HUMAN LOIASIS IN WESTERN NIGERIA

BY

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A B S T R A C T

An attempt has been made in this study to bridge some of the gaps in our knowledge of loiasis in Western Nigeria. At the same time, areas for future research which might lead to a better understanding of the disease pattern in loiasis are highlighted. This thesis is made up of both laboratory and field studies which were carried out at the University College Hospital, Ibadan, and in villages in the Western State of Nigeria respectively.

The sample population for the prevalence study comprised of 10830 school pupils and 1399 adults drawn from villages in divisions all over the Western State. Blood samples were examined parasitologically for microfilariae of Loa loa and the results obtained were analysed and utilised in determining the geographical distribution of loiasis within the state.

Vectors of Loa loa were determined in Ijebu and Remo divisions and the role of the common blood sucking mosquitoes in the transmission of loiasis was assessed in Ibadan city. The observation that Mansonia africana would allow the development of Mf Loa to the mature third stage larva raises the possibility that the Loa loa population in Western Nigeria might be different from the other known populations; therefore the local population was measured and compared with the Kumba (Cameroon) population.

Some of the manifestations often associated with loiasis were discussed on the basis of the observations made in villages during the prevalence studies; furthermore the associations that may exist between loiasis, endomyocardial fibrosis, ABO blood groups and haemoglobin genotypes, elephantiasis and hydrocoele were examined and discussed.

The results of the present study show that loiasis is endemic in the Western State of Nigeria, with most of the transmission taking place in the rain-forest and freshwater swamp zones. It has also been shown that C.silacea and C.dimidiata are the vectors of Loa loa in both the Ijebu and Remo divisions of the State. Even though M. africana supported the development of Mf Loa to the mature larval stage, this mosquito is not important as a vector in nature. The importance of M. africana lies in its possible use as a laboratory vector in preference to Chrysops species because of its short period of larval development. Linear measurements have shown that the Loa loa population in Western Nigeria is slightly smaller than the Kumba population.

There is no preferential infection by Loa loa of any of the blood groups and the haemoglobin genotypes studied. It has also been shown that there is no protective effect in any of the groups studied either in terms of preventing infestation or in the intensity of infection. Therefore none of the blood groups or

haemoglobin genotypes in this study is at an advantage or disadvantage with respect to loiasis. There is yet no conclusive evidence with which to incriminate loiasis with endomyocardial fibrosis, elephantiasis and hydrocoele formation in infected patients.

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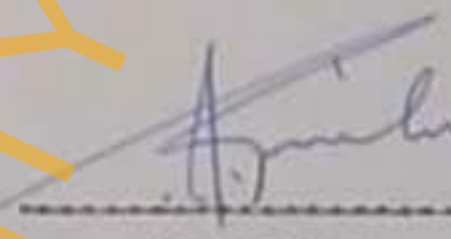
CERTIFICATION BY SUPERVISOR

I certify that this work was carried out by Mr. Eugene O. Ogunbo in the Department of Medical Microbiology, University of Ibadan.



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CHAPTER I

INTRODUCTION

The literature on filariasis shows that Nigeria is an endemic area for most of the human filariae, namely Wuchereria bancrofti, Onchocerca volvulus, Loa loa, Acanthocheilonema perstans and Acanthocheilonema streptocerca. The relative ecological distribution and the incidence of the various human filariae in Nigeria is not yet documented, although there have been reports of their occurrence in many parts of the country (Connal and Connal, 1922; Sharp 1923; Kershaw 1930; 1951; 1955; Kershaw et al 1953; Gordon et al, 1950, Cowper and Woodward, 1961; Gilles, 1965; Nnochiri 1964; 1966, 1968).

Loiasis is the disease caused by an infection with Loa loa, filaria which can live naturally in man and some monkeys. The adults of this worm live in the subcutaneous tissues of man and they often move from place to place causing itching and a creeping sensation. The embryos are called microfilariae and they occur in peripheral blood of infected people where they show a diurnal periodicity in the peripheral circulation. Although human loiasis has been recognised by name for about four centuries, it is only about three decades ago that its economic importance was fully

realised because employees of the former colonial government and business organizations were unwilling to return to Loa loa endemic areas for work.

The epidemiology of human loiasis has been well studied in the Cameroon Republic (Gordon et al 1950; Duke 1954; Crewe 1954) and some studies have been carried out in Congo, Rwanda (Fain 1969) and Southern Nigeria (Kershew 1955). Leiper (1914) suggested that the tabanid fly Chrysops was the probable vector of loiasis, and in 1922, Connal and Connal confirmed that Chrysops silacea and C. dimidiata are the main vectors of loiasis in Sapele, Southern Nigeria. These two flies are now believed to be the main vectors of loiasis in the rainforest zone of Africa where loiasis is endemic.

Experimental studies on loiasis has suffered for a long time partly because of the difficulty in breeding Chrysops for transmission studies. Chrysops has a long larval development of up to 9 months and the adult flies reared from larvae in the laboratory do not thrive very well. Furthermore, the infective larvae of Loa loa will develop naturally only in man and some monkeys but not in the common experimental animals such as mice, rats, guinea pigs, rabbits and even cats. Consequently there are still gaps in our knowledge of loiasis as a human disease.

Very little information is available about the pathology of human loiasis. For example, we do not know anything about the activities in the human body of the third stage (infective) larva

from the time it is innoculated into the blood stream to the time it becomes an adult. It is also not definitely known what symptoms and pathology are developed to the microfilaria and the developing larvae in the human body. Although various reports have been made in the literature about symptoms and pathology often associated with loiasis, it is not conclusive that the recovery of Mf-Loa or the adult worms in a patient suffering from another disease automatically incriminates Loa loa as the causative agent especially in an environment where other infectious diseases abound. Conditions such as encephalitis (Kivita 1982) retinopathy (Toussaint and Davis 1965; Petrithorn et al 1964) proteinuria (Gentilini et al 1963), psychoses (Clathier, 1943; Lambo, 1960) and endomyocardial fibrosis (Ive et al 1967) have been associated with loiasis because of the recovery of microfilariae of Loa loa from some of the vital organs, hence a closer study of loiasis has been stimulated in recent years.

Although animal models such as Dirofilaria immitis in dogs and Litosomoides carinii in cotton rats have been used in an attempt to elucidate some of the problems in filariasis, these have their limitations in respect of the effects of a specific filaria on the human body. A long term detailed study of suitable population groups in both endemic and non-endemic foci for loiasis might present us with more information about this disease if the data collected are carefully analysed and interpreted.

Clinical, parasitological or immunological methods can be used in the diagnosis of filariasis for epidemiological surveys. In loiasis, there is no certainty about the clinical manifestations apart from Calaber swellings. An examination of the literature concerning the clinical manifestations reveals a multitude of signs and symptoms which could be attributed to various other pathogens as well as to Loa loa. The recovery of the adult worm or the microfilariae from the infected individual is the only reliable and specific means of making a positive diagnosis of loiasis. Calaber swellings when present give a definite indication of loiasis although loiasis does not always result in the formation of Calaber swellings. The swelling may also not appear at the time when the diagnosis for loiasis is requested. Immunological tests have been employed in filariasis endemic zones as a supplement to both clinical and parasitological methods of diagnosis, and they are particularly useful in detecting cases of occult filariasis with low microfilarial density in peripheral blood. Some of the immunological tests are more sensitive than the parasitological methods, but there has always been the difficulty of lack of specificity. The antigens often used are those of animal filariae (Dirofilaria immitis and Litosomoides carinii) and these give group reactions for the human filariae and cross-reactions with other helminths. A serological or allergic method utilising specific antigens would

be valuable as a supplement to parasitological examination for the diagnosis of the different human filariae.

1.1 Objectives of the present study

The objectives of the present study developed from the gaps in our knowledge of loiasis especially in Nigeria, and the need to possess adequate information for a better understanding of the disease.

The following specific assignments were undertaken:

1. A review of the literature pertaining to the epidemiology, immunology, and disease associations of Loa loa.
2. A study of the ecological distribution of Loa loa in Western Nigeria from samples of the school children population and adults from the village clinics as well as hospitals.
3. A study of the vectors of Loa loa in Western Nigeria and the possible role of other common blood sucking arthropods in the transmission of Loa loa in Western Nigeria.
4. The measurement of both the microfilariae and adult worms of the Loa loa population in Western Nigeria with a view to finding out whatever differences might exist between it and the description of Loa loa in literature.
5. An assessment of the clinical manifestations to Loa loa amongst school children and an investigation into the possible syndromes occurring in infected people.

CHAPTER II

REVIEW OF LITERATURE

2.1 Historical Sketch

Mongin (1770) first recorded Loa loa from a negro woman at St. Domingo where he extracted the adult worm from between the conjunctive and albuginea.

The first record in Africa was made by Guyot (1777) in Angola. Guyot, a French naval surgeon, made several voyages to Angola and observed many cases of the worm under the conjunctiva of the indigenous people. The word Loa means "eye worm" and it originates from the local language in Angola.

Loa loa was imported with African slaves to Europe and the New world but its transmission is unknown outside Africa. Mercier (1771, 1774) and Arechart (1805) reported many cases of Loa loa from slaves who had recently arrived from Africa in the Western Hemisphere. There was however a decline in the number recorded in the West Indies and South America after 1845 because of the abolution of slave trade. The few cases recorded afterwards outside Africa were among missionaries, former colonial officers and businessmen who had lived in Loa loa endemic zones.

After Guyot's initial record in Angola in 1777, increasing numbers of cases were reported from inhabitants along the West

African coast and the incidence of infection in some villages especially in the Cameroon and Congo was as high as 20% (Blanchard, 1892, Ward 1906). Stoll (1947) estimated the world incidence of Loa loa infection to be 13 millions.

The appearance of the adult worm underneath the conjuctive was very familiar in the endemic areas and Morton (1877) had reported few attempts made in removing the worm from the eye by means of a thorn or a very thin sharp piece of bone.

The oedematous swelling caused by the adult worm occurred frequently amongst the inhabitants of old Calabar and hence were called Calabar swellings. Early workers, Robertson (1895), Plehn (1898) and Thompstone (1932) had already been familiar with, and actually used the name although Ward (1906) believed that Calabar swelling was then regarded as a distinct disease and unrelated to the adult Loa loa.

2.2. Systematic Position

The classification of filariae into families and sub-families is still controversial. Chabaud and Choquet (1953) modified the classification of Yorke (1939) and divided the Family Dipetalonematidae into six sub-families amongst which is Dirofilariinae containing Loa and Dirofilaria. Members of the subfamily Dirofilariinae have short tail and well developed caudal alae in the males. Their oesophagus is divided externally into separate muscular and glandular parts. The genus Loa is found

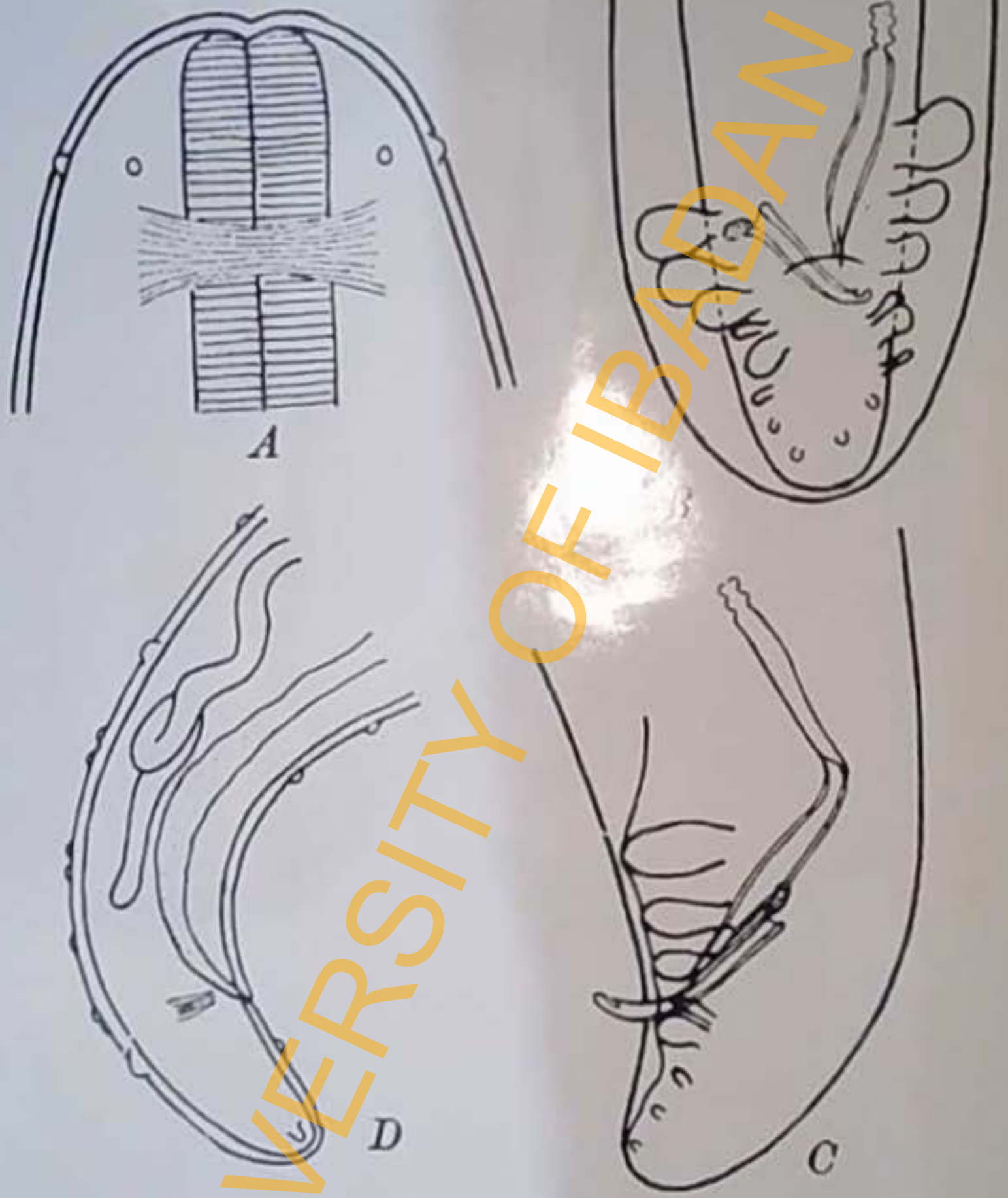

Fig. 1 - Adult Loa - loa (After Yorke and Maplestone, 1926)

A. Anterior extremity of body showing lateral and submedian papillae

B. Posterior end of male worm, ventral view, showing caudal alae, papillae and copulatory spicules.

C. Lateral view of male worm.

D. Caudal extremity of female worm, lateral view.

in Man as Loa loa and a morphologically similar parasite has been recorded in some simian hosts (Treadgold 1920; Vogel 1927; Sandground 1936; Gordon et al 1950).

Phylum	Nematoda
Class	Phasmidia
Order	Spirurida
Superfamily	Filaroidea
Family	Dipetalonematidae
Subfamily	Dirofilariinae
Genus	Loa

2. 2.1 Adult Loa loa

Cobbold (1864) first identified Loa loa although Looss (1904) first studied the worm in detail. The male worm measures 30-34 mm x 0.35 - 0.43 mm, and the female worm measures 50-70 mm x 0.5 mm (Faust 1949). The body of the adult worm is filiform and semi-transparent with numerous round smooth transluscent bosses on the outside which vary greatly in number and arrangement.

The presence of these bosses along the lateral lines on the cuticle easily distinguishes Loa loa from Wuchereria bancrofti which it resembles closely in general anatomy but which has very smooth cuticle. The cuticle of Onchocerca volvulus on the other hand is however ornamented with thickened ridgelike rings which make the worm quite distinct from the other human filariae. The anterior of Loa loa tapers to a small terminal mouth without any papillae (Figure IA). The head bears two

Fig. 2 Microfilaria of Loa-loa showing columns of nuclei(×1000). Note that the nuclei extend right to the tip, and the sheath trails at both ends.

lateral and four small sub-median papillae which lie in one transverse plane just behind the mouth. The tail of the male worm (Figure IB and IC) is curved and provided with lateral alate expansions of the cuticule. The cloacal opening lies mid-ventral in position near the curved tail and is surrounded by five pairs of asymmetrically placed papillae with three pairs of sessile papillae towards the caudal tip. The male worm has two unequal copulatory spicules measuring 123-176u and 88-113u respectively which often extrude through the cloacal openings. The posterior end of the female worm (Figure ID) is broadly rounded and has a pair of terminal papillae. The vulvar opening is situated some 2.5 mm from the anterior end.

2.2.2 Microfilaria Loa

The microfilaria Loa measures 250-300u x 6-8.5u (Faust 1943). It is sheathed and is diurnally periodic in man with peak densities in the peripheral blood between 10 a.m. and 2 p.m. The simian strain is nocturnally periodic. The caudal end is short and relatively thick with the nuclei extending to the tip. The cephalic end is broad and flat (Figure 2). The excretory pore is large. Fulleborn (1913) first observed the points of difference between MF Loa and MF bancrofti but Sharp (1923) studied the differences in details and made careful comparisons in both living and fixed conditions.

Woodman and Bokhari (1941) found differences between the morphology of microfilariae they studied in the Sudan and that

Fig 3 Map of Africa – The Records of Loiasis

described in literature but concluded that the differences were probably not significant in view of the wide range of sizes exhibited by Mf Loa. They also failed to observe the classical diurnal periodicity in Sudan and thus suggested that the Sudanese Loa loa showed a tendency towards aperiodicity. Kershaw (1950) examined prisoners in Kumba for the daily circle of Mf Loa and found that the mid-points and maxima of the swarming of microfilariae in blood occur about 11 a.m. Hawking and Thurson (1951) showed that when microfilariae disappear from human peripheral blood, they accumulate in the capillaries and the small vessels of the lungs from which they emerge 8-12 hours later and back into the peripheral blood. They further postulated that the lungs form the optimum site in the body for the microfilariae to survive. Hawking (1955) showed that physiological changes in the pressure of oxygen and carbon dioxide did not affect appreciably the distribution of Mf Loa between the lungs and the peripheripheral blood, and thus could not be responsible for maintaining the marked diurnal periodicity exhibited by Mf Loa.

2.3 Geographical distribution

Loa loa was believed to be confined to the equatorial rain-forest of Africa which extends approximately from $8^{\circ}N$-$6^{\circ}S$ of the Equator and to $30^{\circ}E$ longitude, with its transmission stretching from the shores of the Gulf of Guinea to the Great Lakes, and covering the rain-forest belt of Central and West Africa. The transmission of Loiasis to man has now been confirmed in Uganda

(Price 1961; Nnochiri 1972; Poltera 1973). The distribution of loiasis is patchy but very heavy infections have been recorded in the Cameroons, the borders of the Congo river, and from Benin and Sapele in Nigeria (Figure 3). Although loiasis is presently known to be acquired in the rain forest belt of Central and West Africa, the disease has been reported from all parts of the world. It was repeatedly introduced into the New World, particularly at the time of the importation of slaves from Africa but it has never become established there and not a single case of autochthonous infection has been proved.

2.4 Epidemiological studies

Until recently, very little was known about the epidemiology of loiasis in comparison with the other important filarial infections of man. Woodman (1935) drew attention to the prevalence of Loa loa in the South-Western districts of Sudan, the adjoining territoes of the Belgian Congo and the French Equatorial Africa, and stressed the lack of information about this filaria. Later Woodman and Bokhari (1941) studied the relationship between Loa loa, the Chrysops species and some disease syndromes in Southern Sudan.

Davidson (1946) found an incidence of 23% in African males in Kumba by blood examination, and Gordon et al (1948) estimated that each individual at Kumba was exposed to the infection of Loa loa once in every five days during the height of the Chrysops season. Following the reports of a series of preliminary studies, the Helminthiasis Research Unit, Kumba,

West Cameroon was set up in 1949 and has since been the seat of research into many aspects of Loa loa infection. Kershaw (1950) discussed the anomalous features of Loa loa infection in the hyperendemic areas such as Kumba where the incidence of infection recorded is far less than expected in view of the repeated exposure of the population for many years to infective bites of Chrysops. Kershaw (1951), Kershaw et al (1953), Kershaw and Nicholas (1954) use standard 50 cmm thick blood films as the index of infection. In their discussion of the results, Kershaw and his co-workers (1953) wondered whether patients with no microfilariae of Loa loa found in the standard 50 cmm blood film were actually free from infection or whether further patients would be found to be positive after a more prolonged and intensive examination. Gordon and Webber (1950) increased the percentage of positive findings from 28 to 46 by filtering lysed blood through a fine wire mesh.

Kershaw (1955) observed in Sapole Rubber Estate that 4-10% of the Chrysops population were infected and he recorded 45% infection in the inhabitants of the estate who lived there for longer than one year. He suggested that the remaining 55% must have received infective bites, and that the failure to find microfilariae after repeated examination of their blood might be due either to failure of the infective larvae to develop to sexual maturity or to the development of resistance which resulted in the death of the adult worms. Further possible reasons suggested by Kershaw were either the suppression of microfilaria production

or their appearance in the peripheral circulation due to immunity in the patients.

The risk of infection is known to vary with the environment in the endemic zones. Kershaw (1955) showed that urbanisation profoundly affects the risks of infection with Loa loa. The incidence of infection is high in small undisturbed villages whereas in towns the incidence of infection is reduced in part by the immigration of uninfected people from places outside the forest range, thus further lowering the already reduced risk of infection.

2.5 Vector Studies

Roche (1948) showed that four species of Chrysops - C.silacea, C.dimidiata, C.longicornis and C.distinctipennis had been recorded up to 1948 from Nigeria and the adjacent Cameroons but only three of these were found in the forest where human loiasis occurs. All the records of C.distinctipennis were from the savannah zone.

Larvae of L. loa were originally shown to develop in C.silacea and C.dimidiata (Leiper, 1914; Connal and Connal, 1922). Both these species are efficient vectors of loiasis and Crewe (1954) showed that the development of the larvae can take place with equal facility in C.langi. Development at a slower rate has been shown to occur in C.distinctipennis (Woodman and Bokhari, 1941), in C. zahni (Duke, 1954) and in C.centurionis (Duke 1955a). But C.langi and C.centurionis which are crepuscular in habit and bite at canopy level, are reluctant to bite man. Duke (1954) showed

that C.zahrai may be capable of transmitting human loiasis, but he considered it unlikely that this species alone could effectively maintain the infection in man. Woodman and Bokhari (1941) had a earlier come to a similar conclusion about C. distinctipennis, a possible vector in the savannah of the southern Sudan.

C.silacea is probably the most important vector of human loiasis in the West African rain forest except where C.dimidiata is locally more common. Both flies seem to hunt by sight and are attracted by movement. Humans are attacked whenever they move about at a point where they can be seen from the forest canopy, and movements of groups of people are more easily detected by the flies than movements of an individual (Davey and O'Rourke, 1951; Duke 1955c). The flies are attracted to moving objects and Duke (1955b) has shown that wood smoke also has a remarkable and pronounced attraction for C.silacea and, by inference, for C.dimidiata. All of these factors are responsible for C.silacea and C.dimidiata coming into intimate contact with man. Their importance as the main vectors of human loiasis arise because they alone have the habit of descending to the ground level in the forest to bite man, the behaviour necessary to complete the chain of transmission, and probably the most important link in this chain.

2.6 Clinical studies

The appearance of adult Loa loa under the conjunctiva is common in endemic areas and the recognition of Calabar swellings,

an allergic inflammatory reaction, in relation to the presence of adult worms was documented by early workers (Robertson, 1895; Plehn 1898; Thompstone, 1899; Ward, 1906). Very little is known about the pathological effects arising as a result of Loa loa infection although vague symptoms of mild fever, generalised muscular pain, paraesthesia, pruritis, urticaria, anorexia and loss of weight may be present. (Edington and Gilles 1969). It is widely known that the eosinophil count rises with Loa loa infection as with other helminth infections. Johnstone (1947) recorded 84% eosinophilia from a European patient with loiasis although the average eosinophil count from infected patients in his experience was between 15% and 30%. Gerbaux et al (1957) ascribed cardiomyopathy with eosinophilia to Loa loa infection.

Transient swellings (Calabar swellings) occurring in patients infected with filarial worms other than Loa loa, were recorded (Rose 1923) but their association with loiasis is so persistent that their occurrence is now regarded at pathognomic of the disease. Connal (1934) noted that in 37 Europeans in loiasis endemic zone, Calabar swellings appeared in 2 within 3 months, in 11 within 6 months and in 24 within 1 year of their first possible exposure and during which microfilariae were not detected from their peripheral blood. It is generally believed that the swelling is associated with the presence of a worm in its immediate vicinity, and Low (1924) described a patient in which the swelling occurred in the penis and a mass "which felt exactly

like a coiled up worm" was seen.

Fulleborn (1913) first suggested that Calabar swelling was an allergic reaction in persons sensitised to Loa loa worm or its excretions. Chandler et al (1930) and Fairley (1931) have shown that the injection of Dirofilaria immitis antigen subcutaneously in persons with Loa loa infection results in the production of a swelling indistinguishable from a Calabar swelling. A similar reaction however followed the injection of same antigen into patients with W.bancrofti although the typical Calabar swellings are not normally recorded.

Chandler et al (1930) have shown further that following the rupture of an adult Loa loa and the escape of its body contents during its removal from the eye, further Calabar swellings appeared in remote areas of the body. Such a reaction is in accordance with Fulleborn's (1913) early suggestion that Calabar swellings occur in those areas of the body where the tissues have absorbed the worms' products to the largest extent.

Occult filariasis was first described by Meyers and Kenwoosar (1939) and Bonne (1939). It has been variously described as eosinophilic lung, tropical pulmonary eosinophilia (Webb et al. 1960) and visceral larva migrans (Manson-Bahr 1960). The main clinical features are hyper-eosinophilia, enlargement of lymph glands, pulmonary symptoms and the absence of the microfilariae in peripheral blood.

Buckley (1958) experimentally infected a human volunteer with Brugia pahangi producing an occult infection with signs and symptoms of eosinophilic lung. However the filarial worm in two other volunteers (Edeson et al. 1960) produced filariasis with normal microfileremia. In a subsequent experiment, Buckley's volunteer developed an occult infection even though the infection (Brugia malayi) was from a human source (Buckley and Wharton 1961). Denaraj et al (1966) reported that in five lung biopsies of eosinophilic lung patients in Singapore, the dead and degenerating microfilariae were found both in exudative and granulomatous lesions. In dogs experimentally immunised with microfilariae of Dirofilaria immitis, freshly injected homologous microfilariae were found trapped in the lung tissue within one hour after the infection. These findings together with those obtained by tests using the Fluorescent antibody technique in which sera of patients with eiosinophilic lung were tested against different species of microfilariae (Jayewardene and Wijayaratnam 1968) indicate that occult filariasis may occur in an individual who is hypersensitive to the microfilarial stage and who acquires a filarial infection in which the worms become mature and produced microfilariae.

Adult worms have never been observed in patients suffering from occult filariasis and the tendency has been to assume that filariae of animal origin are responsible for this disease(Denaraj 1969; Webb et al 1960; Donohugh 1964). Buckley (1958), Buckley and Wharton (1960), however indicated that both human and animal filariae may induce occult filariasis.

Gonnert (1942) observed no symptoms following the transfer of the larvae of L.loa from an infected person to a previously uninfected person although Petithorny et al (1954) suggested that Mf Loa contained a toxic factor which killed a mouse at a dose corresponding to a microfilaremia of 300 microfilariae per mm3 blood in an adult human. Duke (1960) reported active destruction of microfilariae by the spleen of mandrills infected with L. loa and suggested that this phenomen was peculiar to the monkey host as it was not observed in humans.

It has been observed by many workers that a large number of adults in endemic areas may be devoid of clinical and pathological evidence of infection even after long standing infections of up to seventeen years (Ziemann 1926). On the other hand some workers (Thaulon 1923; Elliot 1929; Clothier 1943) quoted many instances in which loiasis caused great distress and was often the cause of invaliding and partial nervous breakdown. Lamba (1960) recorded an association between loiasis and psychosis in some of his patients in Nigeria and Nnochiri (1966) found an infection rate of 10% amongst patients in a mental hospital as against 1% in normal people in the neighbourhood of the hospital. He thus suggested an additional psychological factor with loiasis infection because some patients exhibited several features of mental instability since the presence of Calabar swelling is often attributed to "poison". It is also believed by some workers that in hyper-endemic areas, loiasis could be a common cause of hydrocoeles,

veriocoelus, hernia (Petrithony et al 1964), retinopathy (Tousint and Devin 1956), proteinuria (Gentilini et al 1963), fatal encephaliti (Kivits 1952) and endomyocardial fibrosis (Ive et al 1967).

2.7 Immunodiagnosis

The parasitic methods of demonstrating microfilariae from individual cases are the only reliable and specific means now available for the diagnosis of loiasis. However, it has always been experienced in epidemiological surveys of people in endemic areas that cases with demonstrable microfilariae are only a fraction of the people who are actually infected with the parasite, or who are suffering from the disease, and also that microfilariae can be detected from a large number of people who are apparently healthy. The immune status of the individuals to loiasis in an endemic area is known to vary, and the symptomless period may last for many years or remain throughout life. In new-comers to endemic areas, severe symptoms often occur, and, these may reappear many years after withdrawal from the endemic zones. These variations could be attributed to hypersensitivity (O'Connor, 1932; and Hartman, 1967).

Serological investigations in filariasis have been carried out since 1916 and Kagan (1963) reviewed numerous contributions to the immunodiagnosis of filariasis including loiasis. Serological investigations are particularly useful in cases where microfilariae cannot be found as in occult filariasis, because they measure the types and levels of antibodies being produced to the filarial infectio

Schofield (1957) using D. immitis showed the differences in the level of complement fixing antibodies produced against loiasis at different stages of the disease. Minning (1958) used a saline extract of deep frozen living Loa loa worms and obtained species specificity by complement fixation test. Sera from Wuchereria malayi and Dracunculus medinensis patients gave negative reactions.

Sadun (1953) showed that antibodies may be masked or absorbed by high concentration of excess antigenic substances in sera of infected patients and Ward (1954) showed that microfilarial antibodies were not demonstrable in dog sera by the Fluorescent antibody technique. Franks (1946) demonstrated that microfilaremic serum contains metabolic products of microfilariae which are antigenic, and that such sera gave strongly positive intradermal reactions in patients with filariasis. Other classical hypersensitivity tests such as the Schultz-Dale and passive cutaneous anaphylaxis reactions have been used to demonstrate the presence of microfilarial antigens in the plasma of hosts with high microfilaremia (Guest and Yong, 1965; Guest et al 1967). Various other serological tests have been used including intradermal, precipitin and haemagglutination reactions (Fullaborn, 1926; Chandler et al 1930; Fairley 1931; Rodhain and Dubois 1932; Bruynoghe 1939a; 1939b; Culbertson et al 1944a ; 1944b).

2.8 The Monkey Loa infection

In the strict biological sense, "loiasis" means parasitication of a vertebrate host by a worm of the genus Loa but when the human

host is concerned the term is normally understood to refer to infection with Loa loa, for with two rare exceptions (Mapleston 1930; Skriabin 1940) this is the only species of Loa recorded from man. However in the Budongo forest in Uganda Loa infection was recorded in Papio doguela (baboon), Cercopithecus aethiops, C. mitis, C. C. nictitans and Colobus abyssinicus. The species of Loa in monkeys was described by Treadgold (1920), Vogel (1927) in spider monkey (Ateles paniscus) and Sandground (1936) in Cercocebus mangabey. Gordon et al (1950) recorded infection of Loa in the three species of monkeys Mandrillus leucophaeus, Cercopithecus mona mona and Cercopithecus nictitans martini in the Cameroun Republic. The microfilariae of the monkey Loa are nocturnally periodic. The nocturnal Loa in monkeys is transmitted by different vectors, Chrysops langi and C. centurionis in Kumbe, Cameroons, although experiments have shown that both Chrysops silacea and C. dimidiata (the natural vectors of human loiasis) and C. langi and C. centurionis (the natural vectors of monkey loiasis) will all efficiently sustain the development of the larvae of both human and monkey Loa in the laboratory. Duke (1957) showed that human loiasis can be experimentally transmitted to monkeys after cyclical passage through C. silacea even though monkeys naturally infected with diurnally periodic human strains have not been found. Similarly no human cases of nocturnally periodic monkey loiasis have been observed.

Apart from differences in periodicity, the adult worms of monkey Loa are larger than those of human Loa. Nevertheless the

two strains are extremely similar and hybridisation between them is possible. (Duke and Wijers 1958).

Under natural conditions, there is isolation of the two forms in Kumba Cameroons, not only because of the specificity of the monkey parasite but also because of the different habits of their vectors. Although C. silacea and C.dimidiata are abundant at canopy level, they are exclusively diurnal in bitting habits. C.langi and C.centurionis on the other hand stay exclusively at canopy level and are actively feeding at dusk. Therefore because of their strict crupuscular habit C. langi and C. centurianis are unlikely to carry infections either from monkey to man or vice-versa.

CHAPTER III

GEOGRAPHICAL DISTRIBUTION OF LOA-LOA IN WESTERN STATE

3.1 Introduction

Human loiasis has been known for about 400 years and its transmission is confined almost entirely to the equatorial rainforest of the African continent. Very few studies have been carried out on loiasis in Nigeria and the extent and intensity of the infection in the Western State of Nigeria are still little known. (Connal and Connal 1922; Kershaw, 1955; Cowper and Woodward, 1961; Ngu and Folomi, 1965). A high microfilaremia has been found in blood donors at the University Teaching Hospital, Ibadan (Onunba 1970) and these blood donors are largely drawn from the Western State of Nigeria. In an endemic area for loiasis such as Nigeria, a number of diseases of unknown aetiology are being associated with loiasis. For example, Ive et al (1967) suggested that the distribution of endomyocardial fibrosis corresponds with that of Loa loa in Nigeria but could not postulate more then a geographical association because the basic epidemiological data are not available.

This study was therefore carried out to provide the epidemiological data on loiasis in Western Nigeria. It was also hoped to define the endemic and non-endemic foci for loiasis in some parts of the Ijebu and Remo divisions in the State where

future comparative studies could possibly be carried out on loiasis in relation to other diseases.

3.2 The Western State of Nigeria

The Western State of Nigeria is divided into twelve divisions (Figure 4) (Duze 1970) and it has a population of 9,487,525 (Nigerian Census 1963). It is mainly an agricultural state whose vegetation falls within the normal tropical pattern. Three types of vegetational zones are represented in the state and these are the rain-forest, the savannah and the freshwater swamp The State covers an area of 30,000 square miles and is bounded by Lagos State and the Bight of Benin in the South, the Republic of Dahomey in the West, Kwara State in the North and Mid-West State in the East. Its headquarters, Ibadan is the largest indigenous city in Tropical Africa. The state is peopled by the Yoruba, the largest single ethnic group in the Federation. Nigerians from other parts of the Federal Republic and other nationals are also to be found in large numbers in most cities in the State.

Rainfall varies from 45 inches per year in the northern area to over 100 inches in the South-east of the State. Relative humidity is high ranging from 50 to 95 per cent whilst temperatures range from 65°F to 95°F.

3.3 Sample Population and Survey design

Samples were collected only from villages within each division of the State. Towns were not sampled because they were considered unsuitable in view of the heterogenous nature of people living in them.

In order to obtain uniformity in the samples from the villages, primary school pupils were examined. They are very active within their villages whilst they have restricted mobility outside their villages and their divisions in particular. Any infection recorded in them is therefore most likely to have been contracted within their villages.

Adults would not be so suitable partly because of their great mobility within the State. It is also difficult to obtain uniformity in the adult samples especially in respect of their age and length of residence within the villages because most of the adult villagers are illiterates. Teachers were however examined in all the schools visited in the villages so as to obtain information from this group.

The villages sampled in any division were at least ten miles apart: the number of villages sampled in any division varied, depending both on the size and the type of vegetation represented

in the division. The Ijebu and Remo divisions were more elaborately sampled in order to define the endemic and non-endemic foci for loiasis in these divisions and partly because of their proximity to Ibadan.

The records of the Parasitology Section and the Blood Bank of the University College Hospital, Ibadan were also examined for information on the recorded cases of filarial infection.

3.4 Materials and Methods

3.4.1 Subjects

The bulk of the subjects was from school children between ages of eight and twelve years, and their teachers in the village primary schools. Samples were further obtained from patients attending village clinics at Ishara, Ode-Remo, Ijebu-Igbo and the Military hospital in Ibadan. Further samples were obtained also from patients and blood donors at the University Teaching Hospital, Ibadan.

3.4.2 Blood Samples

All blood samples were obtained between 1000 hours and 1400 hours.

In the villages, two thick blood films, each consisting of 50 cmm blood were made from capillary blood. The blood films were air dried and stored for not more than 24 hours before staining. One of the blood films was stained in Giemsa stain (Appendix I) and examined for the presence of microfilariae, other blood parasites and eosinophils, The other blood film was stained with Mayer's Haemelum (Appendix 2) for microfilaria identification. In order to assess the sensitivity of the method used in the village for detecting microfilariae, twenty individuals were selected, ten positive for microfilariae and ten negative on examination of capillary blood film. 5 ml venous blood were taken from each subject and examined for microfilariae. The blood was lysed in 50 mls of 0.2% Saponin solution (Sawyer and Winstein 1963), incubated for 30 minutes at 37°C and later centrifuged at 1000 r.p.m. for 5 minutes. The deposit was examined for presence of microfilariae. 2 ml venous blood was collected from patients attending the University College Hospital, Ibadan, and from this blood two 50 cmm thick blood films were made and examined as above. The remaining blood was similarly lysed in saponin solution, centrifuged and the deposit was examined for the presence of microfilariae.

In the Ijebu and Remo divisions, a record was made for every individual examined of any history of (1) Calabar swellings (2) Swellings of the limbs and face (3) Tiny worms crossing the eyes and (4) Intense body itchings. Any of these symptoms was regarded as evidence suggestive of Loa loa infection. A record was also made of the attitudes towards and the beliefs about Loa loa symptoms and the various types of remedies applied.

In those divisions of the State where the vegetation is not uniform, samples were collected from villages in the different types of vegetations represented. The data collected were analysed to show the level of transmission of loiasis in the different vegetation zones within the State.

The level of transmission of loiasis was assessed by the microfilaria rate in every vegetational zone within the divisions. The microfilaria rate was assessed as :

$$\text{Microfilaria rate} = \frac{\text{Number of people with microfilariae}}{\text{Number of people examined.}}$$

This assessment, based on the technique of blood examination explained above, is reliable, sensitive, economic and not too time consuming, and conforms with the prerequsites for the design of large scale filarial surveys (Sasa 1967).

3.5 Results

Table I shows the microfilaria counts from capillary and venous blood of ten school pupils from Western Nigeria who had Mf loa in their peripheral blood. The expected number of microfilariae in 5 mls blood and 100cmm blood were calculated both from the finger and venous blood respectively, and found to differ only slightly from the actual numbers recorded for each pupil. The difference was not statistically significant in each case ($P < 0.05$). Ten other school pupils that were negative by capillary blood were also negative for Mf loa on examination of venous blood samples.

Table 2 shows the prevalence of Mf Loa in the different vegetation zones within the divisions in the Western State. 98 villages were sampled varying from four in Ondo division to fifteen in Ijebu division. A total of 10830 school pupils were examined and 356 (3.3%) had Mf loa in their blood. There was no mf bancrofti recorded and only 214 had mf. perstans. The infection rate in the rainforest zone for mf loa ranged from 0.0% in the Ibadan division to 11.3% in Ilesha/Ife division and in the Savannah zone from 0% in Egba, Ibadan and Owo divisions to 1.0% in the Oyo division. The fresh water swamp zone is represented only in two divisions - Egbado and Okitipupa - with 2.5% and 2.1% infection rates respectively. Three vegetational zones are represented in Egbado division and the infection rates with loiasis varied significantly (Table 3) within the rainforest and savannah, and also within the fresh water swamp and rainforest. Ondo, Ijebu and Remo divisions

TABLE I

Numbers of microfilariae recovered from the capillary blood and venous blood of ten pupils with mf. loa in their blood.

Pupil's Number	Capillary Blood: Number of MF Loa in Two 50 cmm Thick Blood Films	Capillary Blood: Expected Number of Mf Loa in 5 ml Blood	Venous Blood: No of Mf Loa in 5 ml Blood	Venous Blood: Expected Number Of Mf Loa in 100 cmm Blood
JO 13^{IV}	20	1,000	1,119	22.38
EIO 10^{IV}	271	13,550	13,852	277.04
IA 13^{III}	26	1,300	1,428	28.56
PI 19^{BEF}	6	300	354	7.08
LW 8^{V}	224	11,200	11,293	225.92
IA 16^{IIIF}	948	47,400	47,951	959.02
IC 9^{V}	16	800	903	18.06
LAO 27^{VF}	52	2,600	2,722	54.44
IL- 36 ^{VIF}	164	8,200	8,297	165.94
PI 20^{VI}	312	15,600	15,920	318.4

There is no significant difference between the number of microfilaria in capillary and venous blood ($P < 0.05$)

TABLE 2

The prevalence of mf.loa in the different vegetation zones within the divisions in Western State of Nigeria.

DIVISION		No of Villages Visited	No of Pupils Examined	Pupils with Mf Loa		No of Adults Examined	Adults with Mf Loa	
Name	Vegetation			Number	%		Number	%
IJEBU	Rainforest	15	1127	[illegible]	[illegible]	305	29	9.5
ONDO	Rainforest	4	452	40	8.9	24	6	25.0
EGBA	Rainforest	7	973	34	3.5	651	41	6.3
OYO	Savannah	11	1195	13	1.09	65	2	3.08
EGBA	Rainforest	4	561	8	1.4	110	1	9.6
	Savannah	2	204	0	0.0	10	0	0.0
EKITI	Rainforest	6	762	30	4.0	27	3	11.1
	Savannah	2	232	1	0.3	15	2	13.3
IBADAN	Rainforest	6	656	6	0.9	36	6	16.7
	Savannah	3	273	0	0.0	21	0	0.0
ILESA/IFE	Rainforest	4	680	70	11.3	28	6	21.4
	Savannah	2	293	2	0.7	11	1	9.1
OKITIPUPA	Rainforest	6	472	25	5.0	28	3	10.7
	Freshwater Swamp	2	191	4	2.1	10	1	9.9
OWUO	Rainforest	6	652	12	2.8	39	3	7.7
	Savannah	4	322	2	0.6	24	3	12.5
OWO	Rainforest	4	328	4	1.2	20	4	20.0
	Savannah	2	246	0	0.0	10	0	0.0
ODODO	Rainforest	4	615	45	7.3	17	2	10.5
	Savannah	4	472	3	0.6	19	1	5.1
	Freshwater Swamp	2	203	5	2.5	11	1	9.1
TOTAL		98	10010	[illegible]	[illegible]	1329	119	8.95

are in the rainforest zones with 8.85% and 3.5% infection rates respectively. Oyo division lying in the Savannah zone has 1.0% infection rate. The other divisions are represented by two vegetational zones with either Rainforest/Savannah or Rainforest/ fresh water swamp combination with varying infection rates recorded from the vegetational zones. Both the villages visited, the number of pupils examined and the number of pupils with mf loa are given in Appendix 3.

1399 adults were examined from the whole State including 651 and 305 from the Remo and Ijebu divisions respectively. They consisted of villagers attending village clinics and teachers in the primary schools visited. Infection with Mf loa was detected in 115 adults (8.22%) from the whole State, and from 29 out of 305 (9.5%) adults in Ijebu division and 41 out of 651 Adults (9.3%) in Remo division.

Table 4 shows the prevalence of Mf. loa infection in 22 villages in Ijebu and Remo divisions. 2100 school pupils were examined and 86 had Mf loa in their blood. In all the villages, the number of pupils showing symptoms suggestive of Loa loa infection was significantly larger ($P < 0.001$) than those with detected microfilaremia. In five villages, Ala, Ibefun, Mobalufon, Imolu and Ijebu-Ijesha there was no Mf loa recorded from any of the school pupils sampled. A higher prevalence of infection was shown in the male pupils, and the difference observed in the infection rate between the male and female pupils was significant ($0.05 > P > 0.02$).

TABLE 3

The prevalence of mf. loa in Egbado division: Test of significant associations (Chi square with Yates correction).

a. Between Rain-forest and Savannah zones

	RAIN FOREST	SAVANNAH	TOTAL
Number with mf Loa	45	3	48
Number without mf Loa	568	399	967
TOTAL	613	402	1015

χ^2_c = 21.99 at 1 d.f.
$P < 0.001$

The infection rates in the two zones are significantly different.

b. Between Rain-forest and Freshwater Swamp zones

	RAIN-FOREST	FRESHWATER SWAMP	TOTAL
Number with mf Loa	45	5	50
Number without mf Loa	568	198	766
Total	613	203	816

χ^2_c = [illegible] at 1 d.f.
$0.02 < P < 0.01$

The infection rates in the two zones are significantly different.

c. Between Freshwater swamp and Savannah zones

	FRESHWATER SWAMP	SAVANNAH	TOTAL
Number with mf Loa	5	3	8
Number without mf Loa	198	399	597
Total	203	402	605

χ^2_c = 1.67 with 1 d.f.
$0.2 > P > 0.1$

The infection rates in the two zones are not significantly different.

TABLE 4

Prevalence of mf. loa in twenty-two villages in Ijebu and Remo divisidns in Western Nigeria.

VILLAGE	Number of Pupils Examined	Number of Pupils with Loa Symptoms	Percentage of Pupils with Loa Symptoms	Number of Pupils with mf. Loa in Blood	Male Pupils with mf Loa in blood Number	%	Female Pupils with mf Loa in Blood Number	%	Number of Adults Examined	Number of adults with mf Loa in Blood	Percentage adults with mf. Loa in Blood
Ikun-Ono	214	53	24.8	6	3	2.4	3	3.4	15	4	26.6
Imu	77	36	46.7	7	4	10.0	3	8.3	12	1	8.3
Ala	35	11	31.4	0	0	0	0	0	9	4	44.4
Mobelufon	18	11	61.0	0	0	0	0	0	6	1	16.7
Idowa	31	15	48.4	1	1	[illegible]	0	0	10	0	0
Ibefun	75	31	41.3	0	0	0	0	0	20	5	25.0
Isosa	46	20	43.5	3	3	11.1	0	0	21	1	4.8
Ishara	143	72	50.3	5	3	3.0	2	4.0	184	17	9.2
Ago-Iwoya	126	35	27.7	4	4	7.0	0	0	22	1	4.5
Oru/Awa	59	19	32.2	6	5	16.0	1	3.1	17	2	11.7
Ikija/Owu	53	28	52.8	3	2	8.3	1	3.4	11	1	9.1
Ijebu-Ife	89	49	55.0	4	3	5.1	1	3.3	15	0	0
Ijebu-Igbo	167	71	42.5	9	8	9.2	1	1.1	134	8	6.0
Falafonmu	26	15	57.7	2	1	5.6	1	11.1	1	0	0
Odogbolu	73	20	27.3	5	3	9.0	2	5.7	10	1	10.0
Aiyepe	90	45	51	3	2	5.0	1	2.0	18	2	11.1
Odo-Remo	128	48	37.5	5	3	4.8	2	3.0	135	20	14.8
Isiro	38	8	21	2	1	5.2	1	5.2	2	0	0
Irolu	55	10	18.2	0	0	0	0	0	3	0	0
Ijebu-Ijee	26	4	15.4	0	0	0	0	0	4	0	0
Iperu	302	142	30.8	7	7	2.3	0	0	13	0	0
Orero	139	80	57.5	14.	7	6.3	7	11.3	6	0	7.8
TOTAL	2100	742	35.3	86	60	5.0	26	2.9	700	68	

TABLE 5

Distribution of loiasis within the ages 9 - 12 years amongst the school pupils in Ijebu and Remo divisions.

Age in Years	Number Examined	Number with mf Loa in Blood	% with mf Loa in Blood
9	519	8	1.54
10	358	6	1.68
11	363	22	6.06
12	877	50	5.70

X^2 = 30.57

P < 0.0001

Infection rates in ages below and above 10 years are Statistically significant

Apart from examining the teachers, villagers attending clinics in Isharo, Ijebu-Igbo and Ode-Remo were examined and they had infection rates of 9.2%, 6.0% and 14.8% respectively.

Table 5 shows a rise in the infection rate from 1.6% in nine year old pupils to 6.1% and 5.7% in eleven and twelve years old pupils respectively in Ijebu and Remo divisions. The difference in the infection rate detected from eleven year old pupils upwards and below eleven years old pupils is highly significant ($P < 0.0001$).

Figure 4 shows the prevalence of Loa loa in the different vegetation zones in the divisions of Western State.

Figure 5 shows the relationship of the 22 villages sampled in Ijebu and Remo divisions with the river and stream systems in the divisions.

Table 6 shows the records of blood filarial infections in Nigeria.

Figure 6 shows the distribution of 222 blood donors with Mf loa according to their town of residence in Western Nigeria.

3.6 Discussion

Kershaw (1950) suggested that the microfilaria counts made from a finger prick blood could be taken as representative of the number of microfilariae in the peripheral circulation. He also showed that blood taken at noon from the thumb has a higher count of microfilariae than that taken from the corresponding vein. Hawking (1958) however showed that the microfilaria count from the finger and venous blood does not differ significantly because microfilariae

are known to pass through blood capillaries as rapidly as the blood cells. The observations of Hawking (1955) have been confirmed in this study (Table 1) and the examination of capillary blood has been shown to be reliable and sensitive. Therefore cappilary blood was examined for the detection of microfilariae in the blood of the village samples. When microfilariae are present in peripheral blood circulation, they are unlikely to be missed after the examination of two 50 cmm samples of blood collected between 1000 hours and 1400 hours; furthermore, counts recorded from two 50 cmm thick blood films would be fully representative of the number of microfilariae in the peripheral circulation of any infected person.

3.6.1 Prevalence of Loiasis in School Pupils

The overall prevalence of Mf loa is 3.3% although the actual infection rate varied from the minimum of 0.6% in the Ibadan division to the maximum of 8.85% in the Ondo division (Appendix 3). The sample size varied from one division to the other partly because of the varying number of vegetation zones represented in the divisions and partly because of the size of the division. The sample size also depended on the number of pupils in attendance at the schools when they were visited.

A sharp rise was observed in the prevalence of loiasis from the Savannah to the rainforest zone within the same division (Figure 4). The rise is more marked especially in Egbado and Ilesha/Ife divisions where 0.3% and 0.70% infection rates were

Fig.4 Showing the prevalence of Loa loa infection in the different vegetation zones within the divisions of Western State of Nigeria

recorded in their savannah zones as against 7.3% and 11.3% infection rates from their rainforest zones respectively. The difference in the infection rates of the two zones is highly significant ($P < 0.0001$). The result thus suggests that the rainforest zones of Egbado and Ilesha/Ife divisions are areas of high loiasis transmission when compared with the savannah zone.

The infection rates for Mf loa detected in the rainforest zones of Egbado and Ilesha/Ife divisions are areas of high loiasis transmission when compared with the savannah zone.

The infection rates for Mf loa detected in the rainforest zones of Ibadan, Owo and Egba divisions are significantly low when compared with those detected in the rainforest Zone in Ilesha/Ife and Egbado divisions ($P < 0.0001$). No infection was recorded in their savannah zones; therefore the Egba, Ibadan and Owo divisions are areas of very low Loa loa transmission. The low transmission could could be explained by the closeness of the divisions to the heart of the savannah where the vegetation is not ideal for the vectors of Loa loa, Chrysops species, which are canopy dwellers. The infection rate of 1.0% recorded in Oyo division which lies entirely in the savannah zone was almost uniform throughout the division and persisted to its northern border (Aha, Shaki, Kishi) villages (Appendix 3). This further confirms the relatively low transmission of loiasis in the savannah region of Western Nigeria.

The fresh water swamp zone is represented only in two divisions (Egbado and Okitipupa) and the overall infection rate recorded in

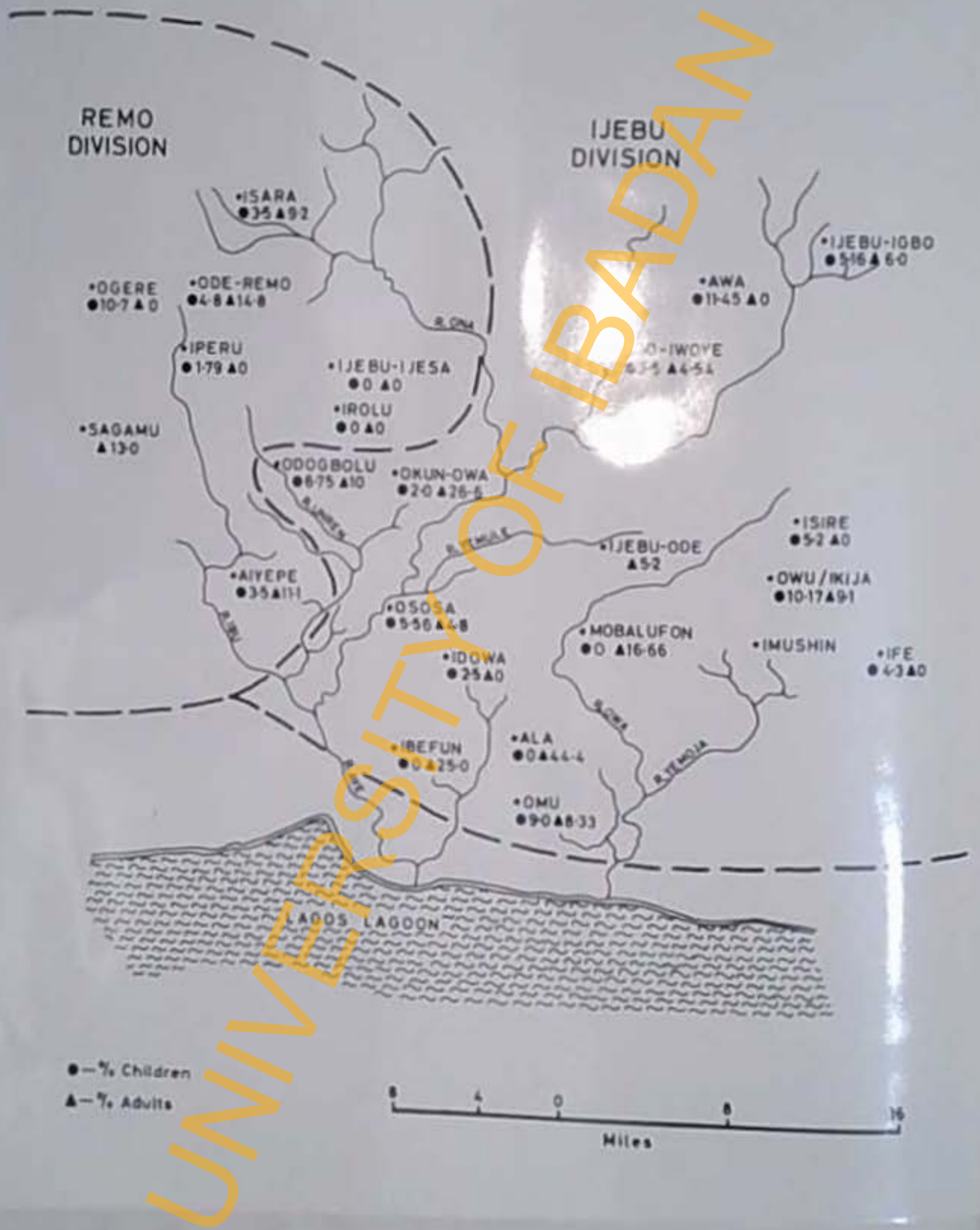

Fig. 5. Prevalence of loiasis in villages in Ijebu and Remo divisions, with positions of the villages in relation to the river systems.

this zone is not significantly different from that recorded from the rainforest zone. The fresh water swamp zone is thus an area of relatively high Loa loa transmission because like the rainforest zone, the vegetation is the type that will favour the development of Chrysops species both at the breeding stages and the tree canopy stages.

Loa loa is endemic in Western Nigeria. The results from the three vegetational zones show clearly that the bulk of Loa loa transmission occurs in in the rainforest and fresh water swamps zones in Western Nigeria with some low level transmission still taking place in the savannah zone. The Ondo division is shown to have the highest Loa loa endemicity closely followed by the Ilesha/ Ife division. The Ijebu, Egbado, Okitipupa, Remo and Ekiti divisions are areas of average endemicity whilst the Oshun, Oyo, Owo, Egba and Ibadan divisions are areas of low Loa loa endemicity.

3.6.2 Prevalence of Loiasis in the Adult population

Table 2 shows that 115 of 1399 (8.22%) adults had Mf loa in their blood. This infection rate may be taken to represent the adult infection rate throughout the Western State. The adults and especially the teachers are highly mobile within the state and could have acquired their infection from any endemic focus they have visited. Their movements can take them from endemic to non-endemic foci for loiasis and vice-versa thus altering their actual exposure to Loa loa transmission.

Mf Loa was recorded from adults in all the divisions of the State although the infection rates varied; there were no positive

cases recorded from villages sampled in the savannah zones of Ibadan, Egba and Owo divisions. These areas have been described above as areas of low Loa loa transmission level. Except in Ijebu-Igbo, Ishara and Ode-Remo, all in Ijebu and Remo divisions, where large adult samples were examined from the schools and clinics, the samples obtained from individual villages were from teachers; the samples are rather small and probably unrepresentative of the adult population in the villages.

9.5% and 9.3% infection rates were recorded from the adult samples from Ijebu and Remo divisions respectively (Table 2). When the infection rates in the school pupils and the adult populations are compared in both divisions it was observed that the infection rate in the adult population is significantly higher ($P < 0.0001$) than that recorded in the school pupil population. The higher infection rate in the adult population is expected in view of the fact that the adult population must have been exposed to Loa loa infection for a longer period than the school pupil population. Infection with Loa loa persists for a long time,(Ziemann 1926) and there is no known age resistance to Loa loa infection. Kershaw (1954) found that Loa loa infection rate rises from nil at birth to about 40% in old age in the former British Cameroons. Similarly in this study, the Loa loa infection rate rises with age, and the higher microfilaremia rate in the adult is clear evidence of higher infection rate. Apart from the longer exposure of the adult population in endemic areas, the adult population who are

Fig. 6 Map of Western Nigeria showing the origin of 232 blood donors with mf loa in 1969. (The figures in brackets indicate the donors examined).

mostly farmers in the villages would be much more exposed to Loa-loa transmission than the school pupil population because the adults spend more time in the bush cultivating their farms, and would consequently have more Chrysops/man contacts.

232 adult blood donors (2.9%) out of 8065 screened in the University College Hospital, Ibadan in 1969 had Loa - loa infection. This prevalence is much lower than the 8.2% recorded from adults in villages in Western Nigeria. 120 (3.5%) of the 3451 blood donors from Ibadan city, an area of low transmission, had Loa loa infection as against 45 (2.7%) out of 1653 from Ijebu-Ode, a high transmission area (Fig 5). In other high transmission areas like Ife, Onda and Ado Ekiti, prevalence rates of 12.8%, 9% and 28.1% were recorded respectively. In Sagamu, a low rate of 0.47% was recorded, whereas Owo, an area of low Loa loa transmission, had a record of 5% infection amongst the blood donors.

The discrepancy in the distribution of infection amongst the blood donors is partly because Ibadan city is the State capital and a big commercial centre which attracts people from all over the State and country. Some of the people would have come from highly endemic areas for loiasis, and thus would swell the prevalence in Ibadan township. The samples from the different towns were not uniform with respect to ages, and a larger number came from Ibadan residents. Therefore the figures recorded in figure 6 do not reflect the level of Loa loa transmission in the towns listed, and

would mislead the unwary and uncritical investigator if they are used for prevalence study.

3.6.3 Endemic and Non-endemic foci in Ijebu and Remo divisions

In five villages (Ala, Ibefun, Ijebu-Ijesha, Irolu and Mobalufon) out of twenty-two villages in Ijebu and Remo divisions, microfilariae were not detected in the school pupil population (Table 4). In Ala, Ibefun and Mobalufon, M. ozzardi were found among the adult (teachers) population, but not in Irolu and Ijebu-Ijesha. Since the teachers who comprise the adult population sampled in most villages in these divisions are not representative of the adult population, only the school pupils population would be useful in deciding the endemic and non-endemic foci for loiasis. Villages with positive Loa loa cases recorded from the teachers would therefore at best be only potential foci for Loa loa transmission provided the vectors breed and feed on humans there. The evidence therefore of Loa loa transmission in a village must be reflected in the population and this can be validly assessed by the infection rate in the school pupil population.

Figure 5 shows the positions of the villages sampled in relation to the rivers and streams in the Ijebu and Remo divisions. Irolu and Ijebu-Ijesha are located away from any stream or river. These two villages prior to the introduction of pipe borne water suffered from severe scarcity of water, and had to live on water collected during the rains and sealed in earthenware pots. Leiper (1914); Connal and Connal (1922) showed that Chrysops

silacea and C.dimidiata are the vectors of Loa loa in West Africa. Gordon et al (1950) found that these insects breed in densely shaded slow flowing muddy streams. Since there are no streams or rivers around Irolu and Ijebu-Ijesha, there could be no chance of Chrysops breeding in these villages, therefore there will be no transmission of loiasis in the villages. Consequently Irolu and Ijebu-Ijesha are non-endemic foci for loiasis because even if infected people come into these villages, there will be no transmission of Loa loa to the villagers because of the absence of Chrysops breeding sites in the villages.

Ala, Ibefun and Mobalufon are close to main streams (Figure 5) which are ideal as breeding sites for Chrysops. There is indirect evidence of breeding of Chrysops taking place in these villages because in the next chapter, C.silacea and C.dimidiata were reported to have been caught under tree canopy near the streams. These villages therefore represent potential areas for Loa loa transmission because of the breeding of Chrysops in and around the villages and also because of the record of infection in the adult (teacher) population.

Odogbolu, Ode-Remo, Ishara, Aiyepe, Omu and Ijebu-Igbo represent endemic foci for loiasis and with transmission occurring at a high level. They are close to streams and rivers which would easily serve as breeding sites for Chrysops, and the high level of transmission is confirmed by the high infection rates recorded both from the school pupil and adult populations. In Ode-Remo

Ishara and Ijebu-Igbo, 14.8%, 9.2% and 6.0% infection rates were recorded respectively from adults including those attending the clinics and the teachers. The adults except the teachers are villagers living a rural life and with no history of travelling outside their division. They consisted of males and females with ages ranging from 20 to 70 years.

In the attempts to find possible aetiological agents for conditions like nephrotic syndromes and endomyocardial fibrosis, many protozoal and helminth infections that are prevalent in our environment are being closely studied, and loiasis has been associated with endomyocardial fibrosis (Ive et al 1967). Ive and his group studied adults who were also sick patients. The unsuitability of adult for correlation studies in loiasis has been discussed above, and sick hospital patients constitute a selected group. It is therefore necessary that correlation studies be carried out in endemic and non-endemic foci between loiasis and some disease conditions of unknown aetiology and especially endomyocardial fibrosis. The present study might be of some value in achieving this objective since both the endemic and non-endemic foci for loiasis have been clearly defined. In this respect, the school pupil population would be ideal because of the uniformity of their samples and their accessibility. Furthermore such a population is easily followed for a few years as might be required in finding out more about the pattern of the association.

3.6.4 Age and Sex distribution

In Table 4 more male pupils were infected in most villages

than female pupils and in both the Ijebu and Remo divisions a significantly higher infection rate was recorded for the male pupils ($P < 0.005$). The difference is possibly caused by the greater exposure of the male pupils to the infective bites of Chrysops. The male pupils are more active than the females and through their constant visits to the bush for farming, firewood collection, and for fetching water from the streams, they are bound to be more exposed to Loa loa infection than the female children. There is probably no sex preference in Loa loa infection.

A higher infection rate has been observed with increasing age from school pupils between 9 and 12 years old. The sharp increase observed in the infection rate after the age of 10 years is highly significant ($P < 0.0001$). It can however be partly explained by the greater activity of pupils of ages eleven and twelve years who are in the senior classes and are more active in their environment than those in the junior classes. Furthermore the cumulative effect of a longer period of exposure to Loa loa transmission might have been partly reflected in the higher infection rate in the eleven and twelve years old pupils.

3.6.5 Prevalence of Symptoms suggestive of Loa loa infection

Any of the conditions below was recorded as Loa loa symptom among the school pupils in Ijebu and Remo divisions.

(i) History of Calabar swellings
(ii) Swellings of limbs and face
(iii) Tiny worm crossing the eyes
(iv) Intense body itching

In Table 4 the figures recorded for Loa loa symptoms are very high and range from 15 to 61% whereas the actual microfilaremia rate ranged from 0-11%. A high percentage of pupils with symptoms was recorded also in Irolu, Ijebu-Ijesha, Ala, Ibefun and Mobelufon where there was no record of Mf Loa from the school pupils. The high prevalence of symptoms is probably explained partly by the fact that such conditions like swelling of face and limbs, and intense body itching which were recorded as Loa loa symptoms might have been caused by other agents. Occult loiasis is also known to be common, and the absence of microfilariae in the blood may thus be due to absence of adult females in the host. When the adult females are present, a possible immunological suppression of microfilariae production may exist or an immediate destruction of microfilariae in the blood by the immune response agencies in the body (Duke 1960) may be responsible for the absence of microfilariae in the blood. Another factor which could have increased the prevalence of symptoms is the possible enthusiasm of school pupils in supplying answers which they hope would please an investigator and probably also make them qualify for other medical facilities and attention.

3.6.6 Incidence of other human blood filariae

The relative prevalence of the various blood filariae as

TABLE 6

Record of blood filariae in Western Nigeria.

STUDY BY	Source of Samples	No. of Samples examined	NUMBER WITH MICROFILARIAE IN BLOOD						
			W.bancrofti		Loa loa		A. perstans.		
			Number	%	Number	%	Number	%	TOTAL
Cowper and Woodward 1961	U.C.H. patients	5150	-	-	38	0.74	14	0.27	52
Agu and Falemi 1964	Hospital patients in Western Nig.	[a]1340	2	0.15	66	4.93	27	2.06	95
Gilles 1965	Akufo-village	828	-	-	34	4.11	12	1.45	45
Parasitology Dept 1967/70	UCH Patients	7118	2	0.03	61	8.6	2	0.03	65
Present study	Military Hospital	[b]121	-	-	11*	9.09	10*	8.26	21
Present study	Adults and School children in villages.	[b]1222	-	-	471	3.86	214+	1.75	685
Present study	Blood Bank records.	10050	-	-	-	-	-	-	285≠(2.8%)

* – 2 Soldiers had both mf Loa and perstans.

a – Night blood examined specifically for W.bancrofti microfilariae

b – Day blood examined specifically for mf Loa.

\+ – mf perstans recorded mainly in Oyo, Owo and Ekiti divisions of Western State.

≠ – Differentiation of microfilariae not carried out.

recorded in the Western State of Nigeria from various types of subjects and by different workers is shown in Table 6. Although these records should not be compared because the conditions under which the studies were carried out varied, it is still possible to make some pertinent comments on the data recorded.

It is observed that not a single case of Wuchereria bancrofti infection was recorded in this study: this however is not surprising because the blood samples examined for microfilariae were collected around mid-day when the microfilariae of W.bancrofti, a nocturnally periodic filaria, is expected to be absent in peripheral blood. Ngu (1962) failed to record W.bancrofti from the night blood of 1000 patients at the University College Hospital Ibadan. Ngu and Folami (1965) also sampled night blood of 1340 patients in all government hospitals in former Western Region of Nigeria and found only two positive cases from Benin hospital. The only 2 positive cases of W. bancrofti infection in the history of U.C.H., Ibadan were recorded from Indian patients (Ogunba 1971b) who arrived in Nigeria less than six months before their admission into the hospital. W.bancrofti is therefore very rare in Western State of Nigeria. An indirect evidence to confirm this suggestion was recorded in Ibadan where wild caught Anopheles gambiae, Anopheles funestus and Culex pipiens fatigans were found to be free from infection with filarial larvae (Ogunba 1971b). A.gambiae and A. funestus are vectors of W.bancrofti in West Africa. Even though the method Ngu and Konstam (1964) employed in assessing the prevalence of W.bancrofti infection in their patients is inefficient, they still showed that the cases of chronic lymphoedema seen in Ibadan are mostly caused by tuberculous adenitis and

chronic pyogenic infections rather then by W.bancrofti.

The prevalence of Acanthocheilonema perstans is lower than that of Loa loa in all the records in Western Nigeria. (Cowper 1967; Gilles 1965). In these studies, A.perstans was found mostly in Oyo, Owo, and Ekiti divisions of the State. It was also recorded in soldiers at the Military Hospital, Oke-Ado, Ibadan but the soldiers with A. perstans infection came originally from Katsina, Makurdi, Onitsha, Owerri and Ogoja in the Northern and Eastern States of Nigeria. The incidence of this infection is known to increase as one goes further East from the Western part of Nigeria and one commonly records concurrent infections with Loa loa and A. perstans (Cowper 1967). 2 of the infected soldiers were found to harbour both Loa loa and A. perstans infections.

Even though both the microfilariae of Loa loa and A. perstans could be seen during examination of blood sample of a patient, the two microfilariae differ so much both in size and nuclear arrangement that it is impossible to fail to distinguish the two from each other. Apart from having a sheath, which is lacking in A. perstans, mf Loa is at least twice as long as A. perstans and its nuclear arrangement is characteristic at both ends.

3.6.7 Examination of monkeys for Monkey Loa

There was very little opportunity to assess the role of monkeys in the epidemiology of loiasis in this study in Western Nigeria. The reasons are partly because of problems in getting monkeys alive from hunters in villages and also because of the

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high cost being demanded for captured living monkeys. 4 monkeys, were however studied in details comprising of two Cercopithecus mona mona, one Cercopithecus aethiops tantalus which came from Ondo division and one rhesus Monkey from Ijebu division.

These monkeys were examined regularly once a week both day and night for blood microfilariae but were negative for periods ranging from 3 to 6 months. Two male monkeys, Cercopithecus mona mona and C.aethiops tantalus became ill having developed swollen masses on right foot pad in C.m.mona and in the head in C.aethiops tantalus respectively. They were then killed and examined for parasites, specifically for adult and microfilarice of Loa in all their tissues. The other two monkeys were also later killed and similarly examined for Loa adults and microfilariae. None of the monkeys had Loa infection. Attempts to examine the large monkey population in the University of Ibadan Zoo were unfavourable because the Zoo keeper believes that monkeys in capitivity are easily disturbed emotionally. Since he believes that monkeys in the Zoo are already subjected to enough stress, he was of the opinion that they should not be further disturbed by blood examinations.

CHAPTER IV

VECTOR STUDIES

4. 1 Introduction

Roche (1948) showed that four species of Chrysops, Chrysops silacea, Chrysops dimidiata, Chrysops longicornis and Chrysops distinctipennis had been recorded in Nigeria but only the first three species were found in the forest where human loiasis occurs. Connal (1921) recorded the infection with Loa loa in the C. silacea and C.dimidiata collected at Sapele while Davey and O'Rourke (1951) observed the breeding of C.silacea and C.dimidiata along the edges of River Okhuo in Benin. Crewe (1952) collected larvae from the breeding sites in the Cameroon rain forest and reared them in the laboratory. From the larvae collected, he obtained not only the expected well-known species of Chrysops but also several species which had not previously been recorded, or had been recorded only from the nests of mason wasps in the Congo (Bequaert, 1932).

There had been many efforts to determine in the past possible development of Loa loa in other insects. A slight development of Mf Loa was shown (Leiper 1914) in Hippocentrum trimaculatum and Haematopota corallipes, but there was no development in Stomoxys nigra, Glossina palpalis, Tabanus par, T.socialis, T.fasciatus T. secedens, Cimex rotundatus and Pulex irritans. Woodman (1949) showed development of mf Loa in Haematopota species up to the third day but failed to obtain development in Stomoxys and Glossina species.

In this study the writer's objectives were to find out the vectors of loiasis in the Ijebu and Remo divisions which were elaborately studied for epidemiological data in the previous chapter. It was also decided to examine the role of mosquitoes that feed on man in the transmission of loiasis since there is yet no record in the literature of any development of mf Loa in mosquitoes.

4.2 Materials and Methods

4.2.1 Chrysops collection and dissection

Chrysops species were collected under the forest canopy along the rivers and streams in the villages visited in the Ijebu and Remo divisions. Collections were also made in the bush close to the village schools by the collecting party consisting of the writer, his assistant and a few school pupils who received monetary reward for their catch. The time spent in looking for Chrysops is expressed as man hours in Table 7. The species and number collected in each village were recorded. Each Chrysops was anaesthetised with chloroform, dissected and examined for developing larvae.

The wings were removed, and the head, proboscis, thorax, fat bodies, abdomen and legs were teased apart separately with fine dissecting needles in physiological saline and examined with a stereo binocular compound microscope for developing larvae of filariae. The results of the dissection were recorded.

4.2.2 Mosquito collection and dissection

Twenty areas representing a cross-section of Ibadan city were selected, and for eighteen months weekly visits were made to these

areas at about 0700 hour to 1000 hours. The houses visited have plastered walls and roofs, and the rooms usually lead to a common hall-way. Each room has an average of 1,200 cubic feet and is fitted with a door and at least one 4 x 3 feet window. Space-spraying was carried out in the selected rooms with a quick acting but non-residual insecticide (Nuvan) which knocked down the mosquitoes that had entered and rested in the rooms during the night. The immobilised mosquitoes were carefully picked and identified, and a record was made of the Culex pipiens fatigens and the Anopheles species [redacted] from each room. The female mosquitoes were dissected and examined with the dissecting compound microscope for filaria larvae.

Adult Mansonia africana were collected twice a week at about 2100 hours around staff quarters close to the fish pond in the University of Ibadan where they breed, and were similarly dissected for filaria larvae.

4.2.3 Maintenance of mosquitoes in the laboratory

4.2.3.1 Aedes aegypti, Anopheles gambiae, An. funestus, and Culex pipiens fatigens

The Ibadan strains of these mosquitoes were maintained from the egg to the adult stage in the laboratory. Wild caught mosquitoes were fed on guinea-pigs for egg-laying. The eggs laid were hatched in straw infusion and the larvae were fed on finely ground rabbit food fortified with powered yeast. Pupae were collected and transferred daily to bowls which were placed in cages for emergence of adults. The adults for each species were kept in separate cages (Appendix 4) and were sustained on sugar lumps and water from moistened cotton wool before blood meal is offered to the mosquitoes.

4.2.3.2 Mansonia africana

A preliminary attempt was made to breed M. Africana from the

egg to the adult stage and substituting the use of expanded polystyrene for the water plants.

Eggs of M. africana were collected from the under surface of leaves of the water plant (Pistis stratiotes) in ponds where M. africana breeds naturally. The eggs were hatched in pond water and the larvae were transferred into bottles fitted tightly with expanded polystyrene (Appendix 5) and filled with the same pond water. The larvae were fed as above. The water in the bottle was replaced with fresh pond water every two days and after the developing larvae had previously been strained. The bottle was covered with mosquito netting and the emerging adults were aspirated daily into a mosquito cage.

Even though some adult M. africana emerged and were collected by this method, the mortality rate at both the larval and pupal stages was very high (over 60%); therefore another method was sought for obtaining a good supply of freshly emerged adults.

Mature larvae and pupae of M. africana were collected daily from the University of Ibadan fish pond where they breed naturally, and they were transferred into a fish tank (Appendix 6) with some water plants(Pistia stratiotus) to which they attached. The larvae were fed as above. The fish tank was covered with mosquito netting and the emerging adults were aspirated daily and transferred

into a mosquito cage. The adult mosquitoes were sustained on sugar lumps and moistened cotton wool like the other mosquitoes.

4.2.4 Experimental feeding of mosquitoes with blood containing Mf Loa

The apparatus (Ogunba 1967) for feeding the mosquitoes was adopted from that of Rutledge et al (1964). They were connected in series to enable many batches of mosquitoes to be fed simultaneously. It is a conical tube with two open ends and an outer water jacket (Fig. 7 and 8). The wider open end with diameter about 4cm is covered during feeding experiments by a membrane which in this series of experiments was Parafilm and which was fastened to the feeder by a rubber band.

Two days old citrated blood containing only microfilariae of Loa loa was obtained from blood bank and introduced into the feeder through the narrower end with diameter of 1/2cm. Kershaw et al (1954) and later Bird and Manson (1961) have shown that the microfilariae in citrated and refrigerated blood would survive in the recipient's blood if transfused within a week, therefore the microfilariae of Loa loa in the citrated blood used for experimental feeding are normal. The outer water jacket was connected to a heating unit and it warmed the blood in the inner glass tube to a constant temperature at 37°C.

Approximately 10 mls of citrated blood containing about 10 microfilariae of Loa loa per ccm was introduced into each artificial feeder already fitted with the Perafilm membrane. In a preliminary study, it was observed that laboratory bred mosquitoes were not ready for a blood meal until after 5 days of emergence as adults. Adult mosquitoes were therefore first sustained on sugar and water

Fig. 7 The artificial feeding apparatus connected in series for feeding of mosquito batches simultaneously. (The water in the bath is heated by the heating unit and circulated into the feeders through the connecting rubber tubes).

Fig. 8 The artificial feeder (enlarged) to show fed mosquitoes. (The glass cylinder is covered on both ends with mosquito netting to enable the mosquitoes to imbibe blood through the membrane).

for the five or six days prior to blood meal. Mosquitoes to be fed blood meal were however starved for a period of twenty four hours before the blood meal so that they can easily feed to repletion when the blood meal is offered.

Mosquitoes were fed in glass cylinders (10 x 7 cm) which were covered at the two ends with mosquito netting and rubber band. Each glass cylinder containing mosquitoes was applied to a feeder for a feeding period of two hours. In preliminary studies, it was observed that those mosquitoes that meant to feed through the membrane usually feed to repletion within the first hour of exposure to the blood meal.

The gorged mosquitoes were transferred singly with an aspirator in 9 x 4 cm glass tubes (Fig. 9). The tubes contained moistened cotton wool to a depth of about 2 cm and on this was placed a circle of filter paper on which mosquitoes laid their eggs. A twig was placed in each tube to provide support for the gorged mosquitoes and to reduce the high mortality rate often suffered by recently fed mosquitoes (Ogunba, 1966). The open end of the tube was covered with mosquito netting held with a rubber band. Each isolated engorged mosquito was provided with a lump of sugar on top of the netting and this sustained the mosquitoes. The isolated mosquitoes were dissected 12 days after an infecting meal and all the mosquitoes that died before the 12th day were similarly dissected. The number and the state of development of the filaria larvae recovered from them were recorded.

Fig. 9 Glass tube containing fed mosquitoes and a twig on which the mosquito can rest. (The cotton wool at the bottom of the tube is moistened, and it controls the humidity in the tube).

4.3 Results

4.3.1. Chrysops species

Table 7 shows the female Chrysops species collected in the Ijebu and Remo divisions, and their infection rate with Loa loa. Chrysops silacea was caught in all the villages except Iroju and Ijebu-Ijesa whilst C.dimidiata was caught in fifteen out of the twenty two villages, C.longicornis was recorded in two villages, Ishara and Ode-Remo, both in Remo division. The overall infection rate with C.silacea and C.dimidiata is 3.0% and 3.7% respectively. No male Chrysops was caught in any of the villages.

Other members of the Family Tabanidae collected along with Chrysops species include Hippocentrum versicolor, Haematopota decora Tabanus biguttatus, Tabanus par and Tabanus pluto. There was no filarial infection recorded in any of the species when the specimens collected were dissected and examined.

4.3.2 Anopheles, Aedes and Culex species

Table 8 shows the prevalence of C. p. fatigans and Anopheles species (An.gambiae and An funestus) caught in sleeping rooms in Ibadan during an eighteen month period. Both Anopheles species and C. p. fatigans were collected throughout the months of the years.

2128 C.p. fatigans, 994 An gambiae and An.funestus, and 740 M. africana caught wild in Ibadan city failed to reveal developing filarial larvae on dissection.

Table 9 shows the dissection results of mosquitoes experimentally fed on blood containing mf Loa. Both Ae. aegypti, Anopheles gambiae and C. p. fatigans failed to support the development of mf Loa even though they ingested enough microfilariae with blood.

Chrysops species collected in Ijebu and Remo divisions and their infection rate with filarial larvae.

VILLAGE	No of Man hours	NUMBER OF CHRYSOPS SPECIES COLLECTED			CHRYSOPS SPECIES INFECTED WITH LARVAE					
		C.silacea	C.dimidiata	C.longicornis	C.silacea		C.dimidiata		C.longicornis	
					Number	%	Number	%	Number	%
Okun-Owa ǂ*	18	46	12	-	2	4.4	0	-	-	-
Omu ǂ	18	35	8	-	2	5.7	0	-	-	
Ala	12	40	-	-	-	-	-	-	-	-
Mobelufon ǂ*	18	41	10	-	1	2.4	1	10.0	-	-
Idowa	12	25	-	-	-	-	-	-	-	-
Ibefun	12	32	2	-	-	-	0	-	-	-
Ososa	12	28	2	-	-	-	-	-	-	-
Ishara ǂ*†	18	51	10	1	3	5.9	-	-	-	-
Ago-Iwoye	18	36	5	-	1	2.9	-	-	-	-
Oru/Awa*	18	49	9	-	2	4.1	-	-.	-	-
Ikija/Owu	12	15	5		-	-	1	20.0	-	-
Ijebu Ife	16	39	6	-	1	2.7	-	-	-	-
Felafonmu[a]	8	15	2	-	-	-	-	-	-	-
Ijebu-Igbo[b]	18	52	9	-	2	3.8	1	11.1	-	-
Odogbolu	18	41	6	-	1	2.4	-	-	-	-
Aiyepe[ab]	12	30	5	-	1	3.3	-	-	-	-
Ode-Remo ǂ*†	20	69	17	1	4	5.8	1	5.9	-	-
Isire	10	8	0	-	-	-	-	-	-	-
Irolu	12	-	-	-	-	-	-	-	-	-
Ijebu-Ijesha	10	-	-	-	-	-	-	-	-	-
Iperu	18	39	-	-	1	2.6	-	-	-	-
Ogere	12	35	-	-	4	11.4	-	-	-	-
TOTAL	322	725	108	2	28	3.5	4	3.7	-	-

* Hippocentrum versicolor collected † H. cm. topot. decore collected ǂ Tabanus par collected a Tabanus biguttatus collected b. Tabanus pluto collected

TABLE 8

Prevalence of Culex pipiens fatigans, Anopheles gambiae and Anopheles funestus caught in sleeping rooms in Ibadan City.

MONTH	No of Premises Visited	CULEX PIPIENS FATIGANS CAUGHT				ANOPHELES SPECIES CAUGHT		
		No. of Rooms Sprayed	No of Females	No of Males	No. of Females Per Room Sprayed	No. of Females	No of Males	No of Females Per Room Sprayed
1968 Nov.	24	49	298	106	6.1	11	4	0.2
Dec.	30	59	787	241	13.3	33	9	0.6
1969 Jan.	42	90	953	340	10.6	64	3	0.7
Feb.	37	65	780	292	12.	82	11	1.3
Mar.	36.	68	305	96	4.5	21	0	0.3
Apr.	31	53	149	56	2.8	37	3	0.7
May	20	36	65	32	1.8	102	18	2.8
June	24	48	34	9	0.7	29	1	0.6
July	34	64	66	20	1.0	77	2	1.2
Aug	24	52	22	5	0.4	16	0	0.3
Sept.	15	38	54	6	1.4	51	1	1.3
Oct.	27	69	129	48	1.9	22	0	0.3
Nov.	-	-	-	-	-	-	-	-
Dec.	-	-	-	-	-	-	-	-
1970 Jan.	53	99	529	223	5.3	242	3	2.4
Feb.	59	120	784	447	6.0	131	7	1.0
Mar.	20	40	123	27	3.1	76	0	1.9

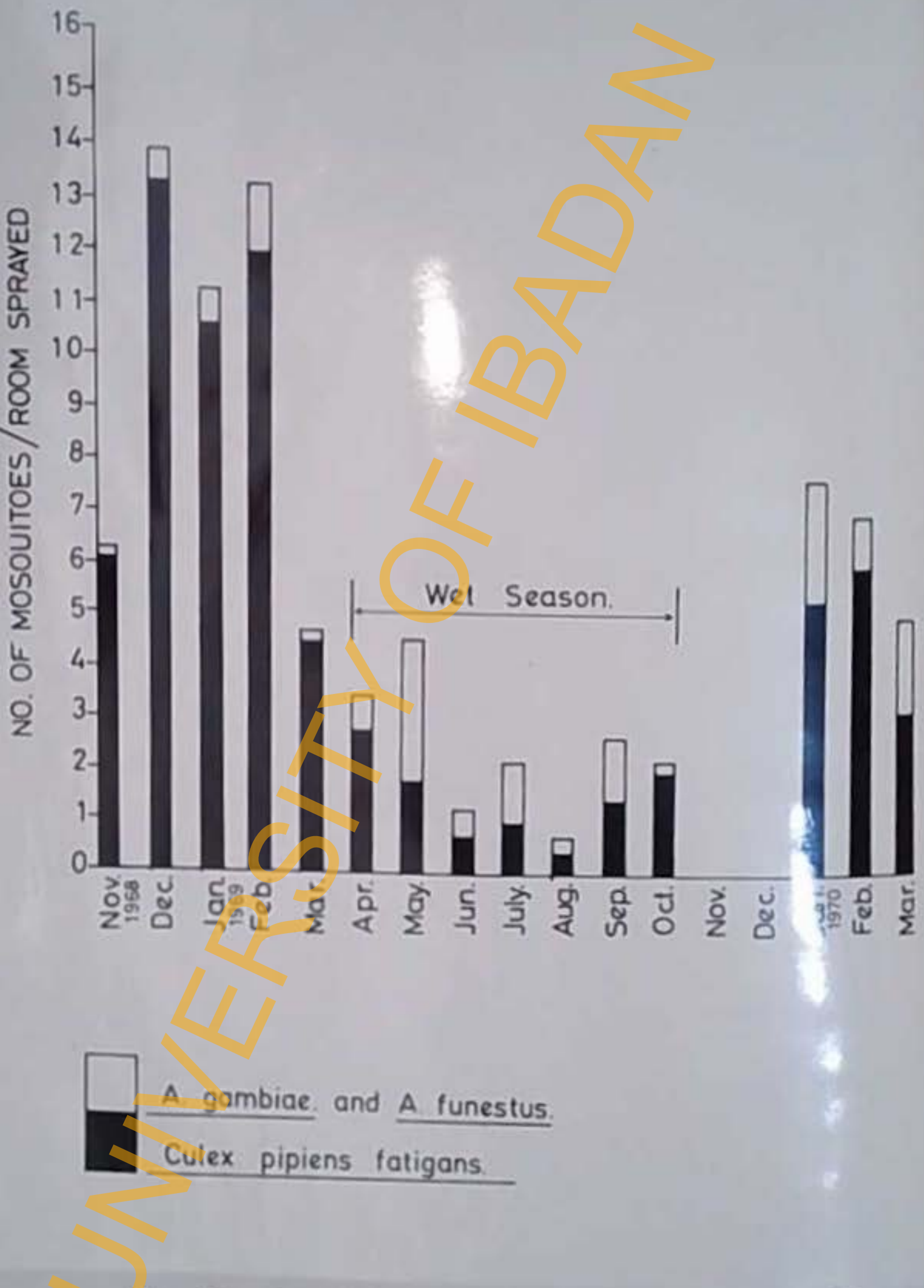

Fig. 10 Population densities of Culex pipiens fatigans Anopheles gambiae and Anopheles funestus caught in sleeping rooms in Ibadan city.

4.3.3 Mansonia africana

A high mortality was experienced in the breeding of this mosquito at the early larval stages and soon after pupation. As much as 50% reduction in number was commonly recorded during these stages hence the other method of collecting mature larvae and pupae for adult production was devised.

Some of the fed and isolated mosquitoes that were later dissected were found to contain eggs in their abdomen which could have been laid if the mosquitoes had the right facilities for oviposition. Twenty seven mature third stage larvae were recovered from the 10 mosquitoes as follows - 12 larvae from the proboscis, 10 from the head, 2 from the thorax and 3 from the abdomen.

The larvae recovered from the mosquitoes varied in size and had the typical head with a pair of amchius on either side of the oral opening. The range of size was between 1634u x 30u to 2210 x 31u. The oesophagus is undivided and is between 10 and 15 per cent of the entire length of the larvae. Three prominent papilla-like structures were attached to the tip of the tail, one dorsal and the other two ventro-laterally placed and each was surrounded by a rounded fleshy structure. These larvae compare favourably with the previous description (Williams 1960) about mature third stage larvae of Lagoa recovered from infected Chrysops species.

Figure 10 shows the marked seasonal variation with high population densities observed during the dry months in Cx. fatigans in Ibadan. The density of the Anopheles species is comparatively low.

4.4 Discussion

Leiper (1914) and later Connal and Connal (1922) showed that C.silacea and C.dimidiata are the vectors of human loiasis. The relative proportions of the two species are known to differ in different areas and Oldroyd (1957) suggested that there may be a geographical alternation between the two species. Both these species have been recorded in the Ijebu and Remo divisions of Western State of Nigeria (Table 7), although C. silacea was commoner in all the villages where both C.silacea and C. dimidiata were caught.

In two villages, Iralu and Ijebu-Ijesha, both in Remo division, no Chrysops species were caught in the bushes near the schools. There are no streams or rivers in either village and therefore the search for Chrysops was limited to the bush; the absence of Chrysops is explained by the absence of breeding sites in these villages. In five other villages, Ala, Idowa, Isiro, Iperu and Ogere, no C.dimidiata were found even though the man hours spent in looking for the C.dimidiata were similar to those spent in Aiyepe and Owu/Ikija where some catches were made. There is thus a strong indication that C.dimidata does not breedd in the former group of villages because a reasonable number of C.silacea was caught in each of the villages at the same time it was being sought.

The infection rate of C.silacea with Loa loa varies from 2.4% in Mobalufon to 11.4% in Ogere, and the overall infection rate is 3.5% whilst that of C.dimidiata is 3.7%. The differences in the infection rates of C.silacea and C.dimidiata in the individual

villages is probably due to the greater relative abundance of C.silacea and thus the greater opportunities for fly/infected man contacts to be made by this species. Mature third stage larvae were recovered mainly from the thorax and head of both species and this record confirmed the observations of Connal and Connal (1922) that the two species are natural vectors of human loiasis in Southern Nigeria. C. longicornis was recorded in Ishara and Odo-Remo but they were not infected with Loa loa.

Ishara and Odo-Remo are only about three miles apart and they probably share the same breeding site for this species. Crosskey and Crosskey (1956) recorded C.silacea, C.dimidiata, C.longicornis and C. distinctipenis in Nigeria and further showed that C.silacea were confined to Southern Nigeria and prevalent throughout the year, thus confirming the year round transmission of Loa loa by the two two species.

There is no record in the literature of mosquitoes supporting the development of mf Loa, although about fifteen mammalian filariae including Wuchereria bancrofti, Brugia malayi and Brugia pahangi are transmitted by some species of mosquitoes (Nelson 1959). Anopheles gambiae, A. funestus and C.P.fatigans would support the development of the microfilariae of W.bancrofti if imbibed with blood (Hawking 1957, Nelson et al 1962). Table 8 shows that both mosquitoes would enter the sleeping rooms in Ibadan and feed on humans in the night hours when mf loa would be absent or at best would be very scanty in the peripheral blood.

The dissection of An.gambiae, An.funestus and C.P.fatigans caught in sleeping rooms in Ibadan failed to reveal any developing filaria larvae. This is primarily because W.bancrofti is rare in Ibadan city (Ngu and Folami, 1965; Cowper, 1967). It was, however, not known whether the non-recovery of the developing larvae of Loa loa in the wild caught mosquitoes was due to lack of contact with the mosquitoes because of the diurnal periodicity of mf Loa The microfilariae of Loa loa are absent or very scanty in the peripheral blood in the night hours when those mosquitoes mentioned above are actively in search of a blood meal. Table 9 shows however that the experimentally fed C.p.fatigans, Ae.aegypti, An.gambiae also failed to support the development of mf Loa even though the blood contained enough microfilariae (at least 10 mf/cmm blood) to infect the mosquitoes if they were susceptible (Macdonald 1962). The failure to recover the developing larvae of Loa loa from the experimentally fed mosquitoes would therefore possibly be a result of a barrier possibly physiological, in the mosquitoes, which makes them refractory to Loa loa infection and thus prevents penetration of the thorax by mf Loa from the stomach (Ogunba 1969). There is further evidence (Table 9) that mf Loa was imbibed with blood during an infecting meal b y the mosquitoes because mf Loa was recorded from the stomach of each of the 31 experimentally fed mosquitoes that died within two days of feeding. Ogunba (1969) showed a gradual decline in the microfilaria content in the stomach of C.p.fatigans with time up to 48 hours when fed with the microfilariae of Brugia pahangi to which it was refractory.

TABLE 9

Showing the dissection results of mosquitoes experimentally fed on blood containing microfilariae of *Loa loa*.

MOSQUITO SPECIES	NUMBER OF MOSQUITOES FED	NUMBER OF MOSQUITOES DISSECTED											
		1-2 days after feeding		3-4 days after feeding			5-9 days after feeding		9-12 days after feeding			With larvae from 2 days after feeding	With mature larvae
		A	B	A	B	C	A	C	A	C	D		
Aedes aegypti	142	0	5	2	0	0	1	0	134	0	0	0	0
Anopheles gambiae	180	0	9	3	0	0	4	0	164	0	0	0	0
Culex pipiens fatigans	230	0	15	8	0	0	4	0	203	0	0	0	0
Mansonia africana	234	0	12	5	0	1	4	0	201	1	10	5.12	4.27

A - With no filaria larva.

B - With microfilaria as in periphera blood.

C - With developing filaria larva.

D - With mature third filaria larvae of *Loa loa*

The mf Loa fed to Ae.aegypti, An. gambiae and C.p. fatigans would therefore have either been excreted from the mosquitoes' stomachs over a period of time or digested after its failure to penetrate the thorax of the mosquitoes.

Twelve out of 234 experimentally fed Mansonia africana supported the development of mf Loa (Table 9). Six mosquitoes died within 3 and 4 days of having an infective meal. Larval development was recorded only in 1 mosquito while in the other 5 mosquitoes there were neither microfilariae nor developing larvae recovered from their tissues. It is possible that these 5 mosquitoes were individually refractory to Loa loa infection. If this is true, the microfilariae that they ingested with blood would fail to develop and would be eliminated from the stomach with time. The failure to recover developing larvae from wild caught M.africana is expected because these mosquitoes feed at night hours with biting peak around 2200 hours, a time when mf Loa is very scanty in or absent from the peripheral circulation. Thus M.africana has nochance of transmiting humen Loa loa in nature

Since simian Loa loa exhibits nocturnal periodicity and M. africana females are actively biting after sunset (Kerr 1933) it would not be difficult for this mosquito to pick up the monkey microfilariae provided it gets up to the canopy level where monkeys usually sleep. Kerr (1933) further showed that Mansonia africana was amongst the most abundant mosquito species caught in Lagos and further observed that it is strongly anthropophilic. These observations have also been confirmed in the campuses of both the University of Ibadan and the University College Hospital, Ibadan

(Okorie 1972). Therefore M. africana will only be attracted to monkeys and other mammalian hosts in the absence of human hosts.

The importance of the finding that M. africana would support the development of Loa loa to the mature third stage larva lies in its experimental value in research. The natural vectors of Loa loa, Chrysops species, undergo a very long larval life cycle and are very difficult to rear in the laboratory, therefore M. africana would serve as a very good substitute for Chrysops species in experimental loiasis because it has the advantage of a short life cycle.

The significance of development of mf Loa to the infective stage in M.africana lies in the fact that both Chrysops species the natural vector of Loa loa and M.africana are not phylogenetically very related, apart from being members of the large Order Diptera.

Nelson (1961) suggests that different orders of arthropods are unlikely to transmit same filaria parasites because of the very strict inter host specificity that had been noticed in the Family Filaroidea. It was also suggested that filarial larvae not only show host specificity but also tissue specificity with marked preference for particular cells. For instance, the Brugia, Onchocerca and Sertaria species would develop in the thoracic muscles

of their vectors while Dirofilaria repens and Dirofilaria immitis would develop in the malphigian tubules, but Loa loa and A.reconditum would develop only in the fat body cells of their insect vectors. It is also generally believed that different species in same genus of filaria would develop in similar tissues of the same members of arthropod family (Nelson 1961).

Exceptions to this strict inter host specificity have been observed and recorded especially in the genus Acanthocheilonema (Dipetalonema) A.perstans and A. streptocerca are known to develop in the thoracic muscles of Culicoides whilst A.arbuta develops in the thoracic muscles of mosquitoes. A.vita however develops in the skeletal muscles of hard and soft ticks. A reconditum and A.menson-bahri develop in the fat bodies of fleas. It is therefore obvious that the strict inter host specificity associated with the Family Filariidae has completely broken down in the genus Acanthocheilonema because its species not only develop in different families of insects but also in different tissues.

Leiper (1914) and later Woodman (1949) had shown some development of of Loa in Hippocentrum trimaculatum and Haematopota cordigera both members of the Family Tabanidae to which Chrysops, the vector of Loa loa belongs. Several other insects including Glossma palpalis, Stomoxys nigra, Cimex rotundatus, Pulex irritans Tabanus par and T.socialis have failed to support the development of Loa loa in their tissues. There had not been any record of development of Loa loa in any mosquito species, but Brugia patei a filaria of cats, dogs, and bush babies has been recorded in Mansoniauniformis and M.africana (Lawrence and Foster 1961).

There is yet no record of B.patei infection in Man in Nigeria. Even though the development of B. patei is similar to that of Loa loa in M. africana with both of them developing in the intersegmental fat cells, the infective, stages of these filariae are easily distinguished from each other. The oesophegus of the infective stage of Brugia species including B.patei is about 30% to 40% of the larval length whereas that of Loa loa larvae recovered from M. africana in this study is less then 15% of the total larval length. The larvae recovered from the M. africana mosquitoes were certainly Loa loa infective larvae because only blood containing microfilariae of Loa loa was fed to the mosquitoes. Measurements of their sizes and their oesophageal ratio were not significantly different from those recorded from Chrysops species (Williams 1960).

CHAPTER V

MEASUREMENTS OF THE LOA LOA POPULATION IN WESTERN NIGERIA

5.1 Introduction

Sharp (1923) reported the various methods of differentiating between the microfilariae of Loa loa and Wuchereria bancrofti in addition to giving the detailed description of mf Loa. Duke and Wijers (1958) studied the relationship between human and simian Loa loa in the rainforest zone of Cameroons, and pointed out the various morphological and behavioural differences between the two strains. As shown in the previous chapter, the Loa loa in the Ibadan area will develop to the third stage larvae in Mansonia africana. In view of the fact that complete development of mf Loa in mosquitoes has not previously been reported, it was considered possible that such development might be a peculiarity of the "Ibadan population." Therefore the morphology of this population was examined to determine whether it differed from other known strains.

5.2 Materials and Methods

Adult worms were recovered from six patients, and these worms were transferred from saline to hot 70% alcohol and preserved. Blood samples containing microfilariae were also collected from the patients and thick blood films were made from them.

TABLE 10

Measurements of adult Loa loa recovered from six patients in Western Nigeria.

PATIENT'S NUMBER	LENGTH OF WORMS RECOVERED IN MICRON UNITS	
	MALE	FEMALE
12205/69	-	46
10008/69	-	42
222599/69	28	55
11224/69	29	69
AT	-	46
	-	52
	?	43
	-	34
	-	42
NI	2.9	70
	-	46
	-	43
	-	57

Thick blood films were made from 35 other infected people from the Savannah and rain forest zones comprising blood donors and school pupils, infected with Loa loa. The thick blood films obtained from the three sources were air-dried, dehaemoglobinised and stained in hot Mayers' haemalum (see Appendix 2).

At least eight microfilariae were selected after every four from the centre of each stained blood film and they were measured by Camera lucida. A pencil line was drawn from the tip of the head down the centre of the body, following all curves, as far as to the tip of the tail of the microfilariae. The sheath of the microfilaria was not measured. A sewing thread was run along the length bof the line drawing and measured. A total of 445 microfilariae were measured from 39 infected people. The range of lengths and the mean length of the microfilariae from each of the 39 infected people was calculated. The overall mean length and mode of the 445 microfilariae from the 39 infected people were also calculated.

5.3 Results

Table 10 shows the measurements recorded for sixteen adult worms(three males and thirteen females) recovered from four patients. Table 11 shows the range measurements and the mean lengths of microfilariae from each of the 39 infected people.

Figure 11 shows the frequency of distribution of the mean lengths recorded from the microfilariae of the 39 infected people. Figure 12 shows the frequency distribution of the lengths of 445 microfilariae.

TABLE 11

Showing the range and mean of lengths of eight microfilariae from thirty nine patients.

Patient's Number	Range of lengths of eight Microfilariae measured	Mean length of Microfilariae
SC 2	220.5u - 308.7u	267.5u
SC 3	200.9u - 289.1u	274.4u
SC 4	205.8u - 284.2u	259.7u
SC 5	186.2u - 298.9u	282.2u
SC 6	205.8u - 274.4u	246.5u
SC 7	180.9u - 284.2u	252.3u
SC 15	156.8u - 254.8u	213.1u
SC 33	206.9u - 278.2u	230.6u
SC 50	176.4u - 249.1u	201.6u
SC 57	176.4u - 259.7u	211.6u
SC 65	171.5u - 220.5u	240.1u
SC 72	201.9u - 274.4u	229.3u
SC 75	156.8u - 289.1u	201.8u
SC 106	206.8u - 274.4u	240.1u
SC 114	225.4u - 345.0u	237.6u
9103/68	156.8u - 289.1u	201.8u
12484/68	225.4u - 254.8u	244.1u
7935/68	230.3u - 284.2u	250.8u
7695/68	210.7u - 289.1u	235.2u
7816/68	220.5u - 254.8u	211.3u
9273/68	171.5u - 240.1u	215.6u
12111/68	196.0u - 259.7u	223.4u
11184/68	201.9u - 249.9u	217.0u
10498/68	186.2u - 249.9u	220.5u
10640/68	230.3u - 264.6u	249.9u
10747/68	181.7u - 284.2u	227.8u
7704/68	210.7u - 259.7u	235.2u
7940/68	249.9u - 284.2u	268.5u
7680/70	215.6u - 254.8u	244.5u
777/70	240.1u - 259.7u	247.4u
12205/68	210.7u - 264.6u	240.1u
10008/69	196.0u - 284.2u	135.2u
222593/69	225.4u - 245.0u	237.6u
11224/69	205.8u - 279.3u	243.5u
NI	156.8u - 217.1u	184.1u
AT	156.8u - 233u	185.6u
NHA VI[T]	132.6u - 222.0u	207.91u
ABH BV	156.8u - 217.1	182.5u
9833FVA	182.8u - 220.5	204.2u

OVERALL MEAN OF THE 445 MICROFILARIAE = 230.4u

5.4 Discussions

The round smooth transluscent bosses on the cuticle of the adult Loa loa are characteristic and diagnostic. The curved posterior end with the pair of copulatory spicules in the male worms were useful in sexing the adult worms. The hot 70% alcohol fixed the adult worms in an extended form and this made their measurements very easy.

Both the nuclear arrangement and the sheath outline are distinctly visible when the microfilariae are stained in hot Mayers haemalum. Therefore it was very easy to identify mf Loa.

Table 10 showed that only sixteen adult worms(3 males and 13 females) were measured. They are all morphologically similar to the description of Loa loa in the literature although their sizes are slightly smaller. The three male worms are slightly smaller than those recorded by Looss (1904) which range from 30-34mm x 0.35-0.43mm. Eight of the female worms also fall short of the range, 50-70mm x 0.5mm, recorded by Looss (1904). Although the observed differences are not significant, the situation might have been different if a larger number of adult worms were measured Adult Loa loa from humans are however difficult to recover in large numbers except when a patient being operated on is heavily infected. Unfortunately, the writer has not met such a patient. Some of the worms measured in this study might have been young adults that could have grown larger, alternatively, they might have been mature adults that are dwarfs in the population or mature adults of normal size in a "small race" population of Loa loa.

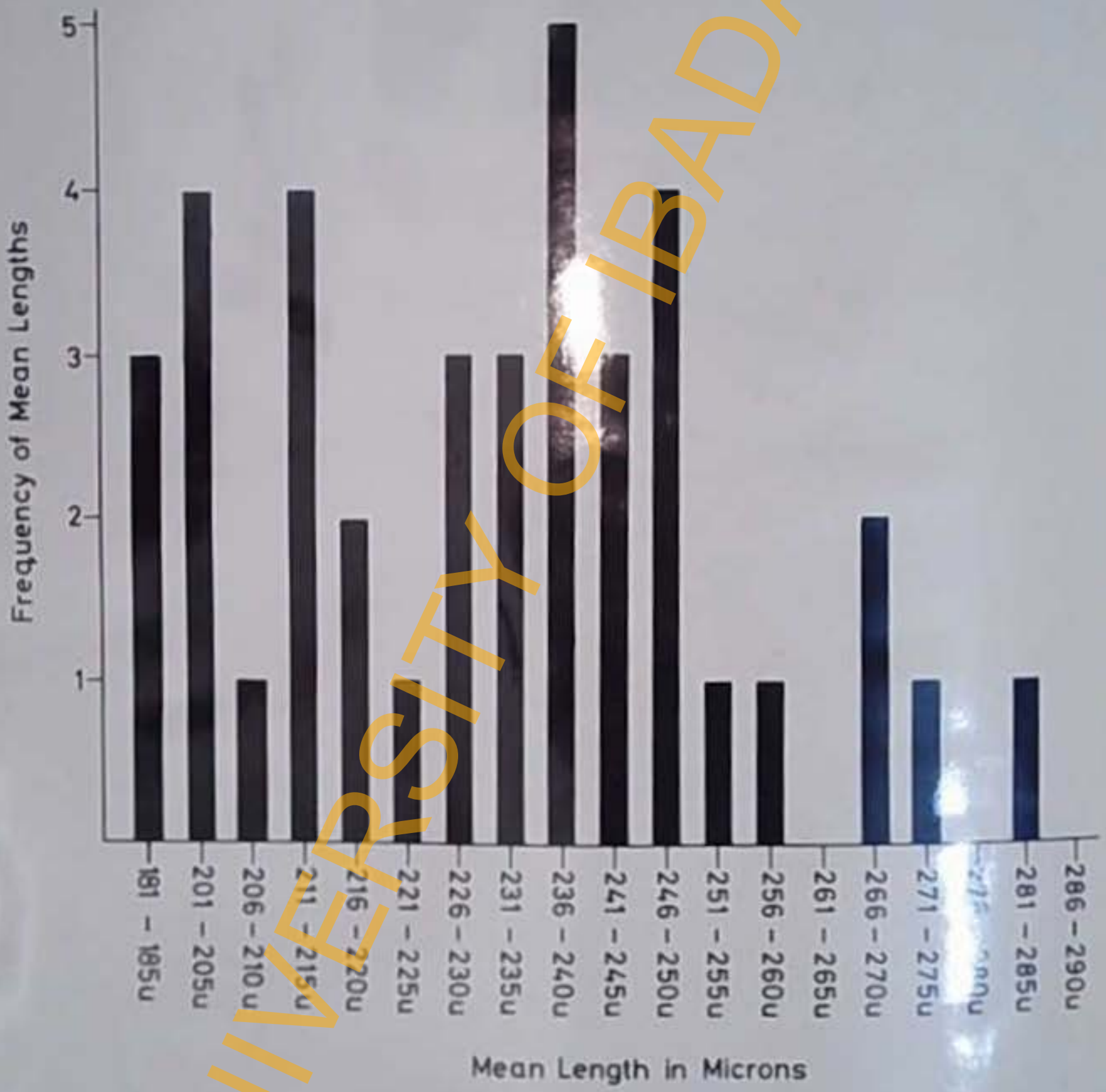

Fig 11. Showing the frequency of distribution of the mean length of microfilariae from 39 infected people.

The effects of premunition on helminths are well known and these might have been manifested in the small size observed in the adult worms in view of the massive helminth infection which is common in this environment. Finally the fixation of the worms in hot alcohol might have resulted in some shrinkage; however the size differences observed in this study could not have been due to shrinkage because the method of fixing employed is similar to Looss' (1904).

The length of the microfilariae showed a very wide range from 156.8u to 308.7u, with the mean as 236.4u and mode from 196 to 200u (Figure 12). Duke and Wijers (1958) recorded a range of 217.5u to 280u from 334 microfilariae from three men and their mode was 247.5u.-250u Looss (1904) recorded length range of 250u to 300u. Statistical analysis using the Student T-test shows that the difference between the sizes of the microfilariae measured in this study and that of Duke and Wijers' (1958) is significant ($P < 0.001$) (Assumed standard Deviation for Duke and Wijers' (1958) study is 30u). By inference therefore the difference in size between mf Loa from Western Nigeria and mf loa measured by Looss (1904) must be significant, because Looss (1904) recorded higher measurements than Duke and Wijers (1958).

The staining technique employed in this study and in that of Duke and Wijers (1958) is similar and could not have been responsible for the observed difference in the microfilaria size. The variation recorded in the individual sizes of the microfilariae (Fig. 12) is that expected from a normal population although the mean lengths of microfilariae from the individual infected people gave a wide scatter (Fig. 11).

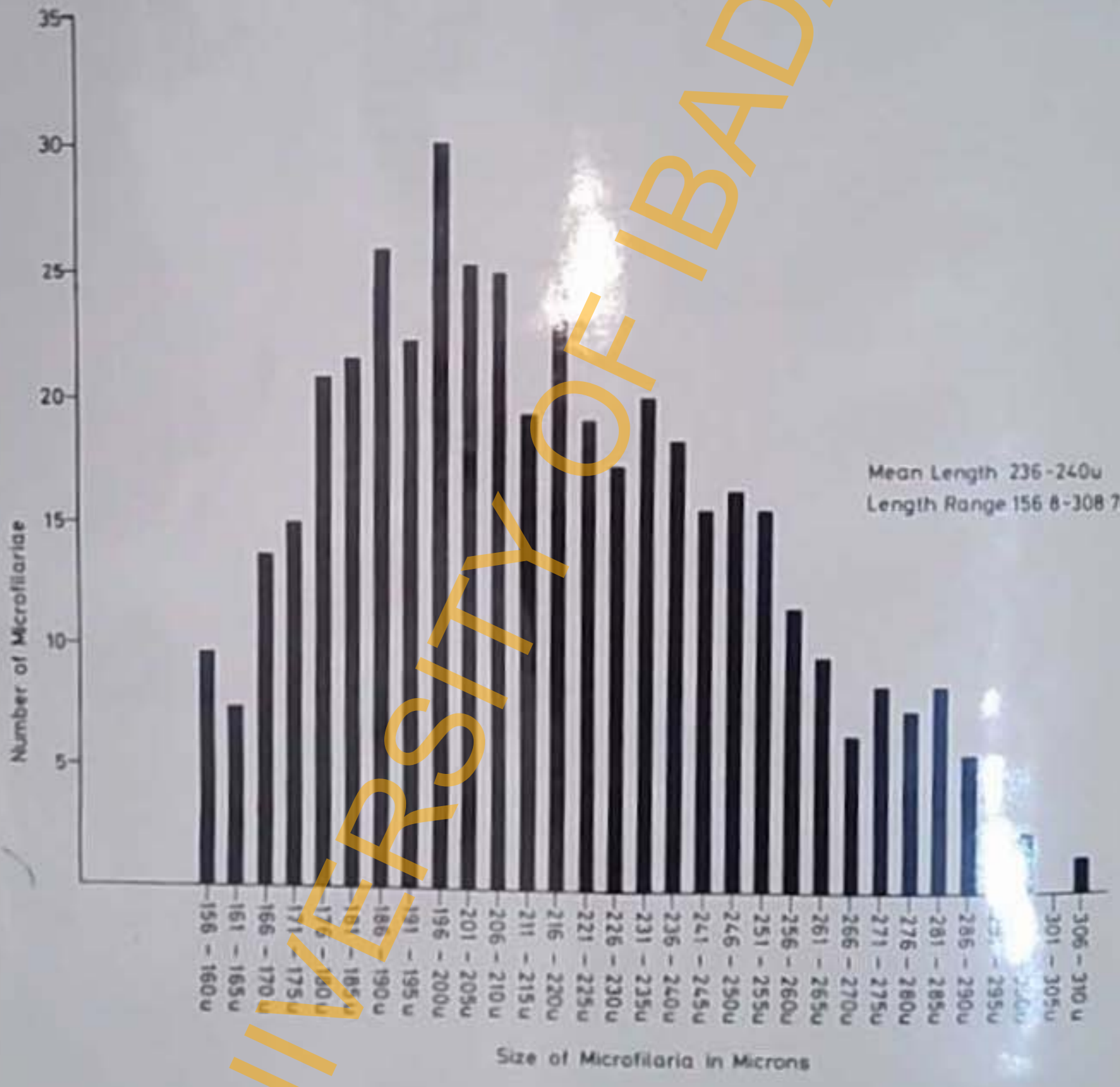

Fig.12. Showing the frequency distribution of the lengths of 445 microfilariae

The mean lengths of the microfilariae from the fifteen school children and fifteen blood donors (Figure 13) were calculated separately and found to be 241-245u and 231-235u respectively. Both these populations of microfilariae were obtained from infected people drawn from all over the State and the difference observed from the mean lengths is probably due to factors other than strain difference. Sodium citrate was used to preserve blood obtained from the blood donors infected with loiasis and this could be partly responsible for the shrinkage in the size of microfilariae from the blood donors. Another possible factor may be premunition.

The school children's exposure to infection would not be as long as that of the blood donors, who are mainly adults, hence the Loa loa in the school children would still be expected to undergo normal development. The adult donors on the other hand, would probably have been exposed to infection for a longer period and the effects of premunition on the worms, both adults and microfilariae, could result in the reduction of their sizes as is observed in this study.

It was shown in the preceding chapter that the Mf Loa from blood donors in Ibadan developed in Mansonia africana to the third stage larva. It is however necessary to comment that although the blood donors were resident in Ibadan at the time of the blood donation, they probably did not all obtain their infection in the Ibadan division alone. Ibadan is a big commercial centre, and it draws the vast majority of its population from the various divisions of the Western State. Furthermore, the blood donors are adults who

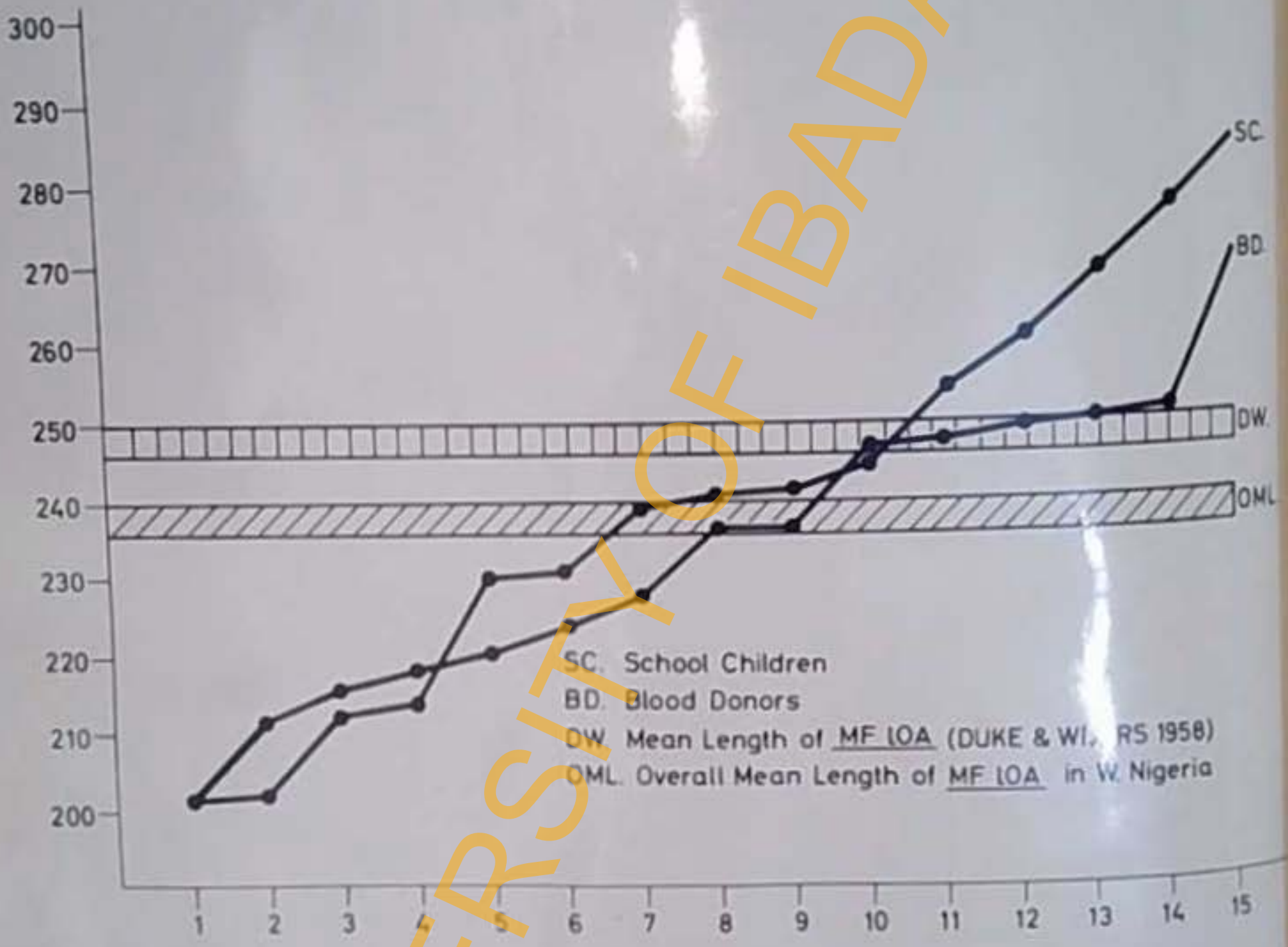

Fig 13 Comparing the mean length of microfilariae of 15 school children and blood donors

are highly mobile, and they could have acquired their infection at any endemic focus for loiasis which they have visited within the State. It is therefore more appropriate to refer to the Loa loa population in this study as coming from Western Nigeria and not from Ibadan, mainly because the blood samples were collected from infected people from all over the State even though some of them, the blood donors, were temporarily resident in Ibadan.

Finally the overall size range and mean length and mode mean of the microfilariae which were measured in this study (Figures 12 and 13) show that the Mf Loa in Western Nigeria is smaller than the rainforest human Mf Loa in the Cameroons. Since the two populations are transmitted naturally by Chrysops silacea and Chrysops dimidiata both in Western Nigeria and in Kumba Cameroons, it would be useful to find out whether development of the Loa loa in Kumba will be allowed to the third stage larvae in Mansonia africana mosquitoes as has been recorded in Ibadan.

CHAPTER VI

MANIFESTATIONS IN LOIASIS

6.1 Introduction

Very little is known in respect of the pathology of loiasis and it is not known whether symptoms occur both to the microfilarial and the developing larval stages. Many symptoms have been ascribed to *Loa loa* infection but some of them such as mild fever, generalised muscular pains, anorexia, and loss of weight may be manifested in many other helminth infections which are prevalent in Nigeria.

Calabar swellings, intense body itching and eye involvement are known to be usually manifested in *Loa loa* infection, and repeatedly as long as the infection persists. On the other hand endomyocardial fibrosis, elephantiasis, hydroceles, and nephrotic Syndrome have been thought to be caused by human filariae, especially *Loa loa* infection in *Loa loa* endemic zone.

The village study in the Ijebu and Remo divisions was utilized in collecting data and information on some symptoms appearing in loiasis and these are discussed below. Studies were also carried out on patients at the University Teaching Hospital, Ibadan, who had diseases often associated with loiasis. The possibility of a relationship existing between the ABO blood group the Haemoglobin AA, AC, AS and loiasis was also examined among blood donors at the University College Hospital, Ibadan.

Fig. 14 Palpebral Oedema (Calabar swelling) of the right eye of a 5 year old boy. The swelling was formed a few hours after a white tiny worm was seen migrating across the conjunctiva.

6.2 Common Symptoms

6.2.1 Calabar Swellings

Calabar swellings are hard tender oedematous swellings of a transient nature, and they often appear on the hands, wrists and face (Figure 14). These swellings are very common among villagers who have never ascribed this manifestation to Loa loa infection or to the adult worm, with which they are very familiar. Calabar swelling is known locally as Okutupo when it occurs on the fore-limbs.

Table 12 shows that 850 (39.9%) of the 2142 villagers in Ijebu and Remo divisions gave a history of Calabar swellings, including swellings of the face and limbs. Of the 101 villagers with microfilaremia, 60 (59.9%) gave a history of Calabar swellings. The effects of the Calabar swellings were found to vary with individuals and with their location on the body. In most cases, it was a painless swelling, but when it formed on a joint, it became very painful and inconvenient, and could impair the free movement of the affected part. The large non-fugitive swellings were often incised by the "local surgeons" and the "bad blood" in them was sucked out with the aid of an inverted bull's horn. When the swellings were fugitive, they caused a lot of anxiety states and were invariably associated with witchcraft in the villages.

Lambo (1960) recorded an association between loiasis and psycosis amongst some of his patients in Nigeria and Nnachiri (1966) observed that some Loa loa patients exhibited several features of mental instability because the presence of Calabar swelling on them was often attributed to poison.

TABLE 12

Prevalence of Loa-loa symptoms among villagers in Ijebu and Remo divisions.

SYMPTOMS	Total Number Examined - 2142		Total Number with mf Loa - 101		
	Number Recorded	%	Number with mf Loa in Blood	%	Percentage infection amongst villagers with Symptoms
1. Calabar Swelling/ Swelling of face and limbs.	850	39.9	60	59.9	7.1
2. Intense Body itching	1,584	74	27	26.9	1.7
3. Adult Worm in eyes/prickly sensation in eye region	156	7.3	82	81.9	52.6
4. None of the above	586	27.4	4	3.9	0.7

Only 60 out of 850 (7.1%) villagers who gave a history of Calabar swellings actually had microfilariae in their peripheral blood, and this low figure illustrate the unreliability of using the presence of swellings alone for the diagnosis of loiasis amongst villagers because there are many other conditions in the environment that could cause oedematous swellings on the body. However, it is possible that occult loiasis also occurs in some villagers because some of the invading worms causing Calabar swellings may be immature or mainly males, in which case they will not produce microfilariae.

In Table 12 it was observed that 59.9% of the villagers with loinsis gave history of Calabar swelling. This observation indicates that the stimulation of the allergic reactions is not automatic in loiasis and it occurs only in special circumstances. Kershaw and Kershaw (1953) recorded the removal of a gravid female worm of Loa loa from a receeding swelling in the hand of one of the authors and suggested that Calabar swelling is a reaction formed against the metabolic products of the adult worm whose movement has been impeded, and that this swelling is usually formed in the vicinity of the worm where the metabolic products have accumulated. It is therefore understandable that some of the infected villagers never gave any history of Calabar swellings.

6.2.2 Intense body Itching

Intense body itching is often associated with filariasis in filarial endemic zones. Itching is not pathognomonic of filariasis because this symptom can be caused by many agents including other helminths and allergens. In the village survey, a history of intense body itching was recorded in 1584 (74%) of 2142 villagers and in 27 (26.7%) of 101 villagers with microfilaremia. It is observed from the record that a low percentage (26.6%) of the villagers with microfilaremia, experienced intense body itching as against 74% of all the villagers, and only 27 villagers (1.7%) had microfilariae out of the 1584 with this symptoms. These low figures strongly indicate that body itching may not be specific to loiasis.

The provocation of allergic reactions has been observed in the treatment of loiasis with Hotrazan (Diethylcarbamazine) as a result of the destruction in the liver of microfilariae withdrawn from the peripheral circulation. Brumpt et al (1969) reported that this drug may also induce encephalitis in patients with heavy microfilaremia. It is however not known whether it is the microfilariae, their metabolic products or both that provoke the intense itching often experienced in some Loa loa patients. It is probable that when a large number of microfilariae die naturally and are withdrawn from the peripheral circulation for destruction, their lysis may provoke an allergic reaction similar to what is observed in the Hotrazan therapy of Loa loa patients.

Non specific reactions such as urticaria, pyrexia and other allergic reactions occurred in 7 (5.7%) of 121 post - transfusion patients observed in 1971

at the University Teaching Hospital, Ibadan. Since Loa loa is endemic in Western Nigeria and a high prevalence of infection is seen among apparently healthy blood donors (Ogunba 1970), it is possible that microfilarine in the donated blood might be responsible for some of these reactions. Usually microfilaremic blood is not transfused into patients, but the screening for microfilarine in blood may not be absolutely fool proof. Therefore there is the possibility of blood with a low microfilaria density being transfused into patients. In such cases, the lysis of the microfilarine could provoke the reactions mentioned above in the sensitised recipients, especially when we realise that microfilarine are proteins and would therefore be antigenic in the recipient when they are eventually destroyed. A quick follow up of the post transfusion non-haemolytic reactions in the seven patients failed to elucidate the role of microfilarine and their metabolic products in the genesis of these reactions.

6.2.3 Eye Involvement

The Adult Loa loa is easily recognised by the villagers mainly because of its frequent migration to the conjunctive. It is known as Arọrọ, and Arọwọ in the Ondo, Ekiti and Owo divisions while it is called Aranju in Ijebu and Remo divisions of Western Nigeria.

82 (81.9%) of 101 infected villagers (Table 12) with microfilaremia gave a history of adult worms crossing the eyes, or of a prickly sensation in orbital tissues, and this represents the highest record amongst the symptoms and manifestations associated with loiasis in Western Nigeria. It is not surprising that this symptom was recorded only in 156 villagers since this worm is

easily recognised and cannot be confused with something else. The adult worms are known to have a great predilection for the tissues surrounding the eyes, and they may cause conjuctivitis, lacrimation, swelling and pain Headache is also frequent (Johnstone 1947).

In Western Nigeria, the villagers associate the presence of the adult worm with many kinds of eye defects ranging from myopia to total blindness. Elliot (1920) reported that the combination of itching, pain and irritation caused by the movement of the adult worm under the conjunctive is maddening, and Clothier(1943) thought that its constant movement within the eye tissues could lead to partial nervous breakdown in a tired overworked individual who needs a peaceful sleep.

Langlois (1962), Toussaint and Davis (1965) however have incriminated Loa loa with some cases of retinopathy and have also reported large numbers of intravascular filariae in the retina and choroid plexus. Owen and Hennessey (1932) reported 33 cases of ocular helminthiasis but were unable to identify the causative agent. Some of the cases referred to as "Bung eye" or "Bulge eye" are similar to the ocular syndrome of the "Kampala Eye worm"which is common in Kampala, Mbale and Masaka districts of Uganda as well as other areas in East Africa. The ocular syndrome consisted of yellow nodules in the bulbar conjunctiva, oedema in the eyelids and face and occasionally proptosis. Nnochiri (1972) has clearly shown that Loa loa is the causative agent in a twelve year old child who had never been outside Uganda. Further confirmation of ocular loiasis

in Uganda has been reported from conjunctival biopsies. (Poltera 1973).

The adult worm can be easily removed while crossing the conjunctiva, and skill in removing it has been perfected by many villagers. The native needle (Ikoti) is often employed in the procedure. Whenever the adult worm disappears into the deeper tissues of the eye before there is the chance to remove it, the villagers apply juices of cassava tubers or onion bulbs and these are claimed to relieve the itchy and prickly sensations in the eye tissues.

The long time effect of the juices on the eye tissues or the worms are however not known. Since the itchy and prickly sensations are relieved, one may suggest that the worms are probably paralysed within the eye tissues by the juices applied. The delicate eye tissues may be equally affected by the constant application of the juices and their effects may be partly responsible for some of the ocular pathological effects often associated with loiasis in Nigeria. The possibility of a simultaneous chronic onchocercal infection in the villagers with its resultant eye involvement cannot however be overlooked because onchocerciasis is also endemic in Western Nigeria (Anchiri 1964).

6.3 Associated conditions

6.3.1 Endomyocardial fibrosis (E.M.F.)

Endomyocardial fibrosis is common in Equatorial Africa and its pathology has been described (Davies, 1948). It is well recognised in its advanced stage, but only retrospective reports have been made of the early stages. (Parry and Abrahams 1965).

Ojo (1970) conclusively showed that neither malnutrition nor consumption of plantains is crucial in the aetiology of endomyocardial fibrosis. Current theories especially amongst French Cardiologists however still incriminate filarial infections (Gerbaux, et al 1957), malaria or streptococcal infections (Shaper 1966) but neither of these has been substantiated. Eosinophilia is often found to be marked in this condition, especially in Europeans in endemic areas (Brockington et al 1957), but this finding is non-specific because eosinophilia can be caused by many factors, especially helminth infections which abound in the tropical region.

Fever, pruritis and swelling of the face in addition to eosinophilia are signs of the early febrile illness in E.M.F. (Parry and Abrahams, 1965), and these symptoms are also shared by many other agents, particularly loiasis. Ive et al (1967) found some evidence of one type of filariasis as the causative agent in ninety one percent of the cases of E.M.F. studied in Nigeria by employing all the various diagnostic techniques for filariasis

They reported that patients with E.M.F. showed 64 percent positive skin test to cattle (Sertaria) filarial antigen while the controls showed only a 36 per cent positive skin test. Sertaria antigens are known to behave as group antigens and would therefore record many non-specific reactions. Therefore, the only conclusion that could be validly drawn from their study is an association with some filaria group of worms which may be of either human or animal origin.

Purified Loa loa antigens found specific for detecting Loa loa infections (Ogunba, 1972) failed to reveal any specific role of Loa loa in the genesis of E.M.F. and other heart conditions (Carlisle et al 1972) although the number of E.M.F. patients in the study was rather small for any firm conclusions to be made. The use of a specific Loa loa antigen is however of obvious importance in narrowing down the possibility of Loa loa being involved in the pathogenesis of endomyocardial fibrosis.

In order to rule out or incriminate Loa loa infection in a causal relationship, it would be necessary to carry out epidemiological studies of the early stages of endomyocardial fibrosis, which fortunately are easily identified, in endemic and non-endemic foci for loiasis.

Such foci have been described in Chapter III. A follow up study of such samples, together with regular Surveillance on loiasis in the foci chosen would provide information which when analysed might elucidate the type of association between loiasis and endomyocardial fibrosis in Nigeria.

6.3.2 A.B.O. Blood groups and Haemoglobin genotypes

There is an increasing interest in research on the association of A.B.O. blood groups, haemoglobin genotypes and diseases, and Anand (1961) has shown that people with blood groups A and B are more susceptible to eosinophilia. However Anand (1965) found no association between Bancroftian filariasis and the blood groups he studied. Loiasis being endemic in Nigeria, it was decided to find out the association that may exist between loiasis, the A.B.O. blood groups

TABLE 13

Distribution of 314 blood donors with microfilariae of Loa loa by ABO blood groups compared with expected distribution from the control group.

BLOOD GROUPS	DONORS WITH MICROFILARIA LOA			CONTROL + (26,027)	
	No. Observed	% Observed	No. Expected	No.	%
A	67	21.33	67	5,544	21.3
B	78	24.84	73.1	6,054	23.3
AB	8	2.54	12.2	1,015	3.9
O	161	51.27	161.7	13,404	51.5

+ Gilles (1965) x^2 = 1.74 d.f. = 3

and the haemoglobin genotypes AA, AC and AS. It was also decided to relate the microfilarial densities in the blood of infected blood donors to the distribution of their blood groups and haemoglobin genotypes.

314 adults (293 males and 21 females) with Mf Loa in their blood, who looked healthy and had blood haemoglobin levels higher than 13mg/100ml, were bled during the day. Blood grouping and haemoglobin electrophoresis were carried out, and two 50cm.blood films were made for microfilariae count and identification. Information on the ABO blood groups and haemoglobin genotypes AA, AC, AS for Yoruba adults were obtained from Gilles (1965) and Esan and Luzzatto (1969) and those were used as controls.

The microfilaria densities were divided into three groups:

i. those with microfilaria count less than 100;
ii. those between 100 and 500; and
iii. those over 500 in 50 cu mm blood.

Table 13 shows no significant difference between the observed and the expected distribution of A.B.O. blood groups in 314 Loa loa blood donors. There is also no significant difference in the distribution of the haemoglobin genotypes AA, AC, and AS when compared with the control group (Table 14). The haemoglobin types SC, SS and CC were not considered because they were not recorded in the donors with loiasis in this study. Though the observed figures for AC and AS are slightly high, the differences are probably due to the smallness of the sample.

The observed and expected data for the AB blood groups within the microfilaria density groups are below the critical level of 5;

TABLE 14

Distribution of 247 blood donors with microfilariae of Loa loa by haemoglobin genotypes, AA, AC, AS compared with expected distribution from control group.*

HAEMOGLOBIN GENOTYPES	DONORS WITH MICROFILARIA LOA			CONTROL * (3,000)	
	No Observ	% Observed	No Expected	No.	%
AA	167	65	169.6	1980	66
AC	20	7.77	14.9	174	5.8
AS	70	27.23	65.7	768	25.6
SC	-	-	2.1	24	0.8
SS	-	-	4.4	51	1.7
CC	-	-	0.2	3	0.08

$x^2 = 2.08$ d.f. = 2

* G.J.F. Esan and L. Luzzatto (1969, personal communication).

TABLE 15

Distribution of 230 ABO blood groups with microfilariae of Loa-loa within microfilarial density groups compared with expected distribution from control group +.

MICROFILARIA COUNT	BLOOD GROUPS										X^2
	A		B		AB		B & AB		O		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
Less than 100/50 cu.mm.blood	16	18.70	23	20.96	2	2.06	25	23.02	45	44.28	0.56
100-500/50 cu.mm.blood	22	21.77	23	25.68	2	2.47	25	28.15	56	52.88	0.53
More than 500/50 cu.mm.blood	11	9.74	6	9.18	-	0.93	6	10.13	24	21.09	2.55

(The figures for A, B + AB, and O blood groups were used to calculate the X^2) d.f. = 2.

+ Gilles (1965).

TABLE 18

Distribution of 239 haemoglobin genotypes with microfilariae of Loa-loa within microfilarial density groups compared with expected distribution from control group†.

MICROFILARIA COUNT	HAEMOGLOBIN GENOTYPES					
	AA		AC		AS	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Less than 100/50 cu.mm. blood	52	56.44	5	4.87	27	21
100-500/50 cu mm. blood	71	70.69	10	6.11	26	26.75
More than 500/50 cu. mm. blood	29	31.68	4	2.88	15	12.5

(There is no evidence of a significant difference between genotype distribution and the microfilarial density groups.)

† G.J.F. Esan and L. Luzzatto (1969) personal communication.

hence it was decided to combine AB and B blood groups in Table 15 in order to avoid introducing large errors in the Chi square test. There is, however, no significant difference in the ABO. distribution within the microfilarial density groups when compared with that expected from the control. The number of donors with haemoglobin genotype AC is small, and when these are arranged according to the microfilarial density groups (Table 16) the figures obtained are too low for analysis. However, no evidence of a significant difference is shown when the observed haemoglobin genotype distribution within the microfilarial idensity groups is compared with that expected from the control.

This study thus suggests that there is no preferential infection by Loa loa of any of the groups of donors whether classified according to their ABO antigens or their haemoglobin types, It was conceivable that a protective effect in one of the groups might be exerted, not in terms of preventing infestation with Loa loa but in terms of reducing the parasite density. However, even when the donors were classified on the basis of the degree of their infestation, no statistically significant difference was found among any of the groups analysed. It must therefore be coacluded that there is no evidence that any of the blood donors is at either an advantage or a disadvantage with respect to infestation by Loa loa. This means that if the difference found in India (Anand 1961) in the rate of eosinophilia between subjects with group A and B compared with group O were confirmed

in Nigeria, the explanation would probably lie in conditions other than filariasis.

6.3.3 Elephantiasis and Hydrocoeles

Lymphadenopathies resulting in elephantiasis and hydrocoeles have always been associated with filariasis in the tropics. Wuchereria bancrofti is the species most commonly associated with these conditions, and a causative relationship between them has been accepted by many investigators on the basis of clinical, epidemiological, and histopathological studies. Much less favoured as causative agents are Onchocerca volvulus and Loa loa

Lowenthal (1934) and Clark (1948) however described a number of cases of elephantiasis from Uganda and Kenya in areas where W.bancrofti are absent. Similarly Cohen (1960) described a small series of cases observed in Ethiopia and Kenya from areas where W. bancrofti was absent. Ngu and Konstam (1964) studied sixty-five case of chronic lymphoedema over a period of five years in Ibadan (Nigeria), where no transmission of W.bancrofti occurs, and found that 25 cases (38.5%) were caused by tuberculous adenitis, 30 (46.1%) by chronic pyogenic infections, 7(10.8%) by malignant or other infiltrations, and 3 (4.6%) were primary or idiopathic lymphoedema. Their findings proved conclusively that in Western Nigeria lymphoedema is often not related to W.bancrofti infections.

Table 17 shows the summary of laboratory investigations and history notes on fifteen patients at the University Teaching Hospital, Ibadan, presenting with lymphoedema from 1965 to 1970.

TABLE 17

Showing the summary of investigations and manifestations in fifteen lymphoema patients.

CASE NUMBER AGE	SEX	TOWN OF DOMICILE	DIURNAL BLOOD FOR MF LOA	NOCTURNAL BLOOD FOR MF BANCROFTI	SKIN SNIP FOR MF VOLVULUS	EOSINOPHILIA	CUTANEOUS MANIFESTATIONS	HISTORY OF ADULT WORM PASSING ACROSS CONJUNCTIVA	OCULAR MANIFESTATIONS	LYMPHADENOPATHIES
1 Adult	M	Ibadan	-ve	-ve	+ve	4%	None	None	None	Bilateral hydrocoele for 8 years Mf Volvulus in hydrocoele fluid.
2 Adult	M	Eruwa	-ve	-ve	+ve	12%	Xeroderma Lichernification Nodules in thigh and scrotum Pruritis	None	None	Elephantiasis of righleg. Right and left Inguinal adenites.
3 Adult	M	Kabba	-ve	-ve3	-ve	8%	Dry and Scaly skin on lower part of right leg.	None	None	Bilateral hydrocoele for 3 years. Elephantiasis of right leg Hydrocoele fluid negative for microfilariae.
4 Adult	F	Ekpom	+ve	-ve	-ve	28%	Peeling of sole Pruritis for 4 years and pain on left leg.	Yes	None	Elephantiasis of right leg and foot.
5 Adult	M	Ibadan	-ve	-ve	-ve	20%	Xeroderma of right lower leg Pruritis.	None	Retinocho-roidal scar ring and choroidal sclerosis	Elephantiasis of right foot and leg for 3 months.
6 Adult	M	Abeokuta	-ve	-ve	+ve	13%	Pruritis. Hard and scarred skin	None	None	Elephantiasis of left leg
7 Adult	M	Ibadan	-ve	-ve	-ve	8%	None	None	None	Elephantiasis of scrotum for 10 years. Chylous hydrocoele fluid negative for microfilariae
8 9 Yrs	M	Ile-Orangun	-ve	-ve	+ve	27%	Pruritis. Skin thickened	None	None	Elephantiasis of left leg.
9 Adult	M	Ibadan	-ve	-ve	-ve	2%	Kebidal scars on right and left legs	None	None	Elephantiasis of left leg and thigh for 4 years.
10 Adult	M	Ibadan	-ve	-ve	-ve	8%	Intermitent Pruritis	None	None	Elephantiasis of right leg for 2 years
11 Adult	M	Ibadan	-ve	-ve	+ve	20%	Onchodermia	None	None	Bilateral hydrocoele with elephanticsis of the scrotum
12 Adult	M	Ibadan	-ve	-ve	+ve	12%	Onchodermia	None	None	Elephantiasis of right leg
13 16 Yrs	M	Ibadan	-ve	-ve	-ve	4%	None	None	None	Elephantiasis of left leg
14 Adult	M	Ibadan	+ve	-ve	-ve	20%	Pruritis	None	None	Bilateral hydrocoele for 5 years Mf log in Hydrocoele fluid
15 Adult	F	Ibadan	-ve	-ve	-ve	3%	Lizard like skin Xerodermia	None	None	Elephantiasis of left leg.

Ten cases were elephantiasis of the legs and the remaining five were hydrocoeles. The investigation aimed at finding parasitological as well as symptomatic evidence of filarial infection in these patients.

Mf bancrofti was recorded in none, but Mf loa was recorded in one of the elephantiasis patients. Mf volvulus was present in the skin snips of four patients. In three other cases, there were cutaneous manifestations suggestive of onchocercal infections. Pruritis was recorded in six patients. Ocular manifestations in the form of retinochoroidal scarring and choloridal sclerosis was recorded in one case in which there were pruritis and xeroderma but no Mf volvulus in the skin snip.

There were no microfilariae recovered from either blood samples or skin snips of patient number 9 and 13 (Table 17); nor did these patients have pruritis or cutaneous manifestations of onchocerciasis. Furthermore their eosinophil counts were low, reading 2% and 4% respectively. This suggests that the elephantiasis in the two patients were of non-filarial origin.

Mf Loa was recovered from one and Mf volvulus from two of the patients with hydrocoele. Neither patient number 3 or number 7 showed evidence of filarial infection and their hydrocoeles were probably not of filarial origin. However patient number 4 had Mf volvulus in the hydrocoele fluid and the skin snip while patient number 14 had Mf Loa in his hydrocoele fluid and in diurnal blood.

Both Loa loa and Onchocerca volvulus are endemic in Nigeria and may occur as adults in the subcutaneous connective tissues. Adult Loa loa are frequently found around the cord and between the tunica vaginalis and dartos muscle during surgery on hernia and hydrocoeles (Duzilleau, 1913; Kivits, 1952; Chesterman, 1958; Woodman and Bokhari, 1941). It was also observed that adult Loa loa were found in the distended lymphatic vessels of the cord while the microfilariae were found in smears of hydrocoele well (Woodman and Bokhari, 1941; Fullerborn, 1922).

If the adult Loa loa is lodged in the visceral layer of tunica vaginalis, an allergic reaction to the metabolic products of the worm, similar to Calabar swelling, could cause considerable damage to the local lymph plexus and produce an irritation of the tunica, resulting in a decreased absorption and an out-pouring of fluid inside the tunica sac. Dubois and Vandenberghe (1942) recorded transient attacks of filarial oedema in the scrotum, while McRobert (1935) noted acute urinary retention and strangury as signs of deep pressure effects of loiasis. Kivits (1952) recorded gelatinous, poorly vascularised whitish thickening of the tunical albuginea and the sac of inguinal hernia as a reaction to the presence of Loa loa.

Local irritation of the visceral layer of tunica vaginalis might occur if the scrotal skin is so heavily infected that M. volvulus are also present in large numbers in the underlying tissues of the dartos muscle and the parietal layer of tunica vaginalis, where they would cause damage to the blood and lymph

capillaries, as well as hypertrophy of the connective tissue. Such a condition might easily be assumed in hydrocoele cases showing a jelly-like oedematous connective tissue swarming with Mf volvulus and lining the scrotal wall (Sharp, 1923; Bryant, 1935). Adult Onchocerca worms free in the dartos muscle or the adjoining tissues may contribute to raising the level of microfilarial concentration. The fibrosis of the dartos muscle and the loss of the elasticity of the scrotal wall would likely cause a decrease in the lymph flow in the vessels draining the sac, while damage to the lymphatic plexus of the parietal layer of tunica vaginalis would interfere with the necessary absorption of fluid from the sac.

Even though the parasitological and other evidence suggest that some of the fifteen cases of lymphoedema under review are of filarial and largely onchocercal origin, it would be essential to confirm that the relationship is not merely incidental. It is therefore necessary that a future prospective comprehensive study be carried out in which many other investigations, including the Mantoux test, Frei test, lymph node biopsy and lymphangiography would be carried out to exclude other causes that may result in lymphoedema in Nigeria.

CHAPTER VII

CONCLUSIONS

7.1 Endemicity of loiasis in Western State of Nigeria

Human loiasis has been shown to be endemic in the Western State of Nigeria and its distribution extends to the three vegetational zones, the rain-forest, freshwater swamp and savannah. Most of the transmission occurs in the rain-forest zone, and this gradually declines as one moves towards the savannah zone where transmission is at its lowest level. The need to sample the right population has been discussed, and the school pupil population has been shown to be ideal for prevalence studies on loiasis because this type of population can provide adequately the requirements for a controlled geographical study, namely uniformity of samples with respect to age, sex and the period of residence in a specific environment. The illiteracy of the majority of the adult population coupled with the protocal problems in enlisting the co-operation of the adult population are some of the factors that make the adult population unsuitable for prevalence studies.

The endemicity of loiasis is possibly a complex interplay of many factors involving both the host, parasite, vector and the environment. Since the transmission level of loiasis varies according to the ecology of the environment, misleading results would be obtained if the wrong environment or group of people is

sampled. Samples obtained from villages have been shown to give reliable information on the prevalence of loiasis in Western Nigeria partly because the villages' natural environments are usually not disturbed, unlike the indiscriminate destruction of trees and surrounding bush often associated with urban development. Furthermore samples from infected adult blood donors were found unsuitable for determining the geographical distribution of loiasis in Western Nigeria.

The techniques often used for detecting infection with loiasis comprise the examination of blood films or large volumes of venous blood, and serology. Both the capillary blood and the venous blood have shown no significant difference in the number of microfilariae they contain, and this finding confirms similar observation made by Hawking (1955). The method of examining two 50 cmm thick blood films used in this study is sensitive, economic and not too time consuming; it is thus suitable for assessing the microfilaraemic rate in Western Nigeria where it is difficult to persuade villagers to give a large quantity of their blood in surveys involving blood sampling.

7.2 Transmission of loiasis

The efficiency of C. silacea and C. dimidiata in the transmission of loiasis is due to the fact that they alone have the habit of descending to the ground level in the forest to bite man.

C.silacea has been shown to be the main vector of loiasis in the villages sampled in the Ijebu and Remo divisions of Western Nigeria. It is more widely distributed and more abundant than C.dimidiata when the two species occur in the same village. C.silacea is probably better adapted than C. dimidiata for breeding in the two divisions, and more persistent in attacking man for blood meal. The breeding of Chrysops species, which are the vectors for loiasis, is a prerequisite for transmission hence the presence or absence of Chrysops silacea and C.dimidiata in the villages has been used partly in determining the endemic and non endemic foci for loiasis. In the non-endemic foci for loiasis there will be no transmission even if Loa loa is introduced by infected people. This is because the vector cycle will be absent owing to the non-availability of the Chrysops breeding sites in and around the foci.

Mansonia africana mosquitoes in Ibadan were found to be partly susceptible to Loa loa when experimentally infected. It is however unlikely that this mosquito would compete with or replace Chrysops in the transmission of human Loa loa in nature because of its night biting habit.

7.3 Population difference

In Western Nigeria the mean length and mode mean of the microfilarial population has been found to be smaller than that of the Kumba population. Furthermore the Mf Loa in Western Nigeria would develop to the infective stage in Mansonia africana mosquitoes, although the size and oesophageal ratio of the infective stage from Mansonia africana mosquitoes did not differ significantly from those reared in Chrysops in Kumba (Williams, 1960). Until comparative biological studies involving the use of Mansonia africana as intermediate host with the Kumba population of Loa loa is carried out, it is not yet possible to ascribe any significance to the observed size difference of microfilariae between the Kumba and the Western Nigerian populations.

7.4 Loiasis and Associated Conditions

There are still many gaps in our knowledge of loiasis as a human disease, and it is also not definitely known what symptoms and pathology are developed to the microfilariae and the developing larvae in the human body. Although many reports have been made in the literature about symptoms and pathology often associated with loiasis, it is not conclusive that the

recovery of Mf Loa or the adult worm in a patient suffering from another disease automatically incriminates Loa loa as the causative agent especially in an environment where other infectious diseases abound.

Little importance should be attached to the history of Calabar swellings in the diagnosis of Loa loa infection because many villagers cannot distinguish Calabar swellings from other swellings that may be caused by other conditions. Besides, a Loa loa infection does not always result in the formation of Calabar swellings. The history of seeing an adult worm and the itchy or prickly sensation caused by the movements of the adult Loa loa in the orbital tissues were found to be specific for loiasis and is of great diagnostic value especially in the villages where any swelling is likely to be regarded as Calabar swelling. Intense body itching alone was found to be of very little value in loiasis diagnosis because it could be caused by a number of agents in the village environment.

It has been shown in this study that there is no relationship between the ABO blood groups, the Haemoglobin genotypes AA, AC, AS and loiasis. It was also shown that there was no protective effect in any of the groups studied either in terms of preventing infestation or in the intensity of infection. Therefore none of the blood groups and haemoglobin genotypes in this study is at an advantage or disadvantage with respect to loiasis.

From the cases of elephantiasis and hydrocoelus studied, the evidence obtained were inconclusive for incriminating loiasis as a possible aetiological agent. It is more in favour of Onchocerciasis. A long time detailed study involving both parasitologists, clinicians and surgeons and carried out in both endemic and non-endemic foci for filariasis may provide the needed information.

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APPENDIX I

Staining procedure of Mf. loa with Giemsa stain

30 ml of distilled water adjusted to pH 7.2. was measured into a staining dish and 30 drops of Giemsa solution (B.D.H., England) was added and thoroughly mixed Thick blood films in staining racks were immersed into the stain mixture for 20 minutes. The staining rack containing the slides was agitated gently at regular intervals to reveal the haemoglobin from the area of the blood film.

After the staining period had elapsed the stained blood films were removed gently from the staining dish and immersed in a dish of tap water for about 5 seconds. The slides were then placed in a semi-upright position to drain and dry in air.

APPENDIX 2

Staining proceedure of Mf. loa with Mayer's acid Haemalum

Materials:

Haematoxylin crystals	-	2gm
Potassium aluminium sulphate	-	50gm
Sodium iodate	-	0.2gm
Thymol	-	One crystal
Methyl Alcohol	-	10 ml
Glacial acetic acid	-	20 ml
Distilled Water	-	1000ml.

The Haematoxylin crystals were added into 10ml of methyl alcohol and allowed to dissolve by constant shaking. The Potassium aluminium sulphate was dissolved in the distilled water by heating, and to the solution was added the sodium iodate and haematoxylin in alcohol. When the mixture was cool, the 20ml glacial acetic acid was added and the whole mixture was thoroughly shaken. The stain was allowed to mature for about 1 month before use.

Thick blood films were arranged with the blood film facing upwards on slide racks after they have been dehaemoglobinised in water, air-dried and fixed with methyl alcohol for 1 minute. The stain was poured on the fixed film and heated gently with flame from a spirit lamp until the stain starts to bubble up when the flame was removed. The flaming procedure was repeated several times during the staining which lasted 10 minutes. The slides were washed in running water for about 2 minutes to blue the nuclei of the microfilariae. The slides were then placed in a semi-upright position to drain and dry in air.

APPENDIX 3

Villages visited within each division and the overall prevalence of Mf loa among the school pupils.

DIVISION	VILLAGES VISITED	VEGETATION	NO. OF PUPILS EXAMINED	NO. OF PUPILS WITH MF LOA
ILESHA/IFE	Wamkin	RF	48	9
	Ipetu	RF	262	26
	Ashipa	RF	186	20
	Ibodi	RF	124	15
	Imesi-Ile	S	149	1
	Esa-Oke	S	137	1
	TOTAL		906	72(7.94%)
OSHUN	Ogbagba	RF	146	0
	Okebara	RF	111	2
	Ejigbo	RF	84	2
	Apomu	RF	82	2
	Awo	RF	46	3
	Ilobu	RF	84	3
	Oalo	S	87	1
	Adekunle	S	90	0
	Iwofin	S	91	1
	Obada	S	94	0
	TOTAL		914	14(1.53%)
OYO	Idode	S	107	1
	Iseyin	S	248	3
	Ipapo	S	116	2
	Agu Are	S	120	1
	Gbogun	S	152	2
	Ipeba	S	16	0
	Fiditi	S	91	0
	Fashola	S	108	1
	Aha	S	20	0
	Sheki	S	136	2
	Kishi	S	81	1
	TOTAL		1195	13(1.04%)

APPENDIX 3

Shows the list of villages visited within each division and the overall prevalence of Mf loa among the school pupils

DIVISION	VILLAGES VISITED	VEGETATION	NO. OF PUPILS EXAMINED	NO. OF PUPILS WITH MF LOA
ONDO	Igba	RF	113	10
	Odigbo	RF	102	11
	Ile-Oluji	RF	134	16
	Alade	RF	103	13
	TOTAL		452	40(8.85%)
EKITI	Oba	RF	189	2
	Emure	RF	99	2
	Erindo	RF	124	4
	Ikarre	RF	150	9
	Ilawe	RF	135	5
	Ilara	RF	65	8
	Aye	RF	144	0
	Ikole	S	178	1
	TOTAL		1084	31(2.85%)
OWO	Amurin	RF	82	1
	Ifon	RF	95	1
	Idoani	RF	89	1
	Ipele	RF	62	1
	Ago-Igbirn	S	129	0
	Oke	S	117	0
	TOTAL		574	4(0.7%)
REMO	Ishara	RF	143	5
	Aiyepe	RF	90	3
	Ode-Remo	RF	128	6
	Iperu	RF	392	7
	Ogere	RF	139	14
	Ijebu-Ijesha	RF	26	0
	Irolu	RF	55	0
	TOTAL		973	34(3.48%)

APPENDIX 3

Shows the list of villages visited within each division and the overall prevalence of Mf loa among the school pupils.

DIVISION	VILLAGES VISITED	VEGETATION	NO. OF PUPILS EXAMINED	NO. OF PUPILS WITH MF. LOA
EGBA	Egbe Obafemi	RF	49	1
	Ododa	RF	160	4
	Orile-Ilugun	RF	345	3
	Abatan	RF	126	0
	Olokemeji	S	105	0
	Imale	S	78	0
	TOTAL		786	8(1.01%)
EGBADO	Ilaro	RF	239	10
	Ishaga	RF	99	11
	Ibeshe	RF	98	5
	Ajilete	RF	177	13
	Igbogila	S	84	0
	Aiyetoro	S	136	1
	Afori	S	90	1
	Aworo	S	92	1
	Ado	FW	98	2
	Ipokia	FW	105	3
	TOTAL		1218	53(4.36%)
IBAOAN	Akingbule	RF	141	1
	Iroko	RF	86	1
	Logun	RF	101	1
	Akanran	RF	90	1
	Lalupon	RF	130	1
	Egbode	RF	108	1
	Ijaiye	S	72	0
	Eruwa	S	80	0
	Igbo-Ora	S	120	0
	TOTAL		929	6(0.64%)

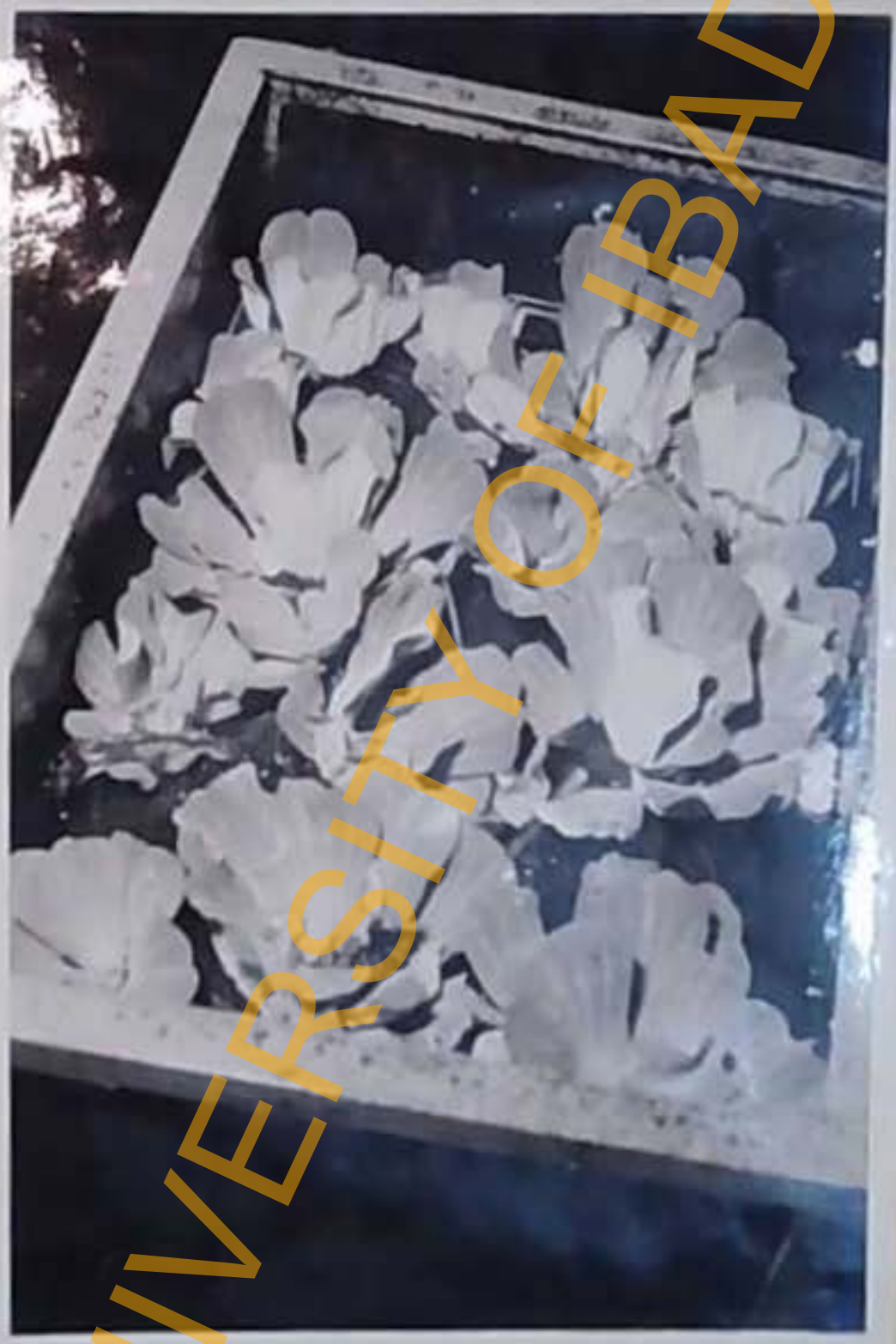
APPENDIX 3

Shows the list of villages visited within each division and the overall prevalence of Mf loa among the school pupils

DIVISION	VILLAGES VISITED	VEGETATION	NO. OF PUPILS EXAMINED	NO. OF PUPILS WITH Mf LOA
IJEBU	Okun-Owa	RF	214	6
	Omu	RF	77	7
	Ala	RF	35	0
	Mobalufon	RF	18	0
	Idowa	RF	31	1
	Ibefun	RF	25	0
	Ooosa	RF	45	3
	Ago-Iwoye	RF	126	4
	Oru/Awa	RF	5[illegible]	6
	Ikija/Owu3	RF	53	3
	Ijebu-Ife	RF	8[illegible]	4
	Ijebu-Inbo	RF	167	9
	Odogbolu	RF	73	5
	Isiro	RF	38	2
	Folafonmu	RF	26	2
	TOTAL		1127	52(4.61%)
OKITIPUPA	Ihititun	RF	130	7
	Aye	RF	131	7
	Igbo-Tako	RF	106	5
	Obade	RF	132	6
	Obekobo	RW	93	2
	Kiribo	RW	98	2
	TOTAL		690	29(4.20%)

Appendix 4. Mosquito cage containing adult mosquitoes. Mosquito pupae were transferred daily into the bowl inside the cage.

Appendix 5: Kilner jar fitted with expanded polystyrene for the laboratory maintenance of larvae and pupae of *Mansonia africana*. (Some larvae are attached to the polystrene).



Appendix 6: Fish tank for breeding Mansonia africana. It is filled with pond water and water lettuce (Pistia stratiotes) to which larvae and pupae of M. africana attached.

Appendix 7: Fish tank covered with mosquito net from which the emerged adult Wansonia africana are attached.