"EPIDEMIOLOGY OF BLOOD PROTOZOAN PARASITES OF DOGS WITH SPECIAL REFERENCE TO PATHOGENESIS, DIAGNOSIS AND CONTROL OF CANINE EHRLICHIOSIS"

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

PUSA (SAMASTIPUR), BIHAR

In partial fulfilment of the requirements

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

(Veterinary Parasitology)

By

Rinki Kumari

Registration No. - M/V. Para/12/2005-2006.

DEPARTMENT OF VETERINARY PARASITOLOGY

BIHAR VETERINARY COLLEGE

PATNA - 800 014

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CERTIFICATE-I

This is to certify that the thesis entitled ***"Epidemiology of Blood Protozoan Parasites of Dogs with Special Reference to Pathogenesis, Diagnosis and Control of Canine Ehrlichiosis"*** submitted in partial fulfilment of the requirements for the award of Master of Veterinary Science **(Veterinary Parasitology)** of the faculty of post-graduate studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar is the record of bonafide research work carried out by **Dr. Rinki Kumari, Registration No.- M/V. Para/12/2005-2006,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

25/5/2007

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Major Advisor

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CERTIFICATE –II

We the undersigned members of the Advisory Committee of **Dr. Rinki Kumari, Registration No.- M/V. Para/12/ 2005-2006,** a candidate for the Degree of Master of **Veterinary Science** with Major in **Veterinary Parasitology,** have gone through the manuscript of the thesis and agree that the thesis entitled ***"Epidemiology of Blood Protozoan Parasites of Dogs with Special Reference to Pathogenesis, Diagnosis and Control of Canine Ehrlichiosis"*** may be submitted by **Dr. Rinki Kumari** in partial fulfilment of the requirements for the degree.

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CERTIFICATE-III

This is to certify that the thesis entitled ***"Epidemiology of Blood Protozoan Parasites of Dogs with Special Reference to Pathogenesis, Diagnosis and Control of Canine Ehrlichiosis"*** submitted by **Dr. Rinki Kumari, Registration No.- M/V. Para/12/2005-2006,** in partial fulfilment of the requirements for the Degree of Master of Veterinary Science **(Veterinary Parasitology)** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar was examined and approved on 03../..10../2007.

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Dedicated

to my

beloved

Parents

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Rinki Kumari

Date : 25/5/2007

Place : Patna

(Rinki Kumari)

CONTENTS

****

LIST OF TABLES

TABLE NO.	DESCRIPTION	PAGE NO.

TABLE NO.	DESCRIPTION	PAGE NO.

****

LIST OF GRAPHS

****

LIST OF PHOTOGRAPHS

****

CHAPTER - I

INTRODUCTION

INTRODUCTION

The dog was certainly the first domesticated animal as was evident from the remnants among the refuse heaps of Paleolithic hunting races. The experience of man with dogs states that this animal is not only pet but it is very useful, faithful and reliable companion.

Our history suggests that the prime utility of dogs in the past was to guard the dwellings of man, protecting household articles from thieves and as night guard against criminals. But in the recent times as we come to know about its wonderful power of smell, it is being used as sniffers to detect and catch the criminals, explosives, narcotics etc. Dogs are also being used as messengers in defense services.

All these qualities of this dumb, faithful friend of human being have increased many fold the responsibilities of veterinarians to protect and treat the ailments of this wonderful friend of human being and out of those ailments parasitic infestations are of prime importance.

There are numerous parasites of dogs which are of major concern for the veterinary profession, however, amongst them blood protozoan parasite constitute special attention as they are widely distributed and may terminate to be fatal if not diagnosed and treated properly. From the available literature, it is evident that the major blood protozoa of canine are

Trypanosoma spp., *Leishmania* spp., *Hepatozoon* spp., *Babesia* spp., *Haemobartonella* spp. and *Ehrlichia* spp.

Canine ehrlichiosis is a tick borne, potentially fatal and enigmatic rickettsial disease caused by an obligate intracellular bacteria of genus *Ehrlichia* of family Rickettsiaceae. Of the several species of *Ehrlichia* (*E. canis*, *E. risticii*, *E. platys* and *E. ewingii*) which can infect dogs naturally, *E. canis* is the most common parasite causing severe clinical illness.

Canine ehrlichiosis is also known as Tropical Canine Pancytopenia, Canine Rickettsiosis, Canine Haemorrhagic Fever, Tracker's Dog Disease, Canine Tick Typhus, Nairobi Bleeding Disease and Idiopathic Haemorrhagic Syndrome.

Donatien and Lestoquard (1935 in Algeria) and Mudaliar (1944 in Madras) were the first to report *E. canis* in the world and in India respectively.

Canine Ehrlichiosis was observed as early as 1967 in British military dogs in Singapore and in the same year, the disease occurred in several Labrador Retrievers trained as tracker's dog and shipped from a British training centre in Malaysia to U.S. Forces in the Republic of Vietnam. In 1968, an epizootic of the disease occurred in Vietnam among German shephered dogs that had originated in the United States. To date, approximately 180 U.S. military dogs have died of TCP (Tropical Canine Pancytopenia) in Southeast Asia.

Canine Ehrlichiosis is a rickettsial disease transmitted by the Brown Dog Tick, *Rhipicephalus sanguineus* (Groves *et al.*, 1975) and possibly the American Dog Tick, *Dermacentor variabilis* (Johnson *et al.*, 1998). Flea and mosquitoes has also been recorded as possible vectors (Troy *et al.*, 1980) Distribution of disease is mainly related to distribution of Vectors (Keefe *et al.*, 1982).

Species of *Ehrlichia* seem to influence severity and manifestations of the disease in the host. *Ehrlichia canis* that infects mononuclear cells, is the classical pathogen associated with severe form of canine ehrlichiosis. *E. ewingii*, a granulocytic *Ehrlichia* (Ewing *et al.*, 1971) exhibit a mild syndrome like polyarthritis in dogs, while infectious thrombocytopenia caused by *E. platys* that infect platelets has been documented (French and Harvey, 1983).

There are various forms exhibited in case of Canine Ehrlichiosis such as acute, subacute and chronic (Van Heerden, 1982) which exhibit wide range of inconsistent clinical manifestations (Huxsoll *et al.*, 1970). It is an important disease with the symptoms of pyrexia, anaemia, vomition, diarrhoea, epistaxis, weakness and lethargy (Kuehn and Gaunt, 1985).

Ehrlichia spp. lives and reproduces in white blood cells but it has particularly devastating effect on the lymphatic system, so called AIDS of the canine world (Ibulaimu, 1996). It affects multiple system organs such as Respiratory,

Circulatory, CNS, Kidney, Liver, Spleen, Endothelium of capillaries etc. Severe depression of immune system enhances the complications due to secondary bacterial infections.

Initially bone marrow hypercellularily is noticed in acute phase : later in the chronic phase; hypoplasia of almost all bone marrow elements leading to pancytopenia is observed (Woody and Hoskin, 1991). Clinical signs of ehrlichiosis resolves without treatment in most cases, with dogs entering the subclinical phase which is often asymptomatic and this is predominant form of Ehrlichiosis too. The dogs that do not successfully eliminate the parasite during subclinical phase may proceed to chronic phase.

Haemorrhagic syndrome in chronic ehrlichiosis in some breeds of dog such as German shepherd is life threatening and improper management leads to fatality. Hemostats currently in use are of little value because of severe thrombocytopenia. In severe cases, epistaxis occur because of thrombocytopenia or due to functional defects of platelets.

Corneal opacity is occasional sign in chronic phase (Harrus *et al.*, 1998a) and opthalmological examination revealed retinal haemorrhage and enlargement of optic disc (Thirunavukkarasu *et al.*, 1994b).

Thrombocytopenia, leucopenia, hypoalbuminemia and hyperglobulinemia are more common in chronic phase whereas leukocytosis, monocytosis and variable thrombocyte count are more common in acute phase of ehrlichiosis. These

variations create diagnostic confusion and make the diagnosis a difficult task in the field. So it is also called 'Silent Killer'.

Though identification of morulae in peripheral blood smear is diagnostic but their transient nature and lower number in blood smear and makes the diagnostic efforts unrewarding. Examination of buffy coat smear seems to be more appropriate method of diagnosis (Greene and Harvey, 1990) under field conditions. In Giemsa stained buffy coat smear, morule and inclusion bodies are important aid in diagnosis of Canine Ehrlichiosis (Elias, 1992).

Concurrent infections of *Ehrlichia* sp. and *Hepatozoon canis* (Smitha *et al.*, 2003) or *Babesia canis* (Matthewman *et al.*, 1993) or *Babesia gibsoni* (Varshney and Dey, 1998) or *Leptospira* spp. (Nambi *et al.*, 2000) have also been documented indicating the possibilities of concurrent infection with other parasites that are not transmitted by Brown Dog Tick which added to clinical complications and treatment failures.

Till now most of the literature regarding Canine Ehrlichiosis is available from Southern states only, and in the Northern parts and in Eastern region in particular, the relevant information is scanty. So there is a need to study in detail the prevalence, clinical diagnosis and therapeutic aspect of this malady.

Unlike all the parasitic infections of dogs, blood protozoan are comparatively difficult to diagnose and treat.

This is because most of the other parasities of canine can be controlled easily with the use of routine deworming and proper antibiotic therapy whenever needed. However that is not in the case of blood protozoan as they remain unaffected by treatment with regular deworming and most of the antibiotic drugs.

From the available literature, it appears that systematic large scale clinical studies on Canine Ehrlichiosis have not attracted enough attention of veterinary clinical scientists particularly in North India. In view of global prevalence of the disease throughout the world and increased traffic of the dogs to different places, it is likely that this ailment may be reported with more frequency in time to come.

Therefore, keeping in view of the above mentioned facts, the present research problem has been undertaken with the following objectives :

(i) To study the epidemiological status of the blood protozoan diseases in dogs.

(ii) To study the clinico-pathological alterations in the host due to Ehrlichiosis.

(iii) To study the comparative efficacy of commonly used drugs against febrile/infectious disease.

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CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Canine Ehrlichiosis was first recognized in 1935 in ticks infested dogs in Algeria by Donatein and Lestoquard. The causative agent was named *Rickettsia canis*. Later on the disease followed more or less similar pattern in other parts of the world. In India the disease was reported by Mudaliar (1944) from Madras for the first time. Subsequently in 1958, Raghavachari and Reddy reported prevalence of *Rickettsia canis* in dogs in Hyderabad. They found that there was severe monocytosis in the dogs suffering from the disease. Ewing (1963) reported the disease in United States in Okalahoma state and morulae of *Ehrlichia canis* were identified in monocytes of infected dogs. In 1967, Wilkins *et al.* described the disease in British military dogs in Singapore. After that in 1968, a highly fatal haemorrhagic disease occurred among military dogs used by United States troops during Vietnam war in South east Asia. The disease was characterized by various clinical manifestations and leading to death of 180 sentry dogs. The disease was named as Idiopathic Haemorrhagic Syndrome (Walker *et al.,* 1970). In 1971, Ewing first time recognized the causative agent of canine granulocytic ehrlichiosis associated with several ailments of dog and the first report of *Ehrlichia platys* as a pathogen of infectious cyclic thrombocytopenia in dogs was given by Harvey *et al.,* (1978) in Florida.

Work Done in India :

Manohar and Ramakrishnan (1982) studied the prevalence of canine ehrlichiosis in Madras. They observed that 2.6 percent of 3684 dogs in the city were infected with *Ehrlichia* parasite.

While reporting the prevalence of *Babesia canis* and *Hepatozoon canis* in pet as well as street dogs from different parts of Haryana and Delhi, Bansal *et al.* (1985) found 5.4 and 2.7 percent dogs to be positive for *Babesia canis* and *Hepatozoon canis* infection respectively. They also encountered mixed infection of *Babesia canis* and *Hepatozoon canis*. They further observed that the prevalence of infection to be more during July to October in street dogs and October to December in dogs brought to the clinics.

Bhaskarao *et al.* (1986) reported the prevalence of haemoprotozoan infection in animals of Andhra Pradesh of which they revealed 13 cases of canine babesiosis, 50 cases of canine trypanosomiasis, while four cases of *Hepatozoon canis* infection.

An unusual case of ehrlichiosis associated with polyarthritis in a dog and its treatment was reported by Thilagar *et al.* (1990). The dog (6 months old Boxer) had acute lameness and pyrexia (105.8^{0}F). Examination of blood smear revealed *Ehrlichia canis* in the monocytes. The dog was successfully treated parentrally with Oxytetracycline at the

dose rate of 20 mg/kg body weight at 8 hours intervals for a week.

While reporting the incidence of *Ehrlichia canis* and *Hepatozoon canis* in naturally infected dogs from Punjab, Juyal *et al.* (1992) observed the occurrence of *Ehrlichia canis* in German shepherd dog first time along with incidence of *Hepatozoon canis* in Cross bred dogs.

In a survey, incidence of canine ehrlichiosis based on the examination blood and buffy coat smear of Thirunavukkarasu *et al.* (1993) examined 5714 dogs between 6 months to 11 years age group and found that 64 (1.12%) were positive for *Ehrlichia canis* with higher prevalence in pedigreed dogs (73.44%) than in the dogs of mixed breed (26.54%). German shepherd and Spitz breed were highly affected. Further they observed higher frequency of infection below 1 year and between 1-5 years age along with highest occurrence of infection in male dogs than in females.

While reporting the prevalence of haemoprotozoans in the domestic animals in Punjab, Juyal *et al.* (1994) found 30 cases were positive for blood protozoans out of 281 dogs examined. They further observed that 18 dogs (6.4%) were encountered for *Babesia gibsoni*, 7 (2.49%) for *Babesia canis*, 4 (1.42%) for *Hepatozoon canis* and 1 (0.35%) for *Ehrlichia canis* infection.

Latha and Joseph (1994) reported the incidence of *Hepatozoon canis* infection in dogs of Madras city. They recorded higher incidence in Exotics and Cross breeds, Males

and in the age group of 1 to 5 years of dog with rare concurrent infection of *Babesia canis* and *Ehrlichia canis.*

While reporting the observations on the occurrence of surra in cattle, buffalo and dogs in Orissa by Sahoo *et al.* (1997), found that 23 dogs harboured *Trypanosoma evansi* in their blood stream.

Das *et al.* (1998) from Pondicherry observed that 6.81 per cent dogs were infected with *Trypanosoma evansi*, 2.27 per cent dogs positive for *Haemobartonella canis* and 2.22 per cent dogs for *Ehrlichia canis* infection.

Incidence of *Trypanosoma evansi* infection in a male hunting dog of two years age was reported by Sharma *et al.* (1998) from Mathura.

Harikrishnan *et al.* (2001a) reported the prevalence of canine ehrlichiosis in Chennai. They examined 6273 dogs by peripheral blood smear examination and revealed 240 dogs positive for *Ehrlichia canis* where in 180 dogs were purebreds (Pomeranian - 6.32%, German shepherd - 5.8% and Mongrels-60 cases). They also found non-significant differences between sex, age and seasonal incidence though the disease was higher during summer. Further they observed concurrent infections with *Hepatozoon canis* in 10 dogs and *Babesia canis* in 9 dogs.

The same study in Chennai was done by Chandrasekar *et al.* (2002). They reported that the most common breeds affected were non-descript (24.31%) and Spitz (23.47%). Young animals (35.69%) and male dogs were commonly affected.

Study on prevalence of *Ehrlichia canis* in dogs at Nagpur was done by Samradhini *et al.* (2003). Infection was detected in 45 out of 238 dogs of one to two years age. They reported that monsoon and post monsoon season showed high infection rate.

In a similar study Lakshmanan *et al.* (2006) described breedwise, agewise and seasonwise prevalence of *Ehrlichia canis* in Chennai. They found that proportion of positive cases were observed to be more in German shepherd breeds (9.09%) and in age group below two years. There was no significant difference in the proportion of positive cases among male and female. There were also no significant differences in prevalence due to constant atmospheric temperature and humidity at Chennai (Sea Coast) favouring ticks except during December and January.

Work done in abroad :

Davidson *et al.* (1975) reported the prevalence of Canine Ehrlichiosis in Thailand. They identified 161 cases of ehrlichiosis serologically, out of 316 dogs examined clinically.

In a study of 355 blood samples Leeflang *et al.* (1976) observed *Babesia canis* in 31 dogs and *Hepatozoon canis* infection in 71 dogs. They also found mixed infection in 17 dogs.

Ezeokoli-CD (1978) reported the occurrence of *Ehrlichia canis* in 11 dogs between 4-8 months age in Nigeria.

In a survey done by Keefe *et al.* (1982), distribution of *E. canis* among military dogs in the world and selected civilian dogs in the United States was reported. They observed that the highest antibody prevalence rate (24%) in military dogs were found in Japan and Okinawa. The predominant form of infection was subclinical. On the other hand, 23 per cent of the seropositive civilian dogs had various signs of disease.

Van Heerden (1982) classified the disease as acute, subacute and chronic infection owing to three different stages of the disease and described the prognostic value of total leukocytic count in canine ehrlichiosis.

In a study on clinical and epidemiological aspects of canine hepatozoonosis in Zaira, Nigeria; Ezeokoli *et al.* (1983) collected blood samples from 354 dogs. Clinically the disease was characterized by a chronic debilitating course, persistant or recurrent fever unresponsive to antibiotics, anaemia, eosinophilia and polychromasia. They found that *Hepatozoon canis* was most prevalent parasite affecting 22 per cent of the dogs examined; *Babesia canis* affected 11 percent and *Ehrlichia canis* affected 5 per cent. They further noticed that *H. canis* affected dogs of all age group while B. canis and E. canis affected predominantly younger dogs within two years of age.

Rajamanickam *et al.* (1985) reported the prevalence of 17.7% of *Babesia gibsoni,* 1.20% of *Hepatozoon canis,* 1.1% of *Babesia canis,* 0.2% of *Ehrlichia canis* and 0.1% of *Trypanosoma evansi* in canines from Malaysia. They also

found that pedigreed and non-pedigreed dogs were equally susceptible to babesia infection.

27 naturally occurring cases of canine ehrlichiosis of dogs from USA was reported by Waddle and Littman (1988).

Winkler *et al.* (1988) reported the incidence of ehrlichiosis in dogs from Switzerland. They screened 25 dogs serologically by indirect immunofluorescence test of which 12 (48%) dogs found positive for *Ehrlichia canis* and mixed infections with *Leishmania donovani* or *Babesia canis* in 4 (16%) dogs.

Prevalence of *Ehrlichia canis* infection in dogs from Oklahoma was reported by Rodger *et al.* (1989). They examined 259 dogs by immunofluorescene test and found 137 (53%) dogs positive for *Ehrlichia canis*. They also found higher percentages of *Ehrlichia canis* in spring to autumn months.

In Tunisia, incidence of *Ehrlichia canis* in male German shepherd aged 1-8 years was reported by Maghreb (1989).

After that Davoust *et al.* (1990) reported prevalence of *Ehrlichia canis* infection in 5 out of 18 military dogs from France.

Jittapalapong and Tipsawake (1991) reported the prevalence of blood protozoa of pet dogs in Thailand. They examined 185 blood samples from dogs 2-5 years age and found *Babesia canis* in seven (3.75%), *Hepatozoon canis* in four (2.16%) and *Ehrlichia canis* in twenty six (14.05%) samples. They also reported the prevalence of mixed infections of *Babesia canis* and *Ehrlichia canis* in two (1.08%) samples

and *Hepatozoon canis* and *Ehrlichia canis* in only one (0.52%) sample.

An epidemiological survey in an ehrlichial zone in Senegal, Dakar on 66 dogs was reported by Parzy *et al.* (1991). They found 78 per cent serologically positive cases in the dog population of the Senegal national police and 37 per cent positive cases in the sample of native dogs belonging to civilians.

A report on prevalence of canine ehrlichiosis in Mongrels in South Africa was given by Rautenbach *et al.* (1991). They examined 220 dogs of which 38 (17.2%) found positive for ehrlichiosis by blood smear examination technique.

Buoro *et al.* (1992) reported that 6.2 and 3.5 per cent of dogs in Kenya were positive for ehrlichiosis and babesiosis respectively. They observed a little monthly variation in the incidence of diseases. In their study, the occurrence of *Ehrlichia canis* was more than *Babesia canis*. In an another study, Elias, E. (1992) reported the prevalence of ehrlichiosis in dogs from Israel. He examined Giemsa stained blood smears of suspected 250 dogs, of which 220 (88%) dogs harboured *Ehrlichia canis* infection.

In a survey of 105 dogs in Veterinary Hospital in Harare, Zimbabwe, Matthewman *et al.* (1993) reported that 55 (52%) dogs had *Ehrlichia canis,* 27 (26%) had *Babesia canis* and 18 (17%) had both the infections.

Subsequently so many studies on prevalence of haemoprotozoan diseases in canines were done in various parts of would. Ulmer *et al.* (1993) reported prevalence of babesiosis among 2 to 10 years of age in 66 dogs from France. After that, Then Salakij *et al.* (1999) reported the rate of prevalence of *Trypanosoma* spp., *Babesia canis*, *Hepatozoon canis* and *Haemobartonella canis* infection as 0.48 per cent, 3.07 per cent, 4.54 per cent, 2.32 per cent and 0.68 per cent respectively in dogs from Thailand. Subsequently O - Dwyer - LH *et al.* (2001) reported the prevalence of *Hepatozoon canis* infection in dogs in Brazil. Out of 250 dogs examined, 98 (39.2%) found positive for *Hepatozoon canis*. They also reported 5.2 per cent and 4.8 per cent dogs positive for *Babesia canis* and *Ehrlichia canis* respectively.

In an another epidemiological study, Chen and Huang (2003) observed that out of 48 cases affected with tick borne disease in Taiwan, *Babesia gibsoni* affected 33 dogs (68.8%), *Babesia canis* affected four dogs (8.3%), *Ehrlichia platys* affected nine dogs (18.8%), *Ehrlichia canis* affected only one dog (2.1%) and *Hepatozoon canis* affected two dogs (4.2%).

CONCURRENT INFECTIONS :

Two dogs showing signs of anaemia, fever, anorexia, weakness and presence of brown dog ticks contained the concurrent infection of *Hepatozoon canis* and *Ehrlichia canis*, reported by Elias and Homans (1988).

In a survey of 67 dogs showing clinical signs typical of babesiosis, plessis *et al.* (1990) examined the blood smears and sera by IFAT and observed the concurrent infection of *Babesia canis* and *Ehrlichia canis* in 55 percent of dogs.

Concurrent infection of *B. canis* and *E. canis* in a terrier puppy with clinical signs of fever, lameness, gingival bleeding, spleenomegaly and lymphadenopathy was diagnosed by Klag *et al.* (1991).

In Punjab, Juyal *et al.* (1992) reported cases of *E. canis* infection with or without concurrent infection of *Hepatozoon canis* in dogs.

Matthewman *et al.* (1993) reported infections with *Babesia canis* and *Ehrlichia canis* in dogs in Zimbabwe. They found that 52, 26 and 17 per cent animals were positive for *E. Canis*, *B. Canis* and both infections respectively.

In similar study, clinical cases of *E. canis* in dogs with or without concurrent infection of *Babesia gibsoni* and *Babesia canis* was reported by Varshney and Dey (1998).

Later on, concurrent infection of *E. canis* with *Leishmania* spp., *Babesia canis, Dirofilaria immitis, Babesia gibsoni* or *Hepatozoon canis* were documented by Gothe (1999).

Kordic *et al.* (1999) diagnosed multiple tick borne infections with *Ehrlichia* spp., *Babesia canis* and *Bartonella vinsonii* among kennel dogs in North Carolina.

Hua *et al.* (2000) reported an outbreak of Canine Ehrlichiosis caused by concurrent infection of *E. canis* and *E.*

platys in China by amplification and sequencing of 165 rRNA genes. They observed two sequences of 1482 (Gzh, 981) and 1483 (Gzh 982) base pairs clearly resembled to that of *E. canis* and *E. platys* respectively.

Nambi *et al.* (2000) reported the incidence of concurrent Canine Ehrlichiosis and Leptospirosis in a mongrel dog aged 6 years from Chennai.

An another case of concurrent ehrlichiosis and trypanosomiasis in a dog was diagnosed by Ramprabhu *et al.* (2001) at Namakkal, India.

Co-infection with three *Ehrlichia* species, *E. canis*, *E. Platys* and *E. equi* in two dogs was documented by Suksawat *et al.* (2001) using PCR technique.

A case of concurrent infection of *Ehrlichia* spp. and *Hepatozoon canis* and its treatment in a dog was reported by Smitha *et al.* (2003) and Ananda *et al.* (2005).

CLINICO-HAEMATOLOGICAL FINDINGS :

The haematological findings by Huxsoll *et al.* (1970) affected with Tropical Canine Pancytopenia were severe anaemia, leucopenia, thrombocytopenia with normal coagulation and prothrombin time but prolonged bleeding time.

In an another study, Pierce (1971) described haematological change in canine ehrlichiosis. He observed marked decrease in packed cell volume and total leukocytic

count with relatively little change in differential leukocytic count.

Apart from the other changes, retinal changes during various stages of experimental infection of *Ehrlichia canis* in dogs was described by Ellett *et al.* (1974).

Some clinical and clinico-pathological findings in canine ehrlichiosis was reported by Ezeokoli (1978). He showed poor appetite, lethargy, elevated body temperature, severe leucopenia, eosinopenia and mild non responsive anaemia.

Neurologic signs in naturally occurring cases of ehrlichiosis was reported by Troy *et al.* (1980). They observed ataxia, arched back, back and neck pain, cranial nerve deficit and intention tremors of head.

Kuehn and Gaunt (1985) described clinical and haematological findings in ehrlichiosis in 56 dogs. The non specific signs were depression/lethargy (59%), weight loss (39%), haemorrhage (34%), lymphadenopathy (27%) and anorexia (27%). The frequency of bleeding disorders thrombocytopenia and pancytopenia was lower than in previous reports.

Later on Collett *et al.* (1987) reported severe anaemia, leukocytosis, neutrophilia, lymphopenia and thrombocytopenia in blood picture of ehrlichiosis affected dogs from South Africa.

Neurologic manifestations of ehrlichiosis in a dog was described in a 5 year old spayed Irish Setter dog having hind limb ataxia by Andreasen - CB (1988).

The clinical signs as reported by Koutinas *et al.* (1989) in canine ehrlichiosis were hyperthermia, pale mucous membrane, pale skin and epistaxis. Low packed cell volume, leucopenia, thrombocytopenia, proteinuria and *Ehrlichia canis* morulae in monocytes were consistent laboratory findings.

Abeygunawardena *et al.* (1990) described patho-physiology of canine ehrlichiosis or TCP (Tropical Canine Pancytopenia). They reported that pathogenesis of TCP involves a wide range of effect or mechanisms targeted to mature platelets. The predominant mechanisms are immunologically mediated resulting in platelet sequestration resulting in decreased number of platelets. Another pathway involving the platelet migration inhibition factor (PMIF) causing platelet sequestration and stasis leading to reduced peripheral platelet count. There was thrombocytopenia, anaemia and lymphopenia as reported by Magnarelli *et al.* (1990).

In 1991, several studies were done on canine ehrlichiosis. Davoust *et al.* described significance of platelet count in case of chronic canine ehrlichiosis. They found that infected dogs had thrombocytopenia. In an another study Klag *et al.* (1991) reported clinico-haematological findings in concurrent ehrlichiosis and babesiosis in a male Terrier cross puppy. The

clinical signs reported were increase in temperature, severe halitosis associated with periodontal disease, right hind limb lameness, occasional gingival bleeding, spleenomegaly and marked submandibular lymphadenopathy. The haematological analysis revealed low haematocrit and low leukocyte counts, decreased number of platelets. In the same year Weiser *et al.* reported haematological analysis in dogs with chronic ehrlichiosis from USA. They found lymphocytosis with azurophilic granulation in lymphocytes with monoclonal gammopathy in affected dogs.

While reporting haematological analysis of naturally infected dogs with *Ehrlichia canis* and *Hepatozoon canis* from Punjab, Juyal *et al.* (1992) revealed 72% N, 18% L, 9% M and 1% E in *E. canis* and 48% N, 36% L, 3% M, 13% E in *H. canis* infection in dogs.

After that Juyal *et al.* (1993) also reported clinical signs of 106-107^{0}F temperature, dullness, anorexia, congestion of eyes in a dog naturally infected with *Ehrlichia canis* from Punjab. Haematology revealed anaemia and thrombo-cytopenia.

Sixty four dogs (Positive for Ehrlichiosis) of different breeds were studied for clinical and haematological findings by Thirunavukkarasu *et al.* (1994a). The clinical signs observed were pyrexia (71.87%), anorexia (71.87%), depression (34.37%), vomiting (29.68%), skin lesions (20.31%), peripheral lymphadenopathy (9.37%) and spleenomegaly (6.25%). There

were lower values of haemoglobin, packed cell volume and total erythrocytic count. Later on, ophthalmic changes were also recognized (Thirunavukkarasu *et al.* 1994b).

Chou, S.R. (1995) done clinicopathological studies on mixed experimental infections with *Babesia gibsoni* and *Ehrlichia canis* in dogs. The clinical signs observed were persistent high body temperature, loss of body weight, anorexia, anaemia, icterus and increased heart and respiratory rates. There was normocytic normochromic anaemia and thrombocytopenia. In an another similar study Tresamol *et al.* (1998) reported reduction in haemoglobin, packed cell volume and total erythrocytic count; anaemia, leucopenia, increase in absolute monocytes and thrombocytopenia in *Ehrlichia canis* infection in dogs from Madras.

A study on haematological changes in case of canine ehrlichiosis was done by Davoust *et al.* (1996). They found that there was thrombocytopenia (100% of cases), anaemia (29%), leucopenia (42%) or leukocytosis (10%) in affected dogs.

Harrus *et al.* (1998a) observed that blood hyperviscosity, elevation of osmotic pressure, vasculities and thrombocytopenia were the important factors in the pathogenesis of acute blindness associated with *Ehrlichia canis*. They later found that spleen played an important role in pathogenesis of canine ehrlichiosis (Harrus *et al.*, 1998b). A case of canine ehrlichiosis showing bilateral uveitis, retinal

edema and perivascular and peripapillar haemorrhages was diagnosed by Bayon *et al.* (1999).

Later on, Gould *et al.* (2000) recorded bilateral anterior uveitis, episcleral and conjunctival congestion and intraocular haemorrhages with signs of ocular pain in a dog showing blindness associated with ehrlichiosis.

Salakij *et al.* (1999) reported anaemia, lymphopenia in babesiosis, ehrlichiosis and hepatozoonosis infections in dogs from Thailand. Characteristically, they observed neutropenia, eosinopenia and hypoproteinaemia in babesiosis; monocytosis, leucopenia, eosinopenia and hypoproteinaemia in ehrlichiosis and leukocytosis and eosinopenia in hepatozoonosis.

While reporting the clinical and haematological findings of concurrent infection of *Ehrlichia canis* and leptospirosis in a dog from Chennai, Nambi *et al.* (2000) found inappettance, vomiting, anaemia, tick infestation, congested mucous membrane, bilateral mucopurulent ocular discharge and salivation. The haematology revealed lowered Hb, PCV and TEC values while DLC revealed 81 per cent neutrophils, 10 per cent lymphocytes and nine per cent monocytes thus detecting monocytosis in the dog.

Histopathological examination of eyes and brain of 27 dogs died of experimental infection of ehrlichiosis, Panciera *et al.* (2001) recorded uveitis and meningitis in dogs.

In a study conducted on 102 cases of uveitis, Massa *et al.* (2002) described *Ehrlichia canis* as most common infectious agent of uveitis in dogs.

In 2003, Smitha *et al.* performed haematological studies in a dog with concurrent infections of *Ehrlichia* spp. and *Hepatozoon canis*. Haemogram revealed values within the normal range except for slight reduction in PCV.

A case report on successful treatment of canine ehrlichiosis with Doxycycline was given by Jain and Gupta (1997). The blood examination revealed drastic reduction in haemoglobin value with prominent leukocytosis.

BIOCHEMICAL FINDINGS :

Manohar and Ramakrishnan (1982) reported that there was reduced albumin and increased gamma globulins in case of canine ehrlichiosis. After that in 1985, Kuehn and Gaunt described the increase in liver specific enzymes, blood urea nitrogen, creatinine, phosphorus and serum proteins in case of ehrlichiosis in dog. In an another study Davoust *et al.* (1991) showed that the infected dogs had hypoalbuminemia and hypergammaglobulinemia. In the same year that is in 1991, Weiser *et al.* described hyperproteinemia in dogs with chronic ehrlichiosis.

Later on, Chou (1995) described the biochemical changes in mixed infection with *Babesia gibsoni* and *Ehrlichia canis* in dogs. They reported that there were increased blood urea nitrogen, total bilirubin, Alanine aminotransferase, Aspartate

aminotransferase and decreased level of albumin, glucose, cholesterol. Examined urine contained some occult blood, bilirubinuria, proteinuria and bilirubin crystals in urine sediments.

While reporting the biochemical changes in a case of concurrent infection of *Ehrlichia* spp. and *Hepatozoon canis* in a dog, Smitha *et al.* (2003), described high serum alkaline phosphatase activity, hypoglycemia, hypoalbuminemia and hyperglobulinemia.

Subsequently, Nambi *et al.* (2000) reported biochemical changes in case of concurrent infection of ehrlichiosis and leptospirosis in a dog. They found serum Alanine Aminotransferase (ALT), 54 IU; Serum Alkaline Phosphatase (SAP), 205.6 IU; Total Bilirubin, 8.1 mg%; Conjugated, 4.5 mg%; Total protein, 5.1 g%; Albumin, 0.8g%; Globulin, 4.3g%; BUN, 26.0 mg% and Creatinine, 2.4 mg%. So, there is increased ALT and SAP activity, increased Bilirubin, BUN and Creatinine, hypoalbuminemia and hyperglobulinemia.

DIAGNOSIS :

Ewing (1965) described that the presence of intracytoplasmic inclusions in circulating leukocytes stained with Romanovsky stain was of diagnostic value in ehrlichiosis.

Huxsoll *et al.* (1970) diagnosed the cases of ehrlichiosis by observing ehrlichial inclusion bodies in impression smears made from lungs, spleen, liver and kidney.

Structure of *E. canis* in blood monocytes of a dog was described by Simpson (1972). He reported that in light microscopy, inclusion (Morula forms) were seen in cytoplasm of monocytes. Electron microscopy indicated that an inclusion was surrounded by a single membrane which contained many organism (elementary bodies) surrounded by a double membrane and contained fine fibrils and granules.

On the basis of clinical signs and pathologic findings, Huxsoll and Hildebrandt (1974) diagnosed canine ehrlichiosis. They confirmed the findings by presence of intracytoplasmic inclusions and demonstration of antibodies to *E. canis* in sera of affected dogs.

Elias (1991) suggested that detection of larger inclusion bodies/morulae in peripheral blood smear seems to be pathognomic for diagnosis of canine ehrlichiosis.

Diagnosis of canine ehrlichiosis was done by Meneses (1997) by detecting inclusion bodies and morulae within stained lymphocytes or monocytes in blood smear.

Waner *et al.* (1997) suggested that diagnosis of subclinical ehrlichiosis should be based on anamnesis, geographic location of the dog, persistant antibody titres to *E. canis,* mild thrombocytopenia and hypergammaglobulinemia.

Comparison of diagnostic efficacy of five cytological methods viz. buffy coat, peripheral blood, lymph node, bone marrow and short term culture cytology was done by Mylonakis *et al.* (2003). They concluded that buffy coat and

lymph node cytology were of diagnostic value in acute canine monocytic ehrlichiosis.

TREATMENT :

Evaluation of therapeutic and prophylactic efficacy of tetracycline in experimentally infected dogs with *Ehrlichia canis* was done by Amyx *et al.* (1971). They reported that tetracycline was effective in remission of clinical and hematological signs of acute disease within 14 days and unable to isolate the organisms from treated dogs. They also suggested low dose of Oxytetracycline therapy at a dose rate of 6.6 mg/kg body weight once daily in endemic areas.

Heerden *et al.* (1979) treated 20 cases of canine ehrlichiosis with Oxytetracycline and Doxycycline orally and found that Doxycycline was effective even in the cases which had not responded to treatment with Oxytetracycline.

An another trial performed by Oqunkoya *et al.* (1985) in a dog infected with *E. canis* with two injections of long acting tetracycline at a dose rate of 20 mg/kg body weight I/M twice at 4 days interval along with 9-Fluoroprednisolone acetate at a dose rate of 2mg I/M and observed 92.3 percent success rate.

Iqbal and Rikihisa (1994b) reported the failure of doxycycline to eliminate *E. canis* from blood and tissues besides clinical recovery of animals as confirmed by cell culture isolation, IFAT, Western immunoblot analysis, plasma gamma globulin concentration and histopathological examination of tissues.

Egenvall *et al.* (1997) reported that doxycycline is found to be effective when given at a dose rate of 5 mg/kg body weight for 10-28 days.

An another successful treatment of canine ehrlichiosis with doxycycline @ 100 mg orally twice daily for 14 days along with hematinics, liver tonics and blood coagulants was done by Jain and Gupta (1997).

Breitschwerdt *et al.* (1998a) reported that doxycycline hyclate was uniformly effective in eliminating the acute infection in experimental canine ehrlichiosis supported by negative culture and PCR findings during post treatment period.

Therapeutic effect of doxycycline @ 10mg/kg body weight at every 24 hours for 42 days in experimental sub clinical canine monocytic ehrlichiosis was reported by Harrus *et al.* (1998c). They showed that a six week doxycycline treatment may not be sufficient to clear *E. canis* parasite from all subclinically infected dogs.

Efficacy of enrofloxacin and doxycycline was compared by Kontos and Athanasiou (1998) for treatment of *E. canis* and *E. platys*. They observed that both the drugs were equally potent in relieving clinical and laboratory abnormalities.

Samradhni *et al.* (2003) treated the dogs infected with *Ehrlichia canis* with oxytetracycline at a dose rate of 10-20 mg/kg body weight intravenously for three successive days with a supportive therapy of oxalgin 1 ml. I/M, Livogen 1ml.

I/M, Hostacortin 1ml S/C and DNS @ 10-20 r weight I/V. Dogs showed complete recovery fro post treatment.

Concurrent infection of *E. canis* and *Hepatozoon canis* was successfully treated by Smitha *et al.* (2003) with doxycycline @ 5 mg/kg body weight orally once daily for 14 days. They further found that particular case was noticed from second day onwards and the animal was found apparently normal with complete recovery in 15 days.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present research work was carried to search out the prevalence of blood protozoan parasitic infection of dogs in and around Patna and identification of clinical cases of Ehrlichiosis and collection of clinico-pathological parameters like hematology and blood chemistry. Selected therapeutic trials were also conducted for evaluating the therapeutic efficacy of commonly used drugs against febrile/infectious disease.

Prevalence :

The study was conducted at the Department of Parasitology, Bihar Veterinary College, Patna in dogs brought to Bihar Veterinary College Hospital, Government Veterinary Polyclinic, private clinics of Patna and in the street dogs during the period between January 2006 to December 2006.

The dogs having febrile conditions, history of tick infestation, bleeding tendencies, ocular lesions, anorexia, depressed, digestive disturbances (diarrhoea, vomition), jaundice, enlarged lymph nodes, neurological disorders, haemoglobinuria, pale/congested mucus membrane, skin abnormalities and unresponsive to routine antibiotic treatment were added in the study.

During the study blood smears from different exotic breeds of dogs viz. German shepherd, Spitz, Pom, Labrador, Doberman, Boxer, Dalmation, Great Dane, Lhasa Apso, Golden Retriever, Dachshund as well as from the dogs of non

descript pet dogs and street dogs were collected periodically during the year 2006. The information with respect to season, breed, clinical signs, sex, age were recorded throughout the year. A total of 212 dogs were examined having febrile condition (temperature more than 104^{0}F).

Blood smears were made on a clean, greese free glass slide from the ear vein. Pinna of the ear was shaved at the site of ear vein and a sterile needle was pricked and a drop of blood was taken on the glass slide and thin blood smear was made. After drying, it was immediately fixed in methanol for two minutes. After that these were stained with Giemsa stain (1:10 dilution) for 45 minutes, then washed properly in running tap water, dried and examined under oil immersion (100 X) for the presence or absence of blood protozoa (Soulsby, 1982).

Buffy coat smears were also examined. Blood was taken in a capillary tube and centrifuged. It was broken at the level of buffy coat and a drop of buffy coat was taken on the glass slide and smear was made, fixed in methanol and stained with Giemsa as detailed earlier. The smears were examined under oil immersion (100 X) objective of the microscope.

Collection of blood :

Blood was collected from the ailing and street dogs (Fig.-2) from cephalic or saphenous vein. Street dogs were firstly tranquilized with the help of blowing gun (Fig.-1) using xylazine as tranquilizer at the dose rate of 1 mg/kg body weight. 2ml. of blood was collected in a vial containing EDTA

(Ethylene Diamine Tetra Acetic Acid) at the rate of 1mg/ml. as an anticoagulant for haematological study and 3ml. of blood in a test tube without anticoagulant for serum separation for biochemical study. The clear (non-hemolysed) serum was collected and kept at – 20°C until used for biochemical study.

A total of 21 cases of ehrlichiosis were used for haemotological, biochemical, clinicopathological studies and for evaluating the therapeutic efficacy of Doxycycline and Oxytetracycline. The 21 positive cases were divided in three groups having 7 dogs each. First group was treated with Doxycycline @ 5mg/kg orally b.i.d. for 15 days, second group was treated with Oxytetracycline @ 22 mg/kg I/V s.i.d. for three successive days and third group was kept as control group (Untreated).

Haematological studies were performed twice weekly starting from pre-treatment period to 21 days post-treatment. Biochemical studies were performed once a week starting from pre-treatment period to 21 days post-treatment and clinico-pathological studies were performed twice a day (morning and evening) starting from pre-treatment period to 21 days post treatment.

Therapeutic trials were studied in clinical cases of Canine Ehrlichiosis for evaluating the therapeutic efficacy of Doxycycline and Oxytetracycline and the evaluation criteria was based on blood/buffy coat smear examination on day 0, 7th, 14th and 21st.

Haematological Study :

The following haematological parameters were studied as per the standard techniques (Jain, 1986).

a. **Haemoglobin (Hb) :** Haemoglobin was estimated by acid haematin method using Sahli's haemoglobinometer. The values were expressed in gram per cent (gm %).

b. **Packed Cell Volume (PCV) :** Packed cell volume was measured by Wintrobe haematocrit method (Wintrobe, 1933). The values were expressed in percentage (%).

c. **Total Leukocyte Count (TLC) :** Total leukocyte count was done using WBC diluting fluid (Curck's solution), WBC diluting pipette and improved Neubauer Haemocytometer chamber. The values were expressed in thousand per micro litre (1000/μL).

d. **Total Erythrocyte Count (TEC) :** It was done using RBC diluting fluid (Hayem's fluid), RBC diluting pipette and improved Neubauer Haemocytometer chamber as per the standard technique. The values were expressed in million per micro litre (10^6/μL).

e. **Diferential Leukocytic Count (DLC) :** Giemsa stained blood smears were used for differential count by Meander/Battlement technique under oil immersion (Jain, 1986). A total of 100 leukocytes were counted and number of Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes were expressed in percentage (%).

f. **Platelets count :** Platelets were counted per 100 WBCs in the Giemsa stained blood smears and following formula was used for calculation.

$$\text{Platelets}/\mu L = \frac{\text{Number of Platelets}}{100\ \text{WBC}} \times \text{Total leukocytic count}$$

The above haematological studies were done twice a week for 3 weeks viz. on 0 day (Pre treatment period), 3rd day, 7th day, 10th day, 14th day, 17th day and on 21st day.

Biochemical Study :

Serum samples collected were used at the earliest for estimation of total protein and total albumin (Biuret method) and Blood Urea Nitrogen (DAM method).

1. Estimation of total serum protein and total serum albumin (Biuret method) :

Principle :

Cupric ion in a alkaline medium forms violet coloured complex with peptide bond nitrogen of peptides and proteins. The intensity of the colour produced is proportional to the number of peptide bonds and hence to the concentration of protein.

Reagents Used :

(i) **Stock Biuret Reagent :** 45 g of sodium potassium tartarate was dissolved in 400 ml. of 0.2N NaOH, 15g of $CuSO_4 . 5H_2O$ was added to this solution and stirred.

Then 5g of potassium iodide was added and the volume was made 1 litre with 0.2 N sodium hydroxide.

(ii) **Biuret Reagent for use :** 200 ml. of stock reagent was diluted to a litre with 0.2 N sodium hydroxide which contained 5 grams of potassium iodide per litre.

(iii) 28 % of sodium sulphite ($Na_2 SO_3$)

(iv) 0.9% sodium chloride (NaCl)

(v) **Standard protein solution :** Bovine serum albumin solution containing 2mg protein/ml.

Procedure : 0.2 ml. of serum was taken into a test tube, 5.8 ml. of sodium sulphite was added and mixed by inversion. Then it was kept for 5 minutes and filtered through a dry filter paper [Whatman no. 44 (40)]. Then the clear filterate was used for estimation of albumin.

Four test tubes were taken. 3ml. of distilled water was taken in the first tube (Blank), in the second tube 3ml. of standard protein solution was taken (Standard/S). In the third tube 0.1ml. of serum and 2.9 ml. of 0.9 %. Sodium chloride was taken (T_{TSP}/Test for Total serum protein). In the fourth tube 3 ml. of filtrate was taken (T_{TA}/Test for Total serum albumin). The 3ml. of biuret reagent was added in all the tubes. Then they were mixed and allowed to stand at 37^0C for 10 minutes.

Reading of the optical Density (O.D.) was taken at 540 nm in a colorimeter.

Calculation :

$$\text{Total serum protein/TSP (g/dl)} = \frac{\text{O.D. of } T_{TSP}}{\text{O.D. of S}} \times 6.0 \times \frac{100 \times 1}{0.1 \times 1000}$$

$$\text{Total Albumin/TA (g/dl)} = \frac{\text{O.D. of } T_{TA}}{\text{O.D. of S}} \times 6.0 \times \frac{100 \times 1}{0.1 \times 1000}$$

Total Globulin / TG = TSP – TA

$$\text{TA : TG, Ratio} = \frac{\text{Total Albumin / TA}}{\text{Total Globulin/TG}}$$

2. Determination of Blood Urea Nitrogen (BUN),

(Diacetyl Monoxime Method/DAM method) :

Principle : Urea gives pink colour when reacts with diacetyl monoxime under acidic medium in the presence of ferric chloride and thiosemicarbazide.

Reagents Used :

(i) Diacetyl monoxime – 1.56g diacetyl monoxime in 250ml distill water.

(ii) Thiosemicarbazide – 41 mg thiosemicarbazide in 250 ml. distill water. It was kept in brown bottle.

(iii) Ferric chloride reagent-324 mg. ferric chloride in 56% orthophosphoric acid, also kept in brown bottle.

(iv) 20% sulphuric acid (V/V).

(v) Acid reagent – In 1000 ml. of 20% sulphuric acid, 1ml. of ferric chloride reagent was added.

(vi) 10% Trichloroacetic acid.

(vii) Preservative diluent for standard – 40mg of phenyl mercuric acetate was dissolved in 250 ml. of distill water with heating. Solution was transferred into measuring cylinder and 0.3 ml. of conc. sulphuric acid was added and volume was made to 1 litre with water.

(viii) Standard used – 10mg. used in 1 litre of preservative diluent (1ml. = 0.01 mg).

Procedure : It was done as follows :

3.4 ml. of distill water was taken in a centrifuge tube, 0.1 ml. of blood was added and mixed. Then 1.5 ml. of 10% Trichloroacetic acid was added, mixed and left for 5 minutes and then centrifuged.

Three test tubes was taken and labeled "Blank", "Test" and "Standard". 1.0 ml. of distill water in "Blank", 1.0 ml. supernatant fluid in "Test" and 1.0 ml. of standard urea in "Standard" was taken.

Then, to each tube 1.0 ml of diacetyl monoxime, 1.0 ml. of thiosemicarbazide and 3.0 ml. of acid reagent was added. They were mixed and placed in boiling water bath for 15 minutes. Tubes were cooled in water and the Optical Density (O.D.) was read at 540 nm in photoelectric colorimeter.

Calculation :

$$\text{Blood urea (mg/dl)} = \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 0.01 \times \frac{100}{0.02}$$

Blood Urea Nitrogen (BUN) = Blood Urea (mg/dl) x 0.467 (mg/dl).

The above biochemical studies were done once in a week for 3 weeks viz. 0 day (pre treatment period), 7th days, 14th day and on 21st day.

Therapeutic Trial :

Selected therapeutic trials would be studied in clinical cases of Canine Ehrlichiosis for evaluating the therapeutic efficacy of Doxycycline and Oxytetracycline as per the protocol given in the following Experimental Design :

Table - 1

Group No.	No. of Animals	Drug used with dose	Parameters of study		
			Hematology	Clinico-Pathology	Blood Chemistry
I	7	Doxycycline @ 5mg/kg orally b.i.d. for 15 days	Performed twice a week starting from pre-treatmnet period to 21 days post-treatment	Performed twice a day (morning & evening) starting from pre-treatment period to 21 days post-treatment	Performed once a week starting from pre-treatment period to 21 days post-treatment
II	7	Oxytetracycline @ 22 mg/kg I/V s.i.d. for 3 days daily			
III	7	Untreated Control			

Evaluation Criteria :

(i) Peripheral blood/buffy coat smear examination on day 0, 7th, 14th and 21st.

All the dogs under treatment were kept under observation and the alteration in symptoms were recorded twice a day for 21

days. The clinical parameters those were given special attention include, changes in body temperature, pulse, respiration, consistency and colour of faeces, urine, etc.

Statistical Analysis :

Data was statistically analyzed as per methods described by Snedecor and Cochran (1967).

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CHAPTER - IV

RESULTS

RESULTS

The study was conducted in the Department of Parasitology, Bihar Veterinary College, Patna during the period between January, 2006 to December, 2006. The dogs having all or any two of the following symptoms like rise in body temperature (> 104°F), history of tick infestation, bleeding tendencies, ocular lesions, anorexia, depressed condition, digestive disturbances (diarrhoea and vomition), jaundice, enlarged lymph nodes, neurological disorders, haemoglobinuria, pale/congested mucous membrane, skin abnormalities and unresponsive to antibiotic treatment were included in the study to search out the prevalence of blood protozoan parasites of dogs (based on blood/buffy coat smear examination) and the dogs found positive for *Ehrlichia* spp. were included for further study.

The results of the present study are presented under the following headings :

I. Prevalence of blood protozoan infection.

II. Haematological changes in Canine Ehrlichiosis.

III. Biochemical changes in Canine Ehrlichiosis.

IV. Therapeutic trial against Canine Ehrlichiosis.

Prevalence of Blood Protozoan infection in Dogs :

During the study a total of 212 dogs, clinically showing any two or more than that of the symptoms of sickness viz., high temperature (>104°F), history of tick infestation, bleeding

tendencies, ocular lesions, anorexia, depressed, digestive disturbances (diarrhoea and vomition), jaundice, enlarged lymph nodes, neurological disorder, haemoglobinuria, pale/congested mucus membrane, skin abnormalities and unresponsive to routine antibiotic treatment, if any etc. were examined for blood protozoan infection throughout the year. Out of which 98 (46.22%) dogs were found positive for blood protozoan infection. The result on monthwise number of dogs examined and found positive is presented in Table-2 and Graph-I.

The monthwise prevalence of blood protozoan infection revealed wide variations in the percentage of infected dogs in each month. The range was between 23.52 per cent in May (minimum) and 83.33 per cent in July (maximum). The overall occurrence of infection was 46.22 per cent.

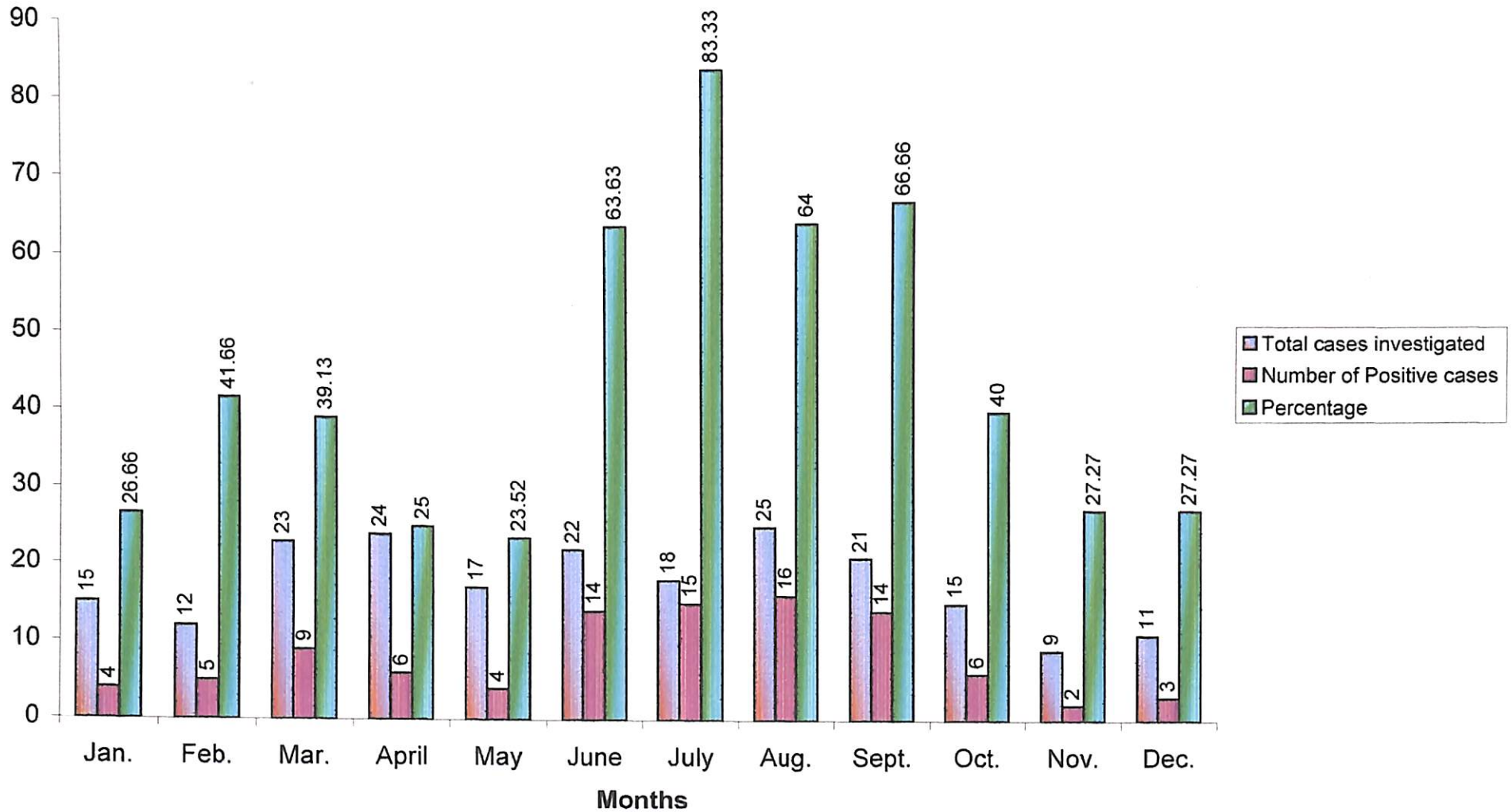
The results of the seasonwise variations of prevalence of blood protozoan infection in dogs is presented in Table-3 and Graph – III. The seasonwise prevalence in monsoon, post-monsoon, winter and summer season was found to be 69.23, 48.88, 31.57 and 29.68 per cent respectively. On analysis of the data, variation in the prevalence of infection was found to be highly significant ($P<0.01$) indicating that the occurrence of infection varied significantly with the seasons.

Age wise prevalence of blood protozoan infection in dogs is presented in Table-4 and Graph-IV. The prevalence was 69.09, 58.33, 40.00, 24.00 and 18.18 per cent in dogs below 1

Table – 2 : Monthwise prevalence of blood protozoan infections in dogs in and around Patna.

Month	Total cases investigated	Number of Positive cases	Percentage	Temperature (°C)		Relative Humidity (%)		Avg. Rainfall (mm)
				Avg. Min.	Avg. Max.	Avg. Min.	Avg. Max.	
Jan.	15	4	26.66	8.65	23.11	56.00	80.11	Nil
Feb.	12	5	41.66	14.72	30.06	51.03	81.46	Nil
Mar.	23	9	39.13	17.25	33.03	35.85	36.96	0.11
April	24	6	25.00	22.22	36.66	34.43	57.13	Nil
May	17	4	23.52	25.77	37.20	49.70	66.88	1.03
June	22	14	63.63	27.10	36.26	60.86	75.51	7.23
July	18	15	83.33	27.04	33.33	72.65	84.68	9.07
Aug.	25	16	64.00	26.80	33.72	69.13	79.96	5.98
Sept.	21	14	66.66	26.17	32.79	74.10	83.89	3.16
Oct.	15	6	40.00	23.08	33.05	61.62	76.55	Nil
Nov.	9	2	27.27	16.39	28.69	62.60	78.56	0.21
Dec.	11	3	27.27	10.51	25.16	54.03	80.65	Nil
Total	212	98	46.22					

Graph - I : Histogram showing monthwise prevalence of blood protozoan infection in dogs in and around Patna.



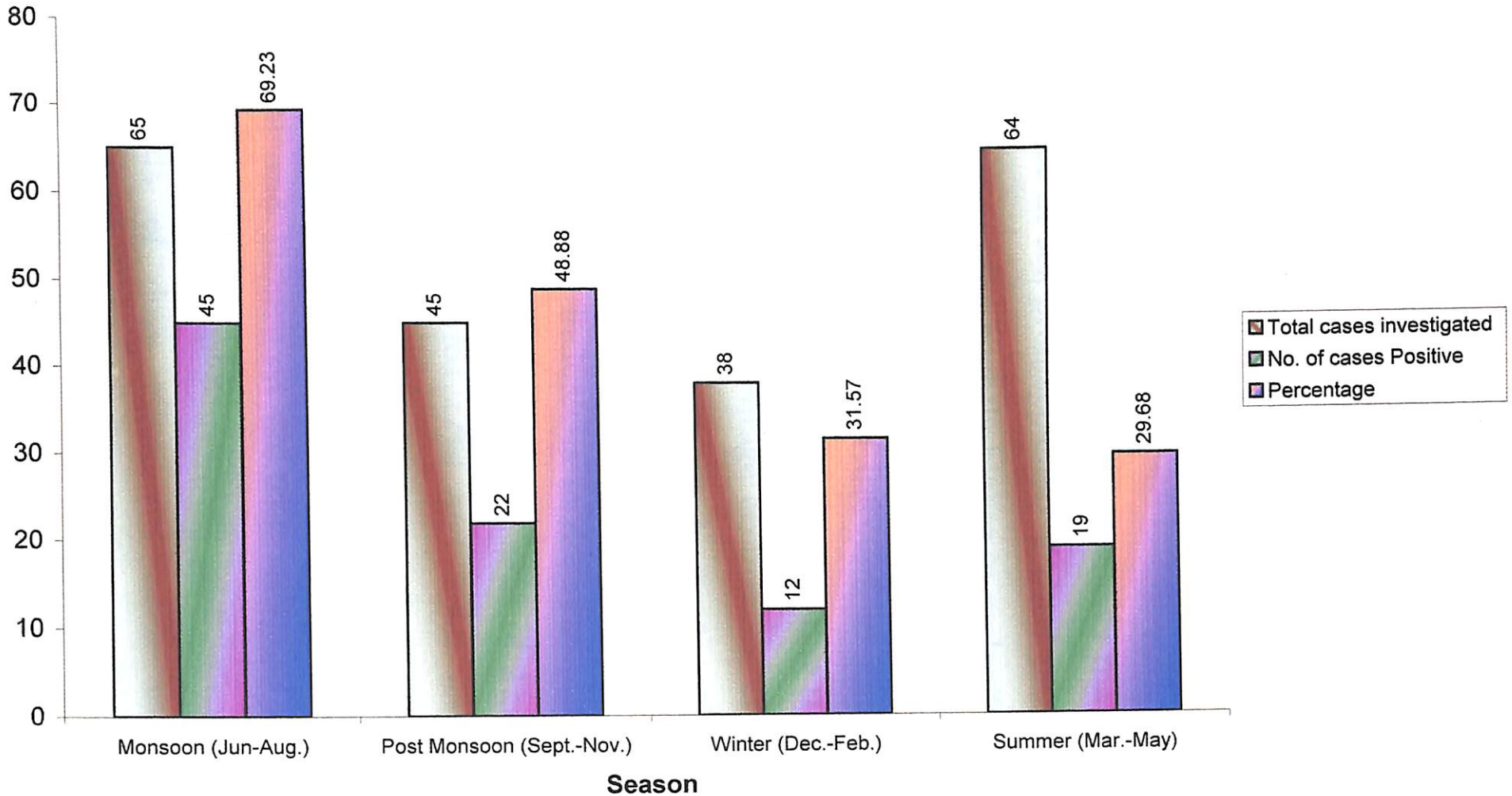
Graph - II : Line diagram showing maximum average temperature and maximum average relative humidity in and around Patna during the year 2006.

Table – 3 : Seasonwise prevalence of blood protozoan infection in dogs in and around Patna.

Season	Total no. of cases	No. of cases positive	%	χ^2_3 df	Temperature (°C)		R. Humidity (%)		Rainfall (mm)	
					Min.	Max.	Min.	Max.	Min.	Max
Monsoon (Jun-Aug.)	65	45	69.23	24.28**	26.80	36.26	60.86	84.68	5.98	9.07
Post Monsoon (Sept.-Nov.)	45	22	48.88		16.39	33.05	61.62	83.89	Nil	3.16
Winter (Dec.-Feb)	38	12	31.57		8.65	30.06	51.03	81.46	Nil	Nil
Summer (Mar-May)	64	19	29.68		17.25	37.20	34.43	66.88	Nil	1.03
Total	212	98	46.22							

** Significant at $P<0.01$

Graph - III : Histogram showing seasonwise prevalence of blood protozoan infection in dogs in and around Patna.



year, 1-2 years, 2-4 years, 4-8 years and above 8 years respectively. The variability in prevalence of blood protozoan in different age group of dogs was found to be highly significant ($P<0.01$), indicating that dogs below one year of age were more susceptible to blood protozoan infection followed by dogs between 1-2 years, 2-4 years, 4-8 years and the least occurrence was above 8 years age group of dogs.

Sexwise prevalence of blood protozoan infection in dogs is presented in Table-5 and Graph-V. The data shows that there is not much difference in sex predisposition of blood protozoan infections. The prevalence was slightly higher in males (48.0%) than that of females (44.64%). On analysis of the data, sexwise variation in the prevalence of infection in male and female dogs was found to be non significant indicating that sex variation does not affect the percentage occurrence of blood protozoan in dogs. The overall sex ratio (male/female) was 1.07.

Prevalence of Ehrlichial inclusions in different breeds of dog is illustrated in Table-7 and Graph-VII. Out of 212 dogs examined, German shepherd (61.22%) and Spitz (56.09%) were most commonly affected breeds followed by non-descript (44.44%), Labrador (40.00%), other exotic breeds (37.5%), Dalmation (25.0%) and Doberman (10.0%). Analysis of the data was found to be significant ($P<0.05$) indicating that variations in breeds of dogs affect the occurrence of haemoprotozoan infection.

Table – 4 : Agewise prevalence of blood protozoan infection in Dogs in and around Patna.

Age group	Total cases examined	No. of positive cases	Percentage	χ^2_4 df
Below 1 year	55	38	69.09	27.81**
1-2 years	60	35	58.33	
2-4 years	50	20	40.00	
4-8 years	25	6	24.00	
Above 8 year	22	4	18.18	
Total	212	98	46.22	

** = Significant at P <0.01

Graph - IV : Histogram showing agewise prevalence of blood protozoan infection in dogs in and around Patna.

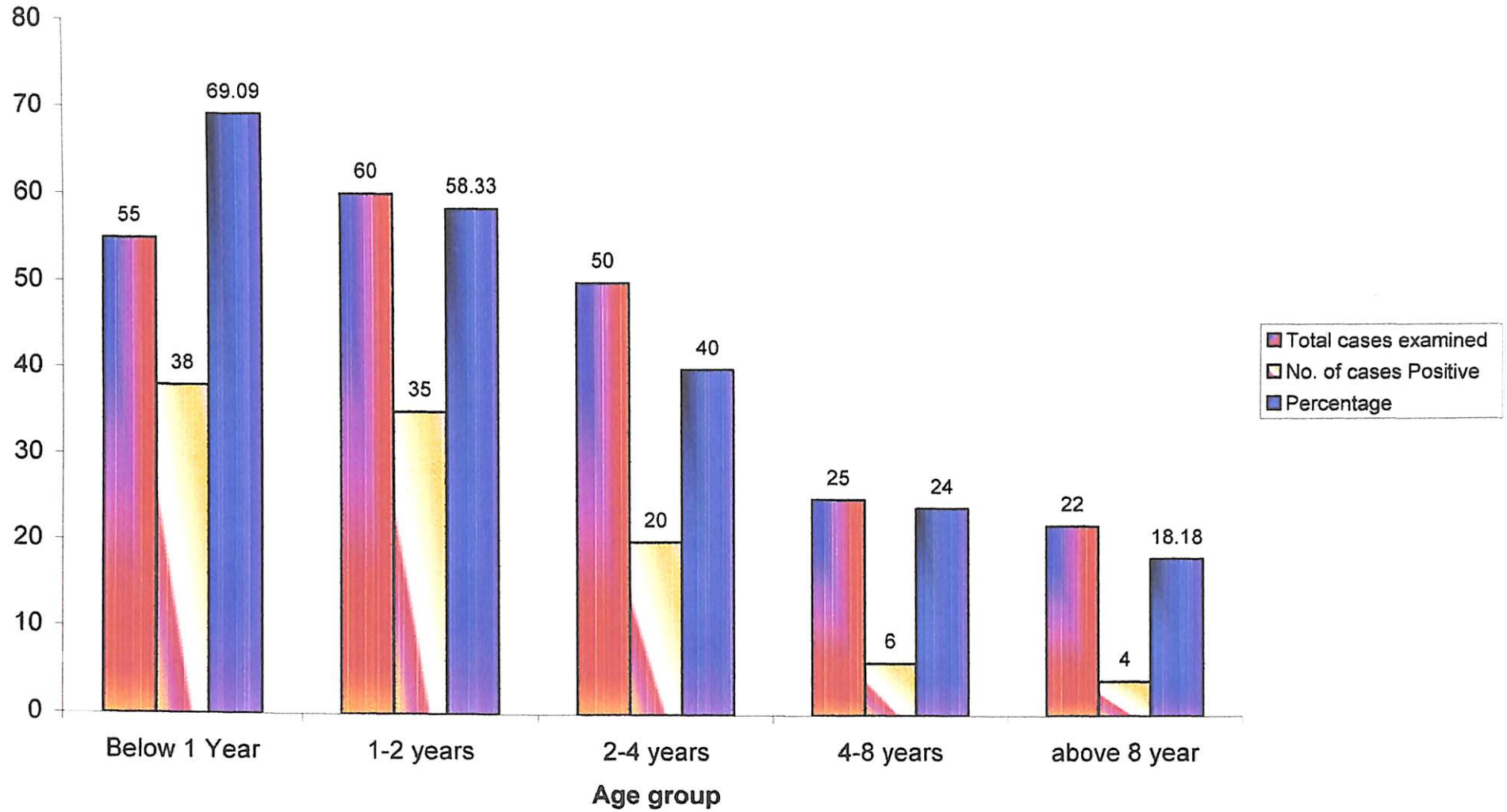


Table – 5 : Sexwise prevalence of blood protozoan infection in dogs in and around Patna.

Sex	Total cases examined	No. of positive cases	Percentage	Sex ratio	χ^2_1 df.
Male	100	48	48.00	48/44.64 = 1.07	0.23^{NS}
Female	112	50	44.64		
Total	212	98			

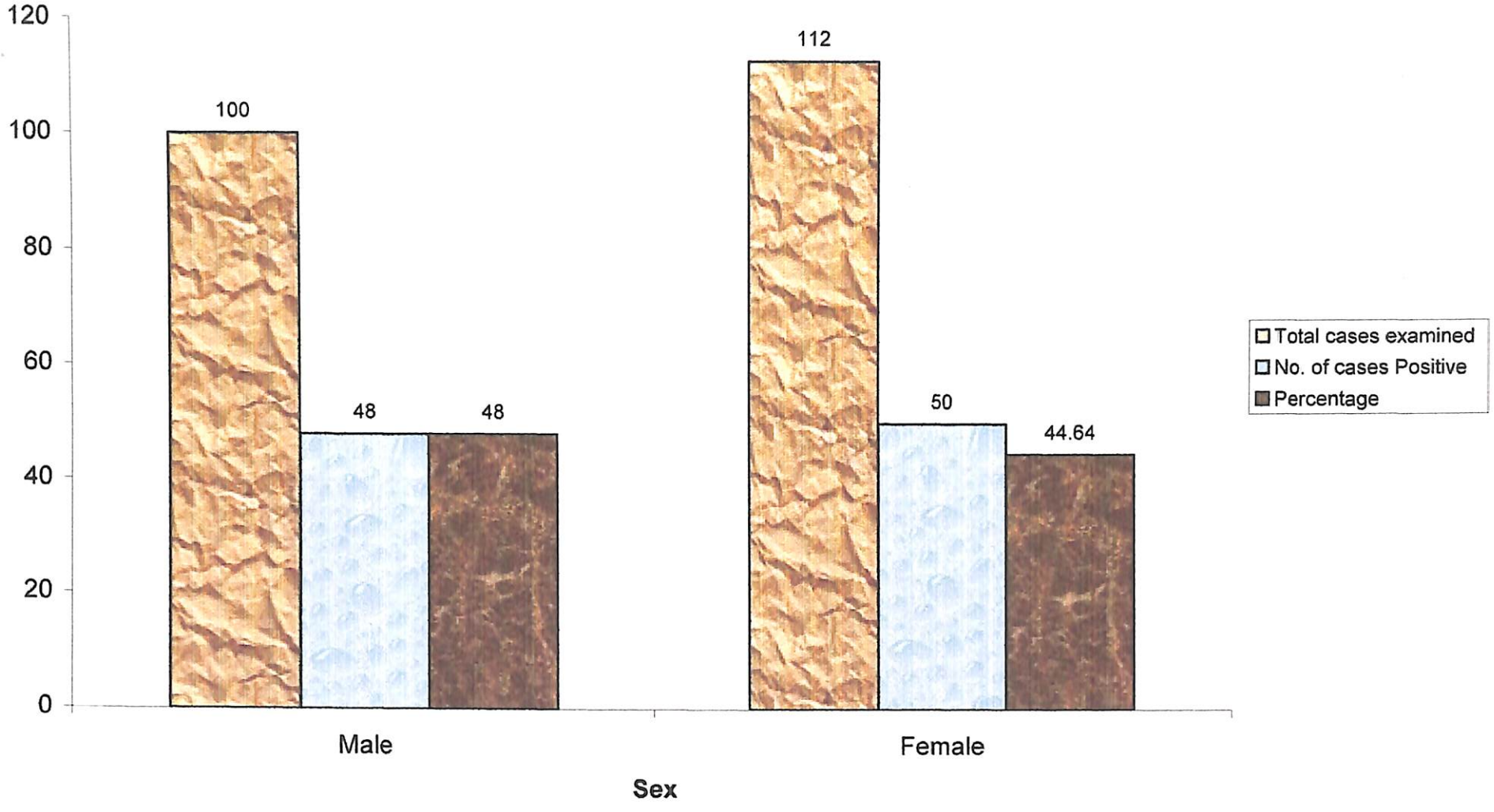
NS = Non Significant.

Table – 6 : Comparative efficacy of Blood and Buffy coat smears in the diagnosis of Canine Ehrlichiosis.

Smears	Total cases investigated	No. of cases diagnosed with Ehrlichiosis	Percentage	χ^2_1 df.
Blood smear	212	79	37.26	2.19^{NS}
Buffy coat smear		94	44.33	

NS = Non Significant.

Graph - V : Histogram showing sexwise prevalence of blood protozoan infection in dogs in and around Patna.



Graph - VI : Histogram showing comparative efficacy of Blood and Buffy coat smears in the diagnosis of Canine Ehrlichiosis.

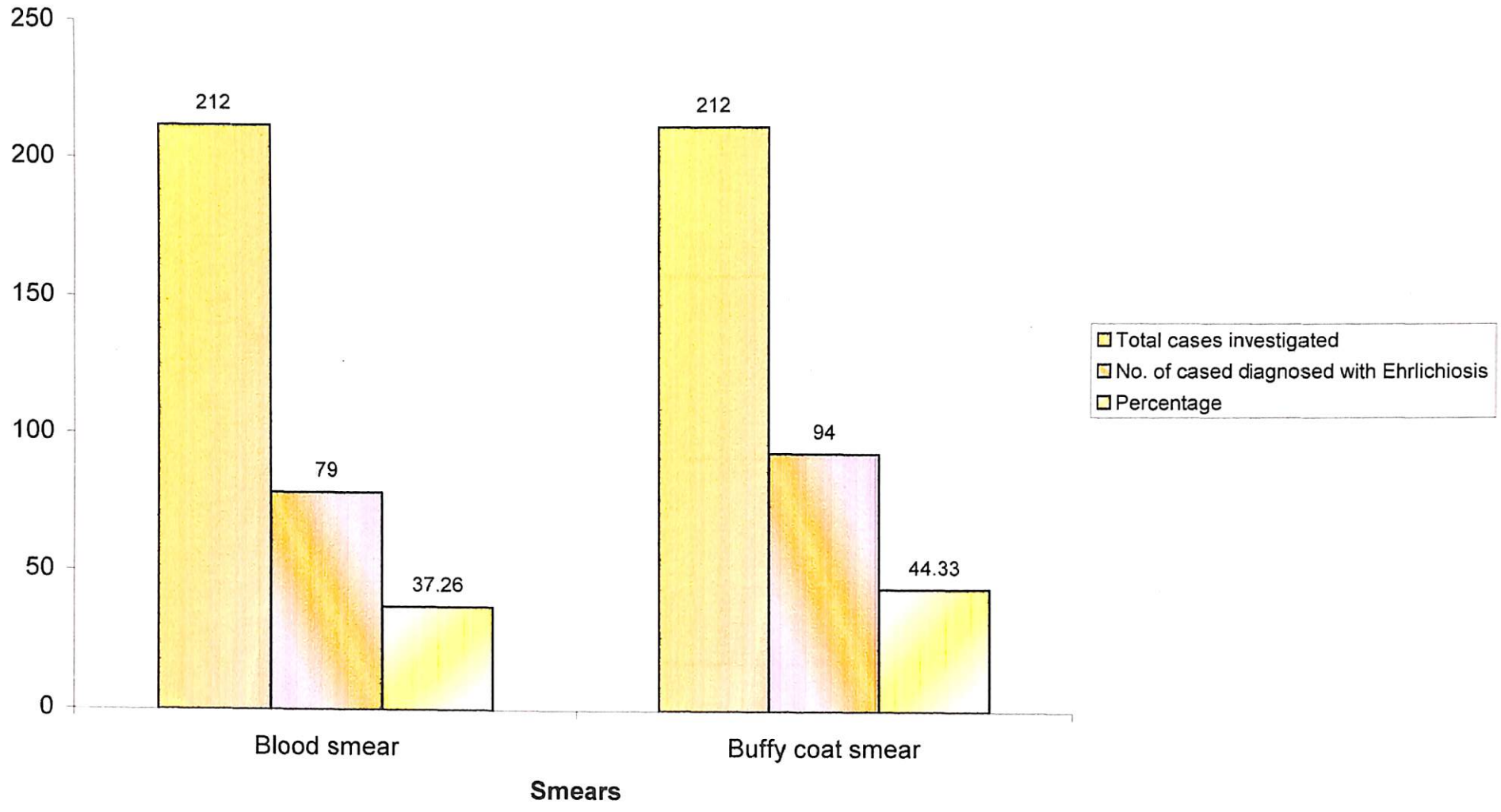
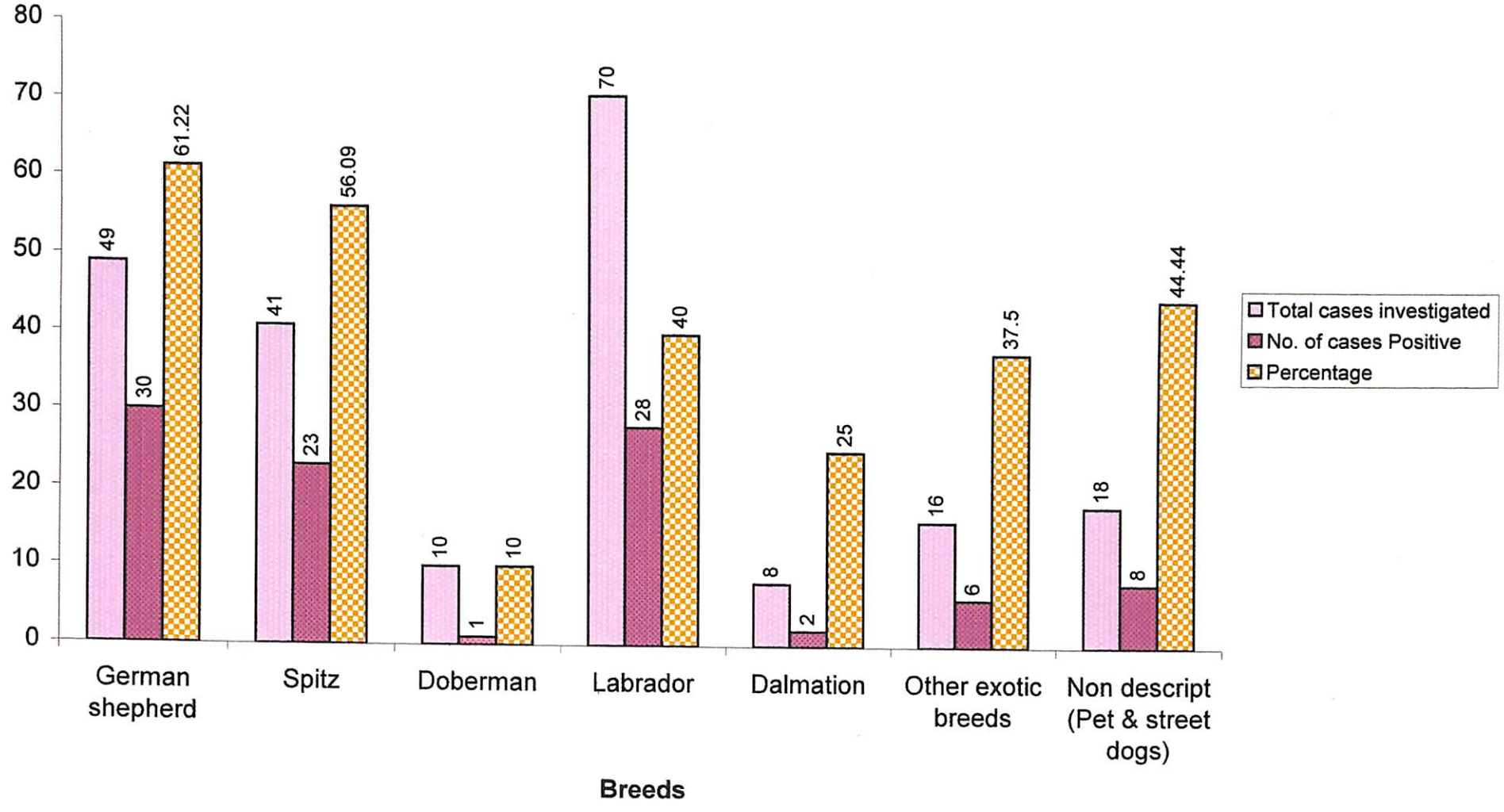


Table – 7 : Prevalence of Blood Protozoan infection in different breeds of dogs in and around Patna.

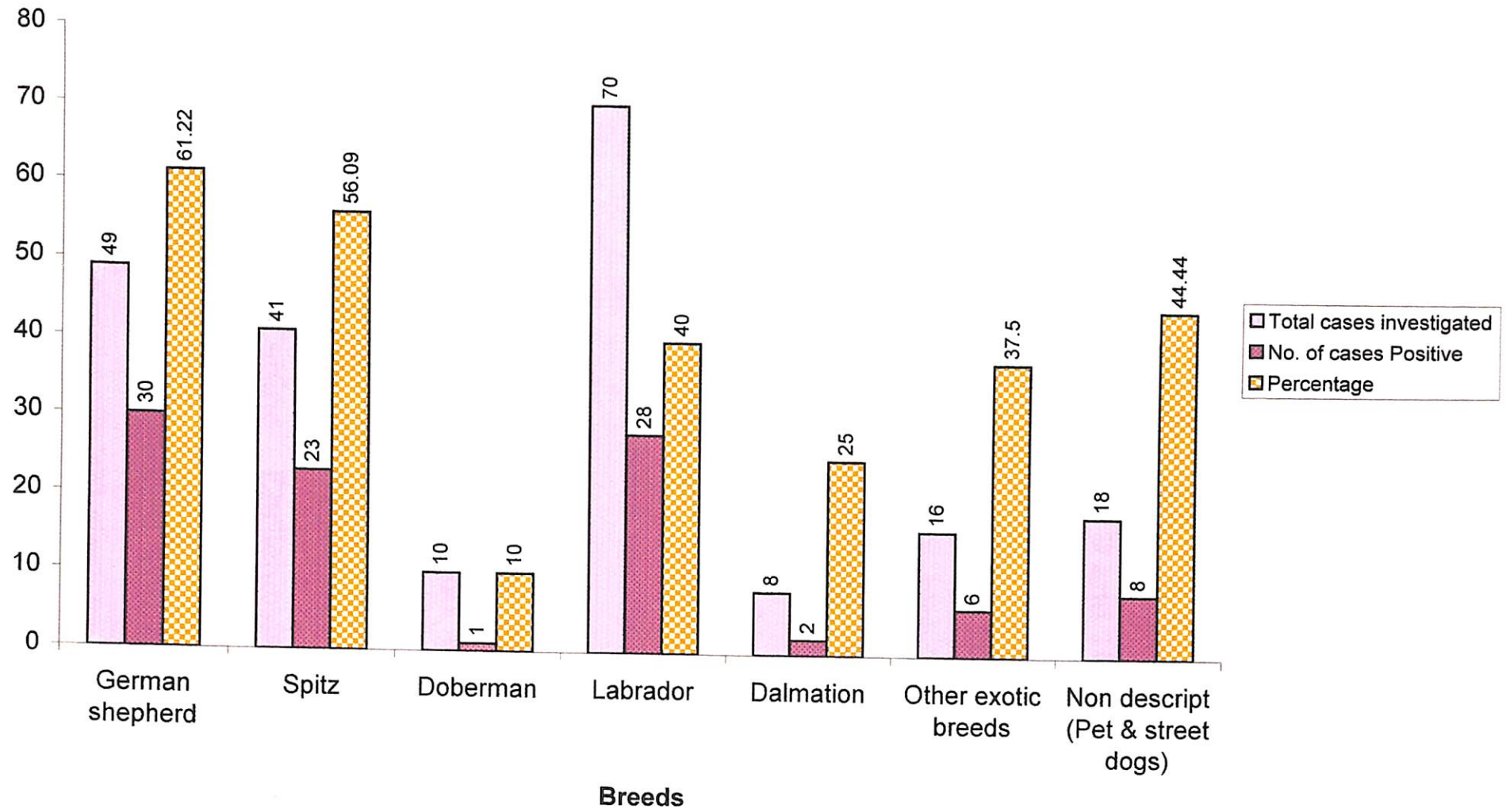
Breeds	Total cases investigated	No. of cases positive	Percentage	χ^2_6 df
German shepherd	49	30	61.22	14.32*
Spitz	41	23	56.09	
Doberman	10	1	10.00	
Labrador	70	28	40.00	
Dalmation	8	2	25.00	
Other exotic breeds	16	6	37.50	
Non descript (Pet & street dogs)	18	8	44.44	
Total	**212**	**98**		

* Significant at $P < 0.05$

Graph - VII : Histogram showing breedwise prevalence of blood protozoan infection in dogs in and around Patna.



Graph - VII : Histogram showing breedwise prevalence of blood protozoan infection in dogs in and around Patna.



Out of 212 clinical cases investigated, peripheral blood smears were found positive in 79 (37.26%) dogs for Canine Ehrlichiosis whereas in buffy coat smear it was found positive in 94 cases (44.33%). Statistically after analyzing the data, the difference was found non significant. The results are presented in Table-6 and Graph-VI.

Ehrlichia canis morulae were found in monocytes (Fig.-5, 6 and 7) whereas granulocytic ehrlichia (*Ehrlichia ewingii*) morulae were found in neutrophils (Fig- 8, 9 and 10).

The result of distribution of different blood protozoan infection in dogs is presented in Table-8 and Graph-VIII. A total of 98 cases were found positive for blood protozoan. Amongst them 56 (57.14%) cases were found positive for *Ehrlichia ewingii* followed by *Ehrlichia canis* in 34 dogs (34.69%), multiple ehrlichial infection (*Ehrlichia canis* and *Ehrlichia ewingii*) in 4 dogs (4.08%) and *Babesia canis* in 4 dogs (4.08 %). Babesia trophozoites were mostly pleomorphic varying from annular and/or oval shaped.

On analysis of the data, variation in prevalence of different blood protozoan infection was found to be highly significant ($P<0.01$).

Clinical manifestations of Canine Ehrlichiosis :

Naturally occurring Canine Ehrlichiosis may be manifested by a wide variety of clinical signs (Fig.-3 and 4). The clinical signs observed in dogs infected with *Ehrlichia* spp. is presented in Table-9. The main clinical signs were anorexia

Table – 8 : Distribution of different Blood Protozoan infection in dogs in and around Patna.

Type of blood protozoan	No. of cases	Percentage	χ^2_3 df
Ehrlichia canis	34	34.69	104.65**
Ehrlichia ewingii	56	57.14	
Multiple ehrlichial infection	4	4.08	
Babesia canis	4	4.08	
Total positive cases	98		

** Significant at $P < 0.01$

Graph - VIII : Histogram showing distribution of different blood protozoan infection in dogs in and around Patna.

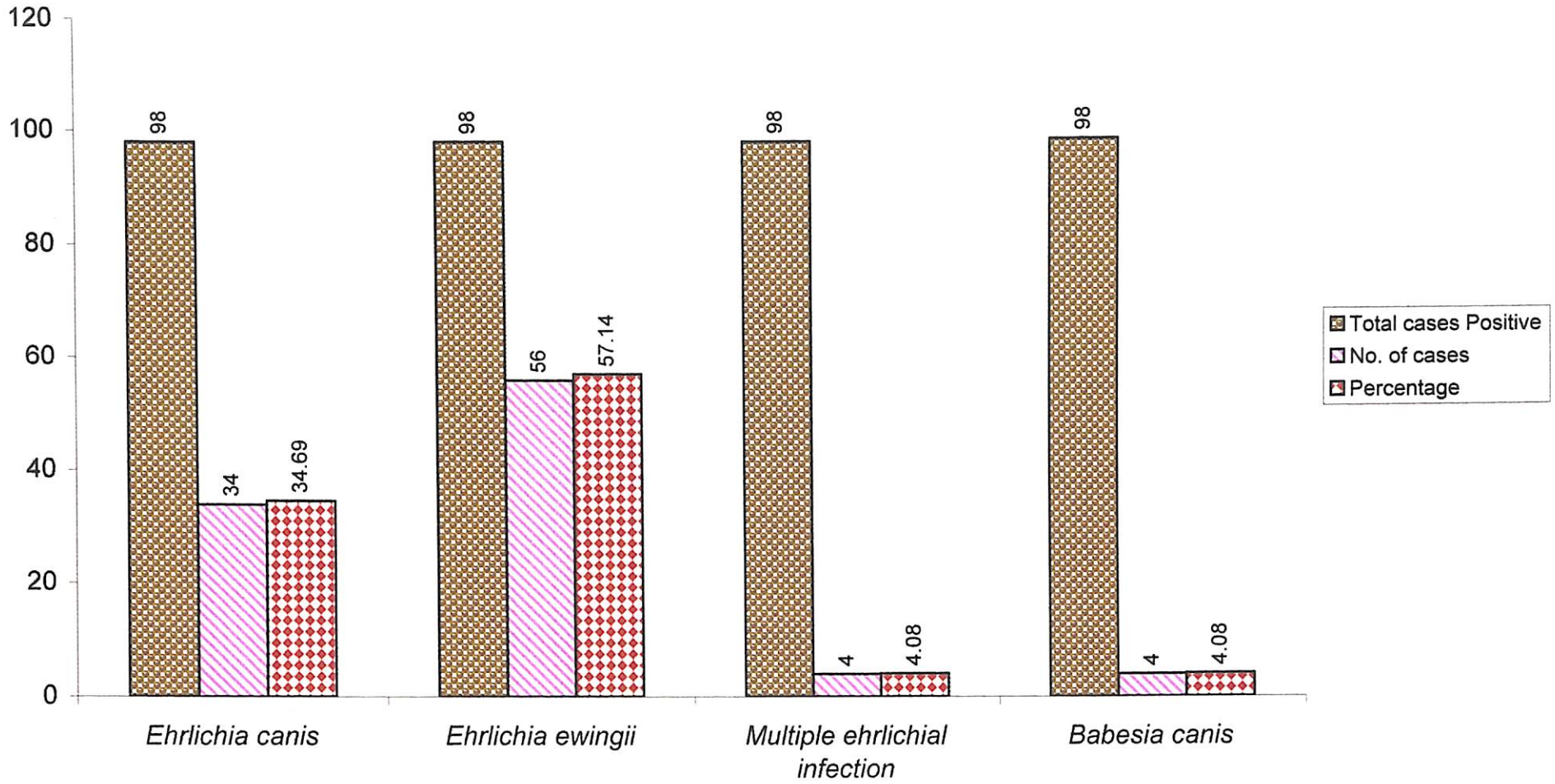


Table – 9 : Clinical signs observed in the dogs infected with *Ehrlichia* spp.

Sr. No.	Clinical Signs	Number of cases	Percentage
1	Anorexia	91	96.80
2	High temperature (>104^{0}F)	94	100.00
3	Ocular lesions	32	34.04
4	Nervous signs	22	23.40
5	Lymphadenopathy	43	43.74
6	Pale mucus membrane	42	44.68
7	Haemorrhage (Nasal & Oral)	52	55.31
8	Weight loss	76	80.85
9	Gastrointestinal signs (Vomition, diarrhoea etc.)	81	86.17
10	Respiratory signs	62	65.95
11	Tick infestation	86	91.48
12	Anaemia	76	80.85
13	Haemoglobinuria	20	21.27
14	Skin lesion	34	36.17

[Plate- 1]

Fig.- 1 : Method of Tranquilizing the Street Dogs by Blowing Gun Method.

Fig.- 2 : Blood Collection from Street Dogs.

[Plate- 2]

Fig. - 3 : Epistaxis in a Labrador dog suffering from Ehrlichiosis.

Fig. - 4 : Corneal opacity in case of Canine Ehrlichiosis.

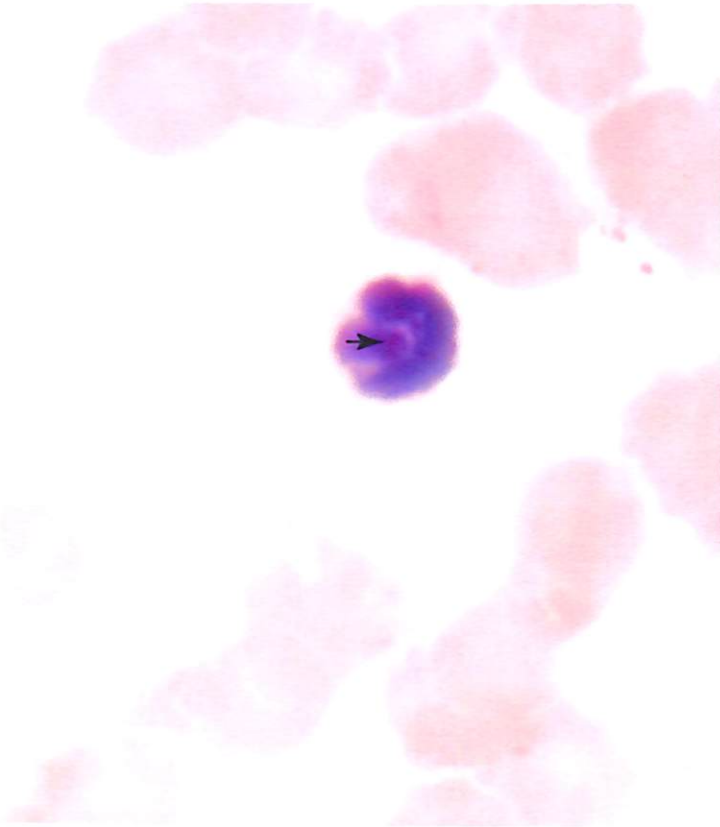


Fig. - 5 : Blood smear of a dog showing *Ehrlichia canis* morulae, Giemsa stain, (X1000).

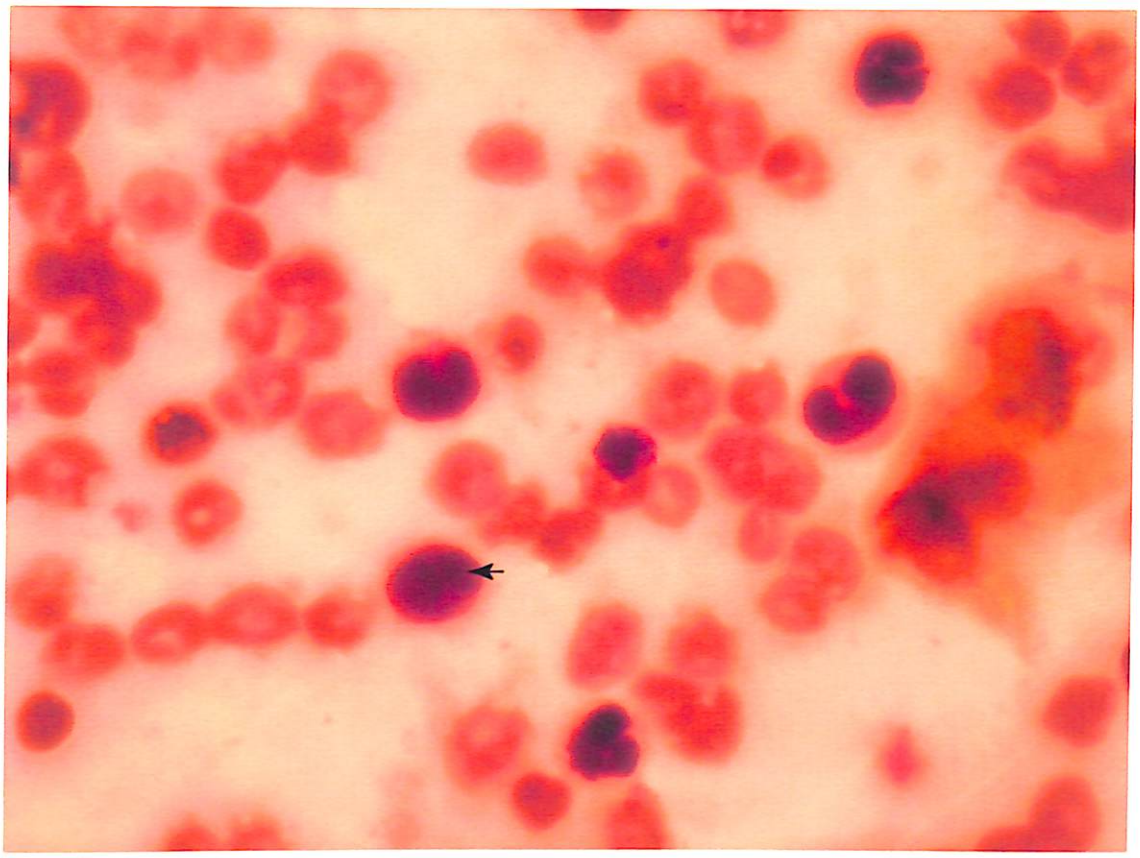


Fig. - 6 : Blood smear of a dog showing *Ehrlichia canis* morulae, Giemsa stain, (X1000).

[Plate- 4]

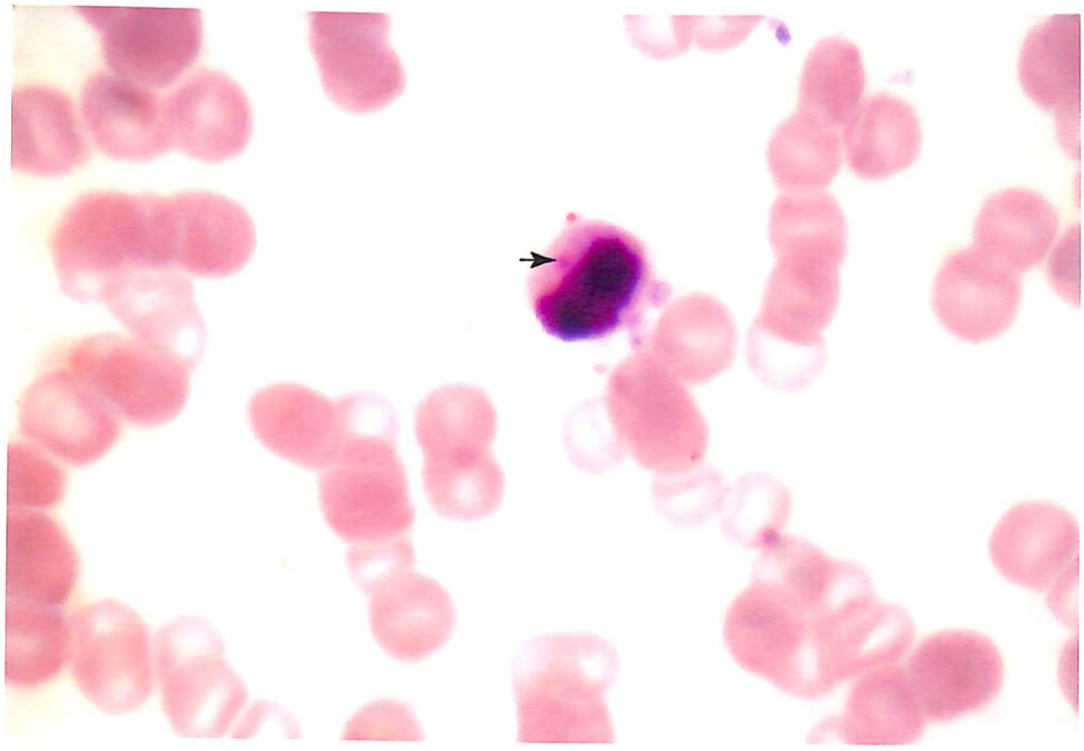


Fig.- 7 : Blood smear of a dog showing initial body of *Ehrlichia canis*, Giemsa stain, (X1000).

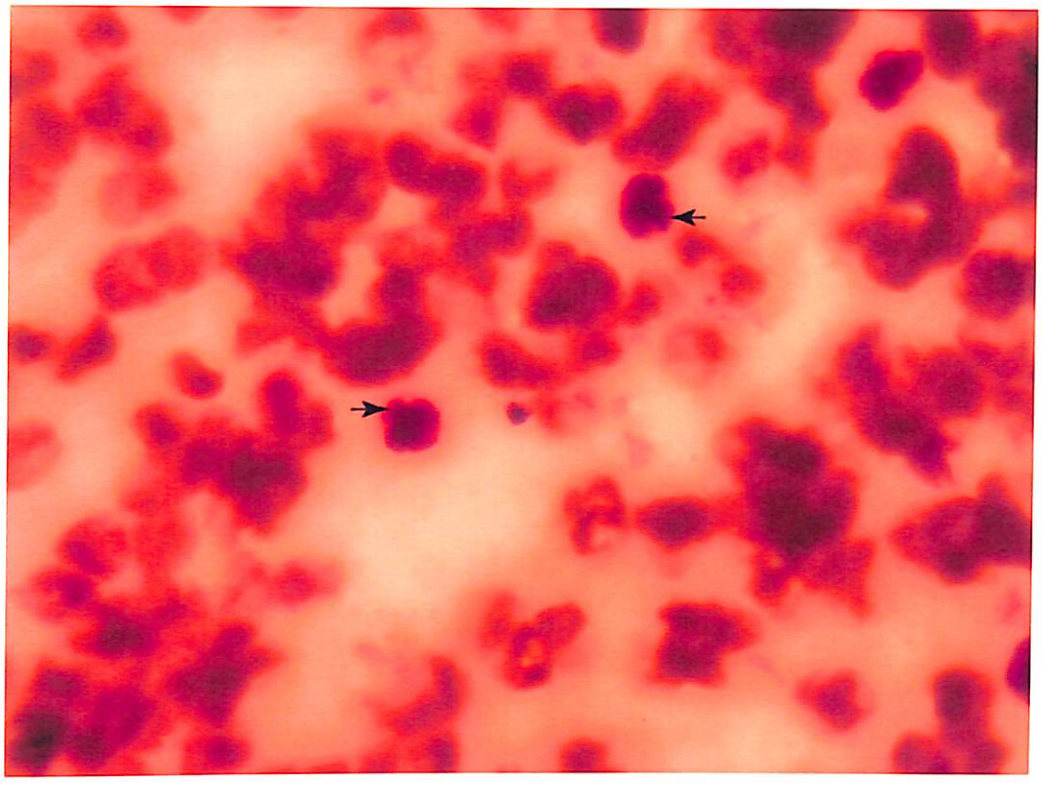


Fig.- 8 : Blood smear of a dog showing granulocytic ehrlichia (*Ehrlichia ewingii*), Giemsa stain, (X1000).

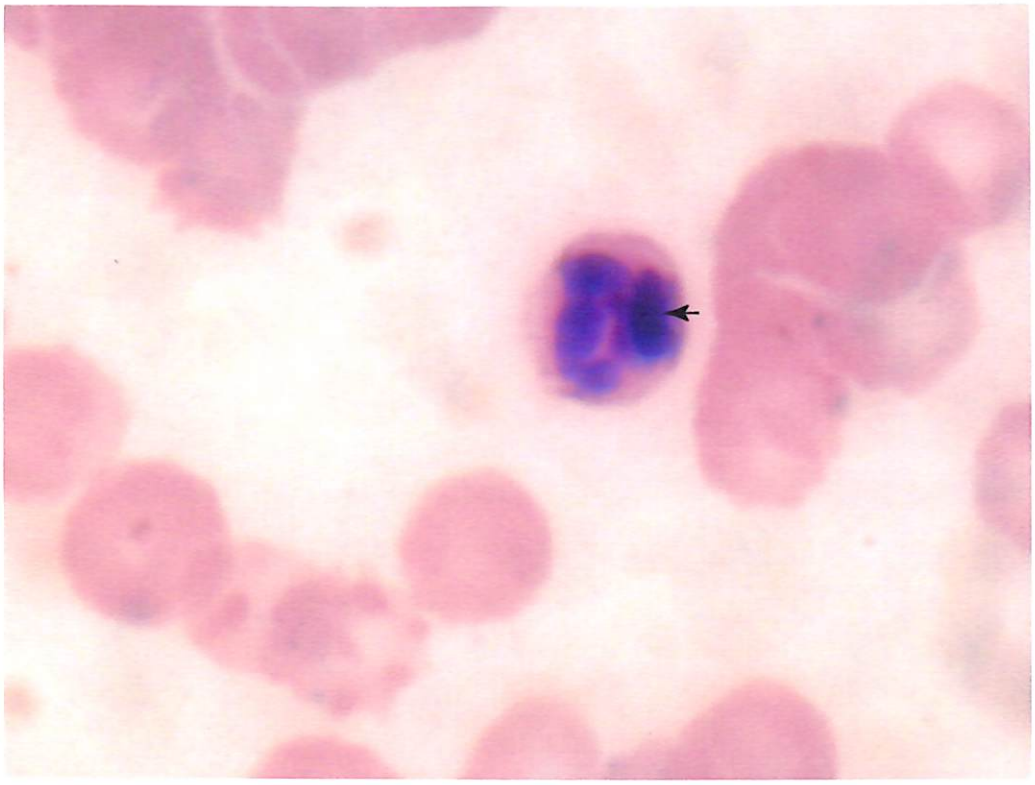


Fig.- 9 : Blood smear of a dog showing granulocytic ehrlichia (*Ehrlichia ewingii*), Giemsa stain, (X1000).

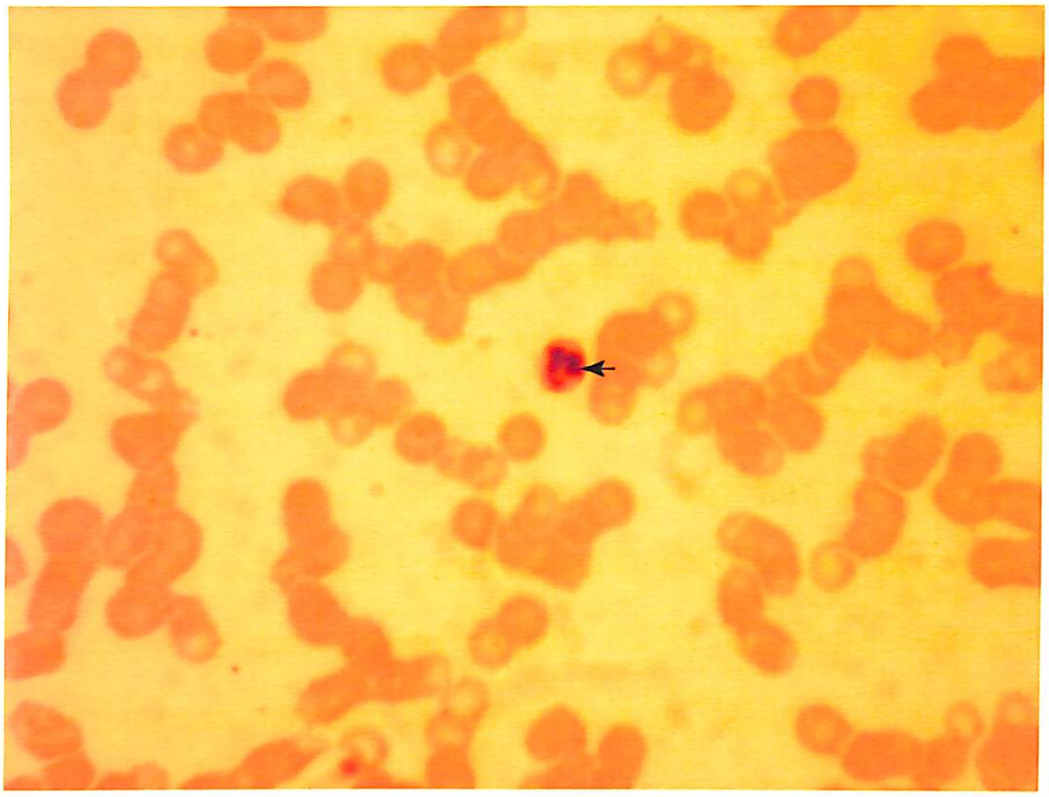


Fig.- 10 : Blood smear of a dog showing granulocytic ehrlichia (*Ehrlichia ewingii*), Giemsa stain, (X1000).

(91/94; 96.8%), high temperature that is more than 104^{0}F (94/94; 100%), ocular lesions (32/94; 34.04%), nervous signs (22/94; 23.40%), lymphadenopathy (43/94; 45.74%), pale mucus membrane (42/94; 44.68%), haemorrhage (52/94; 55;31%), weight loss (76/94; 80.85%), gastrointestinal signs (81/94; 86.17%), respiratory signs (62/94; 65.95%), tick infestation (86/94; 91.48), anaemia (76/94; 80.85), haemoglobinuria (20/94; 21/27%) and skin lesions (34/94; 36.17%).

For detailed haematological, clinicopathological, biochemical study and evaluating the therapeutic efficacy of commonly used drugs (Oxytetracycline and Doxycycline) against Canine Ehrlichiosis, 21 clinical cases of Canine Ehrlichiosis were selected and divided into three groups of seven animals each. First group was treated with Doxycycline @ 5 mg/kg body weight orally twice daily for 15 days. Second group was treated with Oxytetracycline @ 22 mg/kg. body weight I/V once daily for three days. Third group was kept as untreated control. The haematological study was performed twice a week, clinico-pathological observation was performed twice a day (morning and evening), biochemical study and observation of ehrlichial inclusions were done once a week starting from pretreatment period to 21 days post-treatment. The experimental design is presented in Table-1.

Haematological observations :

The haematological studies (Haemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leucocyte Count, Differential Leucocyte Count and Platelets) were done twice weekly starting from pretreatment period to 21 days post treatment.

Haemoglobin (Hb) : The blood haemoglobin value in dogs infected with Canine Ehrlichiosis on 0 day was ranged between 3.0 g per cent to 15.0g per cent (Table-12).

Analysis of variance for the effect of antibiotics on Hb (g%) in dogs suffering from Canine Ehrlichiosis have been presented in Table-10. The Table-10 revealed highly significant ($P<0.01$) effect of drugs on Hb percentage. Mean ± S.E. and range of Hb (g%) of dogs treated for Canine Ehrlichiosis have been presented in Table-13. The overall average estimates of Hb (g%) were found to be ranged from 8.21 ± 0.29 and 11.65 ± 0.29 in the Untreated Control and Doxycycline treated groups respectively. The overall mean values of Hb in the Control group was significantly ($P<0.01$) lowered (3.44 g%) than the Doxycycline treated group but did not differ significantly from Oxytetracycline treated group. Similar findings were also observed on 3rd, 7th, 10th and 14th day post-treatment periods but on 17th day and 21st day post-treatment periods the average estimates of Hb percentage in Doxycycline and Oxytetracycline treated groups have been increased significantly ($P<0.01$) over the Control.

Table – 10 : Analysis of variance for the effect of antibiotics (Doxycycline and Oxytetracycline) on haematological parameters in dogs suffering from Canine Ehrlichiosis.

Source of variation	d.f.	Haemoglobin		Packed Cell Volume		Platelets		Monocytes	
		MS	F	MS	F	MS	F	MS	F
Drug	2	3.87	92.13**	0.18	20.37**	36.67	58.81**	0.005	1.84NS
Day (Period)	6	0.09	0.69NS	0.01	0.93NS	7.03	10.32**	0.006	0.20NS
Error	138	0.04		0.01		0.68		0.003	

* = Significat at P<0.05

** = Significant at P<0.01

NS = Non significant

Table – 11 : Analysis of variance for the effect of antibiotics (Doxycycline and Oxytetracycline) on haematological parameters in dogs suffering from Canine Ehrlichiosis.

Source of variation	d.f.	Total Erythrocyte Count		Total Leucocyte Count		Neutrophils		Lymphocytes	
		MS	F	MS	F	MS	F	MS	F
Drug	2	12.25	7.29**	1.32	0.17NS	0.13	9.52**	0.07	17.47**
Day (Period)	6	1.62	0.96NS	0.05	0.01NS	0.03	2.34*	0.006	1.61NS
Error	138	1.68		7.37		0.01		0.004	

* = Significat at P<0.05

** = Significant at P<0.01

NS = Non significant

Table – 12 : Mean ± S.E. and Range of Haemoglobin of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment								
Doxycycline	9.25[a] ± 1.13 (4-15)	10.88[a] ± 0.78 (6-12)	11.25[a] ± 0.73 (6-12)	11.85[a] ± 1.63 (8-11)	12.14[a] ± 0.61 (9-12)	12.85[a] ± 0.50 (11-12)	13.34[a] ± 0.42 (11-12)	11.65[a] ± 0.29
Oxytetracycline	8.97[a] ± 1.03 (3-15)	9.11[b] ± 1.04 (3-9)	9.34[b] ± 0.96 (3-9)	9.42[b] ± 0.97 (6-9)	9.71[b] ± 0.94 (7-12)	9.94[b] ± 0.84 (7-10)	10.08[b] ± 0.85 (9-10)	9.51[b] ± 0.29
Control (Untreated)	8.67[a] ± 0.70 (3-14)	8.60[b] ± 0.71 (4-14)	8.34[b] ± 0.69 (4-13)	8.22[b] ± 0.70 (4-16)	8.11[b] ± 0.66 (5-12)	7.88[c] ± 0.59 (5-10)	7.62[c] ± 0.52 (5-12)	8.21[b] ± 0.29

Values with different superscripts (Column wise) differed significantly ($P<0.01$).

Vales in parenthesis are range.

2. Neutrophils :

In Table-11, analysis of variance for the effect of antibiotics on Neutrophils (%) in dogs suffering from Canine Ehrlichiosis has been presented. It revealed highly significant ($P<0.01$) effect of drugs on Neutrophil percentage. Mean ± S.E. and range of Neutrophils (%) of dogs treated for Canine Ehrlichiosis have been presented in Table-13. The values of Neutrophils on 0 day (Pre treatment period) varied widely. It ranged between 40-81 per cent. The average estimates of Neutrophils (%) were found to be ranged from 51.06 ± 1.67 to 61.04 ± 1.67 in the Untreated Control and Doxycycline treated groups respectively. The overall mean values of Neutrophils in the control group was significantly ($P<0.01$) lowered by 9.98% and 6.22% from Doxycycline and Oxytetracycline treated groups respectively. Similar findings were also observed on 3rd, 7th, 10th, 14th, 17th and 21st days post-treatment.

3. Monocytes :

Means along with their S.E. and range of Monocytes count in dogs treated for Canine Ehrlichiosis have been presented in Table-14. The average estimates of percentage of Monocytes were found to varied from 7.14 to 11.71 per cent on 0 day that can't be compared and overall percentage varied from 7.48 to 9.48 in Control and Doxycycline treated group respectively. However the analysis of variance did not reveal significant difference from each other (Table-10). The average estimates of Monocytes in Doxycycline and Oxytetracycline

Table – 13 : Mean ± S.E. and Range of Neutrophils of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment	49.85^{Aa}	53.28^{ABa}	57.57^{ABa}	64.42^{BCa}	66.00^{Ca}	68.00^{Ca}	68.14^{BCa}	61.04^{a}
Doxycycline	± 4.69	± 3.93	± 2.31	± 3.15	± 2.90	± 2.42	± 2.46	± 1.67
	(40-80)	(50-70)	(55-70)	(60-70)	(61-70))	(60-68)	(60-68)	
Oxytetracycline	50.57^{Aa}	52.28^{ABa}	55.57^{ABa}	58.57^{BCa}	61.14^{Ca}	61.28^{Ca}	61.57^{BCa}	57.28^{a}
	± 4.42	± 1.22	± 4.25	± 4.01	± 4.16	± 4.23	± 2.92	± 1.67
	(41-78)	(45-78)	(45-72)	(46-70)	(50-70)	(55-68)	(57-68)	
Control (Untreated)	49.28^{Aa}	49.42^{ABb}	50.85^{ABb}	51.85^{BCb}	52.00^{Cb}	51.71^{Cb}	52.28^{BCb}	51.06^{b}
	± 5.08	± 5.15	± 5.68	± 6.00	± 6.12	± 6.25	± 6.91	± 1.67
	(40-81)	(41-79)	(41-79)	(40-80)	(43-81)	(42-78)	(43-78)	

Values with different superscripts (Column wise - a, b, c and row wise – A, B, C) differed significantly.

Vales in parenthesis are range.

Table – 14 : Mean ± S.E. and Range of Monocytes of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment Doxycycline	11.71 ± 5.10 (4-13)	10.85 ± 4.19 (5-12)	9.85 ± 3.41 (6-11)	8.85 ± 2.28 (6-10)	8.42 ± 1.37 (6-9)	8.14 ± 0.96 (6-9)	8.57 ± 0.84 (7-9)	9.48 ± 0.78
Oxytetracycline	9.57 ± 2.95 (4-11)	9.42 ± 2.34 (6-11)	9.57 ± 2.29 (6-11)	8.85 ± 1.35 (6-10)	8.85 ± 1.10 (6-10)	8.85 ± 1.17 (6-10)	8.85 ± 1.12 (6-10)	9.14 ± 0.78
Control (Untreated)	7.14 ± 1.71 (3-10)	7.42 ± 1.36 (4-10)	7.85 ± 1.05 (4-11)	7.42 ± 0.75 (4-11)	7.57 ± 0.97 (3-10)	7.28 ± 1.08 (3-11)	7.71 ± 0.96 (4-11)	7.48 ± 0.78

treated groups were found to be decreased gradually with the advancement of post-treatment periods but the differences were non significant.

4. Lymphocytes :

Means along with their S.E. and range of Lymphocytes percentage of dogs treated for Canine Ehrlichiosis is presented in Table-15. The range varied between 7 to 32 per cent on 0 day (Pre treatment period). Analysis of variance for the effect of antibiotics on lymphocytes (%) has been given in Table-11 and it revealed highly significant ($P<0.01$) effect. The overall estimates of lymphocyte percentage were found to be ranged from 18.11 percent to 26.48 percent in the Untreated Control and Doxycycline treated group respectively. The overall mean value of lymphocytes was significantly ($P<0.01$) lowered by 8.37 percent and 4.95 percent from Doxycycline and Oxytetracycline treated group respectively. Similar findings were also observed on 7th, 10th, 14th, 17th and 21st day post therapy.

5. Total Erythrocyte Count (TEC) :

In table – 11, analysis of variance for the effect of drugs on TEC ($x10^6/\mu l$) in dogs suffering from Canine Ehrlichiosis has been presented. It revealed highly significant ($P<0.01$) effect of drugs on TEC. Mean ± S.E. and range have been presented in Table-16. The overall average estimates were found to be ranged from 4.18 ± 0.18 and 5.12 ± 0.18 in the Untreated and Doxycycline treated group respectively. The

Table – 15 : Mean ± S.E. and Range of Lymphocytes of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment								
Doxycycline	20.57[a] ± 3.99 (8-32)	23.42[a] ± 3.03 (15-32)	25.00[a] ± 2.29 (20-31)	27.42[a] ± 1.02 (25-30)	29.28[a] ± 0.86 (25-32)	29.71[a] ± 0.86 (25-30)	30.00[a] ± 0.78 (25-30)	26.48[a] ± 0.93
Oxytetracycline	20.42[a] ± 3.68 (7-28)	21.28[ab] ± 3.48 (10-27)	22.71[b] ± 2.77 (15-30)	23.00[b] ± 2.60 (20-30)	24.00[b] ± 2.48 (25-30)	24.28[b] ± 2.35 (25-30)	25.71[b] ± 1.75 (26-30)	23.06[b] ± 0.93
Control (Untreated)	19.28[a] ± 3.42 (8-30)	18.00[b] ± 2.49 (9-29)	18.57[c] ± 2.18 (10-30)	18.57[c] ± 2.39 (9-29)	18.42[c] ± 2.49 (9-30)	18.85[c] ± 2.33 (10-30)	19.28[c] ± 2.10 (10-30)	18.11[c] ± 0.93

Values with different superscripts (Column wise) differed significantly ($P<0.01$).

Vales in parenthesis are range.

Table – 16 : Mean ± S.E. and Range of Total Erythrocyte Count (TEC) of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	**3rd day**	**7th day**	**10th day**	**14th day**	**17th day**	**21st day**	**overall**
Treatment Doxycycline	4.33[a] ± 0.57 (1.25-6.12)	4.55[a] ± 0.49 (2.22-6.52)	4.74[a] ± 0.70 (2.81-6.84)	5.26[a] ± 0.44 (3.42-7.00)	5.52[a] ± 0.35 (3.41-7.21)	5.74[a] ± 0.33 (3.41-7.20)	5.74[a] ± 0.33 (3.6-7.20)	5.12[a] ± 0.18
Oxytetracycline	3.81[a] ± 0.58 (1.32-5.92)	4.03[a] ± 0.48 (1.89-5.94)	4.33[a] ± 0.48 (2.51-6.01)	4.37[b] ± 0.48 (3.14-6.52)	4.48[b] ± 0.50 (3.50-6.8)	4.61[b] ± 0.44 (3.5-6.8)	4.63[b] ± 0.44 (3.5-6.8)	4.36[b] ± 0.18
Control (Untreated)	4.22[a] ± 0.56 (1.40-6.01)	5.25[a] ± 0.57 (1.42-5.80)	4.28[a] ± 0.54 (1.52-6.00)	4.24[b] ± 0.53 (1.98-5.20)	4.16[b] ± 0.52 (1.98-5.20)	4.12[b] ± 0.53 (1.88-6.00)	3.98[b] ± 0.54 (1.88-6.00)	4.18[b] ± 0.18

Values with different superscripts (Column wise) differed significantly (P<0.01).

Vales in parenthesis are range.

overall mean values of TEC in the control groups was significantly ($P<0.01$) lower by 0.94 ($x10^6/\mu l$) in Doxycycline treated group but did not differ significantly from Oxytetracycline treated group. Similar findings were also observed on 10th, 14th, 17th and 21st day post-treatment. On 0, 3rd and 7th day the differences were non significant.

6. Total Leucocyte Count (TLC) :

Means along with their S.E. and range of TLC ($x10^3/\mu l$) of dogs treated for Canine Ehrlichiosis have been presented in Table-17. The average estimates of level of TLC were found to be ranged from 8.95 ($x10^3/\mu l$) to 9.17 ($x10^3/\mu l$) on 0 day and overall percentage ranged from 8.94 ± 0.38 and 9.22 ± 0.38 in Control and Doxycycline treated groups respectively. However the analysis of variance did not revealed significant difference from each other (Table-11).

7. Packed Cell Volume (PCV) :

Analysis of variance for the effect of antibiotics on PCV (%) in dogs suffering from Canine Ehrlichiosis have been presented in Table-10. it revealed highly significant ($P<0.01$) effect of drugs on PCV percentage. Mean ± S.E. and range of PCV (%) of dogs treated for canine ehrlichiosis have been presented in Table-18.

The overall average estimates of PCV value were found to be ranged from 13.04 ± 1.36 percent to 31.34 ± 1.36 percent in the Untreated Control and Doxycycline treated groups respectively. The overall mean values of PCV in the control

Table – 17 : Mean ± S.E. and Range of Total Leucocyte Count (TLC) of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment Doxycycline	9.17 ± 1.42 (1.25-12.56)	9.16 ± 1.13 (1.88-11)	9.33 ± 1.01 (2.82-11)	9.21 ± 0.99 (3.48-10)	9.21 ± 0.98 (4.02-10)	9.18 ± 0.94 (4.02-10)	9.26 ± 0.97 (5.2-10)	9.22 ± 0.38
Oxytetracycline	9.31 ± 0.87 (1.15-12.40)	9.26 ± 0.86 (1.92-12.0)	9.32 ± 0.88 (2.51-12)	9.11 ± 0.88 (3.25-11)	9.28 ± 0.75 (4.22-11)	9.21 ± 0.65 (4.22-10)	9.21 ± 0.65 (4.89-10)	9.24 ± 0.38
Control (Untreated)	8.95 ± 1.31 (1.35-12.92)	9.02 ± 1.34 (2.4-12.0)	9.03 ± 1.34 (2.5-12.12)	8.85 ± 1.30 (2.6-11.98)	8.94 ± 1.31 (3.00-11.92)	8.93 ± 1.33 (3.42-11)	8.88 ± 1.31 (3.92-11)	8.94 ± 0.38

Table – 18 : Mean ± S.E. and Range of Packed Cell Volume (PCV) of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment								
Doxycycline	22.42[a] ± 3.85 (8-52)	26.57[a] ± 3.68 (15-50)	29.28[a] ± 2.90 (25-50)	33.00 ± 2.89 (30-51)	34.57[a] ± 0.85 (32-50)	36.00 ± 2.60 (32-50)	37.57[a] ± 2.53 (34-52)	31.34[a] ± 1.36
Oxytetracycline	22.00[a] ± 4.20 (9-50)	23.71[ab] ± 1.24 (14-48)	25.00[b] ± 4.20 (20-50)	25.85[b] ± 4.04 (25-50)	26.71[b] ± 4.02 (30-48)	27.00[b] ± 3.99 (30-50)	28.00 ± 3.98 (30-50)	25.46[b] ± 1.36
Control (Untreated)	21.42[a] ± 4.06 (9-50)	20.57[b] ± 3.98 (10-51)	19.57[c] ± 3.44 (10-51)	18.42[c] ± 3.53 (9-49)	18.14[c] ± 3.40 (9-50)	17.71[c] ± 3.34 (9-50)	17.42[c] ± 3.28 (9-51)	13.04[c] ± 1.36

Values with different superscripts (Column wise) differed significantly ($P<0.01$).

Vales in parenthesis are range.

group was significantly (P<0.01) lowered by 18.30 percent and 12.42 percent from Doxycycline and Oxytetracycline treated groups respectively. Similar findings were also observed on 7th, 10th, 14th, 17th and 21st day post therapy.

8. Platelets :

In Table-10, analysis of variance for the effect of antibiotics on platelets ($x10^5/\mu l.$) in dogs suffering from canine Ehrlichiosis has been presented. It revealed highly significant (P<0.01) effect of drugs on level of platelets. Mean ± S.E. and range of platelets ($x10^5/ml$) of dogs treated for Canine Ehrlichiosis is presented in Table-19. The overall average estimates of platelets were found to be ranged from 1.19 ± 0.11 to 2.84 ± 0.11 in the Untreated Control and Doxycycline treated groups respectively. The overall mean values of platelets in control group was significantly (P<0.01) lowered by 1.65 ($x10^5/\mu l$) and 0.74 ($x10^5/\mu l$) than the Doxycycline and Oxytetracycline treated groups respectively. Similar findings were also observed on 21st day post-treatment. On 7th, 10th, 14th and 17th day post therapy, the number of platelets of Control group was significantly (P<0.01) lower by the Doxycycline and Oxytetracycline treated groups but there was no significant difference between the Doxycycline and Oxytetracycline treated groups.

Biochemical observations :

The biochemical studies (Total Serum Protein, Total Serum Albumin, Total Serum Globulin, Serum Albumin

Table – 19 : Mean ± S.E. and Range of Platelets of dogs treated for Canine Ehrlichiosis.

Particulars	Days							
	0 day	3rd day	7th day	10th day	14th day	17th day	21st day	overall
Treatment								
Doxycycline	0.99^{Aa} ± 0.29 (0.52-0.99)	1.72^{ABa} ± 0.16 (1.50-2.00)	2.32^{Ba} ± 0.27 (2.05-2.52)	2.84^{Ca} ± 0.28 (2.56-3.00)	3.74^{Da} ± 0.23 (3.32-4.22)	3.74^{Da} ± 0.84 (3.48-3.72)	4.56^{DEa} ± 0.13 (3.50-4.80)	2.84^{a} ± 0.11
Oxytetracycline	1.13^{Aa} ± 0.25 (0.62-1.12)	1.53^{ABa} ± 0.24 (1.23-1.62)	1.76^{ab} ± 0.24 (1.32-1.92)	1.94^{Cab} ± 0.24 (1.56-2.01)	2.22^{Dab} ± 0.25 (2.12-2.22)	2.37^{Dab} ± 0.22 (2.22-2.22)	2.56^{Deb} ± 0.18 (2.22-2.60)	1.93^{b} ± 0.11
Control (Untreated)	1.39^{Aa} ± 0.32 (0.63-1.27)	1.24^{ABa} ± 0.24 (0.72-1.26)	1.21^{b} ± 0.24 (0.63-1.27)	1.16^{Cb} ± 0.23 (0.67-1.25)	1.15^{Db} ± 0.23 (0.82-1.17)	1.11^{Db} ± 0.23 (0.71-1.02)	1.10^{Dec} ± 0.22 (0.62-1.10)	1.19^{c} ± 0.11

Values with different superscripts (Column wise - a, b, c and row wise – A, B, C) differed significantly.

Vales in parenthesis are range.

Table – 20 : Analysis of variance for the effect of antibiotics (Doxycycline and Oxytetracycline) on some biochemical parameters in dogs suffering from Canine Ehrlichiosis :

Source of variation	d.f.	Total Serum Protein		Total Albumin		Total Globulin		Albumin Globulin Ratio		Blood Urea Nitrogen	
		MS	F	MS	F	MS	F	MS	F	MS	F
Drug	2	5.86	2.401^{NS}	1.11	1.81^{NS}	2.85	2.83^{NS}	0.49	0.18^{NS}	2417.20	3.21*
Day (Period)	3	0.13	0.005^{NS}	0.12	0.19^{NS}	0.05	0.05^{NS}	2.12	0.78^{NS}	182.07	0.24^{NS}
Error	78	2.44		0.61		1.006		2.69		752.35	

* = Significant at $P<0.05$

NS = Non significant

Globulin Ratio (A:G ratio) and Blood Urea Nitrogen) were done once weekly starting from pretreatment period to 21 days post treatment.

1. Total Serum Protein (TSP) :

Means along with their S.E. and range of TSP of dogs treated for Canine Ehrlichiosis have been presented in Table-21. The average estimates of values of TSP (g/dl) were found to be ranged from 4.70 ± 0.40 in Control to 5.59 ± 0.96 in Doxycycline treated group on 0 day and overall values were ranged from 4.69 ± 0.29 to 5.50 ± 0.29 to 5.50 ± 0.29 in control and Doxycycline treated group respectively. However the analysis of variance did not reveal significant difference from each other (Table-20).

2. Total Serum Albumin (A) :

Mean ± S.E. and range Total Serum Albumin of dogs treated for Canine Ehrlichiosis is presented in Table-22. The overall values of Total serum albumin (g/dl) were found to be rnaged from 2.21 ± 0.14 and 2.52 ± 0.14 in Untreated and Doxycycline treated groups respectively. The analysis of variance table (Table-20) presented that there was no significant difference between the three groups. The average estimates of Total Serum Albumin was found to increased gradually in Group-I and Group-II on 21st day post-therapy.

3. Total Serum Globulin (G) :

Analysis of variance revealed that there was no significant difference between the three groups (Table-20).

Table – 21 : Mean ± S.E. and Range of Total Serum Protein (TSP) of dogs treated for Canine Ehrlichiosis.

Particulars	Days				
	0 day	7th day	14th day	21st day	overall
Treatment					
Doxycycline	5.59 ± 0.96 (2.84-8.28)	5.60 ± 0.95 (2.88-8.84)	5.67 ± 0.93 (3.21-8.92)	5.71 ± 0.93 (3.42-8.94)	5.50 ± 0.29
Oxytetracycline	5.46 ± 0.55 (2.56-8.85)	5.46 ± 0.54 (2.58-8.85)	5.48 ± 0.53 (2.92-8.90)	5.50 ± 0.53 (3.12-8.92)	5.47 ± 0.29
Control (Untreated)	4.70 ± 0.40 (2.72-8.42)	4.69 ± 0.41 (2.74-8.46)	4.69 ± 0.40 (2.76-8.46)	4.69 ± 0.40 (2.77-8.48)	4.69 ± 0.29

Table – 22 : Mean ± S.E. and Range of Total Serum Albumin (A) of dogs treated for Canine Ehrlichiosis.

Particulars	Days				
	0 day	**7th day**	**14th day**	**21st day**	**overall**
Treatment	2.31 ± 0.40	2.47 ± 0.36	2.58 ± 0.41	2.73 ± 0.40	2.52 ± 0.14
Doxycycline	(1.04-3.52)	(1.12-3.54)	(1.22-3.84)	(1.26-3.88)	
Oxytetracycline	2.51 ± 0.30	2.55 ± 0.29	2.60 ± 0.29	2.65 ± 0.29	2.58 ± 0.14
	(1.14-3.65)	(1.16-3.66)	(1.18-3.68)	(1.22-3.65)	
Control (Untreated)	2.22 ± 0.18	2.21 ± 0.19	2.20 ± 0.19	2.20 ± 0.19	2.21 ± 0.14
	(1.08-3.41)	(1.12-3.42)	(1.12-3.42)	(1.18-3.41)	

Table – 23 : Mean ± S.E. and Range of Total Serum Globulin (G) of dogs treated for Canine Ehrlichiosis.

Particulars	Days				
	0 day	7th day	14th day	21st day	overall
Treatment Doxycycline	3.26 ± 0.62 (1.65-5.20)	3.12 ± 0.60 (1.65-5.44)	3.09 ± 0.56 (1.5-5.80)	2.98 ± 0.53 (1.50-6.2)	3.11 ± 0.18
Oxytetracycline	2.95 ± 0.25 (1.70-5.19)	2.90 ± 0.25 (1.68-5.20)	2.88 ± 0.25 (1.62-5.8)	2.84 ± 0.25 (1.68-5.9)	2.89 ± 0.18
Control (Untreated)	2.48 ± 0.25 (1.78-5.21)	2.48 ± 0.24 (1.77-5.22)	2.49 ± 0.24 (1.77-5.20)	2.49 ± 0.23 (1.78-5.22)	2.48 ± 0.18

Mean ± S.E. and range of Total Serum Globulin (G) of dogs treated for Canine Ehrlichiosis is presented in Table-23. The overall average values of Total Serum Globulin (g/dl) were found to be ranged from 2.48 ± 0.18, and 3.11 ± 0.18 in Untreated Control and Doxycycline treated groups respectively. The average values on 0 day was ranged from 2.48 ± 0.25 to 3.26 ± 0.62 in Group III and Group I respectively. There was gradual decrease in the values of Total Serum Globulin in Doxycycline and Oxytetracycline treated groups from pre-treatment period to 21st day post-treatment.

4. Serum Albumin Globulin Ratio (A/G) :

Mean ± S.E. and range of A/G of dogs treated for Canine Ehrlichiosis is presented in Table-24. The average estimates of A/G were found to be ranged from 0.91 ± 0.05 and 0.79 ± 0.11 in Control and Doxycycline treated groups respectively on 0 day and overall values were ranged from 0.90 ± 0.03 and 0.87 ± 0.03 in Group III and Group I respectively. However the analysis of variance did not reveal significant difference from each other shown in Table-20. The average estimates of Serum Albumin Globulin Ratio (A/G) in Doxycycline and Oxytetracycline treated groups were found to be increased gradually with the advancement of post treatment period but the differences were non-significant.

5. Blood Urea Nitrogen (BUN) :

Analysis of variance for the effect of drugs on BUN (mg/dl) in dogs suffering from Canine Ehrlichiosis were

Table – 24 : Mean ± S.E. and Range of Serum Albumin Globulin Ratio (A/G) of dogs treated for Canine Ehrlichiosis.

Particulars	Days				
	0 day	7th day	14th day	21st day	overall
Treatment					
Doxycycline	0.79 ± 0.11 (0.32-0.82)	0.86 ± 0.09 (0.42-0.92)	0.89 ± 0.08 (0.42-0.92)	0.96 ± 0.06 (0.44-0.98)	0.87 ± 0.03
Oxytetracycline	0.84 ± 0.03 (0.34-0.86)	0.87 ± 0.02 (0.42-0.89)	0.90 ± 0.03 (0.42-0.91)	0.92 ± 0.03 (0.42-0.97)	0.88 ± 0.03
Control (Untreated)	0.91 ± 0.05 (0.37-0.89)	0.90 ± 0.05 (0.37-0.89)	0.89 ± 0.05 (0.37-0.89)	0.89 ± 0.05 (0.37-0.89)	0.90 ± 0.03

Table – 25 : Mean ± S.E. and Range of Blood Urea Nitrogen (BUN) of dogs treated for Canine Ehrlichiosis.

Particulars	Days				
	0 day	**7th day**	**14th day**	**21st day**	**overall**
Treatment					
Doxycycline	54.17^{a} ± 13.63 (10.90-110.22)	44.99^{a} ± 9.20 (12.00-80.22)	37.33^{a} ± 7.24 (15.41-40.81)	28.82^{a} ± 4.56 (15.92-22.50)	41.33^{a} ± 5.18
Oxytetracycline	55.94^{a} ± 13.88 (11.00-108.11)	52.27^{ab} ± 12.63 (15.82-85.41)	49.06^{b} ± 12.00 (15.91-45.82)	45.82^{b} ± 10.30 (15.94-23.21)	50.77^{ab} ± 5.18
Control (Untreated)	55.85^{a} ± 10.59 (11.88-109.21)	57.41^{b} ± 9.11 (12.21-100.81)	61.50^{c} ± 9.51 (13.91-100.21)	67.87^{c} ± 9.46 (13.92-99.21)	60.66^{b} ± 5.18

Values with different superscripts (Column wise) differed significantly (P<0.01).

Vales in parenthesis are range.

presented in Table-20. It revealed highly significant (P<0.01) effect of drugs on BUN level. Mean ± S.E. and range of BUN (mg/dl.) of dogs treated for Canine Ehrlichiosis is presented in Table-25. The overall average estimates of BUN level were found to be ranged from 41.33 ± 5.18 and 60.66 ± 5.18 in Doxycycline and Untreated Control group respectively. The overall mean values of BUN (mg/dl) in the Control group was significantly (P<0.01) higher by 19.33 (mg/dl) from Doxycycline treated group but did not differ significantly from Oxytetracycline treated groups.

Therapeutic Trial :

A clinical therapeutic trial with one dose regime of Doxycycline (@ 5mg./kg body weight twice daily orally for 15 days) and Oxytetracycline (@ 22 mg/kg body weight I/V once daily for 3 successive days) was conducted to compare their therapeutic efficacy in the clinical management of Canine Ehrlichiosis.

Evaluation of efficacy of these treatments were based on cytology (Table-26) which revealed that, though, there was progressive decline in positivity in blood/buffy coat smear examination in the two groups on day 7, 14 and 21; ehrlichial inclusions were still persisted in 42.85 per cent and 57.14 per cent cases in Group I and Group II respectively despite clinical recovery indicating that Doxycycline and Oxytetracycline were unable to provide complete parasitological recovery.

Table – 26 : Therapeutic efficacy of drugs used in case of Canine Ehrlichiosis based on blood and buffy coat smear examination.

Group No.	Drugs used with dose	No. of cases showing Ehrlichial inclusions after treatment			
		0 day	7th day	14th day	21st day
I	Doxycycline @ 5mg/kg orally b.i.d. for 15 days	7 (100%)	6 (85.71%)	5 (71.42%)	3 (42.85%)
II	Oxytetracycline @ 22 mg/kg I/V s.i.d. for 3 days	7 (100%)	6 (85.71%)	6 (85.71%)	4 (57.14%)
III	Untreated Control	7 (100%)	7 (100%)	7 (100%)	7 (100%)

The clinico-pathological observations were recorded twice a day (morning and evening) starting from pre-treatment period to 21st day post-treatment. All the clinical alterations were reduced after therapy.

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CHAPTER - V

DISCUSSION

Ramakrishnan, 1982; Thilagar *et al.*, 1990; Thirunavukkarasu *et al.*, 1993; Tresamol *et al.*, 1998; Harikrishnan *et al.*, 2001; Manigandan *et al.*, 2003 and Lakshmanan *et al.*, 2006). In Northern parts of India, Literature regarding Canine Ehrlichiosis is very scanty (Juyal *et al.*, 1992; Jain and Gupta, 1997; Varshney and Dey, 1998; Ashuma *et al.*, 2004 and Kumar and Varshney, 2006).

Concurrent infection of Canine Ehrlichiosis with *Hepatozoon canis* (Elias and Homans, 1988; Juyal *et al.*, 1992; Ananda *et al.*, 2005, Smitha *et al.*, 2003), *Babesia* spp. (Plessis *et al.*, 1990; Klag *et al.*, 1991; Matthewman *et al.*, 1993; Varshney and Dey, 1998; Gothe, 1999), *Leptospira* spp. (Nambi *et al.*, 2000), *Trypanosoma* spp. (Ram prabhu *et al.*, 2001), *Leishmania* spp. and *Dirofilaria immitis* (Gothe, 1999) and strain or species variation of Ehrlichia (Harrus *et al.*, 1997a) leads to variation in clinical sings which makes the diagnosis confusing and treatment unrewarding. Therefore the present study was done to study the epidemiological significance of the blood protozoan diseases in dogs, to study the clinico-haematological and biochemical alterations in the host due to Canine Ehrlichiosis and to study the comparative efficacy of Doxycycline and Oxytetracycline aginst Canine Ehrlichiosis.

Prevalence :

Blood samples from dogs were screened for protozoan parasites round the year at the Department of Parasitology,

DISCUSSION

There are numerous parasites of dogs which are of major concern for the Veterinary Profession, however amongst them blood protozoan parasite constitute special attention as they are widely distributed and may terminate to be fatal if not diagnosed and treated properly. The major blood protozoa of canines are *Trypanosoma* spp., *Leishmania* spp., *Hepatozoon* spp., *Babesia* spp., *Haemobartonella* spp. and *Ehrlichia* spp. (Soulsby, 1982).

The occurrence of blood protozoa infection is mainly related to availability of vectors, which is influenced by the climatic factors and vary according to geographical areas i.e. latitude, longitude and attitude.

Blood protozoan diseases are recognized as emerging disease as they have been found occurring in a greater magnitude among animals with exotic breeds particularly (Bhaskarao *et al.*, 1986).

Canine Ehrlichiosis is a rickettsial disease of canids, transmitted by the Brown Dog Tick, *Rhipicephalus sanguineus* (Groves *et al.*, 1975). It was first recognized in ticks infested dogs in Algeria (Donatien and Lestoquard, 1935). In India the disease was first of all identified in Madras (Mudaliar, 1944).

Detailed investigation on Canine Ehrlichiosis in India has been studied for the first time in southern parts of India (Raghavachari and Reddy, 1958; Murli Manohar and

Bihar Veterinary College, Patna on the basis of cytological examination of peripheral blood smear or buffy coat smears.

The percentage of infection in each month of the year was not constant (Table-2). There was month to month variation and the overall prevalence of blood protozoan infection was 46.22 per cent. During the month of June, July, August and September there was 60.20 per cent infection. During this period the average maximum relative humidity was ranged from 75.51 to 84.68 per cent. In the month of May (37.20^{0}C) the rate or prevalence of the infection was minimum (23.52%) and during this period there was highest ambient temperature (37.20^{0}C), the infection was highest in the month of July (83.33%). In this month the average relative humidity was maximum i.e. 84.68% and the average rainfall was also maximum i.e. 9.07 mm.

Regarding the season, the maximum prevalence was recorded during the monsoon (June to August) season i.e. 69.23 per cent infection while the minimum was recorded in the summer season (March to May) i.e. 29.68% infection as shown in the table-3.

Bansal *et al.* (1985) and Samradhni *et al.* (2003) reported high incidence of blood protozoan in dogs during monsoon and post monsoon season from Haryana and Nagpur respectively. Bhaskarao *et al.* (1986) reported the prevalence of haemoprotozoan infection frequently between May to September in Andhra Pradesh.

In the present study, monsoon season appeared more conducive to prevalence followed by post-monsoon, winter and summer season. The differences observed in respect to seasons were found highly significant (P<0.01).

The infection being vector borne in nature, is directly proportional to the activity of ticks. In the monsoon season, there is increased tick activity due to substantial rainfall and high moisture and humidity (Soulsby, 1982), thus the infection rate being more in monsoon season. On the contrary, during summer season, the tick activity is markedly reduced due to high temperature and dry climate causing low infection during this period.

Regarding the age, the infection below 1 year, 1-2 years, 2-4 years, 4-8 years and above 8 year was 69.09, 58.33, 40.00, 24.00 and 18.18 per cent respectively as shown in Table-4. The effect of age on the prevalence of blood protozoan was found highly significant (P<0.01). The maximum prevalence was found below one year of age and minimum prevalence was found above 8 years of age. The maximum prevalence of infection in younger dogs is in agreement with the findings of Ewing, 1969; Thirunavukkarasu *et al.*, 1993; Latha and Joseph, 1994; Chandrasekar *et al.*, 2002; Samradhni *et al.*, 2003 and Lakshmanan *et al.*, 2006. However according to Nims *et al.*, 1971; Murli Manohar and Ramakrishnan, 1982 and Harikrishnan *et al.*, 2001a; there was no specific susceptible age group of dogs for Canine Ehrlichiosis.

In the present study, it could be interpreted that since the immune system is undeveloped in younger dogs as compared to the adults (Tizard, 1992) and there is low transfer of maternal immunity to new born (Roitt and Delves, 1992), these might be the reasons for more prevalence of infection in dogs below one year age. There is also a general truth that young animals of all kinds are more susceptible to the effect of parasites (Lapage, 1956).

The data presented on sexwise prevalence of blood protozoan parasites shows that males were slightly more susceptible (48%) to infection than the females (44.64%). But there was no significant variation in prevalence of infection in male and female dogs. These are in agreement with the findings of Nims *et al.*, 1971; Waddle and Littman, 1988; Thirunavukkarasu *et al.*, 1993; Latha and Joseph, 1994; Harikrishnan *et al.* (2001a) and Manigandan *et al.*, 2003. This may be due to the effect of androgen hormone in males which makes them more prone to infection than females.

The data presented on the prevalence of haemoprotozoan in different breeds of dogs was found to be significant ($P<0.05$). German shepherd (61.22%) and Spitz (56.09%) were highly affected breeds followed by non-descript (44.44%), Labrador (40.0%), other exotic breeds (37.5%), Dalmation (25.0%) and Doberman (10%). German shepherd has been reported to be more susceptible and more severly affected (Nyindo *et al.*, 1980; Thirunavukkarasu *et al.*, 1993; Harrus *et al.*, 1997a and

Lakshmanan *et al.*, 2006. Different studies have been reported on variations in breedwise prevalence of ehrlichiosis in dogs (Murli Manohar and Ramakrishnan, 1982; Chandrasekar *et al.*, 2002 and Manigandan *et al.*, 2003) possibly because of variations in the population of different breeds in the area.

In the present study more susceptibility of German shepherd and Spitz to infection may be due to more hairy coat which makes the breeds more susceptible to ticks which is the vector of the organism.

On the examination of pheripheral blood and/or buffy coat smear (Harrus *et al.*, 1998d) of dogs showing the clinical symptom/symptoms only 79 (37.26% were found positive for Canine Ehrlichiosis (morule or initial bodies) in blood smear examination while in buffy coat smear examination, cases were positive in 94 dogs (44.33%) as shown in Table-6. The present finding seem to be in agreement with the observations of Elias (1991), Thirunavukkarasu *et al.* (1994a) and Kumar and Varshney (2006). So cytological examination of buffy coat smear appear to be more diagnostic for ehrlichiosis than peripheral blood smear.

Morulae of granulocytic ehrlichia (Fig.-5, 6 and 7) and *Ehrlichia canis* (Fig.- 8, 9 and 10) were found in neutrophils and monocytes respectively. Similar observations were also made by Ewing (1971) and Kumar and Varshney (2006).

The variability of infection in types of blood protozoan in dogs in and around Patna revealed that prevalence of *Ehrlichia*

ewingii was maximum (57.14%) followed by *Ehrlichia canis* (34.69%), multiple ehrlichial infection (4.08%) and *Babesia canis* (4.08%). Juyal *et al.* (1994) reported 8.89 per cent *Babesia* spp., 1.42 per cent, *Hepatozoon canis* and 0.35 per cent *Ehrlichia canis* infection in Punjab. Das *et al.* (1998) reported 6.81 per cent *Trypanosoma evansi*, 2.27 per cent *Haemobartonella* spp. and 2.22 per cent *Ehrlichia canis* infection in Pondicherry. Bansal *et al.* (1985) found 5.4 and 2.7 per cent dogs to be positive for *Babesia canis* and *Hepatozoon canis* infection respectively from different parts of Haryana and New Delhi.

Rajamanickam *et al.* in 1985, reported prevalence of *Babesia gibsoni, Hepatozoon canis, Babesia canis, Ehrlichia canis* and *Trypanosoma evansi* in canines in Malaysia as 17.7, 1.20, 1.1, 0.20 and 0.10 per cent respectively.

The environmental factors prevailing at different geographical locations being variable which is responsible for development of the vector (tick) which regulate the occurrence of infection.

From previous studies, conducted in different parts of the country it was observed that *Ehrlichia canis* is more prevalent in India (Samradhni *et al.*, 2003; Lakshmanan *et al.*, 2006; Kumar and Varshey, 2006). But in the present study, it was found that occurrence of granulocytic *Ehrlichia* i.e. *Ehrlichia ewingii* is more in the area of Patna (Bihar). There were limited cases of *Babesia canis*. Multiple ehrlichial infection was also

observed in dogs agreeing with the findings of Bauer (1995); Meinkoth *et al.* (1998); Breitschwerdt *et al.* (1998b); Pusterla *et al.* (1998); Kordic *et al.* (1999); Hua *et al.* (2000), Suksawat *et al.* (2001) and Kumar and Varshney (2006).

Naturally occurring cases of Canine Ehrlichiosis were manifested by wide variety of non specific and vague clinical manifestations. They were anorexia, high temperature, ocular lesions, nervous signs, lymphadenopathy, pale mucous membrane, haemorrhage, weight loss, gastrointestinal signs, tick infestation, anaemia, haemoglobinuria and skin lesions (Table-9). This was also observed by many workers (Troy *et al.*, 1980; Van Heerden, 1982; Price *et al.* 1987; Waddle and Littman, 1988; Thirunavukkarasu *et al.*, 1994a and Kumar and Varshney, 2006).

The large variations in clinical signs seem to be due to a number of factors including differences in pathogenicity between strains of *Ehrlichia*, breeds of dog, co-infections with other ehrlichial species, concurrent infection with other diseases and immune status of the dog. The physical findings which generated the highest index of suspicion for ehrlichiosis included high temperature (>104^{0}F) not responding to commonly used antibiotics, tick infestation, peripheral lymphadenopathy, weight loss, depressed condition, pale mucosa and bleeding tendencies. The clinical signs of dogs with single infection are well characterized (Troy *et al.*, 1980

and Waddle and Littman, 1988) but remain ambiguous in concurrent infections.

Gastrointestinal signs (vomition, constipation or diarrhoea) were signs in cases of Canine Ehrlichiosis as also been observed by Thirunavukkarasu *et al.*, 1994a; Harrus *et al.*, 1997b and Kumar and Varshney, 2006.

Haemorrhages were manifested as epistaxis (nasal bleeding) as shown in Fig.-3. Hemoptysis were also found. But epistaxis was most common (Troy *et al.*, 1980; Kumar and Varshney, 2006). Present observation of haemorrhage in 55.31 percent cases of ehrlichiosis with or without concurrent infection was higher than the earlier reports (Kuehn and Gaunt, 1985; Thirunavukkarasu *et al.*, 1994a and Kumar and Varshney, 2006) and seems attributable to thrombocytopenia (Harvey *et al.*, 1978; Abeygunawardena *et al.*, 1990; Juyal *et al.*, 1993; Tresamol *et al.*, 1998; Davoust *et al.*, 1991; Woody and Hoskins, 1991; Ristic and Holland, 1993 and Harrus *et al.*, 1996a) and the deposition of immune complexes on vascular wall (Reardon and Pierce, 1981).

Corneal opacity (Fig.-4) and conjunctivtis were the ocular lesions found in the present cases of ehrlichiosis are same as the findings of Thirunavukkarasu *et al.* (1994b); Harrus *et al.* (1998a); Martin (1999); Gould *et al.* (2000) and Kumar and Varshney (2006).

Nervous signs observed in dogs suffering from ehrlichiosis were depression, ataxia, hyperaesthesia, posterior

paresis and convulsions either alone or in combinations were similar with the findings of Troy *et al.* (1980), Van Heerden, (1982), Price *et al.* (1987), Thirunavukkarasu *et al.*, 1994a, Harrus *et al.* (1997b) and Kumar and Varshney (2006).

In the present haematological studies done in the cases of Canine Ehrlichiosis, the values of Hb, PCV, TEC, TLC, Differential count of White Blood Cells and Platelets count were quite variable from mild anaemia to severe anaemia; mild leukopenia to leukocytosis; lymphopenia to lymphocytosis and mild thrombocytopenia in acute stage to severe pancytopenia in chronic stage (Huxsoll *et al.*, 1970; Pierce, 1971; Buhles *et al.* 1974; Kuehn and Gaunt, 1985; Davoust *et al.*, 1996). The anaemic changes (lower values of Hb, PCV and TEC) were significantly ($P<0.01$) well marked in cases of Canine Ehrlichiosis which agrees with the findings of Matthewman *et al.* (1993); Chou (1995) and Davoust *et al.* (1996). The investigations done by Murli Manohar and Ramakrishnan, 1982; Kuehn and Gaunt, 1985; Davoust *et al.*, 1996; Salakij *et al.* 1999; Samradhni *et al.*, 2003; Kumar and Varshney, 2006 shows that the total leukocyte count (1.15 – 12.92 x $10^3/\mu l$), neutrophil (40-81%) and lymphocytes (7-28%) were highly variable. The variations in the values of White Blood Cells could be attributed to different stages of the disease when the ailing dogs were examined.

In the present study the platelet count is significantly ($P<0.01$) lower which is similar with the findings of Huxsoll *et*

al. (1970); Kuehn and Gaunt (1985); Collet *et al.* (1987); Koutinas *et al.* (1989); Abeygunawardena *et al.* (1990); Magnarelli *et al.* (1990); Davoust *et al.* (1991); Woody and Hoskins (1991); Ristic and Holland (1993); Waner *et al.* (1995); Harrus *et al.* (1996a) due to decreased circulating half life of platelets (Smith *et al.*, 1974), increased platelets destruction (Smith *et al.*, 1975; Pierce *et al.*, 1977; Abeygunawardena *et al.*, 1990), Platelets dysfunction (Lovering *et al.*, 1980; Harrus *et al.*, 1996b) or production of antiplatelets antibodies (Waner *et al.*, 1995; Harrus *et al.*, 1996a).

After treatment the haematological values on 21st day come within the range of normal values.

Hypoalbuminemia and hyperglobulinemia were the most common variations in serum protein were found in the present cases of Canine Ehrlichiosis (Troy *et al.*, 1980; Murli Manohar and Ramakrishnan, 1982; Davoust *et al.*, 1991; Smitha *et al.*, 2003; Kumar and Varshney, 2006). The levels of total serum protein were quite variable ranging between 2.56 to 8.85 on pre-treatment period. The level of total serum protein were found normal (Murli manohar and Ramakrishnan, 1982; Chou, 1995), low (Buhles *et al.*, 1974); or high (Price *et al.*, 1987; Harrus *et al.*, 1996c) levels. Similar trend was observed in value of Albumin, Globulin and Albumin-Globulin ratio. The variations in the level of protein depends on the severity or stage of the disease, concurrent infection, intake of protein (Harrus *et al.*, 1997c), blood loss, oedematous inflammatory

fluid (Woody and Hoskins, 1991), proteinuria (Codner and Maslin, 1992) and liver status (Reardon and Pierce, 1981).

The value of mean Blood Urea Nitrogen in infected dogs varied ranging between 10.9 to 110.22 mg/dl in the pre-treatment period. However, Kuehn and Gaunt, 1985; Thirunavukkarasu *et al.*, 1994a observed very high BUN values in dogs infected with ehrlichiosis.

Powers and Meister, (1988), hypothetised the higher value of BUN may be due to increased catabolism owing to prolonged anorexia, dehydration and / or may be due to gastrointestinal haemorrhage leading to reduced glomerular filtration rate (Center, 1994).

After treatment the level of all the biochemical indices came within normal range in the present study.

Tetracycline or Oxytetracycline (Amyx *et al.*, 1971; Davidson *et al.*, 1978; Heerden *et al.*, 1979; Oqunkoya *et al.*, 1985 and Samradhni *et al.*, 2003) and Doxycycline (Heerden *et al.*, 1979; Egenvall *et al.*, 1997; Jain and Gupta, 1997; Breitchwerdt *et al.*, 1998a and Smitha *et al.*, 2003) with different dose schedules have been reported of clinical value in the control of Canine Ehrlichiosis. The present therapeutic trial was undertaken to compare the therapeutic efficacy of Doxycycline (@ 5 mg/kg. body weight once daily orally for 15 days) and Oxytetracycline (@ 22 mg./kg. body weight once daily I/V for 3 successive days). It was observed that in both

the treatments, the dogs started to show disappearance of clinical symptoms.

Iqbal and Rikihisa (1994b) reported the failure of doxycycline to eliminate *E. canis* from blood and tissues besides clinical recovery of animals. Harrus *et al.* (1998c) also reported that a six week doxycycline treatment may not be sufficient to clear *E. canis* parasite from all subclinically infected dogs.

In the present study, the ehrlichial inclusions were examined once a week for 3 weeks for evaluating the therapeutic efficacy of both the drugs. On 7th, 14th and 21st day post therapy, ehrlichial inclusions were found in 85.71, 71.42 and 42.85 per cent cases respectively in Doxycycline treated dogs and in 85.71, 85.71 and 57.14 per cent cases respectively in Oxytetracycline treated group. In the untreated control it was found in cent per cent cases (Table-26).

So, despite clinical recovery ehrlichial inclusions were still persisted after treatment either with Doxycycline or Tetracycline. Similar observations were also reported by Iqbal and Rikihisa (1994) and Harrus *et al.* (1998b). The present study concludes that Doxycycline is superior than Oxytetracycline in the clinical management of Canine Ehrlichiosis which is similar with the findings of Heerden and Immelman (1979).

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CHAPTER - VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present research work was carried in the Department of Parasitology, Bihar Veterinary College, Patna to search out the prevalence of blood protozoan parasitic infection of dogs in and around Patna and identification of clinical cases of Ehrlichiosis and investigation of the clinico-pathological parameters like hematology and blood biochemistry in the infected animals. Selected therapeutic trials were also conducted for evaluating the therapeutic efficacy of Doxycycline and Oxytetracycline.

Out of 212 febrile cases examined, 98 (46.22%) dogs harboured blood protozoan infection.

Month wise prevalence showed that highest infection was in the month of July (83.33%) and lowest in May (23.52%).

The seasonal studies showed that the prevalence of infection was 69.23, 48.88, 31.57 and 29.68 per cent in Monsoon, post monsoon, winter and summer season respectively. So the prevalence of infection was significantly more in monsoon followed by post monsoon, winter and summer season.

The severity of infection in dogs below one year, one to two years, two to four years, four to eight years and above eight years age group of dogs was 69.09, 58.33, 40.00, 24.00 and 18.18 per cent respectively. So, the younger dogs harboured significantly more infection than the adult ones.

The percentage of infection in male and female dogs was 48.00 and 44.64 per cent respectively. The infection was non-significantly more in males than in female dogs. Sex ratio was 1.075.

Cytological examination of buffy coat smears was found superior (44.33%) to peripheral blood smears (37.26%) in the diagnosis of Canine Ehrlichiosis. But the difference was found non-significant.

The disease was significantly more in German shepherd (61.22%) followed by Spitz (56.09%), Doberman (10.00%), Labrador (40.00%), Dalmation (25.00%), other exotic breeds (37.50%) and non descript dogs (44.44%).

Out of 98 positive cases, 56 (57.14%) had *Ehrlichia ewingii*, 34 (34.69%) had *Ehrlichia canis*, 4 (4.08%) had multiple ehrlichial infection and 4 (4.08%) had *Babesia canis* infection.

The naturally occuring cases of ehrlichiosis were manifested by wide variety of clinical signs (anorexia, high temperature, ocular lesions, nervous signs, lymphadenopathy, pale mucous membrane, haemorrhage, weight loss, gastrointestinal signs, respiratory signs, tick infestation, anaemia, haemoglobinuria and skin lesions).

Haematological values seemed to be influenced by the stage of the disease and concurrent infections. The values were quite variable from mild anaemia to severe anaemia; mild leukopenia to leukocytosis; lymphopenia to lymphocytosis and

mild thrombocytopenia in acute stage to severe pancytopenia in chronic stage. The total leukocyte count, neutrophil and lymphocyte were highly variable could be attributed to different stages of the disease when the ailing dogs were examined. The platelet count was significantly ($P<0.01$) lower.

Hypoalbuminemia and hyperglobulinemia were most common variations in serum protein. The level of total serum protein were quite variable. Similar trend was also observed in value of Albumin, Globulin and Albumin-Globulin ratio. The value of Blood Urea Nitrogen in infected dogs varied widely. In some cases the level was so high.

So it can be concluded that the haemato-biochemical indices were more influenced by the stage of the disease, concurrent infections and nutritional status and do not seemed to of specific diagnostic value. After treatment the level of all the haematological and biochemical indices came within normal range.

In both the treatments (Doxycycline @ 5mg/kg body weight twice daily for 15 days, orally and Oxytetracycline @ 22 mg/kg body weight once daily I/V for 3 successive days), the dogs started to show disappearance of clinical symptoms. But the cytological examination done on 7th, 14th and 21st day post therapy revealed ehrlichial inclusions in 85.71, 71.42 and 42.85 per cent respectively in Doxycycline treated group and 85.71, 85.71 and 57.14 per cent respectively in Oxytetracycline treated group.

So despite clinical recovery, ehrlichial inclusions were still persisted after treatment. The present study also shows that Doxycycline is superior than Oxytetracycline in the clinical management of Canine Ehrlichiosis.

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CHAPTER - VII

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