STUDY OF REACTION OF YANKASSA RAMS TO EXPERIMENTAL *COWDRIA RUMINANTIUM* (JAJI STOCK) INFECTION AND CROSS-IMMUNITY TEST BETWEEN IT AND D225 STRAIN

BY

FAISAL MAHMOUD EL-IMAIRI
B.Sc (HONS) (A. B. U., ZARIA)

A THESIS

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CERTIFICATION

This thesis entitled, "Study of Reaction of Yankassa Rams to Experimental Cowdria ruminantium (Jaji Stock) Infection and Cross-Immunity Test Between It and D225 Strain" by Faisal Mahmoud El-Imairi, meets the regulations governing the award of the Degree of Masters of Science (Vet Parasitology and Entomology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

DIGITIZED

.....
Prof. A.A. Ilemobade, DVM; M.Sc.; Ph.D.
Vice-Chancellor, Fed. Univ. of Tech. Akure
External Examiner.

Date: _____

A. Sannusi

.....
Dr. A. Sannusi, DVM; M.Sc.
Chairman
Supervisory Committee

Date: 29/11/92

.....
Prof. E.O. Gyang, DVM; M.Sc.; Ph.D.
Member, Supervisory Committee

Date: 30/11/92

.....
Dr. E.K. Kyewalabye, Ph.D.
Member, Supervisory Committee

Date: _____

DECLARATION

I hereby declare the originality of this research work which was carried out by me in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The Project was supervised by Dr. A. Sannusi, Dr. E.K. Kyewalabye, Professors E.O. Gyang and C.D. Ezeokoli, all of the Faculty of Veterinary Medicine. The work of other investigators are acknowledged and referred to accordingly. No part of this thesis has previously been published or submitted elsewhere for a Degree or Diploma.

F.M. El-Imairi

A.B.U. Zaria
January, 1991.

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DEDICATION

Dedicated to my late Father,
Shiekh M. T. EL-Imairi (may his soul
rest in perfect peace) who provided
every possible assistance for my academic
advancement.

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ABSTRACT

Investigations carried out for this thesis involved:- a survey to determine the case infection rate of heartwater from laboratory records at the Department of Veterinary Parasitology and Entomology, A. B. U. Zaria. The case infection rate was low initially in the years 1973 and 1974; having a case infection rate of 4.17 and 3.13 percent respectively and increased in subsequent years with a peak of 43.59 percent in 1985.

An experiment carried out with two groups of 6 - 12 months old Yankassa Sheep. Group 1 consisted of six experimentally infected animals and group 2 had four uninfected controls. Out of the animals experimentally infected with Jaji stock of Cowdria ruminantium, five were susceptible to the primary infection. The incubation period varied between 10 - 14 days post - inoculation. One animal was found to be solidly immune. The five susceptible animals which recovered with the aid of oxytetracycline/LA[1] treatment on days 11 to 13 post inoculation were immune to both

1 - Pfizer, Nigeria Ltd.

the homologous Jaji stock and the heterologous D225 strain of Cowdria ruminantium. This shows that the D225 strain cross-protected against the Jaji stock.

There were no marked changes observed in the haematological studies, except for a significant drop in the mean eosinophil count.

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

Heartwater (Cowdriosis) is an infectious tick - borne disease of ruminants caused by a rickettsial organism known as Cowdria ruminantium and is transmitted by 3-host ticks of the genus, Amblyomma spp. In Nigeria, Amblyomma variegatum is the proven vector of the disease (Ilemobade, 1976). The disease is characterized by high fever, intermittent convulsion, production losses, nystagmus, respiratory problems and anorexia. Heartwater is confined to Africa south of the Sahara, Malagasy and some of the Caribbean Islands (Uilenberg, 1983, Van Winkelhoff and Uilenberg, 1981 and Gruss, 1981). It has been described in various localities by different names; viz: Heartwater due to its hydrothorax and hydropericardium characteristics, 'Kabowa' meaning trembles (Fulani), 'Daji' (Hausa); both referring to the nervous signs, and in the Sudan it is known as 'Khadar' (Ilemobade, 1976). The first case of suspected heartwater in sheep at Vom in Nigeria was in 1928, but the disease was confirmed in 1931 (Leeflang, 1977).

The organism occurs as clumps of gram-negative cocci within the endothelial cells of renal glomeruli, cerebral cortex and other organs of the host animals

Stewart and Howell (1981). They appear as coccoid forms as well as ring, horseshoe and comma forms, measuring 0.49 to 2. 7um in diameter (Stewart and Howell, 1981).

In the majority of developing countries, there is an acute shortage of animal protein for human consumption. One of the greatest hindrances to increased productivity and steady marketing of livestock in Africa is the problem of animal diseases. Of all the livestock diseases endemic to the African continent, heartwater is one of the most important one limiting the numbers and productivity of cattle, sheep and goats, especially in imported breeds. For susceptible populations of cattle, heartwater is second in importance only to East Coast Fever and tsetse-transmitted trypanosomiasis but it has a wider distribution than either of the two (Uilenberg, 1983). Heartwater has also been implicated as one of the major causes of mortalities in small ruminants in the Eastern Cape Coastal region and the Valley Bushveld in particular (Gruss, 1981). Mortality due to heartwater in an enzootic area was three times as great as that from anaplasmosis and babesiosis (Uilenberg, 1983). In the Nigerian savannah regions heartwater is one of the diseases mentioned to be an obstacle to the importation of exotic breeds of animals for any genetic improvement scheme (Sannusi

& Mohammed, 1986). This was also true in the case of South Africa, Sudan and Tanzania (Uilenberg, 1983 and Karrar, 1960).

The diagnosis of heartwater is presently based on clinical signs and post mortem examinations. Little progress has been made in our knowledge of the immunology of heartwater. This has affected the development of suitable serological test for heartwater diagnosis and survey studies (Barre et. al., 1984). In order to gain further insight into the importance of the disease in sheep, the records of the diagnosis of heartwater using the brain smear technique in the Department of Veterinary Parasitology and Entomology for the past 15 years was examined. The Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, also carried out a project on ticks and tick-borne diseases and one of the aims was to produce vaccines against important tick-borne diseases. The outcome was the isolation of Cowdria ruminantium stock (Jaji stock) in cattle. This stock has not been tested in sheep. It was therefore felt important to test the susceptibility of local sheep to this stock and also to cross-protect this stock with the known Cowdria ruminantium strain (D225).

The aims of this study, therefore, were to;

1) test the susceptibility of local sheep to Cowdria

ruminantium Jaji stock;

2. establish if *Cowdria ruminantium* D225 strain cross-protects against the Jaji stock in intact sheep.

CHAPTER TWO

LITERATURE REVIEW

Introduction to the review:

Haartwater causes severe mortalities in imported breeds of cattle, sheep and goats and is thus one of the main factors preventing the improvement of livestock production in Africa by upgrading local livestock. This review will cover aspects of the disease such as: Distribution and Economic importance, causative agent, clinical manifestations, immunology, immunization and treatment.

In the distant past man has introduced on several occasions domestic ruminants into the endemic regions, especially after the colonization of large parts of subsaharan Africa by Europeans. These animals lacked innate resistance to heartwater and suffered heavy losses until a time came when resistant indigenous breeds emerged, selected by natural disease pressure. Heartwater also became a major disease problem when newly independent countries tried to improve livestock production quickly by using highly productive exotic stock which are susceptible to the infection.

(a) Historical background:

Heartwater was first observed in South Africa in sheep and goats but there seems to be no agreement in the literature about its first record. The disease has been referred to under a variety of names,

viz: Mad Gallsickness, Black lung, Veld poisoning, Heartwater and Dronkgalsiekt by Africans; Pericardite exsudative infectieuse (French), Kaboa (Fulani), Daji (Hausa), Enguruti (Masai) and Khadar or Magak by the Sudanese (Alexander, 1931; Neitz, 1968 and Ilemobade, 1976).

That the disease was possibly associated with tick infestation was surmised by the Pioneer leader Louis Trichardt as early as 1838, in what is almost certainly the first reference to heartwater (Haig, 1955; Neitz, 1968). Alexander (1931) believed that the first authentic reference to heartwater in sheep on record was made by Mr. John Webb on 23rd February, 1877, when giving evidence before the Cattle Disease Commission at Grahamstown as stating that the disease was first observed in the Eastern Province of the Cape of Good Hope about 1858, causing a lot of ravages amongst the small stock. At that time a single farmer lost in one season between 2,000 and 3,000 sheep and goats from a disease which was believed to be heartwater.

It was generally believed that heartwater was caused by an ultravisible virus, and not until 1926, when Cowdry clearly demonstrated the aetiological significance of a rickettsial-like organism present in the tissue of both affected animals and vector ticks, which he named Rickettsia ruminantium. It became apparent that the disease was confined to those areas

where conditions favoured the development of the vector. In Sudan, the first description of heartwater was published in the annual report of the Sudan Veterinary Service in 1903 (Karrar, 1968). The disease existed in the Provinces of Kassala, the Blue Nile and in the Upper Nile Province. In South Africa heavy losses of sheep and goats were recorded (Alexander, 1931). It was also reported in Malagasy and some of the Caribbean Islands (Uilenberg, 1977; Van Winkelhoff and Uilenberg, 1981; Gruss, 1981). In Nigeria, the first case of suspected heartwater in sheep was in 1928 at Vom; but the disease was not confirmed until 1931 (Leeflang, 1977).

(b) Biology of Cowdria ruminantium:

Classification and Characteristics:

In the mammalian host, Cowdria ruminantium is found in the cytoplasm of vascular endothelial cells, in which it occurs singly, in colonies or in clumps. The organism appears in various shapes: coccoid, ring-form, comma, spherical and horse-shoe shaped. They occur as dense masses, vary from 0.2 to 2.5 um. Electron microscopical studies of Cowdria ruminantium show considerable pleomorphism and variation in size (Stewart and Howell, 1981). Coccoid granules measure about 0.2 to 1.7 um in diameter (Haig, 1955). Occasionally diplo-formation is observed (Alexander, 1931). Steward and Howell (1981) reported that each

rickettsia is surrounded by two unit membranes. Electron-dense and electron-pale areas are distinguished within the organism. Studying the ultra-structure of Cowdria ruminantium, Peinaar (1970) showed that there is considerable variation in the size of individual organisms and also in the size of the clumps or colonies. He recognised three distinct types of individual, small, measuring 0.49 um in diameter, large measuring 1.04 um in diameter, and very large which measures up to 2.7 um in diameter.

Cowdry (1925) found that the micro-organisms were gram-negative and stained deep blue with Giemsa. Although those in diplo-formation show no trace of red staining material between the two halves. Sometimes fairly clear halos are visible. When these organisms are stained with Unnu-Pappenheims methyl-green pyronin mixture, they usually stain light red but sometimes take on a slightly greenish tint. They stain easily with Fuchsin and Lofflers methylene blue (Alexander, 1931). These organisms also stained well with iron haematoxylin but on differentiation became bleached. They are likewise easily stained by other basic aniline dyes.

The heartwater organisms occur also in the circulating blood of affected animals, which is infective if inoculated intravenously in susceptible ruminants, though conflicting results were obtained in attempt to localize the organism in the blood (Uilenberg, 1983).

Infection has been associated with the white cell fraction (Ilemobade, 1976), while Alexander (1931) concluded that the organisms were firmly adherent to the red cells and leucocytes. Jackson and Neitz (1932) believed they saw organisms lying free in the plasma. Examination of Giemsa stained choroid plexus tissue cultures showed a considerable number of granules scattered throughout the cytoplasm (Stewart and Howell, 1981). Jackson and Neitz (1932) believed that individual rickettsias in the blood enter endothelial cells, where they grow from a single granule to a large group, causing the cell to rupture and disseminate the organisms once again into the blood, thus repeating the cycle. Some authors have also reported the occurrence of Cowdria ruminantium in various cell types such as macrophages, monocytes, histocytes, kupffer's cells, reticulum cells of lymph nodes, fibroblasts and connective tissue cells of various organs (Du Plessis 1970a, 1975; Ilemobade, 1976). Pienaar (1970) found two cells appearing to be monocytes, and other endothelial cells to be infected. Steward and Howell (1981) confirmed the findings of Pienaar, and using the ultrastructural appearance of Cowdria ruminantium, extended these findings to the kidney, but they failed to detect the organisms in lung macrophages. This suggests that the organism is fairly rare or in a form which is difficult to recognise in these tissues.

In ticks, colonies of Cowdria ruminantium were first described in Amblyomma hebraeum by means of conventional histological sections and Giemsa stained smears (Cowdry, 1925). They were demonstrated in the gut epithelial cells and occasionally within the gut lumen. These findings were recently confirmed by fluorescent antibody staining, light and electron microscopy by Bezuidenhout (1984). In Amblyomma variegatum the organisms were found in the midgut epithelial cells (Kocan, Bezuidenhout and Hart, 1987). Alexander (1931) suggested that under natural conditions infection takes place either by regurgitation or by the excretion of infective material from the gut. Transmission by gut regurgitation has been suggested because organisms were not detected in salivary glands. Homogenates from infected adult ticks did not cause heartwater after intravenous injection into susceptible goats. The first description of Cowdria ruminantium in salivary glands of known infective transmitting Amblyomma hebraeum was reported by Kocan et al. (1987), in which both electron-dense and reticulated forms of the organisms were seen. It was also believed that the development of Cowdria ruminantium in the salivary glands is followed by transmission of the organism via this organ to the vertebrate host. Furthermore, this mechanism of transfer appears to be co-ordinated with the feeding cycle of the tick.

The classification of the causative agent of

heartwater is not yet clear. In the 8th edition of Bergey's Manual of Determinative Bacteriology, Cowdria ruminantium was believed to be the sole representative of the genus Cowdria Moshkovski, but has not been accepted by most authorities (Philip, 1974). Cowdry (1925) originally named the organism Rickettsia ruminantium belonging to the order Rickettsiales, family Rickettsiaceae and tribe Ehrlichieae. Hertig and Wolbach (1924) modified Cowdry's definition (1925), that rickettsiae were gram-negative, intracellular, bacterium-like organisms found in arthropods. Cowdry (1926) proposed criteria for classifying any organism in the genus Rickettsia as being smaller than most bacteria and generally less than half a micron in diameter, pleomorphic, located intracellulary in arthropods and staining rather lightly with aniline. Mohan (1968) regarded Cowdria as Chlamydia. However, studies of electron-micrographs showed differences in the replication and developmental stages of chlamydial and rickettsial organisms but resembled those of some viruses (Du Plessis, 1975). It was therefore proposed that the agent of heartwater be named Cowdria ruminantium and be included in the family Rickettsiaceae.

(c) Host range and susceptibility:

Cowdria ruminantium has been found to occur in invertebrate and vertebrate animals. The invertebrate hosts are exclusively ticks within the genus Amblyomma.

It was first believed that heartwater is transmitted mainly by Amblyomma hebraeum (Alexander, 1931; and Haig, 1955), but later on other Amblyomma species were incriminated to be invertebrate hosts of the organism. In the subsaharan Africa, the tick host incriminated is Amblyomma variegatum (Dardiri, Logan and Mebus, 1987), while in states bordering the Gulf of Mexico and Southern states along the Atlantic Coast, Amblyomma maculatum has been established to be the invertebrate host (Dardiri et al., 1987; Uilenberg, 1982). The distribution of the disease in the Carribbean was attributed to the presence of the ticks, A.variegatum, A. maculatum and A. cajennense (Barre, Camus, Birnie, Burridge; Uilenberg and Provost, 1984). In Sudan the only species found to be the invertebrate host of the organism is A. lepidum (Karrar 1965; 1968). It was stated that only five members of the genus Amblyomma are invertebrate hosts of Cowdria ruminantium namely, A. gemma, A. hebraeum, A. pomposum, A.variegatum, and A. lepidum (Ilemobade, 1976). In another report A. cohaerens, A. astrion, A. tholloni were also incriminated (Bezuidenhout, Jacobsz and Catherina, 1986).

Of the vertebrate hosts, the disease was originally described in sheep and goats and for many years cattle were thought not to be susceptible (Alexander, 1931). But later on cattle was also proved to be infected by the organism obtained from either sheep or goats (Alexander, 1931). Apart from domestic ruminants, natural fatal cases

have been encountered in wild antelopes (Springbuck, eland) and certain species of antelope and deer are susceptible to experimental infection (Uilenberg, 1977, 1983). There was one suspected case of heartwater in a camel found in Sudan (Karrar, 1960). Of the small laboratory animals ferrets have proved susceptible (Haig, 1955). The kumm, Kwanyanga and Noride strains are pathogenic to mice (Jongejan et al., 1988).

Susceptibility of ruminants to heartwater varied within the group and within species. It was found that Persian and African sheep were comparatively resistant to heartwater (Alexander, 1931; and Haig, 1955). This natural resistance was well developed in cross-bred as well as in pure bred Persian sheep. Certain strains of heartwater organisms may kill up to 100% of infected adult Merino and English breeds of sheep, while other strains may kill only 30% (Haig, 1955). Sheep indigenous to South Africa possess a high degree of resistance, while goats under similar conditions are just as susceptible as Merino sheep (Alexander, 1931). In Nigeria, brown goats originating from Zaria and areas north of it, and Friesian-Zebu crossbreds obtained from Shika Agricultural Research Station, were uniformly susceptible to heartwater, while goats, cattle and sheep from different parts of northern Nigeria, showed variable susceptibility (Ilemobade, 1976). Alexander (1931) stated that mortality amongst Angora goats is often higher than that amongst Merino sheep. Mature domestic animals are highly susceptible while calves,

lambs and kids up to the age of three weeks possess a high degree of resistance to heartwater, which is in no way associated with maternal immunity (Neitz, 1968; and Haig, 1955). Karrar (1960) found the organism in one camel, but this evidence is insufficient to generalise the susceptibility of the camel to heartwater. However, heartwater agent is not infective for dogs (Alexander, 1931 and Uilenberg, 1983).

(d) Transmission of Heartwater:

(i) Natural Transmission:

In nature the biological transmission of heartwater is effected by the 3-host tick belonging to the genus Amblyomma. Heartwater has been transmitted by eleven species of Amblyomma. Nine of them are African species while the remaining two are American species. The African species include: Amblyomma hebraeum, A. variegatum, A. pomposum, A. gemma, A. lepidum (Haig, 1955; Neitz, 1968; and Uilenberg, 1983, A. tholloni, A. sparsum, A. astron and A. coharens are also African species (Uilenberg, 1983). The American species are A. maculatum and A. cajennense.

Definite correlation between the presence of the bont tick; A. hebraeum and the incidence of heartwater was established early in 1900. It was demonstrated that infection in ticks was not transmitted through the egg stage but is acquired either in larval or nymphal stages and transmitted in subsequent stages. It was also shown that ticks infected as larvae were not cleansed of

infection by feeding on non-susceptible animals (Uilenberg, 1983).

Heartwater transmission is trans-stadial and attempts at transovarial transmission have invariably failed, apart from one report by Bezuidenhout and Jacobz (1986) which proved transovarial transmission of Cowdria ruminantium by Amblyomma hebraeum, but this has not been repeated. Complete transstadial transmission was reported in A. hebraeum (Alexander, 1931); A. variegatum (Ilemobade & Leefland, 1977); and A. maculatum (Uilenberg, 1982). Transmission was demonstrated only from larvae to nymphae and nymphae to adults in Amblyomma lepidum(Karrar, 1966). In the case of Amblyomma sparsum transmission took place only from larvae to nymphae and as such they are said to be poor vectors. Male ticks, although capable of transmitting heartwater appear to be poor vectors (Ilemobade, 1976; Ilemobade and Leeflang, 1977).

Demonstration of Cowdria ruminantium in other tick species like Argas persicus, Boophilus decoloratus, Rhipicephalus appendiculatus and Rhipicephalus evertsi was reported by Cowdry (1926) to be unsuccessful. Uilenberg (1983) stated that not all African vectors are of equal significance. Amblyomma variegatum is the most important one being perfectly adapted to domestic livestock, being a very efficient vector, and having the widest distribution.

Natural Transmission of Cowdria ruminantium by Amblyomma spp. (Uilenberg, 1983).

Amblyomma spp.	Proven way of transmission
(i) African Species	
A. hebraeum	I, II, III
A. variegatum	I, II, III
A. pomposum	I, II
A. gemma	?b
A. lepidum	I, II
A. tholloni	I, II, III
A. sparsum	I[c]
A. astrion	I
A. cohaerens	I, II, III[d]
(ii) American Species	
A. maculatum	I, II, III
A. cajennense	I

a. I, from larval to nymphal stage; II, from nymphal to adult stage; III, from larval through nymphal to adult stage.

b. The quotation does not appear to be supported by any literature record.

c. Transmission experiments from nymph to adult and from larva through nymph to adult failed (A. sparsum, A. cajenaense).

d. A. cohaerens appears to be an efficient vector; all three transmission attempts were successful.

(ii) Artificial Transmission:

Haartwater can be transmitted by injection of infected blood or homogenized tissues from various organs. Intravenous inoculation of as little as 0.1 ml of virulent blood may produce fatal heartwater (Uilenberg, 1983). Alexander (1931), observed an arbitrary standard of 5ml for small ruminants and 10 ml for cattle. It is not only blood that could cause heartwater, but even milk from reacting animals could be infective, so is spleen suspension (Neitz, 1968).

Apart from the quantity of blood, the route of inoculation is very important. Subcutaneous inoculation of blood was not able to infect more than 25% of susceptible animals (Alexander, 1931, Ilemobade, 1976; Ilemobade and Blotkamp, 1978), whereas intravenous injection of 5ml to 10ml infective blood causes heartwater in over 98% of sheep and goats (Alexander, 1931) but only 9 of 12 susceptible cattle. Uilenberg (1983) stated that 100% cattle reacted to intravenous infection, and

almost 100% of sheep and goats. In the case of ferrets and mice, Neitz (1968) adminstered the infective inoculum by the intraperitoneal route. However, Jongejan et al. (1988), initiated infection by the intravenous injection of tick-derived stabilate, whereas blood stabilate via the same route failed. Du Plessis (1985) stated that the Kwanyanga strain is highly pathogenic to mice, but only when inoculated intravenously and not intraperitoneally, whereas the Kumm strain remains the most pathogenic strain; passaged intraperitoneally in mice to date. Organ emulsions only reliably establish infection if they are injected intravenously, with the exception of brain cortex homogenate of infected animals, which is infective by the subcutaneous route in most cases (Ilemobade, 1976, Ilemobade and Blotkamp, 1978).

Although transmission is mainly by vectors, many outbreaks have been traced to the careless work of humans. Such accidental transmission is attributed to the failure of individuals, especially veterinarians to employ the general methods of asepsis prescribed for routine operations carried out in practice. For example, using contaminated surgical instruments in dehorning operations, drawing of blood and castrations.

(e) Geographical Distribution:

Heartwater is confined to those areas where conditions favours the development of the vectors (Haig,

1955). Heartwater has been reported to occur throughout most of Africa, south of the Sahara, from Cape Province in South Africa to as far north as Kassala in the Sudan, and also from Senegal in the West to Somalia in the East (Uilenberg, 1983). It was estimated that the heartwater endemic area is about thirteen million km^2 in extent. Regions with a very low rainfall and cold climate are free from the disease (Neitz, 1968). Occurrence of the disease in many parts of Africa and the neighbouring island of Malagasy has been confirmed (Haig, 1955).

Heartwater was diagnosed in islands near Africa including La Reunion and Mauritius to the East and also on the Island of Guardeloupe in the Carribean area (Perrean et al. 1980). The disease was also found to occur in Sao Tome in the Atlantic Ocean (Uilenberg et al., 1982). Its existence has been confirmed in Marie-Galante (Uilenberg et al., 1984) and Antigua (Birnie et al., 1985). The distribution of the vector Amblyomma variegatum not only covers Africa but may include the southern parts of the Arabian Peninsula (Uilenberg, 1983). In West Africa the disease has been recognised only in Nigeria, although it is believed to occur in other countries where suitable vectors occur (Ilemobade, 1976).

(f) Pathogenesis:

There is poor understanding of the pathogenesis of

heartwater. In all ruminants, the lesions are fairly consistent but vary with the virulence of different strains. The extent of the lesions show no correlation with the numbers of rickettsias (Uilenberg, 1983). There are rarely any gross lesions in the peracute form. In less severe disease the most characteristic changes are ascites, hydrothorax and hydropericardium (Losos, 1986). In the peracute form, lesions are generally limited to sub-epicardial and subendocardial haemorrhages (Ilemobade, 1976). Infected endothelial cells showed no lesions except that the nucleus might be flattened or pushed to one side by the pressure of the growing cluster of rickettsias (Cowdry, 1926). The pathogenesis of heartwater may be different with different postmortem findings (Abdelrahim and Shommein, 1978; and Ilemobade and Blotkamp, 1978). Pronounced hydropericardium could contribute to cardiac insufficiency and edema of the lungs is sometimes so pronounced (Uilenberg, 1983). The pericardial fluid is usually straw-coloured, but may be blood-tinged (Ilemobade, 1976). The lymph nodes and spleen are generally swollen and edematous (Uilenberg 1983, Ilemobade, 1976). The principal microscopic changes are leukostasis and perivascular infiltration in the liver and kidneys (Losos, 1986).

(g) Clinical Manifestation:

The severity of heartwater varies according to the virulence of the C. ruminantium strain, the

individual or breed susceptibility and the age of the ruminant (Neitz, 1968). The incubation period varies considerably. After natural transmission by ticks the incubation period varies from one to five weeks, with an average of about two weeks (Uilenberg, 1983). In case of sheep and goats which have been infected artificially by intravenous inoculation of virulent material, febrile reactions appear 5 to 35 days with a mean of 9 to 10 days (Alexander, 1931; Uilenberg, 1983). In cattle, however, the average incubation period is slightly longer than that in sheep and goats, being approximately 10 - 16 days with the majority of cases occuring round the 12th day (Alexander, 1931; Haig, 1955; Ilemobade, 1976; and Uilenberg, 1983).

The average incubation period not only varies according to the strain and susceptibility of the animals but also may vary with size of the infective dose. Depending on these, different forms of heartwater have been recognized, which include peracute, acute, sub-acute or chronic form and mild abortive or inapparent forms (Alexander, 1931 and Ilemobade, 1976). These clinical manifestations have been summarized by Alexander (1931), supplemented by Ilemobade (1976); Haig (1955) and Uilenberg (1983).

(i) The Peracute Form: This form is common in exotic breeds of cattle and goats, less so in sheep. It is

characterized by sudden death with the presence of clinical symptoms for periods usually not longer than 36 hours. The affected animals suddenly show febrile reactions and within a few hours temperatures of 41°C or higher may develop. This is usually followed by prostration, collapse and death after paroxysmal convulsions. Cases of this nature present difficulty in diagnosis in either sheep, goats or cattle, whether infection is by artificial or natural means (Alexander, 1931 and Ilemobade, 1976).

(ii) Acute Form: This is the most common form in all susceptible animals. In typical acute cowdriosis sudden rise in temperature, even above 41°C within 1 or 2 days is seen. The temperature may remain high, dropping to subnormal shortly before death. Initially the animals feed and ruminate normally, but later some sluggishness may be observed, and is followed by lack of appetite, cessation of rumination and dyspnea. The animal acquires a tucked appearance and an anxious staring expression of the eyes. In cattle the hair coat is harsh and staring. Rapid pulse is noticed, while normal blood pressure is maintained until just before death, when it becomes thin and thready (Alexander, 1931; Neitz, 1968; and Ilemobade, 1976). The conjunctiva is reddened, but extensive submucous haemorrhages are not usually seen. Hydropericardium is noticed on percussion

and auscultation of the thoracic area; hydrothorax is indicated by the presence of moist rales.

Progressive unsteady gait is seen in sheep and goats. They frequently lean against the wall for support. In severe cases, the affected animals go down on their side, with legs stretched out showing almost continous galloping movements of the legs, masticating movements of the jaws, frothy mouth with continual licking of the lips (Alexander, 1931; Neitz, 1968). Nystagmus and opisthotonus are observed and become more intense with the progress of the disease. One classic pattern of heartwater is the sudden onset intermittent convulsions punctuated by periods of absolute normalcy. Difficulty in respiration and hyperesthesia become more marked (Ilemobade, 1976, Uilenberg, 1983). Temporary recovery may occur, followed by renewed fits. Death usually occurs during these attacks (Uilenberg, 1983). In cattle, the clinical signs are identical to those in goats and sheep except that the nervous reactions are usually more pronounced. A profuse and often hemorrhagic diarrhoea is more frequent in cattle than in sheep and goats. In cattle, a moist cough is frequently noticed associated with bronchial rates.

(iii) Subacute or Chronic Form: The symptoms in this type closely resemble those described for the acute form but are not so intense or well marked.

Diarrhoea develops in cattle and to a lesser extent in sheep and goats. Fever is irregularly intermittent and persist for periods of up to 15 days (Neitz, 1968). Affected animals may collapse and suddenly die, or the temperature may return to normal gradually and the symptoms become progressively less pronounced. However, complete recovery is slow. Pregnant animals in the subacute form are liable to abort (Alexander, 1931; Neitz, 1968).

(iv) Mild Form: This type is characterized by a thermal reaction, inappetence and listlessness for a few days. All other symptoms described above are absent. This form is seen in animals which possess a strong natural resistance, e.g. Persian animals or in those which have acquired a natural immunity by recovery from a previous attack. This form is dangerous because it is not detected easily and serve as a reservoir for the infection of ticks.

(H) Histopathological Findings in Cowdriosis:

The histopathological changes in general are in accord with the macroscopic lesions which are fairly uniform in cattle, sheep and goats, although some local changes are more frequently encountered and more pronounced in one species than in another. These lesions also vary with the virulence of different strains (Neitz, 1968; and Losos, 1986). The rickettsias are detected in the endothelial cells

of the capillaries of the renal glomeruli and in the superficial grey matter of the cortex. They are also found in the spleen, lymph glands, corpus luteum, suprarenals, midbrain, medulla oblongata, corpus striatum, salivery glands, pancreas, and heart muscle (Alexander, 1931).

In the brain common lesions include focal necrosis of blood vessels, hemorrhages, glial changes, edema, swollen axis cylinders, microcavitation, focal necrosis of the granular layer of the cerebellum and subarchnoidal cellular exudation (Ilemobade, 1976; Uilenberg, 1983). Leucostasis of the brains occurred in most animals and also some Purkinje cells and neurones showing cellular degeneration were found in the cerebellum and medulla oblongata (Ilemobade, 1976).

(I) Haematological Observations:

The haematological changes observed in infected animals with Cowdria ruminantium consisted of a drop in hemoglobin with marked leukopenia due to lymphopenia and neutropenia, and a fall in total serum protein (Claman, 1972; Ilemobade, 1976). There was also an increase in alphaglobulins and decrease in gamma globulins. Heartwater organisms were associated with the red blood cells and as such Abdel Rahim and Shommein (1978) associated microcytic hyperchromic anemia with the infection. In sheep

and cattle showing acute fatal cases of heartwater, there were no changes in the blood chemistry except the marked drop in the eosinophil counts (Clark, 1962; Owen et al., 1973). Stress factors e.g. disease lead to increased secretion of adreno-cortical steriods, and eosinopenia has been shown to occur in stress conditions (Schalm et al., 1975). Nervous reactions observed during the terminal phase of heartwater was probably due to a toxin (Pienaar et al., 1966; Cox, 1953).

(J) Diagnosis:

Diagnosis of heartwater in live animals is never a certainty. This is seen in peracute cases where elevated temperature is the only presenting sign, but which is usually unnoticed (Ilemobade , 1976). However, signs helpful in arriving at a tentative diagnosis in acute, subacute and chronic cases are temperature rise, reduced appetite, nervous convulsions with pedalling movements of legs and hyperesthesia, difficult respiration of the abdominal type, presence of the tick Amblyomma spp. on the animal, and the history of heartwater in the area. Jackson and Neitz (1932) distinguished single large rickettsias in blood smears but this has not been confirmed, as such it would not have any practical significance in diagnosis because of their great rarity. In the circulating blood,decrease in

eosinophilic granulocytes is helpful in diagnosis (Ilemobade, 1976). The colour of the blood plasma become progressively darker during fever, often reaching a deep orange colour (Clark, 1962). Gruss (1981) found in the plasma of infected angora goats, an orange-yellow pigment, which appeared with the commencement of the febrile phase. He concluded that this phenomonon to be a useful diagnostic tool.

Definitive diagnosis of heartwater is by microscopic demonstration of the rickettsia in suitable postmortem samples. Microscopic diagnosis was at first based on examination of histological sections of organs, especially the cortex of the brain and kidney, which is believed to be the richest in infected endothelial cells (Uilemberg, 1983 and Losos, 1986). Jackson (1931) showed the organisms to be found with great regularity in intima smears of large blood vessels made from sheep dying from heartwater. In the diagnosis of heartwater, examination of brain squash preparations stained with Giemsa stain or hematoxylin (Uilenberg, 1983; Ilemobade, 1976; and Losos, 1986) is carried out. Examination of smears and sections fixed with methyl alcohol or Carney's fluid and stained with methyl green and Pyronin Y (Losos, 1986) have been done. Lesions which are not considered pathognomonic and usually seen in acute, subacute or chronic cases include abrasions due to mechanical injury sustained during convulsion, ascites, hydrothorax and hydropericardium (Ilemobade, 1976).

For the survey of heartwater, a reliable serological test would be of great value. Several attempts have been made towards achieving this, but has not been successful, mainly because of the lack of suitable antigen. Using infected brain smears as antigen Du Plessis (1970b) failed to adapt the fluorescent antibody technque (IFAT) to diagnosis of heartwater. Ilemobade (1976) obtained inconclusive results with the IFAT test using infected brain smears as antigen. A cappillary flocculation test reported from Nigeria, but was only positive for 2 to 3 weeks (Ilemobade and Blotkamp, 1976). This would not be useful as an aid to clinical diagnosis or in field surveys but only limited to laboratory studies. Neitz et al. (1986b) demonstrated the presence of Cowdria ruminantium in the blood fractions of diseased animals by an enzyme-linked immunosorbent assay. They stated that of all the blood fractions investigated, the red blood cells showed the highest concentration and reached a maximum on the 12th day after infection.

So far there is no accurate method of making a rapid diagnosis of heartwater in the living animal. As such differential diagnosis assume great importance since other diseases with somewhat similar symptoms have to be excluded by a process of elimination. Differential diagnosis includes anthrax, rabies, cerebral babesiosis in cattle, cerebral theileriosis

in cattle, tetanus, strychrine poisoning, hypomagnesamic tetany, East coast fever and anaplasmosis (Alexander, 1931; Haig, 1955; Neitz, 1968; Ilemobade, 1976; and Uilenberg, 1983).

(K) Treatment:

Alexander (1931) stated that no drug or combination of drugs are specific in the treatment of heartwater. He claimed that calcium salts act as curative and prophylactic agents and that good hygiene combined with rational feeding to be of the greatest improtance. It was shown that Uleron, a sulfonamide, has a specific effect on the course of heartwater (Haig, 1955). Other sulfonamides such as sulfapyridine and sulfadimidine were also used later and were active only if administered early in the disease (Uilenberg, 1983). These drugs were almost completely abandoned after the activity of tetracyclines were discovered. Arsenical and antimonial compounds have potentiating effects on the course of heartwater (Ilemobade, 1976). Penicillin and streptomycin do not influence the infectivity of tick-derived heartwater vaccine; neither was chloramphenicol, ampicillin nor imidocarb, were found inactive against heartwater (Haig, 1955; Uilenberg, 1983). Oxytetracycline, given during the incubation period of the disease in sheep, delayed febrile reaction by 4 days and markedly reduced the severity of the disease (Haig, 1955).

Treatment with tetracyclines which are effective against heartwater does not interfere with the establishment of immunity, which is vital to the survival of animals in a contaminated environment (Uilenberg, 1983, and Ilemobade, 1976). Tetracyclines are successfully used in the infection and treatment method of immunization when adminstered in the earliest stage of the disease. A single treatment is not reliable and a second injection either on consecutive days or with an interval of one day starting on the first or second day of the febrile reaction at a dosage rate of 5 to 10mg/kg brings about a return of the temperature to normal and prevents death (Uilenberg, 1983). There has been no proper comparisons made between various formulation and routes of administration or between different tetracyclines(Haig, 1955; Uilenberg, 1983).

(1) Immunity:

The nature of duration of the immunity to heartwater disease has not been clarified. Immunity to heartwater is acquired only when a reaction is set up in a susceptible animal. Alexander (1931) stated that with the exception of certain breeds which possess a degree of natural resistence; no immunity can be demonstrated in any animal which has not passed through an attack of the disease. If a definite reaction is produced, the severity of the disease does not appear to have any influence upon the degree of immunity.

The duration of immunity may be as short as six months or it may persist for periods of up to five years. Cowdria ruminantium persists for periods of up to two months after infection and that this is temporary rickettsemia (Ilemobade Personal Commication, 1991). In sheep the duration varies from 7 - 34 months after recovery or up to at least 4 years if the animals were challenged within 2 months of recovery. This solid immunity afterwards gradually and progressively decreases (Neitz et al., 1986b; Uilenberg, 1983). Challenge during the period of sterile immunity resulted in reinfection (Losos, 1986). In cattle Haig (1955) reported that immunity may wane considerably earlier than in sheep and that there was little or no immunity four months after recovery. However reinfected cattle maintained on tick-infected farms have their immunity reinforced. Du Plessis (1985) related the tick burden with Amblyomma spp. and the Cowdria ruminantium infection rate of the ticks to be the two most important factors to the immune status of cattle. In goats, the duration of immunity is not well documented. Ilemobade (1978) found that blood could be infective up to 50 days following recovery or challenge. In partially and solidly immune animals, reinfections may cause the blood to become temporarily infective (Uilenberg, 1983).

The nature of immunity is quite obscure. Alexander (1931) suggested that the anitgenic

properties are probably cellular and not humoral. The assertion of this cell-mediated immunity is made inspite of the confirmed observation that the parasitized cell undergoes no reaction as a result of the presence of Cowdria ruminantium. To help clarify these aspects, Neitz et al. (1986b) demonstrated by an enzyme-linked immunosorbent assay the presence of Cowdria ruminantium in the blood fractions of diseased animals, and of all the blood fractions investigated the red blood cells showed the highest concentration, which reached a maximum on the 12th day after infection.

Du Plessis (1981) using the indirect fluorescent antibody test in investigating heartwater found the presence of significant concentrations of antibody which failed to influence the development of overt disease, thereby suggesting that immunity is dependent on cellular rather than on humoral factors.

There are various strains of Cowdria ruminantium that have been isolated from different geographical regions, namely the Ball 3 strain and the Zeerust strain, which have been isolated in North Transvaal, South Africa: the Sudanese and Sao Tome strains; D225 strain isolated in Northern Nigeria and recently a new stock called the Jaji stock was isolated from a calf in Jaji (Northern Nigeria). Marked differences existed in the pathogenicity of different strains, although antigenic differences are

not considered to occur between strains (Ilemobade, 1976; Uilenberg, 1983). Cross immunity test of D225 strain from Nigeria with two strains from South Africa were carried out by Van Winkelhoff and Uilenberg (1981). They immunized goats by infection with a particular strain and treatment of the resulting reaction with oxytetracycline and found that the strains were completely cross-protective. Slight differences between strains were reported from the Sudan (Karrar, 1960). Complete cross-protection in sheep between all South African strains tested was reported (Van Winkelhoff and Uilenberg, 1981). Stock of Cowdria ruminantium from Senegal, Zambia and South Africa were compared in cross immunity tests in goats; it was found that the Senegalese stock was the first stock outside South Africa which did not give full cross-protection with the reference stock Ball 3; the Senegal stock was also different from the Zambian stock (Lutale stock), while the Lutale stock appeared to be cross-protective with the Ball 3 (Jongejan, Uilenberg, and Franssen, 1988). Strains of Cowdria ruminantium from Nigeria, Sudan, Sao Tome, Guardeloupe, Mali, and South Africa have been shown to be completely cross-protective (Van Winkelhoff and Uilenberg, 1981, Jongejan et al., 1988).

(m) Immunization:

There has been little progress since the introduction of infection and treatment method of immunization. For active immunization, with virulent

heartwater material, the period of relative unsusceptibility of young calves and lambs was made use of (Haig, 1955). With the discovery of specific chemotherapeutic treatment, immunization was extended to older cattle as well as sheep and goats, entailing close monitoring of animals in order to allow initiation of treatment at the first rise of temperature(Uilenberg, 1983). Haig (1955) stated that with the improvement of therapy, losses have been reduced to a low figure.

Strains vary greatly in virulence and in any vaccination procedure selection of a strain for its low virulence to diminish the high rate of post-vaccinal mortality in sheep and goats has to be an important feature (Logos, 1986). However, it is important to use a strain that causes well defined febrile reactions such as the highly virulent Ball 3 strain used in South Africa (Uilenberg, 1983).

A method for the preparation of Cowdria ruminantium infective tick filtrates which can be used for vaccination of cattle and sheep against heartwater was described by Bezuidenhout (1981). He came to the conclusion that the intravenous use of infective Amblyomma hebraeum filtrates has little advantage for the farmer over the blood vaccine in use at present. The infective tick filtrates are still pathogenic for animals over the age of 4 weeks. And the main advantage of the vaccine lies in its relative low cost, in its preparation; the simplicity of the method for the preparation of the infected

filtrates and the storage of Cowdria ruminantium in Amblyomma hebraeum ticks and filtrates. Neitz and Alexander (1945) reported considerable success by utilizing the natural resistance of calves under the age of three weeks to overcome heartwater produced by the intravenous injection of infective sheep blood; thus developing a durable active immunity.

Alternatively they controlled the disease by the use of Sulphonamides during the incubation period or during the period of detectable clinical symptoms. Tetracycline and oxytetracycline in drinking water at 300mg/11.3Kg body weight for goats, 200mg/11.3Kg for sheep and 200 to 250mg/45.3kg for cattle, were found to be effective if administered early in the cause of the febrile reactions (Karrar and EL Hag Ali, 1965) Hurter (1967) reported the use of oxytetracycline at 8.8 mg/kg in sheep to be effective in the control of heartwater.

Poole (1961) stated that chlortetracycline hydrochloride suspension in oil at 44 mg/kg was effective against heartwater in sheep if treated two to three times.

In Nigeria, Isoun et al, (1974) treated cattle with oxytetracycline at 2.5 mg/kg intravenously for 2 days and sulphamethiazine at 55 mg/kg subcutaneously with 70% success.

The use of infected blood to stimulate immunity

is at present the only available method, but it is far from ideal since the preparation of a blood vaccine is expensive, costly in manpower, cumbersome and dangerous in use. Alternative source of Cowdria ruminantium had to be found and with this aim, other workers studied the propagation of Cowdria ruminantium in mice or tissue cultures. Worsink et.al. (1986) using a stabilate of caprine blood infected with the Kwanyanga stock of Cowdria ruminantium made eight serial passages in groups of mice and eight parallel serial passages in goats and found out that there was no difference between stabilates made after the eighth passage in goats and mice, the Kwanyanga stock being exceptionally of low virulence for Dutch goats. Very low infectivity existed in the saliva collected from infected Amblyomma hebraeum females especially when compared to ground-up tick suspensions made from the same group of ticks (Bezuidenhout, 1981).

(n) Control:

Apart from the immunization of all susceptible animals mentioned previously in this chapter, prophylaxis in infected areas is based on measures aimed at the control of the vectors of heartwater. Heartwater is transmitted by 3-host ticks belonging to the genus Amblyomma and for this reason they are difficult to control because they infest a large spectrum of animals. Also once the infection is acquired in the larval stage, the organism is not

lost irrespective of whether the nymphs feed on susceptible, insusceptible or immune animals. Alexander (1931) found the use of pesticide as an appropriate control measure of the vector of heartwater. He stated that in practice 5-7 day dipping in a fluid containing 21 b. of sodium arsenite per 100 gallons gives excellent result. Alexander (1931) also suggested that dipping at 3 day intervals in a weaker solution is not effective in killing the tick besides having harmful effect on the animal when enforced over a long period. Ilemobade (1976) stated that by the application of pesticide in the effective control of heartwater vectors the interval between treatments must be determined based on the length of time it takes any of the stages to feed on cattle, on their seasonal activity and on the properties of the pesticide used.

Gruss (1981) found that controlled exposure to ticks over a period of 60 days with optimal internal parasite control, and in the total absence of dipping, gave good immunity with repeated challenging. This involved the subcutaneous or ultramascular injection of oxytetracycline every 10 days for 30 days and then every 14 days for a further 30 days, and then dipped. The dosage given is 3mg/kg. For the remaining of the summer the animals are dipped monthly. This also applies to new animals introduced into an enzootic area. Oxytetracycline treatment administered during the incubation period of the disease in sheep delayed the heartwater reaction by about 4 days longer than in

untreated controls (Haig, 1955). The chemoprophylactic method of controlling heartwater on herd basis is uneconomical. However, research in the creation of Amblyomma-resistant breeds, in analogy with Boophilus-resistant Australian cattle may be of great success (Uilenberg, 1983).

CHAPTER THREE

STUDY OF REACTION OF YANKASSA RAMS TO EXPERIMENTAL COWDRIA RUMINANTIUM (JAJI STOCK) INFECTION AND CROSS-IMMUNITY TEST.

INTRODUCTION:

Heartwater causes severe mortality in ruminants and the severity of the disease varies greatly according to the virulence of the Cowdria ruminantium strain, the age, breed susceptibility and the management of the livestock. In order to assess the importance of heartwater in sheep in Zaria, an experimental study was undertaker to assess the susceptibility of a group of adult Yankassa sheep, which were later tested for cross-protection when challenged with a homologous and heterologous strain after treatment of the primary infection.

MATERIALS AND METHODS:

Survey:

The records of the diagnosis of heartwater for the last 15 years (1973-1987), using brain smear technique obtained from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, were examined. The infection rates were determined and used to construct a histogram.

EXPERIMENTAL PHASE:

The ruminants used in this experiment were Fourteen (6 - 12 months old) Yankassa rams which were purchased at Makarfi Market; approximately fifty kilometres away from Zaria, along Zaria-Kano road. On arrival at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, all the animals were sprayed against ticks with Asuntol (Bayer, Germany), at a concentration of 1:800. And treated for helminthiasis with a therapeutic dose of Fenbendazole (Panocur-Hoeschst); 6g powder in 100ml of distilled water and given orally at 5ml of suspension per 15kg body weight. The animals were kept in pens and fed with hay, wheat offal and cotton seed cake. Salt-lick, water and mineral supplements were made available to the rams ad. libitum. The animals were acclimatized for a period of 8 weeks. Faecal samples were examined for helminth parasites and the rams treated accordingly. They were dewormed and sprayed fortnightly because they were housed together with some other animals that were allowed to graze. Rectal temperatures were taken daily.

During the period of acclimatization, two millilitres of blood were drawn from each animal

once a week. The blood was collected before feeding the animals. They were bled through jugular venipuncture, using a disposable syringe with 18 gauge x 2 in needle. Blood was transferred into vacuntainers containing ethyline diamine tetraacetate (EDTA) as an anticoagulant. The blood was for the determination of the Packed Cell Volume (PCV), total serum protein (TSP) and thin blood smear examination. All animals were in apparently healthy condition at the start of the experiments.

The rams were grouped according to their PCV into two groups, ten experimentally infected animals (group 1) and four uninfected controls (group 2).

Animal Inoculation:- After eight weeks of acclimatization, each animal in group 1 received 2ml ground-up tick stabilate (GUTS) of Cowdria rumainantium (Jaji stock) intravenously. The other four animals acted as uninfected controls. Out of the ten original animals that were inoculated with jaji stock of Cowdria ruminantium, four died within 5 - 30 minutes as a result of anaphylactic shock. Daily rectal temperature and blood samples were taken three times a week for the first two weeks of post infection i.e. to the time of the appearance of a febrile reaction. Animals that showed thermal reaction to the infection were treated during the second day of fever (above 40°C) with Terramycin long acting (oxytetracycline, Pfizer Nig. Ltd.) at

20 mg/kg body weight intramascularly.

One month after treatment the animals were challenged intravenously with 2 ml each of a homologous stock (jaji stock) of Cowdria ruminantium. During this month, the animals were bled once a week and daily rectal temperatures taken. Three weeks later, they were re-challenged intravenously with a heterologous strain (D225) at a dose of 2 ml each of infective blood of Cowdria ruminantium.

Haemtological Investigations:

Packed cell volume (PCV) was determined by the micro-haematocrit method. Total serum protein (TSP) was determined using the refractometer. Total leukocyte counts were estimated by the improved Nebacur haemocytometer. Differential leukocyte counts were done by examining one hundred white cells in thin blood films stained with Giemsa stain.

Statistical Analysis:-

Two sample "t" - test (Snedecor and Cochran, 1974) was used to analyse the data of the temperature, packed cell volume, total serum protein, total leukocyte counts and differential leuckocyte counts. The calculated values of the "t"-test were compared with the critical values at 5% level of significance.

R E S U L T S

SURVEY

The case infection rate of heartwater in sheep in Zaria from 1973 - 1987 has been depicted in Figure 1. It can be observed that the case - infection rateswere low initially during the period of survey and increased in subsequent years with a peak in 1985; having infection rate of 43.59 percent. The year 1984 had second highest infection rate of 31.58 percent, followed by 1980, 1987 and 1982 with infection rates of 25.32, 25.00 and 24.24 percent respectively.

EXPERIMENTAL INFECTIONS

Observations on Temperature Reactions:

Phase I (Primary Challenge):

Temperature reaction pattern of Yankassa sheep experimentally infected with jaji stock of *Cowdria ruminantium* for the first 30 days post infection was depicted in Figure 2. All experimentally infected animals, except one, attained high temperatures of

$\geq$ 40°C which responded to treatment with terramycin/LA. Animals that responded to the first treatment were no longer treated, those that did not, were given a second dose of terramycin/LA. The mean reaction time occurred between day 10 and 14 post-infection. The four uninfected animals were in apparently healthy condition throughout the duration of the study.

Phase II (Homologous Challenge):

Figure 3 depicts the second phase of the mean temperature reaction pattern of Yankassa sheep experimentally infected with jaji stock of Cowdria ruminantium. This phase comprised 25 days (31 - 55 days post-infection). The animals were challenged with the homologous stock on day 42 post-infection. It was observed that there was no significant change ($P \geq 0.05$) in the mean temperature value of the infected animals as compared with the control group.

Phase III (Heterologous Challenge):

The third phase reflects the heterologous challenge experiment, which comprised the last

30 days of the experiments (56 - 85 days post-infection). This is depicted in Figure 4, whereby the infected animals were challenged with a heterologous strain (D225 strain) of Cowdria ruminantium on day 64 post-infection. The mean temperature of the infected animals compared with the uninfected animals did not show any significant ($P > 0.05$) variation.

Observations On Haematological Studies:

The mean Packed Cell Volume (PCV) and mean total serum protein (TSP) are depicted in Figure 5. There were no marked changes observed in both the mean PCV and TSP of the infected as compared with the control animals. Although, the control animals had higher overall mean Packed Cell Volume and mean total serum protein than the infected group.

The mean total leukocyte counts has been depicted in Figure 6. In the infected animals, the mean total leukocyte count values were generally lower than those of the uninfected group. However, there was no statistical ($P > 0.05$) variations except on day 64 post-infection, which showed a significant ($P < 0.05$) drop in the mean leukocyte count of the infected group

compared with the uninfected group.

The mean lymphocyte counts are depicted in Figure 7. There was a drop in the mean lymphocyte counts of the infected group but found to be of no significance ($P \geq 0.05$).

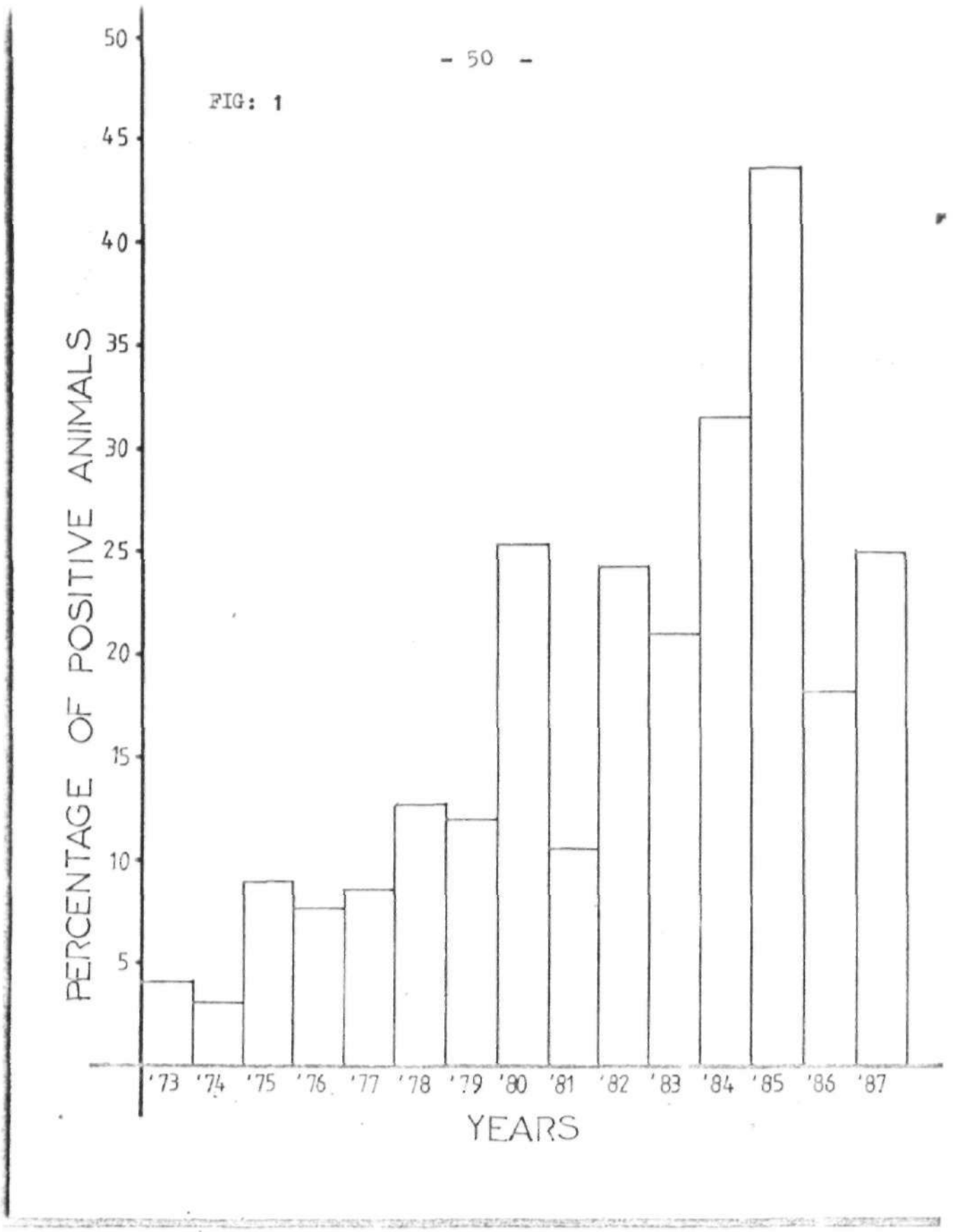
The mean neutrophil count which followed the same trend as the mean total leukocyte counts has been shown in Figure 8.

The mean eosinophil counts are depicted in Figure 9. There was a marked significant drop ($P < 0.05$) in the mean eosinophil count of the infected animals on day 6, 8, 11 and 15 post-infection. Thereafter, the eosinophil count rose, reaching a peak of 0.26 ± 0.24 on day 35 post-infection. There was significant drop ($P < 0.05$) on day 64 post-infection which abruptly rose and finally dropped significantly on day 84 post-infection.

Figure 10 depicts the trend of the mean monocyte counts of the uninfected and experimentally infected animals. In both groups, the mean monocyte counts fluctuated irregularly. As in the case of the mean

total leukocyte and the mean lymphocyte counts, there was no significant variation in the mean monocyte counts of the infected group compared with the control group.

FIG: 1



Histogram showing case infection-rate of heartwater in sheep around Zaria by brain smear examination technique over a period of 15 years (1973-1987).

Fig. 2

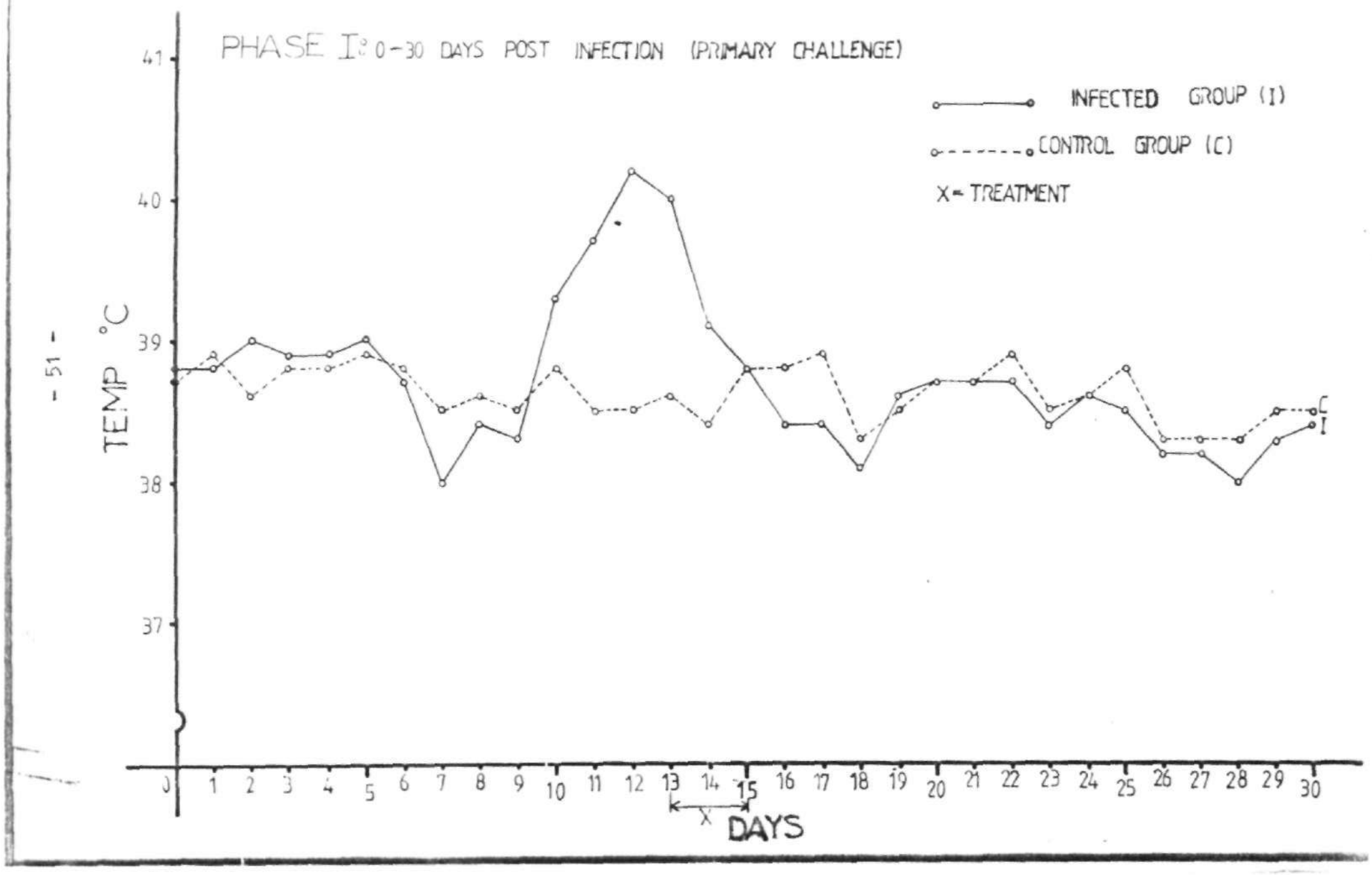

PHASE I: 0-30 DAYS POST INFECTION (PRIMARY CHALLENGE)
INFECTED GROUP (I)
CONTROL GROUP (C)
X= TREATMENT
TEMP °C
41
40
39
38
37
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
X
DAYS

Fig. 3

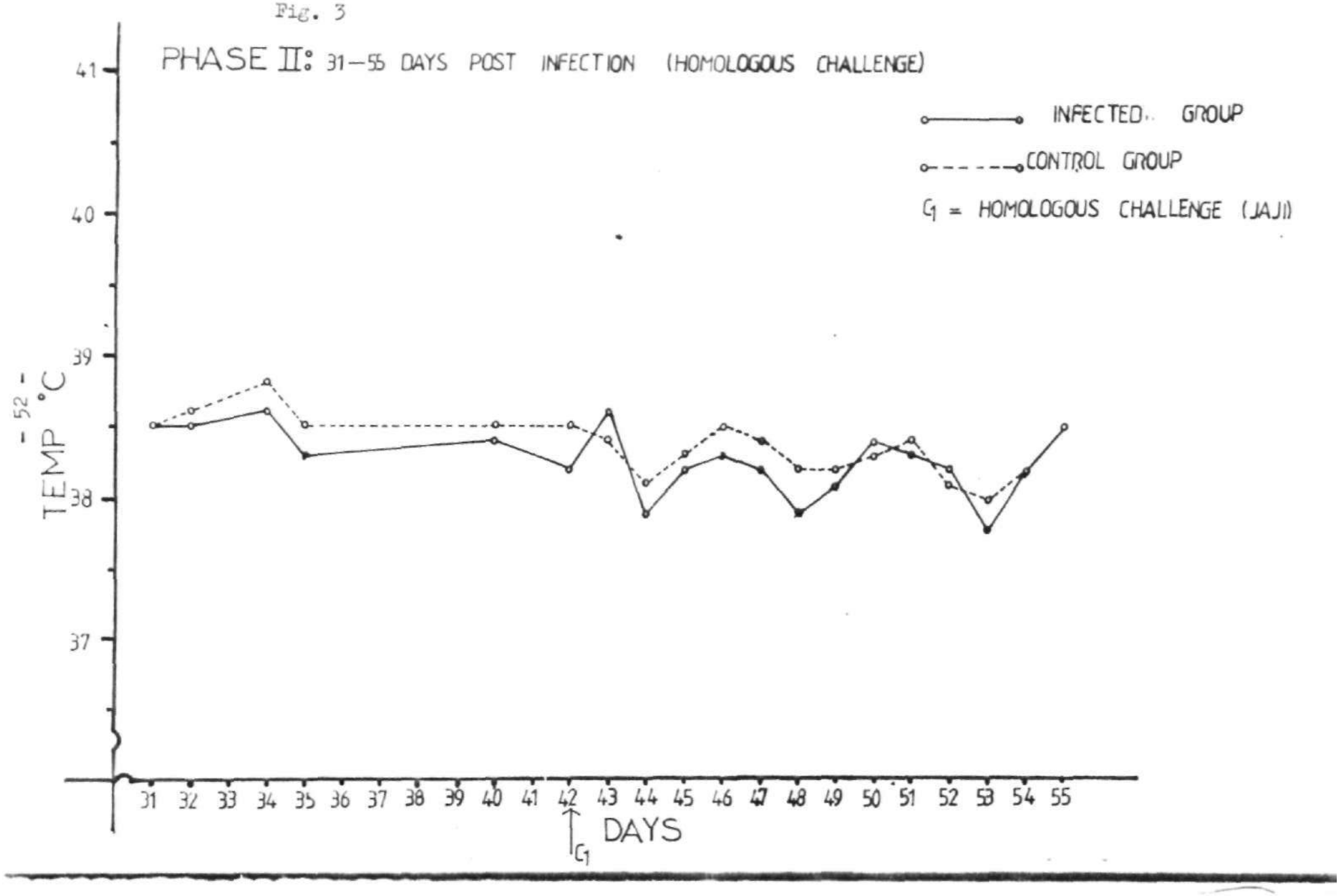

PHASE II: 31–55 DAYS POST INFECTION (HOMOLOGOUS CHALLENGE)
INFECTED GROUP
CONTROL GROUP
C1 = HOMOLOGOUS CHALLENGE (JAJI)
TEMP °C
41
40
39
38
37
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55
DAYS
C1

Fig. 4

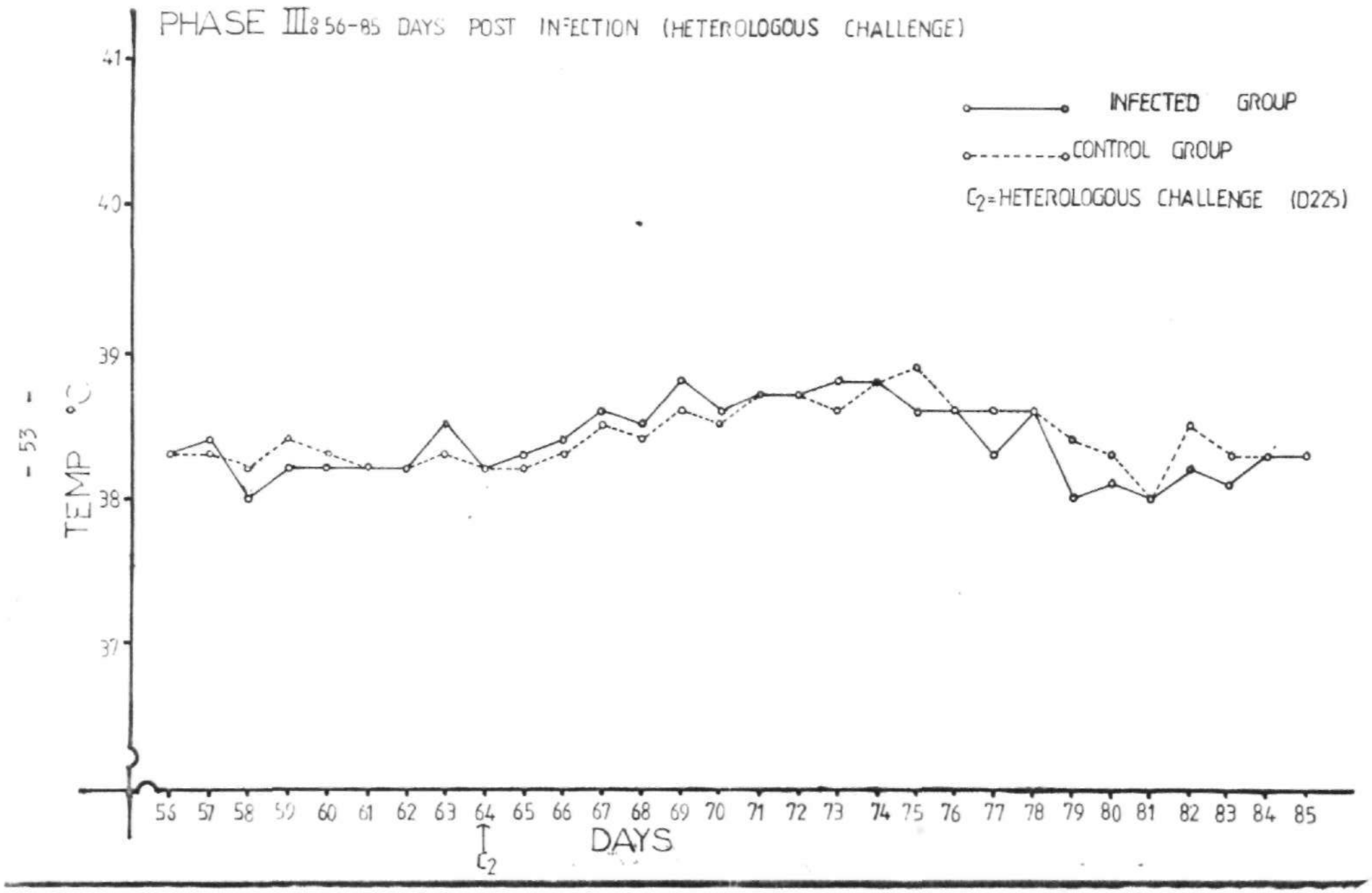

PHASE III: 56-85 DAYS POST INFECTION (HETEROLOGOUS CHALLENGE)
INFECTED GROUP
CONTROL GROUP
C2=HETEROLOGOUS CHALLENGE (D225)
TEMP °C
41
40
39
38
37
56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
DAYS
C2

Fig. 5

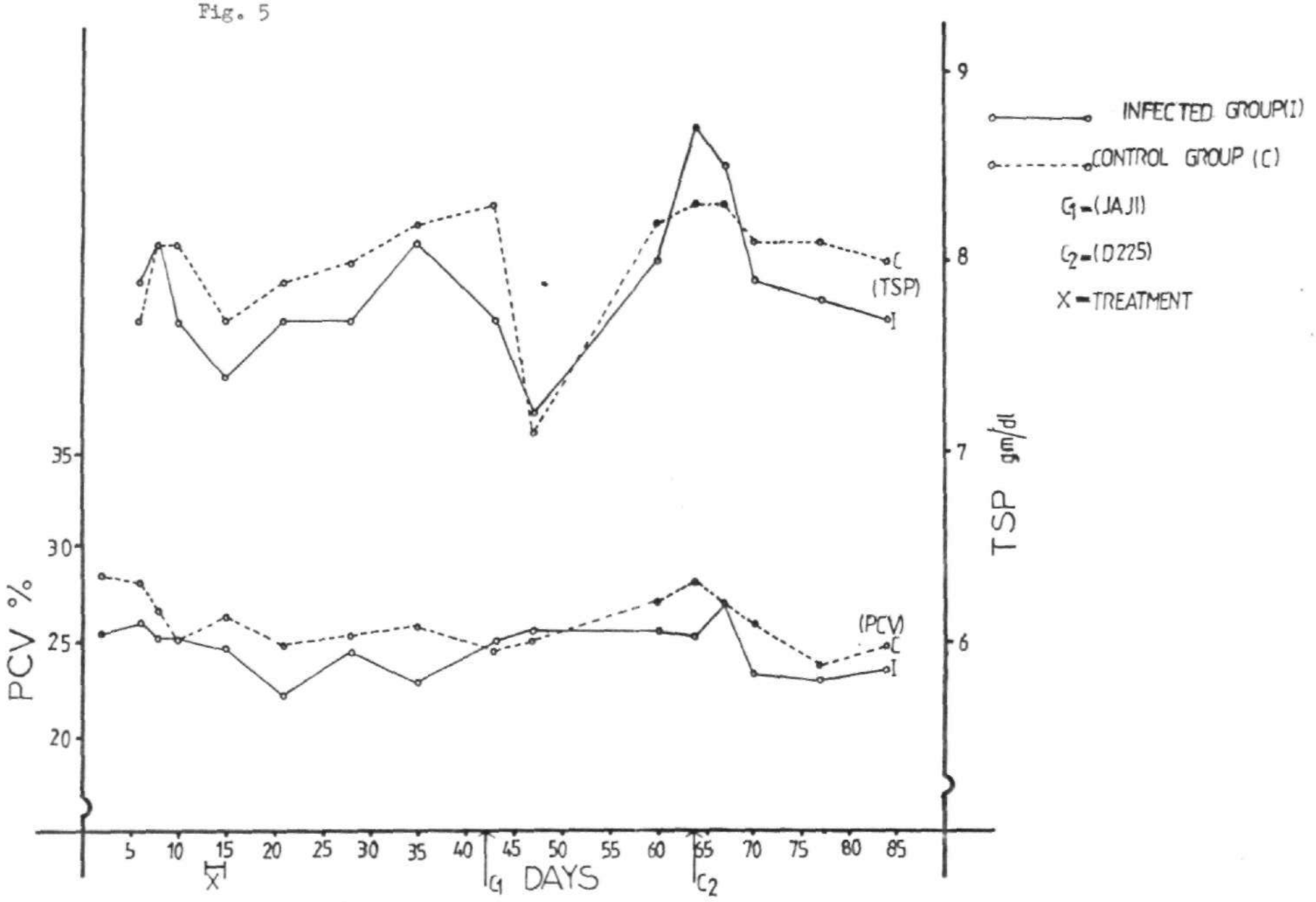
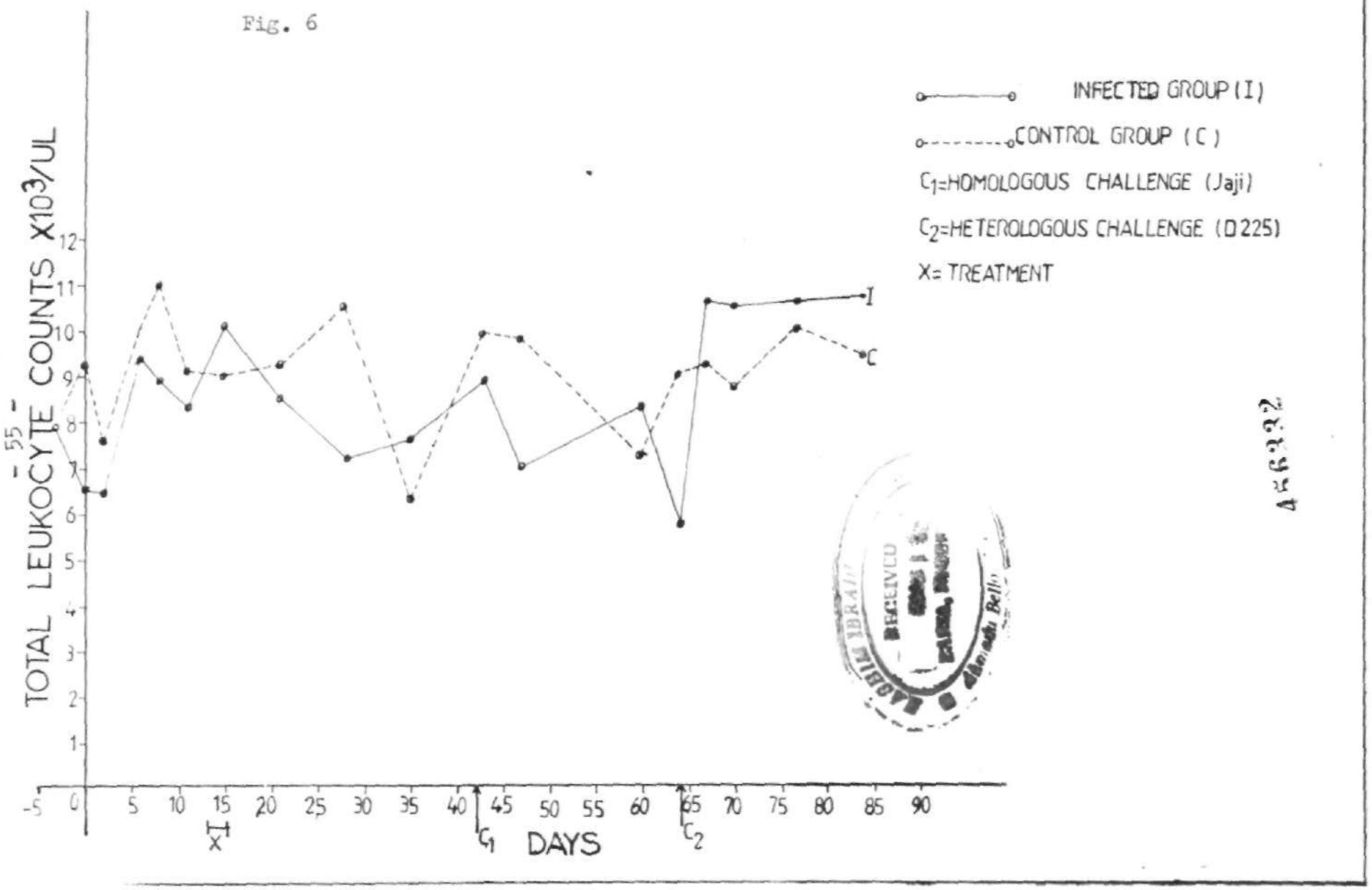

INFECTED GROUP(I)
CONTROL GROUP (C)
G1=(JAJI)
G2=(D225)
X=TREATMENT
PCV %
TSP gm/dl
(PCV)
C
I
(TSP)
C
I
DAYS
X1
G1
G2
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
35
30
25
20
9
8
7
6

Fig. 6


INFECTED GROUP (I)
CONTROL GROUP (C)
C1=HOMOLOGOUS CHALLENGE (Jaji)
C2=HETEROLOGOUS CHALLENGE (D225)
X= TREATMENT
TOTAL LEUKOCYTE COUNTS X10³/UL
DAYS
I
C
X
C1
C2

456332

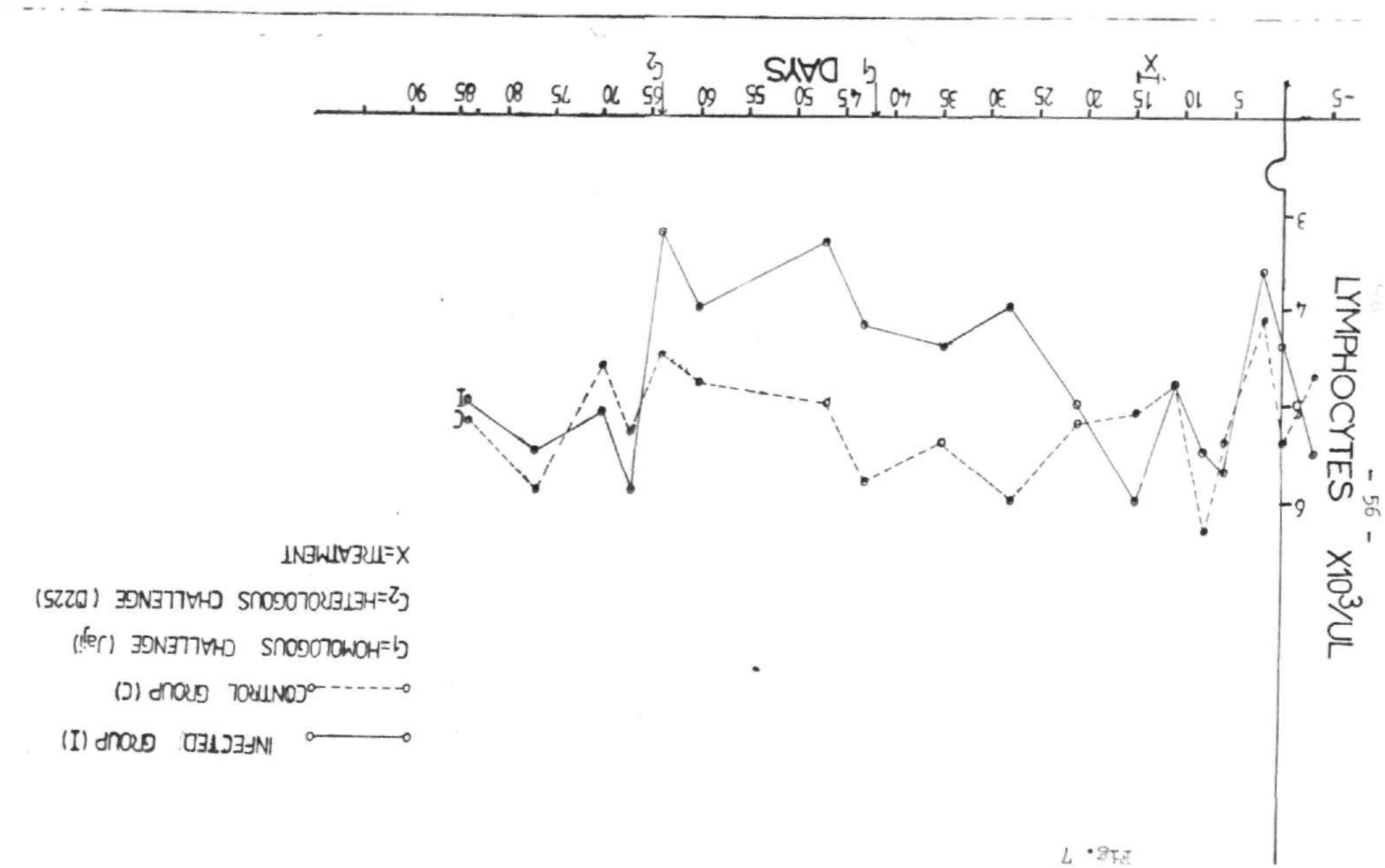

LYMPHOCYTES X10³/UL
DAYS
X=TREATMENT
C2=HETEROLOGOUS CHALLENGE (D225)
C1=HOMOLOGOUS CHALLENGE (Jaji)
CONTROL GROUP (C)
INFECTED GROUP (I)

Fig. 7

Fig. 8

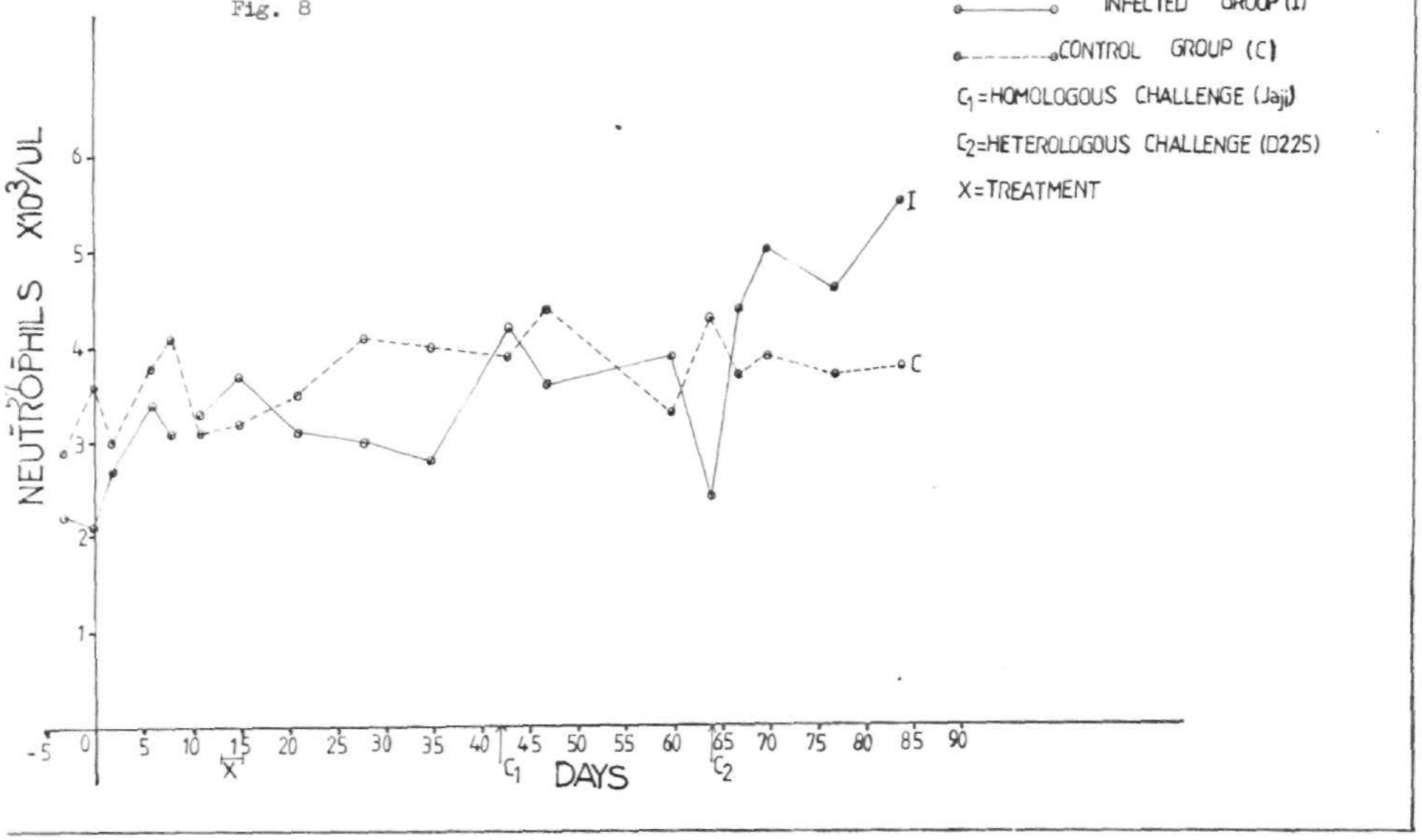

INFECTED GROUP (I)
CONTROL GROUP (C)
C_1=HOMOLOGOUS CHALLENGE (Jaji)
C_2=HETEROLOGOUS CHALLENGE (D225)
X=TREATMENT
NEUTROPHILS X10³/UL
I
C
1
2
3
4
5
6
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
X
C_1
C_2
DAYS

Fig. 9

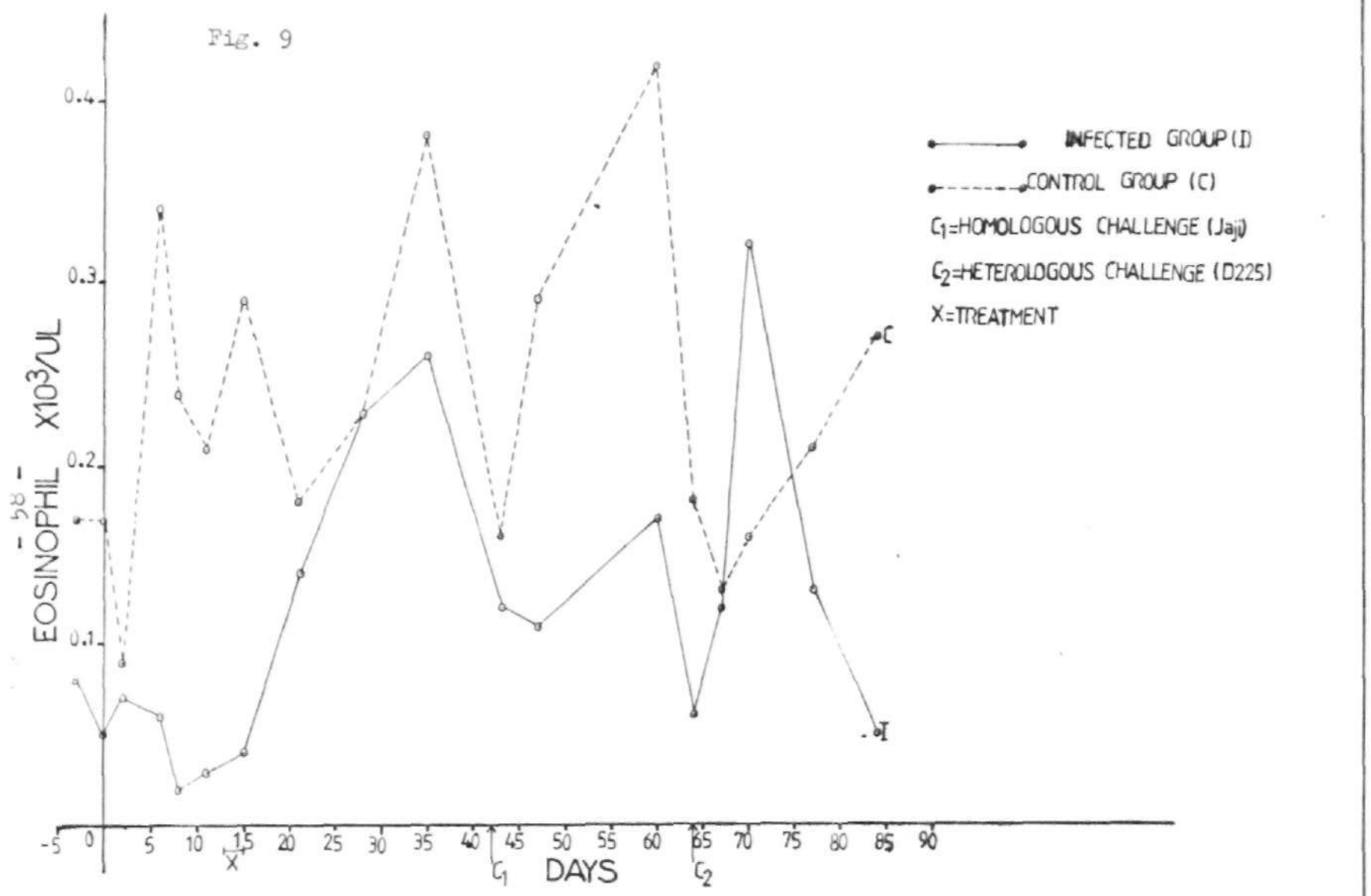
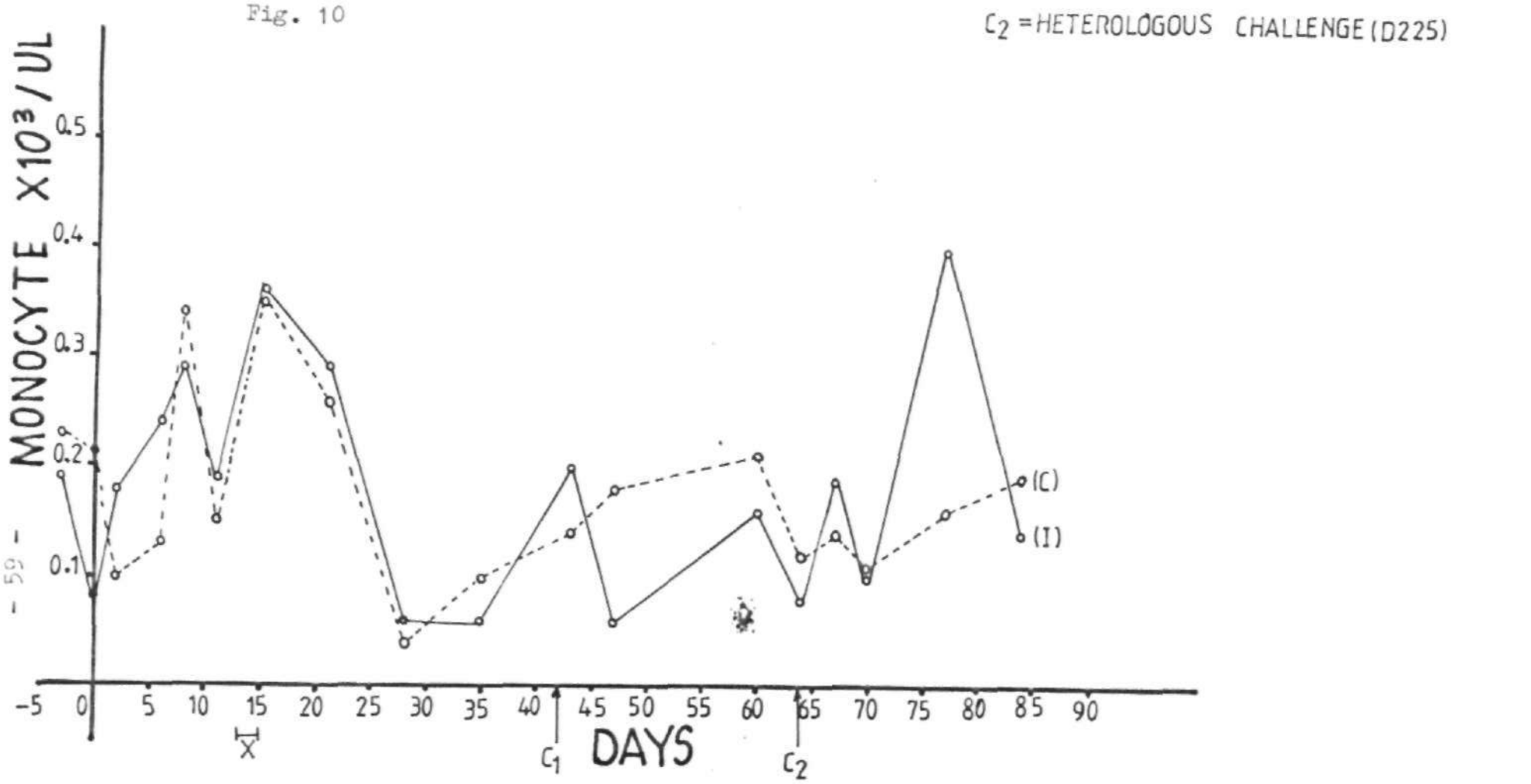

INFECTED GROUP (I)
CONTROL GROUP (C)
C1=HOMOLOGOUS CHALLENGE (Jaji)
C2=HETEROLOGOUS CHALLENGE (D225)
X=TREATMENT
EOSINOPHIL X10³/UL
0.4
0.3
0.2
0.1
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
X
C1
C2
DAYS
C
I

Fig. 10



o------o CONTROL GROUP (C)

o——————o INFECTED GROUP (I)

X = TREATMENT

C_1 = HOMOLOGOUS CHALLENGE (Jaji)

C_2 = HETEROLOGOUS CHALLENGE (D225)

CHAPTER FOUR

DISCUSSION

During the survey, the case infection-rate which represents the percentage of positive brain samples with Cowdria ruminantium infection was determined and used to estimate the relative prevalence over the years. The increase in the case infection-rate of heartwater shown in this study could be attributed to the familiarity with which the clinical staff diagnosed this killer disease with time. It could also mean that the availability of the infected vectors increased over this period or the control of the ticks became relaxed due to economic or other reasons.

In the experimental study, it was demonstrated that five out of six locally used Yankassa rams were susceptible, with incubation periods varying between 10 and 14 days. All susceptible animals had elevated temperatures $\geq 40^{\circ}C$ between days 10 and 14 post-inoculation. They only exhibited thermal reactions during the primary phase, showing that adult rams from Makarfi are highly susceptible. However, they did not react to homologous challenge (Jaji stock), nor the heterologous challenge (D225 strain), which suggests that when the susceptible animals first reacted and were treated with therapeutic levels of oxytetracycline/LA (Pfizer, Nigeria Limited), they developed immunity which protected against further challenges. This

suggests that the Jaji stock is related to the D225 strain and it may be inferred that Cowdria ruminantium stocks in this local geographic area share antigenic similarities. Since the D225 strain of Cowdria ruminantium has been extensively studied, we can use it to immunise susceptible animals in this geographical area by the infection and treatment method.

The non-reacting animal might have been exposed to natural Cowdria ruminantium infection and became immune.

Complete cross-protection between the Nigerian D225 strain, the Ball 3 and Zeerust strains from South Africa were reported by Van Winkelhoff and Uilenberg (1981). They came to the conclusion that, there are no immunological differences between the three strains and that it may be possible to use one strain to immunise in all heartwater areas. However, this is in contradiction with the recent report of Jongejan, Uilenberg and Franssen (1988); who found cross-protection between the Ball 3 from South Africa and the Lutale stocks from Zambia, but both didnot cross-protect against the Senegalese stock. The

latter report shows that we can not use one strain to immunise in all heartwater areas because antigenic differences might complicate immunization against heartwater.

It is possible that the D225 strain of Cowdria ruminantium shares antigenic properties with Jaji stock and that the Jaji stock may be more complex than the D225 strain, although we didnot immunise first with the D225 strain then challenge with the Jaji stock. Based on work done, it is likely that ruminants immunized with Cowdria ruminantium (D225 strain) may be protected against stocks circulating in a large area of the country.

In summary, the work done has demonstrated antigenic similarity of Jaji stock with D225 strain which is a known characterised strain. And that the D225 strain can be used to immunize animals over a large geographical area. It also showed that rams from Makarfi have been proved to be highly susceptible to heartwater.

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APPENDICES

Table 1: Case-Infection rate of Cowdriosis in Sheep by brain Smear Examination Technique (Protozoology Laboratory)

Years	No. of Suspected cases	No. of +ve brain samples	Percentage of tve cases
1973	24	1	4.17
1974	64	2	3.13
1975	44	4	9.10
1976	39	3	7.70
1977	46	4	8.70
1978	55	7	12.73
1979	25	3	12.00
1980	79	20	25.32
1981	58	6	10.34
1982	33	8	24.24
1983	19	4	21.05
1984	19	6	31.58
1985	39	17	43.59
1986	22	4	18.18
1987	8	2	25.00

Table 2: Means and standard deviations of daily rectal temperatures (°C) of Experimental and Control Yankassa Sheep (Primary challenge).

DAYS P.I.	EXPERIMENTAL GROUP	CONTROL GROUP
0	38.83 ± 0.18	38.73 ± 0.08
1	38.82 ± 0.38	38.90 ± 0.07
2	38.95 ± 0.24	38.63 ± 0.15
3	38.87 ± 0.19	38.75 ± 0.11
4	38.92 ± 0.15	38.83 ± 0.11
5	38.97 ± 0.19	38.90 ± 0.07
6	38.68 ± 0.23	38.83 ± 0.08
7	38.03 ± 0.24	38.48 ± 0.23
8	38.43 ± 0.24	38.63 ± 0.24
9	38.30 ± 0.22	38.45 ± 0.51
10	39.32 ± 0.88	38.75 ± 0.17
11	39.72 ± 0.97	38.50 ± 0.33
12	40.20 ± 1.02	38.53 ± 0.37
13	39.98 ± 0.86	38.55 ± 0.35
14	39.08 ± 0.57	38.35 ± 0.29
15	38.82 ± 0.32	38.80 ± 0.25
16	38.35 ± 0.17	38.83 ± 0.20
17	38.42 ± 0.21	38.88 ± 0.19
18	38.05 ± 0.26	38.28 ± 0.22
19	38.60 ± 0.19	38.53 ± 0.18
20	38.72 ± 0.07	38.65 ± 0.29
21	38.73 ± 0.11	38.73 ± 0.22
22	38.72 ± 0.43	38.93 ± 0.22

Table 2 Cont'd.

DAYS P. I.	EXPERIMENTAL GROUP	CONTROL GROUP
23	38.40 ± 0.16	38.50 ± 0.16
24	38.63 ± 0.22	38.58 ± 0.23
25	38.50 ± 0.27	38.75 ± 0.32
26	38.15 ± 0.24	38.33 ± 0.30
27	38.20 ± 0.16	38.28 ± 0.27
28	38.03 ± 0.28	38.30 ± 0.14
29	38.28 ± 0.33	38.50 ± 0.12
30	38.37 ± 0.26	38.53 ± 0.23

Table 3: Means and standard deviations of daily rectal temperatures (°C) of Experimental and Control Yankassa sheep (Homologous challenge)

DAYS P. I.	EXPERIMENTAL GROUP	CONTROL GROUP
31	38.48 ± 0.20	38.48 ± 0.20
32	38.47 ± 0.21	38.60 ± 0.14
34	38.63 ± 0.31	38.75 ± 0.25
35	38.25 ± 0.21	38.45 ± 0.22
40	38.38 ± 0.26	38.45 ± 0.23
42	38.17 ± 0.34	38.48 ± 0.08
43	38.58 ± 0.36	38.43 ± 0.08
44	37.90 ± 0.28	38.13 ± 0.15
45	38.17 ± 0.24	38.28 ± 0.18
46	38.33 ± 0.18	38.48 ± 0.25
47	38.23 ± 0.19	38.35 ± 0.21
48	37.87 ± 0.37	38.20 ± 0.21
49	38.12 ± 0.29	38.20 ± 0.16
50	38.38 ± 0.21	38.30 ± 0.21
51	38.32 ± 0.27	38.38 ± 0.13
52	38.15 ± 0.19	38.13 ± 0.13
53	37.80 ± 0.36	38.00 ± 0.22
54	38.20 ± 0.19	38.18 ± 0.18
55	38.52 ± 0.31	38.48 ± 0.26

Table 4: Means and standard deviations of daily rectal temperature (°C) of Experimental and Control Yankassa sheep (Heterologous challenge)

DAYS P.I.	EXPERIMENTAL GROUP	CONTROL GROUP
56	38.27 ± 0.38	38.25 ± 0.15
57	38.40 ± 0.51	38.30 ± 0.22
58	37.98 ± 0.34	38.18 ± 0.13
59	38.18 ± 0.28	38.35 ± 0.09
60	38.18 ± 0.44	38.33 ± 0.16
61	38.23 ± 0.28	38.23 ± 0.19
62	38.17 ± 0.21	38.18 ± 0.23
63	38.45 ± 0.24	38.33 ± 0.15
64	38.18 ± 0.30	38.18 ± 0.29
65	38.33 ± 0.22	38.15 ± 0.18
66	38.43 ± 0.12	38.25 ± 0.15
67	38.60 ± 0.15	38.50 ± 0.23
68	38.52 ± 0.07	38.40 ± 0.07
69	38.77 ± 0.31	38.60 ± 0.16
70	38.60 ± 0.32	38.48 ± 0.19
71	38.73 ± 0.28	38.65 ± 0.29
72	38.68 ± 0.23	38.68 ± 0.11
73	38.75 ± 0.18	38.58 ± 0.15
74	38.75 ± 0.46	38.80 ± 0.07
75	38.63 ± 0.30	38.90 ± 0.30
76	38.55 ± 0.14	38.55 ± 0.05
77	38.30 ± 0.37	38.55 ± 0.18
78	38.63 ± 0.22	38.55 ± 0.11

Table 4 cont'd.

DAYS P. I.	EXPERIMENTAL GROUP	CONTROL GROUP
79	37.95 ± 0.35	38.38 ± 0.19
80	38.13 ± 0.16	38.33 ± 0.08
81	37.98 ± 0.41	37.95 ± 0.18
82	38.15 ± 0.32	38.45 ± 0.21
83	38.13 ± 0.24	38.28 ± 0.28
84	38.33 ± 0.17	38.33 ± 0.08
85	38.27 ± 0.09	38.25 ± 0.11

Table 5: Temperature reaction time, treatment time and duration of Experimentally infected Yankassa sheep with primary challenge with Jaji stock of Cowdria ruminantium.

Sheep No	Reaction time day P.I.	Treatment time(s) day(s) P.I.	Duration of reaction(days
1. P3018	10	11 & 12	4
2. P3024	-	-	-
3. P3879	10	11 & 12	4
4. P3882	11	12	3
5. P3884	10	11 & 12	3
6. P3885	12	13	3

Table 6: Means and standard deviations of Packed Cell Volume (PCV) of Experimental and Control Yankassa Sheep.

DAYS P.I.	EXPERIMENTAL GROUP	CONTROL GROUP
*Pre-infection	28.33 ± 3.30	28.75 ± 1.64
2	25.42 ± 3.68	28.38 ± 3.19
6	26.00 ± 4.32	28.00 ± 1.87
8	25.33 ± 4.08	26.50 ± 3.28
10	25.33 ± 3.54	25.00 ± 2.45
15	24.67 ± 5.06	26.25 ± 2.28
21	22.17 ± 3.72	24.75 ± 1.92
28	24.50 ± 2.99	25.25 ± 0.83
35	22.83 ± 3.34	25.75 ± 2.38
43	25.00 ± 3.00	24.50 ± 3.20
47	25.50 ± 4.35	25.00 ± 3.08
60	25.50 ± 3.86	27.00 ± 1.87
64	25.17 ± 3.08	28.00 ± 2.12
67	26.67 ± 3.94	27.00 ± 1.87
70	23.33 ± 3.20	25.88 ± 1.43
77	23.00 ± 3.11	23.75 ± 2.28
84	23.50 ± 2.87	24.75 ± 2.28

* 3 days pre-infection.

Table 7: Means and standard deviations of Total Serum Protein (TSP) of Experimental and Control Yankassa Sheep.

DAYS P.I.	EXPERIMENTAL GROUP	CONTROL GROUP
*Pre-infection	8.15 ± 0.39	8.20 ± 0.43
6	7.85 ± 0.48	7.65 ± 0.15
8	8.10 ± 0.77	8.13 ± 0.59
10	7.65 ± 0.64	8.05 ± 0.40
15	7.42 ± 0.47	7.70 ± 0.70
21	7.70 ± 0.64	7.90 ± 0.49
28	7.73 ± 0.50	7.79 ± 0.68
35	8.05 ± 0.53	8.23 ± 0.75
43	7.68 ± 0.90	8.33 ± 0.22
47	7.18 ± 0.52	7.10 ± 0.31
60	8.00 ± 0.26	8.18 ± 0.43
64	8.70 ± 0.65	8.28 ± 0.68
67	8.45 ± 0.63	8.25 ± 0.25
70	7.88 ± 0.46	0.08 ± 0.50
77	7.83 ± 0.36	8.13 ± 0.33
84	7.72 ± 0.90	8.03 ± 0.59

* 3 days pre-infection.

Table 8: Means and standard deviations of Total Leukocyte Counts, Lymphocytes, Neutrophils, Eosinophils and Monocytes of Yankassa Sheep during experimental Cowdria ruminantium infection

Days *Pre-	WBC $X10^3$/ul	Lymphocyte $X10^3$/ul	Neutrophil $X10^3$/ul	Eosinophil $X10^3$/ul	Monocyte $X10^3$/ul
infection	7.94 ± 2.25	5.52 ± 1.90	2.17 ± 0.78	0.08 ± 0.13	0.19 ± 0.13
0	6.63 ± 1.10	4.44 ± 0.82	2.06 ± 0.84	0.05 ± 0.06	0.08 ± 0.10
2	6.53 ± 2.00	3.59 ± 1.43	2.71 ± 0.77	0.07 ± 0.08	0.18 ± 0.14
6	9.42 ± 1.97	5.72 ± 1.57	3.40 ±0.53	0.06 ±0.05	0.24 ± 0.11
8	8.88 ± 2.27	5.46 ± 1.93	3.11 ± 0.78	0.02 ± 0.03	0.29 ± 0.09
11	8.28 ± 1.67	4.77 ± 1.24	3.30 ± 0.63	0.03 ± 0.04	0.19 ± 0.23
15	10.13 ± 2.27	6.00 ± 1.06	3.74 ± 1.50	0.04 ± 0.08	0.36 ± 0.24
21	8.54 ± 2.93	4.99 ± 2.35	3.12 ± 0.97	0.14 ± 0.16	0.29 ± 0.17
28	7.22 ± 1.91	3.99 ± 1.36	2.96 ± 0.92	0.23 ± 0.24	0.06 ± 0.03
35	7.56 ± 1.95	4.43 ± 1.56	2.81 ± 0.66	0.26 ± 0.24	0.06 ± 0.06
43	8.94 ± 2.18	4.20 ± 1.27	4.22 ± 1.20	0.12 ± 0.06	0.29 ± 0.19
47	7.03 ± 2.52	3.30 ± 0.77	3.57 ± 1.70	0.11 ± 0.12	0.06 ± 0.09
60	8.27 ± 2.17	4.01 ± 1.31	3.93 ± 0.93	0.17 ± 0.19	0.16 ± 0.09
64	5.71 ± 1.51	3.17 ± 1.15	2.39 ± 1.03	0.06 ± 0.05	0.08 ± 0.06
67	10.57 ± 3.57	5.88 ± 2.33	4.38 ± 2.13	0.12 ± 0.17	0.19 ± 0.11
70	10.53 ± 3.06	5.07 ± 1.95	5.04 ± 1.72	0.32 ± 0.64	0.10 ± 0.11
77	10.62 ± 3.49	5.47 ± 2.29	4.62 ± 2.03	0.32 ± 0.64	0.10 ± 0.11
84	10.70 ± 1.54	4.98 ± 1.26	5.53 ± 1.47	0.05 ± 0.05	0.14 ± 0.12

* 3 days pre-infection

Table 9: Means and standard deviations of Total Leukocyte Counts, Lymphocytes, Neutrophils, Eosinophils and Monocytes of Control uninfected Yankassa Sheep

Days	WBC X_{10^3}/ul	Lymphocytes $X10^3$/ul	Neutrophil $X10^3$/ul	Eosinophil $X10^3$/ul	Monocytes $X10^3$/ul
*Pre-infection	7.91 ± 3.46	4.66 ± 2.58	2.86 ± 1.04	0.17 ± 0.16	0.23 ± 0.26
0	9.31 ± 3.23	5.39 ± 2.80	3.55 ± 1.27	0.17 ± 0.09	0.21 ± 0.16
2	7.75 ± 3.20	4.08 ± 1.70	3.48 ± 1.73	0.09 ± 0.09	0.10 ± 0.03
6	10.10 ± 3.63	5.86 ± 1.75	3.76 ± 1.95	0.34 ± 0.18	0.13 ± 0.05
8	11.04 ± 4.74	6.33 ± 3.01	4.13 ± 1.55	0.24 ± 0.15	0.34 ± 0.17
11	9.07 ± 3.50	4.81 ± 1.90	3.06 ± 0.63	0.21 ± 0.17	0.15 ± 0.10
15	8.95 ± 3.37	5.10 ± 2.07	3.22 ± 1.03	0.29 ± 0.21	0.35 ± 0.12
21	9.16 ± 3.73	5.19 ± 2.46	3.54 ± 1.29	0.18 ± 0.16	0.26 ± 0.18
28	10.49 ± 5.28	6.00 ± 3.84	4.06 ± 1.48	0.23 ±60.21	0.04 ± 0.04
35	6.33 ± 3.35	5.43 ± 2.12	3.96 ± 1.40	0.38 ± 0.34	0.10 ± 0.06
43	9.94 ± 4.75	5.7 ± 3.17	3.89 ± 1.70	0.16 ± 0.06	0.14 ± 0.13
47	9.80 ± 6.14	4.97 ± 3.07	4.38 ± 2.54	0.29 ± 0.41	0.18 ± 0.14
60	7.21 ± 4.94	4.75 ± 2.53	3.30 ± 0.84	0.42 ± 0.29	0.21 ± 0.16
64	9.01 ± 2.75	4.47 ± 2.03	4.27 ± 1.11	0.18 ± 0.11	0.12 ± 0.08
67	9.24 ± 2.10	5.27 ± 1.21	3.70 ± 0.91	0.13 ± 0.11	0.14 ± 0.09
70	8.73 ± 2.38	4.58 ± 0.91	3.88 ± 1.73	0.16 ± 0.06	0.11 ± 0.09
77	9.99 ± 1.46	5.88 ± 0.94	3.74 ± 0.67	0.21 ± 0.11	0.16 ± 0.05
84	9.39 ± 0.76	5.17 ±0.59	3.76 ± 0.71	0.27 ± 0.19	0.19 ± 0.12

* 3 days pre-infection.

Table 10: Summary of Chi-Square analysis of case-infection rate of Cowdriosis in Sheep

		TIME							
		1973	1974	1975	1976	1977	1978	1978	1980
Disease	Positive	4(3.80)	2(10.15)	4(6.98)	3(6.18)	4(7.29)	7(8.72)	3(3.96	20(9.20)
	Negative	23(20.20)	62(53.85)	40(37.02)	36(32.83)	42(38.71)	48(46.28)	22(21.04)	59(66.48)
	Total	24	64	44	39	46	55	25	79

		1981	1982	1983	1984	1985	1986	1987	Total
Disease	Positive	6(9.20)	8(5.23)	4(3.01)	6(3.01)	17(6.18)	4(3.49)	2(1.27)	91
	Negative	52(48.80)	25(27.77)	15(15.99)	13(15.99)	22(32.82)	18(15.51)	6(6.73)	483
	Total	58	33	19	19	39	22	8	574

( ) = Expected Values.
Calculated X^2 = 51.518**
df = (r-i) (c-l) = 14
Level of significance ( ) = 0.05
Critical Value (CV) = 23.685
**Statistically significant (/0.05)

Table 11: Summary of two samples 't'-test of body temperature of sheep experimentally infected with Cowdria ruminantium.

Days P.I.	't' Value	Days P.I.	't' Value
0	1.019	27	-0.596
1	-0.409	28	-1.765
2	2.349**	29	-1.258
3	1.127	30	-0.995
4	1.025	31	0.000
5	0.695	32	-1.002
6	-1.236	34	-0.643
7	-2.954**	35	-1.448
8	-1.291	40	-0.436
9	-0.6	42	-1.759
10	1.256	43	0.804
11	2.383**	44	-1.485
12	3.087**	45	-0.778
13	3.107**	46	-1.112
14	2.337**	47	-0.939
15	0.105	48	-1.598
16	-4.086**	49	-0.498
17	-3.510**	50	0.590
18	-1.260	51	- 0.373
19	0.583	52	1.068
20	0.583	53	-0.984
21	0.000	54	0.167
22	-0.889	55	0.212
23	-0.439	56	0.099
24	0.346	57	0.330
25	-1.336	58	-1.106
26	-1.052	59	-1.155

CV = 2.306

** Statistically significant.

Table 11 continued

DAYS P.I.	't' values
60	-0.644
61	0.000
62	-0.071
63	0.881
64	0.000
65	1.354
66	2.113
67	0.842
68	2.656**
69	0.998
70	0.669
71	0.446
72	0.000
73	1.558
74	-0.212
75	-1.394
76	0.000
77	-1.237
78	-2.237
79	-2.221
80	-2.278
81	0.136
82	-1.636
83	-0.908
84	0.000
85	0.316

** Statistically significant (P 0.05).

Table 12: Summary of two sample 't'-test of Packed Cell Volume of sheep experimentally infected with Cowdria ruminantium.

Days P. I.	't' value
Pre-infection	-0.233
2	-1.309
6	-0.860
8	-0.477
10	0.161
15	-0.578
21	-1.262
28	-0.481
35	-1.500
43	0.252
47	0.198
60	-0.713
64	-1.589
67	-0.154
70	-1.476
77	-0.411
84	-0.727

Table 13: Summary of two sample 't'-test of Total Serum Protein (TSP) of Sheep experimentally infected with Cowdria ruminantium

Days P. I.	't' value
Pre-infection	-0.171
6	0.795
8	-0.066
10	-1.103
15	-0.765
21	-0.527
28	-0.594
35	-0.448
43	-1.391
47	0.274
60	-0.835
64	0.984
67	0.595
70	-0.652
77	-1.332
84	-0.602

Table 14: Summary of two sample t - test of total leukocytes, lymphocytes, neutrophils, eosinophils and monocytes of sheep during experimental Cowdria ruminantium infection.

Days	WBC X10^3/ul	Lymphocyte X10^3/ul	Neuterophil X10^3/ul	Eosinophil X10^3/ul	Monocyte X10^3/ul
Pre-infection	0.017	0.611	-1.205	-0.982	-0.326
0	-1.921	-0.803	-2.256	-2.547**	-1.598
2	-0.751	-0.494	-0.976	-0.369	1.107
6	-0.388	-0.132	-0.441	-3.708**	1.852
8	-0.980	-0.563	-1.396	-3.588**	-0.615
11	-0.193	-0.041	0.590	-2.558**	0.323
15	00.668	0.917	0.600	-2.708**	0.076
21	-0.295	-0.130	-0.591	-0.387	0.267
28	-1.419	-1.204	-1.467	0.000	0.911
35	0.743	-0.865	-1.775	-0.659	-1.033
43	-0.458	-1.099	0.363	-1.033	0.547
47	-1.009	-1.309	-0.610	-1.041	-1.675
60	0.472	-0.615	1.088	-1.662	-0.740
64	-2.477**	-1.308	-2.745**	-2.383**	-0.911
67	0.664	0.476	0.594	-0.103	0.752
70	0.988	0.463	1.042	0.489	-0.150
77	0.337	-0.334	0.823	-0.867	1.505
84	1.558	-0.278	2.210	-2.771**	-0.646

** Statistically significant (P 0.05).