CLINICAL INVESTIGATIONS ON OEDEMA OF LIMBS IN DOGS

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THESIS

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ETHICS AND JURISPRUDENCE

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

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DECLARATION

I hereby declare that the thesis entitled **"Clinical investigations on oedema of limbs in dogs"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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EXTERNAL EXAMINER

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Dedicated to

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Introduction

1. INTRODUCTION

Dogs were the human companions from the past decades. They had a significant impact and role in human lives. The comfort and function of all the four limbs in dogs are essential for their everyday existence. Since dog's mobility is one of their valuable attribute, the compromise in any one of the limb can result in discomfort. One such common discomfort is swelling or oedema of limbs.

Oedema is an abnormal accumulation of interstitial fluid. "According to Starling hypothesis, the factors regulating fluid movement across the capillary wall are capillary hydrostatic pressure and the plasma colloid osmotic pressure. The net hydrostatic pressure difference is greater than the net colloid osmotic pressure difference, thus a net filtration occurs from the intravascular space into the interstitial space and the filtrate is then removed via the lymphatic system"(Herndon, 2010). Any abnormalities in this process resulted in oedema and is accompanied by swelling.

The tissue oedema are more evident in dependent areas of the body as a result of gravity and hence the occurrence of oedema is more common in ventral aspects of body and distal extremities. The clinical presentations of limb oedema due to various causes are increasing nowadays. Pathophysiology of oedema varies between different conditions like heart failure, renal diseases, hepatic diseases, infections, inflammation etc. and hence finding the exact etiology of limb edema is a must for the proper treatment.

There is paucity of literature on oedema of limbs in dogs. Although many infectious cases are presented with limb oedema, the exact mechanism for the oedema formation is not studied in most cases. In majority of the infectious diseases, immune mediated reactions had an important role in oedema formation. Lymphoedema due to occlusion of lymphatic vessels usually manifested as persistent limb oedema and for

such conditions, proper diagnosis and treatment was not elucidated much in the veterinary literature.

With this background, an investigation on oedema of limbs in dogs was undertaken with the following objectives:

- To identify the etiological factors associated with oedema of limbs
- To identify the clinico-pathological changes associated with oedema of limbs
- To report the response to therapeutic regimens adopted

Review of Literature

2. REVIEW OF LITERATURE

2.1. OEDEMA OF LIMBS

"According to Starling Hypothesis, the main factors regulating fluid movement across the capillary wall are capillary hydrostatic pressure and the plasma colloid osmotic pressure". This theory was extended by Landis to include interstitial hydrostatic and oncotic pressures, physical properties of the capillary membrane and the effect of lymphatic drainage (Chen *et al*., 1976).

Oedema is an abnormal accumulation of interstitial fluid. Normally the fluid and protein that oozing out from the blood capillaries are returned in equal amounts either into the blood or to the lymph capillaries by absorption. Any abnormality in this process resulted in oedema and is accompanied by swelling (Erickson and Detweiler, 2004).

Herndon (2010) stated that the normal delivery of nutrients and removal of byproducts of cellular metabolism were interfered by oedema. The clinical manifestation of oedema varies depending upon the organs affected like central nervous system, lungs, intestines, skin or subcutaneous tissue.

Ware (2011) reported that oedema of tissue was more common in dependent areas of the body as a result of gravity. So the subcutaneous oedema might be evident along the ventral aspect of body, under the mandible or in the lower limbs.

In lymphedema, the rear limbs were commonly affected and usually begins in the distal extremity and progresses proximally. This was manifested as spontaneous onset with pitting oedema and painless swelling of the extremities. All four limbs and the trunk might be oedematous in severe cases (Fossum and Caplan, 2013).

2.2. PHYSIOLOGY AND PATHOPHYSIOLOGY

"According to Herndon (2010), Starling's equation determines the dynamics of transcapillary fluid flow. The net hydrostatic pressure difference (P_c - P_i) is greater than the net colloid osmotic pressure difference ($\pi_c - \pi_i$), thus a net filtration occurs from the intravascular space into the interstitial space and the filtrate is then removed via the lymphatic system".

$$J_v = K_f \left[(P_c - P_i) - \sigma (\pi_c - \pi_i) \right]$$

where J_v– net fluid movement between compartments, K_f– filtration coefficient, P_c and P_i – hydrostatic pressures within the vascular and interstitial space, σ – osmotic reflection coefficient, π_c and π_i - colloid osmotic pressures of plasma and interstitial space.

Stranden (2011) opined that in human patients with venous insufficiency, valvular incompetence and increased ambulatory venous pressure in the lower limb in the upright position were the most important factors for oedema formation. The increased venous pressure increases capillary pressure and resulted in transcapillary filtration.

Innera (2013) reported cutaneous vasculitis as a multifactorial reaction pattern which will impair the vascular function leading to formation of oedema, haemorrhage and purpura. Pitting oedema was the only finding in the early stage of this condition. Type III hypersensitivity reactions resulting from immune complex deposition around the blood vessels was the widely accepted mechanism for this condition.

According to Trayes *et al.* (2013), human patients with unilateral swelling could be resulted from deep venous thrombosis, venous insufficiency, venous obstruction by tumor, lymphatic obstruction, or lymphatic destruction. Other conditions such as congestive heart failure, chronic hepatic or renal disease, protein

losing enteropathies or severe malnutrition might lead to bilateral or generalized oedema.

2.3. ETIOLOGY OF LIMB OEDEMA

2.3.1. Infectious causes

2.3.1.1. Ehrlichiosis

Canine monocytic ehrlichiosis (CME), caused by *Ehrlichia canis*, was an important disease of dogs and other canids. The primary vector of ehrlichiosis was the brown dog tick, *Rhipicephalus sanguineus* and heavy tick infestation was associated with the development of the disease (Mcquiston *et al.*, 2003).

The disease was manifested by various clinical signs and three phases had been recognized: acute, subclinical and chronic (Waner and Harrus, 2013).

2.3.1.1.1. Pathogenesis

In the experimental study with inoculation of *E. canis*, certain dogs developed petechial haemorrhage in subcutaneous tissue, lymphadenopathy and oedema of the limbs. Immunologic dysfunction has been suggested and immune-mediated vasculitis was a consistent finding in tissues of all infected animals which contributed to the pathogenesis of the disease (De Castro *et al*., 2004).

According to Solano-Gallego (2008), ehrlichiosis induced leukocyte and platelet abnormalities, plasma cell infiltration of parenchymal organs, and antigen-Ab complex formation.

Harrus *et al*. (2012) opined that the presence of circulating immune complexes in dogs infected with *E. canis* was one of the hyperimmune mechnisms involved in the pathogenesis of ehrlichiosis.

Among the tick borne illnesses, ehrlichiosis was one of the disease which causes cutaneous vasculitis and was manifested as limb oedema as a clinical sign. The diagnosis was mainly by histological examination of skin biopsies taken from the affected sites (Innera, 2013).

2.3.1.2. Babesiosis

Farwell *et al*. (1982) reported that the two major canine babesia species were *B. canis* and *B. gibsoni* and were transmitted mainly by bite of the ticks belonging to the species *Rhipicephalus* or *Dermatocentor*.

Babesiosis was manifested in the peracute, chronic and subclinical form. The pathogenesis of disease varies depending on the species, other infections, age and immunity of the host (Schoeman, 2009).

2.3.1.2.1. Pathogenesis

A retrospective analysis of 40 dogs with *Babesia gibsoni* infection was conducted in Italy and majority of the dogs revealed concurrent cutaneous lesions associated with Babesiosis. Certain cutaneous manifestations due to vasculitis enhanced by circulating antigen-antibody immune-complexes were previously attributed to *Babesia canis* infection (Tarello, 2003).

Cutaneous vasculitis in babesiosis might be mediated by antigen-antibody complex deposition in the blood vessel walls and activation of inflammatory cells. These inflammatory cells further release free radicals or histamine, which promotes vascular permeability leading to perivascular tissue damage (Tasaki *et al*., 2013).

2.3.1.3. Microfilariosis

Microfilariae were advanced embryos or pre-larvae of filarial parasites. Filarial parasites were highly evolved nematodes which complete their life cycle in

two hosts. Definitive hosts could be either human beings or animals. Intermediate hosts were mostly different species of mosquitoes (Soulsby, 2005).

Ambily (2009) identified the prevalence of *D. repens, D. reconditum, Brugia malayi* and *B. pahangi* microfilariosis in dogs of Kerala, based on the acid phosphatase staining pattern and PCR analysis.

Chirayath (2013) reported the occurrence of microfilariosis in dogs of Thrissur district as 8.12 per cent with higher levels of unsheathed microfilariae.

2.3.1.3.1. Pathogenesis

There was notable pathologic changes in the lymphatic duct of canine rear limbs resulting from *B. pahangi* infection. Limb oedema was variably associated with infection and spontaneous release of histamine and prostaglandin E2. There was also elevated levels of tumor necrosis factor α when the lymph node cells were cultured with Brugia antigen (Orton *et al.*, 1998).

Filarial worm causes dialatation of the large lymphatic vessels and was followed by nodal fibrosis. Oedema might not occur for many years after a surgery or after an infection with filariasis, until the alternative systems which manage the lymph load were affected by any inflammatory process or by failure of venous system (Ryan, 2002).

According to Hardie and Petrus (2003), filariasis was the common cause of human lymphedema. It had a gradual onset and begins from the distal part of the limb and progress proximally. Pitting edema observed initially and in chronic cases pitting was not evident because of tissue fibrosis. The edema might be generalized or might involve one or two limbs.

Ambily (2009) reviewed that limb oedema were more common with sheathed microfilariosis than unsheathed ones and was characterized by lymphangitis.

Symptoms like enlarged lymph nodes, oedema of limbs and scrotum were related to lymphatic system and were more common in Brugian filariosis compared to microfilariosis due to *D. repens*. Lymphangitis was mainly associated with Brugian filariosis (Chirayath, 2013).

2.3.2. Non infectious causes

2.3.2.1. Cardiac diseases

Cardiac failure was the pathophysiologic state wherein the heart was impaired in its ability to eject or receive blood, resulting in physical disability and manifested by a variety of clinical signs (Sisson, 2010).

2.3.2.1.1. Pathogenesis

The ventricular systolic or diastolic dysfunction in heart failure leads to elevation in venous pressure which increases capillary hydrostatic pressure. Oedema resulted when the lymphatic system fails to remove the extra amount of fluid back to the vascular space. This was manifested as pulmonary edema in left side impairment of heart and as peripheral edema in right sided heart failure (Cho and Atwood, 2002).

The term congestive heart failure was used when there will be development of tissue oedema as a result of elevation in capillary and venous pressures. Increased preload occurs when there was hypotension that activates the renin-angiotensin-aldosterone system leading to fluid retention (MacDonald, 2008).

According to Sisson (2010), abnormal cardiac function leads to retention of sodium and water, resulting in the signs of congestion and oedema. With right sided heart impairment, systemic venous pressure rises, resulting in hepatomegaly and ascites, which become apparent when central venous pressure exceeds 15 mm Hg. The development of right sided heart failure was gradual since the lymphatics had an adaptive role in maintaining the increased pressure.

2.3.2.2. Renal diseases

Recognizing kidney disease requires consideration of evidence from multiple sources, including renal function tests, serum electrolyte concentrations and acid-base status, urinalysis, and renal imaging studies (Dibartola, 2010).

"Acute kidney injury (AKI) was characterized by the rapid loss of nephron function, resulting in azotemia and fluid, acid-base and electrolyte abnormalities" (Ross, 2011).

"Chronic kidney disease (CKD) was defined as the presence of functional or structural abnormalities of one or both kidneys that have been present for an extended period, usually 3 months or longer" (Polzin, 2011).

2.3.2.2.1. Pathogenesis

Cardiovascular renal disorders referred to kidney dysfunction arising from a primary disease in the cardiovascular system. There was decreased renal perfusion; activation of the renin-angiotensin-aldosterone system and sympathetic nervous system, formation of reactive oxygen species by injured endothelial tissue and venous congestion of the kidney (Pouchelon *et al.* 2015).

In critically ill patients, venous congestion was manifested as peripheral edema and increased central venous pressures. Hence an increase in renal venous pressure might impair normal renal function leading to renal failure (Chen *et al.*, 2016).

2.3.2.3. Hepatic diseases

According to Raffan *et al.* (2009), as a result of portal hypertension, neurohormonal activation and hypoalbuminemia, there arises many clinical problems in association with chronic hepatitis.

2.3.2.3.1. Pathogenesis

Cirrhosis associated fluid retention in human patients occurs as ascites and peripheral oedema. Increased sodium and water retention by the rennin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS) plays a major part in formation of oedema. Splanchnic arterial vasodilation increases portal hypertension, which further leads to fluid retention (Cardenas and Arroyo, 2003).

In addition to intrahepatic portal venous hypertension with liver disease, there was pooling of blood in the splanchnic circulation and subsequent reduction in the systemic blood pressure resulting in the activation of RAAS leading to retention of sodium by the kidneys which paved the way for ascites and peripheral edema (Watson and Bunch, 2009).

2.3.2.4. Limb oedema secondary to mammary tumors

Chun and Garrett (2010) reported that mammary gland tumours (MGTs) was one of the most frequently seen tumours in female dogs and the third most common tumor overall in cats. A hormonal etiology was well described and spayed animal had relatively lower risk for this condition.

2.3.2.4.1. Pathogenesis

Secondary malignant lymphedema was recently reported after mastectomy in two dogs. Both dogs developed large edematous lesions associated with lameness in the right hindlimb following surgery. Lymphatic obstruction of the right hindlimb was shown with lymphangiography in one dog and lymphoscintigraphy in the other dog (Kang *et al.*, 2007).

MacPhail (2013) reported that inflammatory mammary carcinomas spreads rapidly, invades cutaneous lymphatics and causes marked edema, inflammation, and pain. Dogs became anorectic, weak, and experiences weight loss. The tumors were

poorly demarcated, firm, ulcerated, and might involve both mammary chains. Extensive lymphedema of the limbs might occur secondary to lymphatic occlusion or infiltration. Disseminated intravascular coagulation and thoracic metastasis were seen with inflammatory carcinoma, lameness or limb edema suggests metastasis and this tumor had a poor prognosis.

2.3.3. Idiopathic causes

In veterinary medicine, at least 50 per cent of all vasculitis cases (excluding rabies vaccine-induced lesions) are attributed to idiopathy. Idiopathic vasculitis often presented with pitting oedema and lesions affected the distal extremities (Morris and Beale, 1999; Gaisbauer *et al.*, 2014).

Innera (2013) conducted a retrospective study of vasculitis in dogs and cats and no triggering agent could be detected in 10 out of the 21 cases and they were diagnosed as idiopathic.

2.3.3.1. Pathogenesis

In veterinary medicine, the most widely accepted pathogenesis for cutaneous vasculitis was a type III hypersensitivity response. This results from foreign antigen or altered self-antigen triggering an antibody response and entrapment of antigen-antibody complexes along the vessel walls which in turn activates complement. Complement-derived chemotactic factors further attract inflammatory cells and release lysosomal enzymes that injures blood vessels (Morris and Beale, 1999).

Vasculitis was a reaction pattern and not a disease by itself. It was characterized by an abnormal immune response directed toward blood vessels. The pathophysiology was mostly complex and not fully understood but involves a variety of mechanisms which induce inflammatory changes in the blood vessel wall. Impaired vascular function might have resulted in edema formation, hemorrhage, and purpura (Bloom, 2010; Innera, 2013).

Gaisbauer *et al.* (2014) reported a case of immunological deep dermal vasculitis in a cat with intense pitting oedema on all the four distal limbs. The case was diagnosed with histological examination of punch biopsy samples taken from various sites of the swollen limb and the animal responded to glucocorticoid therapy.

Although type III hypersensitivity reaction was suspected in most cutaneous vasculitis, additional factors like genetics, defects in immune complex clearance, and autoantibodies might also be involved (Shumaker, 2015).

2.4. DIAGNOSIS

2.4.1. Infectious causes

2.4.1.1. Ehrlichiosis

2.4.1.1.1. Blood smear and buffy coat smear

Demonstration of typical intracellular *E. canis* morulae on blood smears was highly specific for ehrlichiosis. However, this method was time-consuming and not very reliable because in blood smears morulae were found in low numbers during the acute phase of infection. This could be improved by evaluation of numerous buffy coat smears (Woddy and Hoskins, 1991).

2.4.1.1.2. Haematology

Thrombocytopenia was the hallmark of acute stage of ehrlichiosis and anemia seen during this phase was classically a mild to moderate normocytic, normochromic and nonregenerative (Harrus *et al*., 2012).

2.4.1.1.3. Serum biochemistry

Elevations in blood urea nitrogen and creatinine might be noted in some dogs infected with canine monocytic ehrlichiosis (Sykes, 2010).

According to Harrus *et al*. (2012), the serum abnormalities include hyperproteinemia, hyperglobulinemia, hypoalbuminemia, and there were elevations in the alanine aminotransferase and alkaline phosphatase activities.

2.4.1.1.4. Abdominal ultrasonography

According to Waner and Harrus (2013), splenomegaly was a prominent finding in both the acute and chronic stages of the disease.

Splenomegaly, lymphadenomegaly and hepatomegaly were the nonspecific clinical signs of ehrlichiosis (Sainz *et al*, 2015).

2.4.1.2. Babesiosis

2.4.1.2.1. Blood smear and buffy coat smear

Tarello (2003) stated that the best test for the diagnosis of Babesia organisms was blood smear examination.

According to Birkenheuer (2012), demonstration of Babesia organisms by light microscopy was highly specific, but it has relatively poor sensitivity because of its limit of detection.

2.4.1.2.2. Haematology

There was no or moderate anaemia had been reported in 30 per cent dogs and severe anaemia in five per cent dogs from a retrospective study of dogs with *Babesia gibsoni* infection with concurrent cutaneous signs in Italy (Tarello, 2003).

A mild, normocytic, normochromic anemia was generally seen during initial infection and becomes macrocytic, hypochromic, and regenerative as the disease progresses. Thrombocytopenia was generally a feature and leukocytosis or leukopenia were inconsistently observed (Birkenheuer, 2012).

Tasaki *et al.* (2013) reported leukocytosis with neutrophilia and regenerative anemia in a dog with babesiosis and generalized alopecia.

2.4.1.2.3. Serum biochemistry

According to Schoeman (2009) those patients with hepatopathies associated with babesiosis, there was elevation in liver enzymes. Based on the severity of anemia, bilirubin concentrations were elevated. There were elevations of BUN and creatinine depending on the renal status of the patient.

Trotta *et al*. (2009) reported hyperproteinemia, hypoalbuminemia and hyperglobulinemia in a *B. gibsoni* infected dog from Italy.

Tasaki *et al.* (2013) stated slightly higher levels of alkaline phosphatase (260 U/l) and BUN (38.3 mg/dl) in a *Babesia gibsoni* infected dog with vasculitis like changes.

2.4.1.2.4. Abdominal ultrasonography

Lobetti (2000) reported that hyperplasia of the mononuclear phagocytic system in canine babesiosis was responsible for splenomegaly.

Tazaki *et al.* (2013) studied a case of dog infected with *B. gibsoni* with cutaneous vasculitis like lesions and ultrasonograghy of abdomen revealed no organ abnormality except for some lesions in the ovary.

2.4.1.3. Microfilariosis

2.4.1.3.1. Peripheral Wet film examination

Wet blood film examination was an important tool in screening of microfilariosis. In a 12 month period of study at Thrissur, 145 positive cases of microfilariosis were detected from screening 8503 dogs (Chirayath and Alex, 2011).

Among the hundred clinically suspected cases of filariasis, 80 dogs were positive for microfilaria on wet blood film examination (Ambily *et al.*, 2014).

2.4.1.3.2. Blood smear and buffy coat smear

Chirayath (2013) reported among the Giemsa stained blood smears of 130 dogs positive for microfilarisis, 90 were having sheathed microfilariae, 24 were having unsheathed microfilariae and 16 were having both infections. The presence of pink coloured sheath that was extending from the body was one of the distinguishing character that distinguish sheathed microfilariae from unsheathed.

Giemsa stained blood smears revealed that 16 out of 80 dogs were positive for sheathed microfilaria and others were unsheathed. The staining characteristics of sheathed and non-sheathed microfilariae were in accordance with the microfilaria of the *Brugia malayi* and *Dirofilaria repens* respectively (Ambily *et al.*, 2014).

2.4.1.3.3. Haematology

According to Baneth *et al.* (2002) pre-treatment blood count of microfilaremic dogs were within the reference ranges.

The major haematological findings in microfilaremic dogs were a mild to moderate anemia, thrombocytopenia, marked leukocytosis with neutrophilia, eosinophilia and monocytosis (Nitwetpathomwat *et al*., 2007).

According to Chirayath (2013), affected animals developed mild anaemia, increased leucocytic count and reduced thrombocytic count.

2.4.1.3.4. Serum biochemistry

Nitwetpathomwat *et al*. (2007) reported that serum values of alanine amino transferase, aspartate amino transferase, alkaline phosphatase, BUN and creatinine were higher in dogs affected with dirofilariasis in Thailand.

Serum ALT and ALP were higher in affected animals. Total protein was within the normal range with lower levels of albumin and higher levels of globulin. Albumin globulin ratio was low. BUN and creatinine were on the upper normal range (Chirayath, 2013).

2.4.1.3.5. Abdominal ultrasonography

According to Baneth *et al.* (2002) ultrasonographic examination of the abdomen was within normal limits.

2.4.1.3.6. Biopsy

Histopathologic findings in chronic lymphedema in human patients with filariasis includes thickened lymphatic vessels, infiltration of fibroblast and inflammatory cells leading to fibrosis of subcutis (Rockson, 2001).

2.4.2. Non infectious causes

2.4.2.1. Cardiac diseases

2.4.2.1.1. Clinical signs

The findings in symptomatic animals with valvular insufficiency include heart murmurs, tachycardia, arrhythmia, weak femoral pulses, pulse deficits, prolonged capillary refill time, pale mucous membranes, tachypnea, dyspnea, orthopnea, respiratory crackles, ascites and jugular venous distension (Haggstrom. 2008).

2.4.2.1.2. Haematology

According to Abbott (1998), the results of complete blood count were often normal in patients with CHF, although stress leukogram was reported in severe failure.

Haggstrom (2008) reported that haematology was usually unremarkable in mild cases of atrioventricular valvular degeneration.

Anemia and thrombocytopenia were the most common abnormalities detected in dogs with pericardial effusion. Anemia was mild, normochromic, normocytic, and nonregenerative in most cases (MacDonald *et al*., 2009).

2.4.2.1.3. Serum biochemistry

In a retrospective study of dogs with heart disease, Boswood and Murphy (2006) found no significant difference in creatinine values at the onset of heart failure.

In severe cases of atrioventricular valvular degeneration, serum biochemistry panels revealed mildly increased liver enzymes and evidence of prerenal azotemia (Haggstrom, 2008).

Sisson *et al.* (2010) observed elevated levels of serum urea and creatinine concentrations in DCM dogs.

2.4.2.1.4. Electrocardiography (ECG)

ECG recordings in a dog with hypertrophic cardiomyopathy revealed elevated R wave, ST coving and bizarre QRS complexes. These were suggestive of left ventricle enlargement with ventricular conduction abnormalities (Marks, 1993).

Notching and widening of the P wave (P mitrale) was suggestive of enlargement of left atrium. Conversely, a tall and peaked P wave (P pulmonale) was associated with enlargement or hypoxia of the right atrium. But many healthy individuals with normal atria had these P wave “abnormalities,” and some individuals with mild or moderate atrial enlargement had normal P waves. ECG was not always an absolute indicator of normalcy or disease (Cote, 2010).

Sinus tachycardia, ventricular arrhythmias, low-voltage QRS complexes, and ST segment elevation were the common findings in those patients with pericardial effusion. There was alternation in the size of the T wave or QRS complex, known as electrical alternans with each beat of the heart and might be due to swinging of the heart back and forth within fluid filled pericardium (Ware, 2011).

2.4.2.1.5. Thoracic Radiography

Rush (2002) observed elevation of the cranial aspect of trachea and increased size of caudal venacava associated with tricuspid regurgitation on lateral thoracic radiography in dogs. Elevated trachea and carina was also detected in right sided or biventricular congestive heart failure. A rounding of cardiac silhouette was observed with right atrial and ventricular enlargement.

Oyama *et al*. (2010) reviewed that thoracic radiography assisted in the determination of size of the heart, chamber enlargement and assessment of the pulmonary circulation. Selective widening of the caudal venacava might be due to right heart failure and that of pulmonary veins due to left heart failure.

2.4.2.1.6. Abdominal ultrasonography

Abdominal ultrasonography of a dog with myocardial dysfunction revealed a large amount of anaechoic abdominal fluid, an enlarged hyperechoic liver with normal hepatic veins (Flood and Hoover, 2009).

According to Ware (2011), abdominal ultrasonography was used for evaluation of organs and abnormal structures which appeared to be normal in X rays. Changes in echogenicity as well as enlargement or shape irregularities of various organs especially liver and spleen, vascular abnormalities, and ascites could be identified in cases of congestive heart failure.

2.4.2.1.7. Echocardiography

Tidholm *et al.* (1998) reported that fractional shortening less than 25 per cent in conjunction with radiographic evidence of cardiac enlargement and typical signs of congestive heart failure might be acceptable for clinical diagnosis of dilated cardiomyopathy in dogs.

Echocardiography was the cornerstone for definitive diagnosis of specific heart diseases. It helps in the evaluation of cardiac function, chamber size, anatomical abnormalities, and valvular competence. Noninvasive estimation of pressures between two chambers was possible using continuous Doppler wave (MacDonald, 2008).

Echocardiographic findings of atrioventricular valvular degeneration include thickening or prolapse of the AV valve and identification of a regurgitant jet on spectral or color flow Doppler (Haggstrom, 2008).

Spier (2008) opined that echocardiography was the best method to confirm DCM. There was decreased systolic function and ventricular enlargement. CHF may occur once the Fractional shortening (FS) was severely reduced (<15 per cent).

Those animals with mitral valve insufficiency had a deformed or thickened mitral valve as well as left atrial and ventricular dilatation and hyperdynamic systolic function. The 2D and M-mode echocardiography revealed anechoic areas surrounding the ventricles in cases of pericardial effusions (James, 2011).

The echocardiographic findings in dilated cardiomyopathy, were left ventricular and left atrial enlargement, hypokinesis of left ventricular free wall and interventricular septum and elevated mitral valve E-point to septal separation (EPSS) and secondary mitral regurgitation. Pericardial effusion was also recorded in advanced cases (Unny, 2014).

2.4.2.1.8. Blood pressure

Brown *et al.* (2005) preferred non-invasive blood pressure measurement methods over invasive blood pressure measurements in clinical situations.

According to Brown *et al.* (2007), hypertensive changes in heart include left ventricular hypertrophy and cardiac failure. On the basis of risk of target organ damage, dogs were classified as minimal risk (less than 150/95 mmHg), mild risk, moderate risk and severe risk (greater than or equal to 180/120 mmHg).

2.4.2.2. Renal diseases

2.4.2.2.1. Clinical signs

Kanaran (2009) observed lethargy, anorexia, vomiting, melena, oliguria/polyuria, muscle weakness and oral ulcers were the common clinical signs of renal failure in dogs.

Clinical signs of acute renal failure (ARF) were not specific and might associated with lethargy, anorexia, vomiting, diarrhea, and dehydration; occasionally with oral ulcers. Unique signs of chronic kidney disease (CKD) include a history of weight loss, poor body condition, polydipsia-polyuria, nonregenerative anemia, and small and irregularly shaped kidneys (Grauer, 2009).

2.4.2.2.2. Haematology

According to Ross (2006), haematocrit of AKI patients might be normal to elevated and consistent with dehydration whereas a normocytic, normochromic anemia might be for CKD patients.

Low haematocrit, RBC count and haemoglobin were observed in dogs with moderate to advanced stages of chronic renal failure (Roudebush *et al*, 2010).

2.4.2.2.3. Serum biochemistry

Serum BUN and creatinine were the indicators of renal damage and elevation of these parameters occurs when the disease was advanced such that 75 per cent of nephrons were damaged. In a study conducted for the evaluation of renal failure in dogs, most of them had serum creatinine levels more than 10 mg/dl, indicating more than 90 per cent of nephrons were damaged (Kanaran, 2009).

Roudebush *et al*, (2010) observed hyperphosphatemia CKD in as a result of decreased glomerular filtration rate (GFR) leading to retention of phosphorus.

According to the Veterinary Acute Kidney Injury (VAKI) scheme, if the creatinine increases 150–199 per cent from baseline, it was considered as stage 1 of AKI and if above 300 per cent from baseline, it was classified as stage 3 (Balakrishnan and Drobatz, 2013).

2.4.2.2.4. ECG

Electrocardiographic examination in canine renal failure revealed peaked T wave indicating hyperkalaemia (Ravindran, 2001).

The ECG also has valuable applications in monitoring patients with systemic abnormalities, including electrolyte disturbances and hypoxemia (Cote, 2010).

2.4.2.2.5. Abdominal ultrasonography

Armbrust *et al.* (2001) observed that the echogenicity of the renal cortex should be less than that of the spleen and less than or equal to that of the liver. The renal cortex was hyperechoic relative to the medulla. The most hyperechoic area was the renal sinus.

Ultrasonographic examination of uremic dogs revealed hyperechoic kidneys with no corticomedullary distinction which was indicative of marked renal fibrosis (Kanaran, 2009).

As per Grauer (2009), renal ultrasonographic findings in dogs with ARF were usually nonspecific, with diffusely normal to slightly hypoechoic renal cortices. Those with CKD revealed diffusely hyperechoic renal cortices with loss of the normal corticomedullary distinction. The increased cortical echogenicity resulted from replacement of damaged nephrons with fibrous connective tissue.

2.4.2.2.7. Blood gas analysis

In those animals with AKI, sodium concentration might be low, normal, or high depending on the degree of vomiting and/or diarrhoea. Hyperkalemia occurs in oliguric or anuric animals. Metabolic acidosis was also reported (Ross, 2011).

2.4.2.2.8. Blood pressure

Doucet *et al.* (2007) observed that most sodium-retaining states were associated with high blood pressure but not with development of oedema or ascites.

Arterial hypertension was a common problem associated with CKD in dogs and cats. The arterial hypertension was assessed by measuring blood pressure by three independent evaluation collected over several days to several weeks (Polzin, 2011).

2.4.2.3. Hepatic diseases

2.4.2.3.1. Clinical signs

Based on the history and clinical signs it was often difficult to diagnose liver disorders because most of the signs were non-specific (Rothuizen, 2000).

Clinical signs of hepatobiliary disease could be extremely variable, which include anorexia, weight loss, abdominal effusion, jaundice, and hepatic coma but none of these signs were pathognomonic (Watson and Bunch, 2009).

2.4.2.3.2. Haematology

Mild to moderate anaemia was common in liver disease and leucocyte count was often normal. Anaemia usually results from chronic illness and/or gastrointestinal bleeding and/or haemostatic disorders secondary to hepatic disease (Hall and German, 2005).

2.4.2.3.3. Serum biochemistry

Dunn (2000) reported that chronic liver diseases could cause hypoalbuminaemia. When the albumin level goes below 1 g/dl, it causes ascites and ascites might also develop with an albumin concentration of 2 g/dl with concurrent portal hypertension.

Evaluation of hepatobiliary enzymes such as ALT, AST, ALP, and GGT were used for the detection of hepatobiliary disease. In end-stage chronic liver disease, these enzymes might be normal or only slightly elevated, because fibrosis of liver tissue and progressive enzyme leakage can reduce total liver enzyme content. Hence these enzymes had low specificity for interpretation of hepatic disease (Webster, 2010).

2.4.2.3.4. Abdominal ultrasonography

According to Webster (2010), ultrasonography enables differentiation between focal and diffuse hepatic disease, assessment of the gallbladder and portal vasculature, and collection of tissue for cytology, histopathology or bacteriologic sampling.

The normal liver was more or less echogenic when compared to the cranial pole of the right kidney and hypoechoic to the spleen. Hepatomegaly was appreciated when the liver extend well beyond the rib cage. Portal veins had more echogenic margins compared to hepatic veins. Cirrhosis or chronic hepatitis results in hyperechogenicity secondary to fibrosis. The liver size might be normal or small with irregular margins with or without ascites (Larson, 2016).

2.4.2.3.6. Echocardiography

Echocardiography was indicated in hepatic disorders with ascites to rule out pericardial effusion, endocardiosis, valvular dysfunction and dilated cardiomyopathy (Dunn, 2000).

2.4.2.4. Oedema of limbs secondary to mammary tumors

2.4.2.4.1. Haematology

No specific laboratory abnormalities were found with lymphedema (Fossum and Caplan, 2013).

Although complete blood count results were nonspecific for mammary neoplasia, it had a role in identifying concurrent geriatric problems or paraneoplastic syndromes (MacPhail, 2013).

2.4.2.4.2. Serum biochemistry

According to Fossum and Caplan, (2013) those animals with lymphedema were not hypoalbuminemic.

Serum biochemistry profile was needed in mammary neoplasia with lymphedema, as it had a role in finding the current status of the animal (MacPhail, 2013).

2.4.2.4.3. Thoracic Radiography

Chun and Garrett (2010) opined that thoracic radiographs were used for evaluating pulmonary metastatic disease and sternal lymphadenopathy. Pleural effusion was a common occurrence in cats with metastatic mammary gland tumour.

Thoracic radiographs should be evaluated for pulmonary metastasis. Thoracic metastasis seen in 25 per cent to 50 per cent of dogs with malignant mammary tumors (MacPhail, 2013).

2.4.2.4.4. Abdominal ultrasonography

Ultrasonography was useful for evaluation of the dermis, subcutaneous tissue and muscle which will be affected by lymphatic obstruction, lymphedema or lymphangiectasia. The hyperechogenic changes of subcutaneous and subfascial tissues of humans with lymphedema correlated with the histopathologial findings of fibrosclerosis that were typical in human lymphedema (Hardie and Petrus, 2003).

Abdominal ultrasonography provided information about soft tissue masses and enlarged lymph nodes and other structures. Lymphography and lymphoscintigraphy were the alternate approach for imaging peripheral lymphatics (Petrie, 2010).

According to MacPhail (2013), abdominal metastasis might detected by abdominal ultrasonography.

2.4.2.4.5. Biopsy

For definitive diagnosis of secondary lymphedema, a cytology or histopathology was required. Biopsy or aspiration of masses or abnormal lymph nodes were performed. In the absence of gross lesions, a routine skin biopsy can identify any diffuse or infiltrative disease (Hardie and Petrus, 2003).

2.4.3. Idiopathic causes

2.4.3.1. Clinical signs

Common cutaneous symptoms of vasculitis include crateriform ulcers, necrosis, haemorrhagic bullae, pustules or papules, plaques and palpable purpurae at distal extremities with dermal oedema (Miller *et al*., 2013).

2.4.3.2. Haematology

According to Foster (2006), haematological changes involved in cutaneous vasculitis included, leucocytosis with left shift, monocytosis, leucopenia, eosinopenia, lymphopenia, neutropenia, normochromic normocytic anaemia and thrombocytopenia.

Outerbridge (2010) reported the complete blood count of dog diagnosed with cutaneous vasculitis revealed normal cell counts with a mild elevation in band neutrophils.

2.4.3.3. Serum biochemistry

Serum alkaline phosphatase and alanine amino transferase concentrations were elevated in a dog diagnosed with cutaneous vasculitis (Outerbridge, 2010).

In a study conducted for cutaneous vasculitis, it was reported that in 50 per cent of the patients there was moderate elevations in liver enzymes (Innera, 2013).

2.4.3.4. Biopsy

Histology was essential to confirm cutaneous vasculitis and it was advised to obtain deep dermal biopsies. Deeper layers of the skin and epidermis was preferred to detect the vascular disease process and early stage biopsies were recommended (Innera, 2013).

Histological findings of vasculitis was characterized by invasion of variable degrees of neutrophilic, eosinophilic and mononuclear cells into the vessel walls with endothelial cell swelling, haemorrhage and fibrinoid degeneration. Occasionally leukocytoclasia was noted within or near the vessels (Shumaker, 2015).

2.5. TREATMENT

2.5.1. Infectious causes

2.5.1.1. Ehrlichiosis

A minimum dose of Doxycycline @ 10 mg/kg daily for 28 days was recommended by The Ehrlichial Consensus Statement from the American College of Veterinary Internal Medicine in 2002 (Harrus *et al*., 2012).

Steroids like glucocorticoids should be considered in those cases with immune-mediated complications and the doses of prednisone should range from 0.5 to 2 mg/ kg/day (Sainz *et al.*, 2015).

2.5.1.2. Babesiosis

A combination of atovaquone @ 13.3 mg/kg body weight, PO, q8h and azithromycin @ 10 mg/kg body weight, PO, q24h hour for 10 days had been effective against *B. gibsoni* infection. For the accompanying immune-mediated erythrocyte destruction, corticosteroid drug administration was recommended by the author (Schoeman, 2009).

Imidocarb dipropionate was an effective drug against *B. canis* given intramuscularly at the dose of 7.5 mg/kg SID or a single dose of 6 mg/ kg given the day after a dose of diminazene (3.5 mg/kg) also been effective (Birkenheuer, 2012).

During therapy with antiprotozoal agents there was reduction in skin lesions which correlated with reduced severity of babesiosis, and after stopping the

medication relapse occurred which suggestived that the cutaneous lesions were associated with babesiosis (Tasaki *et al.*, 2013).

According to Vishnurahav (2014), a combination therapy with Clindamycin @ 11 mg/kg body weight SID and metronidazole @ 25mg/kg body weight SID intravenously, and doxycycline @ 10mg/kg body weight SID orally for 10 days was effective in clearing the *B.gibsoni* infection in dogs.

2.5.1.3. Microfilariosis

Ivermectin @ 100 μg/ kg bodyweight once orally was successful in the treatment of unsheathed microfilariae (Ambily, 2009).

Dogs with *Dirofilaria immitis* infection were treated with adulticide melarsomine @ 2.5 mg/ kg bodyweight i/m twice at 24 hour interval followed by microfilaricidal therapy with milbemycin or ivermectin, 10 days after the adulticide treatment (Atkins, 2010).

Chirayath (2013) reviewed the use of single day oral administration of microfilaricidals such as Ivermectin (100 μg/ kg bodyweight) and milbemycin oxime (0.5 mg/kg bodyweight) for microfilariosis due to *D. repens*. Levamisole @ 10 mg/ kg bodyweight orally for 7 days were recommended for sheathed microfilariosis.

2.5.2. Noninfectious causes

2.5.2.1. Cardiac diseases

Symptoms of heart failure should be reduced with ACE inhibitors and frusemide. The administration of spironolactone should be considered as the heart becomes more refractory (Meurs, 2002).

ACE inhibitors were indicated once CHF develops. They may improve quality of life and cause minimal to mild reductions in BP and Enalapril @ 0.5 mg/kg PO BID was the recommended drug (Haggstrom, 2008).

Along with the β and α_1 blocking properties, carverdilol has some antioxidant effects which reduced the oxidative stress associated with progressive heart failure. In general, beta-blockers were not advisable in acute congestive heart failure (Gordon, 2010).

2.5.2.2. Renal diseases

Consevative management of CKD included dietary change designed to reduce serum phosphorus concentrations and ACEIs designed to normalize systemic blood pressures and reduce proteinuria (Grauer, 2009).

All animals with acute renal failure would not respond to fluid therapy. For advanced renal cases peritoneal dialysis or haemodialysis might be required (Langston, 2010).

Treatment of AKI includes specific therapy for the cause, as well as supportive therapy based on the stage of renal damage. Fluid therapy should be considered based on the level of hydration, electrolyte, and acid-base status of the patient (Ross, 2011).

Maintenance dose of replacement solutions such as Lactated Ringer's solution predisposes the renal patient to hypernatremia and hypokalemia because these solutions contain more sodium and less potassium than the patient normally loses. Hence response to fluid therapy has to be reassessed (Davis *et al*. 2013).

2.5.2.3. Hepatic diseases

Only symptomatic treatment was possible for acute hepatitis. Intravenous fluid therapy to correct hypovolemia, acidosis or alkalosis, hypoglycaemia and electrolyte disturbances was the usual procedure (Rothuizen, 2000).

Treatment of ascites associated with liver failure consists of the use of diuretics like aldosterone antagonists (spironolactone, 1 to 2 mg/kg administered PO ql2h), and then with the addition of furosemide (2-4 mg/kg administered PO ql2h) in refractory cases (Watson and Bunch, 2009).

Steroid therapy was the generally used for chronic hepatitis because of their anti-inflammatory, antifibrotic and immunomodulatory effect but contraindicated for infectious conditions. Silymarin appears to be a strong free-radical scavenger and had hepatoprotective action (Brovida and Rothuizen, 2010).

2.5.2.4. Oedema of limbs secondary to mammary tumors

Therapy was usually unrewarding. Infectious disorders required long-term antimicrobial therapy. Long term heavy bandage application might encourage lymphatic flow and reduce lymphedema. Surgical options might include procedures to facilitate lymph drainage and procedures to excise abnormal tissue. Short-term administration of anti-inflammatory agents or diuretics, bandaging, and physical therapy might be recommended in cases of traumatic and postsurgical induced lymphedema (Petrie, 2010).

Surgery was the mainstay of treatment of MGTs in dogs and cats. A major drawback of this method in case of inflammatory carcinomas was the impossibility to remove the entire tumor, and regrowth might occur within days of the surgery. Those dogs with poor prognostic factors, a doxorubicin or doxorubicin/ cyclophosphamide protocol was the conventional treatment (Chun and Garrett, 2010).

2.5.3. Idiopathic causes

Treatment should be based on history, clinical findings and identification of the inciting cause. If no underlying trigger of vasculitis can be identified, glucocorticoids should be considered as the mainstay of treatment. The author prefers a lower dose of 0.5 to 1 mg/kg of prednisolone. Once remission was achieved, slow tapering with a 25 per cent dose every 14 days was attempted. Alternative therapies may include cyclosporine, azathioprine, chlorambucil, pentoxiphylline, tetracycline/niacinamide combination, Sulfasalazine and dapsone (Innera, 2013).

Miller *et al*. (2013) opined that main goal in the treatment of vasculitis was the correction of underlying cause and immunomodulatory drug therapy. The author has recommended immunosuppressive doses of prednisolone ranging between 2 and 4 mg/kg orally every 24 hours.

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Mannuthy during the period of July 2015- March 2016.

Dogs brought to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with the complaint of limb oedema formed the materials for the present study.

3.1. SELECTION OF ANIMALS

Dogs presented with limb oedema without any orthopaedic abnormalities were selected for the study. They were screened for infectious and non-infectious etiologies based on detailed clinical examination.

Out of the 58 cases screened during this period, 36 cases were selected for the study. Peripheral wet film, blood smear and buffy coat smear examinations were conducted in all animals to identify any haemoparasitic infection. Haematological and serum biochemical values of all cases were assessed. Abdominal ultrasonograghy was performed to assess any organ abnormality. Electrocardiographic, thoracic radiographic and echocardiographic studies were conducted to assess any cardiac disorders. In certain cases blood pressure and blood gas analysis were done. Histopathological examination of biopsy sample from oedematous area was performed in selected cases.

Based on these diagnostic techniques, the cases were classified into eight groups which include oedema of limbs associated with ehrlichiosis, babesiosis, microfilariosis, cardiac diseases, renal diseases, hepatic diseases, mammary gland tumours and idiopathic group. Appropriate therapeutic regimens were adopted for each groups based on different etiologies and response was evaluated on 30^{th} day of observation based on clinical improvement and haematobiochemical analysis.

3.2. PROCEDURES ADOPTED

3.2.1 Clinical Examination of the Patient

Detailed history and results of clinical examination were recorded as per the proforma (Annexure-I).

3.2.2. Investigations for haemoparasites

3.2.2.1. Wet film examination

One drop of blood collected from ear tip, placed on a glass slide and examined immediately under 10x objective of a light microscope after putting coverslip. This is to detect the presence of moving parasites like microfilaria in the blood.

3.2.2.2. Blood smear examination

Thin peripheral blood smears were prepared from ear tip, air-dried and fixed in methanol and stained with Giemsa stain (1:10) and examined under oil immersion objective of a light microscope. This is to detect the presence of haemoparasites like Babesia and Ehrlichia. To differentiate between sheathed and unsheathed microfilaria, thick smears were prepared and examined.

3.2.2.3. Buffy coat smear examination

About two millilitre of blood collected in EDTA vial was centrifuged at 3000 rpm for 15 minutes. The plasma was removed and a smear was prepared from buffy coat layer on a clean glass slide, fixed in methanol, stained with Giemsa and examined under oil immersion objective of a light microscope.

3.2.3. Haematological parameters

Blood samples were collected from all the selected dogs for detailed study. About two millilitres of blood was collected in a clean, dry, test tube with EDTA di potassium salt @ 1mg/ml of blood as anticoagulant for haematologic investigations with the help of automatic animal blood cell counter using standard techniques as described by Schalm *et al.* (1975). The following parameters were observed.

1. RBC count [x 10^6 /mm^3]
2. Haemoglobin (Hb) [g/dl]
3. Volume of packed red cells (VPRC) [%]
4. Total leucocyte count (TLC) [X10^3 /mm^3]
5. Differential leucocyte count (DLC) [%]
6. Platelet count [x10^5 / mm^3]
7. Mean Corpuscular Cell Volume (MCV) [fl]
8. Mean Corpuscular Haemoglobin (MCH) [pg]
9. Mean Corpuscular Haemoglobin Concentration (MCHC) [%]

3.2.4. Serum biochemistry

About four ml of blood was collected from all cases in a clean, dry, test tube without anticoagulant, allowed to clot and centrifuged at 3,000 rpm for 15 minutes. Sera that separated were stored at -20˚C until further analysis and were measured spectrophotometrically by using semiautomatic biochemical analyser Erba Mannheim Chem-5 Plus V_2, Transasia Biomedicals Ltd, Mumbai.

3.2.4.1. Blood Urea Nitrogen

Urea was estimated by GLDH - Urease method using standard kits from Erba diagnostics (Tiffany *et al*., 1972).

3.2.4.2. Serum Creatinine

Creatinine concentration was estimated by Jaffe's alkaline picrate method using standard kits supplied by Erba diagnostics (Slot, 1965).

3.2.4.3. Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) was estimated based on the reference method of international federation of clinical chemistry (IFCC) using standard kits from Erba diagnostics (Bradley *et al*., 1972).

3.2.4.4. Alkaline phosphatase (ALP)

Alkaline phosphatase was estimated by Tris carbonate buffer method using standard kits supplied by Erba diagnostics (Bessey *et al*., 1946).

3.2.4.5. Total protein and albumin

Serum protein was estimated by Biuret method using standard kits supplied by Erba diagnostics (Flack and Woollen, 1984).

Serum albumin was estimated by using Bromocresol green method with standard kits supplied by Erba diagnostics (Doumas, 1971).

3.2.4.6. Total bilirubin and direct bilirubin

Total bilirubin and direct bilirubin was estimated by Diazo method using standard kits supplied by Erba diagnostics (Pearlman and Lee, 1974).

3.2.5. Abdominal ultrasonography

3.2.5.1. Equipment

Selected dogs were subjected to ultrasound scanning of abdomen using Mindray DC 6 Vet Ultrasound machine with 2.5, 3.0, 5.0 and 7.5 MHz transducer (Plate 1).

3.2.5.2. Ultrasound Scanning Procedure

Abdominal ultrasonographic examination was performed with the animal in the dorsal or lateral recumbency. In dorsal recumbency animal was positioned in such a way that cranial portion of the image was oriented to the viewers left on sagittal scan and right side of the animal to viewers left on transverse scan.

Preparation of the site required clipping the hair over the entire abdomen, including midway up the body wall over the right and left caudal intercostal spaces. Acoustic coupling gel was applied to the skin. Liberal amounts of acoustic coupling gel provided sufficient contact for the best image possible.

A sector transducer, which provided a smaller contact zone was preferred for imaging through intercostal space. The frequency of transducer used for medium to large breeds of dogs was 3.5-7.5 MHz and for small breeds was 8-10 MHz. Firm pressure on the transducer was applied to gain maximum contact and displace overlying bowel gas.

The ultrasonograms were reviewed for alterations in the size and echogenicity of various abdominal organs like liver, spleen, kidney etc. The image was recorded on electromagnetic tape and later photographed.

3.2.6. Electrocardiography

Electrocardiograms were recorded using BPL-CARDIART-6108-T machine with the patient in right lateral recumbency as per the procedure of Goodwin (2001) and Martin (2000) (Plate 2). Electrocardiograms were recorded at the speed of 25 mm/second and sensitivity of 1 mv = 10 mm.

3.2.7. Thoracic radiography

Lateral plane radiographs of thorax in diseased animals were taken for cardiac evaluation. Size of X- ray film and radiographic factors varied depending upon the size and chest girth of patients. Abnormal cardiac size and shape were evaluated and correlated with other clinical parameters. Those cases with generalized enlargement of the heart silhouette on plain radiographs were considered for further echocardiographic studies.

3.2.8. Echocardiography

Selected dogs were subjected to echocardiographic studies using Mindray Z6 ultrasound scanner (Plate 3). Both two-dimensional and M-mode echocardiographic views of the heart were obtained from Right parasternal and left parasternal windows. Color flow Doppler echocardiography was used to assess blood inflow pattern across the heart valves. Left ventricular dimensions were measured in the right parasternal

short axis view. Values for each parameter were determined by the average of three to five cardiac cycles (Boon, 2011).

Direct measurements were as follows:

Left ventricular internal dimension at end systole (LVIDs)

Left ventricular internal dimension at end diastole (LVIDd)

From the direct measurements taken, the indirect measurements were calculated as follows:

Shortening fraction (FS) (%) = (LVIDd – LVIDs)/LVIDd

End systolic volume index was calculated according to Teichholz cube formula.

End Systolic Volume (ESV) (ml) = $7(LVIDs)^3/ (2.4 + LVIDs)$

End Diastolic Volume (EDV) (ml) = $7(LVIDd)^3/ (2.4 + LVIDd)$

Stroke volume (SV) in ml = EDV – ESV

Ejection Fraction (EF) (%) = (EDV – ESV) / EDV

3.2.9. Blood Pressure

Non-invasive blood pressure monitoring was carried out in selected cases using BP-Accugard Oscillometric digital blood pressure apparatus (Plate 4). With the animal in lateral recumbency, recordings were made in a calm environment using cuffs of suitable sizes. The cuffs were fixed on the forelimb at the same level as the base of the heart. The first reading was discarded and the next three successive readings were taken and the average values were noted (Carr *et al*., 2008).

3.2.10. Blood gas analysis

Blood pH, Bicarbonate (mmol/L), Base excess (mmol/L), Sodium (mmol/L), Potassium (mmol/L) and Calcium (mmol/L) of selected cases were measured by Epoc blood gas analyser (Plate 5).

3.2.11. Skin biopsy

Biopsy of oedematous limb in selected cases was performed using sterile ACCUSHARP disposable biopsy punch of 6.0 mm diameter (Plate 6).

3.2.11.1. Procedure

The area to be biopsied was gently clipped or shaved to remove the hairs. The animal was properly restrained and 1-2 ml of local anesthetic (2 per cent lignocaine) was injected subcutaneously around the site to be biopsied. Five minutes later, the skin samples were taken by placing the punch vertically over the selected lesion and applied gentle continuous pressure. The punch should only be rotated in one direction. The biopsy sample was carefully grasped at the base with thumb forceps to avoid crushing injuries and all other subcutaneous attachments were severed using scissors. The biopsy site was then closed with simple interrupted sutures (Miller *et al*., 2013).

The sample was then placed on wooden tongue depressor to avoid tissue folding. The sample was fixed in 10 per cent neutral buffered formalin immediately after collection. The sample was then processed for histopathologic examination, embedded in paraffin, four micrometer sections were made, stained with haematoxylin and eosin and subjected for microscopic examination.

3.3. TREATMENT AND RESPONSE TO TREATMENT

For cardiac diseases, treatment was done with ACE inhibitor enalapril @ 0.5 mg/kg bodyweight BID, supported with diuretics like furosemide or combination of

furosemide and spironolactone @ 2 mg/kg bodyweight BID orally. Apart from these, carvedilol was given @ 0.2 mg/kg bodyweight BID in hypertrophic cardiomyopathy.

For hepatic etiology, hepatoprotectant drugs like silymarin @ 150 mg/dog/day and for renal etiology, fluid therapy with 5 per cent dextrose and normal saline were given.

Blood parasitic infections like ehrlichiosis was treated with Doxycycline @ 5 mg/kg bodyweight BID orally for 21 days. Dogs with babesiosis were given a combination therapy of Clindamycin @ 11 mg/kg bodyweight, Metronidazole @ 25 mg/kg bodyweight SID intravenously and Doxycycline @ 10 mg/kg bodyweight SID orally for 10 days. Cases of sheathed microfilariosis were treated with Levamisole @ 10 mg/kg bodyweight SID orally for 7 days and unsheathed microfilariasis were treated with with Ivermectin @ 100 μg/kg bodyweight SID orally.

Apart from these, those cases with Immune mediated vasculitis were treated with prednisolone @ 2 mg/kg bodyweight PO initially followed by tapering doses.

Response to treatment was assessed by follow up examinations done one month after the treatment.

3.4. STATISTICAL ANALYSIS

The data obtained were statistically analysed using computer software SPSS version 21 using repeated measures of ANOVA for comparison of normal and diseased animals on 0^{th} and 30^{th} day of observation. In addition, one of the individual group was analysed by independent t test (Kaps and Lamberson, 2009).

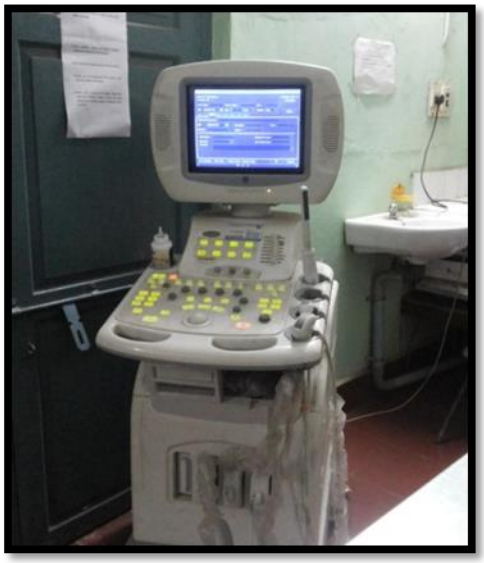


Plate 1. Mindray DC 6 Vet Ultrasound machine

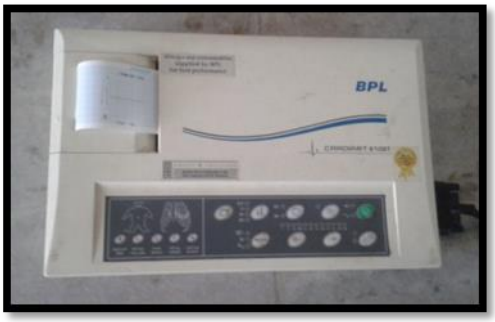


Plate 2. ECG machine-BPL-CARDIART-6108-T

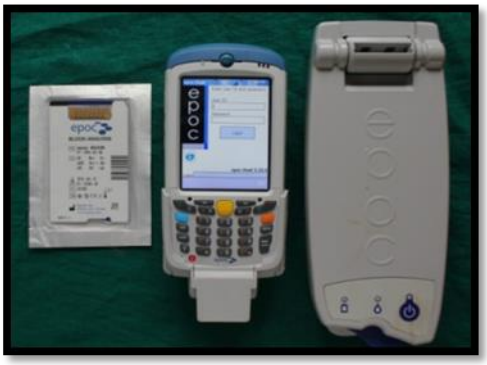


Plate 5. Epoc blood gas analyser

Plate 3. Mindray Z6 Ultrasound machine

Plate 4. BP-Accugard oscillometric digital blood pressure apparatus

Plate 6. ACCUSHARP Biopsy punch 6mm

Results

4. RESULTS

Dogs presented to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with the complaint of limb oedema were selected for the study. All the dogs were subjected to detailed clinical examination and various diagnostic procedures to identify the etiological factor for the limb oedema. Therapy was given based on the etiology and reassessed after 30 days. The data obtained were statistically analysed in comparison with six normal animals using repeated measures of ANOVA and one of the individual group was analysed by independent t test.

4.1. OCCURRENCE OF LIMB OEDEMA

Based on the test results and findings, 36 cases with limb oedema were selected and were classified into eight groups (Table 1, Fig. 1, Plate 7). The dogs presented with oedema were categorized based on the limbs involvement into either unilateral, bilateral or multiple limb affection (Fig. 2). Among the groups, some dogs had pitting oedema while others had non-pitting oedema (Fig. 3).

Table 1. Clinical grouping of limb oedema cases

GROUPS	TYPES	No.
Group I	Oedema of limbs associated with Ehrlichiosis	6 cases
Group II	Oedema of limbs associated with Babesiosis	6 cases
Group III	Oedema of limbs associated with Microfilariasis	6 cases
Group IV	Oedema of limbs associated with Cardiac diseases	6 cases
Group V	Oedema of limbs associated with Renal diseases	2 cases
Group VI	Oedema of limbs associated with Hepatic diseases	2 cases
Group VII	Oedema of limbs due to mammary gland neoplasia	2 cases
Group VIII	Idiopathic limb oedema	6 cases

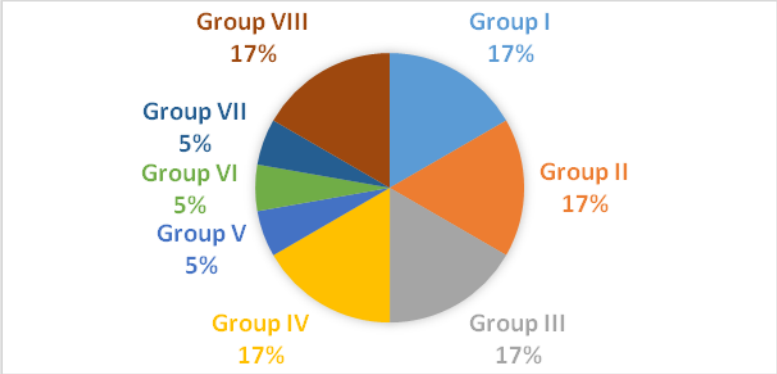


Fig. 1. Etiological classification of dogs with limb oedema

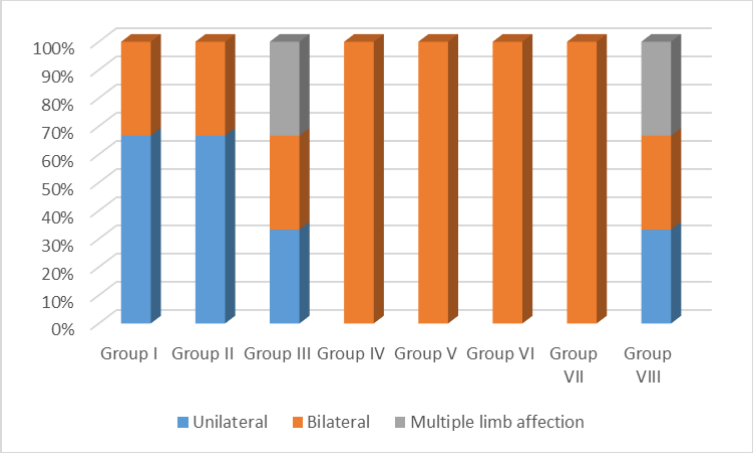


Fig. 2. Number of limbs affected with odema (%)

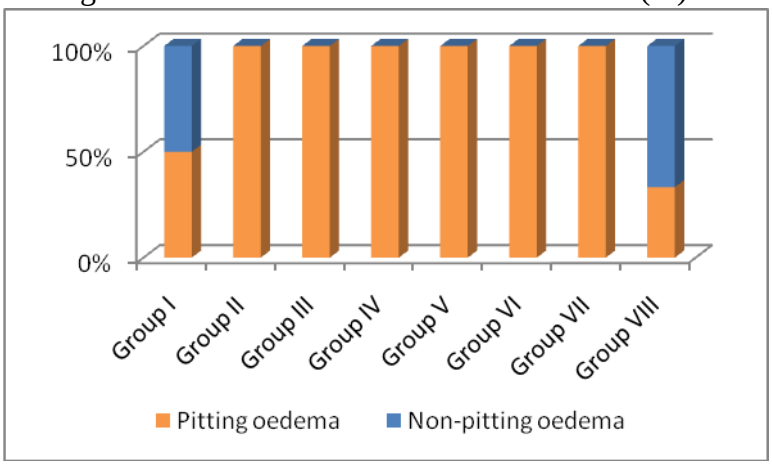
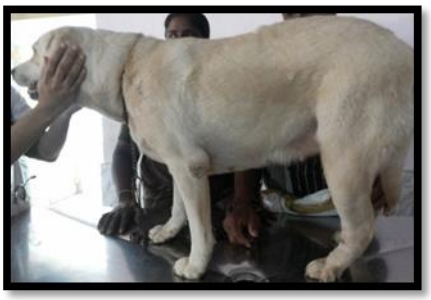


Fig. 3. Occurrence of limb oedema based on pitting (%)

Plate 7. Clinical presentations of limb oedema

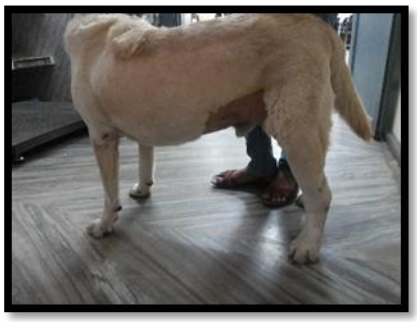
a. Limb oedema due to ehrlichisis



b. Limb oedema due to babesiosis

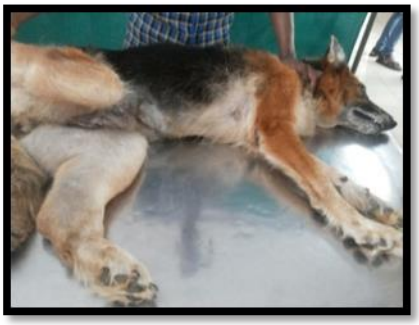
c. Limb oedema due to microfilariasis

d. Limb oedema due to cardiac origin



e. Limb oedema due to renal origin

f. Limb oedema due to hepatic origin



g. Limb oedema due to mammary tumour

h. Idiopathic limb oedema

4.2. DIAGNOSTIC TECHNIQUES

4.2.1. Peripheral wet film, blood smear and buffy coat smear examination

All the 36 cases were screened by peripheral wet film examination and out of that six cases were positive for microfilariae and by detailed blood smear examination they were further classified into sheathed (2 cases) and unsheathed (4 cases) microfilariae. Six cases of babesiosis and six cases of ehrlichiosis were identified by blood smear and buffy coat examination respectively (Plate 8).

4.2.2. Abdominal ultrasonographic findings

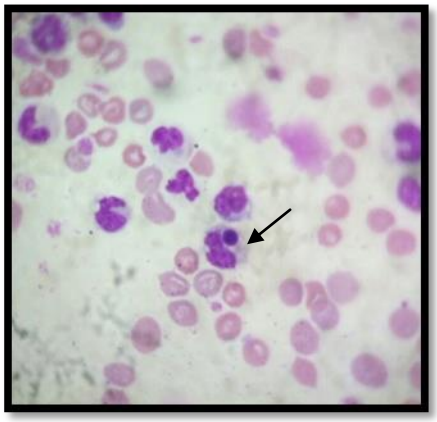
In both group I and II only one dog revealed splenomegaly on abdominal ultrasonography.

In group IV animals, four dogs which were presented with limb oedema in association with ascites revealed large amount of anaechoic fluid in the abdomen with liver lobes and intestinal loops floating in it. Abdominocentesis of ascitic fluid revealed modified transudate in most cases. Also hepatomegaly was evident in two other dogs of the same group.

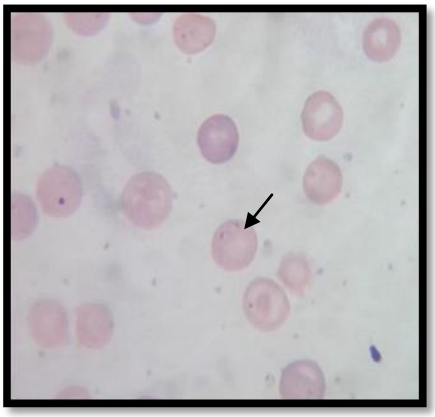
In group V animals, comparative reduction in the size of kidneys was noticed in one case and normal size of kidneys in the other.

In group VI, both the dogs were presented with ascites and ultrasonography revealed large amount of anaechoic fluid with freely floating liver lobes and intestinal loops. The lobes were small and irregular liver border was noticed in one of the dog (Plate 9).

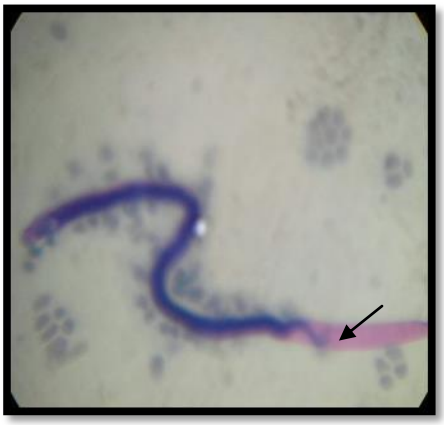
Plate 8. Blood and buffy coat smear examination



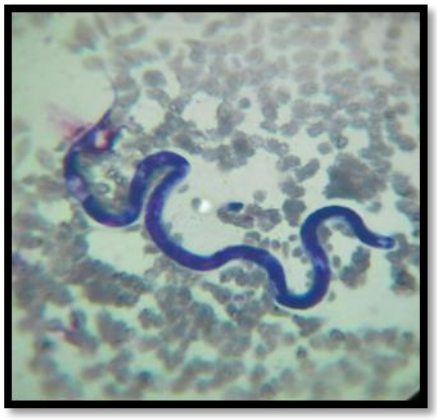
a. Buffy coat smear showing *E. canis* morula within the monocyte



b. Blood smear showing *B.gibsoni* organism within the RBC.

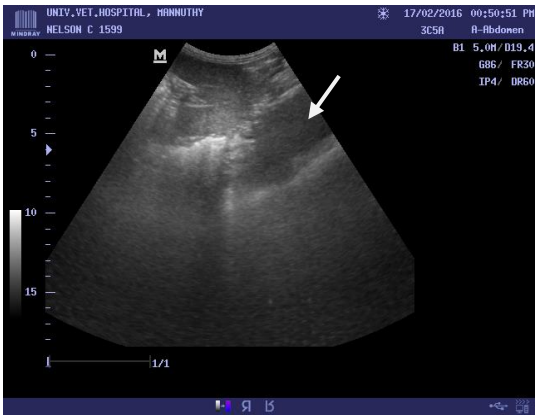


c. Blood smear showing sheathed microfilariae

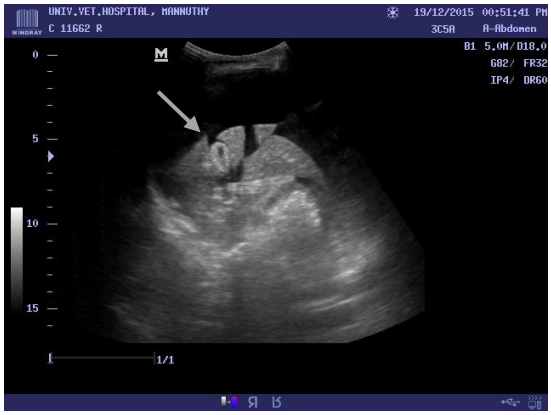


d. Blood smear showing unsheathed microfilariae

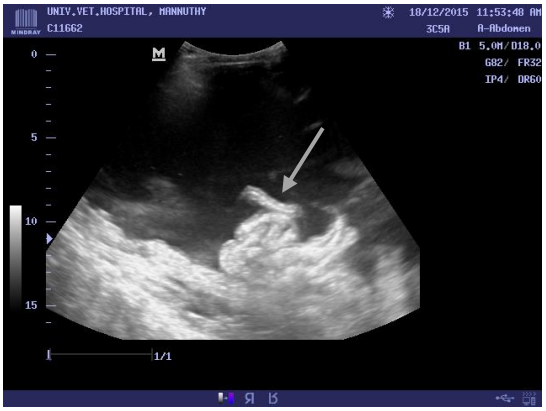
Plate 9. Abdominal ultrasonographic findings



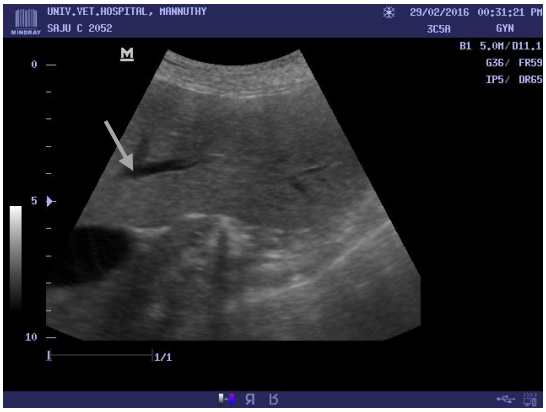
a. Splenomegaly associated with Babesiosis



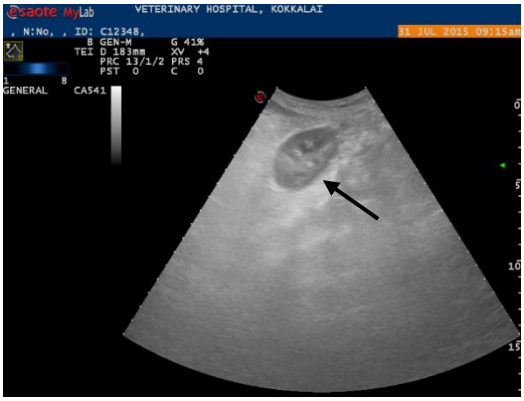
b. Ascites with anaechoic fluid and floating liver lobes in a case of congestive heart failure



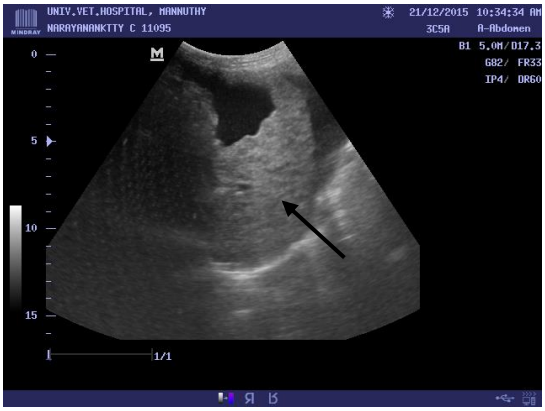
c. Severe ascites and floating intestinal loops in congestive heart failure



d. Hepatomegaly with enlarged hepatic vessels in right sided heart failure



e. Reduced size of the kidney in chronic renal failure



f. Reduced size of liver with irregular borders in chronic hepatitis

4.2.3. Electrocardiographic findings

Abnormalities in the ECG were mainly detected in group IV and VI using the bipolar limb lead II. In group IV, two dogs had small amplitude P waves, low voltage QRS complexes with small or no T waves which were suggestive of fluid accumulation. Electrical alternans were recorded in two cases with pericardial effusion. There was one case with ST coving. Another case revealed reduced amplitude of P wave (0.05 mV) and R wave (0.06 mV) with peaked negative T waves (0.25mV). Among group VI, one of the hepatic case with ascites revealed small P wave with reduced amplitude of QRS complex (Plate 10).

4.2.4. Thoracic radiographic findings

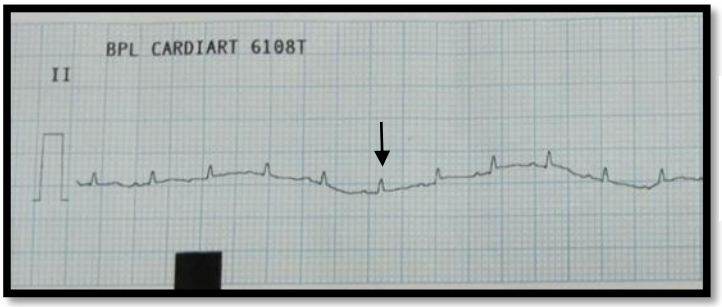
Thoracic radiographic abnormalities were detected mainly in group IV animals. Out of the six cases cardiac silhouette was not clear in lateral radiographs of three cases, which were suggestive of pleural effusion with or without pericardial effusion. Pulmonary oedema was present in two other cases with interstitial or alveolar pattern. In one case, there was enlarged cardiac chambers with tracheal elevation and pulmonary oedema (Plate 11).

4.2.5. Echocardiographic findings

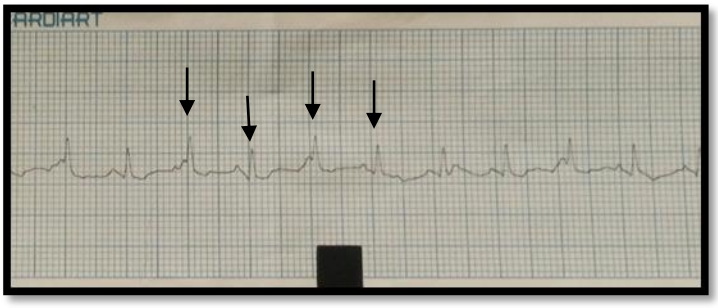
Echocardiography was conducted mainly in group IV and group VI animals. In group VI, two dogs with ascites were subjected to echocardiography and no cardiac abnormalities were revealed.

In group IV, out of the six cases, the right parasternal long axis four chamber view revealed severe dilatation of right atrium and ventricle in two cases. Severe pleural effusion was detected in one case.

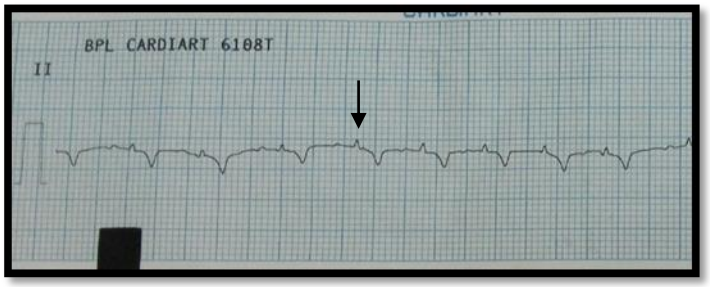
Plate 10. Electrocardiographic findings



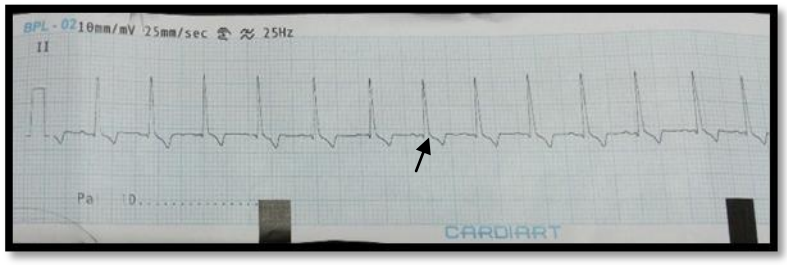
a. Small amplitude P waves, low voltage QRS complexes without T waves suggestive of fluid accumulation



b. Electrical alternans suggestive of pericardial effusion

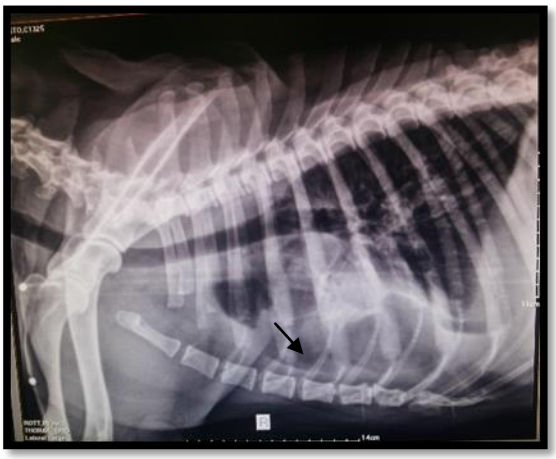


c. Reduced amplitude of P wave (0.05 mV) and R wave (0.06 mV) (arrow) with peaked negative T waves in a 6-year-old crossbred dog

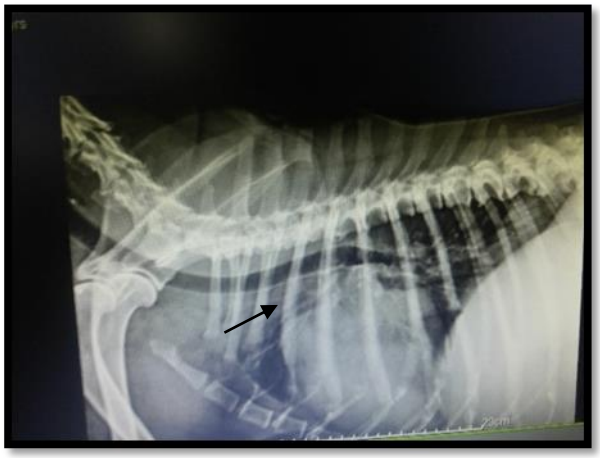


d. A case with ST coving suggestive of myocardial hypoxia

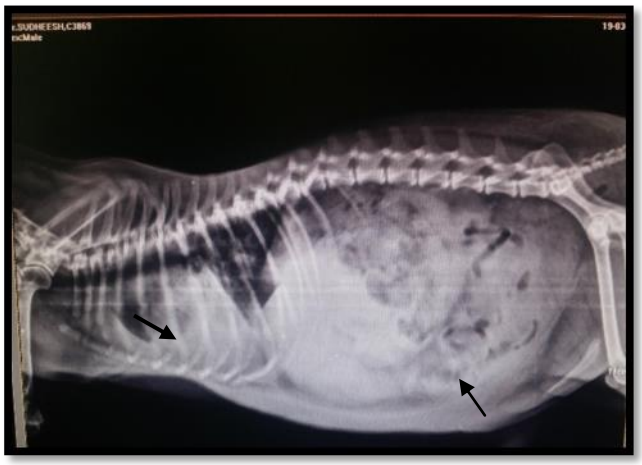
Plate 11. Thoracic radiographic findings



a. A case with severe pleural effusion and the cardiac silhouette was not clear.



b. One case of CHF showing cardiomegaly and tracheal elevation with pulmonary oedema



c. A case of severe pleural effusion along with ascites

In three cases, colour flow doppler echocardiography revealed colour flow jet across mitral, tricuspid and aortic valves indicating severe valvular insufficiency. Pericardial effusion was also detected along with these changes. Three cases showed increased left atrium to aortic diameter (LA/Ao) ratio as 2.23, 1.52 and 1.45 respectively when compared to normal animals which is usually 1.3 or less (Plate 12).

The mean values of M – mode echocardiographic parameters like left ventricular internal dimension during diastole (LVIDd), left ventricular internal dimension during systole (LVIDs), end diastolic volume (EDV), end systolic volume (ESV), stroke volume (SV), ejection fraction (EF) and fractional shortening (FS) were 2.80 ± 0.37 cm, 1.60 ± 0.24 cm, 31.80 ± 11.95 ml, 9.20 ± 3.25 ml, 22.80 ± 8.53 ml, 73.20 ± 3.31 and 40.80 ± 2.56 per cent respectively. Mean values of Echocardiographic parameters are given in table 2. A significant decrease in LVIDs, EDV and ESV were observed in diseased animals when compared to normal animals. A significantly higher value was obtained for FS and EF in diseased animals than normal animals. No significant variation was obtained for LVIDd and SV between the normal and diseased animals.

4.2.6. Blood Pressure measurement

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded mainly for group IV and V animals which are presented in Table 3. The mean SBP and DBP in normal group were 130.17 ± 0.48 and 84.50 ± 0.43 mm Hg respectively. The mean values of SBP in group IV and V were 151.17 ± 3.63 and 152.5 ± 2.64 mm Hg respectively and that of DBP were 90.50 ± 1.65 and 96.6 ± 1.05 mm Hg. It was found that there was significant difference in SBP and DBP between the normal and group IV animals. Since the number of cases were less in group V, that group was exempted from the analysis.

Table 2. M- mode echocardiographic values of normal and group IV dogs

Parameters	Normal animals	Diseased animals	t value
LVIDd (cm)	4.00 ± 0.00	2.80 ± 0.37	2.40^{ns}
EDV (ml)	69.50 ± 0.89	31.80 ± 11.95	3.16^{*}
LVIDs (cm)	3.00 ± 0.00	1.60 ± 0.24	3.73^{*}
ESV (ml)	28.17 ± 0.65	9.20 ± 3.25	5.51^{**}
SV (ml)	38.00 ± 1.13	22.80 ± 8.53	1.75^{ns}
EF (%)	55.50 ± 0.96	73.20 ± 3.31	5.63^{**}
FS (%)	30.50 ± 0.76	40.80 ± 2.56	4.17^{**}

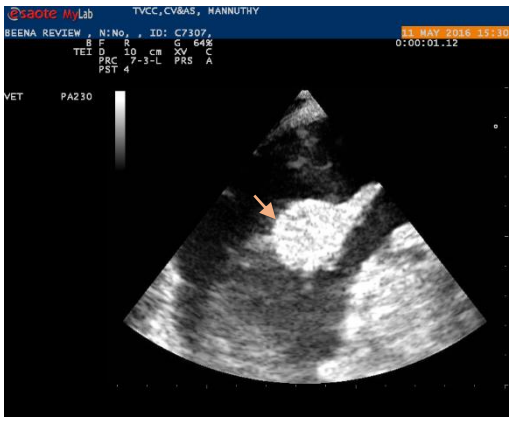
* - significant at 5 % level, ** - significant at 1 % level and ^{ns} - not significant

Table 3. Blood pressure values of normal and group IV & V dogs

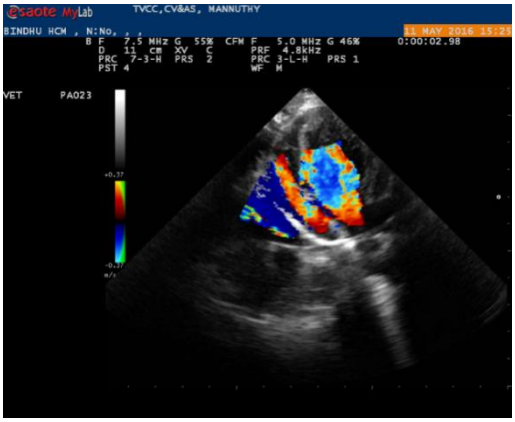
Parameters	Normal animals	Group IV	t value	Group V
Systolic pressure (mm Hg)	130.17 ± 0.48	151.17 ± 3.63	5.74^{**}	152.5 ± 2.64
Diastolic pressure (mm Hg)	84.50 ± 0.43	90.50 ± 1.65	3.52^{*}	96.6 ± 1.05

* - significant at 5 % level, ** - significant at 1 % level and ^{ns} - not significant

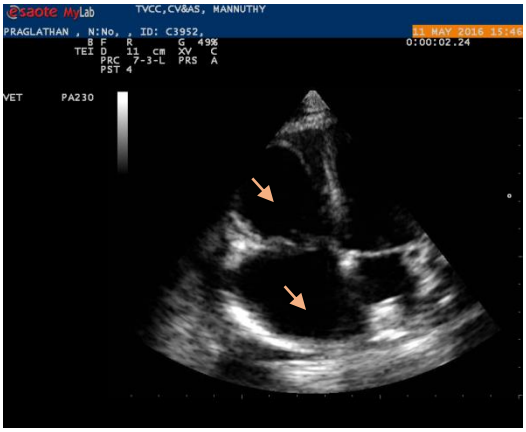
Plate 12. Echocardiographic findings



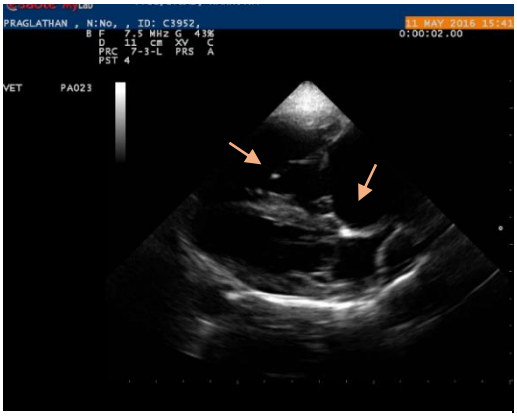
a. A case with pleural effusion with lung lobes floating in it.



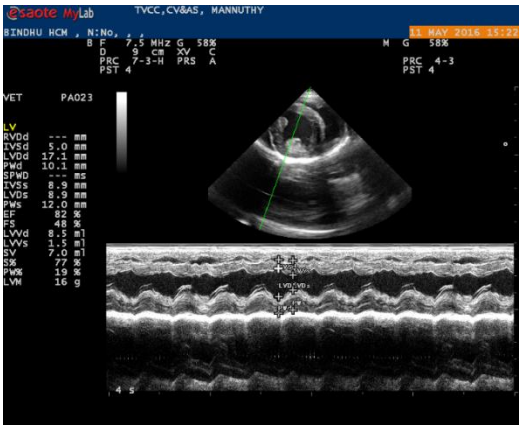
b. A case with severe mitral, aortic and tricuspid regurgitation



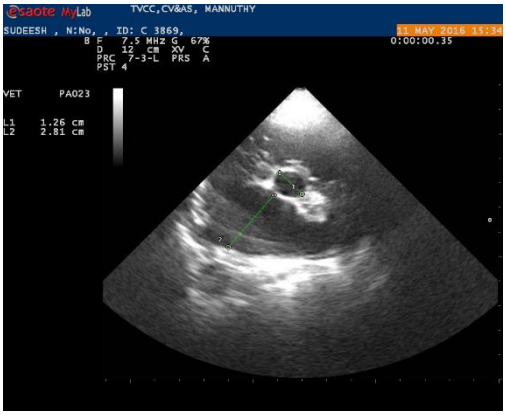
c. A case with right atrial and ventricular enlargement in the left parasternal apical four chamber view



d. A case with dialated right atrium and ventricle in the right parasternal four chamber view



e. Left ventricle at right parasternal short axis view with M-mode at the level of papillary muscle



f. A case with increased LA/Ao ratio (2.23)

4.2.7. Blood gas analysis

Blood gas analysis was conducted only in group V animals. In that, out of the two cases, both the dogs had metabolic acidosis with lowered blood pH (7.28 in both cases) and bicarbonate levels (11.8 & 11.3 mmol/l). The sodium (141, 147 mmol/l), potassium (4.1, 4.4 mmol/l) and calcium (1.34 mmol/l in both) level were normal in both the animals.

4.2.8. Histopathological findings of skin biopsy

Among the eight groups, skin biopsy of the oedematous region was taken from selected animals of group I, II, III, VII and VIII.

In group I with ehrlichiosis as the etiology, biopsy was conducted in two cases. One case revealed diffuse infiltration of inflammatory cells throughout the dermal area with haemorrhage and severe infiltration in the subcutaneous area with focal accumulation of plasma cells. The other case revealed moderate diffuse infiltration of inflammatory cells predominantly mononuclear cells in the subepithelial and subcutaneous area with dilatation of small and medium vessels (Plate 13. a, b). The infiltration was more intense around the blood vessels and these were suggestive of cutaneous vasculitis.

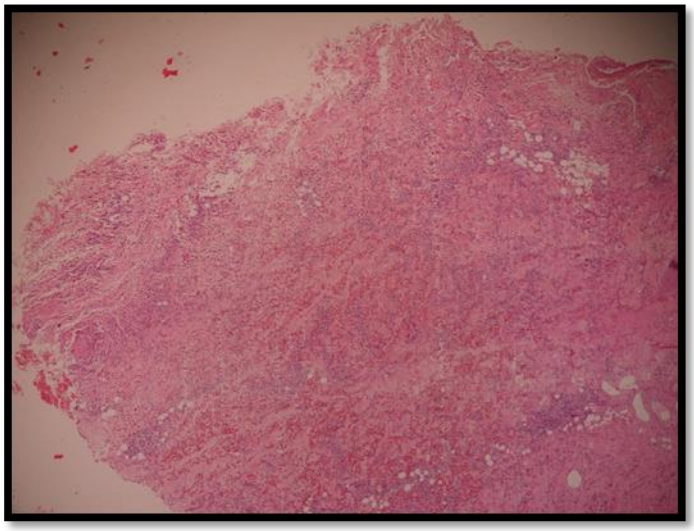
In group II animals with babesiosis, three cases were selected for biopsy. First case revealed dilatation of deep dermal vessels with mild infiltration of inflammatory cells. In the second case, there was moderate perivascular accumulation of inflammatory cells and plasma cells in the subepidermal area and massive accumulation in the subcutaneous area. Haemorrhage and dilatation of lymphatics were also observed in the subepidermal region. In the third case, there was moderate diffuse infiltration of inflammatory cells in the subepithelial area with dilatation of vessels (Plate 13. c, d). These histological features were suggestive of vasculitis.

In group III with microfilariasis, biopsy was taken from four cases in which two were sheathed and others unsheathed. In case of unsheathed microfilariosis, there was extensive dilatation of deep dermal vessels with mild to moderate focal infiltration of inflammatory cells around the blood vessels in the subepithelial area which was suggestive of vasculitis. Those with sheathed microfilariosis, there was moderate dilatation of subepithelial and subcutaneous lymphatic vessels and infiltration of inflammatory cells around small blood vessels with accumulation of pink staining proteinaceous material within small vessels of the dermis which were suggestive of both vasculitis and lymphangitis (Plate 13. e, f, g)

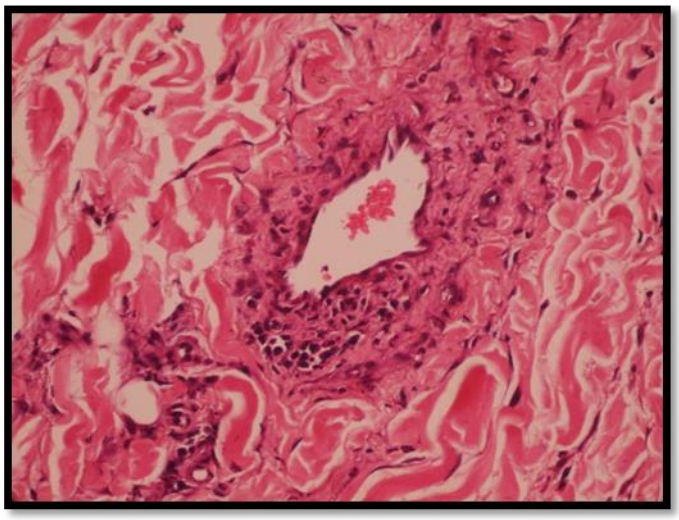
In group VII with limb oedema due to mammary gland neoplasia, skin biopsy taken from the oedematous hind limb of one of the animal was suggestive of enlarged lymphatic duct with pink stained oedematous fluid and severe dermal oedema.

In group VIII, all the six dogs were selected for biopsy since no other etiological agent was identified for these dogs. In the first case there was subepithelial mild infiltration of inflammatory cells around the vessels and dilatation of blood vessels. Second case revealed subepithelial and subcutaneous dilatation of blood vessels with mild infiltration of mononuclear cells into the media of large blood vessels in the deep dermis. In the third case also there was dilatation of subepithelial and subcutaneous blood vessels with moderate to severe infiltration of inflammatory cells around small venules and haemorrhage in the subcutaneous area. The fourth case revealed diffuse accumulation of inflammatory cells predominantly lymphocytes, macrophages and plasma cells in the subepithelial region and around the blood vessels. There was congestion of blood vessels and dilatation lymphatics with mild infiltration of inflammatory cells into the media (Plate 13. h, i). The other two cases also had the same findings and the histopathologic changes were typical of vasculitis.

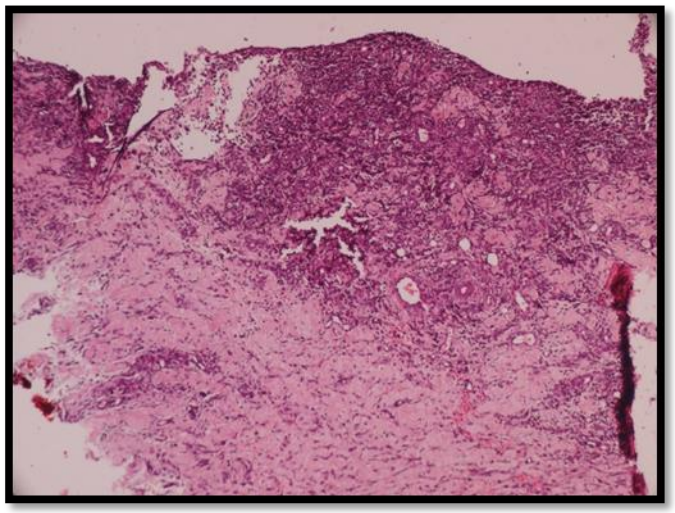
Plate 13. Histopathological findings of skin biopsy



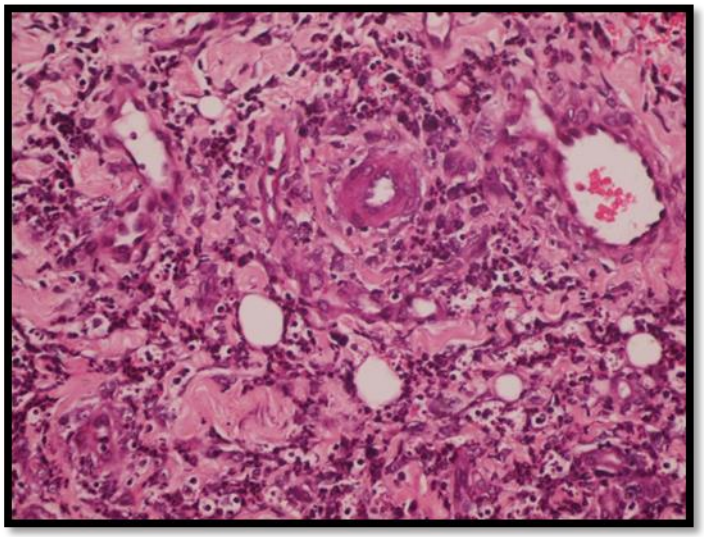
a. Group I - Diffuse infiltration of inflammatory cells throughout dermis with haemorrhage in the subcutaneous area (H & E, 40 x)



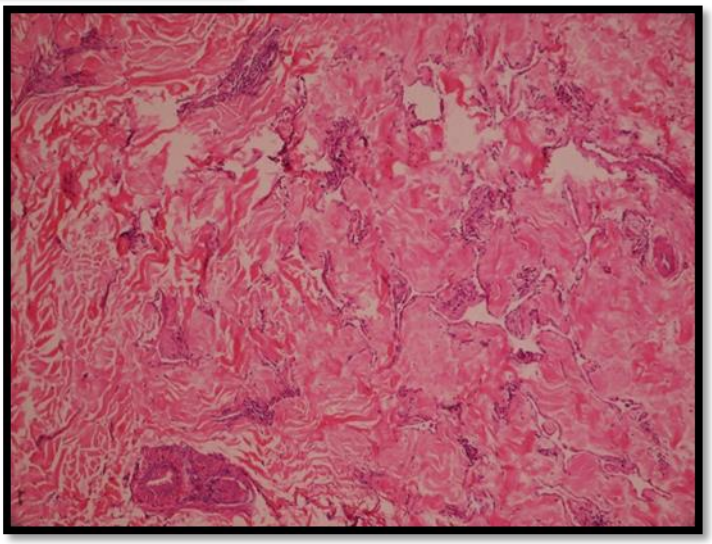
b. Group I - Infiltration of inflammatory cells (lymphocytes) around blood vessels in the subepithelial region (H & E, 400 x)



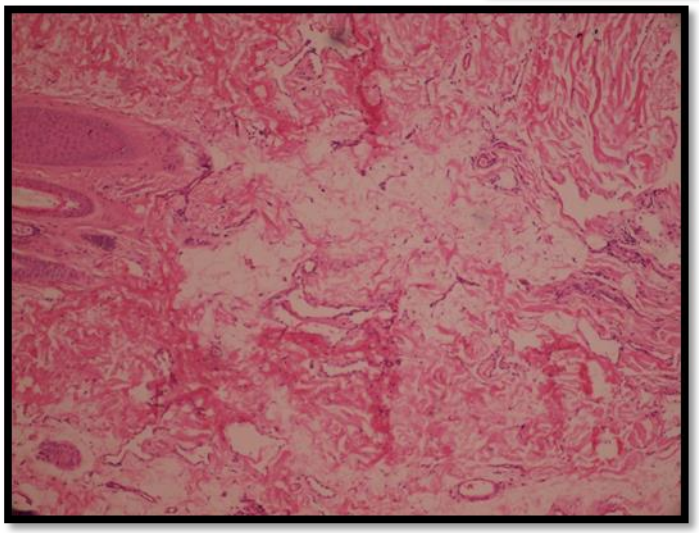
c. Group II - Perivascular accumulation of inflammatory cells and plasma cells in the subepithelial & subcutaneous area with haemorrhage (H & E, 100 x)



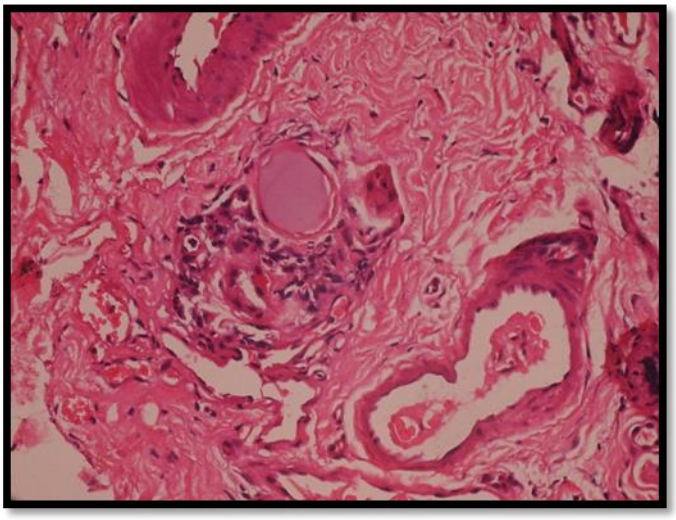
d. Group II - Perivascular accumulation of inflammatory cells and plasma cells in the subepithelial & subcutaneous area (H & E, 400 x)



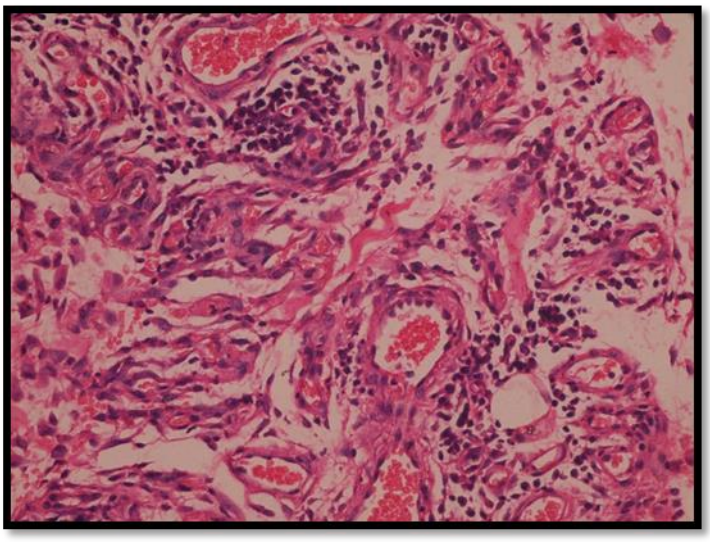
e. Group III - Extensive dilatation of deep dermal lymphatics with focal infiltration of inflammatory cells around vessels (H & E, 100 x)



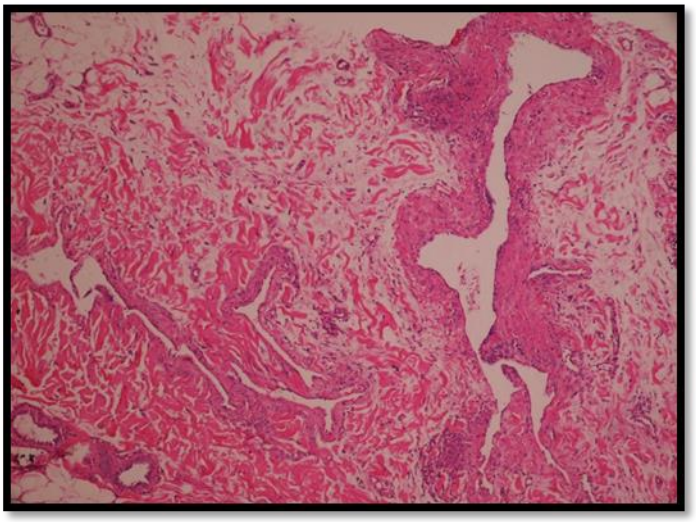
f. Group III - Dilatation of large number of lymphatic vessels in the dermal area (H & E, 100 x)



g. Group III - Moderate dilatation of subepithelial lymphatic vessels, infiltration of inflammatory cells and accumulation of pink staining proteinaceous material (H & E, 400 x)



h. Group VIII - Congestion of blood vessels with severe infiltration of mononuclear cells around small venules of subcutaneous area (H & E, 400 x)



i. Group VIII - Infiltration of mononuclear cells into the media of large blood vessels (H & E, 100 x)

4.2.9. Haematological findings

The mean haematological values of normal and diseased animals on 0^{th} day and 30^{th} day are given in table 4. Since one animal in group V died after few days of therapy, that group was exempted from the analysis.

4.2.9.1. Total Erythrocyte Count (TEC)

The mean TEC of normal animals on 0^{th} day and 30^{th} day were 7.16 ± 0.14 and 6.78 ± 0.28 million/cmm respectively. Out of the eight groups, group I, II, III and VIII were comparable to normal group on 0^{th} day whereas groups IV, VI and VII had significantly lower values. Similarly, on 30^{th} day, all the groups were comparable to normal group except group II and VII. The mean TEC of group I animals showed significant increase after therapy with corresponding means 5.53 ± 0.28 and 5.98 ± 0.22 million/cmm on 0^{th} and 30^{th} day respectively. Similarly, in group III also there was significant increase in TEC on 30^{th} day of observation with corresponding means 5.54 ± 0.34 and 5.96 ± 0.31 million/cmm pre and post therapy respectively.

4.2.9.2. Haemoglobin (Hb)

The mean Hb of normal group on 0^{th} day and 30^{th} day were 13.07 ± 0.43 and 12.77 ± 0.34g/dl respectively. There was statistically significant decrease in the Hb of groups IV and VII whereas all other groups were comparable to normal group on the 0^{th}day of presentation. After 30 days, all the groups were comparable to normal group except group IV. The mean value of Hb in group III before and after treatment was 10.87 ± 0.92 and 12.20 ± 0.61g/dl respectively. It was found that there was significant increase in the mean Hb on this group after treatment.

4.2.9.3. Volume of packed red blood cells (VPRC)

The mean VPRC level of normal animals on 0^{th} day and 30^{th} day were 42.03 ± 0.41 and 38.68 ± 2.04 per cent respectively. Among the groups, group I, II,

III and VIII were comparable to normal group on 0^{th} day of presentation whereas groups IV, VI and VII had lower values than normal group. On 30^{th} day, only group IV showed statistically significant decrease and all other groups were statistically insignificant to normal group. There was no significant variation in any of the groups before and after therapy.

4.2.9.4. Total leucocyte count (TLC)

Mean of TLC in normal group was found to be 12.73 ± 1.31 and 13.10 ± 0.37 thousands/cmm on the 0^{th} and 30^{th} day of observation respectively. The mean of all groups were comparable to the normal group on 0^{th} and 30^{th} day respectively. There was no statistically significant difference noticed in any of the group before and after therapy.

4.2.9.5. Differential leucocytic count (DLC)

The mean lymphocyte count of normal group was 13.15 ± 1.73 and 15.23 ± 1.26 per cent on 0^{th} day and 30^{th} day respectively. There was no significant difference noticed in any of the group with that of normal group on both 0^{th} and 30^{th} day of observation. There was significant increase noticed in group I after therapy with corresponding means 9.05 ± 1.06and 14.08 ± 0.92 per cent respectively.

The mean value of monocyte in normal group on 0^{th} day and 30^{th} day was 5.33 ± 0.90 and 6.82 ± 1.07 per cent respectively. All the groups were comparable with the normal group on both 0^{th} and 30^{th} day of observation. No statistically significant difference was noticed in any of the groups before and after therapy.

The mean granulocyte in normal group on 0^{th} and 30^{th} day was 81.52 ± 2.39 and 76.70 ± 2.11 per cent respectively. No statistically significant difference was noticed in any of the groups with normal group on pre and post therapy. The mean value of granulocyte in group I pre and post therapy was 86.53 ± 1.97 and 81.29 ± 1.77 per cent respectively and showed significant decrease after therapy.

4.2.9.6. Thrombocyte count (TC)

The mean thrombocyte count in normal group was 2.69 ± 0.60 and 3.22 ± 0.33 lakhs/cmm on 0^{th} day and 30^{th} day respectively. All the groups were comparable with normal group on both days of observation. There was no statistically significant difference in any of the groups before and after therapy.

4.2.10. Serum biochemistry

The mean serum biochemical values of normal and diseased animals on 0^{th} day and 30^{th} day were given in table 5. Since one animal in group V died after few days of therapy, that group was exempted from the analysis.

4.2.10.1. Total protein (TP)

The mean value of TP in normal dogs at 0^{th} and 30^{th} day of observation was 6.97 ± 0.25 and 6.56 ± 0.22 g/dl respectively. All the groups were comparable with normal group on 0^{th} day of presentation. On 30^{th} day, group III shows significant increase in total protein with normal group whereas all other groups were insignificant. A statistically significant reduction in TP was noticed in group I after therapy with corresponding means 8.42 ± 0.43 and 7.86 ± 0.30 g/dl on 0^{th} and 30^{th} day respectively.

4.2.10.2. Albumin

The mean albumin value of normal group was 3.30 ± 0.13 and 3.01 ± 0.07 g/dl respectively on 0^{th} day and 30^{th} day of observation. Groups II, III, VI and VII recorded significant decrease in albumin level when compared to normal group whereas no significant variation was recordrd in groups I, IV and VIII on 0^{th} day. On 30^{th} day, all the groups were comparable to normal group. The mean albumin of group II were 2.61 ± 0.16 and 2.81 ± 0.10 g/dl and that of group III were 2.52 ± 0.11

and 2.83 ± 0.19 g/dl before and after therapy respectively. There was significant increase in the albumin level noticed in these groups after treatment.

4.2.10.3. Globulin

The mean globulin value of normal group was 3.67 ± 0.27 and 3.56 ± 0.15g/dl respectively on 0^{th} day and 30^{th} day. When compared with normal animals, a significantly higher value of globulin was recorded in group III before therapy and in groups I and III after therapy. The mean globulin value of groups I, III and VIII before therapy were 5.19 ± 0.38, 6.26 ± 0.77 and 5.25 ± 0.52 g/dl and the corresponding values after therapy were 4.62 ± 0.22, 5.18 ± 0.44 and 4.29 ± 0.22 g/dl respectively. It was found that there was significant reduction in the globulin levels of these groups after therapy.

4.2.10.4. Albumin globulin ratio (AG ratio)

The mean AG ratio of normal group on 0^{th} and 30^{th} day was 0.93 ± 0.10 and 0.85 ± 0.02 respectively. There was a significant decrease recorded in the AG ratio of all groups except groups IV and VI which were comparable to the normal groups on the 0^{th} day. There was no significant variation in any of the groups with the normal group on 30^{th} day. The mean AG ratio of group III was 0.43 ± 0.05 and 0.56 ± 0.06 on 0^{th} and 30^{th} day respectively which indicated a significant increase after therapy.

4.2.10.5. Alanine amino transferase (ALT)

The mean value of ALT in normal dogs was 24.35 ± 3.13 and 20.49 ± 1.20 IU/l respectively on 0^{th} and 30^{th} day of observation. There was no significant variation in any of the groups before and after therapy with the normal group. In group III and VIII, the mean ALT on 0^{th} day of observation was 27.44 ± 4.65 and 48.95 ± 15.56 IU/l respectively. The corresponding values on 30^{th} day of observation were 33.52 ± 5.25 and 56.53 ± 17.21 IU/l respectively. It was recorded that there was significant increase in the mean of ALT in these groups after treatment.

4.2.10.6. Alkaline phosphatase (ALP)

The mean value of ALT in normal dogs was 63.68 ± 12.17 and 39.88 ± 4.54 IU/l respectively on 0^{th} and 30^{th} day. There was no significant variation in any of the groups before and after therapy with the normal group. Among other groups, group III shows significant increase in ALT on 30^{th} day with corresponding means 119.84 ± 5.53and 133.21 ± 6.84 IU/l on pre and post therapy respectively.

4.2.10.7. Total bilirubin (TB) and direct bilirubin (DB)

The mean value of TB among normal dogs on 0^{th} and 30^{th} day were 0.68 ± 0.07 and 0.40 ± 0.03 mg/dl and that of DB were 0.26 ± 0.05 and 0.21 ± 0.03 mg/dl respectively. There was no significant difference noticed in the mean of TB and DB in any of the groups with normal group on 0^{th} and 30^{th} day. Similarly, no significant variation was noticed for TB and DB in any groups before and after therapy.

4.2.10.8. Blood urea nitrogen (BUN)

The mean value of BUN in normal group was 18.49 ± 2.27 and 20.12 ± 1.48 mg/dl respectively on 0^{th} day and 30^{th} day. A significant increase in BUN was recorded in group VII with normal group on both 0^{th} and 30^{th} day of observation, while all other groups were comparable with the normal group. It was found that there was no significant difference in any of the group before and after therapy.

4.2.10.9. Creatinine

The mean value of creatinine in normal dogs was 1.03 ± 0.15 and 1.02 ± 0.09 mg/dl respectively on 0^{th} and 30^{th} day of observation. A significant increase in creatinine was noted only in group VII and all other groups were comparable with that of normal group on 0^{th} day. There was no significant difference in any of the group with the normal group on 30^{th} day. There was no significant variation reported in any of the groups pre and post therapy.

Table 4. Haematological values of normal and diseased dogs before and after treatment

Parameters	TEC (10^6/cmm)		Hb (g/dl)		VPRC (%)	
	0th day	**30th day**	**0th day**	**30th day**	**0th day**	**30th day**
Normal	$7.16^{a} \pm 0.14$	$6.78^{a} \pm 0.28$	$13.07^{a} \pm 0.43$	$12.77^{a} \pm 0.34$	$42.03^{a} \pm 0.41$	$38.68^{a,b} \pm 2.04$
Ehrlichiosis (Group I)	$5.53^{a,b,c} \pm 0.28^{B}$	$5.98^{a,b} \pm 0.22^{A}$	$12.77^{a,b} \pm 0.87$	$13.55^{a} \pm 0.51$	$37.47^{a,b,c} \pm 2.00$	$39.15^{a,b} \pm 1.54$
Babesiosis (Group II)	$5.51^{a,b,c} \pm 0.39$	$5.05^{b} \pm 0.40$	$11.37^{a,b} \pm 0.52$	$10.48^{a,b} \pm 1.14$	$35.27^{a,b,c,d} \pm 1.56$	$32.67^{a,b,c} \pm 2.99$
Microfilariosis (Group III)	$5.54^{a,b,c} \pm 0.34^{B}$	$5.96^{a,b} \pm 0.31^{A}$	$10.87^{a,b,c} \pm 0.92^{B}$	$12.20^{a} \pm 0.61^{A}$	$33.45^{a,b,c,d} \pm 2.72$	$35.98^{a,b} \pm 2.02$
Cardiac diseases (Group IV)	$5.14^{b,c} \pm 0.73$	$5.33^{a,b} \pm 0.71$	$7.70^{c} \pm 1.21$	$8.36^{b} \pm 1.42$	$26.88^{d} \pm 3.18$	$26.86^{c} \pm 2.75$
Hepatic diseases (Group VI)	$4.98^{b,c} \pm 1.07$	$5.52^{a,b} \pm 1.34$	$10.40^{a,b,c} \pm 2.00$	$10.45^{a,b} \pm 2.75$	$31.95^{b,c,d} \pm 5.75$	$32.55^{a,b,c} \pm 6.95$
Mammary tumour (Group VII)	$4.44^{c} \pm 0.32$	$4.56^{b} \pm 0.00$	$9.70^{b,c} \pm 0.10$	$10.50^{a,b} \pm 0.40$	$30.45^{c,d} \pm 0.65$	$31.25^{b,c} \pm 0.05$
Idiopathic (Group VIII)	$6.27^{a,b} \pm 0.52$	$6.69^{a} \pm 0.38$	$12.95^{a,b} \pm 0.78$	$13.58^{a} \pm 0.52$	$40.20^{a,b} \pm 2.21$	$41.08^{a} \pm 1.48$

Means with common superscript (a – d within a column, A – B within rows separate for each parameter) doesnot differ significantly at 5 % level.

Table 4. Haematological values of normal and diseased dogs before and after treatment (continued…)

Parameters	TLC (10^3/cmm)		DLC					
			Lym (%)		Mon (%)		Gra (%)	
	0^{th} day	30^{th} day	0^{th} day	30^{th} day	0^{th} day	30^{th} day	0^{th} day	30^{th} day
Normal	12.73 ± 1.31	13.10±0.37	13.15 ± 1.73	15.23±1.26	5.33 ± 0.90	6.82±1.07	81.52 ± 2.39	76.70 ±2.11
Ehrlichiosis (Group I)	17.42 ± 2.08	14.68± 0.90	9.05 ± 1.06^{B}	14.08 ± 0.92^{A}	4.42 ± 1.05	5.47±1.04	86.53 ± 1.97^{A}	81.29 ± 1.77^{B}
Babesiosis (Group II)	17.95 ± 1.98	16.32±3.33	11.35 ± 0.91	18.55±3.73	7.88 ± 0.90	9.58±2.02	80.77 ± 1.65	71.87±4.94
Microfilariosis (Group III)	16.88 ± 2.91	13.62±1.06	13.63 ± 2.69	15.6±1.24	7.95 ± 2.57	6.07±1.31	78.40 ± 5.19	78.33±2.02
Cardiac diseases (Group IV)	16.35 ± 2.60	15.18±2.00	16.92 ± 2.68	15.82±1.73	7.52 ± 1.67	5.72±1.00	75.58 ± 3.62	78.46±2.31
Hepatic diseases (Group VI)	20.00 ± 4.60	16.70±2.20	6.60 ± 2.10	11.15±1.65	4.00 ± 2.40	5.35±0.15	89.40 ± 4.50	83.5±1.50
Mammary tumour (Group VII)	28.20 ± 7.80	20.90±4.40	6.15 ± 0.35	8.20 ±2.40	4.00 ± 1.20	3.95±0.85	89.85 ± 0.85	87.85±3.25
Idiopathic (Group VIII)	17.13 ± 1.68	14.42±0.60	13.02 ± 2.24	14.02±1.35	6.20 ± 1.36	5.95±0.91	80.78 ± 2.99	80.03±1.77

Means with superscript A and B within row separate for each parameter differ significantly at 5% level

Table 4. Haematological values of normal and diseased dogs before and after treatment (continued…)

Parameters	Platelet (10^5/cmm)	
	0^{th} day	**30^{th} day**
Normal	2.69 ± 0.60	3.22±0.33
Ehrlichiosis (Group I)	2.96 ± 0.40	3.26±0.24
Babesiosis (Group II)	3.00 ± 0.76	3.33±0.55
Microfilariosis (Group III)	4.04 ± 0.28	3.98±0.22
Cardiac diseases (Group IV)	3.48 ± 0.77	3.28±0.82
Hepatic diseases (Group VI)	2.11 ± 0.36	3.04±0.39
Mammary tumour (Group VII)	2.73 ± 0.28	2.65±0.15
Idiopathic (Group VIII)	3.17 ± 0.26	3.73±0.23

Table 5. Serum biochemical values of normal and diseased dogs before and after treatment

Parameters	Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		AG ratio	
	0^{th} day	30^{th} day	0^{th} day	30^{th} day	0^{th} day	30^{th} day	0^{th} day	30^{th} day
Normal	$6.97^{a,b,c} \pm 0.25$	$6.56^{b} \pm 0.22$	$3.30^{a} \pm 0.13$	$3.01^{a,b} \pm 0.07$	$3.67^{b,c} \pm 0.27$	$3.56^{c} \pm 0.15$	$0.93^{a} \pm 0.10$	0.85 ± 0.02
Ehrlichiosis (Group I)	$8.42^{a,b} \pm 0.43^{A}$	$7.86^{a,b} \pm 0.30^{B}$	$3.22^{a,b} \pm 0.08$	$3.25^{a} \pm 0.11$	$5.19^{a,b,c} \pm 0.38^{A}$	$4.62^{a,b} \pm 0.22^{B}$	$0.60^{b,c,d} \pm 0.06$	0.71 ± 0.02
Babesiosis (Group II)	$8.23^{a,b,c} \pm 0.74$	$7.20^{a,b} \pm 0.29$	$2.61^{c} \pm 0.16^{B}$	$2.81^{a,b} \pm 0.10^{A}$	$5.64^{a,b} \pm 0.67$	$4.39^{a,b,c} \pm 0.28$	$0.49^{c,d} \pm 0.06$	12.86 ± 12.23
Microfilariosis (Group III)	$8.78^{a} \pm 0.75$	$8.01^{a} \pm 0.49$	$2.52^{c} \pm 0.11^{B}$	$2.83^{a,b} \pm 0.19^{A}$	$6.26^{a} \pm 0.77^{A}$	$5.18^{a} \pm 0.44^{B}$	$0.43^{d} \pm 0.05^{B}$	0.56 ± 0.06^{A}
Cardiac diseases (Group IV)	$6.36^{b,c} \pm 0.21$	$6.59^{b} \pm 0.18$	$2.83^{a,b,c} \pm 0.14$	$3.11^{a} \pm 0.08$	$3.52^{c} \pm 0.24$	$3.64^{b,c} \pm 0.14$	$0.83^{a,b} \pm 0.09$	0.86 ± 0.04
Hepatic diseases (Group VI)	$6.14^{c} \pm 0.26$	$7.34^{a,b} \pm 0.44$	$2.65^{b,c} \pm 0.15$	$3.29^{a} \pm 0.19$	$3.48^{c} \pm 0.12$	$4.05^{b,c} \pm 0.25$	$0.76^{a,b,c} \pm 0.02$	0.81 ± 0.00
Mammary tumour (Group VII)	$7.69^{a,b,c} \pm 1.44$	$6.95^{a,b} \pm 0.50$	$2.29^{c} \pm 0.02$	$2.53^{b} \pm 0.13$	$5.40^{a,b,c} \pm 1.46$	$4.43^{a,b,c} \pm 0.38$	$0.46^{d} \pm 0.13$	0.57 ± 0.02
Idiopathic (Group VIII)	$8.03^{a,b,c} \pm 0.61$	$7.11^{a,b} \pm 0.36$	$2.79^{a,b,c} \pm 0.24$	$2.83^{a,b} \pm 0.17$	$5.25^{a,b,c} \pm 0.52^{A}$	$4.29^{a,b,c} \pm 0.22^{B}$	$0.55^{c,d} \pm 0.06$	0.66 ± 0.03

Means with common superscript (a – d within a column, A – B within rows separate for each parameter) doesnot differ significantly at 5 % level.

Table 5. Serum biochemical values of normal and diseased dogs before and after treatment (contd…)

Parameters	ALT (IU/l)		ALP (IU/l)		Total bilirubin (mg/dl)		Direct bilirubin (mg/dl)	
	0th day	30th day	0th day	30th day	0th day	30th day	0th day	30th day
Normal	24.35 ± 3.13	20.49±1.20	63.68 ± 12.17	39.88±4.54	0.68 ± 0.07	0.40 ±0.03	0.26 ± 0.05	0.21±0.03
Ehrlichiosis (Group I)	31.01 ± 6.76	32.6±4.97	113.86 ± 21.58	160.03±48.15	0.53 ± 0.06	0.34±5.93	0.25 ± 0.02	0.25±0.02
Babesiosis (Group II)	43.04 ± 10.07	43.81±10.37	119.18 ± 9.11	127.28±10.77	0.53 ± 0.06	0.44±0.04	0.31 ± 0.05	0.28±0.04
Microfilariosis (Group III)	27.44 ± 4.65^{B}	33.52 ± 5.25^{A}	119.84 ± 5.53^{B}	133.21 ± 6.84^{A}	0.49 ± 0.05	0.41±0.05	0.30 ± 0.05	0.25±0.04
Cardiac diseases (Group IV)	47.12 ± 2.51	48.74±0.99	170.89 ± 40.21	171.05±21.81	0.41 ± 0.07	0.34±0.06	0.29 ± 0.06	0.21±0.03
Hepatic diseases (Group VI)	32.60 ± 0.60	76.55±30.65	181.90 ± 31.10	237.6±76.8	0.40 ± 0.05	0.38±0.06	0.26 ± 0.06	0.24±0.05
Mammary tumour (Group VII)	38.10 ± 12.28	45.02±9.34	85.45 ± 56.96	98.77±54.21	0.62 ± 0.06	0.58±0.13	0.34 ± 0.03	0.34±0.08
Idiopathic (Group VIII)	48.95 ± 15.56^{B}	56.53 ± 17.21^{A}	141.18 ± 25.65	193.38±59.99	0.46 ± 0.05	0.38±0.03	0.28 ± 0.05	0.24±0.04

Means with superscript A and B within row separate for each parameter differ significantly at 5% level

Table 5. Serum biochemical values of normal and diseased dogs before and after treatment (contd…)

Parameters	BUN (mg/dl)		Creatinine (mg/dl)	
	0th day	**30th day**	**0th day**	**30th day**
Normal	$18.49^{b} \pm 2.27$	$20.12^{b} \pm 1.48$	$1.03^{b} \pm 0.15$	1.02 ± 0.09
Ehrlichiosis (Group I)	$15.13^{b} \pm 1.37$	$17.83^{b} \pm 1.83$	$1.25^{b} \pm 0.10$	1.30 ± 0.37
Babesiosis (Group II)	$20.21^{b} \pm 3.76$	$23.62^{b} \pm 2.33$	$1.20^{b} \pm 0.18$	1.11 ± 0.16
Microfilariosis (Group III)	$20.95^{b} \pm 2.18$	$22.29^{b} \pm 1.93$	$1.08^{b} \pm 0.11$	1.11 ± 0.07
Cardiac diseases (Group IV)	$30.67^{b} \pm 6.31$	$24.06^{b} \pm 2.90$	$1.06^{b} \pm 0.07$	1.08 ± 0.11
Hepatic diseases (Group VI)	$23.05^{b} \pm 4.96$	$22.52^{b} \pm 2.02$	$1.14^{b} \pm 0.09$	1.30 ± 0.05
Mammary tumour (Group VII)	$59.16^{a} \pm 18.95$	$48.52^{a} \pm 22.74$	$2.67^{a} \pm 0.52$	2.20 ± 0.70
Idiopathic (Group VIII)	$18.70^{b} \pm 3.58$	$21.67^{b} \pm 2.78$	$1.52^{b} \pm 0.12$	1.30 ± 0.14

Means with common superscript (a – d within a column, A – B within rows separate for each parameter) doesnot differ significantly at 5 % level.

4.3. THERAPEUTIC RESPONSE

4.3.1. Oedema of limbs associated with ehrlichiosis

All the six cases were treated with Tab. Doxycycline @ 5 mg/kg body weight bid for 21 days. In addition, two cases were treated with Tab. prednisolone based on the histopathologic findings of vasculitis. Prednisolone was given initially with an immunosuppressive dose of 2 mg/kg body weight in divided doses, followed by tapering to 25 per cent every week. All the cases responded to therapy and the oedema was subsided except in one case where recurrence was reported after stopping the medication.

4.3.2. Oedema of limbs associated with babesiosis

All the six cases were positive for *B.gibsoni* and a combination therapy of three antibiotics was used which included clindamycin @ 11 mg/kg body weight, metronidazole @ 25 mg/kg body weight SID intravenously and doxycycline @ 10 mg/kg bodyweight SID orally for 10 days. Histopathologic findings was suggestive of vasculitis and hence prednisolone was given to all cases at a dose rate of 1 mg/kg body weight bid and tapered to 25 per cent every week. All the dogs responded to therapy and recurrence was reported in one dog after one month post therapy.

4.3.3. Oedema of limbs associated with microfilariosis

Among the six cases, four of them were positive for unsheathed microfilariae and others were sheathed. For unsheathed microfilariae, Ivermectin @ 100 μg/kg body weight SID and for sheathed microfilariasis, Levamisole @ 10 mg/kg body weight SID for 7 days was given orally. In addition, three animals had a history of treatment with diuretics and slight reduction in oedema was reported. All the cases were free of microfilariae on the next week itself, but limb oedema was persisted. Based on the histopathological result, prednisolone was added in four cases and in two cases, a combination of doxycycline @ 5 mg/kg body weight bid and

prednisolone was given and there was reduction in oedema in all the cases. In two cases, lymphatic drainage occurred from the biopsy site and there occurred manual reduction in the oedema.

4.3.4. Oedema of limbs associated with cardiac diseases

Among the six cases, all the cases were treated with Tab. Enalapril @ 0.5 mg/kg bodyweight twice daily and Tab. Furosemide @ 2 mg/ kg body weight twice daily orally. Tab Furosemide was switched over to combination of Furosemide and Spironolactone @ 2 mg/kg body weight twice daily orally after one week of therapy and advised to continue for lifelong therapy along with Enalapril. In addition, ceftiofur injection was given parenterally to one case with severe pleural effusion and amoxicillin-sulbactum injection to two cases with leukocytosis. In addition, two dogs with hypertrophic cardiomyopathy was given carverdilol @ 0.2 mg/kg body weight bid orally. Among these, one dog died after three days of therapy and two dogs after two months of therapy. All other cases well responded to therapy with subsequent reduction in ascites and limb oedema.

4.3.5. Oedema of limbs associated with renal diseases

Both the cases were treated with intravenous fluid therapy with a combination of Normal saline and dextrose 5 per cent in the 1:1 ratio. Sodium bicarbonate was administered after calculating the bicarbonate deficit in both the cases. In one case recombinant human erythropoietin was given @ 50 IU / kg subcutaneously two times per week in combination with iron dextran injection. Aluminium hydroxide was given @ 50 mg/kg/day in both the cases as an intestinal phosphate binder. Other supportive therapies with antiemetics, H2 receptor antagonists, sucralfate and B complex injections were also given. Also advised the owner to gradually change the dog to a renal prescription diet. Among the two cases, one dog died on the fourth day of therapy and other dog showed improvement initially but after one month it also succumbed to death.

4.3.6. Oedema of limbs associated with hepatic diseases

Both the dogs with hepatic disease had ascites and was initially treated with Furosemide @ 2 mg/kg body weight twice daily and then a combination of Furosemide and Spironolactone at the same dose twice daily. Both the dogs were supported with hepatoprotectant drugs like Silymarin @ 150 mg/dog/day, antioxidant vitamin E and oral protein supplementation. Both the dogs responded to the therapy and ascites and limb oedema were reduced. One of the dog died after 2 months when the owner stopped the medication.

4.3.7. Oedema of limbs associated with mammary gland neoplasia

Both the dogs in this group had undergone surgical excision of mammary gland two months before and again presented with ulcerating lesions in the inguinal region with pitting oedema on both hindlimbs. The dogs were supported with dextrose 5 per cent solution, antibiotic Ceftriaxone-tazobactum @ 15 mg/kg, Pantoprazole @ 1 mg/kg and Furosemide @ 2 mg/kg body weight. In one of the dog, Prednisolone was added @ 1 mg/kg body weight bid for 14 days. Both the dogs responded to therapy initially but one dog succumbed to death after one month and the other one was euthanized.

4.3.8. Idiopathic limb oedema

Histopathology of all the dogs in this group suggested idiopathic cutaneous vasculitis and hence an immunosuppressive therapy with prednisolone was considered. All the dogs were treated with prednisolone @ 1 mg/kg body weight bid for first week and then the dose was tapered 25 per cent every week and treatment was given for one month. In addition to prednisolone, doxycycline @ 5 mg/kg body weight bid was added in three cases. All the cases responded to therapy and oedema was reduced within one week itself but recurrence was reported in four cases after stopping the medicines.

Discussion

5. DISCUSSION

In the present study on limb oedema, the various etiological factors and pathological processes involved were investigated in 36 cases. Based on the findings a suitable therapy was adapted and its response was evaluated.

5.1. OCCURRENCE

Based on the etiology, out of the 36 cases, 50 per cent were infectious, 33 per cent non-infectious and 17 per cent were due to idiopathic causes. Among the infectious causes, six cases each were due to ehrlichiosis, babesiosis or microfilariosis. In the non-infectious etiology, six cases of cardiac disease, two cases each of renal disease, hepatic disease or oedema associated with mammary gland neoplasia were investigated. Six cases were studied under idiopathic category.

Based on the physical examination of the oedematous area, majority of the cases had pitting oedema (81 per cent) and others had non-pitting oedema (19 per cent). Ware (2011) reported that painless pitting oedema was due to high capillary hydrostatic pressure, hypoalbuminemia or lymphatic obstruction whereas painful oedema was associated with increased capillary permeability in cases of inflammation or infection.

Based on the number of limbs affected, 56 per cent of the cases had bilateral limb oedema, 33 per cent had unilateral limb oedema and 11 per cent cases had oedema on more than two limbs. Ware (2011) opined that if unilateral limb was affected it might be due to localized vasculitis or regional lymphatic or venous obstruction. Bilateral involvement was usually symmetric and if the hindlimbs were affected it might be due to pelvic inlet mass, pelvic lymphatic obstruction, right sided heart failure or vasculitis. Marked hypoalbuminemia also resulted in bilateral oedema. Also widespread oedema could be resulted from congestive heart failure, cardiac tamponade, systemic vasculitis, anaphylaxis or myxedema.

5.2. DETAILS OF VARIOUS DIAGNOSTIC PROCEDURES

5.2.1. Peripheral wet film, blood smear and buffy coat smear examination

All the six cases of Ehrlichiosis were identified by buffy coat smear examination. According to Woddy and Hoskins (1991) buffy coat smears were preferred for detection of intracellular *E canis* morulae. The present study also identified six *B. gibsoni* positive cases by blood smear examination. Tarello (2003) reported that the best test for the diagnosis of babesia organism was blood smear examination. Six wet film positive cases were selected and blood smear examination revealed four of them with unsheathed microfilariae and two others with sheathed microfilariae. This was in accordance with Chirayath (2013) who opined that wet film and blood smears were important procedures for screening and identification of microfilariae in the peripheral blood.

5.2.2. Abdominal ultrasonographic findings

In the present study, only one animal in group I revealed splenomegaly and according to Waner and Harrus (2013), splenomegaly was a prominent finding in acute and chronic stages of ehrlichiosis and this might be due to diffuse proliferation of lymphocytes and plasma cells in the spleen during the acute stage of infection. Other dogs might be in the subclinical stage of ehrlichiosis and hence splenomegaly was not prominent.

In the present study with six cases of *Babesia gibsoni* infection, only one of the dog revealed splenomegaly. Lobetti (2000) opined that the hyperplasia of the mononuclear phagocytic system in babesiosis was responsible for splenomegaly. According to Birkenheuer (2012), splenomegaly was prominent during the acute and chronic stages of canine babesiosis, while in uncomplicated cases or in subclinical cases, splenomegaly was an inconsistent sign.

In group IV dogs, ascites and hepatomegaly were evident on most of the cases. According to Sisson (2010), ascites and hepatomegaly resulted when the systemic venous pressure rises due to impairment of right side of the heart. Gompf (2001) reported as a result of right sided heart failure, the blood accumulates in the liver and spleen leading to congestion and increased venous pressure further resulting in ascites.

Out of the two renal cases, one dog had reduced kidney size while the other dog had normal size. The reduced size of kidneys was indicative of chronic renal failure and it was in accordance with the findings of Polzin (2011).

The presence of ascitic fluid with small and irregular liver lobes were suggestive of chronic hepatitis or cirrhosis. This was according to Watson and Bunch (2009), who stated that animals with chronic hepatitis had a small diffusely hyperechoic liver and those with cirrhotic liver had a nodular appearance.

5.2.3. Electrocardiographic findings

In group IV, small P wave, low voltage QRS complex and electrical alternans were revealed in cases of pleural and pericardial effusions. In addition ST coving was detected in one case of hypertrophic cardiomyopathy. According to Martin (2000), Cote (2010) and Ware (2011), small voltage QRS complexes were associated with pleural or pericardial effusions, ascites, intrathoracic mass, obesity, hypovolemia and hypothyroidism. Electrical alternans was characteristic of pericardial effusion in which heart swings back and forth in the pericardium. Coving of ST segment was associated with myocardial hypoxia and might indicate ischemia from heart failure (Olsen *et al*, 2010).

5.2.4. Thoracic radiographic findings

The cases with mitral and tricuspid valve insufficiency developed chamber enlargement with elevation of trachea and carina. In severe cases there were pleural

and pericardial effusions. These findings were observed by Rush (2002) who stated that with right sided or biventricular congestive heart failure there was elevation of cranial aspect of trachea and increased size of caudal venacava. Olsen *et al*. (2010) reported pleural effusion, ascites, hepatomegaly and splenomegaly in cases of right sided heart failure. With progression of the disease interstitial and alveolar oedema develops in the lung parenchyma.

5.2.5. Echocardiographic findings

In the current study, six cases with congestive heart failure mainly due to valvular insufficiency were studied. Three of the cases had combined affections of mitral, tricuspid and aortic valves leading to both left as well as right sided heart failure. Boon (2011) opined that as a result of chronic mitral valvular insufficiency, volume overload occurs in the left atrium and ventricle leading to heart failure. Degenerative changes of tricuspid valve usually occur in addition to mitral valve insufficiency. Pleural and pericardial effusions noticed in two of the cases were in accordance with Olsen *et al*. (2010) who stated that effusions might present in dogs with heart failure.

In the present study, there was significant reduction in the values of LVIDs, EDV and ESV, associated with hypertrophic cardiomyopathy. Ware (2009) reported an abnormally thickened left ventricle, enlarged left atrium, with or without left ventricular outflow obstruction and mitral valve regurgitation in hypertrophic cardiomyopathy. According to Kahn (2010) and Gugjoo *et al.* (2013), in hypertrophic cardiomyopathy the M mode echocardiographic values of left ventricular end systolic diameter (LVIDs), end diastolic volume (EDV) and end systolic volume (ESV) were significantly reduced leading to decreased stroke volume and activation of compensatory renin-angiotensin system.

The left atrial to aortic root (LA/Ao) ratio was used to evaluate the atrial size and in the present study three cases showed increased LA/Ao ratio as 2.23, 1.52 and

1.45 and is suggestive of left atrial enlargement. According to Olsen *et al*. (2010), the normal LA/Ao in M-mode was between 0.8 to 1.2 and dogs in congestive heart failure had a ratio of 2 or greater.

Colour flow Doppler echocardiography revealed colour flow jet across mitral valve in all the cases. Based on the degree of mitral regurgitation, the cases were classified into mild, moderate and severe as explained by Boon (2011). The present study revealed an increased left ventricular fractional shortening and ejection fraction in connection with mitral regurgitation. This may be due to reduced left ventricular afterload that occurs in moderate to severe mitral regurgitation. Hence the ejection phase indices like left ventricular fractional shortening, ejection fraction etc. were greater than normal (Ware, 2011).

5.2.6. Blood pressure measurement

Blood pressure was measured in group IV and group V animals by using the non-invasive blood pressure (NIBP) by oscillometric method. The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) of group IV animals were 151.17 ± 3.63 and 90.50 ± 1.65 mmHg respectively. Both the SBP and DBP of this group were significantly higher than the normal group and it indicated mild risk of hypertension. This corroborates with the findings of Brown *et al*. (2007) who stated that hypertensive changes in heart include left ventricular hypertrophy and cardiac failure and on the basis of risk of target organ damage, dogs with <150/95 mmHg were classified as minimal risk and 150-159/95-99 mmHg were classified as mild risk.

The mean value of SBP and DBP of group VI was 152.5 ± 2.64 and 96.6 ± 1.05 respectively which belongs to stage I CKD according to IRIS classification (Polzin, 2011). Dunn *et al.* (2000) opined that the activation of renin- angiotensin-aldosterone system and altered adrenergic system with the release of vasopressor

substances by the kidney, leading to peripheral vascular resistance, retention of sodium and water and hypertension.

5.2.7. Blood gas analysis

Blood gas analysis conducted in group V animals with renal diseases revealed that metabolic acidosis was a consistent sign with decreased blood pH and bicarbonate level. This was explained by Polzin (2011), that metabolic acidosis promotes the progression of chronic kidney disease and alkalinization therapy was recommended when the blood pH and bicarbonate concentration falls below the normal range.

5.2.8. Histopathological findings

Among the eight groups, skin biopsy of the oedematous region was taken from selected animals of group I, II, III, VII and VIII. The histopathological changes associated with vasculitis included variable degrees of neutrophilic, eosinophilic and mononuclear cell invasion of vessel walls, endothelial cell swelling, fibrinoid degeneration, red blood cell extravasation and occasional leukocytoclasia noted within or near the vessel walls.

The histopathology of all the selected cases in group I, II and VIII suggested cutaneous vasculitis which corroborates with the findings of Innera (2013). According to the author, cutaneous vasculitis was an immune mediated dermatosis characterized by an aberrant immune response directed towards the blood vessels and pitting oedema might be the only finding in the early stage of the disease. The clinical presentation depends mainly on the extent of vascular destruction, and both cutaneous and systemic forms have been reported. Palpable purpura, plaques, wheals and serpentine papules, alone or in combination with pitting edema, were the common dermatological signs. Histologic features of a deep biopsy sample only can confirm the diagnosis.

In cases of ehrlichiosis, the infiltration of inflammatory cells are more towards and around the dialated blood vessels which was in agreement with the findings of Innera (2013) who opined it as cutaneous vasculitis. There are many triggering factors for cutaneous vasculitis. Infectious agents cause direct injury to vessel wall or formation of antigen-antibody complexes on the vascular endothelium or activation of B or T cells. Due to these triggering factors tick borne diseases like ehrlichiosis, anaplasmosis etc. cause vasculitis (Outerbridge, 2010). Knottenbelt (2002) reported vasculitis in equine ehrlichiosis either due to direct inflammatory responses mediated by injured vascular endothelial cells or due to immune complex deposition on the damaged area which might further exaggerate the inflammation.

Histopathological findings of babesiosis revealed perivascular infiltration of inflammatory cells in the subepithelial and subcutaneous area which was typical to vasculitis. Tazaki *et al*. (2013) reported cutaneous vasculitis in a dog with babesiosis and the histological examination of skin biopsy revealed perivascular infiltration of neutrophils with mild oedema and marked extravasation in the upper dermis and around the small blood vessels.

The histopathology of skin in microfilariosis cases revealed dilatation of lymphatic vessels with infiltration of inflammatory cells around the lymphatic vessels and blood vessels, which suggested lymphangitis and vasculitis. According to Outerbridge (2010), dirofilariasis could be a reason for infectious vasculitis. In the present study vasculitis was present in both sheathed and unsheathed microfilariosis. In sheathed microfilariosis with more persistent oedema, lymphangitis was noticed, which was in accordance with Ambily (2009) and Chirayath (2013) who reported lymphangitis in canine Brugian filariosis.

In group VII with limb oedema due to mammary gland neopasia, skin biopsy taken from the oedematous hind limb of one of the animal was suggestive of enlarged lymphatic duct with pink stained oedematous fluid. This may be due to lymphatic

obsstruction. According to Fossum and Caplan (2013), inflammatory mammary carcinomas grew rapidly and invades cutaneous lymphatics which resulted in extensive lymphedema of the limbs due to lymphatic occlusion or infiltration. Ware (2011) also reported that obstruction of venous or lymphatic flow by neoplasms in the pelvic inlet could resulted in bilaterally symmetric oedema on the hindlimbs.

In the idiopathic limb oedema group, the histopathology of all cases suggested the presence of cutaneous vasculitis. This was in accordance with the reports of Morris and Beale (1999), Nichols *et al*. (2001) and Innera (2013). According to these authors, at least 50 per cent of all vasculitis cases in veterinary medicine (excluding rabies vaccine-induced lesions) were regarded as idiopathic. Although type III hypersensitivity reaction was suspected in most cases of cutaneous vasculitis, additional factors like genetics, defects in immune complex clearance, and autoantibodies might also be involved (Shumaker, 2015).

5.2.9. Haematological and biochemical findings

5.2.9.1. Oedema of limbs associated with ehrlichiosis

Apart from AG ratio, all other mean haematological and biochemical values of this group were comparable to the normal group on 0^{th} day of observation and a mild anemia was reported but it was insignificant and was indicative of subclinical infection. Similarly, hyperglobulinemia was the only abnormal finding after 30^{th} day of observation. This corroborates with the reports of Harrus *et al*. (2012) who stated that the most of dogs with ehrlichiosis developed hyperproteinemia due to hypergammaglobulinemia. This indicated that hyperimmune mechanisms played an important role in the pathogenesis of ehrlichiosis (Harrus *et al*, 2012).

After 30 days of observation, a significant increase in the levels of TEC was reported which indicated improvement in mild anaemia that was present during the inflammatory phase (Harrus *et al*. 2012). A significant increase in the lymphocyte

per cent and a significant decrease in the granulocyte per cent were also noticed after therapy and the values were within the normal level indicating the suppression of earlier inflammatory processes. There was significant decrease in the total protein and globulin level after therapy which indicated clearance of ehrlichial antigen and reduction in hyperglobulinemia indicated a positive response to therapy (Harrus *et al*. 2012).

5.2.9.2. Oedema of limbs associated with babesiosis (Babesia gibsoni)

According to Tarello (2003), only mild anemia was reported in 30 per cent dogs with concurrent cutaneous signs associated with babesiosis. In the present study, although insignificant, the mean TEC of this group was lower than the normal group on 0^{th} day of observation while it showed significant decrease on 30^{th} day of observation. The reason for anemia on 0^{th} day might be due to intravascular and extravascular hemolysis. According to Schoeman (2009), *Babesia gibsoni* organisms were very difficult to clear by conventional therapy and usually dogs became chronic carriers or developed recurrence. So lack of complete clearance of organisms might have caused anaemia even after the therapy.

The serum biochemical studies revealed a significant decrease in the albumin and AG ratio on 0^{th} day and was comparable on 30^{th} days of observation with normal group. This was in accordance with Trotta *et al*. (2009) who reported hyperproteinemia, hypoalbuminemia and hyperglobulinemia in a *B. gibsoni* infected dog from Italy. In the current study there was a significant increase in the albumin level and a non significant decrease in globulin level after therapy indicating positive response to therapy with glucocorticoids.

5.2.9.3. Oedema of limbs associated with microfilariosis

There was no significant variation recorded in the values of TEC, Hb, VPRC, TLC and platelet count in cases of microfilariosis on both days of observations. According to Baneth *et al.* (2002) pre-treatment blood count of microfilaremic dogs

were within the reference. Although insignificant, a mild anaemia was recorded in the present study which might be due to the haemolysis resulted from the destructive motility of microfilaria as suggested by Kitagawa *et al*. (1989). There was a significant increase in the mean of TEC and Hb after therapy which indicated the positive response of therapy and was in accordance with the findings of Ambily (2009).

In the current study hypoalbuminemia, hyperglobulinemia and low levels of AG ratio were noticed on the 0^{th} day of observation. The increased globulin concentration might be due to the presence of parasitic antigen or due to the release of Hb from the lysed erythrocyte as suggested by Moustafa *et al*. (1991). Kitagawa *et al*. (1989) observed dogs with heartworm disease developed hypoalbuminemia as a result of degenerative changes in the liver. Similar findings were reported by Chirayath (2013) with lower levels of albumin, higher levels of globulin and lower AG ratio in microfilaremic dogs. After therapy, there was significant increase in the albumin and AG ratio and a significant decrease in the globulin level which indicated marked improvement post therapy.

5.2.9.4. Oedema of limbs associated with cardiac diseases

There was statistically significant decrease in the mean TEC, Hb and VPRC in cases with cardiac involvement when compared to normal group on the 0^{th} day of observation. After 30 days of treatment, the value of TEC was comparable to the normal, but Hb and VPRC were significantly decreased. Couto (2009) reported dogs with congestive heart failure develops dilutional anaemia caused by intravascular fluid retention. Apart from anemia there was no significant variation reported in other haematological and biochemical parameters.

5.2.9.5. Oedema of limbs associated with renal diseases

Since only two cases were studied and one of the dogs died after four days of therapy, statistical analysis was not conducted in this group. Both the dogs had normocytic, normochromic anemia. The BUN and creatinine of one dog was 145 mg/dl and 15 mg/dl respectively and the other one had 140 mg/dl and 7 mg/dl respectively. Elevated levels of phosphorus was also found in both cases with corresponding values 20 mg/dl and 10 mg/dl respectively. These were in accordance with the findings of Polzin (2011) who reported a hypoproliferative anemia, hypertension, elevated levels of BUN, creatinine and phosphorus, associated with CKD.

5.2.9.6. Oedema of limbs associated with hepatic diseases

The mean TEC and VPRC of this group were statistically lower than the normal group on 0^{th} day of presentation. This was in accordance with the reports of Hall and German (2005) who opined mild to moderate anaemia in liver disease and this usually resulted from chronic illness and/or gastrointestinal bleeding and/or haemostatic disorders secondary to hepatic disease.

According to Dunn (2000), hypoalbuminemia was a consistent finding in chronic liver disease and ascites might develop if the albumin level goes below 2g/dl with concurrent portal hypertension. The present study also observed a significant decrease in the level of albumin on 0^{th} day of presentation.

5.2.9.7. Oedema of limbs associated with mammary gland tumors

There was significant anaemia, hypoalbuminemia with reduced A:G ratio and elevated BUN and creatinine on 0^{th} day of observation which might be attributed to the weakness, anorexia, or metastasis. According to Fossum and Caplan (2013), lameness or lymphoedema suggests metastasis.

5.2.9.8. Idiopathic limb oedema

All the haematological and biochemical values were insignificant in this group on the 0^{th} day of observation except A:G ratio. Although insignificant, there was an elevated level of serum globulin with normal levels of albumin which lead to a significantly lower A: G ratio. This was in accordance with the findings of Innera (2013). As per the author, antigen-antibody complex deposition on the blood vessels subsequently develops vasculitis and oedema. Apart from the idiopathic causes, vaccinations, drugs, infectious, immune mediated and allergic conditions etc could cause vasculitis (Foster, 2006).

When comparing pre and post therapies, there was a significant increase in the level of ALT on 30^{th} day of observation. This might be due to the administration of glucocorticoids as an immunomodulatory drug. Webster (2010) reported that there was increase in levels of serum ALT, ALP and GGT after corticosteroid therapy.

5.3. THERAPEUTIC RESPONSE

Response to treatment was assessed on 30^{th} day based on clinical improvement, haematobiochemical analysis and blood smear examination.

5.3.1. Oedema of limbs associated with ehrlichiosis

All the cases were treated with Tab. Doxycycline @ 5 mg/kg bodyweight twice daily orally for 21 days according to the treatment protocol recommended by Harrus *et al*. (2012). Tetracyclines exert a variety of anti-inflammatory and immunomodulating properties by themselves or in combination with niacinamide (Innera, 2013). In addition, prednisolone was added in two cases with histopathological lesions of vasculitis @ 1 mg/kg body weight twice daily orally for the first week followed by tapering to 25 percent every week. This was in accordance with Sainz *et al*. (2015) who suggested the administration of glucocorticoids @ 0.5 to 2 mg/kg bodyweight per day if immune mediated complications occur with

ehrlichiosis. All the cases responded to therapy and all the haemato-biochemical parameters were improved after 30 days of observation and blood and buffycoat smear examination were negative. Oedema subsided during the first week of therapy in all cases but one case reported recurrence after one-month post therapy.

5.3.2. Oedema of limbs associated with babesiosis (Babesia gibsoni)

All the six cases were given a combination therapy of three antibiotics which included clindamycin @ 11 mg/kg body weight, metronidazole @ 25 mg/kg body weight SID intravenously and doxycycline @ 10 mg/kg bodyweight SID orally for 10 days which was in accordance with the treatment protocol suggested by Vishnurahav (2014). With the histopathological results awaiting, all the cases were treated in addition with prednisolone @ 1 mg/kg body weight twice daily orally for the first week followed by tapering to 25 per cent every week. According to Tasaki *et al.* (2013), no course of treatment for cutaneous babesiosis had been reported till now. Since the histopathological findings were suggestive of vasculitis, the immunomodulatory dose of prednisolone was administrated (Innera, 2013). All the dogs responded to therapy and clinical remission of oedema was reported within a week itself. Among the cases, one dog developed oedema one month after therapy and treatment with prednisolone was repeated for one more month.

5.3.3. Oedema of limbs associated with microfilariosis

Cases with unsheathed microfilariae were treated with Ivermectin @ 100 µg/kg bodyweight SID and sheathed microfilariae were treated with Levamisole @ 10 mg/kg bwt SID for 7 days orally. This was in accordance with the results of Ambily (2009) and Chirayath (2013). Based on the histopathological result, prednisolone was added to four cases @ 1 mg/kg body weight twice daily orally for the first week followed by tapering to 25 per cent every week. In addition, two cases were treated with a combination of doxycycline @ 5 mg/kg bodyweight twice daily orally and prednisolone. In two cases, lymphatic drainage occurred from the biopsy

site and manual reduction in oedema was reported. Fossum and Caplan (2013) suggested the medical management of secondary lymphedema with bandaging, caring of skin and proper use of antibiotics to prevent cellulitis and lymphangitis. According to the author, steroids and diuretics were used for treating human cases of lymphoedema. Long-term treatment of lymphedema with diuretics was contraindicated since it removes only fluids and proteins get concentrated in the area which further damages the tissue. In the present study, all the cases were negative for microfilariosis on 30th day of observation. All other haemato-biochemical parameters were within normal level whereas hyperproteinemia and hyperglobulinemia persisted. Hyperproteinemia observed in the present study might be due to increase in the globulin concentration which was in accordance with Ambily (2009). Complete reduction in limb oedema was reported in four cases and slight reduction in two cases in the present study.

5.3.4. Oedema of limbs associated with cardiac diseases

Among the six cases, all the cases were treated with Tab. Enalapril @ 0.5 mg/kg body weight twice daily and Tab. Furosemide @ 2 mg/ kg body weight twice daily orally. Tab Furosemide was switched over to combination of Furosemide and Spironolactone @ 2 mg/kg body weight twice daily orally after one week of therapy and advised to continue for lifelong therapy along with Enalapril. The line of treatment was in accordance with Haggstrom (2008). In addition, two cases with hypertrophic cardiomyopathy were treated with carverdilol @ 0.2 mg/kg body weight bid orally as per Gordon (2010). In addition, ceftiofur injection was given parenterally to one case with severe pleural effusion and amoxicillin-sulbactum injection to two cases with leukocytosis. Among these, one dog died after three days of therapy and two dogs after two months of therapy. All other cases responded to therapy with subsequent reduction in ascites and limb oedema.

5.3.5. Oedema of limbs associated with renal diseases

Both the cases were treated with intravenous fluid therapy with a combination of Normal saline and dextrose 5 per cent in 1:1 ratio. Sodium bicarbonate was administered after calculating the bicarbonate deficit in both the cases. In one case recombinant human erythropoietin was given @ 50IU / kg subcutaneously two times per week in combination with iron dextran injection. Aluminium hydroxide was given @ 50 mg/kg/day orally in both the cases as an intestinal phosphate binder. Other supportive therapies with antiemetics, H2 receptor antagonists, sucralfate and B complex injections were also given. Also advised the owner to gradually change the dog to a renal prescription diet. The line of treatment was in accordance with Polzin (2011). Both the dogs had a grave prognosis and one dog died on the fourth day of therapy and other dog died after one month.

5.3.6. Oedema of limbs associated with hepatic diseases

Both the dogs were treated with furosemide @ 2 mg/kg body weight and then a combination of furosemide and spironolactone at the same dose twice daily. The dogs were supported with hepatoprotectant drugs like silymarin, antioxidant vitamin E and oral protein supplementation. According to Rothuizen (2000), aldosterone antagonists like spironolactone was preferred for ascites of hepatic origin and could be supported with glucocorticoids, antioxidants, antifibrotic drugs, silymarin etc. for chronic hepatitis. Watson and Bunch (2009) opined that glucocorticoids never be used without a biopsy in cases of idiopathic chronic hepatitis. Both the dogs responded to the therapy and ascites and limb oedema were reduced. One of the dog died after 2 months when the owner stopped the medication.

5.3.7. Oedema of limbs associated with mammary gland neoplasia

Both the dogs were supported with dextrose 5 per cent solution, antibiotic ceftriaxone-tazobactum @ 15 mg/kg, Pantoprazole @ 1 mg/kg and Furosemide @ 2

mg/kg body weight. In one of the dog, prednisolone was added @ 1 mg/kg body weight bid for 14 days. According to Chun and Garett (2010), surgical excision was the mainstay of therapy in mammary gland tumors. Since there was regrowth of MGTs after excision and concurrent metastasis were suspected, symptomatic therapy was given to these animals. Petrie (2010) suggested bandage application with antimicrobial, anti-inflammatory and diuretic therapy might be helpful in cases of traumatic and post-surgical induced lymphedema. Both the dogs responded to therapy initially but one dog succumbed to death after one month and the other one was euthanized.

5.3.8. Idiopathic limb oedema

Based on the histopathologic finding of vasculitis, all the dogs were treated with prednisolone @ 1 mg/kg body weight bid for the first week and then the dose was tapered to 25 percent every week and the treatment was given for one month. In addition to prednisolone, doxycycline @ 5 mg/kg body weight bid was added in three cases. This was in accordance with Innera (2013) who preferred starting a lower dose of prednisolone @ 0.5-1 mg/kg bodyweight and could be increased if clinical remission was not achieved and slow tapering with 25 percent dose reduction in every two weeks. In addition, alternative therapies like cyclosporine, azathioprine, chlorambucil, pentoxiphylline, tetracycline/niacinamide combination, sulfasalazine and dapsone were also recommended by the author in refractive cases. All the cases responded to therapy and oedema was reduced within one week itself but recurrence was reported in four cases after stopping the medicines. The triggering factor for idiopatic vasculitis was not elucidated and that might be the reason for the maximum number of recurrence reported in this group. Treatment was by avoiding the triggers of vasculitis and immunomodulatory therapy. The success rate of therapy varies between 40 and 88 per cent with a potential for protracted remission after discontinuation of medications (Miller *et al*, 2013).

Summary

6. SUMMARY

The study entitled 'Clinical investigations on oedema of limbs in dogs' was conducted in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Mannuthy during the study period of 2015-2016. Dogs presented to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with the complaint of limb oedema without any orthopaedic abnormalities were selected for the study. The dogs were subjected to wet film, blood smear and buffy coat smear examinations to rule out haemoparasites. Haematobiochemical analysis, abdominal ultrasonography, thoracic radiography and electrocardiography were conducted. In those dogs suspected for cardiac abnormalities, echocardiograghic studies and blood pressure measurement were performed. Those dogs with renal diseases were subjected to blood gas analysis. Punch biopsy of the oedematous area was taken from selected dogs and histopathological examination was done. Based on the various diagnostic techniques, the clinical cases of limb oedema were classified into eight groups. Specific therapy was given to each groups and therapeutic response was evaluated on the 30^{th} day based on clinical improvement, haematobiochemical analysis and by blood/buffy coat smear examinations.

Among the cases, infectious diseases like ehrlichiosis, babesiosis and microfilariosis were the predominant ones causing limb oedema (50 per cent). Non infectious diseases like congestive heart failure, chronic renal failure, chronic hepatic diseases and mammary gland neoplasia were contributing to 33 per cent of the total cases. Idiopathic cases of limb oedema contribute to 17 per cent.

Abdominal ultrasonography revealed splenomegaly in ehrlichiosis and babesiosis. Ascites with hepatomegaly and engorged hepatic vessels were noticed in cardiac diseases. Small sized kidneys and small liver lobes with irregular border were detected in chronic renal failure and chronic hepatic disease respectively.

Electrocardiographic abnormalities were detected mainly in cardiac diseases and in hepatic diseases. Small P wave with low voltage QRS complex were seen in both these conditions due to fluid accumulation. In addition, electrical alternans was seen in case of pericardial effusion and ST coving was seen in myocardial hypoxia associated with heart failure.

Thoracic radiographic abnormalities were detected in cardiac cases which included pleural effusion, pericardial effusion, elevated trachea and pulmonary oedema. Cardiac silhouette was not clear in certain cases.

Echocardiography was conducted in those cases with cardiac diseases. Severe pleural effusion was recorded in one case. In some cases, there was increased left atrial to aortic root (LA/Ao) ratio. The significant lowering of left ventricular systolic diameter, end systolic volume and end diastolic volume indicated the reduced stroke volume associated with hypertrophic cardiomyopathy. The significantly increased ejection fraction and fractional shortening were associated with the reduced afterload in moderate to severe mitral valve insufficiency. Colour doppler studies indicated the various degrees of mitral, tricuspid and aortic valve regurgitations associated with heart failure.

Blood pressure was measured in cardiac and renal cases and mild hypertension was recorded in both the groups. Blood gas analysis of renal diseases revealed metabolic acidosis with normal levels of other electrolytes.

Histopathology of skin biopsy revealed cutaneous vasculitis in all the selected cases of infectious and idiopathic cases of limb oedema. In addition to vasculitis, lymphangitis was also a finding in sheathed microfilariosis.

Haematobiochemical analysis was conducted on 0^{th} and 30^{th} day of observation and the values were compared with the normal animals. Treatment response was also evaluated within groups before and after therapy. There was

significant variation recorded in certain groups when compared to normal animals. Certain groups showed marked improvement after therapy.

Specific therapy was given to each group based on the etiology. In addition, those groups with cutaneous vasculitis were given immunomodulatory therapy with prednisolone @ 1 mg/kg body weight twice daily followed by tapering for a period of 30 days. All the infectious and idiopathic cases well responded to the therapy whereas most of the non-infectious causes had a grave prognosis. Recurrence was more common in idiopathic group since the triggering factor mediating the cutaneous vasculitis was not elucidated.

Based on the present study, it could be concluded that

- There are many etiological factors for the formation of limb oedema
    1) Infectious causes were ehrlichiosis, babesiosis and microfilariosis
    2) Non-infectious causes were congestive heart failure, chronic renal failure, chronic hepatic disease and mammary gland neoplasia
    3) Idiopathic causes of limb oedema
- Histopathological finding of cutaneous vasculitis in infectious and idiopathic limb edema was a main finding in this study. In addition to vasculitis, lymphangitis also constitute oedema in sheathed microfilariosis
- Immunomodulatory therapy using prednisolone should be effectively used for treating cutaneous vasculitis until clinical remission occurs
- Recurrence is common in idiopathic limb oedema, so detailed study of triggering factors should be adopted

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CLINICAL INVESTIGATIONS ON OEDEMA OF LIMBS IN DOGS

JOMY THOMAS

(14-MVM-12)

ABSTRACT

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

(Veterinary Clinical Medicine, Ethics and Jurisprudence)

2016

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

DEPARTMENT OF VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR - 680651

KERALA, INDIA

ABSTRACT

Thrity six dogs presented with oedema of limbs were grouped into eight based on the etiology after detailed clinico-pathological investigations. All the dogs were screened for haemoparasites by wet film, blood smear and buffy coat smear examinations. Haemato-biochemical parameters were evaluated in all cases on both 0^{th} day and 30^{th} day of observation. The dogs were further evaluated using electrocardiography, thoracic radiography and ultrasonography. Further diagnosis was based on the histopathological result of skin biopsy from the oedematous area. A suitable therapy was conducted based on the etiology and response was evaluated.

According to the present study, six cases each of ehrlichiosis, babesiosis and microfilariosis with limb oedema were identified and grouped separately. Six dogs were confirmed with congestive heart failure based on the clinical signs, ECG, thoracic radiographs and echocardiographic evaluation. Two dogs with chronic renal failure and chronic liver diseases were studied and grouped separately. Two cases with limb oedema secondary to mammary gland neoplasia also came under the study. There were six dogs with unknown etiology and they were grouped into the category of idiopathic limb oedema.

Further diagnosis was based on the histopathological result of skin biopsy from the oedematous area. Accordingly in ehrlichiosis, babesiosis, microfilariosis and in idiopathic cases the histopathological findings suggested cutaneous vasculitis. In addition, lymphatic dialatation and lymphangitis were detected in microfilariosis.

Based on the etiology, each groups were treated separately and most of the cases with non-infectious etiology had a grave prognosis. Those groups with cutaneous vascilitis, along with the specific therapy, prednisolone was added in the immunomodulatory doses and tapered off within in a month. All the cases were well responded to therapy and recurrence was reported in few cases after the cessation of therapy.

Academic Form No.19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

FACULTY OF VETERINARY AND ANIMAL SCIENCES

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis:

Clinical investigations on oedema of limbs in dogs.

2a.Title of the departmental/KVASU research project of which this forms a part:

Not applicable

2b. Code No. if any, and order by which the departmental / KVASU research project is approved:

Not applicable

3a. Name of the Student:

Jomy Thomas

3b. Admission No:

14-MVM-12

4a. Name of the Major Advisor:

Dr. S. Ajithkumar

4b. Designation:

Professor and Head,
Department of Clinical Veterinary Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences, Mannuthy

5. Objectives of the study:

(1) To identify the etiological factors associated with oedema of limbs in dogs.
(2) To identify the clinico-pathological changes associated with oedema of limbs in dogs.
(3) To report the response to the therapeutic regimens adopted.

6. Practical / Scientific utility:

Oedema of limbs in dogs is a common clinical problem encountered in Kerala. Pathophysiological conditions from variety of causes may result in the accumulation of interstitial fluid (oedema). Some inflammatory and

anaphylactic conditions may also lead to oedema formation. Therapy for oedema varies depending on the underlying etiology and mechanism of oedema formation. There is paucity of literature on oedema of limbs in dogs. So the present research work is undertaken to identify the clinico-pathological changes associated with oedema of limbs in dogs and to report the efficacy of treatment protocols adopted.

7. Important publications on which the study is based:

Pedersen (1999) studied idiopathic vasculitis on deep biopsies of oedematous hindlimb after ruling out all possible secondary causes in dogs.

Tellier (2001) identified immune complex vasculitis in a Shar-pei dog with swollen hock. Punch biopsy was performed and histopathological and direct immunoperoxide histochemical staining was done. The study demonstrated mixed inflammatory cell dermatitis and vasculitis with vascular thrombosis, perivascular hemorrhage, and severe dermal oedema.

According to Malik *et al*. (2002) immune complexes deposited in the blood vessel wall led to vasculitis and were commonly found in dependent parts like limbs and ventrum with pitting oedema.

Knottenbelt (2002) stated that oedema resulting from vasculitis had some significant differences from other causes of abnormal extracellular fluid accumulation. The oedema was generally more persistent and might not subside simply with gentle exercise.

Foley *et al.* (2007) stated that tick borne diseases were associated with polyarthritis and thrombocytopenia.

Kahn (2010) reported that immune mediated vasculitis led to oedema of limbs in dogs and horses.

Gaisbauer *et al.* (2014) performed punch biopsy of the skin and studied the histopathological changes and suggested deep dermal vasculitis in a cat presented with oedema of limbs.

8. Outline of technical program / plan of study:

A minimum of twenty four dogs presented to the University Veterinary Hospital Mannuthy and Kokkalai, with oedema of limbs will be selected for the study.

Haematological parameters including total red blood corpuscles (RBC) count, total white blood corpuscles (WBC) count, Differential leucocyte count (DLC), thrombocyte count, Volume of Packed Red Cells (VPRC) and haemoglobin concentration will be measured by using auto analyzer as per the methods described by Meinkoth and Allison (2007)

Biochemical parameters including total protein, albumin, globulin, albumin-globulin ratio, blood urea nitrogen, creatinine, alkaline phosphatase, alanine amino transferase, bilirubin (total and direct) will be estimated with semi-automatic biochemical analyzer as per the procedure of Kerr (2002)

Peripheral wet film and blood smear examination will be conducted to identify the presence of haemoparasitic infection, if any.

Electrocardiographic, radiographic and ultrasonographic changes will be recorded as per standard procedures.

Skin Biopsy from the affected site will be taken from selected animals and subjected to histopathological examination and changes will be recorded.

Appropriate therapeutic regimens will be tried depending on the results of clinical investigations.

For cardiac diseases, treatment will be done with ACE inhibitors like enalapril @ 0.5 mg/kg bwt BID, supported with diuretics like furosemide/spironolactone @ 2 mg/kg bwt BID orally. Apart from these, digoxin is given in dialated cardiomyopathy @ 0.22 mg/m^2 BID and carvedilol @ 0.2 mg/kg bwt BID in hypertrophic cardiomyopathy.

For hepatic etiology, hepatoprotectant drugs like silymarin and for renal etiology, fluid therapy with 5% dextrose and normal saline will be given.

Blood parasitic infections like ehrlichiosis, babesiosis and microfilariosis will be treated as per the standard protocol.

Immune mediated vasculitis if present, will be treated with prednisolone @ 2 mg/kg bwt PO initially followed by tapering doses.

9. Main items of observation:

(1) Signalment
(2) Anamnesis and clinical signs
(3) Hematological parameters
    a) Total erythrocyte count
    b) Volume of packed red cells (VPRC)
    c) Haemoglobin concentration
    d) Leukocyte count
    e) Differential leukocyte count
    f) Platelet count
(4) Biochemical parameters
    a) Total protein
    b) Albumin, globulin and (A: G) ratio
    c) Total bilirubin
    d) Direct bilirubin
    e) Alanine amino transferase (ALT)
    f) Alkaline phosphatase (ALP)
    g) Creatinine
    h) Blood urea nitrogen (BUN)
(5) Peripheral wet film and blood smear examination
(6) Electrocardiogram
(7) Thoracic radiogram
(8) Abdominal ultrasonogram
(9) Histopathology of skin biopsy sample
(10) Treatment response

10. Facilities:

a. Existing facilities in the College of Veterinary and Animal Sciences will be utilized
b. Additional facilities required:
    Chemicals, glass wares and test kits

11. Duration of study:

Four semesters

12. Financial estimate:

a. Chemicals and biologicals: Rs.20000/-

b. Contingencies : Rs. 5000/-

Total : Rs.25,000/-

13. Signature of the student:

14. Signature of the Major Advisor:

Place: Mannuthy.
Date: 10-8-2015

Name, Designation and signature of members of the Advisory Committee:

Chairperson:

Dr. Ajithkumar. S
Professor and Head,
Teaching Veterinary Clinical Complex,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651

Members:

1. Dr. Usha Narayana Pillai
Professor and Head,
Department of Clinical Veterinary Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651

2. Dr. Deepa Chirayath
Assistant Professor,
Department of Clinical Veterinary Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651

3. Dr. Ajith Jacob George
Associate Professor,
Department of Veterinary Pathology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651

APPENDIX –I

References:

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APPENDIX-II

Time frame of work:

Semester –I

1. Collection of literature
2. Preparation of project proposal

Semester-II

1. Collection of data from clinical cases of limb oedema in canines presented at University Veterinary Hospital Mannuthy and Kokkalai
2. Collection of literature

Semester-III

1. Hematological, biochemical studies and histopathological study of skin biopsy taken from dogs presented with limb oedema
2. Assessment of treatment response.

Semester-IV

1. Compilation of clinical data and interpretation of results
2. Finalisation, writing and submission of thesis

CERTIFICATE

Certified that the project has been formulated observing stipulations laid down under the Prevention of Cruelties to Animals Act Amendment 1998.

Place: Mannuthy

Date: 10-8-2015

Dr. S. Ajithkumar

(Major Advisor)

Annexure

ANNEXURE – I

PROFORMA

1. Case no: Date:

2. Name & Address of the owner: Place:

3. Phone No:

Animal	Species	Breed	Sex	Age	Colour	Parity	Body wt

4. Owner's complaint:

5. Present and past history:

6. Observations:

Temp (^{0}F)	Respiration (rate/min)	Pulse (rate/min)	Mucus membrane	Lymph node	Wet film	Blood smear	Buffy coat smear

7. Haematology and Serum biochemistry

Parameters	**Result**	**Parameters**	**Result**
RBC (10^6/cmm)		Total protein (g/dl)	
Hb (g/dl)		Albumin (g/dl)	
VPRC (%)		Globulin (g/dl)	
TLC (10^3/cmm)		AG ratio	
Lym %		BUN (mg/dl)	
Mon %		Creatinine (mg/dl)	
Gra %		ALP (IU/l)	
PLT (10^5/cmm)		ALT (IU/l)	
		Bilirubin total (mg/dl)	
		Bilirubin direct (mg/dl)	

8. Clinical signs:

9. Orthopaedic examination:

10. ECG:

11. Thoracic radiography:

12. Abdominal ultrasonography:

13. Echocardiography:

14. Blood Pressure:

15. Blood gas analysis:

16. Histopathology of skin biopsy:

17. Treatment response:

Signature of the chairman Signature of the student

CURRICULUM VITAE

1. Name of candidate : Dr. Jomy Thomas

2. Date of birth : 01.06.1989

3. Place of birth : Kothamangalam, Ernakulam, Kerala

4. Marital status : Single

5. Permanent address : Kochuthottiyil(h), Nazareth Hill P.O, Kuravilangad, Kottayam, Kerala.

6. Major field of specialization : Veterinary Clinical Medicine, Ethics and Jurisprudence

7. Educational status : B.V.Sc & A. H, undergoing M.V.Sc

8. Professional experience : Veterinary Surgeon on contract basis for a period of 4 months at V.H, Peerumedu under AHD.

9. Publications made :

a. Limb oedema associated with canine ehrlichiosis and its therapeutic management - Journal of Veterinary and Animal Sciences (Accepted for publication)
b. An unusual case of *Leptospira hebdomadis* infection with bilateral limb edema in a dog-28th Kerala Science Congress
c. *Babesia bigemina* infection in a neonatal calf-25th Swadeshi Science Congress

10. Membership of professional societies:

a. Member, Kerala State Veterinary Council
b. Member, Indian Society for Advancement of Canine Practice
c. Indian Veterinary Association

Place: Mannuthy

Date :