CLINICO - THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS

By

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THESIS

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DECLARATION

I hereby declare that this thesis, entitled **"CLINICO-THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS"** is a bonafide record of research work done by me during the course of research and that this 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.

Mannuthy
24.4.03

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CERTIFICATE

Certified that this thesis, entitled **"CLINICO-THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS"** is a record of research work done independently by **Smitha .J.P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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SMITHA .J.P.

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Introduction

1. INTRODUCTION

Canine ehrlichiosis is a tick-borne febrile, rickettsial disease of dogs characterized by anaemia, pancytopenia and severe debilitation caused by the infection with *Ehrlichia canis*. The disease is also known as canine typhus, canine haemorrhagic fever, tracker dog disease, idiopathic haemorrhagic syndrome, tropical canine pancytopenia and Nairobi bleeding disease.

Ehrlichia was first discovered by Donatien and Lestoquard (1935) at the Pasteur Institute, Algeria. They noted that experimental dogs maintained in the institute which were infested with the brown dog tick *Rhipicephalus sanguineus*, occasionally developed a severe febrile illness, characterized by anaemia. Blood smears from such infected dogs stained by Giemsa stain showed small rickettsia-like organisms inside canine monocytes. The newly discovered organism was named *Rickettsia canis*. Later on, in 1945, S.D. Moshkovski renamed the organism as *Ehrlichia canis*.

The full pathogenic potential of *Ehrlichia canis* for canines was first recognized during a war in the Republic of Vietnam (1968-70) where a severe epizootic occurred among U.S. military dogs, resulting in heavy mortality and morbidity. This severe and often fatal form of disease known as tropical canine pancytopenia was later on recognized as canine ehrlichiosis.

Ehrlichial infections are known to occur world wide and they are presently classified under Order - Rickettsiales; Family – Rickettsiaceae; Tribe – Ehrlichieae; Genus – Ehrlichia and the type species of the genus being

Ehrlichia canis. All ehrlichia are presumed to be tick-borne and only *E. sennetsu* and *E. chaffeensis* are thought to be pathogenic to humans. Experimental studies have revealed that *Rhipicephalus sanguineus* is a vector, but not a reservoir of *Ehrlichia canis*.

In India, the first report of canine ehrlichiosis was from Madras by Mudaliar (1944). Since then many reports of its occurrence came from different parts of India especially from South India. In Kerala, the first occurrence of canine monocytic ehrlichiosis was reported by Tresamol *et al.* (1995).

Ehrlichia go through three developmental stages. (1) Elementary body – individual ehrlichia organisms, (2) Initial body – immature organismal inclusion, (3) Morula - mature organismal inclusion. Infected monocytes usually contain several morula each containing several dozens of elementary bodies. Morula break-up into elementary bodies when infected cell ruptures and infectious cycle is repeated.

After acquiring the infection, the course of ehrlichiosis can be divided into three phases, acute, subclinical and chronic, based on clinical signs and clinicopathologic abnormalities. The clinical signs in the acute phase may occasionally be mild and non specific. It begins after an incubation period of 8-20 days and lasts 2-4 weeks during which the organisms multiply in mononuclear cells by binary fission and spread throughout the body. Subclinical phase of 40-120 days soon follows. If infected dog is immunocompetent, elimination of infection may occur. Dogs that do not

successfully eliminate the parasite during the subclinical stage may remain in this stage and may subsequently proceed to the chronic phase which is characterized by impaired bone marrow production of blood elements resulting in pancytopenia.

Diagnosis of the disease can be conclusively made by detection of inclusion body or morulae in blood/buffy coat smears. This microscopic demonstration of typical *E. canis* morulae is usually performed in acute phase of the disease and is diagnostic. Indirect fluorescent antibody (IFA) test is still considered to be the gold standard test, though dot-blot enzyme linked immunoassay procedures have been developed and shown to be sensitive for the detection of antibodies to *Ehrlichia canis*.

Canine ehrlichiosis is an emerging disease. Though many suspected cases are being reported from different areas, no systematic study on this disease, has yet been made, in Kerala, so far. The non specific signs and lesions associated with ehrlichiosis often confuse the clinician in arriving at a confirmatory diagnosis. This situation warrants the necessity of a systematic study to be conducted, based on a conclusive diagnostic technique. Under these circumstances, this project is envisaged with the following objectives.

1. To determine the occurrence of ehrlichiosis in dogs.
2. To study the clinico-haematological and biochemical changes in ehrlichiosis.
3. To assess the efficacy of treatment with doxycycline.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Occurrence

Donatien and Lestoquard (1935) described the disease for the first time in Algeria and recovered the causative agent from dogs exposed to tick infestation and named it as *Rickettsia canis*.

Danks (1937) recorded a fatal outbreak of canine ehrlichiosis in Kenya and the disease was called Nairobi bleeding disease.

Carmichael and Fiennes (1942) described the disease syndrome as it appeared in Uganda and designated the illness as "canine typhus".

The incidence of canine ehrlichiosis in India was reported for the first time by Mudaliar (1944) in a Spaniel dog of eight months age.

Bool and Sutmoller (1957) identified *Ehrlichia canis* in the monocytes of severely ill dogs in the island of Aruba (Netherlands Antilles).

Raghavachari and Reddy (1958) confirmed that ehrlichiosis continues to be a problem in India.

McGaughey *et al.* (1962) reported the occurrence of *Ehrlichia canis* from Ceylon.

In the United States, the first case of canine ehrlichiosis was reported in 1963 with the causative agent mistakenly identified as *Babesia canis* (Ewing, 1963).

A new canine disease outbreak that had caused marked mortality among dogs in Singapore was later on identified as ehrlichiosis (Wilkins *et al.*, 1967).

Farrel (1968) reported ehrlichiosis in countries bordering Mediterranean in Europe.

Huxsoll *et al.* (1970) described tropical canine pancytopenia, a fatal disease in tropical and subtropical areas, responsible for the death of large number of military dogs in South East Asia.

Ewing *et al.* (1971) reported a new strain of *Ehrlichia canis*, almost exclusively in neutrophils and occasionally in eosinophils from Arkansas.

In Israel, first report of canine ehrlichiosis was from Klopfer and Nobel (1972).

A higher incidence of canine ehrlichiosis was reported from Kenya by Price *et al.* (1987).

Koutinas *et al.* (1989) reported the occurrence of ehrlichiosis in dogs, for the first time in Greece.

In Connecticut, Magnarelli *et al.* (1990) reported the first case of ehrlichiosis in canines.

First report of canine ehrlichiosis from Costa Rica was reported by Meneses (1995).

The first case of canine ehrlichiosis in Finland is reported by Jarvinen and Taponen (1997).

Suto *et al.* (2001) reported the first confirmed case of canine ehrlichiosis in Japan.

2.2 Epidemiology

The disease has been found to occur in a variety of breeds with varying susceptibility.

2.2.1 Breed

Purebred and crossbred German shepherd dogs were found to be more susceptible to the infection when compared to other breeds and they developed a severe form of the disease (Huxsoll *et al.*, 1970; Walker *et al.*, 1970).

German shepherd dogs were found to be more prone to the clinical signs like haemorrhage especially epistaxis (Huxsoll *et al.*, 1972).

Stephenson and Ristic (1978) reported higher prevalence of ehrlichiosis in crossbred and purebred German shepherds than other breeds of dogs.

Specific and non-specific immunosuppression due to *Ehrlichia canis* occurred in the German shepherd dogs (Nyindo *et al.*, 1980) which was attributed to their increased susceptibility to infection.

In ehrlichiosis no significant difference was observed in the infection rate between the German shepherd dogs and other breeds (Keefe *et al.*, 1982).

German shepherd dogs and other purebred dogs were found to be predisposed to the development of a severe chronic disease (Kuehn and Gaunt, 1985).

No breed prevalence was noticed for canine ehrlichiosis in a study conducted by Waddle and Littman (1988).

According to Elias (1991) breed definitely plays a role in predisposition towards ehrlichiosis with 66.7 per cent of cases being diagnosed in German shepherd dogs.

Matthewman *et al.* (1993) observed no breed susceptibility to ehrlichia infection in purebred dogs.

Higher incidence of canine ehrlichiosis was noticed in German shepherd and Spitz in a survey conducted in Madras city (Thirunavukkarasu *et al.*, 1993).

In an epidemiological study on canine monocytic ehrlichiosis conducted by Harrus *et al.* (1997), German shepherd dogs were significantly over represented.

Based on an epizootiological survey, Harikrishnan *et al.* (2001) concluded that *Ehrlichia canis* was found to be more prevalent in purebred than in Mongrels, Pomeranian breed showed greater susceptibility followed by German shepherd dogs.

2.2.2 Age

Nims *et al.* (1971) carried out an epizootiological study and found that mortality and clinical signs were not related to age.

No age-wise occurrence was noticed in a retrospective study on *Ehrlichia canis* epizootic around Arizona (Stephenson and Ristic, 1978).

No age prevalence was noticed in a retrospective study of 27 cases of naturally occurring canine ehrlichiosis conducted by Waddle and Littman (1988).

Elias (1991) found that there is no evidence for the age predilection towards the incidence of ehrlichiosis.

Younger dogs had lower prevalence than adult or older dogs in a seroepidemiological survey on canine ehrlichiosis carried out in Israel (Baneth *et al.*, 1996).

Canine monocytic ehrlichiosis was found to be distributed unevenly irrespective of age in a retrospective study of 100 cases in Jerusalem (Harrus *et al.*, 1997).

No significant difference between age groups in the incidence of canine ehrlichiosis was observed in an epizootiological work carried out by Harikrishnan *et al.* (2001).

2.2.3 Sex

Sex predilection was not evident regarding the prevalence of canine ehrlichiosis (Stephenson and Ristic, 1978; Waddle and Littman, 1988; Elias 1991 ; Harrus *et al.*, 1997 and Harikrishnan *et al.*, 2001).

Higher incidence was noticed in males (57.81 per cent) than in females in a survey on the incidence of canine ehrlichiosis in Madras city (Thirunavukkarasu *et al.*, 1993).

2.2.4 Transmission

The role of brown dog tick, *Rhipicephalus sanguineus* as a vector was first documented by Donatien and Lestoquard (Ewing, 1969).

Role of *Rhipicephalus sanguineus* ticks (larvae, nymphs and adults) to transmit *Ehrlichia canis* infection was well established by Groves *et al.* (1975).

Lewis *et al.* (1977) reported that engorged nymphs from a dog in the acute phase of ehrlichiosis transmit the infection more efficiently than from a dog in the chronic phase of disease.

Parasites other than ticks like fleas and mosquitoes are also incriminated to be the possible vectors, as evidenced by the prevalence of ehrlichiosis in indoor dogs with minimal exposure to ticks (Troy *et al.*, 1980).

Mathew *et al.* (1996) conducted transmission experiments with Oklahoma isolate of *Ehrlichia canis* and found that cell cultured isolates of ehrlichia might lose its affinity for ticks.

Johnson *et al.* (1998) described a successful trans-stadial transmission of *Ehrlichia canis* experimentally by *Dermacentor variabilis*, another ixodid tick. This was the first report that, tick other than *Rhipicephalus sanguineus* is capable of trans-stadial transmission of this pathogen.

In *Rhipicephalus sanguineus*, strict trans-stadial transmission was noticed and adult ticks were capable of transmitting infection to susceptible population for at least 155 days. Blood transfusion from infected donors could also transmit the ehrlichial organism (Neer, 1998).

2.2.5 Concurrent infection

Though canine ehrlichiosis was recognized as a separate disease entity and characterized as well, natural outbreaks are sometimes complicated by one or more concurrent infections.

Gillain (1942) reported a complicated syndrome caused by co-infection with *Rickettsia canis* and *Babesia canis*.

Mudaliar (1944) reported combined infection of *Hepatozoon canis* and *Rickettsia canis* from Chennai.

Bool and Sutmoller (1957) described concurrent infection with *Ehrlichia canis, Babesia canis* and *Hepatozoon canis*.

Concomitant infection of two dogs with *Ehrlichia canis* and either *Babesia canis* or *Haemobartonella canis* was reported by Kuehn and Gaunt (1985).

Price *et al.* (1987) reported concurrent infection of canine ehrlichiosis and babesiosis.

Mixed infections of *Ehrlichia canis* and *Babesia canis* had been reported by Matthewman *et al.* (1993).

A case of concurrent infection of *Ehrlichia canis* and Leptospira spp. was reported by Nambi *et al.* (2000).

Harikrishnan *et al.* (2001) recorded concomitant infections of *Hepatozoon canis, Ehrlichia canis* and *Babesia canis* in an epizootiological study of canine ehrlichiosis in Chennai.

Mixed infections of ehrlichiosis, babesiosis and hepatozoonosis in a Cocker spanial pup of one month age were reported by Ramprabhu *et al.* (2001).

Ramprabhu *et al.* (2001a) recorded a rare occurrence of concomitant infections of trypanosomiasis and ehrlichiosis in a mongrel male dog of 3½ years.

2.3 Pathogenesis

Nims *et al.* (1971) suspected hypersensitivity or autoimmune mechanism responsible for the development of lesions in tropical canine pancytopenia from the consistent histopathologic finding of perivascular plasma cell infiltration in different organs.

According to Buhles *et al.* (1974) in acute ehrlichiosis, there was increased sequestration and destruction of blood cells which resulted in a transitory pancytopenia while the bonemarrow remained normal, whereas the pancytopenia in chronic ehrlichiosis was associated with bonemarrow hypoplasia.

Smith *et al.* (1975) opined that thrombocytopenia in *Ehrlichia canis* infected dogs results from increased platelet destruction that begins within few days of infection.

Lymphocytes from dogs infected with *Ehrlichia canis* were found to be cytotoxic for autologous monocytes which bore a temporal relationship to the thrombocytopenia (Kakoma *et al.*, 1977).

Pierce *et al.* (1977) stated that accelerated non-antibody mediated destruction of platelets contributed markedly to the development of thrombocytopenia in dogs with acute ehrlichiosis.

Kakoma *et al.* (1978) devised a platelet migration inhibition test to determine the presence of anti platelet activity in serum collected from natural and experimentally induced cases of ehrlichiosis.

Lovering *et al.* (1980) carried out an experimental study in which a qualitative and quantitative deficit in platelet function occurred in ehrlichial thrombocytopenia as evidenced by the reduced platelet adhesiveness and decreased circulating platelet concentration.

According to Pyle (1980) the leukopenia and thrombocytopenia in acute ehrlichiosis were the results of increased destruction or sequestration of circulating elements rather than decreased production.

Increased release of platelet factor and the findings of antinuclear antibodies suggested an immune mechanism in the pathogenesis of canine ehrlichiosis (Codner *et al.*, 1985).

Canine ehrlichiosis elicits a strong immune response in affected dogs which causes a qualitative defect in platelet aggregation and activation (Matus *et al.*, 1987).

In experimentally induced ehrlichiosis, Codner and Maslin (1992) found that an inverse relationship existed between the magnitude of proteinuria and serum albumin concentration that suggested the possible involvement of pathological changes in kidney tubules.

A study by Codner *et al.* (1992) indicated that minimal change glomerulopathy rather than immune complex mediated glomerulonephritis could explain transient proteinuria in the absence of histologic evidence of glomerular disease.

A recent study suggested that the spleen had a key role in the pathogenesis of canine monocytic ehrlichiosis which was substantiated by the finding that the disease process was considerably milder in the splenectomized dogs than in the intact dogs (Harrus *et al.*, 1999).

Harrus *et al.* (2001) concluded that circulating immune complexes had a role in pathogenesis of canine monocytic ehrlichiosis since they were detected in both acute and subclinical phases of experimental infection.

2.4 Clinical signs

Carmichael and Fiennes (1942) classified canine ehrlichiosis into three groups: cutaneous form, septicaemic form and nervous form based on the clinical signs.

Ewing and Buckner (1965) reported a grave illness with severe anemia of normocytic normochromic type, developed in dogs having combined infection of babesiosis and ehrlichiosis.

Ehrlichiosis can be fatal in young puppies and the symptoms commonly noticed were recurrent fever (107°F), photophobia, nasal discharge, vomiting, fetid smell of breath and spleenomegaly (Ewing, 1969).

Unilateral or bilateral epistaxis was described as the most striking clinical sign of tropical canine pancytopenia (Huxsoll *et al.*, 1970).

Tropical canine pancytopenia was characterized by mild/severe pyrexia for 3-5 days, anorexia, severe weight loss, loss of stamina and extreme weakness (Walker *et al.*, 1970).

Huxsoll *et al.* (1972) described the clinical signs of tropical canine pancytopenia in experimentally infected German shepherd dogs as epistaxis, ulceration of the nasal mucosa, corneal opacity, hyphema, petechial and ecchymotic haemorrhages in the mucosa of the penis and buccal cavity, posterior weakness, dyspnoea and oedema of the limbs.

Epistaxis, high body temperature (104°F), subcutaneous haemorrhages on the abdomen, enlarged parotid, sublingual and mammary lymphnodes, listlessness, ataxia and coughing were the physical findings associated with *Ehrlichia canis* infection (Stephenson *et al.*, 1975).

According to Pyle (1980), the only consistent finding among cases of ehrlichiosis was inconsistency and hence diagnosis of ehrlichiosis was difficult since there were no pathognomonic signs.

Manohar and Ramakrishnan (1984) noticed major clinical signs as pyrexia, anorexia, conjunctivitis, mucus discharge from both eyes and loss of weight.

Codner *et al.* (1985) carried out a retrospective study of sixteen cases of canine ehrlichiosis and findings included diffused interstitial pulmonary radio-opacities, normal platelet count, haemorrhage despite platelet counts greater than 1,00,000/mm^3.

Non-specific clinical signs such as depression/lethargy, weight loss, bleeding, lymphadenopathy, anorexia and bleeding tendencies were noticed in 56 cases of ehrlichiosis (Kuehn and Gaunt,1985).

Okin (1985) observed the clinical signs such as bilateral one-half corneal oedema, acute epistaxis and weight loss in a case of ehrlichiosis.

An unusual case of *Ehrlichia canis*-associated polyarthritis with swollen stifle joints was reported in a seven month old Boxer dog (Bellah *et al.*,1986).

Selective appetite, lethargy, weight loss, pallor/congestion of mucous membrane, pyrexia, spleenomegaly, lymphnode enlargement, blood in faeces, haematuria, haematemesis, epistaxis, petechiation/ecchymoses on visible mucous membrane or skin were the main clinical signs recorded in natural cases of ehrlichiosis (Price *et al.*,1987).

Three cases of ehrlichiosis associated with polyarthritis had been reported by Cowell *et al.* (1988). There was pain on walking, lameness and inflammation of synovial joint.

In a retrospective study of 27 cases of ehrlichiosis, the commonly presented complaints were lethargy, weakness, anorexia, depression, weight loss, epistaxis and melena (Waddle and Littman, 1988).

A rare occurrence of ehrlichiosis with seizures lasting 1-2 minutes was reported by Meinkoth *et al.* (1989).

A case of ehrlichiosis associated with polymyositis was reported by Buoro *et al.* (1990). There was progressive tetraparesis, hyporeflexia and generalized muscle wasting.

A rare occurrence of polyarthritis with discharge from preputial sheath, fever, congested mucous membrane and serous nasal discharge was reported in a case of ehrlichiosis (Thilagar *et al.*, 1990).

Non-specific signs such as rise of temperature, dullness, anorexia and congestion of eyes with mucopurulent discharge were reported by Juyal *et al.* (1992) in cases of canine ehrlichiosis.

Clinical evidence of arthritis or muscular stiffness such as lameness, signs of joint pain or swelling, synovial fluid abnormalities indicative of inflammatory joint disease, limb stiffness, stilted gait etc. was observed in a group of dogs with ehrlichiosis (Stockham *et al.*, 1992).

Cervical pain, weight loss, epistaxis and gingival bleeding were observed in a case of canine ehrlichiosis. Signs of cervical hyperaesthesia were also noticed (Maretzki *et al.*, 1994).

The major clinical signs observed in natural cases of ehrlichiosis included pyrexia, anorexia, depression, vomiting, skin lesions, spleenomegaly, peripheral lymphadenopathy and various bleeding disorders such as epistaxis, melena, haematemesis and haematuria (Thirunavukkarasu *et al.*, 1994).

Emaciation, anorexia, pyrexia, anaemia, weakness and depression were the common clinical signs observed in dogs with ehrlichia infection (Meneses, 1995).

Harrus *et al.* (1997) in a study of 100 cases of canine monocytic ehrlichiosis, observed that the most common clinical signs were depression, lethargy, lymphadenomegaly, fever, anorexia, panting, pale mucous membrane and bleeding, of which epistaxis was most common.

Egenvall *et al.* (1998) conducted an experimental study by inoculating seven Beagle dogs with a Swedish isolate of ehrlichia spp. and the most prominent clinical signs were high fever for 2-5 days and depression.

Acute blindness, spleenomegaly, pale mucous membrane and ocular lesions were observed by Harrus *et al.* (1998) in dogs suffering from acute ehrlichiosis.

Neer (1998) opined that canine ehrlichiosis was a multisystemic disorder and the common complaints on presentation included lethargy, mild weight loss, anorexia with or without haemorrhagic tendencies.

Rajguru *et al.* (1998) reported a case of ehrlichiosis with nervous signs such as ataxia and seizures along with other usual nonspecific signs of the disease.

2.5 Haematology

Terminal manifestations of tropical canine pancytopenia were characterized by marked leukopenia and normocytic anaemia (Walker *et al.*, 1970).

The onset of severe chronic tropical canine pancytopenia was heralded by a recurrence of severe thrombocytopenia and leukopenia, 40-80 days after infection (Buhles *et al.*, 1974).

Non-regenerative anaemia, marked rouleaux formation on blood smears, hyperproteinemia and proteinuria were the initial findings observed in *Ehrlichia canis* infected dogs (Hoskins *et al.*, 1983).

Kuehn and Gaunt (1985) described anemia and thrombocytopenia as the most frequent haematologic abnormalities identified with ehrlichiosis.

A prevalence study on subclinical phase of ehrlichiosis in dogs revealed hyperglobulinemia, thrombocytopenia, absolute lymphocytosis and absolute neutropenia (Codner and Farris-Smith, 1986).

In a retrospective study of 27 natural cases of canine ehrlichiosis, Waddle and Littman (1988) found that most common haematological abnormalities were thrombocytopenia and anaemia.

Eight dogs with chronic ehrlichiosis had lymphocytosis and azurophilic granulation in majority of the lymphocytes (Weiser *et al.*, 1991).

Most common haematologic abnormalities detected in dogs with granulocytic ehrlichiosis were thrombocytopenia and normocytic normochromic anaemia (Stockham *et al.*, 1992).

Matthewman *et al.* (1993) reported anaemia and thrombocytopenia as the major consistent findings in concomitant infections of ehrlichiosis and babesiosis.

Non-regenerative anaemia, lymphopenia, neutrophilia with a mild left shift and thrombocytopenia were observed in a case of granulocytic ehrlichiosis (Maretzki *et al.*, 1994).

Common haematologic disorders such as anaemia, severe panleukopenia and thrombocytopenia were observed by Liang *et al.* (1995) in a case of canine ehrlichiosis.

Meneses (1995) observed anaemia, leukopenia and thrombocytopenia as the most common haematological alterations in a study on twelve dogs suffering from ehrlichiosis.

Tresamol *et al.* (1995a) pointed out anaemia, thrombocytopenia, monocytosis and leukopenia as the haematological alterations in ehrlichiosis in dogs.

Davoust *et al.* (1996) observed that acute ehrlichiosis was characterized by thrombocytopenia (100 per cent cases), anaemia (29 per cent), leukopenia

(42 per cent) or leukocytosis (10 per cent), in a retrospective study on the haematologial parameters of three groups of dogs with *Ehrlichia canis* infection.

Thrombocytopenia, anaemia (normocytic and normochromic) and lymphopenia were the predominant haematological findings noticed by Harrus *et al.* (1997).

Waner *et al.* (1997) reported that the most prominent haematological finding in the subclinical phase of ehrlichiosis was mild thrombocytopenia with a concomitant increase in platelet size. None of the dogs were either leukopenic, neutropenic or anaemic.

Egenvall *et al.* (1998) noticed profound changes in haematological parameters such as marked thrombocytopenia and moderate leukopenia in a case of experimentally induced granulocytic ehrlichiosis.

Thrombocytopenia and granular lymphocytosis are consistent findings in all stages of ehrlichiosis (Neer, 1998).

2.6 Serum biochemistry

Burghen *et al.* (1971) observed alterations in serum characterized by hypergammaglobulinemia and concomitant decrease in albumin value in dogs suffering from ehrlichiosis.

Increased alanine amino transferase, decreased total serum protein and albumin concentrations were observed during the first week after infection and

increased gamma globulin concentration after the 3rd week of infection (Reardon and Pierce, 1981).

Hoskins *et al.* (1983) pointed out serum hyperviscosity syndrome associated with *Ehrlichia canis* infection in a dog. The serum sample had a total protein of 9 g/dl, albumin content 2.3 g/dl and globulin 7.3 g/dl.

High alkaline phosphatase and alanine amino transferase activities, high serum urea nitrogen, creatinine and phosphorus were observed in 56 dogs with ehrlichiosis presented at Louisiana State University, SAC, from January 1980 to December 1983 (Kuehn and Gaunt, 1985).

A high total protein concentration of >12 g/dl with hypoalbuminemia (1.8 g/dl) and hyperglobulinemia (>10 g/dl) were noticed in a German shepherd dog with ehrlichiosis (Matus *et al.*, 1987).

Serum chemistry abnormalities noticed were hypoalbuminemia, hyperglobulinemia, elevated serum alkaline phosphatase, elevated serum alanine amino transferase, bilirubinemia, hyperphosphatemia and azotemia in a retrospective study of 27 natural cases of canine ehrlichiosis (Waddle and Littman, 1988).

Biochemical alterations such as hypoalbuminemia, hyperglobulinemia and high levels of alkaline phosphatase and transaminases (alanine transaminase and aspartate transaminase) were observed in twelve cases of ehrlichiosis in Costa Rica (Meneses, 1995).

Hypergammaglobulinemia suggestive of a monoclonal gammopathy which developed secondary to proliferation of a single clone of plasma cells had been reported in the case of a Poodle dog suffering from *Ehrlichia canis* infection (Michels *et al.*, 1995).

Increased alanine amino transferase, aspartate amino transferase, blood urea nitrogen and serum globulin values and low values for serum albumin and albumin-globulin ratio were obtained in a study conducted by Tresamol *et al.* (1995a).

Marked serum protein alterations such as significantly elevated levels of total protein, total globulin, α-2 globulin, β-2 globulin and gamma globulin and significantly lower values of albumin, α-1 globulin and A/G ratio were met within a large group of naturally infected dogs with ehrlichiosis (Harrus *et al.*, 1996).

A persistently high antibody titre to *Ehrlichia canis* was reported in the subclinical phase of ehrlichiosis which can be taken as one of the most reliable parameters for judging possible subclinical ehrlichial infection (Waner *et al.*, 1997).

Serum protein electrophoresis revealed a narrow globulin peak indicating monoclonal hypergammaglobulinemia. Elevated levels of creatine kinase and lactic dehydrogenase were also met with (Harrus *et al.*, 1998).

Serum chemistry abnormalities encountered in canine ehrlichiosis include hyperproteinemia, hyperglobulinemia, hypoalbuminemia and elevated alanine aminotransferase and alkaline phosphatase activities (Neer, 1998).

2.7 Pathology

Ewing (1969) found that in ehrlichiosis of dogs, spleen was invariably enlarged, two to three times its normal size with its capsule swollen and reddish purple in colour. Splenic parenchyma was bulged beyond the cut edge of the capsule and larger trabeculae.

Huxsoll *et al.* (1970) described striking necropsy lesions as lymphadenopathy, petechial and ecchymotic haemorrhages on serosal and mucosal surfaces of numerous organs and subcutaneous tissues in tropical canine pancytopenia. Most prominent histologic finding observed was perivascular infiltration of plasma cells in numerous organs.

Hildebrandt *et al.* (1973) described gross lesions like haemorrhages in cutaneous tissues and major organs, generalized lymphadenopathy especially that of mesenteric lymphnodes in tropical canine pancytopenia. Microscopical changes observed were bone marrow elements and altered structure of the lymphopoetic tissues with plasmacytosis.

Pyle (1980) observed gross lesions like haemorrhages in subcutaneous tissues, major organs and generalized lymphadenopathy in ehrlichiosis. Microscopically, the most characteristic feature was altered architecture of

lymphopoietic tissue with plasmacytosis and generalized perivascular lymphoid and plasma cell accumulation.

Histopathologically, subendothelial aggregates of mononuclear cells in pulmonary blood vessels, renal periglomerular and perivenular plasmacytosis, haemopoietic hyperplasia and perivascular cuffs of lymphocytes and plasma cells in many organs were observed in acute stage of ehrlichiosis in experimentally infected dogs by Reardon and Pierce (1981).

In an experimentally infected ehrlichiosis case, significant histopathological lesions observed were plasma cell infiltration in various organs, like kidney, spleen, liver, lung, urinary bladder and intestine, erythrophagocytosis and haemosiderosis in spleen and lymphnodes and haemorrhages in various organs (Manohar and Ramakrishnan, 1984).

A case of polymyositis associated with *Ehrlichia canis* infection revealed atrophy of skeletal muscle and characterized histologically by plasmacytic, lymphocytic and immature lymphoreticular cellular infiltrates with accompanying areas of necrosis (Buoro *et al.*, 1990).

Codner and Maslin (1992) reported that the morphological changes in experimental ehrlichiosis indicated transient glomerular leakage of protein in sufficient magnitude that contributed to the hypoalbuminemia.

Post mortem lesions in experimentally induced granulocytic ehrlichiosis included reactive splenic hyperplasia and non specific mononuclear reactive hepatitis (Egenvall *et al.*, 1998).

Petechial and ecchymotic haemorrhages on the serosal and mucosal surfaces of most organs including the nasal cavity, lung, kidney, urinary bladder, gastro intestinal tract and subcutaneous tissue were noticed by Neer (1998).

Ocular (uveitis) and meningeal lesions were the most prominent histopathological changes observed by Panciera *et al.* (2001) in dogs suffering from ehrlichiosis.

2.8 Diagnosis

Diagnosis of canine tick typhus by examination of blood smear was described by Carmichael and Fiennes (1942).

Confirmatory diagnosis of suspected cases of ehrlichiosis could be accomplished by demonstration of typical morulae in the cytoplasm of leukocytes (Ewing, 1969).

Acridine orange staining was effectively utilized to demonstrate *Ehrlichia canis* inclusion bodies in buffy coat and other tissue smears (Carter *et al.*, 1971).

A case of ehrlichiosis has been identified by detecting elementary and initial bodies of *Ehrlichia canis* in the cytoplasm of mononuclear cells in coverslip culture (Kaminjolo *et al.*, 1976).

Bellah *et al.* (1986) detected round to oval intracytoplasmic inclusions in approximately one per cent of the neutrophils in suspected cases of ehrlichiosis in dogs.

Ehrlichiosis-associated polyarthritis was diagnosed by detection of morulae/inclusion body in one per cent of neutrophils in blood smear/synovial fluid smear (Cowell *et al.*, 1988).

A case of canine ehrlichiosis was confirmed by microscopic examination of stained blood smear which revealed morulae in monocytes (Buoro *et al.*, 1990).

Elias (1991) described the importance of blood smear examination for the diagnosis of ehrlichiosis in an enzootic region by observing the presence of large intracytoplasmic *Ehrlichia canis* inclusion bodies/morulae in circulating mononuclear leukocytes.

Thirty seven cases of canine granulocytic ehrlichiosis were documented by finding ehrlichial morulae in the granulocytes of dogs from Missouri (Stockham *et al.*, 1992).

Eosinophilic morulae of *Ehrlichia canis* were observed in the cytoplasm of lymphocytes in a 2-year old male Akita dog (Liang *et al.*, 1995).

Tresamol *et al.* (1995) detected *Ehrlichia canis* inclusion bodies in the cytoplasm of monocytes in a female German shepherd dog.

A case of ehrlichiosis was diagnosed in a dog in which inclusions were observed in neutrophils (Vijayan *et al.*, 1997).

Diagnosis of fourteen cases of ehrlichiosis in dogs was made based on the presence of inclusions in granulocytes of dogs in Sweden (Egenvall *et al.*, 1997).

Rajguru *et al.* (1998) diagnosed a case of ehrlichiosis by detection of *Ehrlichia canis* morulae in monocytes.

Harikrishnan *et al.* (2001) examined peripheral blood smears stained with acridine orange and found that yellow coloured morulae could be well differentiated from the cytoplasm of mononuclear cells.

2.8.1 Immunofluorescence

Carter *et al.* (1971) demonstrated *Ehrlichia canis* in buffy coat and other tissue smears by direct immunofluorescence test.

Ristic *et al.* (1972) standardized the indirect immunofluorescence test for detection and titration of antibodies to *Ehrlichia canis* using organism cultured in canine blood monocytes.

Harper (1975) made tentative diagnosis of four cases of ehrlichiosis based on haematologic parameters and confirmed using the indirect fluorescent antibody test.

Serological diagnosis of infectious cyclic thrombocytopenia in dogs using IFA test was reported by French and Harvey (1983).

An indirect immunofluorescent antibody test was described by Paxton and Scott (1989) to diagnose the agent of tick-borne fever.

Detection of antibodies against *Ehrlichia canis* was carried out using indirect fluorescence antibody test by Brouqui *et al.* (1991) which proved the presence of organism in Africa.

Out of 105 dogs examined by indirect fluorescence antibody test, 52 per cent had antibodies reactive with *Ehrlichia canis* (Matthewman *et al.*, 1993).

Tresamol *et al.* (1994) carried out indirect fluorescence antibody test to detect *Ehrlichia canis* antibodies from suspected serum samples.

Harrus *et al.* (1997) confirmed diagnosis of canine monocytic ehrlichiosis by detecting anti-*Ehrlichia canis* antibodies by indirect immunofluorescence test.

Waner *et al.* (1997) opined that a state of mild thrombocytopenia together with a persistently high IFA titre would highly suggest the diagnosis of subclinical ehrlichiosis.

Diagnosis of ehrlichiosis was usually done based on positive results of IFA test which detected serum antibodies as early as seven days after initial infection (Neer, 1998).

A comparative study between two different serological tests i.e., indirect fluorescent antibody test and immunoblot analysis in the diagnosis of granulocytic ehrlichiosis gave comparable result (Magnarelli *et al.*, 1999).

Mason *et al.* (2001) carried out an epidemiological survey to detect *Ehrlichia canis* infection in 316 serum samples of domestic dogs using indirect fluorescence antibody test of which 2.22 per cent reacted positively.

2.8.2 Enzyme Linked Immunosorbent Assay (ELISA)

Rikihisa *et al.* (1992) found that IgG ELISA response using purified *Ehrlichia canis* antigen was optimum in the diagnosis of canine granulocytic ehrlichiosis.

Dot blot enzyme-linked immunoassay was found to be as sensitive and specific as that of indirect fluorescence antibody test for the diagnosis of ehrlichiosis (Cadman *et al.*, 1994).

Waner *et al.* (1996) experimentally infected six beagle dogs with *Ehrlichia canis* and detected antigenemia in their plasma using a sandwich ELISA.

A rapid ELISA test kit was compared with the immunofluorescence test for the detection of *Ehrlichia canis* antibodies in dogs and both tests were found to be equally sensitive (Waner, 1999).

An ELISA for detection of anti-*Ehrlichia canis* IgG was developed which was found to be equally sensitive to indirect immunofluorescence antibody test (Harrus *et al.*, 2001a).

Magnarelli *et al.* (2001) made diagnosis of granulocytic ehrlichiosis using a class-specific recombinant based ELISA in dogs and horses.

2.8.3 Immunoblot assay

Ohashi *et al.* (1998) developed a dot immunoblot assay based on a recombinant protein that provided a simple, consistent and rapid serodiagnosis.

Yu *et al.* (2000) documented protein immunoblotting using recombinant P120 *Ehrlichia canis* which was a potential antigen that proved effective for the serodiagnosis of canine ehrlichiosis.

McBride *et al.* (2001) documented that the recombinant proteins rP43 and rP28 are sensitive and reliable serodiagnostic antigens for *Ehrlichia canis* infections.

2.8.4 Molecular Techniques

Iqbal *et al.* (1994) compared polymerase chain reaction with other tests for early diagnosis of canine ehrlichiosis and found that sensitivity was slightly less than that of other established methods.

Iqbal and Rikihisa (1994) realized application of polymerase chain reaction in the diagnosis of *Ehrlichia canis* infection in tissues using a pair of primers specific to *Ehrlichia canis* 16S rRNA gene sequence.

Engvall *et al.* (1996) developed a polymerase chain reaction-based assay for detecting DNA of granulocytic ehrlichiosis in blood samples from dogs, horses and cattle.

A polymerase chain reaction-based detection assay that specifically detected *Ehrlichia canis* in dogs with acute infection was developed (McBride *et al.*, 1996).

Wen *et al.* (1997) opined that the nested PCR was highly sensitive and specific for detection of *Ehrlichia canis* and might be more useful in assessing the clearance of the organisms after antibiotic therapy than IFA test.

Polymerase chain reaction was performed to determine the carrier state of *Ehrlichia canis* in the subclinical phase of ehrlichiosis and also to determine the significance of persistent IFA titres (Harrus *et al.*, 1998b).

Murphy *et al.* (1998) conducted a molecular and serologic survey of *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs and ticks from Oklahoma. Polymerase chain reaction amplification was carried out using primers ECC and ECB that amplified a portion of the 16S rRNA gene.

Molecular diagnosis was compared with serological test by Magnarelli *et al.* (1999). Polymerase chain reaction could yield more accurate information on development of active acute ehrlichial infections.

Persistent infection of dogs with granulocytic ehrlichia species was detected by single as well as nested PCR using primers targeting regions of the 16S rRNA gene (Egenvall *et al.*, 2000).

Polymerase chain reaction assay using biotinylated *Ehrlichia canis* specific primers was developed for detection of ehrlichiosis in dogs (Mathew *et al.*, 2000).

Meinkoth *et al.* (1998) detected concurrent infection of a dog with two separate species of *Ehrlichia* using nested PCR technique.

Polymerase chain reaction by sequencing of 16S rRNA gene confirmed the first canine case of *Ehrlichia canis* infection in a Pekingese dog in Japan which died due to the disease (Suto *et al.*, 2001).

2.9 Treatment

Carmichael and Fiennes (1942) reported sulfonamides especially sulfapyridine to be very effective in the treatment of canine ehrlichiosis.

Raghavachari and Reddy (1958) found oxytetracycline to be effective in the treatment of ehrlichiosis.

Farrell (1968) also recommended oxytetracycline as the drug of choice for canine ehrlichiosis.

Buckner and Ewing (1967) proved the inefficacy of the drugs chloramphenicol, procaine penicillin, sulfadimethoxine and sulfacetamide to eliminate ehrlichia infection from experimentally infected dogs.

Amyx *et al.* (1971) found that oral administration of the drug tetracycline at the rate of 66 mg/kg body weight for 14 days resulted in remission of clinical signs.

Variable response to tetracycline therapy was reported in dogs affected with *Ehrlichia canis* (Buhles *et al.*, 1974).

A comparative study between the efficacy of the drugs imidocarb dipropionate and tetracycline hydrochloride was carried out which proved imidocarb dipropionate superior in alleviating signs as well as eliminating the disease (Price and Dolan, 1980).

Tetracycline was the drug of choice for ehrlichiosis in dogs and the condition responded dramatically to therapy. Tetracycline hydrochloride or

oxytetracycline was given as a total daily oral dose of 66 mg/kg given in two to three doses (Pyle, 1980).

Adeyanju and Aliu (1982) suggested that imidocarb dipropionate was a potentially useful agent for the treatment of concurrent infections of canine ehrlichiosis and babesiosis.

Therapy with tetracycline hydrochloride per os @ 65 mg/kg body weight thrice daily and supportive treatment with vitamin and iron dextran produced drastic improvement in the condition of a pup suspected to have ehrlichiosis (Okin, 1985).

Price *et al.* (1987) observed that treatment of acute disease of ehrlichiosis resulted in rapid recovery while treatment of chronic disease was not usually successful.

Treatment of a case of ehrlichiosis in dog showing polyarthritis with doxycycline @ 3 mg/kg per os every 12 hrs for 14 days and another case using a combined therapy with tetracycline and prednisolone had been reported by Cowell *et al.* (1988).

A clinical case of canine ehrlichiosis in which oral corticosteroid therapy was instituted along with tetracycline and haematinics, showed marked clinical improvement which was evident from the haemogram obtained after the treatment (Parthasarathy *et al.*, 1989).

Thilagar *et al.* (1990) reported that oxytetracycline at the dose rate of 20 mg/kg body weight at eight hours interval for 14 days cured a rare case of ehrlichiosis associated with inflammation of various joints.

Iqbal and Rikihisa (1994a) proved that some dogs remained as carriers even after treatment by reisolating *Ehrlichia canis* from blood and tissues of dogs after treatment with doxycycline.

A successful report of combined therapy of a case of granulocytic ehrlichiosis with doxycycline @ 10 mg/kg orally for three weeks and prednisolone @ 1 mg/kg orally every 24 hrs gradually tapered to 0.5 mg/kg orally every 72 hrs over a 4 week period came from Maretzki *et al.* (1994).

Treatment with tetracycline hydrochloride orally at a dose rate of 20 mg/kg body weight at eight hours interval for two weeks was found to be successful in treating ehrlichiosis in a dog (Liang *et al.*, 1995).

A case of canine ehrlichiosis was reported to be successfully treated with oxytetracycline given @ 20 mg/kg body weight i/v for 14 days along with liver extract and fluids as supportive therapy (Tresamol *et al.*, 1995).

Egenvall *et al.* (1997) reported rapid recovery of 14 Swedish dogs with ehrlichiosis using doxycycline given @ 5 mg/kg daily for 10-28 days.

A case of canine ehrlichiosis in a six month old puppy has been reported to be treated with parenteral doxycycline therapy successfully (Granholm, 1997).

Successful treatment of ehrlichiosis with doxycycline (100 mg bid) for six consecutive days was reported by Jain and Gupta (1997).

Harrus *et al.* (1997) treated hundred cases of canine monocytic ehrlichiosis with doxycycline @ 10 mg/kg body weight daily orally for three weeks in conjunction with intramuscular injections of imidocarbdipropionate @ 5 mg/kg body weight at 14 days interval.

A hundred per cent elimination of experimentally induced *Ehrlichia canis* infection in dogs was reported after treatment with doxycycline (Breitschwerdt *et al.*, 1998).

The efficacy of doxycycline treatment (10 mg/kg body weight every 24 hours for six weeks) in eliminating *Ehrlichia canis* infection was evaluated in four subclinically infected dogs of which one dog did not respond to treatment (Harrus *et al.*, 1998a).

In a short-term study on the treatment of *Ehrlichia canis* and *E. platys*, Kontos and Athanasiou (1998) observed that enrofloxacin was equally effective as that of doxycycline in the remission of clinical and laboratory abnormalities as well.

Though tetracycline and oxytetracycline have been considered to be the initial drugs of choice, doxycycline and minocycline were also used as drugs of choice. Short term therapy with glucocorticoids may be beneficial early in the treatment period when severe or life threatening thrombocytopenia was present (Neer, 1998).

Tetracycline and doxycyline had been shown to be moderately effective treatments for all forms of canine ehrlichiosis including canine infectious thrombocytopenia (Irwin, 2001).

Ehrlichiosis could be successfully treated with doxycycline since it restores phagosome-lysosome fusion by inhibiting a protein secreted by the bacteria which hinders fusion (Waner *et al.*, 2001).

2.10 Prevention and control

Amyx *et al.* (1971) found tetracycline as an effective prophylactic agent given at the rate of 6.6 mg/kg body weight.

Prophylactic use of tetracycline for tropical canine pancytopaenia @ 3 mg/lb body weight was further emphasized as practical means for controlling the infection in highly endemic areas (Willder, 1977).

Prevention and control of ehrlichiosis could be accomplished through spraying and dipping for ticks, identification of affected animals by IFA test and treatment or elimination of all dogs that were test positive (Pyle, 1980).

Ristic and Holland (1993) carried out a series of immunization studies using inactivated cell culture derived *Ehrlichia canis* antigen, fortified by adjuvants and high levels of antibody response was induced.

Quarantine was advised to dogs imported from ehrlichiosis endemic areas to prevent acquisition and establishment of infection (Sumption and Strachan, 1997).

Chemotherapy, chemoprophylaxis and tick control measures are the primary means of prevention since no vaccines are currently available (Neer, 1998).

Long term tetracycline prophylaxis (6.6 mg/kg once daily) and repositol oxytetracycline (200 mg i/m twice weekly) had been practised in military working dogs to prevent *Ehrlichia canis* infection in highly endemic regions (Breitschwerdt, 2000).

Low doses of doxycycline @ 2 mg/kg orally every 24 hours may be used in endemic areas during tick season (Couto, 2000).

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine with the clinical cases presented at the University Veterinary Hospitals, Mannuthy and Kokkalai, KAU during the period of June 2001 to October 2002.

The dogs showing symptoms like fever, lymphadenomegaly, anorexia, congested mucosa and bleeding episodes suggestive of ehrlichiosis were taken for detailed examination. A group of apparently normal healthy dogs presented to the hospital during the same period, were selected as the control group. Signalment and short previous history of the cases were recorded. Preliminary clinical examination was carried out in these dogs. Blood smear and clinical materials were collected from these dogs and similar investigations were carried out in the experimental and the control group as well.

3.1 Epidemiology

The dogs that were diagnosed positive for ehrlichiosis based on the two diagnostic tests belonging to different age, sex and breed were examined in detail. A detailed signalment, anamnesis and symptoms of each case were recorded as per the proforma (Appendix I).

3.2 Clinical findings

A detailed description of clinical signs suggestive of ehrlichiosis such as elevated temperature, anorexia, selective appetite, generalized lymphadenopathy, congested mucous membrane, chronic wasting, bleeding episodes etc. were recorded.

3.3 Collection of clinical materials

3.3.1 Preparation of blood smear and screening

Blood smear was prepared by collecting one drop of peripheral blood from the ear-tip on a clean grease-free glass slide and was stained using Giemsa stain.

3.3.1.2 Preparation of Giemsa stain

Stock solution

Giemsa stain powder	-	1 g
Glycerol	-	54 ml (warmed to 60°C)

Cooled to room temperature

Added

Absolute methanol	-	84 ml
Azur – II	-	200 mg

Stock solution was diluted 1:9 with a neutral buffer having pH 7-7.2.

Composition of buffer

Disodium hydrogen phosphate – 0.15 M (9.47 g/l)	-	61.1 ml
Potassium dihydrogen phosphate – 0.15 M (9.08 g/l)	-	38.9 ml

Procedure

1. The blood smear was first fixed in methanol for three minutes.
2. The diluted stain was poured onto the blood smear and allowed to act for 1 hour
3. Poured off the stain and washed in buffer
4. Air dried and examined under the oil immersion objective of the microscope.

Buffy coat smear was similarly prepared, stained and examined.

3.3.2 *Collection of blood*

About 10 ml of whole blood was collected from cephalic or saphenous vein using sterile disposable needle and syringe. Three ml of blood was transferred to a sterile vial containing EDTA as anticoagulant at the rate of 1-2 mg/ml of blood.

3.3.3 Collection of serum

The remaining whole blood was transferred to a sterile test tube and kept undisturbed in a slanting position, at room temperature, for 30 min. It

was transferred to a refrigerator at 4°C. The serum, oozed out after clot retraction, was separated, centrifuged at 3000 rpm for 10 min and transferred to a polypropylene serum vial and stored at –20°C for various biochemical estimation.

3.4 Indirect fluorescent antibody test (IFAT)

IFAT was done for all the suspected sera which were stored at –20°C in 2 ml cryo vials as per the method standardized by Ristic *et al.* (1972).

3.4.1 Materials

1. *Ehrlichia canis* antigen coated slides (12 wells) procured from Fuller Laboratories, Fullerton, U.S.A. was used for IFA test.
2. Negative and positive control sera
3. FITC-conjugated rabbit antidog IgG procured from Sigma Aldrich, U.S.A.
4. Phosphate buffer saline (PBS, pH 7.2) .

Potassium dihydrogen phosphate	-	10.2 g
Disodium hydrogen phosphate	-	31.93 g
Sodium chloride	-	17.0 g
Dist. Water	-	4 litres

5. Mounting media (9 parts of glycerol: 1 part of PBS)
6. Cover glass (22 x 50 cm)
7. Distilled water
8. Test sera

3.4.2 Procedure

1. Acetone pre-fixed slides were stored at –20°C until use. Both positive and negative control sera and conjugate were stored at –20°C in aliquots until use.
2. All serum samples including the positive and negative controls were thawed to room temperature and diluted to 1 in 10 using PBS, pH 7.2.
3. The slides were allowed to come to room temperature.
4. Diluted sera were placed so as to form a bubble over each well. Positive control serum was applied to the bottom right hand well and the negative control to the left of the positive control.
5. The slides were then kept in a humid chamber and incubated at 37°C for 30 minutes.
6. The excess sera were removed with one quick downward motion from the slides. The slides were then washed twice in PBS and once in distilled water using a clinical rotator at 60 oscillations per minute.
7. The slides were then air dried.
8. A drop of FITC conjugated rabbit antidog IgG, diluted to 1 in 70 in PBS, was then applied to each well so as to form bubble over entire well.
9. The slides were again incubated in a humid chamber at 37°C for 30 minutes.
10. Washing procedures were repeated as described earlier.
11. Air dried the slides

12. One drop of mounting media was then applied to each well and each slide was covered with a coverslip.

13. The slides were then examined under a fluorescent microscope – Olympus BX 51.

Interpretation

A positive test is indicated by specific fluorescence of the *E. canis* morulae located within the cytoplasm of the monocytes. No specific fluorescence should be observed with the negative control serum or negative samples.

3.5 Haematological estimation

The following haematological parameters were estimated.

3.5.1 Haemogram

3.5.1.1 Erythrocyte sedimentation rate (ESR)

It was estimated using Wintrobe's method by keeping the blood for one hour (Coles, 1986).

3.5.1.2 Packed cell volume (PCV)

Estimated by Wintrobe's method as per Coles (1986) and expressed as per cent.

3.5.1.3 Haemoglobin (Hb)

It was estimated by acid-haematin method using Sahli's haemoglobinometer and was expressed as gram per deciliter (Coles, 1986).

3.5.1.4 Total erythrocyte count

Total RBC count was estimated using Hayem's fluid as per Coles (1986) and value expressed as $x10^6$ cells/mm^3 of blood.

3.5.1.5 Erythrocyte indices

The erythrocyte indices were calculated to identify the type of anaemia using the following formulae (Coles, 1986).

a. Mean corpuscular volume (MCV) $= \dfrac{\text{PCV/1000 ml of blood}}{\text{Total erythrocyte count in millions/mm}^3}$

Value expressed as femtoliters

b. Mean corpuscular haemoglobin (MCH) $= \dfrac{\text{Haemoglobin in grams/1000 ml}}{\text{Total erythrocyte count in millions/mm}^3}$

Value expressed as picogram

c. Mean corpuscular haemoglobin concentration (MCHC) $= \dfrac{\text{Hb in grams/10,000 ml of blood}}{\text{PCV/100 ml of blood}}$

Value expressed as grams/decilitres

3.5.2 Leukogram

3.5.2.1 Total leukocyte count (TLC)

Total WBC count was estimated using Thoma's fluid as per Coles (1986) and value expressed as $x10^3$ cell/mm^3 of blood.

3.5.2.2 Differential leukocyte count (DLC)

Blood smear was stained by Giemsa method and 100 leukocytes were counted under oil immersion objective and differential counts were expressed as percentage (Benjamin, 1985).

3.5.3 Thrombocyte count

Platelet count was estimated by direct method using Rees-Ecker fluid (Coles, 1986) and value expressed as $x10^5$ cells/µl.

Thrombocyte count was also estimated by an indirect method as per Coles (1986). This was performed on a stained blood film prepared for routine haematologic examination.

The number of platelets observed, in tallying 100 leukocytes, were recorded. Absolute number is obtained using the formula,

$$\frac{\text{Number of platelets x total WBC count}}{\text{100 WBC}} = \text{Number of platelets}$$

3.6 Serum biochemical estimation

The following biochemical estimations were carried out.

3.6.1 Serum total protein

The total protein concentration was determined colorimetrically by direct Biuret method (Gormall *et al.*, 1949). The reagent and standard were supplied by Agappe diagnostics. Values expressed as grams/deciliter.

3.6.2 Albumin

Albumin concentration of the samples were determined by Bromocresol green method (Doumas *et al.*, 1971) using the kit from Agappe diagnostics. Values expressed as grams/deciliter.

3.6.3 Globulin

Globulin concentrations were derived from the known total protein and albumin values. Values expressed as grams/deciliter.

3.6.4 Albumin-globulin ratio (A:G ratio)

A:G ratio was calculated.

3.6.5 Serum alanine amino transferase (ALT)

ALT was estimated using commercially available kit (Agappe diagnostics) and the final readings were taken spectrophotometrically at 405 nm. Values expressed as units/ml of serum.

3.6.6 Serum alkaline phosphatase (AP)

AP was estimated using commercial kit from Agappe diagnostics and readings were taken at 405 nm. Concentration was expressed as units/ml of serum.

3.6.7 Serum creatinine

Serum creatinine level was estimated by alkaline picrate method using commercially available kit from Span diagnostics. Values were expressed as mg/dl.

3.7 Therapeutic trial

Treatment was initiated in cases wherein parasitemia was confirmed by detection of morula/inclusion body in the cytoplasm of leukocytes by blood or buffycoat smear examination. Doxycycline (Tab. Vibazine – 100 mg) was given @ 5 mg/kg body weight once daily orally for 14 days and Prednisolone (Tab. Wysolone – 5 mg) @ 1 mg/kg body weight orally for five days followed by 0.5 mg/kg body weight for five days and 0.25 mg/kg body weight for the remaining days.

After 14 days of therapeutic trial, clinical response of each case was recorded. Blood and sera of each animal were collected and subjected to haematological and biochemical evaluation. The data obtained from various parameters were compared between pre treatment and post treatment groups.

3.8 Statistical analysis

The data obtained were analysed statistically as per the procedure described by Snedecor and Cochran (1980).

Results

4. RESULTS

A total of sixty four dogs showing symptoms suggestive of ehrlichiosis were included in the experimental group and were subjected to detailed study. The control group consisted of six apparently normal healthy dogs presented to the hospital during the study period.

Based on two distinct diagnostic tests *viz*, blood/buffy coat smear examination and indirect fluorescent antibody test; the 64 animals were classified into five groups.

Group 1 Six apparently healthy dogs taken as control group

Group 2 Fifteen cases proved positive both by blood smear examination and indirect fluorescent antibody (IFA) test.

Group 3 Twenty seven cases proved positive by IFA test and negative by blood smear examination.

Group 4 One case positive by blood smear examination and negative by IFA test

Group 5 Twenty one cases proved negative both by blood smear examination and IFA test

Values of the infected groups except that of group IV were compared with that of the control group statistically. Group IV was not taken into account because it contained only one animal. Group V could not be considered

because the animals in that group were not conclusively proved as cases of ehrlichiosis.

4.1 Epidemiology

4.1.1 Breed-wise occurrence of *Ehrlichia canis* infection

Among the 64 cases studied, prevalence of *Ehrlichia canis* infection was confirmed by the IFA test, in 42 animals. This included the 15 animals out of 16, that proved positive for ehrlichiosis by the blood smear examination. Positive fluorescence reaction was observed in different breeds of dogs such as German shepherd, Great dane, Golden retriever, Labrador retriever, Dalmatian, Dobermann pinscher, Fox terrier, Boxer and Pomeranian.

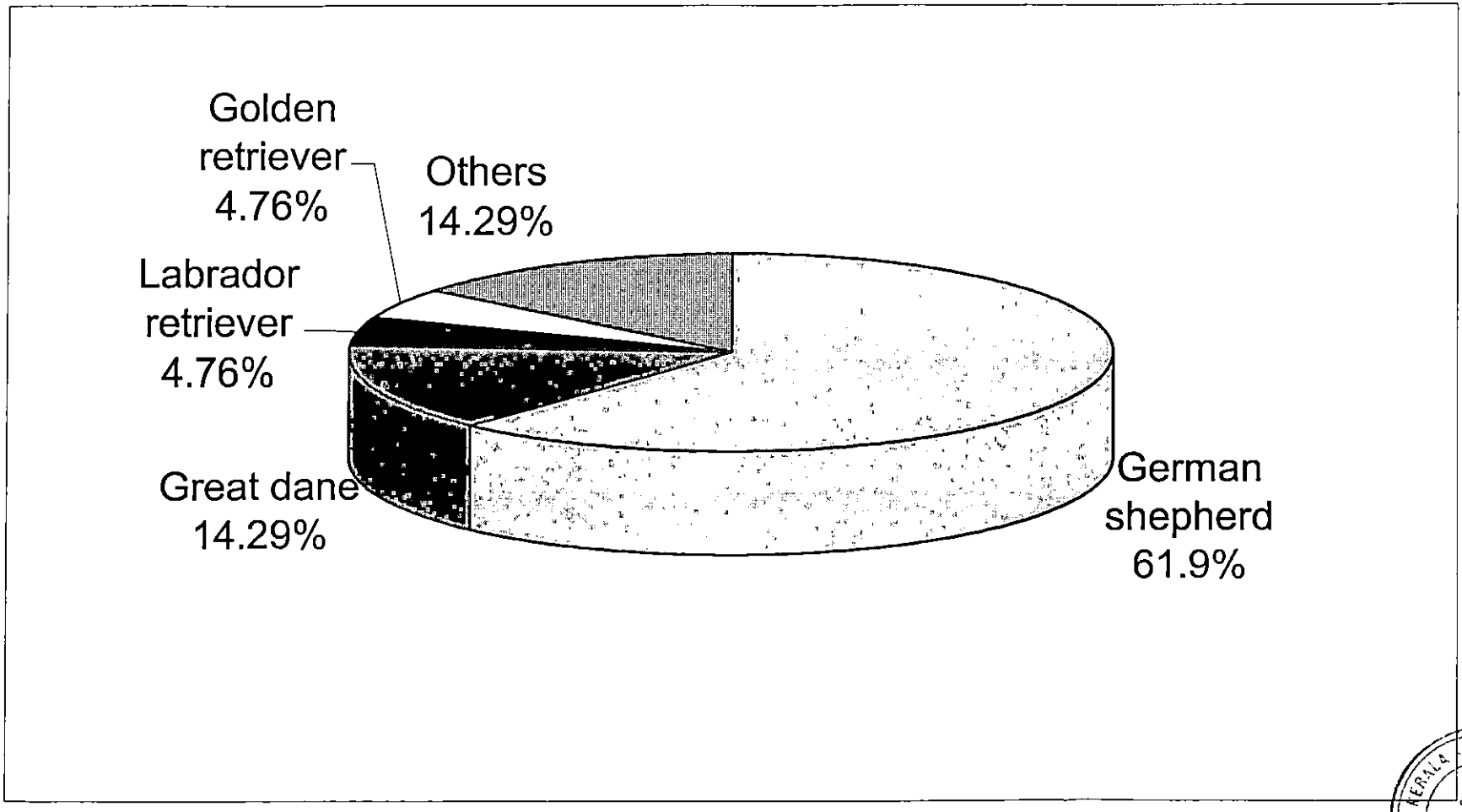
Among the total per cent positives, highest rate of infection was observed in German shepherd dogs where 26 animals out of 42 (61.9 per cent) showed positive reaction followed by Great dane (14.29 per cent), Golden retriever (4.76 per cent), Labrador retriever (4.76 per cent) and others together constituted 14.29 per cent out of the total positives (Fig.1).

Statistical analysis using test for proportions revealed no significant difference between the different breeds for the susceptibility to infection.

4.1.2 Age-wise occurrence of *Ehrlichia canis* infection

Three age groups were categorized. (1) Pups (0-6 months), (2) Growing age (6 months – 2 years), (3) Adults (above 2 years) and the age-wise distribution of cases were studied.

Fig. 1 Breed-wise occurrence of *Ehrlichia canis* infection



Among the total per cent positives, highest rate of infection was observed in the growing age group (42.86 per cent) closely followed by the adult age group (40.48 per cent) and pups showed a comparatively lower rate of infection (16.67 per cent) among the total seropositives (Fig.2).

Statistical analysis showed no significant difference in the proportion of positive cases in various age groups.

4.1.3 Sex-wise occurrence of *Ehrlichia canis* infection

Among the total seropositives, males contributed to the major percentage (61.9 per cent) and females showed a comparatively lower percentage (38.1 per cent) (Fig.3).

Twenty six males were found to be positive, among the total 42 males examined (61.9 per cent) and among the 22 female dogs presented, 16 were positive (72.7 per cent) by IFA test. Statistical analysis, using test for proportions, revealed that no significant difference existed between males and females in the infection rate.

4.2 Clinical findings

The occurrence of clinical signs in the 42 seropositive dogs, in the order of increasing frequency is shown in Fig.4.

The most common clinical signs were lymphadenomegaly, fever, congested mucous membrane, depression and lethargy, selective appetite, panting, ocular and nasal discharge, vomiting, diarrhoea and bleeding episodes.

Fig.2 Age-wise occurrence of *Ehrlichia canis* infection

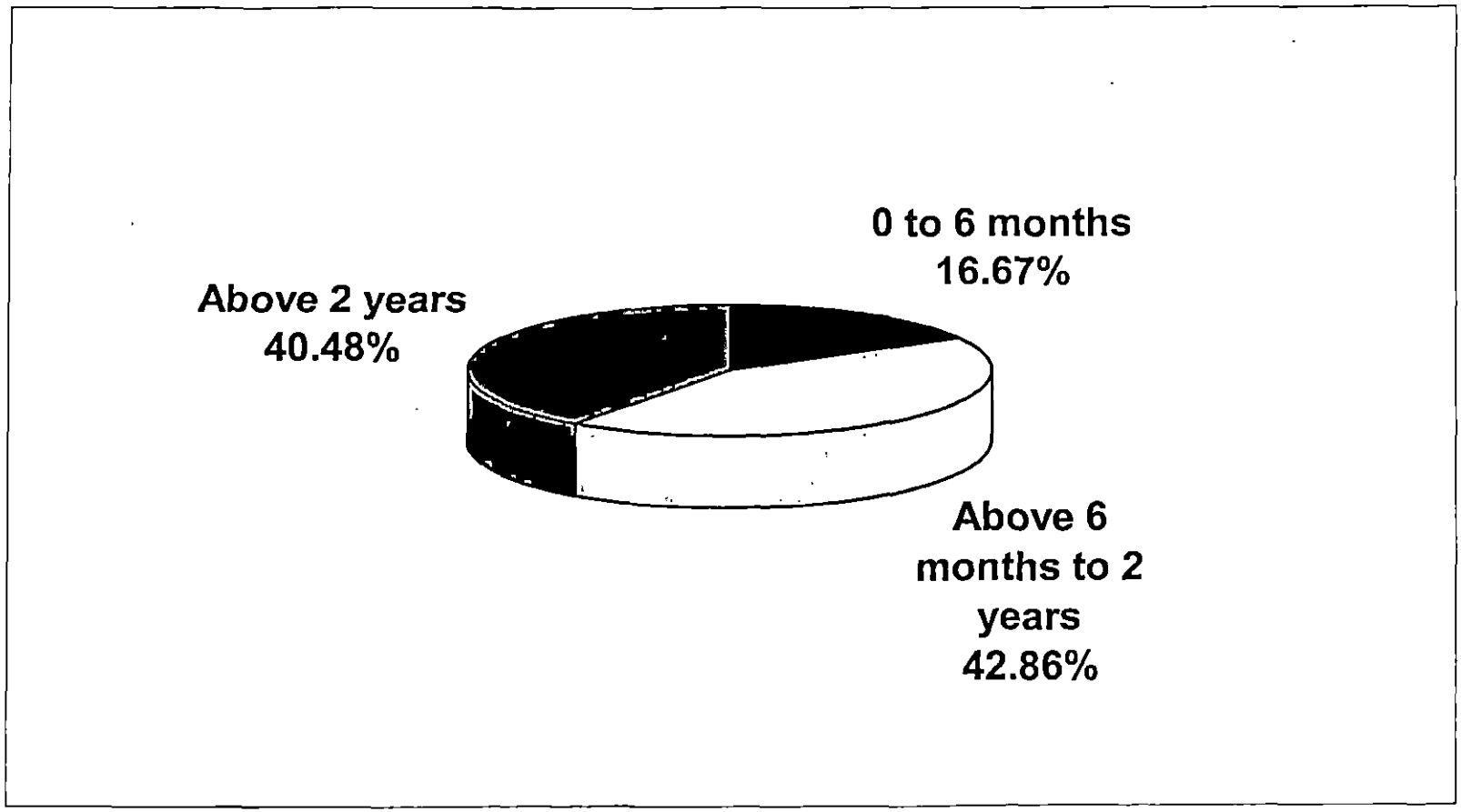
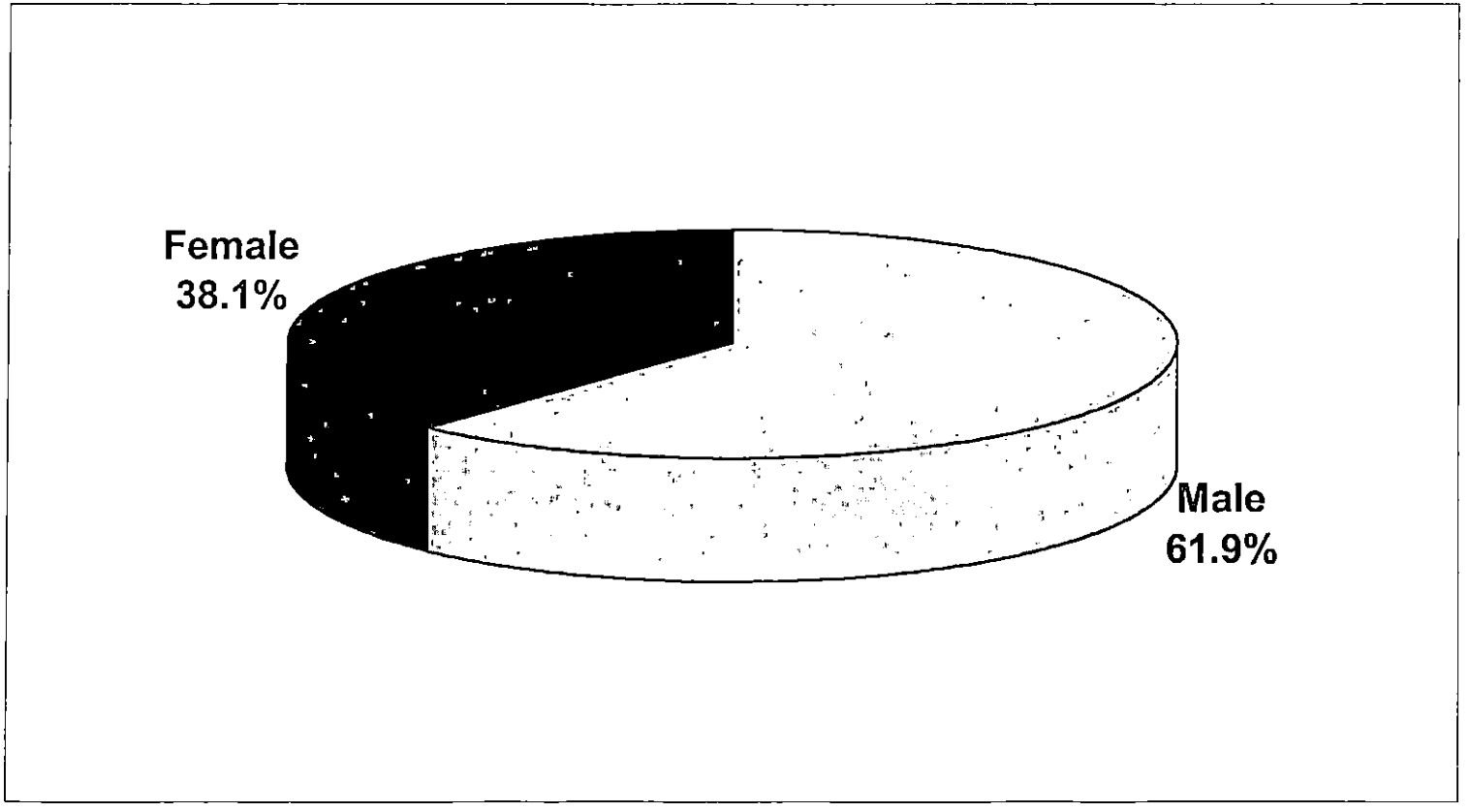


Fig. 3 Sex-wise occurrence of *Ehrlichia canis* infection



Lymphadenomegaly of popliteal lymphnodes and rarely all lymphnodes was noticed in 85.7 per cent, which was the most prominent clinical finding observed in this study. Fever was noticed in 69.1 per cent where the body temperature ranged between 103.8°F to 107°F.

Majority of affected dogs showed a selective appetite (59.5 per cent) rather than anorexia (28.6 per cent). Digestive disturbances such as vomiting (28.6 per cent) and diarrhoea (31.0 per cent) were observed in some cases.

A higher percentage of affected dogs showed signs of depression and lethargy, ocular and nasal discharge.

A major percentage of seropositives showed congested mucous membrane (61.9 per cent) followed by pale mucosa (23.8 per cent) and a few showed icteric mucosa (4.8 per cent).

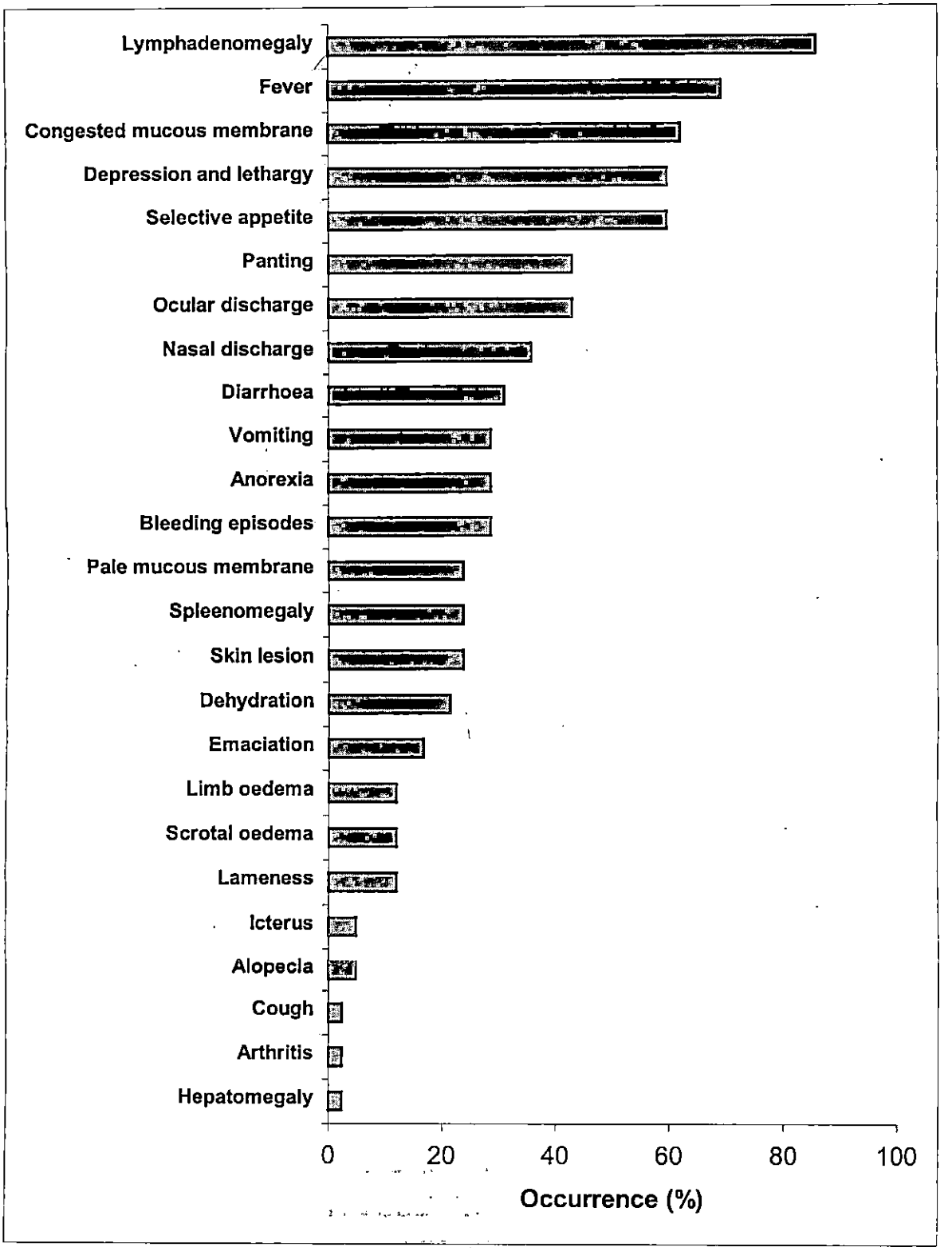
Bleeding episodes were reported in a few cases (28.6 per cent) in the form of epistaxis, melena, haematochezia, haematemesis and ecchymoses of skin.

Many animals showed oozing wounds or similar lesions in between the paw region, joints and inner aspect of limbs (23.8 per cent).

Oedema of limb and scrotum with reddened, moist, scabby lesions on scrotum were shown by a few (11.9 per cent) and some showed lameness (11.9 per cent).

Some seropositive animals were presented with the complaint of chronic weight loss for several months (16.7 per cent).

Fig.4 Percentage occurrence of clinical findings in 42 dogs with ehrlichiosis



Spleenomegaly was an inconsistent finding that contributed to 23.8 per cent. Hepatomegaly was observed in one case (2.4 per cent).

Rare occurrence of cough, alopecia, and arthritis were encountered in this study(Table I)

Among 42 seropositive dogs, 27 animals (64.3 per cent) had a history of ectoparasite infestation, mainly with ticks.

4.3 Examination of clinical materials

4.3.1 Examination of blood smear/buffy coat smear

On examination of blood smears stained by Giemsa from suspected cases, inclusion bodies were observed in the cytoplasm of leukocytes of sixteen animals (25 per cent) (Table 2). The inclusion bodies observed in the leukocytes were in the form of elementary bodies or morulae stained bluish pink or lilac coloured with Giemsa stain (Plate 1). In some cases, inclusion bodies and vacuoles were seen within the leukocytes (Plate 2).

No inclusion bodies or morulae could be observed in blood or buffy coat smears of any of the six animals in the control group.

4.3.2 Concurrent infections

Two cases of concurrent infections of *Ehrlichia canis* and blood protozoan *Hepatozoon canis* could be detected by examination of blood smear. The blood smears revealed gelatin capsule-shaped gamonts of *Hepatozoon*

Table 1. Frequency of clinical signs presented in *Ehrlichia canis* positive cases

Clinical signs		Number	Per cent
Fever		29	69
Depression and lethargy		25	59.5
Appetite	Selective appetite	25	59.5
	Anorexia	12	28.6
Lymphadenomegaly		36	85.7
Mucousmembrane	Congested	26	61.9
	Pale	10	23.8
Panting		18	42.9
Ocular discharge		18	42.9
Nasal discharge		15	35.7
Digestive system	Diarrhoea	13	31
	Vomiting	12	28.6
Bleeding abnormalities		12	28.6
Skin lesions		10	23.8
Alopecia		2	4.8
Spleenomegaly		10	23.8
Hepatomegaly		1	2.4
Dehydration		9	21.4
Emaciation		7	16.7
Oedema	Limb	5	11.9
	Scrotum	5	11.9
Lameness		5	11.9
Icterus		2	4.8
Cough		1	2.4
Arthritis		1	2.4

Plate 1. ***Ehrlichia canis*** **morula – Blood smear - Giemsa stain x 900**

Plate 2. ***Ehrlichia canis*** **morula and vacuolation - Blood smear - Giemsa stain x 900**

Plate 3. ***Ehrlichia canis*** **morula and gamont of** ***Hepatozoon canis*** **– Blood smear - Giemsa stain x 900**

Plate 4. ***Ehrlichia canis*** **morulae in the cytoplasm of monocyte – indirect fluorescent antibody test - x 1000**

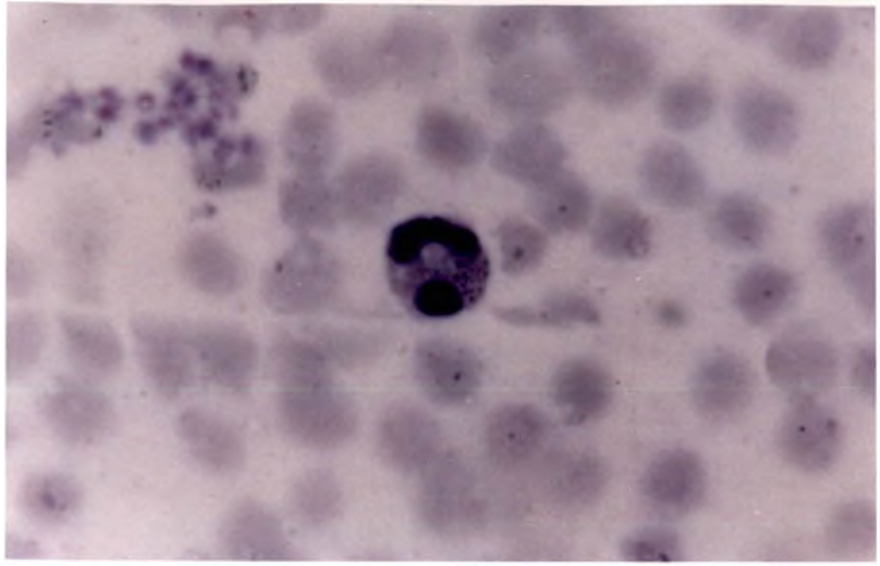


Plate 1

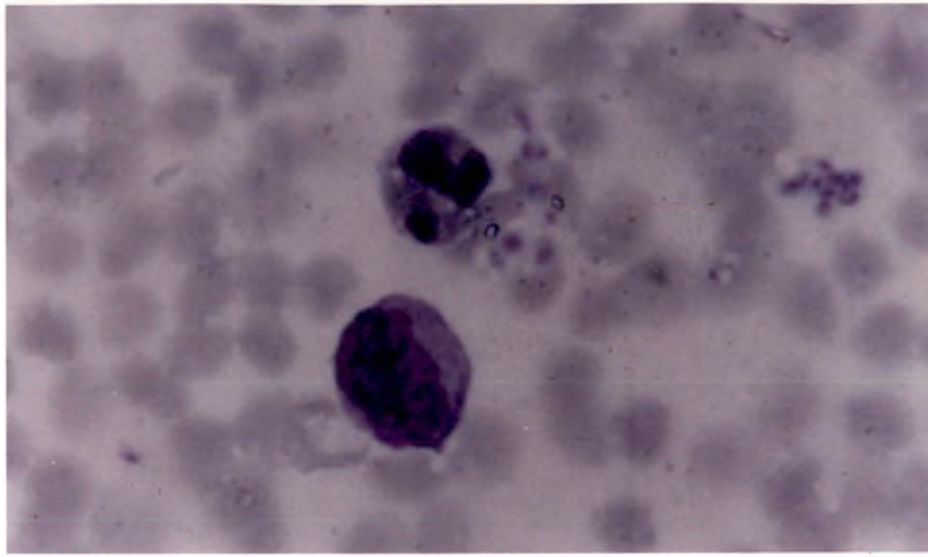


Plate 2

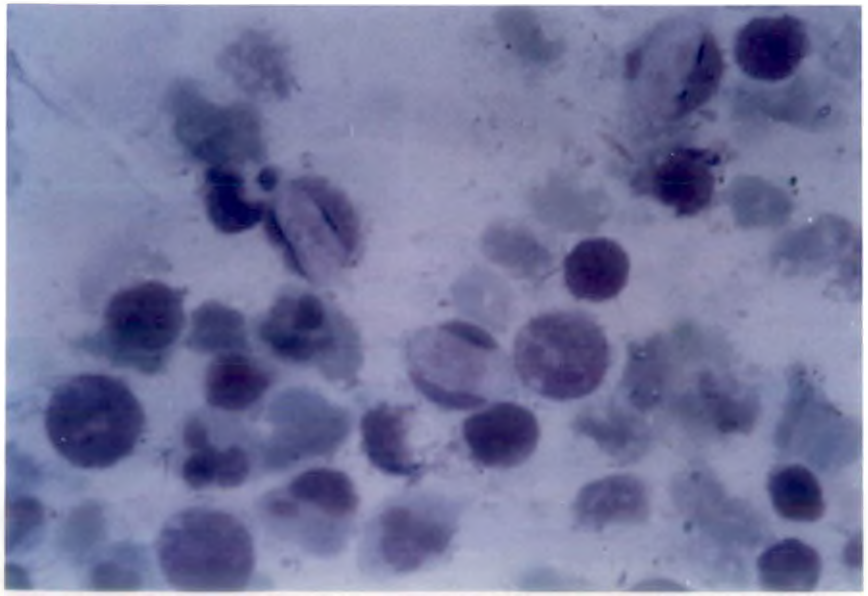


Plate 3

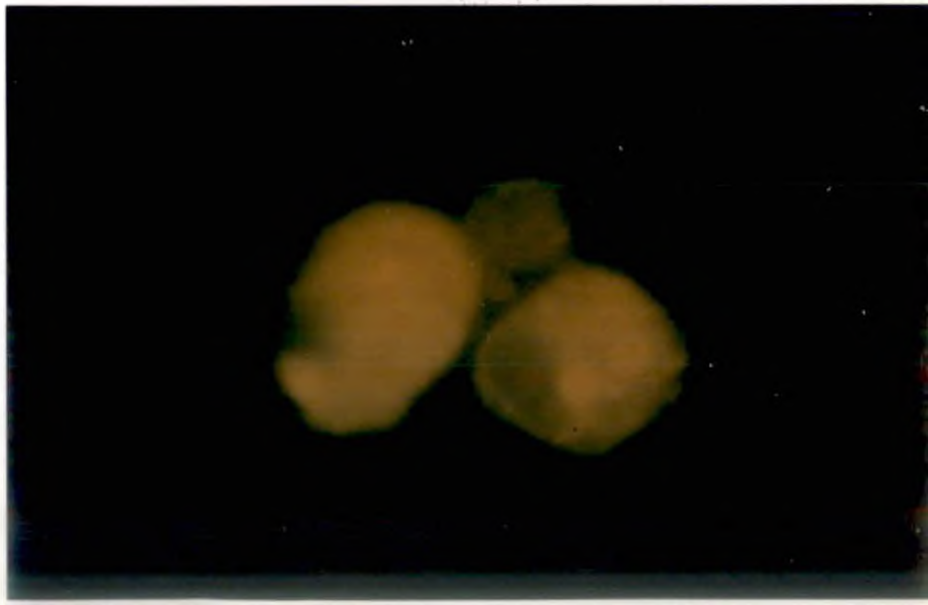


Plate 4

canis in the neutrophils and also *Ehrlichia canis* inclusion body in the leukocyte (Plate 3).

4.4 Indirect fluorescent antibody (IFA) test

A total of 42 animals out of 64 (65.6 per cent) were found to be positive by IFA test which is considered to be the gold standard test (Table 3). Positive reaction in the test was observed by a specific fluorescence of the *Ehrlichia canis* morulae or inclusion body in the cytoplasm of the monocytes.

Antigen was detected in the cytoplasm of cultured mononuclear cells coated on the wells of IFA slides. Fluorescence was observed in populations of elementary bodies, initial bodies and morulae (occurring either singly or in multiples) when positive serum samples were applied (Plates 4 and 5). There were also cells with apparently soluble antigen diffusely distributed through the entire cytoplasm (Plate 6).

Fluorescence observed with in an entire microscopic field, is showed in Plate 6 viewed at lower magnification.

No fluorescence could be observed in or outside the cells when sera from *Ehrlichia canis* free dogs were applied (Plates 7 and 8).

4.5 Haematological estimation

The following haematological parameters were estimated.

Table 2. Result of blood smear examination in the diagnosis of *Ehrlichia canis* infection

Test	No. of animals tested	No. of positives	Per cent of positives
Blood smear examination	64	16	25

Table 3. Result of indirect fluorescent antibody (IFA) test in the diagnosis of *Ehrlichia canis* infection

Test	No. of animals tested	No. of positives	Per cent of positives
Indirect fluorescent antibody test	64	42	65.6

Table 4. Haemogram in different groups of dogs

Group	No. of animals	Mean ± standard error						
		ESR mm/hr	PCV (%)	Haemo globin (g/dl)	RBC x (10^6/mm^3)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group I	6	1.17^{a} ± 0.31	44.93^{a} ± 2.45	14.15^{a} ± 0.37	6.89^{a} ± 0.11	65.24^{a} ± 0.85	20.54 ± 0.33	31.5 ± 0.41
Group II	15	5.9^{b} ± 0.65	34.6^{bc} ± 1.06	10.93^{b} ± 0.30	5.03^{b} ± 0.13	69.11^{ba} ± 2.03	21.81 ± 0.54	31.71 ± 0.64
Group III	27	7.24^{c} ± 0.71	33.06^{c} ± 1.09	9.87^{c} ± 0.31	4.42^{C} ± 0.18	76.5^{cb} ± 2.34	22.79 ± 0.61	29.96 ± 0.48
		**	**	**	**	*	NS	NS

Means having common superscripts column-wise do not differ significantly

NS – Non Significant ($p \geq 0.05$)

* - Significant ($p < 0.05$)

** - Highly significant ($p < 0.01$)

Plate 5. *Ehrlichia canis* aggregates or clusters of inclusions in the cytoplasm of monocyte in IFA test x 1000

Plate 6. Soluble antigens of *E. canis* diffusely distributed in the cytoplasm of monocytes in IFA test x 1000

Plate 7. Negative reaction in IFA test – high magnification x 1000

Plate 8. Negative reaction in IFA test – low magnification x 400

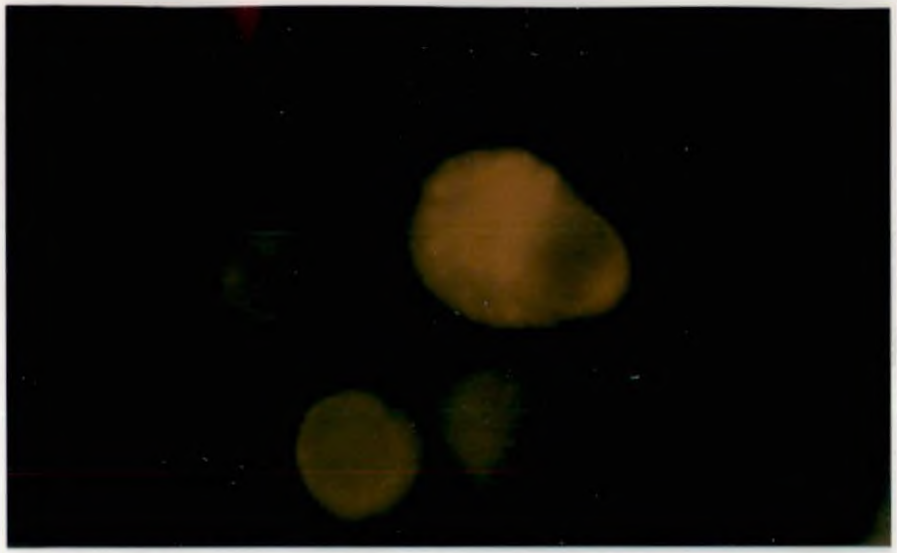


Plate 5

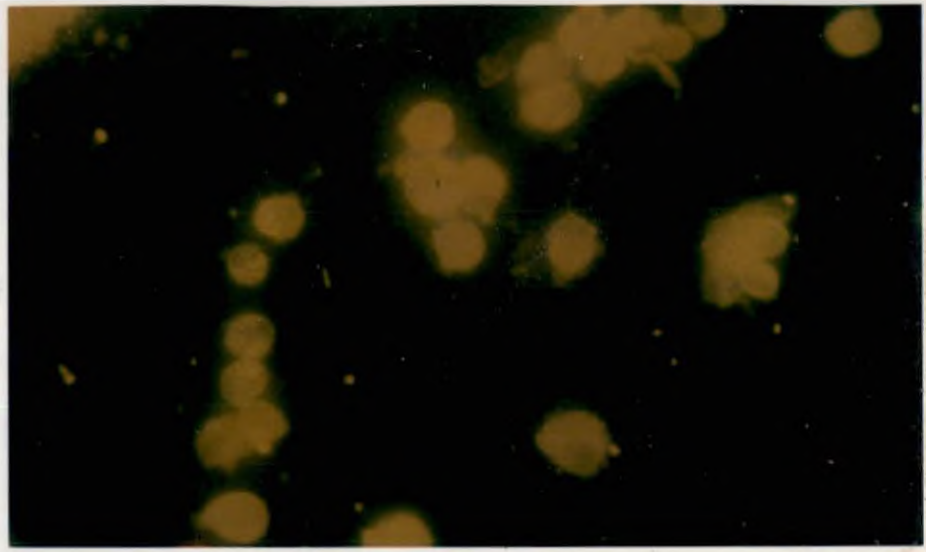


Plate 6

Plate 7

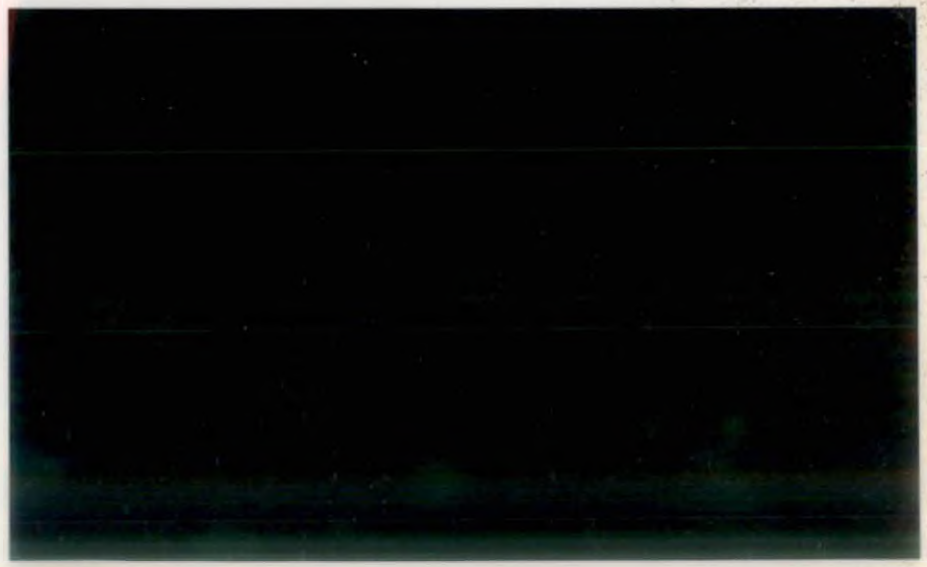


Plate 8

4.5.1 Haemogram

4.5.1.1 Erythrocyte sedimentation rate

The control animals had a mean ESR value of 1.17 ± 0.31 mm/hr. whereas groups II and III showed a higher ESR of 5.9 ± 0.65 mm/hr and 7.24 ± 0.71 mm/hr respectively.

Statistical analysis showed a significant difference in ESR values between group I (control group) and group II and III (diseased groups). However, no significant difference was observed between groups II and III (Table 4).

4.5.1.2 Packed cell volume (PCV)

Statistical analysis of PCV values revealed a highly significant reduction ($p<0.01$) in the mean values of group II and III (34.6 ± 1.06 per cent and 33.06 ± 1.09 per cent) respectively compared to the control mean value of 44.93 ± 2.45 per cent (Table 4).

4.5.1.3 Haemoglobin (Hb)

The haemoglobin values were significantly lowered ($p<0.01$) in the disease groups (II and III) compared to the control group I. The mean values for group I, II and III were 14.15 ± 0.37 g/dl, 10.93 ± 0.3 g/dl and 9.87 ± 0.31 g/dl respectively. The mean value for haemoglobin of group III was significantly low compared to that of group II (Table 4).

4.5.1.4 Erythrocyte count

Low erythrocyte counts were observed for the disease groups II and III when compared to the mean value for group I ($p<0.01$). A significant reduction for mean RBC count was observed in Group III when compared to that of Group II (Table 4). The mean values for Group I, II and III were 6.89 ± 0.11 x $10^6/mm^3$, 5.03 ± 0.13 x $10^6/mm^3$ and 4.42 ± 0.18 x $10^6/mm^3$ respectively (Table 4).

4.5.1.5 Erythrocyte indices

MCV, MCH and MCHC were calculated for different groups and subjected to statistical analysis.

Mean corpuscular volume (MCV)

Statistical analysis revealed no significant difference in the mean MCV values between group I and II and between group II and III. But mean value of group III was significantly higher ($p<0.05$) compared to the mean value of control group. The mean values for group I, II and III were as follows: 65.24 ± 0.85 fl, 69.11 ± 2.03 fl and 76.5 ± 2.34 fl (Table 4).

Mean corpuscular haemoglobin (MCH)

The mean MCH values for group I, II and III (20.54 ± 0.33 pg, 21.81 ± 0.54 pg and 22.79 ± 0.61 pg) do not differ significantly from one another (Table 4).

Mean corpuscular haemoglobin concentration (MCHC)

No significant difference was observed between the mean values of group I, II and III, by statistical analysis (Table 4). Mean values of group I, II and III were 31.5 ± 0.41, 31.71 ± 0.64 and 29.96 ± 0.48 respectively.

4.5.2 Leukogram

4.5.2.1 Total leukocyte count (TLC)

Statistical analysis showed no significant difference of the mean values of groups II and III (9.63 ± 0.58 x 10^3/mm^3 and 9.3 ± 0.49 x 10^3/mm^3) from that of the control group which was 10.15 ± 0.61 x 10^3/mm^3 (Table 5).

4.5.2.2 Differential leukocyte count (DLC)

Neutrophil count (per cent)

There was no significant difference between the mean values of control group (71.67 ± 1.52 per cent) and the groups II and III (66.47 ± 2.85 per cent and 69.15 ± 1.88 per cent respectively) (Table 5).

Lymphocyte count (per cent)

The mean value of lymphocyte count for group II (24.4 ± 2.48 per cent), and group III (22.26 ± 2.14 per cent) did not differ significantly from the control mean value (25.33 ± 1.31 per cent) (Table 5).

Table 5 Leukogram in different groups of dogs

Group	No. of animals	Mean ± standard error				
		TLC x $10^3/mm^3$	DLC (%)			
			Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
Group I	6	10.15 ± 0.61	71.67 ± 1.52	25.33 ± 1.31	2.0^{a} ± 0.26	1.00 ± 0.36
Group II	15	9.63 ± 0.58	66.47 ± 2.85	24.4 ± 2.48	8.4^{bc} ± 0.75	0.6 ± 0.26
Group III	27	9.3 ± 0.49	69.15 ± 1.88	22.26 ± 2.14	7.56^{c} ± 0.51	0.82 ± 0.23
F ratio		NS	NS	NS	**	NS

Means having common superscripts within a column do not differ significantly
NS – Non Significant ($p \geq 0.05$)
** - Highly significant ($p < 0.01$)

Table 6. Mean thrombocyte count in different groups of dogs

Groups	No. of animals	Mean ± standard error
		Thrombocyte count x $10^5/\mu l$
Group I	6	3.67^{a} ± 0.11
Group II	15	1.18^{bc} ± 0.13
Group III	27	1.30^{c} ± 0.10
F ratio		**

Means having common superscripts within a column do not differ significantly
** - Highly significant ($p < 0.01$)

Monocyte count (per cent)

The monocyte counts were significantly higher ($p<0.01$) in the disease groups II and III (8.4 ± 0.75 per cent and 7.56 ± 0.51 per cent respectively) compared to that of control group (2.0 ± 0.26 per cent) (Table 5).

Eosinophil count (per cent)

No significant difference was observed between the control mean value (1.0 ± 0.36 per cent) and the mean values for groups II and III (0.6 ± 0.26 per cent and 0.82 ± 0.23 per cent respectively) (Table 5).

4.5.3 Thrombocyte count

The mean value for control group was 3.67 ± 0.11 x 10^5/µl. Statistical analysis revealed a highly significant reduction ($p<0.01$) in the mean values of Groups II and III (1.18 ± 0.13 x 10^5/µl and 1.3 ± 0.1 x 10^5/µl) from the control mean value (Table 6).

4.5.4 Haematology of group IV

Group IV comprised of only one animal. Its haematological parameters were as follows. Erythrocyte sedimentation rate : 3 mm/hr, Packed cell volume : 32 per cent, Haemoglobin: 11.2 g/dl, RBC count : 5.1 x 10^6/mm^3, MCV : 62.74 fl, MCH : 22 pg, MCHC : 35 g/dl, total leukocyte count : 14.4 x 10^3/mm^3, Neutrophil : 72 per cent, Lymphocyte : 18 per cent, Monocyte : 10 per cent and Thrombocyte count : 2.24 x 10^5/µl (Table 7).

Table 7. Haematological parameters - Group IV

Parameter	Value obtained
Erythrocyte sedimentation rate (mm/hr)	3
Packed cell volume (per cent)	32
Haemoglobin (g/dl)	11.2
Total erythrocyte count (10^6/mm^3)	5.1
Mean corpuscular volume (fl)	62.74
Mean corpuscular haemoglobin (pg)	22
Mean corpuscular haemoglobin concentration (g/dl)	35
Total leukocyte count (10^3/mm^3)	14.4
Neutrophils (per cent)	72
Lymphocytes (per cent)	18
Monocytes (per cent)	10
Eosinophils (per cent)	0
Thrombocyte count (10^5/µl)	2.24

4.5.5 Haematological abnormalities

Percentage distribution of the values for various haematological parameters were calculated.

The most striking haematological abnormalities observed were thrombocytopenia and anaemia. Out of the 42 animals, low haemoglobin value was observed in 66.7 per cent of animals and 61.9 per cent of animals showed a reduced RBC count and PCV.

Erythrocyte indices were within the normal range in majority of animals.

Thrombocytopenia was observed in 76.2 per cent of cases whereas 19 per cent showed a normal thrombocyte count.

Majority of the animals showed normal white blood cell count. Nineteen per cent of the animals showed leukopenia and leukocytosis was observed in 19 per cent of animals. The most frequent leukocyte abnormalities noticed were monocytosis and eosinopenia. Neutropenia was observed in 14.3 per cent cases and lymphopenia was observed in 9.5 per cent cases (Table 8 and Fig.5).

4.6 Serum biochemical estimation

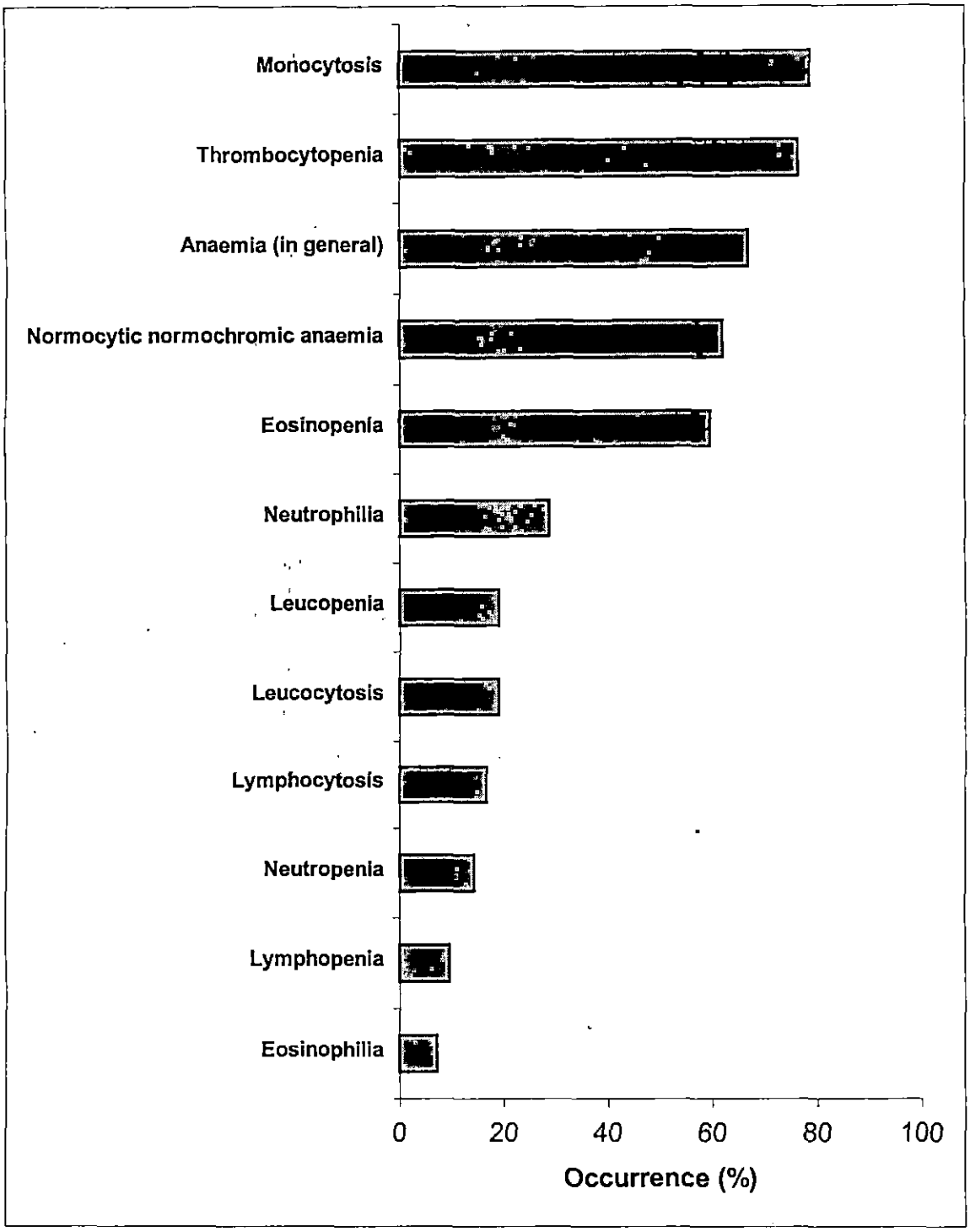
4.6.1 Total serum protein

Significantly higher values ($p<0.05$) for total serum protein were recorded in the disease groups II and III (9.29 ± 0.5 g/dl) and 8.88 ± 0.27 g/dl) compared to the control mean value of 7.1 ± 0.13 g/dl (Table 9).

Table 8. Haematological abnormalities in *Ehrlichia canis* infection

Parameter	Normal range (control group)	Affected range	Number evaluated	Percentage distribution of values		
				Low	Normal	High
PCV (per cent)	41 - 54	16.8 - 55.6	42	61.9	33.3	4.8
Haemoglobin (g/dl)	13.2 - 15.6	4.5 - 16.4	42	66.7	28.5	4.8
RBC count (10^6/mm^3)	6.52 - 7.25	1.3 - 7.96	42	61.9	26.2	11.9
MCV (fl)	62.88 - 68	54.54 - 129.23	42	4.8	73.8	21.4
MCH (pg)	19.6 - 21.52	18.22 - 34.62	42	11.9	78.6	9.5
MCHC (g/dl)	30 - 32.68	26.25 - 35.38	42	16.7	80.9	2.4
WBC count (10^3xmm^3)	8.1 - 12.1	1.8 - 14.1	42	19	62.9	19
Neutrophils (per cent)	67 - 78	40 - 86	42	14.3	59.5	28.6
Lymphocytes (per cent)	20 - 29	6 - 60	42	9.5	73.8	16.7
Monocytes (per cent)	1 - 3	0 - 14	42	2.4	19.0	78.6
Eosinophils (per cent)	1 - 2	0 - 4	42	59.5	33.3	7.2
Thrombocytes (10^5/µl)	2 - 3.84	0.6 - 4.1	42	76.2	19.0	4.8

Fig.5 Percentage occurrence of haematological abnormalities in 42 dogs with Ehrlichiosis



4.6.2 Serum albumin

The mean albumin values for groups I, II and III were 3.7 ± 0.17 g/dl, 2.23 ± 0.16 g/dl and 2.26 ± 0.11 g/dl respectively. Significant decrease ($p<0.01$) in the albumin values was recorded in the disease groups II and III (Table 9).

4.6.3 Serum globulin

The mean serum globulin values of groups II and III (7.07 ± 0.45 g/dl and 6.59 ± 0.22 g/dl) were significantly higher ($P<0.01$) compared to the control mean value of 3.4 ± 0.12 g/dl (Table 9).

4.6.4 Albumin-Globulin ratio (A/G ratio)

The A/G ratio was significantly lower ($p<0.01$) in the disease groups compared to that of control group. The mean values of different groups I, II and III were 1.12 ± 0.08, 0.32 ± 0.02 and 0.35 ± 0.02 respectively (Table 9).

4.6.5 Serum alanine amino transférase (ALT)

The mean value of ALT for the control group was 19.92 ± 1.42 IU/l. A highly significant increase ($p<0.01$) of ALT values was observed for the disease groups II and III (67.07 ± 6.25 IU/l and 89.54 ± 4.32 IU/L) compared to the control mean value (Table 9).

4.6.6 Serum alkaline phosphatase (AP)

The average AP values for groups I, II and III were 67.17 ± 2.36 IU/l, 253.42 ± 16.85 IU/l and 248.5 ± 11.16 IU/l respectively. Analysis of variance

Table 9. Serum profile in different groups of dogs

Group	No. of animals	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALT (IU/l)	AP (IU/l)	Creatinin (mg/dl)
Group I	6	$7.1^{a} \pm 0.13$	$3.7^{a} \pm 0.17$	$3.4^{a} \pm 0.12$	$1.12^{a} \pm 0.08$	$19.92^{a} \pm 1.42$	$67.17^{a} \pm 2.36$	$1.15^{a} \pm 0.07$
Group II	15	$9.29^{bc} \pm 0.5$	$2.23^{b} \pm 0.16$	$7.07^{bc} \pm 0.45$	$0.32^{bc} \pm 0.02$	$67.07^{b} \pm 6.25$	$253.42^{bc} \pm 16.85$	$2.32^{bc} \pm 0.19$
Group III	27	$8.88^{c} \pm 0.27$	$2.26^{c} \pm 0.11$	$6.59^{c} \pm 0.22$	$0.35^{c} \pm 0.02$	$89.54^{c} \pm 4.32$	$248.5^{c} \pm 11.16$	$2.46^{c} \pm 0.16$
		*	**	**	**	**	**	**

Means bearing common superscripts within a column do not differ significantly

* - Significant ($p<0.05$)

** - Highly significant ($p< 0.01$)

Table 10. Serum profile - Group IV

Parameter	Value obtained
Total protein (g/dl)	7.6
Albumin (g/dl)	3
Globulin (g/dl)	4.6
A/G ratio	0.7
Serum ALT (IU/l)	68
Serum AP (IU/l)	122
Creatinine (mg/dl)	1

Table 11. Biochemical abnormalities in *Ehrlichia canis* infection

Parameter	Normal range (control group)	Affected range	Number evaluated	Percentage distribution of values		
				Low	Normal	High
Creatinine (mg/dl)	1.0 - 2.1	0.9 - 4.5	42	2.4	35.7	61.9
ALT (IU/l)	16 - 25	20 - 144.5	42	0	21.4	78.6
AP (IU/l)	56.5 - 72.4	58 - 397	42	0	42.9	57.1
Total protein (g/dl)	6.7 - 8.6	5.5 - 13	42	2.4	50	47.6
Albumin (g/dl)	2.3 - 4	1.1 - 3.7	42	59.5	40.5	0
Globulin (g/dl)	3 - 4.6	4.4 - 11.3	42	0	45.2	54.8
A/G ratio	0.79 - 1.33	0.11 - 1.2	42	69.1	30.9	0

revealed a highly significant increase ($p<0.01$) of AP values between the diseased groups and the control group (Table 9).

4.6.7 Serum creatinine

The mean serum creatinine value of control group was 1.15 ± 0.07 mg/dl. Statistical analysis revealed a significant increase ($p<0.01$) in the mean values of groups II and III (2.32 ± 0.19 mg/dl and 2.46 ± 0.16 mg/dl) compared to the control mean value (Table 9).

4.6.8 Serum profile of Group IV

Serum profile revealed a total serum protein : 7.6 g/dl, albumin : 3 g/dl, globulin : 4.6 g/dl, A/G ratio: 0.7, serum ALT : 68 IU/l, Serum AP : 122 IU/l and creatinine : 1 mg/dl respectively (Table 10).

4.6.9 Biochemical abnormalities

Percentage distribution of values for various biochemical parameters were calculated and the values were studied (Table 11).

The common abnormalities observed were elevated levels of serum enzymes, alanine amino transferase (78.6 per cent) and alkaline phosphatase (57.1 per cent) and serum creatinine level (61.9 per cent).

Other abnormalities observed were elevated levels for total protein (47.6 per cent) and globulin(54.8 per cent) and low values for albumin (59.5 per cent) and A/G ratio (69.1 per cent).

Table 11. Biochemical abnormalities in *Ehrlichia canis* infection

Parameter	Normal range (control group)	Affected range	Number evaluated	Percentage distribution of values		
				Low	Normal	High
Creatinine (mg/dl)	1.0 - 2.1	0.9 - 4.5	42	2.4	35.7	61.9
ALT (IU/l)	16 - 25	20 - 144.5	42	0	21.4	78.6
AP (IU/l)	56.5 - 72.4	58 - 397	42	0	42.9	57.1
Total protein (g/dl)	6.7 - 8.6	5.5 - 13	42	2.4	50	47.6
Albumin (g/dl)	2.3 - 4	1.1 - 3.7	42	59.5	40.5	0
Globulin (g/dl)	3 - 4.6	4.4 - 11.3	42	0	45.2	54.8
A/G ratio	0.79 - 1.33	0.11 - 1.2	42	69.1	30.9	0

4.7 Treatment trial with Doxycycline and Prednisolone

Thirteen animals were found to be positive for ehrlichiosis based on examination of blood/buffy coat smears. They were subjected to treatment trial for fourteen days. All the animals showed clinical cure by 14 day therapy, except for one which showed an elevated temperature even after therapy, for which the treatment was extended to one more week. Various haematological and biochemical parameters of these animals before and after therapy were compared statistically by paired t-test and the results were as follows:

4.7.1 Haematological parameters

4.7.1.1 Erythrocyte sedimentation rate (ESR)

A highly significant reduction ($p<0.01$) in the mean ESR value was observed after treatment (2.31 ± 0.33 mm/hr) compared to pretreatment mean value (6.04 ± 0.71 mm/hr) (Table 12).

4.7.1.2 Packed cell volume (PCV)

The mean PCV values that recorded before and after treatment were 33.69 ± 0.99 per cent and 45.02 ± 1.0 per cent respectively. A significant increase ($p<0.01$) could be observed after therapy (Table 12).

4.7.1.3 Haemoglobin (Hb)

Paired t-test revealed a significant increase ($P<0.01$) for Hb value after treatment (14.64 ± 0.41 g/dl) compared to the mean pretreatment value of 10.81 $\pm$ 0.33 g/dl (Table 12).

Table 12. Haematological parameters of treatment group before and after therapeutic trial

	No. of animals	ESR mm/hr	PCV (%)	Hb (g/dl)	RBC count x $10^6/mm^3$	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet count x $10^5/\mu l$	TLC x $10^3/mm^3$	Differential Leukocyte Count (%)			
											N	L	M	E
Before treatment	13	6.04 ± 0.71	33.69 ± 0.99	10.81 ± 0.33	5.00 ± 0.15	67.86 ± 2.14	21.73 ± 0.6	32.15 ± 0.63	1.23 ± 0.14	9.62 ± 0.65	66.38 ± 3.29	24.62 ± 2.87	8.31 ± 0.80	0.54 ± 0.27
After treatment	13	2.31 ± 0.33	45.02 ± 1	14.64 ± 0.41	6.82 ± 0.09	65.96 ± 1.08	21.44 ± 0.46	32.52 ± 0.54	2.34 ± 0.16	10.44 ± 0.57	69 ± 2.63	27.15 ± 2.41	2.69 ± 0.49	1.08 ± 0.33
		**	**	**	**	**	NS	NS	NS	NS	NS	NS	**	NS

** Bearing column differ significantly ($p<0.01$)
NS: Non significant

4.7.1.4 Erythrocyte count

The mean values for RBC count before and after treatment were 5.0 ± 0.15 x 10^6/mm^3 and 6.82 ± 0.09 x 10^6/mm^3 respectively. Statistical analysis showed significant difference ($p<0.01$) between them (Table 12).

4.7.1.5 Erythrocyte indices

No significant difference was observed in the values of erythrocyte indices after treatment. The mean values of MCV before and after treatment trial obtained were 67.86 ± 2.14 fl and 65.96 ± 1.08 fl respectively, that of MCH were 21.73 ± 0.6 pg and 21.44 ± 0.46 pg respectively and that of MCHC were 32.15 ± 0.63 g/dl and 32.52 ± 0.54 g/dl respectively (Table 12).

4.7.1.6 Total leukocyte count (TLC)

Mean values for TLC before and after treatment, were 9.62 ± 0.65 x 10^3/mm^3 and 10.44 ± 0.57 x 10^3/mm^3 respectively. Statistical analysis revealed no significant difference between the TLC values before and after treatment (Table 12).

4.7.1.7 Differential leukocyte count (DLC)

No significant difference was observed between the values of differential cell counts except for the monocyte count for which a significant reduction ($p<0.01$) was observed after treatment (2.69 ± 0.49 per cent) compared to the mean pretreatment value of 8.31 ± 0.8 per cent. The mean values for neutrophil, lymphocyte and eosinophil before treatment were 66.38 ± 3.29 per cent, 24.62 ± 2.87 per cent and 0.54 ± 0.27 per cent respectively.

Their corresponding mean values obtained after treatment were 69.0 ± 2.63 per cent, 27.15 ± 2.41 per cent and 1.08 ± 0.33 per cent respectively (Table 12).

4.7.1.8 Thrombocyte count

A significant increase ($p<0.01$) was observed in platelet count after treatment. The mean values before and after treatment were 1.23 ± 0.14 x $10^5/\mu l$ and 2.34 ± 0.16 x $10^5/\mu l$ respectively (Table 12).

4.7.2 Serum profile

4.7.2.1 Total protein

Significant difference ($p<0.01$) was observed between the mean total protein values before and after the treatment. The mean values before and after therapy, obtained were 9.23 ± 0.55 g/dl and 6.11 ± 0.17 g/dl respectively (Table 13).

4.7.2.2 Albumin

Before treatment, the mean albumin value obtained was 2.18 ± 0.17 g/dl. Statistical analysis showed a significant increase ($p<0.01$) in the level of albumin (3.1 ± 0.07 g/dl) after treatment (Table 13).

4.7.2.3 Globulin

Mean globulin values revealed a significant difference after treatment. Before treatment, the mean value was 7.07 ± 0.5 g/dl and that after treatment it showed a significant ($p<0.01$) decrease (3.01 ± 0.13 g/dl) (Table 13).

Table 13. Serum profile of treatment group before and after therapeutic trial

	No. of animals	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALT (IU/l)	AP (IU/l)	Creatinin (mg/dl)
Before treatment	13	9.23 ± 0.55	2.18 ± 0.17	7.07 ± 0.5	0.32 ± 0.02	65.42 ± 7.12	264.35 ± 17.54	2.38 ± 0.21
After treatment	13	6.11 ± 0.17	3.1 ± 0.07	3.01 ± 0.13	1.05 ± 0.04	25.04 ± 2.4	113.03 ± 14.34	1.78 ± 0.11
		**	**	**	**	**	**	*

* bearing column differ significantly

* - Significant ($p<0.05$)

** - Highly significant ($p< 0.01$)

4.7.2.4 A/G ratio

The pre and post treatment mean values for A/G ratio were 0.32 ± 0.02 and 1.05 ± 0.04 respectively. Analysis showed significant difference ($p<0.01$) between the values (Table 13).

4.7.2.5 Alanine amino transferase (ALT)

A significant reduction ($p<0.01$) in the mean values of ALT was observed after treatment. The pre and post treatment values for ALT were 65.42 ± 7.12 IU/l and 25.04 ± 2.4 IU/l respectively (Table 13).

4.7.2.6 Alkaline phosphatase (AP)

The mean values for AP before and after therapeutic trial were obtained as 264.35 ± 17.54 IU/l and 113.03 ± 14.34 IU/l respectively. Paired t-test revealed a significant reduction ($p<0.01$) between mean AP values (Table 13).

4.7.2.7 Serum creatinine

Statistical analysis revealed a significant reduction ($p<0.05$) in the creatinine level after treatment. The mean values before and after therapy were 2.38 ± 0.21 mg/dl and 1.78 ± 0.11 mg/dl respectively (Table 13).

Discussion

5. DISCUSSION

Canine ehrlichiosis is a disease of worldwide distribution. In India, many reports of its occurrence and prevalence studies are available. Though detailed and systematic studies on this disease have been conducted in the neighbouring state, no such study has been taken up in Kerala so far. Hence a detailed systematic study was undertaken on the diagnostic and clinicotherapeutic aspects of canine ehrlichiosis.

5.1 Epidemiology

In the present study, no significant breed, age or sex susceptibility was observed for *Ehrlichia canis* infection which concurs with the findings of Troy *et al.* (1980), Kuehn and Gaunt (1985) and Waddle and Littman (1988).

5.1.1 Breed

In the present study, the occurrence of ehrlichiosis was noticed in different breeds of dogs such as German shepherd, Great dane, Golden retriever, Dobermann pinscher, Fox terrier, Boxer and Pomeranian. Though higher proportion of positive cases were observed in German shepherd purebreds and crossbreds (61.9 per cent) compared to others, no significant difference was observed among the breeds (Fig. 1). This concurs with the earlier reports of Stephenson and Ristic (1978), Troy *et al.* (1980), Keefe *et al.* (1982), Kuehn and Gaunt (1985), Waddle and Littman (1988), Matthewman *et al.* (1993) and Harikrishnan *et al.* (2001). However, a significantly high

proportion of ehrlichiosis among German shepherd dogs was recorded by many workers (Nims *et al.,* 1971; Nyindo *et al.,* 1980; Elias, 1991; Harrus *et al.,* 1997).

This variability in breed predisposition may be related to variation in the susceptibility among different breeds to *Ehrlichia canis* infection. German shepherd dogs were described as the most susceptible breed, being more prone to the development of the severe form of the disease (Walker *et al.,* 1970; Huxsoll *et al.,* 1972; Nyindo *et al.,* 1980; Harrus *et al.,* 1997). This increased susceptibility of German shepherds is attributed to impaired immune response to infection with *Ehrlichia canis.* Studies comparing GSD with beagle dogs have indicated that a specific and nonspecific immunosuppression occurs due to *E. canis* infection in GSD. Cell mediated immunity was found to be depressed in GSD experimentally infected with *E. canis* as demonstrated by leukocyte migration inhibition test and decrease in delayed type hypersensitivity response to specific antigens (Nyindo *et al.,* 1980). Ristic and Holland (1993) opined that, GSD breeds are more susceptible to the bleeding episode and epistaxis. In a recent retrospective study, German shepherd dogs were shown to be over-represented, with a greater proportion of this breed of dog dying from the disease, while the under representation of crossbred dogs in the occurrence of canine ehrlichiosis points out to the fact that they are fairly resistant to the infection. Thirunavukkarasu *et al.* (1993) reported higher incidence of ehrlichiosis in German shepherd dogs and Spitz and stated that this may be attributed to their thick hair coat that encourages tick infestation. Stephenson

and Ristic (1978) opined that higher prevalence among German shepherd dogs may be related to sampling errors or due to their higher susceptibility to the ehrlichia infection. All these findings support the hypothesis that in German shepherd breed, the disease is more severe and life threatening, with a poorer prognosis.

5.1.2 Age

In the present study, no significant difference was observed in the proportion of positive cases among different age groups (Fig. 2). Similar findings were reported by earlier workers also. (Stephenson and Ristic, 1978; Keefe *et al.,* 1982; Kuehn and Gaunt, 1985; Price *et al.,* 1987; Waddle and Littman, 1988; Elias 1991; Harikrishnan *et al.,* 2001).

Ewing (1969) reported that pups were more susceptible to infection than adults. Baneth *et al.* (1996) observed that young dogs have a lower seroprevalence than adults. The present study revealed 17 per cent of pups being infected with ehrlichiosis. Forty per cent of the infected animals were above two years and 43 per cent of infected animals were of age between six months to two years.

5.1.3 Sex

No significant difference was observed in the occurrence of infection between males and females in the present study (Fig.3) which agree with the reports of Keefe *et al.* (1982), Kuehn and Gaunt (1985), Waddle and Littman (1988), Elias (1991), Harrus *et al.* (1997) and Harikrishnan *et al.* (2001).

However a higher occurrence of disease was reported in males than in females (Nims *et al.,* 1971) while Greig *et al.* (1996) reported a major proportion of females being infected with ehrlichiosis. In the present study males constituted 62 per cent among the total positives of ehrlichiosis and females 38 per cent.

5.2 Clinical findings

Canine ehrlichiosis is a puzzling disease as far as the clinical signs are concerned. The symptoms are quite nonspecific and variable and none of the signs can be pointed out as specific or pathognomonic. Pyle (1980) critically reviewed the clinical signs of ehrlichiosis in dogs and commented that the only consistent finding among cases of ehrlichiosis was inconsistency.

The common complaints presented in this study included elevated temperature, lymphadenomegaly, congested mucous membrane, depression and lethargy, selective appetite, weight loss, anorexia, ocular and nasal discharge, vomiting and diarrhoea. These nonspecific signs correlated well with the findings of many earlier workers. (Lewis and Huxsoll, 1977; Kuehn and Gaunt, 1985; Price *et al.*, 1987; Waddle and Littman, 1988; Greig *et al.*, 1996; Harrus *et al.*, 1997).

Lymphadenomegaly (85.7 per cent) was the predominant clinical abnormality observed in the current study. This concurs with the finding of Harrus *et al.* (1997) where 72 per cent of the dogs were showing lymphadenopathy. Lesser percentage of lymphadenomegaly was observed in other retrospective studies (Troy *et al.*, 1980; Waddle and Littman, 1988;

Woody and Hoskins, 1991). This difference noticed in the occurrence of lymphadenomegaly in different studies may be due to the fact that the dogs were presented in the clinics at different stages of the disease. Lymphadenomegaly is usually noticed during the acute phase of the disease rather than in the chronic phase. (Harrus *et al.*, 1997). Thus, it is apparent that more acute cases are included in this study. So also it is pertinent to note that certain strains of *Ehrlichia canis* may provoke a more pronounced immune response leading to a higher occurrence of lymphadenomegaly.

Other prominent clinical findings observed in the present study were fever (69 per cent) since the rectal temperature was more than 39.5°C, congested mucous membrane (61.9 per cent), pale mucosa (23.8 per cent) which may be secondary to anemia, depression (59.5 per cent), weight loss (16.7 per cent) and spleenomegaly (23.8 per cent). These clinical signs were suggestive of ehrlichiosis and generated the highest index of suspicion for ehrlichiosis (Pyle, 1980).

In the present study, panting was observed in a comparatively higher percentage (42.9 per cent) of animals which is rarely reported. However this finding correlates well with the findings of Harrus *et al.* (1997). He opined that since ehrlichiosis was mainly occurring during the hot months of the year due to increased tick activity, there was a possibility that panting exhibited by these dogs might be overlooked considering it as a physiological phenomena to combat the higher environmental temperature. Panting associated with

ehrlichiosis might be related to respiratory distress secondary to pulmonary infection which might be present in ehrlichiosis (Hildebrandt *et al.*, 1973).

Cutaneous petechiae and ecchymoses were mainly observed on the ventral aspect of the abdomen in the present study that concurred with the findings of earlier workers (Price *et al.*, 1987; Thirunavukkarasu *et al.*, 1994; Waner *et al.*, 1999). Bleeding disorders, particularly epistaxis were described as a common clinical finding in severe chronic ehrlichiosis, that too in German shepherd dogs (Troy *et al.*, 1980). In the present study, the bleeding episodes were recorded in a few dogs (28.6 per cent) that concur with the observations of Kuehn and Gaunt (1985). The bleeding abnormalities were mainly manifested as epistaxis, melena, petechiae, ecchymoses, haematuria, hematochezia and haematemesis. Harrus *et al.* (1996a) suggested that platelet dysfunction, together with thrombocytopenia might be contributing to the bleeding tendency observed in ehrlichiosis. Haemorrhagic episode even with a mild thrombocytopenia also implies a lack of vascular integrity induced by ehrlichia infection. Platelet dysfunction is assumed to be potentially induced by antiplatelet antibody interaction with the platelet membrane glycoproteins. (Codner *et al.*, 1985; Ruiz de Gopegui and Feldman, 2000).

In the present study, epistaxis was observed in three animals. Fresh blood in faeces was reported in two cases and digested blood in two animals which is an indication of gastro-intestinal bleeding. Similar findings were reported by Walker *et al.* (1970); Hildebrandt *et al.* (1973); Price *et al.* (1987) and Thirunavukkarasu *et al.* (1993). Though in many cases especially in

chronic phase of ehrlichiosis, these bleeding tendencies were met with, it did not occur in all dogs (Huxsoll *et al.*, 1970; Pyle, 1980).

Skin abnormalities were observed in twelve animals in this study, a major proportion of which were in the form of ulcerative skin lesion in between the toes, over the paw region and behind the hock joint. Alopecia was observed in two cases. Pyle (1980) opined that, the dogs with ehrlichiosis being immunodeficient, secondary bacterial infections frequently occurred in the limb that complicated the decubitus ulcers which these affected dogs were more prone to develop. Carmichael and Fiennes (1942) described two types of skin lesions associated with ehrlichiosis, the first being a circumscribed necrotic area with purplish colour at the point of attachment of the tick and the second type being an erythemato-pustular type most clearly seen in unpigmented areas of the axilla and groin. In the present study, the skin lesions resembling the second type were predominantly observed. A kind of moist dermatitis was also reported by Hildebrandt *et al.* (1973). However Price *et al.* (1987) stated that cutaneous lesions commonly encountered in dogs suffering from ehrlichiosis were never directly attributable to *Ehrlichia canis* infection.

Oedema on the limbs and scrotum were observed in 22.1 per cent cases, in the present study, as reported by earlier workers (Hildebrandt *et al.*, 1973; Cowell *et al.*, 1988; Buoro *et al.*, 1990). This may be consequent to the disruption in the vascular integrity leading to increased vascular permeability induced by ehrlichia infection or might be the result of hypoalbuminemia and

proteinuria or a combined effect of both with an ultimate outcome of oedema in the dependent parts.

Ocular and nasal discharge was observed in a total of 33 animals, in the present study. Bilateral mucopurulent ocular and nasal discharge was recorded by Ewing and Buckner (1965).

A single case of arthritis involving several joints was observed in the current study as reported by previous workers (Bellah *et al.*, 1986; Cowell *et al.*, 1988; Thilagar *et al.*, 1990). This might be arising from immune mediated complications associated with ehrlichiosis. Harrus *et al.* (2001) demonstrated the presence of immune complexes in sera of dogs naturally and experimentally infected with *Ehrlichia canis*. They opined that some manifestations documented in canine monocytic ehrlichiosis, such as glomerulonephritis, polyarthritis and uveitis were immune complex mediated.

Spleenomegaly was observed in ten animals in this study which is infrequently reported by many workers (Pyle, 1980; Reardon and Pierce, 1981; Neer, 1998). Spleenomegaly in acute ehrlichiosis was noncongestive and caused by an increase in nonerythrocytic cellularity resulting from diffuse proliferation of lymphoreticular cells in the white pulp and reticuloendothelial cells of the red pulp. This type of spleenomegaly may result in significant nonspecific pooling or sequestration of erythrocytes and platelets. The effects of spleenomegaly and follicular haemorrhage, undoubtedly have a contributory role to the total loss of platelets as seen in acute ehrlichiosis. Thus spleen plays

a major role in the pathogenesis of canine monocytic ehrlichiosis (Reardon and Pierce, 1981; Harrus *et al.*, 1999).

Gastro-intestinal disorders such as vomiting and diarrhoea were noticed in 25 animals in this study and it agrees with the findings of earlier workers (Ewing and Buckner, 1965; Huxsoll *et al.*, 1972; Harrus *et al.*, 1997). So also 21.4 per cent of infected animals showed dehydration which was assessed by skin turgor to be six per cent or more loss of body water.

A history of ectoparasitic infestation was recorded in 27 animals (64.3 per cent) in the present study which points to the possibility of other ectoparasites also acting as vectors for the transmission of this disease as pointed out by Troy *et al.* (1980).

A wide variation observed in clinical signs may be attributed to variation in the virulence of strain, immune status of the host, presence of concurrent infection, stage of the disease, breed etc. that makes the diagnosis difficult (Harvey *et al.*, 1979). This wide variety of symptoms exhibited by dogs suffering from ehrlichiosis may occur either singly or in combination in different breeds of dogs that offer a challenge to the present day practising veterinarians. A German shepherd dog with tick infestation, anaemia, congested mucous membrane, epistaxis, fever and lymphadenopathy may be diagnosed as a case of ehrlichiosis with little experience. On the other hand, a dog that is exhibiting depression, mild weight loss and border line anaemia presents a diagnostic challenge to even an experienced clinician because there

are many other diseases showing these signs. Hence uniformity of clinical signs exhibited by a dog suffering from ehrlichiosis is not a factor to be relied upon in the symptomatic diagnosis of the disease condition. Frequently a practising veterinarian finds empirical therapy against ehrlichiosis and the response of the dogs to treatment, as means for establishing the diagnosis. However it is important that a confirmatory diagnosis, based upon laboratory results is a must and these can help in predicting the prognosis of infection.

5.3 Blood smear examination/Buffy coat smear examination

Examination of blood smear/buffy coat smear for the presence of cytoplasmic inclusions namely initial body or morula could be considered as a conclusive evidence for the presence of this infectious organism and was diagnostic (Waner *et al.*, 1999). Many reports of ehrlichiosis diagnosed by this method alone or in combination with others were available substantiating the significance of this method of diagnosis (Bellah *et al.*, 1986; Elias, 1991; Stockham *et al.*, 1992; Egenvall *et al.*, 1997; Meneses, 1997).

In the present study, 25 per cent of animals revealed inclusion bodies in the cytoplasm of leukocytes, most of them being observed in lymphocytes, some in the neutrophils and few in the monocytes. Greig (2000) opined that morulae were frequently observed in circulation (granulocytes) during granulocytic ehrlichiosis and in contrast were rarely observed in monocytes during monocytic ehrlichiosis. They were in the form of elementary bodies, initial bodies or morulae stained bluish pink or dark purple coloured with

Giemsa stain. The inclusion bodies observed were identical to those described by Simpson (1972). Elias (1991) found that 88 per cent of cases to be positive for ehrlichiosis, based upon the examination of blood smear whereas Meneses (1997) could get only 68.8 per cent of cases to be positive by this technique. But Woody and Hoskins (1991) opined that only 4 per cent of blood smears of dogs with ehrlichiosis had detectable organisms in blood cells making it an unreliable method for diagnosis. Similar observations were also noted by Harikrishnan *et al.* (2001) wherein only 3.8 per cent of cases were found to be positive by this technique.

Ewing (1969) opined that examination of blood smear though confirmatory, was not a reliable technique in the diagnosis of canine ehrlichiosis because of the low parasitemia within the peripheral blood that demanded careful examination of the stained smears by an experienced technician for a long time. Malherbe (1947) recommended the use of first drop of blood that oozed out from a shallow incision or prick in the ear for diagnostic purposes. Ewing (1963) emphasized the searching of the feathered end of stained blood smears, to get the best results. Elias (1991) reported that the inclusion bodies were exclusively observed in acute phase of the disease and that it was observed more frequently in GSD dogs than other breeds. In the present study, no difference was observed among the breeds in the occurrence of inclusion bodies. However, the more number of cases diagnosed as positive were German shepherd's (62.5 per cent). It is mainly due to the fact that

German shepherd dogs are more prone to the disease and more cases that are presented in the clinics suffering from the disease belong to this breed.

Matthewman *et al.* (1993) based on a study on ehrlichiosis carried out at Zimbabwe, reported that, no inclusion bodies could be detected in the blood smears of dogs with serological evidence of infection. However, in the present study, out of 42 animals which were positive by fluorescent antibody test, 15 animals revealed inclusion bodies in the blood smear (35.7 per cent).

Absence of inclusion bodies did not indicate that the animal was free of infection because they could be easily demonstrated in peripheral smears only during the febrile phase of the disease and was difficult to obtain in the chronic or subclinical phase (Ristic *et al.*, 1972; Lewis and Huxsoll, 1977). The reason was attributed to the fact that in persistent infection (subclinical or chronic phase), the number of circulating infectious units of *Ehrlichia canis* could be too low to be detected or viable organisms were harboured in host tissues rather than in blood (Buhles *et al.*, 1974).

It has also been noticed that the appearance of organism in the peripheral leukocytes varied considerably with animals (Manohar and Ramakrishnan, 1984; Egenvall *et al.*, 1997) which also makes the examination of blood smear, a less reliable diagnostic technique. However reports were there, where typical intracytoplasmic inclusions were observed in peripheral blood smears 16-21 days, post exposure, in experimental studies (Buckner and Ewing, 1967; Ewing *et al.*, 1971).

In spite of all these drawbacks, diagnosis based on microscopic demonstration of intracytoplasmic inclusions especially in acute phase remains to be an easy and simple technique that does not demand any sophisticated equipments and it can be performed in any laboratory. According to Greig (2000), the most rapid method for diagnosing ehrlichiosis was finding the intra cytoplasmic morulae within infected circulating leukocytes.

5.3.1 Concurrent Infections

Though canine ehrlichiosis is a distinct disease entity, concurrent infections are likely to occur because of the presence of common vectors involved in the transmission of diseases. The present study revealed two cases of concurrent infections with haemoparasite, *Hepatozoon canis* along with *Ehrlichia canis*. Concurrent infections with *Hepatozoon canis* had been reported by many previous workers. (Donatien and Lestoquard, 1935; Tresamol *et al.*, 1995a; Harikrishnan *et al.*, 2001; Ramprabhu *et al.*, 2001). The pathogenicity of *Hepatozoon canis* alone is still questionable since gametocytes were commonly found in asymptomatic dogs (Baneth *et al.*, 1995). Many opined that hepatozoonosis was not pathogenic, unless the infection was overwhelming or the dog was immunosuppressed or had concurrent infection that triggered the onset of clinical disease. (Ezeokoli *et al.*, 1983; Gosset *et al.*, 1985; Baneth *et al.*, 1995; Macintire *et al.*, 1997).

The clinical manifestations of the two cases, in this study, were mainly associated with ehrlichiosis. This confirms the hypothesis that the concomitant

occurrence of both the haemoparasites might have either increased the susceptibility or promoted the flaring up of other organism. Gosset *et al.* (1985) endorsed this view by stating that the altered immunity consequent to ehrlichiosis might predispose a dog to *Hepatozoon canis* infection or allow manifestation of a subclinical infection.

5.4 Indirect Fluorescent Antibody (IFA) Test

Indirect fluorescent antibody test is regarded as the main stay for diagnosing ehrlichial infections. It is still considered the gold standard test for diagnosing ehrlichiosis, in spite of its inherent drawbacks.

Indirect fluorescent antibody test was employed in the present study which revealed 65.6 per cent (42 animals out of 64) seropositive reaction among the dogs presented with signs suggestive of ehrlichiosis. Similar results were reported by Tresamol *et al.* (1994) and Greig *et al.* (1996) whereas a lower percentage of seropositivity was also reported by Egenvall *et al.* (1997) and Magnarelli *et al.* (1997), which were 13.1 and 9.4 per cent respectively.

In the present study, a higher percentage of seropositive reaction noticed might be due to the fact that the animals brought to the hospital that were showing symptoms suggestive of ehrlichiosis were included in this study. This high percentage also points to the fact that, the organism persists in our environment and indicates the applicability of this test in epidemiological investigations to detect the magnitude and extent of infection prevailing among the dog population in the state. The applicability of this test for the same

purpose had been emphasized by Stephenson and Ristic (1978) and Baneth *et al.* (1996).

The indirect fluorescent antibody test is specific and highly sensitive and is applicable to diagnosis of natural as well as experimentally induced ehrlichiosis. Ristic *et al.* (1972) opined that positive reaction by IFA test could be considered as active persistent infection since successful transmission of the infection from the reactors to susceptible dogs occurred. The reactors may also be considered as positive cases of infection, because the humoral antibody response does not prevent persistent infection and once infected, the animals may remain as carriers of the disease (Buhles *et al.*, 1974; Harvey *et al.*, 1979; French and Harvey,1983). Clinically asymptomatic dogs that were showing high antibody titres to *Ehrlichia canis* were in the subclinical or carrier state of the disease which might ultimately develop to the chronic form of the disease (Codner and Farris-Smith, 1986; Waner *et al.*, 1997). This finding assumes increased importance due to the fact that it is the chronic form of the disease that is more severe and fatal. However, Harrus *et al.* (1998b) documented that IFA test results could not be taken as a reliable indicator of the carrier state because dogs having anti-ehrlichia antibodies might not carry the parasite.

The early appearance of serum antibodies was recorded at seven days after experimental inoculation which were of the IgM and IgA classes. The transition of the IgM class to IgG occurred approximately 14 days after inoculation. Thereafter, this subclass persisted extensively even at 60 days after inoculation (Weisiger *et al.*, 1975). The persistence of antibody might be

attributed to a committed B-cell response to chronic antigenic stimulation by the infective organism and indicating a prolonged duration of infection (Matus *et al.*, 1987). These antibodies of any subclass originating in the course of infection did not seem to be associated with protection against reinfection. Also, this anti-ehrlichia antibody is supposed to play a role in the maintenance of the long term carrier (Weisiger *et al.*, 1975).

In the present study, one animal confirmed positive for ehrlichiosis by blood smear examination, did not show seropositive reaction during IFA test. A similar finding was reported by Tresamol (1992), wherein 15 animals that were positive by blood smear examination failed to give a positive reaction by IFA test. This might be attributed to low titre in the early stage of acute infection when the sero conversion had just started that could not be detected by IFA test. There are reports substantiating this finding. Greig *et al.* (1996) could find only 75 per cent seropositivity in a group of dogs having ehrlichiosis, which turned to 100 per cent when they were tested during the convalescence period. Egenvall *et al.* (1997) commented that seroconversion could occur before, during or after the appearance of inclusions. Dogs in which inclusions could be found might or might not have already seroconverted and hence a rise in titre might be expressed inconsistently. Couto (2000) suggested that antibody titres might be negative or negligible during the acute phase because it takes upto three weeks to develop a significant titre. This is an important disadvantage when IFA test is performed in the early phase of infection.

The serum reaction was considered positive if they showed a specific fluorescence at a minimum serum dilution of 1:10 (Ristic *et al.*, 1972). The same serum dilution was used, in the present study.

A very low titre below 1:10 has been reported during the terminal phase of the disease which could have been due to reduced immunological responsiveness apparently caused by a total exhaustion of the bone marrow as revealed by histopathological examination (Ristic *et al.*, 1972). This finding assumes increased importance due to the fact that a reliable diagnosis cannot be expected for animals in terminal chronic phase of ehrlichiosis by IFA test.

Recently several inherent drawbacks of IFA test have been discussed and depicted. Identification of four-fold seroconversion to the appropriate ehrlichia species is retrospective and hence IFA test serves as a poor tool for therapeutic decision making in acute illness (Greig, 2000). It is not useful for assessing clearance of *E. canis* after antibiotic therapy since dogs remain IFA positive for a long period of time after eliminating the organism (Matus *et al.*, 1987; Waner *et al.*, 2001). A single seropositive test is not diagnostic because healthy dogs may remain seropositive in endemic areas due to multiple exposure to ehrlichia species. In addition, closely related ehrlichiae may induce cross reactive antibodies that pose a potential problem with respect to the specificity of the IFA test (Baneth *et al.*, 1996; Wen *et al.*, 1997; Greig, 2000; Waner *et al.*, 2001). The possibility of multiple tick borne infections can further complicate the credibility of IFA test in detecting *E. canis* infection.

In spite of the above drawbacks, it is still advisable to rely on IFA test to gauge the success or failure of treatment of canine ehrlichiosis. A decline in *E. canis* antibody titres is an indication of successful outcome of the treatment (Waner *et al.*, 2001).

To conclude, the use of IFA test is an important aid in confirming the exposure to *Ehrlichia canis* when equipments for more sophisticated techniques like Western blotting and PCR are not available. Hence an IFA test should be used in combination with collection of complete history, physical examination of the dog, assessment of clinical signs and the results should be interpreted cautiously.

5.5 Haematological findings

5.5.1 Haemogram

The principal haematological abnormalities observed in 42 dogs which were found to be positive for ehrlichiosis, included anaemia, thrombocytopenia and infrequent leukopenia/leukocytosis and monocytosis.

Anaemia was observed as one of the most frequent findings, in the present study, with low haemoglobin values (66.7 per cent), packed cell volume (61.9 per cent) and RBC count (61.9 percent). Similar findings were recorded by many previous workers (Huxsoll *et al.*, 1970; Nims *et al.*, 1971; Buhles *et al.*, 1974; Kuehn and Gaunt, 1985; Price *et al.*, 1987; Waddle and Littman, 1988; Meneses, 1995; Harrus *et al.*, 1997). The anaemia observed was normocytic and normochromic since the erythrocyte indices calculated for

different groups of dogs, in this study, were within the reference range. Same observations were quoted by Waddle and Littman (1988) and Buoro *et al.* (1990). Pyle (1980) attributed the anaemia in ehrlichiosis to the reduced rate of erythropoiesis. This may be due to the apparent decrease in the erythrocytic compartment, lack of reticulocytosis and normal values for the mean corpuscular volume. According to Cotter (2000), anaemia associated with ehrlichiosis is usually nonregenerative as indicated by normocytic and normochromic anaemia, unless it is associated with haemorrhage due to thrombocytopenia. The marrow may be hypercellular with an increased myeloid erythroid ratio in the acute phase, but the chronic phase is characterized by pancytopenia and hypoplasia of the marrow except for plasmacytosis. Non-regenerative anaemia can also result from immune-mediated mechanism, one of the factors contributing to the pathogenesis of canine monocytic ehrlichiosis. The bone marrow may show erythroid hypoplasia or evidence of erythrophagocytosis.

Hildebrandt *et al.* (1973) opined that the prolonged anaemia might be primarily a consequence of the suppressed bone marrow activity. The lack of extramedullary haematopoiesis in spleen and other organs even in dogs with prolonged anaemia might be indicative of a generalized erythropoietic suppressive factor.

Ewing and Buckner (1965) depicted a grave illness in dogs accompanied by severe anaemia of normocytic, normochromic type. They attributed this severity to the concurrent occurrence of two infections *Babesia canis* and

Ehrlichia sp. Babesia contribute to haemolysis and in ehrlichiosis, there suppression of erythropoiesis which makes the anaemia to be longstanding.

Generalized bonemarrow hypoplasia affecting the production of all the three cell lines i.e. the erythroid, myeloid and megakaryocytic cells leading to aplastic anaemia is associated with chronic ehrlichiosis (Buhles *et al.*, 1974; Stephenson *et al.* 1975; Woody and Hoskins, 1991; Rikihisa *et al.*, 1992). With aplastic anaemia less than 25 per cent of the marrow is composed of haematopoietic cells, primarily lymphocytes and plasma cells and the rest is replaced with fat. In ehrlichiosis, the most prominent contributory factor to aplastic anaemia is nothing but the immune-mediated pathogenic mechanism. Affected animals are at risk for bacterial sepsis or secondary bacterial complications from granulocytopenia or bleeding from thrombocytopenia (Cotter, 2000). In the present study one such case of aplastic anaemia with very low values for PCV (16.8 per cent), Hb (4.5 g/dl), RBC count (1.3 x 10^6/mm^3), total WBC count (1.8 x 10^3/mm^3), and thrombocyte count 60,000/µl was reported. Bone marrow biopsy carried out in that particular case conclusively proved the occurrence of aplastic anaemia.

In the present study, two dogs positive for ehrlichiosis, showed microcytic hypochromic anaemia changes that might be suggestive of iron deficiency caused by chronic blood loss. This type of anaemia is consistent with chronic rather than acute external haemorrhage (Rogers, 2000). Such type of anaemia associated with ehrlichiosis, was recorded by earlier workers (Kuehn and Gaunt, 1985; Tresamol, 1992; Matthewman *et al.*, 1993). Harvey

et al. (1982) reported a rare occurrence of ehrlichiosis in dogs with iron deficiency anaemia. Rogers (2000) also opined that this type of anaemia might be consequent to altered iron metabolism rather than its absolute deficiency. The suppression of erythropoiesis might be arising from the relative unavailability of iron owing to its sequestration in the reticuloendothelial system particularly in liver and bone marrow. However Kuehn and Gaunt (1985) could not find any increased amounts of iron in bone marrow on majority of the marrow smears taken from dogs infected with *Ehrlichia canis.*

Conversely, eight dogs showed macrocytic normochromic anaemia, in this study. This may be due to increased activity of the bonemarrow in some conditions usually associated with normocytic anaemia (Benjamin, 1985). Macrocytic anaemia was also reported by Walker *et al.* (1970) in pancytopenic dogs.

In the present study 4.8 per cent of the affected dogs showed PCV and Hb values above the reference range and 11.9 per cent of the affected animals showed polycythemia. This might be consequent to hypovolemia resulting from dehydration and haemoconcentration.

Erythrocyte Sedimentation Rate (ESR)

Though ESR observed in dogs of various groups were within the normal range, in the present study, a significant change in ESR was observed between the control group (1.17 ± 0.31 mm/hr) and infected groups II and III (5.9 ± 0.65 mm/hr and 7.24 ± 0.71 mm/hr respectively). This significant increase in the

ESR in the infected group may be either arising from anaemia in which ESR is accelerated due to small number of cells that can settle more easily in large volume of the fluid or due to alterations in plasma proteins. Increased globulin level increases ESR whereas ESR is inversely related to albumin level (Benjamin, 1985). Since anaemia, hypoalbuminemia and hyperglobulinemia are frequent findings associated with ehrlichiosis, a relative increase in the sedimentation rate of erythrocytes can be naturally expected in ehrlichiosis and is reported by many earlier workers (Huxsoll *et al.*, 1970; Tresamol, 1992; Ristic and Holland, 1993).

5.5.2 Leucogram

5.5.2.1 Total leukocyte count

Total leukocyte count was within the normal range in a majority of *Ehrlichia canis* positive animals (62.9 per cent). Leukopenia was shown by 19 per cent of infected animals whereas 19 per cent of positive cases showed leukocytosis. Also there was no significant difference between the mean total leukocyte counts of the control group ($10.15 \pm 0.61 \times 10^3/mm^3$) and the infected groups II and III ($9.63 \pm 0.58 \times 10^3/mm^3$ and $9.3 \pm 0.49 \times 10^3/mm^3$ respectively).

Normal leukocyte counts in majority of the animals in the present study concur with the reports of Madewell and Gribble (1982) and Tresamol *et al.* (1995a).

Though leukopenia was not a frequent finding in the current study, it has been described as a common haematological abnormality in ehrlichiosis. (Buhles *et al.*, 1974; Pyle, 1980; Reardon and Pierce, 1981; Ristic and Holland, 1993; Harrus *et al.*, 1997; Neer, 1998). Waddle and Littman (1988) reported a lesser per cent of cases associated with leukopenia (22 per cent) in a retrospective study on ehrlichiosis.

Leukocytosis has been reported as a frequent finding in *Ehrlichia canis* infection by Spurling (1977), but the lower percentage (19 per cent) of leukocytosis observed in the present study is in agreement with the findings of Harrus *et al.* (1997).

5.5.2.2 Differential leukocyte counts

In the present study a significant difference was observed only in the mean values of monocyte count of the control and infected groups. (2.0 ± 0.26 per cent for the control group; 8.4 ± 0.75 per cent and 7.56 ± 0.51 per cent for infected groups). Relative monocytosis was observed in 78.6 per cent of cases affected with *Ehrlichia canis* in the current study. Though monocytosis is associated with chronic inflammatory diseases, it can also occur within hours as an early change in the same diseases that cause neutrophilia. In this study, though neutrophilia was observed only in 28.6 per cent cases, monocytosis could be observed in a greater proportion of cases. This may be due to the fact that monocytes have a shorter marrow transit time than neutrophils, allowing monocyte responses to occur much earlier than neutrophilia. This is commonly

noticed in diseases with depletion of marrow reserves or in cases of injury to marrow precursors (Kociba, 2000). Monocytosis is also reported to occur in association with acute stress reactions in dog (Coles, 1986).

Monocytosis associated with *Ehrlichia canis* infection has been reported by many previous workers (Carmichael and Fiennes, 1942; Mudaliyar, 1944; Ewing, 1969; Cowell *et al.*, 1988; Thirunavukkarasu *et al.*, 1994; Harrus *et al.*, 1997). No significant difference was observed among the mean differential counts of neutrophils, lymphocytes and eosinophils in this study.

Lymphocytosis has been reported as a rare finding in acute ehrlichiosis (Codner and Farris-Smith, 1986). In the present study, only 16.7 per cent showed lymphocytosis whereas in 73.8 per cent cases the differential lymphocyte count were within the normal range. This points to the possibility of more acute cases of ehrlichia being presented to the clinics.

One animal in the present study showed a much higher percentage of lymphocytes (60 per cent) compared to others. Absolute lymphocytosis observed in canine ehrlichiosis has been attributed to chronic antigenic stimulation persistent during subclinical and chronic phases of the disease. (Codner and Farris-Smith, 1986; Weiser *et al.*, 1991). Mild lymphocytosis was reported in chronic infectious disease, hypersensitivity reactions or immunologically mediated diseases but profound lymphocytosis has been found associated with ehrlichiosis in acute and subclinical phases of the disease (Breitschwerdt, 2000). Granular lymphocytosis (azurophilic granulation in

majority of blood lymphocytes) has been observed with *Ehrlichia canis* infection. The granularity observed in the cytoplasm was typical of well differentiated lymphocytic leukaemia (Weiser *et al.*, 1991).

Lymphopenia was reported only in 9.5 per cent cases in the present study. This may also be due to the stress associated with the disease. Stress is a factor that produces a moderate to marked decrease in lymphocytes (Coles, 1986). Lymphopenia as observed in this study was reported by previous workers. (Kuehn and Gaunt, 1985; Waddle and Littman, 1988; Harrus *et al.*, 1997).

In the present study, 59.5 per cent of infected animals revealed neutrophil count within the normal range. Neutrophilia was observed in 28.6 per cent of cases and neutropenia was observed in 14.3 per cent of infected animals.

Lesser proportion of neutrophilic leukocyte response in the present study may be indicating that major proportion of cases of ehrlichiosis being in the mild acute phase of the disease or may be reflecting the self-limiting tendency of this disease especially noticed in immuno-competent animals. Neutrophilia has also been reported by Harrus *et al.* (1997). A normal leukogram has been reported by Waner *et al.* (1997) in subclinical phase of infection.

In this study, one case revealed the neutrophil differential count as very low (40 per cent) with a corresponding increase in lymphocyte count (60 per cent). This was diagnosed as a case of aplastic anaemia associated with

ehrlichiosis. According to Cotter (2000), with aplastic anaemia, fat will be replacing a major proportion of haematopoietic cells (>75 per cent) and the remaining cells will be predominantly represented by lymphocytes and plasma cells. This may be contributing to the relative increase in the differential lymphocyte count in circulation and a corresponding sharp decline in the granulocyte count as observed in this particular case. Thus neutropenia and in general, granulocytopenia may be regarded as a very important predisposing factor for secondary bacterial infection to take upper hand in chronic phase of ehrlichiosis. Mild neutropenia has been reported during the subclinical phase of ehrlichiosis by Codner and Farris-Smith (1986) and in acute phase of infection by Harrus *et al.* (1997).

Eosinopenia as observed in 59.5 per cent of infected animals in this study was in agreement with the reports of Kuehn and Gaunt (1985) and Waddle and Littman (1988). Eosinopenia or complete disappearance may be associated with any stress condition (Coles, 1986).

Eosinophilia has been observed in 7.2 per cent of infected animals in the present study. Coles (1986) opined that a relative increase in eosinophils may be observed in the recovery stages of some acute infections. This is usually a reappearance of eosinophils following the eosinopenia that accompanies stress associated with the more acute stages of the disease. Eosinophilia has also been reported by Harrus *et al.* (1997) in a retrospective study on 100 cases of natural canine monocytic ehrlichiosis.

5.5.3 Thrombocyte count

Canine ehrlichiosis is a disease caused by ehrlichia species that affects platelet number and function. Majority of the animals (76.5 per cent) in this study were having thrombocytopenia whereas for 19 per cent of infected animals, thrombocyte count was within the normal range. The thrombocyte count in the control group and infected groups were $3.67 \pm 0.11 \times 10^5/\mu l$, $1.18 \pm 0.13 \times 10^5/\mu l$ and $1.30 \pm 0.10 \times 10^5/\mu l$ respectively.

Thrombocytopenia was considered to be the most salient and consistent haematological abnormality of dogs naturally or experimentally infected with *Ehrlichia canis* (Hildebrandt *et al.*, 1973; Smith *et al.*, 1975; Stephenson *et al.*, 1975; Codner and Farris-Smith, 1986; Waddle and Littman, 1988; Eng and Giles, 1989; Koutinas *et al.*, 1989; Matthewman *et al.*,1993; Waner *et al.*, 1997; Egenvall *et al.*, 1997; Kuffer-Frank *et al.*, 1999).

The mechanism of thrombocytopenia depends on the stage of the disease. In acute phase of the disease, the mechanism involved in the pathogenesis of thrombocytopenia include increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets and immunologic destruction resulting in a significantly decreased platelet life span (Pierce *et al.*, 1977; Smith *et al.*, 1975; Pyle, 1980). Thrombocytopenia is also attributed to qualitative and quantitative deficit in platelet function as evidenced by reduced adhesiveness of platelets due to antiplatelet antibody, plasma inhibiting factor or a direct effect of

Ehrlichia canis on circulating platelets (Lovering *et al.*, 1980). Those dogs in which altered platelet function was demonstrated had decreased release of platelet factor III and decreased platelet adhesiveness. Platelet factor III is a membrane phospholipid with procoagulant activity which is released following aggregation or lysis of platelets. The platelet defects were attributed to the platelet membrane being coated by macroglobulins (Kuehn and Gaunt, 1985).

In an experimental study on acute ehrlichiosis, Harrus *et al.* (1996a) found that platelet aggregation was significantly inhibited in majority of the infected dogs and that a significant increase occurred in the preaggregation lag time. They concluded that platelet dysfunction was equally responsible for bleeding episodes as that of absolute thrombocytopenia. In addition, a platelet migration inhibition factor was proposed to play a major role in enhancing platelet sequestration and stages leading to thrombocytopenia.

Demonstration of serum platelet bindable antiplatelet antibodies in dogs after experimental infection with *Ehrlichia canis* supports the assumption that immune destruction may also contribute to the pathogenesis of thrombocytopenia in acute ehrlichiosis (Waner *et al.*, 1995; Harrus *et al.*, 1996b). *Ehrlichia canis* infection might have altered the immune system resulting in the over production of natural antiplatelet antibodies.

In severe chronic phase of the disease, decreased platelet production due to bone marrow hypoplasia is considered to be the reason for thrombocytopenia (Woody and Hoskins, 1991; Rikihisa *et al.*, 1992).

Though Davoust *et al.* (1996) noticed 100 per cent thrombocytopenia in acute ehrlichiosis, in the current study only 19 per cent of animals had normal thrombocyte count and even 4.8 per cent of positive cases had thrombocytosis. The thrombocyte count of the affected animals showed a wide range of 60,000-4,00,000/µl. The lack of thrombocytopenia observed in some affected dogs might be due to the difference in platelet destruction. This can be attributed to the virulence of strains, immune status of the host, clinical phase of the disease at the time of collection of sample and the difference in breed also.

Thrombocytopenia and haemorrhage have been considered the hallmarks of ehrlichiosis, but may not be prominent during the acute and subclinical phase (Pyle, 1980). Harrus *et al.* (1996a) suggested that platelet dysfunction that may occur in the acute stage of canine monocytic ehrlichiosis, together with thrombocytopenia might be contributing to the bleeding tendency observed in ehrlichiosis.

Haemorrhagic episodes like epistaxis (frequently unilateral), cutaneous or mucosal petechiae, melena, ecchymoses, haematochezia, haematemesis and haematuria may occur during the acute phase of ehrlichiosis. These symptoms may become more evident or more severe during chronic phase of disease. The lower percentage of bleeding episodes, observed in the present study, points out to the fact that most of the animals have been presented in the acute phase of the disease.

Stephenson and Ristic (1978) opined that epistaxis/gastrointestinal bleeding may occur when the platelet count decreases below 20,000/µl whereas petechiae or ecchymoses are likely to develop when the platelet count reaches below 40,000/µl. In the present study, bleeding disorders were noticed in 12 infected animals, among which majority had a thrombocyte count within the range of 60,000-80,000/µl. However, among four animals that exhibited epistaxis, one had only mild thrombocytopenia and one had even normal thrombocyte count (2,20,000/µl). This normal count of thrombocytes in animals with haemorrhagic tendencies may be attributed to the qualitative platelet defects occurring in *E. canis* infection (Kuehn and Gaunt, 1985). Another school of thought is that, haemorrhagic episode even with a mild thrombocytopenia implies a lack of vascular integrity or platelet dysfunction potentially induced by antiplatelet antibody interaction with integrins, the platelet membrane glycoproteins (Codner *et al.*, 1985; Ruiz de Gopegui and Feldman, 2000). Evidence for vasculitis caused by *Ehrlichia canis*, was emphasized by identifying the morulae of *Ehrlichia canis* in endothelial cells of the lungs and prominent perivascular accumulations of plasma cells detected in many tissues (Huxsoll *et al.*, 1972; Hildebrandt *et al.*, 1973). This wide discrepancy in the correlation between thrombocytopenia and bleeding tendency may also be attributed to difference in the strain, breed, immune status of the host and stage of the disease.

Platelet count is a good screening test to identify different stages of ehrlichiosis in dogs, particularly those in the subclinical phase of infection.

Based on a retrospective study on 100 cases of canine monocytic ehrlichiosis, Waner *et al.* (1997) stated that, for judging possible subclinical infection, the most reliable haematological parameter appeared to be a state of mild thrombocytopenia and stressed the importance of its evaluation especially in the subclinical phase as an influential prognostic factor.

5.6 Biochemical analysis

Abnormalities revealed from the serum biochemical profile of infected animals, in the present study, included elevated levels of serum creatinine, serum alanine aminotransferase, serum alkaline phosphatase, serum globulins and low levels of serum albumin and decreased A/G ratio. Similar findings were recorded by previous workers (Kuehn and Gaunt, 1985; Waddle and Littman, 1988; Ristic and Holland, 1993; Meneses, 1995; Harrus *et al.*, 1996; Harrus *et al.*, 1997; Neer, 1998).

5.6.1 Serum total protein

Fifty per cent of infected animals had the serum protein level within the reference range whereas 47.6 per cent showed hyperproteinemia and only 2.4 per cent of positive cases showed hypoproteinemia. Here, the protein values of affected animals showed a wide range varying from 5.5-13 g/dl. Among the 47.6 per cent that showed hyperproteinemia, a few cases were characterized by very high protein level exceeding 8 g/dl. One animal that revealed the highest protein value (13 g/dl), had a corresponding total globulin level of 11.3 g/dl and albumin level of 1.7 g/dl. It had a corresponding PCV value of 38 per cent with

erythrocyte indices within the normal range indicating normocytic normochromic non-regenerative anaemia. This concurs with the findings of Michels *et al.* (1995) who reported a case of chronic ehrlichiosis with total protein value of 11.0 g/dl and a corresponding globulin level of 7.9 g/dl, albumin level of 3.1 g/dl and a PCV of 34 per cent. Weiser *et al.* (1991) reported a case of chronic ehrlichiosis wherein the total protein value was 14.7 g/dl, total globulin 12.4 g/dl and albumin 2.3 g/dl. They correlated this hyperproteinemia with hypergammaglobulinemia and observed that monoclonal gammopathy occurred in a disproportionate number of dogs with ehrlichiosis compared to other infectious diseases. Hoskins *et al.* (1983) reported a serum hyperviscosity syndrome associated with *Ehrlichia canis* infection in a Lhasa apso dog which had a total protein content of 9 g/dl, serum albumin 2.3 g/dl and serum globulin 6.7 g/dl which indicated a gammopathy. In the present study a few positive cases also showed total protein in excess of 8 g/dl. Hyperproteinemia with serum protein concentrations exceeding 8 g/dl has been reported in ehrlichiosis especially in the chronic phase of the disease (Pyle, 1980; Hoskins *et al.*, 1983; Price *et al.*, 1987; Weiser *et al.*, 1991; Michels *et al.*, 1995; Harrus *et al.*, 1997). One of these cases that showed a higher range had PCV of 50.2 per cent and RBC count 7.28 x 10^6/mm^3. These values suggest that the animal may be under dehydration and this hypovolemic state seem to result in relative increase in the total protein value.

Harrus *et al.* (1996) attributed the hyperproteinemia associated with ehrlichiosis to the higher gamma globulin concentrations. According to him,

the reverse also is true, ie., a significant decrease in total protein is associated with significantly low concentration of gamma globulins, noticed mainly in pancytopenic dogs. Weisiger *et al.* (1975) viewed that the gamma globulin response found during the first seven days after infection with *Ehrlichia canis* is IgM and IgA, thereafter the IgG concentration will gradually increase and cause hyperproteinemia.

5.6.2 Serum albumin

Hypoalbuminemia was observed in majority of (60 per cent) positive cases as observed in previous reports (Kuehn and Gaunt, 1985; Price *et al.*, 1987; Waddle and Littman, 1988; Ristic and Holland, 1993; Harrus *et al.*, 1996; Harrus *et al.*, 1997).

The affected range of serum albumin in positive cases was between 1.7 to 3.7 g/dl. The hypoalbuminemia noticed in all stages of canine ehrlichiosis may be attributed to factors like anorexia and related reduction in protein uptake, peripheral loss to oedematous inflammatory fluids as a result of increased vascular permeability and consequent to vasculitis (Woody and Hoskins,1991), decreased protein production due to concurrent mild liver affections (Reardon and Pierce, 1981) or may be due to the minimal change glomerulopathy (Codner *et al.*, 1992). Another school of thought is that the hypoalbuminemia occurs as a compensatory mechanism for the hyperglobulinemic state for maintaining the oncotic pressure thereby preventing an increase in the viscosity of blood (Woody and Hoskins, 1991).

The lowest extreme value for albumin 1.7 g/dl was noticed with a corresponding highest globulin value of 11.3 g/dl, in one case of this study.

5.6.3 Serum globulins

In the present study 54.8 per cent of positive cases, revealed hyperglobulinemia from the serum profile. The affected range was 4.4-11.3 g/dl. A few cases revealed much higher value for globulin, though the same has been reported by Weiser *et al.* (1991) and Michels *et al.* (1995). Hypergammaglobulinemia in canine monocytic ehrlichiosis is usually polyclonal. Monoclonal gammopathy is rarely observed resulting in hyperviscosity and associated clinical symptoms (Hoskins *et al.*, 1983; Michels *et al.*, 1995; Harrus *et al.*, 1996).

According to Ristic and Holland (1993) gammaglobulins are major contributors of hyperglobulinemia. The gammaglobulin concentration increases during the febrile phase of canine ehrlichiosis and persists during the subclinical and chronic phase of the disease. In the present study, five animals showed very high values for total globulins (between 9-11.3 g/dl). Kuehn and Gaunt (1985) reported high globulin content of 9.3 g/dl in dogs suffering from ehrlichiosis. Similar findings were reported by Matus *et al.* (1987), Weiser *et al.* (1991) and Michels *et al.* (1995). The immune mediated pathogenesis involved with ehrlichiosis may be the probable reason for this higher value. The persistence of higher values might be attributed to a committed B-cell response to chronic antigenic stimulation by the infective organism and

indicating a prolonged duration of infection. These immunoglobulins of any subclass originating in the course of infection did not seem to be associated with protection against reinfection (Weisiger *et al.*, 1975; Reardon and Pierce, 1981; Harrus *et al.*, 1996b). This poor correlation between these two parameters and the poloyclonal gammopathy recorded in most sick dogs, suggest that, nonspecific antibody production is induced by *Ehrlichia canis* and that the anti-*Ehrlichia canis* antibodies are not the main source of gamma globulins contributing to the hyper gammaglobulinemia. This phenomenon suggests an exaggerated immune response to occur in ehrlichiosis with inadequate efficacy (Reardon and Pierce, 1981). The above three cases thus might be indicating a prolonged duration of illness.

$Alpha_2$ and $beta_2$ globulin levels were also found to increase in dogs infected with *Ehrlichia canis*. This increase may be due to the consequence of tissue damage and inflammation. Though lower concentration of globulins has not been reported in this study, the same was observed by Harrus *et al.* (1996) in the pancytopenic group of dogs naturally infected with *Ehrlichia canis*. This may be consequent to the pronounced leukopenia in these dogs.

5.6.4 Albumin/Globulin ratio (A/G ratio)

A significant reduction in A/G ratio was observed in 69.1 per cent of positive cases in the present study. Similar findings were reported by previous workers (Burghen *et al.*, 1971; Kuehn and Gaunt, 1985; Waddle and Littman, 1988; Harrus *et al.*, 1996).

Decreased A/G ratio is noticed when there is a relative or absolute increase in globulins and/or a decrease in albumin which is generally associated with chronic inflammation and antigenic stimulation (Benjamin, 1985), which can very well be correlated with ehrlichiosis.

5.6.5 Serum alanine amino transferase (ALT)

Elevated levels of serum ALT was observed in 78.6 per cent of positive cases. This is in agreement with the reports of earlier workers (Kuehn and Gaunt, 1985; Waddle and Littman, 1988; Thirunavukkarasu *et al.*, 1994; Meneses, 1995; Harrus *et al.*, 1997).

Increased ALT in the dog is specific for hepatic disease. As this enzyme is present in large quantities in the cytoplasm of hepatocytes, ALT is increased in serum when cellular degeneration or destruction occurs in this organ (Coles, 1986).

In the present study, the range of ALT values in the infected animals varied between 20-144.5 IU/l. A higher proportion of positive cases (78.6 per cent) showing marked rise in ALT, is indicating pathologic changes in hepatic parenchyma associated with ehrlichiosis. Focally distributed groups of degenerated/necrosed hepatocytes, extensive infiltration of mononuclear cells in many portal areas along with hypertrophied Kupffer cells had been reported in dogs with experimentally induced ehrlichiosis (Ewing, 1969). Reardon and Pierce (1981) noticed that an increase in serum ALT activity occurred simultaneously with the development of many expanding foci of

reticuloendothelial cells in the hepatic sinusoids which compressed and injured the adjacent hepatocytes leading to necrosis of these cells. However, they observed in a study on acute experimental ehrlichiosis that, this hepatic injury was sufficient to increase the levels of ALT in serum, but might persist only for a short period of time (14-21 days). This explains why the serum enzyme abnormalities may not be noticed in all cases of ehrlichiosis. In this study, 21.4 per cent of infected animals showed ALT within the reference range.

5.6.6 Serum alkaline phosphatase (AP)

Increased levels of serum AP were noticed in 57.1 per cent of infected animals that concurred with the previous reports (Kuehn and Gaunt, 1985; Waddle and Littman, 1988; Ristic and Holland, 1993; Meneses, 1995; Harrus *et al.*, 1997; Neer, 1998).

In dogs, the serum alkaline phosphatase level can be utilized as an indicator of hepatic malfunction. The elevation of liver alkaline phosphatase in canine serum is associated with active pathology in that organ. Acute hepatocellular necrosis results in minimal increase in serum alkaline phosphatase though ALT may be dramatically increased (Coles, 1986). This explains why a lower proportion among the positive cases (57.1 per cent) showed elevated levels of AP than those of ALT (78.6 per cent). Estimation of serum AP thus can be used as an adjunct to serum ALT estimation in detecting the liver damage.

5.6.7 Serum creatinine

In the present study elevated levels of serum creatinine were observed in 61.9 per cent of infected animals, though many cases revealed a marginal increase. The range of serum creatinine values in the infected animals varied from 0.9 to 4.5 mg/dl. High blood urea values were recorded by Walker *et al.* (1970) and Price *et al.* (1987). High serum creatinine values were earlier recorded by Kuehn and Gaunt (1985); Waddle and Littman (1988) and Harrus *et al.* (1997).

Though a major proportion of cases in the present study showed marginal increase in creatinine (2-3 mg/dl) that might be related to pre renal causes such as hypovolemia as opined by Kuehn and Gaunt (1985) and Waddle and Littman (1988). There were infected cases that revealed higher creatinine values that ranged between 3-4.5 mg/dl which could be related to renal factors.

Serum or plasma creatinine concentration was used as an index of retention of nitrogenous wastes by the kidneys (Finco, 1997). The assessment of serum creatinine level over BUN, bears an added advantage that creatinine production is not as easily influenced by catabolic factors as affecting urea formation. Therefore conditions such as fever, toxemia, dehydration, infection and drug administration do not readily influence creatinine levels.

Early stages of progressive kidney disease are accompanied by minor changes in creatinine levels though damage to a major portion of renal parenchyma occurs. As the disease advances, a stage is usually reached at

which destruction of a small number of nephrons may be accompanied by a large fluctuation in creatinine concentration. A rapid fluctuation in creatinine level is also influenced by the rate of progress in disease. Thus in acute renal infections, change in creatinine level occurs rapidly than in chronic diseases where renal parenchyma may be destroyed before variations are reflected in creatinine concentration (Coles, 1986). Thus it is apparent that more acute cases are presented in this study.

There are many reports suggesting the pathological involvement of renal parenchyma in ehrlichiosis leading to azotemia that can be reflected as high creatinine level. Hildebrandt *et al.* (1973) observed subcapsular and focal haemorrhages near corticomedullary junction in kidneys of 42 dogs affected with ehrlichiosis. Histologically, these affected kidneys revealed prominent plasmacytosis around glomeruli and interstitium. Codner *et al.* (1992) investigated pathogenesis of proteinuria in six dogs experimentally infected with *Ehrlichia canis* during the acute phase of the disease. Though glomerular lesions were minimal, marked deposition of immunoglobulins (IgG and IgM classes) could be observed in glomerular tufts. Harrus *et al.* (2001) detected circulating immune complexes in sera of dogs naturally and experimentally infected with *Ehrlichia canis*. According to them a longer exposure to *Ehrlichia canis* might result in the development of immune complex mediated glomerulonephritis. Breitschwerdt (2000) stated that chronic antigenic stimulation by *Ehrlichia canis* might induce immune complex

glomerulonephritis. A membraneous glomerulonephritis had been reported by Troy *et al.* (1980) in ehrlichiosis.

Taking into account the fact that, the azotemia might also be related to prerenal factors, interpretation of creatinine values must be made carefully especially when the creatinine values were obtained with a minimal increase.

5.7 Treatment trial

Sixteen animals were confirmed positive for ehrlichiosis by observing morulae or inclusions in the blood or buffy coat smears and were subjected to treatment trial for fourteen days. Doxycycline was administered @ 5 mg/kg body weight orally once daily for fourteen days and prednisolone was administered at the initial dose rate of 1 mg/kg body orally followed by a tapering dose @ 0.5 mg/kg body weight towards the end of treatment trial. Three animals did not turn up, after fourteen days of trial. Response to treatment in the rest of thirteen animals, was assessed by remission of clinical signs, absence of intracytoplasmic inclusions in the leukocytes, improvement of abnormalities (haematological as well as biochemical) in the laboratory findings.

The clinical response after treatment trial, in the present study, was encouraging. All the animals subjected to therapeutic trial showed dramatic clinical improvement with the remission of clinical signs. Only one animal showed slightly elevated temperature (103.8°F) and slight inappetence even after 14 days, for which the treatment was extended to one more week which

had an uneventful recovery. This hundred per cent rate of recovery after treatment with doxycycline or in combination with glucocorticoid therapy had been reported by the previous workers (Cowell *et al.*, 1988; Maretzki *et al.*, 1994; Egenvall *et al.*, 1997; Jain and Gupta, 1997; Breitschwardt *et al.*, 1998).

According to Ristic and Holland (1993), among all the therapeutics tested, tetracyclines are the most effective for treatment of *Ehrlichia canis* and other ehrlichial infections of dogs. Among these, doxycycline is considered as the treatment of choice now a days @ 10 mg/kg body weight orally once daily for a period of three weeks (Harrus *et al.*, 1997; Couto, 2000). In the present study, the infected dogs were treated with doxycycline @ 5 mg/kg body weight orally once daily for fourteen days and apparent recovery was obtained more or less rapidly. A similar result with use of doxycycline at the same rate for same duration was reported by Egenvall *et al.* (1997). This rapid recovery might be indicating that the dogs under trial were only mild or moderately ill. In this study, the choice of doxycycline among the tetracyclines has had an added advantage that the dogs under trial, need not be presented to the clinics every day, since it can be administered at home orally and also the drug was cost effective compared to its injectable counterparts.

Doxycycline successfully eliminates the infection since it restores phagosome lysosome fusion probably by inhibiting a protein secreted by the ehrlichial organism which hinders fusion (Waner *et al.*, 2001).

Short-term prednisolone therapy was also instituted in this study that suppressed the exaggerated immune response which was partially responsible for thrombocytopenia. It may also be helpful in the treatment of other immune-mediated conditions associated with ehrlichiosis such as polyarthritis, vasculitis and meningitis (Neer, 1998). Corticosteroids may improve vascular integrity or platelet function by blocking the dog's immune reaction to *Ehrlichia canis* (Codner *et al.*, 1985). Matus *et al.* (1987) instituted prednisolone therapy to suppress the monoclonal immunoglobulin response and to eliminate the causative organism. They found that within a short span of time treatment response occurred and there existed a correlation between the remission of clinical signs and reduction in immunoglobulin concentration. Short-term administration of anti-inflammatory and immunosuppressive drugs thus may help to relieve from secondary immune mediated complications, characteristic of ehrlichia infection (Ristic and Holland, 1993).

Though many reports are there supporting the efficacy of doxycycline, some reports give evidence for the carrier status of the dogs experimentally infected with *Ehrlichia canis* even after treatment with doxycycline that questions the efficacy, dose and duration of doxycycline therapy in dogs with *Ehrlichia canis* infection (Iqbal and Rikihisa, 1994a; Harrus *et al.*, 1998b). Harrus *et al.* (1998a) evaluated the efficacy of doxycycline treatment in eliminating *Ehrlichia canis* from four subclinically infected dogs. Of these, one dog was found to be positive by PCR after 42 days of treatment suggesting that even six weeks of doxycycline treatment may not be sufficient to clear

Ehrlichia canis from all subclinically infected dogs. The resistance of this organism against doxycycline even in natural infection was reported by Wen *et al.* (1997). The difference in response to treatment between different studies may be related to the stage of the disease, the time at which the treatment is started and the difference in ehrlichia species.

It is suggested that doxycycline can fairly eliminate the ehrlichia infection provided the therapy is given in the acute phase of the infection itself. The earlier the treatment is initiated in the disease process, the more favourable is the prognosis (Price *et al.*, 1987; Breitschwerdt *et al.*, 1998; Neer, 1998; Breitschwerdt, 2000). Dramatic improvement generally occurs within 24-48 hours of initiation of therapy (Neer, 1998). In the present study also, most of the infected animals showed clinical improvement within one to two days, or at the most within a week as reported. The disease, on the contrary, is found to be refractory to treatment, once it enters into the chronic phase of the disease. However, in mild chronic cases of ehrlichiosis fairly fast clinical improvement occurs, though it takes more time for the haematological recovery to occur (Breitschwerdt, 2000).

The response to treatment was found varying with the species. *Ehrlichia canis* and *Ehrlichia chaffeensis* may not be eliminated by doxycycline therapy under certain circumstances, whereas therapeutic elimination of *Ehrlichia ewingii* and *Ehrlichia equi* is possible with more certainty (Breitschwerdt *et al.*, 1998).

In the present study, the response to treatment was in addition assessed by haematological and biochemical evaluation after 14 days. Significant change was observed in the values of haemoglobin, PCV and RBC count before and after therapy. Haematological improvement to therapy in the acute phase of infection was reported by earlier workers (Price *et al.*, 1987; Parthasarathy *et al.*, 1989; Neer, 1998). Mild thrombocytopenia observed in the infected dogs in this study, was relieved after therapy that was evident from the significant change observed between the mean pre and post treatment values ($1.23 \pm 0.14 \times 10^5/\mu l$ and $2.34 \pm 0.16 \times 10^5/\mu l$). Similar results were recorded earlier (Ristic and Holland, 1993; Neer, 1998). Total leukocyte and differential cell counts did not reveal any significant change after treatment except for the monocyte count which showed a significant reduction after treatment (from 8.31 ± 0.8 per cent to 2.69 ± 0.49 per cent).

The serum profile, in the present study revealed significant change in the values of total protein, albumin, globulin, A/G ratio, serum ALT, AP and creatinine after treatment. These values after treatment reflected apparent recovery from the abnormalities in laboratory findings. Price *et al.* (1987) viewed that acute kidney damage was more easily treated with tetracycline and BUN levels returned to reference range with a simultaneous regression of clinical signs whereas the therapy was found unsuccessful for treating chronic kidney damage.

It was apparent from the above findings that, all the dogs under trial might have been presented during the acute phase of infection and that the treatment started in time ensured complete recovery from the disease clinically as well as with respect to the data pertaining to the haematological and biochemical findings.

Summary

6. SUMMARY

The present study is mainly focused on the clinico-therapeutic and diagnostic aspects of ehrlichiosis in dogs in and around Thrissur and also to evaluate the haematological and biochemical abnormalities in the affected dogs.

A total of sixty four animals brought to the University Veterinary Hospitals at Kokkalai and Mannuthy showing symptoms suggestive of ehrlichiosis were included in the study. The control group comprised of six apparently healthy normal animals. A detailed signalment, history, physical data and clinical symptoms of each suspected case were recorded as per the proforma.

Diagnosis of the disease was made based on two techniques. First, by examination of the blood/buffy coat smears taken for the detection of inclusion body or morulae; secondly by the indirect fluorescent antibody test, the gold standard test, for the detection of *Ehrlichia canis* antibodies.

Altogether five groups were categorized based on the two diagnostic tests.

Group I	-	Healthy controls
Group II	-	Positive by blood smear examination and IFA test
Group III	-	Positive by IFA test and negative by blood smear examination
Group IV	-	Positive by blood smear examination and negative by IFA test.
Group V	-	Negative by blood smear examination and IFA test as well

Various haematological and biochemical parameters estimated included erythrocyte sedimentation rate, haemoglobin, packed cell volume, total erythrocyte count, erythrocyte indices, total and differential leukocyte counts, thrombocyte count, serum total protein, albumin, globulin, A/G ratio, serum ALT, serum AP and serum creatinine.

On examination of Giemsa-stained blood smears from suspected cases, inclusion bodies were observed in the cytoplasm of leukocytes of 16 animals (25 per cent). Two cases of concurrent infections, with blood protozoan, *Hepatozoon canis* were also detected by blood smear examination, along with *Ehrlichia canis* infection.

Indirect fluorescent antibody test, which is considered as the gold standard test was employed for the detection of antibodies. A total of 42 animals out of 64 were found to be positive by IFA test. Positive reaction in the test was observed by a specific fluorescence of the *Ehrlichia canis* morulae or inclusion body in the cytoplasm of monocytes.

No age, sex or breed predisposition for *Ehrlichia canis* infection was observed in this study.

Clinical signs were quite non-specific. The most frequent signs noticed in positive cases included fever, lymphadenomegaly, selective appetite/anorexia, depression and lethargy and congested mucous membrane.

Bleeding abnormalities were observed in few cases (28.57 per cent) in the form of epistaxis, melena, haematochezia, haematemesis, ecchymoses of skin.

Skin lesions were observed in 23.81 per cent of cases, mostly observed as oozing wounds in between the paws.

Oedema of limb and scrotum, weight loss, spleenomegaly, hepatomegaly, cough, alopecia and arthritis were encountered as infrequent findings in this study.

Ectoparasite infestation was observed in 64.3 per cent of animals, mostly by ticks.

The most frequent haematological abnormalities noticed were anaemia and thrombocytopenia. Significant low values for haemoglobin, packed cell volume and erythrocyte count were observed in Group II and III compared to Group I.

No significant difference between the control and infected groups was observed in the total leukocyte count and the differential cell counts except for the monocyte count for which a significantly higher value was obtained for the infected groups.

Thrombocytopenia was evident in both the infected groups. A significant difference in the mean ESR values was observed between the control and infected groups.

Most important serum chemistry abnormalities observed in the infected group included elevated levels of serum creatinine, serum ALT, AP, total protein and globulin and low values for albumin and A/G ratio.

Treatment trial with doxycycline @ 5 mg/kg body weight once daily for 14 days orally and prednisolone with initial dose of 1 mg/kg body weight followed by 0.5 mg/kg body weight, gave promising result. All the thirteen animals, subjected to therapeutic trial showed remission of clinical signs and most of the abnormalities in laboratory findings within 14 days of therapy except for one case for which the treatment was extended to one more week. A significant difference was observed for most of the haematological and biochemical parameters evaluated before and after treatment.

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CLINICO - THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS

By

SMITHA. J. P.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The present study was undertaken to get a detailed understanding on clinico-therapeutic, haematological and biochemical aspects of ehrlichiosis in dogs. A total of 64 animals showing symptoms suggestive of the disease were taken as the study group. Diagnosis was based on examination of blood/buffy coat smear and by IFA test as well. Examination of blood/buffy coat smear revealed *Ehrlichia canis* morulae in the cytoplasm of leukocytes in 16 animals. IFA test, detected antibodies in 42 animals. No age, sex or breed predilection for the disease was noticed. Most frequent clinical signs observed were fever, anorexia/selective appetite, lymphadenomegaly, depression and congested mucous membrane. Haematological abnormalities mainly encountered included normocytic normochromic anaemia, thrombocytopenia and monocytosis. Serum biochemical abnormalities observed were elevated levels of serum ALT, AP, total protein, globulin, creatinine and low levels for albumin and lower values for A/G ratio. A therapeutic trial with doxycycline and prednisolone gave encouraging clinical response and good uneventful recovery. The results of the present study confirm that ehrlichiosis is a disease that is prevalent in our area and treatment with doxycycline can be considered as the therapy of choice for ehrlichiosis in dogs.

Appendix

CLINICO – THERAPEUTIC STUDIES ON CANINE EHRLICHIOSIS

Sl. No..... Case No. ..... Date: .....

Name : .....

Address : ..... Phone No. : .....

.....

..... e-mail : .....

Details of the animal

Breed: ..... Colour: ..... Age: ..... Sex: M/F Identification marks: .....

History Past

Present

Presence of ectoparasites : **Past** ☐ Yes ☐ No. **Present** ☐ Yes ☐ No.

If present

Infestation	Heavy	Medium	Low
Ticks			
Fleas			
Lice			

Home confinement

Inside home	Tied outdoors	Free to roam outdoors

Vaccination status

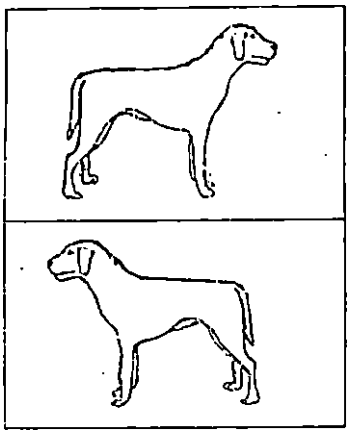
Rabies	CD	CAV_1	CAV_2	Parvo	Pi	Leptospirosis

Food : Type Frequency

Clinical signs

	Yes	No		Yes	No
Fever	☐	☐	Mucosal petechiae/ecchymoses	☐	☐
Anorexia	☐	☐	Haematochezia	☐	☐
Pale mucous membrane	☐	☐	Haematuria	☐	☐
Depression and lethargy	☐	☐	Haematemesis	☐	☐
Panting	☐	☐	Hyphema	☐	☐
Vomiting	☐	☐	Ocular discharge	☐	☐
Spleenomegaly	☐	☐	Diarrhoea	☐	☐
Dehydration	☐	☐	Lameness	☐	☐
Bleeding episodes			Icterus	☐	☐
Epistaxis	☐	☐	Ataxia	☐	☐
Cutaneous petechiae/ecchymoses	☐	☐	Nasal discharge	☐	☐
Melena	☐	☐	Circling/Head tilt	☐	☐
Vaginal/penile bleeding	☐	☐	.....	☐	☐

Lymphadenomegaly *Popliteal* ..... *Prescapular* .....



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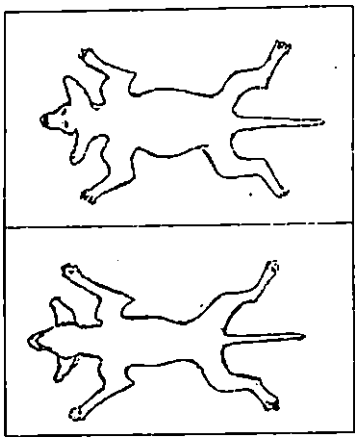

Date of onset of clinical signs: ..... Date of presentation: .....

Clinical data

Temperature	Pulse	Respiration	Mucous membrane	F/s examination

Clinical parameters (Haematological)

	RBC $x10^{12}/l$	WBC $x10^{9}/l$	Hb g/dl	PCV %	Platelets $x10^{9}/l$	DLC				
						N	L	M	E	B
0^{th} day										
7^{th} day										
15^{th} day										

Erythrocyte Indices

MCH	MCV	MCHC

Biochemical

Total protein	Albumin	Globulin	A/G ratio	Serum ALT	Serum AST	Serum AP	Serum creatinine	BUN	Serum CK

Treatment	Dose	Route	Duration	Interval	Result
Doxycycline					
Prednisolone					
Supportive if any					

Cost of treatment Medicine Others