THE PATHOGENESES AND CLINICAL PATHOLOGY OF COWDRIOSIS [HEARTWATER] IN SHEEP

MBITHE MULINGE, B.V.M.
University of Nairobi

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Department of Clinical Studies
Faculty of Veterinary Medicine
University of Nairobi

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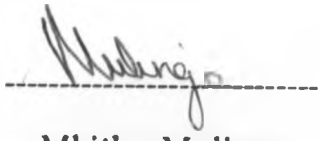
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University



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This thesis has been submitted for examination with my approval as a University supervisor.

Prof. G. M. Mugera, Dip, V.Sc., M.Sc., Ph D.

This thesis has been submitted for examination with my approval as a University supervisor

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Prof. G. M Mugera, Dip., V.Sc., M.Sc., Ph.D.

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ABSTRACT

Heartwater is a disease of ruminants transmitted by ticks. It is mainly found in the African continent and the surrounding islands but lately isolated cases have been reported elsewhere.

The disease occurs in different forms ranging from peracute to inapparent forms. It is associated with a febrile reaction, acute gastroenteritis, hydropericardium and nervous symptoms.

Heartwater is a killer disease and thus effective treatment and control of the disease are of utmost importance. Tetracylines used early have been found to be effective but for this, accurate diagnosis of the disease is important. The only definitive way of diagnosing the disease is at portmortem when the brain smears stained with giemsa reveal the rickettsial organisms. Lately, researchers have been doing a lot of serology work on the disease with the aim of coming up with diagnostic tests in the live animal. Clinical pathology tests can also help as diagnostic aids of the disease.

This work was designed with this in mind, to observe the behaviour of particular blood components of sheep infected with Cowdria ruminantium and to correlate this with the pathogenesis of the cell fraction associated with the organisms in blood.

ABSTRACT

Heartwater is a disease of ruminants transmitted by ticks. It is mainly found in the African continent and the surrounding islands but lately isolated cases have been reported elsewhere.

The disease occurs in different forms ranging from peracute to inapparent forms. It is associated with a febrile reaction, acute gastroenteritis, hydropericardium and nervous symptoms.

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This work was designed with this in mind, to observe the behaviour of particular blood components of sheep infected with Cowdria ruminantium and to correlate this with the pathogenesis of the cell fraction associated with the organisms in blood.

Twenty sheep were used in this work. These were adults, non-pregnant and of Dopper breed (or Dopper cross). They were housed in the animal compound, Kabete, and fed on hay, bran and water. The sheep were mainly purchased from the Faculty Farm, Kabete.

Fifteen sheep were infected while five were used as controls. Initially, blood was collected from all four sheep:- EDTA blood for hematology and clotted blood for biochemistry. This was done for 3 consecutive days before infection to get the baseline values. After this 3 experimental sheep were infected with Cowdria ruminantium intravenously. A blood stabilate obtained from Veterinary Laboratories, Kabete was used as the infective agent.

Body temperatures were monitored twice daily and after the onset of the febrile reaction, blood samples were taken for hematological and biochemical analysis. Blood samples were collected daily in the first 3 days and after that every other day upto death or recovery. In those sheep that died postmoretm was carried out.

Some more work was done to try and identify the cell fraction in blood associated with Cowdria ruminatium.

The animals manifested anorexia, depression, increased respiratory rate, increased pulse rate, harsh lung sounds with moist rales and muffled heart sounds. Nervous signs observed in some animals included circling, high stepping gait, chewing movements, ataxia and incoordination and then lateral recumbency and paddling movements. All animals which manifested nervous signs died of the disease.

The major findings on postmortem were froth in the trachea, congested and oedematous lungs, hydropericardium, ascites, hyrdothorax and congested and oedematous brain.

From the hematology results, sheep infected with Cowdria ruminantium showed anaemia, leukopenia during incubation period followed by a leukocytosis after fever onset. There was a neutrophilia, lymphopaenia and oesinophils disappeared completely from circulation. Statistical analysis using analysis of variance showed these findings were statistically significant.

From the clinical biochemistry results there was an increase in glucose concentration especially terminally which was statistically significant.

Of the enzymes analysed, lactate dehydrogenase, creatinine phosphokinase and aspartate aminotransferase showed the most signifcant change in that their activities increased. Alkaline phosphatase and alanine aminotransferase did not show significant changes.

Blood urea nitrogen increased significantly after the febrile reaction.

The study of the cell fraction associated with the organism revealed the neutrophil as the cell most likely to be associated with Cowdria ruminantium but this is, of course, subject to exhaustive research work.

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DEDICATION

For Mutunga, my husband and our two children, Linda Syokau and Tito Musau.

CHAPTER 1

1:1 INTRODUCTION

Heartwater is an infectious, often fatal non-contagious disease affecting domestic and wild ruminants. It is caused by a Rickettsia, Cowdria ruminantium and is transmitted by ticks of the Amblyomma genus. The distribution of the disease follows that of the vector tick. It is found in Africa, South of the Sahara, Madagascar and the surrounding islands.

All domestic and wild ruminants are susceptible to the disease but only domestic ruminants, viz goats, sheep and cattle suffer the clinical disease while the wild ruminants act more as reservoirs.

The disease occurs in different forms ranging from peracute to inapparent forms (Alexander, 1931 and Neitz, 1968). It is associated with a febrile reaction, acute gastroenteritis and hydropericardium followed by nervous symptoms. Nervous signs are the most distinct symptoms of the disease, although not always present (Neitz, 1978). These signs are commonly confused with other causes of nervous signs (Bruckner, Hurter and Boshoff, 1978).

Until the sixties almost all the work on heartwater was done in South Africa. Now that the seriousness of the disease has been recognised in many countries , this situation has changed.

At present, heartwater is considered primarily an African disease but lately the disease was found in the French West Indies and seems possible that it may spread to the American continent.

The facts which have been established about the causative agent are; the morphology and classification of the organism.

The pathogenesis and clinical pathology of this disease have not been studied fully. There are many hypotheses which have been put forward concerning the pathogenesis of the disease;

1. Increased capillary permeability has been suggested which allows seepage of plasma proteins (Clark, 1962), resulting in the transudation through the serous membranes.

2. Vascular lesions have been explained to be due to the presence of Cowdria ruminantium (Pienaar, Basson and Van der Merwe, 1966).

3 .Jackson and Neitz (1932), proposed that a toxin was the cause of the increased vascular permeability and the nervous symptoms.

4. Release of vasoactive amines (histamine and serotonine) has also been suggested to be the cause of lesions in the mice (Du Plessis, 1975).

1:2 OBJECTIVES

To carry out hematological and serum biochemical studies in sheep experimentally infected with Cowdria ruminantium and find out their relationship with the pathogenesis , clinical manifestations and pathology of the disease.

1: 3. LITERATURE REVIEW

THE CAUSATIVE AGENT

Heartwater is caused by a pleiomorphic rickettsial organism, Cowdria ruminantium measuring about 0.2-0.5 u [Cowdry, 1925a]. The organism belongs to the order Rickettsiales and sub-family Ehrlichiae [Bergey, 1974].

Morphology and staining characteristics

Several researchers [Jackson and Neitz,1932; Piennar,1970; Du Plessis,1970a; Dardiri and Wool,1982; Stewart and Howell, 1981;] have described the pleiomorphic forms of Cowdria ruminantim. Donatien and Lestoguard [1938] observed initial bodies i.e homogenous masses in the cytoplasm which stained purple with giemsa. Their size was between 1-2u and attaining 5u. Apart from the cocci forms described by Cowdry [1925];and the initial bodies described by Donatien and Lestguard [1938], bulky dark blue forms (2-4u) have been seen. Other forms which have been documented are rings, horseshoe, rods, and irregular masses [Camus and Barre,1988].

The morphology of the parasite in the host is similar to that in the vector, [Cowdry,1926]. Electron microscopy enabled the precise morphology to be determined [Du plessis,1975; Stewart and Howell, 1981].

Colonies of Cowdria ruminantim were seen by Cowdry [1925b],in giemsa stained parraffin-sections of tissue. Later studies have utilised epoxy-embedded sections and a variation of Mallory's stain which renders colonies, tissue components and haemoglobin into various shades of blue.

Stability and preservation

Alexander [1931] reviewed his attempts and those of others to preserve the infectivity of C. ruminantium in blood at room temperature and at 4°C. They were able to preseve C. ruminantium for 38 hours. Alexander [1931] stated that the organism does not survive for more than 24 hours at room temperature and that in some, viability is lost within 12 hours. Henning [1956] stated that C. ruminantium in blood remained infectios for 4 days.

Alexander [1931] commented that storage at low temperatures appeared to have a favourable influence on the viability of C. ruminantium. Haig [1952] later noted that Alexander had stored C. ruminantium for a period of 2 years in a dry-ice cabinet. Weiss, Haig and Alexander [1952] reported the details of the successful preservation of C ruminantium in 10% phosphate-buffered spleen homogenates at -74°C. Neitz [1968] reported that C. ruminantium in blood and spleen homogenates maintained at -76°C for 2 years was infectious to animals. Karrar [1960] was unable to preserve citrated or defibrinated blood at -32°C. Abdel Rahim and Shommein, [1978] also failed to preserve the organism in goat blood collected in EDTA and frozen at -70°C.

The use of 10% dimethyl sulphoxide [DMSO] as a cryoprotectant for blood stabilates was reported by Ramisse and Uilenberg [1970].

Bezuidenhout, [1981] successfully produced tick stabilates of C. ruminantium for use as a heartwater vaccine.

Engorged infected nymphae were homogenised in BLP and 5% DMSO, snap frozen and maintained on dry-ice or liquid nitrogen [-196oC]

Brain homogenates made in phosphate buffered saline [PBS] and frozen with or without DMSO have been shown to be infectious to animals, [Ilemobade and Blotkamp, 1978; Ullenberg, 1983].

Logan [1987] and others before her also demonstrated that C. ruminantium can effectively be preserved in a variety of organ suspensions at low temperatures [-70°C to -196°C] for indefinite periods of time.

Culturing

Page [1967] stated that C. ruminantium could not be cultivated in artificial media. Andreasen [1974] reported the successful cultivation of C. ruminantium in a monolayer tissue culture of Amblyomma tick gut cells using blood from sheep suffering from heartwater. In1980, C. ruminantium was kept alive in primary goat kidney cells' culture for thirteen days, the longest period reported [Jongejan, Van Winkelhoff and Uilenberg, 1980].

Calf endothelial cell line have been used for in vitro culturing of C. ruminantium [Benzuidenhout , 1985]. Logan [1985] managed to culture infected neutrophils which she used as antigen in serological studies.

More recently, more work has been done on the adaptation of C. ruminantium in mice and cell cultures, (Parklea, 1990). This was the first successful attempt to culture Kenyan isolates of C. ruminantium. He used both the Tanariver and the Transmara isolates

and was able to propagate these in cell cultures and mice. The parasite was found to persist in mice tissues for at least 196 days.

HOSTS, DISTRIBUTION AND TRANSMISSION.

All domestic ruminants are susceptible to the disease with goats being the most susceptible and cattle the least [Page 1967]. Wild ruminants have also been shown to be susceptible but the disease is not well manifested as in domestic ruminants, (Neitz, 1933; 1935; 1937;). Heartwater occurs in Africa and the surrounding islands and it's distribution follows that of the tick vectors of the Amblyomma species. It is found in Africa south of the Sahara , Madagascar and the surrounding islands. Isolated cases have been reported in other countries e.g Brazil, [Souza and Martins,1937]. North American continent seems to be threatened from the Antilles; (Diamant, 1965; Hoogstraal, 1962;).

Natural transmission of heartwater is by ticks of the Amblyomma species. These include A. habreum , A. variegatum ,A. pomposum , A. gemma , and A. lepidum. Transmission is transtadial [Illemobade et al, 1976]. Transovarial transmission has also been reported but was found to be of low freaguency by Uilenberg [1979]. The Amblyomma ticks are three host ticks. A larvae feeding on an infected animal is able to transmit the disease in it's nymphal and adult stages [Uilenberg, 1983]. Some researchers had earlier shown that the disease can be transmitted by intravenous inoculation with infected blood, [Dixon, 1898; Spreull,1904; and Theiler,1903;.]. More recently Ilemobade and Blotkamp [1978] showed that the disease could be transmitted by subcutaneous

cause any disease as did on intravenous inoculation [Uilenberg,1971]. This showed that the organism existed in different forms in different body tissues.

PATHOGENESIS.

The pathogenesis of heartwater is poorly understood. There seems to be general agreement that increased permeability of smaller blood vessels plays an important role in the pathogenesis of the disease; [Clark, 1962;Owen, Littlejohn, Kruger, and Erasmus,1973;Du Plessis, 1975;Prozesky and Du Plessis, 1985;] The increased permeability is said by some to be due to a toxin , [Steck, 1928; Pienaar,Basson, and Van der merwe, 1966; Neizt, 1968; Piennar, 1970; Ilemobade, 1976;] but the toxin has not been demonstrated. The increased permiability has also been said to be due to the parasites C. ruminantium. Vaso-active substances have also been incriminated [Du PLessis]

Cowdria ruminantium has been demonstrated in endothelial cells of capillaries in fresh brain squash smears and in fresh histological sections of the various organs in domestic ruminants dying from the disease.

Du Plessis,[1970a] described the localization of the organism in lymph nodes where it multiplied by binary fission during the incubationperiod. By use of the elecron microscope he was able to show C. ruminantium as early as 2-4 days after inoculation. The release of elementary bodies that arose from fission of the initial bodies into circulation caused the onset of fever. The organsim parasitised the

reticulo-endothelial cells including peritoneal macrophages and the intima cells of blood vessels including those of the brain capillaries [Du Plessis, 1975]. The organism also parasitised the lung macrophages as shown by Ilemobade and Blotkamp,[1976].

Alexander [1931] hypothesised that the parasite is not evenly distributed in the blood and appear to be attached to erythrocytes and leucocytes. Evidence has been gained that the infective agent of C. ruminantium is associated with leucocyte fraction of blood [Weiss, et al 1952; Logan, 1987].

Jackson and Neitz [1932] suggested that C. ruminantium was free in the blood during parasitaemic stage of the disease.They advanced the theory that rickettsia introduced naturally or artificially into the blood stream enter endothelial cells where they develop from a single granule to a large group until it ruptures, thus releasing the organism into circulation.

CLINICAL SIGNS.

The incubation period of heartwater is influenced by the species of the animal, the route of infection, virulence of the isolate,and the amount of infective material administered; [Alexander, 1931; Neitz, 1968; Uilenberg, 1983.].

Cattle develop fever 12 days after intravenous inoculationof 10 ml of blood infected with the Ball 3 strain of C. ruminantium. The incubation period is reduced by an avarage of 2-4 days when animals are inoculated intravenously with 5 ml of Amblyomma habraem nymph suspension infected with the same isolate, [Van der merwe, 1979; L.Van der merwe, Personal Communication 1986]. In naturally infected animals

incubation period ranges from 9-29 days with an avarage of 18 days, [Alexander,1931].

The incubation period in sheep and goats inoculated intravenously with 10 ml of blood stabilate varied from 5-35 days [avarage 9-10 days]. In naturally infected animals it varied from 7-35 days [avarage 14 days], [Alexander 1931; Uilenbreg,1983].

The course of the disease varies ranges from peracute to innaparent forms [Alexander, 1931; Neitz, 1968].

Cattle

The peracute form has been seen in heavily pregnant Bos taurus breeds and in 6-8 month-old animals of different breeds [Henning, 1956; Van der merwe; 1979]. Fever develops suddenly and animals die within a few hours without overt clinical signs.

Acute heartwater is the most common form of the disease in endemic areas. It is characterised by a fever of 40^{o}C or higher, that usually remains high and drops subnormally shortly before death. In a few febrile animals clinical signs may be absent for 1-9 days, [Alexander, 1931; Uilenberg, 1981.]. Animals appear normal but gradually show innapetance. Petechiae are visible on the mucous membranes of the conjuctiva of most animals. Nervous symptoms ranging from a mild incoordination to pronounced convulsions occur in the majority of acutely affected animals, [Alexander, 1931;]. Other sign are hypersensitivity, aimless wandering, chewing movements, headpressing and high stepping gait.

The incidence of mild form of heartwater is difficult to determine because symptoms are rarely seen in field cases. Calves less than 3 weeks of age [Uilenberg, 1981], animals infected with an isolate of low

virulence [Neitz, 1968], and heartwater-immune animals [Alexander,1931] develop the mild form of the disease. Apathy, fever and a slight tachypnoea are the only signs. Most cases recover within a few days [Camus and Barre, 1982].

Some workers have described diarrhoea as a constant clinical sign of heartwater in cattle [Alexander,1931; Da Graga,1964; Uilenberg , 1981;].

Sheep and goats

Exotic goat breeds commonly suffer the peracute form of the the disease. They die suddenly and have convulsions prior to death [Uilenberg, 1983]

Acute heartwater is the most common form of the disease in sheep. The animals show nervous symptoms but these are generally less pronounced than in cattle [Alexander 1931].They will show unsteady gait initially, stand with their legs wide apart, head down, ears drooping and appear to be listless.Terminally lateral recumbency, galloping , chewing movements, licking of lips and nystagmus maybe seen [Alexander, 1931].

The mild form of heartwater is seen in some breeds e.g. Persian sheep, young animals and animals that have previously been exposed to C. ruminantium [Alexander 1931; Uilenberg, 1983].

CLINICAL PATHOLOGY

Haemoglobin (Hb)

Graf (1933), concluded that heartwater was not associated with any specific change in the blood Hb content. However, significant lowered Hb levels were found in experiments contacted by different researchers [Ilemobade and Blotkamp, 1978; Owen et al, 1973; Abdel Rahim and Shommein , 1977; Van Amstel et al, 1986;]. This decrease in Hb was found to occur shortly after the fever onset.

Haematocrit [Ht], Red blood cell count[RBC], Mean corpuscular volume [MCV] and Mean corpuscular haemoglobin concentration [MCHC].

The following researchers found that there was a general tendency for Ht to fall during the course of the disease [Clark, 1962; Ilemobade and Blotkamp, 1978; Owen et al, 1973; Abdel Rahim and Shommein, 1977,].

Clark (1962;) Owen et al,(1973); Abdel Rahim and Shommein (1977), found the drop in haemoglobin to be proportional to that of the haematocrit. A drop in RBC was demonstrated by Abdel Rahim and Shommein, [1977]. They also demonstrated a drop in both the MCV and MCHC values.

Total and differential white cell count.

A leukopaenia was reported to have developed 3 days after infection by Ilemobade and Blotkamp [1978].This leukopaenia occured prior to the fever onset. Abdel Rahim and Shommein [1977], reported a leucocytosis associated with the clinical disease. Camus and Barre [1982], reported on the presence of a neutrophilia while Abdel Rahim and Shommein [1977], Illemobade and BLotkamp [1978] reported a neutropaenia. A lymphopenia was reported by Ilemobade and Blotkamp [1978] but Abdel Rahim and Shommein [1977] found a marked lymphocytosis.

No changes were observed on monocytes but eosinophils were observed to disappear from circulation [Clark, 1962; Abdel Rahim and Shommein, 1977].

Chemical pathology.

A rise in blood glucose was reported by Clark [1962], Graf, [1933], and Illemobade and Blotkamp [1978]. This rise seems to appear terminally. Van Amstel et al, [unpublished data, 1986], did not support this finding.

Clark [1962], Illemobade and Blotkamp [1978], concluded that there were no significant changes in blood proteins.

Both Clark [1962] and Graf [1933], found increases in the blood urea nitrogen .Several researchers reported that the plasma becomes darker during the course of the disease [Clark, 1962; Gruss, 1981;].

Both sodium and potassium levels were found to be higher in the effused fluid as compared with serum values [Van Amstel et al, unpublished data, 1986].

PATHOLOGY

Lesions in cattle, sheep and goats are fairly similar, although quite variable in extent. Some changes are more common in some species than others [Steck, 1928; Uilenberg, 1981]. A tentative diagnosis of the disease can be made on macroscopic lesions alone , but to confirm the disease C. ruminantium organisms have to be demonstrated in brain smears or histopathological sections [Prozesky, 1987 ;].

The most common change observed is the effussions of body cavities [Steck,1928]. He observed that hydrothorax amounted to several litres in bovines, about 1/2 litre in sheep and about 20 ml in goats [Steck, 1928]. Hydropericadium is more pronounced in sheep and goats than in cattle, [Henning, 1956]. Oedema of the lungs is also a common finding in animals infected with hearwater and appears more severe in animals that die of the peracute form of the disease [Van de Pypekamp and Prozesky, 1987]. The mediastinal and associated lymph nodes are also oedematous.

Spleenomegally has been observed in the majority of animals, [Uilenberg, 1971; Andreasen, 1974; Illemobade, 1976;].

Steck [1928] found out that about 25% of animals examined showed swollen and slightly pale kidneys in sheep and goats and congested in cattle. Prozesky and Du Plessis [1985] found the kidneys to be swollen and pale and that there was oedema of the perirenal tissue during the first day of the febrile stage.

Congestion and oedema of the brain have been described by several researchers; [Van de Pypekamp and Prozesky, 1987; Piennar, Basson and Van der merwe, 1966].

Most histopathological changes have been described as not striking [Steck, 1928, Alexander; 1931]. These include oedema of the lungs, nephrosis, congestion and oedema of the brain , lymph nodes and the spleen.

IMMUNE MECHANISM

All strains of Cowdria ruminantium so far appear to be cross-protective, apart from two South African mouse- infective strains where the differences might be due to rodent passage and in one case to the method of cross-immunity testing (Cammus and Barre, 1988).

Susceptibility of different local breeds of domestic ruminants to heartwater varies. It has been shown that some breeds and species are more susceptible than others which may be an indication of natural resistance [Ilemobade,1977]. It has been shown that the local breeds of animals are more resistant to the disease than newly introduced exotic animals. This resistance has been suggested to be acquired through long natural selection. Very young calves possess a considerable degree of age-resistance to the disease. Solid or partial immunity follows recovery [Uilenberg, 1977].

The nature of the immunity is not properly known. Serum or large quantities of gamma-globulins from immune or hyperimmunized animals, whether given simultaneously with the infective agent, or during the incubation period or during the clinical disease do not influence the outcome of the infection [Alexander, 1931; Du Plessis , 1970b].

Recovery from the acute phase of the disease is followed by a sterile immunity as suggested by Bennett [1986]. The nature of this immunity is thought to be a combination of both cell-mediated and humoral immunity but is not properly known.

Illemobade and Blotkamp [1976], carried out an experiment on the use of capillary flocculation test for the diagnosis of heartwater.They used antigens prepared from the cerebral cortices of brains obtained from animals killed at the terminal stages of the disease, or animals which had died for not more than six hours from the disease. Flocculation was observed with sera from animals approximately one week after clinical recovery on oxytetracycline treatment. The test was positive for a period of 1-3 weeks after which it was not possible to detect any positive reaction.

All recovered animals were immune to challenge even after the flocculation test could not detect any reaction, thus indicating that the antibodies developed were not a reflection of the immune status of the animal and hence not in agreement with those of earlier workers.

On the other hand Rahim and Shommein [1977], carried out research on hematological studies in goats infected with Cowdria ruminantium. Their results showed a lymphocytosis which was possibly due to the body's cellular immune mechanism by increasing lymphoid cells.This may have indicated a kind of cell-mediated immunity.

Local breeds of cattle in endemic areas have been observed to suffer little mortality and this was suspected to be due to a resistance acquired through long natural selection. Neitz and Alexander [1945], found that calves of local Africander breeds were susceptible to the

disease, while Bonsma [1944], had reported earlier that at one particular breeding station only 5% of Afrikandes were lost to the disease against 60% of imported European beef cattle.

The high resistance to the disease in cattle raised in endemic areas was later confirmed by Henning [1956] and Uilenberg [1971]. These later researchers showed that the resistance was not linked to any particular breed but that even the African zebu , Sahiwals and other local breeds in heartwater-free areas were highly susceptible to the disease.

Calves born of highly susceptible stock were found to be relatively resistant to the disease for a 2-3 weeks period immediately after birth. This resistance was independent of the immune status of the dam . Studies in small ruminants have shown that there is no permanent carrier state in animals recovered from heartwater. Blood was negative after 55 days [Ilemobade, 1976].

The persistence of Cowdria ruminantium in the blood of recovered animals was also studied again by Ilemobade (1978). The results from his studies showed that C. ruminantium could be recovered from the blood of infected animals upto to 40 days after clinical recovery irrespective of whether recovery was spontaneous or drug-induced. He also observed that a challenge of the recovered animals with fresh inoculation of the organism was followed by a period when the organism could be recovered from susceptible goats for upto 50 days after the challenge. This showed that recovered animals could remain carriers for at least 40 days and that immune animals could serve as carriers for an even longer period. Thus, the disease could be perpetuated in endemic areas this way, an important factor in the epidemiolgy of the disease.

Alexander [1931] reported that out of 34 recovered sheep from the disease, 2 died , 5 had a severe reaction, and only two had no febrile response to the homologous challenge (apparently given between fourteen and eleven months after recovery). In the experiments of Neitz (1939), all recovered sheep were solidly immune if tested within six months of recovery and only 10 out of 121 responded by a febrile reaction without any further clinical symptoms to homologous or heterogous challenge 7 to 34 months after recovery. Neitz et al [1947] found that immunity was solid if the sheep were challenged within 2 months of recovery, and that afterwards a gradual progressive immunity followed that remained sufficient to protect against a fatal outcome for at least 4 years.

Immunity in cattle is considered by some authors to be short lived unless it is reinforced by repeated reinfection.The data of Neitz and Alexander [1945] , indicated that the occurence of fatal heartwater is upto 20% of cattle in the 3 1/2 years following immunization. Haig [1955] stated that immunity in cattle may wane considerably earlier than in sheep, many instances being reported where there was little or no immunity after 4 months. Henning [1956], in contrast , found that the protection in cattle was generally sufficient to prevent severe clinical symptoms or death when challenged from 12-18 months after immunization. Chabeuf [1976] reported that 5 of 15 friesian cattle immunized by the infection and treatment method died on naturally contracted heartwater within 2 years after being exposed to natural infection in Madagascar.

Several serological tests have been used in the diagnosis of the disease. These include indirect immunofluorescence test [IFAT] which

detects serum antibodies. It is said that these antibodies have a very short life [about 6 months in cattle, 18 months in sheep], and can only indicate a past infection but not the immune status of the animal tested [Ilemobade, 1981]. Logan [1987] cultured neutrophils infected with C. ruminantium and used these as antigen in IFA test.

CONTROL AND PROPHYLAXIS

Heartwater as a disease has proved difficult to control in endemic areas. The control methods which would be employed against this disease would consist of the control of the vector ticks and a prophylactic control involving chemoimmunization. Eradication of the tick vectors has its limitations such as development of tick resistance to the commonly used acaricides . Chemoimmunization is a crude method which involves infecting the animals and then treating them at specified periods using tetracyclines . This method is not very practical in large scale enterprises and also increases the number of carriers thus perpetuating the disease [Neitz and Alexander, 1941].

Immunization is the most practical method in endemic areas where people want to raise improved livestock but where strict tick control measures cannot be implemented. Efforts are being made at present in South Africa and Nigeria to find better ways of immunization.

There are considerable differences in the virulence between the various strains of C. ruminantium [Neitz,1968], while there are no immunological differences [Winkelhoff and Uilenberg, 1981]. Thus a

strain of low virulence can be selected for immunisation purposes since the antibodies or the immune factors produced by the animal host can cross-protect.

CHAPTER 3

MATERIALS AND METHODS

Experimental Animals

Twenty sheep were purchased from well-managed farms in arears adjacent to Nairobi where heartwater is not very common. These sheep were about one and a half years old, of Dopper breed, and not pregnant. The sheep were housed in the medicine unit and fed on hay and wheat bran and given water ad libitum.

The organism

This was obtained from Veterinary Research Laboratories, Kabete, where the organism was obtained from a positive case of heartwater. From these positive cases, blood was obtained [EDTA blood]. For preparation of a blood stabilate a preservative, DMSO [8 dimethyl sulfoxide] was added into the blood then stored in 10 ml vials at -196^{o}C [in liquid nitrogen].

METHODS.

Fifteen sheep were infected while five were used as controls. First a thorough physical examination was carried out noting the vital parameters viz, body temparature, pulse, heart and respiratory rates. After this pre-infection samples were taken for four consecutive days for baseline values. Five ml EDTA blood and 20 ml blood were collected.

Haematology

The following were analysed.

1.Packed cell volume (PCV)

2.Total protein (TP)

3.Hemoglobin concentration (Hb)

4.Red cell count (RBC)

5. White cell count (WBC)

6.Differential cell count of leucocytes.

Neutrophils

Lymphocytes

Eosinophils

Monocytes

Biochemistry.

The following were analysed.

1. Total protein (TP)
2. Albumen.
3. Globulin.
4. Alkaline phosphatase (AP)
5. Alanine aminotransferase (ALT)
6. Aspartate aminotransferase (AST)
7. Serum Blood urea nitrogen (BUN)
8. Creatinine phosphokinase (CPK)
9. Lactate dehydrogenase (LDH)
10. Glucose

After the 4th day,15 of the sheep were infected with 10ml blood stabilate of Cowdria ruminantium. 5 of the sheep was used as a control.

During the incubation period, body temperatures were monitored in all sheep daily.

Blood samples, EDTA and clotted blood, were collected after every two days and analysed in the same way as was done during pre-infection period.

After fever onset the animals were bled daily for three consecutive days, and there after every other day until death or recovery.

Data analysis

Analysis of variance was used in this study. The differences were significant if P was less than 0.05 (at 5% level of significance) or 0.01 (at 1% level of significance).

Determination of the cell associated with C. ruminantium in blood

Two procedures were applied:

1. A method of separating individual peripheral blood leucocytes.
2. Culturing infected neutrophils (Logan, 1985)

Separating individual peripheral blood leukocytes

From the literature review, some authors have documented that Cowdria ruminantium is associated with the leukocytes The particular cell, however, is still a controversial issue.

This procedure was aiming at investigating the cell fraction associated with the organism and if the organism could be cultured in vivo using this cell.

In order to study this parasite-blood-cell association there arose the need to be able to isolate and recover the individual cells in peripheral bovine blood.

STUDY OF THE CELL FRACTION ASSOCCIATED WITH COWDRIA RUMINANTIUM

PROCEDURE;

1. A thorough physical examination was first carried out
2. The sheep was bled for three consecutive days for baseline values; 5ml EDTA blood for hematolgy -PCV, Hb, RBC, WBC, TP and differential white cell count.
3. The sheep was then injected intravenously with a blood stabilate of Cowdria ruminantium obtained from Veterinary Laboratories (kabete), a Tanariver isolate.
4. Body temperature of the sheep was then monitored daily until the fever was 41.5^{o}C

5. Forty ml of EDTA blood was tthen withdrawn (liquid EDTA was used as the anticoagulant).

6.Separation of cell was then done using the ficoll-hypaque technique as follows:

After the blood was taken it was transferred to the laboratory within 30 minutes of collection. The blood was then put into a 125 ml sera bottle and diluted into 1:3 by adding approximately 80 ml of calcium-magnesium free Dulbeccos phosphate buffer (DPB) into each bottle. The diluted blood was carefully layered over the Ficoll-hypaque grantient solution -18.5 ml of diluted blood and 8.0 ml of the ficoll-hypaque solutin per tube.

A distinct interface was formed between the blood and the gradient solution. However, in order to facilitate the smooth delivery of the diluted blood onto the gradient without disturbing the interface, the walls of each tube had been coated with a thin layer of the gradient solution. A small amount of the blood was run gently down the moist tube walls in a smooth broad band reaching the gradient surface as a broad fluid front. The rest of the blood was put into the tube upto the shoulders. The blood was then centrifuged at 1,000 g for 20 minutes. Three layers were formed;

Layer 1, plasma fraction.(p)

Layer 2, mononuclear cells(MN)

Layer 3. polymorphonuclear cells(PMN).

The plasma fraction was aspirated to a point 5 mm above the distinct white band of the mononuclear cells which was formed at the original blood gradient interface. The MN rich fraction was aspirated to a point just 2 mm below the interface and the PMN cell-rich

fraction was then aspirated , leaving the erythrocyte -PMN fraction in the tubes. The PMN cells were recovered by lysing the erythrocytes with distilled water, followed by reconstitution to tonicity with phosphate buffer saline(PBS).The resultant PMN cells were added to those of layer 3. The MN and the PMN cells from layer three were freed of erythrocytes in the same way . One-tenth ml fraction of each leucocyte prepartaion was then mixed with 0.9 ml of 0.05 % of trypan blue vital stain and examined in the Improved Neubauer haemocytometer for viability and concentration of the cells.

To obtain glass adherent and non adherent MN cells, the MN cell preparation was washed twice in DPB and resuspended in the growth medium, fortified with 20% Fetal calf serum and antibiotics (penicillin 100 IU and streptomycin 100 mg).

The cells were passed through a series of transfers in 16 oz glass bottles and incubated for 3 hours before each transfer. The cells that remained in suspension were centrifuged at 150 g for 15 minutes. The cell pellets were resuspended at the appropriate concentrations for the different assays. Adherent cells were recovered from the glass bottles by elution using several changes of buffered trypsin-versene (0.25% trypsin and 0.02% versene). The adherent cell suspensions were washed once before culturing. The cells were then incubated and checked for any growth of the mononuclear cells at intervals of 12 hours for three days. The cells were then harvested and stained using giemsa stain and examined under the microscope for Cowdria ruminantium.

PROCEDURE OF CULTURING NEUTROPHILS

This was aimed also at finding out if the organism is associated with the neutrophil cells in circulation. Logan (1987) did some work with Cowdria ruminantium and showed in her work that the organism was associated with the neutrophil. In this experiment the Logan's procedure was used

Sheep "no 34", a cross between dopper and persian breeds was used. It was female, about 2 years old and was from the faculty farm, Kabete. The sheep was fed with hay and bran and offered water ad libitum.

First a thorough general and physical examination was carried out prior to infection. All body systems were normal. Five ml EDTA blood and 20 ml clotted blood was then collected by venipuncture from the jugular vein for hematology and biochemistry.

The sheep was then injected intravenously with 5 ml blood stabilate which had been prepared from a positive case of heartwater and preserved in liquid nitrogen (-196^{o}C). This was the Tanariver isolate.

The temperature was then monitored daily. At the onset of the febrile reaction the sheep was injected 5 ml of Dexazone^{R} (Dexamethazone). This is a corticosteroid and has the effect of increasing the number of neutrophils in peripheral blood. Eight hours later the animal was bled using ACD as the anticoagulant.

ACD= 8g citric acid

22g sodium citrate

24.5g glucose per 1ml of distilled water

150 ml of blood was collected for culturing and 5 ml for hematology.

SEPARATION OF NEUTROPHILS FROM WHOLE BLOOD

The 150 ml of blood was stored in 4°C immediately it arrived in the laboratory. It was then transferred into 4 by 35 ml aliquotes and centrifuged at 2,000 rpm (700g) for 15 minutes. The plasma part and the buffy coat were discarded. The erythrocytes were lysed by adding 20 ml distilled water and 10 ml 2.7% sodium chloride. This was then centrifuged at 1,000 rpm (200g) for 10 minutes. A pellet was formed at the bottom of the tube. Most of the solution was discarded.

The erythrocytes were lysed again in the same way. The solution was discarded and the pellet resuspended in Hanks BSS - 20ml into each tube- then centrifuged at 1,000 rpm (200 g) for 10 minutes. Most of the solution was then discarded and the pellet (cells) were resuspended and put in cell line bottles. The culture media was then prepared as follows;

10% of fetal bovine serum

10% Hepes buffer

80% RPMI with L-glutamine supplemented with penicillin, streptomycin and Fungizone ^{R} (Amphotericin B) mcg/ml. Into each bottle 5 ml of cells and HBSS and 20 ml of the culture media were put and placed in an incubator at 37°C. These were incubated for 48 hours after which some of the cells were harvested and placed in on slides and stained with giemsa. The harvesting was then done again

in 72 hours and the slides stained using giemsa and kept in a deep freezer (-72°C)

PROCEDURES

HEMATOLOGY

PACKED CELL VOLUME AND TOTAL PROTEIN (PCV AND TP)

The capillary tube was filled with EDTA blood to 3/4 full and centrifuged using a haemofuge for 5 minutes at 10,000 rpm. A microhematocrit reader was used to read PCV and a hand refractometer for TP.

CELL COUNT AND HAEMOGLOBIN CONCENTRATION

A coulter counter ZM was used for these parameters.

White blood cell count.

Whole unclotted blood was diluted with isoton to a dilution of 1: 50,000, then the cells were counted. [principle is given below]

Red blood cell count

Whole blood was diluted with isoton to a dilution of 1:250,000 then the cells were counted [principle is below]

Haemoglobin concentration.

To determine the haemoglobin concentration, 6 drops of zap-o-globin were added into the white cell diluent. The Hg concentration was then read directly on the hemoglobinometer in grams %.

Differential count of white blood cells.

A blood smear was made from the EDTA blood and stained using giemsa. Counting of the cells was done using a microscope and expressed as a %.

BIOCHEMISTRY.

Total protein.

The Biuret method was applied.

1.0.5ml of the test sample was put into a test tube.

2. 9.5 ml of 23% Na_2SO_4 was added into this and mixed. A precipitate was formed.

3.3 ml of the precipitate was transferred into another test tube into which 5 ml of the biuret reagent was added and mixed.

The mixture was left for 30 minutes after which it was read directly on a spectrophotometer (Model ASA 24).

ALBUMIN.

1. Into the 7 ml of the remaining precipitate [procedure number two above] was added 0.4 ml of tween "80" and ether.

2. The test tube was stoppered and mixed by inverting the test tube twenty times.

3. The mixture was then placed in a water bath at 37°c until it separated.

4. This was then spun for 3-4 minutes at 2,000 rpm.

5. To the 3 ml of the bottom solution [the albumin fraction] 5ml of the biuret reagent was added and mixed. The reaction was timed for 30 minutes and read as for TP.

GLOBULIN.

This was calculated from the difference of total protein and albumin.

ALKALINE PHOSPHATASE (AP).

The determination of AP was done according to the optimised kinetic method recommended by the Germany Society for Clinical Chemistry.

PRINCIPLE.

AP was measured by reacting para-nitrophenylphosphate (p-NPP) with the enzyme [serum] which converts this to para-nitrophenol (p-NP) and phosphate. Production of p-NP is proportional to AP-this is shown as increase in absorbance at 405 or 410 nm.

$$\text{p-Npp} + \text{H2O} \xrightarrow[\text{mg++}]{\text{AP}} \text{P-NP} + \text{po4}$$

p-NPP para- nitrophenylphosphate

p-NP - Para-nitrophenol

mg++ -Magnesium ions

There were three reagents in the kit;

Reagent 1: Diethanolamine -HCL buffer

Reagent 2: Para-nitrophenylphosphate

Reagent 3: Reconstituting fluid

To reagent 2, 10 ml of reagent 3 are added. 10 volumes of reagent 1 and 1 volume of reagent 2 make the working solution.

Test procedure

1. 2.0 ml of the working solution was put into the test tube and incubated for several minutes at room temparature.

2.Forty ml of the sample was then put into the test tube , mixed and the activity measured using a spectrophotometer [Model ASA 24]

Alanine Aminotransferase [ALT/GPT]

For the determination of ALT according tothe optimised kinetic method by the German Society for Clinical Chemistry.

Principle

The activity of ALT present in the specimen was measured by the following method;

L-alanine + a -ketoglutanate $\xrightarrow{ALT}$ pyruvate + glutamate

ALT activity was proportional to NADH consumption, measured as a decrease in absorbance at 340 nm.

Pyruvate + NADH + H^{++} $\xrightarrow{LDH}$ lactate +NAD+

Reagent 1: L-alanine solution

Reagent 2: Lyophilised substrate

Reagent 3: a-Ketoglutarate solution

To the contents of the reagent 2, 10 ml of reagent 1 was added.

Test procedure

1. 1.15 ml of the reagent 2 was put into into a test tube .
2. 250 ul of the sample was added, mixed and incubated for 5 minutes at working temperature.
3. 100 ul of reagent 3 was added and mixed .
4. The activity of the enzyme was measured in iu/l using a spectrophotometer-Model ASA 24.

CREATININE KINASE [CK/CPK]

CPK was determined according to the optimimised kinetic method recommended by the German Society for Clinical Chemistry.

Principle

The activity of CPK was measured by the following method.

Creatinine phosphate +ADP _____ creatinine +ATP

ATP + glucose hexokinase_____ ADP +glucose-6-phosphate

glucose -6-phosphate + NADP_____6 -phosphogluconate + NADPH+H+

Therefore the determination of CPK was proportional to the production of NADPH which was shown by the increase in absorbance at 340 nm.

Reagent 1: Imidazole acetate buffer.

Reagent 2: Lyophilised substrate.

Reagent 3: Creatine phosphate.

10 ml of reagent 1 was added into the contents of bottle 2[R2].

Test procedure

1. 1.4 ml of the reconstituted reagent 2 were put in a test tube .
2. 50 ul of sample were added.
3. The mixture was incubated for 5 minutes.
4. 50 ul of the reagent 3 were then added and the activity measured in iu/l using a spectrophotometer-Model ASA 24.

LACTATE DEHYDROGENASE [LDH]

The activity was determined by the following method.

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ lactate +NAD^+

The activity of LDH was proportional to the consumption of NADH which was followed by the measuring of decreased absorbance at 340 nm.

Reagents.

Reagent 1: Buffered NADH.

Reagent 2: Pyruvate substrate

Phosphate buffer, pH 7.5.

Reconstitution of reagents.- Reagent 1: The contents of bottle 1 were reconstituted with 12 ml distilled water.

Test procedure.

1.1.4 ml of reagent 1 were put into a test tube.

2. 50 ul of the reagent were then added and incubated for several minutes at working temperature.

3. 50 ul of the sample were added and mixed. The activity was then measured in iu/l using a spectrophotometer-Model ASA 24.

BLOOD UREA NITROGEN [BUN]

Berthelot's reaction was applied whereby there is cleavage with urease (Fawcett and Scott, 1960])

Principle.

UREA + $2H_2^+$ Urease ——$2NH^+_4$ + CO_3

Ammonium ions [NH4+] react with phenol and lymphochlorite to give a coloured complex.

Reagents:

Reagent 1: Phosphate buffer urease.

Reagent 2: Urea [standard]

Reagent 3: Phenol.

Reagent 4: Sodium lympochlorite + Sodium hydroxide.

Reagents 3 and 4 were reconstituted with double distilled water - amount depending on each kit's instructions.

Test procedure

To 0.10 ml of the reagent 0.1 ml, 0.02 ml of the serum was added. These were mixed and incubated at 37°C (water bath) for 10 mins. After this 5.0 ml of reagent 3 and 4 each were added to the mixture, mixed immediately and incubated for 15 mins at 37°C. The absorbance was then read using a spectrophotometer (Model ASA 24).

GLUCOSE

The principle.

Glucose + O_2 + H_2O $\underline{\text{GOD}}$ Gluconate + H_2O_2

H_2O_2 + ABTS POD coloured complex + H_2O.

It was done according to the calorimetric method recommended by the German Society of Cinical Chemistry.

CHAPTER 4

RESULTS

CLINICAL MANIFESTATIONS

A total of 20 sheep were used in this experiment. Out of the 15 inoculated with infected blood, 5 died, 2 of the five died from the peracute form of the disease while 3 died of the acute disease. The remaining ten were left to run the whole course of the disease upto the convalescing stage. Three of these did not show any clinical signs apart from a fever.

From the results the mean incubation period was 9 days (ranging from 5-10 days) and the mean course of the disease was 7 days. The highest mean temperature recorded was 41.2°C. From the temperature means (FIG.1), the temperature increased steadily from day 9, formed a plateau from day 10 to day 14 after which the temperature dropped to normal values in those animals which did not die at fever

After the fever onset, hardly any other clinical symptom was noted for the first 2 days or so except may be depressed appetite and dullness. Then the animal became very depressed and revealed a very high and thready pulse rate of about 140/min and a high respiratory rate of upto 30/ minute. In some animals no nervous signs were seen prior to death . In those animals the major clinical signs manifested were harsh lung sounds with moist rales, muffled heart sounds and decreased area of cardiac dullness. Inappetance was manifested in all the animals. The few animals which developed nervous signs showed circling, high stepping gait, chewing movements, ataxia and incoordination. Eventually the animals would go into lateral recumbency where paddling was manifested , and die after a few days. All the animals which developed nervous signs died. One sheep was

infected when pregnant (I could not detect the early pregnancy) aborted on the eighth day after fever onset. Diarrhea was noticed in 3 of the terminally-ill sheep.

PATHOLOGY

In those animals which died, similar lesions were observed. Froth in the trachea was an outstanding sign and was thought to be one of the immediate causes of death. The lungs were congested and oedematous and there was hydropericardium, ascites, and hydrothorax in most of the cases. This fluid tended to be straw-coloured . In some cases there were hemorrhages in the spleen, kidney capsule and the lungs. The mesenteric lymph nodes were congested and oedematous. Other intestinal lymph nodes were also congested and oedematous. Brain squash smears were made from the brain capillaries and stained using giemsa to demonstrate the organism. (Figures 2-4).

Histological sections revealed perivascular infiltration, congestion, hemorrhages and oedema of the lungs, congestion and infiltration by inflammatory cells of the brain, lungs and liver and vacuolar degeneration of the brain. (Figures 5-11).

FIG. 1 . Mean Temperature (^{o}C) In sheep infected with Cowdria ruminantium and the controls.

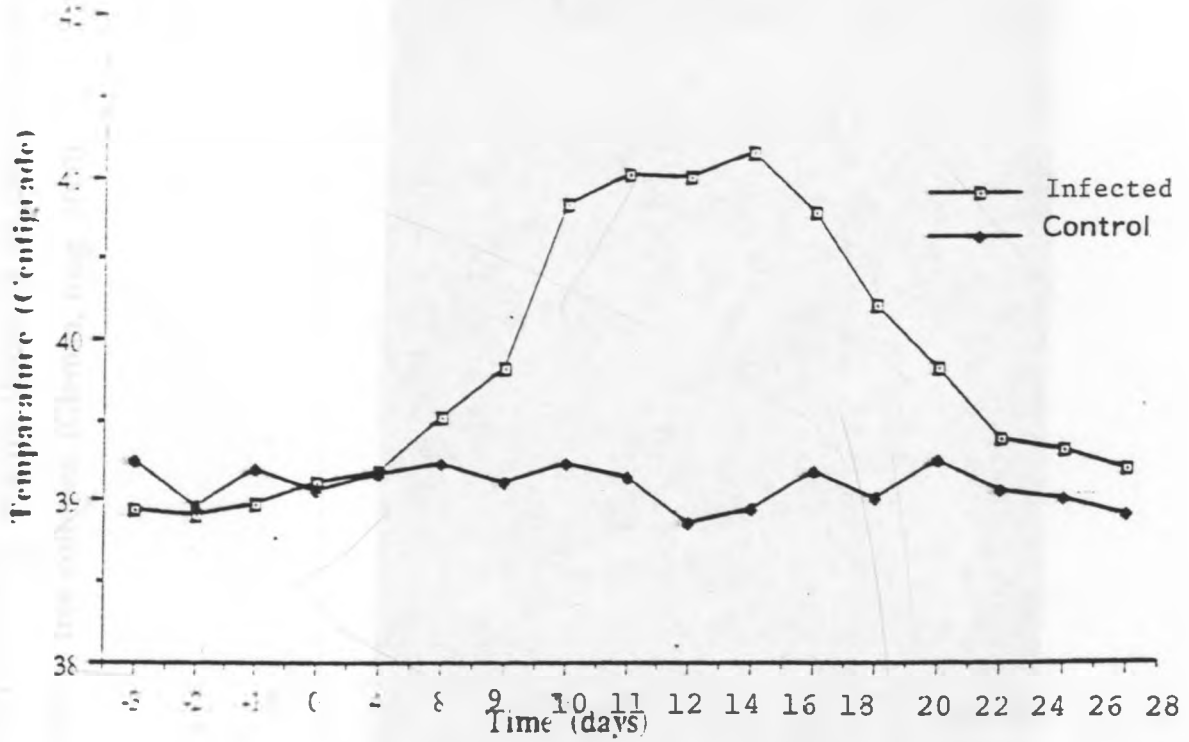


FIG. 2

A brain impression smear showing lymphocytes with Cowdria ruminantium colonies (arrows) in the cytoplasm. The lower cell had ruptured the cytoplasmic membrane releasing the colonies some of which can be seen as free colonies. (Giemsa, mag. 1000).

FIG. 3

A brain impression smear showing a lymphocyte (far right with arrow) about to release one colony of C. ruminantium. (Giemsa, mag. 1000)

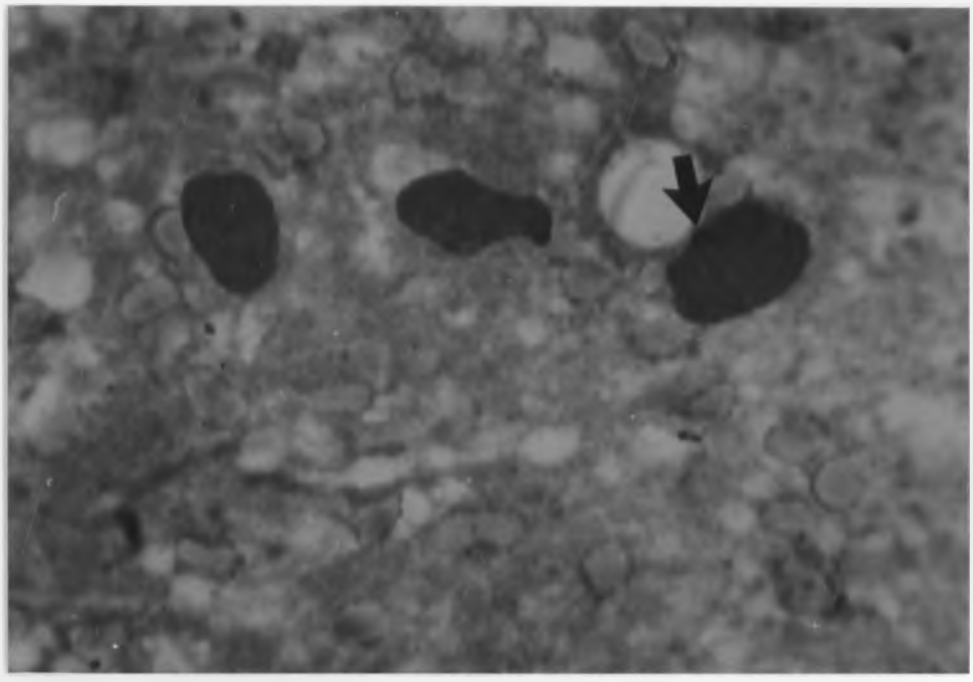


FIG. 4

A brain impression smear showing a lymphocyte releasing C. ruminantium colony (Arrow) which is surrounded by a halo. (Giemsa, mag. 1000).

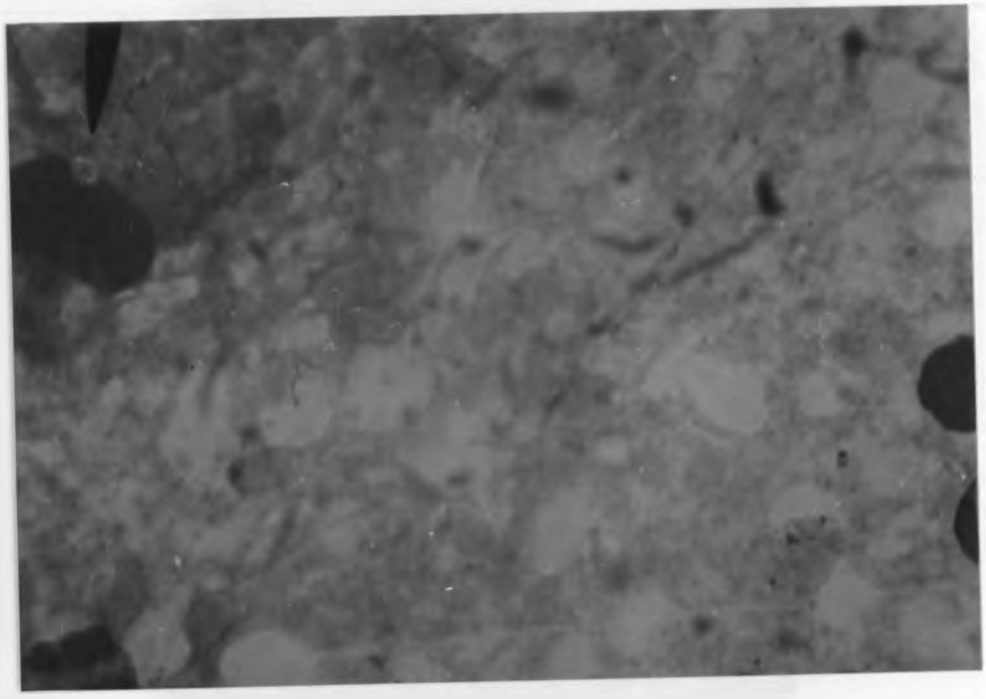


FIG. 5

A heart of a sheep which died of heartwater showing a few petechiations (arrow).

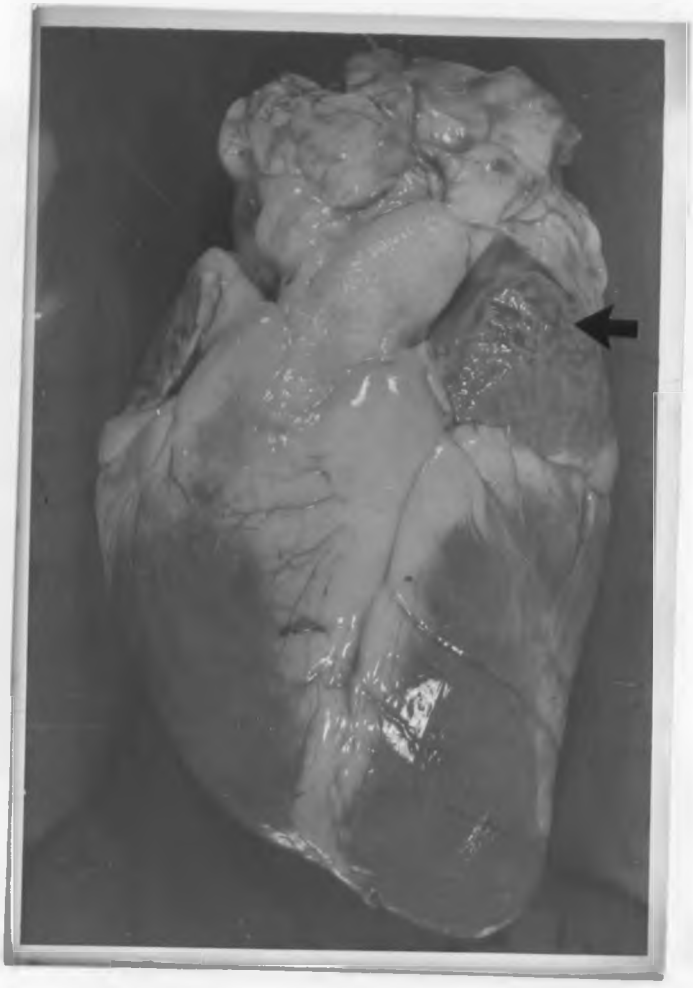


FIG. 6

A lung of a sheep which died of heartwater showing congestion (arrow).

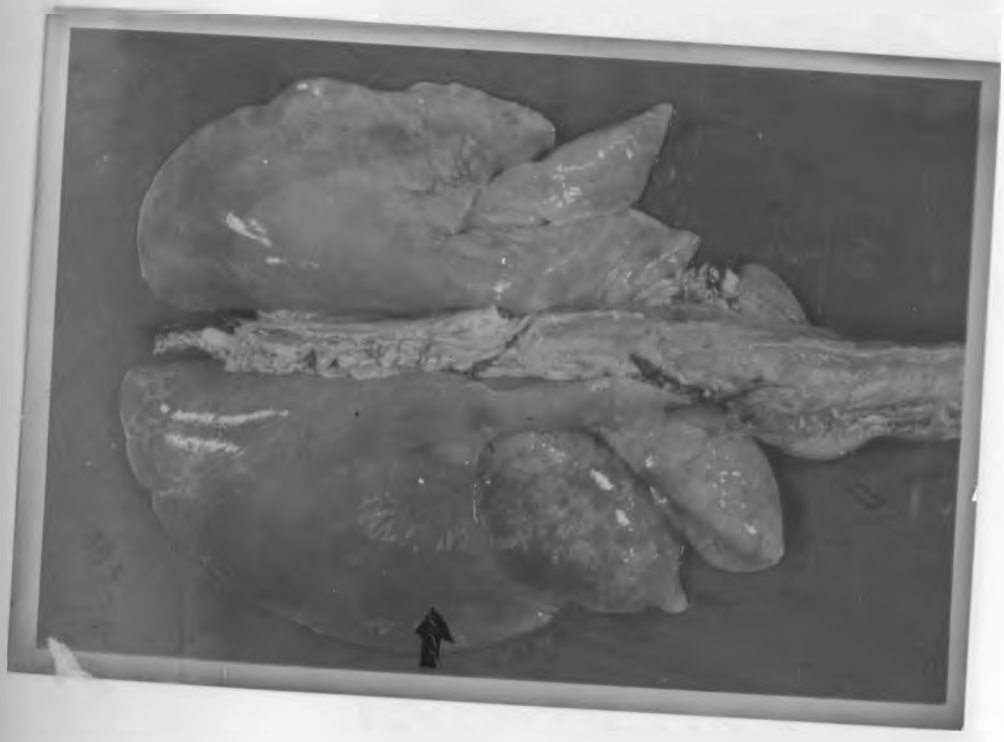


FIG. 7

A brain of a sheep that died of heartwater showing congestion (arrow).

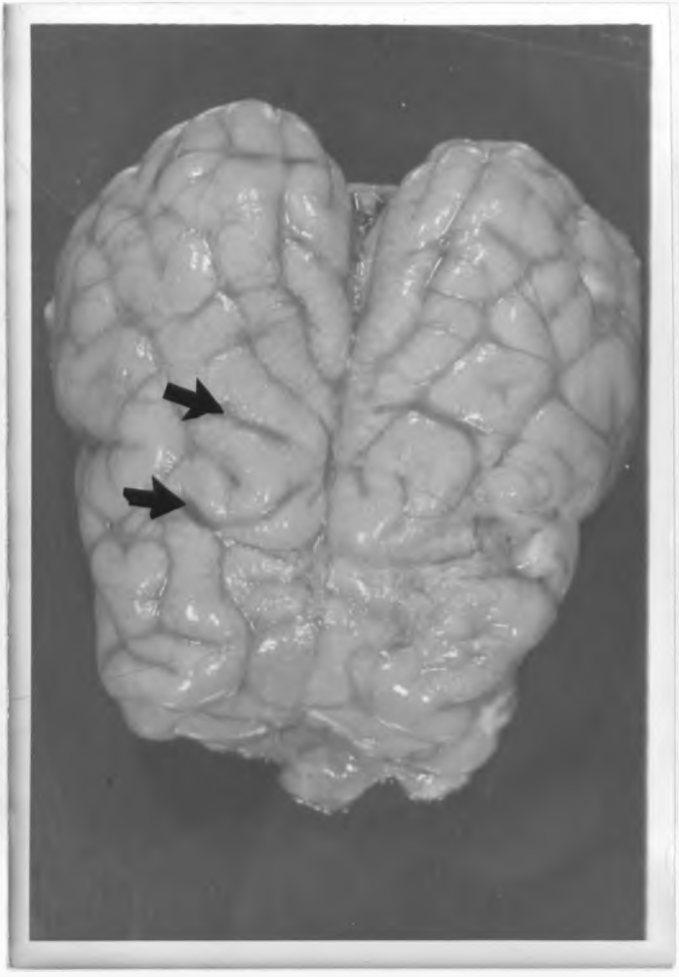


FIG. 8

A histological section of a lung of a sheep which died of heartwater showing congestion (arrow) and oedema (Pink-staining mass). (H.E. 100)

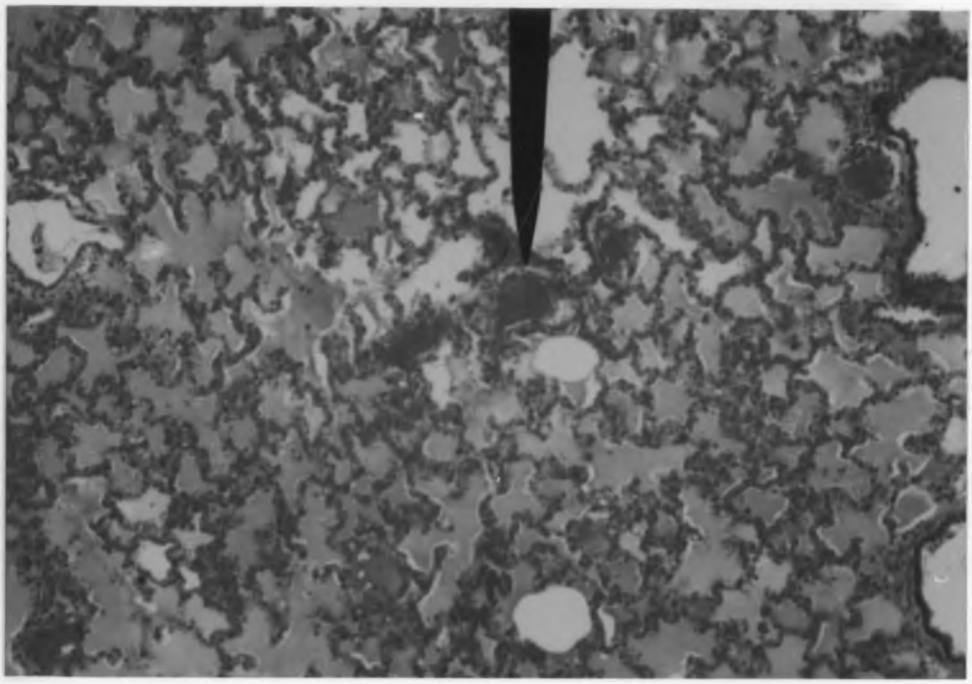


FIG. 9

A higher magnification of figure 7 showing congestion (N), perivascular infiltration (A), oedema (arrow) and haemorhages (H). (400).

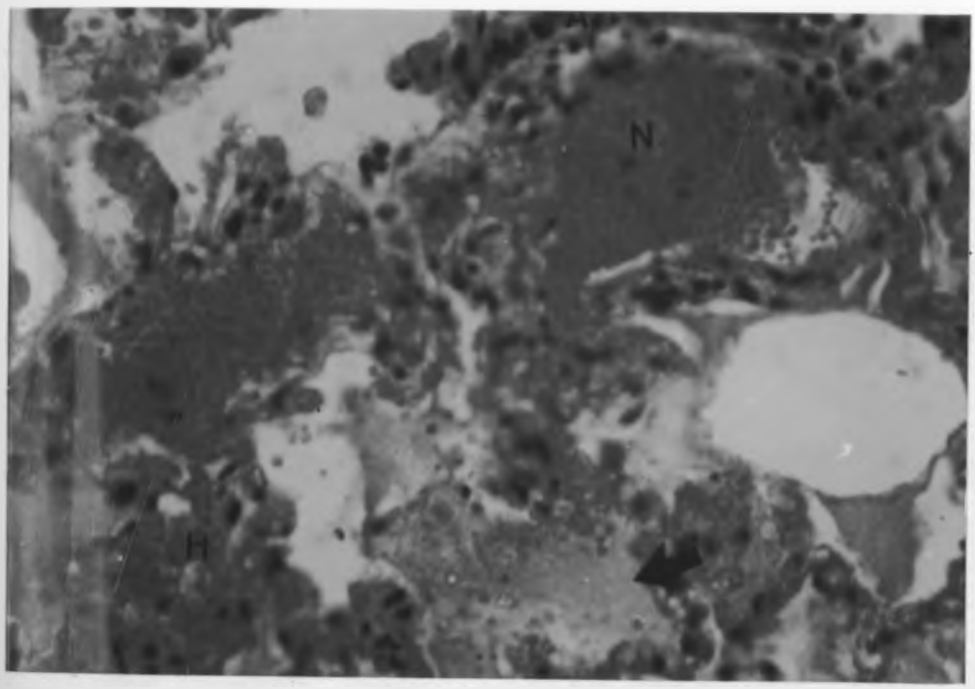


FIG. 10

A histological section of the brain of a sheep that died of heartwater showing vacuolar degeneration (arrow). (H.E. by 400)

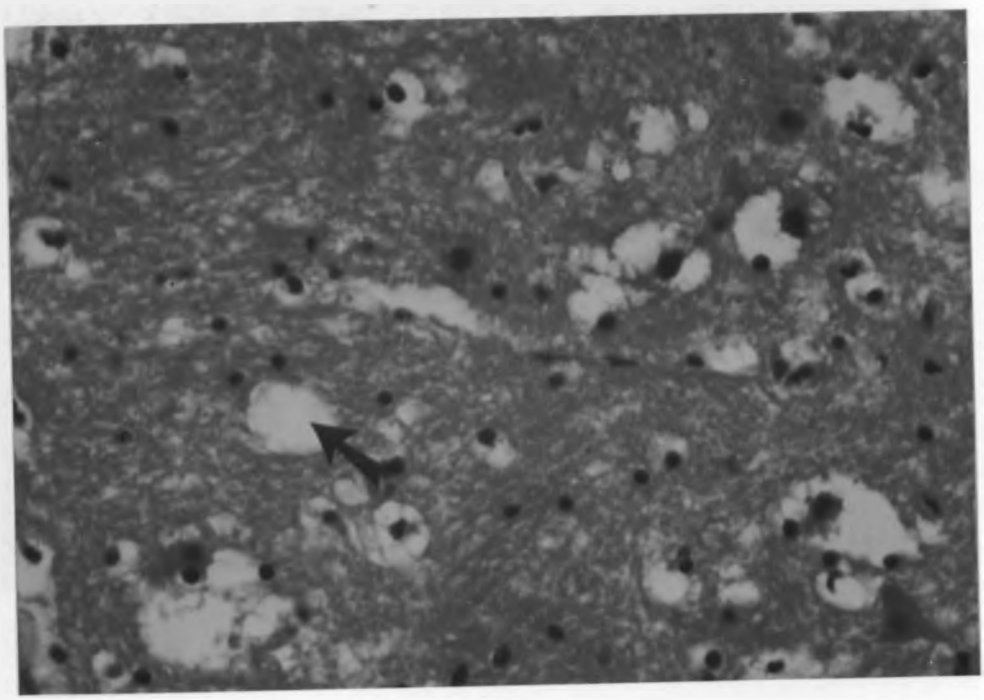
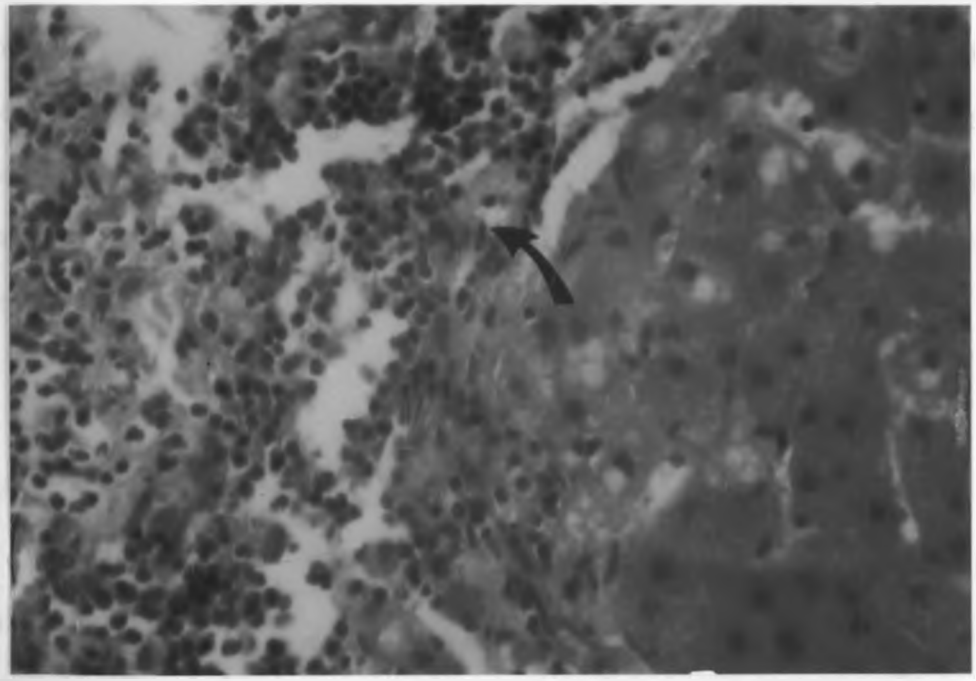


FIG. 11
A histological section of the liver of a sheep that died of heartwater showing infiltration (arrow). (H.E. by 400).



LABORATORY RESULTS.

HAEMATOLOGY

PACKED CELL VOLUME

It was noted that there was a distinct decrease in PCV during the course of the disease. This was noted to start during the febrile stage of the disease and a slight increase was noted just after the temperature came down to normal. The most significant drop was observed on day 9 (the day the febrile reaction commenced) and rose slightly on day 10. The lowest mean PCV was on day 18 after which it started increasing. This was tested statistically and the decrease was found to be of significance at 1% degree of freedom, with a P value of less than 0.01 (figure 12 and table 1).

HAEMOGLOBIN

There was also a drop in the Hb. This was more pronounced in some animals than in others. This decrease correspond to the drop in PCV. The most significant decrease was observed at day 9 (the day there was a febrile reaction). The lowest mean Hb was recorded at day 22 (figure 13 and table 2). On statistical analysis the decrease was significant with a P value of less than 0.01.

RED BLOOD CELL

There was a marked drop in the red blood cell count in all the experimental animals. This drop was noticed to begin just after the fever onset. at day 9 when the most significant drop was recorded. At day 10

there was a slight increase after which there was a decrease upto day 23 when the lowest count was recorded. Statistically this decrease was found to be significant with a P value of less than 0.01. At days 23, 25 and 27 the RBC count was noted to be increasing (figure 14 and table 3).

FIG. 12. Mean packed cell volume (%) in sheep infected with Cowdria ruminantium and the controls.

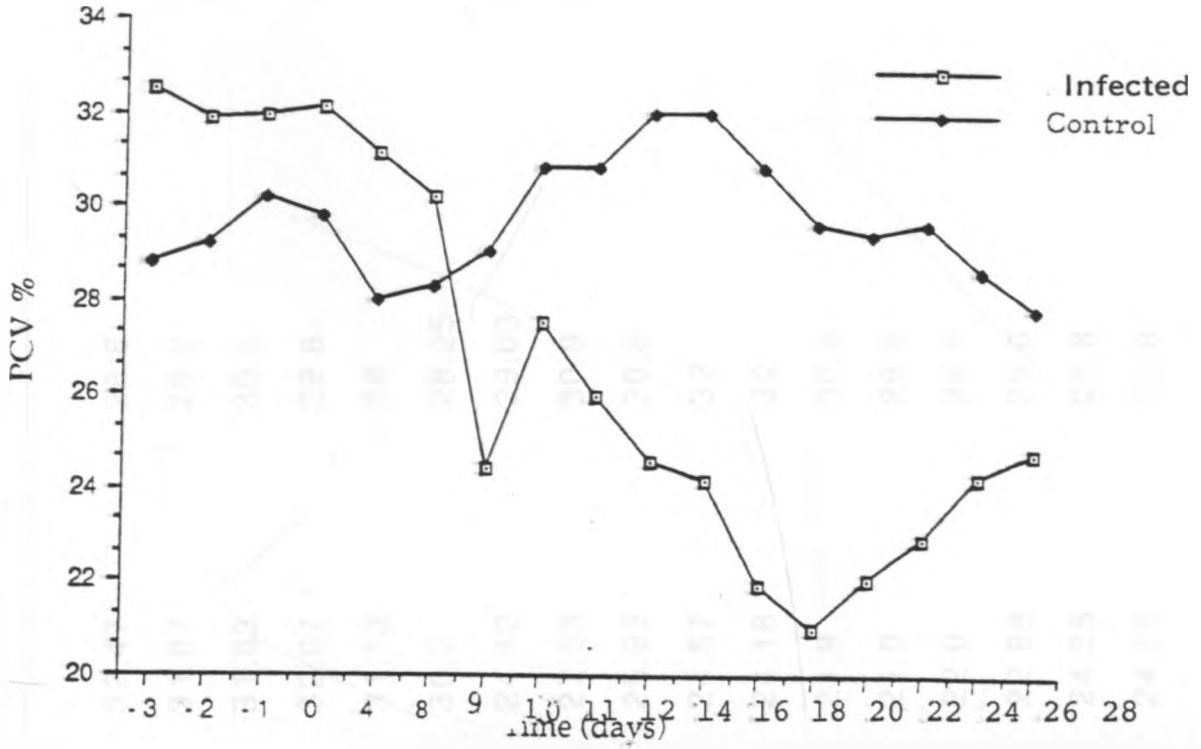


Table 1: Packed cell volume (%) in sheep infected with Cowdria ruminantium and the controls

Period (days)	PCV% (Infected)	PCV% (Control)
- 3	32.47	28.8
- 2	31.87	29.2
- 1	31.93	30.2
0	32.07	29.8
4	31.13	28
8	30.2	28.25
10	24.42	29.03
11	27.53	30.8
12	25.93	30.8
14	24.57	32
16	24.18	32
18	21.9	30.8
20	21.0	29.6
22	22.0	29.4
24	22.88	29.6
26	24.25	28.6
28	24.75	27.8
	27.69 ±0.32	29.73 ±0.22

P <0.01

f= 14.66

FIG. 13. Mean Hemoglobin concentration (mg %) in sheep infected with Cowdria ruminantium and the controls.

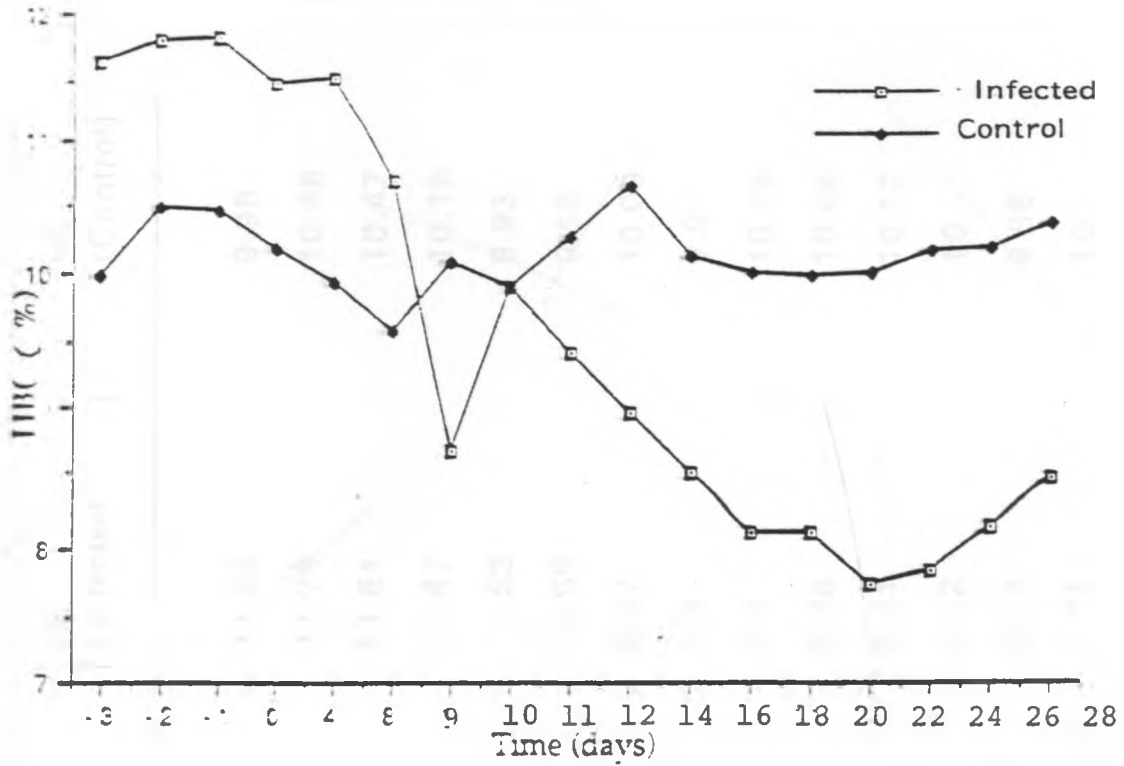


Table 2: Hemoglobin concentration (mg %) in sheep infected with Cowdria ruminantium and the controls

Period (days)	Hb. (Infected)	Hb. (Control)
- 3	11.63	9.98
- 2	11.79	10.48
- 1	11.81	10.47
0	11.47	10.18
4	11.52	9.93
8	10.69	9.58
10	8.67	10.09
11	9.9	9.9
12	9.4	10.26
14	8.96	10.66
16	8.51	10.12
18	8.12	10
20	8.13	9.98
22	7.74	10
24	7.84	10.16
26	8.16	10.18
28	8.46	10.36
	7.74. ±0.21	10.00 ± 0.28

$P < 0.01$

$f=40.19$

FIG. 14. Mean Red blodd cell count ($1o^6$) in sheep infected with Cowdria ruminantium and the controls.

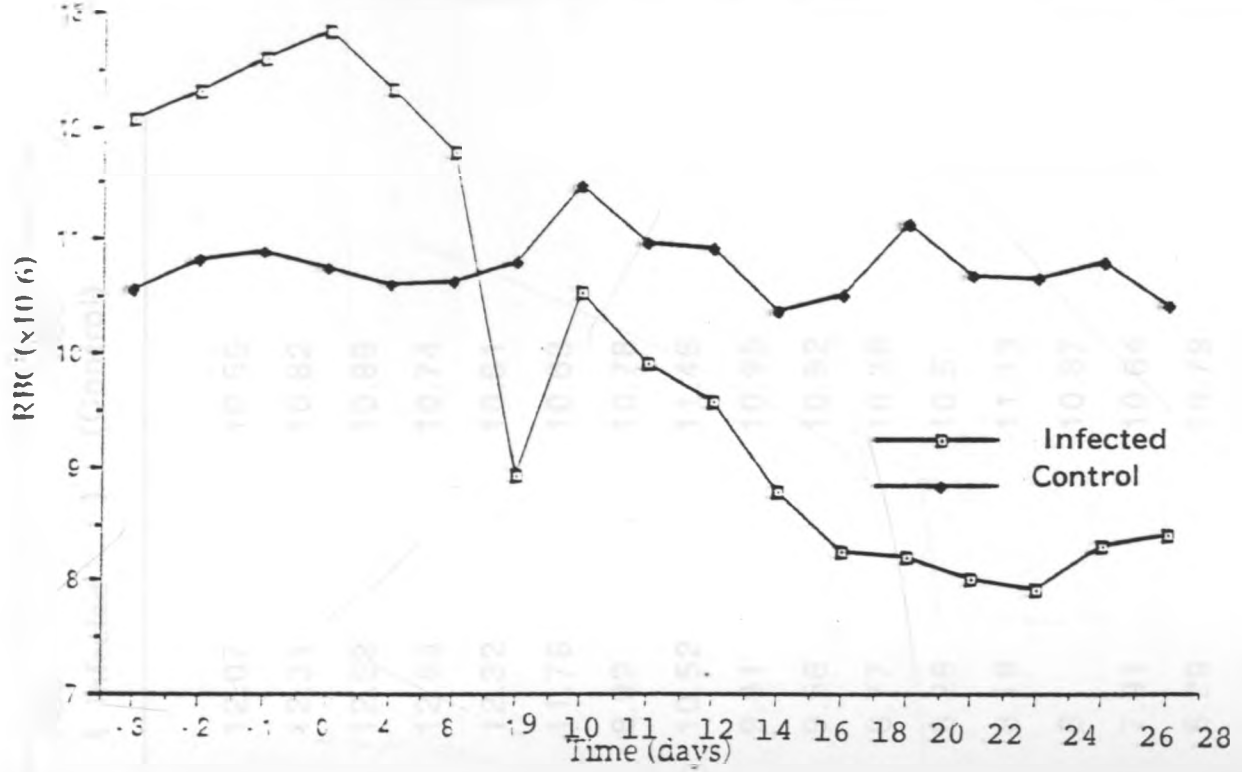


Table 3. RBC (10^6) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	RBC (Infected)	RBC (Control)
- 3	12.07	10.55
- 2	12.31	10.82
- 1	12.59	10.89
0	12.83	10.74
4	12.32	10.61
8	11.78	10.63
10	8.92	10.78
11	10.52	11.46
12	9.91	10.95
14	9.56	10.92
16	8.77	10.36
18	8.26	10.5
20	8.19	11.13
22	8	10.67
24	7.91	10.64
26	8.29	10.79
28	8.39	10.42
	8.00. ± 0.15	10.67± 0.34
	P <0.01	
	f=64.88	

WHITE BLOOD CELL COUNT

Total white blood cells increased markedly in all infected animals. A leukopenia was first noted during the incubation period just prior to the fever onset. A leucocytosis with a neutrophilia and a lymphopenia was noted just after the fever onset. Eosinopenia was also noted with these cells disappearing completely from circulation. The highest mean WBC count was recorded at day 23. On statistcal analysis the changes were found to be significant with a P value of less than 0.01 (figure 15 and table 4).

NEUTROPHILS

Neutrophil count increased significantly as from day 9 after which there was a slight drop noticed on day 10, then an increase recorded continuously upto day 15. At day 17 the mean neutrophil count dropped but rose again to the highest value recorded at day 22 after which there was generally a drop in neutrophil count. (figure 16 and table 5). After statistical analysis the increase was found to be significant with a P value of less than 0.01.

LYMPHOCYTES

There was a decrease in lymphocytes count corresponding to the increase in neutrophil count. This drop was noticed to be significant (with a P value of less than 0.01) first after the febrile reaction after which there

a P value of less than 0.01) first after the febrile reaction after which there was a slight increase at day 10, then a gradual decrease. The lowest value was recorded at day 22 (figure 17 and table 6).

EOSINOPHILS

The mean eosinophil count showed that there was disappearence of circulating eosinophils. Values below 1 indicate that some mean values were 0 at that particular time while others were 1 or more, but later in the disease (around day 16) the group mean was 0. This decrease was also significant with a P value of less than 0.01 (figure 18 and table 7)

FIG. 15. Mean white blood cell count (10^3) in sheep infected with Cowdria ruminantium and the controls.

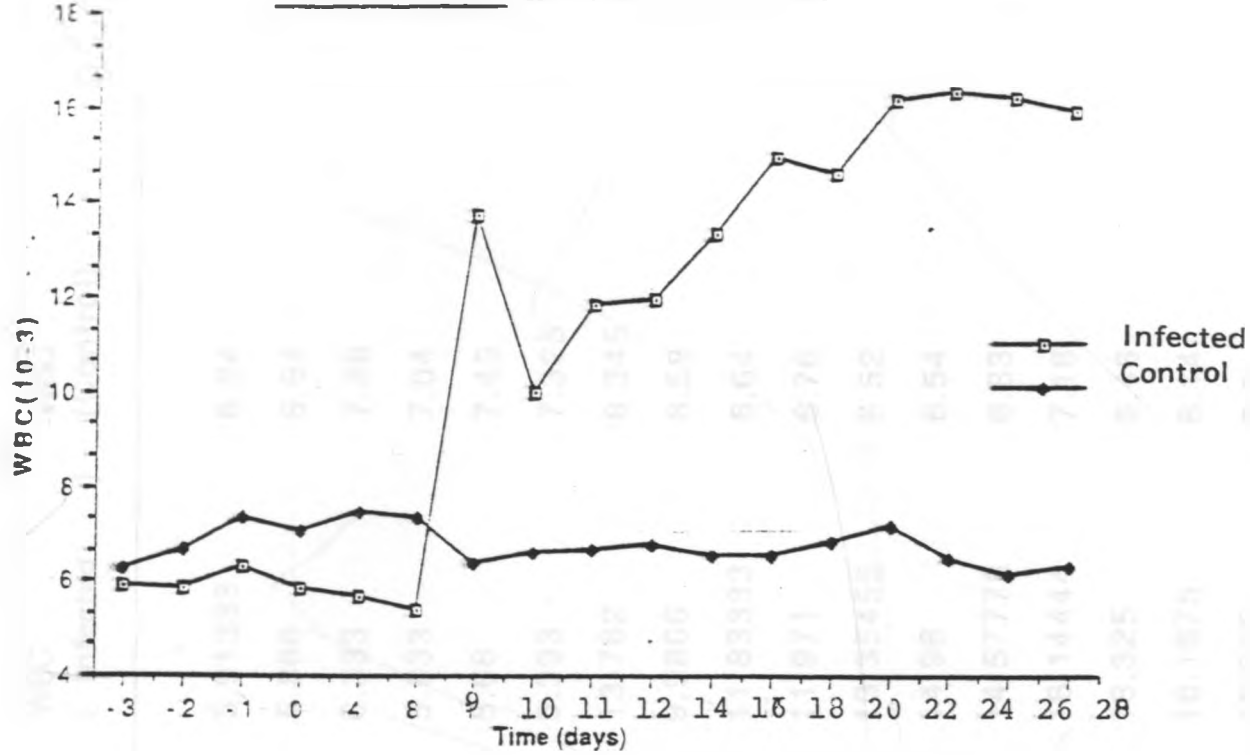


Table 4 White Blood Cells (10^3)In sheep infected with Cowdria ruminantium and the controls

Period (days)	WBC (Infected)	WBC (Control)
- 3	5.91333	6.24
- 2	5.866	6.64
- 1	6.333	7.36
0	5.833	7.04
4	5.66	7.45
8	5.393	7.325
10	13.762	6.345
11	9.9866	6.58
12	11.83333	6.64
14	11.971	6.76
16	13.35455	6.52
18	14.96	6.54
20	14.57778	6.83
22	16.14444	7.18
24	16.325	6.46
26	16.1875	6.14
28	15.925	6.3
	9.882 ± 2.03	6.530 ±3.16
	P <0.01	
	f=41.50	

FIG. 16. Mean Neutrophils (%) in sheep infected with Cowdria ruminantium and the controls.

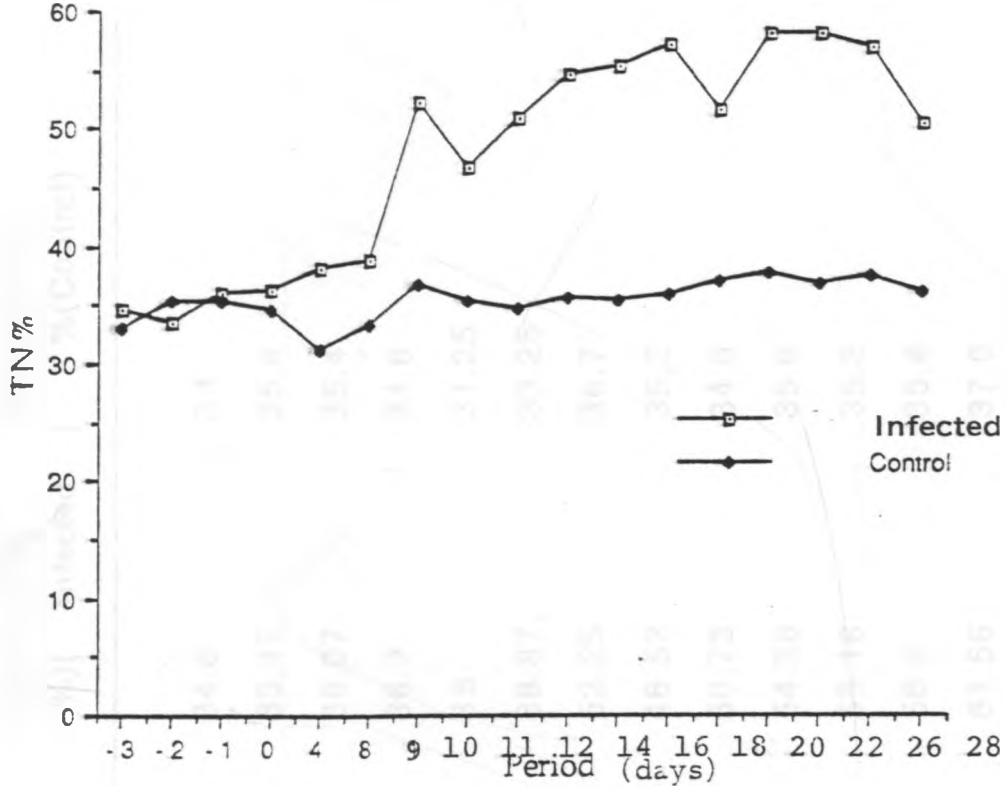


Table 5. Mean Neutrophils(%) in sheep infected with Cowdria ruminantium and the controls

Period (days)	Neutrophils (%)(Infected)	Neutrophils %(Control)
- 3	34.6	33
- 2	33.47	35.4
- 1	36.07	35.4
0	36.2	34.6
4	38	31.25
8	38.87	33.25
10	52.25	36.7
11	46.53	35.2
12	50.73	34.6
14	54.36	35.6
16	55.18	35.2
18	56.9	35.8
20	51.56	37.0
22	58.0	37.6
24	57.88	36.8
26	56.88	37.4
28	50.38	36.0
	45.36.± 0.50	35.34.± 0.82

P<0.01

f=54.87

FIG. 17. Mean lymphocyles (%) in sheep infected with Cowdria ruminantium and the controls.

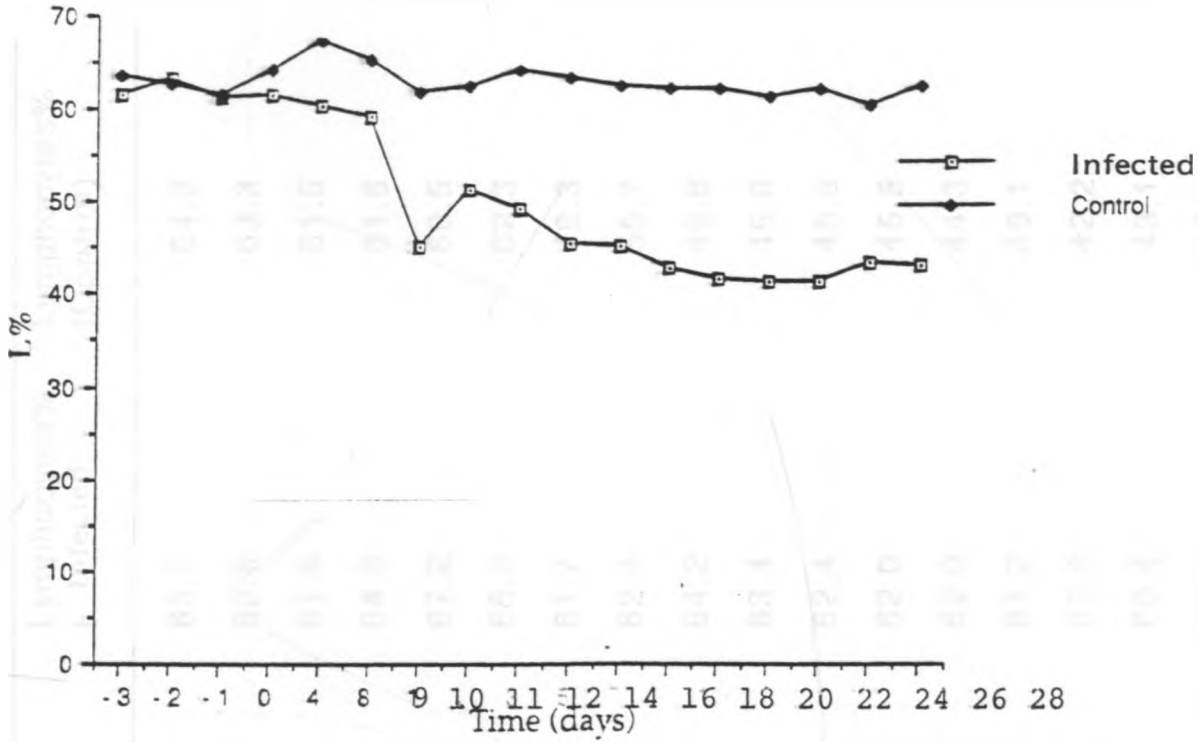


Table 6 Mean Lymphocytes(%) in sheep infected with Cowdria ruminantium and the controls

Period (days)	Lymphocytes% (Infected)	Lymphocytes% (Control)
- 3	63.6	64.3
- 2	62.6	63.3
- 1	61.6	61.5
0	64.0	61.6
4	67.2	60.5
8	65.2	62.3
10	61.7	49.3
11	62.4	55.7
12	64.2	49.6
14	63.4	49.6
16	62.4	46.5
18	62.0	45.5
20	62.0	44.3
22	61.2	49.1
24	62.2	42.2
26	60.4	43.1
28	62.4	49.0
	52.65. ±0.75	62.81 .± 0.53

P<0.01
f= 66.18

FIG. 18. Mean Eosinophils (%) in sheep infected with Cowdria ruminantium and the controls.

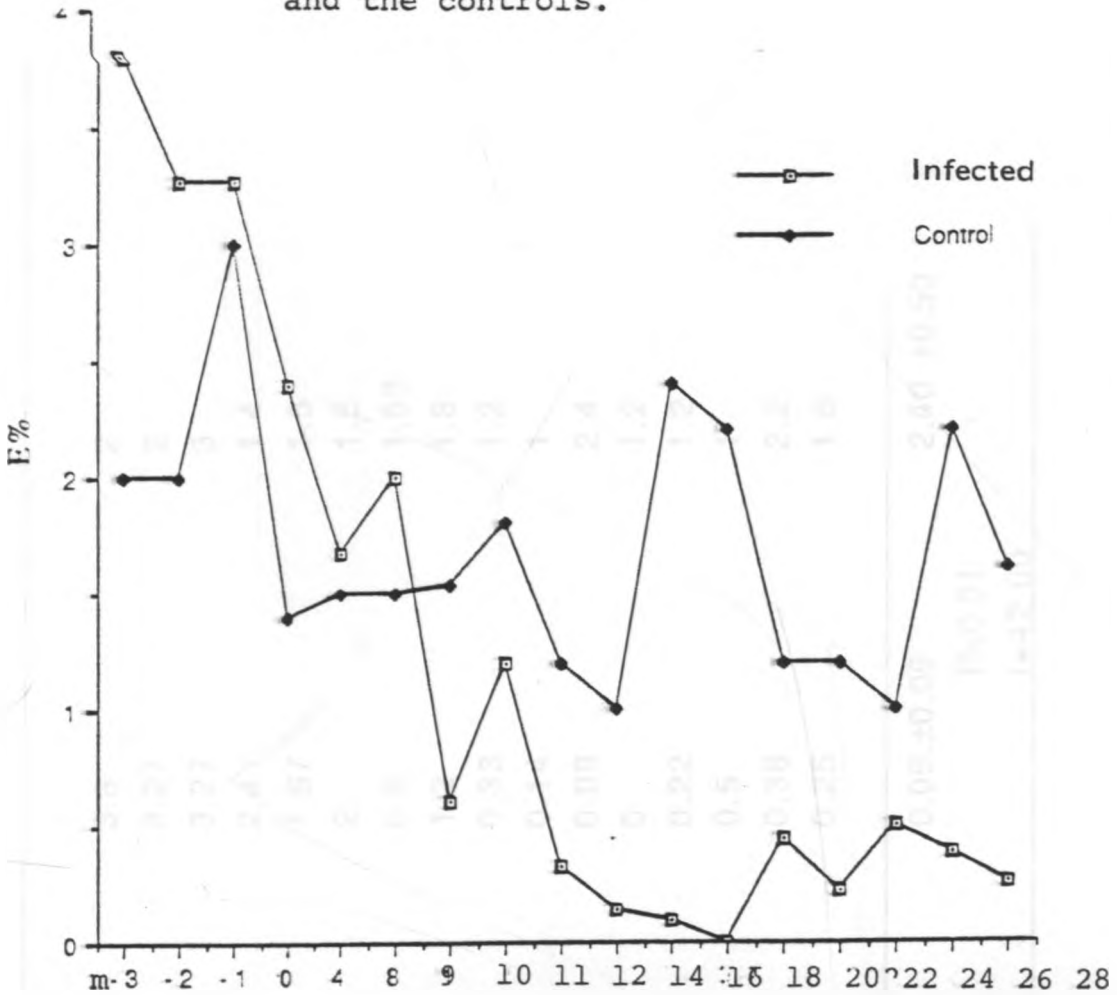


Table 7.Mean Eosinophils (%) in sheep infected with Cowdria ruminantium and the controls

Period (days)	Eosinophils % (Infected)	Eosinophils % (Control)
- 3	3.8	2
- 2	3.27	2
- 1	3.27	3
0	2.4	1.4
4	1.67	1.5
8	2	1.5
10	0.6	1.53
11	1.2	1.8
12	0.33	1.2
14	0.14	1
16	0.09	2.4
18	0	1.2
20	0.22	1.2
22	0.5	1
26	0.38	2.2
28	0.25	1.6
	0.09 ±0.09	2.40 ±0.50
	P<0.01	
	f=42.00	

BIOCHEMISTRY

TOTAL PROTEIN

There was an irregular behaviour of mean total protein. The baseline values of the infected and the controls were totally different, although the animals were housed together. After febrile reaction the experimental sheep showed a decrease in total protein which was significant with a P value of less than 0.01 (figure19 and table 8).

ALBUMIN

There was an irregular behaviour of the mean albumin concentration in both the infected and the control animals. There was a slight drop in the mean albumin concentration at day 9 and day 15 as compared to the controls which was not significant as the P value was greater than 0.01 and 0.05 (figure 20 and table 9).

GLOBULIN

The mean globulin concentration was also irregular in both the infected and the controls. Significant differences were noted with the P value being less than 0.01 (figure 21 and table 10)

GLUCOSE

Glucose concentration increased significantly ($P < 0.01$) after the onset of the febrile reaction i.e day 9, dropped slightly at day 10 then increased steadily from day 11. The highest mean glucose concentration was recorded

at day 22, a time when most animals which died were at the terminal stage and that was when glucose levels were highest. After day 22 there was a general trend for the glucose concentration to decrease for this was a period of convalescence for the surviving animals (figure 22 and table11).

FIG 19. Mean Total protein (mg%) in sheep infected with Cowdria ruminantium and the controls.

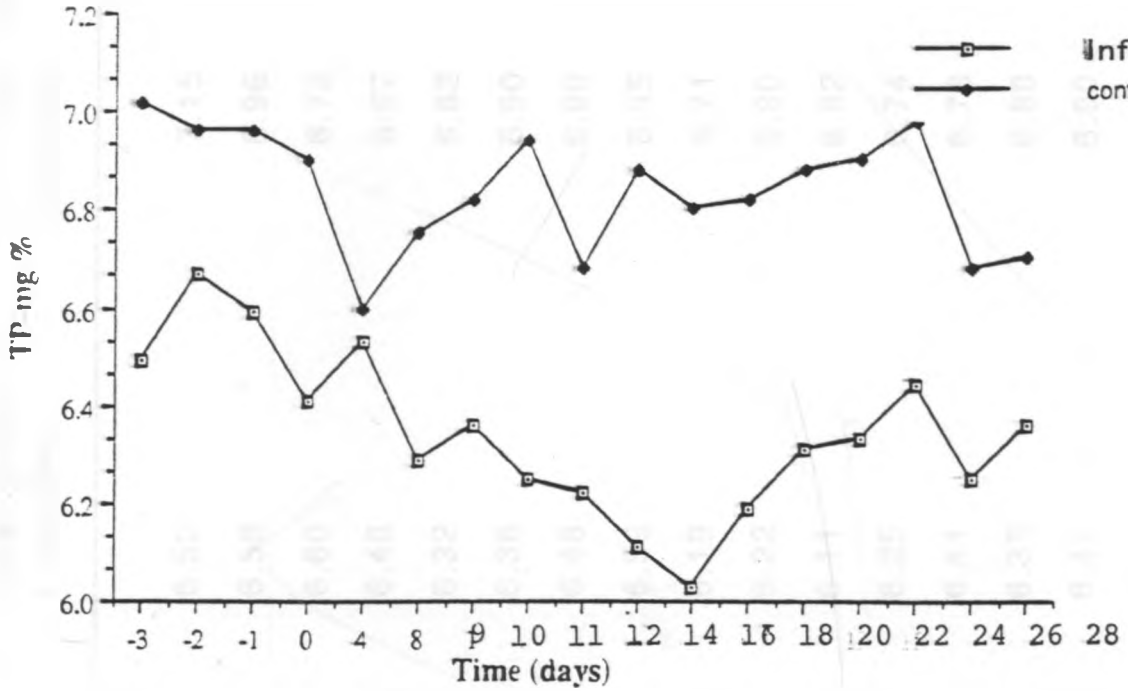


Table 8.Mean Total protein (mg %) in sheep infected with Cowdria ruminantium and the controls

Period (days)	Total protein (Infected)	Total protein (Control)
- 3	6.52	7.15
- 2	6.58	6.96
- 1	6.60	6.72
0	6.48	6.97
4	6.32	6.82
8	6.36	6.90
10	6.46	6.90
11	6.59	6.95
12	6.19	6.71
14	6.22	6.80
16	6.11	6.82
18	6.25	6.74
20	6.41	6.78
22	6.35	6.80
24	6.47	6.90
26	6.41	6.90
28	6.47	6.78
	6.41. ±0.04	6.88 ±0.03

P<0.01

f=38.17

FIG. 20. Mean Albumin (mg%) in sheep infected with Cowdria ruminantium and the controls.

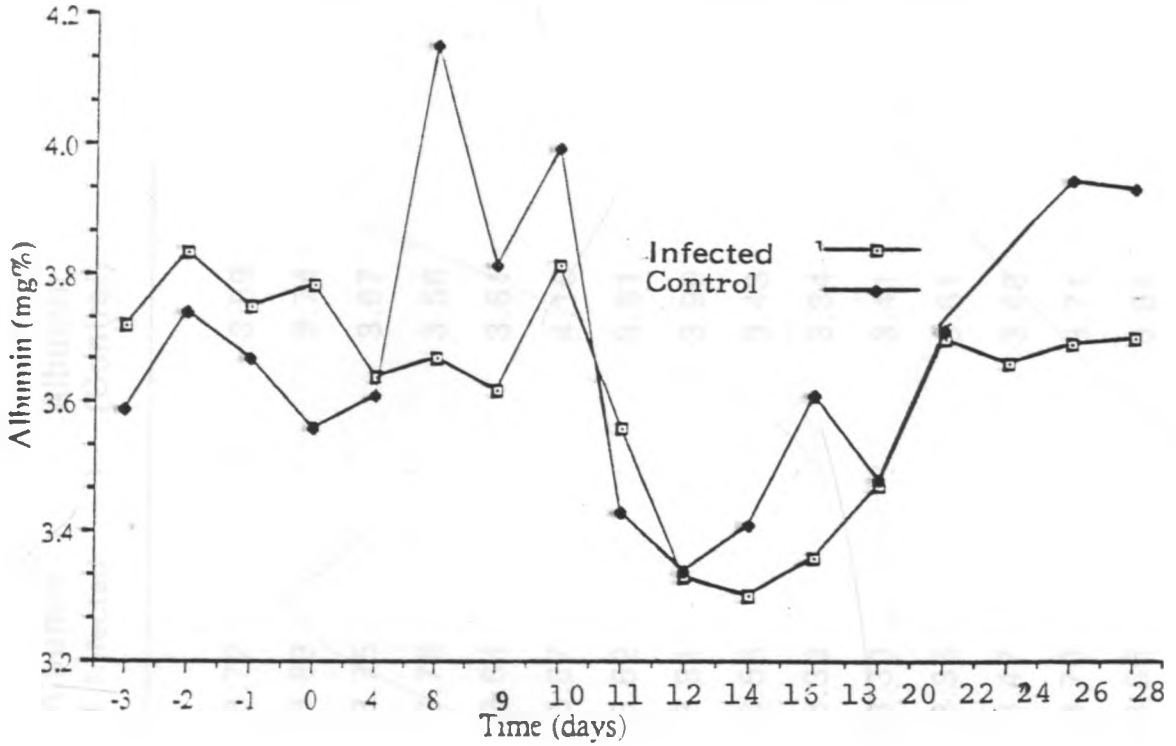


Table 9. Mean albumin (mg %) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	Albumin (Infected)	Albumin (Control)
-3	3.72	3.59
-2	3.83	3.74
-1	3.75	3.67
0	3.78	3.56
4	3.64	3.61
8	3.67	4.15
10	3.62	3.81
11	3.81	3.99
12	3.56	3.43
14	3.33	3.34
16	3.30	3.41
18	3.36	3.61
20	3.47	3.48
22	3.70	3.71
24	3.66	3.84
26	3.69	3.94
28	3.70	3.93
	5.67±0.03	3.70 ±0.04

P>0.05

f=0.36

FIG. 21. Mean Globulin (mg %) in sheep infected with Cowdria ruminantium and the controls.

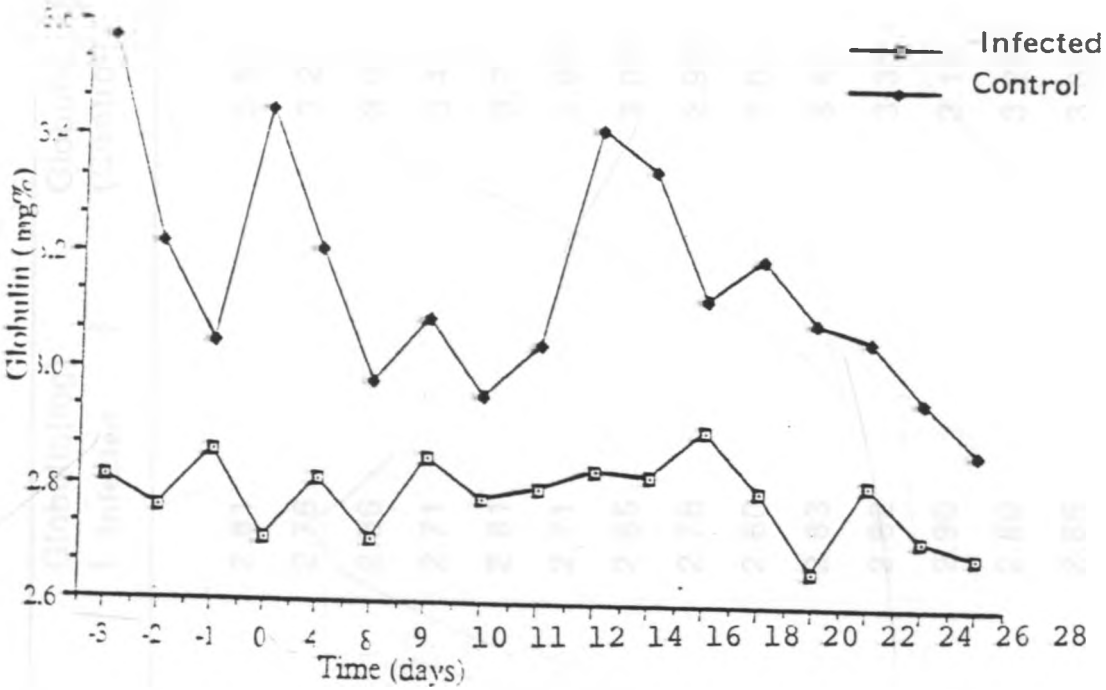


Table 10: Mean Globulin(mg %) in sheep infected with Cowdria ruminantium and the controls

Period (Days)	Globulin(mg) (Infected)	Globulin(mg) (Control)
- 3	2.81	3.57
- 2	2.76	3.22
- 1	2.86	3.05
0	2.71	3.45
4	2.81	3.21
8	2.71	2.98
10	2.85	3.09
11	2.78	2.96
12	2.80	3.05
14	2.83	3.42
16	2.82	3.35
18	2.90	3.13
20	2.80	3.20
22	2.66	3.09
24	2.81	3.06
26	2.72	2.96
28	2.69	2.87
	2.79.± 0.05	3.19. ±0.04

P<0.01
f=40.91

FIG. 22. Mean glucose (Mmol/L) in sheep with Cowdria ruminantium and the controls.

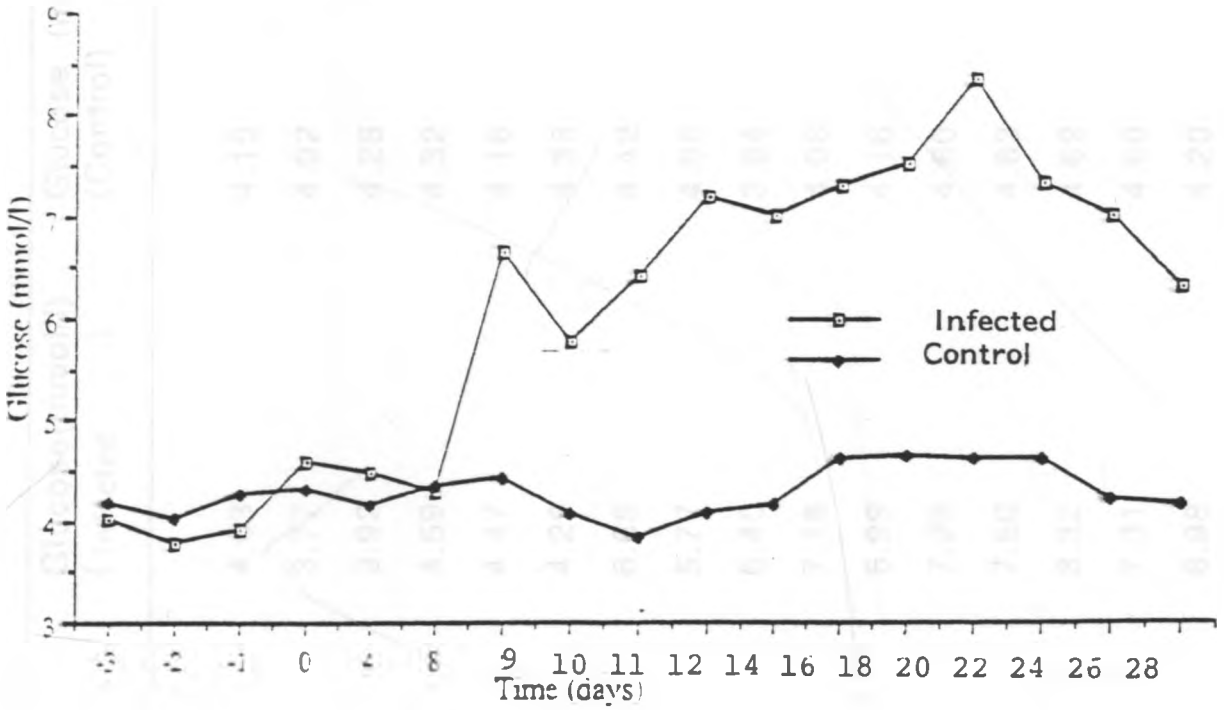


Table 11:Mean Glucose (Mmol/l) in sheep infected with Cowdria ruminanitum and the controls

Period (days)	Glucose(mmol/l) (Infected)	Glucose (mmol/l) (Control)
- 3	4.03	4.19
- 2	3.77	4.02
- 1	3.92	4.26
0	4.59	4.32
4	4.47	4.16
8	4.29	4.33
10	6.65	4.42
11	5.77	4.08
12	6.40	3.84
14	7.18	4.06
16	6.99	4.16
18	7.28	4.60
20	7.50	4.63
22	8.32	4.62
24	7.31	4.60
26	6.98	4.20
28	6.29	4.16
	7.00. ±0.18	4.20. ±0.43

P >0.01
f=23.61

ALANINE AMINOTRANSFERASE (ALT)

In both the infected and the controls this enzyme level in blood was more or less the same except a very slight increase just prior to fever onset and also around day 22. This slight increase in day 22 may be of some significance as this was the mean day at which most sheep died, although there were no significant differences observed and even on statistical analysis ($P > 0.05$) no differences of significance were obtained between the experimentals and the controls (figure 23 and table 12).

ASPARTATE AMINOTRANSIFERASE (AST)

There was an increase in mean AST at day 9. This was found to be statistically significant ($P < 0.01$). There was a decrease at day 10 upto to day 15 when an increase was recorded . This increase was highest at day 22 - mean terminal stage (figure 24 and table 13).

ALKALINE PHOSPHATASE (AP)

The mean AP concentration behaved in a haphazard manner. First the mean concentration values of the infected group and the control groups were very different prior to infection. After infection the values recorded were also irregular. There was a decrease in serum AP. This decrease was noted to be lowest at day 19 and was not significant ($P > 0.05$) (figure 25 and table 14).

FIG. 23. Mean Alanine amino-transferase in sheep infected with Cowdria ruminantium and the controls.

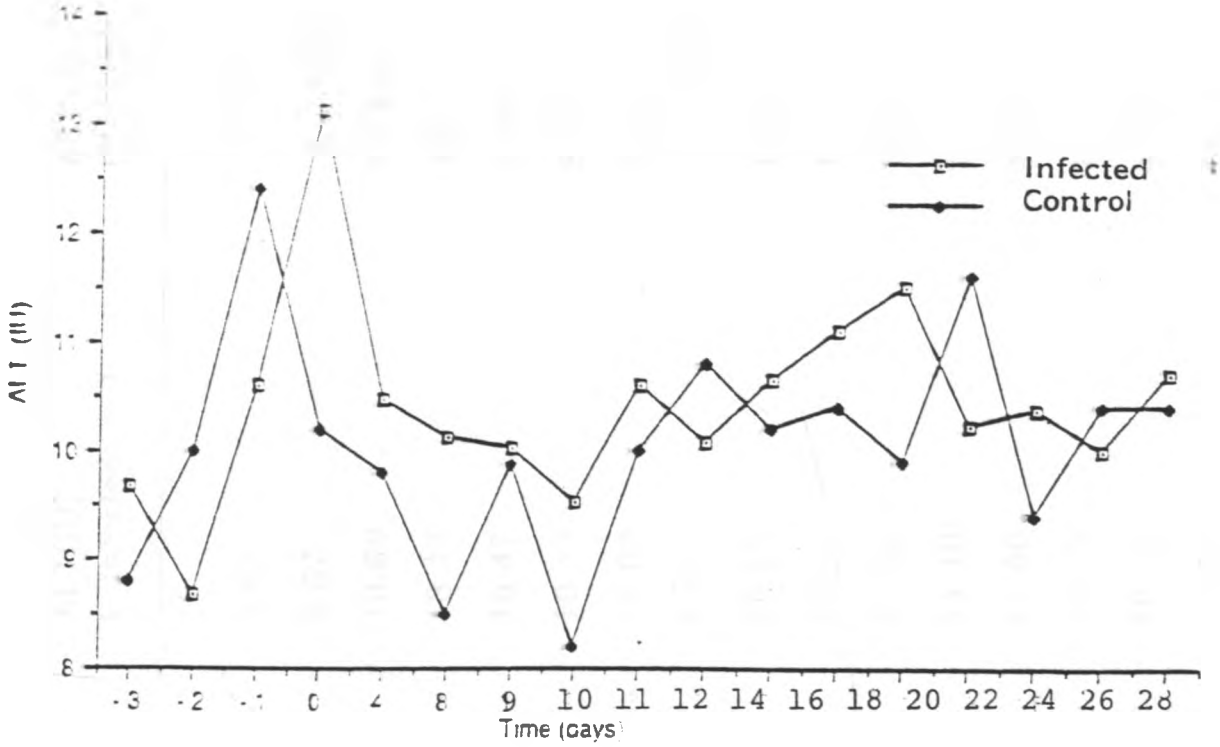


Table 12: Mean Alanine aminotransaminase (IU) in sheep infected with Cowdria ruminantium and the controls

Period (days)	ALT(IU) (Infected)	ALT(IU) (Control)
- 3	9.67	8.80
- 2	8.67	10.00
- 1	10.60	12.40
0	13.13	10.20
4	10.47	9.80
8	10.13	8.50
10	10.02	9.87
11	9.53	8.20
12	10.60	10.00
14	10.07	10.80
16	10.64	10.22
18	11.10	10.44
20	11.50	9.90
22	10.22	11.60
24	10.38	9.40
26	10.00	10.40
28	10.71	10.44
	10.29 ±0.27	9.98 ±0.31

P>0.05
f=0.35

FOG. 24. Mean Aspaitrate amino-transferase(1μ) in sheep infected with Cowdria ruminantium and the controls.

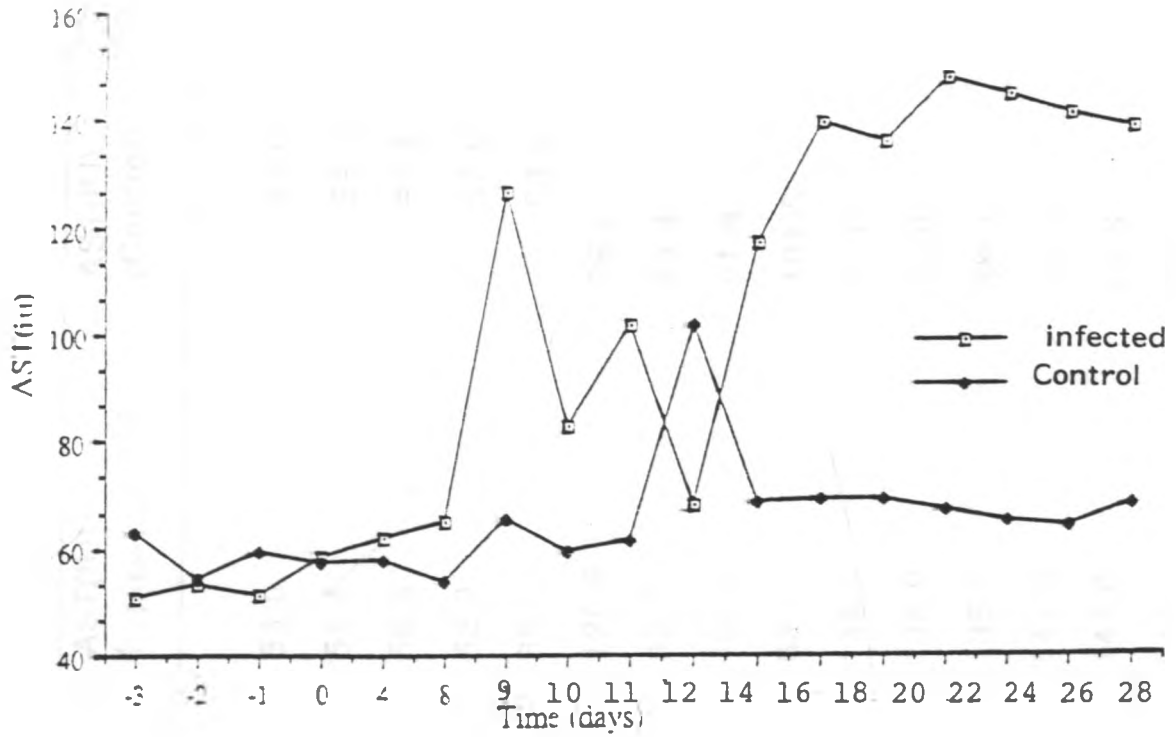


Table 13 Mean Aspartate Aminotransferase (IU) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	AST(IU) (Infected)	AST(IU) (Control)
- 2	53.0	54.0
- 1	51.4	59.2
0	58.3	57.4
4	62.0	57.8
8	65.0	53.5
10	126.6	65.6
11	82.7	59.4
12	101.4	61.6
14	68.2	101.5
16	116.9	68.6
18	138.9	69.0
20	135.7	68.9
22	147.3	66.8
24	144.0	64.8
26	140.7	64.0
28	138.2	67.8
	90.31 ±1.58	62.77 ± 2.93
	P <0.01	
	f=33.19	

FTG. 25. Mean Alkaline phosphatase (1µ) in sheep infected with Cowdria ruminantium and the controls.

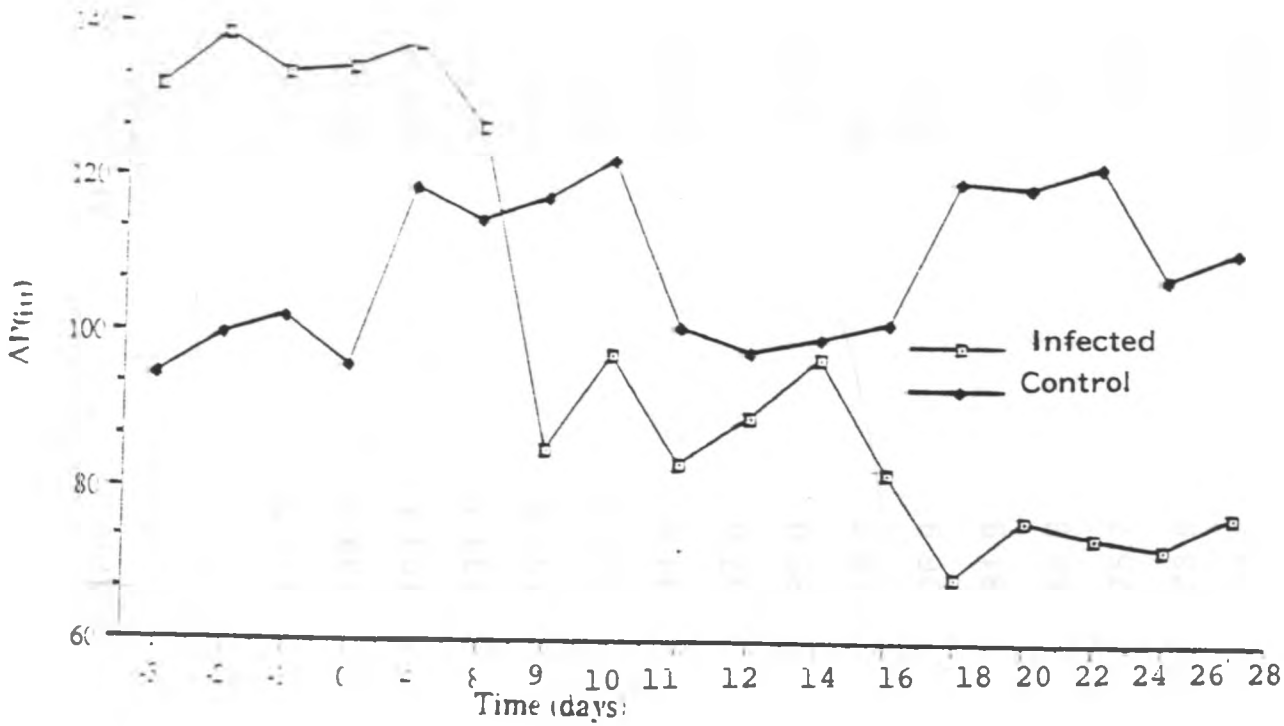


Table 14 Mean Alkaline phosphatase (AP) (IU) in sheep infected with Cowdria ruminantium and the controls

Period (days)	AP(IU) (Infected)	AP(IU) (Control)
-3	131.9	94.4
-2	138.6	99.6
-1	133.4	102.2
0	134.4	95.6
4	137.6	118.8
8	126.4	114.5
10	84.6	117.3
11	97.0	122.4
12	83.0	100.8
14	88.8	97.4
16	96.9	99.4
18	81.9	101.4
20	68.6	119.8
22	75.7	119.2
24	73.8	122.0
26	72.5	107.6
28	76.7	110.8
	107.14.±3.27	107.61±4.38

P >0.05
f=0.01

CREATININE PHOSPHOKINASE (CPK)

Mean serum CPK increased slightly after fever onset (at day 9) and then dropped slightly at day 10. The highest increase was at day 13 after which there was a decrease upto day 22 after which there was a slight increase. This increase at day 13 and 22 also correspond to the time most animals died (figure 26 and table 15).

LACTATE DEHYDROGENASE (LDH)

Mean serum LDH behaved in a peculiar manner in that it increased tremendously between the incubation period and the first day after fever onset. A slight drop was then recorded on day 10 (but not to the baseline values) after which there was a steady increase. The highest increase in infected sheep was recorded at day 22. In individual sheep those terminally ill were showing the highest levels of serum LDH of upto 900 iu This increase ($P< 0.01$) was found to be significant (figure 27 and table 16).

BLOOD UREA NITROGEN (BUN)

There was a general trend of the BUN to increase. During the incubation period the increase was slight and then it was most significant after the febrile reaction, at day 9. There was a slight decrease at day 10 and then an increase was recorded from day 11to day 15 when the mean BUN was at its peak. After day 15 there was a general trend to decrease. In individual cases the highest BUN was recorded in animals at terminal stage. The changes in BUN were significant ($P < 0.01$) (figure 28 and table 17).

FIG. 26. Mean Creahnine phospho-kinase (1µ) in sheep infected with Cowdria ruminantium and the contorls.

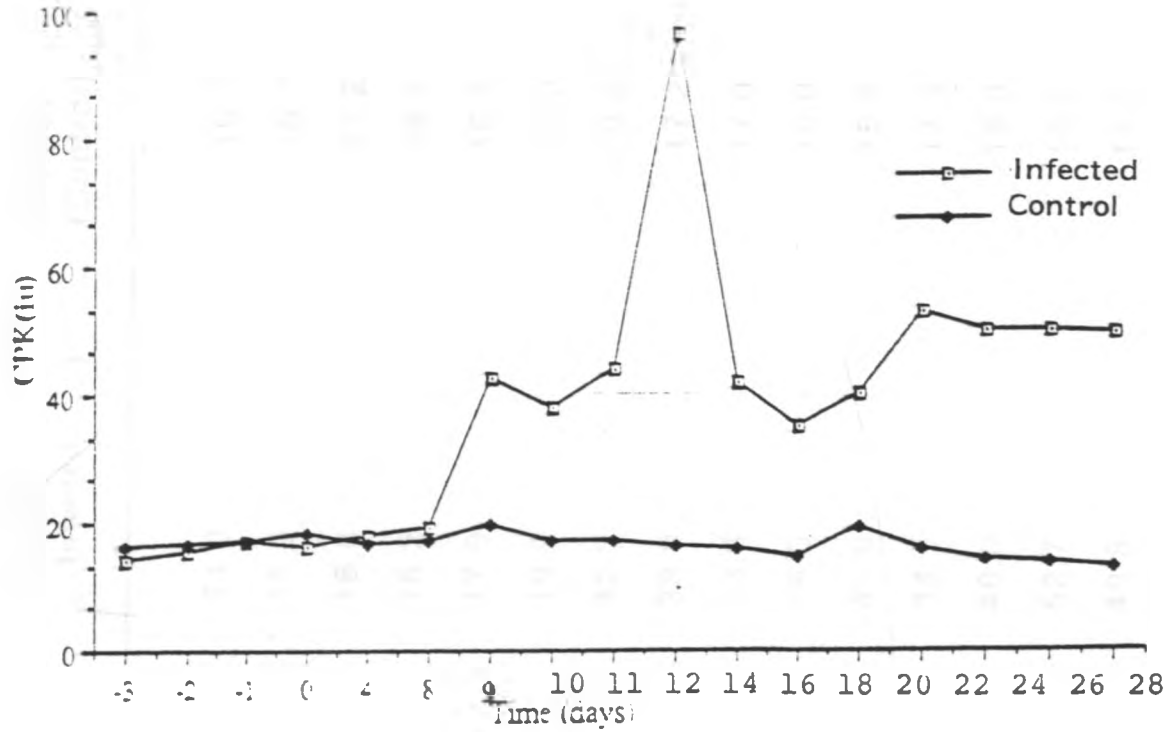


Table 15 Mean Creatinine Phosphokinase (CPK) (IU) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	CPK(IU) (Infected)	CPK(IU) (Control)
- 3	14.0	16.2
- 2	15.5	16.4
- 1	16.8	17.2
0	16.2	18.4
4	17.9	16.4
8	19.0	17.0
10	42.3	19.6
11	38.0	17.2
12	43.6	17.0
14	96.0	16.0
16	41.9	15.8
18	34.9	14.4
20	40.0	19.0
22	52.7	15.8
24	49.8	14.0
26	50.0	13.8
28	49.5	12.8
	34.34 ±1.57	17.72 ±4.55

$P < 0.05$

$f=5.12$

FIG. 27: Mean Lactate dehydrogenase (1μ) in sheep infected with Cowdria ruminantium and the controls.

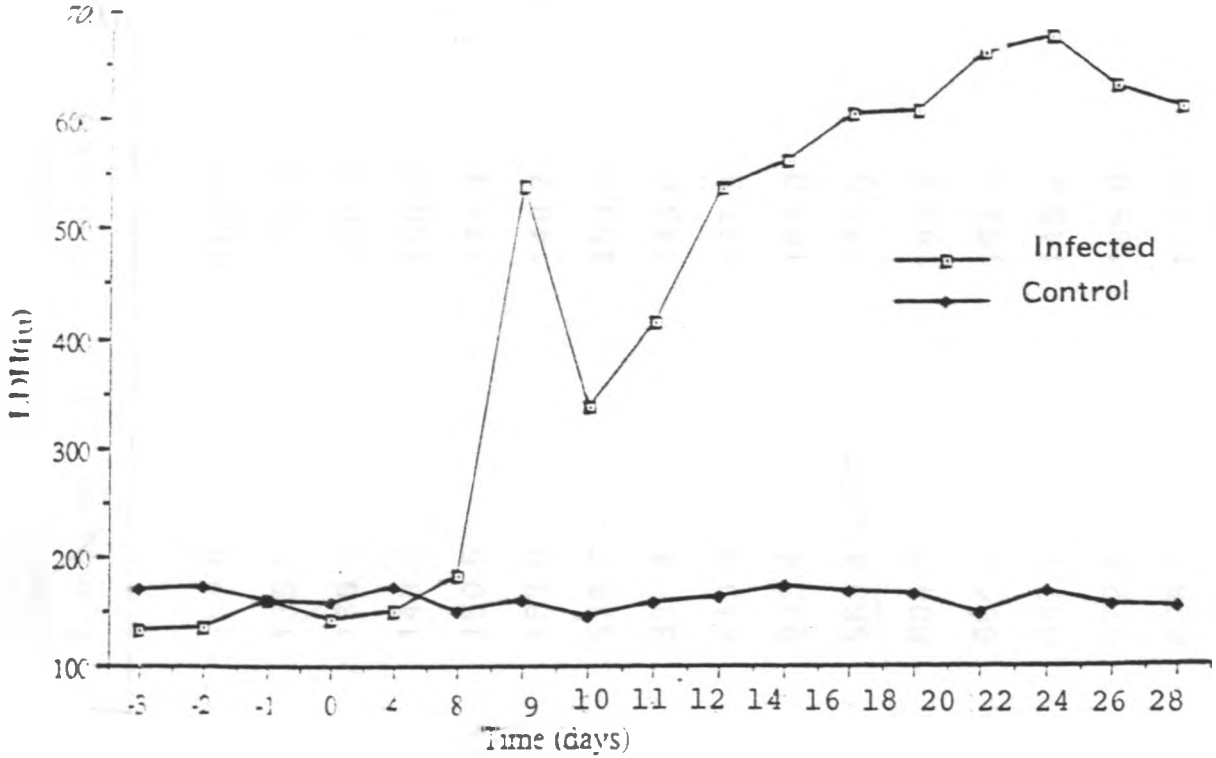


Table 16 Mean Lactate Dehydrogenase (LDH) (IU) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	LDH(IU) (Infected)	LDH(IU) (Control)
-3	132.9	168.6
-2	135.5	172.2
-1	160.2	160.4
0	143.4	156.8
4	150.5	171.4
8	183.0	148.7
10	538.7	158.8
11	336.4	143.6
12	413.9	157.8
14	535.4	163.0
16	560.4	173.0
18	604.0	168.4
20	604.4	164.0
22	657.7	148.4
24	672.5	166.8
26	628.7	153.8
28	608.5	152.6
	354.07±3.43	160.74±17.06
	P<0.01	
	f=50.07	

FIG 28. Mean Blood urea nitrogen (mg%) in sheep infected with Cowdria ruminantium and the controls.

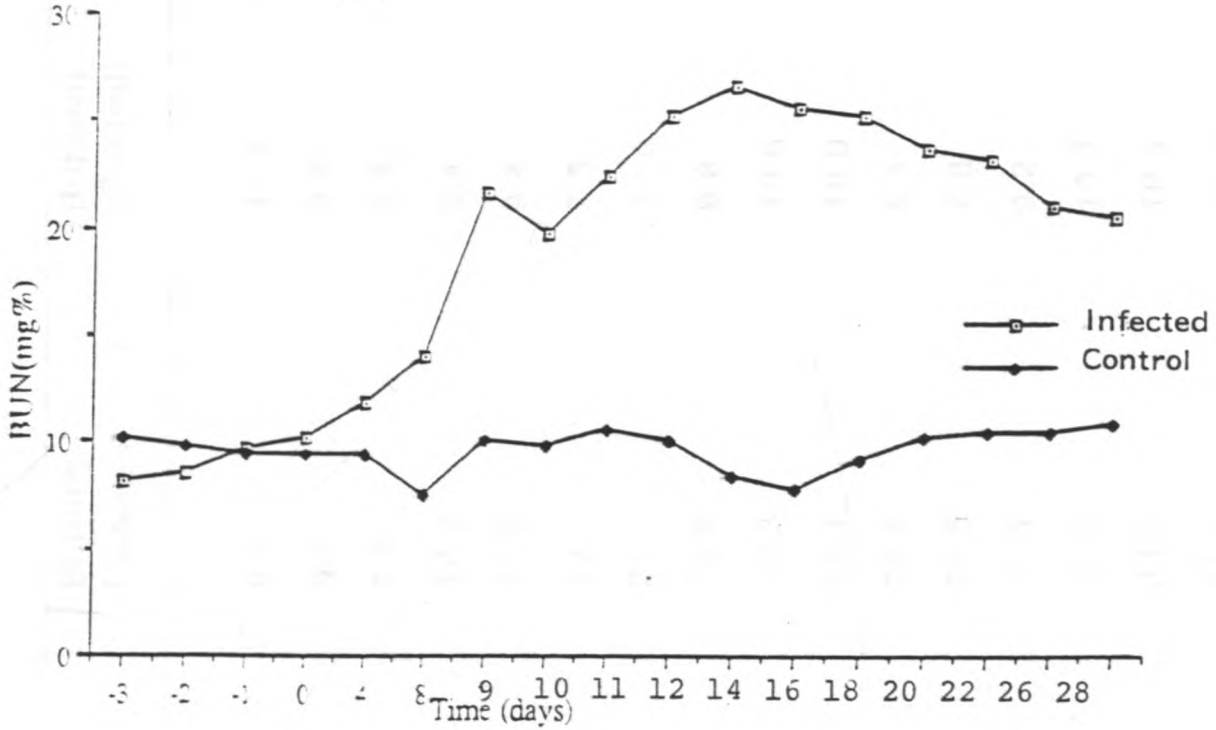


Table-17 Mean Blood Urea Nitrogen (BUN) (mg) in sheep infected with *Cowdria ruminantium* and the controls

Period (days)	BUN(mg) (Infected)	BUN(mg) (Control)
- 3	8.1	10.2
- 2	8.5	9.8
- 1	9.6	9.4
0	10.2	9.4
4	11.8	9.4
8	13.9	7.5
10	21.5	10.0
11	19.6	9.8
12	22.3	10.6
14	25.1	10.0
16	26.5	8.4
18	25.5	7.8
20	25.1	9.2
22	23.6	10.2
24	23.1	10.4
26	21.0	10.4
28	20.4	10.8
	17.39 ± 0.22	9.62 ± 0.51

P <0.01
f=85.35

Study of the cell fraction associated with Cowdria ruminantium in sheep

In the procedure of culturing mononuclear cells, the infected sheep recovered without treatment. The fever lasted for 4 days and the course of the disease was 15 days. Other clinical signs observed were innappetance, depression, dyspnoea, harsh lung sounds on auscultation, and tendency to lie down most of the time.

After incubating the plates for 48 and 72 hours slides of the culture material were made and stained using giemsa. No rickettsia particles could be viewed in any of the mononuclear cells. An attemp to culture the cells for a longer period were fruitless as the cells started dying on the 3rd day.

In the procedure of culturing infected neutrophils, the sheep died during the febrile stage, at day 3. The disease ran an acute course and the signs observed were; fever of 40.5^{o}C to 41.3^{o}C, depression, inappetance, and harsh lung sounds as well as dyspnoea. No nervous signs, apart from depression were observed. On post mortem the lungs were congested and oedematous, there was slight hydropericardium and the brain was slightly congested. Brain smears that were stained with giemsa revealed Cowdria ruminantium colonies in the endothelial cells.

CHAPTER 5

4 :1 DISCUSSION

CLINICAL MANIFESTATIONS

In this study the mean incubation period was 9 days. This agrees with work done by other researchers. Lounsbury (190) recorded an incubation period of 11-14 days using infected ticks. Spreull (1922) noted that if the ticks feed on the body and not on the hind limbs, the incubation period is shortened by 1-2 days.

In experimental infection, the incubation period has been said to vary with the infecting dose, the route of infection, the species, individual and breed susceptibility and the strain of the tick (Camus and Barre, 1988). The incubation period was found to be very long if less than 5 ml of infected blood was used (Currason, 1943), while infection of one ground-up nymph or 1/100 of a nymph gives rise to an incubation period of 9-10 days. It has been found that the incubation period is shortest if the infective material is injected intravenously. As concerns different species incubation period is longer in cattle (11-12 days) while in sheep the mean incubation period recorded was 9 days but can be 5 days (Curasson, 1943) to 35 days (Alexander, 1931).

Clinical signs appeared 2-3 days after the febrile response. This agrees with the findings of Uilenberg (1981b), who noted that the appearence of symptoms is usually seen 1-9 days after a febrile response.

Two sheep died of the peracute form of the disease; one died without showing any clinical signs except a febrile response while the other died on the second day. In most of the animals the temperature

exceeded 41°C. Other clinical signs observed correspond to the documented findings. It has been recorded that abortions are frequent in pregnant animals. One of the infected sheep aborted during the course of the disease. This seemed to be due to the stress condition the disease caused. Ilemobade (1976) stated that there was increased amounts of corticosteroids in animals infected with heartwater. This increase in corticosteroids may possibly account for the abortions in animals suffering from heartwater. Diarrhea was also observed in 3 of the infected sheep in this study. This is in agreement with findings of Uilenberg (1971) who noted diarrhea to occur in most cases.

In this work, 2 of the sheep suffered the peracute form of the disease, 10 from the acute form and 3 of the subacute form. None of the sheep suffered from the documented chronic form of the disease.

It was noted that prognosis was poorest in those animals which showed nervous signs. This was found to be in agreement with findings of Camus and Barre (1988), who stated that all animals which manifest nervous signs almost always die.

In this experiment the mortality rate was 33.3%. This is within the ranges reported by other researchers, although on the lower end, (Alexander, 1931; Neitz, 1939). In pure exotic breeds higher mortalities have been reported; 50 % by Karrar (1960); 20 % by Alexander (1931). Although the sheep used in this experiment were doppers, they were not pedigree material. This may account for the low mortality rate as compared to the documented ones.

LESIONS

The most outstanding lesions observed in this work corresponded to the documented ones. Hydrothorax, hydropericardium and ascites, although not extensive, were recorded, with the fluid being straw coloured. Congestion and oedema of the lungs and brain were also observed. Other findings were enlargement of the mesenteric lymph nodes, hemorrhages in the spleen, kidney capsule and the lungs. All these agree with the findings of several other researchers viz; Steck (1928), Curasson (1943), Curasson and Pelphy (1928), Henning (1956), Pienaar et al., (1966), Uilenberg (1971 and 1981). It has been documented that spleenomegally is common in small ruminants (Alexander, 1931), but others documented that it is a rare finding, (Uilenberg, 1971b and Ilemobade, 1976). This experiment is more in agreement with the latter as only one out of the five cases which died showed slight spleenomegally. Increased size of the lymph nodes was supported by Alexanders findings (1931). He recorded that the lymph nodes generally showed hypertrophy, with focal hemorrhages, especially the mesenteric, the retropharyngeal and the submaxillary lymph nodes. The brain showed oedema and congestion of the meninges. This agrees with the findings of Pienaar et al., (1966), and this pathology in the brain is the cause of the nervous signs observed in heartwater cases.

HEMATOLGY

ANAEMIA

PACKED CELL VOLUME

There was a marked drop in PCV during the course of the disease, which was observed to increase slightly terminally. This drop was observed to start just after the fever onset. This finding was found to agree with most researchers work. Clark (1962), Owen et al (1973) and Abdel Rahim and Shommein (1977) found a similar drop in infected animals which they reported was statistically significant. Ilemobade and Blotkamp (1978), however, observed that this drop in PCV had no significant difference from the control group.

RED BLOOD CELL COUNT

There was a general tendency for the red blood cell count to decrease during the course of the disease. This decrease was found to be statistically significant as compared to the controls ($P < 0.01$). This finding was not in agreement with findings of Abdel Rahim and Shommein (1977) and Van Amstel et al., (unpublished data, 1986) who found the drop not significant.

HAEMOGLOBIN

There was a drop in haemoglobin concentration in infected animals during the course of the disease. This decrease was found to be significant statistically ($P < 0.01$). This agreed with work done by Ilemobade and Blotkamp (1978), Owen, Littlejohn , Kruger and Erasmus (1973). Abdel Rahim and Shommein (1977) did hematological studies in 20 male goats and also demonstrated that there was a progressive drop in Hb. This was later confirmed by Van Amstel and other researchers (1986) in calves. They found the decrease in Hb

concentration started occuring shortly after the fever onset. This indicates anaemia in heartwater.

From the literature review, this anaemia which develops during the course of heartwater raises a lot of questions. There seems to be some controversy as to the exact type of anaemia which develops. Abdel Rahim and Shommein (1977) postulated that it is a microcytic hypochromic anaemia as seen in iron deficiency anaemias. Van Amstel et al., (1986) did some work and concluded that it is a normochromic normocytic anaemia as is seen with bone marrow depression. In this work the bone-marrow-depression school of thought was found to be the most probable cause of the anaemia. This is because there was absence of circulating immature red blood cells and also because of the disappearance of circulating eosinophils. This is of course subject to more research work as I could not explain some of these findings.

Iron deficiency anaemia was also suspected by Abdel Rahim and Shommein (1977). This is also a very possible course of the anaemia as heartwater seems to increase the phagocytic activity which may lead to trapping of iron by macrophages.

Anaemia resulting from acute blood loss can be ruled out as no significant hemorrhage is associated with the disease (Prozesky, 1987). Hemolysis as cause of the anaemia was ruled out by some reseachers.

WHITE BLOOD CELL COUNT AND DIFFERENTIAL COU

There was a slight leukopenia observed at days (incubation period). A leukocytosis was observed just fever onset and continued to increase during the cou disease even after there were no discernable Differential count revealed that there was a neutropl started decreasing at day 24 post infection. No neutrophils were found in circulating blood during the the disease. Lymphopaenia was noticed also during the the disease while eosinophils disappeared from circu after onset of fever.

The leukopaenia observed in this work agreed with of Ilemobade and Blotkamp (1977) who observed that a developed 3 days after infection prior to fever onset. A leukocytosis was also reported by some researchers to associated with the disease; Abdel Rahim and Shommei reported a leukocytosis associated with the clinical dis Camus and Barre (1982) reported the presence of a neu while Abdel Rahim and Shommein (1977) reported a neu A lymphopaenia was reported by Ilemobade and Blotkam but Abdel Rahim and Shommein (1977) found a marked lymphocytosis. No changes were observed in the monocy most of the researchers but the disappearence of eosin observed in this work was found to agree with findings (1962) and Abdel Rahim and Shommein (1977).

The leukopenia before fever onset is probably due to the destruction of phagocytic cells by the organism, or withdrawal of these cells from circulation. After infection C. ruminantium is picked by these cells to the regional lymph nodes where multiplication is by binary fission. The cells then bust releasing these microorganisms (the elementary forms) into circulation. This is probably what triggers off the fever.

The normal leucocyte count in sheep is 4,000-12,000 with an avarege of 7,300 (Fraser, 1929).

In this study there was a profound increase in leucocytes with a neutrophilia. This was thought to be as a result of the disease, heartwater, as well as the stress the disease subjects the animal to. A corresponding decrease in lymphocytes was recorded.

The disapearence of eosinophils was quite prominent. This was suggested to be due to increased corticosteroids in circulation (Ilemobade, 1976). This may also explain decreased lymphocytes as Coles (1967), stated that in response to stress and increased adrenal activity, lymphocytes are destroyed in greater numbers than any other cell. Adrenal activity results in secretion of ACTH which in turn stimulates secretion of increased amounts of corticosteroids into circulation. Thus, the disappearence of eosinophils and decreased lymphocyte count may be related.

BIOCHEMISTRY

TOTAL PROTEIN, ALBUMIN, AND GLOBULIN

There were changes noticed in infected animals. When tested statistically using analysis of variance the differences between the experimental and the control means were significant for total protein ($P < 0.01$), globulin ($P<0.01$) but not for albumin ($P> 0.05$).

In the literature changes in serum proteins may indicate kidney or liver disease and can therefore be used in diagnosing liver and kidney diseases and also help in determining the prognosis of such cases (Coles, 1967). Heartwater does not seem to have a profound effect on the kidneys and the liver and especially as far as their functions are concerned. This agrees with the pathology of these organs in animals dying of the disease. There was no significant change grossly or histologically in either of these organs except some hemorrhages on kidney capsule, congestion and infiltration by inflammatory cells in the liver.

The decrease in total protein which was noticed in some cases was thought to be due to nutritional factors. The highest decrease in the mean total protein in the infected animals was at day 7. This was found to be in agreement with findings of Munene (1987) who noted a decrease also at day 7. It was around this time that the sick animals were almost or completely off feed and this seems to be the most likely explanation of the decrease. On the other hand there was a slight increase in total protein at day 15 and day 19. This could be due to several factors; at this

time some of the animals were at their worst, or were convalescing. In the prostate animals the increase in total protein was thought to be due to the dehydration status of such animals while in the recovering animals there was an increase due to increased dietary intake.

The behaviour of albumin and globulin fractions was irregular and therefore no definite conclusions could be drawn. There was slight decrease in albumin and a corresponding increase in globulin after the febrile response. In the literature, it is said that a decrease in albumin fraction may be due to a direct defect resulting in an inhibition of the synthesis of albumin or it may be due to an increase in the concentration of the globulins, (Cole, 1967). The latter (increase in globulins) was thought in this work to be the more likely cause as invasion of the body with foreign material (bacterial, viral, protozoal or parasitic) will result in an increase in globulins (Coles, 1967). This may be a response to the antibody producing system. According to Ilemobade (1976), the fraction of the globulins which was raised was the alpha-globulins.

GLUCOSE

There was a definite increase in glucose in the infected animals. This agrees with findings of most researchers who have worked on this disease. Graff (1933), Clark (1962), Ilemobade and Blotkamp (1978) and Ilemobade (1976), found that animals infected with Cowdria ruminantium showed a significant increase in glucose. From this work and that of the other researchers this

increase was highest terminally.

Ilemobade (1976), noted that this rise in glucose coincided with a rise in lactate and pyruvate and a drop in the pH.

From the literature hyperglycemia may result from several factors. It may be due to an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar or distubances in the endocrine system upon these processes (Coles, 1967). Consequently, a hyperglycemia may result from :

1. A normal hepatic output of glucose with a subnormal rate of the peripheral removal.
2. An increase in the hepatic production and release of the glucose with normal removal rate by peripheral tissues.
3. A combination of these factors
4. Pancreatic diseases as acute pancreatic necrosis and chronic pancreatitis.
5. Other causes; Convulsions and anoxia. (Coles, 1967).

From the above, an increase in glucose in animals infected with heartwater seems to be due to the convulsions and the decrease in respiratory function. Owen at al., (1973) found out that there was respiratory acidosis, hypoxia and an increased ventilatory equivalent for oxygen which coincided with severe respiratory distress.

Corticosteroids were noted to increase in animals infected with Cowdria ruminantium. (Ilemobade, 1976). ACTH is involved in increasing steroids concentration in the body and it may be this ACTH which causes hyperglycemia as is documented by Coles (1967).

ALANINE AMINOTRANSFERASE (ALT)

There was a slight increase in serum ALT at the onset of the febrile reaction and also at day 13 although statistically this was found not to be significant ($P>0.05$). Therefore heartwater does not seem to cause any profound changes `as far as this enzyme is concerned.

This enzyme is used routinely to detect liver disease in dogs, cats and primates but not in sheep, cattle and horses.

LACTATE DEHYDROGENASE (LDH)

There was an increase in LDH in animals infected with Cowdria ruminantium especially just before the fever onset and also terminally when the highest levels were recorded.

LDH catalyses the irreversible oxidation of pyruvate to lactate and is widely distributed in tissues of the body. In the literature it is said that there is an increase in pyruvate and lactate corresponding to the increase in glucose (Ilemobade, 1976). The highest increase in both glucose and LDH was recorded terminally in this experiment. It therefore seems probable that the increase in LDH maybe as a response to increased sugars and especially pyruvate in the serum, simply as a way of maintaining the body's chemistry balance.

ASPARTATE AMINOTRANSFERASE (AST)

This enzyme is used in determination of neuromuscular disease or liver disease. In sheep there are differences of the normal values associated with age and physical activity.

In this work there was elevation of serum AST in animals infected with Cowdria ruminantium. This increase was mainly observed in animals which showed nervous signs and this was probably due to the convulsions and other causes of increased muscle activity. Otherwise no other cause could be thought of as the muscle fibres looked normal grossly and histologically.

ALKALINE PHOSPHATASE (AP)

Serum AP is used as an indicator of liver insufficiency although in sheep and cattle this has its drawbacks as the normal values have such a wide range so as to sometimes not be able to know what value indicates disease or not.

In this experiment a slight decrease in serum AP was recorded but on statistical analysis it was not significant ($P>0.05$). Also the values were still within the normal range.

CREATININE PHOSPHOKINASE (CPK)

Creatinine phosphokinase is also used in the determination of neuromuscular activity disorders especially in humans. CPK functions in making ATP available for contraction by the phosphorylation of ADP from creatinine phoshate (Coles , 1967). Activities of CPK are significant in skeletal and cardiac muscles and therefore elevations of CPK may indicate disease of the muscles.

There was an elevation of CPK especially terminally in animals infected with Cowdria ruminantium. Elevated CPK, from the literature, indicates that muscle necrosis is present and still progressively active

while elevated ALT accompanied by normal CPK indicates that muscle necrosis is no longer active (Coles , 1967).

Elevations of CPK have been reported in cases of polioencephalomalacia in sheep, among other diseases and it was suggested that the enzyme may be of value in diseases of the central nervous system .

Therefore in conclusion , CPK elevations may be due to myogenic. or neurogenic diseases which affect muscles as neurogenic disease results in secondary changes to the muscle fibre as a result of alterations in its innervation.

BLOOD UREA NITROGEN (BUN)

Increased levels of BUN may be due to renal prerenal or postrenal alterations. Intrinsic kidney factors influencing the BUN levels are primarily associated with nephritis. Levels of BUN are increased in acute, subacute and chronic interstitial nephritis. Also damage to the nephron may result in increased BUN (Coles, 1967).

In heartwater the cause of the increased BUN was thought to be prerenal. Prerenal increase in BUN can be caused by several factors including high nutritional levels of protein, dehydration, decreased glomerular filtration resulting from blood pressure changes or high levels of cortisol resulting in increase in protein catabolism (Ganong, 1975).

In this work elevations started just after the onset of the febrile reaction. Van Amstel et al (unpublished data, 1986) found similar results in calves experimentally infected with. Cowdria ruminantium. He also suggested that there was some interference in the glomerular function in cases of heartwater.

Study of the cell fraction associated with Cowdria ruminantium in sheep

Cowdria ruminantium is associated with blood but there has been some controversy as to the cell fraction it is associated with in blood if any as this would also throw more light to the pathogenesis and immune response of the disease. Whole blood obtained from a positive case of heartwater will cause the disease in a healthy animal if inoculated intravenously but not subcutaneously. Du plessis (1970) did some work which gave rise to the theory that Cowdria is borne by mononuclear cells to the regional lymph nodes where they undergo the first replication. Logan et al; (1987) managed to culture infected neutrophils and used these as antigen in indirect immunofluorescence tests. In summary, most researchers have indicated that the organism is associated with the leucocyte fraction in circulation. From this work results were not conclusive. Cowdria ruminantium particles were not seen in the mononuclear culture but in the neutrophil culture. However this does not rule out the mononuclear cells as also being able to harbour the organism as the form in which the organism is in (elementary, morula etc) may be of importance in this cell-organism association. Also the manner of culturing the cells was different and also the two procedures were run in different laboratories with different states of sterility and these small factors may have had a profound effect in the results obtained. This may also explain why slides stained before culturing in both procedures showed nothing even in the neutrophil culture which revealed the parasite after culture.

Cowdria ruminantium exists in many forms. These forms seem to

occur in different type of cells. Du plessis (1970) and Ilemobade (1976) gave rise to a theory which suggested that the first replication occurs in the lymph nodes after which there is rupture of the cells and release of elementary bodies into circulation.These forms in circulation possibly differ, for examle, with those in the brain as it has been observed that subcutaneous inoculation of the organism is not able to cause the disease when a blood stabilate is used but causes the disease if an infected brain is used.

There may be some relationship between this and the leucocytosis and the neutrophilia noted in the hematology results. It is also possible that many different blood cells are associated with Cowdria ruminantium but that this depends on the form of the organism. This, however, is not enough work to make any conclusive remarks but offers more room for further work on this disease.

CONCLUSION

In all sheep infected with Cowdria ruminantium, hematology results revealed anaemic conditions. Thus, anaemia was a major finding in this work characterised by decreased red blood cell count, packed cell volume and hemoglobin concentration.

A slight leukopaenia was observed during the incubation period while during the course of the disease, a leukocytosis with a neutrophilia, lymphopaenia and eosinopaenia was observed. No immature neutrophils were observed. These changes pointed strongly to the role played by leucocytes in the pathogenesis of the desease.

Neutrophils were found to be the cells most probably assocciated with the organism in blood.

Hyperglycemia was very prominent in animals infected with heartwater especially in terminal cases.

An increase in aspartate aminotransferase (AST), creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) was noted in all animals infected with Cowdria ruminantium.

There was an increase in blood urea nitrogen (BUN) which was noted to be highest after the febrile reaction.

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RESULTS

Body Temperature

GROUP A

	SHEEP NO	
DAY	1	2
1	38.5	38.4
2	38.4	39.0
3	38.6	39.2
4	39.0	38.8
5	39.6	38.7
6	39.5	39.5
7	39.0	39.6
8	40.0	40.0
9	40.7	39.5
10	40.9	40.1
11	41.0	40.6
12	41.5	41.0
13	42.0	41.3
14	40.0	42.2
15	DEAD	40.5
16		39.5
17		39.3
18		39.3
19		39.4
20		39.4

Average incubation period = 7 days

Average course of the fever = 7 days

3	4(control)
38.7	39.5
38.9	39.0
39.2	39.5
39.1	39.1
38.6	39.7
39.4	39.2
39.9	39.5
40.0	39.3
40.1	38.7
41.2	38.4
41.2	39.1
41.5	39.4
42.0	39.0
41.3	39.3
40.5	39.0
39.5	38.9
39.3	38.7
39.6	38.7
39.6	39.0
39.5	39.0

GROUP B

BODY TEMPERATURE

	SHEEP	
Day	1	2
1	39.2	39.2
2	38.9	38.9
3	39.0	39.0
4	39.0	39.3
5	39.0	39.1
6	39.1	39.5
7	39.5	39.6
8	40.0	39.9
9	41.0	40.5
10	42.0	41.5
11	42.3	41.5
12	42.0	42.0
13	42.3	42.1
14	40.5	40.5
15	39.6	39.7
16	39.8	39.9
17	39.5	39.8
18	39.3	38.9
19	39.4	39.0
20	38.1	39.0

Average incubation period = 8 days

Average course of the fever = 6 days

NO

3	4(Control)
38.7	39.1
39.1	38.7
39.1	39.5
39.3	39.1
39.0	39.2
39.4	39.5
39.7	
39.6	39.2
39.7	38.7
40.5	38.7
41.5	38.9
42.0	39.4
42.3	39.3
41.2	39.1
41.0	39.5
39.8	38.9
DIED	39.2
	39.3
	38.9
	39.5

(6.33)

GROUP C

BODY TEMPERATURE

	SHEEP NO			
DAY	1	2	3	4 (control)
1	39.1	38.7	38.3	39.0
2	39.2	39.3	38.9	38.7
3	39.0	39.2	39.4	38.9
4	38.9	39.4	39.5	39.0
5	38.9	39.3	39.3	38.9
6	39.3	39.1	39.0	39.0
7	39.5	39.7	39.6	39.1
8	39.5	39.9	39.9	39.0
9	39.5	40.3	40.0	39.2
10	40.0	40.3	41.2	39.3
11	40.5	41.5	41.5	38.9
12	41.9	41.5	42.0	38.7
13	42.3	42.5	42.6	39.5
14	42.5	41.0	42.0	39.6
15	41.5	39.9		39.6
16	40.3	39.5		39.2
17	39.7	DEAD	DEAD	39.1
18	39.8			38.7
19	39.5			38.9
20	39.4			39.0

Average incuabtion period = 8 days (8.33)
Average course of the fever = 6 days (6.33)

GROUP D

BODY TEMPERATURE

Day	1
1	39.3
2	38.3
3	38.4
4	38.9
5	39.0
6	39.1
7	39.4
8	39.3
9	39.9
10	40.0
11	40.0
12	40.7
13	40.9
14	41.0
15	41.5
16	42.0
17	40.6
18	41.0
19	DEAD
20	
21	
22	
23	

Average incubation period =

Average course of fever =

SHEEP NO		
2	3	4(control)
39.0	39.3	39.3
39.1	39.0	39.3
38.9	39.0	39.4
39.2	39.1	39.1
39.4	38.7	39.0
39.1	38.8	39.0
39.3	39.5	39.3
39.6	39.6	39.2
39.7	39.9	39.4
39.5	39.7	38.9
40.5	40.3	38.5
41.2	40.5	39.1
41.3	40.9	39.2
42.0	42.0	39.3
42.5	42.1	38.9
42.0	42.3	38.7
41.3	41.9	39.7
40.7	40.7	39.7
40.3	40.5	39.3
39.9	39.9	39.3
39.7	39.9	39.1
39.4	39.1	39.0
39.0	39.2	39.3

8 days (8.33)

9 days

GROUP E

BODY TEMPERATURE

	SHEEP NO			
Day	1	2	3	4(Control)
1	38.7	39.1	39.4	39.5
2	39.1	38.9	39.2	39.4
3	39.5	38.6	39.5	39.7
4	39.4	39.2	39.5	39.2
5	38.9	39.3	39.4	38.9
6	39.0	39.5	39.5	39.5
7	39.7	39.5	39.7	39.1
8	39.9	39.4	40.5	38.5
9	40.5	39.5	40.7	38.8
10	41.2	40.5	41.5	39.1
11	41.5	41.6	41.7	39.3
12	DIED	41.5	41.2	39.3
13		40.3	40.7	39.5
14		39.8	41.00	38.9
15		39.5	DEAD	39.4
16		39.4		39.7
17		39.5		39.6
18		38.7		38.8
19		39.4		38.4
20		39.0		39.0

Average incubation period = 8 days

Average course of fever = 4.6 days

GROUP A SHEEP NO. 1

EXPERIMENTAL BIOCHEMISTRY RESULTS

P	TP gm%	Albumin gm%	Globulin gm%	Glucose mmol/l	Alt (iv)	Ast (iv)	Ap (iv)	CPK (iv)	LDH (iv)	BUN mg%
-3	6.7	4.00	2.70	4.00	12	40	140	24	140	9
-2	6.5	3.30	3.20	3.15	11	43	115	10	135	10
-1	6.3	3.30	3.00	3.90	13	42	125	16	98	15
0	6.7	3.50	3.20	4.10	10	38	135	15	151	11
4	6.9	4.00	2.90	5.10	8	60	140	20	140	18
8	7.1	4.20	2.90	4.90	11	80	90	13	140	24
1	6.3	3.20	3.10	5.30	10	95	50	20	225	22
2	6.7	3.30	3.40	5.60	15	88	55	14	230	25
3	6.4	3.30	3.10	4.80	5	80	40	16	300	29
5	6.2	3.00	3.20	7.50	7	95	90	10	450	26
7	6.0	3.10	2.90	8.00	7	115	98	23	500	24
9	6.0	3.20	2.80	8.30	8	129	100	34	470	25
11	6.0	3.15	2.85	8.15	9	144	80	45	620	26
13	6.0	3.20	2.80	9.50	10	142	84	51	950	30
15	D		E		A			D		
17										
19										

GROUP A SHEEP NO. 1 EXPERIMENTAL

HEMATOLOGY

	P	PCV%	T.P gm%	H.B. gm%	RBC x106 /cmm	WBC /cmm	TN%	ST%	L%	M%	E%	B%
Pre-infection	-3	31	6.2	11.9	11.5	8,400	22	0	76	0	2	0
Period	2	32	6.4	11.5	12.5	8,600	27	0	71	0	2	0
	1	33	6.2	10.5	11.5	8,700	25	0	72	0	3	0
Incubation	0	30	6.7	11.0	12.9	7,200	30	0	68	0	2	0
	4	32	7.0	11.5	12.3	6,700	29	0	69	0	2	0
	8	30	7.1	10.2	12.3	6,300	26	0	73	0	1	0
After	1	28	6.5	10.5	12.4	9,700	34	0	64	0	2	0
Fever Onset	2	27	6.6	10.69	10.5	11,900	45	0	55	0	1	0
	3	25	6.2	10.9	9.9	13,400	55	0	45	0	0	0
	5	24	6.0	9.5	9.2	14,500	36	0	45	0	0	0
	7	24	6.0	9.7	8.0	16,000	51	0	49	0	0	0
	9	20	5.9	9.2	8.5	15,500	55	0	45	0	0	0
	11	22	5.9	8.5	8.4	15,500	52	0	48	0	0	0
	13	24	6.0	8.4	8.5	15,700	54	0	46	0	0	0
	15		D		E		A		D			
	17											
	19											

GROUP A SHEEP NO. 2 EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP %	HB gm%	RBC x106 /cmm	WBC /cmm	TN %	ST %	L %	M %	E %	B %
Pre infection Period	-3	33	7.0	13.70	13.4	7,900	30	0	66	0	4	0
	-2	32	7.1	13.80	13.20	5,400	30	0	68	0	20	0
	-1	30	6.9	12.9	14.04	6,800	33	0	64	0	3	0
In-cubation Period	0	32	7.0	13.00	16.00	6,700	30	0	66	0	4	0
	4	33	7.0	12.90	14.05	5,400	33	0	64	0	2	0
After Disease Onset	1	28	6.6	10.70	13.75	11,500	38	0	60	0	2	0
	2	26	6.5	9.20.	12.70	11,900	48	0	52	0	0	0
	3	26	6.4	9.00	10.50	13,700	48	0	46	0	1	0
	5	26	6.2	8.60	10.30	15.200	50	0	46	0	0	0
	7	22	6.0	8.00	8.30	15,700	54	0	47	0	0	0
	9	20	6.9	8.10	9.00	17,700	52	0	44	0	0	0
	11	20	7.0	8.20	8.25	16,200	58	0	41	0	0	0
	13	21	6.9	7.90	7.90	16,700	59	0	42	0	0	0
	15	25	7.0	8.80	7.00	16,900	58	0	40	0	0	0
	17	25	6.8	8.20	8.30	17,500	60	0	44	0	0	0
	19	26	6.9	8.30	8.40	16,400	60	0	45	0	0	0

GROUP A SHEEP NO.2

EXPERIMENTAL BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l	ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LPH (iv)	BUN mg%
-3	7.10	4,60	2.50	4.00	7	52	110	14	138	8
-2	7.0	4.30	2.70	3.75	8	60	130	13	137	9
-1	7.0	5.00	2.00	3.90	12	50	155	13	141	7
	7.0	4.70	2.30	4.60	10	49	112	14	135	10
0	6.8	3.80	3.00	4.50	10	54	90	16	139	11
4	6.7	4.00	2.70	5.30	8	57	110	18	143	15
	6.0	3.00	3.00	5.15	9	65	120	19	200	20
1	6.3	3.30	3.00	4.90	10	68	80	12	285	22
2	6.0	3.20	2.80	5.00	11	106	50	20	250	25
3	6.0	3.10	2.90	7.30	11	109	55	25	420	27
5	6.8	4.20	2.60	7.20	12	143	60	24	385	28
7	6.9	4.40	2.50	9.20	10	140	62	36	390	26
9	7.0	4.30	2.70	9.20	9	155	55	32	500	27
11	7.0	4.30	2.70	10.00	8	150	70	45	650	22
13	6.7	3.70	3.00	8.20	78	98	52	46	700	22
15	6.6.	3.70	2.90	8.50	10	84	75	57	500	24
17	6.8	3.80	3.00	7.30	12	85	63	60	540	20
19										

GROUP A SHEEP NO. 3 – EXPERIMENTAL

HAEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC cmm	TN %	ST %	L %	M %	E%	B%
Preinfection		33	6.8	10.9	9.25	8,500	31	0	65	0	4	0
Period	-2	34	7.0	12.0	10.30	7,500	22	0	76	0	2	0
	-1	32	6.8	12.5	13.20	8,500	42	0	56	0	2	0
Incubation	4	33	6.6.	11.9	11.30	6,200	44	0	55	0	1	0
Period	8	30	6.7	10.3	11.21	5,500	42	0	56	0	2	0
After	1	28	6.5	10.2	11.50	9,500	45	0	53	0	2	0
disease	2	26	6.5	10:0	9.30	11700	50	0	48	0	2	0
Onset	3	24	6.7	9.50	9.70	12600	55	0	45	0	0	0
	5	25	6.0	8.20	10.30	14500	54	0	46	0	1	0
	7	26	6.1	8.7	9.20	13500	60	0	40	0	1	0
	9	20	5.9	8.3	8.05	14700	62	0	38	0	0	0
	11	18	6.2	7.9	7.30	15700	60	0	40	0	0	0
	13	20	6:5	7.8	8.10	17200	58	0	42	0	0	0
	15	22	6.4	8.2	8.30	17500	60	0	40	0	0	0
	17	24	6.7	8.5	9.20	18000	50	0	45	0	0	0
	19	24	6.6	8.7	9.30	16900	52	0	47	0	0	0

GROUP A SHEEP NO. 3

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%
-2	5.9	3.50
-1	5.2	3.70
4	7.0	3.60
8	7.1	3.20
1	7.2	3.15
2	6.7	3.70
3	7.0	3.90
5	6.3	3.30
7	6.2	3.20
9	6.1	3.20
11	5.9	3.00
13	6.4	3.20
15	6.4	3.50
17	6.4	4.00
19	6.7	4.10
	6.6	4.30
	6.8	3.8

GLO gm%	GLU mmol/l	ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
2.40	5.25	10	50	140	14	92	7
1.50	5.50	10	60	132	14	94	11
3.40	4.90	17	55	115	17	171	15
3.90	3.90	15	63	134	18	160	12
4.05	5.20	12	88	125	20	155	18
3.00	6.20	11	90	130	23	110	20
3.10	5.20	8	72	112	22	180	24
3.00	7.40	9	82	110	16	170	27
3.00	7.55	11	88	80	14	38	27
2.90	8.22	13	104	70	22	490	26
2.90	7.50	15	94	92	27	540	28
3.20	7.30	16	96	50	29	600	24
2.90	8.90	10	93	60	40	540	22
2.60	9.00	7	105	65	38	600	24
2.60	7.20	8	142	70	36	720	22
2.30	8.50	10	155	50	45	870	26
3.00	7.20	11	157	51	50	620	24

GROUP A SHEEP NO. 4 — CONTROL

HAEMATOLOGY

P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC cmm
-3	30	7.0	10.00	10.70	8,400
-2	28	6.9	11.00	11.30	6,400
-1	33	6.7	11.05	11.40	7.200
0	32	7.1	9.50	10.00	6,700
4	30	6.8	9.60	9.90	7,000
8	27	6.9	9.50	10.40	8,200
1	30	6.8	9.70	12.00	2,400
2	34	7.0	9.90	12.01	6,700
3	28	7.0	10.30	10.30	6,900
5	29	6.8	9.30	10.10	7,500
7	33	6.8	9.00	9.50	6,200
9	32	6.7	9.50	10.20	6,400
11	29	6.9	9.80	11.90	6,850
13	31	7.0	10.40	10.60	6,900
15	31	6.9	10.10	10.90	5,400
17	28	6.6	9.10	11.40	6,200
19	28	6.7	10.70	10.10	6,700

TN %	ST %	L %	M %	E%	B%
31	0	66	0	3	0
34	0	64	0	2	0
40	0	58	0	2	0
35	0	65	0	0	0
30	0	68	0	2	0
33	0	66	0	1	0
34	0	64	0	1	0
34	0	66	0	0	0
36	0	64	0	0	0
35	0	63	0	2	0
35	0	62	0	3	0
37	0	62	0	1	0
41	0	58	0	1	0
42	0	58	0	0	0
40	0	58	0	2	0
38	0	60	0	2	0
36	0	62	0	2	0

GROUP A SHEEP NO.4 CONTROL

BIOCHEMISTRY RESULTS

P	TP gm(%)	Alb gm%	Glo gm%	Glu mmol\l	Alt (iv)	Ast (iv)
-3	6.9	3.40	3.30	4.35	10	81
-2	6.8	3.80	3.50	4.50	9	68
-1	7.0	4.00	3.00	5.10	8	66
0	6.7	3.70	3.20	5.00	10	69
4	6.9	3.90	3.00	4.90	7	79
8	6.9	4.00	2.90	3.90	7	52
1	6.9	4.10	2.80	4.20	9	40
2	7.0	4.50	2.50	4.70	8	51
3	6.8	3.50	3.3	3.90	10	65
5	6.7	2.90	3.66	3.90	11	82
7	6.8	2.90	3.60	4.20	11	70
9	6.8	3.5	3.3	5.00	9	64
11	6.8	3.6	3.2	5.20	10	75
13	7.0	4.0	3.00	4.90	7	88
15	7.0	4.2	2.8	4.70	6	80
17	6.9	4.0	2.9	3.90	11	75
19	6.7	4.1	2.6	4.00	12	72

Ap (iv)	CPK (iv)	LDH (iv)	BUN (iv)
151	13	130	13
168	12	129	10
150	12	132	11
154	13	134	8
145	12	198	9
142	12	150	7
152	12	163	10
154	12	170	10
139	21	197	12
142	13	116	10
147	13	205	9
145	12	199	8
151	12	175	10
155	12	150	10
167	13	200	9
143	15	165	8
154	9	170	12

GROUP B SHEEP NO. 1 EXPERIMENTAL

HAEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
Pre-infection period	-3	32	5.8	10.4	9.60
	-2	31	6.2	10.8	10.75
	-1	28	6.0	10.1	9.95
	0	30	6.1	9.90	10.30
Incubation Period	4	29	6.3	10.20	9.80
	8	30	6.1	10.10	9.70
After disease	1	28	6.2	10.4	10.50
	2	28	6.4	10.8	10.20
	3	26	6.2	9.5	9.30
	5	24	6.0	9.6	9.20
	7	22	5.9	8.5	9.20
	9	22	6.0	8.2	8.90
	11	20	5.8	8.9	8.70
	13	21	5.9	7.0	8.20
	15	21	6.2	7.8	8.60
	17	22	6.2	7.2	8.50
	19	22	6.3	8.9	8.40

WBC /cmm	TN %	ST %	L %	M %	E %	B %
5700	32	0	68	0	0	0
4800	33	0	62	0	5	0
5500	31	0	66	0	3	0
5700	31	0	67	0	2	0
5400	32	0	65	0	3	0
6000	35	0	63	0	2	0
5610	37	0	61	0	2	0
8600	39	0	60	0	1	0
9500	45	0	55	0	0	0
9900	48	0	52	0	0	0
12400	50	0	50	0	0	0
14300	54	0	46	0	0	0
14700	61	0	39	0	0	0
15200	60	0	40	0	0	0
16100	58	0	42	0	0	0
16500	57	0	43	0	0	0
17000	59	0	41	0	0	0

GROUP B SHEEP NO. 1 EXPERIMENTAL

BIOCHEMISTRY

P	TP gm%	ALB gm%	GLO gm%	CLU mmol/l
-3	6.66	3.93	2.73	3.9
-2	6.35	3.93	2.42	2.4
-1	6.70	4.09	2.61	2.8
0	6.30	4.00	2.30	3.5
4	6.21	3.84	2.37	3.2
8	6.30	3.80	2.70	4.5
1	7.21	4.48	2.73	3.2
2	8.25	4.50	3.75	5.7
3	7.45	4.45	3.00	5.8
5	7.20	4.00	3.20	6.9
7	6.70	3.60	3.10	7.0
9	6.60	3.50	3.10	8.5
11	6.70	4.1	2.60	7.89
13	6.00	3.20	2.809	7.5
15	6.10	3.10	3.00	6.4
17	6.20	3.40	2.80	5.8
19	6.20	3.50	2.10	5.5

ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
5	45	131	13	190	9
8	49	201	14	195	7
10	59	133	15	200	10
17	43	140	15	110	9
12	44	196	17	120	8
10	50	125	25	110	11
15	58	226	20	200	27
10	60	205	18	227	24
15	90	250	20	250	25
8	95	263	37	300	28
9	144	270	52	800	29
14	140	190	40	840	32
16	143	94	44	790	27
18	159	85	37	640	24
19	152	90	32	650	22
10	155	81	52	540	18
11	156	85	40	400	18

GROUP B SHEEP NO. [illegible] EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
Pre-infection period	-3	32	6.7	10.9	10.90
	-2	32	6.6	10.7	11.00
	-1	31	6,6	11.5	10.50
	0	32	6.8	11.7	12.45
Incubation period	4	30	6.7	11.3	10.45
	8	30	6.5	11.2	10.20
	1	32	6.4	10.8	10.75
	2	33	6.0	11.9	12.00
	3	30	6.0	10.5	10.00
	5	28	5.9	10.2	9.95
	7	28	6.0	9.3	8.90
	9	27	6.2	8.5	9.00
	11	24	6.4	8.9	8.30
	13	22	6.4	8.2	8.20
	15	22	6.9	8.0	8.80
	17	24	6.7	9.5	8.90
	19	24	6.6	9.6	8.80

WBC /cmm	TN %	ST %	L %	M %	E5 %	B %
4700	32	0	65	0	3	0
4300	33	0	65	0	2	0
4700	34	0	64	0	2	0
4600	34	0	66	0	0	0
5200	35	0	65	0	0	0
5000	36	0	63	0	1	0
4000	38	0	62	0	0	0
4600	39	0	59	0	2	0
5100	45	0	65	0	0	0
5800	52	0	48	0	0	0
6900	71	0	43	0	0	0
10700	58	0	42	0	0	0
15800	62	0	38	0	0	0
15400	60	0	40	0	0	0
15750	60	0	40	0	0	0
15400	59	0	41	0	0	0
15500	60	-	40	0	0	0

GROUP B SHEEP NO. 2 EXPERIMENTAL

BIOCHEMISTRY

P	TP gm%	ALB gm%	GLO gm%
-3	6.70	3.30	3.40
-2	7.37	4.05	3.32
-1	6.79	3.38	3.41
0	7.26	4.35	2.91
4	6.62	3.57	3.05
8	6.70	3.50	3.20
1	7.50	3.75	3.85
2	5.70	3.70	2.00
3	6.00	3.55	2.45
5	6.00	3.50	2.50
7	6.10	3.10	3.00
9	6.10	3.30	2.80
11	6.30	3.40	2.90
13	6.50	3.70	2.80
15	7.00	3.75	3.25
17	6.90	3.95	2.95
19	6.80	3.90	2.90

GLU mmol/l	ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
2.0	6	39	214	13	100	8
1.5	4	53	211	11	114	8
3.5	6	50	218	11	100	8
2.7	5	59	227	11	110	10
2.6	6	44	217	11	112	9
2.9	10	50	215	10	120	10
3.0	4	69	165	11	150	11
3.4	3	88	101	21	228	22
4.8	10	95	110	25	320	27
5.9	9	94	89	45	640	28
6.4	8	155	90	88	730	31
6.3	7	160	74	70	740	24
7.9	7	161	60	65	860	22
8.5	9	152	54	70	650	24
8.3	9	154	50	54	740	26
7.8	8	160	55	52	720	22
7.5	10	158	58	55	700	20

GROUP B SHEEP NO. 3 EXPERIMENTAL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC /cmm	TN %	ST %	L %	M %	E %	B %
-3	32	6.5	11.4	10.50	4500	40	0	56	0	4	0
-2	30	5.8	11.6	12.50	5600	42	0	58	0	0	0
-1	33	6.8	11.6	13.10	5600	48	0	52	0	0	0
0	34	6.0	12.2	13.15	4400	46	0	54	0	0	0
4	32	6.1	11.9	13.40	4500	45	0	55	0	0	0
8	30	6.0	11.4	13.10	4700	45	0	52	0	3	0
1	34	6.8	12.3	12.00	6800	50	0	50	0	0	0
2	35	6.2	12.7	15.00	6500	48	0	48	0	4	0
3	32	6.9	10.3	13.10	7500	56	0	44	0	0	0
5	28	6.6	10.4	12.30	10400	60	0	40	0	0	0
7	26	66	9.6	10.40	10800	59	0	41	0	6	0
9	D I E D			D U R I N G			F E V E R				

GROUP B SHEEP NO. 3 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	Alb gm%	Glo gm%	Glu mmol/l
-3	6.40	3.50	2.90	4.3
-2	6.44	4.01	2.43	2.8
-1	6.70	3.59	4.11	24
0	6.35	4.22	2.13	4.7
4	6.58	4.01	2.57	2.4
8	6.20	4.10	2.10	3.0
1	7.76	4.15	3.61	4.5
2	6.75	4.00	2.95	5.2
3	7.10	4.10	3.00	6.9
5	6.70	3.40	3.30	7.4
7	6.60	3.70	2.90	8.9
9				

ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN (iv)
17	91	110	14	130	8
5	96	191	14	150	8
8	62	147	17	152	6
50	119	200	17	190	11
30	70	175	18	200	7
20	80	160	15	210	9
10	144	232	91	450	23
18	138	177	84	350	18
20	138	89	79	500	20
8	145	90	69	520	27
10	144	76	110	640	27

GROUP B SHEEP NO. 4 CONTROL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%	RBC x10⁶ /cmm
-3	30	7.4	9.9	10.30
-2	30	7.5	10.1	10.50
-1	28	7.6	10.2	9.95
0	27	7.4	9.4	9.50
4	S A M P L E			
8	"			
1	30	7.0	10.5	10.50
2	30	6.8	9.9	10.00
3	31	6.9	10.2	10.20
5	34	7.2	11.5	10.95
7	32	6.9	10.7	9.70
9	30	6.8	10.8	9.90
11	31	7.1	11.1	10.30
13	30	6.9	10.9	10.40
15	30	7.0	10.7	10.10
17	28	6.6	10.6	11.00
19	29	6.5	11.2	10.50

WBC /cmm	TN %	ST %	L %	M %	E %	B %
6600	40	0	55	0	0	0
6700	40	0	60	0	0	0
7300	36	0	60	0	4	0
6400	39	0	60	0	1	0
L O S T						
4478	40	0	58	0	2	0
4900	30	0	65	0	5	0
4500	35	0	64	0	1	0
4700	37	0	63	0	0	0
5400	38	0	60	0	2	0
4900	40	0	58	0	2	0
4600	41	0	59	0	0	0
4800	40	0	58	0	2	0
5100	42	0	58	0	0	0
5200	39	0	50	0	5	0
4900	38	0	60	0	2	0

GROUP B SHEEP NO. 4 CONTROL
BIOCHEMISTRY

P	TP gm%	Alb gm%	Glo gm%	Glu mmol/l
-3	8.26	3.95	4.34	2.0
-2	7.5	3.60	3.90	3.0
-1	6.0	3.41	2.60	3.7
0	7.95	3.58	4.33	2.8
4	7.21	3.56	3.65	2.8
8	S A M P L E			
1	8.45	3.74	4.5	4.0
2	6.95	3.95	3.00	3.2
3	6.85	3.35	3.50	3.5
5	7.02	3.40	3.58	3.4
7	6.90	3.50	3.40	3.5
9	6.70	3.70	3.00	4.7
11	7.00	3.40	3.60	4.2
13	7.00	3.25	3.75	4.1
15	7.10	3.60	3.50	4.5
17	6.90	3.90	3.00	4.2
19	6.80	3.90	2.90	38

Alt (iv)	Ast (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN (iv)
5	76	144	14	195	7
9	43	147	15	200	9
17	84	128	17	210	7
5	61	119	18	220	10
5	63	205	12	195	5
H E M O L Y S E D					
5	81	167	13	189	10
4	75	154	15	110	7
7	68	125	18	194	9
10	72	134	16	179	8
10	79	134	17	210	10
11	80	145	14	205	8
8	84	167	17	220	7
19	69	164	13	194	9
10	70	170	14	195	10
9	65	114	8	160	9
9	62	120	10	175	11

GROUP C SHEEP NO. 1 EXPERIMENTAL

HAEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
Pre-infection period	-3	38	6.6	13.8	12.25
	-2	37	6.8	13.2	14.25
	-1	37	6.8	13.1	15.00
Infected Incubation period	0	36	6.6	12.6	14.25
	4	32	6.5	13.0	1600
	8	34	6.4	12.0	12.00
After Fever onset	1	30	6.4	12.0	10.00
	2	30	6.4	10.5	10.00
	3	26	6.2	9.0	9.50
	5	24	6.0	8.5	8.50
	7	25	6.0	8.0	8.75
	9	24	6.0	8.2	8.00
	11	24	7.2	8.0	8.90
	13	22	7.0	8.1	8.50
	15	22	6.9	8.0	8.20
	17	24	6.7	8.0	8.00
	19	24	6.8	8.6	8.00

WBC /cmm	TN %	ST %	L %	M %	E %	B
7,700	54	0	42	0	4	0
6400	48	0	52	0	0	0
6,500	54	0	56	0	0	0
6600	41	0	59	0	0	0
5400	45	0	55	0	0	0
4000	50	0	50	0	0	0
8795	52	0	48	0	0	0
13000	52	0	48	0	0	0
13500	60	0	40	0	0	0
15000	62	0	38	0	0	0
15200	60	0	40	0	0	0
14800	62	0	38	0	0	0
14600	56	0	40	0	4	0
15200	54	0	44	0	2	0
16200	50	0	46	0	4	0
15100	52	0	45	0	3	0
15700	54	0	44	0	2	0

GROUP I SHEEP NO. 1 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l
-3	6.60	3.50	3.10	4.30
-2	6.80	3.72	3.08	3.40
-1	6.80	4.00	2.80	4.00
-0	6.60	3.70	2.90	4.10
4	6.60	3.76	2.84	5.80
1	7.17	3.50	3.67	7.90
2	5.24	2.80	2.40	4.95
3	6.00	2.50	3.50	7.10
5	6.50	3.10	3.40	7.50
7	6.60	3.10	3.50	6.80
9	6.54	3.00	3.50	6.50
11	7.03	3.80	3.25	6.4
13	6.9	3.90	3.00	6.6
15	6.7	3.80	2.90	5.2
17	6.7	4.00	2.70	4.9

ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN (iv)
10	40	161	17	168	7
10	36	150	20	187	15
10	40	163	27	152	20
10	42	160	20	160	18
10	43	119	20	169	20
6	42	51	20	200	20
7	64	34	21	201	20
7	84	101	23	325	26
8	95	107	44	420	24
7	112	98	40	420	25
8	127	90	238	420	24
9	145	71	32	490	15
8	130	82	21	560	16
8	122	80	22	540	14
9	120	79	20	500	13

GROUP C SHEEP NO. 2 EXPERIMENTAL

HEMATOLOGY

Pre-infection	Dp	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC /cmm	TN %	ST %	L %	M %	E %	B %
Period	-3	36	6.4	13.1	15.60	4200	44	0	37	0	19	0
	-2	36	6.6	13.4	16.00	6300	40	0	18	0	18	0
	-1	36	6.5	13.1	1600	8000	42	0	42	0	16	0
Infection	0	34	6.4	12.8	16.00	6400	48	0	38	0	14	0
Incubation period	4	33	6.8	11.4	14.40	4000	59	0	36	0	5	0
	8	34	6.4	10.6	14.50	4200	57	0	39	0	4	0
After Fever Onset	1	30	6.4	10.5	12.00	8800	60	0	38	0	2	0
	2	27	6.0	9.5	10.00	12900	58	4	36	0	2	0
	3	25	5.0	9.7	10.00	14700	60	0	37	3	0	0
	5	24	6.0	8.2	9.20	16500	68	4	32	0	0	0
	D		J		E		D					

GROUP C. SHEEP NO. 2 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP	ALB	GLO	GLU	ALT
	gm%	gm%	gm%	mmol/l	(iv)
-3	6.40	3.70	2.70	4.5	9
-2	6.60	3.60	3.00	2.9	9
-1	6.70	3.80	2.9	4.6	8
0	6.60	3.80	2.80	4.8	8
4	6.70	3.70	3.00	5.6	9
8	6.60	3.60	3.00	3.0	6
1	7.70	4.70	3.00	5.7	7
2	4.76	2.70	2.10	8.0	7
3	5.57	3.00	2.57	8.8	7
5					

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
28	136	26	127	5
37	122	25	180	6
40	131	28	129	4
42	129	30	129	7
40	131	27	12	10
36	130	29	229	10
90	52	27	349	10
110	59	209	589	15
120	45	901	661	22

GROUP C SHEEP NO. 3 EXPERIMENTAL

HEMATOLOGY

Pre-infection	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
period	-3	27	7.2	9.5	15.10
	-2	25	9.0	90.6	12.30
	-1	26	7.5	9.6	13.00
Infected	0	27	7.2	9.4	12.80
Incubation period	4	29	7.6	10.7	12.60
	8	28	7.2	9.6	12.40
After disease onset	1	26	8.2	9.4	10.00
	2	26	7.9	9.0	10.20
	3	24	8.4	9.1	10.00
	5	23	7.0	9.9	10.00

WBC /cmm	TN %	ST %	L %	M %	E %	B %
5900	43	0	56	0	1	0
6000	40	0	58	0	2	0
6000	40	0	58	0	2	0
7,600	42	0	56	0	2	0
6600	34	0	65	0	1	0
4600	22	0	77	0	1	0
8349	60	0	39	0	1	0
12000	60	0	39	0	1	0
16100	63	0	37	0	0	0
1800	65	0	37	0	0	0

GROUP C. SHEEP NO. 3 EXPERIMENTAL

BIOCHEMISTRY

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l	ALT (iv)
-3	7.2	3.5	3.7	3.0	4
-2	7.0	4.6	4.4	5.4	4
-1	7.0	4.0	3.0	4.8	8
0	6.9	3.3	3.6	45	10
4	5.3	3.6	3.7	5.6	8
8	6.0	3.2	2.8	5.0	10
1	7.8	2.9	4.9	5.6	2
2	8.0	5.0	3.0	6.7	2
3	8.0	4.9	3.1	9.3	2
5	8.0	4.9	3.1	9.5	2

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
51	76	15	111	10
51	89	20	124	10
40	110	18	110	10
48	90	20	115	10
60	219	38	184	18
62	90	45	300	20
60	85	41	453	20
69	77	55	837	22
71	71	84	851	22
70	75	90	900	26

GROUP C SHEEP NO. 4 CONTROL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%
-3	28	7.0	10.0
-2	30	7.0	10.0
-1	30	7.0	10.0
0	28	6.9	11.0
4	25	6.6	8.7
8	27	6.6	9.0
1	26	6.6	9.2
2	28	7.0	9.3
3	33	6.4	10.7
5	34	6.8	11.0
7	32	6.5	10.8
9	32	7.0	10.3
11	32	7.0	10.3
13	30	7.0	9.5
15	29	6.9	10.0
17	32	6.6	9.9
19	28	6.8	9.8

RBC x106 /cmm	WBC /cmm	TN %	ST %	L %	M %	E %	B %
11.15	6200	36	0	64	0	0	0
11.30	9600	42	0	56	0	2	0
11.20	8700	40	0	58	0	2	0
11.30	9000	41	0	57	0	2	0
10.65	1000	41	0	57	0	2	0
9.60	9000	40	0	55	0	5	0
9.50	7728	41	0	55	0	4	0
12.00	7600	47	0	48	0	2	0
12.00	7000	38	0	60	0	2	0
11.50	7500	38	0	60	0	2	0
10.50	7400	38	0	60	0	2	0
10.06	7900	40	0	58	0	2	0
11.40	8900	40	0	60	0	0	0
1200	8900	42	0	56	0	2	0
12.10	7500	38	0	60	0	2	0
11.50	6900	40	0	58	0	2	0
11.70	6700	38	0	60	0	2	0

GROUP C. SHEEP NO. 4 CONTROL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%
-3	7.00	4.00	3.00
-2	7.00	3.75	3.75
-1	6.90	3.50	3.40
0	6.70	3.70	3.00
4	7.20	4.00	3.20
8	7.40	4.10	3.30
1	7.30	4.00	3.30
2	6.70	3.50	3.70
3	6.60	3.50	3.70
5	6.60	3.50	3.10
7	6.80	3.70	3.10
9	6.60	3.50	3.10
11	6.60	3.60	3.00
13	6.60	3.60	3.00
15	6.60	3.40	3.20
17	6.80	3.80	3.00
19	6.90	3.95	3.05

GLU mmol/l	ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
4.9	8	42	56	25	197	10
5.0	10	40	58	20	190	13
5.0	11	42	60	23	160	12
5.7	11	41	69	28	139	16
4.5	10	45	60	20	160	7
5.1	8	39	62	22	191	7
4.9	10	40	57	25	113	10
4.7	11	41	49	20	138	9
4.7	11	41	49	20	138	9
5.0	10	46	52	25	170	10
5.1	10	44	59	20	160	7
4.9	10	43	50	17	163	7
5.3	12	42	46	29	175	13
5.3	12	40	50	20	150	12
5.4	11	40	52	21	160	11
4.9	10	45	54	19	160	10
4.7	9	50	57	18	140	9

GROUP D SHEEP NO. 1 EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
Pre-infection	-3	38	6.9	10.30	12.00
	-2	34	6.7	10.40	11.80
	-1	32	6.8	11.70	11.90
	0	36	6.6	11.80	12.00
Incu-bation	4	34	6.6	12.40	11.80
	8	28	6.7	9.20	11.30
After disease onset	1	28	6.4	9.10	10.40
	2	27	6.3	8.15	9.20
	3	24	6.0	8.40	8.40
	5	24	5.6	8.00	8.20
	7	25	6.1	7.90	7.50
	9	22	6.4	7.20	6.20
	11	D		E	A
	13				
	15				

WBC /cmm	TN %	ST %	L %	M %	E %	B %
5600	34	0	64	0	2	0
4700	34	0	64	0	2	0
5800	38	0	59	0	3	0
5400	42	0	55	0	3	0
5700	44	0	55	0	1	0
6000	50	0	48	0	2	0
8100	54	0	44	0	2	0
9700	61	0	39	0	0	0
11500	58	0	42	0	0	0
14100	60	0	40	0	0	0
16100	60	0	40	0	0	0
16800	65	0	35	0	0	0

D

GROUP D SHEEP NO. 1 EXPERIMENTAL

BIOCHEMISTRY

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l	ALT (iv)
-3	6.9	4.10	2.80	3.70	11
-2	6.6	3.90	2.70	4.0	11
-1	6.7	3.70	3.00	4.1	13
0	6.6	3.55	3.05	4.0	12
4	6.5	3.70	2.80	3.9	10
8	6.6	3.80	2.80	4.7	9
1	6.4	3.40	3.00	4.8	11
2	6.2	3.50	2.70	5.2	10
3	6.1	3.30	2.80	7.5	10
5	6.0	3.20	2.80	7.4	14
7	6.0	3.10	2.90	8.0	14
9	6.1	3.10	3.00	8.5	13
11			D		E
13					
15					

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
52	121	10	98	10
57	125	8	80	7
60	110	12	100	7
61	125	13	120	8
59	130	10	110	9
54	110	11	199	11
57	98	19	170	12
49	84	15	200	17
148	82	11	540	20
150	70	13	740	22
154	63	27	760	28
150	55	30	980	28
A		D		

GROUP D SHEEP NO.2 EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC /cmm	TN %	ST %	L %	M %	E %	B %
Pre-	-3	38	7.0	12.80	13.10	4600	34	0	63	0	3	0
infection	0	36	7.1	11.50	11.80	4800	36	0	62	0	2	0
Incubation	4	34	6.4	11.70	11.70	7000	38	0	59	0	3	0
period	8	36	6.6	10.50	11.60	6700	44	0	53	0	3	0
After												
disease	1	30	6.2	9.60	10.40	7200	50	0	50	0	0	0
onset	2	24	6.0	8.40	9.80	8400	48	0	50	0	2	0
	3	26	6.1	8.40	8.90	10300	52	0	46	0	2	0
	5	22	6.4	8.20	8.50	12300	54	0	45	0	1	0
	7	24	6.2	7.40	8.60	14100	57	0	43	0	0	0
	9	20	6.5	7.50	8.00	16800	55	0	45	0	0	0
	11	18	6.6	7.00	7.40	16900	60	0	40	0	0	0
	13	18	6.6	6.50	7.00	18400	62	0	38	0	0	0
	15	20	6.8	6.70	7.20	17000	61	0	39	0	0	0
	17	22	5.7	7.8	7.5	16200	60	0	40	0	0	0
	19	24	5.9	7.2	8.0	15600	59	0	41	0	0	0

GROUP D SHEEP NO. 2 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l	ALT (iv)
-3	7.1	4.20	2.90	3.9	11
0	7.0	4.10	2.90	4.1	12
4	7.0	4.20	2.80	4.2	16
8	7.0	4.50	2.50	3.8	15
1	6.7	4.30	2.40	3.7	9
2	6.6	3.70	2.90	3.7	6
3	6.4	3.60	2.80	4.0	11
5	6.0	3.70	2.30	4.5	7
7	6.0	3.15	2.85	4.2	8
9	6.2	3.00	3.20	5.3	11
11	6.2	2.90	3.30	5.4	15
13	6.4	2.75	3.65	5.5	14
15	6.6	3.00	3.60	6.0	15
17	6.6	3.75	2.85	7.9	12
19	6.6	3.50	3.10	7.4	11
	6.2	3.40	2.80	6.9	11

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
60	140	10	190	9
59	170	11	170	7
61	155	17	120	8
62	130	17	90	8
67	135	19	110	9
70	140	20	85	11
75	90	21	98	11
55	84	15	110	20
60	62	16	120	21
62	54	20	170	28
63	44	19	290	27
61	40	25	380	25
67	42	29	350	26
158	38	34	500	22
159	38	30	400	22
150	45	54	420	20

GROUP D SHEEP NO. 3 EXPERIMENTAL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC cmm
-3	28	6.9	10.2	12.50	4700
-2	29	6.8	10.4	11.90	5200
-1	30	6.6	11.5	11.80	5400
0	29	6.7	9.5	12.00	4800
4	28	6.6	10.0	11.30	5500
1	30	6.9	10.20	11.40	6700
2	28	6.5	9.90	10.20	8200
3	27	5.6	9.2	10.10	10100
5	27	5.6	9.2	9.3	10700
7	26	5.4	8.5	8.40	13500
9	24	6.0	8.1	8.00	14700
11	22	6.0	8.0	8.00	16800
13	26	6.1	7.5	7.50	16700
15	26	5.9	7.4	7.20	16200
17	25	5.7	7.9	7.80	16100
19	26	5.8	7.9	8.00	15900

TN %	ST %	L %	M %	E %	B %
34	0	64	0	2	0
28	0	68	0	4	0
29	0	67	0	4	0
30	0	68	0	2	0
35	0	60	0	2	0
40	0	58	0	2	0
40	0	59	0	1	0
38	0	61	0	1	0
44	0	56	0	0	0
51	0	49	0	0	0
56	0	44	0	0	0
60	0	40	0	0	0
62	0	38	0	0	0
61	0	39	0	0	0
60	0	40	0	0	0
59	0	41	0	0	0

GROUP D SHEEP NO. 3 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l	ALT (iv)
-3	6.8	3.90	2.90	3.9	11
-2	6.9	3.90	3.00	4.0	10
-1	6.8	3.80	3.00	3.8	9
0	6.7	3.75	2.95	4.2	7
4	6.7	3.60	3.10	3.8	8
8	6.7	3.70	3.00	3.9	11
1	6.9	4.00	2.90	4.5	7
2	6.6	3.90	2.70	4.6	9
3	6.2	3.50	2.70	5.0	11
5	6.0	3.20	2.80	4.7	10
7	5.5	3.50	2.00	4.8	12
9	5.9	3.50	2.40	5.70	14
11	6.0	3.70	2.30	7.2	14
13	6.0	3.75	2.25	7.9	10
15	6.0	3.55	2.45	7.0	9
17	6.0	3.40	2.60	6.8	11
19	5.9	3.20	2.70	5.2	11

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
60	110	1.5	100	8
54	120	22	107	9
70	130	15	110	10
72	100	16	120	9
64	120	20	175	7
63	146	10	180	8
59	101	11	260	8
49	90	11	240	12
57	98	19	380	17
56	97	20	640	24
58	92	21	730	24
102	80	15	740	22
160	94	40	860	27
165	150	140	650	28
162	154	150	740	28
155	140	84	620	22
142	141	89	700	24

GROUP D SHEEP NO. 4 CONTROL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%	RBC x106 /cmm
-3	28	6.7	9.90	10.30
-2	28	6.6	9.80	9.50
-1	29	6.6	9.90	9.70
0	30	6.7	10.50	11.40
4	28	6.8	10.70	10.70
8	31	6.8	10.10	12.30
1	32	6.6	10.20	12.50
2	28	6.7	10.70	12.50
3	30	6.7	10.10	11.25
5	32	6.7	11.00	11.55
7	30	6.9	10.10	11.90
9	30	6.9	9.20	12.10
11	28	6.8	9.30	11.70
13	27	6.9	9.40	10.40
15	27	7.0	9.70	10.20
17	27	6.8	10.60	9.90
19	28	6.7	10.10	10.30

WBC /cmm	TN %	ST %	L %	M %	E %	B %
4600	28	0	68	0	4	0
4500	30	0	67	0	3	0
5400	31	0	64	0	5	0
6400	29	0	69	0	2	0
5400	26	0	74	0	0	0
5200	28	0	72	0	0	0
4900	30	0	68	0	2	0
4700	34	0	64	0	2	0
4300	32	0	65	0	3	0
4700	35	0	65	0	0	0
4900	36	0	60	0	4	0
5000	34	0	64	0	2	0
5000	34	0	64	0	2	0
6200	29	0	71	0	0	0
5800	30	0	69	0	1	0
4900	38	0	62	0	0	0
5100	37	0	62	0	1	0

GROUP D SHEEP NO. 4 CONTROL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l
-3	6.7	3.40	3.30	3.90
-2	6.7	3.50	3.20	3.50
-1	6.7	3.20	3.50	4.00
0	6.7	3.30	3.40	3.90
4	6.8	3.40	3.40	2.90
8	6.9	4.00	2.9	3.90
1	6.8	3.90	2.9	3.40
2	6.6	3.50	3.10	3.70
3	6.6	3.10	3.50	3.70
5	6.8	3.00	3.80	3.40
7	6.8	3.15	3.65	3.50
9	6.8	3.45	3.35	4.20
11	6.9	3.10	3.80	4.10
13	6.9	3.90	3.00	3.90
15	6.8	4.00	2.8	3.80
17	7.0	4.10	2.90	4.10
19	6.7	3.80	2.90	4.50

ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
10	69	55	30	190	10
12	52	62	15	195	12
12	51	113	17	100	9
14	60	60	20	110	8
15	65	12	12	150	7
10	52	149	14	90	7
9	59	152	16	150	9
11	74	155	24	155	10
13	72	96	18	100	13
12	76	89	18	170	15
8	80	92	12	120	10
9	78	100	14	120	10
11	89	115	18	110	9
11	63	117	22	118	11
10	60	128	10	129	12
12	70	130	14	114	14
13	75	125	17	108	12

GROUP E. SHEEP NO. 1 EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 cmm	WBC cmm	TN %	ST %	L %	M %	E %	B %
Pre-infection	-3	28	5.8	12.00	11.25	5400	28	0	69	0	3	0
	-2	30	6.0	11.95	11.75	5800	28	0	72	0	0	0
Period	-1	30	5.1	11.20	12.30	6500	32	0	66	0	2	0
Incubation	0	29	4.9	11.50	11.40	4300	31	0	67	0	2	0
Period	4	29	5.0	10.80	10.45	3900	30	0	68	0	2	0
	8	28	5.4	10.40	10.50	5700	34	0	63	0	3	0
After fever	1	26	5.9	10.20	10.90	8900	40	0	58	0	2	0
Onset	2	25	5.7	9.80	9.80	10500	41	0	59	0	0	0
	3	26	6.0	9.70	10.00	11600	44	0	56	0	0	0
	5	D I E D D U R I N G F E V E R										
	7											
	9											
	11											
	13											
	15											
	17											
	19											

GROUP E. SHEEP NO. 1 EXPERIMENTAL

BIOCHEMISTRY

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l
-3	5.8	3.2	2.60	4.5
-2	5.9	3.4	2.50	4.2
-1	5.2	3.4	1.80	3.5
0	5.0	3.0	2.00	4.9
4	5.0	3.1	1.90	5.2
8	5.6	3.6	2.00	6.7
1	5.8	3.4	2.4	6.8
2	5.8	3.5	2.3	8.2
3	6.0	3.7	2.3	8.5

ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
11	50	120	8	145	7
8	45	94	18	120	7
10	42	100	14	170	9
10	55	110	9	180	11
7	85	75	10	195	12
9	92	81	13	240	18
11	190	80	25	540	21
15	170	70	40	630	22
20	180	65	60	720	27

GROUP E SHEEP NO. 2 EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106/ cmm	WBC cmm	TN %	ST %	L %	M %	E %	B %
Pre-												
infection	-3	29	5.8	11.20	12.00	5800	30	0	66	0	4	0
Period	-2	27	6.0	11.60	11.15	7500	31	0	67	0	2	0
	-1	28	6.1	12.00	11.45	6200	31	0	67	0	2	0
	0	30	5.7	10.90	12.29	5900	30	0	69	0	1	0
Incubation	4	29	5.2	11.50	11.80	4500	32	0	66	0	2	0
Period	8	26	4.9	11.30	10.50	4200	34	0	63	0	3	0
After												
disease	1	25	5.3	9.10	10.70	5800	37	0	63	0	0	0
Onset	2	24	5.2	8.10	10.80	8700	40	0	59	0	1	0
	3	22	4.8	7.90	9.00	10100	40	0	59	0	1	0
	5	20	5.9	7.50	9.10	11500	44	0	56	0	0	0
	7	18	6.0	8.00	9.20	12700	48	0	52	0	0	0
	9	20	6.1	7.10	8.90	13700	50	0	50	0	0	0
	11	21	5.7	7.80	8.50	14500	51	0	49	0	0	0
	13	24	5.6	8.30	8.40	14800	55	0	45	0	0	0
	15	25	5.4	8.10	8.00	14900	55	0	45	0	0	0
	17	28	5.5	8.20	8.12	14700	52	0	48	0	0	0
	19	28	6.0	8.50	8.22	15000	54	0	46	0	0	0

GROUP E SHEEP NO. 2 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO mmol/l	GLU (iv)	ALT (iv)
-3	5.8	3.4	2.4	4.5	11
-2	5.2	3.2	2.0	5.0	13
-1	5.4	3.1	2.3	4.2	9
0	5.1	3.5	2.2	3.9	10
4	5.8	3.3	2.5	5.0	10
8	5.7	3.3	2.4	4.9	14
1	5.4	3.3	2.2	4.8	15
2	5.4	3.4	2.00	5.1	11
3	5.5	3.7	1.8	5.2	12
5	4.9	3.10	1.80	5.8	12
7	4.0	3.00	2.00	6.2	7
9	5.5	3.50	2.00	6.7	8
11	6.2	3.1	3.1	7.0	8
13	5.7	3.6	2.1	8.2	9
15	5.8	3.7	2.1	7.4	11
17	6.1	3.6	2.5	6.3	12
19	6.1	3.7	2.4	6.4	11

AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
45	110	10	135	10
50	84	18	120	7
40	85	15	165	8
42	90	14	192	9
57	73	9	150	10
64	140	10	220	11
65	121	19	250	11
73	122	17	270	12
100	91	19	350	18
98	85	25	400	18
121	74	26	370	22
139	70	30	480	24
143	43	27	500	25
150	65	28	790	24
155	55	30	870	27
145	54	34	820	22
150	60	33	800	24

GROUP E. SHEEP NO. 3 - EXPERIMENTAL

HEMATOLOGY

	P	PCV %	TP gm%	HB gm%	RBC x106 /cmm	WBC cmm	TN %	ST %	L %	M %	E %	B %
Pre infection Period	-3	32	5.8	12.30	12.40	5100	31	0	67	0	2	0
	-2	32	6.2	12.20	12.45	5200	32	0	64	0	4	0
	-1	33	5.9	12.91	11.85	5800	28	0	69	0	3	0
Incubation Period	0	34	5.5	11.70	11.90	4600	30	0	70	0	0	0
	4	30	5.1	11.50	12.00	8100	34	0	65	0	1	0
	8	29	5.0	11.00	10.50	8200	32	0	66	0	1	0
After disease Onset	1	27	5.4	10.40	10.10	10500	36	0	62	0	2	0
	2	28	5.8	9.9	10.15	11400	39	0	60	0	1	0
	3	26	6.0	10.2	10.20	12600	42	0	58	0	0	0
	5	24	6.4	9.5	9.80	12700	44	0	56	0	0	0
	7		D I E D									

GROUP E. SHEEP NO. 3 EXPERIMENTAL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%
-3	5.8	3.4	2.4
-2	5.9	3.7	2.2
-1	6.0	3.3	2.7
0	5.4	3.5	1.9
4	5.2	3.2	2.0
8	5.1	3.3	1.8
1	5.4	3.5	1.9
2	5.8	3.8	2.0
3	6.0	3.9	2.1
5	6.3	3.5	1.8

GLU mmol/l	ALT (iv)	AST (iv)	AP (iv)	CPK (iv)	LDH (iv)	BUN mg%
4.7	10	55	160	8	130	7
4.5	7	45	145	15	120	7
4.2	10	60	125	18	185	8
4.5	8	80	134	15	190	10
5.4	8	95	120	14	170	11
5.2	12	84	110	18	310	13
5.9	9	110	85	20	520	8
6.3	11	120	97	35	720	22
8.5	10	135	65	42	800	22
8.7	22	140	60	44	840	24

GROUP E. SHEEP NO. 4 CONTROL

HEMATOLOGY

P	PCV %	TP gm%	HB gm%
-3	28	7.0	10.10
-2	30	6.8	11.50
-1	31	6.9	11.20
0	32	6.4	10.50
4	29	6.2	10.70
8	28	6.7	9.70
1	28	7.0	9.80
2	34	7.2	9.70
3	32	6.4	10.00
5	31	6.9	10.50
7	33	6.9	10.10
9	30	6.7	10.20
11	28	6.6	9.90
13	29	6.7	9.80
15	30	7.1	10.30
17	28	6.8	10.10
19	26	6.8	10.00

RBC x106	WBC /cmm	TN %	ST %	L %	M %	E %	B %
10.30	5400	30	0	67	0	3	0
11.50	6000	31	0	66	0	3	0
12.20	8200	30	0	68	0	2	0
11.50	6700	29	0	69	0	2	0
11.70	7400	28	0	70	0	2	0
10.20	6900	32	0	68	0	0	0
10.70	8000	32	0	67	0	1	0
10.80	9000	31	0	69	0	0	0
11.00	10500	32	0	68	0	0	0
10.50	9400	33	0	66	0	0	0
10.20	8700	29	0	70	0	1	0
10.25	8500	28	0	68	0	4	0
10.35	8700	29	0	69	0	3	0
9.95	9100	35	0	63	0	2	0
9.90	8500	34	0	66	0	0	0
10.15	7500	32	0	66	0	2	0
10.00	8100	31	0	68	0	1	0

GROUP E. SHEEP NO. 4 CONTROL

BIOCHEMISTRY RESULTS

P	TP gm%	ALB gm%	GLO gm%	GLU mmol/l
-3	6.9	3.7	3.2	4.9
-2	6.8	3.8	3.0	4.2
-1	6.9	4.0	2.9	3.5
0	6.6	3.7	2.9	4.2
4	6.5	3.5	3.0	4.5
8	6.6	3.7	2.9	5.0
1	7.0	4.1	2.9	5.1
2	6.9	4.0	2.9	3.9
3	6.6	3.7	2.9	3.4
5	6.9	3.9	3.0	4.6
7	6.8	3.8	3.0	4.5
9	6.8	3.9	2.9	4.2
11	6.6	3.7	2.9	4.2
13	6.5	3.8	2.7	4.9
15	7.0	4.0	3.0	4.6
17	6.9	3.9	3.0	3.9
19	6.8	3.9	2.9	3.8

ALT iu	AT iu	AP (iu)	CPK (iu)	LDB %	BUN mg%
10	45	70	10	155	8
12	65	65	15	140	8
15	55	62	20	170	7
11	55	85	18	160	9
11	45	110	18	175	10
7	65	100	22	195	9
7	70	89	24	145	9
8	57	92	10	170	12
9	62	95	8	160	10
11	65	70	8	180	7
12	70	55	17	170	6
13	80	67	15	155	6
8	73	120	19	140	7
9	74	110	12	130	9
10	74	100	12	150	10
10	65	97	13	170	11
9	80	98	10	170	10