IMMUNODIAGNOSIS OF CANINE DIROFILARIOSIS USING MICROFILARIAL ANTIGEN

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2003

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DECLARATION

I hereby declare that the thesis entitled "**IMMUNODIAGNOSIS OF CANINE DIROFILARIOSIS USING MICROFILARIAL ANTIGEN**" is a bonafied 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 the thesis entitled "**IMMUNODIAGNOSIS OF CANINE DIROFILARIOSIS USING MICROFILARIAL ANTIGEN**" is a record of research work done independently by Dr. Priya M. N., 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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ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr. H. Subramanian, Associate Professor and Head, Department of Veterinary Parasitology and Chairman of the Advisory Committee, for his scholarly advice, incessant encouragement, valuable suggestions, constructive criticism and continued interest shown during this study.

I deem it my privilege in expressing my heartfelt gratitude and thanks to Dr. K. Devada, Assistant Professor, Department of Veterinary Parasitology, and a member of the Advisory Committee for her affectionate encouragement, timely advice, kindness and professional guidance.

I owe my deep gratitude to Dr. K. M. Jayakumar, Associate Professor, Department of Clinical Medicine, and a member of Advisory Committee, for his constant support, timely and whole hearted help and concern.

I am very much obliged to Dr. M. Mini, Assistant Professor, Department of Veterinary Microbiology, and a member of Advisory Committee, for her creative suggestions, support, meticulous guidance and continued interest shown at every stage of my work.

I am in short of words to express my heartfelt gratitude to Dr. Chintu Ravishankar and Dr. P.X. Antony, for their timely help and advice which enabled me a lot in overcoming the hardships faced during the period of research work. The support, concern, constant inspiration and help offered by them can never be forgotten.

I am extremely grateful to Dr. Lucy Sabu, Assistant Professor, Department of Veterinary Parasitology, for her words of inspiration, kindness and possible help extended during each and every phase of my research work.

The invaluable help and co-operation rendered by Dr. M.R. Saseendranath, Associate Professor and Head, Dr. K. Vijayakumar, Dr. P.V.

Tresamol, Assistant Professors, Department of Veterinary Epidemiology and Preventive Medicine and Dr. S. Ajithkumar, Dr. Premni Elias, Assistant professors, Department of Clinical Medicine are greatly acknowledged.

I extend my sincere thanks to the non-teaching staff of the University Veterinary Hospitals at Kokkalai and Mannuthy for their kind co-operation and help offered in the collection of sample and data needed for this work.

My sincere thanks are due to Dr. V. Jayaprakashan, Associate Professor and Head, Dr. G. Krishnan Nair, Associate Professor, Department of Veterinary Microbiology for the facilities provided in conducting research work.

I am thankful to staff of the Department of Microbiology for their co-operation and help.

The timely help rendered by Sri. P.R. Chandrashekaran, Associate Professor and Head, Department of Instrumentation are greatly acknowledged.

I owe my sincere thanks to Mrs. Sujatha, Associate Professor and Head, Department of Statistics, for her help in analysing the data.

I sincerely thank all the staff members in the Department of Veterinary Parasitology for their help throughout my research work.

I express my heartfelt thanks to my colleagues Dr. Rejitha T.S., Dr. Shameem. H. and Dr. Beena J.N. for their invaluable help, co-operation and support extended throughout the course of the academic programme.

I remember with gratitude the help rendered by my friends Dr. Mridula V. and Dr. Radhika, R. for literature collection.

I acknowledge '98 batch UG students for their help and concern shown throughout the study period.

I express my heartfelt thanks to my friends Dr. Priya P., Dr. Sindhu, Dr. Thushara, Dr. Renjith, Dr. Bindu Mathew, Dr. Sanjeetha, Dr. Siji, Dr. Priya

Das, Dr. Abida, Dr. Indu, Dr. Devi, Dr. Manju, Dr. Elaiyaraja, Dr. Raju, Dr. Nisha and Dr. Chitra for their tireless help, incessant encouragement and mental support given to me during my study period.

The facilities provided by Dr. E. Nanu, Dean (i/c), College of Veterinary and Animal Sciences, Mannuthy for this work is greatly acknowledged.

I am thankful to the members of M/s Peagles, Mannuthy for efficient execution in the preparation of this thesis.

I am grateful to KAU for awarding me the fellowship for the post graduate study.

With immense pleasure I would like to acknowledge with gratitude the moral support, encouragement and blessings extended by my Achan, Amma, Chechi, Chettan, Brother-in-law, Lakshmi and Appu in fulfilling this endeavor.

I am also grateful to my in-laws for their understanding, co-operation, encouragement and prayers offered to me in the completion of this work.

This task would not have been completed successfully but for the understanding, love, mental support, constant encouragement and help by my beloved husband Mr. Rajkumar. I express my heartfelt gratitude to him for bearing with me all the inconveniences.

Above all I bow my head low before the Almighty whose radiance enabled to complete this work

PRIYA, M. N.

Dedicated

To

Sree Guruvayurappan

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Introduction

1. INTRODUCTION

The most common companion animal, the dog, was first domesticated about 12,000 years ago, probably from scavenging packs of wolves that accompanied human hunters. By the dawn of history, man was employing dogs for three main purposes: hunting, guarding and making war. Over the years, dog rearing has increased tremendously especially in urban and semi-urban areas. In the present century, the dog has maintained most of its historical roles and are also utilised for crime detection and tracking. In addition to these social values or privileges, pet dogs contribute substantially to the income of pet breeders by way of sale of pups. Social attitudes towards dogs ever since their domestication, must have always affected their health and well being. Equally the ways in which dogs are managed today, have a great effect on canine health than most dog owners probably realise. Owing to these reasons health cover to dogs has assumed great importance.

Dogs suffer from innumerable diseases of which those caused by parasites especially nematodes have always been a cause of great concern for millions of pet owners and veterinarians all over the world. It is virtually impossible for any dog to escape worm infestation perpetually. Even the most hygiene conscious and fastidious persons cannot prevent a dog becoming infested, but by timely diagnosis and periodic dosing with an antiworm drug one can control such infections. In most of the cases adult stages of the parasite are most pathogenic but sometimes other stages may also be equally harmful to the host.

Canine dirofilariosis is an important parasitic disease caused by different species of dirofilaria of which *Dirofilaria immitis* and *D. repens* are significant. Adult worms of *D. repens* occur in the subcutaneous tissues and those of *D. immitis* occur in the right ventricle and pulmonary arteries of the host. The problem is likely to occur during the animal's most active years. Natural infection is acquired by the introduction of the infective third stage larvae into the dog,

through a mosquito vector belonging to the genera *Culex, Anopheles* or *Aedes*. In Kerala dirofilariosis is caused by *D. repens* only (Radhika, 1997) with a prevalence rate of 7.59 per cent.

The diagnosis of filarial infection in dog is important in clinical veterinary practice and in selecting dogs for research purposes. Dogs are generally screened for dirofilariosis on the basis of examination of blood samples for microfilariae, either directly or by a concentration technique. However, 5 to 25 per cent of canine infections do not have circulating microfilariae and are termed occult (Knight, 1983). This type of dirofilariosis can occur in (i) in the prepatent period (ii) due to drug induced sterility of adults by prolonged therapy (iii) immune mediated sterile infections with antibodies to the microfilariae and (iv) in unisexual infections (Rawlings *et al.*, 1982). Understanding of this condition and awareness of this form of dirofilariosis are essential in the administration of timely treatment. Serological tests like Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) and dot-ELISA may help us to diagnose canine dirofilariosis accurately.

The major reason for veterinary parasitological research is that parasites can cause disease in animals resulting in loss of production, suffering and even death. The existence of parasitic zoonoses adds to the need for research. Filarids, especially *D. repens* and *D. immitis* pose a zoonotic threat to the humans. A case of subcutaneous dirofilariasis in a woman in Kerala, was reported by Senthilvel and Pillai in 1999. According to Soulsby (1982), one area where 80 per cent of the dogs were infected, 30 per cent humans were reactive to serodiagnostic tests. Hence, study on the immunodiagnosis of canine dirofilariosis becomes significant.

Considering the above factors a detailed study was undertaken,

a) To assess the feasibility and reliability of microfilarial antibody detection by Enzyme Linked Imunosorbent Assay.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PREVALENCE OF INFECTION

Wong *et al.* (1973) mentioned that filariasis is one of the common parasitic diseases in dogs and described the aspects of occult dirofilariasis.

By screening of 92 dogs in Calicut, Kerala, Valsala and Bhaskaran (1974) detected *Dirofilaria immitis* in the peripheral blood of 12 dogs (13.04 per cent)

Martin and Collins (1985) examined the blood samples from 331 grey hounds in Hunter valley and nearby coastal areas of New South Wales and found that 10.9 per cent were infected with *Dirofilaria immitis* and 3.6 per cent with *Dipetalonema reconditum.* They also stated that the prevalence was greater in summer than in winter.

Saseendranath *et al.* (1986) recorded the incidence of canine dirofilariasis in Trichur, Kerala as 24.2 per cent by screening dogs brought to the Kerala Agricultural University hospital, Kokkalai, during a period of six months from September 1984 to February 1985.

Dhaliwal *et al.* (1987) reported a case of dirofilariasis by *Dirofilaria immitis* from Punjab for the first time.

First case of occult form of heartworm disease caused by *Dirofilaria immitis* in a four Retriever female dogs in UK was reported by Matic and Herrtage (1987).

Rojo- Vazquez *et al.*, (1990) conducted a survey in four areas of Spain by examining 1683 dogs, of which 2.1 per cent were positive for microfilaria of *Dirofilaria immitis*, 0.2 per cent for *Dirofilaria repens*, 0.7 per cent for *Dipetalonema reconditum* and 4.1 per cent for *Dipetalonema dracunculoides* and that three dogs carried mixed infection.

Kamalu (1991) in south eastern Nigeria found that microfilariae with the characteristics of *Dirofilaria repens* were frequently observed in the peripheral blood of dogs.

Patton and McCracken (1991) determined the prevalence of heartworms in dogs and cats in Eastern Tennessee, USA. They found that 5.08 per cent of dogs and cats had circulating *Dirofilaria immitis* microfilariae whereas 13.82 per cent of dogs were positive for heartworm antigen by ELISA test.

Lee *et al.* (1996) studied *Dirofilaria immitis* infection in German Shepherd dogs in five areas of Korean Republic and found that the infection rate was 10.2 per cent and 28.3 per cent by modified Knott's test and antigen test respectively.

Liang *et al.* (1996) detected microfilariae in the blood of 5.3 per cent of dogs using ELISA heartworm antigen test kit and the prevalence was found to be 17.7 per cent.

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 (Radhika, 1997).

Field and laboratory studies performed by Aranda *et al.* (1998) in Spain in order to assess the canine dirofilariasis revealed an incidence rate of 12.8 per cent, 3.7 per cent and 2.7 per cent for *Dirofilaria immitis, Dipetalonema reconditum* and *D. dracunculoides* respectively.

Bokaie *et al.* (1998) mentioned the prevalence rate of *Dirofilaria immitis* infection as 34.6 per cent according to their study carried out in dogs in Meshkinshahr area, North-west Iran.

Wang (1998) assessed the current status of *Dirofilaria immitis* infection in North Taiwan and found 60.6 per cent of dogs to be infected. Of these, 55 per cent had *Dirofilaria immitis* and the rest had *Dipetalonema reconditum*.

Razmi (1999) indicated that nine dogs (6.5 per cent) were infected with *Dirofilaria repens* and seven (5 per cent) with *Dipetalonema reconditum* in a study conducted on 138 dogs of Mashhad using Knott's technique.

Necropsis carried out in 179 privately owned dogs from a hyper zoonotic area in North Italy to study on the occult heartworm infection revealed that 62 (34.6 per cent) of the dogs were positive for *Dirofilaria immitis* adult worm (Rossi *et al.*, (1999).

Results of the study conducted by Cancrini *et al.* (2000) demonstrated a very high prevalence of *Dirofilaria repens* at 84.6 per cent and 37.1 per cent respectively in two cities of south eastern Spain.

Fan *et al.* (2001) estimated the seroprevalence of *Dirofilaria immitis* infection in domestic dogs in Taiwan as 13.4 per cent using a commercial ELISA kit (Snap TM, IDEXX, USA) for detecting circulating antigens released by adult female worm.

Lai *et al.* (2001) indicated that the prevalence of dirofilariasis in stray dogs from central Taiwan had increased continuously from 56.93 per cent in 1994 to 60.55 per cent in 1999.

According to the study carried by Seo *et al.* (2001) on the occurrence of canine heartworm disease in three breeding farms in the vicinity of Seoul, South Korea, the infection rates were 50.3 per cent and 20.3 per cent for adult worm and microfilariae respectively.

Baneth *et al.* (2002) reported a case of *Dirofilaria repens* infection in an eleven year old female spayed Boxer admitted to veterinary clinic in the town of

Naharia in the north west of Israel. Microscopical examination of blood smear revealed microfilariae indicative of *Dirofilaria repens*.

An epidemiological study carried out by Kramer and Genchi (2002) showed that 16 per cent of all the tested cats were positive for anti-*Dirofilaria immitis* antibodies with values ranging from 9 to 27 per cent depending on location.

Seven hundred and eighty two blood samples collected from dogs in the city of Buenos Aires and its outskirts, Argentina, were analysed by Rosa *et al.* (2002) who recorded the prevalence rate of canine dirofilariosis as 17.7 and 23.5 per cent in northern and southern outskirts respectively.

Out of the total 263 dogs in Seoul, South Korea, examined by Yoon *et al.* (2002) 1.5 per cent was found to be positive for microfilaria. They also mentioned that females were significantly more infected than males.

In a study carried out by Wu and Fan (2003), in stray dogs of north Taiwan the overall prevalence of adult heartworms were found to be 57 per cent and that of microfilariae were 25 per cent.

2.2 IDENTIFICATION OF MICROFILARIAE

2.2.1 Morphology and Biometry

Taylor (1960) studied the morphology and biometry of *Dirofilaria immitis*, *D. repens* and *D. ethiops* Loa loa , *Wuchereria bacrofti* and *Brugia malayi* using phase contrast microscope.

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

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

2.3 ANTIGEN PREPARATION

Chandra *et al.* (1974) described the preparation of *Wuchereria bancrofti* larval antigen which was found to be highly specific and sensitive in diagnosing microfilaria positive cases.

In a comparative assessment Chandra *et al.* (1978) quoted the usefulness of *Wuchereria bancrofti* and *Brugia malayi* infective larval antigens and suggested that *B. malayi* larval antigens can be used in filarial skin test.

Scholtens and Patton (1983) suggested a method for the preparation of *Dirofilaria immitis* adult worm antigen.

Boreham (1984) explained the activation of some biological systems by extracts of adult worms and microfilariae of *Dirofilaria immitis* in vitro.

Detergent extraction of heartworm antigens for ELISA and Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was explained by Boto *et al.* (1984).

Dirofilaria immitis antigen can be prepared from adult female worms by a method including filtration (Gillis *et al.*, 1984).

Katiyar *et al.* (1985) reviewed the procedure for the preparation of *Brugia malayi* antigen and suggested that L_3 antigen is more specific than the adult whole worm antigen in the detection of filarial infection by filarial skin test.

Weil *et al.* (1985) proposed a method for the preparation of soluble *Dirofilaria immitis* adult worm antigens (DiA) extracted in 0.01 M Phosphate buffered saline, pH 7.2.

Chandra *et al.* (1986) described the concentration technique for microfilaria and used it for microfilariae antigen preparation.

Murthy *et al.* (1988) observed that lyophilised antigen from *Brugia malayi* larvae stored at 37° C retained its biological activity for the assessment period of 18 months as evinced by skin reaction.

Kaneko *et al.* (1990) analysed the excretory secretory products of adult *Dirofilaria immits* cultured *in vitro* by SDS-PAGE and immunoblotting.

Prieto *et al.* (1997) indicated the procedure for the preparation of somatic and excretory/secretory antigens from adult *Dirofilaria immitis* to understand the usefulness of ELISA in the early diagnosis of dirofilariosis.

Utility of adult antigens of *Dirofilaria immitis* for the early detection of dirofilariosis was studied by Prieto *et al.* (1999).

Crude somatic (S), Partial somatic and excretory/secretory (ES) antigens of adult heartworm were identified by Lee and Jee (2000).

Somatic antigens from third stage larvae of *Dirofilaria immitis* (SL_3) were used to detect IgG response against heartworm infection in eight experimentally infected cats (Prieto *et al.*, 2001).

Song *et al.* (2002) studied the immunological response of dogs experimentally infected with *Dirofilaria immitis* and also mentioned the preparation of crude extracts of *Dirofilaria immitis*.

2.4 DIAGNOSIS BY ENZYME LINKED IMMUNOSORBENT ASSAY

Grieve *et al.* (1981) conducted ELISA using a semi purified, adult *Dirofilaria immitis* derived antigen in experimentally infected dogs. They found that there were significant levels of antibody 16 weeks after inoculation, but no apparent correlation between antibody levels and either adult worm numbers or microfilaria counts.

Scholtens and Patton (1983) evaluated the usefulness of ELISA for occult dirofilariosis in a population of naturally exposed dogs. The titers of dogs with

evidence suggestive of occult dirofilariosis were significantly higher than the mean titers of those without evidence of filarial infection, with patent filarial infection or with a history of dirofilariosis.

Boto *et al.* (1984) mentioned the procedure of ELISA for dog anti-heartworm antibodies in their study about the antigens of *Dirofilaria immitis* which are immunogenic in the canine host. Significant antibody titers were first registered three months post infection, and then peaked at 1:30,000 at the end of the prepatent period (six months) in all of the dogs.

Gillis *et al.* (1984) described the criteria for interpreting and reporting the results of an occult heartworm ELISA to practitioners. They suggested that ELISA could be used to support a diagnosis of occult heartworm disease.

Enzyme Linked Immunosorbent Assay was compared with Knott's test result, record of exposure, clinical signs, laboratory results and radiographic changes and identified risk factors for canine heartworm infections (Glickman *et al.*, 1984)

Martin *et al.* (1985) assessed four commercially available ELISA kits for the diagnosis of *Dirofilaria immitis* infection in dogs and observed that the kits varied in sensitivity from 36 per cent to 86 per cent, in specificity from 44 per cent to 70 per cent and in accuracy from 53 per cent to 65 per cent. Kits were most specific while testing young dogs (<= three years)

Wilkins and Giurea (1985) reviewed the diagnostic tests for detection of heartworm infection and suggested that high titers (>1:400) indicated the presence of young or mature adult heartworms. They opined that animals with low titers (<1:400) should be re-evaluated clinically and retested after four weeks.

Parasite antigen detection with monoclonal antibody based ELISA appeared to be superior to previously discussed diagnostic methods for canine

dirofilariosis in terms of sensitivity, specificity and relation to infection intensity (Weil *et al.*, 1985)

Grieve *et al.* (1986) compared the results of direct parasitological examination with ELISA and Indirect fluorescent antibody test (IFAT) results within each age category.

In an enzyme-linked immunosorbent assay conducted by Matsumura *et al.* (1986) for detecting antibodies against *Dirofilaria immitis* in dogs, lack of significant difference between the O.D. value of microfilaraemic and amicrofilaraemic infections was observed. They suggested that the use of immunosorbent chromatography for canine dirofilariasis might be useful for purifying antigens and eliminating cross-reactions against other parasitic infections, when immunological methods are used for serodiagnosis.

Valladares and Lopez (1987) developed ELISA and Indirect fluorescent antibody test (IFAT) for the diagnosis of canine dirofilariosis. In IFAT, out of 120 sera, 34 to 36 microfilaraemic sera were positive and in the ELISA out of 59 sera 20 to 23 microfilaraemic sera were positive. There were some cross reactions with *Toxocara canis*

Tanaka and Atwell (1991) found that in *Dirofilaria immitis* infected dogs, the mean ELISA titers increased directly with the degree of infection.

Camargo *et al.* (1992) compared dot-ELISA with ELISA for the serological diagnosis of toxocariasis and found that dot-ELISA was more specific.

Larsson *et al.* (1992) emphasised the importance of immunodiagnostic tests such as ELISA in the diagnosis of dirofilariosis.

Ata *et al.* (1993) assessed circulating anti-filarial IgM and IgG antibodies by ELISA in 184 serum specimens from *Wuchereria bancrofti* infected patients.

The results denoted the importance of circulating anti-filarial IgM antibody level in the diagnosis of acute filarial infection.

In a comparative study Liu *et al.* (1995) suggested that there were no statistically significant difference between ELISA and dot-ELISA and between dot-ELISA and dot-Immunogold silver staining (dot-IGSS) respectively.

Prieto *et al.* (1997) evaluated the reliability of two ELISA based antibody tests coupled with somatic and secretory/excretory antigens. It was concluded that ELISA with secretory/excretory antigen complex of adult *Dirofilaria immitis* appeared to be highly specific and a suitable tool for the diagnosis of feline heart worm diseases.

Marks and Bloomfield (1998) detected canine heartworm infection by antigen ELISA in 6.4 per cent of red foxes captured in Australia.

Anti-filarial IgG4 – ELISA was seen to be a very useful serological tool for prevalence surveys and diagnosis of *Brugia malayi* (Rahmah *et al.*, 1998)

In a study conducted by Prieto *et al.* (1999) it was emphasised that serological testing with ELISA was useful in the early detection of *Dirofilaria immitis* infection and together with Western blot analysis, assisted in the evaluation of the response to chemoprophylactic treatment of feline dirofilariosis.

El-serougi *et al.* (2000) evaluated IgG_4 response against antigen extracts from *Wuchereria bancrofti* microfilariae and *Dirofilaria immitis* adult worms.

Lee and Jee (2000) used ELISA, silver staining and immunoblot to examine the antigenicity of crude somatic (S), partial somatic and excretory/secretory (E/S) antigens of adult heartworm.

Prieto *et al.* (2002) identified the useful antigenic fractions containing 20 to 30 kDa polypeptide from adult *Dirofilaria immitis* somatic antigen using indirect ELISA for the early detection of feline dirofilariosis.

Song et *al.* (2002) investigated the cross reactivity between *Dirofilaria immitis* and three intestinal nematodes of dogs and explained the partial cross reaction between the sera of *D. immitis* infected dogs and the antigen of *Toxocara canis*.

Song *et al.* (2002) analysed serum samples from dogs experimentally inoculated with *Dirofilaria immitis* by ELISA and Immunoblotting methods and found that antibody titers by ELISA reached high levels at the fourth moulting stage after inoculation of L_3, and higher molecular weight regions are mainly detected by immunoblotting in young animals.

2.5 DIAGNOSIS BY DOT ENZYME LINKED IMUNOSORBENT ASSAY

Matsumura *et al.* (1988) described an enzyme (Horse Radish Peroxidase) linked Immunosorbent assay using nitrocellulose (NC) paper (dot-ELISA) for the detection antibodies against *Dirofilaria immitis* in dogs. They suggested that the dot-ELISA technique may be useful for veterinarians and also a means for field surveying human filariasis.

Pappas (1988) suggested that dot-ELISA is a highly versatile solid phase immunoassay for antibody or antigen detection and can be used in the detection of human and veterinary protozoan and metazoan parasitic diseases.

Tandon *et al.* (1988) developed a dot-ELISA using nitrocellulose membrane as solid support for binding *Brugia malayi* antigen and antihuman IgG- Horse Radish Peroxidase conjugate.

Zheng *et al.* (1990a) compared dot-ELISA with standard sandwich ELISA in detecting parasite antigen in sera from patients with bancroftian filariasis. They concluded that dot- ELISA can be performed in virtually all filariasis endemic areas as it did not require radioactive or sophisticated equipments.

In a comparative study it was found that specificity of dot-ELISA and sandwich ELISA was greater than 95 per cent and in terms of sensitivity, dot-ELISA was better than sandwich ELISA (Zheng *et al.* 1990b).

Montenegro *et al.* (1991) proposed Dacron (polyethyleneterathalate) as a matrix for dot-ELISA procedure and as an alternative to nitrocellulose.

Camargo *et al.* (1992) standardised dot-ELISA using somatic and excretory/secretory antigens of *Toxocara canis* and compared the assay with ELISA and the two tests were found to have similar sensitivity, but dot-ELISA was found to be more specific in the presence of the two antigens studied.

Liu *et al.* (1994) employed dot-IGSS and dot-ELISA using soluble antigens of *Brugia malayi* to detect anti-*Wucheraria bancrofti* antibodies.

Liu *et al.* (1995) emphasised that there were no statistically significant differences between ELISA and dot-ELISA and between dot-ELISA and dot-IGSS.

Ganesh *et al.* (2001) developed a simple inexpensive dot blot Assay using a 66 kDa *Brugia malayi* microfilarial protein antigen for the diagnosis of bancroftian fialarial infection in an endemic area.

Materials and Methods

3. MATERIALS AND METHODS

3.1 PREVALENCE OF INFECTION

Dogs brought to the University Veterinary Hospital, Kokkalai for treatment and vaccinations and blood samples of dogs obtained from the University Veterinary Hospital, Mannuthy during a period of one year from June 2002 to May 2003 were examined for microfilarial infection. Details regarding sex, age and seasonal variation of microfilarial infection were also recorded. The blood samples were screened by wet film examination for the detection of microfilariae in the blood.

3.1.1 Wet Film Examination

A drop of fresh blood from the ear tip was collected on to a clean slide and covered with a cover slip. The film was examined immediately under low power (x 10) objective of the microscope for the presence of microfilariae.

3.2 COLLECTION OF POSITIVE BLOOD SAMPLES

Blood samples that were found positive for microfilariae by wet film examination were collected from the cephalic vein of the dog, allowed to clot and the serum separated. A drop of the serum was taken on to a clean glass slide and examined under low power objective of the microscope for the presence of the microfilariae. Motile microfilaria was found in greater concentration in serum and was generally easier to observe because of the absence of the erythrocytes (Kelly, 1973). The collected serum samples containing microfilariae were then kept at 4°C for the preparation of antigen. Fifty microfilaria positive serum samples were kept at -20°C for the serological tests. Serum was also separated from the blood samples that were collected from thirty six dogs affected with other helminths, but negative for microfilaria and also from thirty two total helminth free dogs and kept at -20°C for the serological tests.

3.3 IDENTIFICATION 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 Giemsa stain to study the morphology and biometry.

3.4 PREPARATION OF MICROFILARIAL ANTIGEN

Antigen was prepared using microfilariae present in positive serum samples. The method used for the preparation of antigen by Matsumura *et al.* (1986) using adult worm was employed with minor modifications.

Microfilariae positive serum samples were stored at 4° C until the preparation of antigen. At the time of antigen preparation, the samples were washed in phosphate buffered saline (PBS) pH 7.4, by adding PBS up to three fourth of the centrifuge tube and inverting it after covering with an aluminum foil and the process was repeated three times. Further, the mixed serum samples were centrifuged for five minutes, the supernatant was discarded and the sediment containing microfilariae was again mixed with PBS (pH 7.4) and then centrifuged at 3000 rpm for five minutes. The washing procedure was repeated six times. After completing the washing procedure the sediment containing microfilariae was collected. Several samples prepared in this manner were pooled together.

The pooled suspension was sonicated in a sonicator (Tomi Seiko Co., Tokyo, Japan) at 10 kHz for five minutes. It was done by placing the beaker containing pooled samples in a container with ice, to reduce the heat generated during the sonication procedure.

The suspension obtained after sonication was then centrifuged at 15000 g for 30 minutes at 4° C in a Beckman cooling centrifuge. The supernatant containing microfilarial antigen was collected after centrifugation. It was then stored in separate aliquots at 4° C until use.

The protein content of the microfilarial antigen was determined by Biuret method using Photometer 5010.

3.5 IMMUNODIAGNOSTIC TESTS

The diagnosis of canine dirofiiariosis in dogs was carried out by Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) and dot-Enzyme Linked Immunosorbent Assay (dot-ELISA) using microfilarial antigen and serum antibodies.

3.5.1 Indirect Plate Enzyme Linked Imunosorbent Assay Using Microfilarial Antigen

3.5.1.1 Buffer and Reagents

Borosil brand of glass and Tarson brand of plastic wares were used in the study. Only Analar grade chemicals were used.

The materials were processed using standard methods and sterilised either in hot air oven or in autoclave depending upon the material to be sterilised.

3.5.1.1a. Phosphate buffered saline (PBS) 10x concentrated solution

Sodium chloride	80 g
Potassium chloride	2 g
Disodium Hydrogen phosphate	15 g
Potassium dihydrogen phosphate	2 g
Distilled water	1000 ml

pH was adjusted to 7.4 by 1N NaOH and sterilised by autoclaving at 121° C for 15 minutes at 15 lbs (1.02 kg/cm) pressure.

3.5.1.1f. Citrate buffer 0.01 M, pH 5.0

0.2 M Sodium phosphate dibasic ($Na_2HPO_4.2H_2O$)	25.7 ml
0.1 M Citric acid	24.3 ml
Distilled water	50.0 ml

Adjusted pH to 5.0 when found necessary and stored at 4° C.

3.5.1.1g. Substrate

O-phenylene diamine dihydrochloride (OPD) obtained from Sigma-Aldrich

3.5.1.1h. Substrate solution

OPD (3.5.1.1g)	4 mg
Citrate buffer (3.5.1.1f)	10 ml

Just prior to use, five µl of 30 per cent hydrogen peroxide was added to the freshly prepared substrate solution.

3.5.1.1i. Stopping solution (1.25 M sulphuric acid)

Concentrated Sulphuric acid	34.7 ml
Distilled water to make	500 ml

3.5.1.2. Conjugate

Antiimmunoglobulin G Horse Radish peroxidase (anti IgG-HRP) conjugate against dog was obtained from Genei, Bangalore.

3.5.1.3. Standardisation

Optimum concentration of the prepared microfilarial antigen, test serum samples and the anti-dog IgG-HRP conjugate (Genei Pvt. Ltd.) was standardised by the checker board titration. One of the serum samples which was found positive on wet film examination and gave highest titre in ELISA was taken as positive control. One of the blood samples which was found negative by wet film examination and gave lowest titre was taken as negative control. Optimum concentration of microfilarial antigen test serum and anti dog IgG-HRP conjugate was found to be 1:100, 1:100 and 1:4000 respectively.

3.5.1.4 Test proper

An indirect plate enzyme linked immunosorbent assay was performed essentially as described by Song *et al.* (2002) with minor modifications.

All the wells of the ELISA plate (flat bottomed ELISA plate with 96 wells-Tarsons Pvt. Ltd.) except two were coated with 50 µl each of 1:100 dilution of antigen in carbonate-bicarbonate buffer and incubated overnight at 4°C. The plate were then washed thrice with PBST (3.5.1.1c) and tapped dry onto a lint free absorbent towel. Then the uncoated sites were blocked with 200 µl of two per cent BSA (3.5.1.1.d) and incubated at 37°C for one hour. After incubation, the BSA solution was drained off and the plate tapped dry. Hundred micro liters of test serum samples diluted to 1:100 in BSA-PBS (3.5.1.1d) were added to the wells in duplicate. Positive and negative controls and a conjugate control without any serum were also kept and the plates were incubated at 37°C for one hour.

It was then washed and dried as before and each well was charged with 100 µl of 1:4000 antidog-IgG HRP conjugate in BSA-PBS and was incubated at 37°C one hour. The wells were washed and dried as mentioned earlier.

Hundred micro liters each of freshly prepared chromogen/substrate solution (3.5.1.1h) was charged into all the wells including control wells. The plates were incubated in the dark at 37°C for 30 minutes.

Reaction was stopped by adding 100 µl of 1.25 M sulphuric acid (3.5.1.1i) to each well.

The OD values were read at 492 nm against in software based Multiscan Ascent ELISA reader.

Average of the OD values of three samples which gave lowest titer value in the test was calculated and twice that OD value was taken as cut off value.

3.5.2 Dot Enzyme Linked Imunosorbent Assay (dot-ELISA)

3.5.2.1 Nitrocellulose Membrane (NCM)

Probind 45 (Amersham Pharmacea) brand of Nitrocellulose membrane having an average pore diameter of 0.45 µm was used.

3.5.2.2 PBST- One per cent

Tween-20	1 ml
PBS (3.5.1.1b)	100 ml

pH was adjusted to 7.4 using 1N HCl.

3.5.2.3 Bovine serum Albumin (BSA) – 3 per cent (Blocking buffer)

BSA	3 g
PBS (3.5.1.1b)	100 ml

A fresh solution was prepared just before use.

3.5.2.4 Chromogen/substrate

Diaminobenzidine (DAB) obtained from Sigma-Aldrich.

3.5.2.5 Chromogen/substrate solution

DAB (3.5.2.4)	5 mg
PBS (3.5.1.1b)	10 ml

The solution was made fresh and 10 µl of 30 per cent hydrogen peroxide was added just before use.

3.5.2.6. Test proper

The plastic template with eight strip-like projections, each with five millimeter width and separated from its adjacent one by a distance of four millimeters were prepared. Nitrocellulose membrane pieces (5 mm x 5mm) were attached to the tip of each projection with adhesive (Fevibond, Pidilite industries Ltd.). Two micro liters of neat/undiluted antigen was deposited on to the nitrocellulose membrane and allowed to dry at room temperature. The unbound sites were blocked with three per cent solution of BSA (3.5.2.3) for one hour. The membrane was rinsed three times in PBST (3.5.2.2), and then incubated at 37°C for one hour in 1:40 positive/negative/test sera diluted in BSA-PBS. Positive and negative controls were the same as used in indirect enzyme linked immunosorbent assay. The membrane was again rinsed as before and incubated in 1:500 antidog-IgG HRP conjugate diluted in BSA-PBS at 37°C for 30 minutes. It was then rinsed and immersed in chromogen/substrate solution (3.5.2.5) for two to three minutes.

The reaction was stopped by rinsing the membrane in PBS. Dried the membrane and noted the colour development.

3.5.2 Statistical analysis

Statistical analysis was done to determine whether any significant difference occurred between the tests in the present study. Z-test was done as described by Rangaswamy (1995).

Results

4. RESULTS

4.1 PREVALENCE OF INFECTION

Out of the total number of 2435 dogs screened from June 2002 to May 2003 at Kerala Agricultural University Hospitals, Kokkalai and Mannuthy for microfilariosis, 172 were found positive giving a prevalence rate of 7.06 per cent. Month wise, the maximum prevalence was observed in April 2002 (14.81 per cent) which was higher than the rate in January (7.46 per cent), March (8.20 per cent), May (11.98 per cent), June (9.43 per cent) and July (8.97 per cent). Comparatively lower prevalence rates were observed in August (4.79 per cent), September (6.47 per cent), October (3.64 per cent), November (4.12 per cent), December (5.33 per cent) and February (5.86 per cent). The month wise prevalence is presented in Table 1 and depicted graphically in Fig. 1.

Out of the 172 positive cases, 116 (67.40 per cent) were male dogs while 56 (32.60 per cent) were females. The prevalence of infection in dogs in the age group six months to six years was higher at 135 (78.50 per cent) than the dogs aged above six years which was 37 (21.50 per cent) (Table 2). In the present study, all the affected dogs were above six months. The comparison of male and female dogs up to six years of age and above is depicted graphically in Fig. 2.

4.1.1 Wet Film Examination

Wet film examination was found to be a convenient method for screening, which enabled better assessment of the motility of the larvae. The microfilariae showed two types of motility viz. wriggling and progressive forward movement.

Table 1. Month-wise prevalence of microfilariosis

Month	No. of dogs examined	No of positive dogs	Prevalence
June 2002	159	15	9.43
July 2002	145	13	8.97
August 2002	146	7	4.79
September 2002	232	15	6.47
October 2002	302	11	3.64
November 2002	291	12	4.12
December 2002	225	12	5.33
January 2003	201	15	7.46
February 2003	222	13	5.86
March 2003	183	15	8.20
April 2003	162	24	14.81
May 2003	167	20	11.98
Total	**2,435**	**172**	**7.06 (Average)**

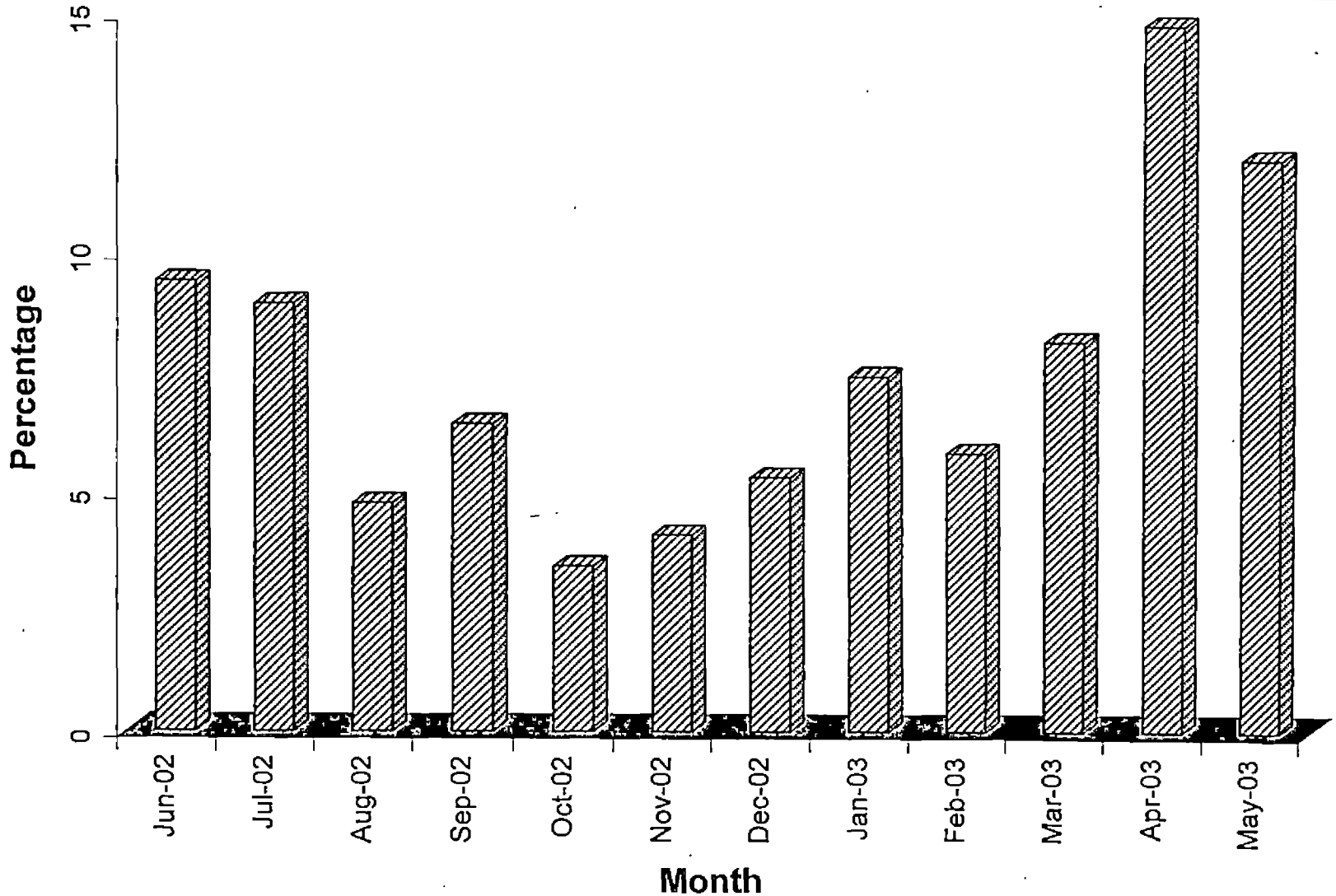

Fig.1 Relative prevalence rate of microfilariosis from June 2002 to May 2003

Table 2. Sex-wise prevalence of microfilariosis in dogs from six month to six years and above six years

Age	No. of males	Per cent	No. of females	Per cent	Total No.	Per cent
6 months-6 years	92	68.10	43	31.90	135	78.5
Above 6 years	24	64.90	13	35.10	37	21.50
Total	**116**	**67.40**	**56**	**32.60**	**172**	**100.00**

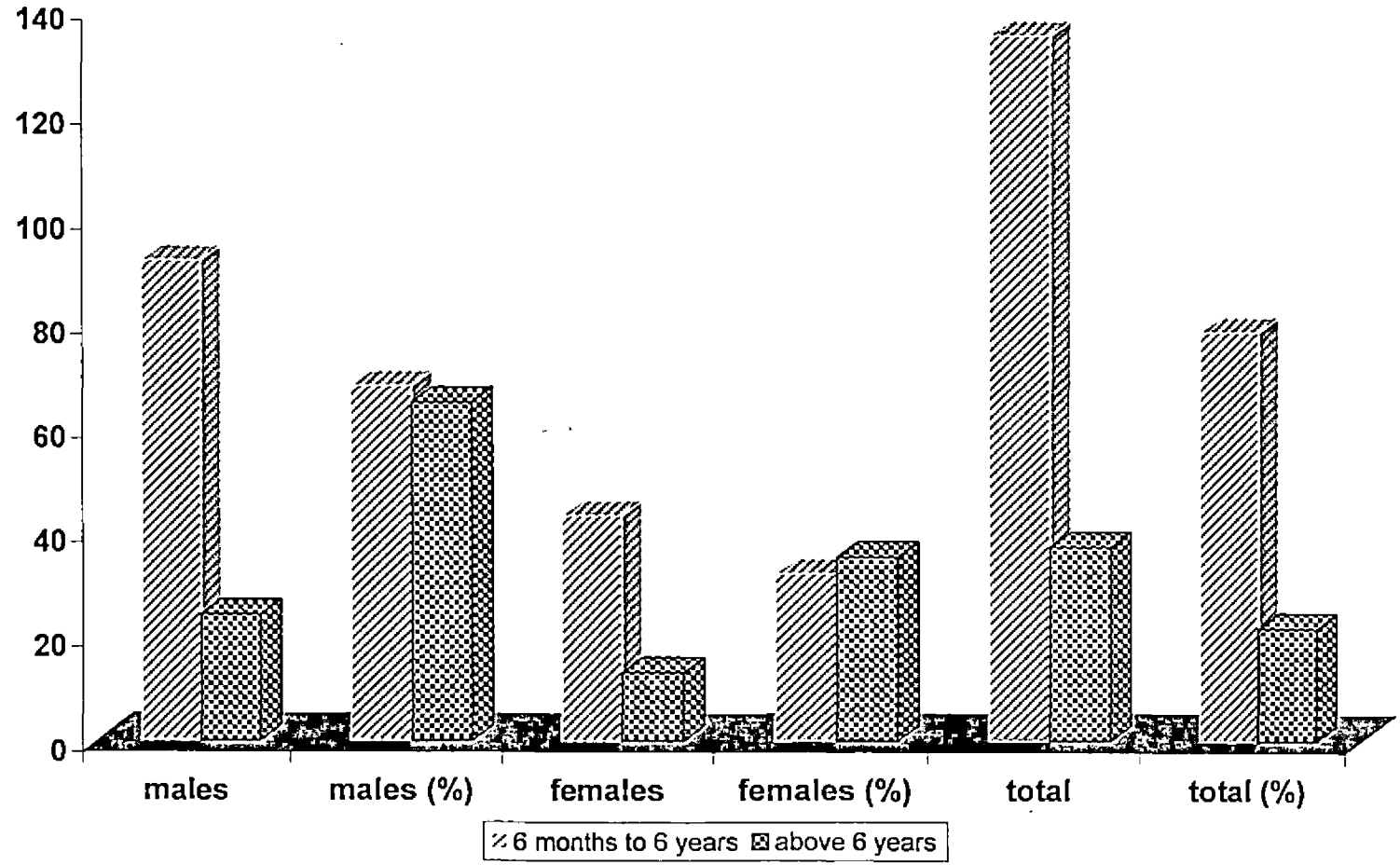

Fig.2 Sex wise and Age wise prevalence of Microfilariosis

4.2 COLLECTION OF POSITIVE BLOOD SAMPLES

Serum was separated from the blood samples collected from 172 microfilariae infected dogs. Motile microfilariae were observed when a drop of serum was examined under the low power objective of the microscope.

Microfilariae in the stored serum were found to be motile up to two weeks, thereafter the motility reduced greatly.

4.3 IDENTIFICATION OF MICROFILARIAE

4.3.1 Morphology and Biometry

Morphological studies using Giemsa staining technique 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 tapering tail. The cuticle appeared to be striated in higher magnification. 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 appreciated well. The G_1 nucleus was oval and large and occupied nearly the whole width of the microfilaria. G_2 and G_3 cells lay close together and were equidistant from G_1 and G_4 cells. The inner body and associated cells or the central body zone were found anterior to the G_1 cell. The tail was long and tapering, leaving a clear space at the end. The 'nuclear column' cells were not seen to extend to the tip of the tail. The camera lucida drawing of microfilaria of *Dirofilaria repens* is illustrated in Plate 1. The staining pattern of microfilaria by the Giemsa staining is demonstrated in Plate 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 3. The findings confirmed that the microfilariae are those of

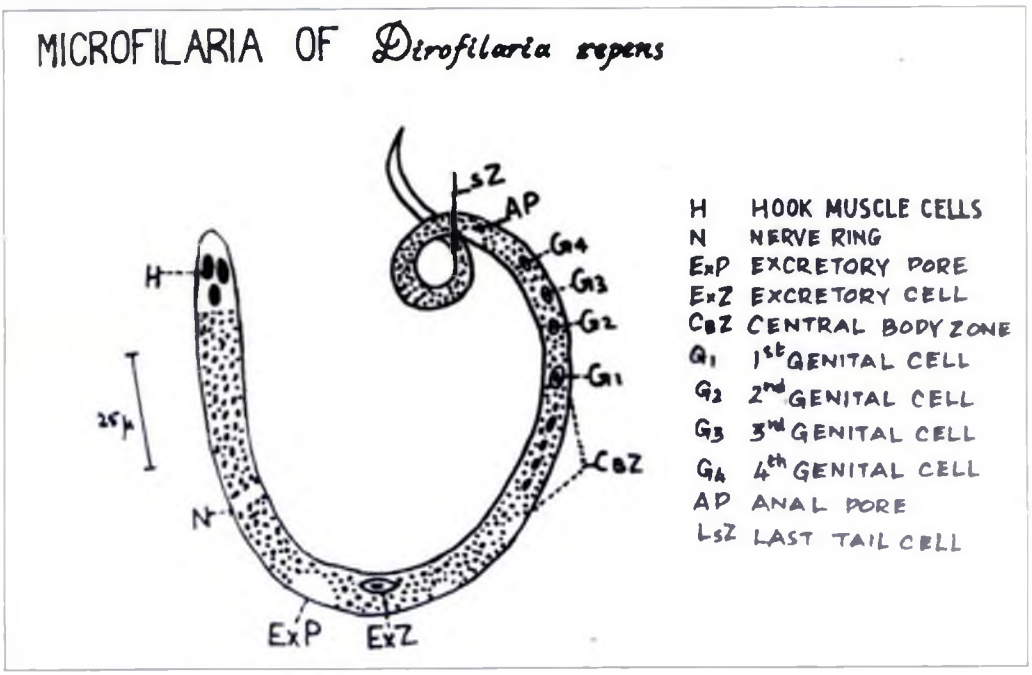

Plate 1. Microfilaria of *Dirofilaria repens* - Camera lucida drawing

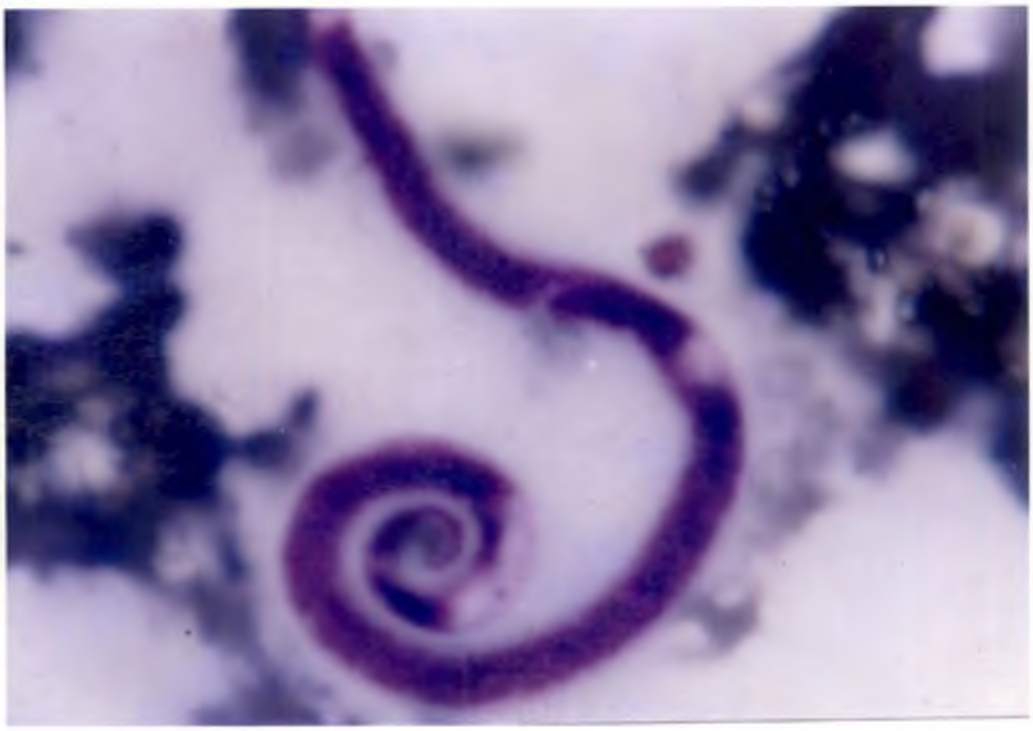

Plate 2. Microfilaria of *D.repens* - Giemsa staining (x1200)

Table 3. Morphology and biometry of microfilaria of dogs in Thrissur

Method	Percentage distance from the anterior end								Total Length (in μ)	Total width (in 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	Anal Pore (AP) Mean ± SE		
Smear stained with Giemsa	23.025 ± 0.198	32.108 ± 0.219	34.496 ± 0.412	61.123 ± 0.192	63.474 ± 0.212	65.011 ± 0.253	67.338 ± 0.302	72.522 ± 0.521	285.134 ± 8.62	6.1 ± 0.3

Dirofilaria repens. In the direct smear, the length and width of microfilariae were 285.134 ± 8.62 and 6.1 ± 0.3 μm respectively.

4.4 PREPARATION OF MICROFILARIAL ANTIGEN

Antigen was prepared using the microfilariae present in positive serum samples which were stored at 4° C.

The protein content of the microfilarial antigen was found to be 1 mg/ml.

4.5 IMMUNODIAGNOSTIC TESTS

4.5.1 Indirect Plate Enzyme Linked Immunosorbent Assay (I-ELISA) Using Microfilarial Antigen

An I-ELISA was performed to detect serum antibodies in 50 known microfilariae infected dogs, 36 dogs positive for other helminths but negative for microfilariae and also in 32 totally helminth free dogs. In this study average of the optical density (OD) values of three samples from the three categories which gave the lowest titre in the test was calculated and twice that OD value was taken as cut off value .In the present study 0.896 was taken as cut off value. The sample was considered positive if their OD value was higher than the cut off point. Using this criterion, 49 (98 per cent) of the serum samples of known microfilaria positive dogs had OD values above 0.896 and were taken as positive for the presence of microfilaria antibodies. In case of serum samples from dogs positive for other helminths but negative for microfilaria 15 (41.67 per cent) samples were tested positive. Accordingly a total of 10 (31.25 pr cent) samples of helminth free dogs were detected as positive.

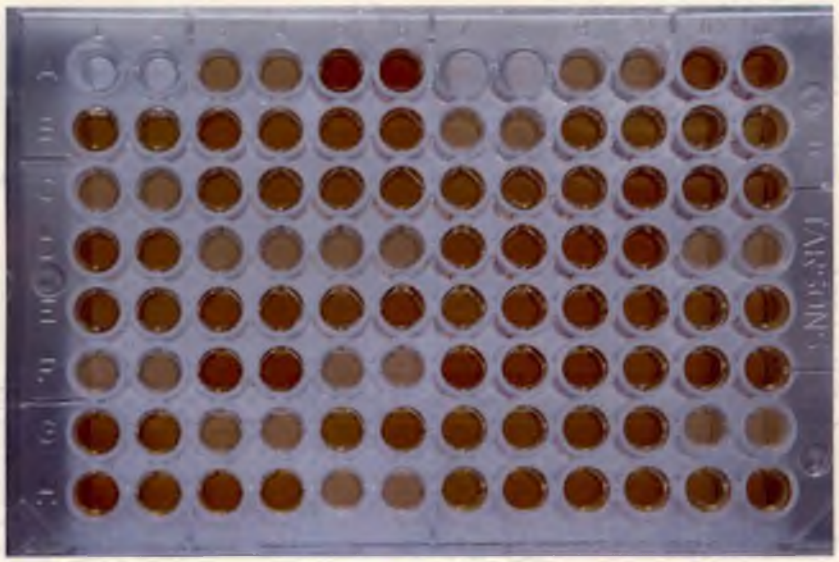

Plate 3. Indirect ELISA

Wells A1 and A2 - Blank
Wells A3 and A4 - Nagative Control
Wells A5 and A6 - Positive Control
Wells A7 and A8 - Conjugate Control
All other Wells - Test sera in duplicate

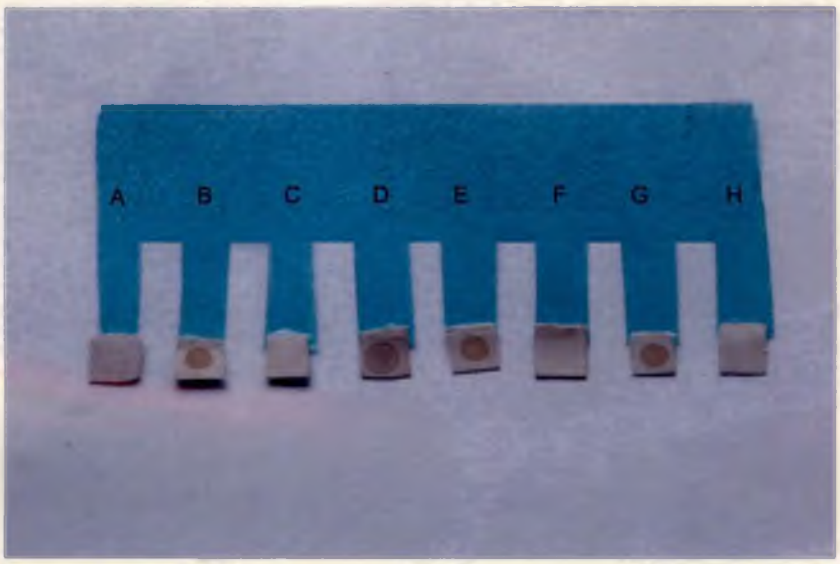

Plate 4. Dot - ELISA

A - Negative Control
B - Positive Control
C to H - Test Sera

4.5.2 Dot-ELISA

Out of the 50 samples tested, 47 (94 per cent) of the serum samples of known microfilaria positive dogs were found positive by dot-ELISA as evinced by the appearance of a brown dot at the site antigen deposition. Similarly, in the case of 36 serum samples from dogs positive for other helminths but negative for microfilariae 19 (52.77 per cent) samples were tested positive. In 32 totally helminth free dogs, 12 (37.5 per cent) serum samples were tested as positive

4.5.3 Comparison of Serological Tests

In the case of known microfilaria positive serum samples 49 (98 per cent) and 47 (94 per cent) samples out of 50 were found to be positive by I-ELISA and dot-ELISA respectively. On comparison of the tests using the z-test, it was found that there was no significant difference between I-ELISA and dot-ELISA in detecting serum antibodies for microfilariae (z value 1.025 at 5 per cent significance). Among the 36 samples tested, 15 (41.67 per cent) and 19 (52.77 per cent) samples of dogs with other helminth infection but free of microfilaria were found to be positive for serum antibodies for microfilariae by I-ELISA and dot-ELISA respectively. There was no significant difference between I-ELISA and dot-ELISA (z value 0.95) in detection of the samples.

Out of the 32 samples which were collected from the totally helminth free dogs 10 (31.25 per cent) and 12 (37.5 per cent) samples were found to be positive for serum antibodies for microfilariae by I-ELISA and dot-ELISA respectively. Comparison of the tests revealed that there was significant difference between I-ELISA and dot-ELISA (z value 7.3029) in detecting serum antibodies (Table 4).

Table 4. Comparison of the serological tests

Category	Number of animals tested	Number found positive	
		I-ELISA	Dot-ELISA
Microfilaria positive dogs	50	49 (98%)	47 (94%)
Dogs with other helminth but negative for microfilaria	36	15 (41.67%)	19 (52.77%)
Totally helminth free dogs	32	10 (31.25%)	12 (37.5%)

Discussion

5. DISCUSSION

5.1 PREVALENCE OF INFECTION

During the present study, out of 2435 dogs screened for microfilaria (from those brought to Kokkalai and Mannuthy University Veterinary hospitals) in Thrissur, 172 (7.06 per cent) were found to be positive for the infection. The infection was encountered throughout the year. The prevalence was higher during the months of January, March, May, June and July with the highest peak in April. This revealed greater prevalence of infection in summer. Higher summer prevalence could be due to seasonal periodicity in the occurrence of circulating microfilariae during summer which has been attributed to the effect of high environmental temperature. In summer large populations of mosquitoes could emerge with consequent immediate transmission of a large number of microfilariae to dogs in the early post monsoon period and the concomitant attainment of adulthood by a large number of filarid worms by the end of winter, resulting in the copious shedding of the larvae in summer. This is in agreement with the six to nine month long prepatent period found for *Dirofilaria repens* by Webber and Hawking (1955) and the resultant higher prevalence of microfilariosis during summer.

A higher prevalence of *Dirofilaria immitis* and its high incidence in the coastal areas of New South Wales was reported by Martin and Collins (1985) and they suggested that it might be due to the water courses and poorly drained areas which provide breeding grounds for mosquitoes. This supports the higher occurrence of microfilaria noticed in the present study conducted in Thrissur, the area which is proximal to the coastal belt and contain marshy water logged places. Hirth *et al.* (1966) also reported the higher percentage of heartworm infection in sea shore areas.

The present study has also revealed that male dogs were more affected than females which agree with the findings of Wallenstein and Tibola (1960),

Thrasher *et al.* (1965) Hirth *et al.* (1966) and Selby *et al.* (1980). But Glickman *et al.* (1984) reported that there was no such increased risk in males when age and outdoor activity were controlled.

Majority of the infected dogs (78.5 per cent) in the present study were below six years of age. This finding tallies with that of Hirth *et al.* (1966), Selby *et al.* (1980) and Glickman *et al.* (1984) who reported that age was a significant factor for heartworm infection, with highest risk in four to seven years of age and a decrease in risk of infection beyond eight or nine years of age. Several mechanisms may contribute to produce these patterns as opined by Selby *et al.* 1980, which are the following: death of the infected dogs, death of the worms, effective treatment of the infected animals, better medical care including vaccination and heartworm cheamoprophylaxis, diminished immune capability and heartworm induced immune suppression.

The finding in the present study that the dogs below six months were negative for microfilariosis is ascribable to the fact that the prepatent period of *Dirofilaria repens* is six to nine months (27 to 34 weeks) as reported by Webber and Hawking (1955).

5.2 IDENTIFICATION OF MICROFILARIA

5.2.1 Morphology and Biometry

In the morphological and biometric studies it was found that microfilarial length and width was 285 $\pm$ 8.6 and 6 $\pm$ 0.3 μm respectively when stained with Giemsa staining technique. This was in accordance with Taylor (1960) and Soulsby (1982).

The fixed point study proved that the microfilaria belong to *Dirofilaria repens* which is in commensurate with the findings of Taylor (1960) and Soulsby (1982).

5.3 INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY (I-ELISA) USING MICROFILARIAL ANTIGEN

Dirofilaria repens is an important canine pathogen and there is well recognized need for improved diagnostic technique for canine dirofilariosis. Only a small proportion of the infection can be detected clinically and the parasitological diagnosis is inconclusive. Recently the ELISA has received considerable attention with respect to its possible use for the serodiagnosis of various parasitic infections. In canine dirofilariosis an ELISA technique was used to quantitate antibody levels in experimentally infected dogs by Grieve *et al.* (1981).

In the present study an indirect plate ELISA (I-ELISA) was performed to detect the serum antibodies in known infected dogs, in dogs positive for other helminths but negative for microfilariae and also in totally helminth free dogs. In the case of 50 serum samples of known microfilaria infected dogs 49 (98 per cent) samples were found positive. The corresponding figures in 36 dogs with other helminths and in 32 totally helminth free dogs were 15 (41.67 per cent) and 10 (31.25 per cent) respectively. These results indicate nonspecific reaction as positive responses were obtained in amicrofilaraemic dogs also.

According to Gillis *et al.* (1984) the ability to detect microfilarial infections serologically depends on a combination of factors including choice of antigen and test system, stage of infection, the concentration, affinity and avidity of specific antibody and the history of the dog. In the present investigation microfilarial antigen without purification was utilised to detect serum antibodies by ELISA which might be one of the important reasons for the nonspecific reactions. This is in agreement with Prieto *et al.* (2002) who indicated that the purification of specific markers was a critical step in the refinement of serological diagnostic tests, applicable in field conditions, since it could allow the elimination or reduction of non-specific reactivity which the complex antigens produced.

Many of the scientists like Grieve *et al.* (1981) observed the cross reaction of *Dirofilaria immitis* with *Toxocara canis* and indicated that antigen refinement will be critical because of the sensitivity of ELISA and cross reactivity typical of filarids. In this regard Matsumura *et al.* (1986) utilised purified antigen in ELISA for detecting antibodies against *Dirofilaria immitis* in dogs and based on the results he suggested that the use of immunoadsorbent chromatography for canine dirifilariosis is especially useful for purifying antigens and eliminating cross reactions against other parasitic infections, when immunological methods are used for serodiagnosis. All these finding indicate that lack of purification might be one of the important reasons for the nonspecific reactions observed in the present study.

Positive reactions obtained in dogs with other helminths but without microfilariae might be due to cross reaction. This finding tallies with that of Matsumura *et al.* (1986) where he found a little cross reactivity in the sera of dogs infected with other parasites like *Toxocara canis* ,*Dipylidium caninum* ,*Ancylostoma caninum* and *Trichuris vulpis* .

This is also in accordance with the findings of Song *et al.* (2002a) who performed a study to investigate whether there was cross reactivity between *Dirofilaria immitis* and other intestinal nematodes of dogs and indicated that there was partial cross reaction between sera of *D. immitis* infected dogs and antigen of *Toxocara canis*.

Occult dirofilariosis might be another factor responsible for obtaining positive reactions in totally helminth free dogs. This concept was supported by the results obtained in the study carried out by Scholtens and Patton (1983). They also suggested that positive results might be also obtained in amicrofilaraemic dogs as the amount of circulating antibody detected in dogs previously infected with heartworms was higher than, but not significantly different from, that found in unexposed dogs, and lower than, but significantly different from, that found in dogs with microfilaraemia.

5.4 DOT –ENZYME LINKED IMMUNOSORBENT ASSAY (DOT- ELISA)

In canine dirofilariosis, the detection of specific antibodies has been used as a serodiagnostic tool. The dot-ELISA was found to be a simple quick and specific test with detection levels comparable to Indirect-ELISA and could be used in the field conditions.

In the present study, out of the 50 serum samples of known microfilaria positive dogs 47 (94 per cent) were found positive by dot-ELISA. Similarly the figure obtained in the case of 36 dogs with other helminths and 32 totally helminth free dogs were 19 (52.77 per cent) and 12 (37.5 per cent) respectively. These results reveal the presence of non-specific reactions. This is in contrary to the observations of Matsumura *et al.* (1988) who reported that dot-ELISA can be specific for the infection, since very low densitometric readings were observed in mouse antisera against different parasite antigens and OD values less than 0.10 were obtained for sera from non-infected and parasite infected dogs. However, they also mentioned the possibility that serum antibody levels in dogs that have been recently treated or have 'self-cured' might elicit positive response which could be one of the reasons for getting positive results in amicrofilaraemic dogs in the present study.

Microfilarial antigen without purification was used in the present investigation which could be another important reason for the positive response obtained in amicrofilaraemic dogs as opined by Matsumura *et al.* (1988).

Cross reaction with other helminths and occult form of the dirofilariosis might be another factor responsible for the positive reaction obtained in the amicrofilaraemic dogs (Scholtens and Patton, (1983) and Song *et al.* (2002a).

5.5 COMPARISON OF SEROLOGICAL TESTS

Number of samples detected as positive by Indirect ELISA and dot-ELISA were 49 (98 per cent) and 47 (94 per cent) in known microfilaria positive

dogs, 15 (41.67 per cent) and 19 (52.77 per cent) in dogs with other helminths and 10 (31.25 per cent) and 12 (37.5 per cent) in totally helminth free dogs respectively. Comparison of the tests revealed the fact that there was not much significant difference between Indirect ELISA and dot-ELISA in detecting serum antibodies in most of the cases of these three groups. This is in accordance with the findings of Liu *et al.* (1995) who emphasised that there was no statistically significant difference between ELISA and dot-ELISA and between ELISA and dot-IGSS respectively. But this is in contrary to the observations of Matsumura *et al.*(1988) and Zheng *et al.* (1990b) who suggested dot-ELISA as a better technique.

According to many scientists purification is an important factor which will affect the feasibility of the assays like ELISA. This could not be carried out in the present investigation due to lack of facilities. The results of the study reveal the need of purification of antigen to get improved specificity and sensitivity. But in most of the known microfilaria positive cases Indirect ELISA and dot-ELISA were also positive. This indicates that these assays can be used to detect dirofilariosis. By adopting methods to avoid non specific reaction these can be used to detect occult dirofilariosis also.

The results of the present study suggests that detection levels of dot-ELISA is comparable to that of Indirect-ELISA , at the same time dot-ELISA was found to be quick, simple and can be carried out at room temperature. Thus dot-ELISA is better test that can be effectively employed in the field conditions. However, further investigations are necessary to evaluate the method with amicrofialrial antibodies.

Summary

6. SUMMARY

A detailed study on the prevalence of canine dirofilariosis in dogs in Thrissur during the period from June 2002 to May 2003, feasibility and reliability of microfilarial antibody detection by ELISA and comparison of sensitivity of Indirect plate ELISA and dot-ELISA in the detection of dirofilariosis in dogs was carried out. Microfilaria of *Dirofilaria repens* was the only microfilaria encountered in the study. The diagnosis was based on morphological and biometrical studies.

Out of the total number of 2435 blood samples of dogs screened by wet film examination, 172 (7.06 per cent) animals were found positive for microfilariae. Generally the infection was found prevalent throughout the year and its incidence happened to be high during the summer months. The incidence was also more in male dogs below six years of age.

Microfilarial antigen was prepared from known microfilaria positive serum samples. The protein content of the antigen was found to be 1 mg/ml. It was stored at 4°C.

The immunodiagnosis of canine dirofilariosis was carried out by an Indirect-ELISA and dot-ELISA for detecting specific antibodies in serum samples.

Blood samples were collected from three groups of animals; 50 known microfilaria positive dogs, 36 dogs with other helminths but negative for microfilariae and 32 totally helminthfree dogs. The sera was separated from these samples and stored at – 20°C for the immunodiagnostic tests.

An Indirect ELISA and dot-ELISA was performed to detect antibodies in serum samples of three groups of animals. In the case of serum samples of known microfilaria positive dogs 49 (98 per cent) and 47 (94 per cent) samples were

found positive by indirect ELISA and dot-ELISA respectively. The corresponding figure in dogs with other helminths were 15 (41.67 per cent) and 19 (52.77 per cent) and that of totally helminth free dogs were 10 (31.25 per cent) and 12 (37.5 per cent) respectively. The results indicated that both Indirect ELISA and dot-ELISA are sensitive tests to detect canine dirofilariosis, but are less specific as the positive reactions were also obtained in few number of amicrofilaraemic cases.

Though sensitivity of dot-ELISA and Indirect ELISA are at par statistically, dot-ELISA is simple, quick and can be carried out at room temperature, hence may be considered more applicable in the field conditions. However questions regarding the specificity and applicability of the test in the field have to be ascertained.

References

REFERENCES

Aranda, C., Panyella, O., Eritja, R. and Castella, J. 1998. Canine Filariasis. Importance and transmission in the Baix Llobregat area, Barcelona (Spain). *Vet. Parasitol.* 77: 267-275

Ata, A.H.A., El-Bassiouny, A.E.I., Shaker, Z.A., Attia, M.M., El-Khashab, M.N., El-ammal, N.E., Mourad, A.A. and El-Ghoneimy, S. 1993. Antifilarial IgM versus IgG antibody determination in the diagnosis of *Wuchereria bancrofti* infection in Egyptians. *J. Egypt. Soc. Parasitol.* 23: 277-288

Baneth, G., Volansky, Z., Anug, Y., Favia, G., Bain, O., Goldstein, R.E. and Harrus, S. 2002. *Dirofilaria repens* infection in a dog: diagnosis and treatment with melarsomine and doramectin. *Vet. Parasitol.* 105: 173-178

Bokaie, S., Mobedi, I., Mobebuli, M., Hosseini, S.H. and Nad, A. 1998. The prevalence of dirofilariosis in dogs in Meshkinshahr area, North West Iran. *J. Fac. Vet. Med.* 53: 23-26

Boreham, P.F.L. 1984. Activation in vitro of some biological systems by extracts of adultworms and microfilariae of *Dirofilaria immitis*. *J. Helminthol.* 58: 207-212

Boto, M.O.W., Powers, K.G. and Levy, D.A. 1984. Antigens of *Dirofilaria immitis* which are immunogenic in the canine host: Detection by immuno-staining of protein blots with the antibodies of occult dogs. *J. Immunol.* 133: 975-980

Camargo, E.D., Nakamura, P.M., Vaz, A.J., Silva, M.V., Chieffi, P.P., Melo, E.O., Da-Silva, M.V. and De-Melo, E.O. 1992. Standardisation of dot-ELISA for the serological diagnosis of toxocariasis and comparison of the assay with ELISA. *Revista-do-Instituto-de-Medicina-Tropical-de-Sao-Paulo* 34: 55-60

Cancrini, G., Allende, E., Favia, G., Bornay, F., Anton, F. and Simon, F. 2000. Canine dirofiiariosis in two cities of south-eastern Spain. *Vet. Parasitol.* 92: 81-86

Chandra, R., Govila, P., Chandra, S., Katiyar, J.C. and Sen, A.B. 1974. *Wuchereria bancrofti* larval antigen in the diagnosis of human filariasis by skin test. *Indian J. med. Res.* 62: 1017-1024

Chandra, R., Katiyar, J.C., Murthy, P.K., George, P.A. and Sen, A.B. 1978. *Brugia malayi* infective larval whole worm antigen in the diagnosis of filariasis by skin test. *Indian J. med. Res.* 68: 61-66

Chandra, R., Katiyar, J.C., Murthy, P.K., Singh, A.K., Tyagi, K. and Sen, A.B. 1986. Comparison of filarial skin test and microfilaria demonstration in the diagnosis of lymphatic filariasis. *Indian J. med. Res.* 83: 171-174

Dhaliwal, P.S., Randhawa, S.S., Kalra, I.S. and Sodhi, S.P.S. 1987. Dirofilariasis in dog – a case report. *Indian J. Vet. Med.* 7: 38-39

El-Serougi, A.O., Ferky, A.A., Farray, A.M.K. and Saleh, W.A. 2000. Evaluation of the IgG_4 in Egyptian Bancroftian filariasis. *J. Egypt. Soc. Parasitol.* 30: 59-67

Fan, C.K., Su, K.E., Lin, Y.H., Liao, C.W., Du, W.Y. and Chiou, H.Y. 2001. Seroepidemiologic survey of *Dirofilaria immitis* infection among domestic dogs in Taipei city and mountain aboriginal districts in Taiwan (1998-1999). *Vet. Parasitol.* 102: 113-120

Ganesh, B.B., Kader, A.M., Agarwall, G.S., Reddy, M.V.R. and Harinath, B.C. 2001. A simple and inexpensive dot-blot assay, using a 66-KDa *Brugina malayi* microfilarial protein antigen, for diagnosis of bancroftian filarial infection in an endemic area. *Trans. Royal Soc. Trop. Med. and Hyg.* 95: 168-169

Gillis, J.M., Smith, R.D. and Todd, K.S. 1984. Diagnostic criteria for an enzyme-linked immunosorbent assay for occult heart worm disease : Standardisation of the test system in naturally exposed dogs. *Am. J. Vet. Res.* 45: 2289-2292

Glickman, L.T., Grieve, R.B., Breitschwerdt, E.B., Grieve, M.M., Patronek, G.J., Domanski, L.M., Root, C.R. and Malone, J.B. 1984. Serologic pattern of canine heart worm (*Dirofilaria immitis*) infection. *Am. J. Vet. Res.* 45: 1178-1183

Grieve, R.B., Glickman, L.T., Barker, A.K., Grieve, M.M., Thomas, C.B. and Patronek, G.J. 1986. Canine *Dirofilaria immitis* infection in a hyperzoonotic area: Examination of parasitologic findings at necropsy and by two serodiagnostic methods. *Am. J. Vet. Res.* 47: 329-331

Grieve, R.B., Johnson, M.M., Jacobson, R.H. and Cypess, R.H. 1981. Enzyme linked immunosrbent assay for measurement of antibody responses to *Dirofilaria immitis* in experimentally infected dogs. *Am. J. Vet. Res.* 42: 66-69

Hirth, R.S., Huizinga, H.W. and Nielsen, S.W. 1966. Dirofilariasis in Connecticut dogs. *J. Am. Vet. med. Ass.* 148: 1508-1516

Kamalu, B.P. 1991. Canine filariasis caused by *Dirofilaria repens* in south-eastern Nigeria. *Vet. Parasitol.* 40: 335-338

Kaneko, H., Hayasaki, M. and Ohishi, I. 1990. Antigenic identification of excretory-secretory products of adult *Dirofilaria immitis*. *Jap. J. Vet. Sci.* 52: 995-1000

Katiyar, J.C., Chandra, R., Murthy, P.K., Tyagi, K. and Sen, A.B. 1985. Specificity of *Brugia malayi* antigens in filarial skin test. *Indian J. med. Res.* 81: 465-470

Kelly, J.D. 1973. Detection and differentiation of microfilariae in canine blood. *Aust. Vet. J.* 49: 23-27

Knight, D.H. 1983. Heartworm Disease. *Text book of Veterinary Internal Medicine* (ed. Ettinger, S.J.) W.B. Saunders, Philadelphia, pp. 1097-1124

Kramer, L. and Genchi, C. 2002. Feline heartworm infection: serological survey of asymptomatic cats living in northern Italy. *Vet. Parasitol.* 104; 43-50

Lai, C.H., Ting, C.H., Tang, K.C. and Wang, J.S. 2001. Variation in the prevalence of dirofilariasis in stray dogs from central Taiwan. *J. Chin. Soc. Vet. Sci.* 27: 69-73

Larsson, M.H.M.A., Preterote, M. and Mirandola, R.M.S. 1992. Diagnosis of occult dirofilariasis by the ELISA test in dogs in the state of Sao Paulo. *Braz. J. Vet. Res. Anim. Sci.* 29: 93-96

Lee, C.S. and Jee, C.H. 2000. Studies on antigenicity and production of monoclonal antibody for diagnosis of canine heartworm *(Dirofilaria immitis)*. *Korean J. Vet. Res.* 40: 130-137

Lee, J.C., Lee, C.Y., Shin, S.S. and Lee, C.G. 1996. A survey of canine heartworm infections among shepherds in South Korea. *Korean J. Parasitol.* 34: 225-231

Liang, S.L., Huang, H.P., Yang, H.L. and Wong, S.S. 1996. Prevalence of canine dirofilariasis in pet dogs from the Taipei area. *J. Chin. Soc. Vet. Sci.* 22: 167-174

Liu, Y.S., Du, W.P., Chen, M. and Wu, Z.X. 1994. Comparative study of dot-immunogold silver staining and dot-ELISA for the detection of serum antibodies against *Wuchereria bancrofti*. *Southeastern Asian J. Trop. Med. Pub. Hlth.* 25: 724-727

Liu, Y.S., Du, W.P. and Wu, Z.X. 1995. Comparison of three immunological assays for detecting specific IgG against *Wuchereria bancrofti* in cases with bancroftian microfilaraemia. *End. Dis. Bull.* 10: 39-41

Marks, C.A. and Bloom Field, T.E. 1998. Canine heartworm *(Dirofilaria immitis)* detected in red foxes *(Vulpes vulpes)* in urban Melbourne. *Vet. Parasitol.* 78: 147-154

Martin, T.E. and Collins, G.H. 1985. Prevalence of *Dirofilaria immitis* and *Dipetalonema recondetum* in grey hounds. *Aust. Vet. J.* 62: 159-162

Martin, T.E., Collins, G.H., Griffin, D.L. and Pope, S.E. 1985. An evaluation of four commercially available ELISA kits for the diagnosis of *Dirofilaria immitis* infection in dogs. *Aust. Vet. J.* 62: 166-169

Matic, S.E. and Herrtage, M.E. 1987. Diagnosis and treatment of occult dirofilariasis in an imported dog. *J. small Anim. Pract.* 28: 183-196

Matsumura, K., Kazuta, Y., Endo, R., Tanaka, K. and Inoue, T. 1986. The Enzyme linked immunosorbent assay for detecting antibodies against *Dirofilaria immitis* in dogs. *Vet. Parasitol.* 21: 165-171

Matsumura, K., Kazuta, Y., Endo, R., Tanaka, K., Inoue, T. and Matsuda, H. 1988. A rapid microassay for dirofilarial antibodies using antigen-coated nitrocellulose paper. *Vet. Parasitol.* 27: 337-344

Montenegro, S.M.L., Almeida, A.M.P., Carvalho, A.B., Carvalho, L.B., De-Carvalho, A.B., De-Carvalho, L.B. and De-Almeida, A.M.P. 1991. The use of Dacron plates for dot-enzyme linked immunosorbent assay (dot-ELISA). *Memorias-do-Instituto-Oswaldo-Cruz*. 86: 461-465

Murthy, P.K., Tyagi, K., Singh, A.K., Chandra, R., Pandey, V.C. and Katiyar, J.C. 1988. Stability of filarial diagnostic antigen (*Brugia malayi*). *Indian J. med. Res.*88: 134-137

Pappas, M.G. 1988. Recent applications of dot-ELISA in immunoparasitology. *Vet. Parasitol.* 29: 105-129

Patton, S. and McCraken, M.D. 1991. Prevalence of *Dirofilaria immitis* in cats and dogs in eastern Tennessee. *J. Vet. Diag. Invest.* 3: 79-80

Prieto, G., Ceciliani, F., Venco, L., Morchon, R. and Simon, F. 2002. Feline dirofiiariosis: antibody response to antigenic fractions containing specific 20 to 30 KDa polypeptides from the adult *Dirofilaria immitis* somatic antigen. *Vet. Parasitol.* 103: 341-353

Prieto, G., McCall, J.W., Venco, L., Genchi, M., Simon, F. and Genchi, C. 2001. IgG response against infective larvae of *Dirofilaria immitis* in experimentally infected cats. *Vet. Res.* 32: 93-96

Prieto, G., Simon, F., Genchi, C., McCall, J.W. and Venco, L. 1999. Utility of adult antigens of *Dirofilaria immitis* for the early detection of dirofilariosis and for the evaluation of chemoprophylactic treatment in experimentally infected cats. *Vet. Parasitol.*86: 5-13

Prieto, C., Venco, L., Simon, F. and Genchi, C.1 1997. Feline heartworm (*Dirofilaria immitis*) infection: detection of specific IgG for the diagnosis of occult infections. *Vet. Parasitol.* 70: 209-217

Radhika, R. 1997. Prevalence, clinical pathology and treatment of microfilariasis in dogs in Thrissur. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p. 122

Rahmah, N., Khairul, A.K., Tengku, R.H., Zurainee, M.N., Noor-A-Shikin, A., Fadzillah, A., Maimunah, A. and Ashraful-Haq, J.1998. Use of antifilarial IgG4-ELISA to detect *Brugia malayi* infection in an endemic area of Malaysia. *Trop. Med. Intl. Health.* 3: 184-188

Rangaswamy, R. 1995. *Textbook of Agricultural statistics.* New Age International Publishers Limited, Wiley Eastern Limited, New Delhi, p. 495

Rawlings, C.A., Dawe, D.L., McCall, J.W., Keith, J.C. and Priestwood, A.K. 1982. Four types of occult *Dirofilaria immitis* infection in dogs. *J. Am. Vet. med. Ass.* 180: 1323-1326

Razmi, C. 1999. A study of the situation of different filarial infection in dogs of Mashhad. *J. Fac. Vet. Med.* 54: 5-7

Rojo-Vazquez, F.A., Valcarcel, F., Guerrero, J. and Gomez-Bautista, M. 1990. Prevalencia de la dirofilariosis canina en cuarto areas geograficans de Espana. *Med. Vet.* 7: 297-305

Rosa, A., Ribicich, M., Betti, A., Kistermann, J.F., Cardillo, N., Basso, N. and Hallu, R. 2002. Prevalence of canine dirofilariosis in the city of Buenos Aires and its outskirts (Argentina). *Vet. Parasitol.* 109: 3-4

Rossi, L., Fezia, G. and Carnevale, M. 1999. Occult heartworm infection in a hyperendemic area of northern Italy. *Veterinaria cremona.* 13: 53-61

Saseendranath, M.R., Varghese, C.G. and Jayakumar, K.M. 1986. Incidence of canine dirofilariasis in Trichur, Kerala. *Indian J. Vet. Med.* 6: 139

Scholtens, R.G. and Patton, S. 1983. Evaluation of an enzyme linked immunosorbent assay for occult dirofilariasis in a population of naturally exposed dogs. *Am. J. Vet. Res.* 44: 861-864

Selby, L.A., Corwin, R.M., hayes, H.M. 1980. Risk factors associated with canine heartworm infection. *J. Am. Vet. med. Ass.* 176: 33-35

Senthilvel, K. and Pillai, K.M. 1999. A case of subcutaneous dirofilariasis in a woman in Kerala. *Indian Vet. J.* 76: 263-264

Seo, Y.W., Shin, S.S. and Kim, J.T. 2001. Occurrence of canine heartworm disease in three breeding farms in the vicinity of Seoul, Korea. *Korean J. Vet. Res.* 41: 79-83

Song, K. H., Hayasaki, M., Cho, K. W., Lee, S. E. and Kim, D. H. 2002a. Cross-reactivity between sera from dogs experimentally infected with *Dirofilaria immitis* and crude extract of *Toxocara canis*. *Korean J. Parasitol.* 40:195-198

Song, K.H., Hayasaki, M., Choliq, C., Cho, K.W., Han, H.R., Jeong, B.H., Jeon, M.H., Park, B.K. and Kim, D.H. 2002b. Immunological responses of dogs experimentally infected with *Dirofilaria immitis*. *J. Vet. Sci.* 3:109-114

Soulsby, E.J.L. 1982. *Helminths, arthropods and protozoa of domesticated animals.* Seventh edition. The English language book society and Bailliere Tindall, London. p. 307-312

Tanaka, K. and Atwell, R.B. 1991. The humoral response in natural *Dirofilaria immitis* infections in dogs. *Aust. Vet. J.* 68: 396-399

Tandon, A., Murthy, P.K., Saxena, R.P., Sen, A.B. and Saxena, K.C. 1988. Dot-ELISA for diagnosis of lymphatic filariasis. *Indian J. med. Res.* 87: 429-433

Taylor, A.E.R. 1960. Studies on the microfilariae of *Loa loa, Wuchereria bancrofti, Brugia malayi, Dirofilaria immitis, D. repens* and *D. aethiops. J. Helminthol.* 34: 13-26

Thrasher, J.P., Ash, L.R. and Little, M.D. 1965. Filarial Infections of Dogs in New Orleans. *J. Am. Vet. med. Ass.* 143: 605-608

Valladares, B. and Lopez, R.R. 1987. Immunodiagnosis of canine dirofilariasis. *Revista-Iberica-de-Parasitologia-Vol. Extraordinario: Enero*: 225-228

Valsala, K.V. and Bhaskaran, R. 1974. Dirofilariasis in dogs. *Kerala J. Vet. Sci.* 5: 74-77

Wallenstein, W.L. and Tibola, B.J. 1960. Survey of canine filariasis in the Maryland area – Incidence of *Dirofilaria immitis* and Dipetalonema. *J. Am. Vet. med. Ass.* 137: 712-716

Wang, L.C. 1998. Canine filarial infections in north Taiwan. *Acta. Topica.* 68: 115-120

Webber, W.A.F. and Hawking, F. 1955. Experimental maintenance of *Dirofilaria repens* and *D. immitis* in dogs. *Exp. Parasit.* 4: 143-164

Weil, G.J., Malane, M.S., Powers, K.G. and Blair, L.D. 1985. Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis* infected dogs. *J. Immunol.* 134: 1185-1191

Wilkins, R.J. and Ciurea, D. 1985. Heartworm infection: reviewing the diagnostic tests. *Vet. Med.* 42-45

Wong, M.M., Suter, P.F., Rhode, E.A. and Guest, N.F. 1973. Dirofilariasis without circulating microfilariae: A protein in diagnosis. *J. Am. Vet. med. Ass.* 163: 135-139

Wu, C.C. and Fan, P.C. 2003. Prevalence of canine dirofilariosis in Taiwan. *J. Helminthol.* 77: 83-88

Yoon, H.Y., Yoon, C.S., Jeong, S.W., Kim, T.J., Park, S.Y., Chung, B.H., Choi, Y.M. and Lee, W.C. 2002. Prevalence and relative risk of canine dirofiiariosis among dogs in Seoul, South Korea. *Vet. Rec.* 151: 576-577

Zheng, H.J., Fuhrman, J.A., Xu, M., Cheng, M.F., Reddy, M.V.R. and Piessens, W.F. 1990a. Comparison of dot-ELISA with sandwitch ELISA in detecting circulating antigen in patients with bancroftian filariasis. *Chin. med. J.* 103: 709-712

Zheng, H.J., Tao, Z.H., Cheng, W.F. and Piessens, W.F. 1990b. Comparison of dot-ELISA with sandwitch-ELISA for the detection of circulating antigens in patients with bancroftian filariasis. *Am. J. Trop. Med. Hyg.* 42: 546-549

IMMUNODIAGNOSIS OF CANINE DIROFILARIOSIS USING MICROFILARIAL ANTIGEN

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Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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2003

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ABSTRACT

A detailed study was conducted on the prevalence on canine dirofilariosis in Thrissur from June 2002 to May 2003, feasibility of microfilarial antibody detection by ELISA and comparison of sensitivity of Indirect ELISA and dot-ELISA in the detection of dirofilariosis. Microfilariae encountered were identified as those of *Dirofilaria repens*.

Out of the total number of 2435 blood samples from dogs screened by wet film examination 172 (7.06 per cent) dogs were found positive for microfilariae. It has been found that canine dirofilariosis was prevalent throughout the year with more incidence during summer. An Indirect ELISA and dot-ELISA were also conducted in three groups of animals using microfilarial antigen prepared from known microfilaria positive serum samples. Out of the total number of 50 samples of known microfilaria positive dogs 49 (98 per cent) and 47 (94 per cent) were found positive by Indirect ELISA and dot-ELISA respectively. The corresponding figures in 36 dogs with other helminths were 15 (41.67 per cent) and 19 (52.77 per cent) and that of 32 totally helminth free dogs were 10 (31.25 per cent) and 12 (37.5 per cent) respectively. The results indicate that both Indirect ELISA and dot-ELISA are feasible for the detection of the microfilarial antibodies though they are less specific.