PREVALENCE, CLINICAL PATHOLOGY AND TREATMENT OF MICROFILARIASIS IN DOGS IN THRISSUR

By
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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DECLARATION

I hereby declare that this thesis entitled "**PREVALENCE, CLINICAL PATHOLOGY AND TREATMENT OF MICROFILARIASIS IN DOGS IN THRISSUR** " is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "**PREVALENCE, CLINICAL PATHOLOGY AND TREATMENT OF MICROFILARIASIS IN DOGS IN THRISSUR**" is a record of research work done independently by **Smt. Radhika, R.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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To My Loving Parents And Husband

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Introduction

INTRODUCTION

The domestic dog (*Canis familiaris* L.) known to have been in the service of man as far back as some 12,000 years ago, has been valued not only as a coveted pet, guard and companion but also as game retriever, smuggling-detector and criminal aprehender. Because of its tremendous multi-faceted values, it has been developed into innumerable breeds and dog-rearing has become a most lucrative enterprise, offering good self-employment opportunities and substantial financial gains. With the popularisation of dog shows, the rearing of exotic breeds has become the status symbol of the society. Such breeds like the German Shepherd, Great Dane, Lhasa Apso, Doberman, Pomeranian etc. are in much greater demand than the mostly non-descript indigenous varieties.

Due to the prevalence of improper managemental practices, extreme climatic variations, high land pressure, heavy monsoon and faulty drainage system, dogs in Kerala are prone to many zoonotic, contagious, parasitic, nutritional and metabolic diseases.

Parasitic diseases constitute the largest group of diseases affecting dogs. It is a well-known fact that in the tropical and subtropical countries it is difficult to find dogs which are free of parasites. Although, most of the

parasites do not produce an acute fatal disease, the growth, work and breeding efficiency of the dog is adversely affected by parasitism. Hence, parasitic diseases in dogs warrant maximum attention in rearing and management of the animals. The large number and variety of parasites and their wide host-range make parasitic diseases most complex and their diagnosis very difficult. Although generally the mature stages of parasites are considered to be the most pathogenic, other stages may also be equally or more pathogenic at times.

Of the several parasitic diseases of dogs, filariases caused by *Dirofilaria immitis*, *D. repens*, *Dipetalonema dracunculoides*, *D. reconditum* and *D. grassi* are of considerable importance. The larval stages shed by these filarial worms at all times into the circulation are called microfilariae. Instances of infection with microfilariae of filarial worms are assuming an increasingly major role in the health-care of dogs. According to Saseendranath *et al*. (1986), more than 24 per cent of the dog population in Thrissur are infected with microfilariae. It is known that microfilariasis is prevalent inspite of the adoption of strict managemental practices and is noticed even in well-maintained dogs. Both exotic and non-descript dogs are highly susceptible to microfilarial infection irrespective of their breed or sex. Microfilariae of filarid worms are normally transmitted to

dogs through the bites of infected mosquitoes belonging to the genera *Culex*, *Anopheles* and *Aedes*.

The adults of *Dirofilaria immitis* occur in the right ventricle as well as pulmonary arteries of the host and of *D. repens* in the sub-dermal tissues, between tendons of legs as also in kidneys and lymphatic nodes. The predilection site of both adult *Dipetalonema dracunculoides* and *D. reconditum* is the peritoneal cavity. The concentration of microfilariae of the worms in the blood varies over a 24 hour period and may reach as high as 10^3 to 10^5 microfilariae/ml of blood. The microfilariae exhibit incomplete nocturnal periodicity and are inbibed by female mosquitoes while sucking blood. Experiments conducted in dogs infected with *Dirofilaria immitis* and *D. repens* revealed a marked seasonal variation in microfilaraemia (Cancrini *et al.*, 1975). The finding suggests that atmospheric temperature significantly affected these variations.

The adult filarid worms have different predilection sites and specific diagnosis is made only by the recovery of the worms after the death or slaughter of the host. Though microfilariae have very little morphological differences, they can be speciated by adopting reliable techniques and this will give a clue to the species of the filarid worm, its predilection site and the harmful effects on the host.

The study on microfilariasis in dogs is all the more important in a filariasis-hyperenzootic area such as Thrissur, as the filarids, especially *Dirofilaria repens* and *D. immitis*, pose a zoonotic threat to the human inhabitants of the area.

Considering the afore-mentioned relevance of the disease and its high frequency of occurrence in Thrissur, a detailed study was undertaken to:

1. Study the prevalence of microfilaria infection in dogs,

2. Speciate the microflariae isolated from the blood of dogs,

3. Assess the clinico-pathological changes in microfilarial infection and

4. Compare the efficacy of Ivermectin, Milbemycin oxime and Levamisole against microfilariasis in dogs.

Review of Literature

REVIEW OF LITERATURE

2.1 Prevalence of infection

The research work conducted by various scientists in the field has revealed that filariasis is one of the most common parasitic diseases in dogs (Wong *et al.*, 1973; Anderson, 1992).

The first case of *Dirofilaria immitis* was reported by Leidy in 1856. Railliet and Henry (1911) established the genus *Dirofilaria* with *D. immitis* as the type species and also described *D. repens* in the same year. *D. repens* was also detected in dogs in the Democratic Republic of Vietnam (Bauche and Bernard, 1911). Webber and Hawking (1955) detected *D. repens* and found that the pre-patent period of the parasite is 27 to 34 weeks (6-9 months). The description of *Dipetalonema reconditum* by Newton and Wright in 1956 threw doubt on the specific validity in cases of prior heartworm reports based solely on the different types of microfilariae.

Wallenstein and Tibola (1960) surveyed the Maryland area for canine filariasis and reported that male dogs were more prone to infection than the females. The finding was endorsed by the study of Thrasher *et al.* (1965) on the prevalence of canine filariasis in New Orleans. Gubler (1966), who made a

comparative study on the distribution, incidence and periodicity of *Dirofilaria immitis* and *Dipetalonema reconditum*, also opined that male dogs had a higher prevalence rate. Hirth *et al.* (1966) studied the prevalence, sex- and age-variations in canine filariasis in Connecticut dogs.

Lewis and Losonsky (1977) studied the sex- and age-distribution of dogs with heartworm disease at Georgia.

Martin and Collins (1985) examined 331 Greyhounds in the Hunter Valley and New South Wales and found that the prevalence rate of *Dirofilaria* species and *Dipetalonema reconditum* were 10.9 and 3.6 per cent respectively. They also stated that the prevalence was greater in summer than in winter.

Doby *et al.* (1986) reported the prevalence of *Dirofilaria immitis* and *D. repens* infection in dogs in Western France. Davoust and Lahitte (1989) reported development of enzootic dirofilariasis involving *D. repens* and *D. immitis* in military kennels in South Eastern France.

Magi *et al.* (1989) studied the distribution of canine filariasis in Tuscany during 1986 and 1987 and found that out of 205 dogs examined, 63.4 per cent had microfilaria of *D. repens*, 23.9 per cent of *D. immitis*, 5.4 per cent of *Dipetelonema reconditum*, and that 7.3 per cent had combined

infection with *Dirofilaria repens* and *D. immitis*. The highest incidence of *D. repens* was observed in the coastal area of Pisa, Lucca, Leghorn and around Grosseto. Perez-Sanchez *et al.* (1989) studied the prevalence of canine filariasis in Salamanca (North West Spain) by examining 293 blood samples of dogs. The overall prevalences of *D. immitis*, *D. repens* and *Dipetelonema reconditum* infection were 12.3 per cent, 0.3 per cent and 2.1 per cent respectively.

Parija (1990) reviewed zoonotic *Dirofilariasis* and opined that *D. repens* is the common parasite of dog in USSR, Europe, Africa, India and Sri Lanka while *D. immitis* occurs in USA, Australia and Japan.

Rojo-Vazquez *et al.* (1990) conducted a survey in four areas of Spain. Out of the 1,683 dogs examined, 2.1 per cent were positive for microfilaria of *D. immitis* , 0.2 per cent for *D. repens*, 0.7 per cent of *Dipetalonema reconditum* and 4.1 per cent for *D. dracunculoides* and that 3 dogs carried mixed infection. Kamalu (1991) described canine filariasis due to *Dirofilaria repens* in South Eastern Nigeria. Martini *et al.* (1991) diagnosed canine filariasis after screening 329 dogs in an hyperendemic area of Northern Italy and found that 163 dogs (44.5 per cent) were positive, of which 160 dogs had *Dirofilaria immitis* microfilariae, 2 had *D. repens* and one, both the species.

Doganay and Biyikoglu (1992) found a prevalence rate of 2 to 12 per cent and 2 to 3 per cent respectively for microfilaria of *D. immitis* and *D. repens* in dogs in various parts of Turkey. *Dipetelonema reconditum* was reported from one dog.

Rossi *et al.* (1993) studied the prevalence of microfilariasis in dogs in Piedmont and found that the rate was 23.8 per cent with regard to *Dirofilaria immitis* and 20.5 per cent for *D. repens*.

Papazahariadou *et al.* (1994) examined 50 dogs with episodic weakness and 50 clinically normal hunting dogs. A prevalence rate of 10 per cent for *D. immitis*, 30 per cent for *D. repens* and 8 per cent for *Acanthocheilonema reconditum* were found in episodic weakness group while in clinically normal group, the figures were 14 per cent for *Dirofilaria repens*, 4 per cent for *Acanthocheilonema reconditum* and none for *Dirofilaria immitis*.

In India, according to Bhalerao (1935), Mudaliar and Alwar (1947) and Singh *et al.* (1958), although evidences indicated filariasis in dogs, there are very few published reports of Dirofilariasis. Dog filariasis due to *D. immitis*, *D. repens* and *Dipetalonema dracunculoides* were reported by Rao in 1938. D'souza (1950) recorded canine microfilariasis and some of its clinical aspects in four Indian dogs in

Madras. Prusty *et al.* (1972) reported Dirofilariasis in dog from Bhubeneswar. *Dipetalonema grassi* and its microfilariae were reported for the first time from dogs in India by Balasubramaniam *et al.* (1975) from Madras. A survey conducted by Chakrabarthy and Choudhary (1983) in 3200 dogs in West Bengal between 1977 and 1981 revealed microfilariae in the peripheral blood of 140 dogs (4.37 per cent) of more than 7 years of age. Among these, *Dirofilaria immitis* was noticed in 3.06 per cent and *Dipetalonema reconditum* in 1.3 per cent of dogs. Sharma and Pachauri (1982) examined 400 dogs from the tarai region and found a prevalence rate of 5.25 per cent for *Dirofilaria* infection on the basis of microscopic examination of blood. Dhaliwal *et al.* (1987) reported *D. immitis* from Punjab for the first time. Patnaik (1989) examined 7 dogs and found a prevalence rate of 44.28 per cent of *D. repens* in Orissa.

In Kerala, Valsala and Bhaskaran (1974) screened 92 dogs in Calicut and detected microfilaria of *D. immitis* in the peripheral blood of 12 dogs (13.04 per cent). Incidence of canine dirofilariasis in Thrissur was reported by Saseendranath *et al.* (1986) by screening dogs brought to the Kerala Agricultural University Veterinary Hospital, Kokkalai, during a period of six months from September 1984 to February 1985. The prevalence rate of *D. immitis* was 24.2 per cent.

2.2 Diagnosis

Newton and Wright (1956) modified the Knott's Method for detecting microfilariae in blood. Lindsey (1961) used the modified Knott's Method to detect and differentiate microfilaria of *Dirofilaria immitis* and *Dipetalonema reconditum*. The results obtained was confirmed by the same author in 1962 from the necropsy findings of 71 dogs that had been examined. Based on this, Lindsey (1965) recommended the Modified Knott's Method for detecting and identifying microfilariae in dog blood. Jackson (1969) used special staining method for the diagnosis and differentiation of canine microfilariasis due to *Dirofilaria immitis* and *Dipetalonema* Sp. and gave the measurements of microfilariae of both the species. The method was claimed to have 90 per cent accuracy. The number of microfilaria in the blood gave no indication to the extent of adult filarial infection.

Altman (1972) compared direct smear technique, microcapillary tube method, Knott's test and multipore filtration for the diagnosis of canine microfilaria. Kelly (1973) reviewed the wet smear, serum, saponin concentration, Modified Knott's Technique, capillary haematocrit technique and filter technique for detection of microfilariae. In a comparative study of the different methods for diagnosis and differentiation of canine microfilariae, Stein and Lawton

(1973) found that the Modified Knott's Test gave much reliable results for the diagnosis and differentiation while the direct wet smear, filtration technique, capillary sedimentation technique and saponin test were of less reliable value.

Watson (1973) supplemented his observations to that of Kelly (1973) for detecting and differentiating microfilaria in canine blood.

Valsala and Bhaskaran (1974) diagnosed microfilariasis by examination of blood films and stained the smear with Wright's-Giemsa method to detect and identify microfilaria.

Whitlock (1978) also compared the efficacy of wet smear, microcapillary haematocrit, serum concentration, Modified Knott's Technique and filtration techniques for the diagnosis of microfilarial infection and opined that Modified Knott's Technique was a reliable method for diagnosing canine microfilaria.

Acevedo *et al.* (1981) used the Knott's Technique to detect microfilariae in blood of dogs in the United States. Courtney *et al.* (1985) examined 2004 Greyhounds using Modified Knott's Test to study the prevalence of *Dirofilaria immitis*, *D. striata* and *Dipetalonema reconditum* infection.

Lahitte *et al.* (1986) compared the efficacy of Modified Knott's Test and direct microscopic examination of blood film in detecting microfilariae.

Saseendranath *et al.* (1986) diagnosed microfilariasis in dogs in Thrissur by examining the wet film of blood. Whiteley (1988) reviewed the various screening tests for microfilariae and used Modified Knott's Technique for concentration and identification of microfilariae. Perez-Sanchez *et al.* (1989) diagnosed microfilariasis by Modified Knott's Test. Rojo Vazquez *et al.* (1990) screened 1683 dogs for microfilariasis using the Knott's Technique. Martini *et al.* (1991) used the direct smear and Modified Knott's Test to assess the sensitivity and specificity of the positive and negative samples. Dhaliwal and Sani (1993) diagnosed *D. immitis* infection by direct smear and the Knott's concentration technique. Blagburn (1994) described the characteristics to differentiate microfilariae of *Dirofilaria* sp. and *Dipetalonema*. Das and Das (1996) conducted direct test, Modified Knott's Test and the histochemical staining method for detection and identification of microfilariae in dogs.

2.3 Differentiation of microfilariae

2.3.1 Morphology and biometry

Taylor (1960) studied the morphology and biometry of *Dirofilaria immitis*, *D. repens*, *D. aethiops Loa loa*, *Wuchereria bancrofti* and *Brugia malayi* using phase contrast microscope. Watson *et al.* (1973) compared the microfilariae isolated by Modified Knott's Test and filter method and found that the length of *D. immitis* and *Dipetalonema reconditum* isolated by filter technique was considerably shorter than that by the Modified Knott's Method.

The morphology and biometry of microfilariae found in dogs have been described by Soulsby (1982) and Sonin (1985).

Saseendranath *et al.* (1986) studied the morphology and length of microfilariae in dogs using Wright's stain method.

Valcarcel *et al.* (1990) described the morphology and biometry of microfilariae of different species affecting dogs.

2.3.2 Staining with Brilliant cresyl blue

In 1965, Sawyer *et al.* found that Brilliant cresyl blue stains the cephalic hook of the microfilariae of *Dipetalonema reconditum*, reveals the structure as a fine barb in the case of *Dirofilaria immitis* microfilariae and that the R-cells and

the excretory cells are also stained with the dye. They (1966) also differentiated canine microfilaria with Brilliant cresyl blue.

2.3.3 Histochemical differentiation

Chalifoux and Hunt (1971) demonstrated the acid phosphatase activity in smears of canine microfilariae by the naphthol AS-TR phosphate method. *Dirofilaria immitis* and *Dipetalonema reconditum* were distinctly and accurately distinguished by this technique. Enzyme activity was restricted to two distinct zones in microfilariae of *Dirofilaria immitis* whereas in *Dipetalonema reconditum* it was uniformly distributed throughout.

Balbo and Abate (1972) also differentiated canine microfilariae by the naphthol AS-TR-phosphate staining method. The acid phosphatase activity was restricted to the excretory and anal pore region of *Dirofilaria immitis* microfilariae and latter region of *D. repens* microfilariae but was distributed throughout the body of *Dipetalonema* sp. microfilariae with anterior end staining more intensely than the posterior end, which also showed irregular concentration of the dye.

Kelly (1973) reviewed the methods of detecting *Dirofilaria immitis* and *Dipetelonema* sp. of microfilariae and observed that acid phosphatase enzyme staining technique is

the most accurate method for speciation of microfilariae. Omar (1977) studied the distribution of acid phosphatase activity in the larval stages of *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi* and *Dirofilaria immitis* and found that accurate differentiation of these microfilariae were possible on the basis of the patterns of the activity.

Schillhorn-Van-Veen and Blotkamp (1978) differentiated histochemically the microfilariae of *Dipetalonema*, *Dirofilaria*, *Onchocerca* and *Setaria* spp. of man and domestic animals in the Zaria area (Nigeria).

Whitlock (1978) described the histochemical differentiation of acid phosphatase enzyme of the microfilariae of *Dirofilaria immitis* and *Dipetalonema reconditum*.

Yen and Mak (1978) differentiated the microfilariae of *Brugia*, *Wuchereria*, *Dirofilaria* and *Breinlia* histochemically. They observed that the acid phosphatase activity in microfilariae gives sufficiently characteristic and consistent results for the differentiation of even closely related species. Ortega-Mora *et al.* (1989) studied the acid phosphatase activity and morphological characteristics of *Dipetalonema dracunculoides* microfilariae in dogs and found that the enzyme activity was concentrated around the central

body, the anal pore and the excretory pore. Rossi and Abate (1990) compared the acid phosphatase activity in microfilariae of *Setaria labiato-papillosa* with microfilariae of dogs. Valcarcel *et al.* (1990) described the morphology and acid phosphatase staining characteristics of *Dirofilaria immitis*, *D. repens*, *Dipetalonema reconditum* and *D. dracunculoides*.

Das and Das (1996) identified canine microfilariae on the basis of their morphological characters and confirmed the findings with histochemical study.

2.4 Clinical pathology

2.4.1 Haematology

Snyder *et al.* (1967) found that the sedimentation rate as well as the total leukocyte and eosinophil counts were greater in dogs with microfilaremia and that the mean levels of haemoglobin, packed cell volume, plasma sodium, phosphate, chloride and bicarbonate were greater in clinically normal dogs. Farnes *et al.* (1972) found neutrophilia in dogs with chronic *Dirofilaria* infection. Weiner and Bradley (1972) observed eosinophilia in both primary and secondary infections in experimentally infected dogs with *Dirofilaria immitis*.

Singh *et al.* (1973) observed decreased erythrocyte count in microfilariasis in buffaloes and attributed this change to

the damage caused by microfilariae to the liver tissues with consequent blood loss and depression of erythropoiesis resulting in decreased cell production.

Perman and Schlotthauer (1977) studied the haematology of dogs with heartworm disease in which anaemia was normochromic and normocytic. In their studies in the haematological values in dogs infected with microfilariae of *Dipetalonema* sp., Wittwer *et al.* (1979) found no significant differences in haematocrit leukocyte counts or differential leukocyte counts between dogs naturally infected with *Dipetalonema* and uninfected dogs and concluded that the parasite does not produce eosinophilia or any other significant change in leucocyte counts.

Kumar (1980) attributed the decreased erythrocyte count to the liver damage caused by the microfilariae.

Rawlings *et al.* (1980) studied the haematology in *Dirofilaria immitis* and *Dipetalonema reconditum* infection and observed that a single eosinophil and basophil count has little diagnostic value in dogs suspected of heartworm infections. Sharma and Pachauri (1982) conducted haematological studies and showed low haemoglobin concentration, increased sedimentation rate and a marked but not statistically significant increase in lymphocyte percentage in infected dogs without any change in total

leucocyte count. Chakrabarthi and Choudhury (1983) found that microfilaria infected dogs had decreased erythrocyte count as well as haemoglobin content and increased eosinophil count.

Kumar *et al.* (1984) studied the clinico-haematological changes in microfilariae-affected buffaloes and attributed the decreased erythrocyte production to the liver damage caused by the microfilariae.

Sharma and Pachauri (1986) studied the haemato-biochemical indices in heartworm disease in dog, and found a marked fall in haemoglobin, total erythrocyte count, packed cell volume, total platelet count and erythrocyte fragility rate coupled with increase in blood clotting time, erythrocyte sedimentation rate and total leucocyte count. The leukocyte picture showed neutropenia, eosinophilia and lymphocytosis.

2.4.2 Biochemical aspects

Tomoda (1962) studied the changes in serum protein, serum compound protein and ascitic protein in canine filariasis. Infection with *Dirofilaria immitis* showed an increase in total protein as also β and r globulin and a decrease in serum albumin. It was also observed that serum protein changes in canine filariasis were characteristic of the host's reaction to infection and not of the disease. He (1962) also studied the serum protein changes in canine filariasis and its

correlation with liver dysfunction and noted that *Dirofilaria* infected dogs showed increased plasma globulin and decreased albumin levels. Snyder *et al.* (1967) found that the total serum proteins and the globulin fractions were significantly higher in dogs with microfilaremia when compared to clinically normal dogs. According to electrophoretic patterns, the increase was due to the gamma globulin fraction. The albumin fraction was higher in uninfected dogs, but there were no appreciable change in the β-globulin fraction in positive cases. Mean levels of plasma glucose and serum glutamic pyruvic transaminase were higher in microfilaria infected dogs. Shibata (1974) observed an elevation on serum enzymes like glutamic pyruvic transaminase, glutamic oxaloacetate alkaline phosphatase, Blood Urea Nitrogen (BUN) and urine bilirubin in dogs infected with *D. immitis*.

Barsanti *et al.* (1977) analysed the serum proteins using agarose electrophoresis in normal dogs and those naturally infected with *D. immitis*. In the latter the β-globulin concentration was significantly higher and resulted in increased concentration of total protein than in the former.

Everett *et al.* (1977) attributed increased values of Alkaline phosphatase (ALP), Aspartate amino transferase (AST) and Alanine amino transferase (ALT) to the liver damage.

Barsanti (1978) also analysed the serum proteins with agarose electrophoresis in subclinically and clinically infected dogs with *D. immitis* and found significantly increased concentrations of ß-globulins in infected dogs.

Keenan *et al.* (1978) also attributed the increase in Aspartate amino transferase (AST) and Alanine amino transferase (ALT) values to the liver damage.

Cortez *et al.* (1980) reported that AST and ALT were 24 per cent and 32 per cent higher respectively in *Dirofilaria*-infected dogs.

Kaneko (1980) studied the clinical biochemistry of domestic animals. He observed increased ALP, AST and ALT and attributed it to the liver damage while elevations in BUN was attributed to the damage to the kidneys.

Yasumoto *et al.* (1981) observed distinct decrease in albumin with increase in β_1, β_2 as also r-globulins, ß-lipoprotein and IgG_2 in canine dirofilariasis. Sharma and Pachauri (1982) found that bilirubin, AST and ALT levels were significantly higher in infected dogs while blood glucose and total serum protein levels do not exhibit any change. A significant increase in serum globulin and a decrease in serum albumin were also observed. They (1986) also studied the biochemical profile of blood of infected dogs which showed an

increased level of glucose, bilirubin, urea nitrogen, creatinine globulin, total lipids, cholesterol, phospholipids, sodium chloride, phosphorus, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, sorbitol dehydrogenase, ornithine carba myl transferase, glutamate dehydrogenase, isocitric dehydrogenase and lactic dehydrogenase. Significant decrease was also noticed in serum albumin, potassium, calcium, copper, iron, zinc and manganese values.

Yousif *et al.* (1990) studied the haematobiochemical changes in microfilariae affected horses under field conditions.

Davoust *et al.* (1991) studied the electrophoretic changes in serum proteins of dogs infected with *D. immitis* and *D. repens* and observed a significant high r-globulin levels in dogs infected with both nematodes.

Chen and Liang (1993) studied the blood chemistry of dogs in the Taipei area. The serum aspartate and alanine amino transferase values were higher than 50 v/l in 14 per cent and 20 per cent respectively and the BUN was higher than 28 mg/dl in 40 per cent of the infected dogs. Gascon *et al.* (1993) found an increase in serum total protein, ß-globulin fraction and fibrinogen and concluded that these were good indicators of inflammation.

2.5 Treatment with microfilaricides

2.5.1 Treatment with Ivermectin

Campbell and Blair (1978) worked on the efficacy of Avermectins against *D. immitis* in dogs and found a profound suppressive effect on circulating microfilariae. Blair and Campbell (1979) studied the efficacy of Avermectin B_1a at dose rate of 0.05 and 0.1 mg/kg body weight and noticed reduction in the circulating microfilariae of *D. immitis* to 90 per cent within 24 hours. Jackson and Seymour (1981) also studied the efficacy of Avermectin against microfilariae of *D. immitis*. A single oral dose of 0.25 mg of Ivermectin/kg body weight eliminated all microfilariae within three weeks.

McCall *et al.* (1981) studied the prophylactic activity of Avermectins at 200 µg/kg subcutaneously against experimentally induced *D. immitis* infection and observed 100 per cent efficacy.

Blair *et al.* (1983) studied the dose response of Ivermectin against *D. immitis* microfilariae in dogs with naturally acquired infections. Dose rates of 0.0125, 0.05 as also 0.2 mg/kg body weight eliminated the circulating microfilariae within 24 hours and 0.00313 mg/kg reduced the microfilariae considerably.

Suderman and Craig (1984) studied the efficacy of Ivermectin against *D. immitis* microfilariae in naturally infected dogs at a dose rate of 200 µg/kg subcutaneoulsy and found the efficacy to be 90 per cent on the 21st day.

Simpson and Jackson (1985) observed 92 to 98 per cent reduction in microfilarial counts in 48 hours after treatment with Ivermectin at a dose rate of 0.2 mg/kg body weight subcutaneoulsy.

Jackson *et al.* (1986) found that single oral doses of Ivermectin at 0.05, 0.1, 0.2 and 0.25 mg/kg were effective against microfilariae of *D. immitis*.

Lok *et al.* (1988) studied the effects of Ivermectin on embryogenesis of microfilariae within female heartworms and indicated that not only the development of early stages is interrupted but also the later development stages into the circulation are affected.

Paul *et al.* (1991) observed 100 per cent efficacy with Ivermectin chewable tablets at 6 µg/kg body weight.

Coskun *et al.* (1992) also studied the efficacy of Ivermectin against *D. immitis* microfilariae in naturally infected dogs at a dose rate of 200 µg/kg body weight. The

efficacy was recorded by counting the microfilariae by Modified Knott's Test.

2.5.2 Treatment with Milbemycin oxime

Kurokawa *et al.* (1985) found that Milbemycin D at a dose rate of 0.1 g/kg was effective in removing *D. immitis* microfilaria. Shiramizu and Abu (1985) studied the efficacy of 5 weekly doses of the drug at a dose rate of 1 mg/kg against *D. immitis* in dogs and found an efficacy of 80 per cent.

Sakamoto *et al.* (1985) observed that Milbemycin D administered over a long period had no untoward effect. Sasaki *et al.* (1986) found no risk in clinical use of Milbemycin D in uninfected dogs but adverse reactions occurred in dogs with microfilaraemia. Sasaki *et al.* (1986) observed a shock-like reaction in 11 per cent of *D. immitis*-infected dogs with Milbemycin D administration at a dose rate of 0.1 to 5 mg/kg body weight; there was recovery in 4 hours.

Field trials conducted by Genchi *et al.* (1993) with Milbemycin oxime at a dose rate of 0.5 mg/kg body weight orally at monthly intervals for 7 months which eliminated all microfilaria. Shutou *et al.* (1993) found that Milbemycin oxime at a dose rate of 0.1 or 0.2 g/kg eliminated microfilariae in the blood of 86 per cent of dogs in 4 days.

Blagburn (1994) reviewed and updated the microfilaricidal therapy. Milbemycin oxime at 0.1 mg or over/kg given orally greatly reduced numbers of circulating microfilariae within 24 hours. Doses of 0.25 and 0.5 mg/kg given orally were more effective for the purpose after adulticide therapy.

2.5.3 Treatment with Levamisole

Carr and Bessmer (1975) used Levamisole at 3-5 mg/lb body weight orally for 6 days and noticed elimination of 50 per cent microfilaria in blood. Bradley (1976) found that Levamisole resinate at 11 mg/kg daily for 10 days made microfilarial counts zero within 10 days. According to Garlick (1976), Levamisole at 5 mg/lb body weight was safe in early and moderate *D. immitis* infection but preliminary treatment with antibiotic and antiinflammatory drugs was essential in advanced cases. Bradley and Alford (1977) studied the efficacy of levamisole resinate against *D. immitis* in dogs at twice daily doses of 4.4 mg/kg body weight for two weeks and 6.6 mg/kg for further two weeks. The treatment was 100 per cent effective against microfilaria. Jackson (1977) studied the activity of Levamisole against the various stages of *D. immitis* in dogs and concluded that it is a good microfilaricide at an oral dose of 5 mg/lb daily.

Bradley (1978) claimed that Levamisole could be used as a microfilaricide and adulticide in dogs.

Atwell and Baldock (1979) used Levamisole at 10 and 20 mg/kg orally for 21 days. Both the dose rates reduced the numbers of circulating microfilaria to fifty per cent. Chaikin (1979) used Levamisole at the dose rate of 11 mg/kg body weight as a simultaneous microfilaricide and adulticide in canine heartworm disease. There was marked drop in microfilarial counts on repeated blood examination and an average of 13 days were needed to eliminate the microfilariae completely.

Review of use of Levamisole against *D. immitis* by Jackson (1980) revealed inconsistent results against adult worms but much effectiveness as microfilaricide. Carlisle *et al.* (1984) studied the effectiveness of Levamisole hydrochloride against microfilariae of *D. immitis* in dogs and found it to be an efficient microfilaricide at a dosage of 10 mg/kg twice daily for 14 days. Dose rate of 20 mg/kg was also effective, but toxic. Hayasaki *et al.* (1984) studied the larvicidal effects of short-term medication of Levamisole hydrochloride in *D. immitis* infected dogs. Complete larvicidal action was observed at 5 mg/kg thrice daily for 5 days.

Materials and Methods

MATERIALS AND METHODS

3.1 Prevalence of infection

Most of the dogs brought to the University Veterinary Hospital, Kokkalai for treatment as also for vaccinations and a large number of blood samples and smears of dogs obtained from the University Veterinary Hospital at Mannuthy during a period of one year from October '96 to September '97 were examined for microfilarial infection. Details regarding the sex, age, seasonal variation, clinical symptoms and intensity of microfilarial infection were also recorded. The blood samples were screened by wet film and Modified Knott's Technique for the detection of microfilariae in the blood.

3.2 Diagnosis of infection

Wet film

A drop of fresh blood from the ear tip was collected on to a slide and covered with a cover slip. Before drying, the film was examined under the low power of the microscope for the presence of microfilariae. The number of microfilariae in a microscopic field was counted to determine the intensity of infection. Infections were graded into mild (+), moderate

(++,+++) and severe (++++,+++++) microfilaraemic groups. The type of motility of the microfilariae was also noted.

Modified Knott's Technique (MKT)

The technique of Knott (1930) as modified by Newton and Wright (1956) was employed for concentration and identification of canine microfilariae. One ml of whole blood was mixed with 10 ml of 2 per cent formalin and the mixture centrifuged for 5 minutes at 1500 rpm. The supernatant solution was discarded and the whole sediment was mixed with a drop of new methylene blue 1-in-1000 solution. The number of microfilariae was counted under the low power of the microscope to assess the severity of the infection.

Serum

The freshly collected blood sample was allowed to clot and serum separated. A drop of the serum was taken on to a clean glass slide and immediately examined under the low power of the microscope for the presence and motility of the microfilaria.

Blood smear

Thick smears stained with Wright's, Leishman's or Giemsa stain were used to diagnose and differentiate the microfilariae.

3.3 Differentiation of microfilariae

3.3.1 Morphology and biometry

The blood smears from microfilaria-positive dogs were used for the differentiation of microfilariae. Thick smears were stained with Wright's, Leishman's and Giemsa stains to study the morphology and biometry.

3.3.2 Staining with Brilliant cresyl blue

Thick blood film on slides were kept overnight at room temperature and then dehaemoglobilized with tap water for 10 minutes. The wet films were transferred to 10 per cent and 1:50 dilution of 1 per cent brilliant cresyl blue and examined after 10 minutes under microscope.

3.3.3 Histochemical differentiation

The method of Chalifoux and Hunt (1971) was followed to study the differences in the acid phosphatase enzyme activity in microfilariae.

Preparation of smears

Approximately 5 ml of blood was drawn from the cephalic vein of each dog and was allowed to clot. The clot was loosened with an applicator stick and was washed with 5 ml of

distilled water. The water and serum were then poured into a test tube and centrifuged for 5 minutes at 1000 rpm. The supernatant fluid was discarded and a drop of the fluid containing the resuspended sediment was placed on a microscopic slide and examined for microfilariae. Smears were prepared from the sediment, air-dried and fixed in absolute acetone at 4°C for 1 minute, further air-dried and stained for the demonstration of acid phosphatase activity.

The reagents employed for the Naphthol AS-TR phosphate method are:

Solution I: Michaelis Veronal Acetate Buffer, pH 10.0

Sodium acetate ($NaC_2 H_3O_2.3H_2O$)	-	9.714 g
Sodium barbital	-	14.714 g
Distilled water	-	500 ml

Stored at 4°C

Solution II: Naphthol AS-TR-phosphate

Naphthol AS-TR-phosphate, sodium salt (Sigma chemical company)	-	0.05 g
N, N-Dimethyl formamide	-	5.0 ml

Prepared fresh

Solution III: Pararosanilin

Pararosanilin hydrochloride	-	1.0 g
Distilled water	-	20.0 ml
Concentrated HCl	-	5.0 ml

The Parasosanilin was added to water, got dissolved by heating and then the hydrochloric acid was added and the solution cooled. Stored at 4°C.

Solution IV: Sodium nitrite, 4%

Sodium nitrite ($NaNO_2$)	-	4.0 g
Distilled water	-	100 ml

Kept at 4°C

Solution V: 1% methyl green in phosphate buffer, prepared from the following stock solutions:

A: 0.2 M sodium phosphate

$Na_2H\ PO_4$	-	28.396 g
Distilled water	-	1000 ml

B: 0.1 M citric acid

$C_3H_4\ (OH)\ (COOH)_3.H_2O$	-	21.011 g
Distilled water	-	1000 ml

Working solution

Sol. A	-	77.1 ml
Sol. B	-	122.9 ml
Methyl green	-	2.0 g

Kept at room temperature

Preparation of substrate

In a beaker, 20 ml of solution I was mixed with 50 ml of distilled water and 4 ml of solution II added to it. 3.2 ml each of solution III and IV were mixed separately and then added to the mixture in the beaker. The pH of the mixture was adjusted to 5.0 with 0.1 N NaOH. The final solution was prepared fresh everytime before the staining procedure.

Staining procedure

The air-dried smears were incubated in the substrate for 1 hour at 37°C, rinsed in distilled water, counterstained in 1 per cent methyl green (Solution V) for 5 minutes and rinsed in distilled water. The slides were then dehydrated in 95 per cent and absolute ethyl alcohol respectively, rinsed in Xylene and mounted in Canada balsam. The smears were examined for the precipitated red azo dye indicating acid phosphatase activity.

3.4 Clinical pathology

3.4.1 Haematology

Blood samples were taken from 36 microfilaria-positive dogs and 15 uninfected dogs of 1-4 years age for the haematological studies. The positive dogs were divided into three groups of twelve each of mild, moderate and severe infection. About 2 ml of blood was collected from each animal and Ethylene diamine tetra acetate (EDTA) was added at the rate of 1 mg/ml of blood as anticoagulant.

3.4.1.1 Haemoglobin (Hb)

The haemoglobin percentage was estimated by acid haematin method, using Sahli's haemoglobinometer (Benjamin, 1985).

3.4.1.2 Packed cell volume (PCV)

The Packed Cell Volume was determined using the microhaematocrit method. The blood was taken directly into a heparinized capillary tube (75 mm x 1.0 mm) one end of which was then sealed with special clay and centrifuged at 10,000 rpm for 5 minutes. The packed cell volume was read using a graphic haematocrit tube reader.

3.4.1.3 Erythrocyte Sedimentation Rate (ESR)

The Erythrocyte Sedimentation Rate was determined by using Wintrobe's tube as mentioned by Wintrobe (1981).

3.4.1.4 Total Erythrocyte Count (TEC)

Total Erythrocyte Counts were estimated as per the method described by Schalm *et al.* (1975).

3.4.1.5 Total Leucocyte Count (TLC)

Total Leucocyte Counts were also estimated as per the method described by Schalm *et al.* (loc. cit).

3.4.1.6 Differential Count (DC)

The Differential Count was done using smears stained with Wright's stain and as per the method described by Benjamin (op.cit).

3.4.1.7 Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration and Mean Corpuscular Haemoglobin were calculated

from the values obtained for packed cell volume, haemoglobin and total erythrocyte count.

3.4.2 Biochemical aspects

The blood samples from microfilaraemic as well as non-microfilaraemic dogs were analysed to study the biochemical aspects. The samples were collected in sterilized test tubes and allowed to clot to get serum separated. The serum was analysed on the same day or stored at 4°C in refrigerator when analysed later.

3.4.2.1 Serum protein assays

Serum total protein was estimated by the Biuret method (Varley, 1988) using Qualigens Diagnostic Kit and absorbance read on spectrophotometer at 555 nm.

Serum albumin was determined by the Bromocresol green (BCG) dye binding method (Doumas, 1978) using Qualigens Diagnostic Kit and read at 630 nm.

Serum globulin was calculated from the difference of serum total protein and albumin (Benjamin, op.cit).

Albumin/Globulin ratio (A/G ratio) was calculated from the albumin and globulin values (Benjamin, op.cit).

3.4.2.2 Serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT)

Serum AST and ALT was estimated by the DNPH method of Reitman and Frenkel (1957) using Qualigens Diagnostic Kit and read at 505 nm.

3.4.2.3 Serum urea and Blood Urea Nitrogen (BUN)

Serum urea was estimated by the DAM method (Wybenga, 1971) using Qualigens Diagnostic Kit and read at 520 nm and BUN was estimated from it.

3.5 Treatment with microfilaricides

Dogs having mild, moderate and severe infections were treated with Ivermectin, Milbemycin oxime, Levamisole hydrochloride and efficacy of each drug evaluated at 1st, 7th, 14th and 21st days on the basis of number of microfilaria in wet film and in the Modified Knott's Technique.

Subcutaneous injections of Ivermectin ("IVOMEC") were given as single dose of 200 µg/kg (1 ml/50 kg) body weight and 333 µg/kg (1 ml/30) kg body weight.

Milbemycin oxime ("INTERCEPTOR") was given orally as a single dose of 0.5 mg/kg body weight.

Subcutaneous injection of Levamisole hydrochloride ("HELMONIL") was given as a single dose of 7.5 mg/kg body weight in mild, moderate and severe groups and the dose repeated daily for upto 7 days in cases of animals in the severe group.

Results

4. RESULTS

4.1 Prevalence of infection

Out of the overall number of 2648 dogs screened during October 1996 to September 1997 from the hospitals at Kokkalai and Mannuthy for microfilariasis, 201 were found to be positive, giving a prevalence rate of 7.59 per cent. Month-war, the maximum prevalence rate was observed in May 1997 (25.77%) which was significantly higher than the rates in January (8.99%), April (11.45%), June (10.44%) and July (9.02%). Comparatively lower prevalence rates were observed in October (4.17%), November (3.97%), December (5.13%), February (5.98%), March (7.58%), August (4.58%) and September (5.38%). The month-wise prevalence in figures is presented in Table 1 and depicted grapically in Graph 1. The graph reveals a steap peak for May and a small peak for January.

Out of the 201 positive cases, male dogs were more affected 124 (62%) than the females 77 (38%). The prevalence of infection in dogs from 6 months to 6 years were higher 146 (72.6%) than the aged dogs 55 (27.4%) (Table 2). The majority of cases affected were above six months of age and only two were diagnosed positive at six months. The comparison of prevalence in male and female upto 6 years of age and above are depicted in Graph 2.

Table 1. Month-wise prevalence of microfilariasis in dogs

Month	No. of dogs examined	No. of dogs positive	Prevalence %
October '96	216	9	4.17
November '96	302	12	3.97
December '96	234	12	5.13
January '97	289	26	8.99
February '97	184	11	5.98
March '97	264	20	7.58
April '97	166	19	11.45
May '97	97	25	25.77
June '97	316	33	10.44
July '97	144	13	9.02
August '97	306	14	4.58
September '97	130	7	5.38
Total	2648	201	7.59

Graph 1. GRAPH SHOWING MONTH-WISE PREVALENCE OF MICROFILARIASIS

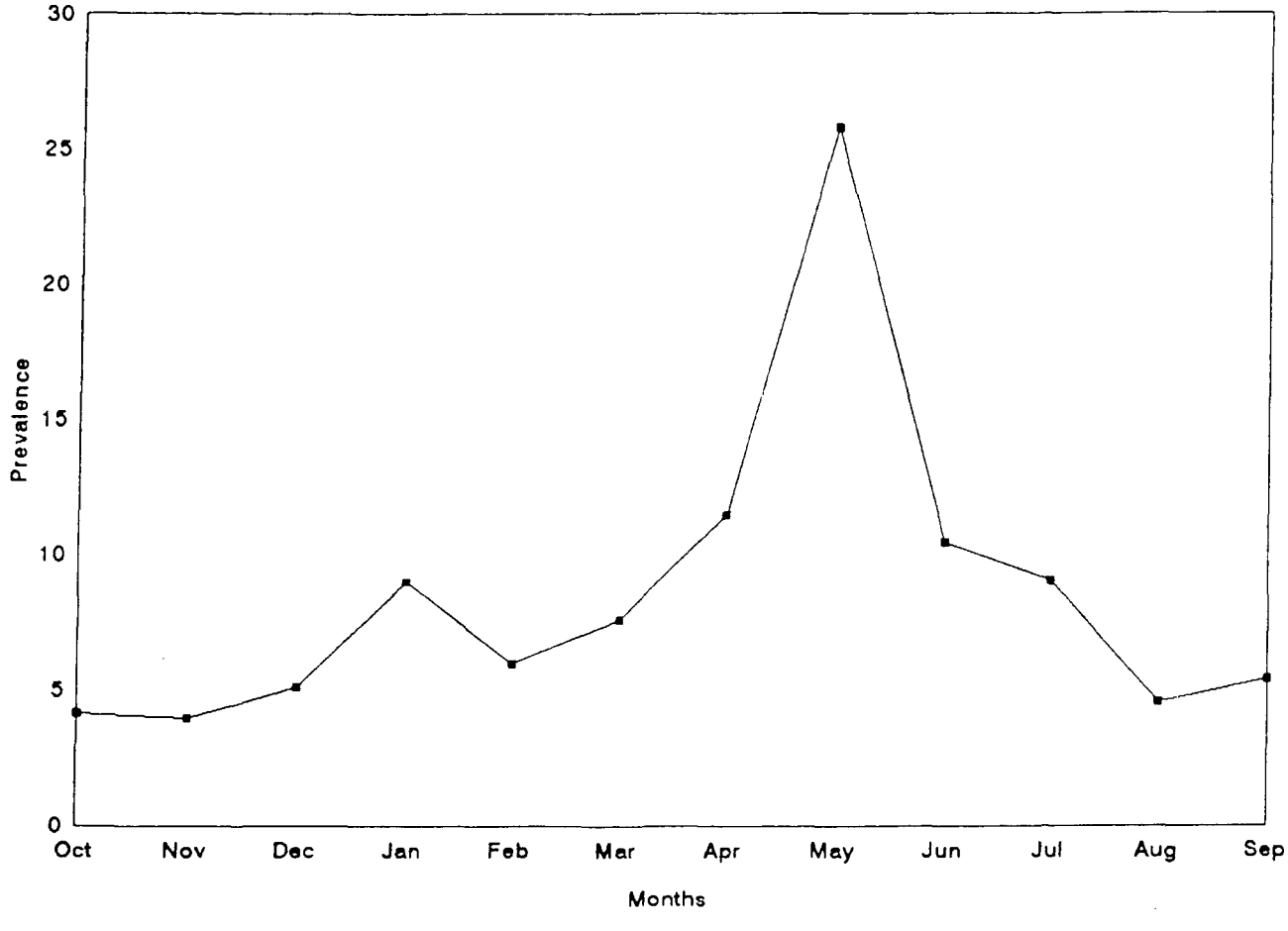
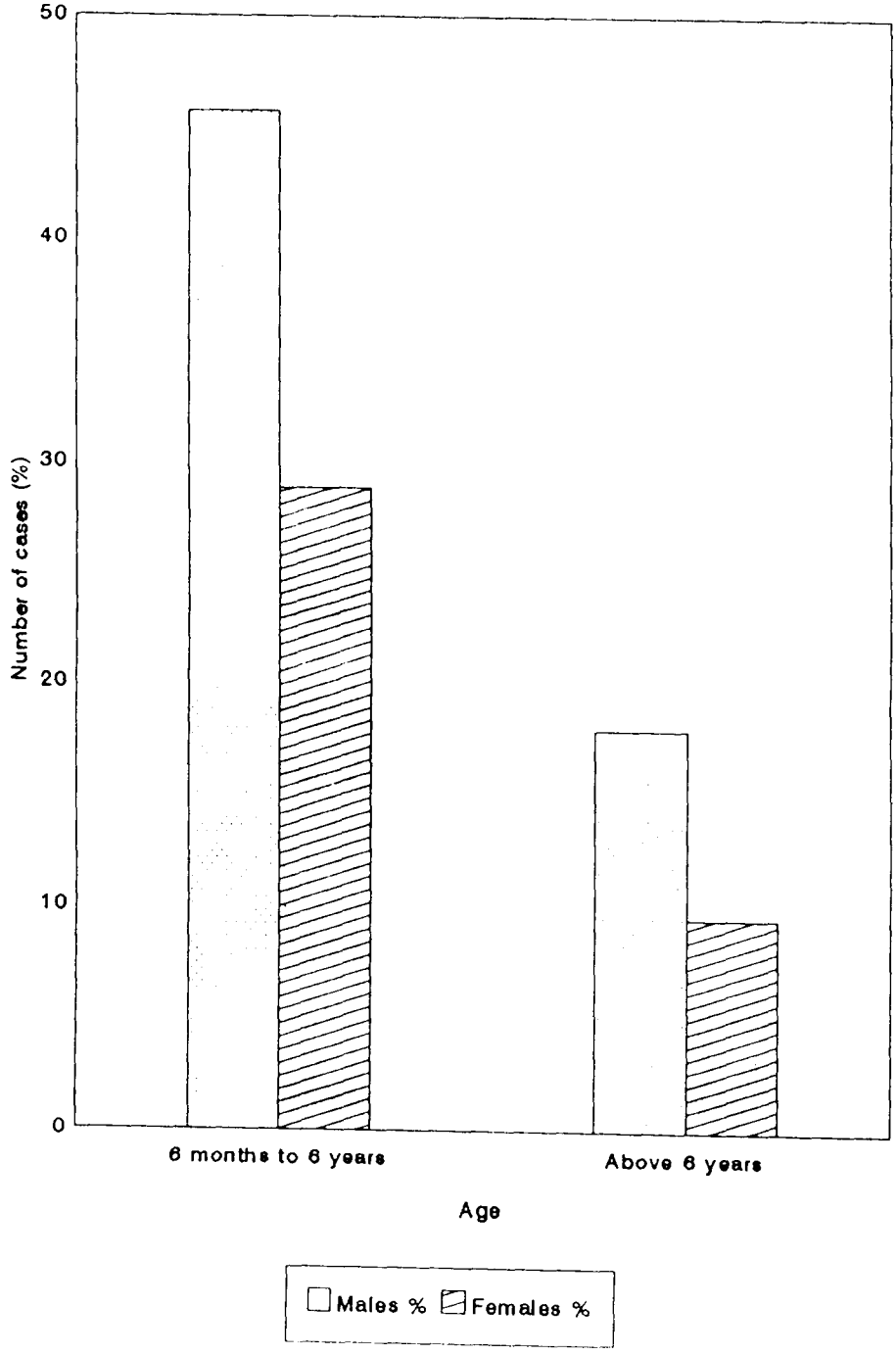


Table 2. Sex-wise prevalence of microfilariasis in dogs from 6 months to 6 years and above 6 years

Age	No. of males	%	No. of females	%	Total No.	%
6 months to 6 years	88	60.3	58	39.7	146	72.6
Above 6 years	36	65.5	19	34.5	55	27.4
Total	124	62	77	38	201	100

Graph 2. GRAPH SHOWING AGE AND SEX VARIATIONS IN MICROFILARAEMIC DOGS



4.2 Diagnosis of infection

Among the different methods used for diagnosing microfilariasis, the wet film examination was found to be more convenient and giving better assessment of the motility of the larvae. The microfilariae showed two types of motility viz. the wriggling and the progressive forward movements. Modified Knott's Technique (MKT) was the most reliable and accurate of the techniques as it revealed the total number of microfilaria per ml of blood, had the advantage of detecting infection of low intensity and also enabled the study of the morphology as well as biometry of microfilaria. Serum examination was a time consuming procedure but was helpful to study the motility of the microfilaria. The smear method was the least accurate as those diagnosed to be positive in other techniques appeared negative with this technique except in cases of heavy infection.

The mean values of MKT, serum and smear methods of diagnosis are given in Table 3, and on comparison of the values by the students t test a significant difference at 1% level was obtained among all treatments pertaining to the mild and moderate groups viz., MKT and serum, MKT and smear as well as serum and smear, while in the severe group a significant difference (at 1% level) was obtained only between the MKT and smear and the smear and serum methods only. Though the comparison between

MKT and serum techniques with regard to severe group yielded only non-significant results, the mean values of MKT were obviously higher than those of the serum methods (Table 4).

The comparison between MKT, serum and smear methods in mild, moderate and severe microfilaraemic dogs are represented graphically in Graph 3.

4.3 Differentiation of microfilaria

4.3.1 Morphology and biometry

Morphological studies using Wright's, Leishman's, Giemsa and Methylene blue staining techniques revealed that the microfilariae from the infected dogs of the present study were those of *Dirofilaria repens*. The microfilariae were sheathless, with a blunt head end and a tapering tail. The cuticle appeared to be striated in higher magnifications. Hook muscle cells could be appreciated at the head end. The nerve ring and the excretory cell at the excretory pore region of the microfilaria could be well appreciated. The G_1 nucleus is oval and large and occupies nearly the whole width of the microfilaria. G_2 and G_3 cells lie close together and are equidistant from G_1 and G_4 cells. The inner body and associated cells or the central body zone lie anterior to the G_1 cell. The tail is long and tapering, leaving a clear space at the end. The "nuclear column" cells not extend to the tip of the

Table 3. Mean values of the different diagnostic methods

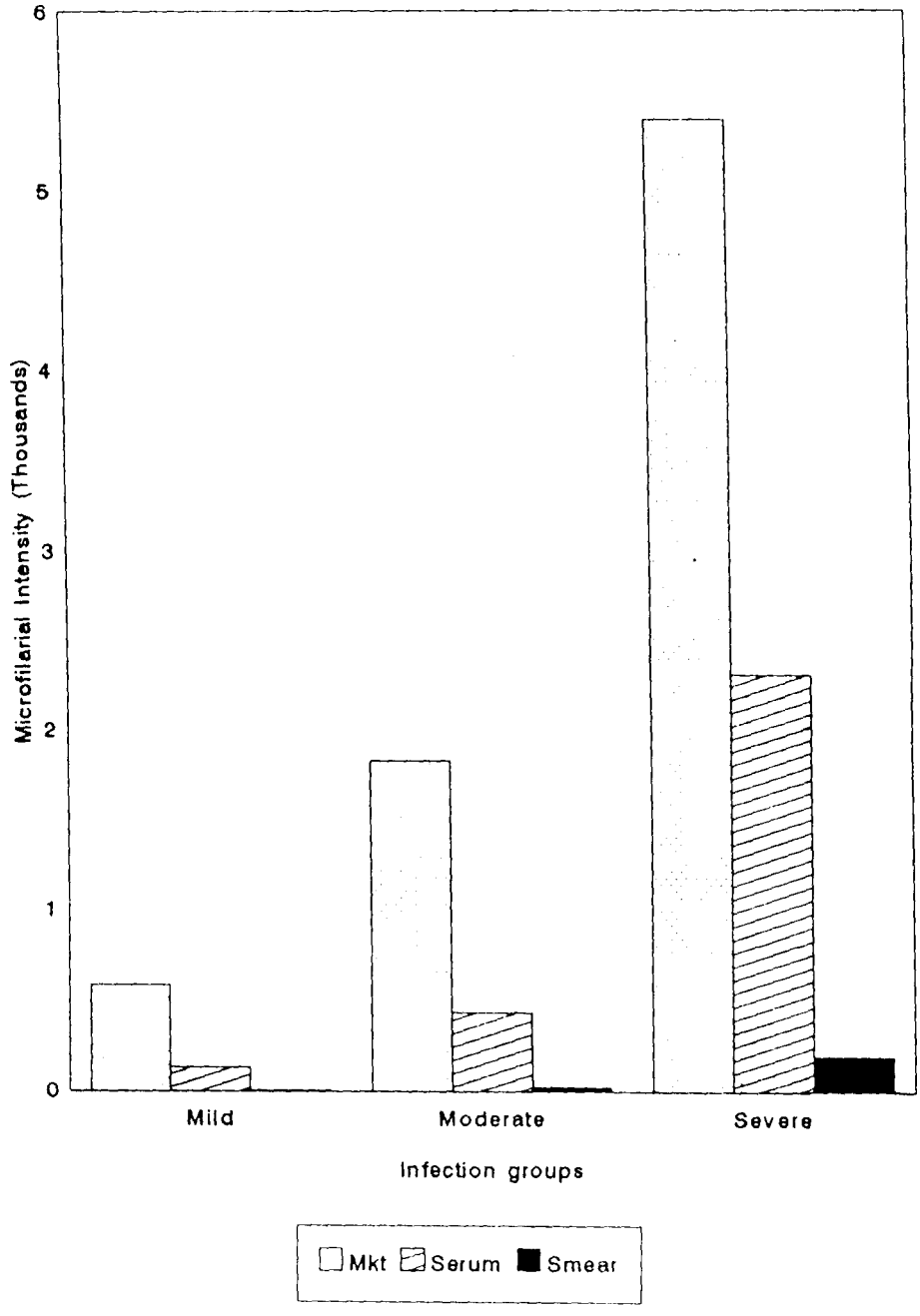
No. of cases	Wet film	MKT Mean±SE No/ml	Serum Mean±SE No/ml	Smear Mean±SE No/smear
22	Mild (-,+)	590.4± 161.7	134.455± 46.7	5.592± 2.4
19	Moderate (++, +++)	1822.8± 316.7	434.840± 78.3	22.790± 8.2
9	Severe (++++,+++++)	5402.0± 1181.9	2300.600± 924.1	198.000± 79.2

Table 4. Comparison between the different diagnostic methods

Intensity	T values		
	MKT & serum	MKT & smear	Serum & smear
Mild	2.7349 **	3.6168 **	3.1612 **
Moderate	4.255 **	5.6823 **	5.2372 **
Severe	2.0673 NS	4.393 **	2.2666 **

** - Highly significant ($P \leq 0.01$)
NS - Non-significant

Graph 3. GRAPH SHOWING THE COMPARISON OF VALUES WITH THE MODIFIED KNOTT'S TECHNIQUE, SERUM METHOD AND SMEAR IN MILD, MODERATE AND SEVERE MICROFILARAEMIC DOGS



tail. The camera lucida drawing of microfilaria of *D. repens* is illustrated in Plate 1, Fig.1. The staining patterns of the microfilariae by the different stains are demonstrated in Plate I Fig.2; Plate II Fig.1 and 2.

The percentage distance of various fixed points viz. Nerve ring, excretory pore, excretory cell, G_1, G_2, G_3, G_4, anal pore and total length are given in Table 5. The findings confirm that the microfilarae are those of *D. repens*.

Biometrical studies revealed that the total length of microfilariae varied significantly in cases of direct smear and MKT methods yet were confined to that of *D. repens*. In the MKT method the microfilaria had a length of 336.95 ± 10.3 μ and width of 6 ± 0.14 μ while in the direct smear these parameters were respectively 285.131 ± 8.66 and 6 ± 0.2 μ.

4.3.2 Staining with Brilliant cresyl blue

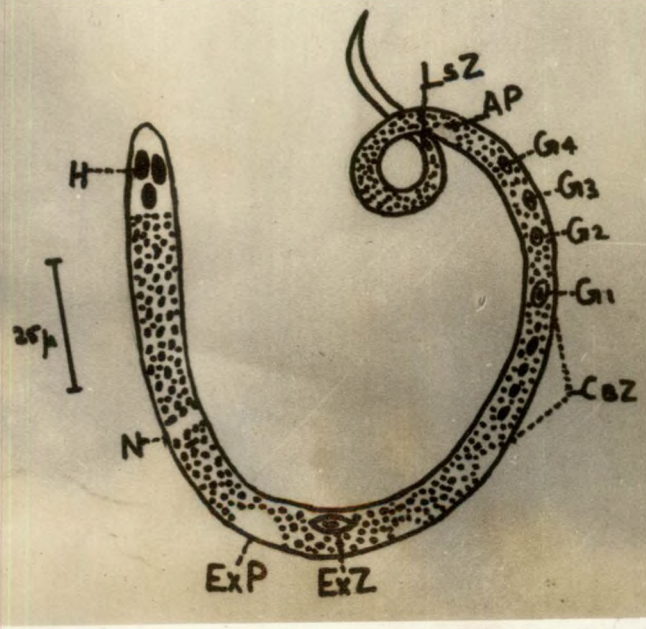
The R cells (genital cells) and the excretory cells could be well stained with 10 per cent Brilliant cresyl blue. The nerve ring, excretory pore and the anal pore could also be well delineated (Head end was blunt and tail end tapering). Staining with 1 in 50 dilution of 1 per cent Brilliant cresyl blue did not clearly reveal the R cells or the excretory cells and constituted the least reliable method for differentiating microfilariae of *D. repens*.

PLATE I

Fig.1 Microfilaria of *Dirofilaria repens* - camera lucida drawing

Fig.2 Microfilaria of *D. repens* - Wright's stained (x 1200)

MICROFILARIA OF *Dirofilaria repens*



H	HOOK MUSCLE CELLS
N	NERVE RING
ExP	EXCRETORY PORE
ExZ	EXCRETORY CELL
CBZ	CENTRAL BODY ZONE
G_1	1st GENITAL CELL
G_2	2nd GENITAL CELL
G_3	3rd GENITAL CELL
G_4	4th GENITAL CELL
AP	ANAL PORE
LsZ	LAST TAIL CELL

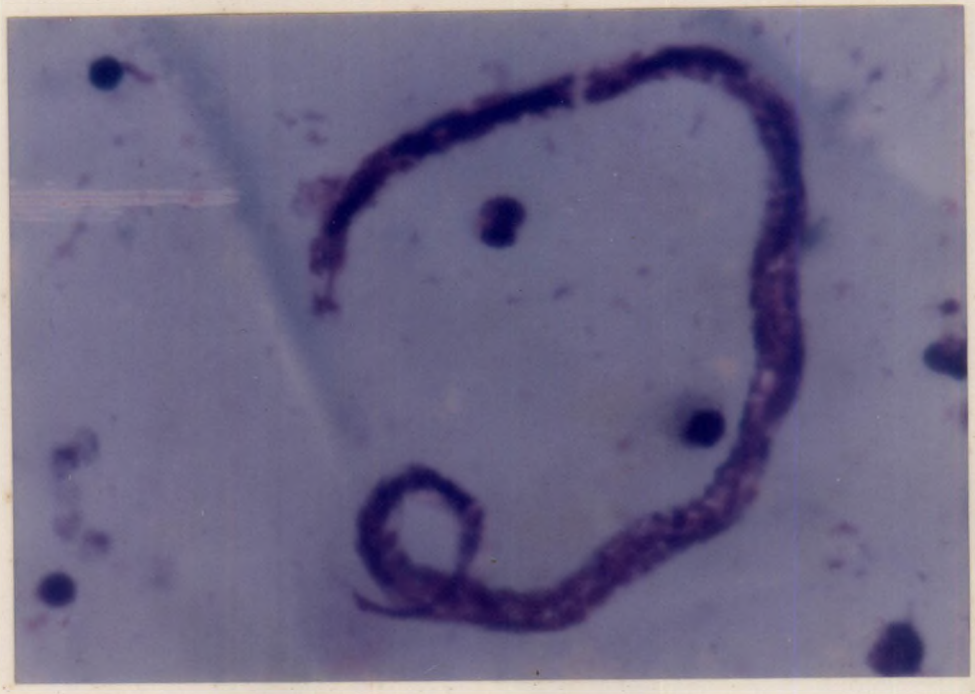


PLATE II

Fig.1 Microfilaria of *Dirofilaria repens* - Leishman's stained (x 1200)

Fig.2 Microfilaria of *D. repens* - Giemsa stained (x 1200)

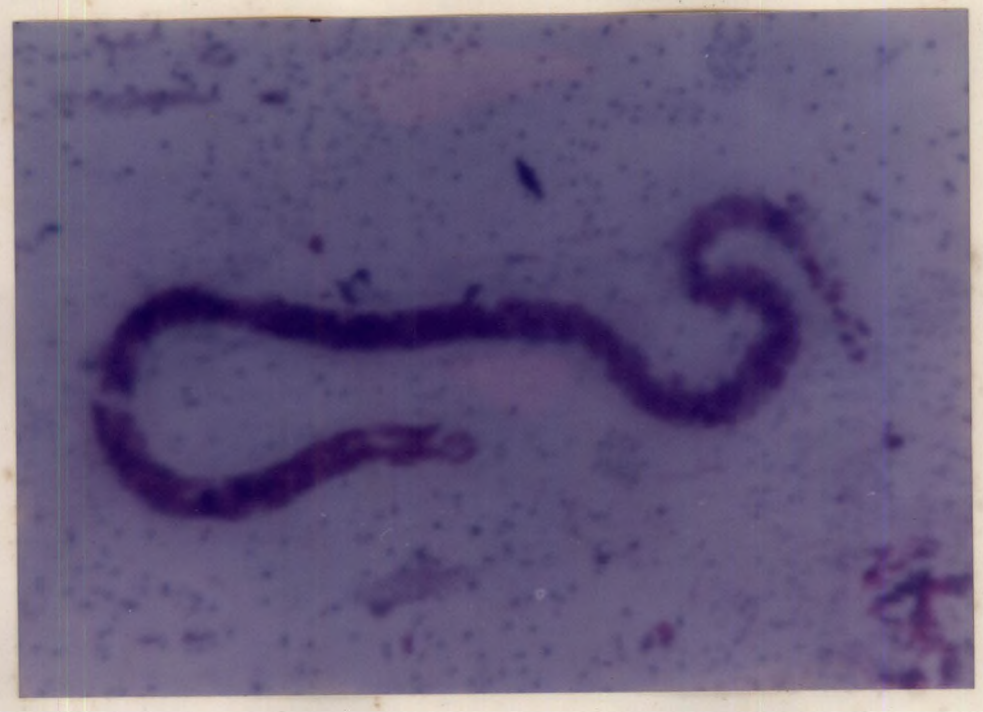


Table 5. Morphology and biometry of microfilaria of dogs in Thrissur

Method	Percentage distance from the anterior end								Total length (μ)	Total width (μ)
	Nerve ring (NR) Mean±SE	Excretory pore (EP) Mean±SE	Excretory cell (EC) Mean±SE	1st genital cell (G_1) Mean±SE	2nd genital cell (G_2) Mean±SE	3rd genital cell (G_3) Mean±SE	4th genital cell (G_4) Mean±SE	Analpore (AP) Mean±SE		
Smear stained with Wright's, Leishman's or Giemsa	23.029± 0.201	32.105± 0.223	34.562± 0.316	61.089± 0.205	63.457± 0.224	65.018± 0.247	67.333± 0.309	72.546± 0.469	285.131± 8.66	6.0± 0.2
MKT (treated with 2% formaline and stained with methylene blue)	23.03± 0.219	32.049± 0.301	34.504± 0.32	61.143± 0.199	63.50± 0.24	65.1± 0.251	66.968± 0.41	70.316± 0.519	336.95± 10.3	6.0± 0.14

4.3.3 Histochemical differentiation

Histochemical differentiation was found to be the most accurate, specific and reliable staining technique and revealed that the microfilaria undoubtedly belonged to *D. repens*.

Depending on the progressively forward and/or wriggling movements of microfilariae during the wet film examination, varying acid phosphatase enzyme activity areas were noted on the microfilariae of *D. repens* (Table 6). Progressively forward-moving microfilariae were stained as a spot at the anal pore region and diffusely at the central body region (Plates III and IV) whereas those with wriggling movement took the stain (spot) at the anal pore region only (Plates V and VI). This differentiation could be well appreciated under the low power of the microscope itself. The procedure also revealed that the proportion of microfilariae having wriggling movement was higher (75.9 per cent) in comparison to those with progressively forward motion (15.5 per cent). A small proportion of microfilariae (8.6 per ent) exhibited both types of movements (Table 6). Furthermore, clotted blood, serum as also 2 ml serum mixed with 5 ml distilled water and stored at 4°C upto 4 weeks did not affect the acid phosphatase enzyme activity. The acid phosphatase activity in stained smears diminished by about a month.

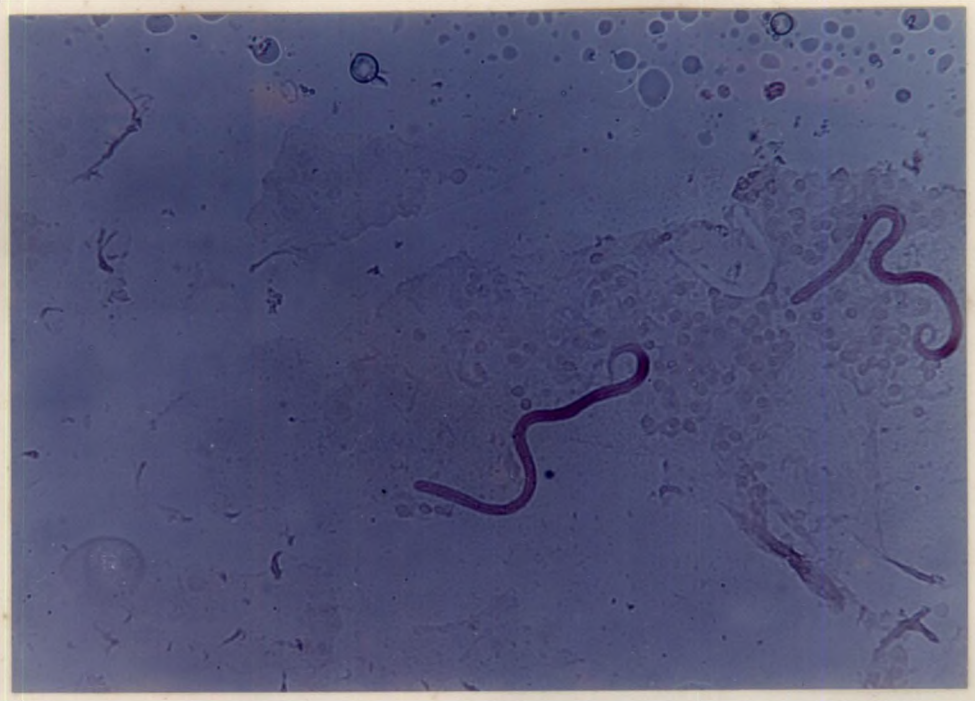
Table 6. Acid phosphatase enzyme activity areas in *Dirofilaria repens* microfilariae in comparison to their motility

Motility in wet film	No.of cases	Percentage	Areas of enzyme activity
Wriggling type	44	75.9	Anal pore
Progressively forward type	9	15.5	Anal pore + central body
Wriggling-cum-progressively forward types	6	8.6	Anal pore & Anal pore + central body

PLATE III

Fig.1 Microfilaria of *Dirofilaria repens* - Progressively forward type - Histochemically stained (x 250)

Fig.2 Microfilaria of *D. repens* - Progressively forward type - Histochemically stained (x 400)



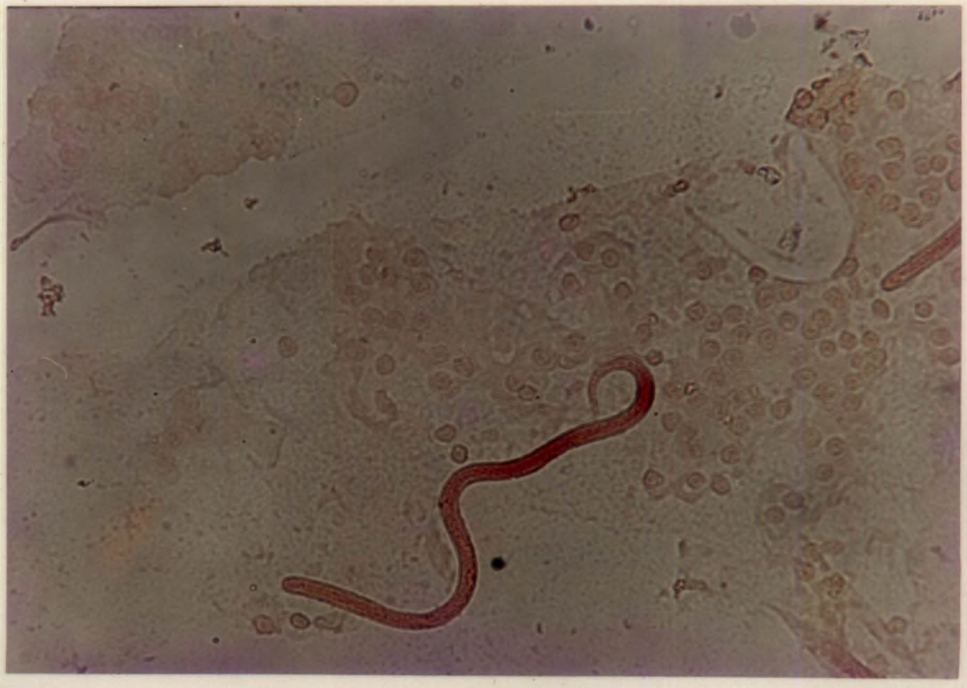


PLATE IV

Fig.1&2 Microfilaria of *Dirofilaria repens* - Progressively forward type - Histochemically stained (x 1000)

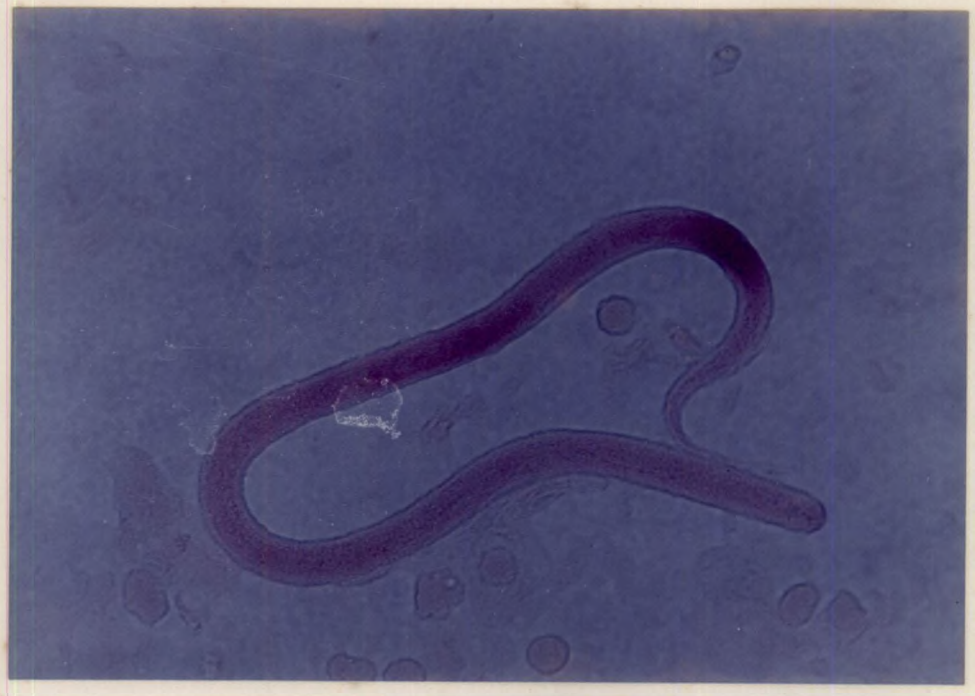
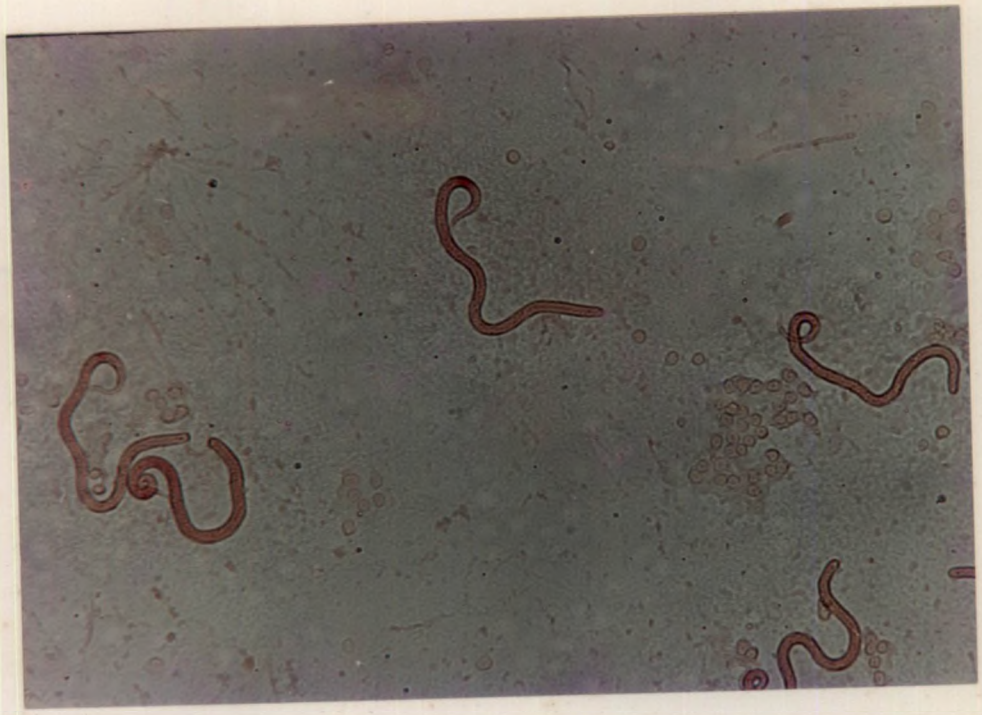


PLATE V

Fig.1 Microfilaria of *Dirofilaria repens* - Wriggling type - Histochemically stained (x 160)

Fig.2 Microfilaria of *D. repens* - Wriggling type - Histochemically stained (x 400)



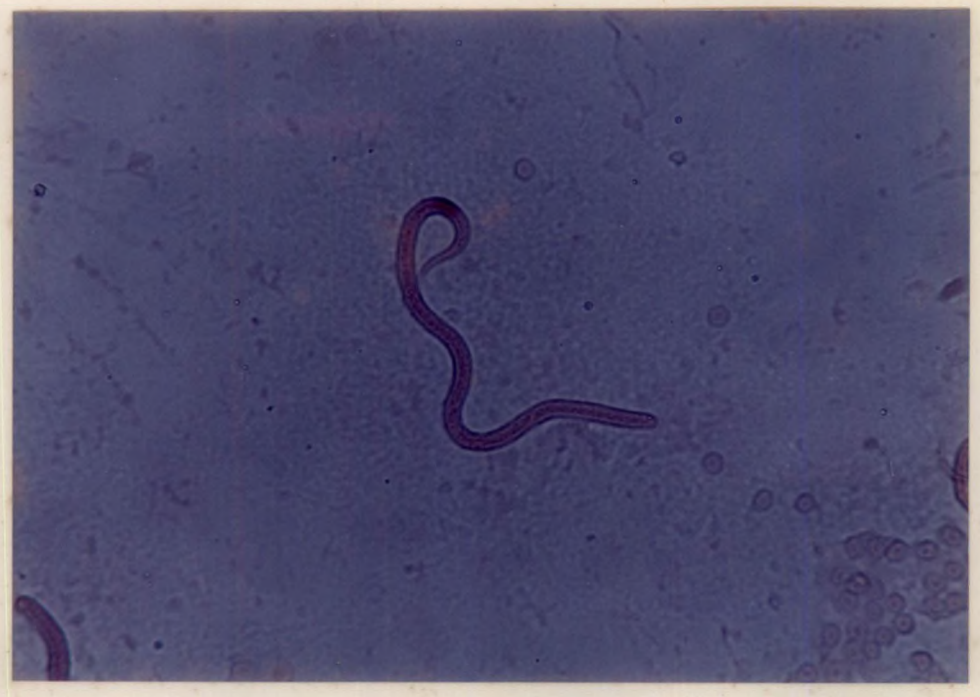
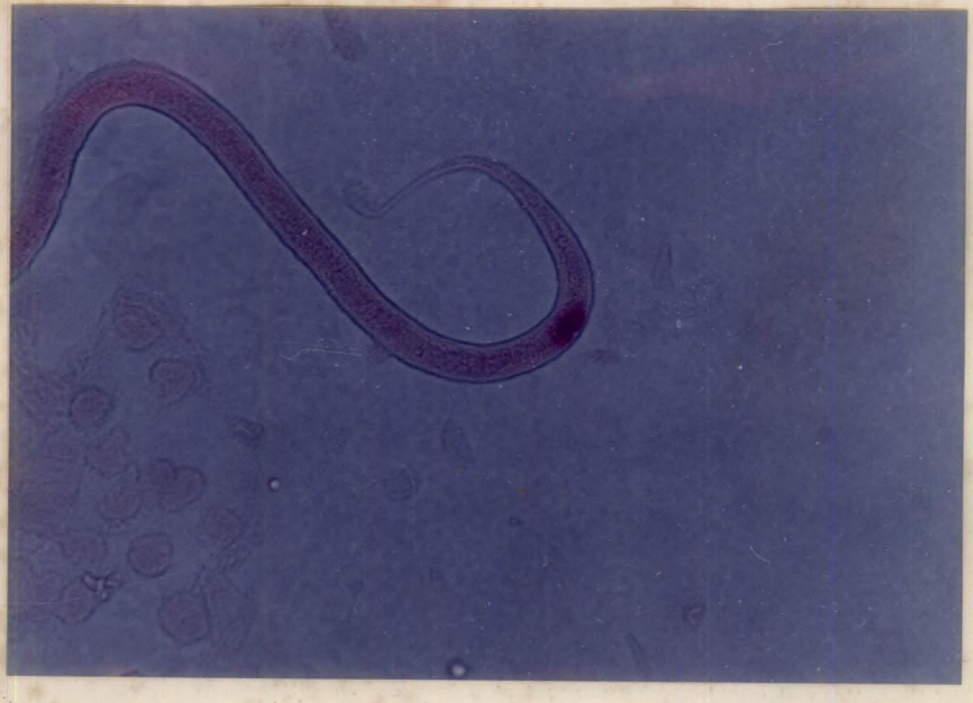


PLATE VI

Fig.1&2 Microfilaria of *Dirofilaria repens* - Wriggling type - Histochemically stained (x 1000)



4.4 Clinical pathology

4.4.1 Haematology

Haematological values of 36 microfilaremic dogs (12 in each in mild, moderate and severe groups) aged 1 to 4 years were compared with a group of 15 non-microfilaraemic dogs of the same age category. The mean and standard error with respect to the values of the various haematological parameters relating to the four groups are furnished in Table 7. The graphical representation of the comparison of the values for Haemoglobin, Packed Cell Volume, Erythrocyte Sedimentation Rate, Total Erythrocyte Count and Total Leucocyte Count of the different groups are given in Graph 4,5,6,7 and 8.

4.4.1.1 Haemoglobin (Hb)

A reduction in the percentage of haemoglobin was observed in the mild, moderate and severe microfilaraemic cases in contrast to the non-microfilaraemic ones. The mean values were 17.627 ± 0.235, 13.408 ± 0.715, 12.783 ± 0.562, 12.517 ± 0.946 g/100 ml respectively for the non-microfilaraemic, mild, moderate and severe groups (Table 7, Graph 4). Comparison using the analysis of variance (ANOVA) revealed significant difference between the uninfected and infected with regard to haemoglobin (Table 8).

Table 7. Haematology of non-microfilaraemic and microfilaraemic dogs

	Category	Non-microfilaraemic dogs	Microfilaraemic dogs		
			Mild	Moderate	Severe
	No. of dogs examined	15	12	12	12
	Parameters	Mean±SE	Mean±SE	Mean±SE	Mean±SE
1.	Haemoglobin (g/100 ml)	17.627±0.235	13.408±0.7159	12.783±0.5629	12.517±0.9465
2.	Packed Cell Volume %	54.200±0.932	43.000±2.333	38.000±1.11	37.083±2.77
3.	Erythrocyte Sedimentation Rate (mm/hr)	0.267±0.1188	14.083±2.7713	29.417±3.464	30.250±6.5356
4.	Total Erythrocyte Count millions/cu.mm	8.565±0.2324	6.842±0.2742	6.128±0.211	6.242±0.427
5.	Total Leucocyte Count (thousands/cu.mm)	12.812±0.9089	22.602±2.217	13.620±1.409	16.828±2.190
6.	Neutrophils %	76.067±1.348	72.167±2.6298	69.667±1.9976	70.000±2.875
7.	Lymphocyte %	22.133±0.7462	22.083±2.234	26.750±1.943	24.917±2.815
8.	Eosinophils %	1.600±0.289	4.333±0.554	3.167±0.297	4.583±0.794
9.	Monocytes %	0.200±0.106	0.500±0.260	0.417±0.193	0.583±0.147
10.	Basophils %	0	0.083±0.084	0	0
11.	MCV (Cu.μ)	63.985±0.600	63.093±1.865	62.488±2.136	59.201±1.051
12.	MCH ($\mu\mu$g)	20.897±0.584	19.606±0.699	21.103±1.152	19.832±0.814
13.	MCHC (%)	32.639±0.568	31.548±1.288	34.188±2.180	33.471±1.331

4.4.1.2 Packed Cell Volume (PCV)

A reduction in the Packed Cell Volume per cent was observed in microfilaraemic dogs of the mild, moderate and severe groups compared with normal dogs. The respective mean values were 54.2 ± 0.932, 43 ± 2.33, 38 ± 1.11, 37.083 ± 2.77 per cent for the non-microfilaraemic, mild, moderate and severe microfilaraemic groups (Table 7, Graph 5). Comparison of values revealed a significant difference between the non infected and infected groups as well as between the mild and severe infection groups (Table 9).

4.4.1.3 Erythrocyte Sedimentation Rate (ESR)

A marked increase in ESR was observed in mild, moderate and severe microfilaraemic dogs when compared with non microfilaraemic dogs. The mean values increased from 0.267 ± 0.1188 mm/hr (non-microfilaraemic dogs) to 14.083 ± 2.7713, 29.417 ± 3.464 and 30.250 ± 6.5356 mm/hr in mild, moderate and severe cases respectively (Table 7; Graph 6). There was a significant difference among all groups except between the moderate and severe groups which showed non-significant difference (Table 10).

4.4.1.4 Total Erythrocyte Count (TEC)

The Total Erythrocyte Count of non-microfilaraemic dogs were greater than that of microfilaraemic dogs, although the

Table 8. Comparison between the different groups in `Haemoglobin' values

Groups	Values g/100 ml	Critical difference	Remarks
GP1 Vs GP2	13.408±0.716 Vs 12.783±0.563	1.961	NS
GP2 Vs GP3	12.783±0.563 Vs 12.517±0.9465	1.961	NS
GP3 Vs GP4	12.517±0.9465Vs 17.627±0.235	1.86	**
GP4 Vs GP1	17.627±0.62 Vs 13.408±0.716	1.86	**
GP4 Vs GP2	17.627±0.62 Vs 12.783±0.563	1.86	**
GP1 Vs GP3	13.408±0.716 Vs 12.517±0.9465	1.961	NS

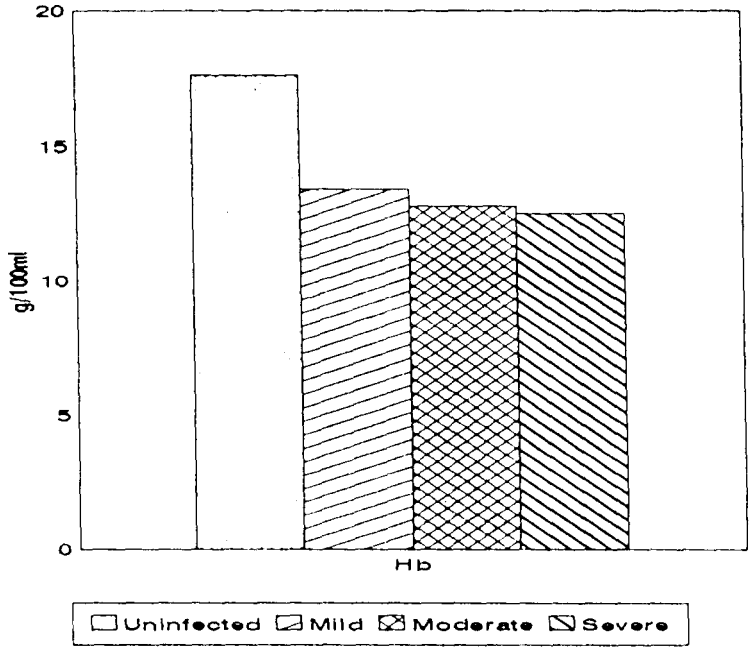
Table 9. Comparison between different groups in `Packed cell volume' values

Groups	Values %	Critical Diff	Remarks
GP1 Vs GP2	43.00 ±2.33 Vs 38.00 ±1.11	5.455	NS
GP2 Vs GP3	38.00 ±1.11 Vs 37.08 ±2.7	5.455	NS
GP3 Vs GP4	37.08 ±2.77 Vs 54.2 ±0.932	5.175	**
GP4 Vs GP1	54.20 ±0.932 Vs 43.00 ±2.33	5.175	**
GP4 Vs GP2	54.20 ±0.932 Vs 38.00 ±1.11	5.175	**
GP1 Vs GP3	43.00 ±2.33 Vs 37.08 ±2.77	5.455	**

** - Highly significant (P≤0.01)
NS - Non significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

Graph 4. GRAPH SHOWING HAEMOGLOBIN VALUES OF NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



Graph 5. GRAPH SHOWING PACKED CELL VOLUME OF NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS

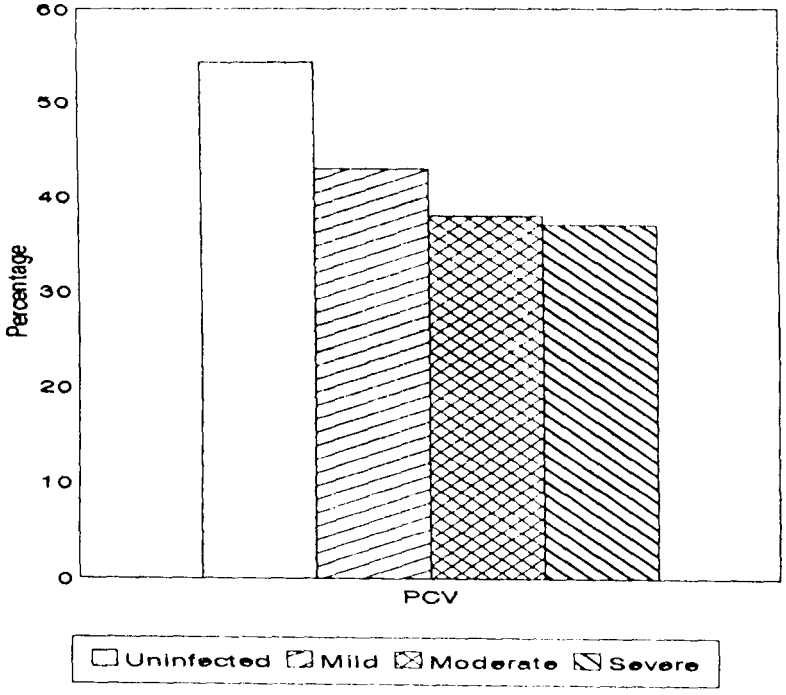


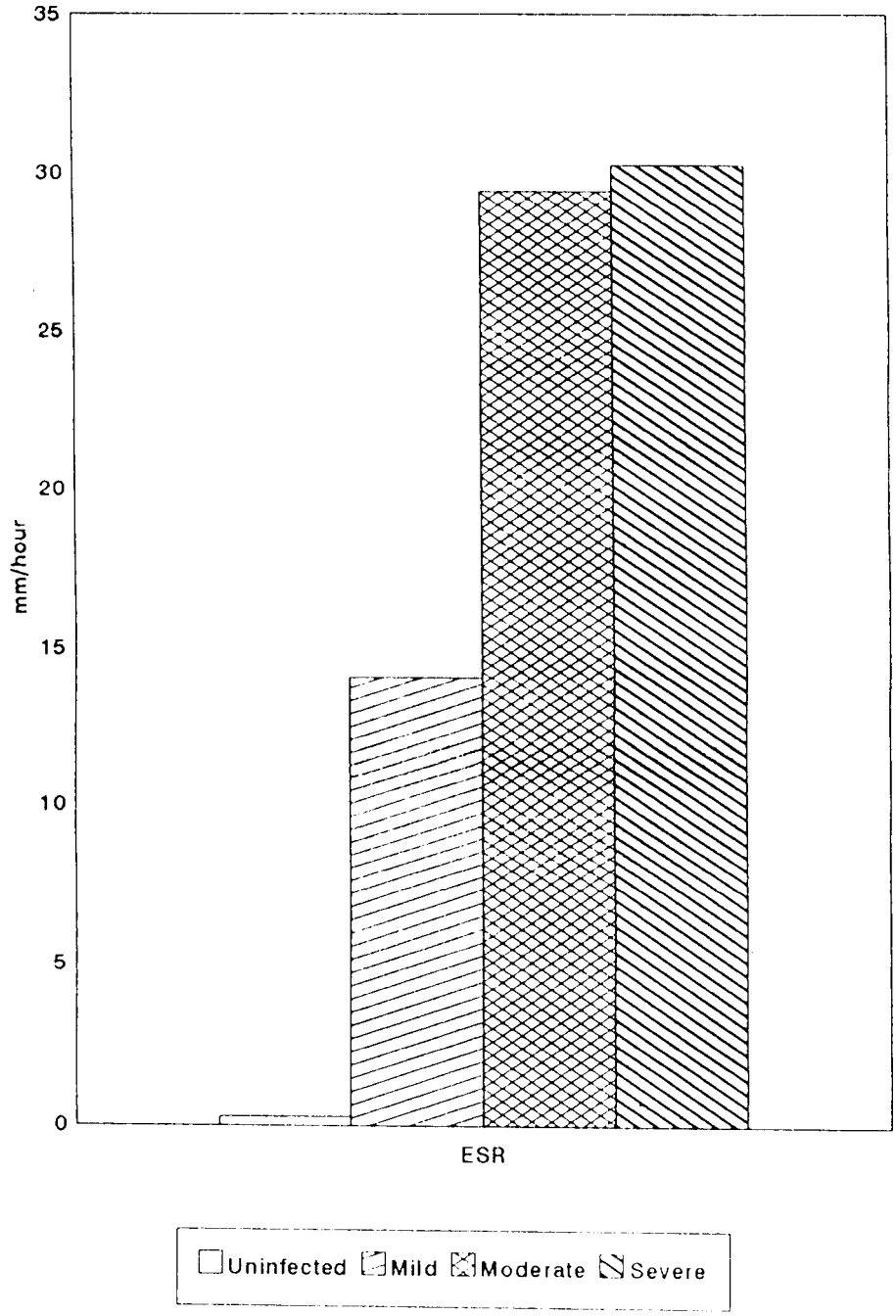
Table 10. Comparison between the different groups in `Erythrocyte Sedimentation Rate' values

Groups	Values mm/hour		Critical difference	Remarks
GP1 Vs GP2	14.083±2.77	Vs 29.417±3.46	10.86	**
GP2 Vs GP3	29.417±3.46	Vs 30.25 ±6.54	10.86	NS
GP3 Vs GP4	30.25 ±6.54	Vs 0.267 ±0.119	10.306	**
GP4 Vs GP1	0.267±0.19	Vs 14.083±2.77	10.306	**
GP4 Vs GP2	0.267±2.199	Vs 30.25 ±6.54	10.306	**
GP1 Vs GP3	14.083±2.77	Vs 30.25 ±6.54	10.86	**

** - Highly significant ($P \leq 0.01$)
NS - Non-significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

Graph 6. GRAPH SHOWING ERYTHROCYTE SEDIMENTATION RATE OF NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



severely infected dogs had a non-significantly higher TEC than the moderately affected ones. The mean TEC values were 8.565 ± 0.2324, 6.842 ± 0.2742, 6.128 ± 0.2107 and 6.242 ± 0.427 millions/cu mm respectively for mild, moderate and severe groups (Table 7; Graph 7). Comparison revealed a significant difference in the values between the uninfected and the infected (mild, moderate and severe) groups while a non-significant difference occurred among the infected three groups (Table 11).

4.4.1.5 Total Leucocyte Count (TLC)

An increase in TLC was noticed in microfilaraemic dogs in comparison to the uninfected and the increase was highly significant in the mild group as compared to the moderate and severe categories (Table 12). The severe group had a non-significantly higher TLC when compared with the moderate group. The mean values were 12.812 ± 0.9089, 22.602 ± 2.217, 13.62 ± 1.409 and 16.828 ± 2.19 thousands/cu mm respectively for the uninfected mild, moderate and severe groups (Table 7; Graph 8).

4.4.1.6 Differential Count

Neutrophil

Non-significant neutropenia was observed in microfilaraemic dogs when compared with uninfected dogs. The mean neutrophil values were 76.067 ± 1.348, 72.167 ± 2.6298,

Table 11. Comparison between the different groups in `Total Erythrocyte Count' values

Groups	Values millions/Cu.mm	Critical difference	Remarks
GP1 Vs GP2	6.842±0.274 Vs 6.128 ±0.211	0.859	NS
GP2 Vs GP3	6.128±0.211 Vs 6.242 ±0.427	0.859	NS
GP3 Vs GP4	6.242±0.427 Vs 8.565 ±0.23	0.815	**
GP4 Vs GP1	8.565±0.27 Vs 6.842 ±0.274	0.815	**
GP4 Vs GP2	8.565±0.27 Vs 6.128 ±0.211	0.815	**
GP1 Vs GP3	6.842±0.274 Vs 6.242 ±0.427	0.859	NS

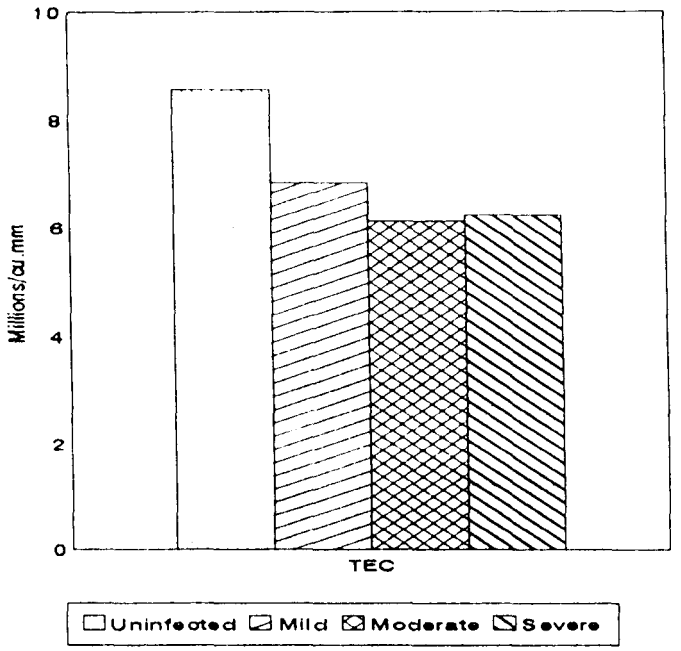
Table 12. Comparison between the different groups in `Total Leucocyte Count' values

Groups	Values 10^3/Cu.mm	Critical difference	Remarks
GP1 Vs GP2	22.602±1.74 Vs 13.62 ±1.409	4.959	**
GP2 Vs GP3	13.62 ±1.409 Vs 16.828±2.19	4.959	NS
GP3 Vs GP4	16.828±2.19 Vs 12.812±0.909	4.705	NS
GP4 Vs GP1	12.812±0.909 Vs 22.602±1.74	4.705	**
GP4 Vs GP2	12.812±0.909 Vs 13.62 ±1.409	4.705	NS
GP1 Vs GP3	22.602±1.74 Vs 16.828±2.19	4.959	**

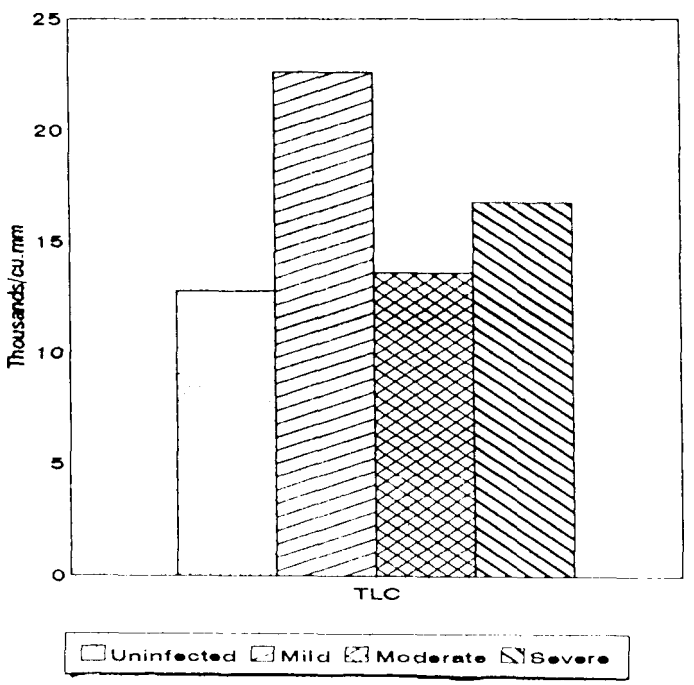
** - Highly significant ($P \leq 0.01$)
NS - Non significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

Graph 7. GRAPH SHOWING TOTAL ERYTHROCYTE COUNT OF NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



Graph 8. GRAPH SHOWING TOTAL LEUCOCYTE COUNT OF NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



69.667 ± 1.9976 and 70 ± 2.875 per cent respectively for the uninfected, mild, moderate and severe infection groups (Table 7). The infected groups also showed comparatively non significant differences (Table 13).

Lymphocyte

Non-significant lymphocytosis was observed in microfilaremic dogs when compared with normal dogs. The mean values were 22.133 ± 0.7462, 22.083 ± 2.234, 26.75 ± 1.943 and 24.917 ± 2.815 per cent respectively for the uninfected,mild, moderate and severe cases (Table 7). The infected groups also showed non-significant difference (Table 14).

Eosinophil

Eosinophilia was observed in microfilaraemic dogs in contrast with uninfected dogs. A significantly higher eosinophil value was obtained in all the infected groups when compared to the uninfected dogs. The eosinophil values of moderate group was non-significantly lower than the mild group. The mean values were 1.6 ± 0.289, 4.333 ± 0.554, 3.167 ± 0.297 and 4.583 ± 0.794 per cent respectively for the uninfected, mild, moderate and severe groups (Table 7). The infected groups showed non significant difference (Table 15).

Table 13. Comparison between the different groups in Neutrophil values

Groups	Values %		F value	Remarks
GP1 Vs GP2	72.167±2.63	Vs 69.667±1.998	1.933	NS
GP2 Vs GP3	69.667±1.998	Vs 70.000±2.9	1.933	NS
GP3 Vs GP4	70.00 ±2.90	Vs 76.067±1.35	1.933	NS
GP4 Vs GP1	76.067±1.35	Vs 72.167±2.63	1.933	NS
GP4 Vs GP2	76.067±1.35	Vs 69.667±1.998	1.933	NS
GP1 Vs GP3	72.167±2.63	Vs 70.00 ±2.90	1.933	NS

Table 14. Comparison between the different groups in Lymphocyte values

Groups	Values (%)		F value	Remarks
GP1 Vs GP2	22.083±2.20	Vs 26.750±1.943	1.192	NS
GP2 Vs GP3	26.750±1.94	Vs 24.917±2.82	1.192	NS
GP3 Vs GP4	24.917±2.82	Vs 22.133±0.75	1.192	NS
GP4 Vs GP1	22.133±0.75	Vs 22.083±2.20	1.192	NS
GP4 Vs GP2	22.133±0.75	Vs 26.75 ±1.94	1.192	NS
GP1 Vs GP3	22.083±2.20	Vs 24.917±2.82	1.192	NS

NS - Non significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

Monocyte

A non-significant monocytosis was observed in microfilaremic dogs when compared with uninfected dogs. The monocyte values of moderate group was non-significantly lower than the mild group. The mean values were 0.2 ± 0.106, 0.5 ± 0.26, 0.417 ± 0.193 and 0.583 ± 0.147 per cent respectively for the uninfected,mild, moderate and severe groups (Table 7). The infected groups also showed non significant difference (Table 16).

Basophil

There was no significant change in basophil values. The basophil values were 0, 0.083 ± 0.084, 0 and 0 per cent respectively for the uninfected, mild, moderate and severe groups (Table 7) and a non significant difference was seen between any group (Table 17).

4.4.1.7 Mean Corpuscular Volume (MCV)

There was a non-significant decrease in Mean Corpuscular Volume in infected dogs when compared to uninfected dogs. The mean MCV values were 63.985 ± 0.06, 63.093 ± 1.865, 62.488 ± 2.136 and 59.201 ± 1.051 cubic microns for uninfected,mild, moderate and severe categories (Table 7). There was also no significant difference between any group (Table 18).

Table 15. Comparison between the different groups in Eosinophil values

Groups	Values (%)	F value	Remarks
GP1 Vs GP2	4.333±0.55 Vs 3.167 ±2.29	1.480	NS
GP2 Vs GP3	3.167±0.297 Vs 4.583 ±0.79	1.480	NS
GP3 Vs GP4	4.583±0.794 Vs 1.600 ±0.289	1.404	**
GP4 Vs GP1	1.600±0.289 Vs 4.333 ±0.554	1.404	**
GP4 Vs GP2	1.600±0.289 Vs 3.167 ±0.297	1.404	**
GP1 Vs GP3	4.333±0.554 Vs 4.583 ±0.794	1.480	NS

Table 16. Comparison between the different groups in Monocyte values

Groups	Values (%)	F value	Remarks
GP1 Vs GP2	0.500±0.26 Vs 0.417 ±0.193	0.912	NS
GP2 Vs GP3	0.417±0.193 Vs 0.583 ±0.147	0.912	NS
GP3 Vs GP4	0.583±0.147 Vs 0.200 ±0.106	0.912	NS
GP4 Vs GP1	0.200±0.106 Vs 0.500 ±0.260	0.912	NS
GP4 Vs GP2	0.200±0.106 Vs 0.417 ±0.193	0.912	NS
GP1 Vs GP3	0.500±0.260 Vs 0.583 ±0.147	0.912	NS

** - Highly significant ($P \leq 0.01$)
NS - Non significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

Table 17. Comparison between the different groups in Basophil values

Groups	Values (%)			F value	Remarks
GP1 Vs GP2	0.083±0.084	Vs	0	1.089	NS
GP2 Vs GP3	0	Vs	0	1.089	NS
GP3 Vs GP4	0	Vs	0	1.089	NS
GP4 Vs GP1	0	Vs	0.083 ±0.084	1.089	NS
GP4 Vs GP2	0	Vs	0	1.089	NS
GP1 Vs GP3	0.083±0.084	Vs	0	1.089	NS

Table 18. Comparison between the different groups in `Mean Corpuscular Volume' values

Groups	Values (Cu.μ)			F value	Remarks
GP1 Vs GP2	63.093±1.865	Vs	62.488±2.136	1.453	NS
GP2 Vs GP3	62.488±2.136	Vs	59.201±1.051	1.453	NS
GP3 Vs GP4	59.201±1.051	Vs	63.985±0.600	1.453	NS
GP4 Vs GP1	63.985±0.600	Vs	63.093±1.865	1.453	NS
GP4 Vs GP2	63.985±0.600	Vs	62.488±2.136	1.453	NS
GP1 Vs GP3	63.093±1.865	Vs	59.201±1.051	1.453	NS

NS - Non-significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

4.4.1.8 Mean Corpuscular Haemoglobin (MCH)

The mean MCH values were 20.897 ± 0.584, 19.606 ± 0.699, 21.103 ± 1.152 and 19.832 ± 0.814 micro micro grams respectively for the uninfected, mild, moderate and severe groups respectively (Table 7) and no significant change was observed between any group (Table 19).

4.4.1.9 Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean MCHC values were 32.639 ± 0.568, 31.548 ± 1.288, 34.188 ± 2.18 and 33.471 ± 1.331 per cent respectively for uninfected, mild, moderate and severe groups (Table 7). No significant difference existed between any group (Table 20).

4.4.2 Biochemical aspects

4.4.2.1 Serum protein assays

The Serum Total Protein of the infected dogs were significantly greater (8.4494 ± 0.9133 g/dl) than that of the uninfected dogs (5.86583 ± 0.1958). The globulin values of infected dogs were also found to be significantly greater (5.6624 ± 0.7815 g/dl) than uninfected dogs (2.92633 ± 0.0987). The albumin of infected dogs were non significantly lower (2.7279 ± 0.1634 g/dl) than that of uninfected dogs (2.94506 ± 0.126). The albumin-globulin ratio was significantly lower in microfilaremic dogs (0.5716 ± 0.1850)

Table 19. Comparison between the different groups in `Mean Corpuscular Haemoglobin' values

Groups	Values ($\mu\mu g$)	F value	Remarks
GP1 Vs GP2	19.606±0.699 Vs 21.103±1.152	0.829	NS
GP2 Vs GP3	21.103±1.152 Vs 19.832±0.814	0.829	NS
GP3 Vs GP4	19.832±0.814 Vs 20.897±0.584	0.829	NS
GP4 Vs GP1	20.897±0.584 Vs 19.606±0.699	0.829	NS
GP4 Vs GP2	20.897±0.584 Vs 21.103±1.152	0.829	NS
GP1 Vs GP3	19.606±0.699 Vs 19.832±0.814	0.829	NS

Table 20. Comparison between the different groups in `Mean Corpuscular Haemoglobin Concentration' values

Groups	Values (%)	F value	Remarks
GP1 Vs GP2	31.548±1.288 Vs 34.188±2.180	0.636	NS
GP2 Vs GP3	34.188±2.180 Vs 33.471±1.331	0.636	NS
GP3 Vs GP4	33.471±1.331 Vs 32.639±0.568	0.636	NS
GP4 Vs GP1	32.639±0.568 Vs 31.548±1.288	0.636	NS
GP4 Vs GP2	32.639±0.568 Vs 34.188±2.180	0.636	NS
GP1 Vs GP3	31.548±1.288 Vs 33.471±1.331	0.636	NS

NS - Non significant

GP1 - Mild GP2 - Moderate
GP3 - Severe GP4 - Uninfected

when compared to uninfected dogs (1.0068 ± 0.1807) (Table 21 and Graph 9).

4.4.2.2 Aspartate amino transferase (AST) and Alanine amino transferase (ALT)

Aspartate amino transferase (AST) and alanine amino transferase (ALT) of microfilaraemic dogs were significantly higher (23.303 ± 0.7438 units/ml, 21.152 ± 1.088 units/ml respectively than uninfected dogs (17.222 ± 0.9585 units/ml and 14.750 ± 1.0774 units/ml respectively) (Table 22 and Graph 10).

4.4.2.3 Blood Urea Nitrogen (BUN) and Serum urea

The Blood Urea Nitrogen of infected dogs were non-significantly greater (15.179 ± 1.017 mg%) than of the dogs (13.342 ± 1.315 mg%). The serum urea of the former was also significantly greater (32.501 ± 2.177 mg%) than that of the uninfected dogs (15.306 ± 1.327 mg%) (Table 22; Graph 11).

4.4.3 Treatment with microfilaricides

4.4.3.1 Ivermectin

Ivermectin at a dose rate of 200 µg/kg (1 ml per 50 kg) body weight and 333 µg/kg (1 ml/30 kg) body weight were given subcutaneously to mild, moderate and severe groups of microfilaraemic dogs. The drug eliminated the circulating

Table 21. Serum protein fractions in microfilaraemic an non-microfilaraemic dogs

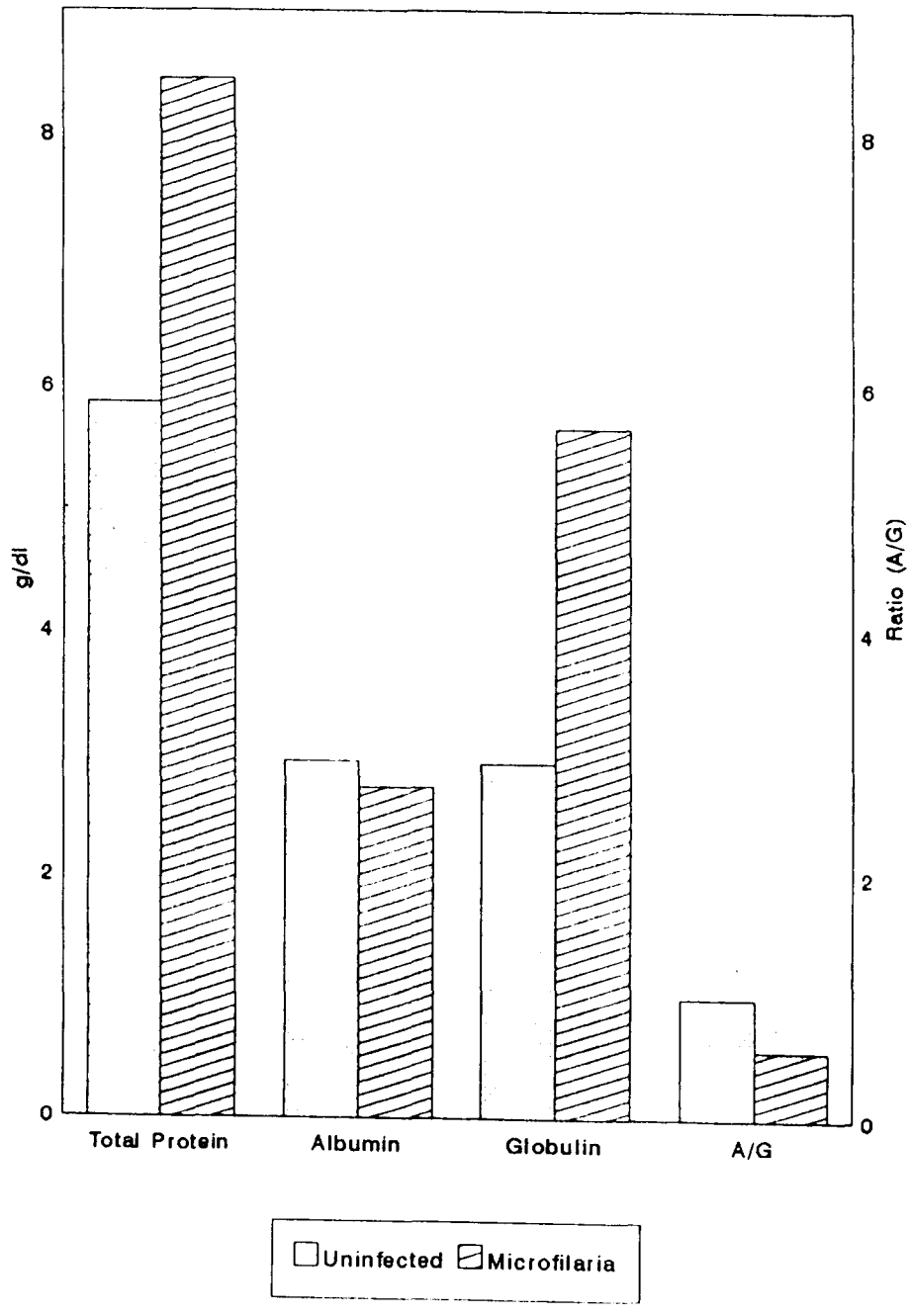
Fraction	Dogs with microfilaremia		Non-microfilaraemic dogs		t value
	No. of dogs	Mean± S.E.	No. of dogs	Mean± S.E.	
Total protein (g/dl)	40	8.44940± 0.9133	18	5.86583± 0.1958	2.7661 **
Albumin (g/dl)	40	2.72790± 0.1634	18	2.94506± 0.126	1.0525 NS
Globulin (g/dl)	40	5.66240± 0.8715	18	2.92633± 0.0987	3.4738 **
Albumin/ Globulin ratio	40	0.5716± 0.029	18	1.0068± 0.0426	8.3473 **

Table 22. Aspartate amino transferase, Alanine amino transferase, Serum urea, Blood urea nitrogen in microfilaraemic and non-microfilaraemic dogs

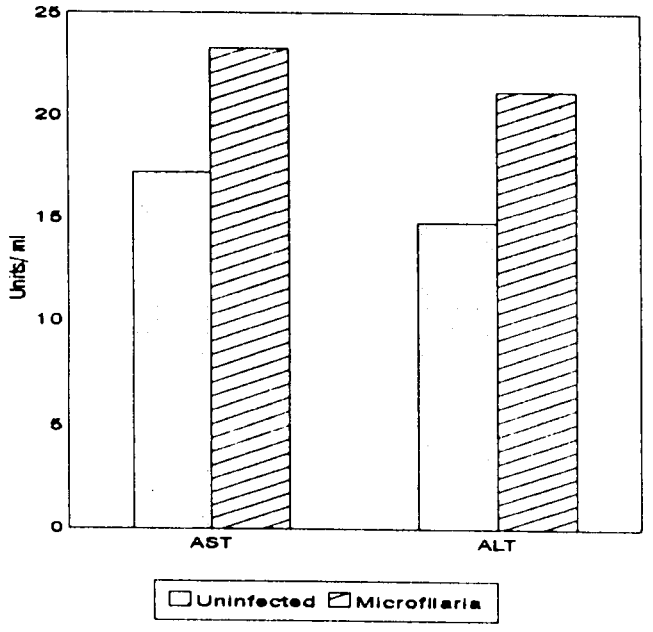
Fraction	Dogs with microfilaremia		Non-microfilaraemic dogs		t value
	No. of dogs	Mean± S.E.	No. of dogs	Mean± S.E.	
AST (units/ml)	33	23.303± 0.7438	18	17.2222± 0.9585	3.6827 **
ALT (units/ml)	33	21.152± 1.088	18	14.750± 1.6774	3.8168 **
Serum urea (mg %)	30	32.501± 2.177	18	15.306± 1.327	6.7441 **
Blood Urea Nitrogen (mg%)	30	15.179± 1.017	18	13.342± 1.315	1.1059 NS

** - Highly significant ($P \leq 0.01$)
NS - Non-significant

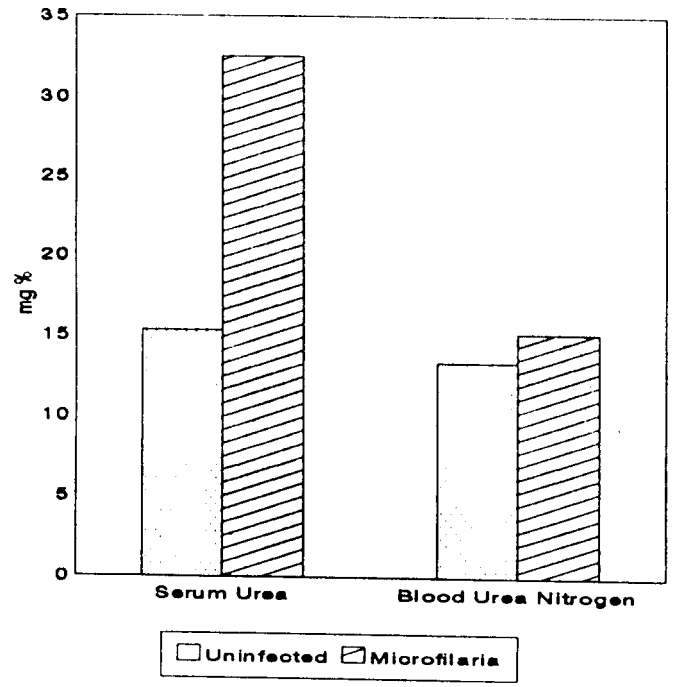
Graph 9. GRAPH SHOWING TOTAL PROTEIN, ALBUMIN, GLOBULIN AND ALBUMIN/GLOBULIN RATIO IN NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



Graph 10 GRAPH SHOWING ASPARTATE AMINO TRANSFERASE AND ALANINE AMINO TRANSFERASE VALUES IN NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



Graph 11 GRAPH SHOWING SERUM UREA AND BLOOD UREA NITROGEN IN NON-MICROFILARAEMIC AND MICROFILARAEMIC DOGS



microfilaria within 24 hours in 76 per cent of the cases. Temperature also returned to normal within this period. The symptoms like reduced feed intake, anorexia, cough, itching were all relieved with a single dose rate of the drug. The mean temperature, mean wet film and mean MKT on the 0th day and the 1st, 7th, 14th as well as 21st days are given in Table 23. The efficacy of the drug was calculated from the decrease in microfilarial count (mean MKT) on 1st, 7th, 14th and 21st days (Table 23).

The efficacy of Ivermectin at 200 μg/kg body weight in the mild group on 1st, 7th, 14th and 21st was found to be 97, 97.3, 98.39 and 99.3 per cent respectively (Table 23 and 26) while in the moderate group it was 99.99, 99.99, 100 and 100 per cent respectively (Table 23 and 28). The efficacy was 99, 99.6, 99.9 and 99.9 per cent for the severe group (Table 23 and 30).

Ivermectin at 333 μg/kg body weight had almost similar efficacy to that of Ivermectin at 200 μg/kg. The efficacy of the drug in the mild group on 1st, 7th, 14th and 21st day were found to be 99.75, 99.88, 99.88 and 99.88 per cent respectively (Table 23 and 26) while for the moderate group it was 99.56, 99.98, 99.98 and 99.98 per cent respectively (Table 23 and 28). In the severely infected group, the efficacy was 98.33, 99.5, 99.8 and 99.8 per cent respectively (Table 23 and 30).

Table 23. Result of treatment of mild, moderate and severe microfilaraemic dogs with Ivermectin

Dose	Intensity	No. of dogs examined	0th day			1st day				7th day				14th day				21st day			
			Mean Temp. (°F)	Mean WF	Mean MKT	Mean Temp. (°F)	Mean WF	Mean MKT	Mean clearance %	Mean Temp. (°F)	Mean WF	Mean MKT	Mean clearance %	Mean Temp. (°F)	Mean WF	Mean MKT	Mean clearance %	Mean Temp. (°F)	Mean WF	Mean MKT	Mean clearance %
200 µg/kg b.w. s/c single dose	Mild	10	103.0	+	281.2	102	-	8.4	97.0	102	-	7.5	97.3	102	-	4.5	98.39	102	-	2	99.3
	Moderate	6	104	+++	1651.0	102	-	0.17	99.99	102	-	0.17	99.99	102	-	0	100.0	102	-	0	100
	Severe	8	102	++++	39583.9	102	-	38.125	99	102	-	15.375	99.6	102	-	2	99.9	102	-	2	99.9
333 µg/kg b.w. s/c single dose	Mild	6	103.2	+	277.67	102	-	0.67	99.75	102	-	0.33	99.88	102	-	0.33	99.88	102	-	0.33	99.88
	Moderate	6	104.5	+++	1366.0	102	-	6	99.56	102	-	0.33	99.98	102	-	0.33	99.98	102	-	0.33	99.98
	Severe	6	105.0	++++	4136.0	102	-	69.17	98.33	102	-	21	99.5	102	-	6.5	99.8	102	-	6.5	99.8

4.4.3.2 Milbemycin oxime

Milbemycin oxime (INTERCEPTOR) at a dose rate of 0.5 mg/kg body weight orally was also tried in the mild, moderate and severe groups of microfilaraemic dogs. This drug also eliminated the circulating microfilariae within 24 hours in 86 per cent of cases and brought down the temperature to normal within this period. The symptoms were relieved with a single dose of the drug. The mean temperature as also the wet film and mean MKT values on the different days of observation are given in Table 24. The efficacy for the mild group as on 1st, 7th, 14th and 21st days were found to be 99.97, 100, 100 and 100 per cent respectively (Table 24 and 26) while for the moderate group it was 99.74, 100, 100 and 100 per cent respectively (Table 24 and 28). In the severely infected group the efficacy was 98.86, 99.22, 99.93 and 99.93 per cent respectively (Table 24 and 30). Adverse reactions like prostration, dyspnoea, weak pulse coldness of skin, paleness of mucous memberane, referable to a shock-like syndrome was observed in two dogs, one in the mild group and the other in the moderate group. These dogs recovered within 4 hours without any treatment.

4.4.3.3 Levamisole

The treatment carried out with Levamisole included a single dose 7.5 mg/kg body weight subcutaneously to mild,

Table 24. Result of treatment of mild, moderate and severe microfilaraemic dogs with Milbemycin oxime

Dose	Intensity	No. of dogs examined	0th day			1st day				7th day				14th day				21st day			
			Mean Temp.	Mean WF	Mean MKT	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %
0.5 mg/kg b.w. s/c orally	Mild	10	103.4	+	477.1	102	-	0.1	99.97	102	-	0	100.0	102	-	0	100.0	102	-	0	100.0
	Moderate	10	104	+++	1398.1	102	-	3.6	99.74	102	-	0	100.0	102	-	0	100.0	102	-	0	100.0
	Severe	8	104.8	++++	3373.5	102	-	38.30	98.86	102	-	26.25	99.22	102	-	2.5	99.93	102	-	2.5	99.93

moderate and severe microfilaremic dogs and repeated daily dose of 7.5 mg/kg body weight subcutaneously for seven days to those of the severe group. The efficacy was calculated on 1st, 7th, 14th and 21st days.

The single dose showed a gradual reduction in microfilaremia with persistance of much of the clinical signs. The temperature did not return to normal even after the 21st day of treatment in 93 per cent of cases. The efficacy for the mild group as on 1st, 7th, 14th and 21st days were found to be 48.34, 66.25, 67.7 and 68 per cent respectively (Table 25 and 26). The efficacy for the moderate group was 20.76, 62.77, 66.95 and 67.3 per cent respectively (Table 25 and 28) and for the severe group, 50.3, 68.1, 69.5 and 69.95 per cent respectively (Table 25 and 30).

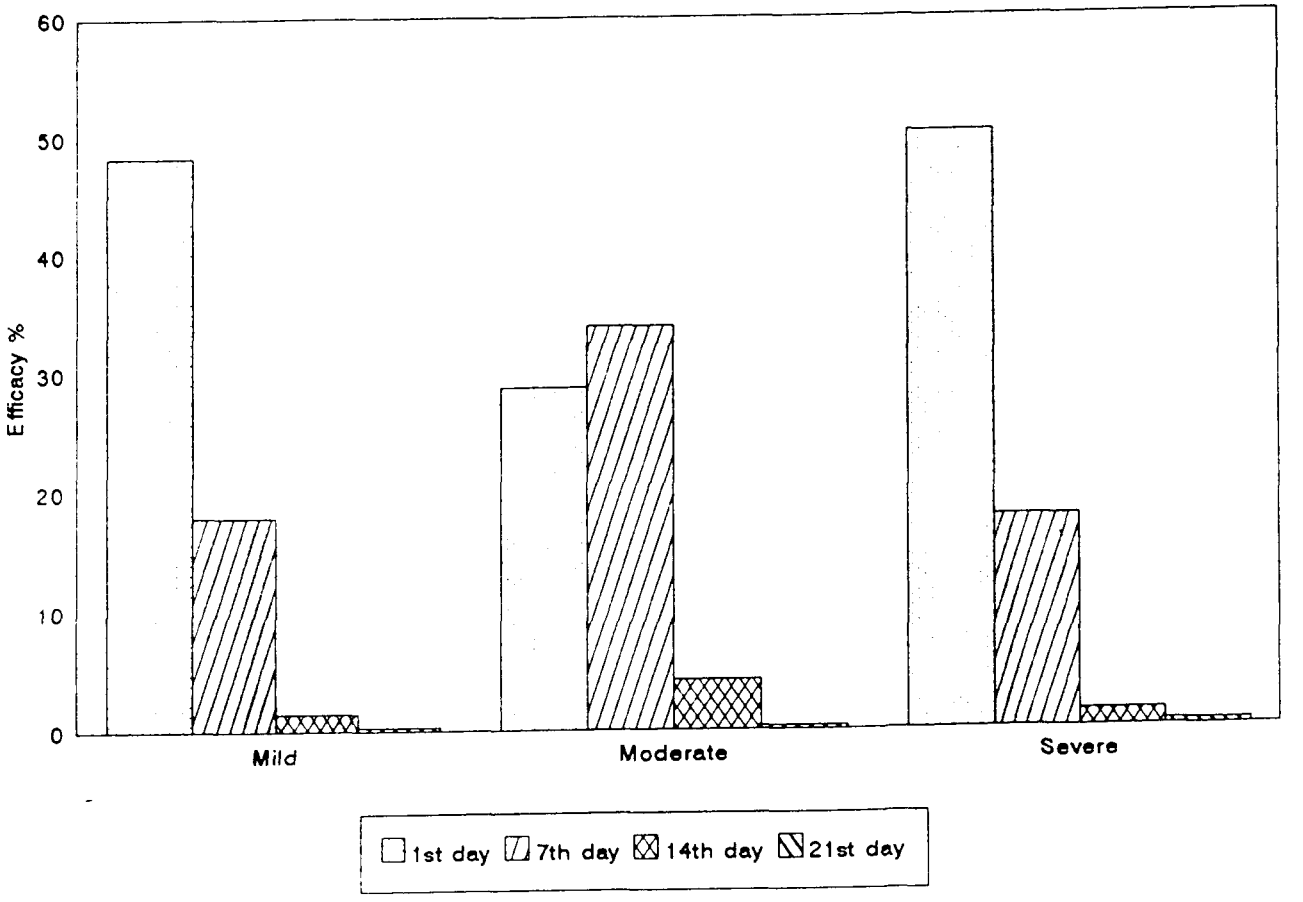
The dose rate of 7.5 mg/kg daily for seven days was more effective in clearing the microfilariae. The efficacy of the treatment regimen was 42.58 per cent on the 1st and ascended to 99.38 per cent 99.6 and 99.7 per cent by the 7th, 14th and 21st days respectively (Table 25 and 30). The temperature returned to normal and symptoms disappeared by 7th day.

Maximum clearance of the microfilariae was found to occur by the 1st and 7th days of treatment with the single dose of 7.5 mg/kg body weight in the mild, moderate and severe microfilaraemic dogs (Graph 12). The maximum clearance in the

Table 25. Result of treatment of mild, moderate and severe microfilaraemic dogs with Levamisole hydrochloride

Dose	Intensity	No. of dogs examined	0th day			1st day				7th day				14th day				21st day			
			Mean Temp.	Mean WF	Mean MKT	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %	Mean Temp.	Mean WF	Mean MKT	Mean clearance %
7.5 mg/kg s/c since dose	Mild	6	103.2	++	521.0	130	++	269.17	48.34	102	+	175.8	66.25	102	+	168.17	67.7	120	+	166.5	68.0
	Moderate	6	104	+++	1703.8	104	+++	1213.17	28.76	103	++	634.8	62.77	103	++	563.0	66.95	103	++	557.5	67.3
	Severe	6	104.2	++++	5746.2	104	++++	2855.5	50.3	103	+++	1832.3	68.1	103	+++	1751.0	69.5	103	+++	1726.3	39.95
7.5 mg/kg s/c daily for 7 days	Severe	6	104.0	++++	3826.0	104	++++	2197	42.577	102	-	23.5	99.38	102	-	11.6	99.6	102	-	11	99.7

Graph 12. GRAPH SHOWING THE EFFICACY OF SUBCUTANEOUS SINGLE DOSE OF LEVAMISOLE IN MILD, MODERATE AND SEVERE MICROFILARAEMIC DOGS



case of dosage reported for seven days was noticed on the 7th day, in severely affected dogs (Table 25).

4.4.3.4 Comparison between different drugs

Mild microfilaraemic group

The study revealed that Milbemycin oxime is the most effective drug when compared to the other drugs with regard to the reduction of microfilaraemia on 1st, 7th, 14th and 21st days followed in order by Ivermectin at 333 µg/kg body weight and Ivermectin at 200 µg/kg body weight. Levamisole was the least effective drug and did not totally clear the microfilaria (Table 26; Graph 13). There was no significant difference between Ivermectin at 200 µg/kg, ivermectin at 333 µg/kg or Milbemycin at 0.5 mg/kg body weight but when compared with Levamisole and the effectiveness of these drugs showed significant difference at $P \leq 0.01$ (Table 27).

Moderate microfilaraemic group

Milbemycin oxime as well as Ivermectin at 200 µg/kg body weight showed 100 per cent efficacy on the 21st day although Ivermectin was more effective than Milbemycin oxime on the first day. Ivermectin at 333 µg/kg body weight was the next in effectiveness than the single dose of Levamisole at 7.5 mg/kg body weight subcutaneously (Table 28; Graph 13). It was also found that there was no significant difference among

Ivermectin at 200 μg/kg, Ivermectin at 333 μg/kg and Milbemycin oxime at 0.5 mg/kg but these drugs showed significant difference ($P \leq 0.01$) in comparison with Levamisole (Table 29).

Severe microfilaraemic group

Milbemycin oxime was found to be the most effective drug on the basis of the result on the 21st day in the severely affected dogs followed by Ivermectin at 200 μg/kg. Ivermectin at 333 μg/kg body weight was the next effective drug followed by Levamisole at 7.5 mg/kg body weight daily for seven days. The single dose of Levamisole at 7.5 mg/kg was found to be the least effective. Also, Ivermectin at 200 μg/kg body weight was found to be more effective than Milbemycin oxime as on the 1st day (Table 30, Graph 13). There was no significant difference among Ivermectin at 200 μ/kg, Ivermectin at 333 μg/kg and Milbemycin oxime at 0.5 mg/kg body weight while these three drugs were more effective in comparison to Levamisole, showing significant difference ($P \leq 0.01$) (Table 31).

Table 26. Efficacy of different drugs on 1st, 7th, 14th and 21st days of treatment in the mild microfilaraemic dogs

	Drugs	Efficacy (%)			
		1st days	7th days	14th days	21st days
1.	Ivermectin 200 µg/kg b.w s/c single dose	III 97.00	III 97.30	III 98.39	III 99.30
2.	Ivermectin 333 µg/kg b.w s/c single dose	II 99.75	II 99.88	II 99.88	II 99.88
3.	Milbemycin 0.5 mg/kg b.w oral single dose	I 99.97	I 100.00	I 100.00	I 100.00
4.	Levamisole 7.5 mg/kg b.w s/c single dose	IV 48.34	IV 66.25	IV 67.70	IV 68.00

Table 27. Comparison between different drugs in the mild microfilaraemic dogs (efficacy on 1st day)

Drugs	Mean clearance rate on 1st day (R sin values)	Critical difference	Remarks
1 Vs 2	88.529±1.520 Vs 84.488±5.560	18.98	NS
2 Vs 3	84.488±5.560 Vs 88.848±1.200	16.43	NS
3 Vs 4	88.848±1.200 Vs 46.431±7.760	18.98	**
4 Vs 1	46.431±7.760 Vs 88.529±1.520	21.22	**
4 Vs 2	46.431±7.760 Vs 84.488±5.560	18.98	**
1 Vs 3	88.529±1.520 Vs 88.848±1.200	18.98	NS

Drug 1 - Ivermectin 200 µg/kg b.w s/c single dose
Drug 2 - Ivermectin 333 µg/kg b.w s/c single dose
Drug 3 - Milbemycin 0.5 mg/kg b.w orally single dose
Drug 4 - Levamisole 7.5 mg/kg b.w s/c single dose
** - Highly significant ($P \leq 0.01$)
NS - Non-significant

Table 28. Efficacy of different drugs on 1st, 7th, 14th and 21st days of treatment in the moderate microfilaraemic dogs

Drugs	Efficacy (%)			
	1st days	7th days	14th days	21st days
1. Ivermectin 200 µg/kg b.w s/c single dose	I 99.99	II 99.99	I 100.00	I 100.00
2. Ivermectin 333 µg/kg b.w s/c single dose	III 99.56	III 99.98	II 99.98	II 99.98
3. Milbemycin 0.5 mg/kg b.w oral single dose	II 99.74	I 100.00	I 100.00	I 100.00
4. Levamisole 7.5 mg/kg b.w s/c single dose	IV 28.76	IV 62.77	III 66.95	III 67.30

Table 29. Comparison between different drugs in the moderate microfilaraemic dogs (efficacy on 1st day)

Drugs	Mean clearance rate on 1st day (R sin values)	Critical difference	Remarks
1 Vs 2	88.382±1.660 Vs 89.832±0.212	9.22	NS
2 Vs 3	89.832±0.212 Vs 88.951±1.094	8.25	NS
3 Vs 4	88.951±1.094 Vs 32.673±4.430	8.25	**
4 Vs 1	32.673±4.430 Vs 89.832±1.620	9.22	**
4 Vs 2	32.673±4.430 Vs 89.832±0.212	9.22	**
1 Vs 3	88.382±1.660 Vs 88.951±1.094	8.25	NS

Drug 1 - Ivermectin 200 µg/kg b.w s/c single dose
Drug 2 - Ivermectin 333 µg/kg b.w s/c single dose
Drug 3 - Milbemycin 0.5 mg/kg b.w oral single dose
Drug 4 - Levamisole 7.5 mg/kg b.w s/c single dose
** - Highly significant ($P \leq 0.01$)
NS - Non-significant

Table 30. Efficacy of different drugs on 1st, 7th, 14th and 21st days of treatment in the severe group

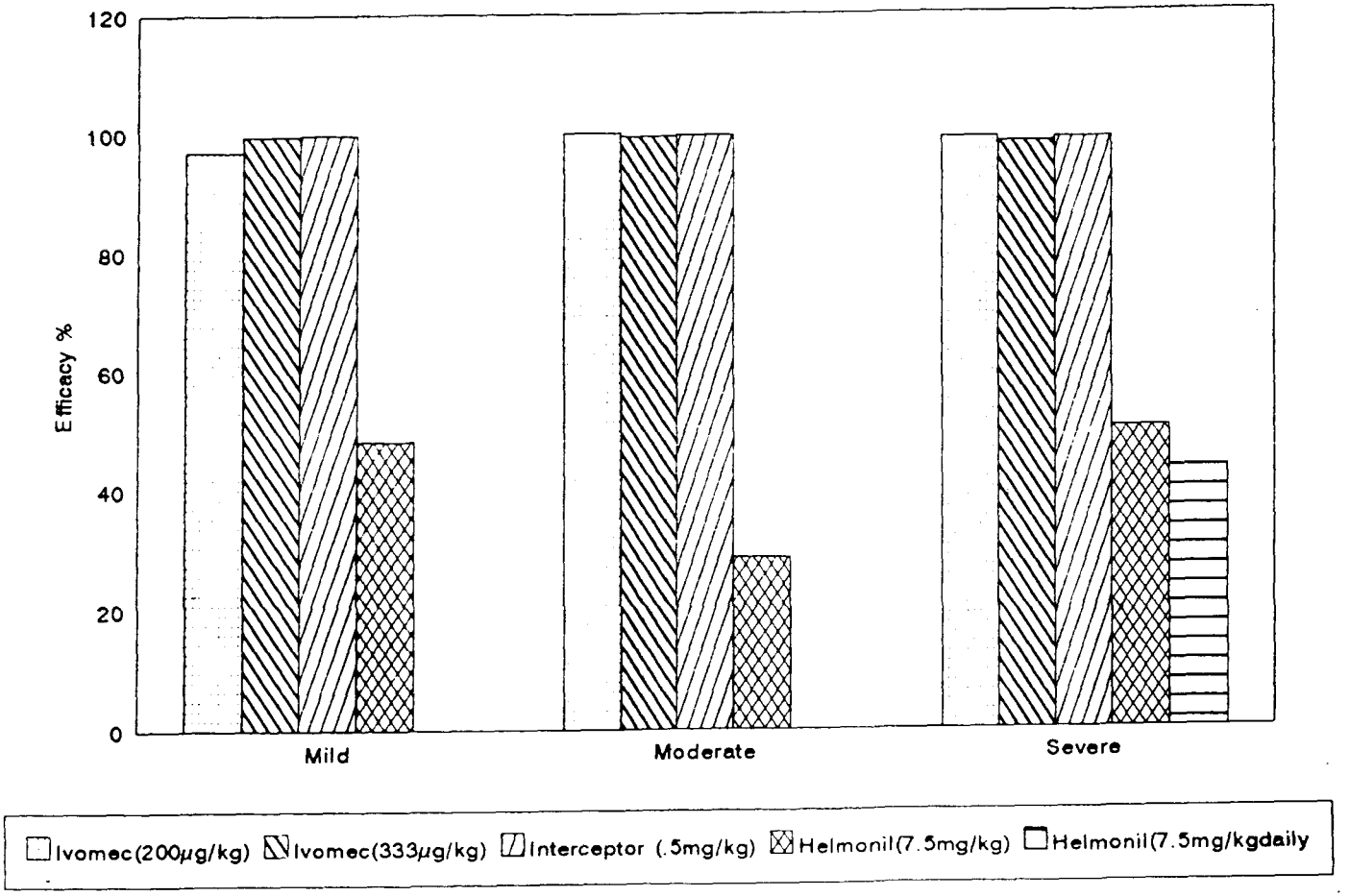
	Drugs	Efficacy (%)			
		1st days	7th days	14th days	21st days
1.	Ivermectin 200 µg/kg b.w s/c single dose	I 99.00	I 99.60	II 99.90	II 99.90
2.	Ivermectin 333 µg/kg b.w s/c single dose	III 98.33	II 99.50	III 99.80	III 99.80
3.	Milbemycin 0.5 mg/kg b.w oral single dose	II 98.86	III 99.22	I 99.93	I 99.93
4.	Levamisole 7.5 mg/kg b.w s/c single dose	IV 50.30	V 68.10	V 69.50	V 69.95
5.	Levamisole 7.5 mg/kg b.w s/c daily for 7 days	V 43.58	IV 99.38	IV 99.60	IV 99.70

Table 31. Comparison between different drugs in the severe microfilaraemic dogs (efficacy on 1st day)

Drugs	Mean clearance rate on 1st day (R sin values)	Critical difference	Remarks
1 Vs 2	86.527±2.670 Vs 87.517±2.030	11.53	NS
2 Vs 3	87.578±2.030 Vs 87.177±2.670	10.68	NS
3 Vs 4	87.177±2.670 Vs 44.178±4.280	11.53	**
4 Vs 1	44.178±4.280 Vs 86.527±2.670	12.33	**
4 Vs 2	44.178±4.280 Vs 87.517±2.030	11.53	**
1 Vs 3	86.527±2.671 Vs 87.177±2.670	11.53	NS

Drug 1 - Ivermectin 200 µg/kg b.w s/c single dose
Drug 2 - Ivermectin 333 µg/kg b.w s/c single dose
Drug 3 - Milbemycin 0.5 mg/kg b.w oral single dose
Drug 4 - Levamisole 7.5 mg/kg b.w s/c single dose
** - Highly significant ($P \leq 0.01$)
NS - Non significant

Graph 13. GRAPH SHOWING THE EFFICACY OF DIFFERENT DRUGS IN MILD, MODERATE AND SEVERE MICROFILARAEMIC DOGS (DAY ONE)



Discussion

DISCUSSION

5.1 Prevalence of infection

Among the, 2648 dogs screened for microfilaria (from those brought to Kokkalai and Mannuthy University Veterinary Hospitals) in Thrissur, 201 (7.59%) were found to be positive for the infection, which was encountered in every month of the year. The prevalence was higher in the months of January, April, June and July, with the highest peak in May. This revealed greater prevalence of infection in summer months. The higher summer prevalence of microfilariasis was in agreement with the findings of Martin and Collins (1985), who reported that the prevalence of infection was greater in summer than in winter. The higher summer prevalence could be attributed to the greater emergent population of mosquitoes with consequent immediate transmission of large numbers of microfilaria to dogs in the early post-monsoon period and the concommittant attainment of adulthood by a large number of filarid worms by the end of winter, resulting in copious shedding of the larvae in summer. This corraboration is in agreement with the 6-9 month (27-34 week) long pre-patent period found for *Dirofilaria repens* by Webber and Hawking (1955) and the resultant higher prevalence of microfilariasis during summer.

Magi *et al.* (1989) found highest incidence of *D. repens* in coastal areas of Pisa, Luccca, Leghorn and around Grosseto. The higher incidence of *D. repens* microfilariae, noticed in Thrissur in the present study may be attributed to the proximity of the area to the coastal belt as also the existence of marshy, water-logged places in the area.

The present study has also revealed that male dogs were more affected with *D. repens* microfilariae than females which agrees with the findings of Wallenstein and Tibola (1960), Thrasher *et al.* (1965), Hirth *et al.* (1966), Gubler (1972) as also Lewis and Losonsky (1977).

It has also been found that 73 per cent of dogs below six years of age were affected which tallies with the findings of Hirth *et al.* (1966) and Lewis and Losonsky (1977) who reported that majority of infected dogs were between 2 and 7 years in age at the time of diagnosis.

The finding in the present study that dogs below 6 months were negative for microfilariasis is ascribable to the fact that the prepatent period of *D. repens* 6-9 months (27-34 weeks) as reported by Webber and Hawking (op.cit).

The microfilariae of *D. repens* were the only microfilariae encountered in this study. This is in agreement with the report on prevalence of *D. repens* in India

by Rao (1938) and Patnaik (1989) but contrary to the observations of Valsala and Bhaskaran (1979) as well as Saseendranath *et al.* (1986).

5.2 Diagnosis of infection

The finding in the present study that for diagnosing microfilariasis, the Modified knott's technique (MKT) is more realiable in comparison to the Direct Wet Smear Examination is in agreement with the observations of Lindsey (1965) Altman (1972), Stein and Lawton (1973), Whitlock (1978), Lahitte *et al.* (1986), Whiteley (1988) as also Dhaliwal and Sani (1993).

Clinical examinations of blood films and staining of smears with Wright's-Giemsa method adopted by Valsala and Bhaskaran (1974) for diagnosing microfilariasis and identifying the microfilaria has been found to be less accurate and reliable in comparison to the MKT, in the present study.

Direct test, MKT and histochemical staining method were useful for detection and identification of microfilariasis in dogs in the present study which was in accordance with the work done by Das and Das (1996) in Orissa.

5.3 Differentiation of microfilaria

5.3.1 Morphology and biometry

In the morphological and biometrical studies, it was found that the microfilarial length and width were 285 ± 8.6, 6 ± 0.2 μm respectively when stained with Wright's, Leishman's or Giemsa while the corresponding values were 336.95 ± 10.3 μ, 6 ± 0.14 μ with Modified Knott's Technique. This was in accordance with the finding of Watson *et al.* (1973) who found that the length of microfilariae of *Dirofilaria immitis* and *Dipetalonena reconditum* isolated by filter technique were considerably shorter than those studied by the Modified Knott's Method. The biometry of microfilariae was found to be in agreement with Taylor (1960), Soulsby (1982), Sonin (1985) and Valcarcel *et al.* (1990).

The fixed point study proved that the microfilaria belonged to *Dirofilaria repens* which was commensurate with the findings of Taylor (1960), Soulsby (1982) and Sonin (1985).

The morphology and movements of the microfilariae in this study were identical those observed by Taylor (1960).

5.3.2 Staining with Brilliant cresyl blue

The R cells and the excretory cells could be stained well only with 10 per cent brilliant cresyl blue which was contrary to the demonstration of these cells with 1 in 50 dilution of 1 per cent Brilliant cresyl blue by Sawyer *et al.* (1965 and 1966).

5.3.3 Histochemical differentiation

Histochemical studies revealed that the microfilaria undoubtedly belonged to *D. repens*.

The present study revealed that microfilariae of *D. repens* showed enzyme activity at the anal pore region (spot) only, in agreement with the work done by Balbo and Abate (1972) Yen and Mak (1978) and Valcarcel *et al.* (1990).

Valcarcel *et al.* (loc.cit) further reported that certain microfilariae of *D. repens* stained at two areas i.e., at the anal pore region (spot) and diffusely at the central body region.

In the present study, these two types of microfilariae of *D. repens* were not only noticed but also could be classified on the basis of their movement in wet film. It was found that microfilariae having wriggling motion stained only at the anal

pore region while those having progressively forward motion stained at the anal pore as well as central body region.

The motility in wet film alone could mislead the differentiation of microfilaria. The wriggling type of microfilariae is mostly those of *D. immitis* while the progressively forward type is of *Dipetalonema* sp. Blagburn (op.cit). The present study revealed both wriggling and progressively forward moving types of *D. repens*, which suggests the importance of differentiating the micirofilariae using histochemical method.

5.4 Clinical pathology

5.4.1 Haematology

Haematological findings showed a significant increase in ESR and TLC and a significant decrease in haemoglobin, TEC and PCV in microfilaraemic dogs. These findings were in agreement with those of Snyder *et al.* (1967), Chakrabarthy and Choudhury (1983) as also Sharma and Pachauri (1986).

The circulating larvae of *D. repens* may result in tissue damage and destruction of RBCs due to their circulation and lead to low total erythrocyte count and Haemoglobin content. This finding has been established by Yousif *et al.* (1990). Singh *et al.* (1973), Kumar (1980) and Kumar *et al.* (1984) also

associated the decrease in erythrocyte production in microfilariasis due to the damage caused by microfilaria to the liver tissues resulting in loss of blood and depression of erythropoiesis. Increase in ESR level could also be associated with the extensive tissue damage in this condition. Increased ESR level can be considered as a valuable diagnostic test in the diagnosis of microfilariasis in dogs according to Yousif *et al.* (op.cit).

The differential leucocyte picture of the microfilaraemic dogs showed a non-significant neutropenia, lymphocytosis and monocytosis with a significant eosinophilia. These findings were in accordance with those of Sharma and Pachauri (1986) who observed that neutropenia, eosinophilia and lymphocytosis accompanied heartworm disease in dogs. Contrary to this finding, Farnes *et al.* (1972) found neutrophilia in dogs with chronic *Dirofilaria* infection while Wittwer *et al.* (1979) found that microfilariae of *Dipetalonema* does not produce any significant change in the differential leucocyte counts.

5.4.2 Biochemical aspects

5.4.2.1 Serum protein assays

In the present study, it was found that Serum Total Protein and Globulin fraction were greater in microfilaraemic dogs while Albumin fraction was greater in normal dogs.

Albumin/Globulin ratio was lower in dogs with microfilaraemia. This was in confirmity with the results obtained by Tomoda (1962), Snyder *et al.* (1967), Shibata (1974), Barsanti *et al.* (1977) and Barsanti (1978). The increase in Serum Total Protein is due to the increased globulin fraction in the serum of infected dogs, (may be as a mechanism) to combat infection.

5.4.2.2 Aspartate amino transferase (AST) and Alanine amino transferase (ALT)

It was found that AST and ALT values were greater in infected dogs. This could be attributed to the liver damage caused by microfilaria (Kaneko, 1980; Everett *et al.*, 1977; Keenan *et al.*, 1978 and Yousif *et al.*, 1990).

5.4.2.3 Blood Urea Nitrogen (BUN) and Serum urea

There was also a slight increase in Blood Urea Nitrogen and Serum Urea. The increase in BUN value is in agreement with the finding of Snyder *et al.* (1967), Shibata (1974), Cortez *et al.* (1980), Kaneko (1980) as well as Sharma and Pachauri (1982 and 1986). No published information was traceable to interpret the significance of serum urea.

5.5 Treatment with microfilaricides

5.5.1 Ivermectin

Treatment of microfilaraemia with Ivermectin at a dose rate of 200 µg/kg and 333 µg/kg eliminated the circulating microfilaria within 24 hours in 76 per cent of cases. This was in accordance with the findings of Blair *et al.* (1983) who found that Ivermectin at a dose rate of 0.0125, 0.05 and 0.2 mg/kg b.w. eliminated the circulating microfilariae within 24 hours while at 0.00313 mg/kg the drug only reduced the microfilariae considerably.

A single treatment with Ivermectin which is a macrolide antibiotic was found to suppress microfilaraemia for several weeks. This was in accordance with the studies of Lok *et al.* (1988) who observed that the effects of Ivermectin on embryogenesis of microfilariae within female heartworms indicated that not only the development of early stages is interrupted but also the later developmental stages into the circulation are affected.

The efficacy of Ivermectin at 200 µg/kg b.w. was found to be 99.3, 100 and 99.9 per cent for the mild, moderate and severe microfilaraemic dogs at 21st day of treatment. This was in accordance with the findings of Jackson and Seymour (1981) as well as Suderman and Craig (1984) who found

100 per cent and 90 per cent efficacy within 3 weeks. Simpson and Jackson (1985) observed 92-98 per cent efficacy within 48 hours.

Ivermectin at a dose rate of 333 μg/kg b.w. S/c was found to be 99.88, 99.98 and 99.8 per cent effective for the mild, moderate and severe groups of microfilaraemic dogs. It was found that Ivermectin at 333 μg/kg body weight had almost similar efficacy as that of Ivermectin at 200 μg/kg b.w.

5.5.2 Milbemycin oxime

Milbemycin oxime at a dose rate of 0.5 mg/kg b.w. orally also eliminated the circulating microfilariae within 24 hours in 86 per cent of cases. This was in accordance with the findings of Blagburn (1994). A single treatment with Milbemycin oxime which is also a macrolide antibiotic similar to Ivermectin in chemistry and spectrum of activity was found to suppress microfilaraemia for several weeks.

The efficacy of this drug on 1st day after treatment was found to be 99.97, 99.74 and 98.86 per cent respectively for mild, moderate and severe microfilaramic dogs while the efficacy reached 100, 100 and 99.93 per cent for mild, moderate and severe groups on the 21st day. The efficacy of Milbemycin oxime in the present study was better than the findings of Shutou *et al.* (1993), who observed that at 0.1 and

0.2 g/kg b.w. eliminated microfilaria in 86 per cent of dogs treated in 4 days period. Genchi *et al.* (1993) got 100 per cent efficacy with Milbemycin oxime at 0.5 mg/kg b.w. orally at monthly intervals for 7 months.

Some adverse reactions like prostration, dyspnoea, weak pulse, coldness of skin, pale mucous membrane referable to a shock like syndrome which were observed in two dogs with microfilaraemia recovered within 4 hours without any treatment. The experience was in agreement with those of Sakamoto *et al.* (1985) and Sasaki *et al.* (1986). These reactions could be ascribed to the individual differences in dogs for drug acceptability and not due to the increased microfilariae in the circulation.

5.5.3 Levamisole

In the present study, dogs treated with Levamisole at 7.5 mg/kg b.w. S/c as single dose was found to be less effective. The efficacy of the drug was 68, 67.3 and 69.95 per cent respectively for the mild, moderate and severe groups.

Levamisole at 7.5 mg/kg S/c daily for 7 days was found to clear microfilariae more effectively. The efficacy reached 99.38, 99.6 and 99.7 per cent on 7th, 14th and 21st days after treatment which suggested that the drug should be used continuously for 7 days to attain maximum efficiency.

Levamisole was tried at lower dose rates by various workers. Carr and Bessmer (1975) used Levamisole at 3-5 mg/lb b.w. orally for 6 days which eliminated only 50 per cent of microfilaria from blood. Bradley (1976) used Levamisole resinate at 5.5 mg/kg b.w. daily for 10 days and complete clearance was not obtained after the trial. Bradley and Alford (1977) found 100 per cent efficacy within 4 weeks with Levamisole given at 4.4 mg/kg b.w. twice daily for 2 weeks, followed by 6.6 mg/kg twice daily for two weeks. Hayasaki *et al.* (1984) observed complete larvicidal action for Levamisole at a dose rate of 5 mg/kg thrice daily for 5 days.

Levamisole has also been tried at higher dose rates. Bradley (1976) used Levamisole at 11 mg/kg b.w. daily for 10 days which eliminated microfilaria completely. Atwell and Baldock (1979) used Levamisole at 10 and 20 mg/kg orally for 21 days and both dosages reduced the numbers of circulating microfilaria only to 50 per cent. Chaikin (1979) used the drug at the dose rate of 11 mg/kg b.w. and found that an average of 13 days were needed to eliminate the microfilaria completely. Carlisle *et al.* (1984) found 100 per cent efficacy with Levamisole at 10 mg/kg and 20 mg/kg b.w. twice daily for 14 days but the higher dose rate was found to be toxic.

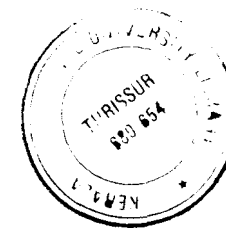
The present findings suggests that the use of Levamisole at an optimum dose rate of 7.5 mg/kg b.w. daily for 7 days is only required to attain maximum efficacy against the microfilaria of *D. repens*.

Comparison between different drugs

Milbemycin oxime was found to be the most effective drug in clearing the microfilariae of *D. repens* in mild, moderate and severe microfilaraemic dogs, followed by Ivermectin and Levamisole.

Oral tablets of Milbemycin oxime are easy to administer while Ivermectin and Levamisole are administered as subcutaneous injection. Further the dogs under Levamisole treatment have to be brought to the clinic daily for 7 days for the continuation of treatment. The practical snags and shortfalls of the other drugs make Milbemycin oxime the drug of choice against microfilaria of *D. repens*.

Summary



SUMMARY

A detailed study on the prevalence, clinical pathology and treatment of microfilariasis in dogs in Thrissur was carried out during the period from October 1996 to September 1997 which yielded the under mentioned salient findings:

1. Microfilariasis was prevalent in dogs throughout the year and its incidence happened to be high during the summer months. The incidence was also more in male dogs below 6 years.

2. For the diagnosis of microfilariasis, the Modified Knott's Technique was more reliable and accurate even though the wet film examination was very much useful for preliminary screening.

3. Microfilaria of *Dirofilaria repens* was the only microfilaria encountered in the study. The specific diagnosis was based on morphological and biometrical studies and confirmed by the histochemical study.

4. Haematological studies showed that the infection caused marked increase in Erythrocyte Sedimentation Rate, Total Leucocyte Count, Eosinophils and Lymphocytes while Haemoglobin, Packed Cell Volume, Total Erythrocyte Count and Neutrophils were reduced.

5. Biochemical studies revealed that there were marked increase in Serum Total Protein and Globulin levels in microfilaraemic dogs while the Albumin fraction and the Albumin/Globulin ratio were greater in non-microfilaraemic dogs. There were also significant increase in AST and ALT values in microfilariasis. Blood Urea Nitrogen and Serum Urea also showed slight elevations.

6. Treatment with Ivermectin at 200 μg/kg b.w. was found to be equally effective as at 333 μg/kg b.w. and there were total clearance of microfilariae within 24 hours in majority of the cases. The treatment also resulted in marked reduction in body temperature, increased appetite and reduced respiratory distress (cough).

7. Chewable tablets of Milbemycin oxime at the dose rate of 0.5 mg/kg b.w. was found to be the most effective drug against microfilaria of *D. repens*. Clinical signs like increased body temperature and deprived appetite were got corrected with a single treatment within 24 hours. Adverse reactions occurred only in very rare cases.

8. Levamisole injection given at the dose rate of 7.5 mg/kg b.w. as single dose was found to be the least effective against microfilaria of *D. repens*. Moreover clinical signs persisted even after treatment.

Levamisole at 7.5 mg/kg b.w. daily for 7 days was found to be more effective than the single dose. Maximum microfilarial clearance was attained on the 7th day treatment. The efficacy was almost similar to those of Ivermectin and Milbemycin oxime.

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PREVALENCE, CLINICAL PATHOLOGY AND TREATMENT OF MICROFILARIASIS IN DOGS IN THRISSUR

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ABSTRACT OF A THESIS

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ABSTRACT

In a detailed study conducted on prevalence, clinical pathology and treatment of microfilariasis in dogs in Thrissur, it has been found that canine microfilariasis is prevalent throughout the year with more incidence during summer. The most reliable and accurate diagnostic method for detecting microfilariae, was noted to be the Modified Knott's Technique while wet film examination was more useful for preliminary screening. Microfilariae encountered were identified as those of *Dirofilaria repens*.

The affected dogs revealed haematological changes like increase in ESR, TLC, eosinophils as well as lymphocytes and decrease in Hb, TEC, PCV as also neutrophils. Biochemical studies revealed increase in Serum Total Protein, Globulin, AST, ALT, BUN as well as Serum Urea in microfilaraemic dogs and higher levels of Albumin, Albumin/Globulin ratio in non-microfilaraemic dogs.

Single oral dose of Milbemycin oxime at 0.5 mg/kg b.w. was found to be the most effective drug for microfilariasis, followed by single s/c dose of Ivermectin at 200 µg/kg b.w. and 7 consecutive daily s/c dose of levamisole at 7.5 mg/kg.