CLINICOPATHOLOGICAL STUDIES ON DIROFILARIASIS IN DOGS IN ORISSA

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Dedicated
To
My Parents
Beloved Wife
&
Son Ishu

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Dated--10.10.11

CERTIFICATE-1

This is to certify that the thesis entitled **"CLINICO-PATHOLOGICAL STUDY OF DIROFILARIOSIS IN DOGS IN ORISSA"** submitted for the degree of "**MASTER OF VETERINARY SCIENCE**" in the subject of **VETERINARY PATHOLOGY**, of the **ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR** is a faithful record of bonafide and original research work carried out by ***Dr. Prasana Kumar Rath***, under my guidance and supervision and that no part of the thesis has been submitted for other degree or Diploma.

The assistance and help received during the course of investigation have been fully acknowledged.

10.10.11

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CERTFICATE-II

This is to certify that this thesis entitled **"CLINICO-PATHOLOGICAL STUDY OF DIROFILARIASIS IN DOGS IN ORISSA"** submitted by ***Prasana Kumar Rath*** to the **ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR** in partial fulfillment of the requirements for the degree of **"MASTER OF VETERINARY SCIENCE"** in the subject of Veterinary Pathology has been approved by the student's Advisory Committee after an oral examination on the same in collaboratrion with an External Examiner.

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Prasana Kumar Rath.

PRASANA KUMAR RATH.

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Abbreviations

ALT = Alanine amino transferase

AST = Aspartate amino transferase

ALP = Alkaline phosphatase

DTPA = Diethylene triamine penta acetic acid

t. = Delta t in seconds

EDTA = Ethylenediamine tetra acetic acid

F = Dilution factor

FR = Final reading

GSH-Px = Glutathion peroxidase

HNO3 = Nitric acid

H_2O_2 = Hydrogen peroxide

HOCL = Hypochlorite Freeradical

IR = Initial Reading

L^* = Lipid radical

$\overline{Hb}$

LOOH = Lipid hydro peroxides

LPO = Lipid peroxidase

M = mole

MDA = Malondialdehyde

Mec = Molar extinction coefficient

Mf = microfilariae

mg/L = milligram per liter

mM = Milli mole

nM = Nanomole

O_2^- = Superoxide

OD = Optical density

PBS = Phosphate Buffer Saline

PCV = Packed cell volume

RBC = Red blood corpuscle

ROS = Reactive Oxygen Species

Rpm = Revolutions per minute

SOD = Superoxide dismutase

TBA = Thiobarbituric acid

TCA = Trichloro acetic acid

TP = Total protein

Vc = Total volume of cuvette

CHAPTER - I

INTRODUCTION

CHAPTER I

INTRODUCTION

Dirofilariasis popularly known as 'Heartworm Disease' is a common and important disease affecting dogs, cats, foxes and wolves in tropics, sub-tropics and temperate regions of the world (Soulsby,1982).*Dirofilaria immitis* is the causal agent of heartworm disease in dogs and cats (Genchi et al., 2001). Dog is considered as a definitive host. The disease has a worldwide distribution. There is apparent increasing prevalence of heartworm disease in canine in India (Sharma and Pachuri,1978 a,b). Canine Dirofilariasis now recognized not only a veterinary problem but also a zoonosis in many parts of the world (Robinson et al., 1977; Simon *et al.*, 1991; Orihel and Eberhard, 1998; Pampiglione *et al.*, 2001; Miyoshi *et al.*, 2006.). Human is the "Dead end host" of *D.immitis* (Dissanaike, 1977) since worms can not reach maturity.

Dirofilaria species considered as the important pathogenic nematode of dogs. Canine Dirofilariasis caused by *Dirofilaria immitis, Dirofilaria ripens, Dipetalonema reconditum.* Important killer species among them is *Dirofilaria immitis*, commonly called as 'heart worm' of dogs. The heart worm lives mainly in the right ventricle, in the pulmonary artery but also found in posterior venacava less often in the right auricle, anterior chamber of eye, inter digital cysts and other parts of body. The males and females copulate in these sites. *Dirofilaria immitis* has an indirect life cycle. Females are viviparous (producing living young instead of eggs), producing motile unsheathed microfilaria which circulating in the peripheral blood at any time but there is a tendency towards nocturnal periodicity. The vectors of *Dirofilaria immitis* larvae are many mosquito species. Approximately in toto,70 species of mosquitoes shown to be capable of supporting larval development of *Dirofilaria immitis* to the 3rd (infective) larval stage.Mostly *culex,Aedes, Anopehles, Mansonia* etc.are incrimanted as intermediate host of the parasite (Arellano *et al*, 2002). The motile microfilaria taken up from the peripheral circulation by the mosquito vector from infected dogs during their blood meal. Within the mosquito vector it molts twice i.e. (L1 microfilariae) will progressively

mature to second stage larvae (L2) and then to the infective third stage larvae (L3) in the malpighian tubules and then migrates to the proboscis through the body cavity for sub subsequent infection to dogs. Development to the L3 stage is directly dependent on temperature. For maturing larvae to L3 stage within 10-14 days, it is necessary to have the temperature consistently above 80^0F. If at any time the temperature falls below 57^0 F, larval development will be retarded. The infected mosquitoes again during their blood meal leaves the infected L_3 larvae to the new vertebrate host which molts to L4 larvae within 3 days. After two months the L4 larvae molt to L5 larvae and relocate from the subcutaneous tissue to the pulmonary arteries. The parasite remains dormant in the vertebrate hosts muscle tissue for 85 to 120 days. After this time period, the parasite enters the host's blood stream, where they carried to the heart. Completion of the life cycle in the heart requires 7 to 9 months. Microfilariae can be seen in the blood approximately 6-7 months after exposure to the infective L3 larvae. Adult parasite remains for 5-7 years in the dog and 2-3 years in cat. The circulating microfilariae may persist for about two years in dog. With a single patent infection with L3 larvae, the infected dogs may develop the disease or serve as reservoir capable of infecting to other non infected dogs. Therefore a dog may remain positive for microfilaria up to 7 years after a single infection with L3 larvae (Pachauri, 1999). The adult worms are thin, almost thread like with males 12-30 cm long and females 25-31cm long. Microfilariae are approximately 300 µm and 7µm wide.

The primary damage in heartworm infection occurs in the pulmonary arteries and lungs. Severity of disease related to the age of infection (stage of life cycle), host's immune response, and the load of worms in host's body. Presence of the immature adult worms initiates vascular damage and possibly lung disease by causing eosinophilia with eosinophilic infiltrates and signs of respiratory disease. Heartworms release vasoactive substances that result in vasoconstriction and hypoxia, which lead to pulmonary hypertension and compromised cardiac output. Pulmonary hypertension causes pressure overload of the right ventricle, resulting in compensatory, concentric ventricular hypertrophy (thickening of the ventricular walls).In high worm burdens, chronic pulmonary hypertension with

tricuspid insufficiency results in elevated cardiac filling pressures and congestive heart failure. Thromboembolism may cause acute decompensation by producing or aggravating pulmonary hypertension, right heart failure, or pulmonary infarction Heartworm infection may also lead to glomerulonephritis and proteinuria secondary to antigen–antibody complex formation

Acute infection in dirofilariasis characterized by heart and kidney failure, blindness, seizures with death which occurs as a result of massive death and disintegration of the parasites, or with extremely heavy parasitic load. Chronic microfilariaemic condition seen mostly in dogs. Canine diorofilariasis categorized clinically as of three types such as Class I, Class II, and Class III. Dogs with class I heartworm disease are often remain asymptotic or may exhibit occasional cough. Class II category dogs show the signs like coughing and exercise intolerance. Dogs with Class III category show symptoms like anemia, fainting spells, right sided heart failure, hypertension, and labored breathing with tachycardia.

Lesions in canine dirofilariasis mainly attributed by the adult worms, which interfere with circulation through the right heart. Insufficiency of the right heart results in congestion of the lungs, liver and spleen as well as ascites. Worms may die and transported to lungs through the pulmonary artery causing pulmonary embolism leading to infraction. In heavy infestations, *D.immitis* may occupy the venacava, resulting phlebosclerosis of venacava and hepatic veins. The microfilariae though circulate freely in the blood, appear to produce little tissue damage. However, some microfilaria die and a small granuloma forms around them which are seen in the kidney of infected dogs. Immune complex glomerulonephritis is also seen in canine dirofilariasis.

Diagnosis of dirofilariasis in dogs is based on morphologic features as well as histochemical staining of circulating microfilariae, serological detection of circulating antigen, and application of molecular tools like ELISA and PCR. Detection of dirofilaria through wet blood smear is the easiest diagnostic method but needs professional expertise to differentiate between *D. immitis and D. reconditum.* Several concentration techniques being employed for detection of dirofilariasis in low level infections (Wylie,1970). Besides all these methods

thoracic radiograph, C-arm examination with history also helps in arriving the diagnosis of dirofilariasis in canine. Post mortem examination and detection of adult worms in the heart followed by histopathology are very important diagnostic tools in animals that die due to heavy heart worm infestation.

Oxidative stress index plays a vital role in pathogenesis of different diseases and give a fair idea about the degree of severity of the disease. SOD, Catalase and glutathione are the major antioxidant enzymes present in RBC to counteract the toxic effects of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxides (Moral *et al.*1977) which are also considered as indicators of oxidative stress in dirofilaria affected dogs.

The worldwide distribution and apparent increasing prevalence of dirofilariasis have prompted studies of this disease in dogs. The aim of the present study was to assess clinicopathological alterations and oxidative stress in dirofilariasis affected dogs in Orissa. The study will help in early diagnosis of clinically affected animals as well as demonstration of dirofilaria worms with its effect in various organs to establish the cause of death due to this.

Studies were conducted on the basis of following parameters.

- Screening of dogs for dirofilaria infection on the basis of clinical signs, thoracic radiography, image intensification TV system, wet blood smear examination along with other concentration methods.
- Haematological and biochemical examination for any alterations in dirofilarial affected dogs.
- Detailed post mortem examination followed by histopathology of the animals died due to heavy load of heart worm.
- To study the oxidative stress indices in dirofilaria infected dogs.

CHAPTER - II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Heartworm infection in dogs has been recognized for over 300 years. Canine Dirofilariasis caused by *Dirofilaria immitis, Dirofilaria ripens, Dipetalonema reconditum*. Important killer species among them is *Dirofilaria immitis*, commonly called as 'heart worm' of dogs. Diagnosis of canine dirofilariasis is based on history of occurrence of the disease in the area and clinical signs and their development. Auscultation of chest can be of great help in evaluating the cardio-pulmonary state of dogs suffering with dirofilariasis. Microfilariae in the peripheral blood can be demonstrated by wet blood smear, thick blood smear, and also with various concentration techniques or with stained blood smear examinations. Besides this radiography, echocardiography, electrocardiography, angiography, C-arm examination, detection of dirofilarial antigen and other molecular or serological technique also being employed in arriving a diagnosis of dirofilariasis. Pathological findings i.e. both gross and microscopic changes of different organs along with presence of adult parasites in the right ventricle or in other places helps a lot in arriving the diagnosis in dirofilariasis during necropsy examinations.

Epidemiology

Schlotthauer, J.C. (1966) studied the host-parasite relationships of Dirofilaria immitis in the dog.

Prusty et al., (1972) reported a case report on Dirofilariosis in dogs.

Mackenzie and Waldie (1991) examined 360 dogs out of which 20 (5.5%) were positive for *D.immitis* infection in the oliver-osoyoos area of the Okanagan valley in British Columbia.

Rao and Acharjyo., (1993) studied the prevalence of D.immitis in wild carnivores maintained and necropsied at Nandankanan park, Orissa over a period 1962-1991 and only in one case of *Vulpes bengalensis* the cause of death was attributed to dirofilariasis.

Liang *et al.*, (1996) reported about no significant difference in the positive rate of dirofilariasis with dog sex. Also the study in opinion that large breeds (German Shepherd dog 61.5%, Rottweiler 30.8%) had higher prevalence than the small breeds (Pomeranian 11.4% ,Miniature Poodle 0%) among the 361 pet dogs subjected for screening to the National Taiwan University Veterinary Hospital.The majority of dogs under one (<1) year age found negative for dog heartworm using microscopic and serological techniques. However, the dogs adult dogs >1 years age had positive rate of 19.7%.

Eslami, 1998 reported that heartworm disease is sporadic or endemic in some parts of Iran and is reported from different domestic and wild carnivores as well as man.

Su (1999) examined 94 stray and 54 house dogs in middle-Southern Taiwan by microscopic examination for microfilariae and ELISA for adult worms and found 36.2% of strays and 20.4% of housed dogs were infected. The study found no significant differences in the positive rates both for adult worms as well as microfilaria relative to dog sex.

Theis *et al.*, (2001) studied the potentiality of *Aedes taeniorhyunchus* and *Culiseta incedens* mosquito species as a vector for *D .immitis* in a dog with known levels of such microfilariae. Though Aedes taeniorhyunchus readily blood fed on infected dog, ingested microfilariae but did not become infected. Simultaneously Culiseta incedens fed on infected dogs became infected and microfilariae developed to L_3 stage. It was concluded that the wide distribution of the mosquito species Culiseta incedens as well as its long and annual period of reproductively might be contributing to its potentiality as a vector of *D .immitis.*

Reiful *et al.*, (2001) described about the role of biological factors in the existence and spread of heartworm disease.

Georgieva *et al.*, (2001) studied the incidence of dirofilariasis in carnivores to reveal their role in epizootiology of the disease The microfilariae possitive samples further checked with Pet Check HTWMPT test (IDEXX) for detection of D.immitis antigen. High incidence found in the stray and rural dogs, followed by working and shepherd dogs. The companion animals were the least invaded .Necropsy examination showed the presence of mature D.immitis organism in the heart and pulmonary artery in many cases.

Rosa *et al.* (2002 and) Reifur *et al.* (2004) reported infection with this parasite in carnivores, especially dogs is worldwide and has been reported from different regions of the world in Brazil.

Ching-chen and Ping-chin.,(2003) studied the prevalence of canine dirofilariasis between the period 1993-1997 in Taiwan in stray dogs and house dogs through necropsy and thick blood smear examination. The overall prevalence of adult worms in the dog population was 57% found from 837 stray dogs necropsied .The prevalence of microfilariae was 25% out of 1228 house dog's blood sample taken for microscopic examination. They observed the increased prevalence may be related to the wind speed, temperature, relative humidity and altitude in different areas surveyed.

Eslami *et al.*, (2005) studied the clinical presentation of heartworm disease with total of 198 household dogs and guard dogs brought to the small animal clinics in Tabriz, west of Iran. 15.1% of the dogs were found to harbor *Dirofilaria immitis*. No relationship was found between the number of microfilaria in the blood and clinical signs.

Bothakur *et al.*, (2006) reported prevalence of *D.immitis* in North eastern states by doing necropsy in dogs from a local abattoir in Mizoram. Prevalence report of 33.75% for *D.immitis* was reported. North eastern part of India was reported to be endemic area for *D.immitis*. On the basis of Modified Knott test, occult dirofilariosis was reported to be 35.89%.

Yildirim *et al.*(2007) studied the prevalence and epidemiological aspects of *D.immitis* in dogs from Kayseri province, Turkey and were found a prevalence value of 9.6 %.The highest heartworm prevalence were observed in 7–10 age

group (28.6%) followed by 4–6 (17.1%) and 0.5–3 (4.8%) age groups. The differences between 0.5–3 and other age groups were found significant, whereas no statistically significant difference was observed between 4–6 and 7–10 age groups. The infection was more prevalent in males, larger breeds and the dogs not on prophylaxis. No statistically significant difference was observed between stray and owned dogs.

Oranto *et al.*, (2009) reviewed the changing distribution patterns of canine vector borne diseases in Italy with special reference to Leishmaniasis and Dirofilariasis which attributed to several biological and ecological factors such as vector distribution, dog movements, improved diagnostics, higher awareness of research and practioneers.

Donato Traversa *et al* (2010) reported that Male and large sized dogs resulted more likely to be infected by Dirofilaria spp., possibly due to the fact that animals living outdoor and of large size are more exposed to mosquito bites.

Clinical signs

Vaughan,(1952) described regarding the cutaneous manifeastations which include an eczematous dermatitis associated with intense irritation and itching.

Chakrabarti *et al.,* (1983) reported the Incidence and clinical profile of dirofilariasis in West Bengal.

Atkin *et al.*, (1988) described the Vein cava syndrome in the massive infestation of *D.immitis* in dogs which evolves into a very serious hemolytic syndrome or a lethal obstruction of the vein cava.

Ludders, J.W., *et al.*, (1988).reported about the functional alterations in the glomerular basement membrane (GBM) leading to proteinuria in dirofilaria positive dogs.

Mackenzie and Waldie (1991) reported a positive case of *D.immitis* mf infection in a dog showing the clinical signs of severe respiratory distress and cardiac murmur.

Mehlhorn *et al.*, (2001) reported that Dogs with mild exercise and low worm burden remain asymptomatic. But chronic infection with exercise intolerance, cough, cor pulmonale, allergic pneumonitis with vena caval syndrome may be seen in dogs.

Eslami *et al.*, (2005) studied the clinical signs in dogs harbored *D.immitis* in the pulmonary artery and heart. The clinical signs observed were mild anorexia, inappetance and sporadic cough, weight loss, depression, dysponea at time of exercise, nasal discharge and systolic murmur. In another group of dogs in study the signs like fatigue exercise intolerance, weight loss, haemoptysis (one case), ascites, right sided heart failure, pulmonary crackles, tachypnea, dyspnea, pulmonary thromboembolism and hepatomegaly seen.

Hodges and Rishniw, (2007) reported the occurrence of *D.immitis* microfilariae in the synovial fluid of two dogs presented with clinical and cytological evidence of polyathritis in one case and with severe effusion in other dog. This study concluded that *D.immitis* infection should be considered a differential diagnosis in patients with polyathritis.

Steven Hodges and Mark Rishniw.(2008) reported about the presence of *Dirofilaria immitis* microfilariae in two dogs in the synovial fluid of the joints.

Radiography:

Thoracic radiographs alone are not diagnostic for heartworm infection but are useful for detecting heartworm disease, determining disease severity, and evaluating cardiopulmonary parenchyma changes. Radiographic changes associated with heartworm disease include right ventricular enlargement, increased prominence of the main pulmonary artery segments, increased size and density of the pulmonary arteries, arterial tortuosity, and pruning. The size of the caudal lobar pulmonary vessels is best evaluated on the dorsoventral projection. The vessels are considered abnormal if they are larger than the diameter of the ninth rib where the rib and the artery intersect. The cranial lobar pulmonary artery is best evaluated on the left lateral projection and should not be larger than its

accompanying vein or the proximal one third of the fourth rib. Thoracic radiographs can also be used to evaluate the pulmonary parenchyma for infiltration, nodules, lymphadenopathy, and pleural effusion. Pulmonary parenchyma changes may include a mixed interstitial to alveolar pattern that is typically most severe in the caudal lung lobes. In eosinophilic nodular pulmonary granulomatosis, the pattern may appear nodular. Radiographic changes may be transient and do not always indicate an active infection.

McCall *et al.*, (2004) reported about enlargement of the right heart, tortuousity of pulmonary arteries in severe and chronic dirofilariasis.

Radiograph is regarded as the most useful method for assessing the severity of canine dirofilariasis. As this is primarily a cardio-pulmonary disease, the typical signs in radiograph usually show heart enlargement and swelling of the pulmonary artery leading to the lungs from the heart which helps in predicting the possibility of complications related to treatment. The earliest and most subtle pulmonary arterial changes are found in the dorsal caudal wedge of the diaphragmatic lung lobes which seen in successive larger branches in severe and chronic cases. Radiographic study reveals the enlarged, tortuous and often truncated peripheral intralobar and interlobar branches of pulmonary arteries, particularly in the diaphragmatic lobes.

Detection of microfilariae:

Mature female worms are viviparous releasing unsheathed motile vermiform embryos into the host's blood stream. Depending on the number of adult parasites they reach concentrations from 103 to 105 microfilariae per milliliter of blood. The microfilariae appear in the blood of experimental infected dogs 190-197 days after infection. They can circulate for years in the blood stream of canidae species and infect mosquitoes. The microfilariae circulate in the blood for as long as two years, waiting for the next stage in their life cycle in the gut of a blood sucking mosquitoes.

Microfilariaemic dogs easily detected by microscopically examining fresh blood containing the motile unsheathed microfilariae through wet blood smear examination which is regarded as the simplest technique used for the diagnosis.

But a false negative result may occur when microfilariae are scarce and also in single gender infection. A stationary rather than a migratory pattern of movement is indicative of a dirofilaria species (Mc Call *et al.*, 2004). The larvae will be found only when the blood sample is fresh and the microfilariae are still active. Particularly in feline dirofilariasis where only transient microfilariamic condition seen, this wet blood smear examination may yield false negative results. *D.immitis* exhibits stationary, writhing movement in the wet blood smear and the anterior end of the parasite tapers in the Modified Knott's method preparation. In contrast, *A.reconditum* exhibits rapid, directional movement in the wet blood smear and the anterior end of the parasite is blunt (like a broomstick Handel) in the modified Knott's rest.

Newton and Wright (1956) demonstrated the modified Knott's method for easy diagnosis and differentiation of *D.immitis* and *D.reconditum* microfilariae in the blood. The authors also studied the morphology of microfilariae of *D.immitis and D.reconditum.* The anterior extremity of *D.immitis* was tapered and tail was straight but in case of *D.reconditum*, the anterior extremity was blunt and tail is curved botton-hook like manner.

Lindsay, (1965) reported that females worms are longer approximately 25-31 cm long and males are 12-30 cm long. Females are viviparous releasing motile unsheathed microfilariae which can cross the capillary and so are found throughout the vascular circulation. Microfilariae are of 314µm (286-340), Width 6.8µm (6.1-7.2) with tapering anterior extremity, straight body and tail.

Sawyer *et al.*, (1965) described a method of differentiating the two species of microfilaria of *D.immitis and D.reconditum.* A thick blood film from the marginal ear vein was dehemoglobinised in tap water for 10 minutes and transferred without drying to a 1: 50 dilution of 1% brilliant cresol blue in 0.8% saline for a further 10 minutes and the slides were rinsed and mounted in saline. Microfilariae are then examined by both "high dry" and oil immersion objectives for the presence of a cephalic hook by staining which was readily seen in *D reconditum* but absent *in D.immitis.*

Wyile, (1970) developed a method for concentrating microfilariae by lysing the anticoagulated blood sample and forcing it through a Millipore filter which claimed to be superior than Modified Knott's technique for recovery of microfilariae.

Dennis and Kean (1971) described about the "Nucleopore Filter" technique for isolation of microfilariae. A thin, transparent plastic membrane of 25mm.diameter with uniform cylindrical perforations with pore sizes of 3.2 or 5 µm were used for separating microfilaria of *D.immitis* and other filarid species from 4-10 ml blood samples.

Wylie, (1970) reported the concentration techniques appeared to be the best in detection of low level infections.

Wylie, (1970) compared wet smear examination , modified Knott's method and capillary tube technique to detect microfilariasis caused by *D.immitis* in canines and found that the efficacy was 56, 55, 69 percent respectively.

Chlifoux and Hunt (1971) accurately diagnosed and studied the morphology of *D.immitis* and *D.reconditum* by acid phosphatase activity. An approx. 5 ml of blood was drawn from a infected dog and allowed to clot. The clot was loosened with an applicator stick and washed with 5 ml distilled water. Then water and serum poured into a test tube and centrifuged for 5 minutes at 1000 rpm. The supernatant fluid was discarded leaving a drop in the bottom of the tube and placed in a microscopic slide and a smear was prepared. It was air dried, fixed in absolute acetone at 4^{o} c and air dried for 1 minute and stained for the demonstration of acid phosphatase activity. By staining, the enzyme activity was restricted to two distinct zones in microfilariae of *D.immitis* where as in *D.reconditum*; this activity was uniformly distributed throughout the organisms.

Stein and Lawton (1973) reported an efficacy of 66.6% and 87.55 respectively of wet smear examination and modified Knott's method in detection of canine microfilariasis caused by *D.immitis.*

Sharma and Pachauri,(1982) in the opinion that since the infected dogs remain asymptomatic for several years, a systematic attempt to find circulating first stage larvae (L_1) will help in identifying the diseased dogs and greatly enhancing the prospects for safe and successful treatment .

Brown and Barsanti (1988) observed the 100 % efficacy of quantitative Buffy coat analysis technique to detect the microfilariasis caused by *D. repens* in dogs which was employed for the first time tor the diagnosis of this species.

Nuchaprayoon *et al.* (2005) reported about detection and differentiation of filarial parasites by universal primer and polymerase chain reaction-restriction fragment length polymorphism analysis.

Ananda *et al.*(2006) described the methods for identification of microfilaria of *Dirofilaria repens* and *Dipetalonema reconditum. J.Vet. Parasitol.*, 20 (1): 45-49.

Ananda and E.D'Souza (2006) reported a comparative efficacy of different techniques to detect the microfilaria in the blood of man and animals. Out of the 80 possitive samples screened the percent efficacy was 66.25%, 63.75%, 92.5%, 100% and 100% by wet film examination, Giemsa's stained blood smear, modified knot's method, citrate saponin acid method and quantitative Buffy coat methods respectively.

Anand *et al.* (2006) reported specific methods for detection and differentiation of *D.repens and Dipetalonema reconditum* from Karnataka on the basis of morphology, micrometry and histochemical staining.Two types of microfilaria *of D.repens* were reported, one stained at the anal pore only and the second one with stain at anal pore as well as at the central body region in acid phosphatase staining, where as *D.reconditicum* microfilariae showed uniform enzyme activity.

Rishnew *et al.*, (2006) studied the usefulness of a single polymerase chain reaction for discrimination between six species of canine microfilariae.

Hematological Alterations

Sharma and Pachauri (1982) reported about a mild anaemia in dirofilaria infected dogs.

Atwell and Buoro (1983), Sevimli *et al.*, (2007) reported a decrease in RBC, Hb and PCV levels in dirofilaria infected dogs.

Biswas *et al.*, (2005) studied the haemato-biochemical alteration and gross pathologic changes in Dirofilaria affected dogs. On necropsy, cardiac dilatation leading to round heart appearance, hypertrophy and thrombosis of vena cava and pulmonary arteries was seen. It was observed that there was a significant reduction in hemoglobin, total erythrocyte count, packed cell volume.

Niwetpathomwat *et al.*, (2007) reported that a major hematological findings in dogs infested with *D.immitis* showed a mild to moderate anemia, mild to severe thrombocytopenia, marked leukocytosis, moderate to marked neutrophillia, eosinophillia and monocytosis.

Biochemical Alterations

Biswas *et al.*, (2005) observed the haemato-biochemi alterations in dogs affected with dirofilariasis and found there is a reduction in serum protein and serum albumin level and increase in alkaline phosphatase, ALT, serum billirubin and MDA indicating oxidative stress and liver damage.

Niwetpathomwat *et al.*,(2007) reported a marked increase in AST and ALP in dirofilaria infected dogs, but difference between infected animals and control groups was not significant. Variations in hematological and biochemical values compared with reference values can give information related to the health status of animals.

Oxidative Stress

Oxidative stress indices are a secondary phenomenon in most of the diseases (Halliwell and Gutteridge, 1984). It is reported to be involved in the pathogenesis of several viral diseases like damage and enhanced erythrophagocytosis in dogs infected with *Babesia gibsoni* and significantly increased level of both met-Hb and MDA in erythrocytes as compared to those in uninfected dogs.

In case of idiopathic dilated cardiomyopathy (IDCM), the concentration of glutathione peroxidase increased significantly from healthy control group, but vitamin-A and SOD concentration were not significantly different (Freeman *et al.*,

1999).The blood glutathione and plasma retinol decreased non-significantly and the plasma MDA level was significantly higher in dogs suffering from visceral leishmaniasis suggesting enhanced lipid peroxidation and of oxidative stress (Bildik *et al.*, 2004). Plasma glutathione peroxidase and SOD activities were significantly higher in bitches which had a previous report of mammary tumor (Szczubia *et al.,* 2004; Kumaraguruparan *et al.*, 2005) indicating the contribution of oxidative stress in malignancies. Peterhans (1997) and Schwarz (1996) reviewed oxidative stress during viral infections.

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Panda et al. (2009) reported altered erythrocytes lipid peroxidation as evident by estimation of malonaldehyde (MDA) concentration in acute cases of gastroenteritis in dogs. The activities of antioxidant enzymes, catalase and superoxide dismutase were also altered.

Postmortem findings

Kume and Itagaki (1955) proposed that immature heartworms migrate to the heart via the veins.

Rao and Acharyo (1971) studied the pathological changes in some of the organs in heartworm infection in an Indian Fox *(Vulpes bengalensis*) in Orissa. On post-mortem examination they found both lungs were grey in colour, consolidated and present wedge shaped infracts. The cut surface of lungs revealed an adult parasite in one of the branches of pulmonary artery. Liver was dark red in colour and kidneys and heart were pale.

Rawlings *et al.* (1977) reported about the right ventricular enlargement in heartworm diseases with round heart appearance.

Rawlings, (1986) reported that dirofilariasis is a disease that affects many organs, including the lung, heart, liver, and kidney

Mackenzie and Waldie (1991) observed ascites, a large nutmeg liver, excess pleural fluid and mottled lungs with some consolidation *in D.immitis* infected dogs.

M.Hayasaki, (1996) studied the re-migration of the Fifth-stage juvenile *Dirofilaria immitis* into pulmonary arteries after subcutaneous transplantation in dogs, cats and rabbits. Live 5^{th} stage juvenile worms recovered from the pulmonary arteries of infected dogs, cats, and rabbits. A mean of 45%, 61% and 18% of the transplanted worms were recovered from the pulmonary arteries of dogs, cats, and rabbits respectively 1 and 3 month later

Katoch and Jithendran, (1999) did the necropsy of a dirofilaria infected male Doberman dog, aged 5 years and 9 month. Post-mortem observations like cyanosed visible mucus membrane, pale and enlarged liver, grey and heavily anthracosed lungs with patchy pneumonic areas, cardiac dilatation with slender white worms in the right ventricle found. Later the worms indentified as *D.immitis*.

Samanta *et al.*(2007) reported a case of heartworm infection in a leopard from Buxa Tiger Reserve, West Bengal. Bunches of adult worms were recovered from the right ventricle of the heart on postmortem. There was complete blockage of lumen of right ventricle which attributed the cause of death by mechanical obstruction in circulation. Macroscopically no significant lesion, except congestion of organs was found. Signs of petechial hemorrhage in sub epicardium and scattered necrotic foci of liver (nutmeg liver) were observed. Besides it, adult worms found in the pulmonary artery and right ventricle of heart.

Histopathology:

Rawlings, C.A., (1986).is in opinion with right ventricle congestive heart insufficiency subsequent to progressive pulmonary artery disease in some dirofilaria affected dogs.

Lombard, C.W.,(1987).reported about the lung, heart, kidney and liver were of major organ damaged much in dirofilaria affected dogs. The most important damage found in the pulmonary arteries where adult parasites locate much of the time.

Grauer *et al.*, (1987).reported about the clinic-pathologic and histologic evaluation of *Dirofilaria immitis*-induced nephropathy in dog.The study in opinion of thickening of GBM with electron dense deposits and foot process effacement of the epithelial cells.

Ludders *et al.*, (1988).reported about the functional alterations in the glomerular basement membrane (GBM) leading to proteinuria in dirofilaria positive dogs.

Grauer *et al.*, (1989) studied about experimental *Dirofilaria immitis*-associated glomerulonephritis induced in part by in situ formation of immune complexes in the glomerular capillary wall.

Gupta *et al.*, (2001) reported the presence of microfilaria and adult worms in association with neoplastic lesions in cytological smears which is regarded as a chance association. But some authors in opinion about such parasitic infection may be a causative factor in tumor genesis.The report regarding association of *D.immitis* in neoplastic lesions in cytology literature very less.The presence of microfilaria in routine cytology smears from one benign and four malignant tumours were reported. Microfilariae could not be indentified on histopathology available in four of these cases.

Paes de Almeida *et al.*, (2003) necropsied five male dogs with spontaneous dirofilariasis and tissue samples were taken from the heart, lung, liver and kidney for histopathology. The histopathology result showed the thickening of the glomerular basement membrane (GBM), presence of dense deposits in the GBM and foot process effacement as most common lesions in dirofilaria infected dogs. If the infection period is longer, these lesions are more severe. The presence of dense deposit indicate that pathologic lesions in dirofilariasis caused by deposits of immune complexes in the membrane. Also the study indicated that immature worms as well as microfilariae and even adult worms can cause glomerulonephropathy.

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Kramer. (2004), reported about evidence that Wolbachia, an endosymbiontic bacterium present in *D.immitis*, may play a role in the immunopathogenesis of heartworm disease.

Kramer *et al.*, (2005,a.b) reported the substantial evidence of presence of Wolbachia in renal tubular cells, glomeruli or inflammatory pulmonary cells by the use of immunohistochemistry techniques employing a polyclonal antibody against the Wolbachia surface protein (WSP) in dirofilaria affected dogs. Also reported about the presence of high titers of IgG antibodies in dogs with dirofilariasis.

Niwetpathomwat et al., (2007) reported several kinds of kidney damage in dirofilaria infected dogs i.e. immune mediated glomerulonephropathy, glomerulosclerosis and chronic interstitial nephritis.

Simon *et al.*, (2007) studied the immunupathology of *Dirofilaria immitis* infection.Report in conclusion about the pivotal role of Wolbachia in the inflammatory pathology in dirofilariasis.Wolbachia responsible for inflammation during infection. Even though Wolbachia lacks LPS but other molecules like WSP, hsp60 or DNA is able to stimulate the inflammatory response. For this reason Wolbachia become the target of antibiotic treatment which not only affects worm fecundity but may decrease inflammatory pathology.

CHAPTER - III

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

The present investigation was carried out for a period from April, 2010 to August, 2011. In Toto 1119 numbers of dogs of both sexes and various age groups and breeds including pet and stray dogs were taken in the study from different sources viz. Teaching Veterinary Clinical Complex and Department of Veterinary Pathology of the College mostly dealing with pet dogs of private owners and Police Dog Squad, Animal birth control (ABC) programme of Bhubaneswar Municipal Corporation (BMC) at Veterinary polyclinic, Sahidnagar dealing with sterilization of stray dogs.

The dogs were screened for dirofilaria on the basis of wet blood smear examination, thick blood smear examination, modified knot method, and Buffy coat smear method and also morphological identification of microfilariae was done. The positive dirofilaria cases were subjected for detailed hematology, biochemical and radiographic examination along with observation of clinical signs. Further few positive cases were also studied for oxidative stress due to dirofilariasis. Some of the dogs were found to be positive during necropsy on the basis of recovery of parasites in heart and lungs followed by histopathological examination of those cases. Morphological parameters of the recovered parasites were recorded.

Screening for dirofilariasis:

Blood samples (5ml from each animal) were collected from all the dogs for screening. Wet blood smear examination, thick blood smear examination, modified knot method, and Buffy coat smear method were employed for screening of dirofilaria.

(a) Wet blood smear examination (Direct mount method)- One drop of blood from each sample was taken on a grease free glass slide and a cover slip was put

on the drop of blood. It was then immediately examined under low power objective of microscope for the presence of microfilariae. Five wet films were similarly prepared and examined to have a better efficacy.

(b) Thick blood smear examination- Thick blood smears were prepared and fixed in methanol, and stained with 1:20 Giemsa's stain for 45 minutes. The slides were then washed, air dried and examined under low power and then under oil emersion to study the morphological characteristics in detail.

(c) Modified Knott's method- This method as per the procedure of Lindsey, (1965) was followed. One ml blood from each sample was taken in a sterilized centrifuged test tube and 9 ml of 2% formalin solution was added to it. Then it was centrifuged in an electrically operated centrifuged machine at 1000 rpm for 5 minutes. The supernatant fluid was poured off and 2 drops of 1: 1000 aqueous methylene blues was mixed to the sediments and shaken well and examined under low power objective of microscope for the presence of microfilariae. (d) Quantitative Buffy coat smear method- The quantitative method was carried out as per the procedure of Brown and Barsanti, (1988). The blood sample after centrifugation, smears taken from Buffy coat area was subjected to see microfilariae under low power objective in microscope.

Morphological Identification:

Morphometrical measurement was also done to identify the species of the parasite. The adult parasites recovered from heart during necropsy examination were measured against a centimeter scale. The microfilariae found in the modified knot's method measured through micrometry.

C-arm examination (Image Intensifier TV System):

Ten nos. of dirofilaria positive dogs brought to Department of Surgery for C-arm examination to evaluate the severity of the disease by seeing the patho-morphological alterations in relation to heart, pulmonary artery and lungs through image intensifier TV system.

Fifty dogs each from dirofilaria positive and dirofilaria negative cases (both Pet and Stray) were sampled randomly for hematological examination. In addition, serum samples of 25 dogs and 25 normal (dirofilaria negative) dogs were subjected to various biochemical tests. For oxidative stress indices study, 16 samples from positive and 10 samples from normal animals as control were utilized. For each examination, separate animals were selected to reduce the stress of excess blood collection from individual animal as 5ml of blood collected in EDTA vial for hematological examination, 3ml collected for serum biochemical analysis and 2ml in heparinised test tube for oxidative stress indices study.

Haematological study:

The peripheral venous blood was collected aseptically in sterile test tubes from cephalic vein by using 22 gauge needles. For hematological study blood was collected in sterile vial using EDTA as anti-coagulant @ 1mg/5ml of blood as recommended by Jain (1986)

- Sahli's acid hematin method was employed for estimation of haemoglobin by usinhg N/10 HCl (Schalm, 1965)
- Total Leukocyte count and total erythrocyte count was done by using Thomas fluid and Haem's fluid as diluents respectively in haemocytometer. (Schalm,1965)
- Geimsa's stain was used for differential count, morphological study of blood cells and identification of microfilariae.
- Wintrob's haematocrit method was used for PCV estimation.

RBC indices were calculated by using value of Haemoglobin, PCV and TEC count.

- Mean corpuscular volume (MCV). The average size of the red blood cells expressed in femtoliters (fl). MCV was calculated by dividing the hematocrit (as percent) by the RBC count in millions per microliter of blood, then multiplying by 10.

- Mean corpuscular hemoglobin (MCH). The average amount of hemoglobin inside an RBC expressed in pictograms (pg). The MCH was calculated by dividing the hemoglobin concentration in grams per deciliter by the RBC count in millions per micro liter, then multiplying by 10.
- Mean corpuscular hemoglobin concentration (MCHC). The average concentration of hemoglobin in the RBCs expressed as a percent. It was calculated by dividing the hemoglobin in grams per deciliter by the hematocrit, then multiplying by 100.

Serum biochemical analysis:

For separation of serum, the blood samples in the test tubes were allowed to stand in slant position at room temperature for about 3-4 hours. The blood clot was separated from the walls of the test tube by carefully running a clean applicator stick around the inner surface of the tube. Care was taken to check haemolysis. The supernatant serum was then removed with auto pipette. The serum samples were stored in deep freeze at a temperature of –20 °C in properly capped and labeled glass vials for analysis. Serum AST, ALT, ALP, Total Protein, Albumin, Cholesterol, Triglycerides, Creatinine and glucose estimated as per the procedure provided in the kits by Acurex Biomedical Pvt Ltd. Serum Calcium and Phosphorus estimated by as per the procedure provided in the kits by Nice Chemicals Pvt Ltd.

Oxidative Stress study:

Processing of blood samples

1. Separation of plasma-The heparinized blood was centrifuge in 2000 rpm for10 minutes to remove plasma. Plasma was collected in a clean eppendorf tube. Packed cells were processed to prepare the RBC haemolysate for estimation of lipid peroxide level, supper oxide dismutase (SOD) and catalase (CAT) enzyme activity.

2. Preparation of RBC- haemolysate

The packed cells then washed with equal amount of Phosphate Buffer Saline (PBS), pH 7.4 and centrifuged to remove supernatant. The process was repeated thrice. 0.1ml of packed washed RBC was taken in a test tube and diluted with 0.9ml of chilled distilled water to get 10% RBC haemolysate. The RBC haemolysate was used for the estimation of antioxidant enzyme level such as superoxide dismutase and catalase, in transition cows.

Oxidative stress indices

1. Lipid peroxidation

Membrane peroxidation damage in the erythrocyte was determined in terms of malondealdehyde (MDA) production by modified method of Stock and Dormandy (1971) as described Placer *et al.* (1966).

Reagents:

* Phosphate buffer saline (pH 7.4) –

NaCl –8.1 gm.; Na_2HPO_4 -2.302 gm.; KH_2PO_4 –0.194gm. ; Distilled water-1lt.

* 30% Trichloro acetic acid (TCA) – 30gm TCA in 100ml of distilled water.

* 0.1M EDTA Solution – 3.722 EDTA disodium in 100ml of distilled water.

* 1% Thiobarbituric acid (TBA)/0.05M NaOH- 1gm TBA in 100ml of 0.05 M NaOH solution (viz. 0.2gm NaOH in 100ml distilled water). The solution was warmed up to dissolve TBA.

PROCEDURE:

An amount of 0.2 ml washed packed cell was suspended in 0.8ml of PBS in a test tube.0.5ml of 30% TCA was added to the suspension. Tube was vertexed and allowed to stand in ice for at least 2hrs. After that the tubes were centrifuged at 2000 rpm for 15 minutes. 1ml of supernatant was transferred to another tube and added with 0.075ml of 0.1M EDTA and 0.25ml of 1% Thiobarbituric acid (TBA)/0.05M NaOH solution. The solution was kept in a boiling water bath for 15 minutes and then allowed to cool at room temperature. Absorbance was read at 532 nm in UV / Visible Spectrophotometer (Lambda 25 UV-VIS-Spectrophotometer, manufactured by M/S Perkin Elmer Pvt. Ltd.). Calculation was done using molar extinction coefficient of MDA-TBA complex at 532 nm i.e.1.56 x 10^{-5} / cm per molar conc. (Utley *et al.* 1967). The amount of lipid peroxidation is expressed as nano mole of MDA formed per ml of packed cell.

Nano mole / ml of packed cell = O.D x F x Vc /Mec.

Where F= Dilution factor

Vc = Total volume of cuvette

Mec = Molar extinction coefficient

O.D = Optical density

2 Antioxidant enzymes

2.1 Catalase

Estimation of Catalase activity in 10% RBC hemolysate was done as per the procedure described by Cohen *et al.* (1970).

Reagents:

* Phosphate buffer 0.05 M of pH 7.0

*Hydrogen peroxide (H_2O_2) 10nM

Phosphate buffer - H_2O_2 was prepared just before use. 0.12ml of 30% hydrogen peroxide v/v was added to 100 ml of Phosphate buffer and standardized to give an O.D. of 0.6 to 00.7 at 240 nm during experiment.

Procedure:

3ml of Phosphate buffer - H_2O_2 was taken in a cuvette. The reading was set at 0 and it was kept as blank. 0.2ml of 10% RBC hemolysate was added to Phosphate buffer - H_2O_2 solution and optical density was recorded for 3 minutes in an interval of 30 sec at 240nm against blank after addition of sample. The time required for O.D to decrease by 50 units or 0.05 was noted.

Calculation:

Catalase (unit/ assay mixture) =(**X**) = Log (IR/FR) x2300/6.93x1/ t

Catalase (unit/ml) = **(Y)** =[X x1000/volume of sample taken (200 micro's)] x F

Catalase (unit/mg of Hb) =Y/hemoglobin (mg/ml)

Where: IR= Initial Reading =O.D at 30 sec.

FR= Final reading = O.D after fall of 50 units.

t. = Time taken for decrease of 50 units

2. Superoxide dismutase (SOD)

The superoxide dismutase (SOD) activity was estimated by methods of Merklund and Merklund (1974) with certain suggested modification by Menami and Yoshikawa (1979) using diluted nitro blue tetrazolium as substrate.

Reagents:

* 0.2 mM Pyrogalol in 10mM HCl – Kept in amber colored bottle under refrigeration.
* Cacodylic acid – 800mg.
* DTPA (Diethylene triamine pentaacetic acid)- 39.335 mg.
* Nitro blue tetrazolium (0.1mM)- 8.1765mg.
* Triton-x-100-0.001%
* Tris- buffer

50mM Tris-Cacodylic acid was prepared by adding the above ingredients from sl.no.2 to 5 in the quanty specified for preparation of 100ml of buffer. The pH was adjusted at 8.2 using the tris-buffer.

Procedure:

3ml of Tris-Cacodylic acid buffer (pH 8.2) was taken in a cuvette and the optical density (OD) was adjusted to zero at420nm and was used as reference blank. In another cuvette 2.95ml buffer and 50 µL of pyrogalol was added. Then the OD was measured at 30 and 90 seconds at 420nm. After each running of sample the cuvette was washed with 10% HNO_3. For the test sample

2.9ml of buffer was taken in a cuvette. 50 µL of sample was added to it and shaken well followed by addition of 50 µL of pyrogalol. OD was recorded at 30, 60, 90 and 120 seconds. In first case the optical density was increased rapidly in 30 and 90 seconds. But addition of samples slowed down the process so that the OD increased in a slower rate.

Calculation:

One unit of SOD is defined as the enzyme activity that inhibits pyrogalol auto oxidation by 50%.

SOD (unit/assay) –X= 2(B-T)/B

SOD (unit/ml) -Y = 1000x X/volume of sample taken (50µL)

SOD (unit/mg of Hb) = Y/hemoglobin in mg/ml.

Where

B (blank) - difference of OD in 1 minute.

T (Test) - difference of OD in 1 minute in test solution.

Post-mortem Examination:

During routine Postmortem examination of dogs in the Dept of Veterinary Pathology 6 no's of dirofilaria positive cases were encountered from which adult worms were recovered from heart. Systematic post-mortem examination of was conducted and special care was taken to record the detailed gross lesions in different organs.

Histopathological Examination:

For histopathological study of the dirofilaria cases, representative portions of different organs with or without gross lesions were collected in 10% formal saline solution. The formalin fixed tissues were processed by routine histological techniques. The fixed tissues were washed overnight in running tap water and dehydrated in ascending grades of alcohol and cleared in xylene. Paraffin blocks were prepared as per the routine procedure and sections were cut at 5 micron thickness and stained by routine haematoxylin and eosin method. Stained slides were examined under microscope for histopthological interpretation.

CHAPTER - IV
RESULTS

CHAPTER IV

RESULTS

In the present study 1119 dogs of various age and sex were screened by blood smear examination, thick blood smear examination, modified knot method, and Buffy coat smear method for presence of dirofilarial infestation spanning a period of around one year. Out of these 467 animals of pet dogs of different breeds presented to the Teaching Veterinary Clinical Complex and Department of Pathology of Veterinary College for various reasons. In addition, they were screened for dirofilariasis and 51 nos. of cases (10.92%) were found to be positive. Further, 1862 stray nondescript dogs of both sex and various age were brought to the Animal birth control (ABC) programme of Bhubaneswar Municipality Corporation (BMC) at veterinary polyclinic, Sahidnagar during the study period. Because of limitation in handling the stray dogs, only 652 no's of dogs could be screened. On screening 127(19.47%) no's of animals were found to be positive for dirofilariasis.

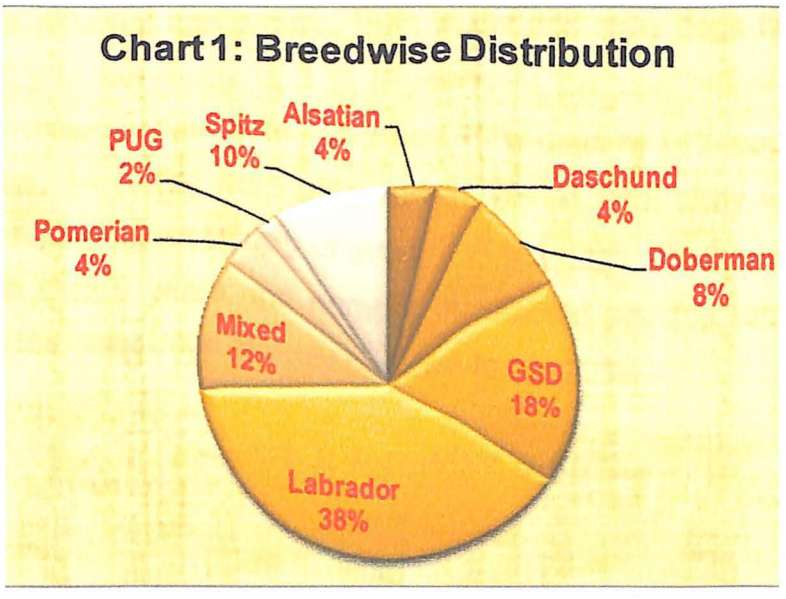
Out of all the screening methods employed, wet blood smear examination (Fig-1) was specific in 114 (64.04%) no of cases and Giemsa stained thick blood (Fig-2) smear examination in 110 (61.79%) no's of cases. The microfilariae identified through the wet blood smear technique as per their motility pattern. The *D.immitis* had slow wriggling movement i.e. undulate at one place and *D. reconditum* had quick progressive movement across the field. Modified knot method and quantitative Buffy coat smear method (Fig-3) were found to be sensitive and specific in detecting all the dirofilaria positive cases 178 (100%) and It was also found that modified knot test as the preferred method for observing morphology and to differentiate *D.immitis* from *D.reconditum*. The *D.immitis* showed longer length of around 296µm , long width and presence of straight tail with tapering anterior extremity (Fig-4) while *D.reconditum* was of short length of around 260µm , short width and presence of bottom hook tail with blunt anterior

extremity (Fig-5). It was seen that the dogs presented to the Veterinary College, 51 found positive for dirofilariasis out of which 18(35.29%) were of *D.reconditum* and 33(64.70%) were of *D.immitis*. Similarly stray dogs of ABC programme under screening found 127 positive for dirofilariasis, i.e. 69(54.33%) were identified as *D.immitis* and 58(45.666%) were *D.reconditum*.

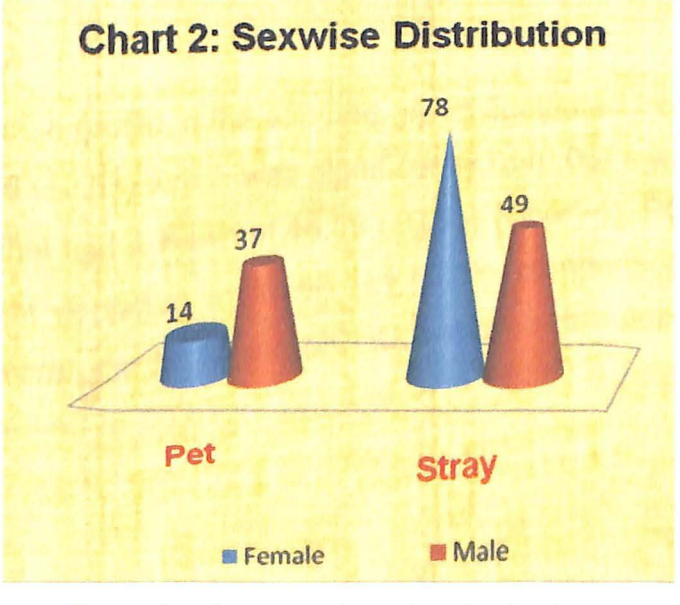
Majority of the positive cases 107 no's (60.11%) of dirofilariasis were asymptomatic which was predominant in stray dogs. However some positive cases 71(39.88%) showed clinical signs like coughing, slight pyrexia, exercise intolerance, inappetance and haemoptysis etc.

Radiographic evaluation through C-arm examination(Fig-6) of 10 affected animals with heavy load of microfilariae in blood during screening revealed cardiomegaly, round heart appearance suggestive of right ventricular hypertrophy, tortousity of the pulmonary artery and darkening of lungs(Fig-7). Radiographic changes associated with heartworm disease include right ventricular enlargement (Fig-8), increased prominence of the main pulmonary artery segments, increased size and density of the pulmonary arteries, arterial tortuosity,and pruning. Thoracic radiographs can also be used to evaluate the pulmonary parenchyma for infiltration, nodules, lymphadenopathy, and pleural effusion. Pulmonary parenchyma changes may include a mixed interstitial to alveolar pattern that is typically most severe in the caudal lung.

Results of breed susceptibility of dirofilarisis revealed highest incidence in Labrador (38%) followed by GSD (18%), mixed breed (12%) and Spitz (10%) among pet dogs. PUG breed comprised two percent while Pomerian, Alsatian and Daschund had incidences 4% of each. About 8% of Doberman breed suffered from dirofilariasis (Chart 1).

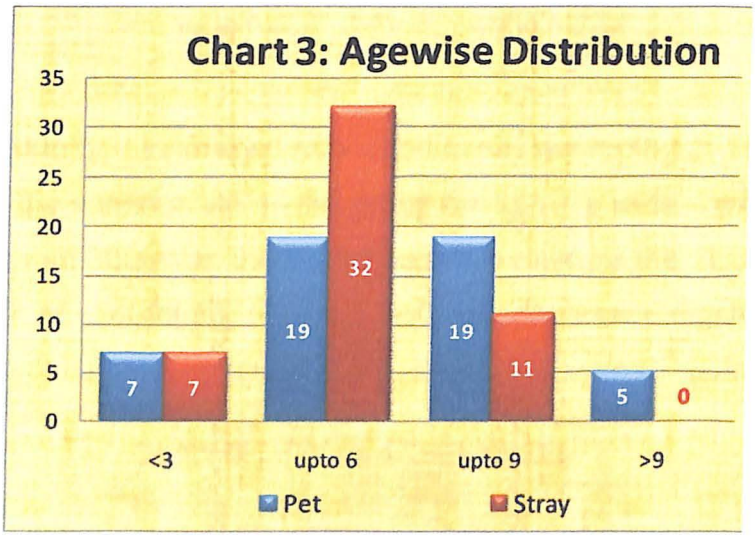


Sex wise distribution revealed that 37(64.70%) nos. of males and 14 nos. (27.45%) of females out of 51 nos. of positive pet dogs and 49(38.58%) were males and 78(61.41%) were the females out of 127 positive stray dogs. This may be due to the fact that denizens of Bhubaneswar and surrounding prefer to keep males; hence the male is higher in number. But in case of stray dogs the ABC program emphasizes sterilizing more females than the male. Hence the number of females is higher. (Chart-2)



According to age the animals are categorised into 4 groups viz. 6 months to 3 years, 3 to 6 years, 6 to 9 years and above 9 years. It may be noted here that in stray there were no animals above 9 years. Chart 3 depicts the status of

dirofilariasis in different age groups. Both in pet and stray dogs the prime age of susceptibility in our study is 3 to 9 years. At the age below 3 years the susceptibility remains same both in pet and stray dogs at 14% each. From 3 to 6 years of age, 19 (38%) from pets and 32 (64%) from stray were affected suggesting a higher susceptibility of stray over pet dogs. On the other hand, in animals within 6 to 9 years, the number of affected pet dog remained same (19) whereas the stray dogs sharply reduced to 11 (22%).



HAEMATOLOGY:

The haemoglobin profile in the affected group fluctuated from 2.4 to 19 g% with means 10.66 (±0.53) which was significantly ($p<0.05$) lower than the non-affected control that had a mean of 14.19 (±0.53) (Chart-4). PCV also similarly showed significant decrease to 28.96 (±1.49) in comparison to the control counterpart. However, the MCV and MCHC, values in the positive cases were significantly increased to 86.67±(7.28) and 37.45 (±0.76) respectively (Chart-4).

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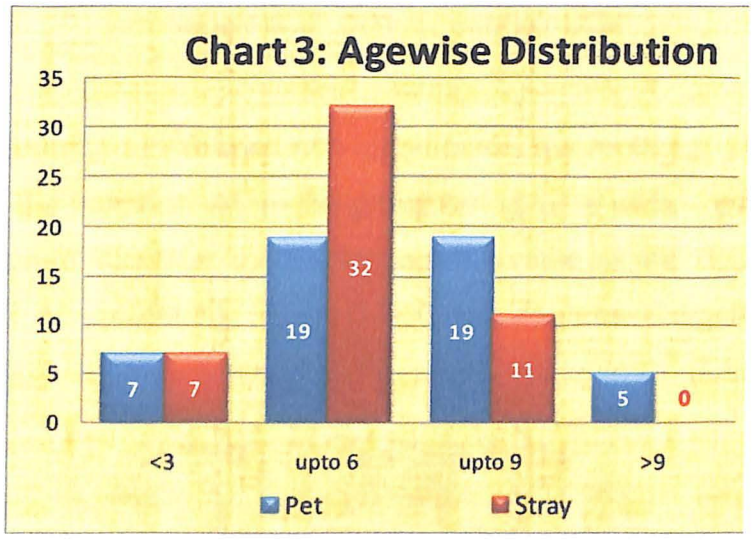

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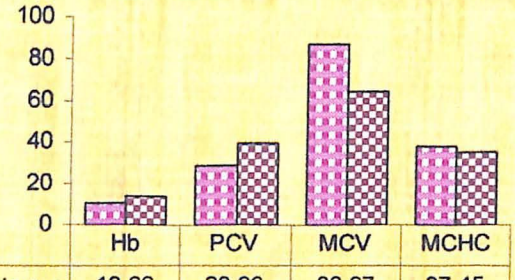
Chart 4: Hb, PCV MCV and MCHC profile



	Hb	PCV	MCV	MCHC
Filaria +ve	10.66	28.96	86.67	37.45
Filaria -ve	14.19	39.88	63.88	35.45

The TLC value had increased non-significantly averaging at 16230.39 (±1143.82) /mm^3, the variation within the group being quite wide – with minimum of 2000 and maximum 30000. In the filarial negative animals the TLC value averaged at 14627.45 (±4083.53) (Chart 5 TEC has decreased significantly in clinical cases of dirofilariasis – average 3.77 (±0.21) million/mm^3 (Chart 5).

Chart 5: TLC and TEC profile

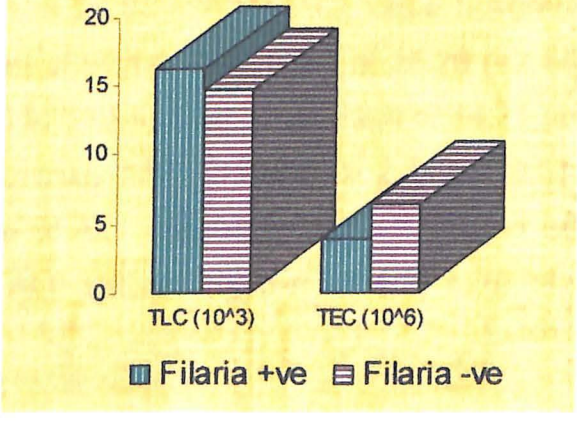
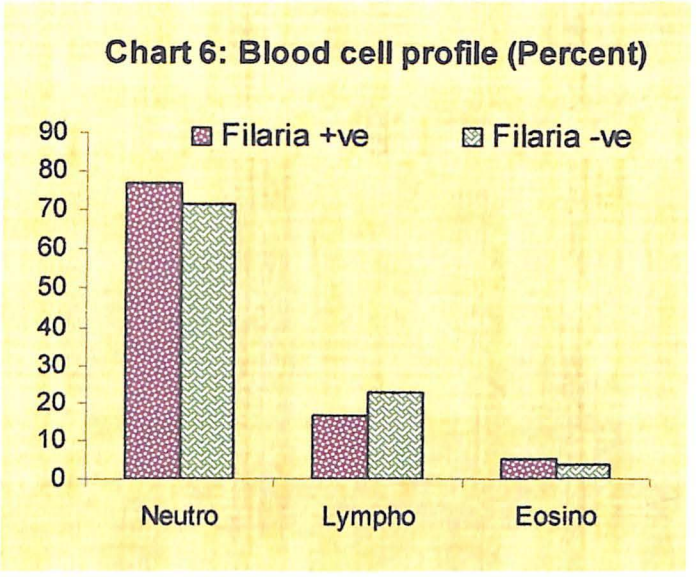


Chart 6 shows the DC profile of infected and control animals. The neutrophil and eosinophil percentage shows an increasing trend while lymphocyte obviously shows a decreased percentage. The average percentage of Neutro, Eosino and Lymphocytes were 77.05 (±1.60), 5.19 (±0.49), and 17 (±1.55) respectively.



BIOCHEMICAL STUDY:

Serum enzymatic analysis particularly AST, ALT and ALP was done in 25 dirofilaria positive and equal number of negative dogs. All of them showed significant ($p<0.05$) rise in the affected animals. As shown in Chart 7 and Table 1 AST in positive animals varied from a minimum 8.90 to maximum of 44.52 with a mean value of 24.83 (±2.15). ALT value hovered between minimum of 3.33 and maximum of 60.1 with a mean of 28.26 (±2.44). Similarly ALP value ranged from 16.86 to 110.43 with an average of 44.21 (±4.81). However, in the negative control animals AST varied from 2.44 to 19.58 with average of 7.61 (±0.74). The value of ALT ranged between 6.23 and 36.06 with average16.4 (±1.79). Similarly ALP value ranged from 4.17 to79.96 with a mean value of 14.38(±2.96).

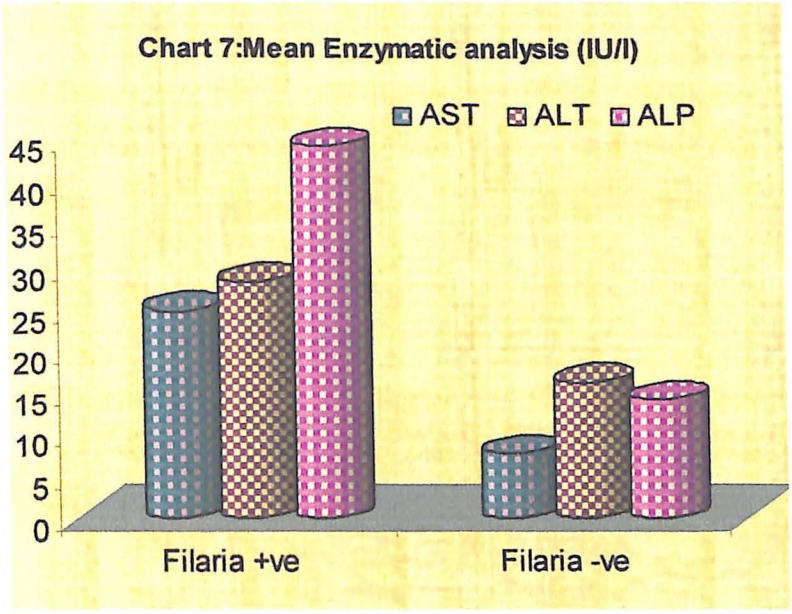
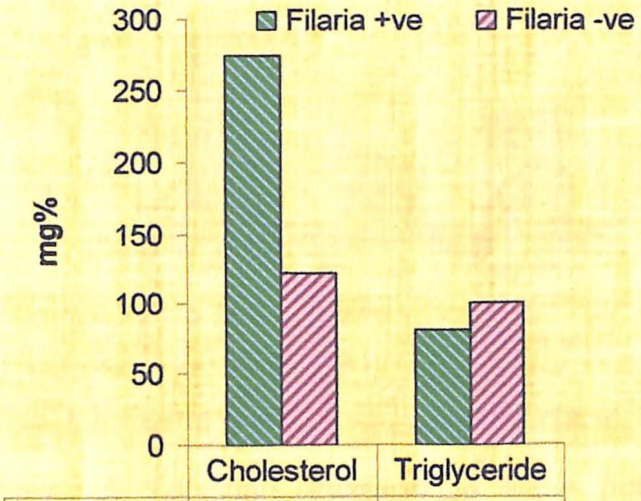


Table 1: Enzymatic analysis (Mean values)

	Filaria + ve	Filaria - ve
AST	24.83 (±2.15)	7.61(±0.74)
ALT	28.26 (±2.45)	16.40 (±1.79)
ALP	44.22 (±4.81)	14.38 (±2.96)
	SE in parenthesis	

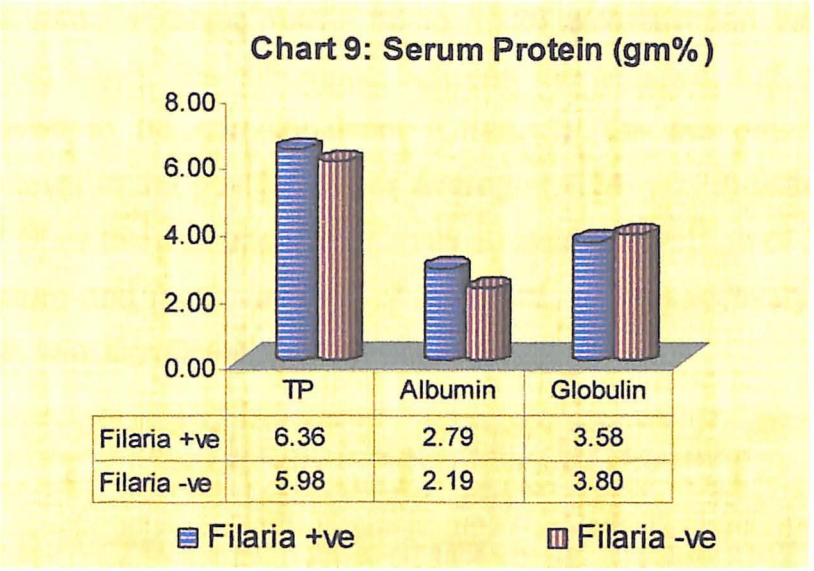
The average cholesterol value in filarial positive dogs was 274.14 (±2.57) ranging from 250.15 to 293.78. The triglyceride value ranges from 71.07 to 91.87 averaging 81.15 (±1.40). In contrast, the negative control showed cholesterol to vary from 89.17 to 159.46 with average of 122.381(±3.30) while triglyceride fluctuated between.71.70 and 119.44 with the average 101.06 (±3.21). The cholesterol showed significant rise in positive animals in comparison to the negative animals. But Triglyceride showed a reverse trend – there was a significant decrease in the value in the affected dogs (Chart 8).

Chart 8: Cholesterol & Triglyceride Value



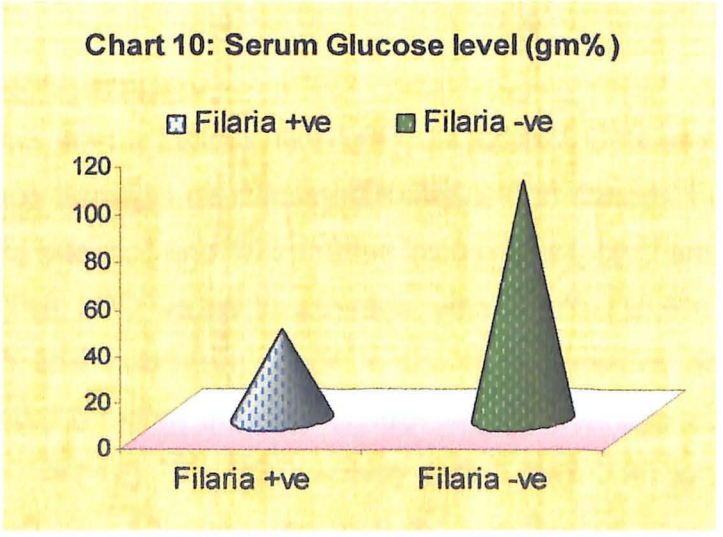
	Cholesterol	Triglyceride
Filaria +ve	274.14	81.15
Filaria -ve	122.38	101.06

The Total Protein value in positive dogs varied between 3.31 and 11 with average of 6.36 (±0.37) while in negative dogs the same value ranged from 4.76 to 7 with a mean value of 5.98 (±0.13), which shows non-significant (Chart-9). There was a significant rise ($p<0.05$) in albumin level in dirofilaria affected dogs with a value ranging between 2.62 and 3.13 with an average value of 2.78 ±0.12.than in non-infected dogs where value ranges from 1.4 to3 with averaging at a value of 2.18 (±0.08). In other hand, the Globulin level showed a significant increase in non-affected dogs with values hovering from 3.28 to 4.16 with a mean value of 3.79(±0.05)(chart-9).



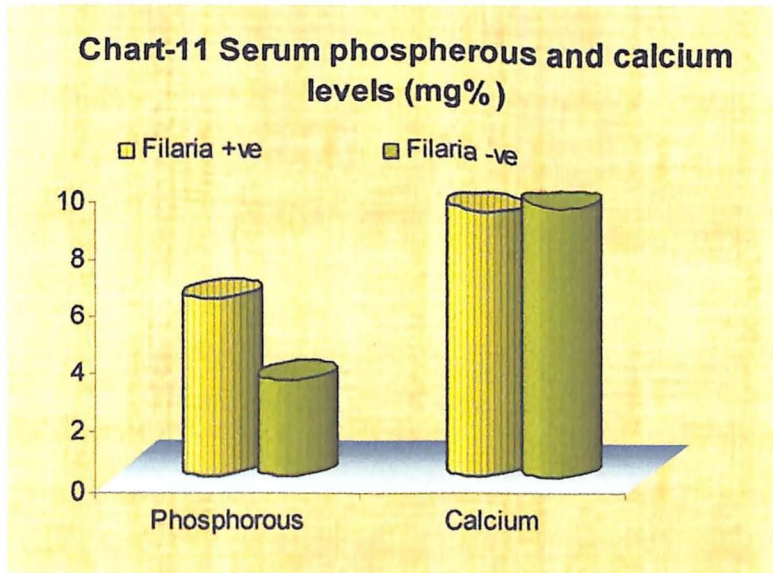
Creatinine showed a significant increase in filaria positive animals in comparison to that of negative animals.

Serum glucose profile revealed a significant reduction in the filarial affected dog with a mean value of 38.71 (±4.17) against the negative animal with an average level of 100.79 (±3.32) (Chart.10).The maximum and the minimum values of affected and non-affected dogs were 81.81 to 12.29 and 129.04 to 69.54 respectively.



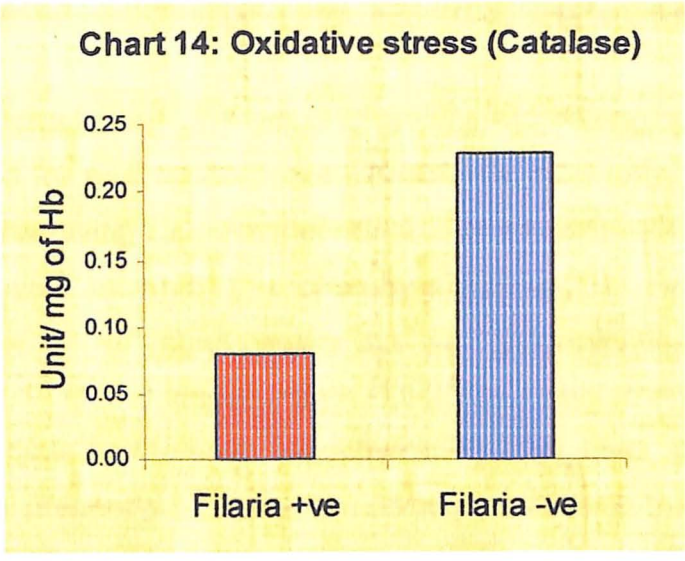
Calcium concentration in serum in filarial positive dogs hovered between 7.52 and 11. 3 mean being 9.34 (±0.25) where as in the non-affected animals

the value covered a range from 7.83 to 11.26 with a mean value of 9.86 (±0.16). Nevertheless, the difference between the affected and non-affected animals proved to be non-significant (Chart.11). On the other hand, the phosphorus level in the positive cases averaged 6.14 (±0.28) with a range of 3.85 to 8.17 while the non-affected animals showed an average of 3.27 (±0.08) with a minimum and maximum limit of 2.48 and 4.10 respectively (Chart.11). This variation was significant at 5% probability.



OXIDATIVE STRESS STUDY:

Oxidative stress indices like LPO, SOD and Catalase were done to assess the tissue damage caused by dirofilariasis in canine. For this purpose 16 samples from affected and 10 samples from non-affected animals as control were utilised. The LPO value in average was 27.76 (±0.53) with range of minimum 22.55 and maximum 31.64 in the filaria positive dogs which was significantly ($p<0.05$) higher than the control with an average level of activity at 19.46 (±1.22) (Chart 12). The SOD activity varied from 0.36 to 2.72 with mean ±SE at 1.96 (±0.19). Although this activity was higher than in the control counterpart showing an average of 1.47 (±0.24) of activity the difference was non-significant (Chart 13). The Catalase level in the affected dogs, on the other hand, hovered between 0.04 and 0.21 mean ±SE being 0.08 (±0.01). The



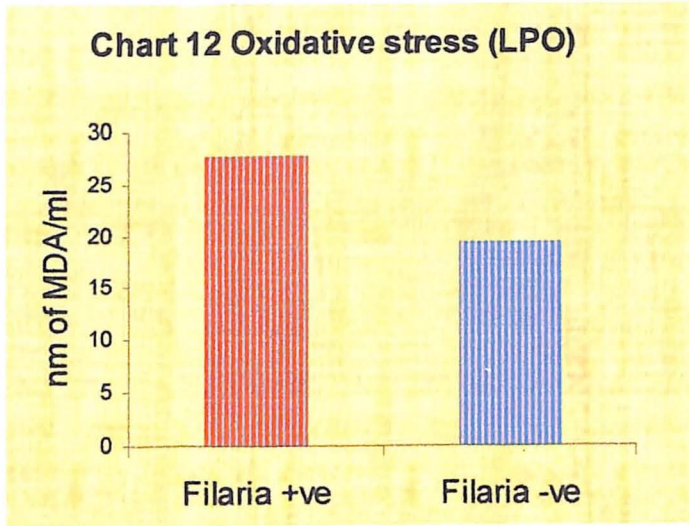
Post-mortem Findings:

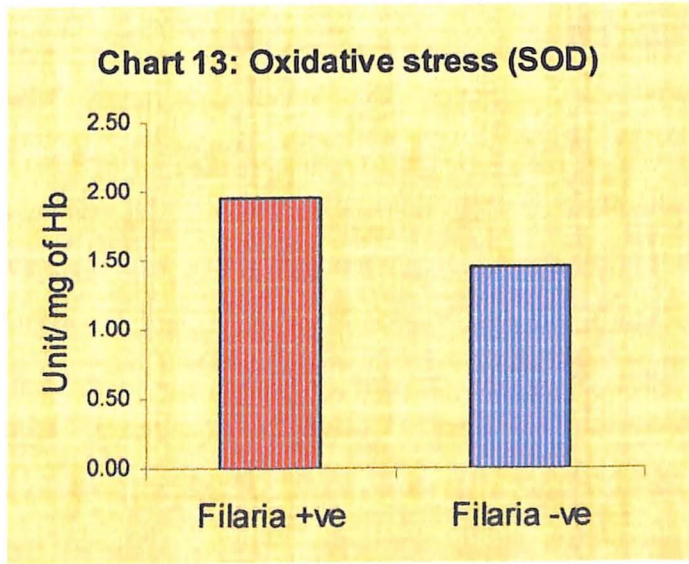
Postmortem examination of 6 no's dogs conducted in the Dept of Veterinary Pathology.

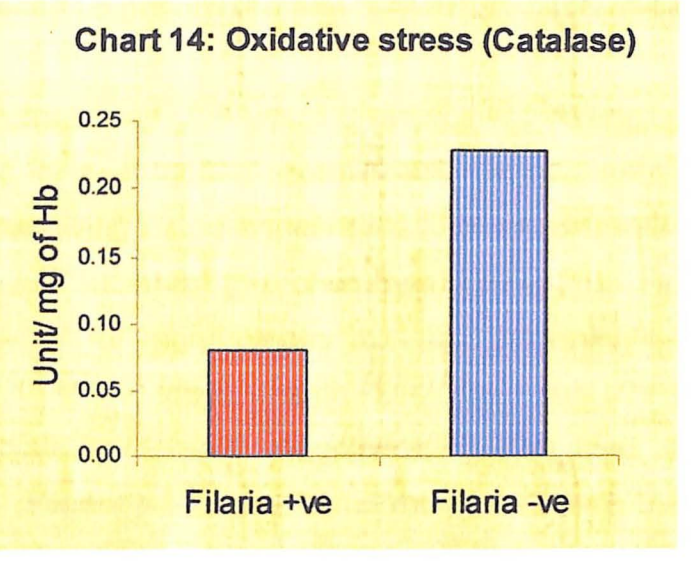
A deshi non-descript female stray dog aged around 5 years brought to the ABC programme died after sterilisation. Externally the dog looked healthy with pendulous abdomen with shrunken eyes and pale oral mucosa (Fig-9). On necropsy there was presence of bloody fluid in the abdominal cavity. Liver was pale and cirrhotic (Fig-10). Lungs showed moderate congestion with oedema and presence of few dirofilaria in the bronchi (Fig-11/12). There was round heart appearance (Fig-13) with right ventricular hypertrophy showing thickening of ventricular walls. (Fig-14) and presence of adult dirofilarial worms. Other post-mortem findings were proliferative, hemorrhagic and ulcerative stomach (Fig-15), proliferative enteritis and haemorrhagic mesentery. Kidney was pale with easy peeling of capsules. Case was diagnosed as dirofilariasis

Necropsy of a female police dog of breed Labrador aged around 9 years revealed congestion, haemorrhage and marked oedema along with presence of dirofilarial worms in the lungs (Fig-16). Trachea was filled with frothy exudates (Fig-17). Heart revealed round appearance and filled with few adult heart worms. Splenomegaly was also evident (Fig-18). Hemorrhagic ulcers noticed in the stomach mucosa and there were linear hemorrhagic streaks in the intestine (Fig-

control non-affected animals showed the activity within 0.09 and 0.41 with an average of 0.23 (±0.03). The difference was significant at α 0.05 (Chart 14).







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19). Case was diagnosed as pneumonia and hemorrhagic enteritis associated with dirofilariasis

A Doberman female dog of 9 years 8 months old belonging to police dog squad was presented for post mortem examination. On necropsy, heart showed ventricular hypertrophy giving round appearance to the heart) with bunch of adult dirofilaria worms found in both the chambers713(Fig-20). Recovered adult parasites were subjected for morphometry and sex determination. Short length (12-16 cm) with coiled tail and longer length (25-30 cm) with straight tapering tail were determined as male and female respectively(Fig-20a). Liver showed cirrhotic changes. There was presence of linear hemorrhagic steaks in the stomach with hemorrhagic enteritis. Case was diagnosed as hemorrhagic enteritis associated with dirofilariasis

Another male police dog of Labrador breed aged around 6 years brought for necropsy. External examinations showed pale mucosa with corneal opacity(Fig-21). There was soiling of anus with reddish colured liquid faeces. P.M findings showed presence of adult worms in the heart. Congested and hemorrhagic lungs (Fig-22) along with pale kidney with rough surfaces (Fig-23) were noticed. There were hemorrhagic contents in the intestine (Fig-24) along with haemorrhages in the urinary bladder mucosa (Fig-25). There was Recovery of few hook worms from the intestinal contents. Liver showed cirrhotic changes. Case was diagnosed as hemorrhagic cystitis and hemorrhagic enteritis compounded with dirofilariasis.

Necropsy of a female pug dog aged around 7 years of a private owner revealed pale mucous membrane of oral cavity. Right ventricular hypertrophy with adult worms recovered from the right ventricle. Liver showed congestion with pale patches (Fig-26). Lungs were oedematous and there was thickened wall of the stomach and intestine with catarrhal exudates(Fig-27). Case was diagnosed as catarrhal gastro enteritis compounded with dirofilariasis.

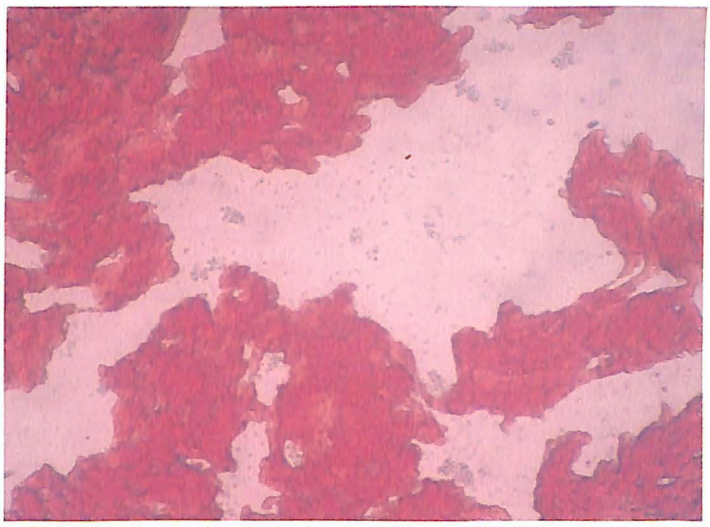
Similarly another male Labrador dog of about 6 years age belonging to police dog squad brought for P.M. examination. Carcass was weak and emaciated (Fig-28).with atrophied muscles and prominent skeleton(Fig-29). Heart was slightly enlarged with few adult worms along with epicardial haemorrhage (Fig-30). Lungs

were congested and oedematous with hemorrhagic patches. There was ulcerative and hemorrhagic gastroenteritis. Case was diagnosed as hemorrhagic gastro enteritis compounded with dirofilariasis

Histopathological Findings:

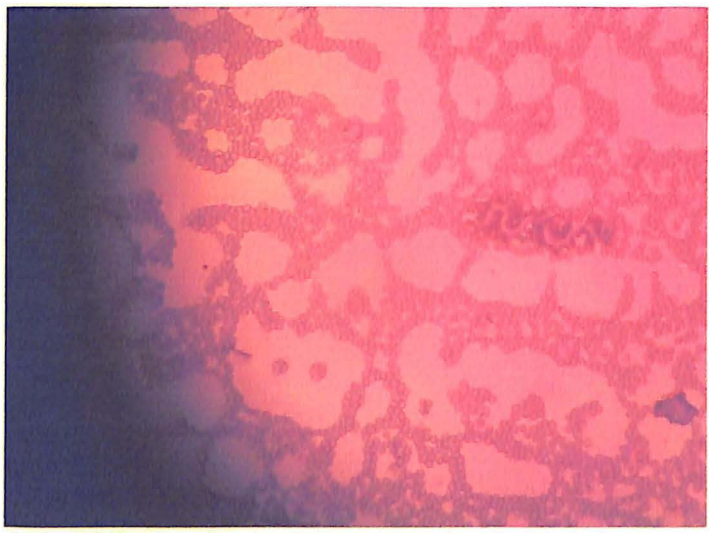
The histopathological alterations in all the animals were more or less similar except stomach, intestine and urinary bladder. In liver there was diffuse infiltration of mononuclear cells through out (Fig-31/32), fibrotic proliferation at the peripcrtal area (Fig-33) and in some cases centrilobular necrosis with individualisation of hepatocytes with loss of hepatic cord arrangement (Fig-34). In some cases there was fibrotic proliferation of the hepatic parenchyma with indivisualation of hepatocytes and disruption of hepatic chords (Fig-35). There was congestion, haemorrhage and oedema of myocardium (Fig-36) and in one case in the epicardium (Fig-37) of heart. Lungs revealed interstitial congestion and haemorrhages. Presence of edematous fluid in the alveoli (Fig-38) was also evident. In some cases, lungs revealed thickening of interalveolar spaces with oedema and infiltration of inflammatory cells predominantly with plasma cells and monocytes (Fig-39). Few cases of lungs showing interstitial pneumonia with thickened septal wall due to congestion, fibrosis and cellular infiltration and hemorrhage (Fig-40) In a singular case cellular infiltration in the peribronchiolar area was noticed (Fig-41). In another case thickened blood vessel wall of the lungs with proliferation of T. media (Fig-42). The kidney showed atrophy of glomeruli with few glomeruli were empty(Fig-43). Glomerular degeneration and atrophy of glomerular tuft with increased bowman's spaces. Besides this there was thickened glomerular basement membrane with infiltration of mononuclear cells (Fig-44)).Interstitial infiltration of chronic inflammatory cells like plasma cells and mononuclear cells also seen in kidneysFig-45). Oedema of the spleen around the trabeculae (Fig-46) was also evident. Besides these other histopathological changes were hemorrhagic exudates on the mucosa of the intestine with desquamation of epithelial linings(Fig-47, 48).

Figure-1



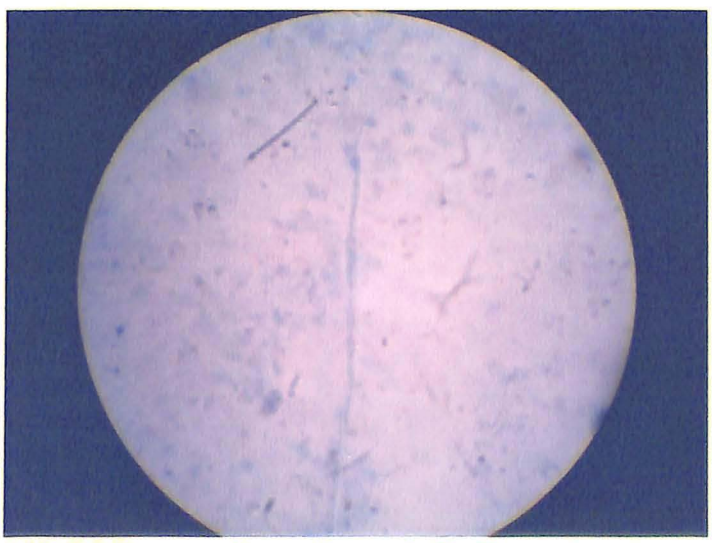
Microfilaria of *D.immitis* in Wet blood smears examination.

Figure-2



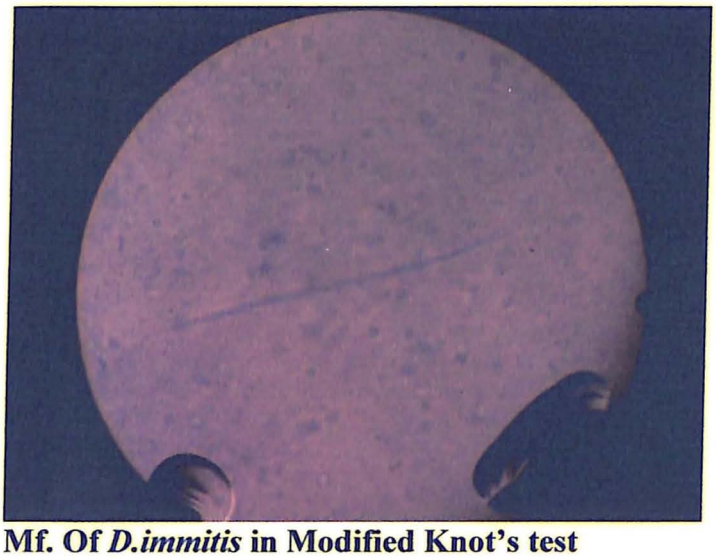
Mf. Of *D.immitis* in Giemsa stained thick blood smear

Figure-3



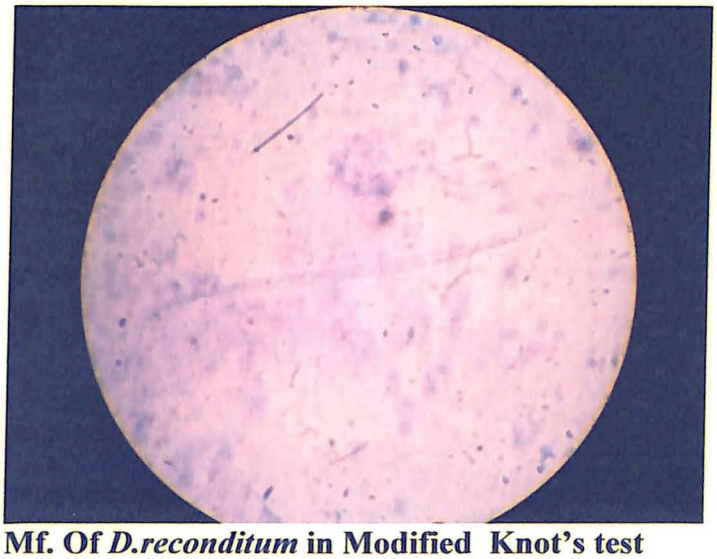
Mf. Of *D.reconditum* through Buffy coat smear

Figure-4



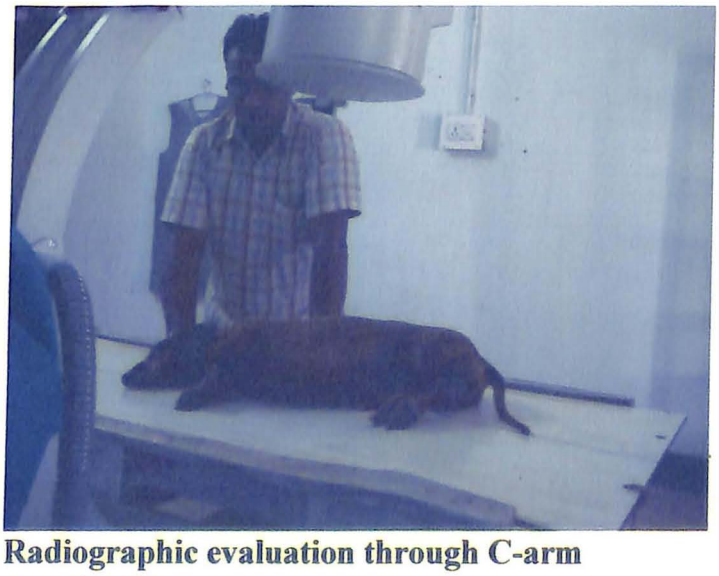
Mf. Of *D.immitis* in Modified Knot's test

Figure-5



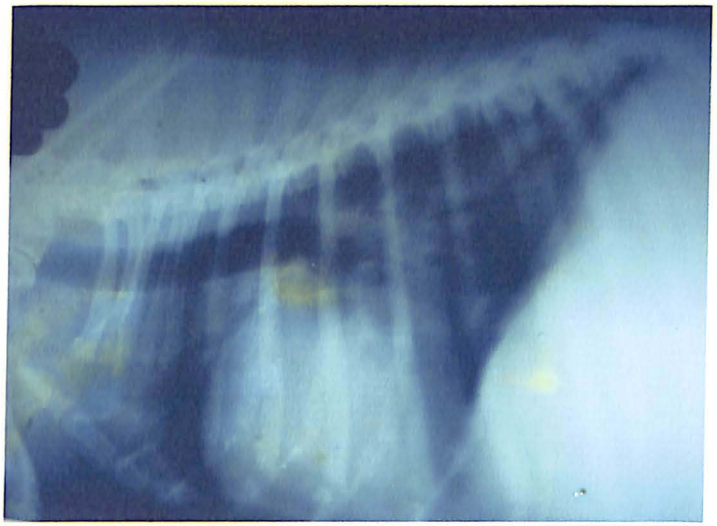
Mf. Of *D.reconditum* in Modified Knot's test

Figure-6



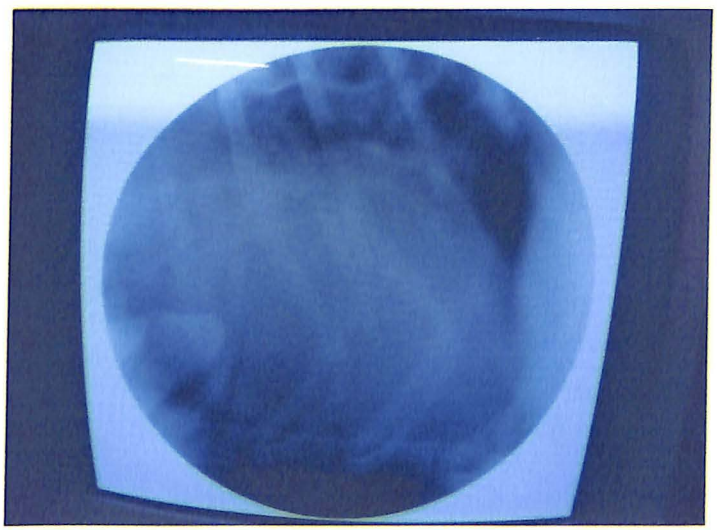
Radiographic evaluation through C-arm examination

Figure-7



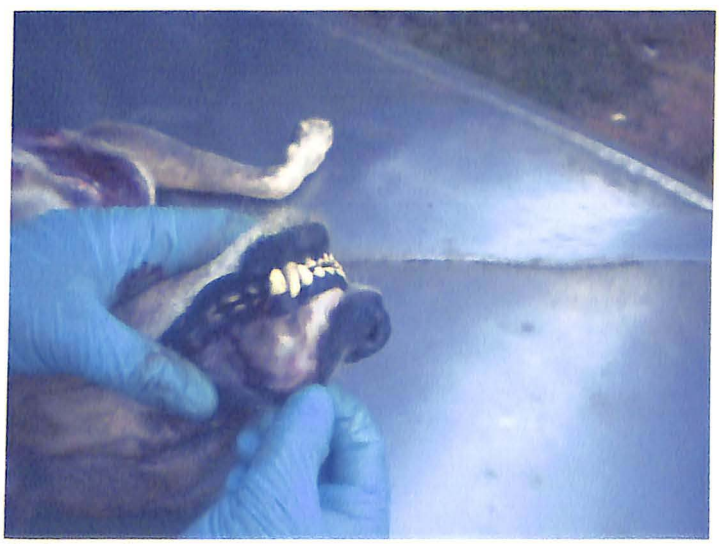
Cardiomegaly,round heart suggestive of right ventricular hypertrophy through C-arm examination

Figure-8



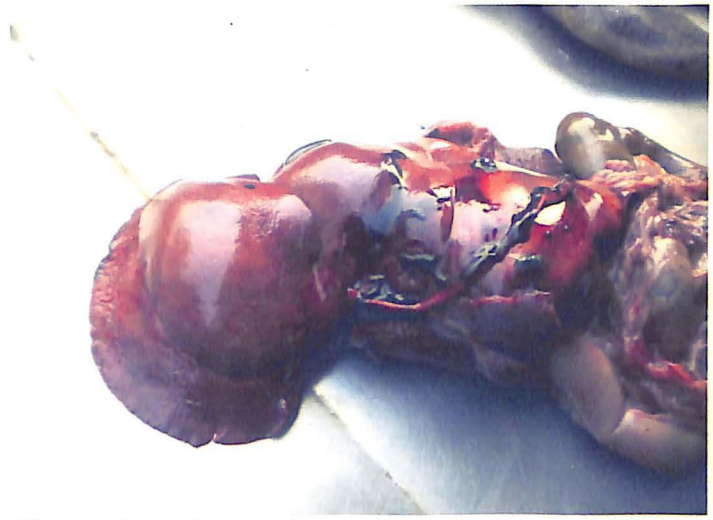
Right ventricular enlargement revealed by C-arm

Figure-9



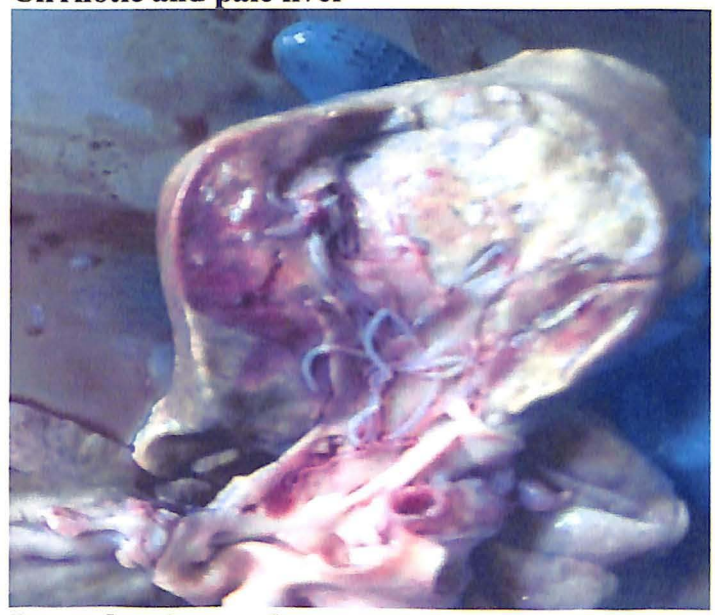
Pale oral mucosa

Figure-10



Cirrhotic and pale liver

Figure-11



Lung showing moderate congestion, edema and presence of few dirofilarial worms in the bronchi

Figure-12

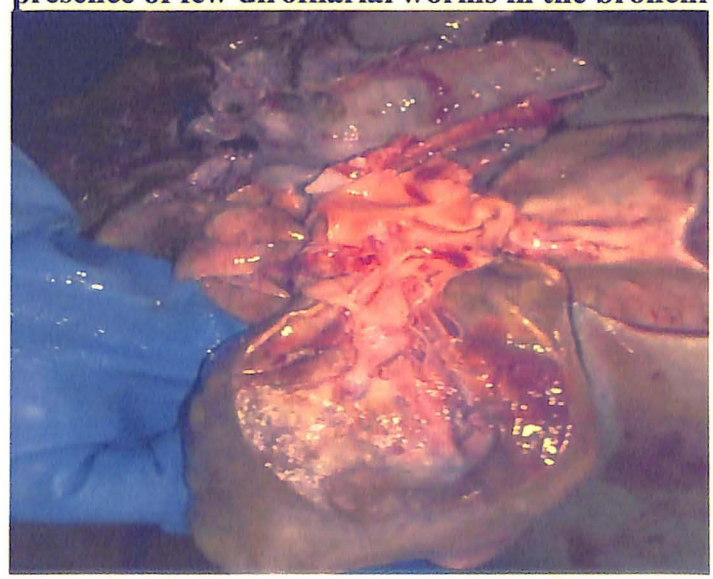


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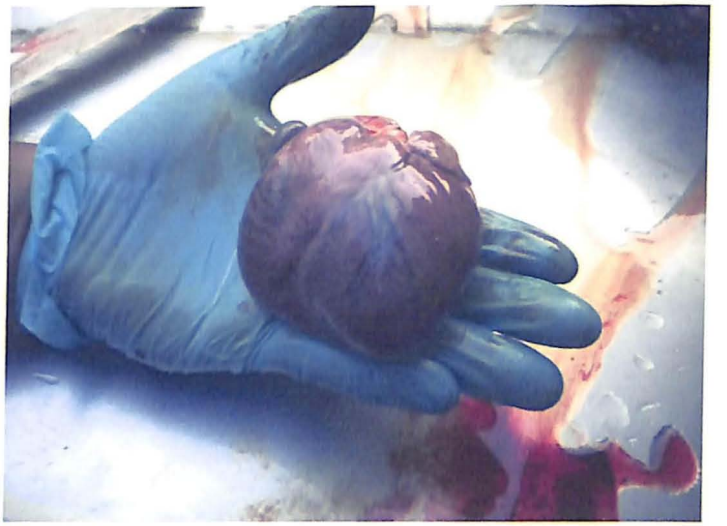
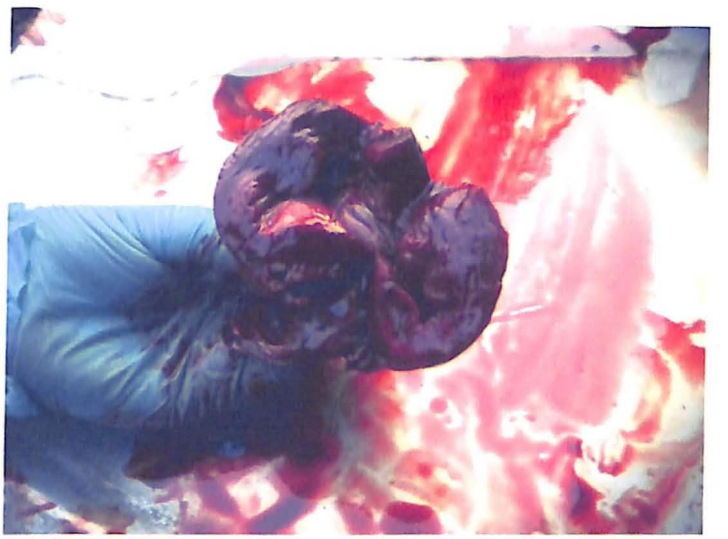
Figure-13



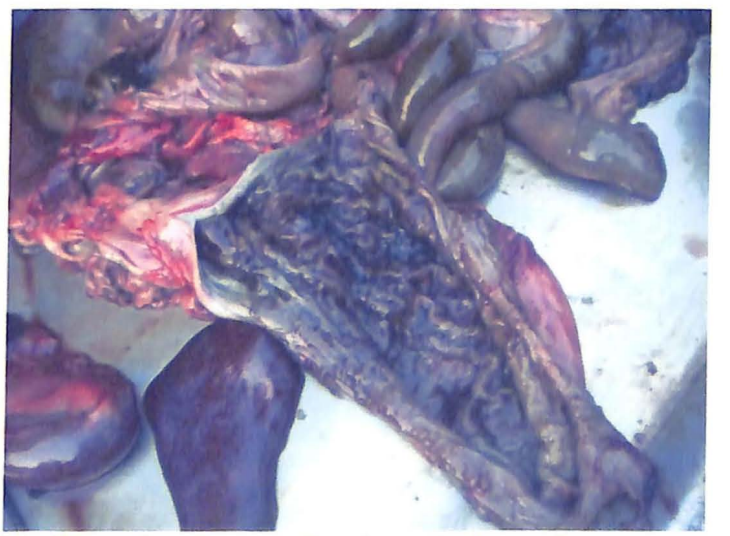
Round heart appearance

Figure-14



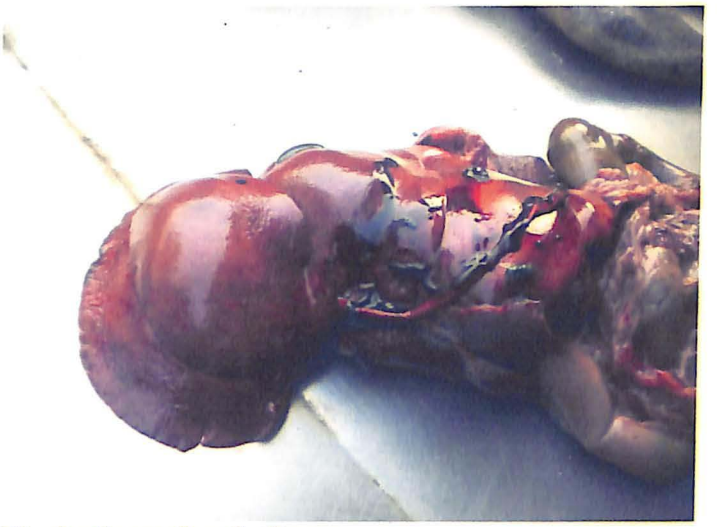
Right ventricular hypertrophy

Figure-15



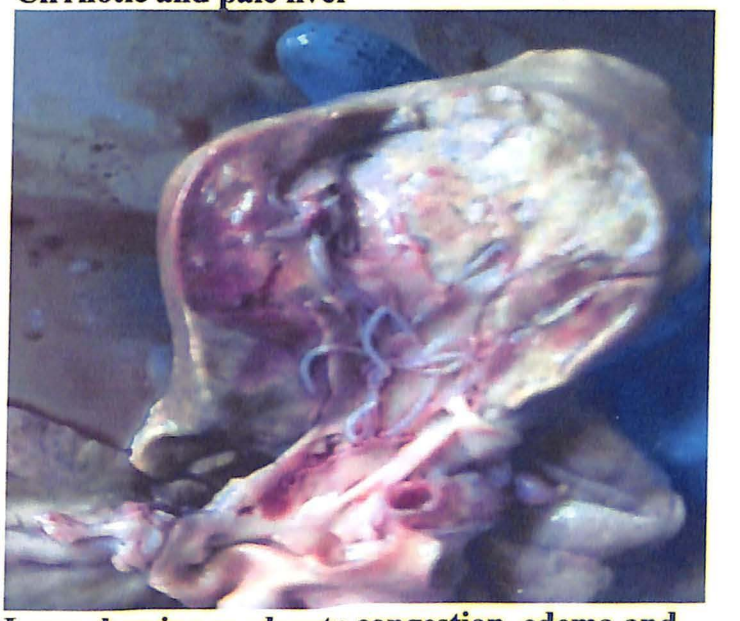
Proliferative, hemorrhagic and ulcerative stomach

Figure-10



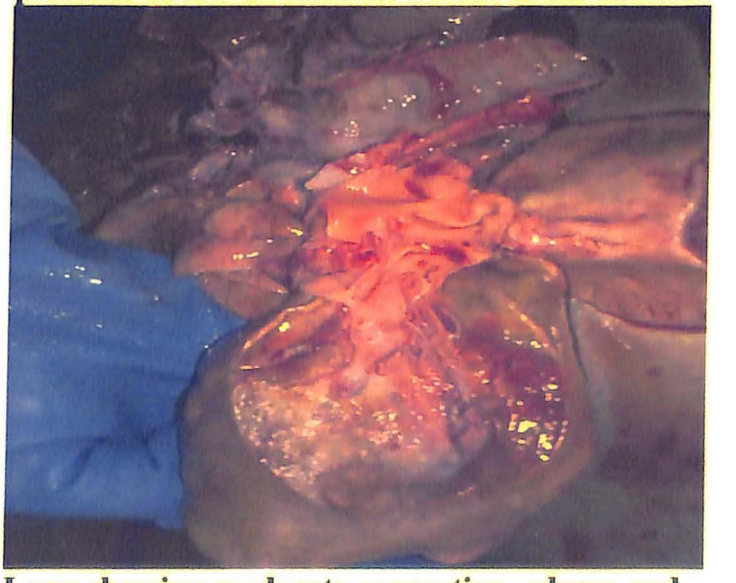
Cirrhotic and pale liver

Figure-11



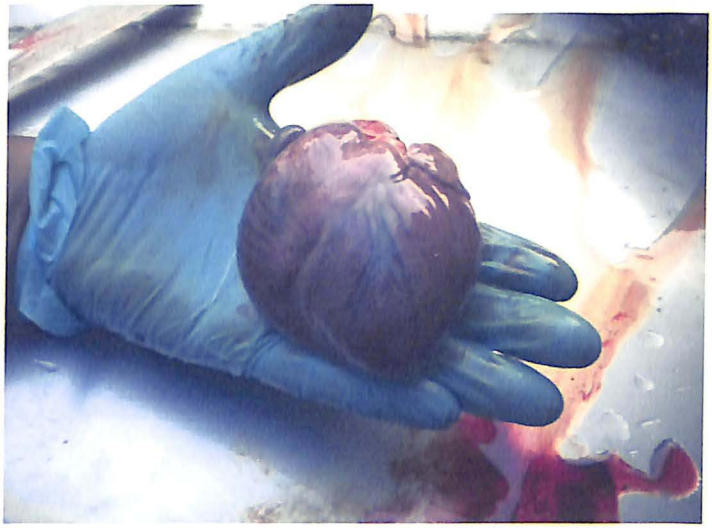
Lung showing moderate congestion, edema and presence of few dirofilarial worms in the bronchi

Figure-12



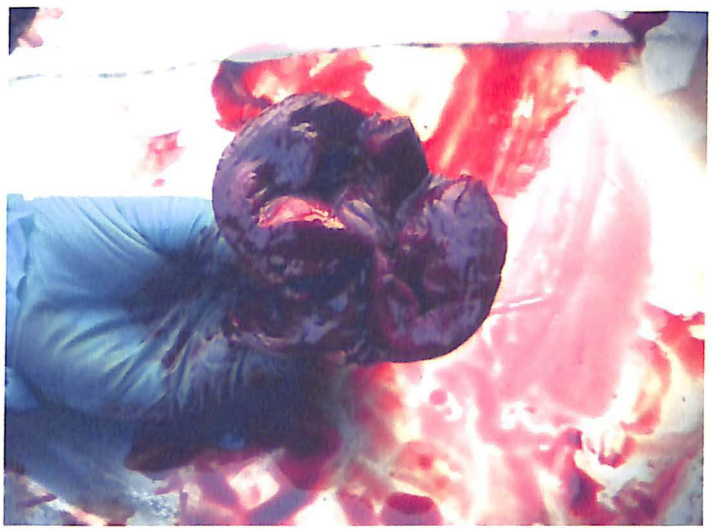
Lung showing moderate congestion, edema and presence of few dirofilarial worms in the bronchi

Figure-13



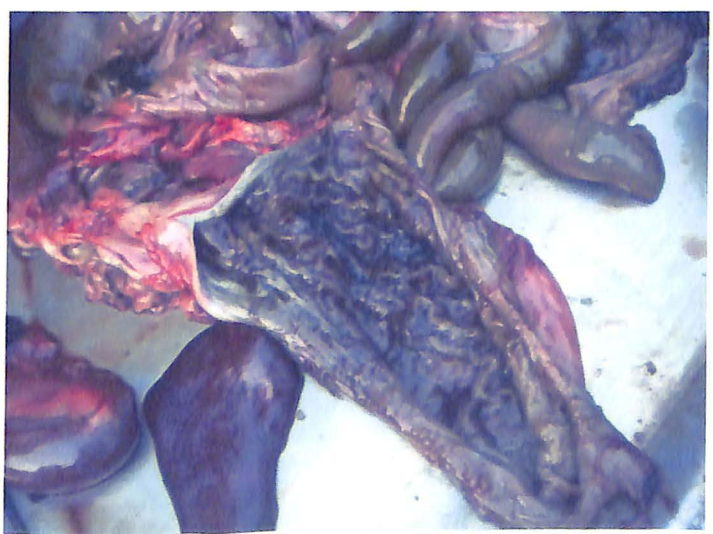
Round heart appearance

Figure-14



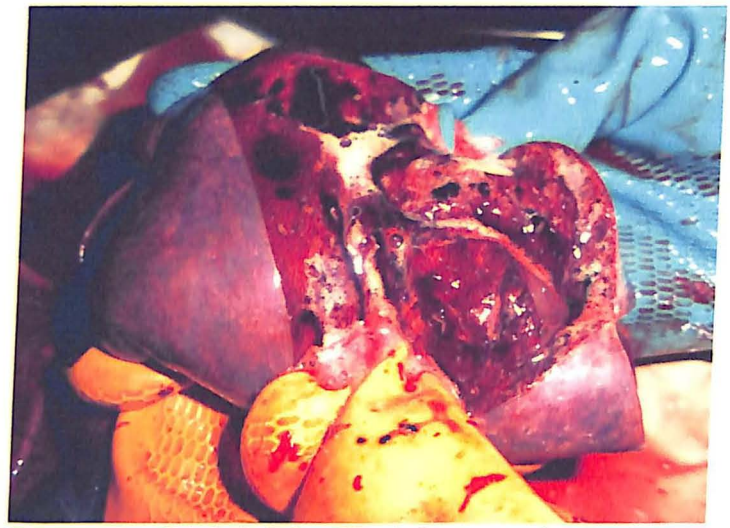
Right ventricular hypertrophy

Figure-15



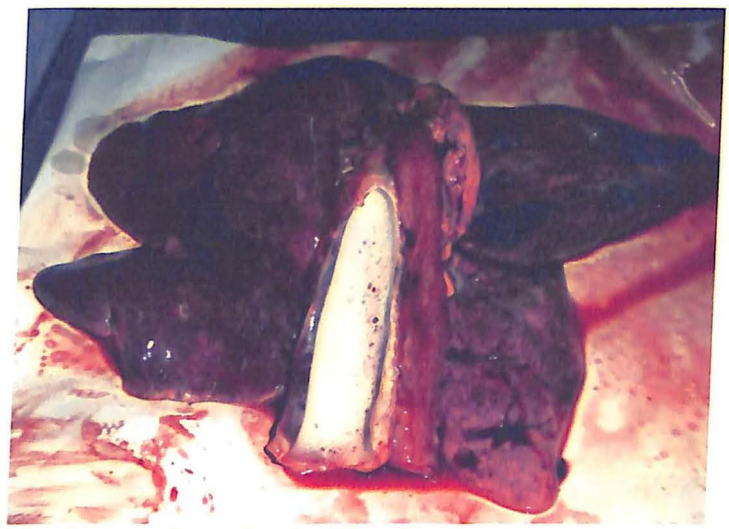
Proliferative, hemorrhagic and ulcerative stomach

Figure-16



Presence of Adult Dirofilarial worms in the lungs

Figure-17

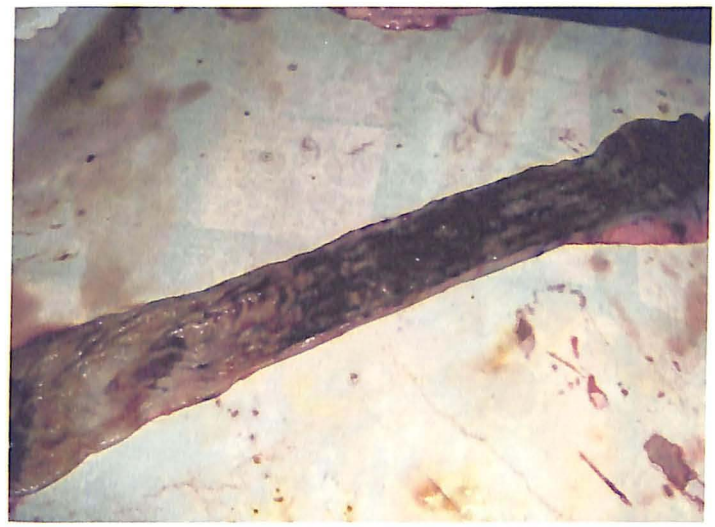


Frothy exudates in the Trachea

Figure-18

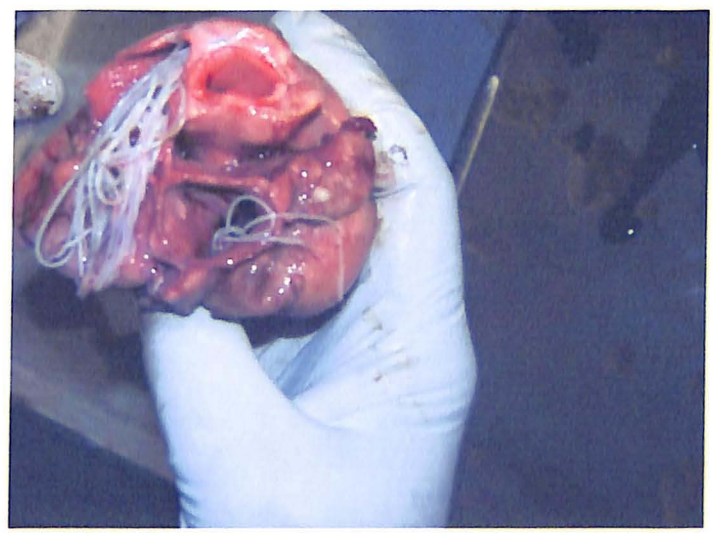
Splenomegaly

Figure-19



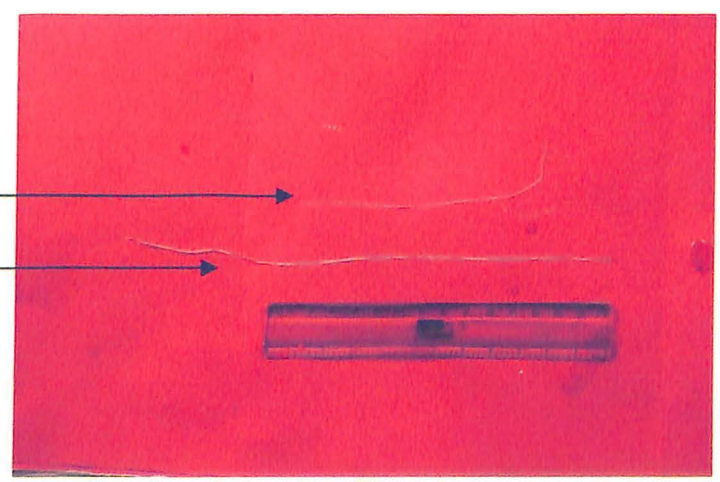
Hemorrhagic streaks in the intestine

Figure-20



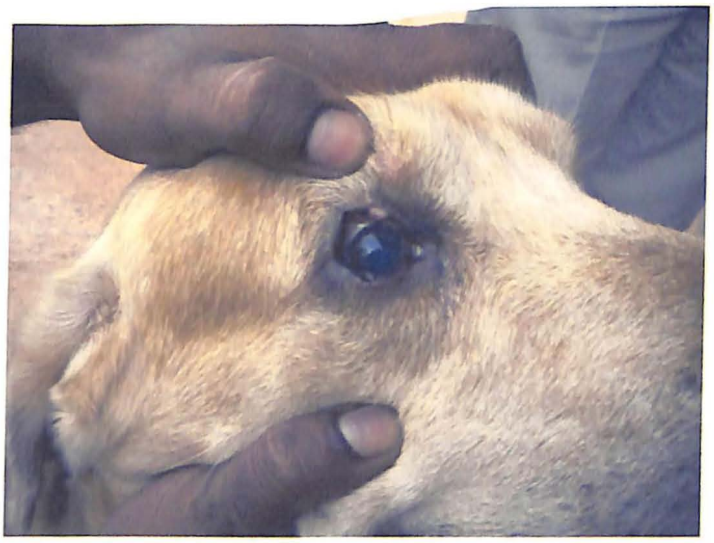
Bunch of adult dirofilarial worms in both chambers of heart with ventricular hypertrophy & Morphometrical measurement of adult parasite

Figure-20(a)



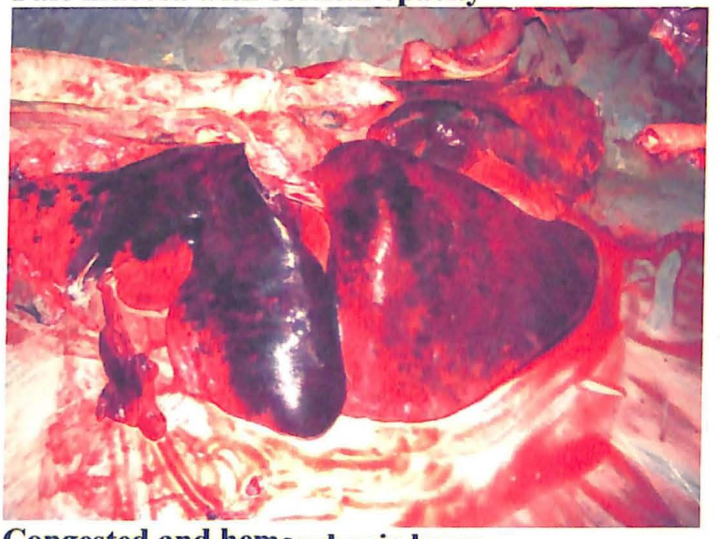
Bunch of adult dirofilarial worms in both chambers of heart with ventricular hypertrophy & Morphometrical measurement of adult parasite

Figure-21



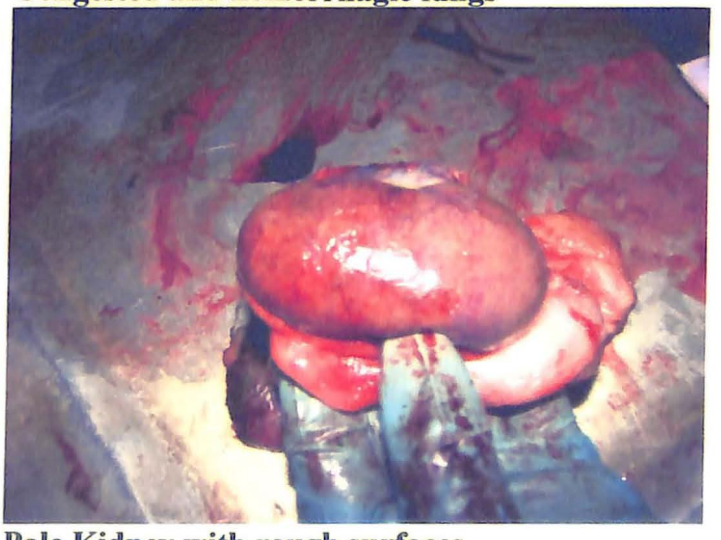
Pale mucosa with corneal opacity

Figure-22



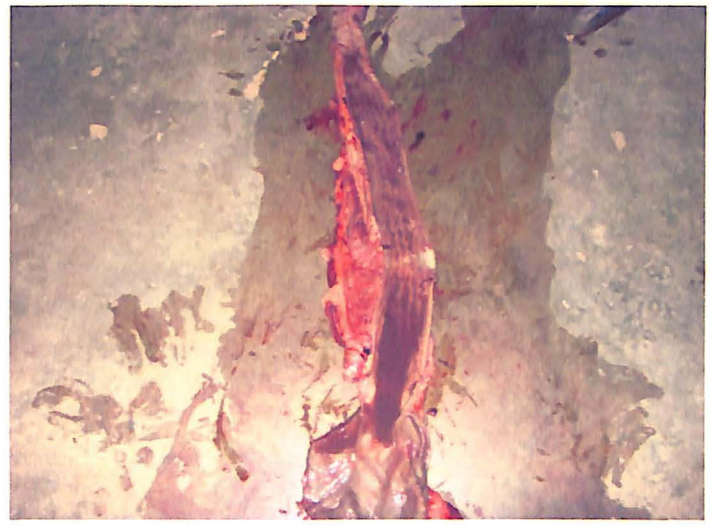
Congested and hemorrhagic lungs

Figure-23



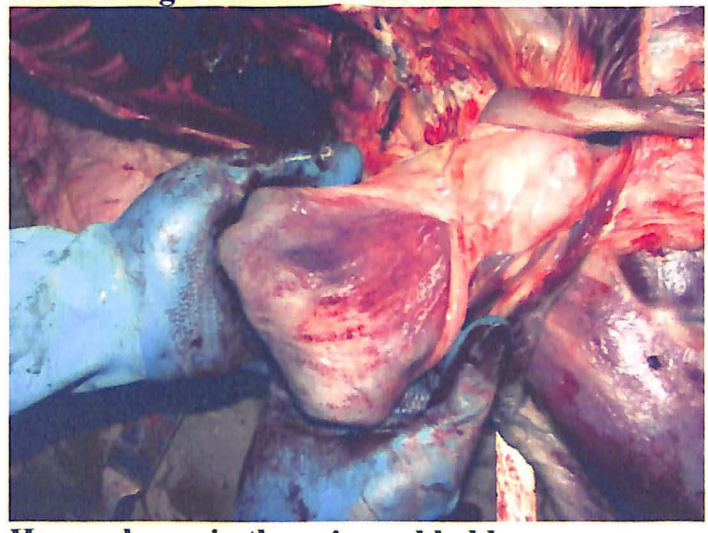
Pale Kidney with rough surfaces

Figure-24



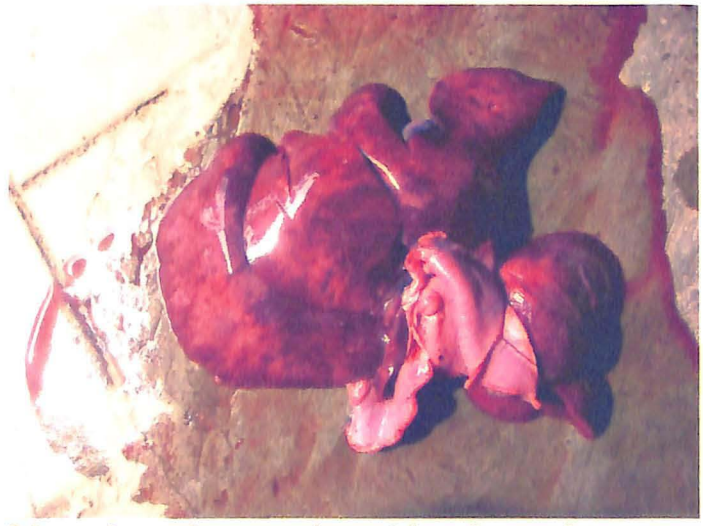
Hemorrhagic contents in the intestine

Figure-25



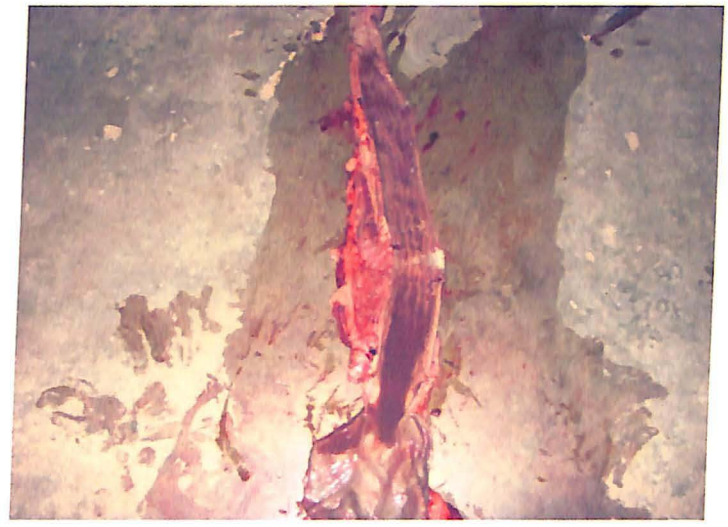
Hemorrhages in the urinary bladder mucosa

Figure-26



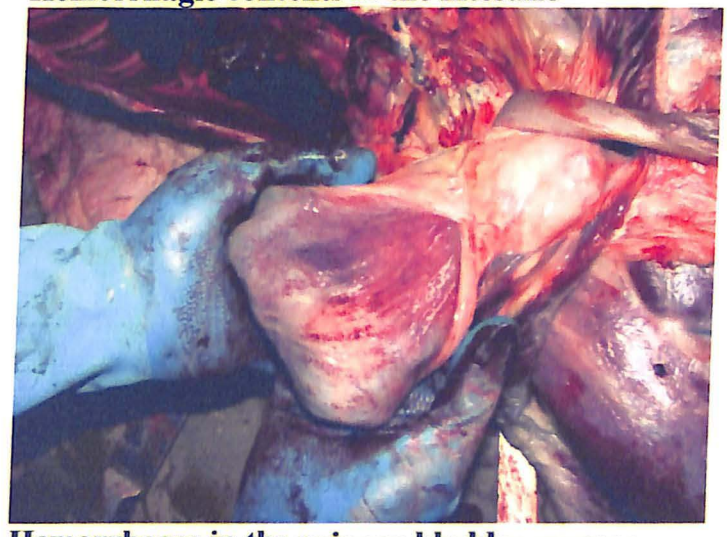
Liver showed congestion with pale patches

Figure-24



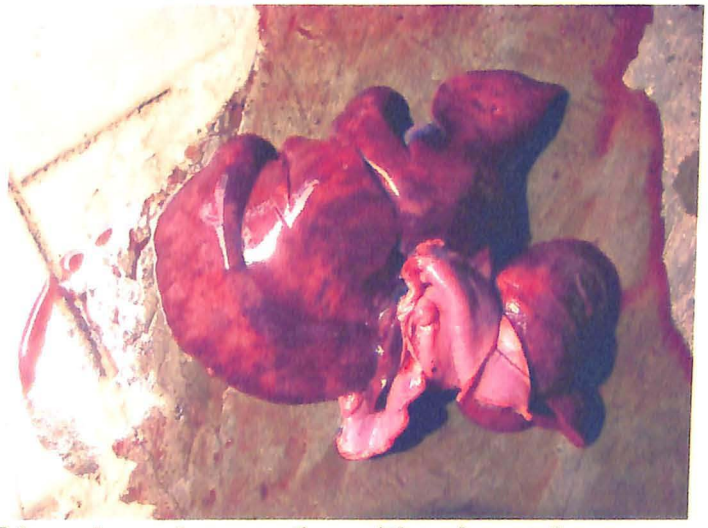
Hemorrhagic contents in the intestine

Figure-25



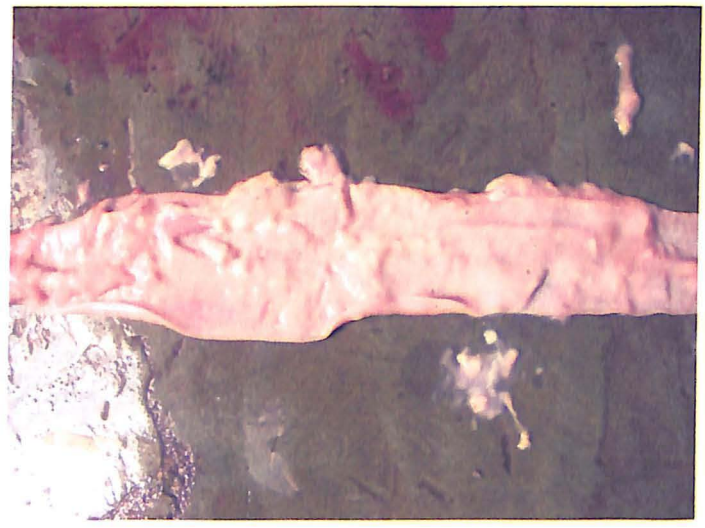
Hemorrhages in the urinary bladder mucosa

Figure-26



Liver showed congestion with pale patches

Figure-27

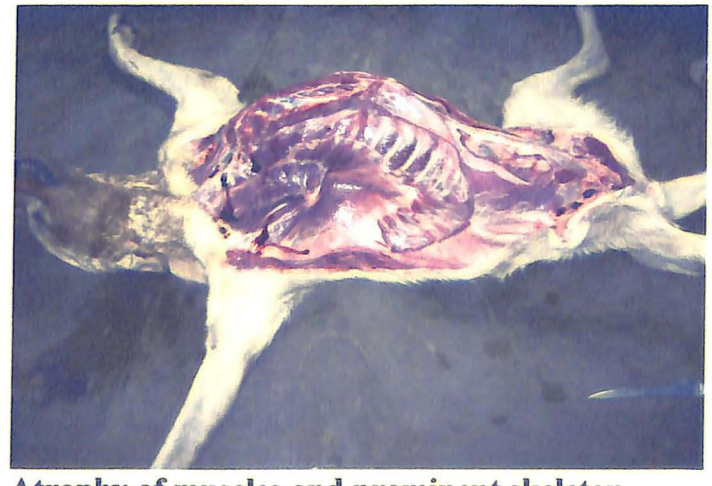


Catarrhal exudates in the intestine

Figure-28

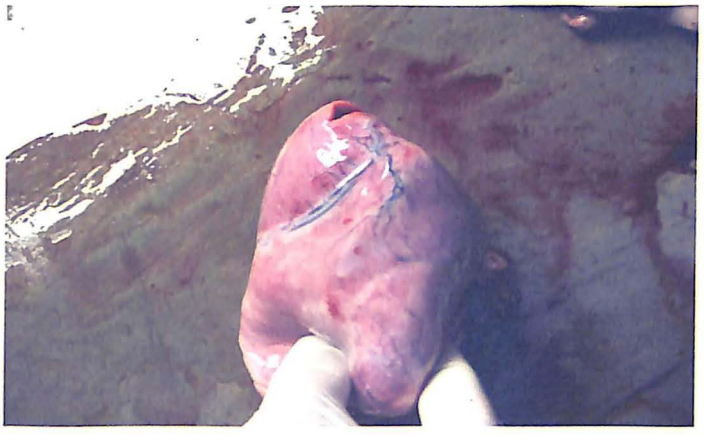
Weak and emaciated carcass

Figure-29



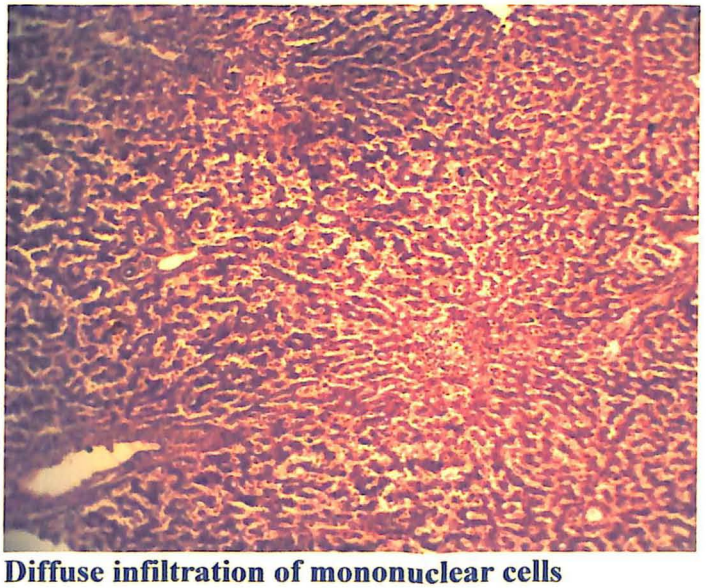
Atrophy of muscles and prominent skeleton

Figure-30



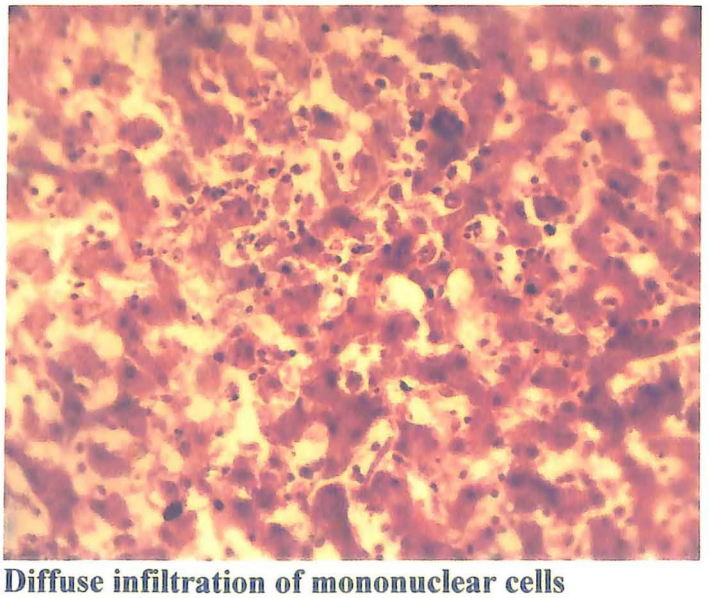
Epicardial hemorrhage

Figure-31



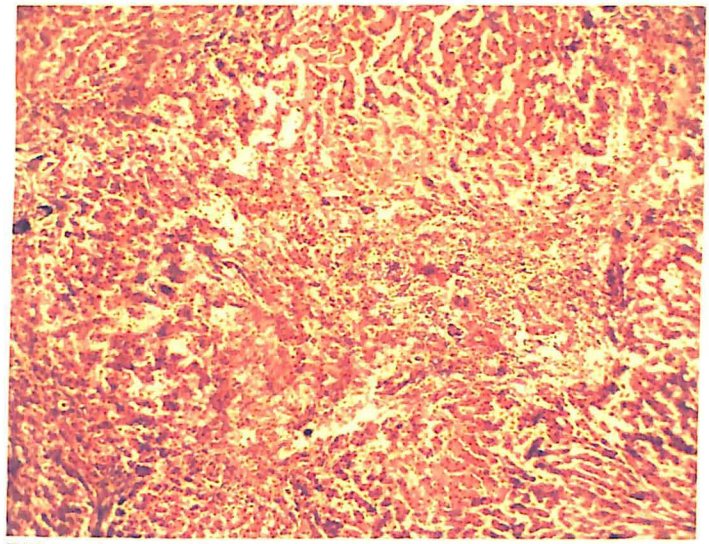
Diffuse infiltration of mononuclear cells throughout Liver H&E x100, H&E x400

Figure-32



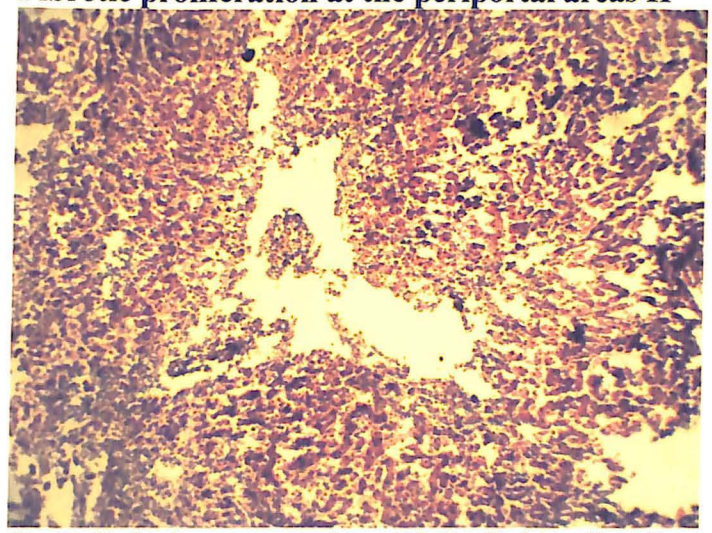
Diffuse infiltration of mononuclear cells throughout Liver H&E x100, H&E x400

Figure-33



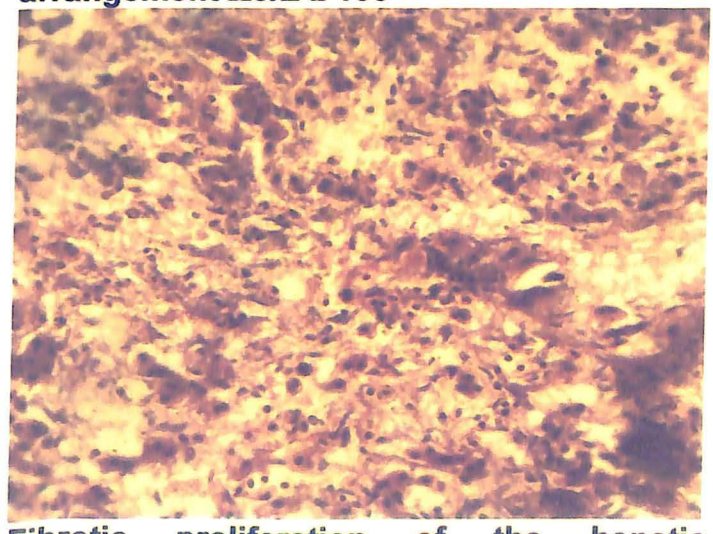
Fibrotic proliferation at the periportal areas H

Figure-34



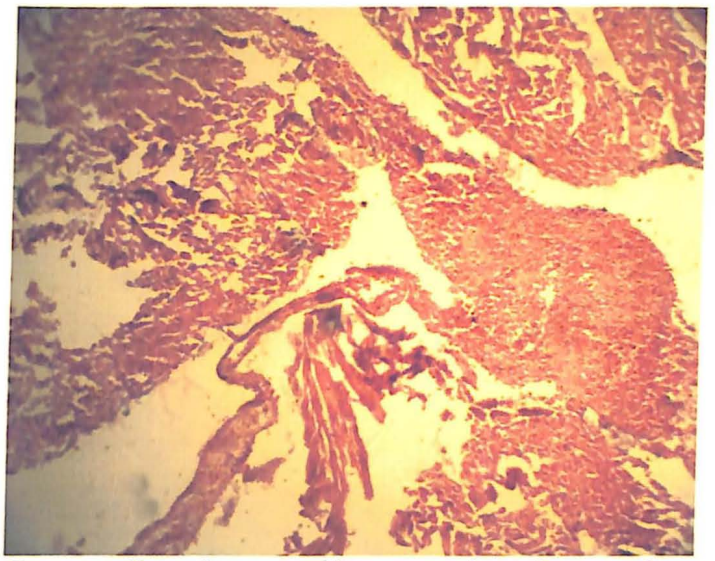
Centrilobular necrosis with individualisation of hepatocytes with loss of hepatic cord arrangement H&E x 100

Figure-35



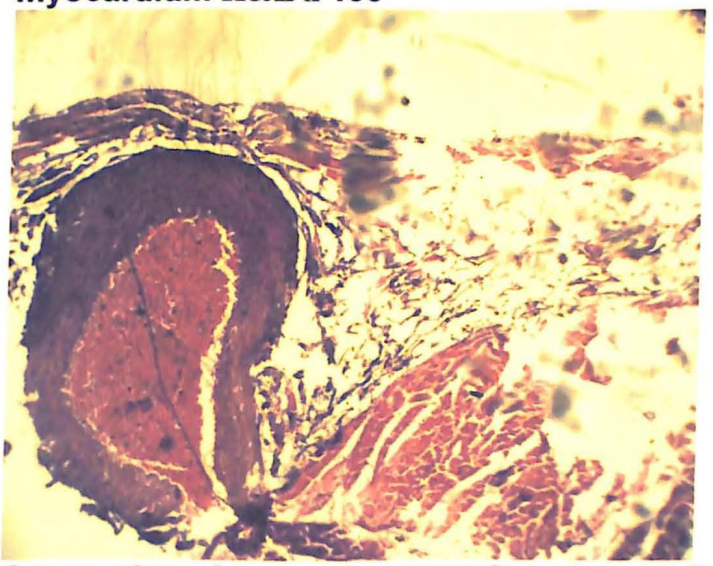
Fibrotic proliferation of the hepatic parenchyma with indivisualation of hepatocytes and disruption of hepatic chords H&E x 400

Figure-36



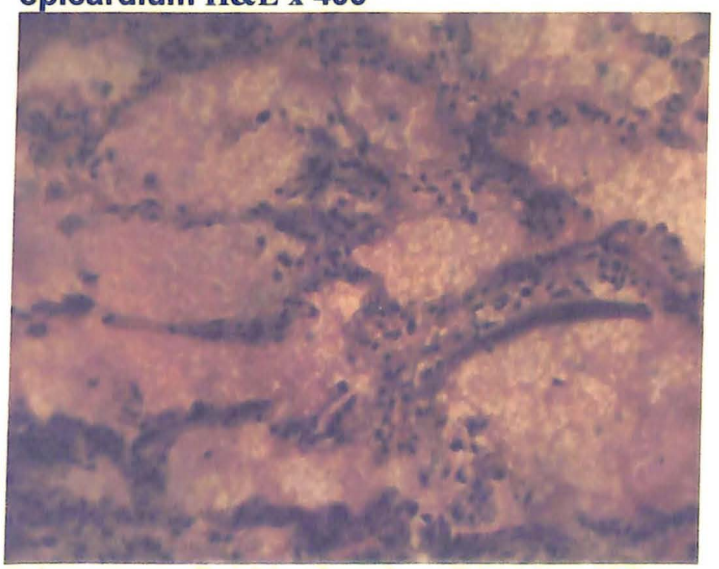
Congestion, haemorrhage and oedema of myocardium H&E x 100

Figure-37



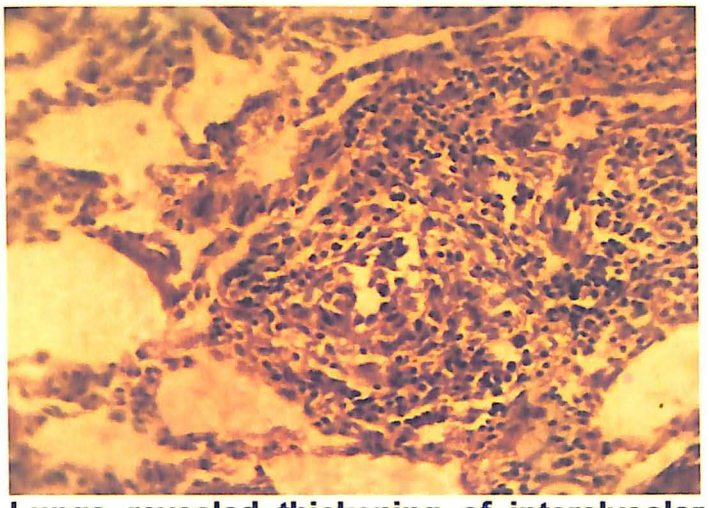
Congestion, haemorrhage and oedema of epicardium H&E x 400

Figure-38



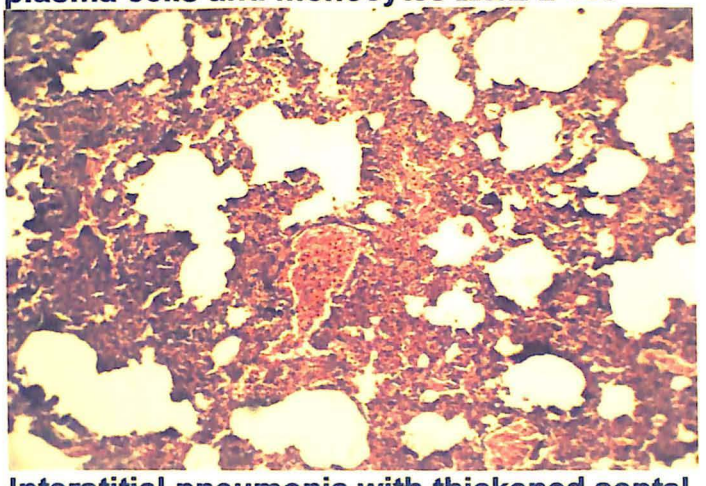
Edematous fluid in the alveoli H&E x 400

Figure-39



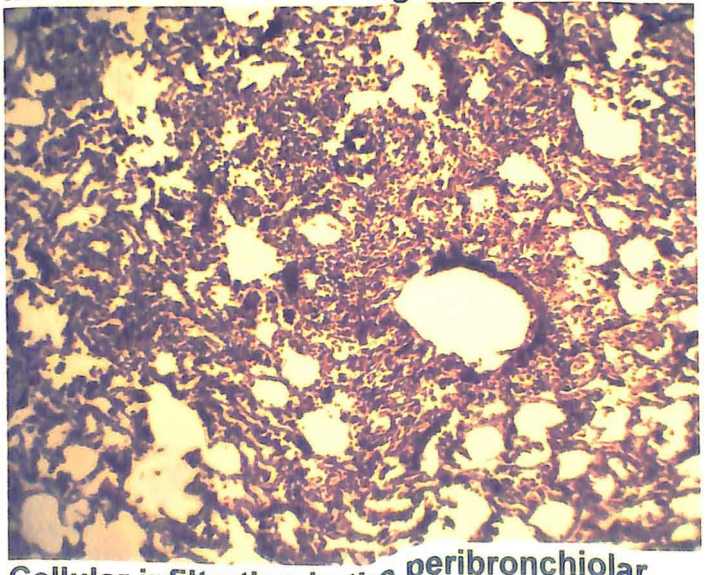
Lungs revealed thickening of interalveolar spaces with oedema and infiltration of inflammatory cells predominantly with plasma cells and monocytes H&E x 400

Figure-40



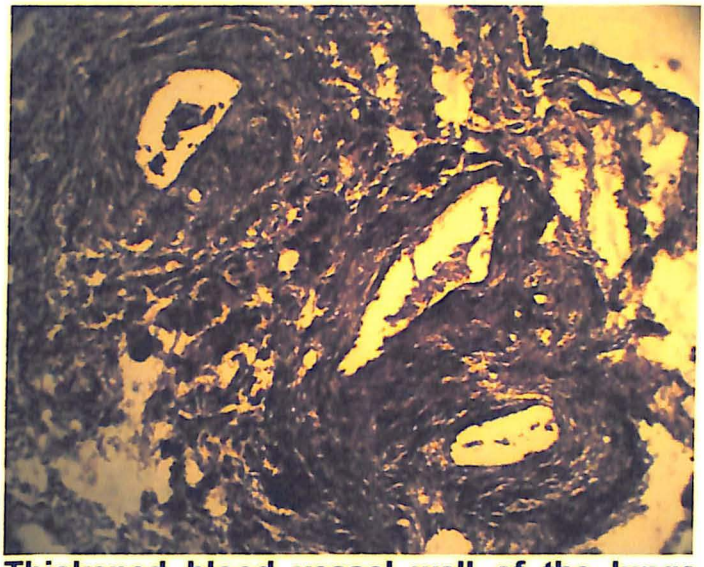
Interstitial pneumonia with thickened septal wall due to congestion, fibrosis and cellular infiltration and hemorrhage H&E x 100

Figure-41



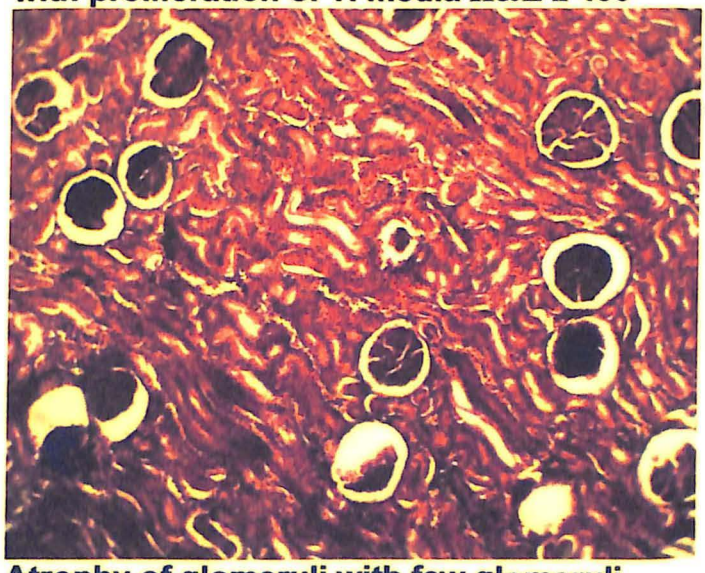
Cellular infiltration in the peribronchiolar area H&E x 100

Figure-42



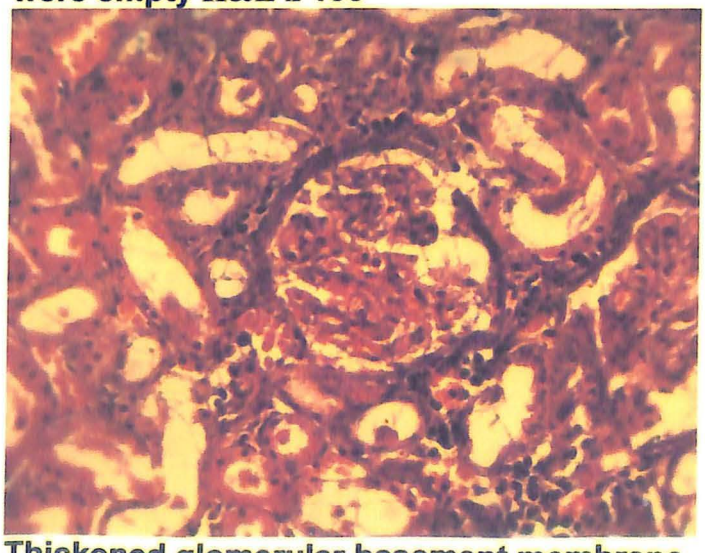
Thickened blood vessel wall of the lungs with proliferation of T. media H&E x 400

Figure-43



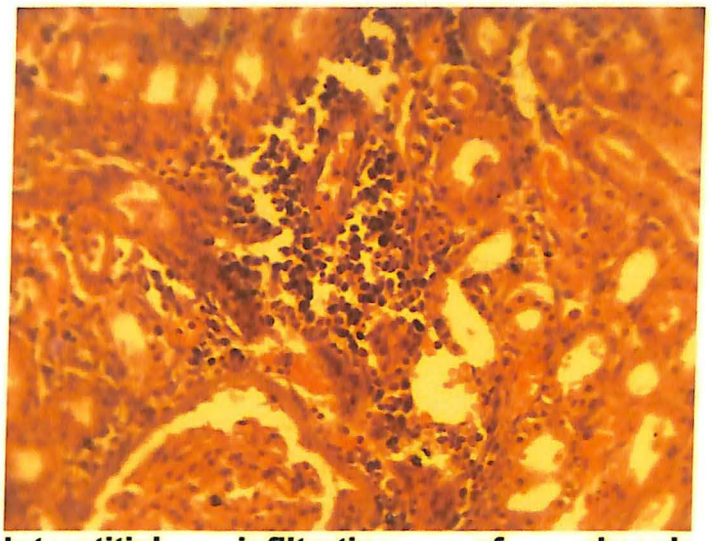
Atrophy of glomeruli with few glomeruli were empty H&E x 100

Figure-44



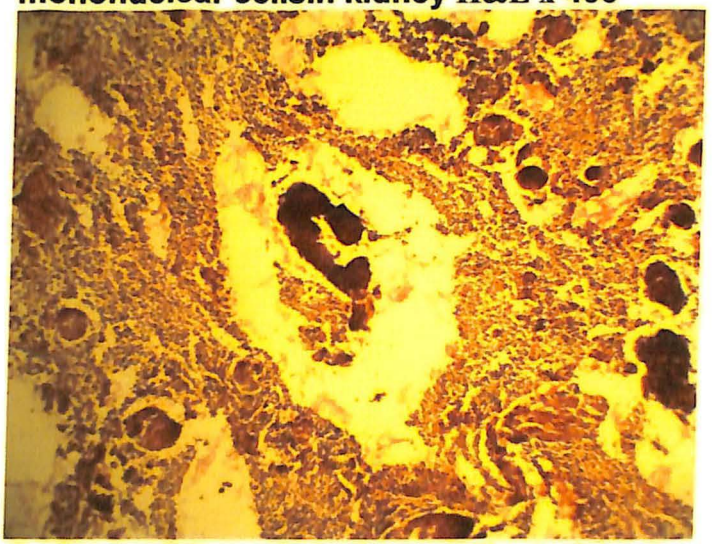
Thickened glomerular basement membrane with infiltration of mononuclear cells H&E x 400

Figure-45



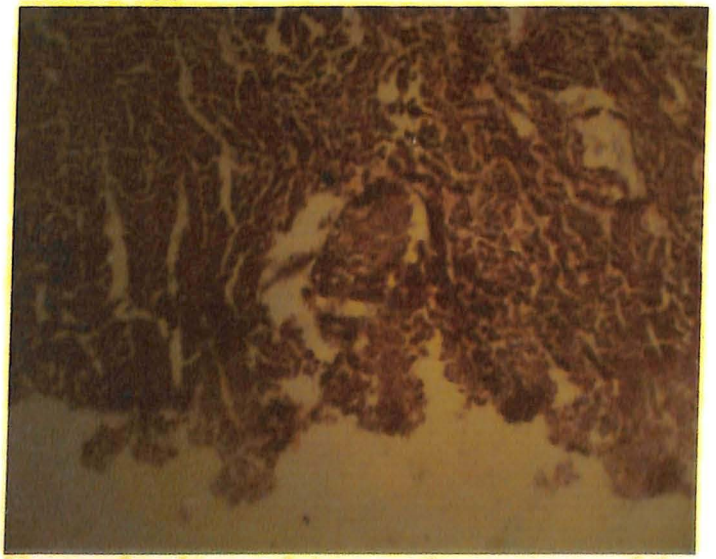
Interstitial infiltration of chronic inflammatory cells like plasma cells and mononuclear cellsin kidney H&E x 400

Figure-46



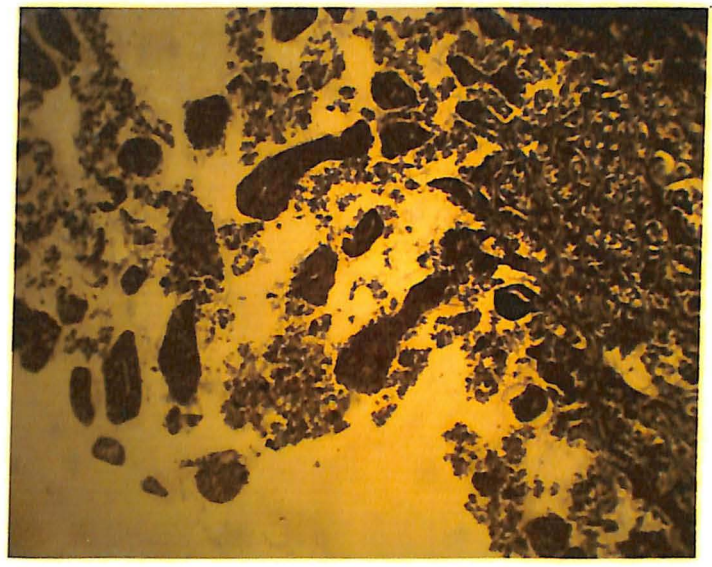
Oedema of the spleen around the trabeculae H&E x 100

Figure-47



Hemorrhagic exudates on the mucosa of the intestine H&E x 100

Figure-48



Desquamation of epithelial linings of intestine H&E x 100

CHAPTER - V
DISCUSSION

CHAPTER V

DISCUSSION

The worldwide distribution and apparent increasing prevalence of microfilariasis have prompted numerous studies of this disease in canines. Dirofilaria is recognised as a potential cause of serious disease in dogs especially in endemic areas. The aim of the present study was to assess the prevalence of dirofilariasis and its clinical as well as pathological alterations in dogs in Orissa. In the present study 1119 dogs of various age and sex were screened by blood smear examination, thick blood smear examination, modified knot method, and Buffy coat smear method for presence of dirofilarial infestation spanning a period of around one year. Out of these 467 animals of pet dogs of different breeds presented to the Teaching Veterinary Clinical Complex and Department of Pathology of Veterinary College for various reasons. In addition, they were screened for dirofilariasis and 51 nos. of cases (10.92%) were found to be positive. Further, 1862 stray nondescript dogs of both sex and various ages were brought to the Animal birth control (ABC) programme of Bhubaneswar Municipality Corporation (BMC) at veterinary polyclinic, Sahidnagar during the study period. Because of limitation in handling the stray dogs, only 652 no's of dogs could be screened. On screening 127(19.47%) no's of animals were found to be positive for dirofilariasis.

Out of all the screening methods employed, modified knot method and quantitative Buffy coat smear method were found to be sensitive and specific in detecting all the dirofilaria positive cases. It was found in this study that modified knot's test as the preferred method for observing morphology and to differentiate *D.immitis* from *D.reconditum* which in agreement with Lindsay (1965) and Newton and Wright (1956).

Majority of the positive cases 107 no's (60.11%) of dirofilariasis were asymptomatic which was predominant in stray dogs. However some positive cases 71(39.88%) showed clinical signs like coughing, slight pyrexia, exercise intolerance, inappetance and haemoptysis etc. The clinical symptoms found during

the present study corroborates with the findings of Eslami *et al.*, (2005).

Radiographic evaluation through C-arm examination of 10 affected animals with heavy load of microfilariae in blood during screening revealed cardiomegaly, round heart appearance suggestive of right ventricular hypertrophy, tortousity of the pulmonary artery and darkening of lungs. McCall *et al.*, (2004) also observed similar findings in his study.

Results of breed susceptibility of dirofilarisis revealed highest incidence in large breeds like GSD and Labrador. However, in present study this distribution may not be taken as conclusive to the susceptibility of specific breeds as the breed wise population varied greatly in the city. It may be noted here that all the dogs from ABC program were of nondescript breed stray dogs.Yildirim *et al* (2007) reported about the increased susceptibility in large breeds.The findigs in agreement with Donato Traversa *et al* (2010).

Out of 100 positive dogs taken for studying the age susceptibility the prime age for infection found as from 3 to 9 years i.e. 81%. It may be explained that after around 6 years that stray dogs die or are killed due to many a reasons varying from diseases to accidents. By the age 9 years and above their population becomes negligible. Ching-cheng Wu and Ping-Chin fan (2003) reported about the age of infected dogs found to be more than 1 years. Where as Yildirim *et al.*(2007) reported significant differences between 0.5–3 and other age groups.

Sex wise distribution revealed that 37(64.70%) nos. of males and 14 nos. of (27.45%) females out of 51 nos. of positive pet dogs and 49(38.58%) were males and 78(61.41%) were the females out of 127 positive stray dogs. This may be due to the fact that denizens of Bhubaneswar and surrounding prefer to keep males; hence the male is higher in number. But in case of stray dogs the ABC program emphasises sterilising more females than the male. Hence the number of females is higher. Donato Traversa *et al* (2010) reported that Male and large sized dogs resulted more likely to be infected by Dirofilaria spp., possibly due to the fact that animals living outdoor and of large size are more exposed to mosquito bites. Yildirim *et al* (2007) reported more prevalent in males.

Serum enzymatic analysis particularly AST, ALT and ALP showed significant ($p<0.05$) rise in the affected animals. This study in agreement with the findings of Niwetpathomwa *et al* (2007) who reported about the significant increase of AST, ALT and ALP in dirofilaria affected dogs. Biswas *et al* (2005) also observed that there was significant increase in AST, ALT values in dirofilaria affected dogs.

The cholesterol showed significant rise in positive animals in comparison to the negative animals. But Triglyceride showed a reverse trend i.e. there was a significant decrease in the value in the affected dogs.

The Total Protein value in positive dogs varied between 3.31 and 11 with average of 6.36 (±0.37) with a non-significant increase than the negative cases. There was a significant rise ($p<0.05$) in albumin level in dirofilaria affected dogs with an average value of 2.78. In other hand, the Globulin level showed a significant increase in non-affected dogs with a mean value of 3.79.This study not in agreement with Biswas *et al* (2005) who reported about significant decrease in serum protein and serum albumin values in dirofilaria affected dogs.

Creatinine showed a significant increase in filarial positive animals in comparison to that of negative animals suggesting certain amount of renal and cardiac dysfunction. This study in agreement with the findings of Niwetpathomwa *et al* (2007) who reported about the significant increase of creatinine values in positive dogs.

Serum glucose profile revealed a significant reduction in the filarial affected dog with a mean value of 38.71 against the negative animal with an average level of 100.79.

The difference between the affected and non-affected animals regarding calcium concentration in serum proved to be non-significant. The average values for calcium concentration were 9.34 and 9.86 in negative and positive dirofilaria affected dogs. On the other hand, the phosphorus level in the positive cases averaged 6.14 while the non-affected animals showed an

CHAPTER - VI
SUMMARY

BIBLIOGRAPHY

CHAPTER VI

SUMMARY AND CONCLUSION

The increasing prevalence of canine dirofilariasis prompted the quick attention of the veterinarians as well as other biologists globally to have an open thought on this matter. The present study has been designed to assess clinicopathological alterations and oxidative stress in dirofilariasis affected dogs in Orissa.

Overall prevalence of this disease was found to be around 15.90% out of 1119.The present study showed an overall prevalence of 10.92% and 19.47% in pet and stray dogs respectively which may be due to risk status of stray dogs and lack routine prophylaxis. Modified knot's test regarded as the preferred method for indentifying the species of dirofilaria as per the present study.

Positive dogs randomly selected were subjected for determining the age, sex and breed suspectibility for dirofilariasis. There was no significant difference between the sex though overall 48.31% were males and 51.68% were females affected with dirofilariasis. The present study found the prime age for susceptibility was between 3-9 years. As far as breed suspectibility is concerned the study opined about the higher prevalence rate seen in larger breed dogs (Labrader-38%,GSD-18%,Mixed-12%) followed by less prevalence in smaller breed dogs (Pug-2%,Pomerinian-4%).

The haematological alteration in dirofilaria affected dogs revealed a significant decrease in Hb, PCV and TEC values in comparison to non affected dogs taken in the study. However there was an increasing trend seen in TLC, MCV and MCHC values with leukocytosis in the positive dogs. The study resulted neutrophilia, eosinophilia and lympphopaenia in dirofilaria affected dogs.

Serum biochemical analysis in the present study showed a significant increase in AST, ALP, ALT, cholesterol, serum albumin and phosphorous values with significant decrease in Triglyceride values in affected dogs than negative control animals. However there was a non significant increase in calcium, total

protein values in the affected than the non-affected dogs as seen in this investigation.

In the present study, oxidative stress indices like LPO and catalase showed a significant rise of values in the dirofilaria affected dogs with a non significant increase in SOD values than the non affected dogs.

Gross and histopathological changes of dirofilaria affected animals reveal more or less similar findings with right ventricular hypertrophy and recovery of adult heart worms in ventricles and lungs along with chronic inflammatory changes in liver, lungs and kidney.

Following conclusions were drawn:

1. There was a significant prevalence of dirofilariasois both in pet and stray dogs in the age group of 3-9 years in Orissa.
2. Wet blood smear and Modified knot's test can be easily applied for routine screening of dirofilariasis even the animal is sick for any disease conditions.
3. The clinico-hemato-biochemical alterations and to be taken into consideration for evaluation severity of the disease.
4. Image intensifier TV system (C-arm) can be utilized as a diagnostic tool in clinically affected dogs.
5. An attempt has been made to evaluate the oxidative stress indices which will help in disease management.
6. As the disease is chronic in nature causing anemia with increasing stress indices, it predisposes the dogs for various secondary bacterial and viral infections which may complicate the disease process.

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