SUSCEPTIBILITY OF WEST AFRICAN DWARF (WAD) GOATS TO EXPERIMENTAL *Cowdria ruminantium* INFECTION

BY

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DECLARATION

The work presented in this thesis is original and carried out under the supervision of Prof. E. O. Akerejola, Prof. J. U. Umoh and Prof. S. U. Abdullahi in the Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria. The work of others referred to have been acknowledged. No part of this thesis has previously been submitted for a degree or diploma.

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D A T E.

CERTIFICATION

This thesis entitled: "Susceptibility of West African Dwarf (WAD) Goats to Experimental *Cowdria ruminantium* infection" by S. O. Okaiyeto meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my late father, Mr. John Olorunpomi Okaiyeto and to my beloved mother, Mrs Sarah Okaiyeto for bringing me to this world, also to my beloved wife, Mrs Bolanle Okaiyeto for her truest love which helped and encouraged me while I struggle with a pleasurable task of acquiring knowledge.

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Q U O T E

"See yourself as you want to be; know you can reach the level to which you aspire, and through the 'I will' spirit, build upon the premise yet to be described, take the simple steps necessary to bring your plans to fruition".

OKAIYETO

ABSTRACT

The susceptibility of West African Dwarf (WAD) goats to experimental C_1 ruminantium infection was evaluated over a period of 9 weeks using ten infected and four control animals. All the 10 infected goats reacted following intravenous inoculation of *C. ruminantium* infected blood. The incubation period varied from 9-11 days.

Two of the WAD goats infected reacted hyperacutely 12 days post-infection high temperature (41°C + 0.5) was maintained for 3 days, the animals then collapsed and died in convulsion without obvious clinical signs.

Three of the 8 infected WAD goats reacted acutely 15 days post-infection, the temperature remained high for 4-6 days and then became subnormal before the animals died. Fever, incordination intermittent convulsion circling and paddling movements where the main clinical features seen in these acutely affected animals. Five of the 8 infected WAD goats which reacted acutely recovered from the infection after maintaining a high temperature (41°C + 0.5) for 8-9 days suggesting the mild form of the disease. No other clinical signs were observed at 20 days post-infection.

Since some of the clinical signs of the disease manifested by these infected WAD goats were severe leading to death in 5 of the 10 infected goats, it is hereby concluded that, WAD goats are susceptible to *C.ruminantium* infection, and that both mild and fatal cases of the disease also exist in experimental WAD goats.

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CHAPTER ONE

1.1 Introduction

Heartwater (cowdriosis) is a non-contagious septicemic disease of cattle, sheep, goats and infrequently some wild ungulates (Neitz, 1944) caused by a rickettsial organism known as *Cowdria ruminantium*. It is transmitted by a 3-host tick of the genus *Amblyomma*. In Nigeria, *A.variegatum* is the proven vector of the disease (Ilemobade, 1976). The disease is characterised by high fever, nervous signs, hydrothorax and hydropericardium. The disease is confined to Africa, Madagascar, Reunion and some of the Carribean islands (Losos, 1986). The first case of suspected heartwater in sheep was at Vom in Nigeria in 1928, but the disease was only confirmed in 1931 (Leeflang, 1977). Electron microscopic studies have shown a great variation in size of single organism from approximately 0.2 to 2.5 nm. Small, medium, and large size organisms are usually distinguishable. In stained smears viewed with a light microscope under oil immersion objective, the small coccoid forms appear to be uniform, whereas the larger ones are pleomorphic but within the same colony the rickettsia appear fairly uniform in size (Uilenberg, 1983).

The organism occurs in groups also called colonies or clusters and are found in the cytoplasm of vascular endothelial cells (Cowdry, 1925, 1926). Electron microscopic studies have shown that groups of rickettsia are situated inside a membrane-line vacoule in cytoplasm of the endothelial cells and that each rickettsia is surronded by two unit membranes (Pienaar, 1970;

Stewart and Howell, 1981). Apart from vascular endothelial cells, *Cowdria* organisms occur in the circulating blood of affected animals (Pienaar, 1970) and has been demonstrated in circulating neutrophils (Logan *et al.*, 1987).

In many developing countries, there is an acute shortage of animal protein for human consumption. One of the greatest hinderances to increase of productivity and steady livestock supply in Africa is the problem of animal diseases.

The economic significance of cowdriosis as production disease in ruminants lies not only in the direct losses such as high mortality associated with clinical form of the disease, but also in the indirect insidiuous losses such reduced feed efficiency. Heartwater is one of the most important limiting factors to the production of cattle, sheep and goats, especially the exotic breeds in Africa (Uilenberg, 1983). For susceptible population of cattle, heartwater is second in importance only to East Coast fever and tse-tse-transmitted trypanosomiasis, but it has a wider distribution than either of the two (Uilenberg, 1983).

Heartwater has also been incriminated as one of the major causes of mortality in small ruminants in the Eastern Cape Coastal region and the Valley Bush Veld in particular in South Africa (Gruss, 1981). Mortality due to heartwater in enzootic areas was reported to be three times as great as that from anaplasmosis and babesiosis (Neitz, 1968).

In Nigeria, heartwater was reported to be responsible for major losses in both exotic and indigenous breeds of cattle, sheep and goats (Isoun *et al.*, 1974; Ilemobade, 1976).

Although this disease appears to be wide-spread, efforts made at recording the actual prevalence rate have been hampered by difficulty in making a diagnosis of the disease in live animals other than subinoculation of blood into susceptible ruminants, or microscopic examination of brain materials obtained by biopsy according to the method of Synge (1978). This method is risky and cannot be used routinely in the field.

The susceptibility of the West African Dwarf (WAD) goats, the major breed of goats in southern Nigeria, to heartwater is not known. The breed is predominantly found in Southern Guinea and the forest zones in Nigeria. Of recent, there was an increased effort to boost small ruminants production in Nigeria by the Federal Government. Several goat farms were established all over the country by Directorate of Food, Road and Rural Infrastructures (DFRRI) ranches of WAD goats were established in the Southern Guinea and Forest zones.

Information on the susceptibility of heartwater in WAD goats will be very useful in planning disease control scheme in these ranches.

The aims and objectives of this research therefore are to:-

Study the reaction of WAD goats to experimental *Cowdria ruminantium* infection by:

a) studying the nature of the clinical syndromes and the sequential clinical changes after infection;

b) studying the haematological parameters, serum electrolytes and plasma glucose levels in the goats.

CHAPTER TWO

2.0 COWDRIOSIS: A REVIEW OF THE LITERATURE

2.1 Historical Background

The first incidence of what probably could have been heartwater was noticed in South Africa in sheep by the Voor-trekker Pioneer, Louis Trichardt in 1838, but there seems to be no agreement in the literature about its first record (Neitz, 1968).

Heartwater has been described under a variety of local names such as mad gallsickness, blackleng, veld poisoning, heartwater and dronkgalsiekt by Afrikans; pericarsite exoudative infectieuse (French), Kaboa (Fulani), Daji (Hausa) Enguruti (Masai), and Khadar or magak by the Sudanese (Alexander, 1931; Neitz, 1968, Ilemobade, 1976).

The association of the disease with tick infestation was surmised by the pioneer leader, Louis Tricharst as early as 1838 in what is almost certainly the first reference to heartwater (Haig, 1956, Neitz, 1968).

The first authentic reference to heartwater in sheep on record was made by John Webb in 1877, when giving evidence before the Cattle Disease Commission of Grahamstown in South Africa stating that the disease was first observed in the Eastern province of the Cape of Good Hope about the time that William Bowkar found a bont tick on a cow which was imported from Zululand. Subsequently,

according to Hening (1956), the disease was reported from various parts of South Africa, but due to confusion with other conditions such as verminosis and pasture deficiencies, all the earlier information regarding the incidence of heartwater could not be fully relied upon.

It was not until 1925 and 1926 when Cowdry clearly demonstrated the aetiological significance of a rickettsial-like organism present in the tissue of both affected animals and vector ticks, which he named *Rickettsia ruminantium*. The name was later changed to *C.ruminantium* (Moshkovski, 1947). The discovery by Cowdry inspired other workers to develop an easy and practical method for the diagnosis of the disease (Jackson, 1931; Purchase, 1945). It is significant to note that the brain squash technique described by Purchase (1945) is today still the most widely used in diagnosis of heartwater.

2.2 Classification

The taxonomic classification of the causetive agent of heartwater has been a subject of debate; however, *C.ruminantium* (Cowdry, 1925) is only representative of the genus *Cowdria* (Moshkovski, 1947). The organism is placed in the order: Rickettsiales, Family; Rickettsiacase and Tribe Ehrlichiese with the genera *Ehrlichia* and *Neorickettsia*.

A toxonomic position near to that of *Ehrlichia* seems logical as both organisms are tick-borne agents, which develop within intracytoplasmic vacuoles of reticulo-endothelial cells. The close relationship between *Cowdria* and *Ehrlichia* is supported by the finding that *Cowdria* also multiplies in vitro within granulocytes, the same host cells for several Ehrlichia species (Logan *et al.*, 1987).

Comparison of the 16s RNA of *Cowdria* with that of the ehrlichial species showed that *Cowdria* forms a tight phylogenetic cluster with *Ehrlichia canis* and also more distantly related to *Anaplasma marginale* (Anderson *et al.*, 1991).

2.3 Morphological Characteristics of the Organism

Cowdria ruminantium is found in the cytoplasm of vascular endothelial cells in which it occurs in groups called Colonies or Clusters (Uilenberg, 1983). Cowdry (1925, 1926) noticed differences in particle size. Jackson (1931) first reported on the extensive variation in size and shape as seen in smears. The size of the individual particles varies from 0.2 to 2.5 um; the organisms are pleomorphic with small coccoid forms and larger rings, horseshoe shapes, but within the same colony the rickettsia appear fairly uniform in size (Uilenberg, 1983). Apart from vascular endothelial cells, *Cowdria* also occurs in the circulating blood (Pienaar, 1970; Stweart and Howell, 1981) and has been demonstrated in circulating neutrophils (Longan *et al.*, 1987).

Cowdry (1925, 1926) first described the colonies of *C.ruminantium* in *Amblyomma hebraeum* by means of conventional histological sections and Giemsa stained smears. His findings still stand today. *C.ruminantium* were demonstrated in the epithelial cells of the intestine and also in the lumen of the gut of the tick. These findings were confirmed by fluorescent antibody staining, light and electron microscopy by Bezuiderhout (1984). In *A.variegatum* the organisms were found in the mid gut epithelial cells (Kocan *et al.*, 1987). The ultra-structure of *C.ruminantium* has been studied using electron microscope (Pienner, 1970; Duplessis, 1975; Neitz *et al.*, 1986; Prozesky *et al.*, 1986; Kocal *et al.*, 1987). Duplessis (1975) reported that the organisms were not separated from the cytoplasm. However, Prozesky *et al.* (1986) and Kocan *et al.* (1987) revealed that the organisms were membrane-bound and a few were surrounded by a capsule.

The multiplication of *C.ruminantium* by budding does not take place, however, it is believed that the multiplication is essentially by binary finsion (Prozesky *et al.*, 1986; Kocan *et al.*, 1987).

2.4 Staining Characteristics

Cowdry (1925) found that the micro organisms were gram-negative and stained deep blue with Giemsa. In some cases relatively clear halos are visible. When these organisms are

stained with Unna-Pappenheim's methyl-green pyronin mixture, they appear light red sometimes taking on a slightly greenish tint. They are easily stained with Fuchin and Lofflers methylene blue (Alexander, 1931). These organisms stained well with iron haematoxyline but became bleached on differentiation. They are also easily stained by other basic aniline dyes. Steck (1928) found that Giemsa stain gave good Romanowsky effect in histological sections. Purchase (1945) showed that brain squash amears could be stained with Giemsa. Burdin (1962) demonstrated the selective staining characteristics of the organism using methyl green and Pyronin Y while Duplessis (1970) obtained good results with the use of haematoxylin and eosin in tissue sections. In stained squash smears, Ilemobade (1976) categorised *C. ruminantium* into four different types of colonies. Viz. type 1, type 2, type 3 and type 4. This classification was based on the staining characteristic and size the colonies.

2.5 Host Range and Susceptibility

Cowdria ruminantium infects both invertebrate and vertebrate animals. The invertebrate host of *C.ruminantium* are exclusively ticks within the genus *Amblyomma*. Alexander (1931) and Haig (1955) believed that heartwater is transmitted mainly by *A.hebraeum* but later other *Amblyomma* species were incriminated to be invertebrate host of the organism.

Of the vertebrate hosts, heartwater was originally thought to be restricted to goats and sheep while cattle were not thought to be susceptible (Alexander, 1931). It was, however, proven later on that cattle can be infected by the organism obtained from either sheep or goats (Alexander, 1931). Apart from domestic ruminants, heartwater has been reported to occur in some species of wild bovidae living in endemic areas (Neitz, 1944; Young and Basson, 1973). Karrav (1960) described a suspected case of heartwater in a camel in an endemic area of Sudan.

Of the small laboratory animals, ferrets have proved susceptible (Haig, 1955). While the Kumm, Kwanyanga and Nonile strains are pathogenic to mice (Jongejan *et al.*, 1988).

The susceptibility of ruminants to heartwater varies within groups and species. Persian and African sheep were found to be comparatively resistant to heartwater (Alexander, 1931 and Haig, 1955).

Matheron (1987) found the possibility of natural selection among Creole Guadeloupean goats to *C.ruminantium* infection and that in endemic heartwater areas, each flock will have developed resistance at a definite rate according to age and the extent of past and present exposure to the disease.

Alexander (1931) stated that mortality amongst Angora goats is often higher than that amongst Merino sheep. Mature domestic animals are highly susceptible while calves, lambs and kids up to the age of three weeks possess a high degree of resistance to heartwater, which is in no way associated with maternal immunity (Haig, 1955; Neitz, 1968). In Nigeria, brown goats originating from Zaria and areas north of it and Friesian-Zebu cross breeds obtained from Shika Agricultural Research Station, were uniformly susceptible to heartwater (Ilemobade, 1976).

Karrar (1960) reported the organism in one camel but this evidence is insufficient to generalise the susceptibility of camel to heartwater. Heartwater agent is not infective for dogs (Alexander, 1931 and Uilenberg, 1983). Bezuidenhout (1987) reported that crowned guinea fowl and the leopard tortoise can be subclinically infected, and act as a source of infection for *Amblyomma* ticks.

2.6 Transmission and Infection

2.6.1 Natural Transmission

Heartwater is transmitted in nature by a 3-host tick belonging to the genus *Amblyomma*. Twelve species of *Amblyomma* transmit heartwater, ten of them are African species while the remaining two are American species (Table 2.1). The African species include *A.hebraeum*, *A.variegatum*, *A.pomposum*, *A.gamma*, *A.lepidum*

(Lounsbury, 1900; Daubney, 1930; Neitz, 1947; Lewis, 1949; Karrar, 1966; Mackenzie and Norval, 1980). Other African species include *A.sparsum* (Norval and Mackenzie, 1981) *A.cohaerens* (Uilenberg, 1983) and *A.marmoreum* (Bezuidenhout, 1987). The American species are *A.maculatum* and *A.cajennense* (Uilenberg, 1982; Uilenberg, 1983). In tropical sub-saharan Africa, *A.variegatum* is the most important tick host incriminated in the transmission of the organism (Uilenberg, 1983) while in the states bordering the Gulf of Mexico and Southern States along the Atlantic Coast, *A.maculatum* has been established to be the invertebrate host. *A.lepidum* and *A.gemma* are found in parts of eastern and north-eastern sub-saharan Africa *A.pomposum* occurs mainly in Angola while *A.cohaerens* occurs in Ethiopia (Walker and Olwage, 1987).

In 1900 a definite correlation was made between the presence of the bont tick, *A.hebraeum*, and the incidence of heartwater. It was also established that ticks infected as larvae transmit the disease as nymphs, and for some vectors it has been shown that the infection may be carried from the larval stage through the nymphal stage, (even when the nymph feeds on a non-susceptible animals) (Uilenberg, 1983).

Transmission of heartwater is transstadial and attempts of transovarial transmission have invariably failed, apart from one report by Bezuidenhout *et al.* (1986) which proved transovarial transmission of *C.ruminantium* by *A.hebraeum*. Alexander (1931)

reported complete transstadial transmission in *A.hebraeum*. *Amblyomma variegatum* (Ilemobade and Leeflang, 1977) and *A.maculatum* (Uilenberg, 1982) have also been proven to transmit the parasite transstadially. Transmission was demonstrated only from larval to nymphal stage and from nymphal to adult stage in *A.lepidum* (Karrar, 1966) while in the case of *A.sparsum*, transmission is from nymph to adult and the transmission from larva through nymph to adult failed (Norval and Mackenzie, 1981). Male ticks, although capable of transmitting heartwater (Alexander, 1931), appear to be poor vectors (Ilemobade and Leeflang, 1977).

Demonstration of *C.ruminantium* in other tick species like *Rhipicephalus evertsi, Boophilus decoloratus, Ornithodoros savignyi* was reported by Lounsbury (1902) to be unsuccessful. Circumstantial evidence also indicates that heartwater is not transmitted by ticks other than those belonging to the genus *Amblyomma* and not all proven African vectors are of equal significance. *A.variegatum* is the most important one, being an efficient vector and perfectly adapted to domestic livestock with a wide distribution (Uilenberg, 1983).

Under natural conditions, infection takes place by excretion of infective materials from the gut or regurgitation by the tick (Alexander, 1931). Transmission by gut regurgitation has been suggested because the organism were not detected in salivary

glands. Kocan *et al.* (1987) first described *C.ruminantium* in salivary gland of a known infectived *A.hebraeum* in which both electron-dense and recticulated forms of the organism were seen. The development of *C.ruminantium* in the salivary glands is believed to be followed by the transmission of the organism through this organ to the vertebrate host.

2.6.2 Artificial Transmission

Heartwater can be transmitted by injection of infected blood or homogenized tissue from various organs. Intravenous inoculation of as little as 0.1 ml of blood from infected animal may produce fatal heartwater in susceptible ruminants (Uilenberg, 1983). According to Alexander (1931), intravenous injection of 5 ml of infective blood to small ruminants and 10 ml to cattle can cause heartwater. Heitz (1968) reported that milk and spleen suspension from reacting animals could cause heartwater if injected into susceptible animals.

In transmission of heartwater, apart from the quantity of blood, the route of inoculation is very important. Blood injected subcutaneously was unable to cause infection in more than 25% of susceptible animals (Alexander, 1931; Uilenberg, 1971, 1976; Ilemobade, and Blotkamp, 1978). In mice, many attempts at establishing *C.ruminantium* infection have been carried out. Neitz (1968) administered the infective inoculum by the intraperitioneal route. Jongejan *et al.* (1988) initiated infection by intravenous

injection of tick-derived stabilate, whereas blood stabilate via the same route failed to cause infection.

Duplessis (1985) stated that the Kwanyanga strain is highly pathogenic to mice but only when inoculated intravenously and not intraperitoneally whereas the Kumm strain remains the most pathogenic strain passaged intraperitoneally in mice to date.

Organ emulsion will only reliably establish infection if they are injected intravenously; the only exception known is brain cortex homogenate of infected animals which is infective by the subctutaneous route in most cases (Ilemobade, 1976; Ilemobade and Blotkamp, 1978).

White cell fraction has been associated with infection (Ilemobade, 1976). That the organisms were firmly adherent to the red cells and leucocyte were concluded by Alexander (1931). Examination of choroid plexus tissue cultures stained with Giemma showed a considerable number of granules scattered throughout the cytoplasm (Stewart and Howell, 1981). Jackson and Neitz (1932) believed that individual rickettsias in the blood enter endothelial cells where they grow from a single granule to a large group; causing the cell to rupture and disseminate the organisms again into the blood thus repeating the cycle. While Duplessis (1970; 1975) proposed that following infection, initial development of the

Table 2.1: Natural Transmission of *Cowdria ruminantium* by *Amblyomma species*.

Amblyomma spp	Proven Mode of Transmission
African species:	
A.hebraeum	I, II, III
A.variegatum	I, II, III
A.pomposum	I, II
A.gemma	?
A.lepidum	I, II
A.tholloni	I, II, III
A.sparsum	I
A.astrion	I, II
A.cohaerens	I, II
A.marmopreum	I, II
American species:	
A. maculatum	I, II, III
A. cajennense	I

I = From larval to nymphal stage
II = From nymphal to adul stage
III = From larval through nymphal to adult stage
? = Stage(s) not reported

Source: Uilenberg G. (1983) Heartwater (*Cowdria ruminantum*): Current Status. *Advances in Veterinary Science and Comparative Medicine*. 27: 428-480.

organism appears to be mainly, but not exclusively, confined to reticulo-endothelial cells which eventually rupture and the organisms are relased into general circulation to invade other endothelial cells. Depending on the host, the organisms appear to have predilection for endothelium in certain organs. In ruminants the highest concentration of these organisms are found in the brain followed by the kidneys (Prozesky and Duplessis, 1985).

2.7 Distribution

Heartwater is known to occur in areas where condition favour the development of the vectors (Haig, 1955). The disease has been reported to occur throughout Africa south of the Sahara, from the Cape Province in South Africa to as far north as Kassals in the Sudan and from Senegal in the West to Somalia in East (Uilenberg, 1983). Neitz (1968) estimated that the heartwater endemic area is about 5 million square miles (13 million km^2). Its distribution includes the Island of Madagascar, and it has recently been diagnosed on other islands near Africa, La Reunion and Mauritius to the East (Parreau *et al*. 1980) and Sao Tome in the Atlantic Ocean (Uilenberg *et al*. 1982). Perean *et al*. (1980) also confirmed its existence on the island of Guadeloupe in the Caribbean area. Regions with a very low rainfall and cold climate are free from the disease (Neitz, 1968). The distribution of the vector, *A.variegatum*, not only covers Africa but may include the Southern

parts of the Arabian Peninsula (Uilenberg, 1983). In West Africa, the disease has been recognised in Nigeria, Cotta De'voure (Ivory Coast), Senegal and Cameroon, and is believed to occur in other countries where suitable vectors are found (Ilemobade, 1976).

2.8 Pathogenesis

The pathogenesis of heartwater is poorly understood. In all ruminants, the lesions are fairly consistent but vary with the virulence of different strains. The extent of the lesion shows no correlation with the number of rickettsias, and cellular infiltration is not a local reaction to the presence of the organisms (Uilenberg, 1983). There is rarely any gross lesions in the peracute form. The most characteristic changes in less severe forms of the disease are ascities, hydrothorax and hydropericardius (Losos, 1986). Lesions in the peracute form are generally limited to sub-epicardial and subendocardial haemorrhages (Ilemobade, 1976).

Cowdry (1926) noted that the infected endothelial cell showed no lesions except that the nucleus might be flattened or pushed to one side by the pressure of the growing cluster of rickettsis. Abdel Rahim and Shommein (1978) and Ilemobade and Blotkamp (1978) suggested that the pathogenesis of heartwater may be different in animals with different postmortem findings. Uilenberg (1983) also reported that pronounced hydropericardium could contribute to

cardiac insufficiency and edema of the lungs is sometimes pronounced. The pericardial fluid is usually straw-coloured but may be blood-tinged (Ilemobade, 1976).

2.9 Clinical Manifestation

2.9.1 General

Clinical syndromes of heartwater are based upon the disease being peracute, acute, subacute or chronic and mild. The severity of the disease varies with the virulence of the *C.ruminantium* strain, the individual or breed susceptibility and the age of the ruminant (Neitz, 1968). Ilemobade (1976) reported 100% susceptibility of Savannah brown goats around Zaria and Friesian-Zebu crossbred calves from Shika Agricultural Research Station. The incubation period after natural transmission by ticks varies from one to five weeks with an average of about two weeks (Uilenberg, 1983). After artificial infection by intravenous inoculation of infective material, sheep and goats show a febrile reaction between 5 and 35 days with mean of 9 to 10 days. It is slightly longer in cattle, about 12 days, but it may be as long as 35 days (Alexander, 1931; Uilenberg, 1983).

The average incubation period not only varies according to the strain and susceptibility of the animals but also varies with the size of the infective inoculum and on the pathogenicity of the causative organism (Uilenberg, 1983).

2.9.2 Peracute Form

This form is rather common in exotic breeds of cattle and goats, but less so in the sheep. It is characterised by high fever of 41°C or higher, the animal collapses and dies in convulsion without premonitory signs. Cases of this nature present difficulty in diagnosis in either sheep, goats or cattle, whether infection is by artificial or natural means (Alexander, 1931 and Ilemobade, 1976).

2.9.3 Acute Form

The acute form has been described to be the most common clinical form in all susceptible animals with sudden rise in temperature, which usually rises above 41°C within 1 or 2 days. It may remain high, dropping to subnormal shortly before death. Initially, feeding and rumination are normal, but later some sluggishness may be observed and is followed by lack of appetite, cessation of rumination and dyspnea. They acquire a tucked up appearance and an anxious staring expression of the eyes. In goats and cattle, the hair coat in harsh and stary. Rapid pulse is notice

while normal heart beat is maintained until just before death, when it becomes thin and thready (Alexander, 1931; Neitz, 1968 and Ilemobade, 1976). The conjunctiva is reddened but extensive submucous haemorrhages are not usually seen. Hydropericardium is noticed upon auscultation of the thoracic area and hydrothorax is indicated by the presence of moist rales.

Sheep and goats show progressive unsteady gait. They frequently lean against the wall for support or stand with their legs apart in an apparent attempt to steady themselves. In severe cases, affected animals go down on their side with legs stretched out showing almost continuous galloping movements of the legs, masticating movement of the jaws, frothy mouth with continual licking of the lips have been reported (Alexander, 1931; Neitz, 1968). Nystagmus and Opisthotonus are observed and become more intense with the progress of the disease.

One classic pattern of heartwater is the sudden onset of intermittent convulsions punctuated by periods of absolute normalcy. Difficulty in respiration and hyperesthesia become more marked (Ilemobade, 1976; Uilenberg, 1983). Temporary recovery may occur followed by renewed fits. Death usually occurs during these attacks, or the animal may linger for a few days in a state of virtual non-reactivity (Uilenberg, 1983). In cattle, the clinical signs are identical to those in goats and sheep except that the

nervous reactions are usually more pronounced. A profuse and often hemorrhagic diarrhoea is more frequent in cattle than in sheep and goats.

2.9.4 Subacute or Chronic Form

The signs are less severe and may be characterised by diarrhoea in cattle and to a lesser extent in sheep and goats. Fever is irregularly intermittent and persists for periods of upto 10 days (Neitz, 1968). Affected animals may collapse and die suddenly or the temperature may return to normal with signs becoming less pronounced. However, complete recovery is slow. pregnant animals in the subacute form are liable to abort (Alexander, 1931; Neitz, 1968).

2.9.5 Mild Form

This form is common in very young animals, animals of genetically resistant population and partially immune individuals. The only symptom may be fever and an increase in the respiratory rate. This form is significant because it is not detected easily and affected animals serve as a reservoir for the infection of ticks.

2.10 Histopathological Findings

Heartwater organisms were detected by Cowdry (1926) in the capillary endothelial cells of the renal glomeruli and brain, and were rare or absent in the liver and lungs of cattle, sheep and goats. They are also found in the spleen, lymph glands, corpus luteum, suprarenals, midbrain, medulla oblongata, salivary gland, pancreas and heart muscle (Alexander, 1931). In the brain, common lesions include focal necrosis of blood vessels, haemorrhages, glial changes, oedema, swollen axis cylinders, microcavitation, focal necrosis of the granular layer of the carebellum and subarachnoidal cellular exudation (Ilemobade, 1976; Uilenberg, 1983). Leukostasis of infected brain occurs in most animals and also some parkinje cells and neurons showing cellular degeneration were found in the cerebellum and medulla oblongata (Ilemobade, 1976).

2.11 Haematological Observation

Haematological changes observed in ruminants infected with heartwater have been fairly divergent (Uilenberg, 1983). Decrease in haemoglobin concentration was observed and occurs shortly after the onset of the febril reaction (Van Amstel *et al.*, 1987). There is also the tendency for the haematocrit value to fall during the course of the disease (Clark, 1962; Ilemobade and Blotkamp, 1978). Abdel Rahim and Shommein (1977) found the drop in haematocrit value

to be proportional to that of haemoglobin concentration. Ilemobade (1978) observed marked leukopenia due to lymphopenia and neutropenia, and a fall in total serum protein. Van Amstel *et al.* (1987) recorded a significant decrease in eosinophil level which correspond with the febrile reaction. Abdel Rahim and Shommein (1978) reported microcytic hyperchronic anemia in heartwater infected animals.

A rise in blood glucose was reported by Clark (1962), and Ilemobade (1978). This rise seems to appear terminally and can result in glucosuria (Clark, 1962).

Clark (1962) found an increase in the blood urea nitrogen. This increase was caused by pre-renal factors such as high nutritional levels of protein, dehydration, decrease glomerular filtration resulting from blood pressure changes, or high levels of cortisol resulting in an increase in protein catabolism (Ganong, 1975).

Owen *et al.* (1973) reported a slight rise in sodium and chloride during the febrile phase of the infection while Van Amstel *et al.* (1987) did not find the change. However, they found sodium levels in the effused fluid to be in excess of those in the serum. Since both sodium and chloride are distributed mainly extracellularly, increases of these ions may occur with primary

water loss (Ganong, 1975) as can occur during febrile state with inadequate water intake. Owen *et al.* (1973) reported a terminal drop in blood potassium (K^+) levels while Van Amstel *et al.* (1987) found K^+ level in the effused fluid to be far in excess of those in the serum.

Ilemobade *et al.* (1978) found terminal increases in blood pyruvate and lactate levels and a simulteaneous drop in venous blood pH. They specualted that these changes indicate the presence of a metabolic acidosis. This may be supported by a terminal drop in bicarbonate (HCo_3) as demonstrated by Clark (1962). The existence of a metabolic acidosis in terminal cases of heartwater is likely to be as a result of drop in blood volume prior to death which will result in anaerobic muscle metabolism giving rise to these changes (Ganong, 1975).

Ilemobade *et al.* (1978) reported a drop in the globulin level during the course of the disease. Van Amstel *et al.* (1987) found a reduction in all protein fractions in their study of experimentally-induced heartwater in calves. This reduction in protein was reflected mostly in the albumin fraction which seems probable as albumin is a smaller protein than globulin and would pass through the endothelium with greater ease (Ganong, 1975).

2.12 Diagnosis

The diagnosis of heartwater is often difficult in the live animal because clinical signs of the disease are not pathognomonic and the course of the disease is fast (Camus and Barre, 1987). There is no way of demonstrating the organisms during life other than sub-inoculation of blood into subsceptible ruminants, or microscopic examination of a brain material obtained by biopsy according to the method of Synge (1978). Blood smears are of no value. The claim of Jackson and Neitz (1932) that they were able to distinguish single large rickettsias in blood smears have not been confirmed. Xenodiagnosis, the uptake by and demonstration of the organism in clean vector fed upon affected hosts, may be used to confirm heartwater infection. Camus and Barre (1987) reported that uninfected larva *A.variegatum* were able to pick up *C.ruminantium* from febrile goats and transmit it, as nymphs, to susceptible animals.

Diagnosis is based on clinical signs, aided by herd or flock history, including the presence of known *Amblyomma* vectors. The disease may sometimes be tentatively diagnosed when typical nervous signs are present, but the clinical signs are variable and very often not typical. Because the course of the disease is rapid, treatment must be quickly instituted and on clinical suspicion only (Uilenberg, 1983).

Antemortem laboratory diagnosis is difficult because there are no obvious stages circulating in the blood of infected animals and the principal area of proliferation and pathogenesis of the organism is in the capillary blood vessels of the brain (Parnell, 1984). Cattle and sheep that have recovered from heartwater after oxytetracycline therapy have been shown to remain carriers of the disease for up to 246 and 223 days respectively after recovery (Andrew and Norval, 1980).

The differential diagnosis includes rabies, cerebral babesiosis in cattle, cerebral theileriosis in cattle, tetanus, strychine poisoning, and hypomagnesemic tetany in the event that nervous disorders are present (Uilenberg, 1983).

Enzyme-linked immunoadsorbent assays or (ELISA) test have been developed for diagnosis of heartwater infected materials. This ELISA test was used to trace the development of antibodies, as well as the appearance of antigen in the blood of infected animals. Use of percoll density gradient or cellular affinity chromatography to separate *C.ruminantium* antigen from infected blood and *A.hebraeum* ticks has been performed by Neitz *et al.* (1987), Neitz and Vermeulen (1987) and Viljoen *et al.* (1987).

Heartwater can usually be diagnosed after death by microscopic demonstration of the rickettsia in suitable postmortem samples. The postmortem lesions, combined with observation made before death,

may be of help in arriving at the definitive diagnosis (Uilenberg, 1983). Romanovesky type stains, particularly Giemsa stain, are used for staining *C.ruminantium* after fixation in methanol or absolute ethanol (Cowdry, 1925; 1926). In brain smear, the groups of *Cowdria* are reddish purpule (small forms) to blue (Large forms) and can be distinguished from the endothelial nuclei by their morphology.

2.13 Treatment

In heartwater, treatment is possible but frequently too late under field conditions (Uilenberg *et al.*, 1983). The sulfonamides were the first drugs shown to have a curative effect. Neitz (1939, 1940) found that Uleron [(4-(4-aminobenzolsulphanamids) bensolsufonedimethylkamide)] was effective if administered early in the disease. Other sulfonamides such as sulfapyridine and sulfadimidine were also used later.

The activity of chlor-tetracyclines against the disease was discovered by Simpson and Wiley (1951) and Weiss *et al.* (1952) followed by oxytetracycline (Haig *et al.*, 1954) and rolitetracycline (Poole, 1961). Experimentally doxycycline at 2 mg/kg given intravenously has been used to treat sheep (Immelman and Dreyer, 1982).

Tetracyclines have been most widely used in the chemotherapy of heartwater. Karrar and El Hag Ali (1965) reported that oxytetracycline in drinking water at dose rate 300 mg/11.3 kg for goat, 200 mg/11.3 kg for sheep and 200-250 mg/45.3 kg for cattle was effective if treatment was started early in the thermal reaction. During the febrile period a single treatment is not reliable, and a second injection is often needed to bring about a return of the temperature to normalcy (Uilenberg, 1983).

The use of long-acting formulation of oxytetracycline (Terramycine L.A.), administered intramuscularly at 20 mg/kg, does not always offer advantages over a normal formulation given by the same route at 10 mg/kg because two or more treatments may also be necessary with Terramycin L.A. Karrar and El Hag Ali (1965) reported good results with oral oxytetracycline treatment for several days if started early in the course of the disease.

However, failure of specific treatment in cases of heartwer has been reported. Clark (1962) suggested that failure of specific therapy in several cases of heartwater was probably due to irreversible brain damage since brain squash smear from such animals were found negative. This probably explains why treated goats which died after showing clinical signs of heartwater were negative on brain smear examination (Ilemobade, 1976). Ilemobade (1976) treated 4 goats with oxytetracycline and a balanced electrocyte (Eltrad) intravenously at the onset of nervous

reactions. Three of the goats died on day 4, 12 and 49 post treatment and were positive for *C.ruminantium* on brain squash examination. This suggests that electrolyte preparation lengthened the course of the disease without preventing death.

2.14 Immunity and Immunization

2.14.1 Immunity

Although it has been reported that under field conditions, domestic ruminants are capable of developing immunity against heartwater following infection (Neitz, 1939), the nature of the immunity is still not clear.

After recovery from the disease, sheep and goats are immune against homologous challenge and only partially against heterologus strains (Henning, 1956). Post-recovery immunity consisted of a short period of premunition followed by a period of gradually decreasing sterile immunity (Losos, 1986). Ilemobade (1976) found that 10 goats given homologous challenge 2-8 weeks after oxytetracycline-induced or spontaneous recovery were immune.

Immunity in cattle is considered by some authors to be short-lived unless it is reinforced by repeated reinfection (Uilenberg, 1983).

It is still not known whether the immunity is linked to the persistence of *Cowdria* in the host or whether sterile immunity occurs (Uilenberg, 1983). Ilemobade (1978) found that blood could be infective up to 50 days following recovery or challenge. *C.ruminantium* has also persisted in the brain of goats dying of heartwater up to 9 weeks after oxytetracycline treatment but the blood was not infective (Ilemobade, 1976).

2.14.2 Immunization

Neitz and Alexander (1941, 1945) first established the basis for a practical and effective method of immunization of animals against heartwater using virulent *C.ruminantium* infected sheep blood administered intravenously to cattle under 1 month of age.

Early immunization efforts in cattle using infection and treatment methods have been reported from Swaziland (Pick and Schius, 1952; Barnard, 1953). There is no difference in clinical reactions caused by immunization by the infection and treatment methods using oxytetracycline in various breeds of cattle (Sutten, 1960). The treatment of cattle has to be started early in the reaction in highly susceptible imported breeds (Uilenberg, 1971b) but in indigenous breeds, delay of treatment has been beneficial because the later the therapy, the better the immunity (Erasmus, 1976).

The infection and treatment method is expensive, laborious, time consuming and to some extent risky because of the irregular reactions especially in cattle and goats, as well as failure of treatment.

2.15 Control

Heartwater control is a multifaceted approach involving the use of chemotherapy, vaccination and partial tick control.

In the treatment of heartwater, sulphonamides and especially tetracyclines have long been the only drugs with significant effect on the disease (Shommein and Abdel Rashim, 1977).

Vaccination of young animal is probably the best and most practical way to stimulate immunity and create enzootic stability. Vaccination is also advisable to protect animals if they are moved from a minimal to a stable disease condition (Bezuidenhout, 1985). The organism has been successfully attenuated in culture (Jogenjan *et al.*, 1993). However, attempts to produce an attenuated vaccine against heartwater has proved largely unsuccessful (Uilenberg, 1983). Natural resistance of livestock to heartwater may prove, in the long run, to be the most effective means of dealing with the heartwater problem. Such natural genetic resistance has been documented in the Caribbean in creole cattle and to a lesser extent in creole goats. In heartwater enzootic areas of Zimbabwe, the

natural resistance of young calves is used to advantage, ranchers are advised not to use acaricide on the calves so that they may develop specific immunity upon exposure to ticks (Andrew, 1990).

Use of parasites and pathogens to control *Amblyomma* species has not been successful, but natural predators are believed to play a significant role. The yellow-billed oxpecker birds (*Buphagus* species) consume considerable numbers of ticks (the equivalent of 13,000 larvae or 100 engorged adults a day) (Stutterheim *et al.*, 1988). Spickett (1987) notes that, in general, biological control management regimes have not proven to be a sound replacement for acaricides.

Human, socio-economic or management factors also mitigate against effective control or eradication of heartwater, such as reliance on intensive use of acaricides which results in a loss of herd immunity, insistence on the introduction of exotic breeds in heartwater areas and willingness to absorb losses rather than carry out control measures (Yunker, 1990).

CHAPTER THREE

3.0 SUSCEPTIBILITY OF WEST AFRICAN DWARF (WAD) GOATS TO EXPERIMENTAL *Cowdria ruminantium* INFECTION

3.1 Materials and Methods

3.1.1 Experimental Animals

Fourteen WAD goats (9-18 month-old; 6 males and 8 females) were used for this experiment, the goats were purchased from Kafanchan located 9° 30¹ North, 8° 20¹ East of Kaduna State, Nigeria. On arrival at Zaria, the animals were dewormed using "Worm Care*" a brand of Levamisole at a therapeutic dose of 7.5 mg/kg body weight, orally. The animals were sprayed with 20% w/v Supona**** at 10 ml in 5 litres of water against ectoparasites, and were also vaccinated against Peste des petits ruminants (PPR) using Tissue Culture Rinderpest Vaccine (TCRV) at 1.0 ml per animal, subcutaneously. Blood samples were taken and screened for haemoparasite.

The animals were kept in a tick-proof pen and pre-conditioned for a period of 6 weeks during which they were fed with hay, wheat offal, and cotton seed. Salt-lick and water were made available to the goats *ad libitum*. Rectal temperatures were taken daily.

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* (Sam pharmaceutical products Nig. Ltd.)

** 2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate (Pfizer Products Ltd., Ikeja, Nigeria).

3.1.2 Pre-infection Blood and Serum Samples

During the period of pre-conditioning, fifteen mililitres of blood were drawn from each animal through jugular venipuncture, using a disposable syringe with 19 x 1.5 inch gauge needle. Three millitres of the blood were transferred into vacutainer tubes containing ethylene diamine tetra-acetate (EDTA) as anticoagulant while the remaining twelve millilitres of blood were transferred into Universal bottles without anticoagulant and allowed to stand for 6 hours for serum collection. The serum was separated using Pasteur pipette.

3.1.3 Animal Inoculation

After six weeks period of pre-conditioning, the animals were divided into two groups made up of Group I consisting of ten animals (infected group) and Group II consisting of four uninfected animals (control group). The *Cowdria* stabilate (Cr 151) was a stock of *Cowdria* originally isolated from crushed adult *A.variegatum* ticks collected from cattle in Basawa, Zaria, Nigeria. It has been cryopreserved in liquid nitrogen at the Department of Veterinary Parasitology and Entomology of Ahmadu Bello University, Zaria, Nigeria.

Two millilitres of the stabilate were injected into a donor goat. At reacting temperature of 41°C, 50 ml of the blood was taken by jugular venipuncture and the animals in Group I were inoculated with 2.0 ml each.

The donor goat later died and Giemsa stained brain squash smear was clearly positive for *C.ruminantium*.

Rectal temperature of the experimental animals was taken daily and those that showed febrile reaction to the infection were allowed to go through full course of the infection without treatment. Blood samples were taken twelve days post-infection for haematological and serum mineral analyses.

3.1.4 Haematological Evaluation

Haematological evaluation were done by determining the following parameters:-

(a) Packed cell volume (PCV) by the microhaematocrit method (Dacie *et al.*, 1975); (b) Total serum protein (TSP) using refractometer; and (c) Haemoglobin concentration by the cyanmethaemoglobin method (Dacie *et al.*, 1975). (d) Total leukocyte counts were estimated by improved Nebuar haemocytometer method (Dacie *et al.*, 1975). (e) The differential leukocyte counts were also done by examining one hundred white cells in blood films stained with Giemsa.

3.1.5 Serum Biochemical Analysis

The biochemical analysis of the serum was also done for the following:-

(a) Serum glucose level determination was as described by Fales (1963); (b) Total protein using Biuret method; (c) Albumin concentration by Doumasi and Biggs method (1972); (d) Urea concentration by the method of Wybengo *et al.* (1971); and (e) Flame photometry was used to determine the electrolyte levels (sodium, chlorine, potassium and bicarbonate).

3.1.6 Postmortem Examination

Postmortem examination was performed on each animal that died. The pericardial sac, thorax, kidney, liver, gall bladder and intestines were examined for postmortem lesions. Brains were removed from dead animals and squash smears made as described by Purchase (1945). The smears were stained with Giemsa and examined using light microscopy under oil immersion objective for *Cowdria* granules.

3.1.7 Statistical Analysis of Haematological and Serum Biochemical Values

The Paired Samples test (Hernett and Murphy, 1976) was used to analyse data for PCV, total serum protein, total leukocyte counts, differential leukocyte counts, serum glucose level, albumin, globulin, urea, and electrolyte levels before and after the onset of fever.

CHAPTER FOUR

4.1 RESULTS

All the infected goats reacted by showing pyrexia as from day 9 post-infection. The intensity and duration of the reaction varied greatly. Two goats reacted hyperacutely and died while 8 reacted acutely out of which three died and five recovered.

The clinical reactions of WAD goats to experimental *C.ruminantium* infection are presented in Table 4.1.

4.2. Observation on Rectal Temperature Patterns

The rectal temperature patterns of the reacting animals are shown in Figures 1.1, 1.2, and 1.3, while the mean rectal temperatures are presented in Tables 4.3, 4.4, 4.5, and 4.6.

All the infected animals showed a febrile reaction on day 10 post-infection (PI) except for Goat No. 290 that reacted on day 11 (Table 4.1). The control animals maintained a normal rectal temperature range.

Table 4.1: Incubation period, extent and duration of pyrexia in West **African Dwarf goats experimentally infected with *C.ruminantium***

Goat Number	Forms of the Disease	Incubation Period (days)	Maximum Temp. (°C)	Duration of Fever (days)	Remarks
277	A	9	41.8	3	Died of hyperacute cowdriosis
284	A	9	40.9	3	"
274	B	9	42.0	5	Died of acute cowdriosis
280	B	9	41.3	5	"
296	B	9	41.3	5	"
276	C	9	41.7	8	Recovered
278	C	9	41.3	9	"
279	C	9	41.5	8	"
283	C	9	41.6	9	"
290	C	11	41.6	8	"

KEY: A = Hyperacute
B = Acute
C = Mild

Hyperacute reactions were noticed in Goats No. 277 and 284 both of which died 3 days after the onset of pyrexia. Those that reacted acutely (Goats No. 274, 280, and 296) also died after maintaining pyrexia for 5 days but the temperature dropped to a subnormal level before death.

The recovered animals (Goats No. 276, 279, 290, 278, and 283) maintained high temperature values for 8-9 days.

Thus, 10 reacting goats were divided into hyperacute, acute, and mild based on the types and intensity of the clinical signs observed.

4.3 Clinical Signs

Hyperacute:

Goat No. 277 showed a high rectal temperature with a peak of 41.8°C on day 14 PI, sudden and violent convulsions which lasted for about 20 minutes. The animal suddenly collapsed and died.

Goat No. 284 had a peak rectal temperature of 40.9°C. It was restless, exhibited some jerky convulsions on standing, charged at objects such as the wall of the pen, and was shivering. The animal went on lateral recumbency with paddling movements of the hind-leg, with occasional bleating and backward deviation of the neck. The animal remained in that position until it finally died after prolonged tetanic convulsive seizures (Plate 1).

Acute:

Three goats (Table 4.1) exhibited the acute form of the disease and died. All the animals had an average incubation period of 9.5 ± 0.6 days with the maximum temperature reaching a peak average of 41.5 ± 0.2°C and was maintained for 7.1 ± 1.81 days. One animal aborted twin fetuses at 2nd trimester (Plate 1.6). Clinical signs exhibited are shown in Table 4.2.

Mild:

Five of the animals (No. 276, 278, 279, 283 and 290) later recovered 18 days post-infection with fever as the only presenting sign.

4.4 Post-mortem Findings

No visible gross lesions were seen in animals that died hyperacutely. However, microscopic examination of brain squash smears revealed colonies oc *C.ruminantium* (Plate 1.7).

The postmortem examination of animals that died acutely revealed congested liver and kidney. The gall bladder was distended and the spleen enlarged. Ten, 15, and 35 mls of straw-coloured fluid, which clotted on standing, was found in the pericardial sac, respectively. Microscopic examination of the Giemsa stained brain squash smear revealed colonies of *C.ruminantium* (Plate 1.8).

4.5 Haematological Parameters

Values of pre-infection haematological parameters are shown in Table 4.7 while post-infection values are shown in Table 4.8. There was marked fall in the packed cell volume (PCV),

Table 4.2: Clinical signs, their onset and duration in WAD goats experimentally infected with *C.ruminantium*

Clinical Signs	Onset of Signs	Duration (Days)
High Fever Excitaion Raised Hair Coat	Day 10 PI	4
Dullness Reluctance to move Tucked in appearance Rapid eye movement Anorexia Cessation rumination Head shaking Incoordinated gait	Day 14 PI	2
Circling	Day 16 PI	1
Collapsing on lateral recumbency Intermittent convulsions Paddling movements Subnormal temperature Death	Day 17 PI	2

haemoglobin (Hb) and Red blood cell (RBC) in infected goats. The decrease in the PCV, Hb, and RBC values in the infected goats when compared with the pre-infected values were statistically significant ($P<0.05$).

There were however no significant differences between the post-infection and pre-infection values of total and differential white cell counts.

4.6 Serum Biochemical Changes

Results of analyses of biochemical parameters are presented in Tables 4.9 and 4.10. There were no significant statistical differences between the pre- and post-infection values of urea, potassium, chloride, and bicarbonate. Post-infection values of sodium, total serum protein, albumin, and globulin were significantly different from the respective pre-infection values ($P<0.05$). The post-infection glucose values were significantly higher compared to the pre-infection values ($P<0.05$).

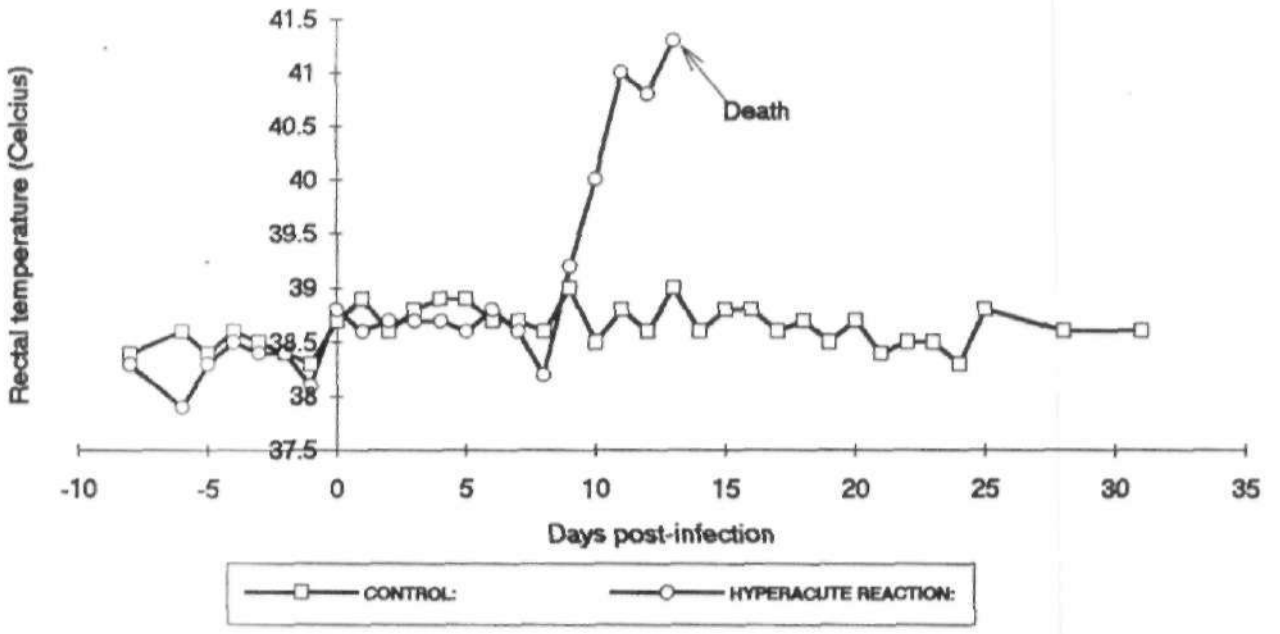


Fig 1.1 Average rectal temperature in two WAD goats showing hyperacute course after being experimentally inoculated with *C.ruminantium*.

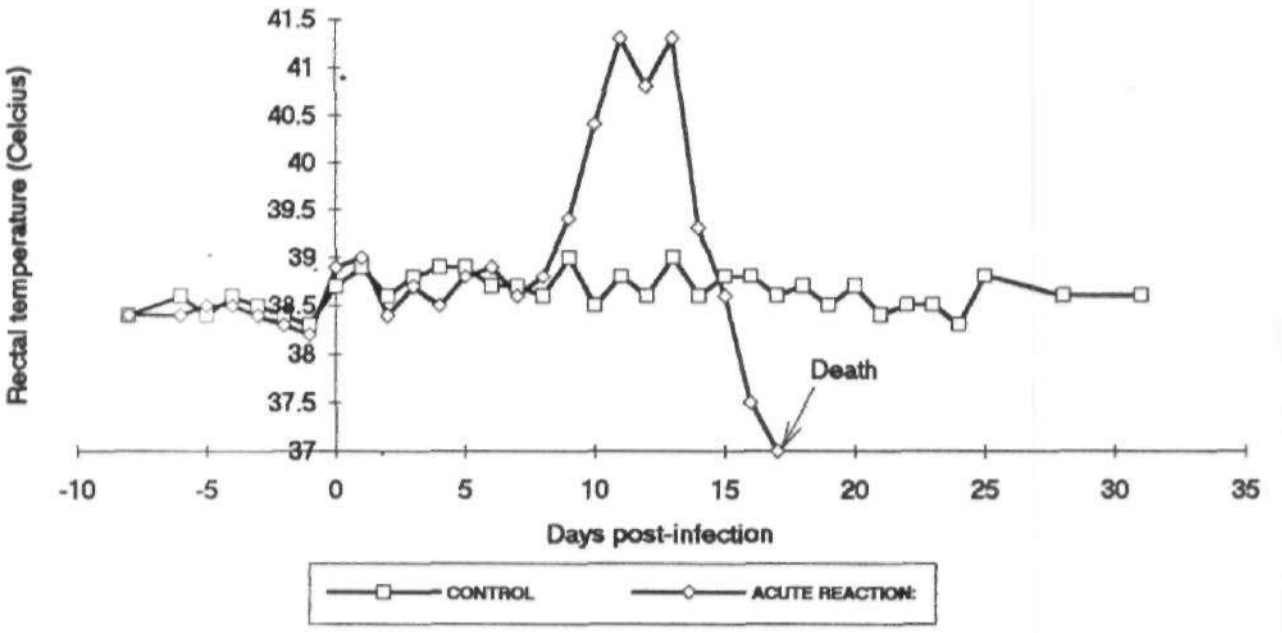


Fig. 1.2 Average rectal temperature in three WAD goats which died from acute form of heartwater after being experimentally inoculated with *C.ruminantium*.

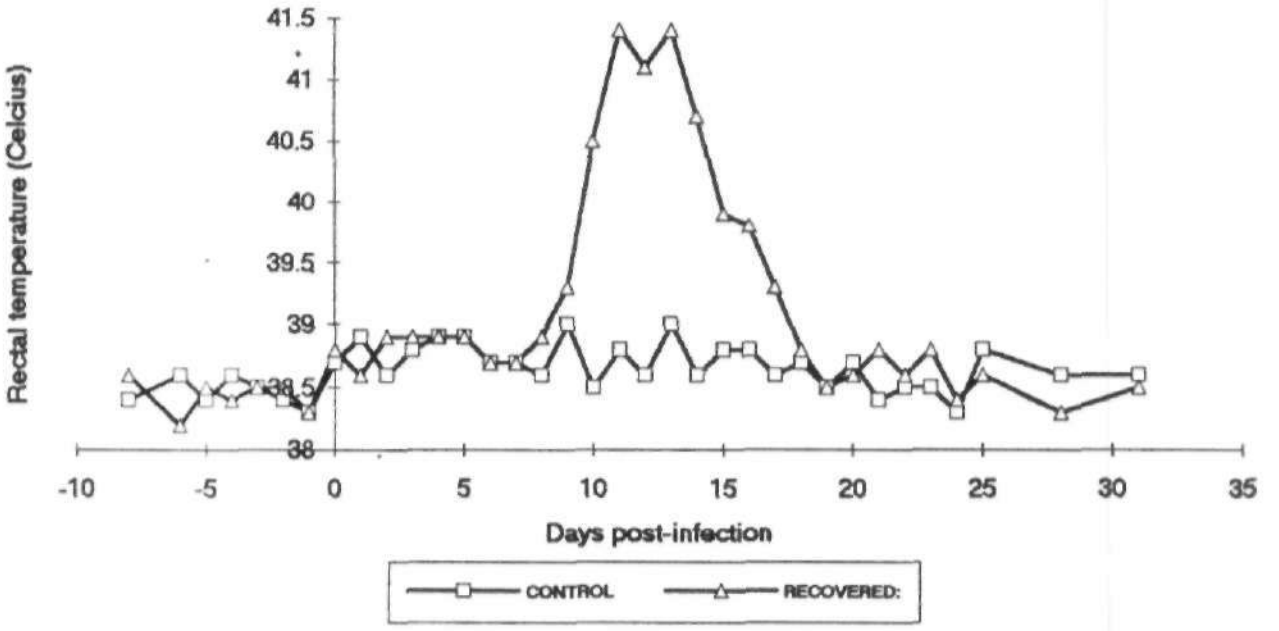


Fig. 1.3 Average rectal temperature in five WAD goats which recover after being experimentally inoculated with *Cowdria ruminantium*.



Plate 1.1 Goats No. 284 Showing severe nervous signs with opisthotonus shortly before death.



Plate 1.2 Goats No. 274 Showing nervous signs.



Plate 1.3 Goats No. 296 Showing aborted fetus as a result of severe reaction to *Cowdria ruminantium* infection.

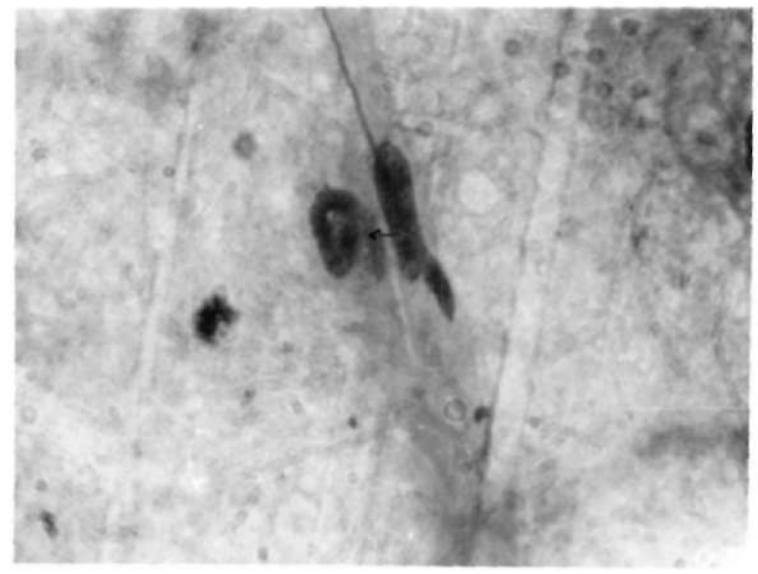


Plate 1.4:*Cowdria ruminantium* granules in a brain squash smear (Arrow). Magnification x 1000

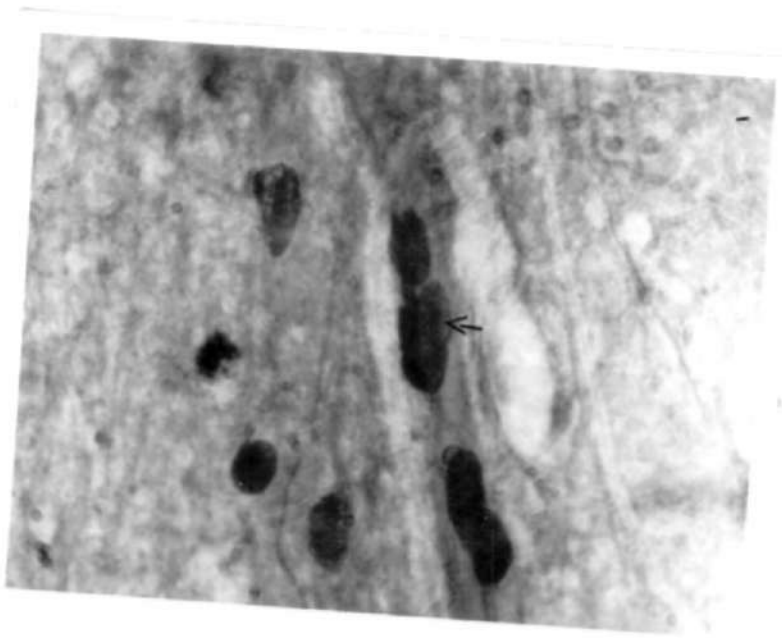


Plate 1.5 *Cowdria ruminantium* granules in the brain squash smear (Arrow). Magnification x 1000

Table 4.3: Mean Rectal Temperature (°C) in West African Dwarf Goats that Reacted Hyperacutely following Experimental Infection With *C.ruminantium*

Days Post-infection	Rectal Temperature (°c) ± Standard Deviation
0	38.4 ± 0.15
1	38.8 ± 0.14
2	38.5 ± 0.21
3	38.8 ± 0.07
4	38.8 ± 0.01
5	38.6 ± 0.14
6	38.5 ± 0.01
7	38.9 ± 0.77
8	38.9 ± 2.10
9	39.0 ± 0.84
10	40.5 ± 1.13
11	41.3 ± 0.63
12	41.3 ± 0.75
13	41.2 ± 0.50

Table 4.4: Mean Rectal Temperature (°C) in West African Dwarf Goats that Reacted Acutely following Experimental Infection With *C.ruminantium*

Days Post-infection	Rectal Temperature (°C) ± Standard Deviation
0	38.9 ± 0.66
1	38.8 ± 0.40
2	38.4 ± 0.11
3	38.6 ± 0.20
4	38.8 ± 0.34
5	38.7 ± 0.35
6	38.8 ± 0.26
7	38.8 ± 0.23
8	39.0 ± 0.75
9	39.9 ± 1.19
10	40.5 ± 1.21
11	41.0 ± 0.37
12	40.3 ± 0.80
13	40.3 ± 1.58
14	38.9 ± 0.84
15	38.1 ± 0.14
16	37.5 ± 0.49
17	37.0 ± 0.63

Table 4.5: Mean Rectal Temperature (°C) in the Five West African Dwarf Goats that Recovered following Experimental Infection With C.ruminantium

Days Post-infection	Rectal Temperature (°c) ± Standard Deviation
0	38.7 ± 0.10
1	38.6 ± 0.49
2	39.1 ± 0.35
3	38.8 ± 0.38
4	38.9 ± 0.46
5	38.9 ± 0.20
6	38.7 ± 0.11
7	38.7 ± 0.29
8	38.8 ± 0.35
9	39.3 ± 0.53
10	40.1 ± 1.15
11	41.1 ± 1.01
12	40.8 ± 0.68
13	41.4 ± 0.15
14	40.5 ± 0.53
15	39.9 ± 0.82
16	39.9 ± 0.67
17	39.3 ± 0.65
18	38.8 ± 0.45
19	38.5 ± 0.44
20	38.9 ± 0.64

Table 4.6: Mean Rectal Temperature (°C) in the Control West African Dwarf Goats

Days Post-infection	Rectal Temperature (°C) ± Standard Deviation
0	38.6 ± 0.06
1	38.9 ± 0.21
2	38.6 ± 0.45
3	38.8 ± 0.42
4	38.9 ± 0.43
5	38.9 ± 0.45
6	38.7 ± 0.51
7	38.7 ± 0.63
8	38.6 ± 0.54
9	39.0 ± 0.54
10	38.5 ± 0.52
11	38.8 ± 0.51
12	38.6 ± 0.50
13	39.0 ± 0.53
14	38.6 ± 0.49
15	38.8 ± 0.48
16	38.8 ± 0.47
17	38.6 ± 0.35
18	38.8 ± 0.43
19	38.9 ± 0.45
20	38.5 ± 0.56

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Table 4.7: Haematological Values in Normal West African Dwarf Goats

Goat No.	PCV (%)	Hb (g/dL)	RBC (10^{12}/L)	Total Protein (g/dL)	Total WBC (10^9/L)	Differential White Blood Cell Counts				
						Neutr-ophils (%)	Lymph-ocytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
274	28	9.3	7.85	7.0	11.8	18	80	0	2	0
276	33	11.0	10.05	7.3	19.9	28	67	0	5	0
277	27	9.0	7.25	7.2	13.1	30	70	0	0	0
278	27	9.0	7.00	7.0	16.2	28	69	0	3	0
279	32	10.7	9.95	7.3	13.0	31	68	0	1	0
280	31	10.3	9.45	7.4	14.4	44	56	0	0	0
283	30	10.0	8.95	7.0	14.2	20	78	0	2	0
284	31	10.3	9.10	7.5	12.2	17	69	0	0	0
290	27	9.0	6.95	6.7	12.8	14	85	0	0	0
296	28	9.4	7.65	6.5	12.2	41	58	0	1	0
Average	29.40	9.80	8.42	17.09	13.98	25.00	70	0	1.4	0

Table 4.8: Haematological Values in West African Dwarf Goats 12 days following infection with *C.ruminantium*

Goat No.	PCV (%)	Hb (g/dL)	RBC (10^{12}/L)	Total Protein (g/dL)	Total WBC (10^9/L)	Differential White Blood Cell Counts: Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
274	22	7.3	5.10	6.5	16.3	33	67	0	0	0
276	25	8.4	6.95	6.5	10.0	33	63	0	0	0
277	19	6.6	4.65	7.0	13.6	28	72	0	0	0
278	19	6.3	4.85	6.5	14.9	25	75	0	0	0
279	27	9.0	7.85	7.0	12.5	23	77	0	0	0
280	26	9.0	7.45	7.0	12.8	31	69	0	0	0
283	19	6.4	4.55	6.5	13.8	30	70	0	0	0
284	27	8.0	7.80	7.0	11.8	30	65	0	0	0
290	23	7.6	5.65	7.0	13.1	17	83	0	0	0
296	25	8.3	6.95	6.5	11.0	59	41	0	0	0
Average	23.20	7.69	5.39	6.75	12.98	30.9	62.50	0	0	0

Table 4.9: Serum Biochemical Values in Normal West African Dwarf Goats

Goat No.	BUN (mg/dL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	HCO3 (mEq/L)	Sugar (mmol/L)	Albumin (g/dL)	Glob-ulin (g/dL)
274	13.6	144	6.1	102	21	2.0	29	34
276	11.8	142	5.2	106	22	2.5	33	35
277	9.1	154	5.9	104	27	3.5	33	42
278	11.8	150	5.1	101	25	2.5	32	39
279	11.8	153	5.6	110	23	3.0	29	39
280	7.3	150	5.0	106	23	2.8	30	39
283	11.8	146	6.1	100	25	2.5	31	37
284	10.0	148	5.0	103	23	2.5	33	27
290	10.9	144	7.5	102	21	4.0	33	30
296	10.0	149	6.3	106	19	1.3	29	21
Average	10.81	148	5.78	104	22.90	2.70	31.20	34.30

Table 4.10: Serum Biochemical Changes in West African Dwarf Goats 12 days following infection with *C.ruminatium*

Goat No.	BUN (mg/dL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	HCO3 (mEq/L)	Sugar (mmol/L)	Albumin (g/dL)	Globulin (g/dL)
274	10.0	142	7.3	100	20	4.5	24	26
276	15.5	146	6.3	112	18	3.0	30	24
277	11.8	146	7.2	108	20	3.0	31	30
278	8.6	144	5.0	102	20	2.5	29	30
279	8.6	146	5.0	100	25	4.0	26	28
280	10.9	140	5.3	100	22	6.0	27	30
283	16.4	146	5.8	106	22	4.5	28	40
284	10.0	144	7.5	102	25	4.5	25	29
290	10.9	140	10.0	103	23	3.0	20	28
296	8.2	144	6.6	104	25	4.0	25	23
Average	11.09	143.80	6.60	94.70	22.0	4.0	26.50	28.80

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The fact that all the ten experimentally infected West African Dwarf (WAD) goats reacted within 9 - 11 days of infection showed that this breed of goat is susceptible, and the varying degree of susceptibility and their classification into hyperacute and acute forms has also been noticed by some workers in other breeds of goats (Alexander, 1931; Rousselot, 1953; Henning, 1956; Neitz, 1968; Ilemobade, 1976; Uilenberg, 1983 and Losos, 1986).

The onset of pyrexia occurred at the same period in all the infected animals. It is suggestive therefore that this isolate can be used as a vaccine because the onset of reaction can be predicted for treatment to be instituted.

In the Southern Guinea zone where these WAD goats predominate, semi-intensive management system is practised whereby the goats are not allowed to go on free grazing but are usually localised within their settlements tied to trees or other objects. They are therefore less exposed to ticks and subsequent development of immunity. This must have probably contributed to the observed high susceptibility of the animals in this study. This finding agrees with similar observation made by Ilemobade (1977) that the method of animal husbandry in Northern Nigeria whereby goats are confined within the villages reduces their chance of being exposed to ticks. In addition, he also suggested

that goats are not preferential hosts of adult *Amblyomma* ticks which feed more on cattle. This further reduces their chances of development of immunity.

One could also speculate that the clinical recovery by some of the animals could be attributed probably to previous exposure to milder strain of *C.ruminantium* which did not offer a complete protection against the stock of *C.ruminantium* used in this experiment.

The marked drop in the PCV, Hb and RBC counts during the course of the disease could be due to febrile reaction, as marked decrease of PCV coincided with the peak of the temperature.

In a similar investigation, Clark (1962) observed a drop in PCV and Hb in heartwater infected animals. The elevation of body temperature might have led to the destruction of RBC and subsequent reduction of the PCV. Davies and Topley (1956) cited by Kalre (1968) observed anaemia following thermal injury and haemolysis as described in heat stroke.

A slight and steady fluctuation was observed in total and differential cell counts, the neutrophils and monocyte count were however not statistically significant. This findings could probably be as a result of bone marrow depression due to the infection. Clark (1962) found no alteration in total or differential cell counts in sheep infected with *C.ruminantium*.

The drop in the total serum protein concentration observed in this study could be attributed to the fall in the albumin and globulin due to capillary leakage because of a concurrent reduction in blood volume. In similar investigation, Van Amstel *et al.* (1987) found a disproportionate loss of fluid and protein from the vascular compartment in heartwater infection.

A significant increase in the level of serum sodium ions in infected animal was observed. The increase in temperature and subsequent reduction in water intake could cause an increase of this ion. Ganong (1991) reported that increases in the concentration of sodium ions could occur due to febrile condition and subsequent inadequate water intake.

The noticeable increase in blood glucose level in the infected animals could be due to severe stress as Lording *et al.* (1991) have showed that severe stress could cause the release of endogenous corticosteroid into the circulation leading to mobilization of body glucose.

5.2 Conclusion

In this study, all the ten infected animals reacted to the infection with *C.ruminantium*. The reacting animals exhibited a high temperature profile reaching a maximum of 42°C and a minimum of 40.9°C.

Fifty percent of the infected animals died after showing various clinical signs, while the remaining 50% recovered with pyrexia being the major presenting sign.

Apart from slight anaemia observed in infected animals during the febrile reaction, the haematological parameters showed no significant change during reaction and after recovery.

However, the serum biochemical analysis showed a significant drop in the total serum protein and a significant rise in sodium ions and blood glucose of infected goats.

This study shows that WAD goats are susceptible to experimental *C.ruminantium* infection. The susceptibility of this breed of goat to *C.ruminantium* used could be attributed to the fact that management practices of goat production in the Southern Guinea zones of Nigeria does not allow extensive free grazing, consequently, they have less chances of coming in contact with *Amblyomma* ticks.

With the susceptibility of WAD goats confirmed from this study, there is the need for proper control measures, probably through the development of an effective vaccine to immunize these animals against the disease.

Appendix 1: Serum Biochemical Analyses

Method:	Procedure:
Modified Doumas and Biggs (1972)	For determination of albumin levels in the serum. Albumin reacts with Bromocresol green dye which has a spexcific affinity for its acidic solution to produce a coloured complex which measured clorimetrically. The values represent the concentration of albumin in the sample. The level of globulin is indirectly determined by subtracting the value of albumin from the total serum protein concentration.
Fales (1963)	For the determination of glucose level in the serum. Glucose oxidase is a specific enzyme which promotes the oxidation of glucose to acid with the production of an equivalent amount of hydrogen peroxide. In the presence of a second enzyme peroxidase oxygen from peroxide can be transfered to a suitable acceptor with the production of a colour complex. The intensity is proportional to the concentration of glucose in the sample.
Wybenga (1971)	For the determination of urea concentration in serum. When urea is in strongly acidic condition with substances such as diacetyl containing two adjacent carboxyl groups or its amonoxine, yellow condensation compounds are formed. The reaction is intensified by the presence of polyvalent ions such as ferric ions. And then a red colour complex is formed which is more linear with concentration than the yellow one. The intensity of the red complex is measured colorimetrically and its intensity is proportional to the concentration of urea in the sample.

Appendix 2: Results of Paired Sample Analysis for PCV

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	29.4	23.2	26.3
Variance	5.15556	10.8444	8
Standard Deviation	2.27058	3.29309	2.82843
Median	29	24	27

Difference between means = 6.2

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 = 3.54187 8.85813 18 D.F

(Unequal Var) Sample 1 - Sample 2 = 3.51757 8.88243 16.0 D.F.

Ratio of Variances = 0.47541

Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 4.90153
vs Sig. Level = 1.14947E-4
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

Appendix 3: Results of Paired Sample Analysis for Haemoglobin

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	9.8	7.69	8.745
Variance	0.568889	1.03878	0.803833
Standard Deviation	0.754247	1.0192	0.896568
Median	9.7	7.8	9

Difference between means = 2.11
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = 1.26741 2.95259 18 D.F
(Unequal Var) Sample 1 - Sample 2 = 1.26222 2.95778 16.6 D.F.

Ratio of Variances = 0.547652
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 5.26241
vs Sig. Level = 5.27983E-5
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

Appendix 4: Results of Paired Sample Analysis for Red Blood Cells

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	8.42	6.175	7.2975
Variance	1.474	1.83125	1.65262
Standard Deviation	1.21408	1.35324	1.28554
Median	8.4	6.275	7.35

Difference between means = 2.245
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = 1.03686 3.45314 18 D.F
(Unequal Var) Sample 1 - Sample 2 = 1.03584 3.45416 17.48 D.F.

Ratio of Variances = 0.804915
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 3.90494
vs Sig. Level = 1.03793E-3
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

Appendix 5: Results of Paired Sample Analysis for Serum Total Protien

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	7.09	6.75	6.92
Variance	0.0987778	0.0694444	0.0841111
Standard Deviation	0.314289	0.263523	0.290019
Median	7.1	6.75	7.0

Difference between means = 0.34
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = 0.0674427 0.612557 18 D.F
(Unequal Var) Sample 1 - Sample 2 = 0.0668472 0.613153 17.5 D.F.

Ratio of Variances = 1.4224
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 2.62142
vs Sig. Level = 0.0173014
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

Appendix 6: Results of Paired Sample Analysis for White Blood Cells

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Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	13980	11980	12980
Variance	6.0462286	1.44262E7	1.02362E7
Standard Deviation	2458.91	3798.19	3199.41
Median	13050	12950	13050

Difference between means = 2000
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = -1006.78 5006.78 18 D.F
(Unequal Var) Sample 1 - Sample 2 = -1043.31 5043.31 15.4 D.F.

Ratio of Variances = 0.419113
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 1.3978
vs Sig. Level = 0.179163
Alternate hypothesis: NE so do not reject H_o.
at Alpha = 0.05

Appendix 7: Results of Paired Sample Analysis for Neutrophils

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Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	27.1	31.2	29.15
Variance	101.211	124.4	112.806
Standard Deviation	10.0604	11.1535	10.621
Median	28	30	29

Difference between means =	-4.1		
Conf. Interval For Diff. in means:	95 Percent		
(Equal Var) Sample 1 - Sample 2 =	-14.0815	5.88151	18 D.F
(Unequal Var) Sample 1 - Sample 2 =	-14.0891	5.88908	17.8 D.F.

Ratio of Variances =	0.813594	
Conf. Interval for Ratio of Vars:	0	Percent
Sample 1 v Sample 2		

Hypothesis Test for H_o:	Diff = 0	Computed t statistic = 0.863184
vs		Sig. Level = 0.399392
Alternate hypothesis:	NE	so do not reject H_o.
at Alpha = 0.05		

Appendix 8: Results of Paired Sample Analysis for Lymphocytes

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	70	68.2	69.1
Variance	82.6667	126.622	104.644
Standard Deviation	9.09212	11.2527	10.2296
Median	69	69.5	69

Difference between means = 1.8

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 =	-7.81367	11.4137	18 D.F
(Unequal Var) Sample 1 - Sample 2 =	-7.84417	11.4442	17.2 D.F.

Ratio of Variances = 0.652861

Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o:	Diff = 0	Computed t statistic = 0.393459
vs		Sig. Level = 0.698602
Alternate hypothesis:	NE	so do not reject H_o.
at Alpha = 0.05		

Appendix 9: Results of Paired Sample Analysis for Serum Urea

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	10.81	11.09	10.95
Variance	3.141	7.92767	5.53433
Standard Deviation	1.7777229	2.81561	2.35252
Median	11.35	10.45	10.9

Difference between means = -0.28
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = -2.49087 1.93087 18 D.F
(Unequal Var) Sample 1 - Sample 2 = -2.5209 1.9609 15.2 D.F.

Ratio of Variances = 0.396207
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = -0.26614
vs Sig. Level = 0.793156
Alternate hypothesis: NE so do not reject H_o.
at Alpha = 0.05

Appendix 10: Results of Paired Sample Analysis for Serum Sodium

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Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	148	143.8	145.9
Variance	15.7778	5.73333	10.75556
Standard Deviation	3.97213	2.39444	3.27957
Median	148.5	144	146

Difference between means = 4.2
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = 1.11789 7.28211 18 D.F
(Unequal Var) Sample 1 - Sample 2 = 1.06899 7.33101 14.8 D.F.

Ratio of Variances = 2.715194
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 2.86364
vs Sig. Level = 0.0103242
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

Appendix 11: Results of Paired Sample Analysis for Serum Potassium

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	5.78	6.6	6.19
Variance	0.610667	2.30667	1.45867
Standard Deviation	0.781452	1.51877	1.20775
Median	5.75	6.45	6

Difference between means = -0.82

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 = -1.95503 0.315035 18 D.F

(Unequal Var) Sample 1 - Sample 2 = -1.98318 0.343178 13.5 D.F.

Ratio of Variances = 0.26474

Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0
vs
Alternate hypothesis: NE
at Alpha = 0.05

Computed t statistic = -1.51817
Sig. Level = 0.146339
so do not reject H_o.

Appendix 12: Results of Paired Sample Analysis for Serum Chlorine

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Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	104	6.175	104.15
Variance	9.11111	14.2333	11.6722
Standard Deviation	3.01846	3.77271	3.41646
Median	103.5	103.5	103.5

Difference between means = -0.3
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = -3.51076 2.91076 18 D.F
(Unequal Var) Sample 1 - Sample 2 = -3.52188 2.92188 17.2 D.F.

Ratio of Variances = 0.64125
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 0.196349
vs Sig. Level = 0.846537
Alternate hypothesis: NE so do not reject H_o.
at Alpha = 0.05

Appendix 13: Results of Paired Sample Analysis for Serum Bicarbonate

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	22.9	22	22.45
Variance	5.43333	6.22222	5.82778
Standard Deviation	2.33095	2.49444	2.41408
Median	23	22	22.5

Difference between means = 0.9

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 =	-1.36873	3.16873	18 D.F
(Unequal Var) Sample 1 - Sample 2 =	-1.36947	3.16947	17.9 D.F.

Ratio of Variances = 0.873214

Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0
vs
Alternate hypothesis: NE
at Alpha = 0.05

Computed t statistic = 0.833635
Sig. Level = 0.415413
so do not reject H_o.

Appendix 14: Results of Paired Sample Analysis for Serum Glucose

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	2.66	3.9	3.28
Variance	0.558222	1.1	0.829111
Standard Deviation	0.747413	1.04881	0.910555
Median	1.5	4	3

Difference between means = -1.24

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 = -2.095573 -0.384268 18 D.F

(Unequal Var) Sample 1 - Sample 2 = -2.10233 -0.377671 16.3 D.F.

Ratio of Variances = 0.507175

Conf. Interval for Ratio of Vars: 0 Percent

Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0

vs

Alternate hypothesis: NE

at Alpha = 0.05

Computed t statistic = -3.04509

Sig. Level = 6.96663E-3

so reject H_o.

Appendix 15: Results of Paired Sample Analysis for Serum Albumin

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	31.2	26.5	28.85
Variance	3.28889	10.5	6.89444
Standard Deviation	1.81353	3.24037	2.62573
Median	31.5	26.5	29

Difference between means = 4.7

Conf. Interval For Diff. in means: 95 Percent

(Equal Var) Sample 1 - Sample 2 = 2.23237 7.16763 18 D.F

(Unequal Var) Sample 1 - Sample 2 = 2.18307 7.21693 14.1 D.F.

Ratio of Variances = 0.313228

Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 4.00252

vs Sig. Level = 8.35134E-4

Alternate hypothesis: NE so reject H_o.

at Alpha = 0.05

Appendix 16: Results of Paired Sample Analysis for Serum Globulin

Sample Statistics:	Pre-infection:	Post-Infected:	Pooled:
No. of Observations	10	10	20
Average	34.3	28.8	31.55
Variance	42.4456	21.7333	32.0944
Standard Deviation	6.51579	4.6619	5.6652
Median	36	28.5	30

Difference between means = 5.5
Conf. Interval For Diff. in means: 95 Percent
(Equal Var) Sample 1 - Sample 2 = 0.175901 10.8241 18 D.F
(Unequal Var) Sample 1 - Sample 2 = 0.135832 10.8642 16.3 D.F.

Ratio of Variances = 1.95348
Conf. Interval for Ratio of Vars: 0 Percent
Sample 1 v Sample 2

Hypothesis Test for H_o: Diff = 0 Computed t statistic = 2.17086
vs Sig. Level = 0.0435599
Alternate hypothesis: NE so reject H_o.
at Alpha = 0.05

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