HAEMATOLOGY OF EXPERIMENTAL CONTAGIOUS CAPRINE PLEUROPNEUMONIA

BY

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DECLARATION

I certify that this work was carried out in the laboratories of Clinical Pathology and Mycoplasmology, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. It was supervised by Professor I. D. I. Saror, Professor D. S. Adegboye and Dr. C. D. Ezeokoli.

The thesis is original and no part of it has been submitted elsewhere for a degree or diploma. Where use was made of other investigators' work, it was referenced and acknowledged.

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CERTIFICATION

This thesis entitled "HAEMATOLOGY OF EXPERIMENTAL CONTAGIOUS CAPRINE PLEUROPNEUMONIA" meets the regulation governing degree of Master of Science of Ahmadu Bello University, Zaria, and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my mother, late Madam Esther Oloruntomola who was called to the great beyond on 23rd October, 1980.

A mother in a billion! You were simply superb! Sleep on sweet Mum till we meet to part no more.

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ABSTRACT

The haematological picture of contagious caprine pleuropneumonia (CCPP) induced in Savannah Brown (SB) goats using the virulent Smith's strain of Mycoplasma mycoides subsp capri was determined. In the first animal experiment, 10 adult SB goats were each inoculated intranasally with a total of 2.6×10^7 organisms contained in 5ml broth culture; 5 goats serving as controls received 5ml sterile broth intranasally, while another 4 goats were kept as stand-by to replace any goat that died of CCPP. The following haematologic parameters were determined 2 weeks and 1 week pre-inoculation, on day of inoculation (day 0), 3 days, 7 days, 11 days, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks 10 weeks, 12 weeks, 14 weeks, and 17 weeks post-inoculation: Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Total Red and White Blood Cell Counts (RBC and WBC), relative and absolute differential counts and total protein level. Some goats survived infection for only 1 week and this first study was terminated 17 weeks post-inoculation, providing data on both acute and chronic stages of CCPP,, In a repeat animal experiment, 6 SB goats received 1.0×10^{10} organisms intranasally each,

also in 5ml broth culture, and 4 goats received 5ml sterile broth culture as control. Unfortunately-all infected goats died per-acutely or acutely within 7 days, such that the chronic blood picture could not be assessed, but these goats provided blood picture of a typical severe CCPP.

Clinical signs of disease were observed and post mortem examination was carried out on all animals that died and on those killed at the termination of each study. Mycoplasma re-isolation attempts were made from the trachea and lungs of each goat, and lung sections were prepared routinely for histopathologic examinations.

In animal experiment I, all infected goats showed elevated body temperature, coughing, inappetance, typical pleuritic sound on auscul-tation, and 3 died at 5 and 6 days post-inoculation. In animal experiment II, all infected goats showed similar clinical signs that were more marked and they died within 7 days post-inoculation, some as early as 48 hours post-inoculation. A mycoplasma confirmed by the growth-inhibition test as M. mycoides subsp. capri was isolated, from the trachea and lungs of all goats that died per-acutely or acutely, and

from three goats killed 17 weeks post-inoculation. No mycoplasma was isolated from any control goat. Gross lesions observed in goats that died were of classical CCPP. They included heavy fibrinous deposits on lung and thoracic cage, pleural adhesions to pericardium and thorax, severe pericarditis and hydropericadium (about 30mls) and hydrothorax (about 200mls), darkened consolidation of all lobes of the right lung and the ventral side of the left diaphragmatic lobe, enlargement of the bronchial and mediastinal lymph nodes. Histologically, there was cellular infilteration of the alveoli, bronchioles and bronchi. Cells were mainly neutrophils, but there were some lymphocytes and plasma cells. A lot of fibrin strands infilterated the alveoli, bronchioles and the interlobular septae. There was vascular congestion, and dilation of the lymphatic vessels.

The haematologic parameters were subjected to statistical analysis using the student t-test. Values of P 0.05 were considered significant. During the acute stage of infection, the mean values of total WBC counts, neutrophils and bands were higher in the infected goats than in control goats (P 0.05). This indicates a leukocytosis and neutrophilia with

a regenerative left shift. During later stages, the values gradually returned to normal level, i.e. comparable to values from control goats or pre-inoculation data. On the other hand, PCV, Hb, RBC counts, and also total blood protein values were not significantly affected during CCPP ($P>0.05$).

It is concluded that acute CCPP results in leukocytosis and neutrophilia with a regenerative left shift, but PCV, Hb, RBC and total protein values are not significantly affected. Moreover, the Smith's strain of M. mycoides subsp. capri is confirmed to be very highly pathogenic to Savannah Brown goats of Nigeria.

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ABBREVIATIONS

PCV = Packed Cell Volume

Hb = Haemoglobin

RBC = Red Blood Cell

WBC = White Blood Cell

gm = gram

mg = milligram

dl = decilitre

ul = microlitre

ml = millilitre

Fig. = Figure

P.I. = Post Inoculation

c.f.u.= Colony forming units

SB = Savannah Brown goats

CHAPTER 1: GENERAL INTRODUCTION

Small ruminants (sheep and goats) play a vital role in the livestock industry. According to the International Livestock Centre for Africa (ILCA) in 1979, approximately 22.3 million goats and 7.6 million sheep are kept mostly under the traditional village husbandry system and in few government or institutional farms (Ojo, 1983).

The advantage of their production is attributed to their sizes, compared to cattle. Less space and finance is needed to raise them such that many low income farmers can engage in their trade.

The goats in Nigeria do not receive the attention they deserve (Anon, 1966). But they unquestionably fit better into climatic and ecological systems of Nigeria than do sheep.

Three types of goat breeds are recognised in Nigeria: the West African Dwarf goat is confined to the humid forest belts of the South, the large-sized and long legged Fulani breed, and the Sokoto Red goats which are located in the Sahelo-Sudan area. The latter types are the most popular of the three. The main goat types that are found in the Savannah

region are crosses of the Sokoto Red and the Kano Brown. They are therefore referred to as Savannah Brown goats (Molokwu and Igono, 1978). Plate I.

Goats constitute about 30% of Nigeria's meat supply. By tradition, they have been found to fit the local circumstances, while their sizes make them specially useful for family consumption and the entertainment of guests in areas where meat cannot be kept for long due to inadequacy of preservation means (Anon, 1966). They are major sources of meat since they are accepted for consumption in all parts of the country. Their value does not reside only in their meat; their skins form a useful article in overseas trade, at prices far exceeding those of sheep skins. The skins are also useful as good quality materia for the local leather industries.

The economic production of sheep and goats are hampered by disease, aggravated by poor management and inadequate nutrition (Akerejola, Schillhorn Van Veen and Njoku, 1979). Ojo (1977) observed that the most important classical pulmonary disease of goats widely reported in textbooks and literature is contagious caprine pleuropneumonia (CCPP). Mohan and Uzoukwu (1983) also reported that the front ranking health problem of goats in Nigeria is

pneumonia, which may be caused by either Mycoplasma alone or in complications with other pathogens.

A lot of work has been done on the nature of the causative agent of CCPP, that is, Mycoplasma mycoides subsp. capri, the clinical signs of the disease, the gross pathological changes and the histopathological lesions, but less attention has been given to the haematological picture and the clinical chemistry. A knowledge of these parameters may lead to a better understanding of the pathogenesis of the disease and possibly add to the list of diagnostic criteria already known.

This work was undertaken to determine the haematological changes associated with experimental Mycoplasma mycoides subsp. capri (Smith's strain) infection. Haematological parameters evaluated included Packed Cell Volume (PCV), haemoglobin concentration (Hb), Erythrocytes (RBC) and leukocyte (WBC) counts, and the WBC differential counts.

In addition, the gross and histologic changes were evaluated. It is hoped that the information derived will assist in the diagnosis and management of goats afflicted with this disease in this area.

CHAPTER 2: REVIEW OF LITERATURE

I. Mycoplasmas in general

Mycoplasmology came into being indirectly as early as 1713 when a severe cattle disease was recognised in Germany and Switzerland. The disease spread throughout Europe, South America, Australia and United States of America in the 1840's and 1850's. The disease was eventually named Contagious Bovine Pleuropneumonia (Foster, 1934). It was eradicated from the United States by means of a comprehensive program of destruction of affected animals, and by 1900, it had been eradicated from both United States and Britain, but continued to be a problem in Australia and Africa. Lately, it was eradicated from Australia (Clay and Lloyd, 1975) and Africa probably remains the focus of infection.

Nocard and Roux (1898) were the first to isolate a microorganism that caused the disease Contagious Bovine Pleuropneumonia (CBPP). Dujardin-Beaumetz (1900) was able to grow the organism on solid medium and gave it the description of a light periphery and dark centre as it is characteristic of most mycoplasmas today. The organism was later described in clearer morphological details

by a number of scientists and they gave the name Pleuropneumonia Organisms (PPO). As organisms with similar characteristics were later isolated from other animals, they became known as Pleuropneumia-like Organisms (PPLO). But in 1956, Edward and Freundt proposed the generic name Mycoplasma. The bovine organisms became known as Mycoplasma mycoides subsp. mycoides as time went on.

Longley (1951) established that the causal organism of contagious caprine pleuropneumonia was similar to the causal organism of bovine pleuro-pneumonia agent and he called it Borrelomyces peri-pneumoniae caprae. The name was later changed to Mycoplasma mycoides subsp. capri (Edward, 1955) and that of contagious agalactia of sheep and goats identified as Mycoplasma agalactiae (Bridré and Donatien, 1923).

Mycoplasme was first isolated from man by Dienes and Edsall (1937) who isolated a species from Bartholin's abscess. Eaton, Meiklejohn, and Herick (1944) were able to isolate the organism of primary atypical pneumonia in man and this organism is known today as Mycoplasma pneumoniae. The first isolation of mycoplasmas in dog was done by Shoetensack (1934). This was from a dog with canine distemper,

The organisms reported by Nelson (1935) to be found in infectious fowl coryza in poultry were later described as being mycoplasmas by Markham and Wong (1952), and the organism is now called Mycoplasma gallisepticum and it is a causal agent of Chronic Respiratory Disease of chickens and turkeys.

There is a group of mycoplasmas which require urea for growth, these are called Ureaplasmas (Shepard, Lunceford, Ford, Purcell, Taylor-Robinson, Razin and Black, 1974). They produce ATP through an electrochemical ion gradient produced by ammonia liberated during intracellular hydrolysis of urea by the organism's urease (Romano, Tolone, Ajello and La Licata, 1980).

Saprophytic forms of Mycoplasmas have been identified in soil, leaves and manure. One of such is Mycoplasma laidlawii which does not require sterol for growth and multiplication, and has now been grouped with similar organisms as Acholeplasmas (Razin, 1981).

Mycoplasmas have been described as the smallest, simplest, self-replicating prokaryotes, that are capable of autonomous growth (Razin, 1981). They are pleomorphic and of about 125-150mu in size (Bordet,

1910) while Maniloff (1969) and Whittlestone (1972) reported sizes of 330nm and 250nm respectively. These characteristic small sizes and thus their ability to pass through bacterial filters, and also their lack of complex cell wall differentiate them from bacteria. Their ability to reproduce independent of living cells distinguish them from viruses. Taxonomically, they are members of the class Mollicutes (Edward and Freundt, 1956) and the order Mycoplasmatales. Important families of the order include Mycoplasmataceae, Acholeplasmataceae and Spiroplasmataceae. The former family has two genera: Mycoplasma and Ureaplasma while the latter has only one genus, Acholeplasma. Several species are known in the different genera. More than sixty species of Mycoplasmas have been identified and they have wide range of habitat (Razin, 1978).

The nutritional and growth requirements of mycoplasmas are important factors that are considered in their isolation and identification (Razin, 1981). They have limited biosynthetic abilities, reflecting their small genome and parasitic mode of life. Consequently, they require complex media for growth consisting mostly of heart infusion, peptone, yeast extract and serum. The serum provides fatty acids

and cholesterol required by most mycoplasmas for membrane synthesis. This requirement of most mycoplasmas for cholesterol, or related sterols is unique among prokaryotes. The main function of the cholesterol appears to be in the regulation of membrane fluidity (Razin, 1981). Glucose or any other metabolizable carbohydrate can serve as energy source for fermentative mycoplasmas possessing the glycolytic pathway. Among non-fermentative mycoplasmas, the source of ATP is by the breakdown of arginine through the arginine dihydrolase pathway (Razin, 1981).

Mycoplasmas have predilection for mucosal surfaces (Gourlay, 1981). Most mycoplasmas infecting man and animals adhere firmly to the epithelial linings of the respiratory or urogenital tracts and rarely invade the tissues and blood stream. Hence they may be considered as surface parasites (Razin, 1981). Their adherence is so firm that they are not eliminated by the mucous secretions or by urine stream. This intimacy of mycoplasmas with their host cells provides an environment in which local concentrations of mildly toxic by-products excreted by the parasites (eg. hydrogen peroxide and ammonia) can build up and cause tissue damage. Also the lack of cell wall in

mycoplasmas enables a close contact of their plasma membrane with the host cells. The resultant host reactions play an important role in pathogenesis. Thus the host may be responsible to a large extent for the appearance of pneumonia by mounting a local immune and phagocytic response to the mycoplasmas.

The Disease Contagious Caprine Pleuropneumonia (CCPP)

Introduction

Mycoplasmas have a predilection for mucosal surfaces and thus the disease with which they are associated are generally those involving the respiratory, urogenital tracts, the mammary glands, joints and eyes. CCPP affects the respiratory tract as the name shows. It is considered to be the most serious epizootic disease of goats, causing high mortality and consequently a great economic loss in areas where goat is important for livelihood (Cottew, 1970). CCPP is widespread in Sudan, Chad, India, Iran, Italy, Spain, Saudi Arabia, Pakistan, Greece, Mexico and Nigeria.to mention a few.

Shirlaw (1949) and Longley (1951) were the first to survey the disease and they emphasized the serious nature, its occurrence and preventive measures.

Longley (1951) established that the causal organism of CCPP was similar to the contagious bovine pleuropneumonia agent and he named it Borrelomyces peripneumonia caprae. He mentioned morbidity and mortality rates of up to 100%. The mortality and incidence rates are apparently influenced by weather, being highest when conditions are cold and wet.

Aetiological Agent of CCPP

Provost, Villemot, Perreau, Queval and Borredon (1967) have indicated that a range of antigenic types are present among the strains represented as being CCPP agents. The representatives strain of the species, M. mycoides subsp. capri was isolated by Chu and Beveridge (Edward, 1953) at Cambridge, from samples of pleural fluid and lung from a goat affected with CCPP in Turkey (Cottew, 1970). This strain was designated as PG3 by Edward (1955). A goat was infected intranasally with atomized culture of the organism, and it developed classical pleuropneumonia. The strain had some similarity to Longley's strain because they both formed large colonies and liquefied serum (Edward, 1953 and 1954), but they apparently were not compared serologically.

A strain from Nigeria (N108), said to be Longley's strain, was compared with M. mycoides subsp. capri (PG3) by El Nasri (1967). There was a cross reaction in gel diffusion test between the two but neither provoked CCPP in experimental goats. Several other strains (G1/61 from Sudan, the 'Vom' strain and OSB-42 from Nigeria were compared with strains known to produce CCPP in Turkey. The pathogenic Turkish strains were shown to be serologically interrelated. They were also serologically related to strain N108 of Nigeria. But OSB-42, 'Vom' and PG3 were antigenically different from the Turkish strains. Some of these strains were still capable of producing CCPP in susceptible goats. At present, not all of the possible combinations of serological interrelationships among these strains have been studied, but it is known that they have relationships with the bovine pleuropneumonia organism, M. mycoides subsp. mycoides. For example, the Sudan G1/61 is apparently identical to this bovine pleuropneumonia organism (Hudson, Cottew and Adler 1967; Jonas and Barber, 1969), and some degree of relationship was shown by the 'Vom' strain, while others were apparently not related.

Lemcke (1965) showed by gel diffusion test that M. mycoides subsp. capri shared antigens with M. mycoides subsp. mycoides, although there was no confirmation by the complement fixation or agglutination tests.

It is worthy of note here that only strains with demonstrable serological identify with the PG3 strain are recognised as M. mycoides subsp. capri. Solution must be found in naming other strains that cause CCPP and yet differ serologically from the PG3 strain (Cottew, 1970).

The organism can be observed by microscopy with dark ground illumination. It may be isolated and grown in either liquid or solid media containing serum. The colonies appear slightly larger and faster growing than those of M. mycoides subsp. mycoides (LC). It is resistant to adverse conditions and will remain viable for long periods of time if suitably preserved and particularly if lyophilized and stored at temperatures below zero (Cottew and Leach, 1970).

Little is known of the factors influencing the pathogenicity of the organism, but is produces glucan which is shed into the medium of growth (Cottew, 1979).

It is speculated that the glucan produced would form an extracellular layer on the organisms and play a role in pathogenicity. It is not known whether these layers or capsules inhibit any of the body defence mechanisms or whether they have a function in attachment to host cells (Cottew, 1979). Moreover, Cherry and Taylor-Robinson (1970) demonstrated that the organism produces hydrogen peroxide when grown in tracheal organ cultures. The peroxide causes a cilia-immobilizing effect of the respiratory tract.

The discussion on the causal agents of CCPP will be incomplete if mention is not made of the caprine strain of M. mycoides subsp. mycoides referred to as the large colony (LC) type (Cottew and Yeats, 1978). Recent work has shown that M. mycoides subsp. mycoides is of two types based on growth rate and behaviour in certain biochemical tests. The first is the large colony (LC) type, most of which are from goats and are pathogenic for sheep and goats but apparently not for cattle (Cottew and Yeats, 1978; Bar-Moshe and Rapapport, 1981; DaMassa, Brooks and Adler, 1983). This LC form has been shown to produce a condition indistinguishable from CCPP (Ojo, 1976c). It is not pathogenic for cattle, nor

does it protect against subsequent challenge with a virulent strain (Cottew, 1979). However more lately Ojo, Kasali and Ozoya (1980) showed that calves immunosuppressed by trypanosome infection (T. vivax) were susceptible to a caprine strain designated Ib9. Thus under appropriate conditions, cattle may be susceptible to M. mycoides subsp. mycoides (LC) of caprine origin.

The small colony types (SC) include the classical Contagious Bovine Pleuropneumonia (CBPP) strains and four other strains from goats; both types (LC and SC) are pathogenic for goats and sheep (Cottew, 1979).

A third mycoplasma designated strain F38 has been shown to play a similar role in the production of CCPP in goats (MacOwan and Minette, 1976 and 1977). The organism has been isolated from a number of natural outbreaks of CCPP in Kenya and has been found to be able to produce the disease experimentally in goats. Infact, the authors feel that the strain F38 produces a more classical CCPP than the M. Mycoides strains.

Table 1 shows the list of mycoplasmas of goats and sheep as well as those isolated and identified in Nigeria, according to Ojo (1983).

Table 2 also shows the list of mycoplasmas of goats and associated diseases according to Cottew (1982). The list shows that mycoplasmas cause diseases other than CCPP in goats.

Epidemiology of CCPP in Nigeria

The disease was first recognised in Nigeria by Sainsbury (1928) and it was common among goats in Northern Nigeria. Beaton (1928 and 1931) was the first to describe the symptons and lesions of the disease, and he isolated an infective agent as being the cause. However, Longley (1951) confirmed the existence of the disease.

Ojo (1971) in his review of microbial diseases of goats in Nigeria considered pneumonia as an important disease. In a study of the bacterial flora of normal and diseased respiratory tracts, Ojo (1976a) observed that the prevalence of caprine pneumonia in Nigeria is high during the rainy and "harmattan" seasons. The author also indicated that the important bacteria isolated from both normal and

Table 4: MYCOPLASMAS OF GOAT AND SHEEP (Ojo, 1983)

M. agalactiae (N)

M. arginini (N)

M. bovis (N)

M. mycoides subsp. capri (N)

M. mycoides subsp. mycoides (N)

M. ovipneumoniae (N)

M. capricolum

M. conjunctivae

M. putrefaciens

M. feliminutum

A. granularum

A. oculi

A. laidlawii

Ureaplasma sp.

Mycoplasma sp F 38

Mycoplasma sp A 1343

Mycoplasma sp G 145

Mycoplasma sp D 2

Mycoplasma sp G

Mycoplasma sp U

Mycoplasma sp V

(N) = Isolated and identified in Nigeria.

Table 2: LIST OF MYCOPLASMAS OF GOATS AND ASSOCIATED DISEASES (Cottew, 1982)

Mycoplasma species		Disease
M. agalactiae	-	Contagious agalactiae
M. arginini	-	Pneumonia, mastitis
M. bovis		Pneumonia, mastitis
M. capricolum	-	arthritis, mastitis
M. conjunctivae	-	Keratoconjunctivitis
M. mycoides subsp. mycoides (LC type)	-	Pleuropneumonia, mastitis, arthritis
M. mycoides subs. capri	-	Pleuropneumonia
M. ovipneumoniae	-	Pneumonia
M. putrefaciens	-	mastitis
A. granularum		
A. laidlawii		
A. oculi	-	keratoconjunctivitis
Ureaplasma spp		
Mycoplasma sp. F 38	-	Pleuropneumonia
Mycoplasma sp. A 1343		
Mycoplasma sp. G 145	-	arthritis

diseased respiratory tracts of goats were Pasteurellae and Mycoplasmata; no mycoplasma was isolated from normal lungs and trachea but mycoplasmas were isolated from the nasopharynx and 30% of diseased lungs. These mycoplasmas were later characterised by biochemical and serological means (Ojo, 1976b): on the basis of biochemical characteristics, the strains were divided into three groups. (A) Eighteen strains which fermented glucose and mannose, digested serum and reduced tetrazolium, (B) 10 strains which reduced tetrazolium, formed "film and spots" and possessed phosphatase activity and (C) two strains which catabolised arginine only. Serologically group A strains were identified as M. mycoides subspecies, group B strains could no be fully identified but on the basis of immunofluorescence and biochemical reactions were put in the M. agalactiae subspecies, to be further investigated and group C strains were identified as M. arginini. In this study, only one of the group A strains was identified serologically to be M. mycoides subsp. capri (M. capri), twelve strains were identified to be M. mycoides subsp. mycoides and the remaining four were difficult to assign to a specific subspecies of M. mycoides. This showed that M. capri had a low recovery by serological tests and may explain why Goni and

Onoviran (1973) could not detect antibodies to the organism in the samples obtained from slaughter houses in Nigeria (Ojo, 1976b).

The natural disease does not affect sheep, although they are susceptible to experimental infection (Longley, 1951; Cottew et al., unpublished data), by subcutaneous injection of virulent material from local form of the disease.

CCPP accounts for about 70% average mortality in goats in Nigeria (Beaton, 1937) but this may have decreased considerably over the years. The accurate incidence of the disease is difficult to ascertain because of inaccurate record keeping. However up to 100% mortality has been reported in literature (Longley, 1951; Ojo, 1976c).

Clinical signs of the disease

The incubation period is said to vary according to the routes of experimental inoculation. The shortest incubation period recorded by Ojo (1976c) was 2-6 days by endobronchial route using the Smith's strain. Goni and Onoviran (1972) who also used the Smith's strain recorded an incubation period of about 3 days (72 hours) by the same route.

In the older literature, Longley (1940) reported another incubation period of 8-28 days (in India) but later reported another period of 10-14 days in 1951 (in Nigeria). Following intranasal spraying, Pillai (1965) found the incubation period to be (14-15 days) and Solana and Rivera (1967) reported 3-8 days using intravenous routes. However, the incubation period of natural disease is said to be 3-5 weeks (Longley, 1951) even though this has not been well documented.

In experimental infections, severely affected animals become anorexic and dull; they stand with hanging heads and are unsteady in their gait. The rectal temperature rises sharply to 40°C - 41.1°C within 24 to 48 hours post inoculation. Animals which are not affected still eat, though not as well as the control animals. Serous to mucoid discharges from the nose is a characteristic sign (Ojo, 1983).

Respiratory symptons are marked in both acute and subacute cases. This include heavy abdominal breathing with audible groaning at frequent intervals and twitching of the nostrils during each breath. Coughing is not common but could be induced by forced exercise. On auscultation, harsh respiratory sounds are heard over the bronchial area.

In chronic state, there are no marked clinical symptoms. The animals loose weight despite eating well and cough occasionally.

The mortality rates vary with different strains. This is dependent on the level of passage and thus virulence. About 50% of mortality is said to occur within 5 days post-injection, though the Smith's strain which is highly virulent gives mortality of up to 100 percent (Ojo, 1983). Animals with high temperature and respiratory involvement rarely recover.

Curasson (1946) described the peracute, acute and chronic forms of the disease. The course of the peracute form is completed in two to five days while that of the acute pneumonic condition may be between fifteen and thirty days. The chronic form which follows the acute is thought to be a phase of slow resolution and recovery from the non-fatal acute form. The chronic lesion is thought to be non-infective.

Gross Pathology

Lesions are confirmed to the lungs, pleura and pericardium and the extent of involvement depends on the duration and severity of disease (Pillai, 1965).

In majority of cases, the lesion is unilateral with involvement of the right lung usually (Ojo, 1976c) and when both lungs are affected, they are equally involved.

The pulmonary lobes show various degrees and stages of hepatization, together with dilation of interlobular septa (Longley, 1940, 1951; Curasson, 1946; Shirlaw, 1949). On sectioning, the lung is oedematous, the pleura membrane of affected lung is intensely inflamed and covered with a thick layer of spongy fibrin containing meshes of yellowish exudate. The thoracic cavity in many cases contains 1-2 litres of fluid which vary from uniformly cloudy, grey to blood-tinged colour. The fluid is very rich in mycoplasmas. The pleura in many cases adhere to the diaphragm, lung, chest wall and pericardium. Fibrinous pericarditis is common but the extent varies from animal to animal. Retropharyngeal, mandibular, mediastinal and bronchial lymph nodes may be enlarged, haemorrhagic and oedematous. This is observed in the acute stages.

In peracute cases multiple focal lesions are seen as described by Pillai (1965). Sections of the lung may show variegated mosaic appearance with lemon-yellow deposit containing much pleural exudate.

in lacunae over the affected lung. This variegated appearance is as it is seen in contagious bovine pleuropneumonia (Cottew and Leach, 1969). The organisms remain viable at the periphery of the lesion longer than in the centre (Longley, 1951) which may account for some of the failures to isolate the organism.

The lesions in the chronic stage are said to be regressive and noninfective (Cottew and Leach, 1969). There is no equivalent of the sequestration as seen in the bovine disease, and this is why recovered animals are believed not to be carriers. Affected lungs are seen in various stages of resolution with encapsulation of acute lesions. Firm and fibrous adhesions attach the lungs to the chest wall. The pericardial sac may contain excess fluid. There may be rhinitis with nasal discharge and congestion of the pharynx and larynx. Lesions are seldom found in the abdominal viscera (Shirlaw, 1949).

Histopathology of CCPP

The pulmonary lesions originate from a primary lymphangitis in the lungs (Shirlaw, 1949). This is started as a fibrinocatarrhal inflammation of the bronchioles (bronchiolitis). There is venous congestion

in the alveolar septae and thrombi in the vessels (Pillai, 1965). The perilobular, perivascular and peribronchial tissue is distended with exudates containing numerous histiocytes, lymphocytes and polymorphs (Cottew and Leach, 1969). An acute pyogenic confluent broncho-pneumonia is seen in 33% of experimental cases and 50% of natural cases (Ojo, 1977). Fibrinous exudate in the alveoli and pulmonary fibrotic lesions are seen in natural infection but not in experimental infection (Ojo, 1977). Central areas of coagulative necrosis bordered by pulmonary oedema could be prominent features in both experimental and natural occurences.

Diagnosis of CCPP

Laboratory diagnosis of mycoplasma infections present several problems not encountered in diagnosis of other bacterial infections. Thus the examination of stained smears of sputum, pus or other excretions is of little diagnostic value due to the minute size, polymorphism and poor stainability of the mycoplasmas, and the presence in the clinical material of mycoplasmas belonging to the normal flora (Razin, 1981) when staining is attempted, mycoplasmas do better with Giemsa than by Gram's stain (Razin, 1981).

Russel et al (1975) and Chen (1977) have demonstrated specific staining by fluorescent dyes of DNA as a means for demonstrating mycoplasmas in contaminated cell cultures. Transmission and scanning electron microscopy have also been employed successfully for this purpose, but these are most inadequate for routine diagnosis due to the financial expense involved. In the light of the above, cultivation and serological identification are very essential for definitive diagnosis (Razin, 1981).

Different mycoplasma types require complex and rich media for growth. These types of media are very conducive for fast-growing bacteria to grow in. To avoid this, antibacterials such as penicillin and thallium acetate are added to the media for growing mycoplasmas.

Adegboye (1981) presented a format for the composition of mycoplasma growth medium, which he has used to propagate successfully a number of both pathogenic and non-pathogenic species.

This consists of:

1. PPLO Broth (powder) - 5.25gm/169ml (70%) deionised distilled water

2. Fresh Yeast Extract - 25ml (10%)
3. Horse serum - 50ml (20%)
4. Benzyl penicillin sodium (10^6 I.U/ml) - 0.25ml (1000 IU/ml)
5. Thallium acetate (10% solution) - 0.5ml (0.02%)
6. Phenol red (1% solution)- 0.5ml (0.002%)
7. Substrate: either glucose (10% solution) - 2.5ml (0.1%)
   or arginine (10% solution)- 2.5ml (0.1%)
   or urea (50% solution) - 0.5ml (0.1%)
   depending on the metabolite form been sought.

The final volume is approximately 250ml.

The pH of the medium is adjusted to 7.6 if glucose substrate is used and to 6.0 if arginine or urea is used.

If solid medium is desired, Ionagar No. 2 (Oxoid Ltd., London, S.E.I) or any highly pure agar such as Agar Noble (Difco Laboratories, Detroit, Michigan, U.S.A.) is added to the above composition at 2.5gm concentration.

Colonies of Mycoplasma mycoides subsp. capri have a "fried-egg" appearance, like most mycoplasmas, consisting of a central zone of growth embedded in the agar. Colonies can be identified by staining

directly on agar with homologous fluorescein conjugated antibody, or by demonstrating inhibition of their growth by specific antiserum (Adegboye, 1981). A more detailed characterisation of isolates can be done by a variety of routine biochemical and serological tests.

Serodiagnosis consists of the demonstration in the patients or animal sera of antibodies that inhibit growth and metabolism of the mycoplasma or fix complement with the antigens (Razin, 1981). But this is not used in the diagnosis of CCPP possibly because the rapidity of onset of the disease would preclude the formation of significant amounts of antibody before the disease is recognised clinically (Cottew, 1970).

In the field, the lesions of CCPP may give a tentative diagnosis of up to "pneumonia" only (Ojo, 1983) and this may be confused with other diseases like acute pasteurellosis. Confirmation is only by the laboratory examinations that must be done. For this purpose, fresh materials such as pleural exudate, mediastinal lymph node and diseased lung tissue should be submitted preserved in 50% glycerin. Lung in formalin should be used for histopathologic evaluation.

The complement fixation test is unsatisfactory (Ojo, 1976b).

The characteristics which appear to be shared by pathogenic strains of the CCPP agent are: good growth in liquid medium, in which branching filaments may or may not be produced; large to very large colonies (up to 1.7mm diameter) on suitable solid media; liquefaction of inspissated horse serum; hemolysis of sheep, horse, and chicken blood agar medium (with a characteristic opaque, whitish-green appearance); formation of branching, filamentous forms in pathological exudates; production of acid in glucose broth; and the production of CCPP in goats inoculated intratracheally with young cultures of the organism (Cottew, 1970).

Control and treatment of CCPP

Solid immunity was not demonstrated in all animals following experimental infection (Shirlaw, 1949; Longley, 1951). Since a number of antigenic types of mycoplasmas appear to be incriminated as causal agents of CCPP, an effective vaccine should be able to protect against all the different types. Formalized

vaccines have been used in Madras (Provost *et al.*, 1967) and this protected about 83% of vaccinated goats.

Young goats are relatively resistant to CCPP but kids of 4-6 months are susceptible to experimental infection (Ojo, 1977). Immunity after recovery from natural infection is not understood. It does not appear to be of high quality because serum from actively immunized goats has no protective value against experimental infection even when simultaneously given. At present in Nigeria, no vaccine is being used, this is because CCPP responds to therapy (Goni and Onoviran, 1973). The vaccine if it should be prepared should contain both *M. mycoides* subsp. *mycoides* and *capri* if it should be efficient. Vaccination will be difficult here because goats are not reared either semi-intensively or intensively in Nigeria and many parts of Africa (Ojo, 1982).

Tylosin has been found effective but it is considered expensive (Ojo, 1983). Terramycin has been used too. A new drug called Tiamulin (R) developed by Biochemie, Vienna, with Sandoz as the representative in Nigeria is being tried in experimental infections (Ojo, 1983). It is effective *in vitro* and *in vivo*.

Lastly it is known that attenuation of mycoplasma strains by laboratory passage tends to reduce both virulence and immunogenicity (Razin, 1981). In most cases, attenuated viable vaccines did not confer the level of protection required from commercial vaccine. A unique exception exists for M. mycoides subsp. mycoides, where subcutaneous inoculation of live organisms in the tail protects cattle effectively against CBPP.

Haematology of caprine mycoplasmosis

In a short communication on the haematology and plasma protein changes in caprine pleuropneumonia in Sudan, Gameel (1976) observed that there was a steady rise in the leucocyte counts in diseased animals kept under close observation. He observed that the rise in leucocytes was due to increases in neutrophil and lymphocyte counts of the blood samples. He also observed that monocytes and eosinophils were not affected. Erythrocyte counts, haemoglobin levels, packed cell volumes and erythrocyte sedimentation rates declined progressively. Plasma protein and fibrinogen also decreased slowly and later fell rapidly, becoming virtually undetectable after 4 weeks of confinement.

Rosendal (1983) in his work on the susceptibility of goats and calves after experimental inoculations or contact exposure to a Canadian strain of Mycoplasma mycoides subsp. mycoides isolated from a goat observed that the total white blood cell (WBC) count dropped from an average of 7.0-17.8 x 10^9/litre to 3.4-1.2 x 10^9/litre four days post inoculation. He also noted that the drop in leucocyte count was due to a neutropenia. Erythrocytes, haemoglobin, packed cell volume remained unchanged after inoculation.

The experimental infection of goats, sheep, and calves with large colony type of M. mycoides subsp. mycoides intravenously resulted in leukopenia and granulocytopenia (Rosendal, 1981). Coagulopathy was reported in some goats. The fibrinogen titre, prothrombin and partial thrombopiastin increased with the progress of the disease. The RBC count, PCV and haemoglobin yielded no values outside established normal ranges.

In a report on the milk borne outbreak of Mycoplasma mycoides subsp. mycoides in a commercial dairy goat, East et al (1983) observed a high WBC count (13,000 cells/mm^3) with neutrophilia (7,200 cells/mm^3) increased fibrinogen (400mg/dl) and monocytosis (530 cells/mm^3).

The foregoing has presented conflicts in the haematologic studies of contagious caprine pleuropneumonia. But one must also take into consideration the differences in the species and strains of Mycoplasma mycoides used by the different authors. For these reasons therefore, this project involves the production of classifical CCPP (using Smith's strain of Mycoplasma mycoides subsp. capri) and establishing the haematological parameters of acute and chronic stages of the disease as might be encountered in the field.

Caprine Haematology

The haematological parameters of healthy Nigerian breeds of goats (and sheep) have been studied; it was found that most values in the Nigerian goats are similar to those of temperate breeds, although the haemoglobin concentration and the mean corpuscular haemoglobin concentration (MCHC) are lower (Oduye, 1976). He also reported that the total leucocyte counts were considerably higher in the Nigerian goats and sheep than those reported for the temperate breeds of animals (Oduye, 1976). This is in agreement with the finding of Edwards, Judd and Squire (1955) who recorded a mean white blood cell count of 14.54×10^3

and 14.23 x 10^3 per microlitre of blood in the West African Dwarf goats and sheep, respectively, in Gold Coast (Ghana) as against the record of Holman and Dew (1963) of 8 x 10^3 per microlitre of blood, and Millson, West & Dew (1960) who reported a range of 9 x 10^3 to 15 x 10^3 in the goat.

Nigerian goats are reported to have a higher differential eosinophil counts than those of the temperate (Oduye, 1976). This might be explained by the uncontrollable exposure of these animals to a host of different intestinal parasites, which are known to encourage high count of eosinophils in blood.

Erythrocyte series

The red blood cells of the goat is the smallest among domestic animals (Schalm, 1975). They are round to blunty triangular in stained blood films. Such films made from blood samples of immature goats (about the first month of life) present a lot of prominent anisocytosis (Holman and Dew, 1964). Nucleated RBC and reticulocytes are not present in the peripheral circulation of healthy animals. Their presence in circulation is in response to anemia (Holman and Dew, 1964).

The assessment of the red blood cells is based on the determination of the Packed Cell Volume (PCV), Haemoglobin concentration (HB), and the total red cell counts. The PCV is determined by the microhaematocrit method, the haemoglobin by the cyanmethaemoglobin method using the Coulter haemoglobinometer and the total red cell count is done by the use of the haemocytometer method. These methods are described by Schalm (1975).

The goat has the lowest PCV of the common domestic animals (Schalm, 1975). The normal value of the PCV of a properly centrifuged goats blood ranges between 22 to 38 percent, although some apparently clinically normal goats could have values below 22 percent.

Oduye (1976) reported that the mean values of PCV, RBC and mean corpuscular volume (MCV) in the Nigerian goat did not differ significantly from the values obtained for 2-year-old female goats as reported by Holman and Dew (1963).

Esievo, Saror, Alafiatayo and Abubakar, (1984b) reported that the PCV and Hb of the male and female Sokoto Red kids increased with age to adult values at 8 to 10 weeks of life. The same pattern was also observed in Yankasa lambs (Esievo et al, 1984b).

These workers explained that the protracted decrease of these parameters very early in life, particularly of the males suggested rapid growth, accompanied by increased in plasma volume without concommittant increase in the red cell mass during this phase.

The erythrocytic indices

These include the Mean Corpuscular Volume (MCV), the Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Haemoglobin Concentration (MCHC). These indices are estimated from the values of PCV, Hb and total RBC counts - per microlitre (ul) of blood (Wintrobe, 1969). The validity of the indices is influenced by the accuracy of the red-cell count, the haemoglobin determination, and the haematocrit or PCV value (Schalm, 1975).

The MCV which expresses the average size in terms of volume, of a single red blood cell, is determined by dividing the volume of packed red cells per 1000ml of blood by the total red cell count in millions per cubit millimeter i.e. PCV of 1000ml of blood/RBC count in ul of blood. The unit of expression is in cubit microns. The value in goats ranges from 15-30 with a mean of 23 as given by

Schalm (1975); 19-37 mean of 27, as given by Coles (1974) and the values for the different ages have been given by Oduye (1976), reproduced in Table 3. The MCV values is noted to increase with age by Oduye (1976), and not significantly affected by sex.

The MCH measures the weight of the haemoglobin in the average erythrocyte of a population of cells (Schalm, 1975) and it is calculated by dividing the haemoglobin present in grams per 1000 millilitres blood by the total erythrocyte count in millions per cubic millilitre (Coles, 1974) and it is expressed (Coles, 1974) and it is expressed in micro micrograms. i.e. Hb in 1000ml of blood RBC count in microlitre of blood. The normal value in goats is given as 5-7(6) micromicrograms (Coles, 1974).

The MCHC measures the ratio of weight of the haemoglobin to the volume of the erythrocyte (Schalm, 1975) and it is determined by dividing the haemoglobin in grams per 10,000ml of blood by the volume of PCV per hundred millitres of blood, i.e. Hb in grams per 10,000ml of blood/percent PCV. It is expressed in percent of haemoglobin of the total weight of an erythrocyte. The normal value in goats is given as 30-35(32) percent (Coles, 1974) and 35-42(38) by Schalm (1975). Hypochromic anaemia is characterised

by a lower than normal MCHC.

Errors may arise in the determination of the erythrocytic indices through the erythrocyte counts obtained by haemocytometer as well as lack of skill of the person making the counts. Both MCV and MCH are influenced by errors in erythrocyte counts. Also, an excess of anticoagulant (EDTA) produces shrinkage of erythrocytes. This results in the reduction of PCV. Erythrocyte count and haemoglobin concentration are not altered. This results in a reduction in MCV and an increase in MCHC. Significant haemolysis of a sample leads to erroneously increased values of MCH and MCHC because the free haemoglobin in the plasma is mathematically incorporated in the remaining non-haemolysed erythrocytes, while the PCV and the total RBC counts are decreased (Schalm, 1975).

Leukocyte series

Esievo et al (1984a) reported a dramatic increase in the WBC of kids with age and this is mostly influenced by increases in absolute lymphocyte counts, and partly by eosinophils. The higher level of lymphocytes than neutrophils observed in the work agreed with the observation in Yankasa lambs (Esievo

et al, 1984b) and cattle (Schalm et al, 1975). The WBC, lymphocyte and eosinophils are said to increase with age generally to adult values at 8 to 10 weeks of life (Esievo et al, 1984a). The absolute neutrophil counts remain lower than lymphocyte counts in both sheep and goats, but the total WBC counts of the kids are about 2 times higher than the WBC count of the lambs, as from 6 weeks of age. However, sex is said to have only a modest effect on these parameters in both Yankasa lambs and the kids of Sokoto Red goat (Esievo et al, 1984a).

Total white blood cell count is done by the use of electronic coulter counter or the manual haemocytometer. The value obtained in one count is expressed in microlitre of blood.

The differential leucocyte count is done by making a thin blood smear on a glass slide, and with the use of immersion oil, a hundred leucocytes are counted. These are expressed in percentages. To obtain the absolute values of the different leucocytes, the percentage of each leucocyte is multiplied with the total leucocyte count for a particular blood sample, as described by Schalm, (1975) and Coles (1974). In clinical diagnosis, the absolute values must be calculated to make good inferences. In the work of

Esievo et al (1984a), no consistent observation was made for band neutrophils and monocytes. The mean absolute eosinophil counts increased from the first week of life up to 5 weeks, thereafter showing no definite pattern of change.

The mean absolute neutrophil could was also reported to have regular waves of increment in the peripheral circulation, but it remains lower than the lymphocyte count. This is in agreement with the reports of Millson et al (1960) and Wilkins and Hodges, (1962). However some other workers have reported higher neutrophil counts (Schalm, 1975).

Band neutrophils occur fairly commonly in peripheral blood in association with neutrophilia in response to disease in the goat (Schalm, 1975). A left shift to metamyelocytes is uncommon, and myelocytes have rarely been encountered.

The basophil is very rare in the healthy goat blood, as it is in other domestic animals (Coles, 1974).

Table 3: MEAN BLOOD VALUES IN CLINICALLY NORMAL NIGERIAN DOGS (Oduye, 1975)

Animals	No. Examined	PCV	Hb g%	RBC ($x10^6$)	MCV	MCHC	Total WBC	Differential Leucocyte Count in Percentage: Segmented Neutrophil	Banded Neutrophil	Eosinophil	Lymphocyte	Monocyte
0-6 months	17	25.1	8.43	13.4	19.8	33.9	18.626	43.8	0.7	1.4	52.9	1.2
		±3.4	±0.94	±3.3	±4.8	±3.9	±3,945	±12.9	±1.4	±1.7	±13.7	±0.9
6-12 months	12	27.0	9.07	12.9	21.2	33.9	17.556	44.9	0.2	4.3	50.2	1.0
		±4.6	±1.41	±2.1	±3.4	±3.3	±4,109	±7.6	±0.4	±4.6	±7.4	±0.9
12-24 months	20	26.9	8.70	11.9	22.9	32.4	16,581	48.4	1.3	6.0	43.4	0.8
		±3.8	±1.31	±1.7	±3.5	±3.2	±4,196	±11.3	±2.0	±5.3	±12.8	±0.9
24 months	36	25.9	8.45	11.8	22.4	32.9	13,542	48.0	0.7	6.4	44.3	0.7
		±4.4	±1.50	±2.3	±4.4	±3.6	±4,219	±11.0	±0.7	±4.7	±10.0	±0.9
All female	40	26.1	8.52	12.2	21.8	33.0	16,141	48.4	0.5	4.3	45.9	0.9
		±4.5	±1.28	±2.2	±3.7	±4.0	±4,766	±9.6	±1.2	±3.3	±10.4	±1.0
Pregnant female	9	26.9	8.56	11.3	23.9	32.3	13,906	48.1	1.0	3.8	46.1	1.0
		±4.0	±1.59	±2.0	±3.6	±2.9	±4,407	±13.8	±1.2	±3.2	±14.8	±0.9
All males	36	25.9	8.64	12.7	21.3	33.5	16,600	44.8	0.9	5.4	48.5	0.8
		±3.9	±1.30	±2.7	±4.8	±2.9	±4,316	±11.2	±1.6	±5.7	±12.2	±0.9
	85	26.1	8.59	12.3	21.8	33.1	16,098	46.8	0.7	4.7	47.0	0.9
		±4.1	±1.31	±2.4	±4.4	±3.4	±4,554	±10.8	±1.4	±4.5	±11.6	±0.9

*West African Dwarf breed

CHAPTER 3: EXPERIMENTAL PRODUCTION OF CONTACIOUS CAPRINE PLEUROPNEUMONIA AND DETERMINATION OF BLOOD PARAMETERS

INTRODUCTION

It is obvious from the literature review that the diagnostic value of haematologic parameters in CCPP has not been fully explored. The available data had been mainly on exotic breeds of goats, and the organism used had either been M. mycoides subsp. mycoides of caprine origin, or strains of M. mycoides subsp. capri, other than the Smith's strain.

In view of this situation, attempts have been made in this study, to evaluate the blood picture of SB goats experimentally infected with the Smith's strain of M. mycoides subsp. capri. This strain is known to produce very severe CCPP similar to what is often encountered in natural CCPP outbreak in Nigerian goats (Onoviran, personal communication). Thus, it was felt that whatever data is obtained on the haematologic parameters would be reminiscent of typical severe CCPP. Moreover, a breed of goat widely distributed in the Savannah belt of Nigeria has been used for the study.

MATERIALS AND METHODS

Goats

In the first animal experiment (Animal Experiment I), 20 Savannah Brown goats were purchased when 6 to 12 months old. Each goat was eartagged (P. 3601 - P. 3620) and dewormed with Ranide(R) (Merck & Co. Inc., Rahway, New Jersey, U. S. A.) at 7.5mg/kg body weight. They were dipped in 10% solution of Pfizona(R) (Pfizer Product Limited, Ikeja, Nigeria) to eliminate ectoparasites. The goats were confined in the same pen for further acclamatization. Rectal temperatures were monitored daily.

Hay, concentrates (in form of cotton seed cake, wheat offal and maize), and salt-lick were supplied together with water ad libitum.

In a repeat study (Animal Experiment II), 10 goats were purchased also (6 to 12 months old). They were eartagged (M. 205, M. 207, M. 216, M. 217, M. 218, M. 303, M. 308, M. 310, M. 311 and M. 312). Hay, concentrate and water were supplied to them and were also confined for acclamatization.

The goats were assigned to pens as follows:

Animal Experiment I

Goats to be inoculated with mycoplasma culture	Control goats	Standby goats
No. P. 3602	No. P. 3603	No. P. 3608
No. P. 3604	No. P. 3615	No. P. 3611
No. P. 3605	No. P. 3616	No. P. 3612
No. P. 3606	No. P. 3617	No. P. 3619
No. P. 3607	No. P. 3620	
No. P. 3609		
No. P. 3610		
No. P. 3613		
No. P. 3614		
No. P. 3618		
Total 10	5	4

Animal Experiment II

Goats to be inoculated with mycoplasma culture	Control goats
M. 216	M. 205
M. 217	M. 207
M. 218	M. 308
M. 303	M. 311
M. 310	
M. 312	
Total 6	4

Pre-inoculation blood samples

During acclamatization, two sets of blood and sera samples were taken at two-weekly intervals to establish a base-line data for the haemogram of the goats. On each occasion, 5ml of blood was collected from each goat into tubes containing heparin, and another 5ml was collected into tubes without heparin by jugular venepuncture using 10ml sterile disposable syringes and 19 gauge x 1½" sterile needles. The former samples were used for haematological studies, while serum samples were collected from the latter aseptically after clotting. The serum samples were stored at -20 C until screened for antibody to M. mycoides subsp. capri (see later).

Production of contagious caprine pleuropneumonia (CCPP)

(a) M. mycoides subsp. capri strain

Ampoules of M. mycoides subsp. capri (Smith's strain) was kindly supplied by Dr. O. Onoviran of the National Veterinary Research Institute (NVRI), Vom. These ampoules were originally supplied by Dr. G. R. Smith of Nuffield Institute of Comparative Medicine, London. It was originally isolated from

cases of CCPP in Turkey and has been proved to be a virulent strain by Cottew and Leach (1969). Ojo (1976c) and Onoviran, Majiyagbe, Molokwu, Chima and Adegboye (1984) have shown that the strain is pathogenic for Savannah Brown goats of Nigeria.

(b) Preparation of inoculum

One ampoule at a virulent passage level was reconstituted in glucose-supplemented mycoplasma broth (GB) as described by Adegboye (1981). (The composition had earlier been summarized in Chapter 2). 0.2ml of the reconstituted organism was introduced into 1.8ml GB in a 10ml screw-cap tube. This was serially diluted down ten-fold to 10^{-5} in four other tubes, similar to cultural procedures of Adegboye, Fasanya, Bwala and Hassan (1982). A medium control tube was set up and all tubes were closed tighly and incubated at 37°C. All the five dilutions showed a pH shift from 7.6 to less than 7.2 within 2-3 days with culture remaining homogenous, i.e. free of precipitates indicative of bacterial contaminants. This pH shift was indicated by the colour change in the medium from pinkish (7.6)

to yellowish (7.2) coloration. This culture represented the first passage.

0.5ml of the 10^{-2} dilution of that passage at a pH of approximately 7.0 was inoculated into universal bottle containing 4.5ml of Glucose broth to provide a 10^{-1} dilution. A 10^{-2} dilution in the same quantity was made. Both dilutions with a 5ml GB as medium control were incubated as usual. The two dilutions showed good growth by the change of the glucose broth from pink to slight yellowish coloration (pH 7.6 to 7.2) within 24-48 hours. The medium control remained pinkish. This represented the second passage in our laboratory.

From the third and final passage, each 5ml culture was inoculated into 45ml broth, to provide two bottle of 50ml culture each. 40ml fresh GB was dispensed aseptically into a third bottle as inoculum control and all three bottle were incubated at $37^{o}C$.

After 24 hours of incubation, the 50ml cultures in each flask had shown desired colour change while the medium control showed no colour change. The two 50ml cultures were pooled together and were gently mixed to produce 100ml inoculum for Animal Experiment I. Inoculum for Animal Experiment II was prepared in

the same three-passage steps using a fresh ampoule, in a different batch of broth. At each passage, the inoculum was subcultured onto agar to check for characteristic colonial morphology. The sterility of the inoculum, in terms of bacterial contaminants, was tested by inoculating one drop each of the inoculum and control broths onto blood and MacConkey agar plates. The inoculum and the control gave no bacterial growth on either medium even up to one week incubation period.

Finally, the number of colony-forming units per ml of each inoculum was determined by standard method (Rodwell and Whitcomb, 1983). Briefly, 0.1ml of the inoculum was diluted accurately down to 10^{-10}. 0.1ml of each of dilution 10^{-5}, 10^{-6} and 10^{-7} was inoculated onto mycoplasma agar plate and incubated approximately for 48 hours in glass anaerobic jar 10% carbon dioxide, humid atmosphere (Adegboye, 1981). Any dilution showing moderate number of colonies was selected; the total number was counted, and c.f.u. determined from this formula: c.f.u./ml = Nx 10x 10^{a}, where N = total colonies counted from selected dilution a. The inoculum for Animal Experiment I contained 5.2×10^{6} c.f.u./ml, while that of Animal Experiment II contained 2.0×10^{9} c.f.u./ml. The batch of media used for the second batch was unusually very good.

(c) Goat Inoculation

5ml each of the incubated inoculum control was aseptically drawn into 5 syringes in the laboratory and taken to the control pen. The rectal temperature and blood samples for "Day 0" were first taken from the control goats. Then each goat was inoculated intranasally by distributing 5ml inoculum into both nostrils in squirts as the goat inhaled with the neck held upright and stretched (Plate II).

After all control goats had been so attended to, the procedure was repeated on the 10 goats to be infected, with each goat receiving 5ml inoculum containing a total of 2.6×10^7 c.f.u. intranasally. This served as the inoculation for Animal Experiment I.

In Animal Experiment II, which was carried out 10 months after Animal Experiment I, 4 control goats also received 5 ml incubated inoculum control intranasally each on Day 0, while 6 goats received 5ml inoculum which contained a total of 1.0×10^{10} c.f.u. intranasally.

Disease monitoring

The animals were observed for clinical manifestation of disease two or three times per day and rectal temperatures were taken daily.



Plate I: A group of Savannah Brown goats just before inoculation of M. mycoides subsp. capri cultures



Plate II: The process of nasal instillation of cultures.

In Animal Experimental I, blood samples for haematology and serum were taken on days 3, 7, 10, 15, 21 and 28 post inoculation and subsequently at 2 weekly interval until termination of experiment at 17 weeks post inoculation (P.I).

In Animal Experiment II, blood samples were collected on Days 0, 1, 2, 3, 4 and 5, as all infected animals had died by day 7 post-inoculation.

Post-mortem examination

Routine post-mortem examination was carried out on all goats that died. The thoracic cavity was carefully exposed. Swabs of the trachea, lungs and pleural fluid were taken aseptically and snapped off into glucose broth for re-isolation of mycoplasma. Sections of the lungs and pleura were preserved in 10% buffered neutral formalin, sectioned at 6 microns and stained with H & E for histopathology. Photographs were taken as necessary.

In Animal Experiment I, where some goats survived into the chronic stage, all goats (including controls) above at post mortem.

Mycoplasma re-isolation and identification

Broth containing each swab was taken into the laboratory nearby. Each tube was gently shaken to disperse any exudate. 0.2ml of the suspension was diluted ten-fold down to 10^{-3} in broth and the tubes were incubated at 37°C. A drop of any dilution showing any colour change was inoculated onto glucose agar plate and incubated routinely to check for mycoplasma colonies. The plates were examined after 48 hours of incubation using stereoscopic microscopes[a] at a magnification of x 25 to x 80.

Any isolate was cloned (purified) by picking single, well isolated colonies and subculturing in broth. The procedure was repeated twice to give a pure isolation of the organism.

The cloned isolates were typed and identified by the growth-inhibition test as described by Mohan, Adegboye and Orajaka (1979). The test was carried out as follows: one drop of 10^{-4} broth culture dilution was allowed to run down the agar medium, in a 90mm petri dish and air-dried. A 6.35mm diameter paper disc impregnated with the antiserum

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a. American Optical Instrument Division, Buffalo 15, N.Y., U.S.A. or Olympus Optical Co., Tokyo, Japan.

to M. mycoides subsp. capri was placed on the inoculum. Another disc impregnated with ethanolic solution of digitonin was placed on another point on the inoculum and a third disc impregnated with normal horse serum placed lastly as a negative control. Discs were arranged to be approximately 20mm apart and the plates incubated routinely. Incubation varied from 24 hours to 72 hours at 37°C and inhibition of growth around any disc was noted. This procedure was carried out on each cloned isolate.

Serological screening of pre-inoculation sera and post-inoculation sera using the agar gel-diffusion test.

To ensure that all goats used were M. mycoides subsp. capri free, pre-inoculation serum samples were screened using the agar gel diffusion test as described by Dada, Adegboye and Mohammed (1981). The test was similarly used to demonstrate sero-conversion in infected goats.

The thoracic fluid from one of the infected animals was used as antigen. After preliminary runs, it was noted that better results were obtained if the thoracic fluid was concentrated by centrifuging

lightly (eg at 600g for 10 minutes). This antigen was added to the central wells of all the slides. Serum samples to be screened were added to the peripheral wells. Slides were set in humid chambers and they were incubated at 37°C. They were observed for development of precipitin lines at 24 hours. The antigen and antisera wells were often re-filled at 24 hours and results read again at 48 hours. This test was conducted on all pre-inoculation serum samples from the total 30 goats used in Animal Experiments I and II, and on sera collected at 3, 7, 10, 15, 21, 28 days and subsequently 2 weeks until 17 weeks, post-inoculation. As positive control, rabbit hyperimmune serum (PG3) used in growth inhibition test was included in the set up.

Haematologic Parameters

Packed Cell Volume (PCV)

This was done by the microhaematocrit capillary tube method as described by Schalm (1975).

Haemoglobin Concentration (Hb)

Determination was done by the use of cyanmethaemoglobin method using the coulter haemoglobinometer.

Blood Cell Counts (Red and White)

This was done by using the haemocytometer counting method as described by Coles (1974).

Differential Counts

This was also carried out by the use of the routine laboratory methods of counting the lymphocytes, neutrophils, eosinophils, monocytes and band cells on Giemsa stained thin blood smear (Schalm, 1975).

Total Protein

This was determined by the use of the Total solids (T.S.) refractometer method (Schalm, 1975).

All results obtained from haematologic studies were subjected to statistical analysis. Means and standard deviations were calculated and values subjected to student t-test. Values of $P<0.05$ was considered significant.

RESULTS

All the goats used in this study were serologically negative for M. mycoides subsp. capri by the gel diffusion test and were in apparent good health prior to inoculation. Their rectal temperatures monitored daily for 2 weeks prior to inoculation were within normal range.

Clinical Observation

No clinical signs were noticed in any animals on days 1 and 2 after inoculation. In Animal Experiment I, pyrexia (40°C) was recorded in all goats inoculated with the mycoplasma as early as 3 days P.I., and this persisted for approximately 6 days (Fig. 1a). The body temperature of those animals that survived the pyrexia returned to normal values by the 8th and 10th day of inoculation. In the repeat experiment (II), very high temperature rise (41°C) was recorded for the animal inoculated with mycoplasma by 72 hours post inoculation. This pyrexia persisted until death of these goats (Fig. 1b). Details of the temperature chart for the experiments are presented in Tables 4A and 4B respectively.

By the fourth day after inoculation, all the goats were anorexic, they would not come out of their housing, some had mucopurent nasal discharges (Numbers P. 3605, P. 3613 and P. 3614) while others were found coughing (Numbers P. 3604, P. 3606 and P. 3610). They were all depressed and insensitive to their environment, sluggish and dull.

Many of them showed abdominal breathing with stretched neck. Two animals (Numbers P. 3614 and P. 3618) died 5 days after inoculation. Another one (Number 3602) died a day later and these were replaced by standby goats numbers P. 3611 and P. 3619.

Auscultation of the others revealed a harsh dry rales. No other clinical signs were observed except that the infected goats were cachetic despite their good appetite at termination of experiment (Plate III).

One of the in-contact goats (Number P. 3611) had an elevated temperature (40°C) approximately 4 days after introduction to the infected pen. The other in-contact goat did not show any clinical sign.

The rectal temperatures of the control goats remained within the normal range (38-39.5°C) and none exhibited any clinical signs of illness. However,



Plate III: Chronically infected cachetic goats 17 weeks P.I.



Plate IV: Control goats 17 weeks P.I.

approximately 5 weeks post inoculation, there was flea infestation in the control pen. All the control goats and the pen were sprayed with Triatix® (The Wellcome Foundation Limited Berkhamsted, Herts, England). Even then the control goats numbers P. 3603, P. 3617 and P. 3620 died on days 35, 38 and 70 of study respectively of flea infestation. They all had anaemia and dry seborrheic dermatitis as a result of the flea infestation.

Post-mortem findings

The gross lesions observed in goats that died acutely included deposits of fibrinous exudates on the pleura and pericardium (Plate V) and about 125ml of the fibrinous fluid was collected from the thoracic cavity while about 18ml was collected from the pericardial sac (Plate VI); the fluid coagulated rapidly on exposure to air. There was consolidation of the lungs (Plate VII) and enlargement of the bronchial and mediastinal lymph nodes. There was distension of the interlobular septae with oedematous fluid giving the surface of the lung a "marbling" appearance (Plate VIII). The trachea was highly congested and it contained a lot of frothy exudate (Plate IX). The same lesions were observed for all animals inoculated with mycoplasma cultures in



Plate V: "Marbling" of the left lung (arrow) darkened, consolidated lung (forcep) of goat No. 217.

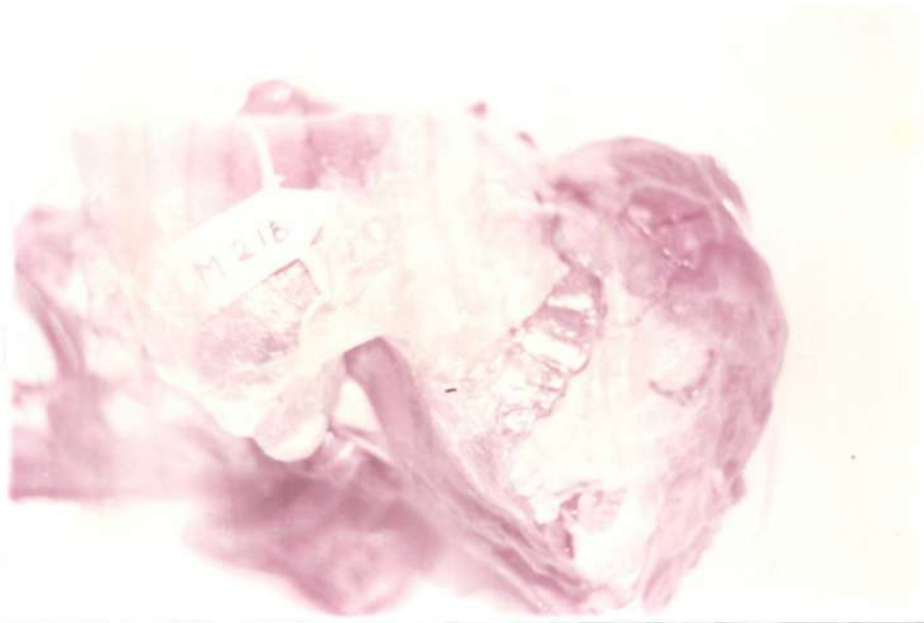


Plate VI. Severe fibrinous deposits on the viscera pleura of goat No. M. 218 that died 3 days P.I. (Animal Exp. II).

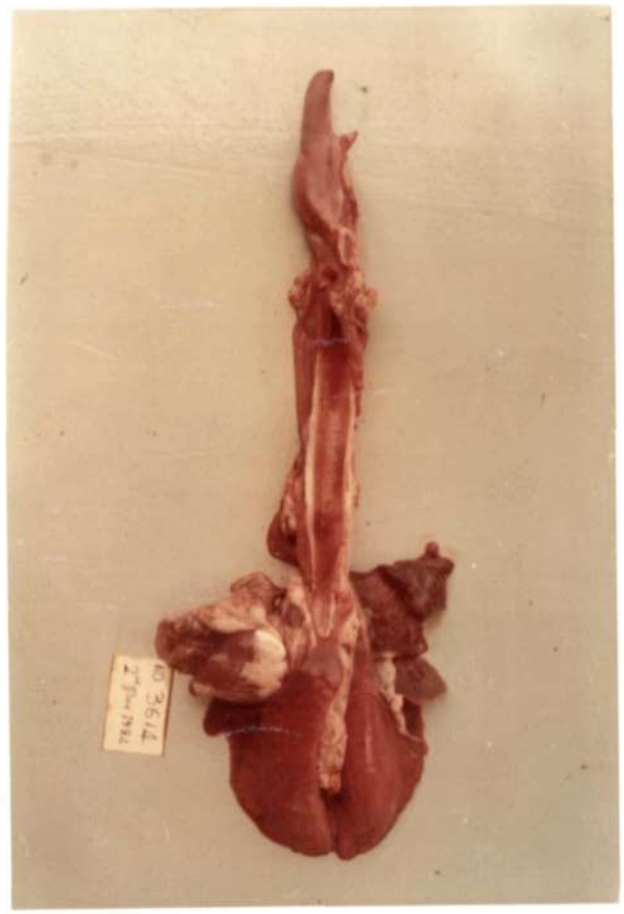


Plate VIIa: Thoracic viscera from infected goat No. P. 3614 showing consolidation of the cardiac, apical and intermediate lobes of the lung (Animal Exp. I)



Plate VIIb: Normal lung from a control goat No. P. 3615 killed 17 weeks P.I. (Animal Exp. I)



Plate VIII: Frothy fluid and petechiae haemorrhages (arrow) in the trachea of goat No. P. 303 that died 4 days P.I. (Animal Exp. II)



Plate IX: Fluid accumulation in the thoracic cavity of goat No. 217 that died days (3) P.I. (Animal Expt. II)



Plate X: Chronic adhesions of the lung to the thoracic cage and contracted lung in goat No. P. 3605 killed 17 weeks P.I. (Animal Exp. I).

Animal Experiment II.

At the termination of the experiment 17 weeks later (Animal Experiment I), the post-mortem lesions of some of the inoculated animals (numbers P. 3604, P. 3605 and P. 3607) were typical of chronic CCPP. These lesions included adhesions of the lung pleura to the thoracic cage (Plate X). Only one of the in-contact goats (P. 3611) showed consolidated points on the right lung.

Histopathological findings

The lesions observed in the lungs in the acute disease included congestion of blood vessels, dilation of the lymphatic vessels in the interlobular septa, neutrophilic infilteration of inter-alveolar septae, bronchioles and the alveoli. Few lymphocytes and plasma cells were seen. There was oedema fluid and fibrin accumulation in the interalveolar septae, alveli and bronchiolar lumen (Plates IX and XII).

The portions of the lungs that showed chronic lesions had mononuclear (lymphocyte and macrophage) infilteration, and obliteration of some alveoli and bronchioles with fibrous connective tissues.

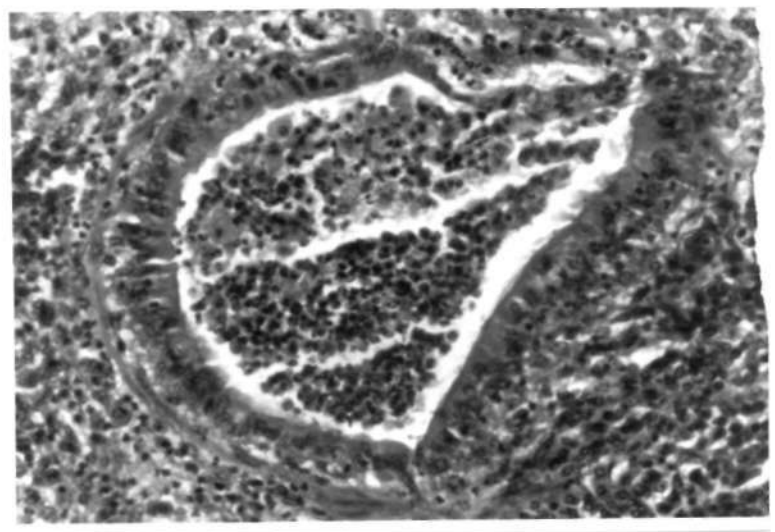


Plate XI: Histological section of a bronchiole showing cellular and fibrin accumulation in the lumen, and in the peribronchiolar Area x400 H & E stain

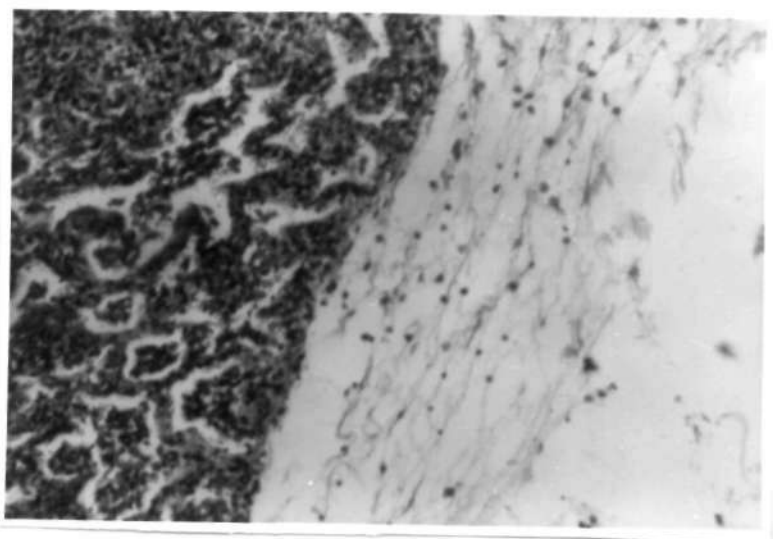


Plate XII: Histological section of cellular infiltration into the alveoli, inter septal hyperplasia and fibrin within the interlobular septa. x 400. H & E Stain

Mycoplasma re-isolation and identification

A mycoplasma confirmed as M. mycoides subsp. capri (Plates XIII and XIV) by growth inhibition test was re-isolated from the trachea and lungs of goat numbers P. 3602, P. 3614 and P. 3618 that died acutely in Animal Experiment I, and in all animals inoculated with mycoplasma culture in Animal Experiment II.

At the termination of experiment, M. mycoides subsp. capri was also recovered from the trachea of goats numbers P. 3604, P. 3605, P. 3607 and the in-contact goat P. 3611. The organism was not recovered from the lungs at this stage.

None of the control goats had lesions in the respiratory tract at post-mortem so no mycoplasma was recovered from the trachea or lungs.

Agar gel diffusion test

A positive reaction to this test is indicated by a precipitin line formation between the serum well and the antigen well.

All the pre-inoculation and week 1 post-inoculation sera samples of all the goats were negative to the test. However, some positive reactions were obtained

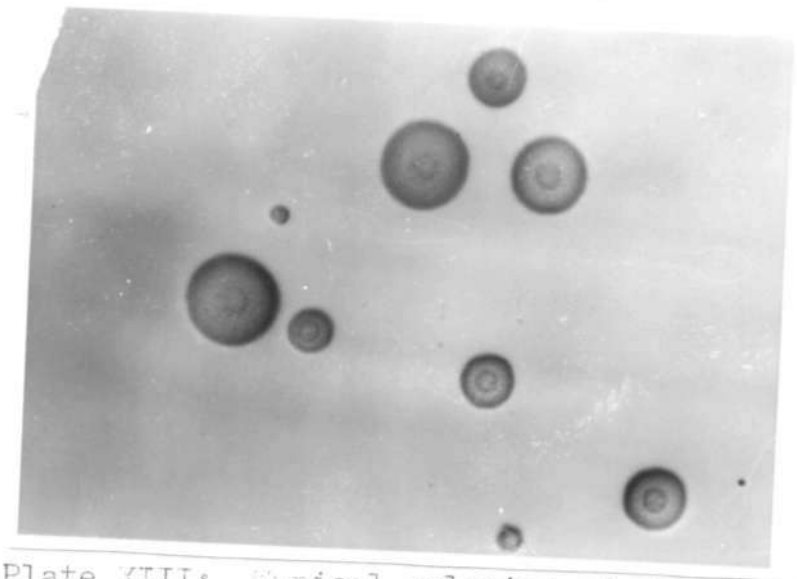


Plate XIII: Typical colonies of M. mycoides subsp. capri (Smith's strain). x 400

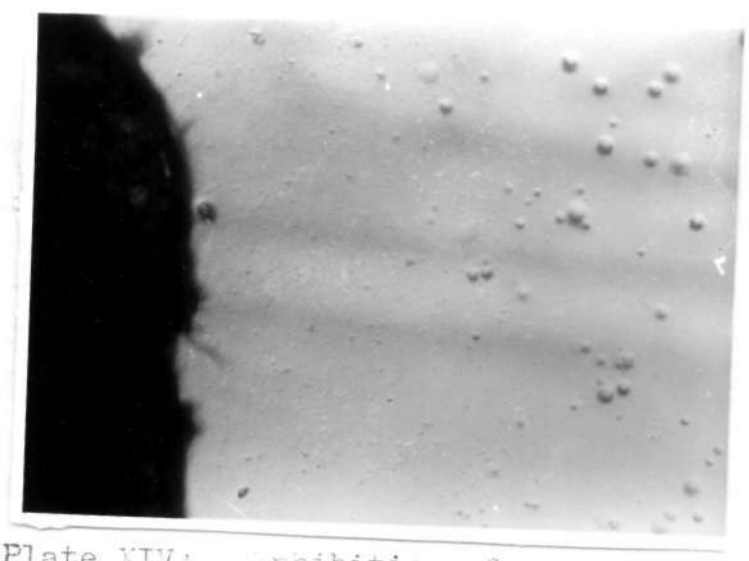


Plate XIV: Inhibition of a mycoplasma re-isolated from an infected goat by a paper disc impregnated in hyperimmune serum against M. mycoides subsp. capri. x 100.

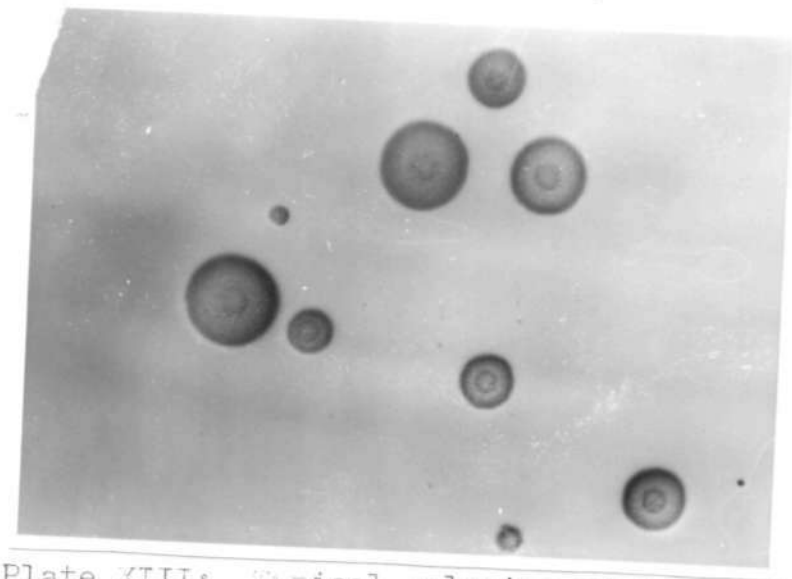


Plate XIII: Typical colonies of M. mycoides subsp. capri (Smith's strain). x 400

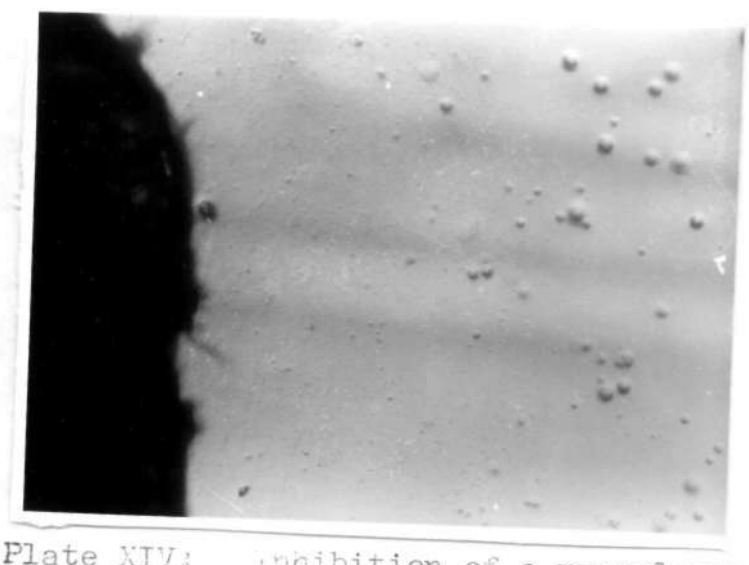


Plate XIV: Inhibition of a mycoplasma re-isolated from an infected goat by a paper disc impregnated in hyperimmune serum against M. mycoides subsp. capri. x 100.

with serum samples obtained 2 weeks (14 days) and 3 weeks (21 days) after inoculation in all the infected goats (P. 3604, P. 3605, P. 3606, P. 3607, P. 3609 P. 3610). The precipitin lines where more distinct at 48 hours of incubation as the wells were re-filled at 24 hours. At week 4 (28 days P.I.), only sera from goats P. 3606 and P. 3616 were positive. As from week 5 (35 days) up to 17 weeks none of the animals showed positive reactions.

Haematological Parameters

Packed Cell Volume, PCV (%)

The data obtained in Animal Experiments I and II are summarized in Figures 2A and 2B respectively, and the detailed data are presented in Tables 5A and 5B respectively. The student t-test that was utilized to test the difference in means ($P>0.05$). This shows that the PCV is not significantly affected by CCPP.

Haemoglobin concentration (gram/deciliure)

The data for the haemoglobin in Animal Experiments I and II are summarized in figures 2A and 2B respectively, and the details of the data are shown in Tables 6A and

6B respectively. There is no significant difference between the means of the control and infected goats ($P>0.05$) and this shows that the haemoglobin concentration is not significantly affected by CCPP.

Red Blood Cell Counts (10^6/microlitre)

The data obtained for the RBC counts in Animal Experiments I and II are summarized in Figures 2A and 2B respectively; and the details in Table 7A and 7B respectively. There was no significant ($P>0.05$) changes in the RBC with CCPP.

Total White Blood Cell Counts (10^3/microlitre)

The mean total white blood cell counts for Animal Experiments I and II are summarized in Figures 3A and 3B respectively; the detailed results are presented in Tables 8A and 8B respectively. There was a significant increase ($P<0.001$) in the total WBC counts within 3 days P.I., and this rise reached a peak at about day 7 P.I. (Animal Experiment I)

In the repeat experiment (Animal Experiment II), there was a significant rise in WBC counts ($P<0.001$) within 3 days of inoculation. This persisted until all the animals inoculated with mycoplasma culture

died. Thus, the disease is accompanied by leucocytosis during the first week, after which the WBC gradually decreased to the pre-inoculation value.

Neutrophil Counts (10^3/microlitre)

The data obtained for Animal Experiments I & II are summarized in Figures 3A and 3B respectively, and the details in tables 9A and 9B respectively. There was a significant rise ($P<0.001$) in the neutrophil counts within 3 to 7 days P.I., and a decline thereafterin both experiments. Thus the inoculation with M. mycoides susbsp. capri caused a neutrophilia, which might be responsible for the leucocytosis.

Band Counts (10^2/microlitre)

The data obtained for the two experiments I and II are as summarized in figures 3A and 3B, and the details in Tables 9A and 9B respectively. There was a significant increase ($P<0.05$) in the first week of inoculation. This rise in band count indicates a regenerative left shift with CCPP. It might also be contributing to the leucocytosis in both experiments.

Monocyte Counts per microlitre

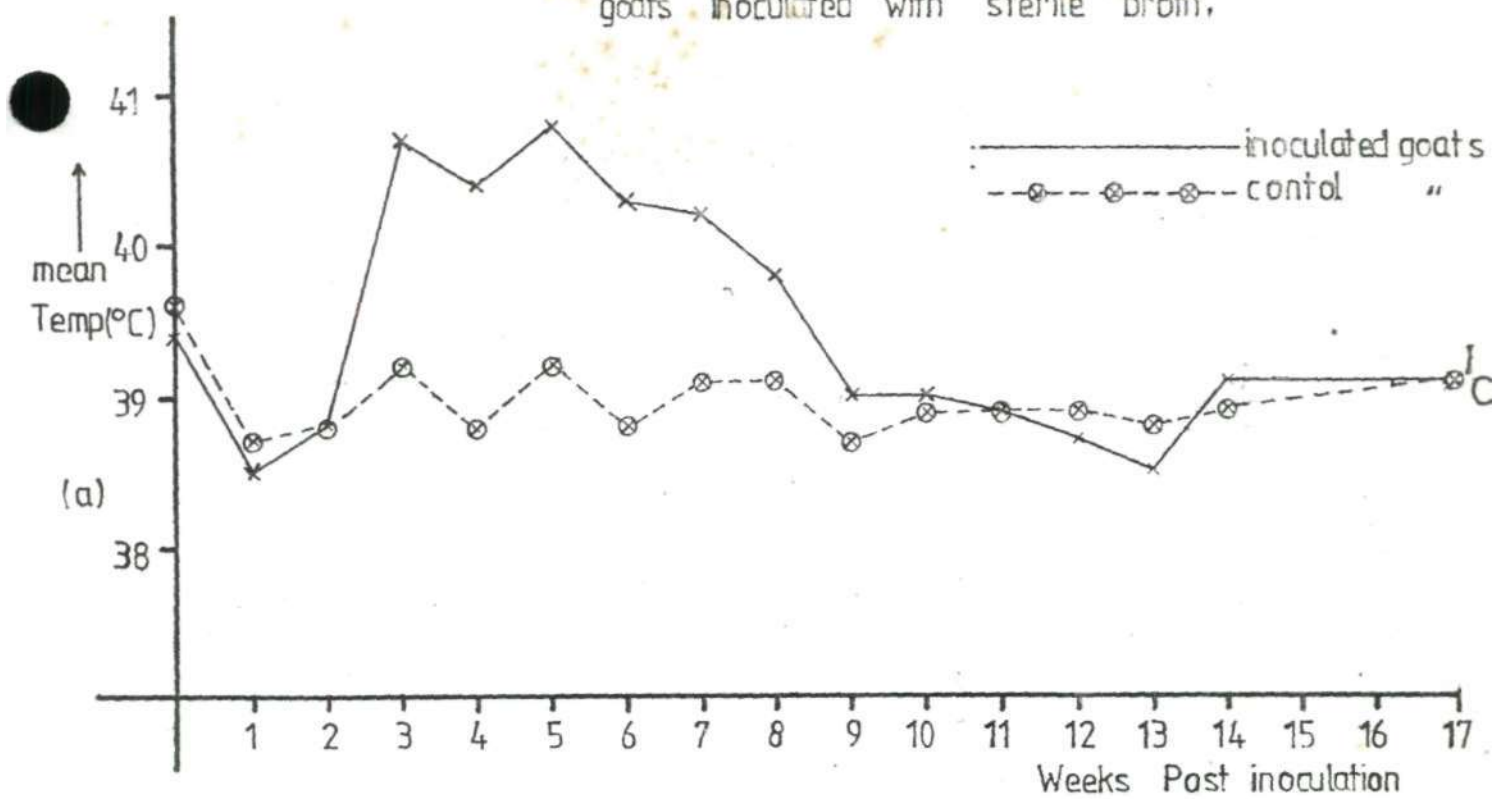
The data for Animal Experiments I and II are represented in Figures 4A and 4B respectively, and, the detailed results are presented in Tables 13A and 13B. The results show a significant increase ($P<0.05$) at the termination of the experiment only. This might be attributed to those animals that showed chronic lesions (Animal Experiment I). There was no significant increase ($P>0.05$) in Animal Experiment II, since the infection was highly acute.

Total Protein (gram/decilitre)

The data obtained for the total protein in Animal Experiments I and II are presented in Figure 5, and the detailed results in Tables 14A and 14B. There is no significant ($P>0.05$) alteration in the values of total protein in CCPP.

FIG 1.

ANIMAL EXPERIMENT I: Rectal temperature chart of Savannah Brown goats which were each inoculated intranasally with cultures containing approximately 2.6×10^7 cfu of *Mycoplasma mycoides* subsp *capri* (Smiths strain) and control goats inoculated with sterile broth.



ANIMAL EXPERIMENT II: Rectal temperature chart of Savannah Brown goats which were each inoculated intranasally with cultures containing approximately 1.0×10^6 cfu of *Mycoplasma mycoides* subsp *capri* Smiths strain and control goats inoculated with sterile broth.

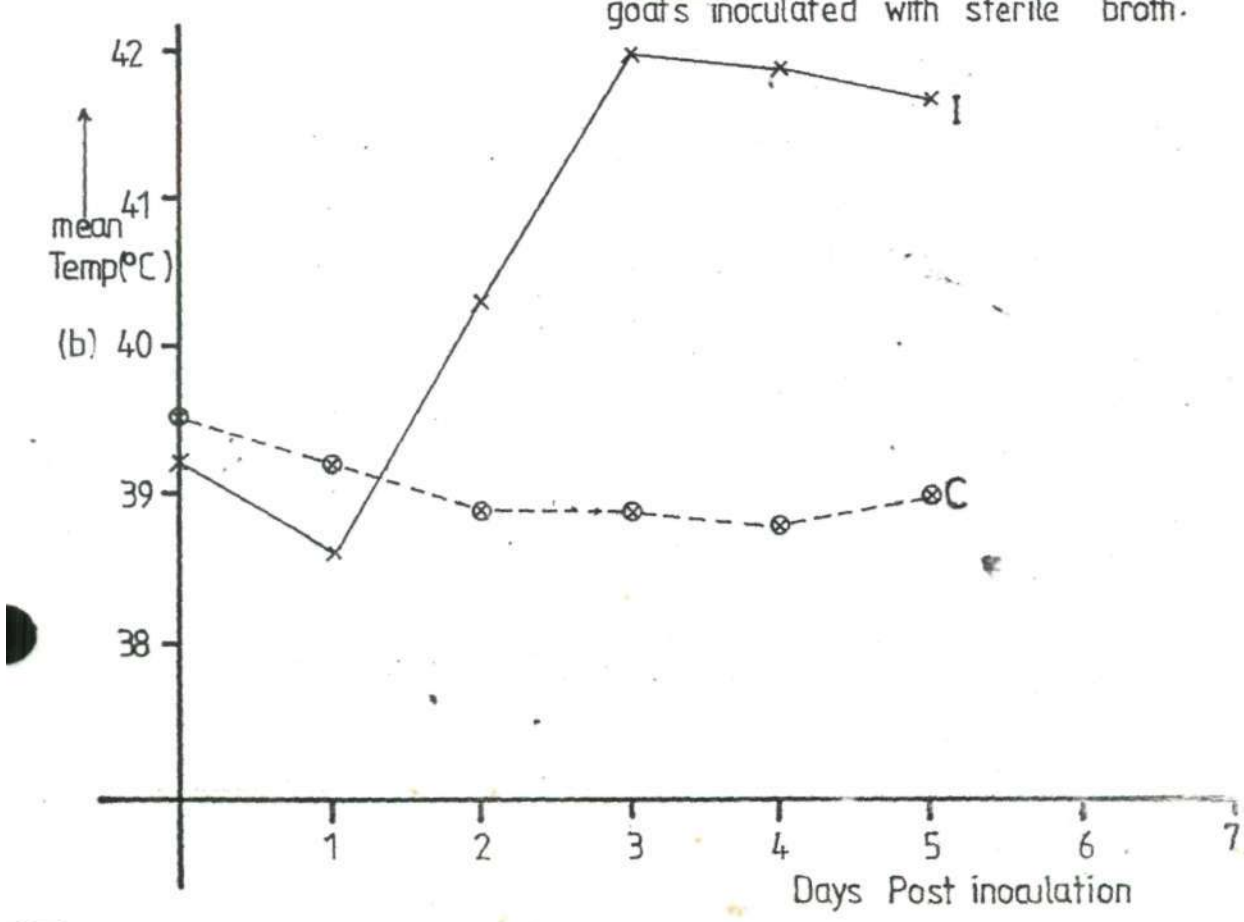


FIG 2A

ANIMAL EXPERIMENT I: The mean PCV, Hb and total RBC values of Savannah Brown goats which were each inoculated intranasally with cultures containing approximately 2.6×10^7 cfu of *Mycoplasma mycoides* subsp *mycoides* (Smiths strain) and control goats inoculated with sterile broth.

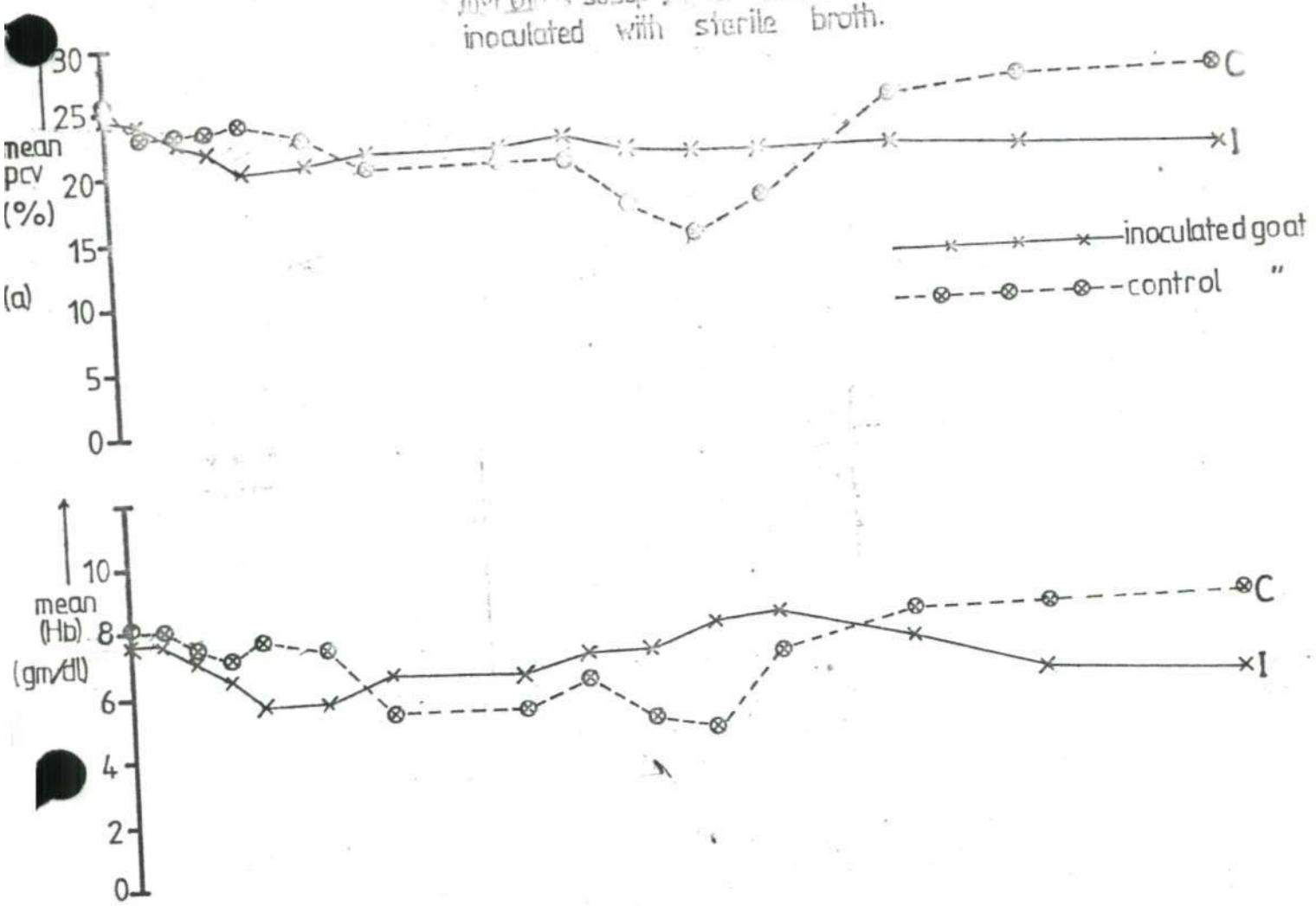


FIG 2B

ANIMAL EXPERIMENT II The mean PCV, Hb and total RBC values of Savannah Brown goats which were inoculated intranasally with cultures containing approximately 10×10^{8} cfu of *Mycoplasma mycoides* subsp *capri* (Smith's strain) and control goats inoculated with sterile broth.

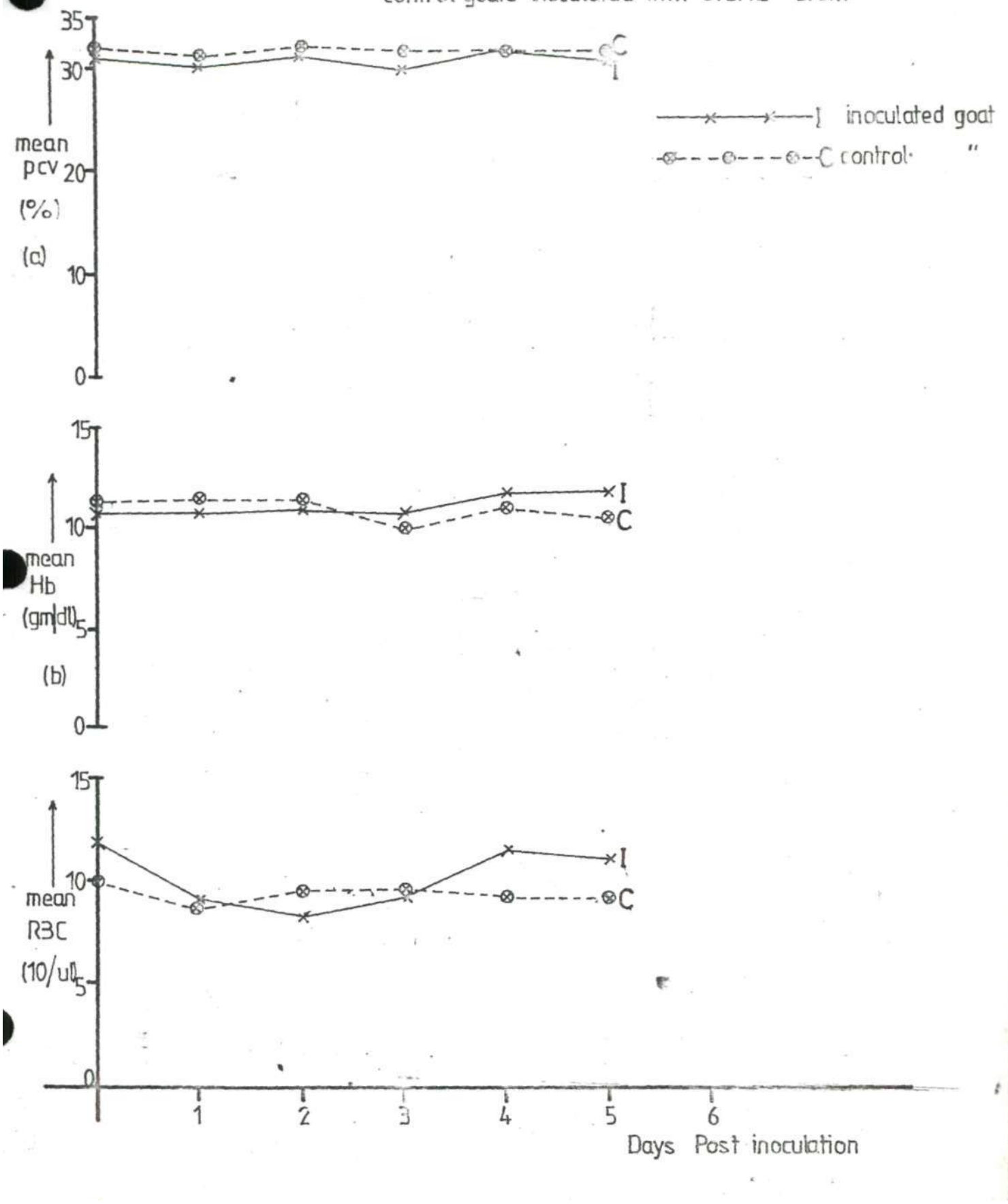


FIG 3A

ANIMAL EXPERIMENT I The mean WBC, Neutrophils and Band counts in Savannah Brown goats inoculated each intranasally with cultures containing approximately 2.6×10^7 cfu of Mycoplasma mycoides subsp capri (Smiths strain) and control goats inoculated with sterile broth.

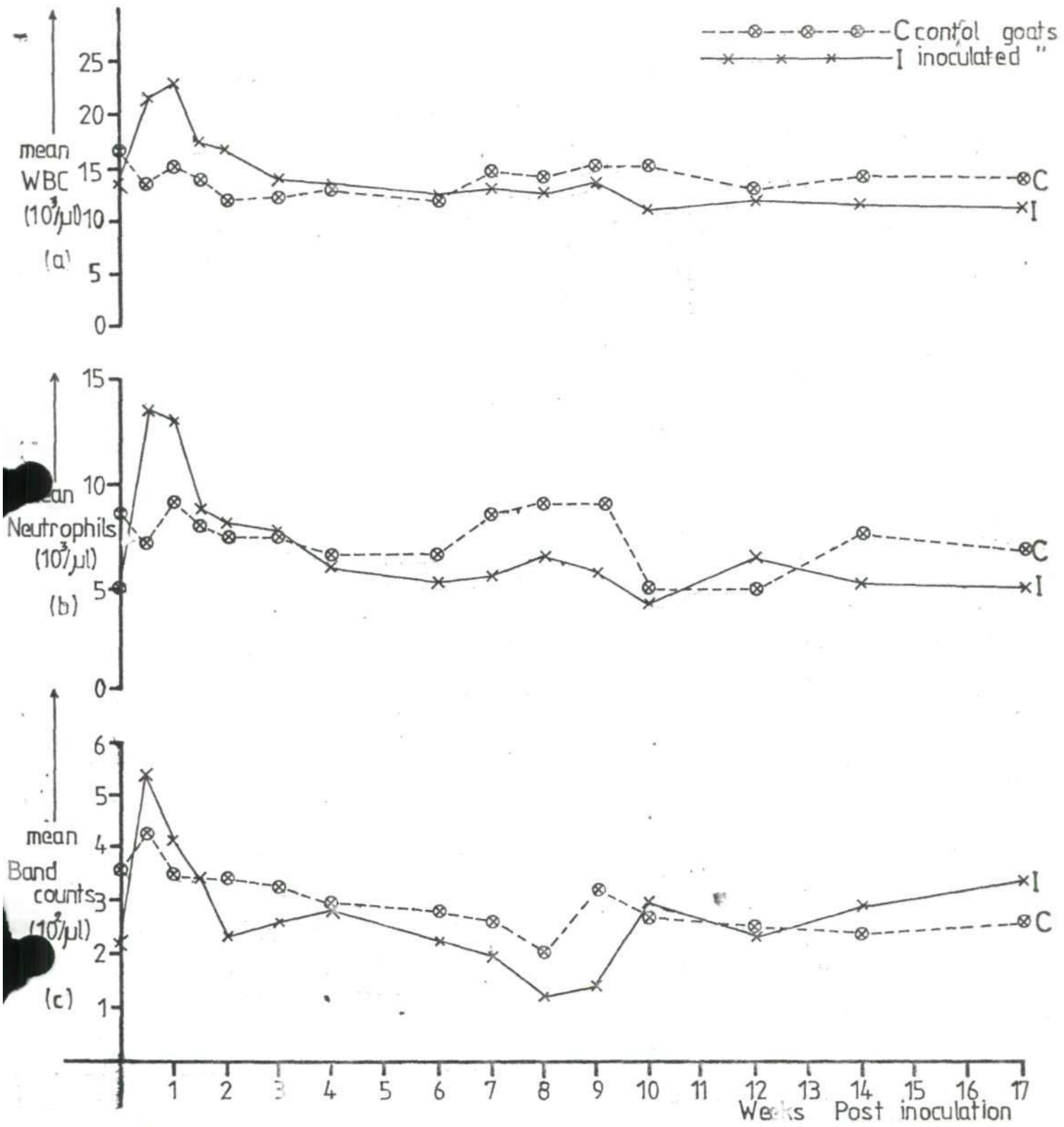


FIG 3B

ANIMAL EXPERIMENT II The mean WBC, Neutrophils and Band counts in Savannah Brown goats each inoculated intranasally with cultures containing approximately 1.0×10^{10} cfu of Mycoplasma mycoides subsp capri (Smiths strain) and control goats inoculated with sterile broth.

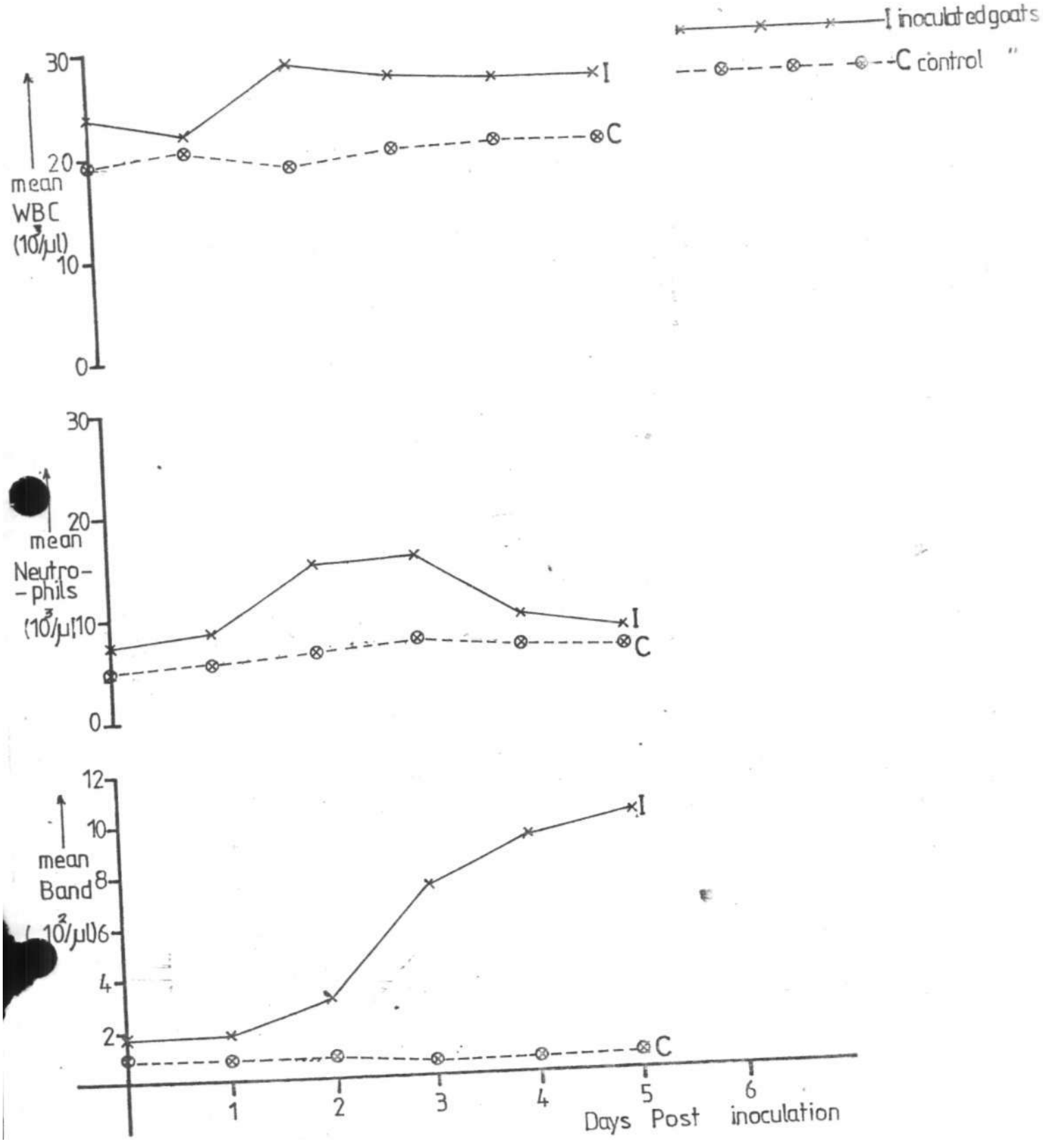


FIG 4A

ANIMAL EXPERIMENT I The mean Lymphocyte, Monocyte, Eosinophil counts in Savannah Brown goats which were each inoculated intranasally with cultures containing approximately 26×10^7 cfu Mycoplasma mycoides subsp capri (Smiths strain) and control goats inoculated with sterile broth.

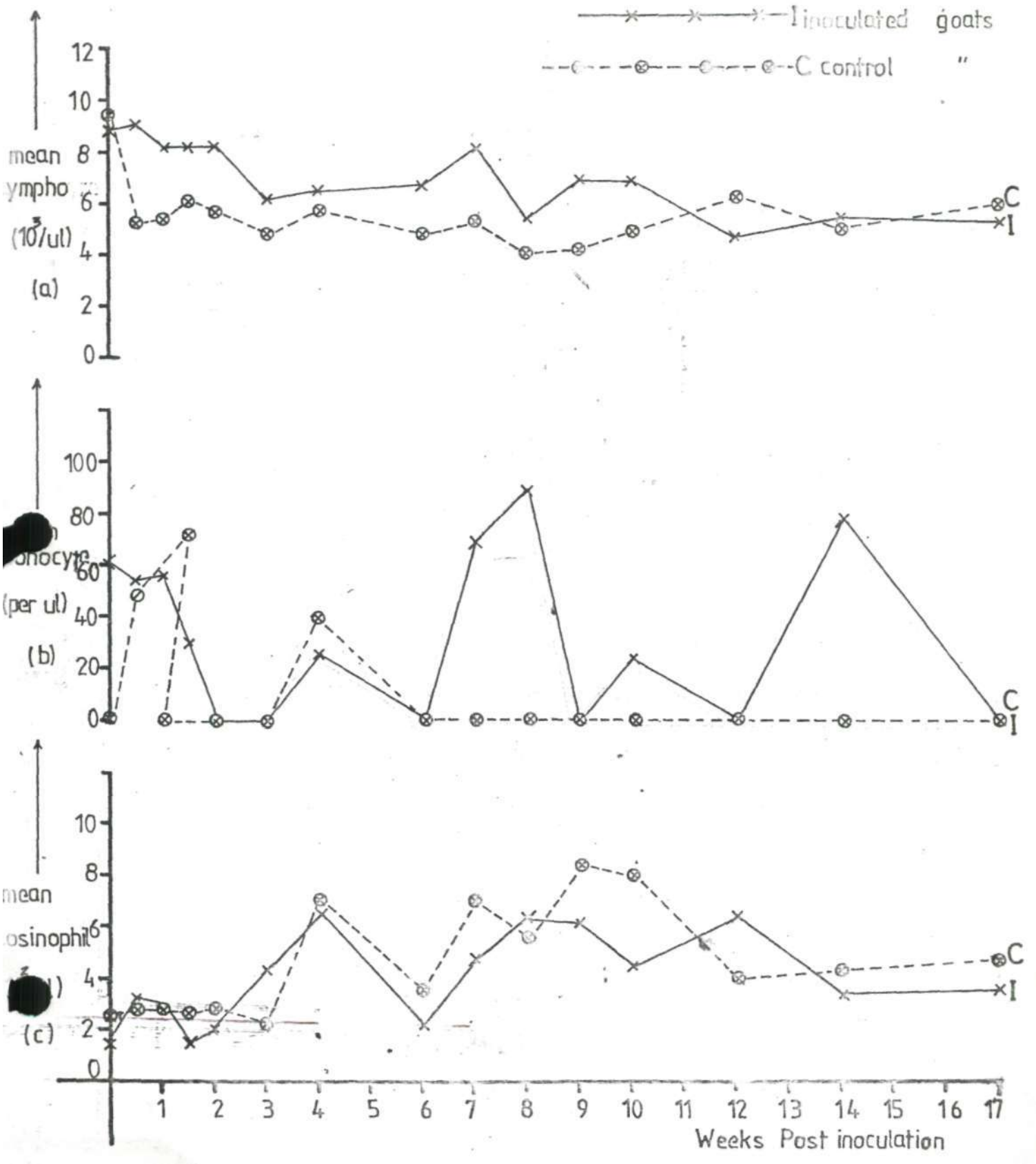


FIG 4B

ANIMAL EXPERIMENT II The mean Lymphocyte, Monocyte, and Eosinophil counts in Savannah Brown goats which were each inoculated intranasally with cultures containing approximately 10×10^{9} cfu of Mycoplasma mycoides subsp capri (Smiths strain) and control goats inoculated with sterile broth.

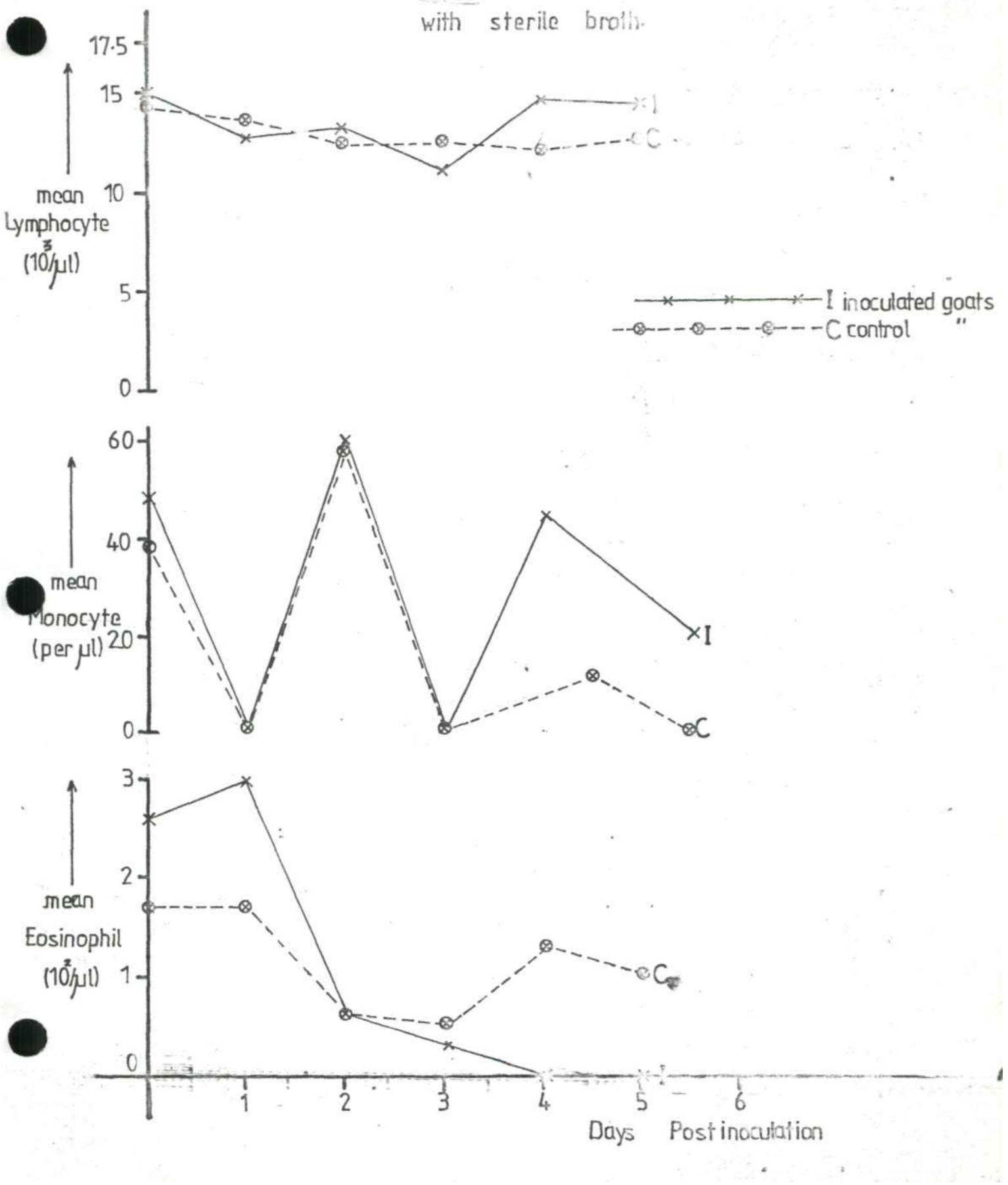
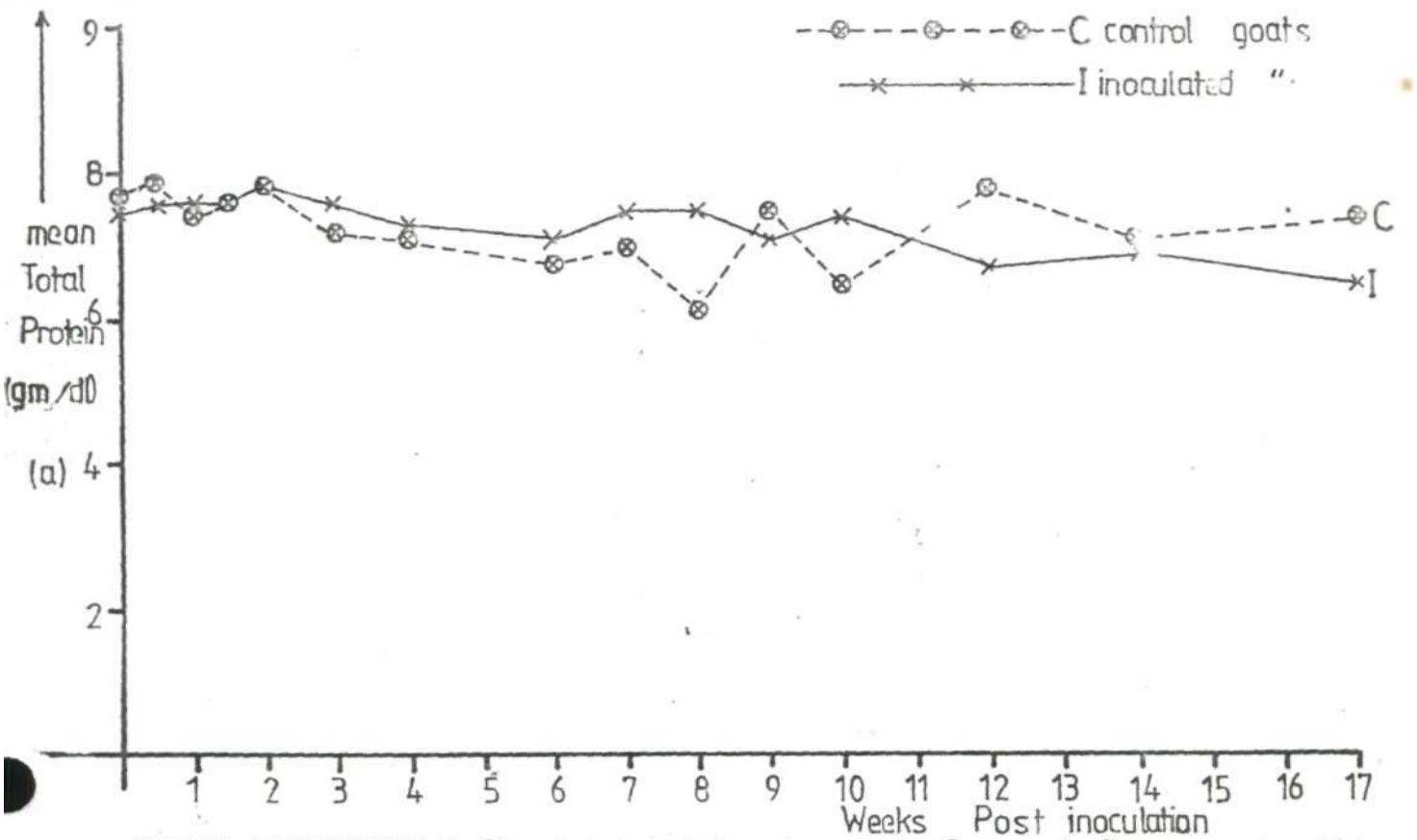
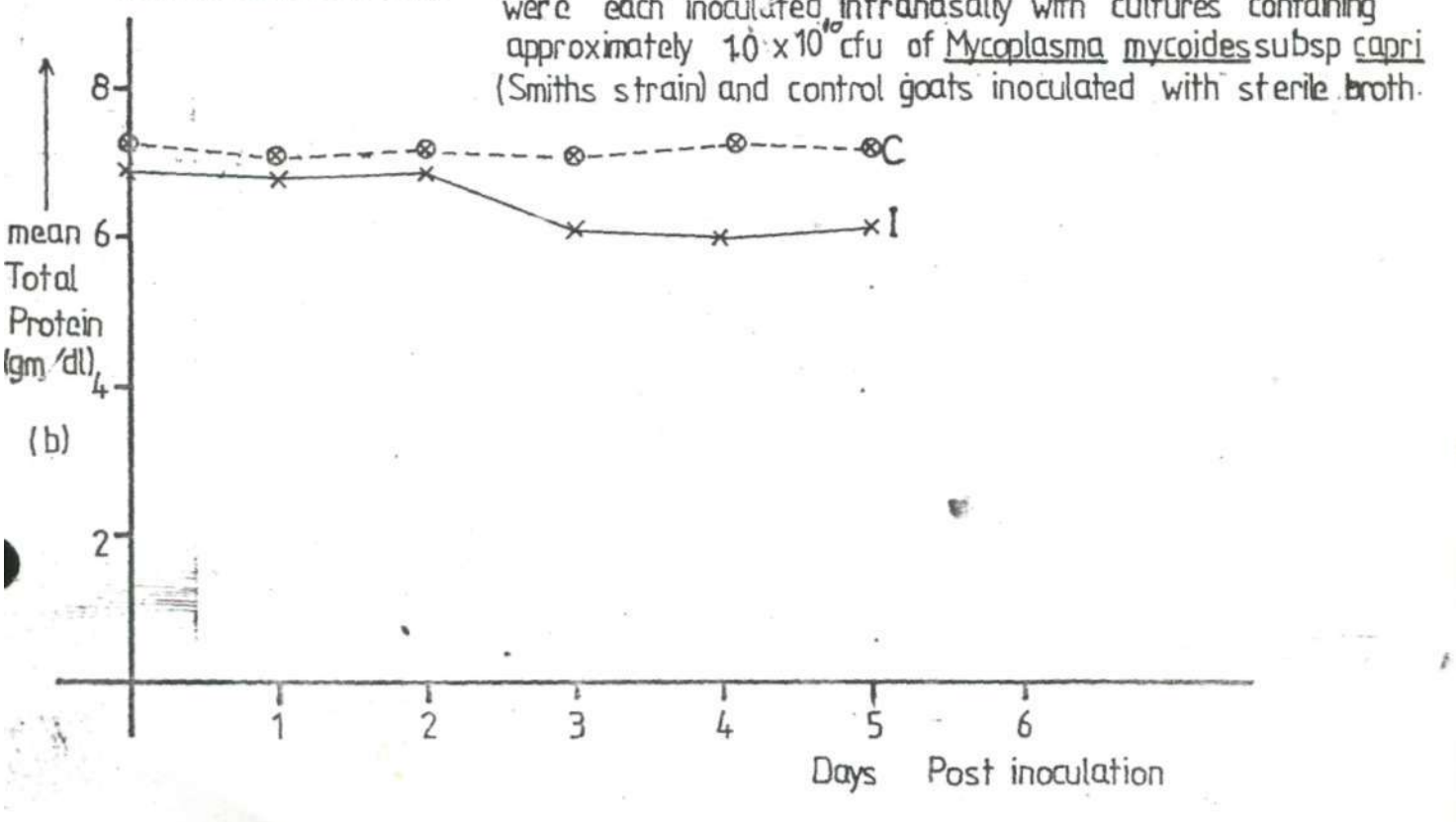


FIG 5

ANIMAL EXPERIMENT I The total protein values of Savannah Brown goats which were each inoculated intranasally with cultures containing approx $2 \cdot 6 \times 10^7$ cfu of Mycoplasma mycoides subsp capri (Smithis strain) and control goats inoculated with sterile broth.



ANIMAL EXPERIMENT II The total protein values of Savannah Brown goats which were each inoculated intranasally with cultures containing approximately $1 \cdot 0 \times 10^{10}$ cfu of Mycoplasma mycoides subsp capri (Smiths strain) and control goats inoculated with sterile broth.



DISCUSSION

The clinico-pathological response of the Savannah Brown goats used in this study to Smith's strain of M. mycoides subsp. capri agrees with the picture of CCPP reported by Goni and Onoviran (1972) and Ojo (1976c), as well as the general textbook descriptions of natural cases (Cottew and Leach, 1969; Cottew, 1970; Cottew, 1979). In the study, Koch's postulate was fulfilled in that acclamatized and apparently healthy goats that were previously free of disease (serologically-free) developed a disease from which the agent introduced was re-isolated in pure culture. There was therefore no doubt that any haematological change detected in any goat post-inoculation was as a result of the disease that was induced. The pyrexia and cough observed in one of the in-contact animals (P. 3611) 4 days after its introduction into the infected pen indicated transmission by aerosol means and herding, and the re-isolation of mycoplasmas from this animal together with the infected animals that died acutely also confirm actual transmission of the disease. The inability to re-isolate the organisms from some of the recovered animals indicates recovery from infection, as was also reported by Curasson

(1946). It seems therefore that goats which recovered fully from CCPP may not be carriers. This is logical since the sequestrum often seen in chronic cases of contagious bovine pleuropneumonia is not a characteristic of CCPP (Longley, 1951).

The inhibitory zones shown around the paper disc soaked in the M. capri hyperimmune serum in the growth inhibition test depicts the specific organism being used in the experiments, and this also fulfils Koch's postulates because: (1) the organism was found in association with the lesions in all cases of the diseased animals; (2) it was a pure culture of the CCPP organism; (3) there was seroconversion during the course of the disease as shown by the gel diffusion test results.

Besides the study has shown the diagnostic value of thoracic fluid harvested from acute CCPP cases. It contains enough organism, especially when slightly concentrated by spinning, to be able to form precipitin lines with either hyperimmune serum or serum from acutely affected animals. It was better than lung homogenate which was found to be invariably blood-tinged, masking the precipitin lines in the gel.

None of the animals used was refractory to the disease unlike what was observed in the studies of Goni and Onoviran (1972). All infected goats in Animal Experiments I and II had elevated rectal temperature from approximately 48 to 72 hours post-inoculation and showed sero-conversion. The goats that actually died acutely must have been overwhelmed by the disease while those that survived were able to withstand the infection. The 100% mortality in the second experiment as against the 30% mortality in the first, is most likely due to a difference in the number of mycoplasmas contained in each inoculum. Animal Experiment I goats received a total of approximately 2.6×10^7 c.f.u. of Mycoplasma mycoides subsp. capri while those of Animal Experiment II received approximately 1.0×10^{10} c.f.u. The batch of the media used in Animal Experiment II was apparently better than that used in Animal Experiment I.

The demonstration of typical chronic lung lesions in some recovered animals (P. 3605 and P. 3607) at 17 weeks post inoculation showed that not all acutely affected animals died. The chronically affected goats were cachetic, despite their good appetite, as also previously reported (Ojo, 1983).

The haematological data hereby presented is that of CCPP induced in Savannah Brown goats with Smith's strain of M. mycoides subsp. capri, a highly virulent strain. Previous similar reports have either been on un-named mycoplasma (Gameel, 1976), or on M. mycoides subsp. mycoides of caprine origin (Rosendal, 1981, 1983; East et al, 1983), or on M. mycoides subsp. capri (Smith's strain) and M. mycoides mycoides of caprine origin (Ojo, 1976c). While the latter study, one of the earliest study of this kind in Nigeria, showed no significant haematological changes in infected goats, Gameel (1976) and Rosendal (1981, 1983) indicated that certain parameters may be affected in infections caused by the strains they used as earlier reviewed. These latter studies gave the impetus for this work. The Smith's strain used produced very severe CCPP, with mortality of 100% in one study. Thus, whatever data has been obtained reflects blood picture of typically severe CCPP.

The results on the PCV, haemoglobin, and total erythrocyte counts agree with those of Rosendal (1981, 1983) and Ojo (1976c) as there were no significant differences in the means of these parameters in infected and control goats. Thus, it appears that

M. mycoides capri and M. mycoides mycoides of caprine origin do not adversely affect the erythrocytic series. This is logical, since these mycoplasmas are not particularly associated with haemagglutinating properties, unlike the avian mycoplasmas M. gallisepticum, M. synoviae and M. meleagridis or the human pathogen M. pneumoniae (Adegboye, personal communication). It might be interesting to carry out similar studies on diseases caused by these mycoplasmas.

A different result was obtained for the leucocytic series. While Rosendal (1981, 1983) recorded a leucocytopenia and granulocytopenia, the present work showed a leucocytosis (during the acute or per-acute stage of infection) that was statistically highly significant ($P<0.001$). There was a steady rise in leucocyte level as from the second day of infection and a peak was reached at approximately 1 week. Thereafter the level gradually declined. This observation is similar to the report of Gameel (1976) and East et al (1983). The leucocytosis was due to a neutrophilia and increased number of band neutrophils indicating a regenerative left shift. By curiosity, thoracic fluids from goats that died acutely were spun down and the sediment examined.

The sediment contained high concentration of neutrophils which has swollen nuclei indicative of degenerative changes.

Although the appearance of monocytes in the peripheral circulation of normal animal could be erratic, the overall summation and means among the infected and control goats in this work showed a significant difference (P 0.05) in the Animal Experiment I. There was no significant difference in this parameter in the second experiment which terminated acutely. This can be explained by the fact that the first set of animals developed the chronic form of the disease thus requiring these large cells for defence, whereas there was no such time lapse in the second experiment. Monocytosis was also reported by East et al (1983).

As for the lymphocyte values, although there was no significant difference in the means of the control (5,073 $\pm$ 0.97) and infected (6,734 $\pm$ 1.24) goats, (P 0.05), the values in the infected goats was persistently higher than those of control goats up to the 10th week of infection. This apparent lymphocytosis in the infected animals may be related to defence mechanisms. Lymphocytosis was also reported by Gamell (1976). By the 17th week of infection, the values were 5,227 $\pm$ 1.42 and 5,874 $\pm$ 1.9 in the

infected and the control goats respectively. In the repeat experiment lasting for one week, there was no difference in the means of the lymphocyte counts of infected and control goats, possibly for the same reason that monocytosis was not observed.

The eosinophil levels generally were unaffected by CCPP. This observation also agrees with Gameel (1976).

The total protein was not significantly affected by the disease in spite of the exudative inflammation. The values were 7.4 $\pm$ 0.4 and 7.3 $\pm$ 0.5 for the infected and control goats respectively (Animal Experiment I). This is rather surprising because one would expect a low blood protein level in infected goats considering the massive fibrinous deposition within the thoracic cavity. Perhaps the host has a special way of rapidly re-adjusting to this obvious pathology of the blood protein. Fibrin is composed primarily of protein. This accounts for its coagulation on exposure to air as was reported earlier.

Finally, it is felt that the few differences in the haematologic records of various workers depend on the species or strain of the mycoplasma

used, the breed of goat and the severity of the disease produced. It is also felt that this type of study should be encouraged in mycoplasmology as it could provide additional diagnostic criteria and lead to further understanding of pathogenic and body defence mechanisms in mycoplasma infections.

SUMMARY AND CONCLUSION

The main objective of this work was to establish haematological data of CCPP caused in Savannah Brown goats by the highly virulent Smith's strain of M. mycoides subsp. capri. The study has been carried out within the limits of financial and material resources available.

The literature search and the results of the work have shown that goat blood responds to contagious pleuropneumonia. The data obtained agrees with some previous data and differs with others. Where differences occur, these might be due to the species or host involved, and the severity of the disease itself.

The haematological data can supplement criteria for ante-mortem diagnosis of CCPP caused by the classical strain of M. mycoides subsp. capri, i.e. when coupled with the already known clinical signs and lesions produced by the disease. During peracute to acute stage of the disease, there is a leucocytosis caused by a neutrophilia with regenerative left shift. During the chronic stage there is a monocytosis with relative lymphocytosis, even though these changes were mild compared with the neutrophilia of the

peracute/acute stage. Erythrocytes appear unaffected by the disease.

More work is indicated on:

(1) Haematology of mycoplasmosis in other species of animals, especially with Mycoplasma species that have haemagglutinating properties.

(2) Current status of CCPP in Nigeria, as the disease does not appear to be a rampant as before.

(3) Possible spread of the disease by direct contact. This should be done in order to clarify whether the disease caused by M. mycoides subsp. capri (Smith's strain) is contagious or not, in view of the reports of MacOwan and Minette (1977) on Mycoplasma strain F38.

(4) The use of thoracic fluid in agar gel diffusion test for ante-mortem diagnosis of the disease.

REFERENCES

Adegboye, D. S. (1981), "Outline of techniques for isolation and identification of Mycoplasma". Lecture Note delivered at the School of Veterinary Medicine, Kansas State University, Kansas, U. S. A.

Adegboye, D. S., Fasanya, O. O. A., Bwala, M. S., and Hassan, M. K. (1982, "Preliminary studies on bovine urogenital mycoplasmosis in Nigeria". Bulletin of Animal Health and Production in Africa. 30(2); 95-99.

Akerejcla, C., Schillhorn, van Veen, T. W., and Njoku, C. O. (1979), "Ovine and Caprine Diseases in Nigeria: A review of economic losses". Bulletin of Animal Health and Production in Africa. 27(1); 65 - 70.

Anon, (1966). FAO on Agricultural development in Nigeria. 1965 to 1980.

Bar-Moshe, B., Rapapport E. (1981), "Observations on M. mycoides subsp. mycoides infection in Saanen Goats". Israel Journal of Medical Science. 17(7); 537-539.

Beaton, W. G. (1928), Annual Report, 1927: Department of Veterinary Services, Nigeria; Pg. 52-53.

Beaton, W. G. (1931), "Disease of goats in Nigeria. II. Notes on a fatal septicaemic infection". Journal of Comparative Pathology and Therapeutics, 44; 192-201.

Beaton, W. G. (1937), Annual Report, 1937. Department of Veterinary Services; Pg. 3.

Bordet, J. (1910), "La morphologie due microbe de la peri pneumomie des bovides". Annale des Institutc Pasteur (Paris), 24; 161-167.

Bridre, J. and Donatien, A. (1923), Le Microbe de l'agal axie contagieuse et sal culture in vitro. Comptes Rendus de l'Academic des Sciences. 177; 841-843,

Chen, T. R. (1977), "In situ detection of Mycoplasma contamination in cell cultures by fluorescent Hoeschst 33258 strain". Experimental Cellular Research. 104; 255-262.

Cherry, J. D. and Taylor-Robinson, D. (1970), "Growth and pathogenesis of Mycoplasma mycoides var. capri in chicken embryo tracheal organ cultures". Infection Immunology 2; 431-438.

Clay, A. L. and Lloyd, L. C. (1975), "The eradication of contagious bovine pleuropneumonia from Australia". Bulletin de l'office internationale des Epizooties. 84, 4634475.

Coles, E. H. (1974). Veterinary Clinical Pathology. In: W. B. Saunders Company. Philadelphia, London, Toronto; Pg 40-148.

Cottew, G. S. (1970), "Diseases of sheep and goats caused by Mycoplasma". In: The Role of Mycoplasmas and L-forms of bacteria in Diseases. Charles C. Thomas. Springfield Illinois. Pg. 198-211.

Cottew, G. S. (1979), "Caprine-Ovine Mycoplasmas". In: Tully J. G. and Whitcombs, R. S. (ed.) The Mycoplasmas, II. Human and Animal Mycoplasmas. New York, Academic Press Inc. Pg. 103-132.

Cottew, G. S. "Significance of Mycoplasmoses in Goats". Proceeding of the 3rd International Conference on Goat Production and Disease. Tucson, Arizona, U. S. A. January 10-15, 1982. Pg. 221-225.

Cottew, G. S. and Leach, R. H. (1969), "Mycoplasmas of cattle, sheep and goats. In: Hayflick, L. (ed.) The Mycoplasmatales and the L-phase of bacteria. New York, Appleton-Century Crofts. Page 552-569.

Cottew, G. S. and Yeats,,F. R. (1978), "Subdivision of Mycoplasma mycoides subsp. mycoides from cattle and goats into two types". Australian Veterinary Journal, 54; 293-296.

Curasson, G. (1946), Maladies Infectiouses des animaux domestiques. 1, Paris, Vigot.

Dada, B. J. O., Adegboye, D. S. and Mohammed, A. N. (1981), "Experience in Northen Nigeria with countercurrent immunoelectrophesis, double diffusion and indirect haemagglutination tests for diagnosis of hydatid cyst in Camels". Journal of Helminthology, 55(3); 197-202.

DaMassa, A. J., Brooks, D. L., Adler, H. E. (1983) "Caprine Mycoplasmosis: Widespread infection in goats with M. mycoides subsp. mycoides (Large Colony type)". American Journal of Veterinary Research, 44(2), 322-325.

Dienes, L. and Edsall, J. (1937), "Observations on L-organisms of Klieneberger". Proceedings of Society for Experimental Biology and Medicine, 36: 740-744.

Dujardin-Beaumetz, E. (1900), "Le microbe de la peripneumonie et sa culture". These de Paris, Paris, O. Doin.

East, N. E., DaMassa, A. J., Logan, L. L., Brooks, D. L., and Mcgowan, B. (1983), "Milkborne outbreak of Mycoplasma mycoides subsp. mycoides infection in a commercial Goat Dairy". Journal of the American Veterinary Medical Association, 182(12); 1338-1341.

Eaton, M. D., Meiklejohn, G., and Herrick, W. V. (1944), "Studies on the etiology of primary atypical pneumonia. A filterable agent transmissible to cotton rats, hamsters and chick embryos". Journal of Experimental Medicine. 79; 649-668.

Edward, D. G. ff (1953), "Organisms of the Pleuropneumonia group causing disease in goats". Veterinary Record, 65; 873-875.

Edward, D. G. ff (1954), "The pleuropneumonia-like group of organisms: a review together with some new observations". Journal of General Microbiology, 10; 27-64.

Edward, D. G. ff (1955), "A suggested classification and nomenclature for organisms of the pleuropneumonia group". International Bulletin of Bacterial Nomenclature and Taxonomy, 5; 85-93.

Edward, D. G. ff, and Freundt, E. A. (1956), "Classification and nomenclature of organisms of the pleuropneumonia group". Journal of General Microbiology, 14; 197-207.

Edwards, E. E., Judd, J. M. and Squire, F. A. (1955), "Blood counts in domestic animals in the Gold Coast". Nature, 176; 549-550.

El Nasri, M. (1967), "Mycoplasma from Contagious Caprine Pleuropneumonia". Annalsof New York Academic of Science, 143; 298-304.

Esievo, K. A. N., Saror, D. I., Alafiatayo, R. A., and Jamu, A. S. (1984a), "Effects of age and sex on some haematological values of Sokoto Red goats". Nigerian Veterinary Journal, 13(2); (in press).

Esievo, K. A. N., Saror, D. I., Alafiatayo, R. A., and Abubakar, A. (1984b), "Effects of age and sex on some haematological values of Yankasa lambs". Nigerian Veterinary Journal, 13(2); (in press).

Foster, J. P. (1934), "Some historical notes on Contagious Pleuropneumonia". Journal of American Veterinary Medical Association, 84; 918-926.

Freundt, E. A. (1958), "The Mycoplasmataceae". In: The Pleuropneumonia group of organisms. Copenhagen, Munksgaard.

Gameel, A. A. (1976); "Haematological and plasma protein changes in caprine pleuropneumonia". Tropical Animal Health and Production, 8, 37:

Gourlay, R. N. (1981), "Mycoplasmosis in cattle, sheep and goats". Israel Journal of Medical Sciences, 17(7); 531-536.

Goni, M. and Onoviran, O. (1972), "Artificial reproduction of CCPP". Nigerian Veterinary Journal. 1, 23-26.

Goni, M. and Onoviran, O. (1973), "Contagious Caprine Pleuropneumonia; a re-assessment of the disease situation in Nigeria". Tropical Animal Health and Production, 5; 114-118.

Holman, H. H. (1944), "Studies on the haematology of sheep. I. The blood picture of the healthy sheep". Journal of Comparative Pathology and Therapeutics. 54; 26-40.

Holman, H. H. and Dew, S. M. (1963), "The blood picture of the goat. I. The two year old female goat". Research in Veterinary Science, 4; 121-130.

Holman, H. H. and Dew, S. M. (1964), "The blood picture of the goat. II. Changes in erythrocyte shape, size and number associated with age". Research in Veterinary Science, 5; 274-285.

Hudson, J. R., Cottew, G. S., and Adler, H. E. (1967), "Diseases of goats caused by Mycoplasma". A review of the subject with some few findings Annal of New York Academy of Science, 143, 287-297.

International Livestock Centre for Africa (ILCA) (1979), "Small ruminant production in the humid tropics", ILCA, Addis Ababa. Pg. 4.

Jonas, A. M. and Barber, R. L. (1969), "Mycoplasma mycoides var. capri isolated from a goat in Connecticut". Journal of Infectious Diseases, 119; 126.

Lemcke, R. M. (1965), "A serological comparison of various species of Mycoplasmas by an agar gel diffusion technique". Journal of General Microbiology, 38; 91-100.

Longley, E. O. (1940), "Contagious Pleuropneumonia of goats". Indian Journal of Veterinary Science and Animal Husbandry, 10; 127-197.

Longley, E. O. (1951), "Contagious Caprine Pleuropneumonia: a study of the disease in Nigeria". Her Majesty's Office, London.

MacOwan, K. J. and Minette, J. E. (1976), "A Mycoplasma from acute contagious caprine pleuropneumonia in Kenya". Tropical Animal Health and Production, 8; 91-95.

MacOwan, K. J. and Minette, J. E. (1977), "The Role of Mycoplasma strain F38 in contagious caprine pleuropneumonia (CCPP) in Kenya". Veterinary Record, 101 (1077); 380-381.

Maniloff, J. (1969), "Pathology of Mycoplasmas". Journal of Bacteriology. 100; 1402-1408.

Markham, F. S. and Wong, S. C. (1952), "Pleuropneumoniae-like organisms in the etiology of turkey sinusitis and chronic respiratory disease in chickens". Poultry Science 31; 902-904.

Millson, G. C., West, L. D. and Dew, S. M. (1960), "Biochemical and haematological observation on the blood and cerebrospinal fluid of clinically healthy and scrapie affected goats". Journal of Comparative Pathology, 70; 194-198.

Mohan, K. and Uzoukwu, M. (1983), "Mycoplasma species and pneumonia of goats and sheep in Eastern Nigerian. University of Nigeria, Nsukka, Nigeria. Yale Journal of Biology and Medicine, 56(5-6); 93.

Mohan, K., Adegboye, D. S. and Orajaka, L. J. E. (1979), "Mycoplasma meleagridis from Turkeys. A first report of isolation from Nigeria". Nigerian Veterinary Journal. 8(2); 27-29.

Molokwu, E. C. I. and Igono, M. O. (1978): "Reproductive performance and pattern in brown goat of Nigerian Savannah zone". Proceedings of the 4th World Conference on Animal Production. 2, 578-590.

Nelson, J. B. (1935). "Coccobacilliform bodies associated with an infectious fowl coryza". Science, 82; 43-44.

Nocard, E. and Roux, E. R. (1898). "Les microbe de la peripneumonie". Annale des Institut Pasteur, (Paris), 12; 240-262.

Oduye, O. O. (1976), "Haematological values of Nigerian goats and sheep". Tropical Animal and Pro uction, 8(3); 131-136.

Ojo, M. O. (1971), "A review of microbial diseases of goats in Nigeria". Bulletin of Epizootic Diseases in Africa, 19; 5-15.

Ojo, M. O. (1975), "Caprine pneumonia. III. Biochemical characterisation and serological types of Pasteurellae". Nigerian Journal of Animal Production, 2; 216-221.

Ojo, M. O. (1976a), "Caprine Pneumonia in Nigeria. I. Epidemiology and bacterial flora of normal and diseased respiratory tracts". Tropical Animal Health and Production 8; 85-89.

Ojo, M. O. (1976b), "Caprine Pneumonia II. Biochemical characterisation and serological identification of Mycoplasmas. Tropical Animal Health and Production, 8, 137-146.

Ojo, M. O. (1976c) "Caprine Pneumonia IV. Pathogenicity of Mycoplasma mycoides subsp. capri and caprine strains of Mycoplasma mycoides subsp. mycoides for goats. Journal of Comparative Pathology. 86, 519-529.

Ojo, M. O. (1977), "Caprine Pneumonia". Veterinary Bulletin, 47; 573-578.

Ojo, M. O. "Contagious Caprine Pleuropneumonia in Africa". Proceedings of the 3rd International Conference of Goat Production and Disease. January 10 to 15, 1982. Tucson, Arizona, U. S. A. Pg. 209-211.

Ojo, M. O. "Mycoplasma Diseases of Small ruminants in Nigeria." A paper presented at the National Workshop on Diseases of Livestock and Poultry. National Research Institute, Vom, Jan. 24-27, 1983. Pg. 13-17.

Ojo, M. O., Kasali, O. B., and Ozoya, S. E. (1980), "Pathogenicity of a caprine strain of M. mycoides subsp. mycoides for cattle". Journal of Comparative Pathology, 90; 209-215.

Pillai, C. P. (1965), "Studies on pneumonia of goats in Sudan". Thesis, Royal College of Veterinary Surgery, London.

Provost, A., Villemot, J. M., Perreau, P., Queval, R. and Borredom, C. (1967), "Some concepts on the antigenic relationships of different Mycoplasma both within and outside". Annal of New York Academy of Science, 143; 38-47.

Rahman, A., Ahmed, M. U., and Mia, A. S. (1976), "Studies on the disease of goats in Bangladesh i Mortality of goats in farm and rural conditions". Tropical Animal Health and Production, 8: 90.

Razin, S. (1978), "The Mycoplasmas". Microbiology Review". 42(2); 414-470.

Razin, S. (1981), "Mycoplasmas: The smallest Pathogenic Prokaryotes". Israel Journal of Medical Science, 17; 510-515.

Razin, S., Kahane, I., Banai, M. and Bredt, W. (1981), "Adhesion of Mycoplasmas to Eukaryotic cells". In: Elliot K, O'Conner M., and Whelan J. (Eds.) "Adhesion and Microorganism Pathogenicity". Ciba Foundation Symposium. 80, Pitman Medical, London.

Rodwell, A. W. and Whitcomb, R. F. (1983). "Methods for direct and indirect measurement of mycoplasma growth. In: Methods in mycoplasmology. Vol. I. Mycoplasma characterisation ed. Razin and Tully, Pg. 185-196. Academic Pres.

Romano, N., Tolone, G., Ajello, F. and LaLicata, R. (1980), "Adenosine 5'-triphosphate synthesis induced by urea hydrolysis in Ureaplasma urealyticum". Journal of Bacteriology, 144; 830-832.

Rosendal, S. (1981), "Experimental Infection of goats, sheep and calves with LC type of Mycoplasma mycoides subsp. mycoides". Veterinary Pathology, 18; 71-81.

Rosendal, S. (1983), "Susceptibility of goats and calves after experimental inoculation or contact exposure to a Canadian strain of Mycoplasma mycoides subsp. mycoides isolated from a goat. Canadian Journal of Comparative Medicine. 47; 484-490.

Russel, C. W., Newman, C. and Williamson, D. H. (1975), "A simple cytochemical technique for demonstration of DNA in cells infected with Mycoplasmas and Viruses". Nature, 253, 461-462.

Sainsbury, R. W. (1928), Annual Report, 1927. Department of Veterinary Services, Nigeria. Pg. 31.

Schalm, O. W. (1965), Veterinary Haematology- Lea and Fiebiger, Philadelphia p. a. Pg. 1-654.

Shepard, M. C., Lunceford, C. D., Ford, D. K., Purcell, D., Taylor-Robinson, S., Razin, S. and Black, F. T. (1974), "Ureaplasma urealyticum (gen. nov., sp. nov.). Proposed nomenclature for human T(T-strain) mycoplasmas". International Journal of Systemic Bacteriology. 24; 160.

Shirlaw, J. F. (1949), "Studies on Contagious Pleuropneumonia of the goat in India". Indian Journal of Veterinary Science and Animal Husbandary. 19; 181-213.

Shoetensack, H. M. (1934), "Pure cultivation of the filerable virus isolated from canine distemper". Kitasato Archives of Experimental Medicine, 11; 277-290.

Solena, P. and Rivera, E. (1967), "Infection of goats in Mexico by Mycoplasma mycoides var. capri". Annals of New York Academy of Science. 143; 357-363.

Whittlesone, P. (1972), "The role of Mycoplasmas in the Production of Pneumonia in the Pig". In: Pathogenic Mycoplasmas. A Ciba Foundation Symposium, Pg. 263-283.

Wilkins, J. H. and Hodges, R. E. D. H. (1962), "Observations on normal goat blood". Royal Army Veterinary Corps. Journal. 33,7.

Wintrobe, M. M. (1965). Clinical Haematology. Lea and Fiebiger, Philadelphia p.a. Pg. 85-275.

APPENDIX

Table 4A: Mean rectal temperature chart (^{o}C) of Savannah Brown goats which were each inoculated intranasally with $2.6x10^{7}$ c.f.u. cultures of *Mycoplasma mycoides* subsp. *capri* (Smith's strain)aand control goats inoculated with sterile broth (Animal Experiment I)

Days Post-inoculation	Infected	Control
0	39.4 ± 0.1	39.6 ± 0.1
1	38.5 ± 0.1	38.7 ± 0.2
2	38.8 ± 0.1	38.8 ± 0.2
3	40.7 ± 0.2	39.2 ± 0.1
4	40.4 ± 0.2	38.8 ± 0.2
5	40.8 ± 0.1	39.2 ± 0.3
6	40.3 ± 0.3	38.8 ± 0.2
7	40.2 ± 0.5	39.1 ± 0.1
8	39.8 ± 0.2	39.1 ± 0.1
9	39.0 ± 0.5	38.7 ± 0.5
10	39.0 ± 0.4	38.9 ± 0.3
11	38.9 ± 0.5	38.9 ± 0.3
12	38.7 ± 0.5	38.9 ± 0.3
13	38.5 ± 0.5	38.8 ± 0.5
14	39.1 ± 0.1	39.1 ± 0.1

Table 4B: Mean rectal temperature chart (^{o}C) of Savannah Brown goats which were each inoculated intrasally with 1.0×10^{10} c.f.u. cultures of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment II).

Days Post-inoculation	Infected	Control
0	39.2 ± 0.35	39.5 ± 0.21
1	38.6 ± 0.46	39.2 ± 0.55
2	40.3 ± 1.071	38.9 ± 0.59
3	42.0 ± 0.38	38.9 ± 0.69
4	41.9 ± 0.50	38.8 ± 0.81
5	41.7 ± 0.40	39.0 ± 0.32

Table 5A: Mean PCV Values (%) of Savannah Brown goats which were each inoculated with 2.6×10^7 c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) cultures and control goats inoculated with sterile broth (Animal Experiment I)

Time	Infected	Control	
Pre-Inoculation Value (Day 0)	24.5 ± 1.60	25.2 ± 2.0	
Post-Inoculation (P.I.)			
3 days P.I.	24.2 ± 4.4	23.4 ± 4.5	
7 days P.I	22.3 ± 4.0	23.0 ± 4.5	
11 days P.I	21.7 ± 4.9	23.4 ± 4.4	
2 weeks P.I.	20.0 ± 7.6	23.8 ± 4.3	
3 weeks P.I.	20.1 ± 7.5	22.6 ± 4.5	
4 weeks P.I.	21.1 ± 5.6	20.0 ± 4.8	FLEA INFESTATION
6 weeks P.I.	21.0 ± 5.5	20.1 ± 4.7	
7 weeks P.I.	21.9 ± 5.0	20.0 ± 4.8	
8 weeks P.I.	20.6 ± 5.9	16.7 ± 5.5	
9 weeks P.I.	20.3 ± 6.0	14.0 ± 6.0	
10 weeks P.I.	20.3 ± 6.0	16.5 ± 5.5	
12 weeks P.I.	21.1 ± 5.6	24.0 ± 3.0	
14 weeks P.I.	19.7 ± 7.3	25.0 ± 2.9	
17 weeks P.I.	19.0 ± 7.3	26.0 ± 2.8	
	$\bar{X}$ = 20.6 ± 1.80	21.3 ± 3.6	

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Student t - value = 1.87

$P > 0.05$

Table 5B: Mean PCV (%) of Savannah Brown goats which were each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment II)

Days Post inoculation	Infected	Control
0 days P.I.	30.8 ± 3.4	32 ± 5.4
1 days P.I.	30.3 ± 3.4	31.0 ± 5.6
2 days P.I.	31.3 ± 2.7	32.3 ± 5.9
3 days P.I.	29.8 ± 2.5	31.5 ± 5.9
4 days P.I.	31.7 ± 1.5	31.5 ± 5.8
5 days P.I.	30.8 ± 1.0	31.5 ± 5.9
$\bar{X}$ =	30.8 ± 0.9	31.6 ± 0.5

t - Value = 2.15

P > 0.05

Table 6A: Mean Haemoglobin Values (Hb) (gm%) of Savannah Brown Goats which were each inoculated intranasally with 2.6×10^7 c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) cultures and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-Inoculation Value (Day 0)	7.7 ± 0.5	8.0 ± 0.77
Post-Inoculation (P.I.)		
3 days P.I.	7.7 ± 1.6	8.0 ± 1.5
7 days P.I.	6.9 ± 1.4	7.4 ± 1.4
11 days P.I.	6.4 ± 1.8	7.1 ± 1.3
2 weeks P.I.	5.8 ± 2.0	7.6 ± 1.5
3 weeks P.I.	5.8 ± 2.0	7.3 ± 1.2
4 weeks P.I.	6.4 ± 1.8	5.3 ± 2.0
6 weeks P.I.	6.2 ± 1.9	4.7 ± 1.7
7 weeks P.I.	6.9 ± 1.4	6.0 ± 1.9
8 weeks P.I.	6.8 ± 1.5	4.8 ± 1.7
9 weeks P.I.	7.6 ± 1.6	4.5 ± 1.7
10 weeks P.I.	7.9 ± 1.7	6.6 ± 1.8
12 weeks P.I.	6.9 ± 1.4	7.7 ± 0.5
14 weeks P.I.	5.8 ± 2.1	7.8 ± 0.5
17 weeks P.I.	5.4 ± 1.9	7.9 ± 0.5
$\bar{X}$ =	6.6 ± 0.8	6.6 ± 1.3
t - value = 0.13		
P > 0.05		

Table 6B: Mean Haemoglobin values (gm%) of Savannah Brown goats which were each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile Broth (Animal Experiment II)

Days Post-inoculation	Infected	Control
0	10.9 ± 1.2	11.4 ± 2.3
1	10.9 ± 1.2	11.5 ± 2.1
2	11.0 ± 0.8	11.5 ± 2.5
3	10.3 ± 1.2	10.1 ± 1.4
4	11.7 ± 0.4	11.2 ± 2.3
5	11.6 ± 0.5	10.0 ± 1.1
$\bar{X}$	11.0 ± 0.6	11.1 ± 0.7

t - Value = 0.36

P > 0.05

Table 7A: Mean Total RBC Values ($X10^6$/ul) of Savannah Brown goats which were each inoculated intranasally with 2.6×10^7 c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-Inoculation (Day 0)	11.4 ± 2.4	12.8 ± 2.0
Post-Inoculation (P.I.)		
3 days P.I.	11.7 ± 3.4	11.3 ± 1.4
7 days P.I.	10.3 ± 3.0	11.2 ± 2.3
11 days P.I.	9.1 ± 2.5	9.7 ± 2.4
2 weeks P.I.	7.8 ± 2.5	9.7 ± 2.4
3 weeks P.I.	7.8 ± 2.5	9.7 ± 2.4
4 weeks P.I.	6.4 ± 1.6	5.2 + 2.7
6 weeks P.I.	4.4 ± 2.2	3.9 ± 1.8
7 weeks P.I.	6.0 ± 2.3	5.5 ± 2.7
8 weeks P.I.	6.4 ± 2.3	4.4 ± 2.4
9 weeks P.I.	7.5 + 2.5	4.0 + 2.5
10 weeks P.I.	6.6 ± 2.3	4.9 ± 2.4
12 weeks P.I.	6.8 ± 2.4	6.6 ± 2.5
14 weeks P.I.	6.4 ± 2.3	8.1 ± 2.4
17 weeks P.I.	6.5 ± 2.3	9.0 ± 2.4
$\bar{X}$	7.5 ± 1.9	7.1 ± 2.7

t - Value = 0.03

$P > 0.05$

Table 7b: Mean RBC (10^6/ul) of Savannah Brown goats which were intranasally inoculated with 1.0×10^{10} c.f.u. of M. mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment II)

Days Post Inoculation	Infected	Control
0	11.7 ± 1.7	10.0 ± 2.7
1	9.0 ± 2.2	8.8 ± 3.5
2	8.3 ± 2.2	9.6 ± 2.5
3	9.2 ± 3.6	9.5 ± 2.5
4	11.5 + 2.2	9.1 ± 2.1
5	11.0 ± 3.0	9.0 ± 2.0
$\bar{X}$ =	9.5 ± 1.4	9.25 ± 0.4

t - value = 0.4

P > 0.05

Table 8A: Mean Total WBC ($X10^3$/ul) of Savannah Brown goats which were intranasally inoculated with 2.6×10^7 c.f.u. of *Mycoplasma mycoides* subsp. *capri* (Smith's Strain) and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-Inoculation (Day 0)	13.7 ± 0.8	16.8 + 1.2
Post-Inoculation		
3 days P.I.	21.8 ± 5.2	13.6 ± 2.3
7 days P.I.	22.2 ± 5.2	15.3 ± 3.4
11 days P.I.	17.6 ± 2.8	13.9 ± 2.9
2 weeks P.I.	17.1 ± 2.8	12.0 ± 3.0
3 weeks P.I.	13.9 ± 2.8	12.4 ± 3.1
4 weeks P.I.	13.6 ± 2.8	12.9 ± 3.0
6 weeks P.I.	12.6 ± 3.0	12.2 ± 3.0
7 weeks P.I.	13.2 + 2.7	14.9 ± 3.4
8 weeks P.I.	12.6 ± 3.0	14.0 ± 3.4
9 weeks P.I.	13.7 ± 2.8	15.0 ± 3.4
10 weeks P.I.	10.5 ± 2.5	15.0 ± 3.4
12 weeks P.I.	12.2 ± 2.8	12.9 ± 3.1
14 weeks P.I.	11.4 ± 2.6	14.1 ± 3.3
17 weeks P.I.	11.2 ± 2.6	13.9 ± 3.3
$\bar{X}$ =	14.4 ± 3.5	13.7 ± 1.1

t - Value (at peak) = 5.37

$P < 0.001$

Table 8B: Mean WBC values (10^3/ul) of Savannah Brown goats which were each intranasally inoculated with 1.0×10^{10} c.f.u. of M. mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth. (Animal Experiment II)

Days Post Inoculation	Infected	Control
0	23.8 ± 7.0	19.4 ± 2.4
1	22.1 ± 5.2	20.5 ± 6.6
2	28.4 ± 5.7	19.1 ± 4.4
3	27.1 ± 6.6	20.1 ± 3.0
4	26.6 ± 7.2	20.2 ± 3.5
5	26.5 ± 6.5	20.0 ± 3.0
$\bar{X}$ =	26.1 ± 2.8	20.0 ± 0.62

t - value at peak) = 6.12

P<0.05

Table 9A: Mean Neutrophil Counts ($X10^3$/ul) of Savannah Brown goats each intranasally inoculated with 2.6 x 10^7c.f.u. of Mycoplasma mycoides subsp. (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-Inoculation (Day 0)	5.04 ± 0.4	8.6 ± 1.6
Post-Inoculation (P.I.)		
3 days P.I.	13.5 ± 5.8	7.2 ± 2.0
7 days P.I.	13.1 ± 5.8	9.2 ± 2.7
11 days P.I.	8.8 ± 2.5	8.2 ± 1.8
2 weeks P.I.	8.4 ± 2.5	7.6 ± 2.0
3 weeks P.I.	7.7 ± 2.0	7.0 ± 2.0
4 weeks P.I.	6.0 ± 2.0	6.7 ± 1.9
6 weeks P.I.	5.4 ± 3.2	6.7 ± 1.9
7 weeks P.I.	5.6 ± 3.2	8.6 ± 1.8
8 weeks P.I.	6.5 ± 3.0	9.5 ± 2.7
9 weeks P.I.	5.8 ± 2.5	9.6 ± 2.7
10 weeks P.I.	4.3 ± 2.9	4.1 ± 2.9
12 weeks P.I.	6.7 ± 3.0	4.9 ± 2.8
14 weeks P.I.	5.2 ± 3.2	8.2 ± 1.5
17 weeks P.I.	5.0 ± 3.1	6.8 ± 3.0
$\bar{X}$ =	7.3 ± 2.8	7.4 ± 1.6

t - Value (at peak = 4.6)

$P < 0.001$

Table 9B: Mean Neutrophil values (10^3/ul) of Savannah Brown goats which were each intranasally inoculated with $1.0x10^{10}$ c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile Broth (Animal Experiment I)

Days Post - Inoculation	Infected	Control
0	7.7 ± 3.2	5.2 ± 0.8
1	8.9 ± 3.1	5.6 ± 1.9
2	14.9 ± 2.4	6.4 ± 1.2
3	15.4 ± 6.8	7.2 ± 1.9
4	9.7 ± 8.0	6.3 ± 7.4
5	8.1 ± 7.0	6.1 ± 7.0
$\bar{X}$ =	12.23 ± 3.4	6.42 ± 6.77

t - value (at peak) = 4.8

$P < 0.001$

Table 10A: Mean Band Count ($X10^2$/ul) of Savannah Brown goats which were inoculated intranasally with 2.6 x 10^7 c.f.u. of M. mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-inoculation (Day 0)	2.2 ± 0.8	3.5 ± 1.4
Post-Inoculation		
3 days P.I	5.4 ± 3.1	4.3 ± 2.1
7 days P.I.	4.1 ± 1.1	3.5 ± 1.8
11 days P.I.	3.4 ± 2.1	2.0.± 0.9
2 weeks P.I.	2.3 ± 2.0	3.4 ± 2.2
3 weeks P.I.	2.6 ± 2.0	3.3 ± 2.2
4 weeks P.I.	3.8 ± 2.1	2.9 ± 2.3
6 weeks P.I.	2.2 ± 2.0	2.8 ± 2.3
7 weeks P.I.	1.9 ± 1.6	2.6 ± 2.3
8 weeks P.I.	1.2 ± 1.0	1.7 ± 1.6
9 weeks P.I.	1.4 ± 1.1	3.2 ± 2.2
10 weeks P.I.	3.0 ± 2.1	2.7 ± 2.3
12 weeks P. I.	2.3 ± 2.0	2.5 ± 2.0
14 weeks P.I.	2.9 ± 2.0	2.4 ± 3.0
17 weeks P.I.	3.4 ± 2.1	2.6 ± 2.1
$\bar{X}$ =	2.5 ± 1.4	3.2 ± 1.0

t - value = 1.6

P > 0.05

Table 10B: Mean band count (10^2/ul) of Savannah Brown Goats each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's Strain) and control goats inoculated with sterile broth (Animal Experiment I)

Days Post Inoculation	Infected	Control
0	1.7 ± 2.1	0.9 ± 1.1
1	1.7 ± 1.8	0.8 ± 0.9
2	3.1 ± 2.3	0.8 ± 0.9
3	7.4 ± 0.7	0.5 ± 0.6
4	9.3 ± 0.7	0.6 ± 0.5
5	10.0 ± 0.7	0.7 ± 0.6
$\bar{X}$ =	5.37 ± 2.38	0.7 ± 0.2

t - value = 3.7

$P < 0.05$

Table 11B: Mean lymphocyte (10^3/ul) of Savannah Brown goats which were each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile Broth (Animal Experiment II)

Days Post Inoculation	Infected	Control
0	15.1 ± 5.8	14.2 ± 3.5
1	12.7 ± 4.7	13.6 ± 5.0
2	13.2 ± 5.3	12.4 ± 3.6
3	11.0 ± 3.3	12.5 ± 3.6
4	14.6 ± 4.3	12.1 ± 1.8
5	14.3 ± 4.0	12.5 ± 2.0
$\bar{X}$ =	13.5 ± 4.6	12.9 ± 3.3

t = 0.3

P > 0.05

Table 12A: Mean Absolute Monocyte Counts of Savannah Brown goats which were inoculated intranasally each with 2.6 x 107 c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth

	Infected	Control
Pre-Inoculation (Day 0)	60.3 ± 4.1	0
Post-Inoculation (P.I.)		
3 days P.I.	54.1 ± 5.3	48 ± 108
7 days P.I.	57.0 ± 150	0
11 days P.I.	31.0 ± 82	72 ± 55
2 weeks P.I.	0	0
3 weeks P.I.	0	0
4 weeks P.I.	27 + 50	41 + 70
6 weeks P.I.	0	0
7 weeks P.I.	69 + 30	0
8 weeks P.I.	88 + 53	0
9 weeks P.I.	0	0
10 weeks P.I.	25 ± 80	0
12 weeks P.I.	0	0
14 weeks P.I.	78 ± 40	0
17 weeks P.I.	0	0

t - value = 4.5

$P < 0.05$

Table 12B: Mean Monocyte counts/ul of Savannah Brown goats which were each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment I)

Days Post Inoculation	Infected	Control
0	48.3 ± 40.1	39 ± 60.8
1	0	0
2	62.8 ± 119.0	60.5 ± 21.0
3	0	0
4	42.4 ± 42.8	10.5 ± 28.3
5	0	0
$\bar{X}$ =	26.3 ± 31.49	42.8 ± 48.5

$t = 0.75$

$P > 0.05$

Table 13A: Mean Absolute Eosinophil Count ($X10^2$/ul) of Savannah Brown goats which were each inoculated intranasally with 2.6 x 10^7 c.f.u. of M. mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth (Animal Experiment I)

	Infected	Control
Pre-Inoculation (Day 0)	1.5 ± 0.4	2.6 ± 1.9
Post-Inoculation (P.I.)		
3 days P.I.	3.2 ± 4.3	2.8 ± 4.1
7 days P.I.	3.1 ± 4.1	2.8 ± 2.5
11 days P.I.	1.4 ± 2.2	2.7 ± 3.0
2 weeks P.I.	2.1 ± 4.5	2.9 ± 4.1
3 weeks P.I.	4.2 ± 3.0	2.2 ± 4.9
4 weeks P.I.	6.5 ± 2.0	7.1 ± 1.0
6 weeks P.I.	2.2 ± 3.8	3.6 ± 3.5
7 weeks P.I.	4.8 ± 2.5	7.0 ± 0.9
8 weeks P.I.	6.4 ± 2.0	5.7 ± 1.9
9 weeks P.I.	6.3 ± 2.5	8.5 ± 0.5
10 weeks P.I.	4.5 ± 3.0	8.1 ± 0.5
12 weeks P.I.	6.5 ± 2.0	4.0 ± 3.0
14 weeks P.I.	3.5 ± 4.4	4.4 ± 2.9
17 weeks P.I.	3.6 ± 3.4	4.9 ± 1.1
$\bar{X}$ =	4.2 ± 1.8	4.5 ± 2.5

$t = 1.27$

$P > 0.05$

Table 13B: Mean Eosinophil count (10^2/ul) of Savannah Brown goats which were each inoculated intranasally with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri.(Smith's strain) and control goats inoculated with sterile broth (Animal Experiment II)

Days Post Inoculation	Infected	Control
0	2.6 ± 1.4	1.7 ± 1.4
1	3.0 ± 3.4	1.7 ± 1.3
2	0.6 ± 1.2	0.6 ± 1.2
3	0.3 ± 0.8	0.5 ± 2.5
4	0	1.3 ± 2.5
5	0	1.0 ± 1.0
$\bar{X}$ =	1.0 ± 1.4	1.1 ± 0.52

$t = 0.004$

$P > 0.05$

Table 14A: Mean total protein (gm%) of Savannah Brown goats which were inoculated intranasally with 2.6×10^7 c.f.u. of M. mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth

	Infected	Control
Pre-Inoculation (Day 0)	7.5 ± 0.4	7.7 ± 0.5
Post-Inoculation (P.I.)		
3 days P.I.	7.6 ± 0.5	7.9 ± 0.5
7 days P.I.	7.6 ± 0.4	7.5 ± 0.5
11 days P.I.	7.6 ± 0.4	7.6 ± 0.5
2 weeks P.I.	7.8 ± 0.5	7.8 ± 0.5
3 weeks P.I.	7.6 ± 0.4	7.2 ± 0.6
4 weeks P.I.	7.3 ± 0.4	7.1 ± 0.7
6 weeks P.I.	7.1 ± 0.3	6.8 ± 0.7
7 weeks P.I.	7.5 ± 0.5	7.0 ± 0.7
8 weeks P.I.	7.5 ± 0.4	6.1 ± 0.4
9 weeks P.I.	7.1 ± 0.3	7.5 ± 0.5
10 weeks P.I.	7.1 ± 0.3	7.5 ± 0.5
12 weeks P.I.	7.4 ± 0.4	7.8 ± 0.5
14 weeks P.I.	6.9 ± 0.4	7.1 ± 0.7
17 weeks P.I.	6.5 ± 0.4	7.4 ± 0.5
$\bar{X}$ =	7.4 ± 0.4	7.3 ± 0.5

t - value = 0.75

$P > 0.05$

Table 14B: Mean total protein (gm%) of Savannah Brown goats which were each inoculated with 1.0×10^{10} c.f.u. of Mycoplasma mycoides subsp. capri (Smith's strain) and control goats inoculated with sterile broth

Days Post Inoculation		Infected	Control
0		6.9 ± 0.5	7.3 ± 0.6
1		6.8 ± 0.5	7.1 ± 0.5
2		6.9 ± 0.4	7.2 ± 0.7
3		6.1 ± 0.5	7.1 ± 0.7
4		6.0 ± 1.5	7.3 ± 0.6
5		6.1 ± 1.0	7.2 ± 0.5
	$\bar{X}$	6.5 ± 0.5	7.2 ± 0.1

t - value = 0.4

P > 0.05