gave the name A. marginale var centrale, to this organism. Later, Theiler (1911) indicated that A. centrale should be considered separate and distinct from A. marginale. He also reported that double

infections of A. marginale and A. centrale were commonly encountered in natural infections on the veld of South Africa. The character of the disease produced by A. centrale in pure infections was considered milder than that produced by A. marginale (Theiler, 1911b). Theiler also observed that recovery from A. centrale resulted in immunity to subsequent exposure to A. centrale, and an increased resistance, although not an absolute immunity to A. marginale infections.

Carpano (1932); Sergent, Donatien, Parrot and Lestoquard (1933, 1935); Omlin (1934); Legg (1936); and Donatien (1952) have confirmed Theiler's observation that the infection produced by A. centrale was milder than that produced by A. marginale. In 1966, Kuttler in his comparative study of the two species, also demonstrated that A. centrale was responsible for a milder clinical manifestation than East African A. marginale infections. However, he observed minor differences in virulence when A. centrale infections were compared with Nevada and Beltsville A. marginale infections in splenectomised calves.

Transmission

Although A. marginale has been constantly demonstrated to be present in the blood of infected or recovered animals,

the exact means by which the disease is perpetuated in nature is still not fully understood. It is generally believed that agents capable of transmitting minute quantities of blood from infected to susceptible animals are responsible for its spread. These include: ticks, horse-flies, mosquitoes, deer-flies, stable-flies and other biting and blood sucking insects. Some of these are considered to be biological vectors, while others are regarded as mechanical transmitters.

Mechanical transmission, apart from that by arthropod vectors has been reported to occur through accidental or artificial inoculation of susceptible animals by means of contaminated needles and surgical instruments (Ristic, 1960; McCallon 1976). This mode of transmission usually accounts for disease episodes out of season. Similarly, Swift and Paumer (1976, 1978) have proved that vertical (transplacental) transmission of A. marginale in pregnant carrier heifers could occur.

Biological Transmission: Ticks are the only recognised biological vectors of A. marginale.

Dikmans (1933a), while analysing results of the transmission studies reported by Smith and Kilborne (1893a,b) on arthropod transmission of piroplasmosis in cattle, had

concluded that they had transmitted both anaplasmosis and piroplasmosis with Boophilus annulatus. However, the failure to eliminate anaplasmosis in the United States of America by eradication of B. annulatus had shown that the disease is perpetuated by agents other than B. annulatus. Consequently, various other ticks have now been demonstrated experimentally to transmit A. marginale through transstadial, or transovarian transmission. (See Table III). In most of these tick species transstadial transmission had been reported (Rees, 1933, 1934; Boyton, Herms, Howell and Woods, 1936; Sanborn, Stiles and Moe 1938; Rezeboom, Stiles and Moe, 1940; Howell, Stiles and Moe, 1941a).

According to Dikmans (1950), transovarian passage of A. marginale from an engorged female tick through the egg to the larval stage has been accomplished under experimental conditions by Boophilus annulatus, B. decoloratus, B. microplus, Dermacentor andersoni, D. occidentalis, Ixodes ricinus and Rhipicephalus sinus. However, there are conflicting reports about the possibility of hereditary transmission of A. marginale by ticks, especially with D. andersoni and D. variabilis. Howell et al (1941a) first reported hereditary transmission with larval progeny of engorged female D. andersoni. However,

TABLE III

Biological Transmission of A. marginale
By Ticks

Vector	References
Boophilus annulatus	Smith and Kilborne (1893); Rosenbusch and Gonzalez (1927); Brumpt (1931).
Boophilus decoloratus.	Theiler (1912b; Rees (1934).
Rhipicephalus simus.	Theiler (1912b).
Ixodes ricinus.	Helm (1924); Zeller and Helm (1923).
Dermacentor andersoni.	Rees (1933, 1934); Boynton et al. (1936); Howell et al. (1941a).
Dermacentor variabilis	Rees (1932, 1934); Sanders (1933).
Dermacentor occidentalis.	Boynton et al. (1936); Herms and Howell (1936); Christensen and Howarth (1966)

various workers (Rees and Avery, 1939; Rozeboom et al; 1940; Ristic, White and Sanders, 1957a; Anthony and Roby, 1966; Howarth and Hokamma, 1973) have failed to demonstrate hereditary transmission of A. marginale with D. andersoni and D. variabilis.

The failure to demonstrate anaplasmosis transmission with Amblyomma americanum, as earlier reported by Rees (1934), Samborn and Moe (1934), Piercy and Schmidt (1941), was also confirmed by Anthony and Roby (1966). After testing all stages of its development with both clinically infected and carrier cattle they then concluded that A. americanum cannot be considered an important vector of anaplasmosis.

In Africa, especially in Kenya (Kane, 1976), Nigeria (Mohammed 1974; Mohammed and Ilemobade, 1976) and Zambia (Akafekwa, 1976), only the Boophilus sp and Rhipicephalus sp. have been linked with the transmission of anaplasmosis. No critical studies to prove their mode of transmission have been reported.

Mechanical Transmission: Ticks, as well as other biting and sucking arthropods have also been regarded to transmit A. marginale mechanically, through their habit of feeding intermittently on both infected and uninfected

susceptible animals. Through accidental or artificial means, human beings have also been incriminated to mechanically transmit the organism by the use of contaminated equipment. Ristic et al. (1957a), in their attempt to trace Anaplasma in adult D. variabilis, using Feulgen and Flourescent Antibody Techniques, demonstrated that ticks can also transmit anaplasmosis mechanically. The organism, A.marginale, was demonstrated in the ingested erythrocytes for 4 days following feeding by the ticks during which apparent degestion of the erythrocytes had taken place. Anthony and Roby (1966) reported that at least 9 species of horse-flies (Tabanus sp) and deer-flies (Stomoxys sp), two genera of mosquitoes and stable-flies, transmitted A. marginale mechanically under experimental conditions.

Experimental evidence of successful transmission of anaplasmosis by Tabanus spp. was first reported by Sanborn, Stiles, Hoe and Orr (1930) and Lotze and Yiengst (1941). Their findings were later corroborated and summarized by Howell et al. (1941a). They showed several species of horse-flies capable of transmitting the disease: Tabanus sulcifrons, T. abactor, T. venustus, T. equalis, T. erythraeus, and T. oklahomenis. Transmission was most readily accomplished if tabanids were allowed to feed partially on the blood of animals affected with clinical anaplasmosis and transferred

shortly thereafter to susceptible animals to engorge themselves. Carrier cattle apparently can also serve as a source of infection to horse-flies (Lotze, 1944).

Sanders (1933) showed T. fumipennis and Stomoxys calcitrans as experimental vectors of anaplasmosis. Morris et al. (1936) added T. atratus to the list of vectors.

Experimental transmission of anaplasmosis by mosquitoes was demonstrated by Howell et al. (1941b). They selected for their study, the genus Psorophora, which was suspected of being capable of transmitting the disease because of its habit of breeding in transient pools in the vicinity where cattle graze and because of large size and voracious feeding habits. In one instance, 1,500 specimens of P. columbiae and P. ciliata, which were permitted to feed alternately on a clinical case of anaplasmosis and a susceptible animal, transmitted anaplasmosis. Positive transmission to other susceptible animals resulted from a similar procedure using 3 species of mosquitoes, P. ciliata, P. columbiae and, Aedes aegypti.

Bhat and Singh (1971) and Mazzola, Amerault, and Roby, (1976) first succeeded in demonstrating A. marginale in cell culture of Aede albopictus. Mazzola et al. (1976) further reported that the parasite remained viable, as

determined by calf inoculations, for 21 days.

Accidental transmission of A. marginale involves means other than by arthropod vectors, excluding deliberate transmission for the purpose of premunization. This however, involves man, as the principal agent. Man accidentally transmit the organism through the use of contaminated surgical and other penetrating instruments.

The ease with which minute quantities of infected blood can cause infection in susceptible cattle by manual manipulation has been demonstrated by Rees (1930b). He transmitted the disease with a lancet used to puncture the ear vein of an infected animal which was subcutaneously inserted into a susceptible animal.

Occurrence of anaplasmosis following unsanitary dehorning has been reported by Hilts (1928), Stiles (1936), and Moe Stiles and Howell, (1940), respectively. Evidence was also adduced by Boynton (1932) that contaminated surgical instruments could aid the spread of the disease. This, he proved by using the same needle to collect blood for brucellosis test, as well as in the administration of vaccines in a herd that contained carrier animals for A. marginale.

Intra-uterine Transmission: Abortion in cattle as a sequella to anaplasmosis has been reported by Fincher, Gibbons, Mayer, Karl and Park (1956), and Gansanov (1968). In one report from South Africa (Bird, 1973) and another from East Africa (Mandara and Munyua, 1971) active infections by A. marginale in neonatal calves were noted. Fincher et al. (1956), therefore, suggested that transplacental transmission of the organism occurred in these cases, since anaplasmosis requires a lengthy period of incubation lasting up to two months. This fact had earlier been indicated by Dykstra, Lienhardt, Pyle and Farley (1938), that calves may become infected with anaplasmosis in-utero.

Piercy (1956) and Mott (1957) studied a number of calves born of infected dams, but failed to prove the existence of in utero transmission in anaplasmosis. Recent studies conducted by Fowler and Swift (1975), Swift and Paumer (1976) conclusively proved that transplacental transmission of the organism occurs. These authors concluded that the organism apparently crosses the placenta within 15 days following inoculation into pregnant heifers or cows. They, however, suggested that acute infection during gestation may be a necessary requirement for transplacental transmission to occur.

They opined that, if the foetus becomes infected with A. marginale and survives until parturition the neonatal calf may serve as a reservoir of infection. In conclusion, they believed that this may become important in the transmission of anaplasmosis, contrary to Ristic's (1960) opinion that in utero transmission is apparently unimportant in the spread of the disease.

Cultivation of Anaplasma in cell cultures

There have been many unsuccessful attempts to cultivate A. marginale in cultured cells derived from several species of animals, including cattle (Veglia, 1915; de Faria, 1928; Dikmans, 1933b; Dykstra, Roderick, Farley, McMahan and Splitter, 1948; Rossi and Triozon, 1953; White and Ristic, 1957; Ristic et al. 1957b; and Dimopoullos, 1959). More recent studies have indicated some successes in this direction.

Marble and Hanks (1972) reported infecting an intact calf with material that had been continuously propagated (20 passages, 1 passage each week) in primary rabbit bone marrow cell culture. In 1973, they reported recovery of A. marginale isolates in both primary and stable rabbit bone marrow cell cultures. Success of these isolations was based on infection of susceptible cattle after 5 to 16 once - a - week passages. They, however, failed to demonstrate the

organism in the culture by Romanowsky and acridine orange stains.

Trueblood and Bear (1973) reported on the multiplication of A. marginale in bovine lymph node (LN) explants and organ cultures. Unlike, Marble and Hanks (1972), they demonstrated the organism in culture by examining Giemsa - and Fluorescent antibody-stained smears. They, however, stated that the reliability of these smears for demonstrating the organism was poor.

In 1975, Hidalgo propagated A. marginale in cell cultures derived from bovine lymph node, similar to that of Trueblood and Bear (1973). The host cells were, however, treated with diethly-lamine-ethyl dextran before inoculation. This resulted in significant numerical increases of A. marginale. The direct flourescent antibody technique (FAT) was used to detect the organism in culture, while the standard microscopic count procedure was employed in obtaining numerical estimates of the organism in cell culture. The success recorded here seems to be an improvement on the earlier reports by Marble and Hanks (1972, 1973a,b) and Trueblood and Boar (1973) where difficulties had been reported in demonstrating multiplication of the organism through staining methods.

Although various arthropods have been incriminated as vectors of A. marginale over the past one century, attempts to propagate it in insect cell cultures was not successful until 1967, when Singh derived cell cultures from larvae of mosquitoes (Aedes albopictus and Ae. aegypti). Bhat and Singh (1971) in their characterization studies of cells in Ae. albopictus cell cultures, demonstrated A. marginale bodies in an infected erythrocyte. Mazzola et al; (1976) introduced infected bovine erythrocyte with A. marginale into cell cultures from the mosquito Aedes albopictus. They demonstrated that A. marginale enters cell of Ae. albopictus in cell culture through phagocytosis of infected erythrocytes. The parasite was demonstrated to be viable for 21 days, based on calf inoculation.

From the foregoing, it could be said that laboratory animals, as well as other blood sucking arthropods may in future assume greater importance in the propagation of A. marginale. In fact, it may become more difficult in Nigeria, to control the disease, since mosquitoes and other biting arthropods abound in far higher proportion than even ticks. Any efforts to control the propagation of A. marginale, may require the control of these vectors, as well.

The Pathogenesis of Anaplasmosis in Cattle

Anaplasma marginale has been recognised as the main pathogenic species of bovine anaplasmosis. The severity of the disease in cattle, could be described as mild, peracute, acute and chronic. Very minute quantity of an infected blood is required to cause infection in a susceptible animal. Roby (1962), has described that as little as 0.001 ml. of blood from a carrier animal has resulted in infection of a susceptible animal. In most cases the resulting disease is mild in young animals, while greater severity has been noticed in adults. The morbidity rate is usually high in outbreaks, but the mortality rate varies widely depending on susceptibility, and may be up to 50 per cent or more in animals newly introduced from anaplasma-free area into an endemic one. Animals that recover from the disease are usually emaciated and have a prolonged recovery period.

Incubation Period: An incubation period of between 21 to 42 days, averaging about 30 to 40 days, have been described (Mott, 1957; Roby, 1962; Jones and Norman, 1962; McCallon, 1976). Mott (1957) defined the incubation period of anaplasmosis as the interval between the time of exposure and the time the first marginal bodies are detectable in stained blood films which is the prepatent period. This

period correlates highly with the size of the infecting dose. The higher the infecting dose the shorter the incubation period and vice versa. Under field conditions, the incubation period averages 35 days.

Clinical Signs: The onset of the disease is characterised by increase in body temperature, inappetence, ruminal stasis, weakness and lower production. Temperature as high as 105°F (40.5°C) may be observed. The continuous destruction of erythrocytes by the invading parasites leads to progressive, severe anaemia and icterus. Venipuncture usually reveals thin watery blood. The mucous membranes of the eyes and the vulva of the female become icteric, and marked pallor of the skin could be observed as well. These observations are particularly apparent after the acute stage is passed, but hemoglobinuria is usually absent.

In advanced cases, restlessness, muscle tremors, weight loss, dehydration and recumbency may occur. The faeces are frequently hard and bile stained. Although diarrhoea has been reported (Jones and Norman, 1962) to occur early in the disease, such an observation appears inconsistent with other clinical signs seen in anaplasmosis.

Animals infected in advanced pregnancy, frequently abort (Henning, 1956; Jones and Norman, 1962; Christensen,

1963; Fowler and Swift, 1975), while temporary infertility has been observed in bulls (Jones and Norman, 1962).

Convalescent Period: Clinical convalescence occurs prior to the development of maximal anaemia and period of maximal parastaemia. It commences with drinking of water and return of appetite and body temperature to normal. Icterus usually persist in some animals during this period (Brock, 1958), while some animals may have deprived appetites and eat dirt (Henning, 1956; and Christensen, 1963).

Pathological Findings: The pathological findings of the disease usually include hematological observations, as well as gross anatomical and histological changes of the disease.

Haematological findings, include anaemia, hemoglobinemia and reduction in the oxygen carrying capacity of the blood. During the anaemic crisis, increased osmotic and mechanical fragility of the erythrocytes have been reported (Ristic, 1960; Jones and Brock, 1966). These are said to be associated with anisocytosis, poikilocytosis, hypochromasia and polychromasia that are observed at the peak of the disease. A close correlation between severity of the disease and the percentage of parasitised erythrocytes, particularly during the clinical stage have also been described (Ristic, 1960).

Leukocytosis is commonly observed during the acute stage of the disease. During this stage, bilirubinemia is consistently observed, while faecal urobilinogen value is also elevated.

In some cases nitrogen content, as well as uric acid nitrogen of the blood are greatly reduced, while non-protein nitrogen is usually increased. Increase in blood urea nitrogen as well as sugar content was also reported by Graf (1933), while increase in amino acid usually occur shortly before death. Rossi et al. (1956) reported a reduction in calcium and a rise in cholesterol level of the blood.

Commonly observed gross changes consist of anaemic and icteric mucous membranes, enlarged spleen, and distended gall bladder. The subcutaneous tissues have a yellow, gelatinous appearance. The cut surface of the enlarged spleen shows dark, protruded parenchymatous tissue. The liver may be enlarged and mottled with rounded borders, while the cut surface varies from brownish-yellow to saffron-yellow.

Petechial hemorrhages may be observed on the epicardium, pericardium, pleura, and diaphragm. The lymphatic glands are generally moderately swollen and moist

on section. The colon and rectum may contain a considerable quantity of thick, mucous material together with hard pellets of mucus-coated faeces. Other organs, including the lungs, kidneys, and genitalia are usually normal, except for the icteric condition.

Histologically, various findings involving the bone marrow, splenic corpuscles and prominent hepatic changes have been discussed by Ristic (1960). Various stages of proliferation are usually seen in the reticular cells, composing the stroma of the bone marrow. The splenic corpuscles are seen with a decreased lymphoblasts, with increased vacuoles and degenerated reticular cells. Leukocytic infiltration of Glisson's capsule, dilation of the portal veins, and swollen endothelial cells, are amongst the prominent hepatic changes usually seen.

Diagnosis: The diagnosis of acute anaplasmosis is usually based on clinical signs, history and age of the affected animal, as well as, microscopic demonstration of the organism in Giemsa-stained, thin blood smears. In chronic infections, microscopic detection of the organisms may fail to establish the disease, since the organisms are not so readily detectable or identifiable due to very low parasitaemia, if any. In such cases, diagnosis may depend on serological tests.

Serological Tests: Serological tests have been successfully employed in anaplasmisis. These serological tests are, the complement fixation (CF; Mohler and Gates, 1952; Gates, et al 1954a,b; 1955; Rogers, 1971; Todorovic, 1977), the capillary tube agglutination (CA; Ristic, 1962; Rogers, 1971), and the rapid card agglutination test (CT; Amerault and Roby, 1968, 1971; Amerault et al, 1972; Amerault, 1973).

The CT is the most recently described of all these serological tests (Amerault and Roby, 1968). However, only the agglutination reactions reported in both the CA and the CT appear to be particularly suitable for field diagnosis of A. marginale, due to their simplicity and practicability. The CF and FA tests are restricted to laboratory diagnosis, due to the complexity of the techniques and equipment necessary for their performance (Mohler and Gates, 1952; Price, Poelma, Hastings and Mitchell 1953; Gates, Mohler, Mott, Poelma, Price and Mitchell 1954a; Gates, Mohler, Mott, Schoening, 1954b; Gates, Roby and Mott, 1955; Pearson, Brock and Kliewer, 1955; Merriman, Bucker and Hobbs, 1962; and Rogers, 1971).

Through various comparative and field evaluation studies, the CT has proved to be the most rapid, simple and

adequate serological test for diagnosing both acute and chronic anaplasmosis and for field survey (Christenberry and Alley, 1972; Alley and Christenberry, 1973; Tedorovic, Long, and McCallon, 1977; Patarroyo and Diazgranados, 1978). In Nigeria, the successful utilization of the CT in studying the disease prevalence has been reported (Obi, 1978; Jagun, 1979). Therefore, the CT has been proved to be an acceptable serological technique for diagnosing bovine anaplasmosis.

Control of the Disease

The control of the disease in endemic areas is directed against the control of the vectors on the host, as well as, immunization of the host against further challenge by the vectors. In some areas a combination of elimination and or treatment of the carriers have been practised, while prophylactic feeding of tetracyclines has been used in controlling the disease in some carrier cattle.

Control of the vectors on the host: The control of species of ticks and blood-sucking arthropods responsible for transmission of A. marginale to cattle on open ranches and farms is based on the use of acaricides. These are usually employed in spray races or dip vats.

Other methods of applying these chemicals include, dusting, fogging and hand-dressing. Fogging, apart from

being expensive, is not efficient; while dusting is not effective for controlling ticks on cattle, its use is restricted to only domestic pets, chickens and sheep. Only hand-dressing, dipping and spraying are generally effectively used in many parts of the World.

In Nigeria, hand-application and hand-spraying are the most widely accepted methods practised by the nomadic cattle rearers, who control the majority of the cattle population. However, machine spraying and dipping have been used mostly on Government ranches. These latter methods are gaining grounds with some individuals who now set up modern cattle farms. This notwithstanding, greater efforts are still required to educate and persuade majority of the cattle owners on the need to regularly control these vectors.

Immunization of the host: In most parts of the World where anaplasmosis is endemic, greater attention is now been given to the control of the disease through immunization of cattle.

At present premunization constitutes one of the most reliable means of immunizing against anaplasmosis. Premunization can be achieved artificially by infecting blood from reacting or carrier animal into susceptible ones. Severe reactions frequently occur following attempted premunization

in cattle, but these reactions can be controlled by drugs. The objective of treatment is to achieve premunity, which is referred to as non-sterile immunity, which persists as long as the infectious organism remains present.

Theiler (1911) described the use of Anaplasma centrale to induce premunition and protect against A. marginale infection. This subsequently led to the use of A. centrale as a premunizing vaccine in some parts of the tropics, especially in Asia and Africa. Schmidt (1937) successfully used field isolates of A. marginale for premunizing cattle against anaplasmosis in the United States of America. This method consisted of injecting blood from known carriers into susceptible Anaplasma-free cattle.

In endemic areas of the tropics, field premunization by natural vectors is known to occur at an early age in cattle (Kuttler, Adams, and Zaraza, 1970). These young animals because of their innate resistance, normally develop premunition following their recovery from such infection. Conscquently, they are said to be protected from future acute infections. However, any introduction of Anaplasma-free adult cattle into such areas is often met with severe losses (Kuttler and Todorovic, 1973). To prevent

such losses some form of immunization are required. Consequently, vaccination of cattle, using various forms of the organism was developed.

Chemo-immunization in anaplasmosis: The process of chemo-immunization is the inoculation of susceptible animals with virulent strains of the causative organisms, where reactions that follow are controlled by specific chemotherapy.

In some tropics, where A. marginale is endemic, this method has been recommended for immunizing cattle against babesiosis, anaplasmosis, theileriosis, and heartwater (Kuttler and Todorovic, 1973; Todorovic, Lopez, Lopez and Gonzalex, 1975; Ilemobade, 1978; Aliu, 1980).

Imidocarb, Gloxazone and Oxytetracycline have been employed in controlling post premunization reactions in A. marginale. Recent studies have shown that the combination of gloxazone-oxytetracycline therapy is more effective than either Imidocarb or Gloxazone when administered alone (Todorovic and Tellez, 1975). However, the use of this combination is not practicable under field condition. Consequently, oxytetracycline therapy is generally used to control post inoculation reactions against anaplasmosis.

Vaccination as a means of control: Vaccination involves the introduction of living, attenuated or killed

strains of the organism (A. marginale) into susceptible cattle. In response, a nonfatal, but latent infection or carrier state is produced, which protects such vaccinated cattle against further A. marginale infection.

An attenuated Anaplasma marginale vaccine which has been reported in the United States did not protect animals against challenge. It produced clinical signs of the disease in some vaccinated animals, particularly aged, lactating dairy cows (Norman 1973). In view of this fact, and because of possibility of the organism reverting to its original virulence, the vaccine was not licensed by the USA government..

An inactivated vaccine against anaplasmosis in cattle had been reported (Kuttler, 1961; Brock, Kliewer and Pearson, 1965). The vaccine contains disintegrated Anaplasma material prepared from the erythrocytes of acutely infected cattle, incorporated with an oil adjuvant suspension immediately before use. This vaccine is available commercially in the United States of America, where its inability to prevent development of the carrier state in cattle naturally infected has been observed (Hidalgo, 1974). McHardy and Simpson (1973) reported another killed vaccine, prepared from a Kenyan and a South African strain of A. marginale, which they suspended in Saponin as adjuvant.

They reported that these preparations delayed the onset and in some cases reduced the severity of the disease, but did not prevent the occurrence of the disease.

The greatest disadvantage of the inactivated vaccine is that inoculated cattle produce isoantibodies directly against erythrocytic antigens in their erythrocytes. The isoantibodies are concentrated in the colostrum of any vaccinated pregnant cows. When calves suckled such dams, severe hemolytic anaemia with accompanying icterus is usually produced. This disease is commonly called neonatal isoerythrolysis (NI) and was first reported in the United States of America in 1970 by Dennis, O'Hara, Young and Dorris. Therefore, the use of inactivated vaccines as means of controlling anaplasmosis has been limited by the threat of high mortality of calves associated with neonatal isoerythrolysis.

Treatment of acute anaplasmosis: Of all the drugs reportedly used in treating acute anaplasmosis (Table IV) only the tetracyclines have been found consistently effective.

Other chemotherapeutic compounds used, as adjunct to the therapy of acute anaplasmosis include a variety of hematinics, blood transfusions and good nursing care.

These are used mainly to eliminate stress and alleviate anaemia, until such a time when the immune mechanism and hemopoietic system of the host could adequately respond to the infection. However, there are conflicting reports as regards the importance of blood transfusions in the treatment of the clinical disease. Despite successes recorded by some workers (Lotze, 1947; Dykstra et al, 1948; Koger, 1954). Others like Carricaburu (1956; Brock, 1959), and Franklin and Schmidt (1954) suggested that its use must be with great caution to avert any eventual loss. While careful nursing of the ailing animal throughout the course of the disease, with a minimum of disturbance, has been suggested to have a favourable influence on recovery.

The Tetracycline Compounds: These compounds include, Chlortetracycline (Aureomycin), Oxytetracycline (Terramycin) and Tetracycline hydrochloride (Polyotic).

Chlortetracycline has been reported to possess inhibitory effect on numerical increase of anaplasma body, when administered during the first few days of the infection (Foote, Brock and Gallgher, 1951a; Miller, Levy, Torbert and Oglesby, 1952; and Oglesby, 1954). It has been found ineffective when administered late in the course of the infection. However, elimination of the carrier state of

TABLE IV

Various drugs that have been employed in the Chemotherapy of acute Anaplasmosis

Drug	References
Arsenicals, antimalarials, antimony derivatives & dyes.	Miller (1956)
Sodium cacodylate.	Boynton et al. (1937)
Neoarsphenanine.	Sotomayor (1948)
Paludrine and quinoline diphosphate.	Farley et al. (1949a, 1950)
Acaprin.	Pulling (1949)
Aralen	Pearson (1950).
Aricyl.	Smith and Howell (1944); Farley et al. (1949b).
Tetracycline compounds:	
Chlortetracycline (Aureomycin).	Foote et al. (1951a); Miller et al (1952); Foote and Wulf (1952); Brock et al. (1953); Oglesby (1954).
Oxytetracycline (Terranycin).	Miller et al. (1952); Farber (1955); Christensen and Harrold (1956); Carricaburu (1956).
Tetracycline hydrochloride.	

the infection through massive feeding of the drugs has been reported (Franklin, Cook and Anderson 1966; Franklin, Cook, Anderson and Kuttler, 1967; Franklin, Huff and Grumbles, 1965).

Oxytetracycline has also been used with success both experimentally and in treating natural cases of anaplasmosis. Miller et al. (1952), Farber (1955), Christensen and Harrold (1956), and Carricaburu (1956) reported inhibition of anaplasma organism, similar to the effect recorded for Chlortetracycline. In addition, they reported that it must be administered early in the course of the infection, if any success is to be achieved.

Carricaburu (1956) indicated considerable success in treating naturally occurring field cases with Oxytetracycline at 3 mg/lb. body weight, given intramuscularly (im). Similar success had early been recorded by Brock et al. (1955) with tetracycline hydrochloride, when used at the rate of 3 mg/lb. im. In the recent times, Oxytetracycline (Terramycin) has been administered successfully either iv or im, at the rate of 10 mg/kg body weight for 2 to 3 days for combating acute anaplasmosis.

Although these tetracyclines have been in use for the past three decades; their use in the therapy of anaplasmosis

has been limited to only routine practice, due to their high cost. Consequently, the quantity usually administered for therapeutic purposes, against acute infection, is such that it produces only a suppression of the causative agent, thus, leading to the production of carriers capable of spreading the disease.

Treatment of Carrier Infections to eliminate Anaplasma

Soon after the tetracyclines have been observed to inhibit the growth of Anaplasma, other experiments were conducted to evaluate the possibility of employing them in eliminating carrier infections (Foote and Wulf, 1952; Splitter and Miller 1953; Brock et al; 1953, 1958; Pearson et al. 1957; Franklin, Cook, Anderson, and Kuttler 1967; Roby, Amerault and McCallon, 1970). These experiments indicated that such therapy would eliminate carrier infection; but only after prolonged administration of fairly large amounts of the drugs.

Pearson et al. (1957) have reported success in eliminating the carrier status of anaplasmosis through the iv or the im injections of 5 mg/lb. tetracycline given 10 times at daily intervals. The injection of oxytetracycline and chlortetracycline has also been reported to be effective when administered for 12 to 16 days, at the rates of 5 mg/lb. and 15 mg/lb. iv, respectively, (Splitter and Miller, 1953).

Oral application of chlortetracycline for a prolonged period has been reported. Franklin et al. (1967) reported that the smallest quantity of chlortetracycline that has been effective in eliminating infection was 0.5 mg/lb., when fed orally for a 120 - day period. However, a similar experiment, using 0.5 mg/lb. orally for 90 days failed to eliminate the organism, but led to containment of the disease (Collins, Scales, Hoffman and Roberts, 1962; Scales, Collins, Hoffman and Roberts, 1962).

Although oral treatment with low levels of chlortetracycline may be effective, its effectiveness is dependent on the quantity consumed, as well as whether the animal is continuously exposed to the infection or not. For these reasons Chlortetracycline levels higher than 0.5 mg/lb. daily are generally used. Thus, a treatment consisting of 5 mg/lb. daily for 30 to 60 days has been reported generally successful (Franklin, Huff, and Crumbles, 1965; Twiehaus, 1962; Roby et al. 1970). However, the prolonged periods of treatment, the expense of these drugs and labour required in the supervision during treatment led to the search for a better system of carrier elimination.

Apart from the tetracyclines, other compounds that have been developed and tried in the treatment of carriers

TABLE V

Drugs that have been tried in the elimination of carrier infection for Anaplasmosis

Drugs	References
Oxytetrecycline	Splitter and Miller (1953); Pearson et al. (1957).
Chlortetracycline	Splitter and Miller (1953); Collins et al. (1962); Scales et al. (1962); Twiehaus (1962); Franklin, Huff & Grumbles (1965); Franklin et al. (1957); Roby et al. (1970).
Gloxazone	Barret et al. (1965); Brown et al. (1968); Roby et al. (1968); Kuttler & Adams (1970; Kuttler & Zaraza (1970); Kuttler (1971a,b 1972).
Imidocarb	Kuttler (1971b); Roby (1972); Roby & Mazzola (1972).

include, Gloxazone and Imidocarb, which are described below:

Gloxazone (Alpha-ethoxyethylglyoxal-dithiosemi carbazone)

Gloxazone is an insoluble powder which is prepared as an aqeous suspension. It has been reported to have specific activity against Anaplasma (See Table V).

The use of Gloxazone only at the rate of 5 mg/kg. iv met with several disappointing results. Many of the animals so treated died of toxicosis. However, the addition of oxytetracycline (11 mg/kg? to gloxazone (5 mg/kg) given iv simultaneously was reported to be successful (Kuttler, 1971a,b; 1972). Despite the success of this combination, the difficulty to be encountered in practice and possibility of after-effect following its administration may not lead many practitioners to accepting it. In fact due to its toxicity in experimental animals the drug was never introduced into the market.

Imidocarb (3',3-bis-C_2 imidozalin-2yl carbanilide)

Imidocarb is a white power, readily soluble in water; originally recognised for its babesiacidal avtivities. Its use in anaplasmosis has been reported to be promising (Kuttler, 1971b; Roby, 1972; Roby and Mazzola, 1972). Imidocarb given at the rate of 4,5, and 6 mg/kg im, on three successive days, has successfully eliminated the Anaplasma carrier status in splenectomised calves (Kuttler,

1971b). Similarly, Roby and Mazzola (1972) had reported that Imidocarb at 5 mg/kg im, was successful in adult carrier cattle using two injections at 2 weeks intervals.

The addition of Gloxazone (at 5 mg/kg, iv) to Imidocarb (at 2 mg/kg, im) appeared compatible and has been effective in eliminating Anaplasma with reduced dose rates of Imidocarb. However, the combination of Oxytetracycline with Imidocarb was reported unsuccessful in eliminating the carrier status of anaplasmosis (Kuttler, 1971b).

From the foregoing, it is pertinent to infer that no single drug has yet been found suitable in the elimination of carriers for anaplasmosis. Even the technical procedure involved in the use of their resultant combination (gloxazone - Oxytetracycline; and gloxazone - imidocarb) has seriously limited their practical application.

Bovine anaplasmosis in Nigeria

In Nigeria, there is dearth of data on the epidemiology of bovine anaplasmosis. However, few clinical cases have been reported throughout the country, mostly in exotic breeds and those cattle raised under rigid tick control programme (Folkers and Kuil, 1967; Folkers, Kuil and Perie, 1967; Buys and Folkers, 1970; Folkers, Buys, and Blotkamp, Van Hoeve and Kuil, 1970; Dipeolu, 1975).

Anaplasma marginale was first detected In Nigeria in 1927 at the Federal Department of Veterinary Research at Vom near Jos (Leeflang, 1977a). However, there is no report on the occurrence of A. centrale, although circumstantial evidence indicates it may be present (Leeflang, 1977a). Anaplasmosis was also reported to be very common in cattle suffering from other diseases or recently immunized against rinderpest; though uncomplicated anaplamosis has occasionally been observed in young calves. Congenital infections of A. marginale have also been described (Leeflang, 1977a).

On the basis of microscopic detection method, Folkers et al. (1970) reported a prevalent rate of 9.9% of A. marginale from a total of 656 White Fulani (Bunaji) cattle presented for treatment against trypanosomiasis in Kaduna State. On the other hand, using similar method as Folkers et al. (1970), Dipeolu (1975) recorded a higher prevalence rate of 17.7% in a total of 1175 samples collected from slaughter cattle at abattoirs in Ibadan, Ilorin, Kaduna, Kano and Jos; showing that there is difference between pastoral herd and those usually sent to slaughter (Trade cattle, mostly). Obi (1978), using the rapid card agglutination technique reported prevalence rate of 34% in 573 trade cattle (Zebu) slaughtered in Ibadan; While, Jagun (1979) employing the same technique obtained 40.5%

prevalence rate in 294 samples examined at the National Animal Production Research Institute, (NAPRI), Shika.

Anaplasmosis is believed to be transmitted among Nigerian cattle by ticks (Mohammed and Leeflang, 1975; Ilemobade, 1978). These authors also reported that the Boophilus sp. of ticks are involved in the transmission of A. marginale in Nigeria. Mohammed and Leeflang (1975) reported that B. decoloratus is one of the most abundant tick species available on the NAPRI Station, and occurs throughout the year. This probably means that A. marginale is constantly being transmitted all the year round. The low clinical cases recorded, might have been due to rigid tick control regimen obtainable therein (Mohammed and Leeflang, 1975).

No evidence of transmission by any other biting flies is yet available, despite the existence of these blood sucking insect vectors (Tabanus, Stomoxys, Haematobius, Sarcophage and Culicodies sp.) in the country. Similarly, the possible involvement of wild ruminants (deers, antelopes, etc.) in the transmission and as carriers of A. marginale, has not been studied despite our large wild life concentration. Transmission through carrier cattle, as the main reservoirs of the disease, is more favoured. No report of possible transmission by means of vertical or transplacental transmission is available.

However, the possibility of man and his contaminated instruments acting as agents of mechanical transmission of the disease, cannot be ruled out.

The most commonly employed means of controlling the disease is through the control of ticks and other actoparasites. Hand-dressing (especially among the nomadic cattle rearers), dipping and spraying with broad-spectrum acaricides, are the methods used in controlling the ticks. Premunization against anaplasmosis is not common. There are reports of successful premunization of some Friesian cattle imported into the country (Ilemobade, 1978). Oxytetracycline (Terramycin Q50) at 11 mg/kg body weight (i.v or i.m), has been used to control the post-immunization reactions, in such cases (Leeflang and Schillhorn van Veen, 1972).

Generally, in most clinical cases, Oxytetracycline at 11 mg/kg body weight, given for two or three consecutive days is commonly employed in the treatment of affected cattle (Jagun, 1979). Also available in Nigeria is the newly introduced "Long acting" Oxytetracycline (Terramycin/LA); a single injection of which is administered at the rate of 20 mg/kg body weight, intramuscularly. Although, it's very active against Anaplasmosis, its very high cost makes Terramycin Q50 more readily acceptable. Imidocarb and Gloxazone are not yet in wide use in Nigeria; their use is mainly restricted to experimental studies of the disease.

CHAPTER III

A SURVEY OF BOVINE ANAPLASMOSIS IN SHIKA AND ENVIRONS

INTRODUCTION

Although bovine anaplasmosis is known to exist in Nigeria (Folkers and Kuil, 1967; Folkers et al. 1970; Dipeolu, 1975; Leeflang, 1977a; Ilemobade, 1978), its prevalence has not yet been clearly documented. Cattle which recover from the clinical disease are known to become carriers for many years or even for life (Ristic, 1960) and therefore, serve as reservoirs of infection. While previous workers have used blood smear examination to determine prevalence of infection, it is clear that serological tests are necessary in order to obtain a complete picture of the prevalence in any given locality. This is particularly so since a carrier animal may not necessarily have detectable parasitaemia. In view of the good results obtained with serological tests (Chapter II), it was decided to use one of these tests. The rapid card agglutination test (CT) was chosen because it requires a minimum of time and equipment for its application and possesses the capability of being performed on the farm, ranch or in the laboratory.

Area Surveyed: The area surveyed was restricted to National Animal Production Research Institute (NAPRI) Shika, some herds around it, and the Zaria abattoir.

Materials and Methods

Cattle: the cattle used were obtained from 5 sources. These are the National Animal Production Research Institute (NAPRI) farm at Shika; Ahmadu Bello University farm, about 3 kilometers south of NAPRI; the Kaduna State cattle multiplication centre and a private farm, both situated in Shika and approximately 1 kilometer from NAPRI, and the Zaria abattoir at Zango, about 17 kilometers south of NAPRI. The animals comprised of different breeds including Zebu (Bunaji and Sokoto gudali), N'dama, Friesian, and Friesian x Zebu crosses. Altogether, 1181 animals were examined. Apart from these, some other breeds, like Wadara, Azoak and the Rahaji were sampled, but because their number was very small the results have been omitted.

Collection of Serum: On the different farms about 10 ml. whole blood was collected from each animal via the jugular vein into a clean universal bottle, using the venipuncture method. At the abattoir, 10 ml. whole blood was collected directly into universal bottle from the severed jugular veins of the slaughtered animals.

The blood samples were allowed to stand and coagulate at room temperature (25°C) for about 12 to 24 hours. Thereafter serum samples were separated from the clotted blood by spinning at 5,000 g for 5 to 10 minutes. The serum samples were then dispensed into sterile 3 ml. vials and stored at -20°C in a deep freezer for not more than 3 weeks.

Performance of the Card Test (CT): The CT was performed according to the manufacturers instructions and described by Amerault et al. (1972).

Scoring of results: Results were scored at 1+, 2+, 3+, or 4+ according to the degree of agglutination, as positive reactors. Those that showed no agglutination were scored as negative.

Statistical Analysis: The data obtained were subjected to chi-square analysis where appropriate.

Results

Out of a total of 1181 samples tested, 528 (44.7 per cent) were positive, while 653 (55.3 per cent) were negative. The degree of agglutination is shown in Table VI.

The highest prevalence of anaplasmosis was recorded in cattle from the private Farm. This was closely followed by the Zaria abattoir and Shika cattle multiplication centre,

TABLE VI

Sero-epidemiological survey of bovine anaplasmosis on National Animal Production Research Institute (NAPRI) Farm Shika and its environs, 1980/81

Place Surveyed	No of samples tested	Number positive	Grade of positivity				% + ve reactors
			4+	3+	2+	1+*	
1. Zaria abattoir Zango	299	218	66	36	48	68	72.9
2. NAPRI, Shika	602	177	32	21	30	94	26.0
3. University Farm Shika	129	78	17	5	12	44	60.5
4. Shika Cattle multiplication centre, Shika	57	41	16	5	10	10	71.9
5. Private Farm (Alh. Kano Shika)	14	14	10	-	-	4	100.0
Total	1181	528	141	67	100	220	44.7

All test areas were significantly higher than NAPRI ($P<0.001$; X^2 = 190.5, 59.8, 53.6 and 38.4 respectively).

with 72.9, and 71.9 per cent respectively. The Ahmadu Bello University Farm recorded 60.5 per cent prevalence, while NAPRI with over half of the total samples tested,had the lowest prevalence of 26.0 per cent (Table VI). The prevalence of anaplasmosis in animals at Zaria abattoir was found to be significantly higher than that of NAPRI ($P<0.001$), but not significantly different from the other surveyed areas ($P>0.05$). Similarly, there was no significant difference in the prevalence of anaplasmosis between the University Farm, Shika cattle multiplication centre and the Private Farm ($P>0.05$).

A further breakdown of the results of all the reactors on NAPRI Farm into four age groups (Table VII), shows that there was a significant difference among all age groups ($P<0.001$). The percentage of reactors in cattle above 24 months of age was significantly higher than each of the other age groups, with those in the 13-24 months age group being significantly higher than those in the 7-12 and 0 - 6 months age groups ($P<0.001$). However, there was no significant difference between reactors in the 7 - 12 and 0 - 6 months age groups ($P>0.05$).

An examination of reactors, in each cattle breed on NAPRI Farm, (Table VIII) shows that the pure Friesian cattle have the highest percentage (44.4 per cent). The percentage

TABLE VII

Anaplasmosis reactors on NAPRI Farm based on age

Age group (Months)	Number of samples tested	Number of Positive reactors	Percentage of +ve reactor.
0 - 6	51	1	1.96
7 - 12	72	3	4.17
13 - 24	94	20	21.27
Above 24	465	153	32.90
All age groups	682	177	25.95

Animals in the age 24 months and above have significantly higher reactors than all the other age groups ($P<0.001$; $X^2 = 21.02$)

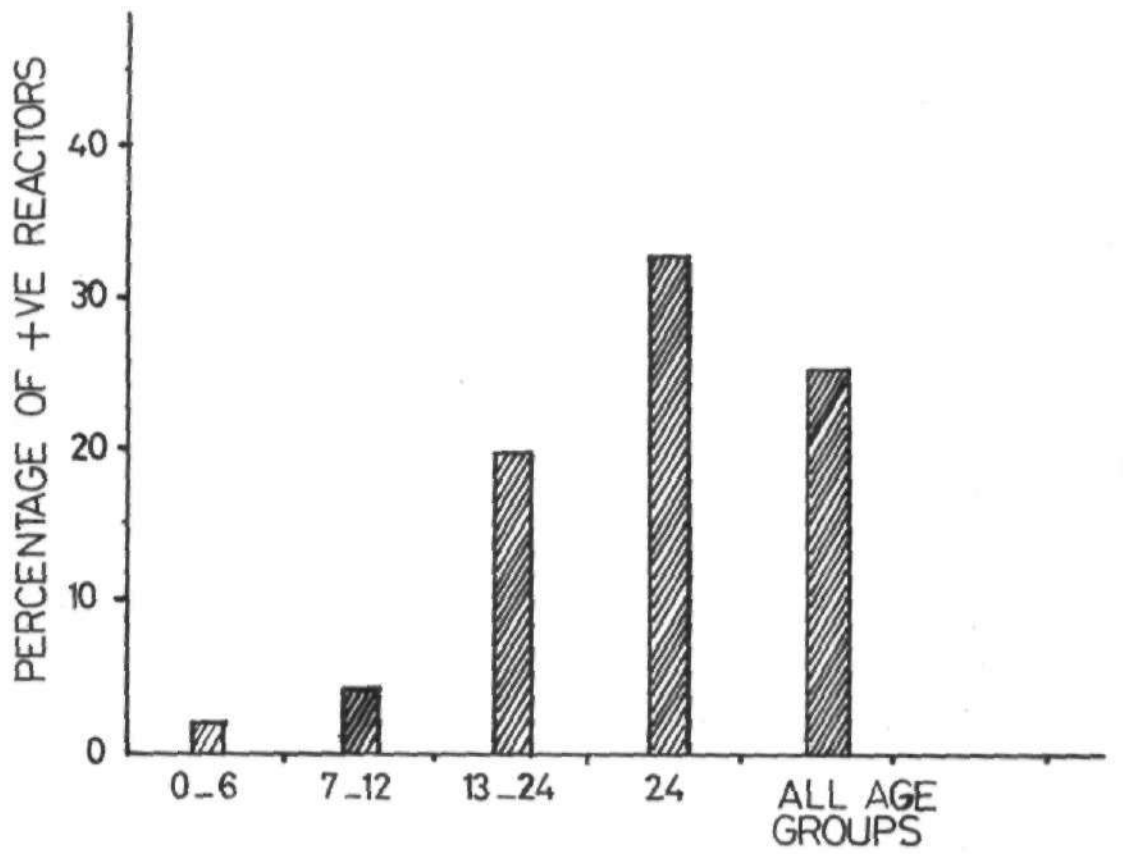


FIG_1 HISTOGRAM SHOWING PERCENTAGE OF ANAPLASMOSIS REACTORS TO RAPID CARD AGGLUTINATION TEST (CT) ON NAPRI FARM SHIKA.

TABLE VIII

A summary of anaplasmosis survey results on NAPRI Farm, depicting breed and age differences of reactors

Cattle Breed	No of samples	No of reactors according to age (month)				Percentage of reactors
		0-6	7-12	13-24	24	
Crossbreds[#]	297	-	2	14	46	20.9
White Fulani	246	-	-	4	52	22.8
Sokoto Gudali	102	1	1	1	41	43.1
N'dama	28	-	-	1	10	39.3
Friesian	9	-	-	-	4	44.4
Total	682	1	3	20	253	26.0

[#]Crossbreeds = Friesian x White Fulani
Charolais x White Fulani
Charolais x N'dama.

of reactors in Sokoto gudali, the N'dama and the White Fulani breeds were 43.1, 39.3 and 22.8 per cent, respectively; the exotic crosses had the lowest percentage of reactors (20.9 per cent). The exotic crosses are products of cross-breeding between the pure Friesian, or Charolais and various indigenous breeds, which include the White Fulani, Sokoto gudali and the N'dama cattle. When the percentage of reactors in all the breeds were statistically analysed, no significant differences were observed ($P<0.001$). No significant difference was observed between the Friesian and the Sokoto gudali ($P>0.05$); between the Friesian and the N'dama ($P>0.05$); as well as between the Sokoto gudali and the N'dama cattle. Similarly, the White Fulani cattle was not significantly different from the exotic crosses ($P>0.05$). Although there was an apparent difference in the percentage of reactors between the Friesian, the Sokoto gudali and the N'dama cattle on one hand, they were not significantly higher than those of the White Fulani and exotic crosses ($P>0.05$). However, when the actual number of reactors in each breed were statistically analysed, the White Fulani was shown to be significantly higher than the Sokoto gudali ($P<0.001$) and the N'dama cattle ($P<0.01$); but not significantly higher than the

Friesian (P>0.05); despite the disparity in number of reactors obtained. Similarly, the number of reactors recorded in the exotic crosses was significantly higher than that of the Friesian, the Sokoto gudali and the N'dama (P<0.001).

Discussion and conclusion

This study demonstrates that the prevalence of anaplasmosis in cattle under different types of husbandry methods varies. In NAPRI where the intensive management pattern exists, the disease prevalence was the lowest. The cattle are adequately protected against any serious tick challenge by rigid acaricide treatment at weekly intervals during the rainy season and fortnightly during the dry season. The cattle are thus prevented from having enough contact with ticks and hence, their exposure to A. marginale infection is limited.

In the other test areas, the management pattern is less intensive. At the Zaria abattoir, the majority of cattle come from private local farms, where regular tick control is not practised. Similarly, the tick control regimen practised in Shika cattle multiplication centre, the University Farm and the Private Farm, situated some few kilimeters from NAPRI, is less rigid. Consequently the cattle in these areas have

greater exposure to A. marginale infection through tick bites, and therefore, have higher prevalence of anaplasmosis than on NAPRI Farm. Based on the premunition factor, it follows therefore, that cattle from NAPRI run a higher risk of coming down with the disease than those from other test areas, should they suddenly be exposed to equal tick challenge. From observation and record, animals in both University Farm and at the private farms were in comparison to NAPRI animals, in good bodily and healthy conditions and, had not suffered from anaplasmosis apart from occasional cases. This calls for a thorough re-evaluation of the present tick control regimen in NAPRI in order to allow cattle from NAPRI to be exposed to Anaplasmosis and therefore sufficient level of tick challenge early in life so as to acquire adequate immunity to subsequent challenge in later life, without adverse effect to the health of the animals. Since eradication of ticks is not practically or economically feasible, the need for an integrated measures, involving premunization/immunization (Ilemobade, 1978; Aliu, 1980), along with moderate tick challenge is desirable. To achieve this, according to Ilemobade (1978), moderate tick challenge is essential after premunization or immunization, to prevent disease outbreak. This necessarily means that the intervals between spraying or dipping must be increased.

Such measures if implemented will, apart from reducing the amount of money spent annually on acaricides, reduce the amount of labour involved in frequent spraying or dipping, and minimize the development of resistance of ticks to acaricides.

The prevalence rate of anaplasmosis obtained in this study (44.7 per cent) is higher than that (34.0 per cent) recorded by Obi (1978). This discrepancy might be due to the fact that Obi (1978) used mainly indigenous (trade) cattle slaughtered at Ibadan abattoir; while animals for this study comprised indigenous, as well as pure Friesian and different exotic crosses with differences in their susceptibility to anaplasmosis (Tables VI & VIII). The samples from Zaria abattoir could be regarded as trade cattle just as those reported by Obi (1978) for Ibadan abattoir. When, these samples are compared, results from Zaria abattoir showed significantly higher prevalence rates ($P<0.001$) than those obtained in Ibadan. For example in the Zaria abattoir 218 out of the 299 animals (72.9 per cent) were reactors; and In Ibadan, Obi (1978) found that 195 out of 573 cattle (34.0 per cent) tested were positive for anaplasmosis. Assuming that anaplasmosis prevalence is greater in indigenous cattle under less intensive management practice, then a correlation would be expected between Obi's (1978) result and the present finding

in indigenous cattle (excluding NAPRI). This, however, is not the case. While both reports point to the importance of anaplasmosis in Nigeria, the difference underlines the desirability of having a national anaplasmosis survey, to reveal the actual prevalence of the disease in different geographical areas of the country.

It has also been shown in this study that cattle below 12 months of age have lower anaplasmosis reactors, than those older than 12 months (Table VII); and that the prevalence to the organism increases with age, thus indicating that animals younger than 12 months are less susceptible to A. marginale than older ones. (Fig I). This observation is in agreement with the findings of Jones and Brock (1966), Jones et al. (1968a,b) who showed that young animals are less susceptible to A. marginale infection than adults. The implication of this is that premunization may be carried out early in life when animals are relatively resistant to clinical infections. However, it may be pertinent here to note that not all calves on NAPRI farm are usually grazed before they are 9 months. This might have added to the reason why calves below the age of 12 months are more resistant than those above 12 months of age.

With regards to cattle breeds tested in NAPRI, anaplasmosis has been shown to be more prevalent in Friesian, the Sokoto gudali and the N'dama cattle, than in White Fulani and the various exotic crosses. Since all the cattle on this farm are assumed to be equally exposed to A. marginale infection, then the Friesian, the Sokoto gudali and the N'dama

cattle would be regarded to be more resistant to the disease than the White Fulani and the exotic crosses. But this interpretation will be grossly misleading if the actual number of animals tested in each cattle breed, as well as the corresponding reactors are not considered. On these two premises it would be pertinent to disregard the results obtained for both the N'dama and the Friesian cattle, since the number of samples obtained in these breeds are very small compared with the others. The Sokoto gudali could then be regarded as having the highest prevalence for anaplasmosis, followed by the White Fulani and the exotic crosses, consequently the Sokoto gudali could be said to be less susceptible to anaplasmosis than the White Fulani and the exotic crosses.

Summary

A total of 1181 serum samples (from NAPRI farm Shika, University farm, Cattle Multiplication Centre Shika, Zaria Abattoir and a private farm in Shika) were tested for antibodies against A. marginale. The rapid card agglutination test (Amerault et al. 1972) was used; the result was analysed using Chi-square analysis. The percentage of positive reactors was 44.7 (528), while 55.3 per cent were scored negative. All test areas were significantly higher than NAPRI ($P<0.001$). On NAPRI farm, a significant difference was shown among all age groups ($P<0.001$); animals less than 12 months were shown to be less susceptible to A. marginale. However, the disease prevalence was not restricted to any particular cattle breed.

CHAPTER IV

THE EFFICACY OF TETRACYCLINE THERAPY AND DURATION OF TREATMENT IN THE COURSE OF BOVINE ANAPLASMOSIS

INTRODUCTION

The use of oxytetracycline in the treatment of clinical anaplasmosis has already been reviewed (Chapter II). In Nigeria the drug is used very widely, and the treatment regimen followed is based on the experience in other countries, for example the U.S.A. (Brock, 1959; Miller, 1956; Franklin et al. 1962), where conditions are different from those of Nigeria. Since it is probable that such regimen may not be valid under Nigerian conditions, especially in relation to parasite strain and husbandry practice, it is important that an effective treatment regimen be established in Nigeria based on control studies. In cattle oxytetracycline is commonly used based on manufacturer's recommendation, of 10 mg/kg body weight.

Apart from oxytetracycline, two other drugs, Imidocarb and Gloxazone, are now known to be efficacious in the therapy of anaplasmosis. Both drugs have already been reviewed (Chapter II), but their use in Nigeria has been limited; however, gloxazone has already been withdrawn from the market by the manufacturers.

Based on the foregoing, it was thought of importance to study the duration of therapy in anaplasmosis in Nigeria, under controlled situation. In order to reduce variables to minimum, it was decided to use the commonly available drug, oxytetracycline and the manufacturer's recommended dose i.e. 10 mg/kg. body weight, but vary the duration of treatment.

Materials and Methods

Experimental animals: The animals used in this study were those shown to be negative for A. marginale by microscopic examination and the rapid card agglutination test (Amerault and Roby, 1968; Amerault, 1973), after herd screening (Chapter III). Such animals were retested immediately prior to their use in the experiment, and were found to be negative.

A total of 27 crossbred calves were used. Their average age was 13.1 months (range: 10 - 17 months). They were randomly divided into 3 groups.

Group A consisted of 12 intact calves; group B had 9 splenectomised calves, and group C 6 intact calves. Group C served as uninfected control. All the animals in groups A and B were later sub-divided into 3 sub-groups based on the duration of treatment with oxytetracycline (Table IX).

Surgical Procedures: Splenectomy was performed via a left paracostal laparatomy, using paravertebral anesthesia.

TABLE IX

Protocol showing division of experimental animals into subgroups according to duration of treatment

Group	Subgroups		
	One Treatment	Two Treatments	Three Treatments
A (Intact)	4	4	4
B (Splenectomised)	3	3	3

Collection of Infected Blood: Two hundred millilitres (200 ml.) of whole blood were collected from a selected donor which had previously been proved to be a carrier of A. marginale (more than 0.1 per cent) by microscopic detection method and the rapid card agglutination test. The blood was collected in a sterile clean bottle containing 20 ml. of 3 per cent Sodium citrate solution, as anticoagulant.

Infection and Post-inoculation Care: All the animals in groups A and B were each inoculated subcutaneously (S.C) with 5 ml. of the carrier blood.which gives approximately $2x10^7$ infected erythrocytes. Prior to inoculation all the animals, especially the splenectomised ones, were kept under close observations for 14 days to exclude any other infections. The observations, included haematologic evaluation, microscopic examination of stained blood smears and daily rectal temperature. The animals were found to be free of any blood parasites.

Prior to and after inoculation, all the animals were kept in enclosed pens and were sprayed weekly with acaricide to prevent tick infestation. They were fed on silage and legume hay rations, occasionally supplemented with groundnut cake; while water and mineral salts were provided ad.lib.

Collection of Samples and Evaluation: The rectal temperatures and packed cell volumes (PCV), of the animals

were monitored 3 times a week until A. marginale was detected microscopically. The PCV was determined using microcapillary method.

Blood samples (2.5 ml.) were collected from each animal, 3 times a week, using disodium ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. Thin blood smears were then made, stained with Giemsa solution and examined microscopically for the presence of A. marginale.

Once A. marginale was detected, rectal temperature, determination of PCV and degree of parasitaemia were carried out daily. The degree of parasitaemia was based on the percentage of parasitised cells out of 500 erythrocytes.

Therapy of Clinical Disease: Treatments were carried out when the PCV had dropped to 16 per cent or below, using oxytetracycline at 10 mg/kg body weight intravenously (iv). A PCV of 16 per cent or below was chosen because field observations have shown that most cases of clinical anaplasmosis are brought to the notice of Clinicians at that point for treatment. Three treatment regimens were followed. One group was treated once, the second group 2 consecutive days and the third group 3 consecutive days (iv) (Table IX).

Assessment of Antibody Response: Serum samples were regularly collected from all infected animals and tested for the presence of antibodies to A. marginale, using the

rapid card agglutination test (Amerault and Roby, 1963; Amerault, 1973).

Results

Clinical manifestation: Anaplasma bodies (A. marginale) were first detected microscopically in the infected animals between 16 and 33 days (mean: 22.78) postinoculation (pi). Figs 1 to 3 show the pattern of development of anaemia and parasitaemia in both splenectomised and intact animals given different treatment regimens.

The pattern of parasitaemia, irrespective of whether treatment was given once, twice or thrice, appeared to be similar in both intact (group A) and splenectomised (group B) animals, except that the degree was more pronounced in group B. In group A, parasitaemia increased gradually from day 20 pi, until it was interrupted by therapy about day 30, when treatment was given. In group B, the parasitaemia increased so rapidly that it required earlier therapeutic intervention. The average of highest parasitaemia reached before treatment in groups A and B were 13.26 and 35.41 per cent respectively.

The fluctuation in the PCV of the infected animals followed similar pattern. However, group B animals were observed to suffer more anaemic crisis than group A.

Tables X and XI summarise the mean PCV values prior to exposure, at the time of treatment and 2 weeks after treatment in both intact (group A) and splenectomised (group B) animals. The average PCV values at the time of treatment were similar in each group. However, the average PCV values were very low in all the splenectomised calves at the time of treatment (Table XI). In the intact calves it was observed that 2 weeks after treatment the average PCV of all surviving animals had returned to almost pre-infection levels (Table X). In contrast, in the splenectomised groups only those calves treated twice showed similarity to the intact calves: while those that were treated once all died, before 2 weeks of treatment and 2 of those treated thrice also died.

Survival Rates: The survival rate based on duration of treatment of clinical anaplasmosis is summarized in Table XII.

In group A (i.e. intact animals) all animals treated once survived, as were those treated twice. One of those treated thrice died due to rapid drop in its PCV from 18.5 to 10 per cent, which occurred overnight and made drug treatment less effective: the animal died soon after treatment. Two animals (one in the group to be treated once and one in the group to be treated thrice) were not treated as their PCV remained above 16 per cent.

TABLE X

Packed cell volume (PCV) of intact calves (group A) before inoculation, at point of treatment and two weeks after treatment with Oxytetracycline

	Packed cell volume (Mean ± S.D.)		
	One treatment	Two treatments	Three treatments
Prior to exposure	30.5 ± 3.9 (27.5 - 36	31.8 ± 2.3 (28 - 33.5)	33.2 ± 1.8 (31 - 35.5)
At point of treatment	15.0 ± 0.4 (14.5 - 15.5)	15.3 ± 0.8 (14.5 - 16.5)	15.2 ± 0.5 +(14.5 - 15.5)
2 weeks after treatment	23.5 ± 3.1 (20 - 27.5)	24.4 ± 4.1 (20.5 - 29)	*27.5 ± 1.0 *(26.5 - 28.5)

Figure in parenthesis represents the range.

*Values for only 2 of the 3 treated calves (Nos 2176 and 2204).

TABLE XI

Packed cell volume (PCV) of splenectomised calves (group B) before inoculation, at point of treatment and two weeks after treatment with Oxytetracycline

	Packed cell volume (Mean ± S.D.)		
	One Treatment	Two Treatments	Three Treatments
Prior to exposure	30.2 ± 2.7 (26.5 - 33)	30.7 ± 2.1 (28 - 33)	29.0 ± 4.5 (25.5 - 36)
At point of treatment	13.5 ± 1.1 (12.5 - 15)	12.6 ± 1.0 (11.5 - 14)	11.5 ± 1.0 (10.5 - 12.5)
2 weeks after treatment	**	24.7 ± 3.0 (20.5 - 27.5)	(20.5)#

#PCV value for only one surviving calf (no 2195) at 2 weeks after treatment;

##All the calves died before 2 weeks of treatment.

Figure in parenthesis represents the range.

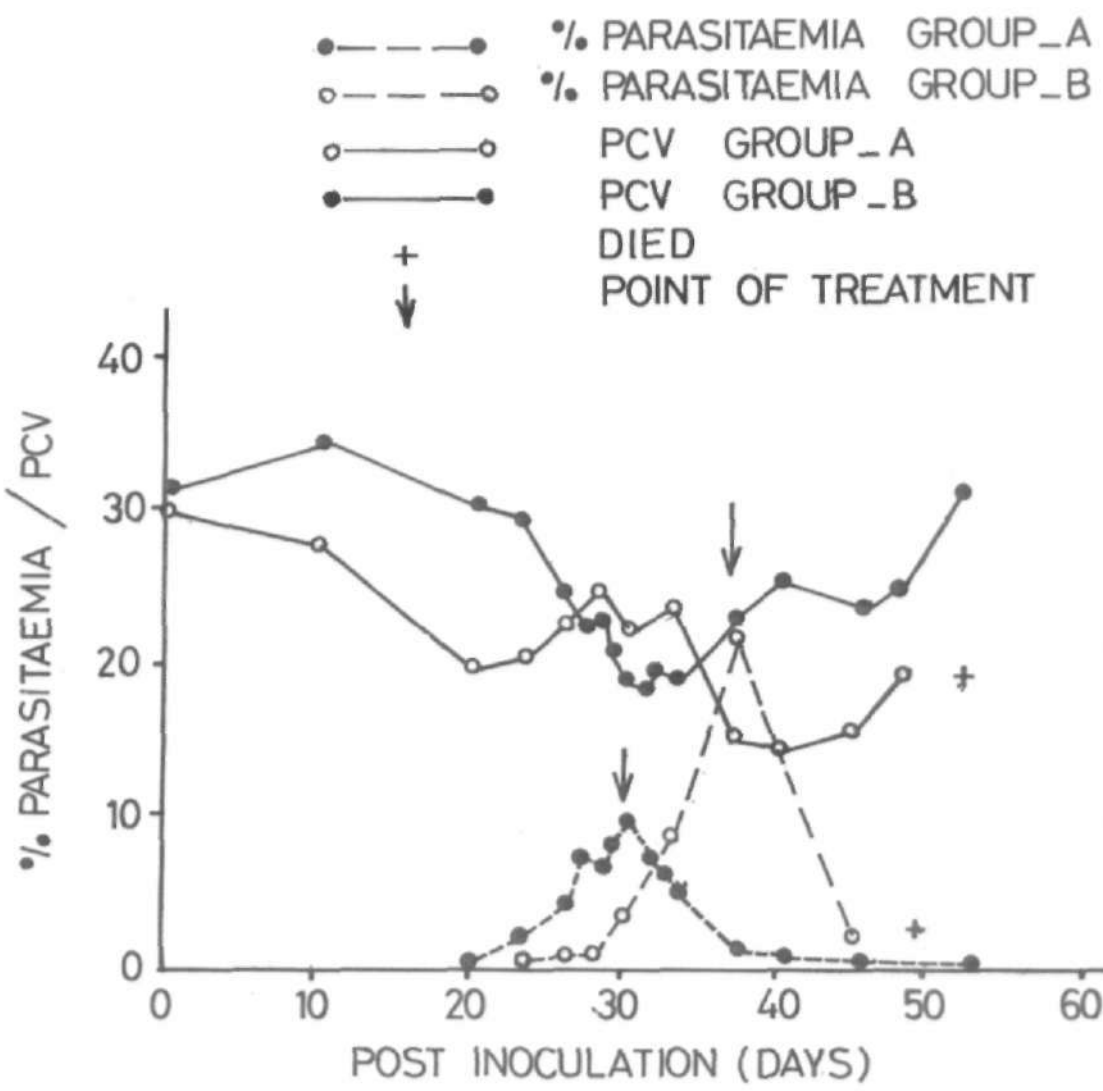


FIG_2 MEAN PARASITAEMIA AND PACKED CELL VOLUME (PCV) IN BOTH INFECTED INTACT (GROUP_A) AND SPLENECTOMISED (GROUP_B) ANIMALS TREATED ONCE WITH OXYTETRACYCLINE.

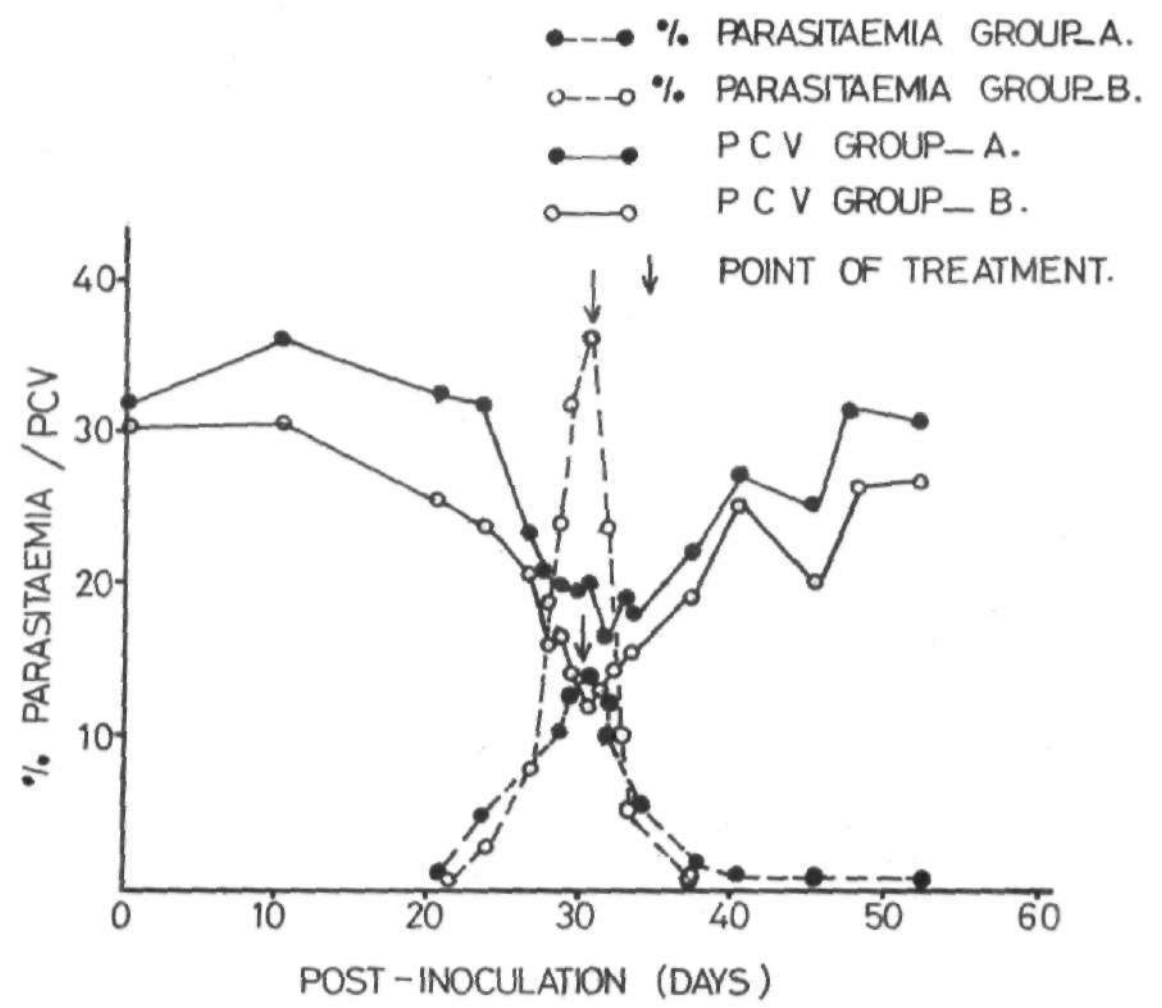


FIG_3 MEAN PARASITAEMIA AND PACKED CELL VOLUME (PCV) IN BOTH INFECTED INTACT (GROUP_A) AND SPLENCTOMISED (GROUP_B) ANIMALS TREATED TWICE WITH OXYTETRACYCLINE.

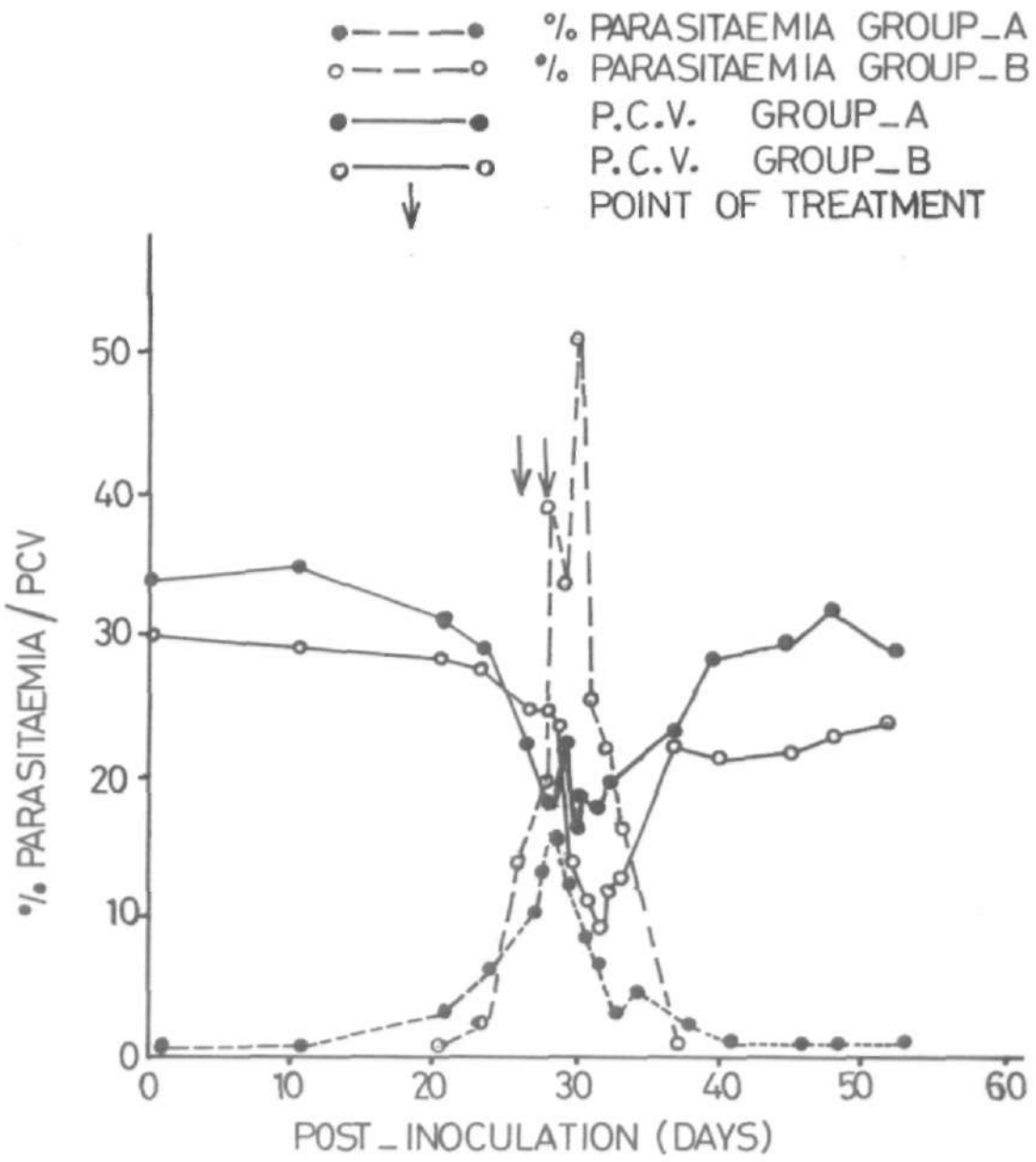


FIG_4 MEAN PARASITAEMIA AND PACKED CELL VOLUME (PCV) IN BOTH INFECTED INTACT (GROUP_A) AND SPLENECTOMISED (GROUP_B) ANIMALS TREATED THRICE WITH OXYTETRACYCLINE.

In group B (i.e. splenectomised) animals all those treated once died, while those treated twice all survived. However, 2 out of the 3 animals treated thrice died, because drug intervention was too late as the PCV drop was so rapid that by the time treatment was given the PCV values were 8.5 and 9 per cent respectively.

Serological Response: Majority of the animals became serologically positive within 20 days of inoculation (Table XIII). By day 35 pi, all animals had become positive. The presence of detectable parasitaemia coincided with development of antibody response. Maximum serological response (+4) was not obtained early during the patent disease until day 52 pi, in the majority of the animals. There was no difference in the response between intact and splenectomised animals, either in the degree of reaction or persistence of antibody within the period of the experiment. This observation agrees with the results reported by Jones et al. (1968a,b) using the complement fixation test; they observed similar pattern of response in both intact and splenectomised calves. However, in this study all the animals remained serologically positive throughout the duration of the experiment, even though no parasite was detected after day 52 pi.

TABLE XII

Effect of duration of treatment on clinical anaplasmosis using Oxytetracycline at 10 mg/kg body weight

Group	No of times treated	No of animals treated	Outcome	
			No cured	No died
A (intact animals)	Once	3+	3	-
	Twice	4	4	-
	Thrice	3+	2	1*
B (Splenectomised).	Once	3	-	3
	Twice	3	3	-
	Thrice	3	1	2#,##

*One animal was not treated since its PCV was consistently above the range specified for treatment (i.e. 15%).

#The PCV of this animal dropped too abruptly for early treatment to be instituted; it died soon after treatment.

##The PCV was persistently low (between 8.5 and 9.0) despite treatment.

TABLE XIII

Serological response in infected, intact and splenectomised animals, using the rapid card agglutination test

Group	Eartag No		Card test scores*								
		Days (pi)	0	20	28	35	45	52	58	65	72
		Treatment									
A	2156	T1	-ve	+2	+4	+4	+4	+4	+4	+4	+4
"	2170	"	-ve	-ve	+1	+1	+2	+3	+2	+4	+1
"	2169	"	-ve	+3	+4	+4	+4	+4	+3	+4	+4
"	2149	"	-ve	+2	+3	+3	+3	+4	nd	+4	+4
"	2188	T2	-ve	+3	+4	+4	+3	+4	+4	+4	+4
"	2177	"	-ve	+2	+3	nd	+2	+3	+4	+4	+4
"	2163	"	-ve	+1	+1	+1	+1	+3	+3	+4	+2
"	2172	"	-ve	+2	+2	+4	+2	+4	+3	+4	+4
"	2176	T3	-ve	+3	+3	+2	+2	+3	+4	+4	+4
"	2352	"	-ve	-ve	+1	+2	D				
"	2190	"	-ve	+3	+3	+4	+4	+4	+4	+4	+3
"	2204	"	-ve	+2	+3	+4	+2	+4	+4	+4	+4
B	2210	T1	-ve	-ve	+1	+1	+2	+4	+4	+4	+4
"	2349	"	-ve	+1	+1	+3	+3	+4	+4	+4	+4
"	2333	"	-ve	+1	+1	+2	+2	+3	+4	+4	+4
"	2182	T2	-ve	+1	+1	+1	+2	+2	+2	+4	+3
"	2185	"	-ve	-ve	+1	+1	+2	+4	+3	+4	+4
"	2237	"	-ve	+1	+1	+4	+1	+4	+4	+4	+4
"	2199	T3	-ve	+1	+1	D					
"	2178	"	-ve	+1	+2	D					
"	2195	"	-ve	+1	+2	nd	+3	+4	+4	+4	D

*Card test scores: -ve = negative; +1 = minimal positive reactor;

Nd = not done (blood clotted;

D = died (no test done);

(pi) = postinoculation in days;

T1 = one treatment, T2 = two treatments, T3 = three treatments.

Necropsy Findings: The most prominent gross lesions observed included, distended gall bladder, anaemic or pale tissues and enlarged mottled liver, with rounded borders and brownish-yellow cut surface. The subcutaneous tissue had a yellow and gelatinous appearance. Petechial haemorrhages were observed on the epicardium, pericardium, pleura and the diaphragm. Some hard pellets of faeces, coated with mucus, were found in the colon and sometimes in the rectum.

Discussion

The results of the experiment clearly show that cattle with clinical anaplasmosis could effectively be treated with oxytetracycline given on two consecutive days, at the rate of 10 mg/kg body weight, without any adverse effect. This is clearly shown in Table XII, where the splenectomised calves treated once died, while those treated on two consecutive days survived. However, it is not possible in this study to compare those with two treatments, with those that received three treatments, because of the unexpected mortalities recorded in the latter group (Table XI). Nevertheless, since two treatments gave 100 per cent cure, it should be expected that if therapeutic intervention occurred early before severe anaemic crisis, as was observed in the animals that died in

group 3, then 3 treatments shojld also result in complete cure with probably faster recovery period. This was in fact demonstrated in Table X, where the PCV of intact animals given 3 treatments showed significant improvement over those which received 1 or 2 treatments.

Although one treatment resulted in 100 per cent cure in intact calves (Table XII), a similar result may not be expected in adult cattle so treated, since it has been shown by various workers (De kock and Quinlan 1962; Roby et al. 1961; Jones and Brock 1966; Jones et al; 1968a,b) that young animals are less susceptible to A. marginale infection and have greater ability to regenerate erythrocytes better than adults. This position is further supported by the finding of 100 per cent mortality in the splenectomised calves treated once. This then agrees with the fact that splenectomy overcomes innate resistance in infected calves and thus induces a situation similar to primary anaplasmosis in adult or aged cattle (De kock and Quinlan, 1926; Roby et al. 1961; Jones and Brock, 1966). This does not mean that splenectomised animals are comparable in all respects with the adults, because the absence of the spleen does not make them immunologically incompetent. However, with 2 treatments, both intact and splenectomised calves were all cured of infection, suggesting that these could well be applicable to adult animals.

Although faster recovery was obtained in those intact animals treated thrice (Table X and Fig 3), it is unlikely

that the difference in PCV, 2 weeks after treatment, justifies the additional expense. Taking the current market price of Oxytetracycline (Terramycin 050 - Pfizer) as ₦0.0194/kg; it will then require ₦11.64 to treat an average adult cattle, weighing 300kg, for 2 consecutive days, while it will cost ₦17.46 to treat the same animal for 3 consecutive days. It is, therefore, economical to give 2 consecutive treatments in clinical bovine anaplasmosis.

It is, also, evident from this study that for effective therapy in clinical anaplasmosis, treatment must be started as early as possible, preferably when the PCV is about 20 per cent or slightly below. Any delay may lead, not only to persistently low PCV and partial recovery, but death, when therapy is finally instituted. However, this does not mean that animals with clinical anaplasmosis and those with very low PCV may not survive when treated. This may require some supportive treatments, such as administration of blood transfusions (Jones and Norman, 1962; Franklin et al. 1962) or some haematinics (Brock et al. 1959), to enhance recovery rate. But more often than not, most supportive treatments do not have time to stimulate erythropiesis sufficiently enough to effect survival. For example, blood transfusion as an adjuvant to therapy induces recrudescence of the parasitaemia and its benefit has been questioned (Jones and Norman, 1962).

Haematinics, on the other hand, are believed to reduce the time required for convalescence (Brock et al. 1959) From this study, it is apparent that 2 treatments of Oxytetracycline, at 10 mg/kg body weight (iv) for 2 consecutive days are best to cure clinical bovine anaplasmosis. This could not be said to give a hundred per cent cure; but from economic point of view, is better than giving for 3 consecutive days. However, this study being a laboratory work, is not completely comparable to the field condition.

Summary and Conclusion

A total of 27 crossbred calves (12 intacts, 9 splenectomised and 6 uninfected control) were subcutaneously inoculated with $2x10^7$ infected erythrocytes. Oxytetracycline (at 10 mg/kg body weight) was administered intravenously when the PCV dropped to 16 per cent or below. The pattern of development of parasitaemia, irrespective of the duration of treatment, appeared to be similar in both intact and splenectomised calves. The splenectomised calves, however, gave higher percentage of parasitaemia. Fluctuation in the PCV followed the same pattern in both groups, but the splenectomised calves were observed to suffer more anaemic crisis than the intact ones.

In the intact calves, all those treated once and for 2 consecutive days survived, while one died amongst those treated

for 3 consecutive days, due to late administration of the drug. In the splenectomised calves, all those treated once died, while all those treated for 2 consecutive days survived. But, due to late treatment 2 out of those that received 3 consecutive treatments died (Table XII).

Since 2 consecutive treatments cured both intact and splenectomised calves, then it could be expected that 3 consecutive treatments would also have cured them. However, from the economic point of view (of the cost of the drug), 2 consecutive oxytetracycline treatments (at 10 mg/kg body weight) would be better to administer in cases of clinical bovine anaplasmosis.

CHAPTER V

GENERAL DISCUSSION

Over the past 55 years, when anaplasmosis was first recognised in Nigeria, only the tick (Boophilus spp) transmission of the disease has been given a lot of attention. But from the different work reported all over the world (chapter II), it is evident that other sources of transmission require some attention. It is, therefore, pertinent, to assume that in future other blood sucking arthropods may become greater sources of propagation of the disease, especially in endemic areas. In fact, in Nigeria, it may become more difficult to control the disease, since mosquitoes, and other biting arthropods, as well as wild ruminants abound in far higher proportion than even ticks. Future investigation in this direction may require evaluation of the role of intra-uterine transmission as another latent source of the disease, especially in cases of abortion in cows and sterility in bulls.

Through the various reports reviewed, on the control of the disease (chapter II), it has been apparent that no complete suitable vaccine has yet been established. The only option

left for most endemic areas, where total eradication of ticks is not feasible, is perhaps the use of artificial premunization. Post-immunization reactions could then be controlled by chemotherapy. However, despite various experiments carried out on both imidocarb and gloxazone, the tetracycline (especially, Oxytetracycline) compounds are still generally used in the control of post-immunization reactions against anaplasmosis.

Although, it is the general belief that splenectomy overcomes innate resistance in infected calves, and thus induces a situation similar to primary anaplasmosis in adult or aged cattle; this should not be assumed that an animal without a spleen is completely empty immunologically. For instance, in this study, if some of the intact calves were left untreated they would have possibly died (Appendices 1-12), just like an adult would have done. Therefore, there is need to review this overstated fact, that the young calves are more resistant to anaplasmosis than the adults. The absence of the spleen in a young animal, does not therefore makes it comparable to the adult in all respects; it only makes it close to the situation that exists in the adult.

In this study, maximum serological response (4+) was not obtained early during the patent disease in the majority of the animals, until day 52 pi, using the rapid card agglutination

test (CT). Using other serological tests, such as complement fixation test (CF), indirect fluorescent technique (IFT) and the capillary tube agglutination test (CA), various workers (chapter II) had reported early rapid rise in antibody titre, than with the CT. This may then suggest that these tests, especially the CF, although very complex and time consuming, may be more sensitive to early antibody production than the CT. Since these tests were carried out in many different countries (especially the U.S.A.) where conditions were quite different from those of Nigeria, then slight variation in the results may be expected. However, it may be assumed that the delay in obtaining maximum serological response (4+), may not be unrelated with the possibility of differences in the strains of A. marginale used in making the antigen as compared to the local strains available in Nigeria. For this assumption to be regarded as untrue, there is need to investigate the possibility of having different strains of A. marginale in the country. Similarly, there is need to investigate methods of preparing antigen locally, instead of our continuous dependent on foreign antigens. Apart from the fact that these foreign antigens, may not always give the desired results, their frequent importation will continue to drain the country's dwindling foreign reserves.

The very low anaplasmosis prevalence (26%) obtained on NAPRI farm, as compared to other test areas, has indicated the need to review the existing tick control method now in practice. Animals should be allowed to acquire premunity early in life, through regular tick challenge. However, chemotherapy may be required in cases of post-immunization reactions, if artifitial premunization is adopted. In addition, there may also be need to control tick infestations through acaricide spray or dips, with greater intervals between spraying or dipping period, than hitherto. For national control of the disease, it may be suggested that all Governments of the Federation should further establish more control posts, where spray races or dip vats will be available for regular control of the disease vectors, especially in trade cattle. Apart from controlling anaplasmosis, this measure will reduce incidences of other tick-borne diseases. However, for this to be effective, it may require government legislation to make it compulsory for all cattle owners in the different parts of the country, especially at border posts, to dip their animals regularly.

The great disparity in the prevalence of anaplasmosis by various workers in Nigeria (chapter III) underlines the desirability of having a national anaplasmosis survey, to reveal the actual prevalence of the disease in different geographical areas of the country.

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Appendix 1.

Eartag no: 2156

Age, breed & sex: 16 months, intact crossbred heifer calf.

Duration of treatment: Once.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	-	36.0	npf
20	39.1	29.0	"
23	38.5	32.0	0.12
26	39.5	26.0	2.57
27	39.1	21.5	3.28
28	39.0	27.5	2.91
29	40.1	12.5	6.19
30	39.5	21.0	5.58
31 T	39.1	15.5	2.49
32	38.8	21.5	1.70
33	38.2	21.0	1.23
37	37.6	26.0	0.25
40	37.7	27.0	0.23
45	39.1	27.5	npf
48	38.6	38.0	"
52	37.4	34.0	"

T = point of treatment; npf = no parasite found.

Appendix 2

Eartage No: 2170

Age, breed & sex: 15 months intact crossbred bull calf.

Duration of treatment: Once.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	-	27.5	npf
20	37.7	30.5	"
23	38.2	28.0	0.12
26	38.9	23.0	1.60
27	38.5	26.0	1.27
28	38.2	24.0	1.96
29	390	25.0	1.87
30	39.5	18.0	8.42
31	39.5	21.5	8.13
32	38.5	18.0	11.07
33	39.2	17.0	12.44
34 T	39.2	15.0	17.05
37	38.1	14.5	1.71
38	39.8	19.5	1.63
39	-	21.5	1.47
40	38.0	22.0	0.73
45	38.7	22.5	npf
48	37.0	20.0	"
52	37.6	25.5	"

T = point of treatment; npf = no parasite found.

Appendix 3

Eartage No: 2169*

Age, breed & Sex: 15 months, intact crossbred bull calf.

Duration of treatment: Once.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	-	34.0	npf
20	38.7	31.0	"
23	38.7	32.0	0.27
26	39.0	25.0	1.32
27	39.5	20.5	2.53
28	38.0	29.0	1.97
29	40.2	29.0	2.01
30	39.7	25.0	4.17
31	39.3	22.0	5.11
32	38.2	24.0	3.81
33	38.0	24.0	1.71
34	37.8	24.5	1.47
35	39.0	22.5	1.53
37.	37.5	27.0	1.56
40	37.9	24.0	0.67
45	39.4	27.0	npf
48	38.4	24.0	"
52	38.5	30.5	"

*No treatment was administered throughout the course of the experiment; npf = no parasite found.

Appendix 4

Eartag No: 2149

Age, breed & Sex: 16 months, intact crossbred heifer calf.

Duration of treatment: Once.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	-	28.0	npf
20	39.1	32.0	0.60
23	37.2	27.5	5.00
26	40.0	24.0	10.59
27 T	40.0	14.5	21.71
28	39.7	12.0	18.89
29	40.3	12.5	19.01
30	39.0	12.5	19.29
31	38.7	13.5	12.17
32	39.3	15.5	7.29
33	38.3	16.5	5.70
37	38.0	23.5	0.59
40	38.0	23.0	npf
45	39.1	15.5	"
48	38.1	21.0	"
52	38.3	34.0	"

T = point of treatment; npf = no parasite found.

Eartag No: 2138

Age, breed & Sex: 15 months intact crossbred bull calf.

Duration of treatment: Twice.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.0	32.0	npf
20	38.5	33.0	0.19
23	38.1	31.0	4.17
26	40.2	16.5	7.80
27	38.2	19.0	5.14
28	39.0	20.0	4.97
29	38.3	19.0	6.21
30	38.3	20.0	7.54
31 T	39.1	15.5	5.33
32 T	38.3	23.0	2.77
33	38.4	18.0	1.04
37	38.7	26.5	npf
40	38.2	32.0	"
45	40.0	28.0	"
48	38.3	32.0	"
52	38.2	30.5	"

T = point of treatment; npf = no parasite found.

Appendix 6

Eartag No: 2177

Age, Breed & Sex: 14 months, intact crossbred bull calf.

Duration of treatment: Twice

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	38.6	33.5	npf
20	38.7	31.0	1.00
23	38.0	28.5	4.27
26	39.1	22.0	11.32
27	38.0	19.5	14.57
28	39.1	15.5	19.01
29	39.0	17.0	15.28
30	39.2	18.0	12.80
31 T	39.5	14.5	5.17
32 T	38.8	16.5	6.12
33	38.3	14.5	1.49
37.	38.2	17.0	0.34
40	38.0	23.5	npf
45	39.3	20.5	"
48	38.0	31.0	"
52	37.2	33.0	"

T = point of treatment; npf = no parasite found.

Appendix 7

Eartag No: 2153

Age, Breed & Sex: 15 months, intact crossbreed heifer calf.

Duration of treatment: Twice.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.0	33.5	npf
20	38.8	30.5	0.17
23	38.0	30.0	1.60
26	39.2	27.0	5.92
27	39.5	24.0	7.24
28	39.3	24.0	8.57
29	40.4	20.5	23.97
30	40.1	20.5	28.00
31 T	40.1	14.5	32.63
32 T	39.4	17.0	22.69
33	39.5	16.5	13.37
37.	38.9	18.5	2.29
40	39.1	24.5	1.33
45	39.1	20.0	npf
48	37.6	31.0	"
52	37.8	29.0	"

T = point of treatment; npf = no parasite found.

Appendix 9

Eartag No: 2172

Age, Breed & Sex: 15 months, intact crossbred heifer calf.

Duration of treatment: Twice.

Postinoculation period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.9	28.0	npf
20	39.2	33.5	0.80
23	38.1	26.0	5.44
26	40.3	26.0	5.37
27	39.7	17.5	9.01
28	39.2	18.0	7.28
29	39.0	19.5	5.17
30	40.0	18.5	4.50
31 T	39.5	16.5	2.62
32 T	39.2	17.0	2.17
33	38.8	20.0	0.80
37	38.0	24.0	0.20
40	38.0	26.5	npf
45	39.0	29.0	"
48	38.2	30.5	"
52	38.2	28.0	"

T = point of treatment; npf = no parasite found.

Appendix 9

Eartag No: 21/6

Age, Breed & Sex: 14 months, intact crossbred heifer calf.

Duration of treatment: Thrice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.4	33.0	npf
20	39.0	30.5	0.09
23	39.3	29.0	3.41
26	40.6	20.0	8.33
27	39.0	19.5	21.35
28	39.3	17.0	23.08
29 T	41.0	15.5	16.67
30 T	40.0	17.0	14.00
31 T	39.7	16.5	9.92
32	-	19.5	2.34
33	38.7	26.5	1.97
37	38.0	28.0	0.94
40	37.7	30.5	0.57
45	40.2	29.5	npf
48	37.8	29.5	"
52	37.5	26.5	"

T = point of treatment; npf = no parasite found.

Appendix 10

Eartag No: 2352

Age, Breed & Sex: 10 months, intact crossbred bull calf.

Duration of treatment: Thrice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	-	35.5	npf
20	-	30.5	"
23	38.7	29.5	0.19
26	38.4	29.5	2.14
28	38.4	34.5	3.27
30	38.2	18.5	7.14
33	38.1	17.5	11.25
35	38.1	16.0	5.42
37 T	38.2	15.5	3.54
38 T	38.7	15.0	3.15
*39 T	37.6	15.0	2.06

*Died on day 39 pi, on the day the final treatment was given; T = point of treatment; npf = no parasite found.

Appendix 11

Eartag No: 2190*

Age, Breed & Sex: 12 months, intact crossbred bull calf.

Duration of treatment: Thrice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	38.9	33.5	npf
16	38.4	31.5	1.20
20	38.6	25.0	9.89
23.	38.6	26.5	14.02
26	40.0	16.0	17.53
27	39.4	17.5	19.21
28	37.7	16.5	21.27
29	39.0	18.0	12.19
30	40.1	23.0	4.03
31	39.3	23.5	3.64
32	38.2	25.0	2.09
33	37.0	25.5	0.51
37	37.8	25.0	0.46
40	37.0	26.5	0.41
45	38.9	27.0	npf
48	37.7	32.5	"
52	37.5	25.0	"

*No treatment was administered throughout the course of the experiment; npf = no parasite found.

Appendix 12

Eartag No: 2204

Age, Breed & Sex: 12 months, intact crossbred bull calf.

Duration of treatment: Thrice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	38.3	31.0	npf
20	38.9	36.5	"
23	38.8	25.0	3.20
26	39.3	19.0	9.68
27	38.6	18.5	11.01
28	37.5	19.0	10.69
29	39.1	16.0	5.21
30	39.1	16.5	4.77
31 T	39.2	14.5	3.95
32 T	39.0	16.5	2.19
33 T	37.7	17.0	1.15
37	37.0	23.5	0.70
40	37.7	24.5	npf
45	39.0	28.5	"
48	38.2	29.5	"
52	38.7	30.0	"

T = point of treatment; npf = no parasite found.

Appendix 13

Eartag No: 2210

Age, Breed & Sex: 11 months, splenectomised crossbred bull calf.

Duration of treatment: Once.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.5	31.0	npf
20	39.2	16.0	"
23	37.6	18.0	"
26	38.2	19.5	"
28	38.3	26.0	"
30	38.6	21.5	1.38
33	38.2	20.0	13.13
35	38.2	17.5	22.56
37 T	38.0	12.5	30.51
38	37.9	9.0	34.72
39	37.4	6.5	29.21
40	38.9	6.5	25.53
42	38.8	10.5	13.89
*43	-	Died	-

*Died on day 43 pi, 6 days post - treatment; T = point of treatment; npf = no parasite found.

Appendix 14

Eartag No: 2349

Age, Breed & Sex. 10 months, splenectomised crossbred bull calf.

Duration of treatment: Once.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.8	33.0	npf
20	38.7	25.0	"
23	37.7	25.5	"
26	38.0	31.0	0.18
28	38.5	28.0	0.92
30	38.5	25.0	8.84
33	37.9	27.5	10.39
35	38.4	20.0	40.79
37 T	38.5	13.0	31.04
38	37.7	13.0	20.13
39	37.2	13.0	18.80
40	37.8	20.5	6.98
42	38.4	18.5	2.84
43	39.8	18.5	1.42
*44	38.5	18.5	0.31

*Died on day 44 pi, 7 days post-treatment; T = point of treatment; npf = no parasite found.

Appendix 15

Eartag No: 2333

Age, Breed & Sex: 11 months, splenectomised crossbred bull calf.

Duration of treatment: Once.

Postonoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.1	26.5	npf
20	38.7	17.5	"
23	38.8	17.0	"
26	37.8	17.0	"
28	37.9	20.0	"
30	38.0	20.0	"
33	37.8	23.0	0.91
35	38.5	23.0	1.30
37	38.0	20.5	3.26
38	38.5	17.5	5.05
39	37.8	17.5	14.65
40 T	38.2	15.0	8.05
42	39.1	11.0	8.73
43	38.1	9.0	5.13
44	38.7	15.5	3.13
45	39.4	15.0	1.40
46	38.6	16.5	0.67
48	39.6	19.0	npf
*52	-	Died	-

*Died on day 52 pi, 10 days post-treatment; probably due to failure to respond to treatment; npf = no parasite found; T = point of treatment

Appendix 16

Eartag No: 2182.

Age, Breed & Sex: 13 months, splenectomised crossbred heifer calf.

Duration of treatment: Twice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.0	31.0	npf
20	39.3	29.0	"
23	37.9	24.5	3.88
26	40.5	16.0	6.30
27 T	39.9	11.5	18.27
28 T	38.6	11.0	25.35
29	40.0	8.5	32.75
30	40.0	10.0	37.50
31	39.5	13.0	19.28
32	38.2	16.0	7.23
33	38.4	16.0	3.16
37	38.9	22.5	npf
40	37.4	27.5	"
45	38.8	22.5	"
48	37.5	25.5	"
52	37.6	18.0	"

T = point of treatment; npf = no parasite found.

Appendix 17

Eartag No: 2185.

Age, Breed & Sex: 13 months, splenectomised crossbred heifer calf.

Duration of treatment: Twice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.8	33.0	npf
20	39.2	26.5	"
23	38.3	24.5	0.17
26	39.5	28.5	5.38
27	39.2	16.5	13.53
28	38.4	18.5	15.14
29	40.1	17.0	19.87
30 T	40.2	12.5	26.63
31 T	38.9	13.0	18.22
32	38.5	14.0	7.32
33	38.7	15.0	3.05
37	38.9	18.5	npf
40	38.4	26.0	"
45	38.6	20.5	"
48	38.0	21.5	"
52	38.2	29.0	"

T = point of treatment; npf = no parasite found.

Appendix 13

Eartag No: 2237.

Age, Breed & Sex: 10 months, splenectomised crossbred bull calf.

Duration of treatment: Twice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.1	20.0	npf
20	38.7	21.5	"
23	39.1	19.0	2.18
26	39.1	16.5	10.16
27	39.2	18.5	23.76
28	39.0	19.5	29.05
29 T	40.5	14.0	40.59
30 T	40.3	13.5	42.64
31	40.2	11.0	32.35
32	38.7	12.0	13.64
33	38.3	13.5	5.84
37	39.8	15.5	npf
40	39.2	20.5	"
45	38.7	15.0	"
48	38.7	29.5	"
52	37.5	31.0	"

T = point of treatment; npf = no parasite found.

Appendix 19

Eartag No: 2199.

Age, Breed & Sex: 12 months, splenectomised crossbred heifer calf.

Duration of treatment: Thrice.

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	38.9	28.0	npf
20	38.0	24.0	"
23	38.2	25.0	0.69
26	38.5	24.0	8.62
27	39.2	24.5	22.97
28	38.5	23.0	27.62
29 T	41.0	12.5	29.11
30 T	39.0	8.5	31.96
31 T	36.7	9.0	25.73
#32	-	Died	-

#Died on day 32 pi, a day after the last oxytetracycline treatment; T = point of treatment; npf = no parasite found.

Appendix 20

Eartag No: 2178.

Age, Breed & Sex: 14 months, splenectomised crossbred heifer calf.

Duration of treatment: Thrice

Postinoculation Period (days)	Temperature °C	% PCV	% Parasitaemia
0	39.0	36.0	npf
20	38.8	26.5	"
23	38.5	25.5	2.33
26	39.1	23.0	12.96
27	39.5	23.0	14.37
28	39.1	24.0	15.02
29	40.7	16.0	37.93
30 T	40.5	11.5	85.80
#31	-	Died	-

#Died on day 31 pi, a day after the first oxytetracycline treated; T = point of treatment; npf = no parasite found.

Appendix 21

Eartag No: 2195

Age, Breed & Sex: 12 months, splenectomised crossbred heifer calf.

Duration of treatment: Thrice.

Postinoculation (pi) - days	Temperature °C	% PCV	% Parasitaemia
0	39.0	25.5	npf
20	38.6	31.5	"
23	37.6	31.0	1.20
26	-	24.5	16.36
27	38.2	24.0	17.26
28	37.3	20.0	70.91
29 T	40.5	10.5	30.60
30 T	39.4	10.0	29.95
31 T	38.2	7.5	22.38
32	37.0	11.0	21.13
33	37.5	11.5	15.64
37	37.4	21.0	npf
40	38.0	20.0	"
45	38.2	20.5	"
48	38.5	21.5	"
52	38.0	22.5	"

T = point of treatment; npf = no parasite found.

SEROLOGICAL SURVEY OF BOVINE ANAPLASMOSIS ON SHIKA FARM AND TREATMENT OF THE CLINICAL DISEASE.

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AN ABSTRACT OF THESIS SUBMITTED TO

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The occasional clinical anaplasmosis experienced on the National Animal Production Research Institute (NAPRI) farm Shika, coupled with the absence of adequate data on the epidemiology of bovine anaplasmosis in Nigeria stimulated this study. The programme was designed to investigate the prevalence of the disease among cattle on NAPRI farm, selected cattle herds around Shika, as well as in slaughter cattle at the Zaria abattoir. It was also aimed at evaluating the duration of treatment required to cure the clinical disease by using Oxytetracycline.

The rapid card agglutination test was used in the sero-epidemiological survey, because of its simplicity and convenience to use even on the field.

The results of the investigation revealed that the prevalence of anaplasmosis under different types of husbandry methods varies. It was shown that NAPRI with over half of the total samples tested had the lowest percentage of reactors and hence the lowest disease prevalence. The existing rigid tick control regimen in NAPRI was suggested to be reviewed, in such a way that cattle on the farm will be allowed better and adequate exposure to the disease and hence obtain higher degree of premunity. Chemotherapy was also suggested as a means of controlling post immunization reactions, where artificial premunization is feasible.

The prevalence of anaplasmosis in slaughter cattle, as well as on a private farm, was considerably higher than in NAPRI cattle, the University farm and a government farm around Shika. The need to conduct a nationwide survey to determine the prevalence of anaplasmosis in Nigeria was also highlighted.

In the second part of the study, the duration of treatment of the clinical disease in cattle was investigated. Oxytetracycline Q50, being the most widely accepted as the drug of choice in Nigeria, was used at 10 mg/kg body weight. It was administered intravenously. A total of 27 crossbred calves (12 intacts, 9 splenectomised and 6 uninfected control) were subcutaneously inoculated with 5ml. of 1% (2x107) infected erythrocytes, from a known carrier. The calves were divided into 3 groups for treatment purposes. The first group was treated for only one day. The second and third groups were treated for 2 and 3 consecutive days respectively. Treatment was administered when the packed cell volume (PCV) had dropped to 16 per cent or below. The degree of parasitaemia, as well as the extent of the anaemic crisis were observed in all the groups.

The pattern of the development of parasitaemia, irrespective of the duration of treatment, appeared to be similar

in both intact and splenectomised calves. However, the splenoctomised calves had greater percentage of parasitaemia. The PCV fluctuation followed the same pattern in both groups. But the splenectomised calves were observed to suffer more anaemic crisis than the intact calves.

A study of the survival rates, with therapeutic intervention, showed in the intact calves, that all those treated once and those for 2 consecutive days, recovered. In the splenectomised calves, all those treated once died, while all those that were treated for 2 consecutive days survived. Late drug intervention was noticed as the cause of death in one intact and two splenectomised calves treated for 3 consecutive days.

It was concluded that 2 consecutive treatments of cattle with anaplasmosis using oxytetracycline at 10 mg/kg body weight was sufficient to cure the disease. It was also found to be more economical.