"PARASITOLOGICAL AND SEROLOGICAL STUDIES OF THE CANINE F1LARIID NEMATODES [DTROFILARIA SPP] IN ZARIA; AND THEIR SIGNIFICANCE TO PUBLIC HEALTH."

BY

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DECLARATION

This is to certify that I carried out the investigation reported in this thesis at the laboratory of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, under the supervision of Professor, J.U. Umoh; Dr. V.C. Ogbogu of Faculty of Veterinary Medicine and Dr. E. Essien of Department of Community Medicine, Faculty of Medicine, Ahmadu Bello University, Zaria.

The thesis is an original work carried out by myself, and no part of it has been presented elsewhere for a degree or diploma.

The works of other investigators therein are duly acknowledged in this presentation.



Anyanwu, I.N.
(January, 1994)

CERTIFICATION

This work entitled "Parasitological and Serological Studies of the Canine Filariid Nematode (Dirofilariae spp) in Zaria, and Their Significance to Public-Health," by ANYANWU, IKECHUKWU NDUBISI, meets the regulation governing the award of the "Masters Degree" of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is completely dedicated to our 'Lord Jesus Christ' for His wonderful guidance and provisions for me during the course of this study, and particularly for leading me safely through the numerous hazards involved in this project.

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ABSTRACT

This survey showed the prevalence of confirmed canine filariae infection in Zaria to be 12.7%. Three species of *Dirofillaria* were isolated using parasitologic methods involving the use of sample concentration technique of Knott, morphological characteristics, microfilariae behaviour, and the use of averarage size of microfilariae in Giemsa stained preparations.

Out of 158 canine blood samples collected and examined, 20 were positive, with a percentage of infections of 8.9%, 2.5% and 4.4% of *D. repens,* D. *immitis-like microfilariae*, and an unidentified species respectively. *D. immitis-like parasite,* has not been previously reported in Nigeria. All cases of *D. immitis*-like infections were mixed infections with the more predominant species, *D. repens*.

There was a statistical significant association ($P<0.05$) between the existence of antibody to *Dirofi* lari.a antigen in the dog population under study and canine dirofilariasis.

Although, the males had somewhat higher antibody titer than the females, this did not show any protective value.

A concurrent survey also revealed high exposure risks of the public to the Dirofilarial parasite, thus it imposes a threat to public health.

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CHAPTER ONE

INTRODUCTION

The filarial parasites of the family Filarioidea, depend for survival on a close association between their arthropod intermediate, and vertebrate definitive hosts. Infection of the vertebrate host occurs when the arthropod vector containing the infective third-stage larvae takes a blood meal.

Species of Dirofilaria are natural filarial parasites of dogs throughout the tropical and sub-tropical areas of the world. The Dirofilarial worms have been reported in man at an increasing rate in the tropical regions; (Beaver and Orihel, 1965; Hubbert, 1975; Faust, et al. 1970). As with many other filariid nematodes parasitising man, several species of mosquitoes have been implicated as the intermediate host for these zoonotic parasites.

It has been shown that unlike the typical human filarial parasites caused by Wuchereria bancrofti, B. malayi, Loa loa, etc., dirofilarial infection in man shows many atypical filarial symptoms and lesions (Faust, et. al., 1970). In fact, a range of clinical signs has been documented and the clinical signs presented depend on the anatomical location of the parasite (Yoshimura and Akao, 1985).

Pulmonary, abdominal, breast, and orbital tumours, etc have been ascribed to the Dirofilaria species

infections in man (Yoshimura and Akao, 1985). Also, the most likely explanation of Tropical pulmonary Eosinophilia (T.P.E) is zoonotic filariasis (Webb, et. al. 1960; Lie Kian, 1962).

The genus Dirofilaria comprises two important parasites, namely Dirofilaria immitis, and Dirofilaria repens, both of which have dogs in particular as natural host in various regions of the world. It may be worth mentioning that apart from the dog heart-worm D. immitis which is highly pathogenic to both man and the natural host; D. repens has also been documented to be pathogenic in man where it may form cutaneous nodules on various parts of the human body, especially at the region of the orbit (Yoshimura and Akao, 1985; Wright, et. al., 1989). It is now believed that the previously ascribed infections of D. conjuctivae could in fact, be due to Dirofilaria repens, (Faust, et. al., 1976).

Dirofilarial infection has been observed to have no age barriers. D. repens has been isolated from a dense nodule around the orbit of a 4-year old boy in Canada (Khan and Meerovitch, 1968). Besides, in the parasite's natural host, infections of both the fetal and new-born pups have been reported (Beaver, 1970).

Filariasis is among the endemic parasitic diseases that have received much recognition by the World Health

Organisation, who in cooperation with the United Nations Development Programme and the World Bank, have taken measures to encourage work by scientists to reduce the depredation of filariasis particularly on the African continent.

Nonetheless, the filarial situation in most endemic regions, especially the West African sub-region, has remained more or less cloudy. Moreover, in spite, of the probability that D. immitis may cause acute reactions in man, as well as the earlier reported chronic cases, there has not been much interest in the study of the prevalence of dirofilariasis particularly in Africa, (Welch and Dobson, 1974).

Thus, the filarial situation particularly in the African sub-continent is such that it looks relevant and urgent for entomologist, parasitologist and Immunologists to cooperate in order to eliminate or drastically reduce the parasite burden on the inhabitants of these endemic territories.

Indeed, zoonotic filariasis is of special interest, and perhaps should assume urgent attention, since the known intermediate host and transmitters are abundant here. Besides, the natural host of the parasites (the dog) is also available here and readily exposed to the arthropod vectors, the mosquito.

Therefore, the public health implications of the presence of Dirofilaria species seems obvious, since man is in constant contact in this region with the

vectors of the Dirofilarial parasite, and also with the parasite natural host.

The objectives of this work were: (1) To elucidate the status of canine Dirofilariasis in Zaria, and (II) To assess possible threat to public health.

It was hoped that the results would also shade more light on the unsubstantiated belief or reports for decades on the absence of the dog heart-worm (D. immitis) in this part of the world, particularly Nigeria.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. Epidemiology of Dirofilariasis

2.1.1. Distribution of Dirofilariae Species:

Species of Dirofilaria parasitise many land mammals, particularly the dogs, and the parasite has been isolated from cats and foxes, (Faust, et. al., 1970; Wright, et. al., 1989).

Dirofilaria immitis which is a common parasite of dogs throughout the tropical and the sub-tropical areas of the world, has been found as a human parasite with several species of mosquitoes serving as natural intermediate hosts and vectors, (Otto and Jackson, 1975, Faust, et al. 1970).

Dirofilaria repens is found in Africa, Europe, and Asia, being a natural parasite of dogs and cats, transmitted also by mosquitoes of the Culex, Anopheles and Aedes genera (Hubbert et. al., 1975).

Dirofilaria tenuis, a subcutaneous parasite of the raccoon in the United States of America has also been recovered from man, (Beaver and Orihel, 1965).

Faust, et. al., (1976); Beaver and Orihel (1965), gave 24 cases of subcutaneous Dirofilarial infections in man in the United States in which the worms were formerly ascribed to D. conjunctivae. Beaver and

Orihel, (1965) observed that this parasite has been reported in man on numerous occasions in several countries of Europe, Africa, and Asia. The worms were removed from tumours and abscesses in various anatomical locations like the arm, breast, upper lip, etc, and with the palpebral conjuctivae and other tissues of the eye being, of course, the most favoured sites. Beaver, and Orihel (1965) further indicated that this parasite in the United States of America is D. tenuis, which is naturally a raccoon parasite, while those infections in the former U.S.S.R., Europe, etc, are probably caused by D. repens, which is a natural parasite of dogs in these areas.

The distribution of Dirofilaria species in the infected host is equally important. In the dog, D. immitis adult is usually found in the regions of the chambers and connecting large vessels of the right heart, and pulmonary vessels, while D. repens lives under the skin especially in the region of the trunk, thighs and neck, peritoneal cavity, and the eye, (Benbrook and Sloss, 1961; Wright, et. al., 1989). Adult D. immitis has also been isolated from the lungs and bronchioles (Benbrook and Sloss, 1961).

Yoshimura, and Akao (1985) reported dirofilariasis in humans Japan. The highest prevalence occurring in the 50 - 69 year age group. The youngest patient, however, was an 18-year old female. All cases but one with D. repens, were due to D. immitis.

Several cases of pulmonary and extra-pulmonary infections in humans have been reported world-wide and documented in the U.S.A., Japan, Australia, and Brazil (Yoshimura and Akao, 1985). They also found a statistically significant difference between the distribution of noduler in the right and left lungs of dirofilarial infected cases. About 70% of the nodules were located in the right lungs.

Thirteen cases of extra-pulmonary dirofilariasis in man have been reported from Japan (Yoshimura, and Akao, 1985). One of them was an autopsied case of 36 year old man, incidentally harbouring two mature female worms in the right ventricle of the heart and inferior vena cava. Of the remaining 12 cases, seven of them were found harbouring D. immitis adults in the subcutaneous tissues, while, 3 cases had theirs in the abdominal cavity. The remaining 2 patients harboured the parasites in the eye. It was indicated that, a case of D. repens was included in these Japanese cases (Yoshimura and Akao, 1985).

The microfilaria of Dirofilaria species are present at all times in the peripheral circulation, although according to Hawking (1956), the maximum number of D. immitis microfilariae are found in the peripheral circulation towards the evening at about 6.00 p.m.; and the minimum is found about the morning period, at about 6.00 a.m., while D. repens has its

peak period between 10.00 p.m. and 3.00 a.m.

The heart-worm, Dirofilaria immitis is primarily a parasite of domestic dog and all breeds are equally susceptible. D. immitis is widely distributed the world over, and in the United States, it is most prevalent along the Atlantic and Gulf coasts, and farther inland in more circumscribed areas often associated with high density of mosquitoes, (Otto, 1969).

2.1.2. Factors Affecting the Distribution of Dirofilaria Species

According to Smyth, (1976) many species of mosquitoes can act as intermediate host of D. immitis and D. repens. Wright, et. al. (1989) observed that about 70 species of mosquitoes of the Culex, Anopheles and Aedes genera are competent vectors.

D. repens has a prepatent period of 25 weeks in its vertebrate host, whereas Dirofilaria immitis shows a prepatent period of 32 weeks both exhibiting sub-periodicity. Their microfilariae are present in maximum number in the peripheral blood when they could easily be picked up, and transmitted by evening-biting mosquitoes, (Smyth, 1976; Hubbert, et al. 1975).

Ecological factors, rather than phylogenetic factors are foremost in the relationship between filarial nemtodes and their arthropod vectors (Lavoipierre, 1958). He further, indicated that environmental factors exert an important influence on

the rate of development in the arthoropod of the filarial organism, and also the survival of the infected intermediate host. Host preferences are also an important influence on the effectiveness of a vector, and affect the possibilities of zoonotic infections.

Wong, et. al (1973), observed that 15% to 20% of dogs with D. immitis infection do not have microfilaraemia, and thus, the characteristic clinical findings such as alterations in cardiovascular, pulmonary, hepatic systems, etc. are important in making diagnosis of such cases.

Richard (1979), reported a case of occult feline dirofilariasis on which diagnosis was based on suspicion of clinical similarities to the disease in dog. The diagnosis was confirmed by angiocardiography. It was also indicated that, at the time of diagnosis, a canine occupant of the house-hold, a 10-year male mixed beagle (dog), was examined and found to have microfilaria of D. immitis.

Dogs continually exposed to infected mosquitoes may be expected to become infected and reinfected with heart-worms year after year, (Jackson, 1969).

Microfilariae of some species of filariae are known to show transplacental crossing. This has been observed in both D. repens, and D. immitis, (Beaver, 1970; Mantovani and Jackson, 1966).

The average incidence of Dirofilariae infection in stray dogs in Japan is 50%, higher than in less exposed dogs to vector mosquitoes, and this is even higher (68%) in older dogs of above 5 years of age, (Yoshimura and Yokogawa, 1970).

In the natural host, the microfilariae of Dirofilaria are incapable of further development to the infective third-stage larvae which normally occurs in the mosquito (Otto, 1969).

2.2. Clinical Manifestations/Pathology

In the dog, heart worm disease is a multiple organ disease with alterations occurring in the cardiovascular, pulmonary, hepatic, renal and hematopoietic systems, (Richard, 1979).

Low levels of infection may not produce obvious pathological manifestations (Collins and Pope, 1987; Bundesen, et. al., 1990). Bundesen et. al, (1990) has shown that presence of one to three worms to produce minimal pathology.

The following clinical signs have been reported in dogs and cats with heart-worm disease: dyspnea, tachypnea, tachycardia, auscultable moist rales, and extra-systoles suggesting cardiopulmonary embarrassment, (Richard, 1979; Jackson, 1969). Other findings include; absolute eosinophilia, immune-complex glomerulopathy, hyperproteinemia, hyperglobulinemia,

and elevated S.G.P.T. value, which is indicative of hepato-cellular degeneration (Morgan, 1969; Richard, 1979).

Also, neurologic signs have been reported in three cases which had adult-worms in the lateral ventricles of the brains, (Donahue, and Holzinger, 1974).

In man, clinical signs of dirofilariasis include; eosinophilia, sub-cutaneous swelling, lung lesions similar to those of malignant neoplasm, (Hubbert, et al, 1975). Also, cough, metastasis of oesophageal cancer, coin lesion, chest pain, oesophageal varix, liver cirrhosis, and lung shadows have all been reported in man, (Yoshimura, and Akao, 1985).

The differential diagnoses of the clinical signs of dirofilariasis in man are lung cancer, (Yoshimura and Akao, 1985), and tropical pulmonary eosinophilia (T.P.E.), (Lien Kian, 1962).

The main clinical features of Tropical pulmonary eosinophilia (T.P.E.) or occult filariasis in man are lymph gland enlargement, blood eosinophilia, and bronchitis with asthmatiform attacks. Lymph gland enlargement is often reported, and located in the axillary, inguino-crural, cervical, or epitrochlear regions.

2.3. Diagnosis

Parasitological Diagnosis

A number of methods have been employed for the diagnosis and characterisation of filarial infection. Dogs are generally screened for dirofilariasis on the basis of examination of blood samples for microfilariae (Wong, et. al., 1973). The condition in which a patient does not show any microfilariae in the blood even though found to be infected with gravid adult female worms is known to present a diagnostic problem.

In a study of 98 dogs in which microfilariae and adult worms were counted, a consistent ratio was not found between the number of the microfilariae and the number of adult worms (Jackson, 1969).

The detection of microfilariae in the blood, is the most dependable and practical method of diagnosis. However, 5 to 20% of dogs with dirofilariasis were found not to show microfilaraemia, although found at necropsy to harbour adult gravid female worms (Wong et. al., 1973; The American Heart-Worm Society, 1978).

Failure to demonstrate microfilariae in the animal may be due to (i) presence of immature adults, (ii) the presence of only one adult sex or, (iii) an immunologically mediated response in which microfilariae are removed from the circulation (Richard, 1979).

The methods for parasitological diagnosis are the

modified Knotts method, the wet smear, filtration technique, capillary sedimentation technique and, the saponin test. The modified Knotts technique has been found to be more reliable in diagnosing the disease than the other methods (Stein and Lawton, 1973).

2.3.1. Differentiating of Dirofilaria species

In the differentiation of the two canine filarial worms, it was found that while D. immitis microfilaria has a tapered cranial end and a straight body shape, that of Dipetalonema spp has a blunt cranial end and a crescent body shape (Stein and Lawton, 1973; The American Heart-Worm Society, 1978).

Also in the fresh blood smear diluted with saline, those of Dirofilaria undulate in one place and never migrate, whereas those of D. reconditum have intervals of progressive movements (The American Heart-Worm Society, 1978).

In wet preparation, the microfilariae of D. repens wriggle very vigorously, while those of D. immitis do not move much, never migrates and stays straight. Also while some species of microfilariae live for long time in water e.g. those of Brugia, Loa, Wuchereria and D. repemns, those of D. immitis always die in water (Denham, personal communication).

2.3.2. Immunological Diagnosis

Immunological methods have also been employed in the diagnosis of filarial infections and have been found useful in sero-epidemiological surveys. These tests include Indirect hemagglutination antibody test, fluorescent antibody technique, skin-snip test, Immuno-diffusion technique, complement fixation and Enzyme-linked immunosorbent assay (ELISA).

The most commonly used filarial antigen is Dirofilaria immitis antigen. The problems of skin-snip test are lack of uniformity in antigen concentration and the possibility of cross reaction with other nematodes.

The enzyme-linked immunosorbent assay technique, although found useful in some parasitic infections such as Chagas disease and trypanosomiasis, (Voller, et. al., 1975), it has not been found dependable for filariasis. This technique was found to give a high antibody titre, even for ordinary saline, (Voller, et. al., 1975).

Indirect fluorescent antibody technique has been carried out by either using soluble antigen or cut sections or whole microfilariae of D. immitis or D. vitae. Frozen sections of Dipetalonema vitae adults have been found to be of considerable value in diagnosing filarial infection in man using the IFA-test, to achieve 90% positive result, (Ambrose, 1974). Using Indirect fluorescent antibody test, four different species of microfilariae were used in testing

sera of human patients suffering from occult filariasis, and the result revealed species specific antibodies, (Jayewardene, and Wijayaratnam, 1968); and tests using microfilariae and larval stages as antigens revealed stage-specific antibodies, (Wong and Guest, 1969).

Ambrose, (1974) observed that the complement fixation technique is the longest serodiagnostic procedure for filariasis and that recent workers appear to attach less importance to it. Apart from being cumbersome, the skin test technique often shows a more reliability in epidemiological surveys than the use of complement fixation test. In utilising Dirofilaria immitis as antigen, a 75% positive reactions were achieved in a skin test for Wuchereria infections, whereas using the same antigen, only 10.4% positive result was obtained in complement fixation test, (Kogan, 1963).

The "Vet Red^{Tm} Antigen test" is based on a new and simple immunoassay system which is capable of probably detecting circulating D. immitis antigen in canine whole blood, thus eliminating the need for any sample preparation to obtain serum or plasma, (Bundesen, et al, 1990). In a population of dogs, "Vet Red^{Tm} test" has been shown to achieve a specificity of 96.8%, and a sensitivity of 96.9% in dogs infected with 3 or more adult female worms; and a sensitivity of 100% in

infections of 4 or more adult females. However, test sensitivity of Vet Red [Tm] decreased when there were fewer than 3 adult female worms present per animal.

The Indirect haemagglutination Antibody (IHA) technique has been found to be a fairly reliable serodiagnostic test, both highly specific and sensitive by a number of workers, (Kagan, 1963; Tada, et al, 1979). Bryceson, et. al., (1976) using IHA technique observed that antigenic diversity among filarial species could be ascertained. Under field conditions, Tada, et. al., (1979), found the IHA technique most reliable method of seroepidemiological survey is that blood samples could be collected from finger prick on filter papers, instead of venepuncture.

Gell et. al., (1975) indicated that in the indirect hemagglutination test, using antigen adsorbed on erythrocytes, not all cells have same agglutinability. That the human red cell is the most agglutinated carrier, but cells of other species may also be used although the reaction is a little less sensitive. Sheep red cells are perhaps the most commonly used in hemagglutination test. Tannic acid-treated red cells (Boyden's test) involves the adsorption of protein antigens onto red cells which have been mildly treated with tannic acid. Proteins are not adsorbed on to red cells. Then the antigen-coated tanned red-cells, are agglutinated by extremely small amounts of homologous antibody. For routine

diagnostic work, the antigen-coated red-cells may be treated with formalin, which then allows them to be kept for long periods, (Campbell, et. al., 1964; Gell, et. al., 1975).

The immune response to nematode infections is known to be of short duration, and is dependent upon current or recent stimulation from living infective agents (Otto, 1969).

It may be necessary to point out that besides parasitologic and serological diagnosis, physical examination, etc, may be diagnostic in nature in identifying heart-worm disease. Jackson, (1969) observed that a dog suspected of having heart-worm disease (dirofilariasis) should be carefully observed, while being walked towards the examination room, and on the floor before it is placed on the examination table. Such an observation permits better evaluation of the general attitude of patient with respect to gait, tail wagging, head carriage, and lameness.

Patients with chronic heart-worm disease seem to labour to force air in and out of the lungs. As the work of respiration becomes more difficult, the ribs increase their angulation with the spine, (Jackson, 1969). The rear limbs are palpated in an attempt to detect obscure lameness often associated with advanced heart-worm disease.

Also pericardial friction rubs may sound like

heart murmurs, and are frequently found in dogs with emboli from heart-worms. Auscultation the heart and lungs is the most important part of the examination of the heart-worm patient, especially when microfilariae cannot be demonstrated in a blood sample, (Jackson, 1969).

It has been observed that dogs with vena cava or liver failure syndrome of dirofilariasis differ in many respect from the typical heart-worm disease. Thus, the syndrome is often characterised by lack of history of any clinical signs, and sudden onset. Although, the syndrome may occur in dogs of any age, those of 3 to 5 years old are most often affected, (Jackson, 1969). The principal signs are known to be sudden weakness, anorexia, hemoglobinuria, and bilirubinuria; and hemoglobinuria is considered pathognomonic for the vena cava syndrome of heart-worm disease.

Jackson, (1969) further observed that in one survey, dogs with vena cava syndrome were seen to harbour twice as many worms as those with chronic heart-worm disease. That the right atrium was invariably enlarged and the liver was also seen to be enlarged and congested. The onset of the syndrome was sudden that death would result in most cases within 12-72 hours.

Morgan, (1969) observed that dogs infected with heart-worm, with multiple organ involvement, may not present any great problem in diagnosis of the disease,

but will, however, cause some concern in making decision regarding prognosis and treatment.

2.4. Public Health Considerations

Information concerning the prevalence of dirofilariasis in man has been restricted largely to reports concerning the surgical recovery and identification of subcutaneous parasite largely Dirofilaria tenuis; a species in the Americas), pulmonary and cardiac (D. immitis) parasites from individuals, (Harrison and Thompson, 1971).

Medical reports of 65 cases have shown that, this zoonosis occurs throughout Southern Europe particularly in the Eastern Mediterranean countries, Russia, India, Africa, North-East Asia, Australia, the Pacific Island, Japan, and in the United States of America, (Welch and Dobson 1974; Harrison, and Thompson, 1971; Yoshimura, and Akao, 1985).

The geographical distribution of dirofilariasis in man, closely parallels the occurrence of D. immitis in carnivores, particularly dogs, (Soulsby, 1968)

Reports of human infections with species of Dirofilariae dating 1887 was mentioned by Faust, et al. (1976); and out of 37 cases, three were found in the heart, or vena cava, and the remainder in subcutaneous tissues of the upper limbs, trunk, face or orbit of the eye.

In 1965, 36 additional human cases of dirofilariasis were reported in the United States. 25 of these cases were Dirofilaria obtained from superficial tissues, while 11, cases were from the heart or lungs. No cases occurred in children, (Beaver, and Orihel, 1965).

The major cause of subcutaneous dirofilarial infections in the United States is Dirofilaria tenuis, a common filaria parasite of raccoons, (Hubbert, et al, 1975; Orihel, and Beaver, 1965).

Lung lesions recognised in radiographs (coin lesions) are caused by the presence of adolescent or adult parasites - D. immitis, whose natural hosts are dogs. Wong, (1974), observed that in none of the human infections with Dirofilariae, was the microfilariae found in the peripheral blood, although gravid females have been observed in at least 2 instances.

Where dogs are associated with vector mosquitoes for long periods of the year, namely in the tropics, infection rates are high, and there is a corresponding high prevalence of antibody, and elevated antibody titre to Dirofilarial antigen in the human populations, (Welch and Dobson, 1974).

The problems imposed by Dirofilaria sp. to human population were first recognised in Indonesia and India, just before the world war-II; the lung involvement consisting of asthma-like attacks of cough, and dyspnea, coupled with an eosinophilia drew the first

attention Hubbert et. al., 1975). Cases of similar characteristics were observed in many tropical regions, and all were cured when treated with arsenicals, or diethyl-carbamazine, (Hubbert, et al, 1975).

Many cases of T.P.E. showed high titres of filarial complement fixation test, and also showed improvement after the administration of diethyl carbamazine, (Lie Kian, 1962).

There is accumulating evidence in recent literature that Tropical pulmonary eosinophilia (T.P.E.), presents a syndrome similar to occult filariasis, which has been ascribed to infections with forms of filaria parasites, (Webb, et al, 1960; Lie Kian, 1962).

It may be mentioned that, the Dirofilariae sp. have been implicated in many instances of clinical diseases in man, some of which called for emergency. For instance, Kumadi, (1980), observed a case of pulmonary infarction in a Nagoya man due to zoonotic filariasis.

"Filarial acute abdomen in pregnancy", was observed in a 32-week old pregnant lady in India, (Charles, 1976).

Reviewing case reports of pulmonary, and extra-pulmonary dirofilariasis found in humans in Japan, Yoshimura, and Akao, (1985) observed that the pulmonary cases of the worms were mostly young adult worms

located in no dules more often at the lower lobes of the right lungs, with patients presenting a range of clinical symptoms such as: cough, chest pain, lung shadows, coin lesions, fever, fatigue, and dyspnea, which raised the suspicion of lung cancer. They observed that in the extra pulmonary cases, one case (an autopsied case), harboured two matured female worms in the right ventricles of the heart, and the inferior vena cava. That, the remainder of the extrapulmonary cases presented a range of symptoms which included: subcutaneous nodules, breast tumour, uterine and liver malignancy (cancer?); visual disturbances, and orbital tumours.

In an endemic region, people begin to acquire filarial infections early in life, which could start as early as 5 to 6 years of age (Guptavanij, 1977; Khan, and Meerovitch, 1968).

Furthermore, a single female worm of D. repens was documented to have been recovered 30 years ago from a painless firm lump, above the right eyebrow in the frontalis muscle of a European who was said to have by then recently visited Nigeria, (O'grady, 1962).

2.5. Treatment, and Control

The only drug that has been shown to be consistently effective in killing adult D. immitis, is thiacetarsamide. The preferred treatment schedule is 0.1 of a 1% solution/0.45kg of body weight twice for 2

or 3 days. This is a short, effective regimen with a minimum of undesirable side effects, and the drug is slowly injected intravenously, (Jackson, 1969).

Dogs continually exposed to infected mosquitoes may be expected to become infected and remain infected with heart-worms year after year. Therefore, Jackson suggested that in enzootic areas, where mosquitoes occur much of the year, thiacetarsamide be given to outdoor dogs every 6 months, regardless of the presence or absence of microfilariae.

Thiacetarsamide-sodium (a trivalent arsenical) has been most thoroughly evaluated and most extensively used chemotherapeutic agent, and has a very high order of activity against the adult filariae of all species of man, or animals, except the genus Setaria, (Otto, 1969).

However, because of the problem in making decision regarding prognosis and treatment of dirofilariasis with multiple organ involvement, Morgan (1969) proposed simple laboratory tests to be essential in assisting with these decisions. Naturally, the liver is an organ of major consideration because it is so deeply involved in the pathologic response to the disease and, it is also the most sensitive organ to arsenical therapy used in filarial treatment (Morgan, 1969).

When it is possible to demonstrate the exixtence of a hepatic disorder, it is unwise in most instances

to cause further damage with arsenical compound. An exception would in fact be the case of "liver failure syndrome" or "venae cavae syndrome" where mortality is probably 100% without treatment (Morgan, 1969). In cases of this nature, the risk of therapy is of minor concern when death is the alternative.

Acute liver damage may be detected by the demonstration of an enzyme in serum, glutamic pyruvic transaminase(SGPT) which is released to the circulation because of cellular destruction. Also another specific enzyme, serum glutamic oxaloacetic transaminse (SGOT) is available in test kit (Morgan, 1969).

One of the side effects with the course of treatment with thiacetasamide is that associated with massing of dead worms in the pulmonary artery and its branches (Otto, 1969). It was however observed that serious reactions from the deat worms after treatment is best prevented by a reduction in exercise including close confinement for 1 or 2 months (Otto, 1969; The American Heart-Worm Society, 1978).

Prednisone given intramuscularly in a single dose of 20mg body weight/dog appears to offer some relief from the pulmonary complications, while an antibiotic may sometimes be used to prevent secondary bacteria pneumonitis (Otto, 1969). Diethyl carbamazine (DEC) is effective preventing infective larvae of D. immitis from reaching the heart in dogs, (Jackson, 1969). DEC, is recommended at a rate of 1.5mg/1b (0.45kg) body

weight, and given daily mixed with food, and given a month prior and continued 2 months after the mosquito season, (American Heartworm Society, 1978; Jackson, 1969), and it was further indicated that untoward reactions may occur in about 5% of dogs given diethylcarbamazine, if they have large amount of microfilariae in their blood, (Jackson, 1969).

Campbell, and Blair, (1978) suggested the use of antiparasitic agents such as the Avermectins, active against the immature worms, for prophylaxis against dirofilariasis.

The surgical treatment of the heartworm infected dog has been proposed as a means of avoiding massive thrombo-emboli, which follows chemotherapy in heavily infected dogs. Dogs suspected of harbouring more than 50 worms are candidates for surgery, (Jackson, 1969). A number of surgical techniques have been described. The most reliable surgical technique is pulmonary arteriotomy, with occlusion of the venae cavae, (Jackson, 1969).

According to Hubbert, et al (1975), the major method of filariasis control is mosquito control through application of insecticides, and also mass treatment of the population with diethylcarbamazine.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Blood Sample Collection

Blood sample (4 - 6 ml) were collected from dogs brought to the Small Animal Unit of Veterinary Hospital, Ahmadu Bello University, Zaria. The sex, breed, age, location in Zaria, and the owners address were recorded.

The blood sample taken from each animal as immediately distributed thus:

(i) 2 or 3 drops of blood mixed with 2 drops of water on a clean slide, and spread evenly to about three-quarters of the slide.

(ii) 1.0ml of the blood was discharged into 10ml 2% formalin, in a centrifuge tube for the modified knott's concentration technique.

(iii) The remaining blood sample in the syringe was discharged into a sterile centrifuge tube or sample bottle which was allowed to clot, in order to obtain the serum for serological investigation.

The collection of sample was also done at Zaria Army Depot (Kasua Doles), where adult dogs were brought from different parts of the town for slaughter. Collection of samples at this site was modified from that of Ahmadu Bello University Teaching Hospital Clinic to make collection of sample possible.

At Kasua Doles, clean universal bottles containing 0.5 to 0.7 ml Alsever's solution were used for the

collection of blood samples. A 5ml syringe without needle was attached to each numbered universal bottle by means of adhesive tape. The sample bottles were given to two of the dog dealers on special arrangement, and instructed on how to collect, and keep the samples fron the slaughtered animals.

Each sample bottle, apart from a syringe being attached to it, carried label (or sticker) to indicate the sex, age, and part of Zaria, from where the slaughtered animal was brought.

The blood samples were mixed with the Alserver's solution in the sample bottles which are then put into a plastic container with lid, and stored by the dog dealers in their refrigerator at $4^{o}C$, until they were collected for laboratory work.

Only 2ml of blood from the slaughtered animals was mixed with the Alsever's solution. Averagely 3 samples (as returns), were collected from the dog dealers weekly, out of an average of 10 sample bottles issued out.

3.2. Parasitologic Examinations:

3.2.1. Blood Smears, and Staining:

The thick smears and the modified Knott's concentration technique were employed. In the direct thick blood smears, 2 to 3 drops of fresh blood samples were mixed with 2 drops of distilled water which were already dropped on a clean 75 x 25cm glass slide. A rectangular smear of the blood sample to about 20 x

25mm size was usually made on the glass slide, using either the tip of the syringe, or the needle used in collecting the blood sample.

The smears were allowed to dry for about 2 hours, and then dehaemoglobinized for about 2 minutes by dipping the slides into a beaker of tap water and allowing them to stand upright. The dehaemoglobinized smear was allowed to dry before being fixed in absolute methanol for about one and half minutes.

In the modified concentration technique of Knott, about 1.0ml of the blood sample was usually discharged quickly into 10ml of 2% formalin in a 15ml centrifuge tube, mixed thoroughly, and centrifuged for 5 minutes at 3,000 r.p.m. The supernatant was decanted, and one or two drops of the sediment was placed on a clean glass slide by means of a Pasteur pipette. The sediment was mixed with one or two drops of distilled water, and spread to about 2 x 3cm wide. The smear was allowed to dry, and then stored in this form until needed for staining. Staining was done for 30 - 40 minutes, using freshly prepared solution from a stock solution of giemsa, diluted to 1:10 in phosphate buffer solution (P.B.S.) of pH 7.2. The slides were washed in distilled water and P.B.S., allowed to dry and examined under the microscope for microfilariae. Microfilariae thus found was measured by means of micrometry (Appendix 2).

Several measurements were taken on a number of parasites in each infected sample, which had been duplicated into many stained slides, for the purpose of taking as many measurements as possible. Representatives of parasite groups isolated were also photographed in colour slides, using Kodak Ektachrome - 100, EPN 135.

Besides taking measurement of the parasites, the morphological characteristics of the microfilariae in the stained preparations were equally noted.

In addition to size, and morphology of the parasites in each infected sample being noted, kthe behaviour of the live parasites was observed. All were used as identification tools for the parasites. When ever observation of giemsa-stained preparation raised suspicion of the presence of D. imintis - like microfilariae, the animal was recalled to the clinic for the observation of evening blood for live microfilariae in haemolysed blood.

3.3. Serological Examinations, (Indirect or Passive Haemagglutination Antibody Technique).

3.3.1. Washing, Tanning, and Coating of Sheep Blood Cells (Erythrocytes).

The sheep blood was mixed with Alserver's solution and kept at $4^{o}C$ in the refrigerator for 1 week to stabilize. It was then washed three times using Phosphate Buffer solution (P.B.S.); PH 7.2, to obtain packed washed red cells.

Three mls of fresh dilute tannic acid solution, (1:20,000) was mixed with 3mls of the washed red cell suspension in P.B.S., incubated, and centrifuged. The resulting packed cells from the tanning process were resuspended in P.B.S. for the antigen coating.

Purified lyophilized D. immitis antigen diluted 1:200 in P.B.S., PH 7.2, was used in coating the tanned erythrocyte. The steps followed in the washing, tanning, and coating of the erythrocytes are outlined in Appendix 3.

3.3.2. Indirect (Passive) Haemagglutination Procedure Using Purified D. immitis Adult antigen

A serial two-fold dilutions of the test serum was performed, and the round bottomed micro-titre plates were used. Besides, 0.025ml of diluent (P.B.S., P^H 7.2) was added into the second microtitration well through to the 11th well, by means of microtitration pipette.

Then, into wells numbers 1, 2, and 12; 0.025ml of the test serum was added. Prior to this, the stored sera were defroze and heated in a water-bath at 56°C for 30 minutes (to inactivate complement), and allowed to remian at room temperature before testing.

Using microdiluters of capacity 0.025ml, serial dilutions were prepared starting from well number-2, through to well number-11.

By means of micropipette delivering also 0.025ml

per drop, 0.025ml of the coated red-cells was added to the wells, starting from well number-1, through to well number-11; while 0.025ml of the control cells was delivered to well number-12.

The titration plates were now shaken gently to mix the contents and covered using clean empty titration plate, and kept to stand at room temperature, and the result read after 2 hours. Also, some of the readings were made the following day, the plates having been stored under $4^{o}C$.

The end-point was taken to be the last well to show a distinctly smooth-mat of agglutinated cells with a crenated rim. Negative results showed closely packed button of cells. [Titre level, is the reciprocal of the dilutions. In the preliminary test-run, positive sera were used to determine the antigen concentration used in the serology].

3.4. Adminstration of Questionnaire Items/Direct Questioning of Operators of Dog (Parasite's Natural Host) Market, in Zaria.

3.4.1. Assessing the Exposure Risk of the Public to the Canine Filarial Parasite.

Questionnaires were administered to dog owners visiting the A. B. U. Veterinary Clinic, between January and June, 1992. The questionnaire comprised 7 questions, and translation of items into vernacular was made in the case of clients illiterate in English language.

The questioning assessed among others:

1. The number of dogs/cats kept by clients or by their neighbours.

2. The period of the year clients experienced mosquito bites, the dry or rainy season or both, and so on. (The questioning items are shown in the Appendix section.

3.4.2. A Direct Questioning of Dog Dealers at the Zaria Army Depot (Kasuan Doles), Dog Slaughter Market

At this dog market the questioning assessed:

1. The year of commencement of the dog business or market in Zaria.

2. The number of adult dogs slaughtered per week/month, and

3. To know why the dog slaughter business has progressed for years, i.e. the resaons for the success of the dog market in Zaria.

CHAPTER FOUR

RESULTS

The results of the survey are displayed in a number of Tables, Plates and Figures.

A. Parasitological Investigations:

The prevalence of confirmed cases of canine dirofilariae infection was 12.7%, while no infection was detected in the 0 - 3 months age group, the prevalence of infection was found to increase with age, with the 3 yr - old and above, showing highest prevalence (Table 1).

Table 2, shows that although there is a somewhat higher prevalence of canine dirofilariasis in the female population than in the males, the infection does not follow any pattern. A near 50-50 rate of infection (2.3% and 2.8%) between the males and the females by the more pathogenic species - D. immitis (dog heart - worm) was also observed.

The canine filarial infections were detected in all parts of Zaria, except Basawa (Table 3 and Fig. 1). Dogs in Samaru area are apparently more infected than those of the other areas. Also the highly pathogenic filaria D. immitis, was detected in most of the areas (Fig. 1).

Table 5, shows that on the average, D. immitis microfilariae are larger in size than those of the predominant species D. repens.

Morphologically, while the D. immitis microfilariae have straight body and tapered anterior end, those of D. repens and its related species were observed to have crescent (curved) body with blunt anterior, in stained preparations (Plates 1, 2 and 3).

It was also observed that dogs infected with D. repens and its related species showed no overt clinical signs, while those infected with D. immitis (the dog heart worm) were found to exhibit pronounced clinical/pathological features. Besides, all D. immitis infections occurred as mixed infections with D. repens (Table 6).

Table 1: Number and percentage of dogs positive for microfilariae according to age, (1992).

Age of Dogs	No. Examined	No. Positive(%)
0 - 3 months	24	0 (0)
4 - 6 months	23	1 (4.4)
7 - 11 months	22	2 (9.1)
1 - 2 Years	42	7 (16.7)
≥ 3 years	48	10 (20)
Total	159	20 (12.6)

Numbers in parenthesis indicate % positive.

Table 2. **Number and percentage rate of dogs positive for microfilaria-type according to sex, (1992).**

(No. of Dogs Examined: M=88; F=70)		
Filaria Species	No. Positive M(%)	F(%)
D. repens	6(6.8)	8(11.4)
D. immitis	2(2.3)	2(2.9)
unidentified sp.	5(3.7)	2(2.9)
Total	13(14.8)	12(17.1)

No. in parenthesis indicate percentages

Table 3. *Dogs positive for microfilariae according to location, (1992)*

Location	No. Examined	No. Positive(%)
GRA/Sabon Gari	46	4(8.7)
Samaru Area	91	13(14.3)
Kongo/T. Wada	5	2(40)
Basawa	7	0(0)
Wusasa Area	5	1(20)
Total	154	20(13)

No. in parenthesis indicate % positive

NB: Mixed infections occurred between the different species.

Table 4. Number of positive infections of the species according to location, (1992).

Filaria Spp.	GRA/Sabon Gari	Samaru Area	Kongo/ T. Wada	Wusasa	Total
D. repens	4(8.7)	8(8.8)	1(20)	1(20)	14
D. immitis	1(2.2)	2(2.2)	1(20)	0(0)	4
Related spp.	0(0)	5(20)	2(40)	0(0)	7
Total	5	15	4	1	25

No. in parenthesis indicate % positive for the species of parasite.

Table 5. Size and prevalence of microfilariae by species in the study area, (1992).

Species	Range of Size(u)	Mean±SD (u)	Prevalence in population	Prevalence in 20 +ve cases
D. immitis	348.75-382.5	359.1±9.37	4(2.5%)	20%
D. repens	307.5-363.75	338.2±9.72	14(8.9%)	70%
Unidentified spp.	206 - 294	257±27.63	7(4.4%)	35%

Table 6. Cases of infection with filarial species and the level of clinical/pathological features (abnormalities) in the host according to ABU Vet. Teaching Hospital clinic case-record, (1992).

Sex	Age at 1st visit to clinic	Current age ofpatient/ date of collection of blood	Clinical/Path. features recorded**	Filaria species
M	3.5 mnths. 3-6-91	1 yr./(15-7-92)	Pronounced	D. repens D. immitis
F	2.5 yrs. 13-6-84	>8 yrs/(3-6-92)	Pronounced	D. repens D. immitis Rel. spp
M	4 mnths. 8-4-82	>8 yrs/(15-7-92)	Pronounced	D. repens D. immitis
F	2 yrs. 26-3-92	2.25 yrs/(3-7-92)	Moderate	D. repens D. immitis
M	5-6 yrs. 6-1-88	10 yrs/(14-5-92)	Nil	D. repens
F	1 yr. 18-3-92	1yr/(23-3-92)	Nil	D. repens
M	10 months.	2 yrs/11-5-92	Nil	unidentified spp.
F.	1.5 yrs	7.6 yrs/1-4-92	Nil	D. repens

** Clinical/pathological features often seen here, in the D. immitis - like infections included: coughing, limping of hind limb, Anorexia, enlarged external lymph nodes, weakness and emaciation, septic lacerated open wounds, alopecia, marked eosinophilia, high total protein levels (7.2 - 9.3g/100ml or above) and, haemaglobinuria, etc.

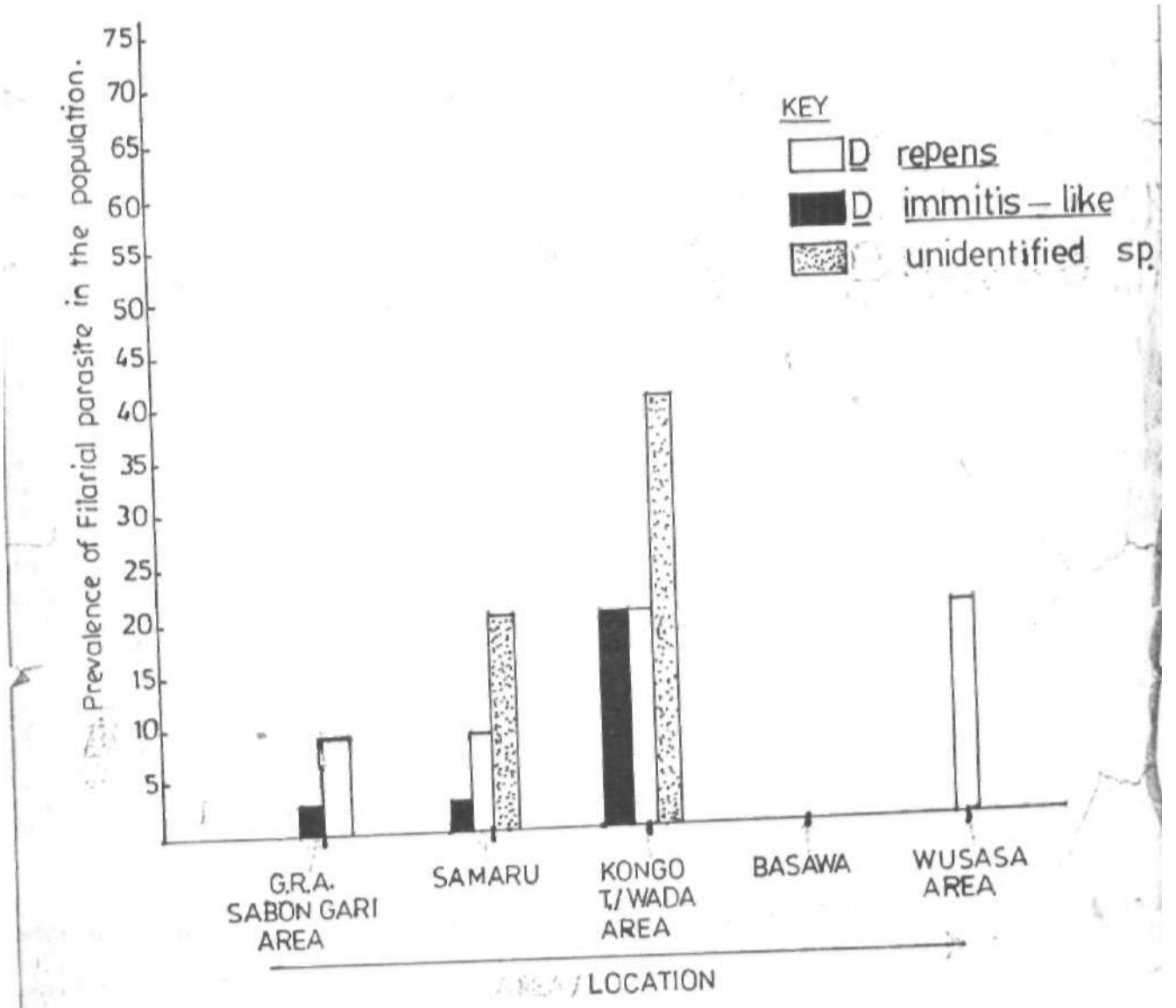


Fig. 1. percentile prevalence of species of canine filarial parasite in the dog population, according to location in Zaria.

PLATE 1: Dirofilaria repens microfilaria:
(Photomicrograph on Opposite page).

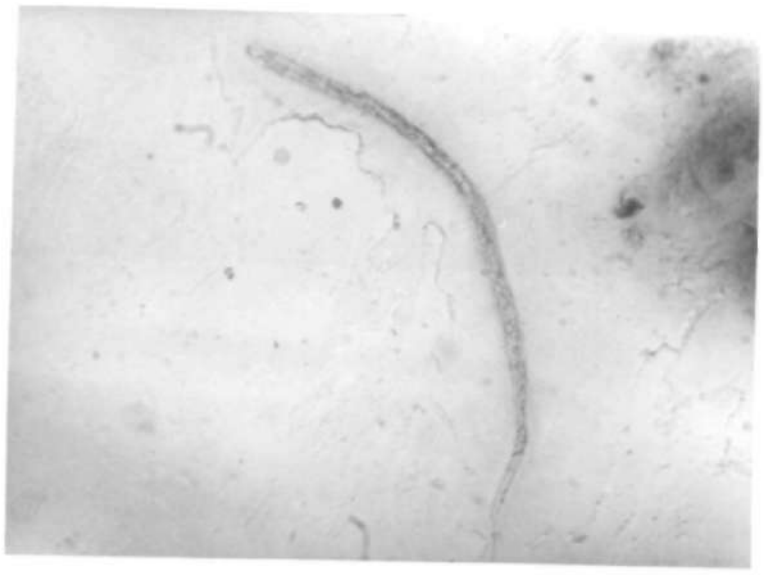
Features of Parasite (in knott concentration method).

1. Crescent or curved body shape

2. Blunt anterior end

3. Size of parasite: 338.2±9.7 millimicrons

Behaviour of live parasite: Wriggles very vigorously and migrates.



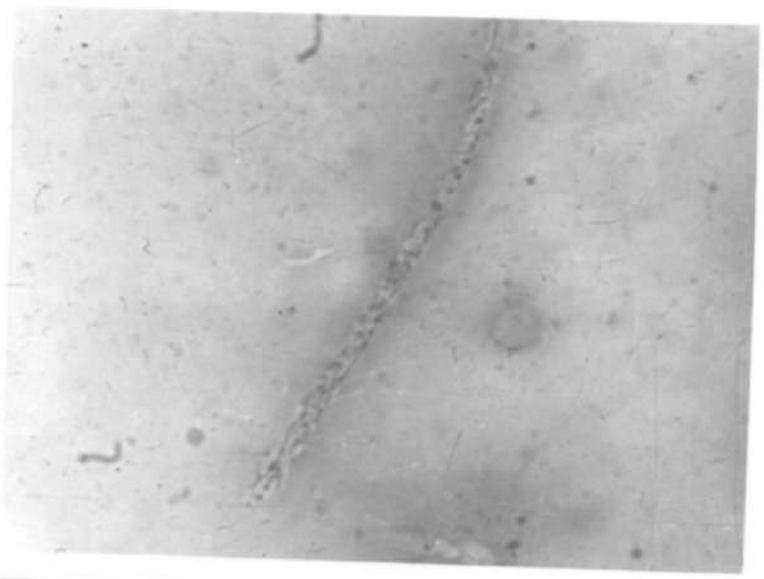


PLATE 2: Dirofilaria immitis- like microfilaria:
(Photomicrograph on Opposite page).

Features of Parasite (in knott concentration method).

1. Straight body shape

2. Tappered anterior end.

3. Size of parasite: 359.1±9.4 millimicrons

Behaviour of live parasite: Microfilaria moves merely sluggishly only in one plane, and never migrates.

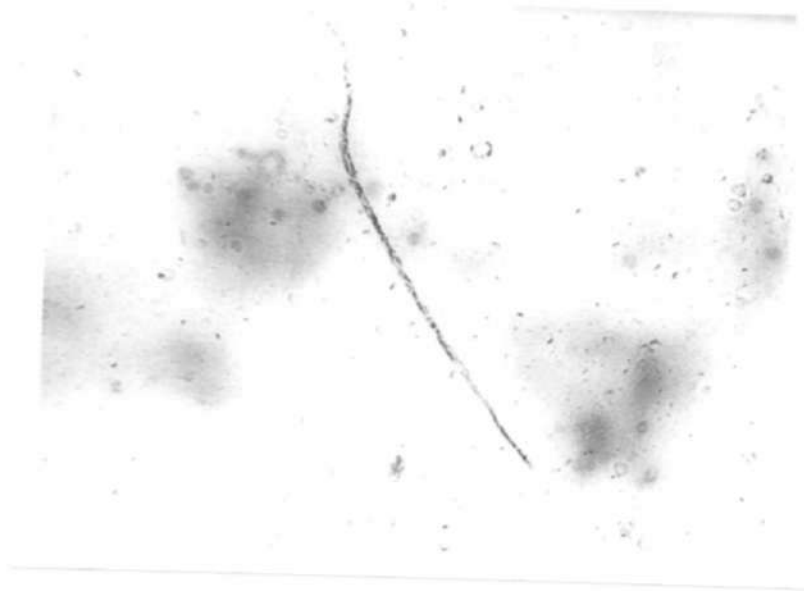




PLATE 3: unidentified spp
(Photomicrograph on Opposite page).

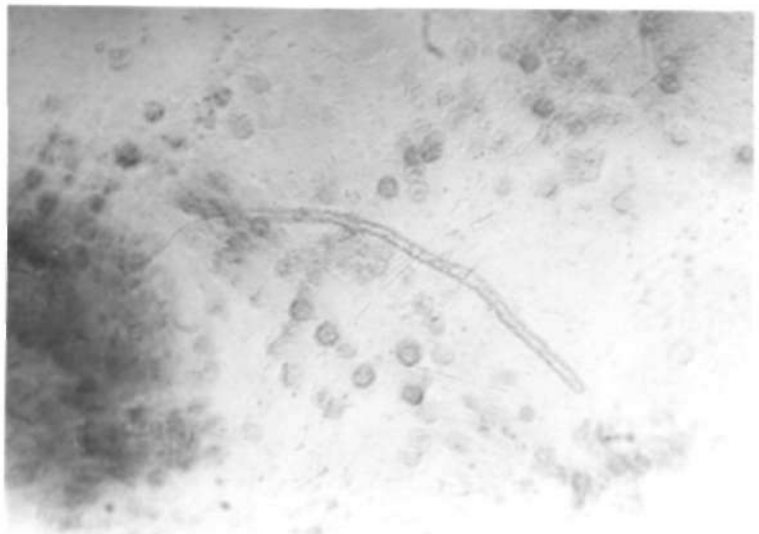
Features of Parasite (in knott concentration method).

1. Crescent or curved body

2. Blunt anterior

3. Size of parasite: 257±27.6 millimicrons

Behaviour of live parasite: Wriggles very vigoriously, and have intervals of short migration.



B. SEROLOGICAL INVESTIGATIONS

There was no consistent pattern observed in the antibody titre either among the respective age-groups, or sex of the hosts (Table 7 and 8), or parasite species, (Table 2).

Table 9, showed that the antibody level of 8.8 ±11.9 in Basawa area of Zaria where no microfilariae was detected in the canine hosts tested, did not differ appreciably from antibody titre of the other areas/locations, particularly that of 8.3±9.8 obtained from the GRA-Sabon Gari area where the infection rate was 8.7%.

The geometric mean titre for the male population of the established cases of infection, was found to be higher than that of the female with 16±5.7 and 8±10.3 antibody titres respectively (Table 10).

Also, whereas the Geometric mean titre (G.M.T) of the male population of confirmed cases was observed to be higher than that of the males in the general population (16±5.7 and 9.7±13.5 respectively), the GMT of the females in both the confirmed and also the general population (8.0±10.3 and 8.9±8.9) respectively did not differ appreciably (Tables 7 and 10).

Besides the Geometric mean titre (GMT) of 12.0±8.0 given by the population of confirmed cases of infection is also higher than the GMT of 9.4±11.9 observed for the general canine population. The seroprevalence of canine

filariasis in the population was estimated as 23.6%, using the geometric mean titre of 16 from the established cases, as the cut off point of titre. Also, the validity test showed that 7.4% of the surveyed population were infected with the parasites, although they did not show microfilariae in the blood. (See appendix 1b).

Table 7: Indirect Haemagglutination Antibody Titre in Respect of the Age of The host.

AGE GROUP	TITRE LEVEL
0 - 3 months	7.2±14.8
4 - 6 months	13.2±19.9
7 - 11 months	7.0±10.2
1 - 2 years	9.5±9.4
≥3 years	11.7±11.10

Geometric Mean Titre for entire population. 9.4±11.9

Table 8: **Indirect Haemagglutination Antibody Titre according to sex of the age - groups.**

AGE GROUP	TITRE LEVEL	
	M	F
0 - 3 months	6.7±18.3	7.7±6.1
4 - 6 months	17.4±22.9	9.2±18.8
7 - 11 months	6.2±10.0	7.7±11.3
1 - 2 years	9.4±8.8	9.5±4.9
≥3 years	12.8±12.1	10.6±8.8
G.M.T. per sex	9.7±13.5	8.9±8.9

Table 9: Indirect Haemagglutination Antibody Levels to *D. immitis* according t0 area/location in Zaria.

AREA	TITRE LEVEL
GRA/Sabon Gari	8.3±9.8
Samaru	10.8±18.0
Kongo/T. Wada	12.0±11.3
Basawa	8.8±11.9
Wusasa Area	16.0±11.3

Table 10: **Antibody titre of the confirmed cases according to sex of individual host.**

Serum No.	Titre Level	
	M	F
087	32	-
098	32	-
106	-	32
109	32	-
130	4	-
131	-	8
136	4	-
132	-	8
133	-	16
118	4	-
142	-	0
149	32	-
GMT per sex	16±6.7	8±10.3
GMT per pop.	12±8.0	

Table 11: Indirect Haemagglutination Antibody Levels of confirmed cases according to the infecting parasite species.

SERUM NO.	TITRE LEVEL	PARASITE SPECIES
087	32	D. repens
098	32	unidentified species
106	32	D. repens D. immitis unidentified species
109	32	D. repens D. immitis
130	4	unidentified species
131	8	D. repens D. immitis
136	4	D. repens
132	8	D. repens
133	16	D. repens D. immitis
118	4	unidentified species
142	0	unidentified species
149	32	unidentified species

C (i) Assessment of exposure risk of the public to the canine filarial parasite (Dirofilaria)

Both the parasite's natural host and vector were found to be substantially present in this community all year round, and were in close association/contact with man. It was shown that 64.4% of respondents kept more than I dog in their homes, while 59.9% of them indicated that so many families other than theirs keep dogs in the area were they live.

In the survey it was also found that various types of mosquitoes are present here, the day and night feeders. While 80% of the respondents had mosquito bites only at nights, 20% received bites either in the day time or both day and night. Furthermore, 60.5% of the public sampled experienced mosquito bites at all seasons of the year (both the dry and rainy seasons).

Also, while the survey showed that 56.8% of families do not take any measures at all to discourage mosquitoes from breeding in and around their homes, 69.4% of respondents were found to be ignorant of filaria parasite, and its possible mode of transmission.

(ii) Assessment of the scope of dog business or market in Zaria

The practice of selective depopulation of adult dogs (the parasite natural host) for meat was found to have been in operation in parts of Zaria for the past 10 years.

At the main centre of this dog market it was estimated that about 516 adult dogs were slaughtered yearly.

The success of the dog business in Zaria was understood to be due to the cheapness of the dog meat compared to beef, and the medicinal values attached to the meat by the patronizers of the business.

CHAPTER FIVE

DISCUSSION

The infection rate of canine filariasis being observed to increase progressively with age of the host is likely due to the period of time it takes the parasite to mature and produce microfilariae which may appear in the general circulation, and also the length of exposure of the host to the vector and transmitter of the parasites. Inability to detect infection in the age-group, 0 - 3 months, is not unconnected with this period it takes the infective 3rd stage larval filarial parasite to mature, (Table 1).

The only infection recorded in the puppies was in the 4 - 7 months age-group, from a 5 months old female infected with D. repens. The observation made here is corroborated by the work of Wright, et al (1989), and Wong, et al (1973) which indicated that D. immitis larvae take 6 months, and 70 days (Two and one third months) respectively to full length, at which time the worms reach the right ventricle or pulmonary artery. The youngest case of the dog heart-worm (D. immitis infection), occurred in a one-year old male dog owned by a well-to-do family, (higher social income class). It is believed that, while the 5 months old D. repens infected puppy could have been inoculated by an infected mosquito probably at the pup's first few days of life or so, the one-year old male dog infected with the

heart-worm may have picked up the infection perhaps between a few days of life to a month, through an infected mosquito taking a blood meal. The one year-old dog was first treated as a puppy on 3rd June, 1991, at the age of three and half months (according to Ahmadu Bello University Veterinary Clinic record). Although the attending clinician's presumptive diagnosis was Ehrlichiosis and Acariasis, the features presented by the patient at that age were likely unconnected with the early stages of dirofilariasis of the heart-worm origin. D. immitis was diagnosed by isolation of the dog heart worm-like microfilariae from the animal at the age of 1 year, being the period of sampling the animal. Furthermore, a close following-up of the clinic case-record for this patient at the later stage of this investigation pointed strongly to the diagnosis of possible heart-worm. The patient exhibited clinical and pathological symptoms of heart-worm disease.

As there was no previous report on the 'heart worm' in Nigeria or perhaps in the sub-region, the organism named D. immitis - like parasite, which exactly possessed both the morphological and behavioural features of D. immitis, was infact, preferred at that stage pending the external confrimation of the isolated specimens. Also its clinical and pathological signs in the hosts reinforced this suspicion.

The rate at which the puppies are being infected here is probably much higher than what is currently being observed, and it is believed that this points likely to the activities of the vectors in our community. It suggests that dogs of all ages, including even humans are at high risk of being inoculated with the infective larvae of this zoonotic parasite, irrespective of social class, as the vectors and transmitters are readily available and feed on both man and his pet.

The prevalence of infection between the two sexes did not differ widely. Moreover, there was no definite pattern observed between the different species of infecting parasites and the sexes, (Table 2). Thus, all sexes are apparently equally susceptible to the parasites.

The non-detection of infection in Basawa area of Zaria probably does not mean non-existence of the parasites in this area, (Table 3). The confirmation of infection by detection of microfilariae in the canine blood usually represents only a fraction of the acutal number of dogs infected. This phenomenon has been observed by Wong, et al (1973), and The American Heartworm Society, (1978) that 15 - 20% of dogs infected with canine filariae do not have microfilariae in the blood. The inability to find any infection in Basawa or in 7.4% of the population estimated to be

infected without microfilariae might primarily be due to what Richard (1979) refereed to as immunologically mediated response in the hosts in which microfilariae are removed from the circulation, or the presence of either a unisex or immature parasite in the dogs sampled. It might also have been as a result of fewer samples examined in Basawa area.

It is likely that contrary to previous widely held belief about the non-existence of the dog heart-worm (D. immitis) in Nigeria, the distribution pattern of the parasite suggests that the detection of the dog heart-worm in Zaria area is perhaps no accident. The infection was detected in most of the areas, (Fig. 1). The non-localization of the infections could raise the suspicion that the parasite has been in this area, or even in Nigeria for quite sometime. This Zaria situation may in fact, be a tip of the iceberg of what is happening in parts of the country, particularly in our Coastal States and towns, since the vector density is usually greater in the Coastal areas. A number of workers had observed a much higher prevalence of D. immitis at the Coastal regions of Australia, and United States, than States far removed from the Coastal areas, (Welch and Dobson, 1974; Wright, et al, 1989).

The mean size of microfilaria has been used as a differentiation tool for filarial parasite. The general observation made was that in any given sample of mixed infection, D. immitis had a much bigger mi-

crofilariae than those of D. repens, (Table 5). This corroborates with the findings of Jackson, (1969), and Soulsby (1982), regarding size of D. immitis, and D. repens. Also the measurements taken by Van veen, (1974) for D. repens in Zaria appears to be in conformity with the observations or measurements taken in this survey, but the measurements differed greatly from those of the industrialized world, like the United States.

It may be difficult to explain the reasons for the much difference in the sizes of the microfilariae of each species found here, and those of other geogrphical areas, particularly the Western world. However, it is probably that the limiting factors that determine the average size of each parasite species among other things could include, climatic conditions, and the general state of health of the natural host of the parasites.

It is to be suggested that dogs with good nutrition might be better equiped immunologically, and thereby mounting sufficient immune pressure on the parasite such that, the parasites are hindered on the average from attaining their potential sizes. In this line of thought, most malnourished canine pets, particularly in the developing countires would perhaps not be in a better position to mount this type of

sufficient immune pressure on the infecting parasites, and as such the parasites tend to grow unhindered by

the host, to full size.

Apart from the use of size to distinguish between canine filarial species, morphological and behavioural features are equally diagnostic in character. While, the live microfilariae of D. repens wriggle very vigorously and migrates, those of D. immitis do not wriggle much, they undulate at a place and never migrate. This observation has been made also by other authors: Stein and Lawton, (1973); Jackson, (1969); The American Heart-worm Society, (1978).

The length of time in which a host harboured the heart-worm (D. immitis) increased the chances of pathological damage to the host, (Table 6). The greatest clinical and pathological abnormalities were recorded for the two 8 year-old host infected with D immitis. Both dogs had several years ago been diagnosed as harbouring microfilariae (according to clinic records), although the species of filaria was not indicated, and perhpas assumed to be the predominant D. repens. All the D. repens-infected hosts, appeared to conveniently tolerate the parasite irrespective of age of host.

The mixed infection which occurred between the dog heart-wrom (D. immitis) and D. repens here, could be a measure of high vector and transmitter activities in this survey area. Besides, this mixed infection phenomenon observed here could possibly have been the

mystery behind the previous faulty reports for decades on the absence of the dog heart-worm in this part of the world, as other species might have been mistaken for the more predominant species, D. repens.

The importance of serological examination lies on the fact that in dirofilariasis, there are cases in which although there is an infection, this is not demonstrable in the canine blood. Wong, et al, (1973), and The American Heart-wrom Soceity, (1978) had observed this situation.

In a validity test (using both negative and positive predictive values) aimed at estimating the number of cases actually infected and seropositive, but showed no microfilariae, shot up the prevalence of canine filariasis in the population from 12.7% to 20.3%, (Appendix 1b). Also, employing the percentile false negative cases to estimate the actual number of host infected in the population, gave similar outcome to the estimations for the negative and positive predictive values.

Apart from the usefulness of validity tests in sero-epidemiological survey, "cut off point" has also been employed to assist decide at what titre an animal is to be assumed infected or not. Although, the cut off point has some value in sero-epidemiological survey, it has its own lapses in event of cross-reactions. Moreover, in dirofilariasis using cut off point in Indirect Haemogglutination Antibody technique

has further set-back, because cases abound as observed in this study, in which the titre is either too low or absent altogether, in spite of, established infections (Table 10). Nonetheless, using the geometric Mean Titre (G.M.T.) of the established cases as the cut off point, the estimated prevalence of filariae infection in the canine population on the basis of immunological reaction (serology) alone, did not differ widely from the estimates obtained through the knowledge of the confirmed cases plus the validity tests.

It is therefore suggested from the analysis of this survey that seroepidemiological investigation could be a useful and fairly reliable method of assessing the prevalence of canine filariasis in the population, utilizing the Geometric Mean Titre (G.M.T.) of the established infections as the cut-off point, (Appendix 1b).

Age of host apparently does not have any influence on the antibody level. However, a higher standard deviation in the puppies (0 - 11 months old), is a reflection of wider variations of titre from the respective mean in the puppy population than in the adult dogs, (Table 7). Although the sex of the different age-groups did not show any titre pattern, the Geometric Mean Titre (G.M.T.) is seemingly slightly higher in the male population than the females. A much lower standard deviation in the adult females, is a

measure of lesser variation from the mean titre in the females than their male counterparts, (Table 8).

Detection of appreciable antibody in Basawa area of Zaria as high as those of G.R.A./Sabon Gari area where the presence of the canine filariae has been confirmed, is suggestive that, although no microfilaria was demonstrated in the canine blood, in the Basawa area, the infection was likely present there. The infection here could occur as occult cases, or as either immature or unisex infection, which can not be picked up by parasitologic examination of the sampled animals.

The higher antibody titre observed in the male population of the established cases than female population, did not provide any protective value for the males, because the prevalence of confirmed infections was higher in the male population than the female. It has been observed by other workers, Welch and Dobson, (1974) that high canine antibody titres do not necessarily reflect immunity of dogs to infection with Dirofilaria, but rather indicates relative rates of parasite transmission in the dog population.

The infecting parasite species does not appear to have any influence on the antibody titre, either as single or mixed infections. It is likely that the titre level observed is a measure of the individual host reactions to the parasite, i.e. host-parasite

relationship for individual dog.

The exposure of the public in this survey area to the canine filarial parasites is high, and is largely due to constant vector activities throughout the year. That a large number of the public received the vector bites (mosquito bites) throughout the year here, is not unconnected with the fact that about 56.8% of the homes here are breeding the vector and transmitter of these parasites. One of the most effective methods according to Faust et al, (1970), used to control the vector mosquito is by reducing the vector threshold to 1.0% of all the breeding sites or homes. It is suggested that this high vector activity likely accounts for the disease in very young pups and also the mixed infection phenomenon found between the predominant D. repens and the dog heart-worm (D. immitis).

Furthermore, the keeping of more than one dog by so many people in Zaria also increases the exposure risk to the public, and thus making the presence of the dangerous dog heart worm in particular a threat to public health, as the parasite could be easily transmitted by the vector between dogs and then to man. The size of dog population, and prevalence of the parasite according to Wright, et al, (1989), are the risk factors for transmission of dirofilariasis in human beings.

Besides, a good number of the public being ignorant to this canine filarial disease and the

possible ways of its transmission is a further threat to public health. Thus, it seems obvious that public education and information about this disease could be profitable in reducing the prevalence of the disease not only in man, but in his canine pets too.

The moderate prevalence of canine filariasis (dirofilariasis) in Zaria might in part be due to an on-going dog business (dog market) in the Zaria area, which was uncovered during the survey period. Selective depopulation of adult dogs for the past decade here, is believed to have contributed in no small measure to keeping down or maintaining the prevalence of canine dirofilarial infections to a moderate level.

It is suggested that if it were not for this slaughter business going on in parts of Zaria, the prevalence of canine dirofilariasis would have been higher than the present figure. This observation might be equally useful in assessing other areas in the country. However, for the fact that the parasites are around and the public is highly exposed, it looks relevant that screening of dogs, and accurately diagnosing this zoonotic parasites and properly treating all cases are all indispensable in reducing or preventing disease transmission.

CONCLUSION/SUGGESTIONS FOR FURTHER RESEARCH

From this study, it can be concluded that:

1. The predominant Dirofilaria species in Zaria is D. repens. However, a dangerous dog heartworm -like filaria (D. immitis-like) which had been reported for decades to be absent in Nigeria, is also present. The distribution of the dog heartworm-like filaria in Zaria seems to suggest that this parasite has been in Zaria or Nigeria for quite sometime now.

2. The presence of the canine filariae is not only a threat to the canine and felid pets, but to human beings (public health) since the activity of the vectors and transmitters in Zaria is very high.

3. A good number of the public is ignorant of this zoonotic disease (dirofilariasis), and its possible mode of transmission. Thus, both education and information to the public nationwide look inevitable in order to reduce or prevent the transmission of the disease, either in man or his pets.

4. Parasitological investigations, although a more practical means of diagnosing the infections, it cannot give a true picture of the actual filarial infection in the population. A combination of the two diagnostic methods, serological and parasitological examinations are very useful and more reliable epidemiological tools.

5.) D. repens infection has relatively no noticeable effect on the natural host (although it also requires adequate treatment as it is zoonotic). However, infection with D. immitis-like filaria has an overwhelming clinical and pathological effects on the host. The clinical and pathological damage increased progressively with time, as observed in the clinic here. The animals were observed to have become a complete liability to their owners.

6.) Since there has never been a previous report on the D. immitis-like parasite either in Nigeriia or in the sub-region, it is likely that the parasite encountered in this study was the dog heart-worm, D. immitis. It may be worth mentioning that a report from the 'International Institute of Parasitology' at St. Albans, United Kingdom, where the parasite was identified later, (see attached identification letter in Appendix 4, last page), indicated that, "the parasite appeared to be D. immitis."

Further work is also required in the following areas:

1. A survey of dirofilariasis in human population is necessary, since the public had been exposed for quite sometime.
2. Surveying and screening of dogs in parts of the country are equally essential, to enable us assess the

true picture of the disease in the country.

3. Also vector survey could be helpful in knowing the offending vector for the parasite.

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APPENDIX 1a

Detectable *Dirofilaria* spp. infection, (Exposure factor) or (independent variable)	(Dependent variable) *Dirofilaria* Antibody in serum (outcome) Present (+Ve)	Absent (-Ve)	Total
Present (+Ve)	11	1	12
Absent	84	10	94
Total	95	11	106

The number of parasite's natural hosts (dogs) tested serologically = 106

No. of +ve sera with no detectable mff in the blood=84

No. with detectable canine filaria spp. = 12

No. with detectable canine filaria spp. - infection with +Ve serum = 11

No. with negative sera and also showing no microfilaria in the blood = 10

(i) Incidence rate of immunoglobin (antibody) in the parasites natural host infected with canine filariae = 92%

(ii) Incidence rate of antibody (Ig) in the dogs not presently showing any infection with the filarial agent (the exposure factor) = 84%

(iii) Relative risk (RR)=

$$\frac{\text{Incidence rate of "Ig" (antibody in dogs showing detectable mff.}}{\text{Incidence rate of antibody in dogs not showing presence of mff. in blood.}}$$

RR = 1.05

Inference: The relative risk (RR) of 1.05 (greater than unity). indicates that an association apparently does exist between exposure factor (canine filarial spp. infection i.e. the independent variable) and the presence of detectable filarial antibody i.e. the outcome or the dependent variable.

(iv). The test of statistical association if any

To test for any statistical association between the exposure factor and antibody detection in the canine host, a 95% confidence interval (CI) is set on the RR, to see if the value on RR is greater than unity. In other words, if there is a statistical significant association between the exposure factor (infecting canine filaria) and the outcome (i.e. immunoglobin or antibody detection).

$$\text{Thus, } \log_e RR = \log_e \frac{a(c+d)}{c(a+b)}$$

DDDDDD

$$\log_e RR = \log_e = 1/a+1/c-1a+b-1/c+d$$

S.E=0.176
95% CI = 0.354 and 0.336.

Inference: From the value of the CI on RR (not including I), there is a statistical significant association between the canine filarial infections in the host and the detection on immunoglobulins or antibody to Dirofilaria antigen in the hosts.

(v) The Attributable Risk (AR): This is the amount of canine fialria antibody attributable to the presence of the filarial parasite infection.

AR = Incidence rate of demostrable immunoglobulin (antibody) in the microfilariae positive cases minus the incidence rate of antibody in the microfilaria negative cases.

AR = 3.6%

(vi) The estimated population attributable risk (PAR):

This is the fraction of all cases of immunoglobulin (antibody) detected in the canine population which were due to exposure to the factor - demonstrable canine filarial infection.
PAR = 0.57%

(vii) Chi square (X^2 concept This can also be set up to see if any statistical significance of cause-effect (factor-outcome) relationship exist between canine filarial infection as observed and detectable antibody to the parasites.

Null Hypothesis: "Thereis no association between the canine filarial infection and antibody detection".

N.B. Level of rejection if $X^2 > 3.841$.
Chosen alpha level = (1-a) = 0.05
Degree of fredom = 1; from (r-1)(c-1)
where r = no. of rows
and c = columns in the 2 by 2 contigency table.

Thus $X^2 = 363$

The derived value of X^2 is much more greater than3.84 (at P<0.05) and thus the Null Hypothesis that there is no significant association between canine filariasis (Dirofilarial infection) and detection of anibody to Dirofilarial antigen in the host is rejected.

Inference: Hence there is statistically significant association at (P<0.05) existing between detection of antibody to Dirofilarial antigen and canine filarial infections.

(viii) Also Odd Ratio (OR) may be set up to further asses the situation that is if statistical association exists.

This is taken diagonally from the 2 by 2 table.

Thus OR = 1.3

OR is greater than unity and further goes to stress a statistical significant association existing between antibody detection and canine filarial infection.

Validity: This is the accuracy with which a test reflects the reality; in other words, it is a measure of how closely the observation taken in an experiment(work), corresponds to the actual state of affair (i.e. the real life situation).

Here both sensitivity and specificity are a measure of validity.

Sensitivity: This is that ability of a screening test to correctly give positive result, when the individuals are truely infected or diseased; while specificity measures the ability of the test proceedure to give negative results when the individuals examined are truely not infected or diseased.

Serological Test	Diagnosis		
	Diseased (Microfilaremic)	Not diseased (No mff. found	Total
Positive	(a)11	(b)71	(a+b)82
Negative	(c) 1	(d)23	(c+d)24
Total	(a+c)12	(b+d)94	(a+b+c+d)106

From the contigency table:
a = Number of canine hosts confirmed to be infected with canine microfilariae and showed antibody to the parasites in the blood (True positives).

b = Number of canine hosts who did not demonstrate mff. but showed antibody to the canine filariae (false positives).

d = Number of canine hosts who did not demonstrate mff. and were also sero negative (true negatives).

c = Number of canine hosts that did demonstrate mff. but were sero negative. (false negatives).

Total serum tested = 106
Total positives = 83

$$\text{Sensitivity} = \frac{a}{a+c} \times 100 = 92\%$$

$$\text{Specificity} = \frac{d}{b+d} \times 100 = 25\%$$

N.B. Factors accountable for low specificity include any all the following, resulting to not finding microfilaria in event of an infection.

(i) Immature adults

(ii) Unisex infection

(iii) Occult cases i.e. immunological events in the host animal which prevent mff. from being released in the circulation.

iii. % false negatives

$$\frac{c}{a+c} \times 100 = 8.3\%$$

Predictive value for +ve test

$$\frac{a}{a+b} \times 100 = 13.4\%$$

Predictive value for negative test

$$\frac{d}{c+d} \times 100 = 96\%$$

N.B. The predictive value for the positive test = 13.4%, indicates the percentage of the 83 canine host which were sero-positive in the serological survey that were actually positive to the Dirofilarial antibody.

While the negative predictive value of the test is the probability indicating that 95% of the dogs which test negative in the test (i.e. 23 true negative) were actually not infected with the canine filariae.

APPENDIX 1b

Estmation of the total number of dogs infected with canine filariae in the population (n = 158).

A) *Using microfilaria confirmed cases + validity test of expected positive cases; (+ve and -ve predictive values)*

		Comments
(i)	Number of dogs with *detectable mff. = 20*	Confirmed infection by *microfilariae.*
(ii)	Number of dogs without *mff.* but truly infected (by virtue of +ve predictive value i.e. 13.4% of 83 dogs=11	Number actually showing antibody to Dirofilaria by validity test (and without mff. in blood.

plus

(iii)	No. of dogs without mff. but truly infected by virtue of -ve predictive value i.e. 96% of 23 = 1	No. of dogs in the group which are seronegetive but not actually seronegetive *to Dirofilaria* antigen (and without mff. in blood).

Total number of infected cases in the population is about 32 = 20.3%.

About 20.3% of the population is infected with the canine filariae.

B) Using the number of mff confirmed cases + % false negative cases to estimate the total number of infected cases in the population

(i) Number of dogs showing mff. in blood = 20

plus

(ii) % false -ves (i.e No. of dogs that did not show mff. but truly had antibody to Dirofilaria antigen i.e. they are truly infected = 8.3% of (158-20)=11.5
i.e number of false -ves = 12 from validity test.

Total number of infected cases = 32 or 20.3% in the population.

Summary:
1) About 20.3% of the population is estimated to be infected with the canine filariae.

2) 20.3-12.65=7.35% of infections showing no microfilariae could be due to (i) occult cases (ii) Immature worms in the infected cases and(iii) unisex infection.

Where 12.65% is the percentage in the population showing microfilaramia; or prevalence of infection in population by virtue of microfilaraemia.

C) Determination of the prevalence of canine filaria infection in the population by virtue of only serological (seroepidemiological) survey.

Cut off point of antibody titre = 12; This is the geometric mean antibody titre (GMT) for the established (comfirmed) cases.

However, the reciprocal of serum dilution close to GMT = 12 is chosen as 16.
Thus, the cut off point for the population titre is chosen as *r*16 for any positive sera (That is any titre less than 16 is assumed to be negative).
Therefore number of cases with antibody titre *r*16=25.
No of sera tested = 106
Therefore prevalence of infection = 23.6%;
and seroprevalence of canine filariasis = 23.6%.
Comment: Seroprevalence of 23.6% (determined using GMT of established cases) is closer to 20.3%, being estimated disease prevalence in the population, than 12.7% which is the prevalence by virtue ofdetection of mff. in canine blood.

Thus, in epidemiological survey, sero-surveys are useful and more reliable tools in dirofilariasis to estimate the extent of infection in the population.

APPENDIX 2

3.2.2. Micrometry/identification of parasites

This involves the use of stage and occular micrometer to determine the "microscopic factor" of the microscope in use.

The steps are:

1. The stage micrometer was focused under x10 power of the microscope's objective lens and the number of the big spaces of the stage micrometer recorded.

2. The microscope eye piece was repalced with an occular micrometer eye piece of x40.

3. Now the eye-piece and the stage micrometers were allinged to determine the number of small spaces of the eye-piece micrometer falling inside the big spaces of the stage micrometer and noting the point at which the small and big lines correspond to one another. You may start reading from the zero calibration the moving upwards to find out where the and small lines correspond with one another to indicate how many lines of each correspond with one another. This is necesary for the estimation of the size of the parasite to be measured.

N.B.: In the stage micrometer used, its whole length was 1 mm, i.e. from 0 to 10 subdivisions.
Therefore one subdivision = 0.1 mm
but 1 mm = 1000 microns
Therefoer one single division of the stage micrometer = 100 microns.
Therefore 0.1 mm = 100 microns.
Lets say while using x40 magnification of the microscope, $1^{1}/_{2}$ (0.15 mm) of the stage micrometer allings or corresponds with 4 small spaces of the occular micrometer.
That is 40 small spaces of occular micrometer=1.5x100 u
Therefore the magnification factor (Mf) of the microscope = 3.75

Step 4. Now the stage micrometer was removed completely and replaced with the prepared slides containing the parasites (microfilaria) to be measured.
However, the eye-piece micrometer still remains in use after calibration.

Step 5. If the parasite (mff) lenghth gave 86 small divisions (or spaces) in the eye-piece micrometer, the actual length of the parasite is 86 x MF = 322microns.
From literature the size ranges of canine microfilariae are:

1. D. repens: 280 - 300 microns
   D. immitis: 307 - 322 "
   Dipetalonema reconditum: 271 microns.
   (Soulsby, 1982)

2. D. repens: 300 - 369 microns (Schillhorn, 1974)

3. D. immitis: 298 - 314 u
   D. reconditum 264 - 298 u
   (Jackson, 1960)

4. D. reconditum: 230 - 290 u (Korkejian & Edeson, 1968).

Appendix 3

3.3.2 Preparation of phosphate buffer solutions (P.B.S.), for antigen, and sera diluents, and for Erythrocyte washing.

Phosphate buffer solution (P.B.S.) was an essential component of the haemagglutination procedure, for erythrocyte tanning, and washing, and also for antigen and serum diluents.

Two types of P.B.S. were prepared for different purposes, for antigen coating, diluents and erythrocyte washing. The two types differed in terms of salt constituents and the final pH of the solution. The resulting solutions were sterilized in an octoclave.

The PBS constituents used are:-

A) PBS for tanning Erythrocyte

(i) Potassium dihydrogen phosphate; (kH_2PO_4): (0.02M)
Qty = 12.2 gm

(ii) Disodium hydrogen phosphate; (Na_2HPO_4): (0.06M)
Qty = 40.4 gm

(iii) Sodium chloride; (Nacl): (0.12M)
Qty = 36gm

*All dissolved in 5 litres of water (distilled) H_2O) and final pH = 6.4; (then sterilized in octoclave)

B. P.B.S. for diluents and washing erythrocytes

(i) Sodium chloride; Qty = 8g/litre
(ii) Potassium chloride (Kcl); Qty = 0.2g/litre
(iii) Disodium Hydrogen phosphate; Qty = 1.15g/litre
(iv) Potassium dihydrogen phosphate; Qty = 0.2g/litre

All dissolved in 1 litre of distilled water and with a final pH = 7.2.
The resulting solution is octoclaved for sterility.

3.3.3 Washing of sheep blood cells (erythrocyte)

Sheep blood cells (erythrocytes) here act as carriers of antigens in the haemagglutination test. In the washing procedure, the sheep blood cells were collected into sterile Alsevers solution, (4 parts of sheep blood to 1 part of Alsevers), which was allowed to stabilize for at least one week at $4^{o}C$ in a refrigerator before use.

Now, the sheep blood was gently mixed with the Alsever after having stablized for one week by gentle stirring, and 3 ml of the blood was pipetted into a sterile 15 ml centrifuge tube, and then 10 ml P.B.S. (pH 7.2) was added.

By means of an applicator they are mixed thoroughly by gentle stirring.

The solution was centrifuged at 3,000 r.p.m. for 5 mins.

The supernatant was decanted by means of a pasteur pipette.

This washing was repeated 3 times.

Now, 0.5 ml of the packed washed red cells was added to 20 ml P.B.S. (pH 7.2) in a large test tube. This solution is thoroughly mixed by gentle stirring with an applicator stick.

3.3.4 Tanning of washed red cells and coating with antigens

(Tannic acid-treated red cells, Boyden's Test). After treating erythrocytes with "tannic-acid" acting as a mordant, they can absorb many protein antigens, and this constitutes an important step in passive haemagglutination test.

In the tanning procedure, 3 ml of the cell suspension from step 5 of the erythrocyte washing above, was pipetted into a centrifuge tube.

3 ml of fresh dilute tannic acid (1:20,000) solution was added to the centrifuge tube, mixed thoroughly by gentle stirring, and placed in an incubator for 10 mins at 37°C. (Note: The tannic acid here is acting as a mordant).

The suspension above is now centrifuged for 5 mins at 3,000 r.p.m., and the resulting supernant decanted by pouring.

The resulting packed cells are resuspended in 3 ml of P.B.S. (pH 7.2), mixed thoroughly using a pasteur pipette, and again centrifuged for 5 mins at 3,000 r.p.m. Also the supernatant was decanted.

Now, the erythrocytes are resuspended in 3 ml P.B. S. (pH 7.2) and then mixed thoroughly.

The next important step to follow was to coat the tanned cells with the lyophilized adult D. immitis

antigen. (N.B.: The erythrocytes here act as carriers of soluble antigens).

In this coating procedure, 4 ml of P.B.S. (pH. 6.4), was pipetted into a centrifuge tube 1 ml of the soluble D. immitis antigen was added to it, followed by 1 ml of the cell suspension from step 5 of the tanning process.

The content was mixed thoroughly by gentle stirring, and the tube allowed to stand at room teprature for 10 mins.

At the same time with step 7 above, "control cells" were prepared also by adding 4 ml of P.B.S. (pH 6.4) to another centrifuge tube, then instead of adding D. immitis antigen solution, 1 ml of saline (sterile) was added, followed by 1 ml of the cell suspension from step 5 of the tanning process. The content was mixed thoroughly by gentle stirring and this tube was also allowed to stand at room temperature for 10 mins as done to the test material.

Both tubes (test materials and the control) were now centrifuged for 5 mins of 3,000 r.p.m. The supernatants were decanted.

The cells were resuspended in 2 ml of P.B.S. (pH 7.2), mixed thoroughly using pasteur pipette.

The cell suspension were centrifuged for 5 mins at 3,000 r.p.m., and the supernatant decanted.

Then finally, these cells are resuspended in 1 ml P.B.S. (pH 7.2).

Now, the coated test cells and the control cells are ready for use for the passive haemagglutination serology.

N.B.: Purefied lyophilized D. immitis antigen used in coating the erythrocytes was obtained from George S. Tilloch & Associates, Santonio Texas 78249, USA; (Supplied by Dr. Galadima, M; Dept. of Microbiol, A.B.U.)

Appendix 4

4.2 Results of the immunological reactions of the parasites natural host (canine host), to purified Dirofilaria immitis antigen (1:200 lyophilized antigen concentration)

Serum number, Sex, Age-group of host, and titre levels in passive haemagglutination antibody test

0.3 months old				4-6 months				7-11 months			
Serum No.	Titre Level M	:	F	Serum No.	Titre Level M	:	F	Serum No.	Titre Level M	:	F
001			0	006	4			019	2		
003			4	016	8			028	16		
004	0			017			0	033	32		
008	0			018	8			041			2
011			2	021			4	046	0		
014	2			024	2			071			8
020	0			026			0	086	0		
030	0			044	32			097	0		
040	2			047	64			113			4
042	4			074			64	130	4		
045	0			084			4	140	2		
054	0			116			2	142			0
064	2			120			0	143	0		
077			8	124			4	146			0
123	64			132			8	150			32
151			16	135	4						
155			16	147			4				
Mean Titre	6.7		7.7		17.4		9.2		6.2		7.7

Mean POPn	= 7.2		13.2		7.0	
Std. Deviat. POPn	= 14.8		19.9		10.2	
S.D: Sex	18.30	6.1	22.9	18.8	10.0	11.3

Serum No.	1-2 years Titre Level M	: F	Serum No.	r 3 years Titre Level M	: F
009	0		002	32	
010		8	023	2	
015		8	025		8
031	8		038		2
039		4	049	0	
043		8	050	8	
048	2		053		8
052		16	061	8	
069		16	078		16
081	8		080	4	
082	8		083		4
095	8		085		8
098	32		087	32	
105	2		104	16	
109	32		106		32
110	0		107		8
111	4		118	4	
112		4	121	32	
114	8		131		4
115	8		134		16
125		8	136	4	
126	8		138	4	
127		0	145	0	
128		16	149	32	
129	2		161	0	

133		16	162	32	
148	16				
154	8				
160	16				
Mean Titre	9.4	9.5		12.8	10.6
Mean Popn		9.45			11.7
Std. Dev.		7.4			11.1
S.D. per Sex	8.8	4.9		12.1	8.8
G.M.T. (Popn)	9.4 ± 11.9				

G.M.T. per sex = Males = 9.7 ± 13.5; Females = 8.9 ± 8.9

Appendix 5

Serial/Code No.:____

QUESTIONNAIRE (FOR DOG OWNERS)

Instructions:

Please read carefully before answering any question. Answer these questions, by simply ticking the correct answer in the appropriate box provided, e.g. [ ]

1. The number of dogs in your family of about 1 year old or more is:

1. [ ] Between 2 and 3 dogs.

2. [ ] Between 4 and 5 dogs.

3. [ ] Only one dog

4. [ ] Is more than 5 dogs.

2. Apart from the dog, does your family keep cats?

1. [ ] No ; [ ] Yes

3. Which of these statements is correct concerning your own village?

1. [ ] only my family keeps dog.

2. [ ] only a few families keep dogs.

3. [ ] so many families keep dogs.

4. What steps do you often take in your own family to prevent mosquitoes from causing sickness to you? (Tick one or two answers only).

1. [ ] We rely on drugs, (Medicine)

2. [ ] We use insecticides, (e.g. Shelltox, etc.)

3. [ ] We attempt to make sure that water is now left open or provided for the mosquitoes to breed (multiply) in our premises.

4. [ ] We don't usually do anything because we are already tired of these mosquitoes.

5. Considering the place you live in Zaria, what time of the day does the mosquito bite you?

1. [ ] only in the day time

2. [ ] only at night

3. [ ] they bite both in the day and at night.

6. *By your own experience, what period of the year do you* have mosquito bites?

1. [ ] only during the dry season of the year

2. [ ] only during the rainy season of the year

3. [ ] Both the rainy and dry seasons of the year

7. Finally, apart from the common malaria, indicate one other sickness or disease you think that the mosquitoes may help to cause? (Tick only one answer).

1. [ ] No other sickness apart from malaria.

2. [ ] Blindness

3. [ ] Filariasis, (occasional body itchings and swellings on parts of the body).

4. [ ] Dysentry

5. [ ] I don't know.

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21 April 1993

Mr I N Anyanwu
Department of Public Health and
Preventive Medicine
Faculty of Veterinary Medicine
Ahmadu Bello University
Zaria
NIGERIA

Dear Mr Anyanwu

I am sorry for the time it has taken for the slides which you sent to the Natural History Museum to be identified.

The slides were passed on to me but unfortunately many were broken and the tubes had completely dried up.

The only slide on which I could find a microfilaria was 102 and this appeared to be *Dirofilaria immitis*.

Yours sincerely

R Muller
Director