CLINICO-HAEMATO-BIOCHEMICAL AND THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
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VETERINARY MEDICINE
(Minor Subject: Veterinary Parasitology)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"Clinico - haemato - biochemical and therapeutic studies on ehrlichiosis in dogs"** submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Medicine** (Minor Subject: **Veterinary Parasitology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Manasa R. Kottadamane (L-2013-V-33-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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ABSTRACT

The present study was conducted to study the prevalence, clinico-haemato-biochemical changes and therapeutic management of ehrlichiosis in clinical cases of dogs presented at Small Animal Clinics of GADVASU, Ludhiana, Punjab (India). Overall prevalence of canine ehrlichiosis recorded among the presented cases by blood smear examination was 7.74 per cent, serology was 86.90 per cent and PCR was 78.57 per cent. Main clinical signs were congested/pale mucus membrane, fever, anorexia, melena, epistaxis, weakness, lethargy and tick infestation, hind limb weakness respiratory distress, corneal opacity, seizures, oedema of legs, bleeding tendencies and petechieal haemorrhages. Main haemato-biochemical findings were anaemia, thrombocytopenia, leucocytosis, lymphopenia, eosinophilia, hypercreatinemia, hyperglobulinemia, hypoalbuminemia, decrease in albumin & globulin ratio and increase in AST, ALT and ALKP activity. Dogs positive for *Ehrlicha canis* were divided into two groups and were treated with oral doxycycline for a period of 21 days and injectable tetracycline for a period of 5 days followed by oral combination of doxycycline upto 21 days. After treatment, significant increase in the mean values of Hb, PCV, platelets and significant decrease in the mean values of eosinophils, ALT, ALKP and Creatinine were recorded. Blood smear examination was found to be less sensitive technique when compared to PCR and serology. Serology is more sensitive and less time consuming technique and it can be used by clinician in clinics for early diagnosis of disease. Most of the cases treated with oral doxycycline were still positive by PCR on 15 day but negative after 21 day post treatment as compared to injectable tetracycline. Hence affected dogs should be treated up to 21 days. Oral doxycycline showed highest therapeutic efficacy as compared to injectable tetracycline.

Keywords: Ehrlichiosis, dog, PCR, serology, doxycycline, tetracycline.

Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

%	:	Per cent
<	:	Less than
>	:	More than
±	:	Plus minus
ºC	:	Degree Celsius
@	:	At the rate of
ALKP	:	Alkaline phosphatase
ALT	:	Alanine transaminase
AST	:	Aspertate transaminase
APA	:	Anti-platelet antibodies
BC		Buffy coat
BM	:	Bone marrow
BUN	:	Blood urea nitrogen
bp	:	Base pair
CBC	:	Complete blood count
CCI	:	Cell culture isolation
CE	:	Canine ehrlichiosis
CC r-I		Cell culture re-isolation
CGE	:	Canine granulocytic ehrlichiosis
CH	:	Chemiluminescent hybridization
CME	:	Canine monocytic ehrlichiosis
CRP	:	C reactive protiens
DLC	:	Differential luecocyte count
DNA	:	Deoxyribo Nucleic Acid
Ec-PCR	:	*E. canis* species specific PCR assay
nEc-PCR	:	*E. canis* nested PCR assay
EDTA	:	Ethylene diamine tetra acetic acid
EI	:	Experimentally infected
EIA	:	Enzyme immuno assay
ELISA	:	Enzyme linked immunosorbent assay
ESI-MS	:	Electrospray ionization mass spectrometry
et al	:	and others
g/dl	:	Gram per deciliter

Hb	:	Haemoglobin
HPLC	:	High-performance liquid chromatography
IFAT	:	Indirect fluorescent antibody technique
Ig M/G	:	Immunoglobulin M/G
IU/L	:	International units per liter
Kg	:	Kilogram
LN	:	Lymphnode
MCHC	:	Mean corpuscular haemoglobin concentration value
MCV	:	Mean corpuscular value
mg/L	:	Milligram per liter
Min.	:	Minute(s)
ml	:	Millilitre
mM	:	Millimolar
MPFC	:	Multiparametric flow cytometric
NI	:	Naturally infected
no.	:	Number
nPCR	:	nestedPCR
OIFs	:	Oil immersion field’s
PB	:	Peripheral blood
PCR	:	Polymerase chain reaction
PCV	:	Packed cell volume
P-D	:	Short term culture
PE	:	Post exposure
PI	:	Pre and post infection
pmol	:	Picomolar
PMIF	:	Platelet migration inhibition factor
RBC	:	Red blood cell
RLB	:	Reverse line blot
rRNA	:	ribosomal RNA
rpm	:	Revolutions per minute
rMAP2	:	Recombinant major antigenic protein 2
SAC	:	Small animal clinic
SH	:	Southern hybridization
sec	:	Second(s)

TAE	:	Tris-acetate EDTA
TCP	:	Tropical canine pancytopaenia
TEC	:	Total erythrocyte count
TLC	:	Total luecocyte count
TPP	:	Total plasma protien
TVCC	:	Teaching veterinary clinical complex
U/L	:	Units per liter
UV	:	Ultraviolet
viz	:	Videlecit
WBC	:	White blood cells
WE	:	Witness ehrlichia
WI	:	Western Immunobloting
µg/ml	:	microgram per millilitre
µl	:	Microliter
µM	:	Micromlar

CHAPTER I

INTRODUCTION

Dogs have taken the centre of attraction of almost all class of people under the nick name “Man’s Best Friend” as they perform many important activities for humans such as hunting, herding, pulling loads, protection, assisting police and military, companionship and many including aiding handicapped individuals. Loyalty, intelligence, devotion and affection are incredibly rewarding. Owning a dog can raise spirits and engender a sense of well-being like almost nothing else. The number of canine patients is increasing day by day in the veterinary hospitals owing to increased concern and attachment of the owners for alleviation of sufferings of their beloved pets.

Among the various haemoprotozoan diseases suffered by dogs, canine ehrlichiosis has been reported to be the most prevalent in Punjab, India. Over the past several decades, tick-borne diseases caused by obligate intracellular organisms have emerged as important threats to mammals worldwide, and have gained notoriety because of growing concern that changing climate conditions may favour vector-borne disease transmission. Canids not only become clinically affected with specific ehrlichiae and rickettsiae, but may also serve as reservoir hosts for organisms that cause disease in humans.

Species that are able to produce infection in dogs are *Ehrlichia canis, E. ewingii, E. chaffeensis, Anaplasma platys, A. phagocytophilum, Neorickkettsia risticii and N. helminthoeca* (Dumler *et al* 2001, Inokuma *et al* 2001 and Headly *et al* 2006). Amongst these species, *E. canis* is the most important species causing serious and potentially fatal disease called as canine monocytic ehrlichiosis (CME). It is a rickettsia belonging to family Anaplasmataceae, gram-negative, highly pleomorphic alpha protobacterium that resides as a micro colony within a membrane-lined intracellular vacuole (morula), primarily within monocytes and macrophages of dogs (Simpson 1972) and consists of a circular chromosome containing 1,315,030 nucleotides (Mavromatis *et al* 2006).

Though canine ehrlichiosis, caused by a number of small obligatory intracellular pleomorphic organisms belonging to *Ehrlichia* species, is prevalent

worldwide but most commonly has been reported from tropical and subtropical areas (Keefe *et al* 1982, Mathewman *et al* 1993, Baneth *et al* 1996, Waner *et al* 1996, Harrus *et al* 1998a, Pretorius and Kelly 1998, Batmaz *et al* 2001, Suto *et al* 2001, Singh *et al* 2011a and Dutta *et al* 2013).

Canine ehrlichiosis previously has been known as canine rickettsiosis, canine hemorrhagic fever, tracker dog disease, canine tick typhus, Nairobi bleeding disorder and tropical canine pancytopenia due to affinity of the parasite to haemopoietic cells of the body that results in leucopaenia and thrombocytopaenia (Price and Sayer 1983). The disease is acknowledged as an important and potentially fatal infectious disease of dogs and other members of the canidae family. It was initially identified in Algeria by Donatein and Lestoquard (1935).

Ehrlichia canis is mainly transmitted by the brown dog-tick *Rhipicephalus sanguineus* (Smith *et al* 1975) has also recently been shown to be transmitted experimentally by the tick *Dermacentor variablis* (Johnson *et al* 1998). The distribution of canine ehrlichiosis related to the distribution of the vector and has been reported to occur in various parts of the world including Asia, Africa, Europe and America (Keefe *et al* 1982 and Baneth *et al* 1996).

Infection occurs when the infected tick ingests a blood meal and salivary secretions contaminate the feeding site. The incubation period of CME is between 8 to 20 days. During this period the organism multiply in macrophages of the monocytic phagocytic system spreading throughout the body. The subsequent course of ehrlichiosis has been divided into three phases: acute, subclinical and chronic cases (Harrus *et al* 1997a). Signs in the acute stage include depression, lethargy, anorexia, pyrexia, lymphadenomegaly, spleenomegaly and weight loss. Some dogs presented with bleeding tendencies have mainly petechiae and ecchymoses of the skin and mucus membrane and occasional epistaxis. Ocular signs include anterior uveitis, corneal opacity, hyphema, tortuous retinal vessels and focal chorioretinal lesions with central pigmented spots and subretinal haemorrhages, resulting in retinal detachment and blindness. Other signs are vomiting, serous to purulent occulonasal discharge, lameness, ataxia and dyspnea. In chronic form, weakness, depression, anorexia, weight loss, pale mucus membrane, fever, peripheral edema mainly in hind limbs and scrotum and platelet related bleeding are also seen. Neurological signs of

meningoencephalitis in acute and chronic stage of the disease are arched back, severe pain, ataxia, paraparesis or tetraparesis, cranial nerve deficits and convulsions.

Varela (2003) mentioned along with thrombocytopaenia, anaemia and leucopaenia also support for diagnosis of canine ehrlichiosis. Biochemical abnormalities included hyperproteinemia (due to hyperglobinemia and hypoalbuminemia), increased alanine aminotransferase and alkaline phosphatase activity. Hematological abnormalities in acute stage are thrombocytopenia, mild leucopaenia and normocytic, normochromic, non – regenerative anaemia. Severe pancytopenia is the hallmark of chronic form of ehrlichiosis. Positive Coomb's test suggests immune damage due to circulating erythrocyte antibodies which lead to hemolytic crisis (Ettinger and Feldman 2005). Eljadar (2010) found leucopaenia, thrombocytopaenia and hypergammaglobulinaemia in *Ehrlichia.* positive cases. Definitive diagnosis of ehrlichial disease can be made by demonstration of morulae in tissue aspirates such as spleen, lung and lymph node.

Diagnosis of the disease also becomes difficult due to its different stages and varied clinical manifestations. Further, the microscopic evaluation of stained blood smears is not sensitive as the organisms are not readily demonstrable in smears. Various serological diagnostic tests such as indirect immunofluorescene assay, latex agglutination test, ELISA and western blotting techniques have been employed by research workers. But it is very essential to take into account the range of cross – reactivities that may confound the diagnosis. Though these tests are sensitive but fail to distinguish the current infection or exposure without establishment of infection. So in this regard, molecular biology technique like PCR using specific primers provides a better diagnostic tool in terms of both sensitivity as well as specificity (Iqbal *et al* 1994a).

For the treatment, various drugs like doxycycline, minocycline, tetracycline, oxytetracycline, imidocarb dipropionate, amicarbalide has been used with variable efficacy (Neer *et al* 2002). Breitschwerdt *et al* (1998) treated experimentally inoculated *E. canis* infected dogs with doxycycline hyclate and found this drug is an effective treatment for acute *E. canis* infection. Harrus *et al* (2004) presented a report indicating an evidence that dogs recover from acute canine monocytic ehrlichiosis after 16 days of doxycycline treatment (@10 mg/kg of body weight every 24 h).

Since not much work has been done on the diagnostic and therapeutic response aspects of this disease in this region so, the present study was planned while keeping in view following objectives:

1. To study the clinico - hemato biochemical changes associated with ehrlichiosis in canine patients.
2. To study the therapeutic management of ehrlichiosis in canine patients.

CHAPTER II

REVIEW OF LITERATURE

The detailed review of literature pertaining to various aspects of clinico-hemato-biochemical changes and therapeutic studies on ehrlichiosis in dogs is described under the following heads:

2.1. History

These ehrlichiae were first discovered at the Pasteur Institute in Algeria by Donatien and Lestoquard (1935). They observed that experimental dogs infested with ticks, *Rhipicephalus sanguineus,* occasionally developed severe illness characterised by anaemia and the blood smears of the infected dogs stained by Giemsa technique, showed small rickettsia like organisms inside the monocytes which they named as *Rickettsia canis*.

2.2. Etiology

E. canis is a spherical body approximately 0.4µm in diameter, occurring intracytoplasmically as a subunit of an inclusion body (Morula) in the monocytes of infected dogs (Nyindo *et al* 1971). *E. canis* is an obligate intracellular rickettsia which is usually seen in canine mononuclear cells but has been reported infrequently in neutrophils and eosinophils. The neutrophils strains are thought to be less pathogenic than the monocytic strains (Rikihisa 1991).

2.3. Taxonomic Classification

At present *Ehrlichia* belong to family Anaplasmataceae (Dumbler *et al* 2001). *Ehrlichia* species is mainly divided into 3 classes based on the host cells they infect, such as monocytic, granulocytic and thrombocytic cells. Based on 16S rRNA sequence homology and antigenic relationship, *Ehrlichia* have been characterized into three genogroups I. *E. canis* genogroup includes *E. canis, E. chaffeensis* and *E. ewingi.* II. *E. phagocytophila* genogroup, which includes E. *phagocytophila, E. equi,* HGE agent and *Anaplasma platys*. III. *E. senestu* genogroup (*E. sennetsu, E. resticii and E. bovis*) (Kruss *et al* 2003). Then after the study on 16S rRNA sequences family Anaplasmataceae consists of genus *Ehrlichia, Anaplasma, Neorickettsia* and *Wolbachia* (Dumbler *et al* 2001).

2.4. Morphology, life cycle and Transmission

Donatien and Lestoquard (1940) described the lifecycle of *Ehrlichia canis*. At

the onset the organisms appeared as large dark homogeneous circular masses and these inclusions were referred to as initial bodies and were confined to monocytes. The initial bodies apparently underwent reproduction or fragmentation or both and passed through a mulberry or morula stage which in turn broke to form elememtary bodies then penetrated the monocytes and each developed into massive initial bodies.

Dubie *et al* (2014) also mentioned in his review that life cycle of *Ehrlichia* spp. goes through the three developmental stages of elementary bodies, initial bodies and morulae. Inside the cell small elementary bodies are 0.2-0.5µm in size, which develop into larger initial bodies of size 1.0 to 1.5µm. Finally formed to intracytoplasmic inclusion bodies called as morula having a size of 2 to 5µm containing approximately around 100 elementary bodies.

Worldwide, there is growing recognition of the importance of rickettsial pathogens in dogs and humans, which are transmitted by a number of different ixodid tick species (Nicolson *et al* 2010). *Rhipicephalus sanguineus*, the brown dog tick, is the most widespread tick in the world and is a well recognised vector of tick-borne pathogens affecting dogs and occasionally humans (Dantas-Torres 2008).

Natural transmission of canine ehrlichiosis is by the brown dog tick, *R. sanguineus* (Donatien and Lestoquard 1935, Mudaliar 1944, Ewing 1969). Kordick *et al* (1999) studied coinfection with multiple tick-borne pathogens in both dogs and humans. They investigated a kennel of sick Walker Hounds. Out of 27 dogs, 26 were seroreactive to *Ehrlichia* species. Detection of DNA from any *Ehrlichia* species was associated with clinical illness and with concurrent *B. canis* infection by PCR. Death seen in those dogs which are living in a dirt lot rather than the concrete kennel. Futher studies on male brown dog tick in transmission of *E. canis* either transstadially or intrastadially by conducting experimentation was done by Bremer *et al* (2005).

The main desire for this study is to determine if male tick can experimentally acquire and transmit *E. canis* in the absence of female tick. Two trails were conducted in which nymphal and male tick both get the infection at a same time on a same infected donor hosts, and transstadially or intrastadially exposed male ticks were fed on separate pathogen-free dogs as a test for transmission. They stated that, this tick development stage could be important in transmission of *Ehrlichia canis*.

Rhipicephalus sanguineus is a three-host tick, with endophilous nidicole behaviour, known as the “kennel-tick”. It is a main vector for *E. canis*, which causes

discomfort and anaemia. Studies on its seasonal dynamics have been carried out in many parts of the world. So Louly *et al* (2007) designed the study to determine the seasonal dynamics of larval, nymphal and adults of *R. sanguineus* infestations on seven naturally infested dogs. Their study revealed that tick generation can develop upto four years in Goiania. *Rhipicephalus sanguineus* feeds primarily on dogs. Ticks are widely distributed around the world and they are known vectors of pathogens, such as *Babesia canis, Ehrlichia canis* and *Rickettsia conorii*.

By considering these points in view, Dantas-Torres *et al* (2008) reviewed the medical and veterinary importance, taxonomy, biology and ecology of *R. sanguineus,* featured, the potential risks associated to the improper use of acaricides, such as environmental pollution and toxicity to humans and other non-target organisms.

Rani *et al* (2011) designed the programme to investigate the occurrence and geographical distribution of canine vector borne diseases of veterinary and public health importance in India. By using PCR assays detected *E. canis* in 20.6% cases. Potential tick vectors, *Rhipicephalus* and *Haemaphysalis* ticks were found on 278 (53%) out of 525 dogs examined. They concluded that at least 6 species of canine tick borne pathogens are found in India. Polymerase chain reaction was more sensitive in detecting circulating pathogens compared to peripheral blood smear examination.

Fourie *et al* (2013) did a work on transmission of *E. canis* by *R. sanguineus* ticks. They injected a strain of *E. canis* to laboratory-bred beagle dogs. *R. sanguineus* nymphs, which fed on this dog, moulted to adult ticks which carried infection rates of *E. canis* between 12% and 19% and were used in the series of experiments. Animals were monitored for fever and thrombocytopenia and were considered infected if they became serologically positive for *E. canis* antibodies as well as PCR positive for *E. canis* DNA. Their findings indicated the need for acaricides to provide either a repellent or a rapid killing effect against the ticks in order to decrease the risk of transmission of *E. canis*.

2.5. Prevalence

As we all know that *E. canis* as a worldwide distribution, but clinical signs may change geographically. Batmaz *et al* (2001) observed 20.77% (59/284) dogs of various breeds seropositive for *E. canis* by IFA test. The occurrence of CME was more common in males (30.83%) than in females (17.68%).

Dagnone *et al* (2003) determined the prevalence of ehrlichiosis in dogs having

tick infestation, anaemia and thrombocytopenia in South Brazil. Out of 129 dogs, 68 dogs were having the brown dog tick, 61 dogs showed thrombocytopenia, 19 had anaemia and 20 dogs had more than one inclusion criteria. For diagnosis they performed positive amplification of ehrlichial DNA by PCR by using specific primers. They found that 21% dogs having tick infestation, 20% dogs with thrombocytopenia and 21% anaemic dogs had ehrlichiosis.

Yabsley *et al* (2008) conducted a serologic survey for *E. canis.* In 2004 around 42.3% dogs and in 2006 43.8% dogs were seropositive for *E. canis*. All of the diagnosed species were transmitted by *R. sanguineus*. They concluded that tick borne pathogens should be considered as differentials for dogs exhibiting thrombocytopenia, leucopaenia, fever and lethargy. Kumar *et al* (2009) conducted prevalence study on haemoprotozoan diseases in canines from Chennai city. The various infections identified were *Babesia gibsoni* (84.9%), *Ehrlichia canis* (6%), *Hepatazoan canis* (4.8%), *Babesia canis* (3.9%) and *Trypanosoma evansi* (0.4%). High rate of prevalence seen in adults (63.1%) mainly during summer and winter.

Nicolson *et al* (2010) reviewed the ecology and epidemiology of ehrlichiosis and proposed directions for future investigation. They stated that canine infections are subclinical or mild. Antibodies to *Ehrlichia* species, induced by exposure to *E. canis* were more common in southern states (1.3%) than in the rest of the country (0.6%). Melo *et al* (2011) conducted a seroprevalence study and risk factors associated with *Ehrlichia* species. Serum of 320 dogs from urban and rural areas of a Pantanal region of Brazil positive by IFA assay. Out of 70.9% dogs, 74.3% from urban area and 67.5% from rural area showed positive reaction to *Ehrlichia* species. Most of the urban dogs were found parasitized by the tick *Rhipicephalus sanguineus.*

Singh *et al* (2011a) a prevalence of haemoprotozoan infections as 8.60% from 488 blood samples from dogs at Ludhiana. Among all prevalence rate of *Ehrlichia canis* was 0.82%.

Milanjeet *et al* (2014) evaluated 214 blood samples from Ludhiana, Punjab. Performed a PCR-based assay for the presence of *E. canis* targeting a portion of the 16S rRNA gene. The morulae of *E. canis* were detected in 2.34% samples. 41.59% of samples produced amplicons of expected size (389bp) specific for *E. canis.* Results of analysis showed high rate of prevalence in summer than in winter.

2.6. Clinical findings

Infection with *E. canis* results in acute, subclinical and chronic disease stages with dogs having a variety of clinical signs and laboratory abnormalities including fever, lethargy, lameness, oculonasal discharge, thrombocytopenia, anaemia, leucopaenia, hyperglobulinemia and proteinuria during various stages of infection. (Gaunt *et al* 2010).

The pathogenesis of ehrlichiosis begins with an incubation period of 8 to 20 days, followed by acute, subclinical and chronic phase of the disease. Acute phase may be mild and non specific and include depression, lethargy, moderate weight loss, anorexia, fever, lymphadenomegaly, splenomegaly, superficial bleeding, vomiting, nasal and ocular discharge, ataxia and dyspnea. Signs will decrease spontaneously within one to four weeks, but dogs can remain sub-clinically infected (Waner *et al* 1999). The clinical signs of chronic phase include depression, weakness, anorexia, weight loss, peripheral edema, anaemia, fever, bleeding due to platelet reduction, secondary bacteriological infections, pneumonia, glomerulonephritis, arthritis, reproduction disorders, neurological signs and ocular alterations (Harrus *et al* 1997b).

Oria (2001) studied 88 animal eyes positive by serology for *Ehrlichia canis*, 63 presented exclusively uveitis, 22 presented uveitis and secondary glaucoma and the remaining three were phthisical. Ocular discharge was observed in 45.4% of the dogs, there was episcleral congestion in 69.3%, ciliary injection in 28.4%, corneal edema in 61.3%, keratic preciptates in 18.1%, flare in 22.7%, hyphema in 10.2%, iris edema in 36.3%, Rubeosis iridis in 25%, iris pigmentation and retinal detachment in 11.3%, tortuous retinal vessels in 27.2%, hemorrhage in 12.5% and retinal hyperreflexia in 9% of the cases.

Beugnet *et al* (2002) observed that 31% of the clinically suspected dogs were seropositive for CME. Chandrasekar *et al* (2002) reported lymphadenopathy, vomition, pyrexia, anorexia, congested mucus membrane, epistaxsis, diarrhea, petechiae, ecchymosis and tick infestation. Bressler *et al* (2003) revealed portal vein and aortic thromboses and Luckschander *et al* (2003) reported renal amyloidosis in chronic ehrlichiosis. Dog had developed proteinuria and renal failure, indicate the presence of glomerulopathy. Moreira *et al* (2003) observed fever, anorexia, apathy, abdominal pain, lymphadenopathy and dyspnoea as the clinical signs in clinical cases of ehrlichiosis in dogs. Pasa and Azizoglu (2003) and Sacchini *et al* (2007) observed

anorexia, weight loss, weakness, lethargy, pyrexia, lymphadenopathy and anaemia in seropositive dogs.

Castro *et al* (2004) observed pale mucous membranes, generalized lymphadenopathy, splenomegaly, oedema and ascitis in *E. canis* infected in dogs. Mylonakis *et al* (2004) observed severe bleeding tendency in dogs with chronic ehrlichiosis in Greece. Leiva *et al* (2005) observed ocular lesions like exudative retinal detachment as the most common ocular manifestation other prevalent findings included anterior exudative uveitis and optic neuritis. Ajay and Varshney (2006) observed anorexia, vomiting, pyrexia, weight loss, melena, arrhythmia, tachypnoea, haemorrhages, pale mucus membrane, nervous deficit, lymphadenopathy, ascitis and uveitis.

Komnenou *et al* (2007) in their study discussed the ocular manifestations associated with CME. Unilateral (24.5%) and bilateral (75.5%) uveitis was most common findings. In addition corneal ulceration (13.3%), necrotic scleritis (11.1%), low tear production (8.9%) and orbital cellulitis (3.3%) were seen. Finally they concluded that, CME should be considered a major differential for a wide range of ocular manifestations exhibited by dogs residing in the endemic areas of the disease.

Sasanelli *et al* (2009) made a detailed study on combined infection of *E. canis*, *B. canis* and *H. canis*. They discussed a clinical case of dog presented to clinical unit of the Faculty of Veterinary Medicine of Bari, Southern Italy. A 3.5 month-old crossbreed dog presented with mild depression, dysorexia, diarrhoea and ascites. On the day of arrival to clinic dog was having severe depression, pale mucus membrane and ascites. But the diagnostic aid does not revealed *E. canis* infection at that stage. Animal was treated symptomatically.

At same time of treatment the dog unexpectedly presented with a cough and pyrexia of 39°C. On day 16 the hemato-biochemical findings, radiographs of lungs, even the microscopic examination of blood and buffy-coat smears revealed the presence of *E.canis* infection and also combined infection of *B.canis* and *H. canis*. By this case they mentioned some of atypical signs (diarrhoea, ascites, cough, involvement of respiratory system) and laboratory features associated with this multiple tick borne diseases.

Van der Krogt (2010) overviewed the clinical picture of dogs presented to veterinary clinics, over a period of ten weeks. According to his findings among 60

dogs main problems seen are anorexia, lethargy, tick infestation, unstable, weight loss, dirty eyes, bleeding/epistaxis, fever ≥39°C, dyspnoea. After physical examination of dogs, he found other signs such as pale mucous membrane, lymphadenopathy, fever, splenomegaly, petechiae/ecchymoses, bleeding/epistaxis, tick infestation conjunctivitis.

Procajlo *et al* (2011) discussed that CME often has an asymptomatic course. The acute form of CME proceeds usually with fever, apathy, weakness and accompanying respiratory symptoms, lameness and disturbances in blood coagulation. In laboratory examinations thrombocytopenia, anaemia and leucopaenia are ascertained. The chronic form of CME proceeds among gentle, unspecific symptoms which may last even 5 years.

2.7. Diagnosis

2.7.1. Hemato-biochemical findings

Huxsoll *et al* (1970) correlated the sign of epistaxis with thrombocyte count. He found that most of the dogs with tropical canine pancytopaenia did not develop epistaxis and other signs of haemorrhage. However, hematological findings in these dogs were similar to those observed in dogs with epistaxis in case of sever pancytopaenia. Thrombocytopaenia in canine ehrlichiosis occur due to increased platelet destruction as a result of decreased life span in the platelet life in chronically infected dogs. Thus platelet release in infected dogs is very slow. While increased destruction of newly produced labelled platelets diminish their number in peripheral blood was observed by Smith *et al* (1975).

Abeygunawardena *et al* (1990) discussed the pathogenesis of TCP. The predominant mechanisms are immunologically mediated resulting in the platelet sequestration coincident with the significant reduction in the number of circulating platelets. Another pathway play a main role in the pathogenesis of TCP by enhancing platelet sequestration and stasis, leading to reduced peripheral platelet count and probably hemorrhagic diathesis.

Harrus *et al* (1996a) performed serum protein electrophoresis in 42 dogs with naturally occurring *E. canis* infection and in 15 clinically healthy dogs (control dogs). The infected dogs were found to have a significant hypoalbuminaemia, hyperglobulinaemia and hypergammaglobulinaemia compared to the control dogs. The pancytopenic group revealed lower concentrations of total protein, total globulin

and gammaglobulin. The lower concentrations of the gammaglobulins coupled with the pancytopenia suggest that the immune state of the pancytopenic *E. canis* infected dogs is more compromised and therefore secondary infections should be expected more frequently in these dogs.

Harrus *et al* (1997b) diagnosed the 100 cases of CME in Israeli dogs. The disease occurred in all age groups and there was no sex predilection. All the dogs having similar signs of CME. Hematological findings included thrombocytopaenia, anaemia and lymphopaenia. To examine the effect of host, environment and hematological prognostic factors on survival, a cox proportional hazards regression model was done. Finally they found that severe anaemia, leucopaenia, pancytopaenia, epistaxis and specially in the dog breed German shepherd were important indicators of poor survival in cases of CME.

Waner *et al* (1997) examined the beagle dogs during the subclinical phase of disease. All dogs were clinically healthy throughout the 6 month examination period. IFA antibody titers of all subclinically infected dogs had *E. canis* (1:2560 to 1:20480). The most prominent hematological finding was mild thrombocytopaenia seen in eight of the nine dogs examined. Leucopaenia in 78% of the dogs and neutropaenia in 71% of dogs. 6/9 dogs had increased serum gamma-globulin concentrations. The most reliable parameters for judging possible subclinical phase was mild thrombocytopenia with a persistently high antibody titer to *E. canis*. Hypergammaglobulinemia would increase the suspicion further.

Stiles (2000) mentioned the sub-clinical phase occurs 6-9 weeks after infection without overt clinical signs despite persistence of thrombocytopaenia, variable leucopaenia and anaemia with progressive hyperglobulinmia. Due to suppression of erythrocyte production and accelerated erythrocyte destruction more severe form of anaemia was seen in chronic phase of infection. Thrombocytopaenia and anaemia are common hematological findings observed by Batmaz *et al* (2001) in *E. canis* seropositive dogs. Ramprabu *et al* (2001) recorded *E. canis* in a 3.5 years old mongrel male dog at Madras Veterinary College, India with neutrophilia and elevated levels of creatinine kinase and aspartate.

Shimada *et al* (2002) observed that concentration CRP markedly increase in the plasma of *E. canis* inoculated dogs. The peak concentrations were ranged from 217.8 to 788.8 µg/ml. *E. canis* antigens and antibodies were detected between 18 to

27 days and 5 to 15 days after inoculation of *E. canis*, respectively. Finally they concluded that the timings of seroconversion and start of the increase in CRP were similar. The higher concentration of CRP become apparent when the PCR product of 16 S rRNA of *E. canis* became detectable.

Moreia *et al* (2003) conducted a retrospective study of clinical cases of dogs examined from March 1998 to September 2001 in Brazil. Demographic aspects clinical characteristics and hematological characteristics were analysed. Out of 194 clinical records 31 animals were infected with *E. canis* and 21 animals with *E. Platys*. The most common signs were fever, anorexia, apathy, abdominal pain, lymphadenapoathy and dyspnea. Animals were having anaemia (70.3%), thrombocytopaenia (50%), leucopaenia (30%) and most *E. canis* morulae were seen in monocytes. Thus 35.9% of dogs suspected for hemoparasitic diseases were infected with *E. canis* or *E. platys*.

Varela (2003) mentioned that thrombocytopaenia is the most common hematologic change and that anaemia and leucopaenia also support for the diagnosis. Biochemical abnormalities included hyperglobinemia, hypoalbuminemia, increased alanine aminotransferase and alkaline phosphatase acitivity.

A common screening test for an *E. canis* infection in endemic area is the use of peripheral blood platelet counts. Bulla *et al* (2004) evaluated platelet counts as a screening test for *E. canis* in an endemic area. They took 217 whole blood samples from dogs which were divided into three groups: 71 non-thrombocytopenic samples (group A, platelet counts >200000/µL) and 146 thrombocytopenic samples (<200000/µL). The thrombocytopenic group was further divided into 62 with platelet counts between 100000–200000/µL (Group B) and 84 samples with <100000 platelets/µL (Group C). All samples were examined for the presence of a segment of the *E. canis* 16S rRNA gene using nPCR. 30.9% were positive for the presence of the *E. canis* 16S rRNA gene, 63.1% of the group C samples and 21% of group B. Only 1.4% of samples from Group A were positive.

De Castro *et al* (2004) in his study inoculated 4 dogs with *E. canis* and four were used as uninfected controls. After a 10–14-day incubation period, infected dogs developed pyrexia up to 41°C for 6–8 days. CBC, platelet count and TPP of infected and control groups are done. RBC, PCV and Hb values differed significantly between the two groups. Hasegawa *et al* (2005) observed that the infection by *E. canis* has no

influence on neutrophils oxidative metabolism even though during the remission period of the acute phase of the disease the neutrophils seem to be more reactive under stimulation. Ehrlichiosis is a multisystemic disease with the potential to cause cardiomyocyte injury in naturally infected dogs.

Niwetpathomwat *et al* (2006) studied the clinical hematology and serum biochemistry in dogs with ehrlichiosis. Hematology showed mild to moderate anaemia and severe thrombocytopenia with decreased levels of RBC, WBC, PCV, platelet count and TPP. 20% of the cases showed elevated levels of BUN, ALKP, ALT and AST values. Shipov *et al* (2008) studied prognostic indicators for CME. He observed anaemia, leucopaenia, thrombocytopenia and serum biochemical abnormalities like hypoalbuminaemia, increased activities of AST, ALKP and LDH, hyperglobinaemia, hypokalemia.

Shipov *et al* (2008) studied clinical records of 40 cases of CME in order to identify prognostic factors for survival in CME. Most of the clinical signs including pale mucous membranes, bleeding tendencies and weakness were more prevalent in the non-survivors compared to survivors. Leucopaenia, thrombocytopaenia and low hematocrit count were seen in non-survivors. Severe pancytopenia was found as a risk factor for mortality. Severe leucopaenia, severe anaemia, prolonged activated partial thromboplastin time and hypokalemia were found to predict mortality with a probability of 100%. Whereas WBC counts, platelet counts and serum potassium concentration were above and each provided 100% prediction for survival.

Eljadar (2010) collected a total of 951 blood samples of suspected dogs from SAC, GADVASU, Ludhiana and reported 7.80% microscopical prevalence for *Ehrlichia* spp. Further in *Ehrlichia* positive cases major signs included were leucopaenia, thrombocytopaenia and hypergammaglobulinaemia. Shah *et al* (2010) diagnosed ten cases of ehrlichiosis presented to SAC, GADVASU, Ludhiana by commercially available dot-ELISA kit (Immunocomb, biogal, Kibbutz, Gated, Isreal). They observed moderate to severe anaemia and thrombocytopenia and elevated BUN in 20% of the dogs, hypoalbuminemia and ALKP was raised in 70% cases. Singh *et al* (2011b) observed anaemia, leucopaenia and thrombocytopaenia in dogs affected with *E. canis* infection.

Asgarali *et al* (2012) determined the prevalence of *E. canis* in stray dogs in north Trinidad. 41 out of 92 stray dogs were seropositive for *E. canis*. The odd ratio

showed seropositive dogs likely to have low platelet counts and elevated serum protein concentrations than seronegative dogs. Villaescusa *et al* (2012) evaluated the lymphocyte population in 28 dogs are naturally infected by *E. canis*. A MPFC study was performed to analyse the distribution of the main lymphocyte subset in the peripheral blood and said that dogs with clinical signs showed lower relative and absolute values of B lymphocytes than dogs without clinical signs.

Das and Konar (2013) evaluated the clinical and hematological aspects of dogs naturally infected CME. A total of 47 cases of ehrlichiosis in dogs were reported with babesiosis (8.51%) and hepatozoonosis (6.38%). *E. canis, E. ewingii, B. canis, B. gibsoni* and *H. canis* were observed under oil immersion lense of microscope. Marked anaemia and neutrophilic leucocytosis were observed.

2.7.2. Microscopic Examination

The first case of CME from Punjab was diagnosed by Juyal *et al* (1992) by conventional giemsa stained thin blood smear microscopy. A prevalence study of 0.35% *E. canis* infection from Punjab by observing the morula in 752 blood samples of the dogs suspected to be infected with hemoprotozoans (Juyal *et al* 1994).

Mallapur *et al* (2002) detected *E. canis* morula in monocytes of the stained blood smear and reported a prevalence rate of 55% in stray dogs of Maharastra. Moreira *et al* (2003) found that 52 dogs were positive for *Ehrlichia* spp. out of 52, 31 dogs were infected with *E. canis*.

Mylonakis *et al* (2003) dicussed the role of cytology in diagnosis of CME. It was a comparison of the diagnostic sensitivity between BC, PB, LN, BM and P-D cytology based on the detection of *E. canis* morulae, in the acute phase of CME. Up to 1000 OIF were screened for *E. canis* morulae in order to assess their diagnostic sensitivity.

De Castro *et al* (2004) studied clinical signs, humoral and cellular immune responses and microscopic and gross tissue alterations resulting from acute experimental *Ehrlichia canis* infection in dogs. Rodriguez-Vivas *et al* (2005) carried out a study in four veterinary clinics located in Merida, Yucatan and Mexico. They found that 44.1% dogs were seropositive to *E. canis* and 5% dogs had typical morulae of *E. canis* present in their monocytes. Lakshmanan *et al* (2007) revealed morulae of *E. canis* in 19.38% (19/98) of samples from Chennai.

Banerjee *et al* (2008) studied a case of dog, with the age of 3.5-month-old, male

in Veterinary Teaching Hospital, Pantnagar (India), with lethargy, fever, anorexia, lymphadenopathy, anaemia and thrombocytopaenia. Cytology revealed *Ehrlichia* spp. intracytoplasmic morulae in 0.99% neutrophils and 3.12% in monocytes.

Sacchini *et al* (2007) described an outbreak of CME in 9 male Labrador Retrievers dogs between October and December 2006 from the eastern region of Saudi Arabia. The majority of dogs presented had severe lethargy, acute anorexia, fever and generalised lyphadenopathy. Dogs were having anaemia, leucopaenia, hypoalbuminemia and thrombocytopaenia. Only 4 dogs out of 9 were having *Ehrlichia* morulae in blood films.

Flacke (2010) collected Blood and faecal samples from wild dogs immobilized for collaring or translocation purposes. 21% dogs showed presence of *E. canis* morulae within monocytes. Eljadar (2010) reported a prevalence rate of 7.8% CME by microscopic examination of 952 blood smears from Ludhiana, Punjab. Kumar *et al* (2010) based on blood and BC smear technique reported 2.94% prevalence of CME in Ranchi.

Dhankar *et al* (2011) screened 203 dogs of different breeds for ehrlichiosis. 23 dogs found positive based on blood and BC technique. Singh *et al* (2011b) examined 776 canine blood samples from Ludhiana, Punjab and recorded 1.54% prevalence of *E. canis*. Singla *et al* (2011) examined blood samples of 60 privately owned dogs suspected to be infected with *E. canis* from Ludhiana, Punjab and found only two samples revealing typical morulae. Singh *et al* (2011a) 8.60% was found to be prevalence of *E. canis* as 1.43% with sex and age had no significant effects in the prevalence.

Dutta *et al* (2013) examined the blood smears of 1029 dogs brought to teaching veterinary complex, Khanapura, Guwahati and recorded 7.58% cases of CME. 4.37% was recorded in pre monsoon (March-May) followed by monsoon 1.46% (June-August) and Post monsoon 1.46% (September-November) and least in winter 0.29% (December-February).

2.7.3. Serological studies

Keefe *et al* (1982) detected *E. canis* antibodies by IFA in sera from 11% of military working dogs and 57% in civilian dogs in the Unitied States during a period of 1 year study. Infection rate from tropical and temperate Zones was 13% to 8% in

cold Zones. Seropositive military dogs did not had much clinical signs compared to civilian dogs. Thus indicates the subclinical form of infection in military dogs.

Botros *et al* (1995) did sero - epidemiological survey on CME in Egypt. A total 374 dogs, 252 from five military kennels and 122 privately owned, were tested for *E. canis* antibody. Prevalence among military dogs (29%) was significantly lower than among privately owned dogs (41%). Dogs from higher sanitary and hygienic kennels were having lower prevalence. 3 dogs with epistaxis had *E. canis* antibody titres >1:320.

Waner *et al* (1997) examined dogs during the subclinical phase of CME. All subclinically infected dogs had IFA antibody titers to *E. canis* at a dilution varying from 1: 2560 to 1: 20480. The most reliable parameters for judging possible subclinical ehrlichial infection in dogs was mild thrombocytopenia, together with a persistently high antibody titer to *E. canis*.

Oliveria *et al* (2000) detected anti-*Ehrlichia canis* antibodies by Dot-ELISA in naturally infected dogs. Most of the clinical signs were similar to the CME. Haemogarm analysis evidenced reduction in RBC (78.85%), Hb (76.92%), and thrombocytopaenia (69.23%) of the animals. Antibody levels of diagnostic value were detected in 92.31% of dogs. DOT-ELISA was efficient in detecting anti-*E. canis* antibodies in sera from naturally infected dogs presenting symptoms.

Waner *et al* (2000b) conducted a study to compare clinic-based ELISA test kit with the IFA test for detection of *E. canis* antibodies in dogs. A good correlation was found between the 2 techniques. Evidence for the sensitivity of the ELISA technique for the early detection of *E. canis* IgG antibodies was demonstrated by comparing the appearance of *E. canis* antibody titers by the IFA and ELISA techniques after artificial infection of 2 sets of dogs. The results were correlated with the appearance of fever and clinical signs. Proposed application of the in-clinic ELISA test is to aid in the diagnosis of CME.

Waner *et al* (2001) presented a review of the ehrlichial diseases affecting dogs with reference to their immune responses, host specificities, cross-reactivites and diagnosis. The review emphasizes that the disease process, cross-reactivites with other ehrlichial species, multiple tick-borne infections and persistent IFA antibody titers post-treatment, should all be considered when interpreting *E. canis* serological results.

Harrus *et al* (2002) compared three different ELISA assays (rMAP2-ELISA,

the Immunocomb and the Snap 3Dx assay) with the indirect IFA test in detecting anti-*Ehrlichia canis* immunoglobulin-G (IgG) antibodies. When qualitative results were compared, there was an overall agreement of 81% between the indirect IFA test and the rMAP2-ELISA. 94% was found between the IFA test and the Immunocomb and 91% was found between the IFA test and the Snap 3Dx assay. 74.6% samples tested, complete agreement in the qualitative results was found in all four tests.

Gallego *et al* (2006) investigated 206 sick and 260 clinically healthy dogs from three different regions in northeastern Spain for antibodies to *R. conorii, E. canis, A. phagocytophilum, B. henselae, B. vinsonii subsp. L. infantum and B. burgdorferi* and for antigen of *D. immitis.* Among all seroprevalence for *E. canis* was 16.7%.

Yabsley *et al* (2008) conducted a serologic survey for *E. canis* in 2004 (104 dogs) and a comprehensive serologic and molecular survey for a variety of tick-borne pathogens in 2006 (73 dogs). In 2004 and 2006, 44 and 32 dogs (42.3% and 43.8%) were seropositive for *E. canis*, respectively. Baticados *et al* (2011) in an attempt to establish the serological status of CME and examined total of 169 canine blood samples by ELISA assay. 95.3% were found seropositive for *E. canis* antibodies using Immunocomb® *Ehrlichia canis* test kit. Out of the total seropositive animals, 59.0% exhibited significant antibody titers whereas 41.0% displayed low antibody titer levels. Only 4.7% (8/169) of the blood samples showed seronegative results.

Muhairwa *et al* (2012) undertook a cross-sectional study to determine the seroprevalence of *E. canis* in dogs in Morogoro Tanzania. A total of 100 randomly selected dogs were tested for the presence of *Ehrlichia canis* antibodies using the Immunocomb® dot-ELISA tests (Biogal, Israel). *E. canis* antibodies were detected in 25% of the dogs. Davoust *et al* (2014) did a rapid immune-migration test for the serological detection of CME (WE, Zoetis, France). Collected 528 serum samples from blood of dogs living in endemic areas of West and East Africa. The WE test results were compared with those obtained by IFA test. The sensitivity of WE was 97% with a specificity of 100%. In military working dogs, the seroprevalence by IFA was 7%, whereas in native dogs it reached 77.1%. By this, WE test represents a simple, fast and reliable test for the detection of anti-*E.canis* antibodies.

2.7.4. Molecular studies

Iqbal *et al* (1994a) studied and compared the sensitivity of PCR with the

serological methods for detecting antibodies against *E. canis* and cell culture methods for isolation of *E. canis* in the early diagnosis of CE on five German shepherd dogs by infecting them with 10^7 *E. canis* infected DH82 cells intravenously. The IFAT and WI could detect *E. canis* antibodies as early as 2 to 8 days of PE. Though CC r-I proved to be the most sensitive and definitive. The sensitivity of PCR is slightly less than the other established methods. But convenience, quickness, and direct nature of detecting *E. canis* DNA through PCR made it more useful for early diagnosis of ehrlichiosis.

McBride *et al* (1996) conducted PCR based detection assay that specifically detected *E. canis* in dogs. A region of the 16S ribosomal RNA gene of *E. canis* was targeted for PCR amplification and CH with a complementary internal 287- base pair oligonucleotide probe. The CH improved the PCR assay sensitivity 1000 - fold as compared with visualization on ethidium bromide-stained agarose gels. A diagnosis of *E. canis* using this PCR/CH assay can be made in 2 days as compared with 14 weeks for CCI. The PCR/CH assay appears to be an acceptable alternative or complement to current diagnostic techniques.

Breitschwerdt *et al* (1998) evaluated the speciation of *Ehrlichia* spp. infected sick dogs reffered to hospital based upon PCR amplification with species-specific primers. The dogs sero-reactive to *E. canis* antigens were determined to be infected with 4 *Ehrlichia* species: *E. canis*, *E. chaffeensis*, *E. equi* and *E. ewingii*. In addition, their findings supported the efficacy of doxycycline for treatment of these infections but indicated that, based upon the persistence of *E. chaffeensis* DNA for one year following treatment, *E. chaffeensis* infection in dogs may be more refractory to doxycycline treatment.

Harrus *et al* (1998b) in order to determine whether dogs in the subclinical phase of CME are carriers of *E. canis* as persistent indirect IFA titers occurred during this phase. PCR was performed with blood, BM, and splenic aspirates collected 34 months postinoculation from 6 clinically healthy beagle dogs experimentally infected with *E. canis*. At least 1 of the 3 samples from 4/6 dogs was PCR positive. The spleens of all 4/6 dogs were PCR positive, and the BM and blood of 2/4 dogs were PCR positive. Indirect IFA titers increased (5month) progressively, remained high for period of more than 11 months, and declined thereafter, suggesting that the dogs were recovering from the disease. Findings suggest that the spleen is the organ most likely to harbor *E. canis* parasites during the subclinical phase and the last organ to

accommodate the parasite before elimination.

Murphy *et al* (1998) used PCR and Southern hybridization to survey for the presence of *E. canis* in blood samples of 65 dogs that harbored ticks from northcentral and northeastern Oklahoma. Dog blood samples were also examined for antibodies against *E. canis* using an IFA test. 10 of 65 dogs (15.4%) examined were positive for *Ehrlichia* spp. by PCR. Only 3.1% of dogs were for *E. canis* by PCR. 10.8% were seropositive for *E. Canis.* Stich *et al* (2002) developed a sensitive PCR assay based on oligonucleotide sequences from the unique *E. canis* gene *p30* to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. The assay was used to detect *E. canis* in canine carrier blood and in experimentally infected *R. sanguineus* ticks. Their results indicated that *p30*-based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

Aguirre *et al* (2004) reported the first isolation and culture of *E. canis* in Spain from a naturally infected dog using the DH82 cell line. After DNA extraction and PCR amplification, a nearly complete sequence of the 16S rRNA gene of the new *E. canis* strain was obtained. This sequence was aligned with the 16S rRNA gene sequences of other *Ehrlichia strains* accessible in gene bank. The 16S rRNA gene sequence of the reported *E. canis* strain showed a high percentage of similarity with the 16S rRNA gene sequence of the *E. canis* from different geographic areas including Japan, Venezuela and Israel which confirmed the presence of *E. canis* in Spain.

Bulla *et al* (2004) evaluated platelet counts as a screening test for *E. canis* in an endemic area, whole blood samples were taken from 217 dogs. All samples were examined for the presence of a segment of the *E. canis* 16S rRNA gene using nPCR reaction. Samples 30.9% were positive for the presence of the *E. canis* 16S rRNA gene. 98.5% of positive samples were having thrombocytopaenia. Doyle *et al* (2005) identified *E. canis* from natural and experimental infections, previously confirmed by PCR and serological and microscopic evidence. The positive cases were detected by developing the tricolor TaqMan real-time PCR assay capable of simultaneously detecting and discriminating medically important ehrlichiae in a single reaction. They mentioned that this assay provides a powerful tool for prospective medical diagnosis

for human and canine ehrlichiosis and for ecologic and epidemiological studies involving arthropod and mammalian hosts.

Lakshmanan *et al* (2007) performed nested PCR assay for the detection of 16S rRNA gene fragment of *E. canis* from the blood of dogs. The genus-specific primers amplified a 477 bp band of *Ehrlichia* sp. in the first PCR. The nested PCR assay using species-specific primers produced a 387 bp band of *E. canis.* The nested PCR detected *E. canis* organisms in 50% of samples which revealed morulae in only 19.38% of samples. This procedure will detect the presence of *E. canis*, even one month after specific antibiotic therapy.

Siarkou *et al* (2007) studied to characterize, the *E. canis* strains involved in naturally occurring CME in Greece, and to investigate if any sequence diversity exists between the 16S rRNA genes of those involved in the mild non-myelosuppressive or the severe myelosuppressive form of CME. The 16S rDNA analysis based on secondary structure revealed that all sequences of the Greek strains were identical to each other and indicated 100% identity among some American (Venezuelan and Brazilian), European (Greek), Middle Eastern (Turkish) and Asiatic (Thailand) strains. The results of this study suggest that the *E. canis* strains involved in the non-myelosuppressive and myelosuppressive forms of CME in Greece share an identical 16S rRNA genotype.

Aguiar *et al* (2008) obtained an *E. canis* isolate was obtained from a naturally infected dog exhibiting clinical signs of ehrlichiosis in Sao Paulo Municipality, Brazil. The isolate was characterized by PCR and DNA sequencing of portions of the ehrlichial genes dsb, 16SrRNA, and p28. Partial dsb and 16S rRNA sequences were identical to 3 and 5 other *E. canis* strains, respectively, from different countries and continents. Conversely, the p28 partial sequence for this *E. canis* (Sao Paulo) differed by 1, 2, and 2 nucleotides from the corresponding sequences of the *E. canis* strains from Jake, Oklahoma and Venezuela respectively. The results in this study indicate that *E. canis* is the only recognized *Ehrlichia* species infecting dogs in Brazil.

Carvalho *et al* (2008) conducted an epidemiological and molecular study of *E. canis* and evaluation of associated risk factors in dogs. Blood samples were collected from 153 dogs and DNA was extracted and analyzed by the nested PCR, using one pair of primers to detect *Ehrlichia* spp. and another pair to detect the presence of *E. canis*. Of the 153 animals, 7.8% were positive for *E. canis* by PCR, indicating the

presence of the parasite in dogs. The associated risk factors were exposure to tick-infested habitats and the fact that the dogs lived in the countryside.

Gal *et al* (2008) carried out molecular study for the detection of *E. canis* by using tissues obtained at necropsy from randomly selected dogs with the intention of investigating naturally-occurring canine ehrlichiosis. The tissues evaluated for the presence of *E. canis* included are LN, spleen, liver, BM and blood. In this study, 8 out of the 18 dogs were found to be positive for *E. canis* by PCR.

Matjila *et al* (2008) collected a total of 1138 blood specimens for a period of 6-year from domestic dogs in South Africa. All specimens were screened for *Babesia*, *Theileria*, *Hepatozoon* and *Ehrlichia/Anaplasma* species using PCR and RLB assays. On RLB, 560/1137 domestic dog-specimens were positive for one or more parasites. Of the positive domestic dog-specimens, 3% specimens were infected with *E. canis*. Mixed infections were also found. The results indicate that a wide range of tick-borne pathogens are circulating in the canine populations in South Africa.

Nakaghi *et al* (2008) did a study to compare the direct detection methods of *E. canis.* They took blood samples of 30 dogs having the signs like anorexia, pale mucus membrane, fever, lymphadenopathy, spleenomegaly, haemorrhages and uveitis. About 63.3% of sera were IFAT positive, 70% were Dot- ELISA positive and 53.3% of the samples are positive for nested PCR.

Mylonakis *et al* (2009) analysed serum samples obtained from 38 dogs with non-myelosuppressive CME and 12 healthy dogs retrospectively. Each serum sample was analyzed in triplicate using an *E. canis* specific nested PCR assay targeting a 389 bp sequence of the 16S rRNA gene. *E. canis* DNA was amplified 63.1% affected dogs and all samples from healthy dogs were negative. Serum-based PCR analysis is suggested for the early diagnosis of CME when whole blood samples are not available.

Alexandor *et al* (2008) reported antibodies against *E. canis*, previously in clinically ill and stray dogs from Portugal. In this study, 22% of dogs with suspected tick-borne disease were detected by nPCR with the same primers. *E. canis* infection was detected by nested PCR in 7/12 dogs that were seronegative by indirect IFA results, emplacing the value of molecular techniques in diagnosis of CME.

Rajgopal *et al* (2009) evaluated the techniques PCR and IFAT in diagnosis of canine ehrlichiosis. PCR was done by using ECC and ECB for primary amplification.

ECAN5 and HE3 for nested PCR. Amplification of 387-bp product in the nested reaction in the samples as well as in the known positive control was confirmatory for *E. canis* infection. Of the 50 samples tested, 44 gave positive PCR results. Of the 50 serum samples tested, 48 were found to be positive by IFAT. Finally concluded that PCR was to be the more suitable than IFAT in the diagnosis of early and subclinical infections of canine ehrlichiosis.

Santos *et al* (2009) performed PCR and nested PCR to study the presence of *E. canis*, in thrombocytopenic and non-thrombocytopenic dogs from Brazil. High incidence (25.4%) of *E. canis* infection in non-thrombocytopenic dogs. Although infection with *E. canis* should be considered in thrombocytopenic dogs. They concluded that final diagnosis needs to be confirmed by complementary tests such as blood smears and PCR to avoid the unnecessary use of antibiotics.

Cardoso *et al* (2010) carried out a study on 4 dogs from the north of Portugal, in which an ehrlichial disease was suspected clinically, by molecular methods. After DNA extraction from blood on filter paper, a 345 bp fragment of the *Ehrlichia* spp. 16S rRNA gene was amplified by PCR. Sequence analysis of PCR products revealed one dog infected with *E. canis.*

Eshoo *et al* (2010) to detect and identify *Ehrlichia* species directly from blood specimens, they described an assay that employs multilocus PCR/ESI-MS. The results were compared to those of a colorimetric microtiter PCR -EIA used as a diagnostic assay. Among 213 whole-blood samples collected from patients who were clinically suspected of ehrlichiosis, were positive for an *Ehrlichia* species by PCR/ESI-MS, giving a positive rate of 18.8%. The 38 specimens that were positive for *Ehrlichia* by both PCR/ESI-MS and the PCR-EIA were further characterized to the species level, with 100% agreement between the two assays. The PCR/ESI-MS assay can be completed within 6 hr, providing another laboratory tool for the diagnosis of ehrlichiosis.

Hsieh *et al* (2010) stated that the genetic diversity of *E. canis* strains worldwide is currently poorly defined. So, the present study aimed to characterize *E. canis* strains in naturally infected dogs in Taiwa, using a combination of PCR and sequence analysis of the 16S rDNA and two antigen encoding, *gp19* and *gp36.* Genomic DNA was extracted from 34 parasitemic dogs and the genes of the pathogen were separately amplified, sequenced and aligned with corresponding sequences

available in gene bank. All 16S rDNA sequences amplified from the Tiawanese isolates were identical and had high similarity with previously reported *E. Canis* sequences.

Carlos *et al* (2011) worked to study the clinical disorders and risk factors of canine ehrlichiosis. Blood samples were collected from 200 dogs. Each dog was clinically examined. The blood samples were analyzed using the Dot-ELISA test, hematometry, platelet counts and searches for morulae on blood smears were performed. Nested PCR was carried out on 50 serologically positive samples and 50 negative samples. 3 positive PCRs were sequenced. 36% were serologically positivity and 5.5% from blood smears. Nested PCR identified 11 positive dogs of which 9 were serologically positive and two were negative. The DNA sequencing was consistent with the presence of *Ehrlichia canis*.

Rani *et al* (2011) studied the prevalence of blood haemoprotozoa in canines from four cities of India. They investigated the pathogen by conventional method using stained blood smears and by molecular techniques using PCR on a total of 525 dogs. They reported that the blood smear examination of every sample was negative. In contrast, using PCR, 49.7% dogs were found to be infected with one or more pathogens. They reported *E. canis* in 20.6% of cases.

Hernandez *et al* (2012) worked to detect the presence of *E. canis* in 91 dog blood samples in Colombia, by molecular and serological techniques. They also performed sequence alignment to indicate the identity of the parasite species infecting these animals. The present work shows the first molecular detection of *E. canis* in dogs from Colombia. Immunoglobulin-G antibodies to *E. canis* in 82.4% cases whereas 5.5% of dogs were positive by PCR.

Dos Santos *et al* (2013) evaluated the presence of *Ehrlichia* DNA in the blood samples of 320 dogs from the urban and rural areas of the municipality of Pocone, Pantagal region and Mato Grosso state by PCR targeting the ehrlichial dsb gene. 48 dogs were found to be positive, 25 from the urban area and 23 from the rural area. Partial DNA sequence obtained from PCR products of 18 samples from the urban area and 16 samples from the rural area were 100% identical to *E. canis* from Brazil and USA.

Eiras *et al* (2013) collected a total of 86 blood samples from dogs with suspected rickettsial disease and 28 non-suspected dogs from Argentina. PCR for

Ehrlichia and sequencing of the positive PCR products were done. *E. canis* was detected in the blood of 6 dogs. Nazari *et al* (2013) collected a total of 500 canine blood samples from veterinary clinics and dog shelters in Malaysia. They performed molecular screening by PCR using genus-specific primers followed by PCR using *E. canis* species specific primers. 10 out of 500 dogs were positive for *E.canis*.

Milanjeet *et al* (2014) evaluated blood samples collected from 214 dogs from Ludhiana, Punjab (India) for the presence of *E. canis* by PCR based assays targeting a part of 16S rRNA gene. An amplicon of desired size 389bp was produced. *Ehrlichia* genus specific PCR was applied on all the samples and an amplicon of 478 bp was detected in 6.54% samples whereas when nested PCR assay specific for *E.canis* was employed using a product of primary PCR assay, an amplicon of 389 bp was produced in 41.59% of the samples indicating that nested PCR, when coupled with primary PCR, resulted in increased sensitivity in detection of *E. canis* infection.

2.8. Therapeutic studies

Wen *et al* (1997) performed a comparative study of nested PCR with IFA assay for detection of *E. canis* infection in dogs treated with Doxycycline. 105 blood samples from dogs (Arizona and Texas) and 30 blood samples from dogs (Ohio) were examined by nested PCR and IFA test. Around 84% of dogs from Arizona and Texas had been treated with doxycycline before submission of blood specimens. Among Arizona and Texas specimens, 44% samples were PCR positive and 76% were IFA positive. 54% IFA-positive samples were positive by PCR, and 88% IFA-negative samples were negative in the nested PCR. None of the Ohio specimens were IFA positive, but 17% specimens were PCR positive.The results indicate that the nested PCR is highly sensitive and specific for detection of *E. canis* and more useful in assessing the clearance of the organisms after antibiotic therapy than IFA, especially in areas in which *E. canis* is endemic.

Breitschwerdt *et al* (1998) experimentally inoculated dogs with *E. canis* to assess the efficacy of doxycycline hyclate for the treatment of acute ehrlichiosis. Treatment with doxycycline eliminated infection in eight of eight dogs. Untreated infected control dogs appeared to eliminate the infection or, alternatively, suppress the degree of ehrlichiemia to a level not detectable by tissue culture isolation or PCR or by transfusion of blood into recipient dogs. They concluded that doxycycline hyclate is an effective treatment for acute *E. canis* infection. However, these results may not

be applicable to chronic infections in nature.

Harrus *et al* (1998b) evaluated the efficacy of doxycycline treatment (10mg/kg of body weight every 24h for 42 days) in eliminating *E. canis* from four subclinically infected dogs. One dog remained PCR positive, suggesting that 6 weeks of doxycycline treatment may not be sufficient to clear *E. canis* parasites from all subclinically infected dogs. Serology was shown to be unreliable in assessing recovery from the carrier state, as anti- *E. canis* antibodies persisted after elimination of the parasite. Their findings suggest that an increase in the platelet count may be an important indicator for dogs that recover from subclinical ehrlichiosis.

Sainz *et al* (2000) reported the clinicopathologic responses of 93 dogs with spontaneously occurring ehrlichiosis to 3 different treatment protocols. 32 dogs were treated with doxycycline (10 mg/kg/day for 28 days), 31 were treated with imidocarb dipropionate (5 mg/kg given 15 days apart in 2 separate injections), and 30 were treated with both drugs simultaneously, at the doses as specified. The dogs underwent clinicopathologic evaluation before and after treatment, and were examined periodically during the 24-month period after the treatment. No differences were found in the clinical responses among the dogs in the 3 treatment groups. As for the clinicopathologic response, obtained with the 3 protocols were similar, the platelet count and serum protein electrophoresis results returned to normal more slowly in dogs that received imidocarb dipropionate as compared to those given the other 2 treatments. Mylonakis *et al* (2003) took a total of 50 dogs (33 males and 17 females, 40 purebreds and 10 mongrels) with natural acute CME. The inclusion criteria were the clinical and clinicopathological compatibility with the acute phase of the disease. All 50 dogs were IFA- and PCR-tested positive for *E. canis*. The full clinical recovery after a 4-week treatment with oral doxycycline (5 mg/kg BW, q12h).

Harrus *et al* (2004) presented a report indicating an evidence that dogs recover from acute CME after 16 days of doxycycline treatment (10 mg/kg of body weight every 24 h). Blood PCR was as valuable as splenic aspirate PCR for early diagnosis of acute CME. Splenic aspirate PCR was, however, superior to blood PCR for the evaluation of ehrlichial elimination.

Sousa *et al* (2004) evaluate the clinical response of doxycycline, preceded or not by the imidocarb, for treating canine ehrlichiosis. 2 groups of 9 dogs were composed. The first was treated with doxycycline, whereas the second was treated

with doxycycline and imidocarb. Results showed that both treatments were satisfactory, demonstrating that the clinical response didnot depend on the use of imidocarb in the experiment.

Davoust *et al* (2005) had made an epidemiological and laboratory studies to validate a programme of chemoprevention of CME. The dogs were given 100mg of doxycycline per os daily. HPLC was used to determine plasma levels of doxycycline. Seropositve dogs were asymptomatic and generally became seronegative after treatment. Blind doxycyclinaemia tests done on dogs living in Africa, showed that minimum observed concentration was always greater than 0.2µg/ml. Minimum inhibitory concentration of doxycycline is ≤0.03µg/ml.

Lakshmanan *et al* (2007) detected *E. canis* organisms in 50% (49/98) of samples by nPCR as against the routine blood smear examination, which revealed morulae in only 19.38% of samples. They also detected the presence of *E. canis*, even one month after a combined parenteral oxytetracycline and oral doxycycline treatment in 2 dogs out of those 49 dogs which were found positive for *E. canis* by nested PCR.

Chipde *et al* (2008) observed anaemia, neutropaenia, low PCV and thrombocytopaenia in clinical ehrlichiosis. Hematological alterations analyses of hyperproteinuria, hypoalbuminaemia, increase in serum alanine aminotransferase, serum aspartate aminotransferase, lactate dehydrogenase, urea and creatinine levels. Dogs treated with oxy-tetracycline @ 22mg/kg I/V and doxycycline @ 10mg/kg orally resulted in elimination of infectious agent and improvement was observed in hemato-biochemical findings with complete recovery.

Banerjee *et al* (2008) studied occurrence of concurrent hepatozoonosis, monocytic and granulocytic ehrlichiosis in a dog. 3.5-month-old, male, German Shepherd dog was brought to the Veterinary Teaching Hospital, Pantnagar, India. *Ehrlichia* spp. intracytoplasmic morulae was present in 0.99% and 3.12% of neutrophils and monocytes, respectively. Treatment with doxycycline resulted in both clinical and parasitologic cure in 72 h from initiation of treatment.

Baneth *et al* (2009) evaluated the quantitative real-time PCR for the detection of *E.canis* in naturally (NI) and experimentally infected (EI) dogs pre and post infection (PI) during doxycycline therapy and from blood and conjunctivas of naturally infected dogs. The primers and probe were designed to amplify a 93 bp fragment of the single copy of *E. canis* gene with the TaqMan system. All EI dogs

were positive by 7 day of PI to 10 and 12 day PI ($p < 0.05$). Even the conjunctivas of all EI dogs were positive by 12 day of PI, only 60% NI dogs were positive by conjunctival PCR. Finally they concluded that kinetics of *E.canis* during acute experimental infection with complete pathogen clearance following doxycycline treatment was demonstrated for the first time by real-time PCR.

Moreira *et al* (2009) made this study, to evaluate clinical, hematological and immunological parameters of dogs with acute monocytic ehrlichiosis before and after treatment with tetracycline. 9 dogs were experimentally infected with a Brazilian strain of *E. canis* and were treated with tetracycline hydrochloride @ 22mg/kg /day for 21 days, beginning at the thirteth day after infection. Their immune responses were evaluated before and after the treatment. Clinical and hemato-biochemical observations were similar to that of CME. Most parameters of immunophenotyping that were altered during the acute phase returned to normal, demonstrating the favourable response after treatment. This indicated that *E. canis* infection promotes important alterations, which seem to modulate the immune response, allowing persistence of the agent and eshtablishment of the acute phase. The 21-day treatment regime, results in restoration of immune condition.

Perea *et al* (2009) demonstrated the high prevalence of *E. canis*. They identified clinically, normal, *E. canis* seropositive dogs. All dogs were evaluated for the presence of *E. canis* DNA by PCR on whole blood before, during and after treatment. On day 0 all 24 dogs in the study were positive by $SNAP^{+}$. All dogs remained clinially healthy with normal CBCs for the durations of the time that they were in the study. Importantly, only one of the initial 24 $SNAP^{+}$ healthy dogs was positive on day 0. This dog was in the doxycycline treatment group and was PCR negative by day 21 post treatment. Finally they suggested that most clinically normal, *E. canis* seropositive dogs in a highly endemic geographic area are not concurrently infected and thus routine treatment of clinically normal, seropositive dogs is not warranted.

Van der Krogt (2010) investigated the clinical signs, diagnosed and treated the dogs with ehrlichiosis. Blood samples were taken from all 52 dogs, 32 out of 50 dogs were tested for hematological abnormalities of which 84% had thrombocytopenia, 69% had anaemia and 3% had leucopaenia. The SNAP 3Dx test was done on 42 dogs, out of which, 30 were positive and rests 12 were negative. Infected dogs were treated

with doxycycline and imidocarb dipropionate in combination with atropine sulphate against possible side effects.

Davoust *et al* (2013) studied morbidity and mortality associated with the diseases transmitted by the ticks, in Darkar, Capital of Senegal, located in Africa. They took two kennels having 34 dogs. In the first day of study, out of 34 dogs, 18 (53%) dogs were positive for *E. canis* by PCR. After one month of doxycycline treatment, the numbers of PCR positive dogs were decreased to 2 (5.9%).

Gaunt *et al* (2010) assessed the effects of either simultaneous or sequential experimental infections with *E. canis* and *A. platys* on hematological and serological parameters, duration of infection, and efficacy of doxycycline therapy in dogs infected with one or both organisms. 6 dogs per group were either uninfected, *A. platys* infected, *E. canis* infected, *A. platys* and *E. canis* co-infected. *A. platys* infected and *E. canis* challenged or *E. canis* infected and *A. platys* challenged at day 112 post-infection (PI). Doxycycline treatment was initiated at 211 days PI, followed by dexamethasone immunosuppression beginning 410 days PI. *E. canis* infected dogs, whether single or co-infected, remained thrombocytopenic and *E. canis* PCR positive in blood for 420 days. When treated with doxycycline, all *E. canis* infected dogs became PCR negative and the thrombocytopenia resolved. Despite immunosuppression, neither *A. platys* nor *E. canis* DNA was PCR amplified from doxycycline-treated dogs.

CHAPTER III

MATERIALS AND METHODS

3.1 Place of study

The present study entitled **"CLINICO-HAEMATO-BIOCHEMICAL AND THERAPEUTIC STUDIES ON EHRLICHIOSIS IN DOGS"** was carried out at Small Animal Clinics, Teaching Veterinary Hospital, GADVASU, Ludhiana, Punjab.

3.2 Selection of animals for study

At the teaching veterinary hospital, on an average more than fifty dogs are presented everyday for the treatment of various ailments. Dogs irrespective of age and breed showing clinical signs suggestive of fever, tick infestation, anaemia, vomiting, melena, lethargy, inappetance, anorexia, hind limb weakness, epistaxis, lymphadenopathy, ocular discharge, corneal opacity, and respiratory distress were selected and were subjected to detailed clinical and laboratory examination for identification of *Ehrlichia canis*. The study was conducted as follows:

3.3 Physical and clinical examination

3.3.1 Client information

Case number, date of presentation, name, address and phone number of the dog owner were recorded in 'Canine ehrlichiosis case record' to follow up the case at least for a period of one month.

3.3.2 Signalment

Patient specific data which included case number, age, breed, sex, weight was recorded in 'Canine ehrlichiosis case record'. This helped to sort out the age wise and breed susceptibility.

3.3.3 Patient History

A detailed history regarding the prior treatment given, tick infestation, fever, vomiting, melena and hind limb weakness, epistaxis was recorded.

3.3.4 Detailed physical examination

A complete physical examination of the animal was carried out. Various parameters like rectal temperature, respiration rate, heart rate, pulse rate, hydration status, general attitude, posture, body condition and colour of the visible mucous membrane, presence of ticks on skin coat of the dogs was examined.

3.4 Blood examination

3.4.1 Blood collection

The animal was properly restrained on the lateral recumbency. For hematological and DNA extraction for PCR assay, two millilitres of blood was collected from the cephalic or recurrent tarsal vein, using a dry sterile syringe aseptically and was immediately transferred to a vial containing Ethylene-diamine tetra-acetate (EDTA) as an anticoagulant. Thin blood smears of each blood sample were prepared. The blood smears were stained by the standard Leishman staining method and examined immediately under oil immersion (100X) lens. Remaining sample was preserved at -20ºC for PCR. For estimation of biochemical parameters 2 ml of blood was collected without anticoagulant in sterile syringes and was allowed to clot for 2 hours and centrifuged at 2500 rpm for 3 minutes for the separation of serum. Serum samples were collected and preserved at -20°C (if necessary) for estimation of biochemical parameters. Blood samples were collected on day 0^{th}, 15^{th} and 21^{th} from dogs which were infected with *Ehrlichia canis* for hematological, PCR and biochemical analysis.

3.4.2 Blood smear examination

Microscopic examination of blood smear was done after staining the prepared thin blood smears with Leishman Stain as per standard protocol (Mundim *et al* 2008).

3.4.3 Hematology

Whole blood was used to determine the following parameters using fully automatic hematology analyser[1]

Haemoglobin

Packed cell volume (PCV)

Total leucocyte count (TLC)

Total erythrocyte count (TEC)

Platelets

Differential leucocyte count (DLC) was done using Leishman's stain as per the method suggested by Jain (1986).

3.4.4 Biochemical analysis

Blood samples were analyzed for various biochemical parameters like BUN, creatinine, albumin, globulin, total protein, ALT (Alanine transaminase), ALKP

(Alkaline phosphatase), and total bilirubin. These analyses were performed using fully automated chemistry analyzer[2], using reagent Kits[3].

3.5. Serological detection of IgG Anti- *E. canis* antibodies by ImmunoComb® canine ehrlichia antibody test kit (Biogal, Galed Labs.)

Serum samples obtained from the 0^{th} day blood samples of ehrlichiosis suspected dogs used for this study. ImmunoComb® canine *ehrlichia* antibody test kit (Biogal Galed Lab., Israel) was performed at room temperature (20°-25°C) and carried out following the manufacturer's instructions. An equivalent intensity of the color reaction in comparison with a positive reference point was used as guide in order to denote the level of antibodies in each sample: intense color reactions as compared to the reference spot were considered positive for antibodies against *E. canis* whereas a colorless or faint gray color reaction indicates either a negative result or undetectable levels of antibodies.

The ''gold standard'' for the detection of antibodies to *E. canis*, is the indirect immunofluorescence antibody (IFA) test. In the clinic or laboratory, ELISA units can be translated to IFA IgG *E. canis* antibody titers by using the color scale provided in the kit. S3 is considered the "cutoff" level of IgG antibody, which is roughly equivalent to a positive immune response at a titer of 1:80 by IFA assay. Antibody titers for the different "S" levels (ImmunoComb® scores (IC)) were as follows; S1 and S2 (1:20-1:40), S3 and S4 (1:80-1:160) S5 and S6 (1:320-1:1280) (Baticados A M and Baticados W N. 2011).

3.6. Materials for PCR studies

3.6.1. Kits and reagents for DNA studies

Maxima Hot start PCR master mix, Gene Ruler^{TM} 100bp DNA ladder and 6X gel loading dye (Genetix), ethidium bromide (Sigma), agarose-Low EEO (SRL, INDIA), 10X TBE buffer (Genetix), ethanol (Merck), nuclease free water (Thermo Scientific), PCR primers (Axygen), DNA isolation kit (QIAGEN).

3.6.2. Glasswares and Plasticwares

The glasswares and plasticwares, used for the present study, were purchased from Borosil, Axygen, Tarsons and Axiva.

3.6.3. Equipments

Refrigerated circulating liquid bath (Macro Scientific works Pvt. Ltd.) Thermal cycler (Applied Bioystem), SYNGENE gel documentation system (UK), Sartorius BP 2215 weighing balance, SPINWIN Microfuge spin apparatus (Eppendorf), Horizontal gel electrophoresis apparatus (Atto), Micropipettes (Eppendorf), Deep freezer (Vestfrost).

3.6.4. Genomic DNA extraction for analysis of blood samples by PCR

For PCR assay, whole genomic DNA was isolated from 200 µl of whole blood by using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per the manufacturer's recommendations. Briefly, 20 µl QIAGEN protease was pipetted into the bottom of an autoclaved 1.5 ml eppendorf tube and then 200 µl whole blood sample was added. Thereafter, 200 µl lysis buffer (AL) was added to the eppendorf tube for lysis of blood sample and was mixed properly by pulse vortexing for 20 sec. The tube was then incubated at 56^{o}C for 15 min in a hot circulating water bath and then briefly centrifuged at 8000 rpm for 1 min in order to remove the extra drops from inside of lids. Further 200 µl of ethanol (96 -100%, molecular grade) was added in the eppendorf tube containing the sample and was mixed properly by pulse vortexing for 20 sec. After that the sample was centrifuged at 8000 rpm for 1 min in order to remove the extra drops from lids of eppendorf tube. The sample was then transferred from eppendorf tube to the QIAamp mini spin column without wetting its rim; column cap was closed and the mini spin column was centrifuged at 8000 rpm for 1.5 min. The mini spin column was placed in a clean 2 ml collection tube and the filtrate in previous collection tubes was discarded; the mini spin column was opened and 500 µl of washing buffer (AW1) was added to it without wetting its rim. The cap of mini spin column was closed and centrifuged at 8000 rpm for 1.5 min and then the mini spin column was transferred in clean new 2 ml collection tube discarding the filtrate of older collection tubes. Then 500 µl of washing buffer (AW2) was added in the mini spin column containing sample without wetting the rim and the cap was closed and centrifuged at 13000 rpm for 4 min. Thereafter, the mini spin column was transferred in a clean 1.5 ml eppendorf tube and the filtrate of older collection vials was discarded. In the end 150 µl of elution buffer (AE) was added and incubated at room temperature for 1 min, centrifuged at 8000 rpm for 1.5 min discarding the mini spin

column and the extracted DNA in elution buffer was stored at -20^{o}C for further use.

3.6.5. Standardization of *Ehrlichia* spp. PCR assay (E-PCR)

The E-PCR assay was standardized by using following set of primers as described by Murphy *et al* (1998) targeting a portion of 16S rRNA gene to amplify all *Ehrlichia* spp. The sequences of primers employed in PCR assay are as follows:

Forward primer (ECC): 5′AGAACGAACGCTGGCGGCAAGC3′

Reverse primer (ECB): 5′CGTATTACCGCGGCTGCTGGCA3′

Briefly, the PCR was set up in 25 µl reaction consisting of 1X molar concentration of 12.5 µl Maxima hot start PCR master mix, 1.5 µl of 10 pmol each of the respective primers and 5 µl of template as DNA source. The final volume was made up to 25 µl by adding the requisite amount of nucleus free water.

The cycling conditions adopted for reaction with specific primers in PCR protocol were described as under:

After an initial denaturation at 95°C for 4 min, 35 cycles of denaturation (94°C for 3sec), annealing (64°C for 45 sec) and extension (72°C for 1min) were conducted and the final extension was performed at 72°C for 8 min. These conditions have been tabulated as under:

Table 1: E-PCR reaction cycling conditions

Stage	**Temperature and Time**	**No. of cycles**
Stage 1 (Initial denaturation)	95^{o}C for 4 min	1
Stage 2 (Denaturation)	94^{o}C for 30 sec	35
Stage 3 (Annealing)	64^{o}C for 45 sec	
Stage 4 (Extension)	72^{o}C for 1 min	
Stage 5 (Final extension) Stage 6	72^{o}C for 8 min 4^{o} C for ∞	1

3.6.6. Standardization of *E. canis* species specific nested PCR assay (nEc-PCR)

Standardization of nested Ec-PCR was carried out by using *E. canis* species specific primers amplifying a portion of 16S rRNA gene of *E. canis* as described by Murphy *et al* (1998). The sequences of primers employed in PCR assay are as follows:

Forward primer (ECAN5): 5′CAATTATTTATAGCCTCTGGCTCTGGCTATAGGA3′
Reverse primer (HE3): 5′TATAGGTACCGTCATTATCTTCCCTAT3′

The nested PCR was set up in 25 µl reaction consisting of 1X molar concentration of 12.5 µl Maxima hot start PCR master mix, 1.5 µl of 10 pmol each of the respective primers are used with only difference that instead of using whole genomic DNA sample, 2 µl of the primary PCR product obtained from the E-PCR assay was used as source of DNA. The final volume was made up to 25 µl by adding the requisite amount of nucleus free water.

The cycling conditions adopted for reaction with specific primers in PCR protocol were described as under:

After an initial denaturation at 94°C for 3 min, 37 cycles of denaturation at 94^{o}C for 1 min, annealing at 55^{o}C for 2 min and extension at 72^{o}C for 1.5 min were conducted and final extension was performed at 72^{o}C for 8 min.

Table 2: nEc-PCR reaction cycling conditions

Stage	**Temperature and Time**	**No. of cycles**
Stage 1 (Initial denaturation)	94^{o}C for 3 min	1
Stage 2 (Denaturation)	94^{o}C for 1 min	37
Stage 3 (Annealing)	55^{o}C for 2 min	
Stage 4 (Extension)	72^{o}C for 1.5 min	
Stage 5 (Final extension) Stage 6	72^{o}C for 8 min 4^{o} C for ∞	1

3.6.7. Detection of the Ec-PCR amplified product

The PCR amplicons were checked for amplification by electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) at 70-90 V for 1h. Briefly, agarose (1.5%) (w/v) suspension was made in 1X TBE (Tris-acetate-EDTA) buffer and heated in microwave oven until the agarose was completely dissolved to give a clear transparent solution. Ten microlitres of ethidium bromide (10 mg/ml) was added to make final concentration of 0.5µg/ml and solution was allowed to cool. After fitting the comb in a balanced and sealed tray, the gel was casted. The solidified gel was placed in horizontal gel electrophoresis apparatus and was immersed in sufficient amount 1X TBE buffer. Comb was removed to load the sample DNA under test. PCR

amplified product (25µl) mixed with 5µl of 6X loading dye (Genetix), was loaded in the wells of the gel for electrophoresis and run for 1-2 hr at 70-90 V. Along with PCR products Generuler DNA™ ladder 100 bp (Genetix) was also run. DNA bands were visualized under UV light and were photographed using gel documentation system and molecular size of PCR product was estimated. Suitable positive and negative controls were also run alongside. DNA of *E. canis* isolated from the blood of clinically infected dog found to be positive by Leishman staining served as positive control, while leucocyte DNA isolated from healthy dogs not suffering from any haemoprotozoan infections along with a non-template control was used as negative control.

1. ADVIA® 2120 Hematology system, Siemens Healthcare Diagnostics Inc., USA
2. Johnson & Johnson VITROS 750Xrc & Fully automated chemistry system.
3. Johnson & Johnson diagnostic kits, Mumbai, India.

3.7. TREATMENT AND MANAGEMENT

Dogs with ehrlichiosis were divided into two groups viz, **A and B**. and these **two** groups were subjected to the treatment with oral doxycycline and injectable tetracycline with oral combination of doxycycline. Response of therapy was evaluated on the basis of clinical improvement, absence of ehrlichial infection from blood smear, PCR and laboratory findings.

Therapeutic regimens

Group **A** (n=20) dogs with ehrlichiosis were treated with oral doxycycline and Group **B** dogs (n=15) were treated with injectable tetracycline along with the oral combination of doxycycline and conventional therapy for canine ehrlichiosis.

3.7.1. Treatment with doxycycline

a) 20 dogs were subjected to treatment with oral doxycycline at a dosage of 10mg /kg body weight, for a period of 21 days until blood smear and PCR results become negative.

3.7.2. Treatment with tetracycline

a) 15 dogs were subjected to treatment with injectable tetracycline at a dosage of 20-22mg /kg of body weight, daily for a period of 5 days along with the oral combination of doxycycline until the reduction in clinical signs and until blood smear and PCR results become negative.

3.7.3. Conventional medical therapy for all two groups of dogs.

a) If the dog was anorectic for many days, DNS were used intravenously at the dose rate of 15-20 ml/kg body weight to rehydrate and provide energy source.
b) Metoclopramide at the dose rate of 0.2-0.4 mg/kg I/M bid were given as an antiemetic.
c) Ranitidine at the dose rate of 2 mg/kg I/M bid were given daily as H_2 Blocker.
d) Maintenance fluids were continued intravenously at the dose rate of 40-60 ml/kg/day.
e) Vitamin B complex injections were also given once a day during the course of treatment.
f) Adrenaline (local application), Adrenochrome (5-10mg), Ethamsylate (250-500mg QID), haemocoagulase (0.5-1ml total dose) as anti-haemorrhagics. Some other drugs are vitamin K and Revici (5-10ml, IV or IM). Tab Cadisper-C @ 2 tabs TID).
g) Oral supplementation of hematenics (haem up, dexorange) and livertonics (liverolin, liv-52).
h) Ivermectin injection @ 0.2mg/kg body weight against high tick infestation.

3.8 Patient evaluation

After collection of the pertaining data, the evaluation of the patient was done for a period of one month to assess the improvement in hemato-biochemical parameters and absence of *E. canis* infection in blood smear and PCR.

3.9. Statistical analysis

Prevalence of the disease was determined: prevalence of *E. canis* infection with regards to months and season in the affected animals and possible hemato-biochemical alterations and possible associations between the evaluated variables and positive reaction to the agents were determined. Further, in order to see any statistical significant differences amongst various hematological-biochemical parameters between the positive groups and the control group and comparison within the treatment group and between the treatment groups were analyzed by one way analysis of variance at 5 per cent level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted at Small Animal Clinics, Teaching Veterinary Hospital, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. A total 155 dogs showing signs of fever or tick infestation, anorexia, vomiting, lethargy, hind limb weakness and epistaxis were examined for ehrlichiosis over a 12 month period from January 2014 to December 2014. Out of 155 dogs, 12 dogs were found to be positive for ehrlichiosis by blood smear examination. Among 155, 42 dogs which are having clear signs of ehrlichiosis with thrombocytopenia are subjected to complete laboratory, PCR and serology based analysis to know the clinico-hemato-biochemical changes and to assess the therapeutic management of disease. Ten apparently healthy dogs were kept as control group.

4.1 Prevalence of ehrlichiosis in dogs

4.1.1. Blood smear Examination

Thin blood films were prepared and stained with wright leishman's stain as per the standard protocol and were examined under oil immersion objective of microscope for detection of morulae or initial bodies of *Ehrlichia canis*. When the morula was found in at least one of the monocytes or lymphocytes, the dog was considered to be positive for *E. canis* infection (Hildebrandt *et al* 1973 and Mylonakis *et al* 2003).

In the present study overall prevalence of canine ehrlichiosis based on blood smear examination was 7.74 per cent (12/155). Juyal *et al* (1994) did a prevalence study of 0.35% *E. canis* infection from Punjab by observing the morula in 752 blood samples of the dogs suspected to be infected with haemoprotozoans.

Eljadar (2010) reported 7.9 per cent (75/951) of the cases were positive for *Ehrlichia* spp. Out of these, 28 per cent (21/75) cases were positive by Wright Giemsa stained thin blood films, 36 per cent (27/75) cases were positive by buffy coat smear . Milanjeet (2013) found 2.34 per cent of cases to be positive for *E. canis* morulae in the same region of Punjab. Singh *et al* (2011a) made a prevalence study on canine parasitic infections in and around Ludhiana, Punjab. Among all prevalence rate of *Ehrlichia canis* was 1.43 per cent. Lakshmanan *et al* (2006) had observed 5.66 per

cent of dogs positive when screened for the presence of inclusion bodies of *E. canis* by blood smear examination in the Small Animal Clinic of Madras Veterinary College, Chennai.

Similar type of work done by Dhanakar *et al* (2011) found 11.35% dogs positive for ehrlichiosis in Haryana and Delhi States. From South-Western regions the republic of Korea (South Korea) morula of *Ehrlichia canis* was found in 6.1% of the screened canine blood smears by Lim *et al* (2010).

The lack of morula in the examined samples does not exclude suspected ehrlichiosis thus the final diagnosis should be supported by serological and molecular test (Nyindo *et al* 1980, Harrus *et al* 1997a, Pusterla *et al* 2000). Most commonly used serological test are ELISA and IFAT allowing to find IgM antibodies from 7^{th} day after infection and IgG from 14-15 days after the infection (Waner et al 2000a, Okewole and Adejinmi 2009).

In this present study out of 155 screened samples, 42 dogs which are showing clear signs of ehrlichiosis were subjected for molecular, serological and therapeutic studies. Total of 78.57 per cent (33/42) of molecular prevalence was noted from the suspected cases. PCR amplification of individual DNA sample (*E. canis*) using their specific primers produced expected fragments of size 389bp by nested PCR. Milanjeet *et al* (2014) performed *Ehrlichia* genus and species specific PCR and found 41.59 per cent of samples to be positive for *E.canis* by nested PCR assay. Choudhary (2009) diagnosed 3.12 per cent (218/6990) of cases for ehrlichiosis. Of the 90 clinically suspected cases 40 cases were diagnosed positive for canine ehrlichiosis based on blood smear, Buffy coat examination and nested PCR. Simplest method in diagnosis of ehrlichiosis by microscopic smear staining test using the Giemsa, Wright-Leishmann or Diff-Quick methods (Harrus *et al* 1997c and Inokuma *et al* 2005).

Shimada *et al* (2002) did an experimental inoculation on beagle dogs with *E. canis* infected DH82 cells, to know the plasma concentration of CRP. After inoculation infection was confirmed by both presence of antibody titers (IFA test) and by amplification of *E. canis* 16s RNA gene with same primers what we used in our study and increased plasma concentration of CRP were noted. Detected antigen 18-27 days after infection and antibodies between 5-15 days.

In this present work out of 84 suspected dogs 73 (86.90%) dogs were seropostive to *E. canis* antibodies and results are agreeing with the similar work previously done by Eljadar (2010) in the same region of Punjab, found that 93.33 per cent (70/75) cases were positive by serology. Harikrishnan *et al* (2009) detected *E. canis* antibodies in sera from 21/56 dogs (37.5%) in ELISA and 23 dogs (41.1%) in Dot-ELISA. They stated that ELISA is a valuable tool for diagnosing the subclinical and chronic forms of canine ehrlichiosis. Akhtardanesh *et al* (2010) found overall seroprevalence of ehrlichiosis was 14.63% which was determined as 13.8% and 10.6% using immunofluorescence antibody test (IFA) and rapid immunochromato-graphy (ICA), respectively. Baneth *et al* (1996) assayed 410 serum samples by IFA test and serum reactive at a 1:20 dilution or greater was considered positive. They found overall seroprevalence was 30 per cent with 23.9 per cent in pet dogs and 37.5 per cent in stray dogs. Seroprevalence in apparently healthy dogs was (17.6 per cent) lower but did not differ significantly from sick animals (26.6 per cent). Amusategui *et al* (2008) analysed 649 canine serum samples by IFA test. 57.83 per cent of total seropositivity was found in dogs.

De Castro *et al* (2004) in their work stated that after 30 days of inoculation all the infected dogs showed positive titers for *E. canis* by testing all the samples for specific IgG response to *E. canis* with dot-blot ELISA kit (Immunocomb®, BIOGAL). Sasanelli *et al* (2009) reported a case of *E. canis* infected dog with an antibody titer of 1:160. Castro (1997) and Oria (2001) used the Immunocomb test – (Biogal) based on the DBELIA technique, to determine IgG antibodies specific for the organism.

Variable prevalence of ehrlichiosis has been reported from various parts of India. Kumar *et al* (2009) reported over all positivity for *Ehrlichia canis* 6 per cent (29/485) in canines from Chennai city. Chipde *et al* (2007) had shown 42.85 per cent prevalence of canine ehrlichiosis in Nagpur city.

4.1.1. Month wise prevalence of ehrlichiosis in dogs

4.1.1.1. Month wise prevalence of ehrlichiosis in dogs by blood smear examination (n=12/155).

In the present study maximum prevalence by blood smear examination 40 per cent in the month of October, 20-25 per cent in the months of March, June and

November, 16.66 per cent in the month of September and minimum of 2.85 per cent in the month August whereas in the months of December, January, February, April and July no cases were observed. (Table 3, Figure 1).

4.1.1.2. Month wise molecular prevalence of ehrlichiosis in dogs by Polymerase chain reaction (PCR) (n=33/42).

Maximum prevalence (100%) found in the months of June and October, 88.88 per cent in the month of August, 83.33 per cent in the month of September, 80 per cent in the month of March, Minimum of 75 per cent in the months of July and November. No cases were reported in January, February, April and December. Majority CME occurs in endemic area during the spring and summer months when the tick population is more active (Waner and Harrus 2000a) (Table 3, Figure 2).

4.1.1.3. Month wise serological prevalence of ehrlichiosis in dogs by ImmunoComb® canine ehrlichia antibody test kit (Biogal Galed Labs) (n=73/84).

In the present study maximum (100%) seroprevalence noticed in the months November, 96 per cent in August, 92.85 per cent in June, 90 per cent in September, 87.5 in the month of May, 83.33 per cent in July, 66.66 percent in April, 42.86 per cent in March minimum of 33.33 per cent in October. No cases were found in the months of January, February and December. (Table 4, Figure 3).

4.1.1.4. Month wise molecular prevalence (n=33/42) in comparison with month wise serological prevalence (n=36/42) of same cases positive for ehrlichiosis.

Molecular prevalence 100 per cent in the months of June and October, 80-88.88 per cent in the month of March, August and September, 75 per cent in the months of July and November and minimum of 40 per cent in the month of May. Serological prevalence seen maximum (100%) in the months of April, August and November, 80-83.88 per cent in the months of March, May, June and September, 75 per cent in July and minimum of 50 per cent seroprevalence in the month of October (Table 3, Figure 4).

Table 3: Months wise prevalence of ehrlichiosis in dogs

Months	Blood smear examined n=155	Positive by smear n=12 (%)	Blood samples tested n=42	Positive by PCR n=33 (%)	Serum samples tested (n=42)	Positive by serology n=36 (%)
Jan	7	0	0	0	0	0
Feb	5	0	0	0	0	0
Mar	10	2 (20)	5	4 (80)	5	4 (80)
Apr	14	0	1	0	1	1 (100)
May	17	0	5	2 (40)	5	4 (80)
Jun	15	3 (20)	6	6 (100)	6	5 (83.33)
Jul	20	0	4	3 (75)	4	3 (75)
Aug	35	1 (2.85)	9	8 (88.88)	9	9 (100)
Sep	12	2 (16.66)	6	5 (83.33)	6	5 (83.88)
Oct	5	2 (40)	2	2 (100)	2	1 (50)
Nov	8	2 (25)	4	3 (75)	4	4 (100)
Dec	7	0	0	0	0	0
Total	**155**	**12 (7.74)**	**42**	**33 (78.57)**	**42**	**36 (85.71)**

Figures in parentheses indicate percentage & (n) indicates number of dogs

Table 4: Months wise seroprevalence of ehrlichiosis in dogs

Months	Serum samples tested n=84	Positive for *E. canis* antibodies n=73 (%)
Jan	0	0
Feb	0	0
Mar	7	4 (80)
Apr	3	2 (66.66)
May	8	7 (87.5)
Jun	14	13 (92.85)
Jul	6	5 (83.33)
Aug	25	24 (96)
Sep	10	9 (90)
Oct	3	1 (33.33)
Nov	8	8 (100)
Dec	0	0
Total	**84**	**73 (86.90)**

Figures in parentheses indicate percentage & (n) indicates number of dogs

4.1.2. Season wise prevalence of ehrlichiosis in dogs

4.1.2.1. Season wise prevalence of ehrlichiosis in dogs by blood smear examination (n=12/155).

Maximum prevalence (30.76%) observed in autumn (October & November), 9.33 per cent cases were found positive in summer (May & June), 8.33 per cent in spring (March & April) and 4.47 per cent in rainy (July, August & September). (Table 5, Figure 5).

4.1.2.2 Season wise molecular prevalence of ehrlichiosis in dogs by PCR (n=33/42).

Maximum prevalence (84.21%) in the month of rainy season (July, August & September), 83.33 per cent in autumn (October & November), 72.72 per cent in summer (May & June), 66.66 per cent in spring (March & April), whereas no cases were reported in winter (December, Janvaury & February) (Table 5, Figure 6).

4.1.2.3. Season wise seroprevalence of ehrlichiosis in dogs by ImmunoComb® canine ehrlichia antibody test kit (Biogal Galed Labs) (n=73/84).

Overall 86.90 per cent of seroprevalence was reported and distributed as follows, maximum seroprevalence was reported in summer (100%) and rainy (92.68%) season, 75 per cent in autumn, 50 per cent in spring, no prevalence found in winter season (Table 6, Figure 7).

4.1.2.4. Season wise molecular prevalence (n=33/42) in comparison with season wise serological prevalence (n=36/42) of same cases which are positive for ehrlichiosis.

Overall 78.57 per cent of molecular prevalence was reported with maximum prevalence (84.21%) in rainy season, followed by 83.33 per cent in autumn, 72.72 per cent in summer, 66.66 per cent in spring, whereas no cases were reported in winter, whereas 88.09 per cent of total seroprevalence was found among maximum prevalence in rainy (89.47%) season, 83.33 per cent in spring and autumn, 81.81 per cent in summer and no cases were reported in winter (Table 5, Figure 8).

In the present study apparently blood smear examination, PCR and serology showed that maximum prevalence was in the months of March, June, July, August, September, October and November. Acording to the season wise, mainly in summer,

rainy, autumn and least in spring season. The probable reason behind this trend may be correlated to the seasonal activity of the brown dog tick, *Rhiphicephalus sanguineus* which is in its abundance in hot and humid period of the year (Soulsby 1982) thus resulting in the higher incidence of haemoprotozoan infections in warm months during warmer seasons (Harrus *et al* 1997b).

Further, Keefe *et al* (1982) observed that *E. canis* infection was more likely to occur with higher rates in tropical and temperature zones below 45°latitude while the rates of infection decreased in the cold zone north of 45° latitude. Similar seasonal variation has been reported from Guwahati with maximum prevalence (4.37%) in pre monsoon (march-may) followed by monsoon (1.46%) (june-august) and post monsoon (1.46%) (september-november) and least prevalence percent of 0.29% was recorded in winter (december-february) (Dutta *et al* 2013). Kumar *et al* (2009) found 8.2% of *E. canis* infection from july to november. Similarly, Eljadar (2010) from Ludhiana, Punjab recorded maximum prevalence of the disease during the summer season with prevalence rate of 56% followed by rainy season (37%).

Table 5: Season wise prevalence of ehrlichiosis in dogs

Months	Blood smear examined n=155	Positive by smear n=12 (%)	Samples used for PCR n=42	PCR positive n=33 (%)	Serum samples tested n=42	Serology Positive n=36 (%)
Winter (Dec, Jan & Feb)	19	0	0	0	0	0
Spring (Mar& Apr)	24	2 (8.33)	6	4 (66.66)	6	5 (83.33)
Summer (May & June)	32	3 (9.33)	11	8 (72.72)	11	9 (81.81)
Rainy (Jul, Aug & Sep)	67	3 (4.47)	19	16 (84.21)	19	17 (89.47)
Autumn (Oct & Nov)	13	4 (30.76)	6	5 (83.33)	6	5 (83.33)
Total	155	12 (7.74)	42	33 (78.57)	42	36 (85.71)

Figures in parentheses indicate percentage & (n) indicates number of dogs

Table 6: Seasonwise seroprevalence of ehrlichiosis in dogs

Months	Serum samples tested n=84	Positive for *E. canis* antibodies n=73 (%)
Winter (Dec, Jan & Feb)	0	0
Spring (Mar& Apr)	10	5 (50)
Summer (May & June)	21	21 (100)
Rainy (Jul, Aug & Sep)	41	38 (92.68)
Autumn (Oct & Nov)	12	9 (75)
Total	84	73 (86.90)

Figures in parentheses indicate percentage & (n) indicates number of dogs

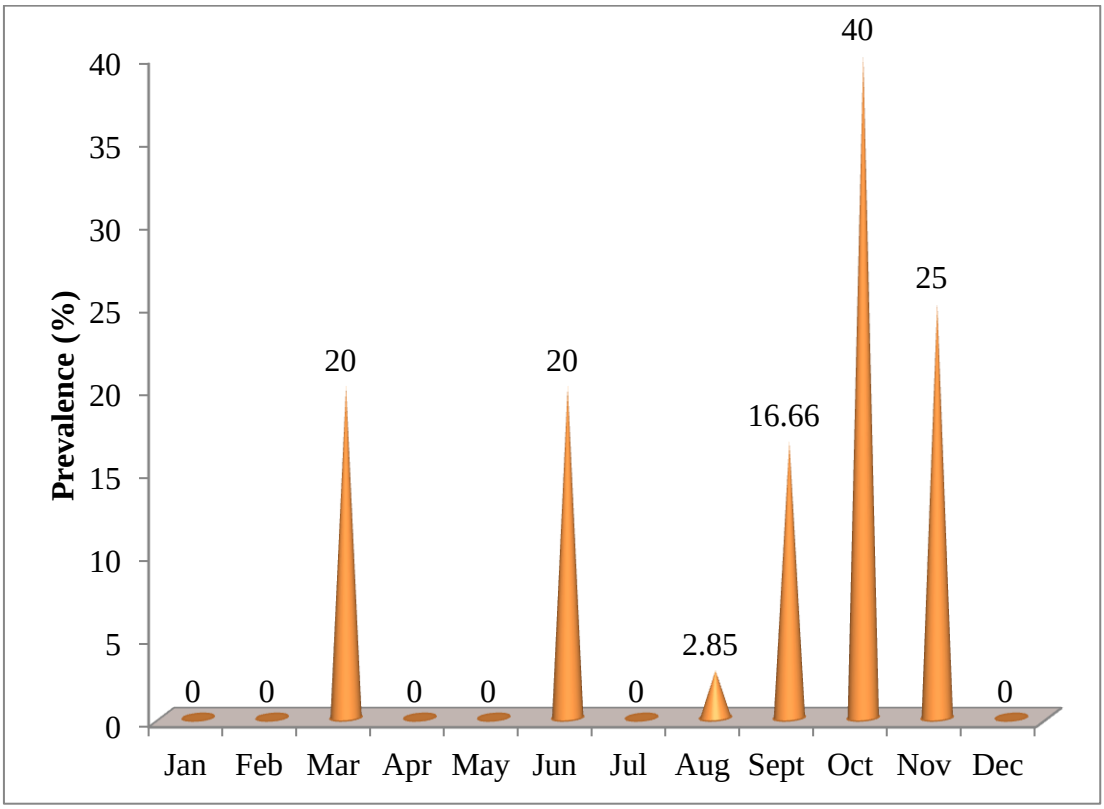


Figure 1. Month wise prevalence of ehrlichiosis in dogs by blood smear examination

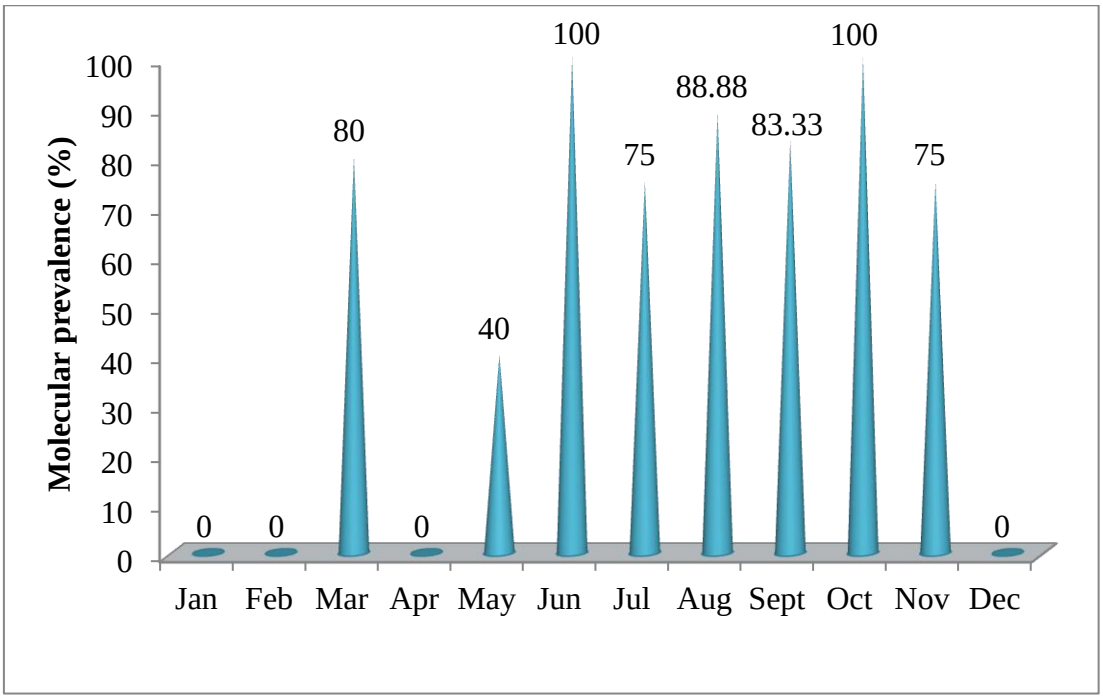


Figure 2. Month wise molecular prevalence of ehrlichiosis in dogs by PCR

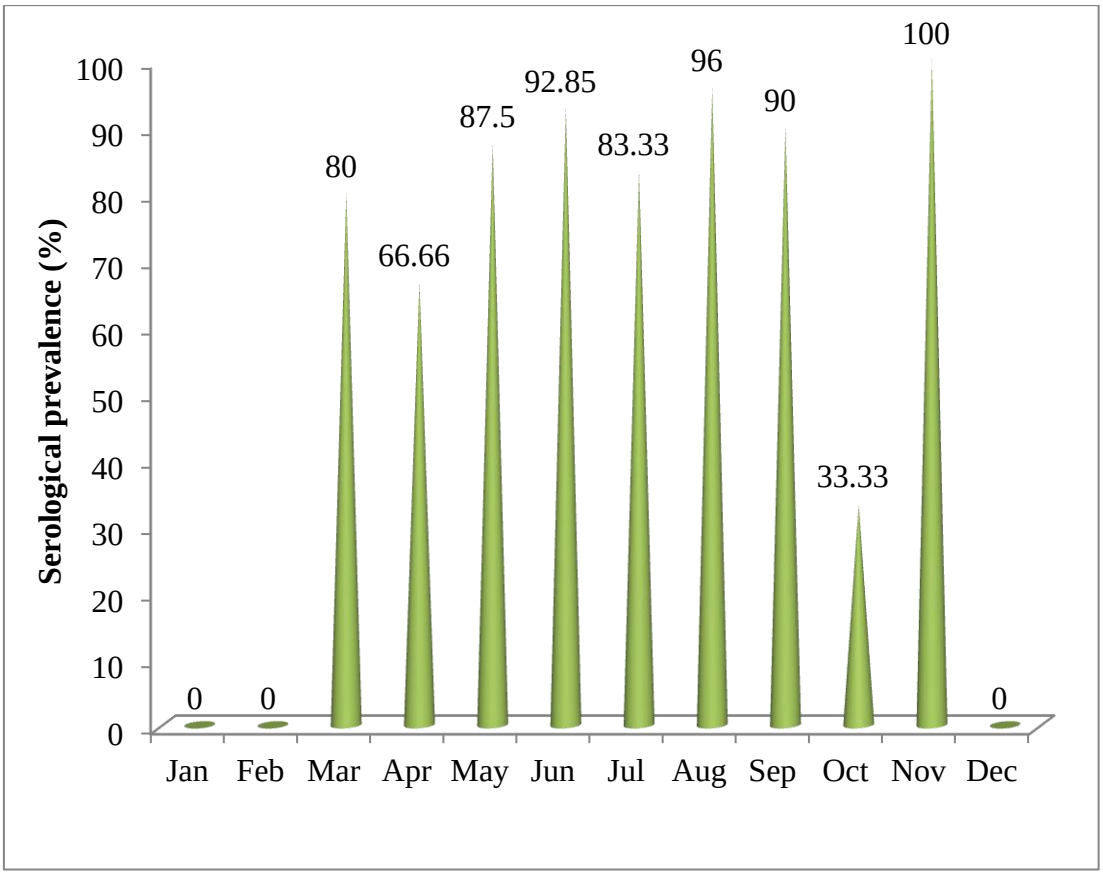


Figure 3. Months wise seroprevalence of ehrlichiosis in dogs

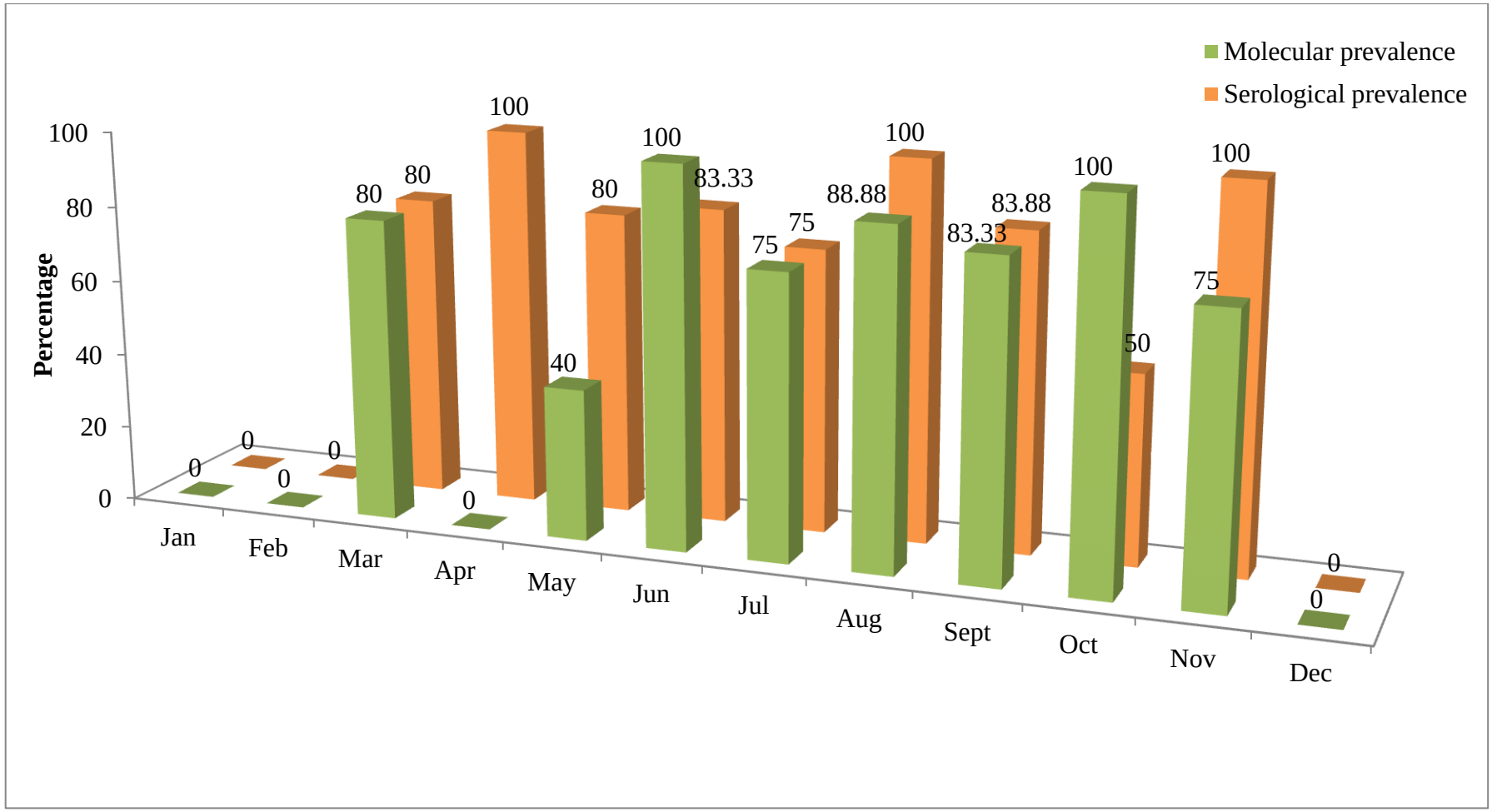


Figure 4. Month wise molecular prevalence in comparison with month wise serological prevalence of ehrlichiosis in dogs

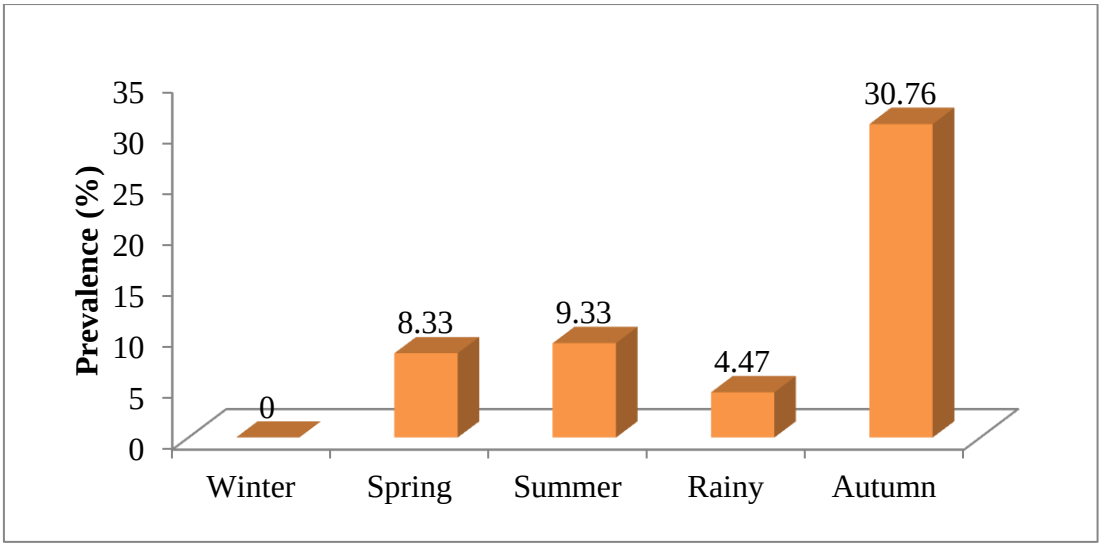


Figure 5. Season wise prevalence of ehrlichiosis in dogs by blood smear examination

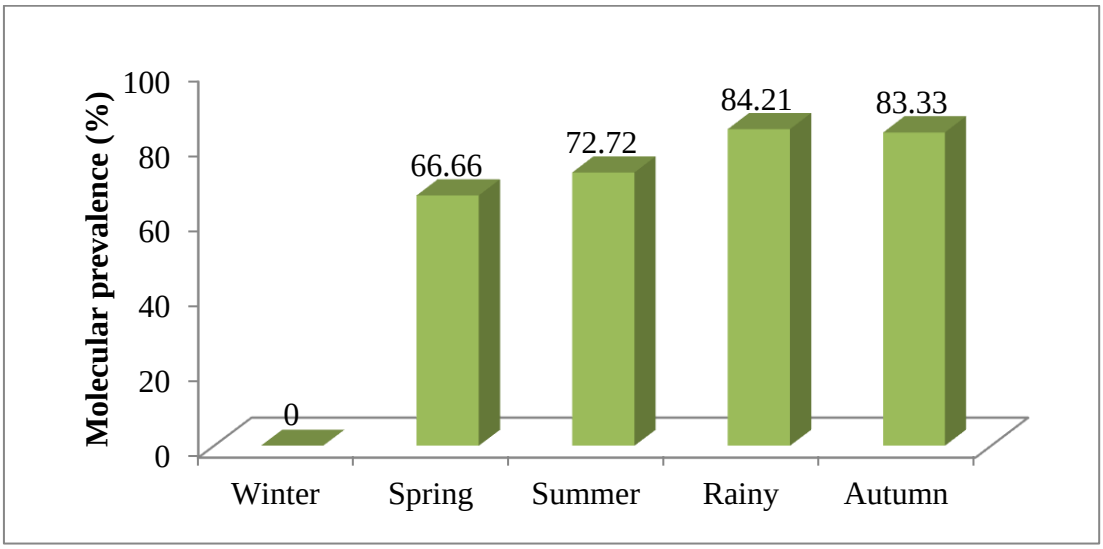


Figure 6. Season wise molecular prevalence of ehrlichiosis in dogs by PCR

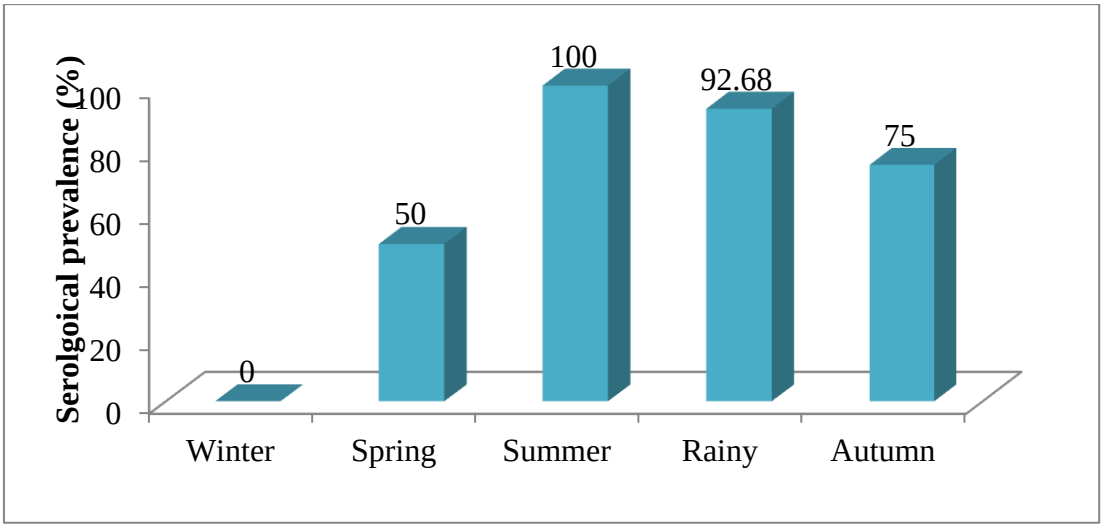


Figure 7. Season wise seroprevalence of ehrlichiosis in dogs

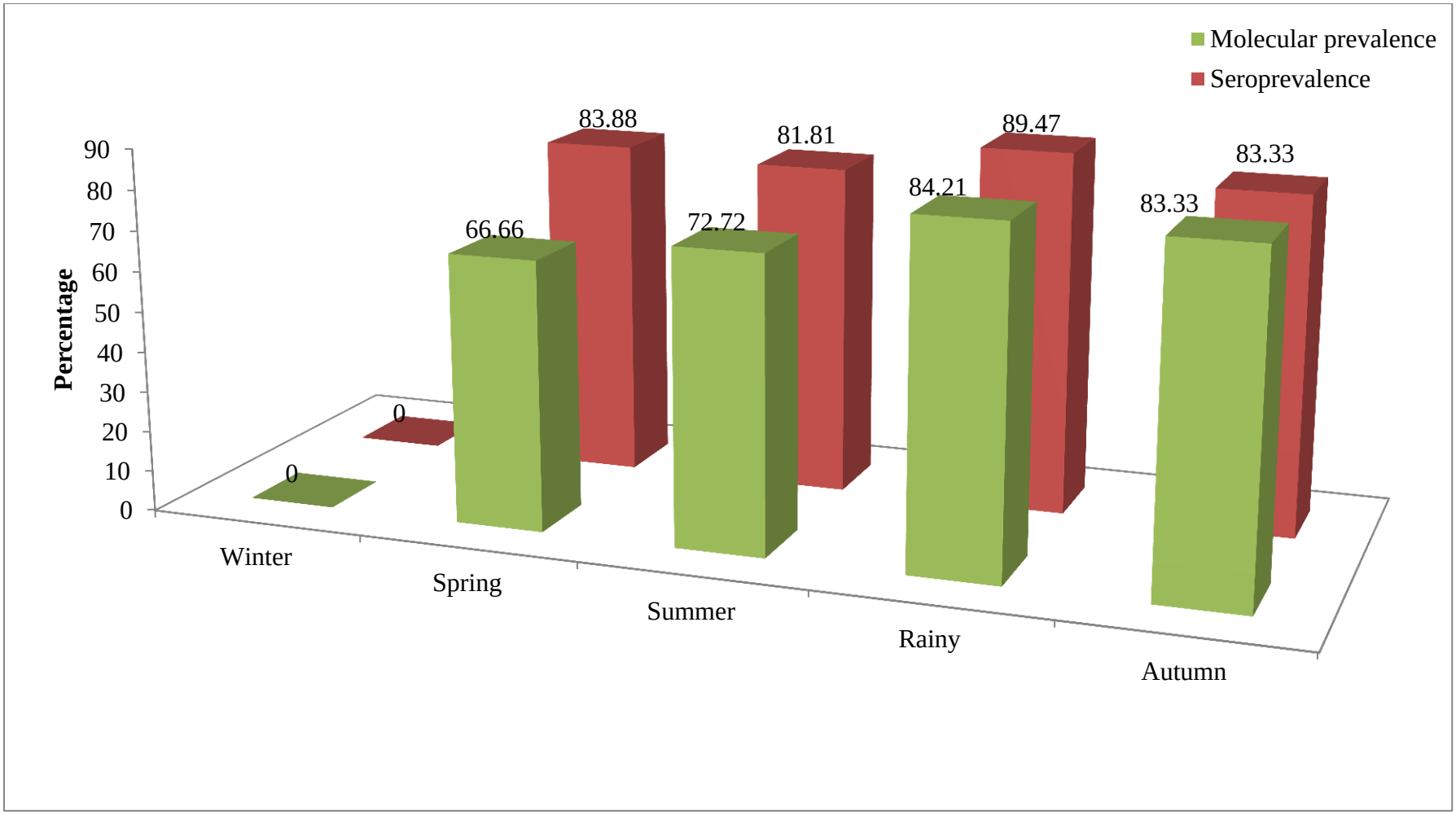


Figure 8. Season wise molecular prevalence in comparison with season wise serological prevalence of ehrlichiosis in dogs

4.2 Clinical signs/findings

All the dogs which are positive for ehrlichiosis of different breeds and of both sexes, showed varied clinical signs such as, congested, pale mucus membrane, inappetance, anorexia, tick infestation, fever, weakness, depression, lethargy, vomiting, melena, respiratory distress, lymphadenopathy, loss of weight and epistaxis etc. Most of the cases were having common history of fever, anorexia and tick infestation.

4.2.1. Clinical findings of dogs which are positive for ehrlichiosis by Blood smear examination (n=12/155)

Most common clinical signs observed in dogs which are positive by routine blood smear examination were fever (66.66%), congested mucus membrane, tick infestation and melena (58.33%), anorexia and lymphadenopathy (50%), pale mucus membrane and loss of weight (41.66%), inappetance, vomiting and respiratory distress (25%), epistaxis and petechieal haemorrhages (16.66%), depression, bleeding tendency, ocular discharge, oedema of legs and normal feed intake (8.33%), lethargy (3.33%) (Table 7, Figure 10). These findings in confirmatory with the findings of Dhanakar *et al* (2011) found that dogs with ehrlichiosis were having the signs such as tick infestation, depression, pyrexia, anaemia, vomition, bleeding tendencies. Waner and Harrus (2000a) discussed similar findings in different phases of ehrlichiosis in dogs. Other signs like corneal opacity, bleeding tendencies, vomiting, ocular discharge, lameness in acute stage. In chronic stage fever, peripheral edema of hind limbs, severe epistaxis, interstitial pneumonia, renal failure and arthritis in severe form of disease. Other signs like ataxia, convulsions, hemorrhages, extensive plasma cell infiltration and perivascular cuffing of the meninges was seen. Nose bleeds and melena were observed by Pyle *et al* (1980) and Cockwill *et al* (2009).

4.2.2. Clinical findings of dogs which are positive for ehrlichiosis by Polymerase chain reaction (n=33/42)

Most common clinical signs reported in *E. canis* infected cases were having tick infestation and weakness (54.54%), congested mucus membrane (51.51%), fever (48.48%), followed by anorexia and melena (45.45%), pale mucus membrane and epistaxis (42.42%), inappetance and lymphadenopathy (36.36%), loss of weight (27.27%), respiratory distress (21.21%), lethargy and vomiting (15.15%), hind limb weakness (12.12%), depression (9.09%) bleeding tendency, ocular discharge,

petechieal haemorrhages, seizures (6.06%), corneal opacity, oedema of legs (3.03%) (Table 7, Figure 10). Walker *et al* (1970), Troy and Forrester (1990) observed high fever, anorexia, lymphadenopathy, respiratory disturbances, nervous and ocular alterations. Petechial hemorrhage in subcutaneous tissue and most organs, generalized lymphadenopathy and edema of the limbs (Hildebrandt *et al* 1973). Bleeding tendency in the course of the *E. canis* infection is the result of the rickettsial affinity to the cells of the endothelium of the blood vessels, damage of blood vessels and activation of platelets which will aggregate at the damaged spot and leads to excessive utilization resulting in thrombocytopaenia (Harrus *et al* 1996b and Cockwill *et al* 2009).

4.2.3. Clinical findings of dogs which are positive for ehrlichiosis by Serological examination (n=73/84)

All most all clinical signs are similar as mentioned above with slight difference in their percent prevalence. Affected dogs were having tick infestation (58.66%), weakness (46.66%), anorexia and melena (41.33%), Lymphadenopathy (40%), inappetance and fever (37.33%), congested (33.33%) and pale mucus membrane (32%), hind limb weakness (29.72%), vomiting and epistaxis (24%), respiratory distress (14.66%), seizures and petechieal hemorrhages (10.81%), pink conjunctiva (10.66%), normal intake of food (8%), loss of weight, bleeding tendency and ocular discharge (6.66%), corneal opacity (5.33%), oedema of legs (2.7%) (Table 7, Figure 10).

The above mentioned clinical findings are agreeing with the findings of Manohar and Ramakrishnan (1982) observed symptoms such as pyrexia, anorexia, conjunctivitis, mucus discharge from both the eyes, loss of weight and corneal opacity. Kuehn and Gaunt (1985) noticed symptoms like depression/lethargy, weightloss, epistaxis, melena, petechiae, lymphadenopathy and anorexia. Meinkoth *et al* (1989) reported seizures are one of the notable nuerological sign should not be neglected in diagnosing ehrlichiosis. He specifically mentioned signs seen in acute phase were mild and non specific. Most of the signs reduce spontaeneously within 1 to 4 weeks, but dogs can remain sub-clinically infected.

In chronic phase along with weakness, depression, anorexia, fever, other signs like peripheral edema, anemia, bleeding due to platelet reduction, secondary bacteriologic infections, pneumonia, glomerulonephritis and lameness due to arthritis were mentioned by Harrus *et al* (1997a) and Neer (1998).

Table 7: Clinico-symptomatology in dogs with ehrlichiosis

Clinical signs/findings	Smear positive n=12 (%)	PCR positive n=33 (%)	Sero-Positive n=73 (%)
Fever	8 (66.66)	24 (72.72)	28 (37.33)
Tick infestation	7 (58.33)	18 (54.54)	44 (58.66)
Anorexia	6 (50)	17 (45.45)	34 (46.57)
PMM	5 (41.66)	17 (51.51)	33 (45.21)
CMM	7 (58.33)	16 (48.48)	32 (43.83)
Weakness	7 (58.33)	18 (54.54)	35 (46.66)
Melena	7 (58.33)	15 (45.45)	31 (41.33)
Lymphadenopathy	6 (50)	12 (36.36)	30 (40)
Inappetance	3 (25)	14 (36.36)	35 (47.95)
Epistaxis	2 (16.66)	14 (42.42)	18 (24)
Loss of weight	5 (41.66)	9 (27.27)	5 (6.66)
Vomiting	3 (25)	5 (15.15)	18 (24)
Respiratory distress	3 (25)	7 (21.21)	11 (14.66)
Petechieal haemorrhages	2 (16.66)	2 (6.06)	4 (10.81)
Lethargy	4 (3.33)	5 (15.15)	6 (8)
Bleeding tendency	1 (8.33)	2 (6.06)	5 (6.66)
Ocular discharge	1 (8.33)	2 (6.06)	5 (6.66)
Depression	1 (8.33)	3 (9.09)	3 (4)
Hind limb weakness	0	4 (12.12)	11 (29.72)
Oedema of legs	1(8.33)	1 (3.03)	1 (2.7)
Seizures	0	2 (6.06)	4 (10.81)
Corneal opacity	0	1 (3.03)	4 (5.33)
Mucous membrane normal	0	0	8 (10.66)
Normal feed intake	1 (8.33)	2 (12.12)	4 (5.48)

Figures in parentheses indicate percentage & (n) indicates number of dogs

Chandrasekar *et al* (2002) observed congested mucus membrane, epistaxis, diarrhea, petechiae and tick infestation. Pasa and Azizoglu (2003) and Sacchini *et al* (2007) observed anorexia, weight loss, lethargy, pyrexia, lymphadenopathy and anemia in seropositive dogs. Ajay and Varshney (2006) observed arrhythmia, tachypnoea and uveitis. Sasanelli *et al* (2009) diagnosed a case of *E. canis* by serology having atypical signs such as diarrhoea, cough and involvement of respiratory system.

Fever and other clinical signs were observed in all infected dogs may have been caused by increased production of interleukin-1 (IL-1) by antigen presenting cells and B cells or exogenous pyrogen products of parasite (Gershwin *et al* 1995). Increased lymphocyte numbers in medullary and paracortical regions of lymphnodes, medullary cord hyperplasia and increased number of histiocytes and plasma cells in the lymph nodes, white pulp and splenic cord hyperplasia in the spleen are reasons for lymphadenopathy and spleenomegaly De castro *et al* (2004).

Immunohistochemistry of lymphoid organs revealed increased number of IgM and IgG immunolabled cells in dogs with ehrlichiosis. Such findings associated with an increase in specific anti- *E. canis* IgG antibody titers, indicate that humoral responses play a main role in CME (Cadman *et al* 1994).

Table 8. Clinical signs in acute phase of ehrlichiosis

Clinical findings	**Acute phase**		
	Smear positive n=12 (%)	**PCR positive n=33 (%)**	**Seropositive n=73 (%)**
Fever	8 (66.66)	24 (72.72)	28 (37.33)
Tick infestation	7 (58.33)	18 (54.54)	44 (58.66)
Anorexia	7 (58.33)	17 (45.45)	34 (46.57)
PMM	5 (41.66)	17 (51.51)	33 (45.21)
CMM	7 (58.33)	16 (48.48)	32 (43.83)
Weakness	7 (58.33)	18 (54.54)	35 (46.66)
Melena	7 (58.33)	15 (45.45)	31 (41.33)
Lymphadenopathy	6 (50)	12 (36.36)	30 (40)
Epistaxis	2 (16.66)	14 (42.42)	18 (24)

Figures in parentheses indicate percentage & (n) indicates number of dogs

Table 9. Clinical signs in chronic phase of ehrlichiosis

Clinical findings	Chronic phase (Including signs of acute phase)		
	Smear positive n=12 (%)	PCR positive n=33 (%)	Seropositive n=73 (%)
Loss of weight	5 (41.66)	9 (27.27)	5 (6.66)
Vomiting	3 (25)	9 (27.27)	18 (24)
Respiratory distress	3 (25)	7 (21.21)	11 (14.66)
Petechieal haemorrhages	2 (16.66)	2 (6.06)	4 (10.81)
Lethargy	4 (3.33)	5 (15.15)	6 (8)
Bleeding tendency	1 (8.33)	2 (6.06)	5 (6.66)
Ocular discharge	1 (8.33)	2 (6.06)	5 (6.66)
Depression	1 (8.33)	3 (9.09)	3 (4)
Hind limb weakness	0	6 (18.18)	11 (29.72)
Oedema of legs	1(8.33)	1 (3.03)	1 (2.7)
Seizures	0	2 (6.06)	4 (10.81)

Figures in parentheses indicate percentage & (n) indicates number of dogs

4.3 Vital body parameters in dogs with erhlichiosis

4.3.1 Rectal temperature

The mean ± SD values of rectal temperature (^{o}F) of dogs which are smear positive for *E. canis* were 103.96±1.53 ^{o}F, PCR positive were 104.15±1.42 ^{o}F, seropositive were 104.13±1.52 ^{o}F (Table 10). Infected dogs positive by above three diagnostic methods had significant difference in rectal temperature as compared to control group. Shipov *et al* (2008) mentioned in their study 37.5 per cent of positive cases were having rectal temperature more than 107.25^{o}F.

4.3.2 Heart rate

The mean ± SD values of heart rate (per minute) in dogs affected with *E. canis* were having 84.83±6.48 (Smear positive), 90.78±11.14 (PCR positive), 89.17±11.01 (Seropositive) beats per minute (Table 10). In heart rate no significant difference noted between the affected and control group.

4.3.3 Respiration rate

The mean ± SD values of respiration rate (per minute) in dogs infected with *E. canis* were 25.33±5.25 (smear positive), 28.19±6.49 (PCR positive) and 29.36±7.13 (seropositive) per minute (Table 10). In respiration rate no significant difference noted between the affected and control group.

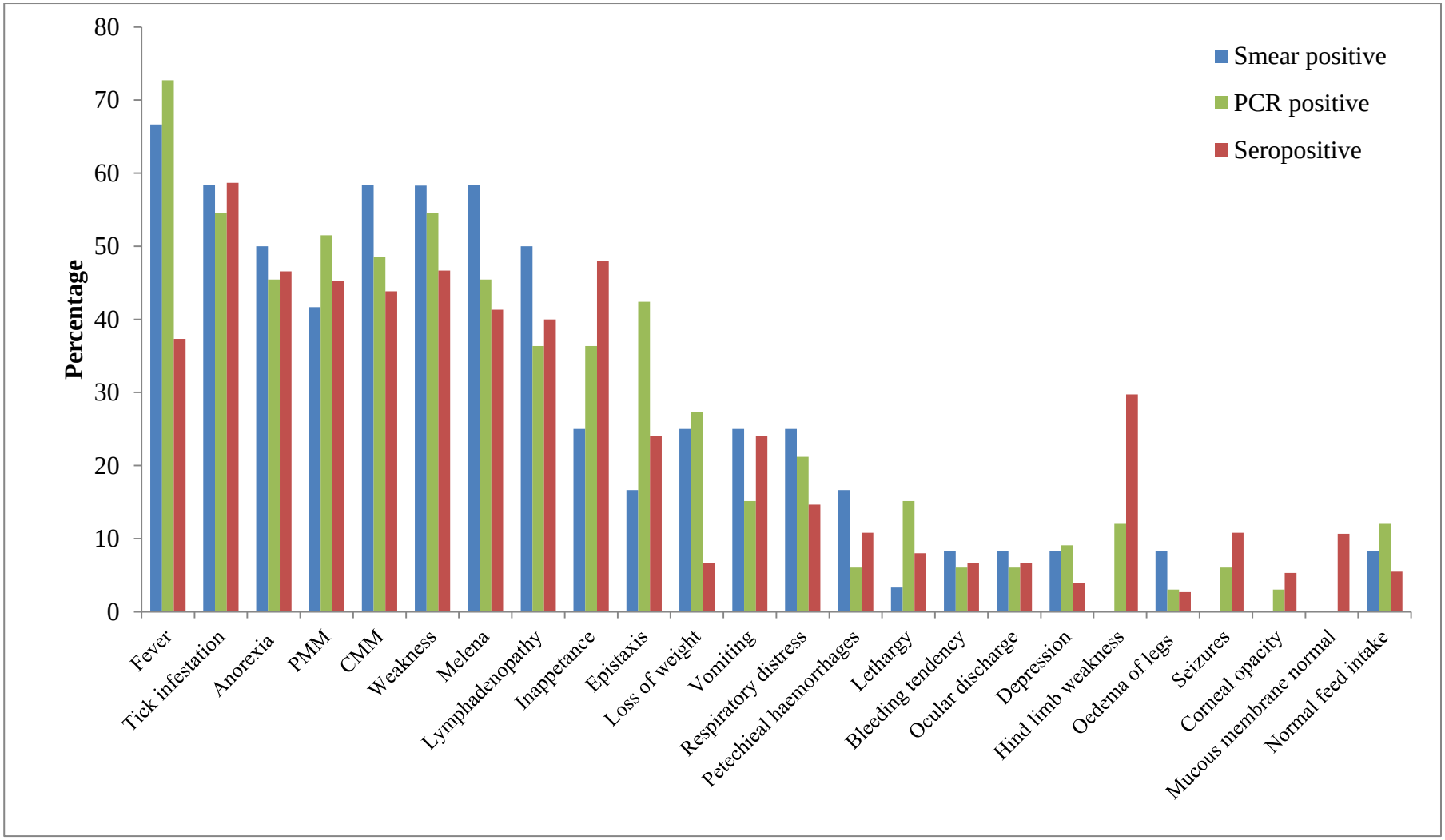


Figure 9. Comparative Clinico-symptmatology of dogs positive for ehrlichiosis by three different diagnostic tests

4.3.4 Pulse rate

The mean ± SD values of respiration rate (per minute) in dogs infected with *E. canis* were 83±6.49 (smear positive), 88.78±10.710 (PCR positive) and 87.58±10.97 (seropositive) per minute (Table 10). No significant difference noted between the affected and control group.

Table 10: Comparative diagnostic study of vital body parameters of *E. canis* affected dogs by one way Analysis of variance.

Parameters (0 day)	Rectal Temperature (°F)	Heart rate (per minute)	Respiration rate (per minute)	Pulse rate (per minute)
Smear positive (n=12)	103.96±1.53[b]	84.83±6.48[a]	25.33±5.25[a]	83±6.49[a]
PCR positive (n=33)	104.15±1.42[b]	90.78±11.14[a]	28.19±6.49[a]	88.78±10.79[a]
Sero positive (n=36)	104.13±1.52[b]	89.17±11.01[a]	29.36±7.13[a]	87.58±10.97[a]
Control (n=10)	92.01±0.71[a]	90.3±11.69[a]	29.8±2.15[a]	88.8±11.50[a]

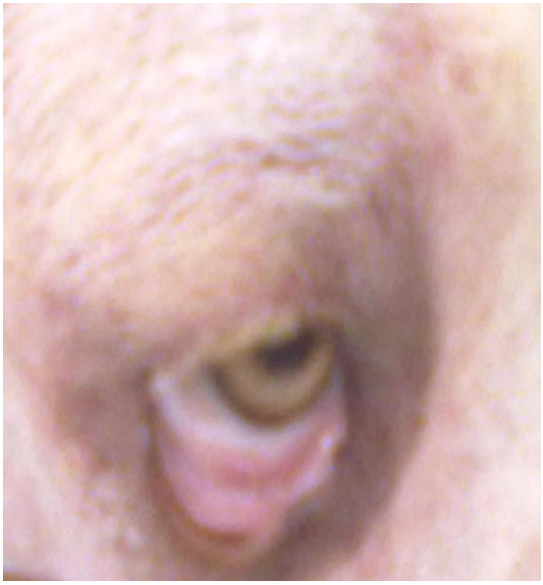
Figures with different superscripts in a column differ significantly ($p<0.05$)
Figures with same superscripts in a column do not differ significantly ($p<0.05$)

4.4 Hemato-biochemical findings in dogs with ehrlichiosis

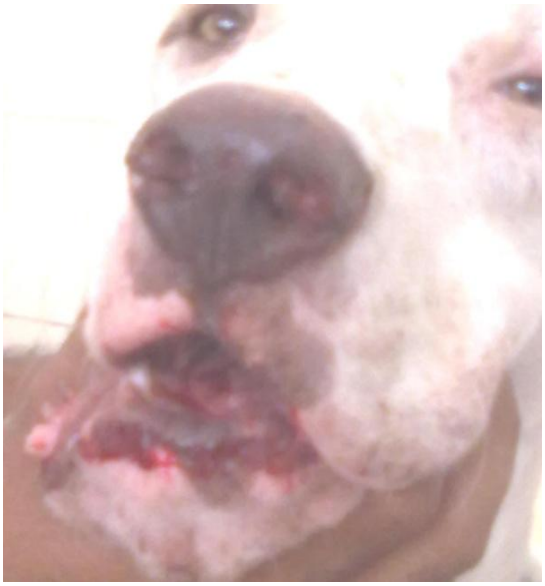
Most common hemato-biochemical findings in dogs with ehrlichiosis were anaemia, thrombocytopenia, leucocytosis, neutropenia and lypmphopenia followed by hyperproteinaemia, hypoalbuminaemia, hyperglobulinaemia, decrease in albumin & globulin ratio, increased level of ALT, increase in ALKP, hyperbilirubinaemia, slight increase in BUN and creatinine.

4.4.1. Hemato-biochemical findings in dogs positive for ehrlichiosis by blood smear examination

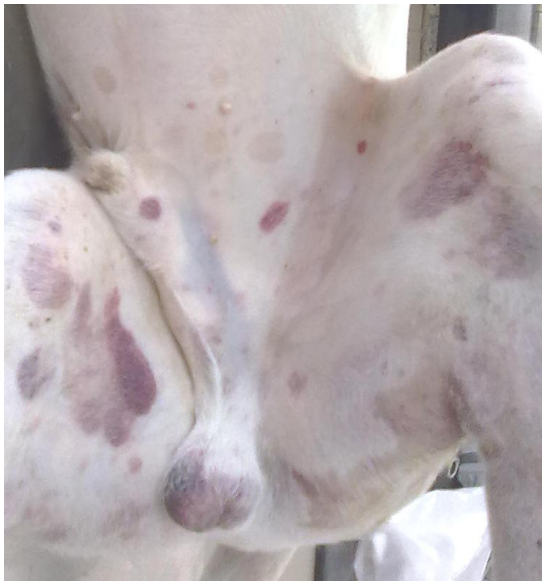
Most common hematological findings reported in *E.canis* infected dogs diagnosed by blood smear (n=12) were having anaemia (100%), thrombocytopenia (100%), lymphopenia (75%), leucocytosis (58.33%), leucopaenia (41.66%), neutropenia (25%). Biochemical findings were hyperbilirubinaemia (41.66%), increased level of AST (83.33%), increase in ALT (66.66%), increase in ALKP



Pale mucus membrane



Epistaxis with bleeding tendency



Petechieal haemorrhages and large circular red spots on ventral aspect of abdomen

Figure 10. Indian bully breed of dog showing signs of ehrlichiosis

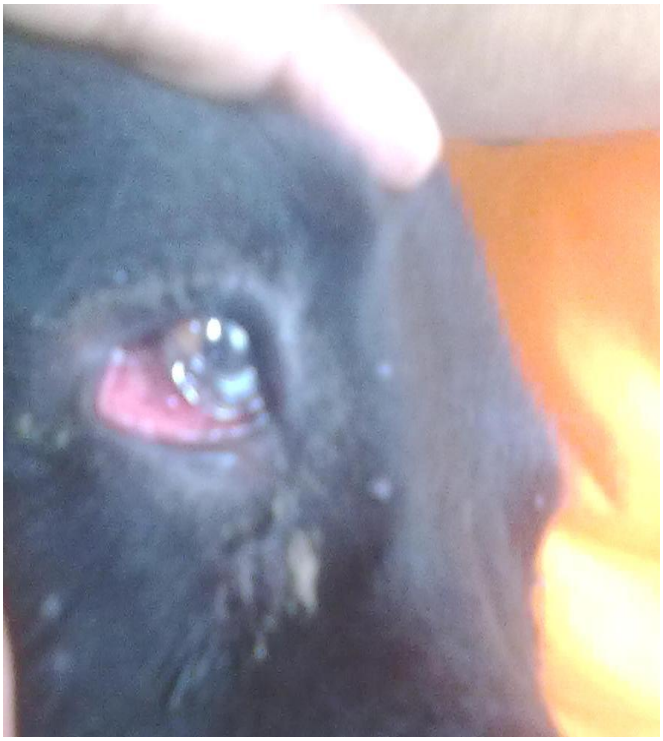


Figure 11. Labrador retriever breed of dog showing congetsed mucus membrane

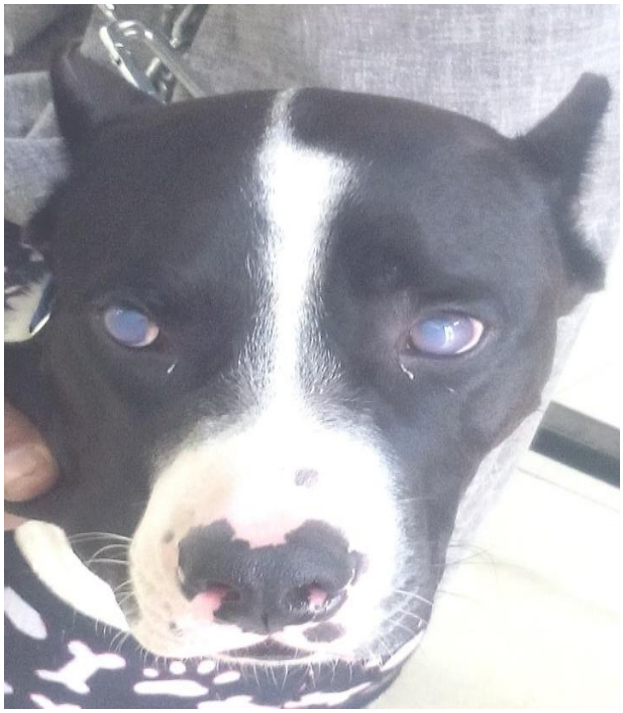


Figure 12. Indian bully breed of dog showing corneal opacity with oedema

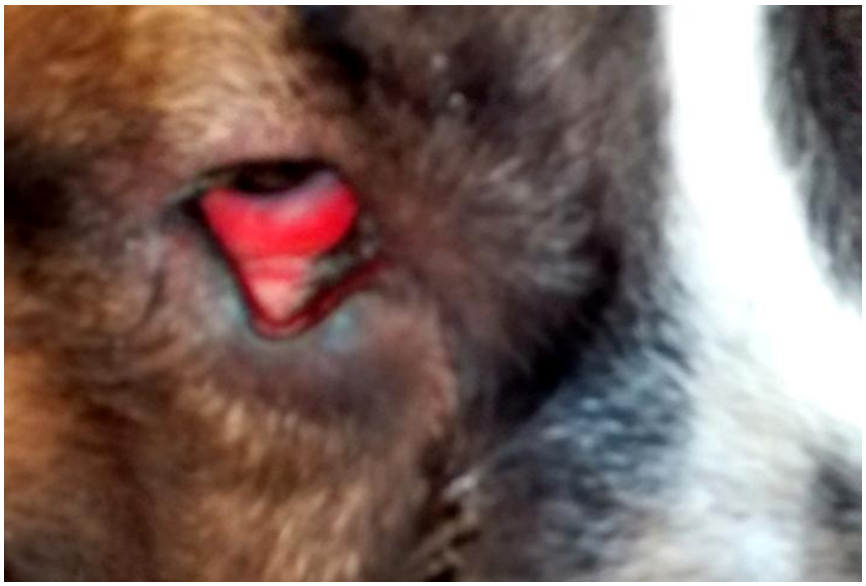


Figure 13. St. Bernard breed of dog showing congested mucus membrane

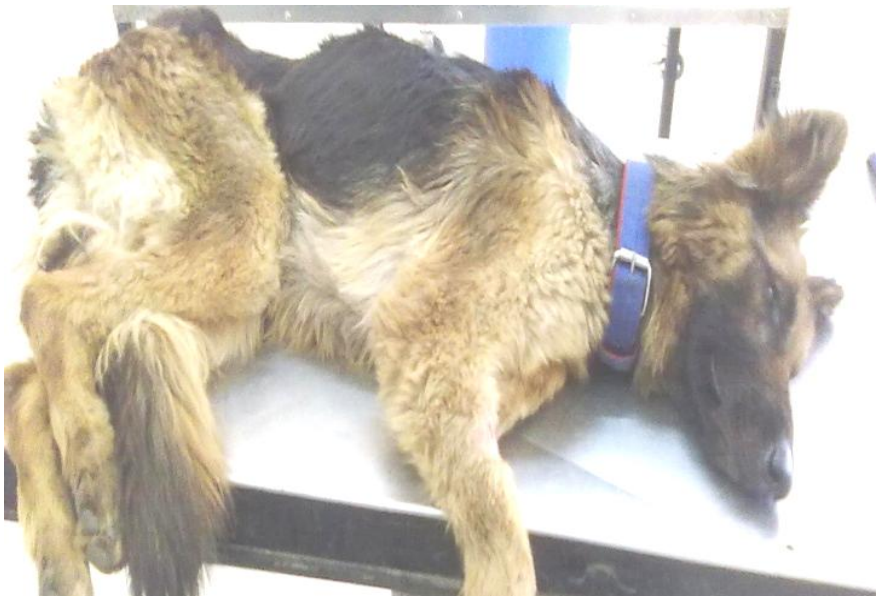
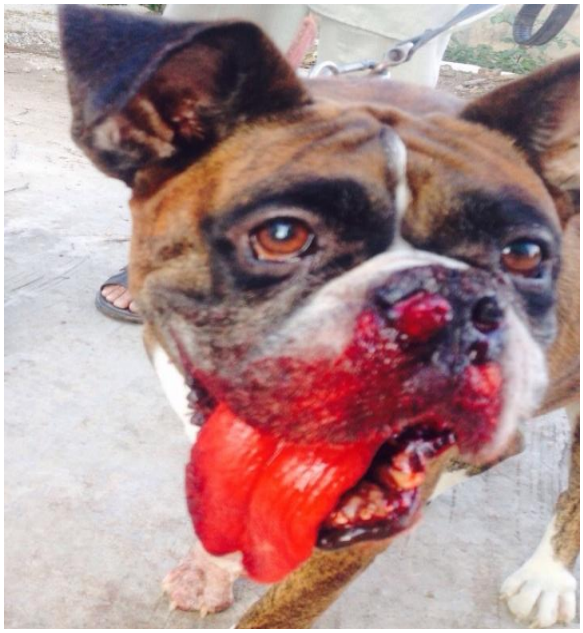
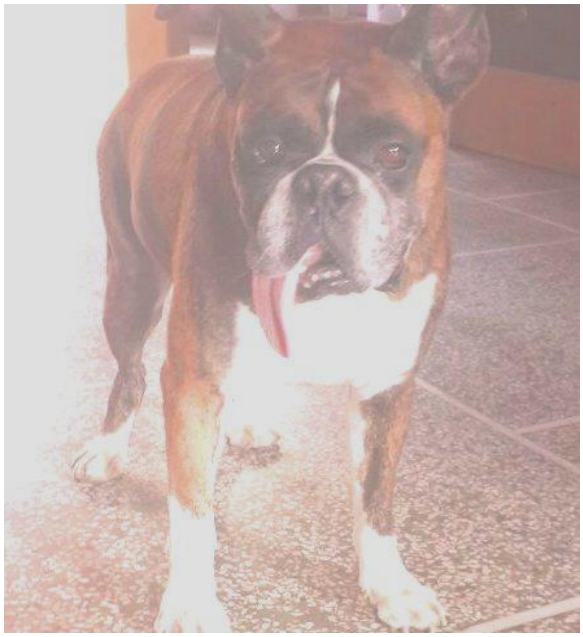


Figure 14. Chronic case of dog with epistaxis, depression and lethargy



Before treatment

Figure 15. Boxer breed of dog epistaxis before treatment



After treatment

Figure 16. Boxer breed of dog recovered after treatment

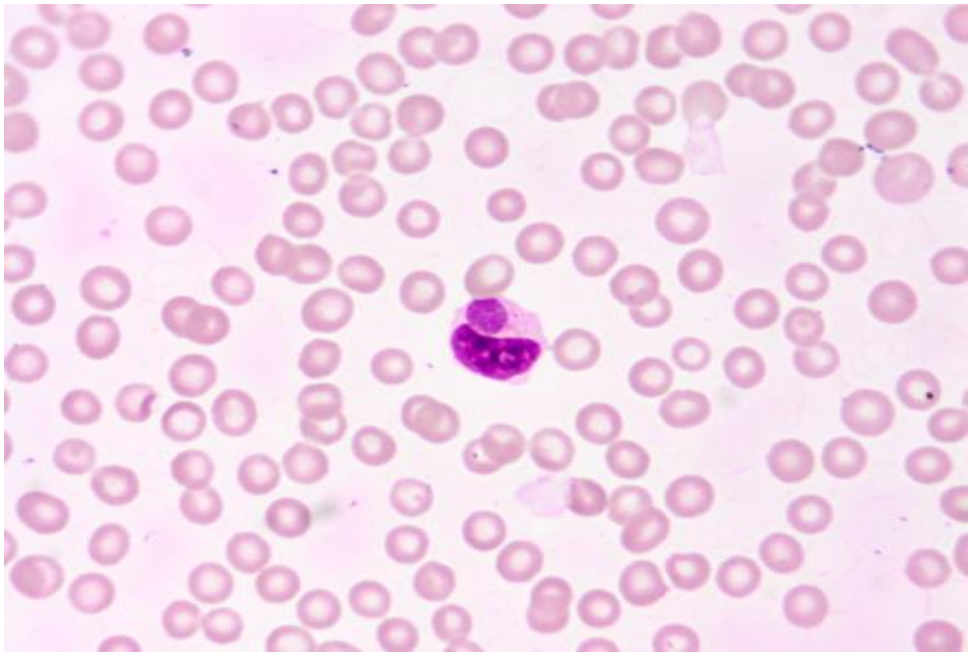


Figure 17. Morula in the cytoplasm of monocyte with severe thrombocytopenia

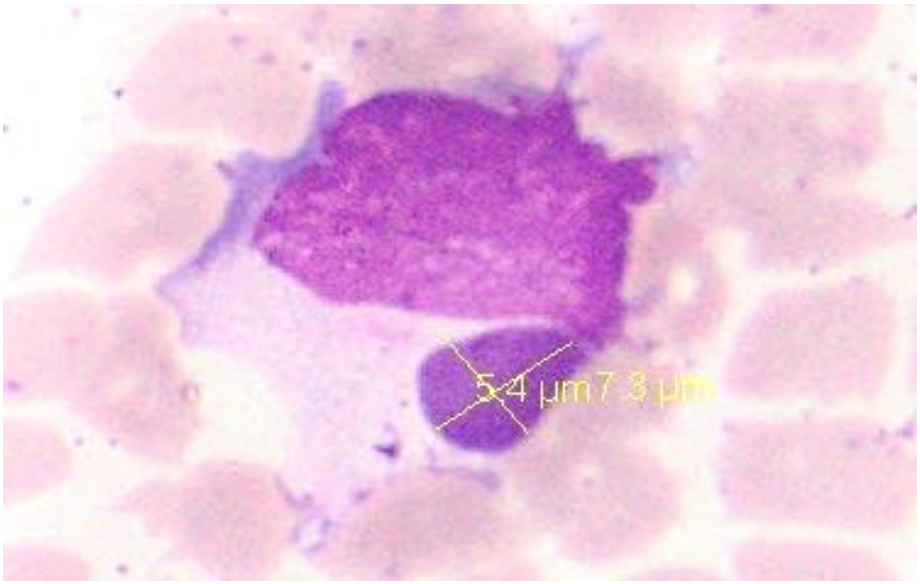


Figure 18. *Ehrlichia canis* morula of size 5.4µm in monocyte of dog

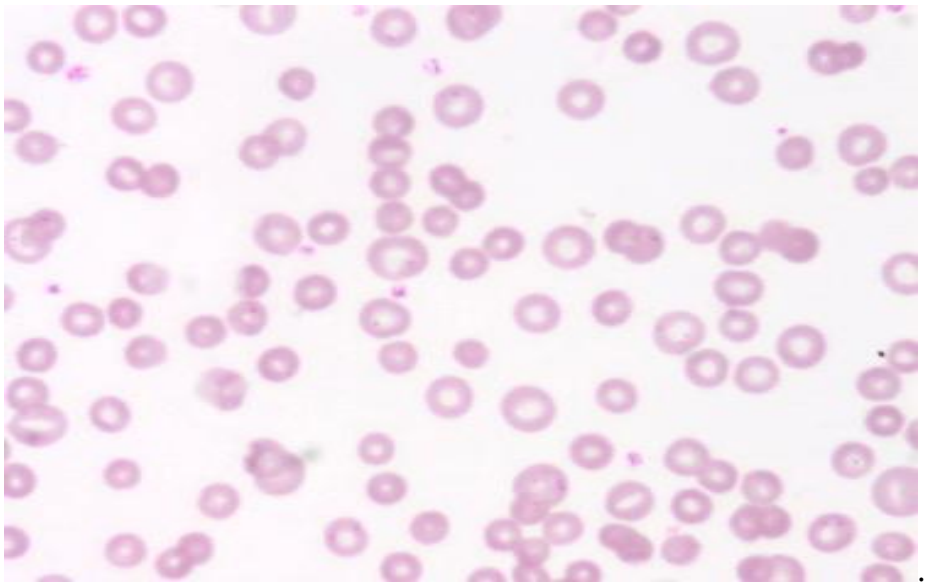


Figure 19. Leishman stained canine thin blood smear showing anaemia

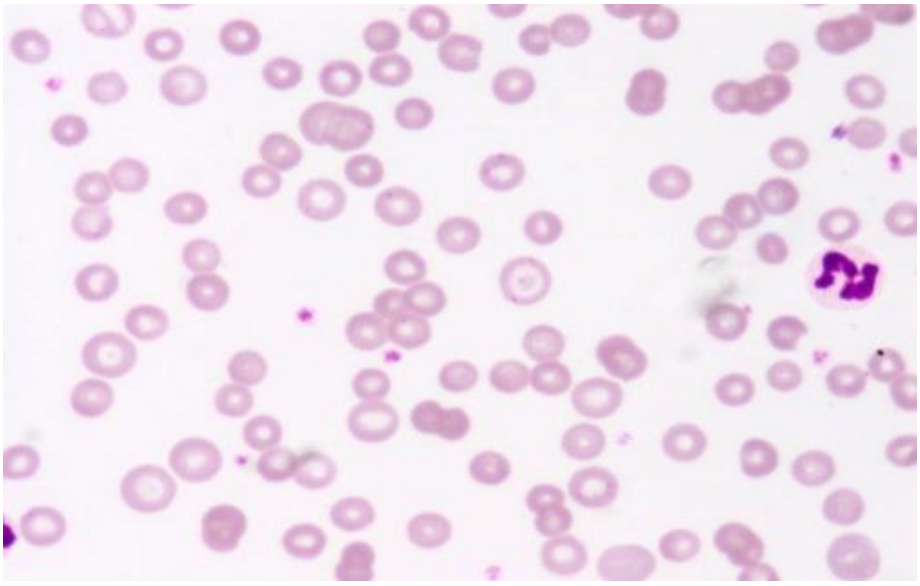


Figure 20. Leishman stained canine thin blood smear showing thrombocytopenia

(66.66%), hyperproteinaemia (50%), hypoalbuminaemia (83.33%), hyperglobulinaemia (58.33%), decrease in albumin & globulin ratio (75%), increase in BUN (66.66%) and creatinine (75%) (Table 11, Figure 21). Leucopaenia, mild anaemia and thrombocytopenia were common in acute stage, whereas severe pancytopenia was the hall mark of chronic phase of disease. Earlier hypoalbuminemia, hyperglobulinaemia and mild increase in ALT and ALKP acitivities in dogs with ehrlichiosis have been reported by (Waner and Harrus 2000a). Thrombocytopenia in acute phase of disease may be due to increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased spleenic sequestration of platelets and immulogic destruction resulting in a significantly decreased platelet life span (Kakoma *et al* 1978, Pierce *et al* 1977, Smith *et al* 1975).

Abeygunawardena *et al* (1990) proposed platelet migration inhibition factor play a role in enhancing platelet sequestration and stasis, leading to reduced peripheral - blood platelet counts. Harrus *et al* (1996b) and Reardon *et al* (1981) did demonstrated serum platelet bindable antiplatelet anti-bodies (APA) in dogs with experimental inoculation with *E.canis* supports immune destruction contribute to the pathogenesis of thrombocytopaenia. After development of thrombocytopaenia significant increase in the mean platelet volume is seen and reflects active thrombopoiesis.

Woody *et al* (1991) reported in chronic phase, decreased production of platelet due to bone marrow hypoplasia results in thrombocytopenia with pancytopaenia. Lovering *et al* (1980) found platelet adhesiveness was shown to decrease in dogs acutely infected with *E. canis.* Harrus *et al* (1996b) shown sera of infected dog with *E. canis* inhibit platelet aggregation when incubated with platelets of healthy dogs. Above all these findings suggest that platelet dysfunction together with thrombocytopaenia, were contributing factor for bleeding tendency observed in the disease. Interaction of APA with platelet membrane glycoprotiens was proposed to cause the platelet dysfunction and presence of maximal concentrations of serum APA with platelet dysfunction, suggesting main role of APA in causing platelet dysfunction in acute stage of canine ehrlichiosis.

4.4.2. Hemato-biochemical findings in dogs positive for ehrlichiosis by PCR

Hematological findings found in dogs which are positive by PCR included thrombocytopenia (100%), anaemia (87.87%), lymphopenia (84.84%), leucocytosis (69.69%), neutropenia (24.24%), leucopaenia (21.21%) and eosinophilia (21.21%).

Biochemical parameters revealed hyperbilirubinaemia (45.45%), increased level of AST (93.93%), ALT (66.66%), ALKP (75.75%), hyperproteinaemia (66.66%), hypoalbuminaemia (63.63%), hyperglobulinaemia (72.72%), decrease in albumin & globulin ratio (72.72%), increase in BUN (48.48%) and creatinine (75.75%) (Table 11, Figure 21). Suksawat *et al* (2001) found that out of 49 dogs from Thailand, anaemia was seen in 31dogs, thrombocytopenia in 27dogs, fever in 5 dogs and 38 dogs (75%) had antibodies against *E. canis* and PCR yielded positive amplification of *E. canis* in 10 (20%) dogs. Hypoalbuminemia, hyperglobulinemia and hypergammaglobulinemia are predominant findings (Burghen *et al* 1971, Harrus *et al* 1996a, Keefe *et al* 1982). Hypoalbuminaemia may be due to the peripheral loss of albumin to edematous inflammatory fluids as a result of increased vascular permeability (Woody *et al* 1991), blood loss, or decreased protein production due to concurrent mild liver disease (Reardon 1981) or due to minimal-change glomerulopathy Codner *et al* 1992a. Shipov *et al* (2008) have reported leucopaenia in monocytic ehrlichiosis. Mendonca *et al* (2005) observed that hematological alterations caused due to canine ehrlichiosis were anaemia (77.98%), thrombocytopaenia (87.15%) and lymphopaenia (22.02%).

As albumin synthesis is regulated by oncotic pressure (Rothschild *et al* 1984), the decrease in albumin concentrations may act as compensatory mechanism for the hyperglobulinemic state, there by maintaining the oncotic pressure and preventing increase in blood viscosity (Woody *et al* 1991). Gamma globulin concentration increase during the subclinical and chronic phases of the disease (Ristic *et al* 1993). During the *E. canis* infection, organism produce antibodies against glycoproteins of the dogs own platelets, which leads to their malfunction (Burghen *et al* 1971). Rikihisa (1991) mentioned that platelet migration inhibited by PMIF (Platelet Migration Inhibition Factor) produced by activated lymphocytes. Normocytic nonregenerative anemia with slight leucopaenia preceded by lueko- and monocytosis, hypoalbuminemia, hperglobulinaemia and increased level of AST, ALKP, BUN and creatinine (Harrus *et al* 1997c). Nakaghi *et al* (2008) observed leucoytosis in dogs infected with *E. canis* during the acute stage of infection.

4.4.3. Hemato-biochemical findings in dogs positive for ehrlichiosis by serology

Seropositive dogs were also having related findings thrombocytopenia (100%), anaemia (75%), lymphopenia (72.22%), leucocytosis (66.66%). Biochemical findings includes hyperbilirubinaemia (44.44%), increased level of AST (86.11%),

increase in ALT (63.88%), increase in ALKP (72.22%), hyperproteinaemia (58.33%), hypoalbuminaemia (61.11%), hyperglobulinaemia (66.66%), decrease in albumin & globulin ratio (75%), increase in BUN (52.77%) and creatinine (72.22%) (Table 11, Figure 21). The presence of anemia in *E. canis* seropositive dogs varied from 43 per cent (Frank and Breitschwerdt 1999) to 90 per cent in the US (Troy 1980). Baneth *et al* (1996) observed 41% (26/63) of dogs with ehrlichiosis were having anaemia.

Troy (1980) observed thrombocytopaenia in all 30 seropositive dogs for ehrlichiosis. In United States Frank and Breitschwerdt (1999) did retrospective serologic study found 77 per cent dogs were positive for ehrlichiosis were having thrombocytopenia. In Israel, thrombocytopenia was present in 27% dogs in a serosurvey (Baneth *et al* 1996). In a serologic survey of Switzerland, 26.7% thrombocytopenic dogs seroreacted to *E. canis* (Pusterla *et al* 1997).

In present study 100 per cent prevalence of thrombocytopenia were found in dogs which were seropositive to *E. canis*. Sasanelli *et al* (2009) reported a case of ehrlichiosis, showed increased levels of ALT (67 u/l), AST (84 u/l), ALKP (829 u/l), BUN (24.6 mg/dl), creatinine (0.7 mg/dl) and total bilirubin (0.4) on day 0. On 16 still anemia, thrombocytopenia and low serum albumin levels were still persisted. Bressler *et al* (2003) observed elevated liver enzymes and hypoalbuminaemia in a chronic case of ehrlichiosis with portal vein and aortic thromboses.

Neer *et al* (2002) stated that thrombocytopenia is a common finding in dogs with ehrlichiosis. Difference in prevalence may reflect diversity in strain pathogenicity or be a bias of selection, because thrombocytopenic dogs are more likely to be tested for ehrlichiosis and concluded that dogs with history of ticks, anemia or thrombocytopenia with amplification of ehrlichial DNA in about 20% can be differentiated for ehrlichiosis (Dagnone *et al* 2003). Akhtardanesh *et al* (2010) found 16.66 per cent dogs positive for typical morulae of *E. canis* were observed in monocytes and seropositive for *E. canis* and displayed obvious hyperglobulinemia, thrombocytopenia, leucopaenia, anemia and high ALKP level. Cowell *et al* (1988) observed low packed cell volume, lymphopenia, monocytosis, thrombocytopenia and eosinophilia in cases of polyarthritis associated ehrlichiosis. Kuehn and Gaunt (1985) reported low albumin globulin ratio as serum biochemical abnormality in natural infection with *E. canis*. Mylonakis *et al* (2004) observed hypoalbuminemia and increased level of ALT activity in dogs with ehrlichiosis.

Table 11. Hemato-biochemical findings in dogs with ehrlichiosis

Parameters	Smear positive n = 12 (%)	PCR positive n = 13 (%)	Seropositive n = 73 (%)
Anaemia	12 (100)	29 (87.87)	27(75)
Thrombocytopenia	12 (100)	33 (100)	36 (100)
Leucocytosis	7 (58.33)	23 (69.69)	24 (66.66)
Lymphopenia	9 (75)	28 (84.84)	26 (72.22)
Leucopaenia	5 (41.66)	7 (21.21)	9 (25)
Neutropenia	3 (25)	8 (24.24)	9 (25)
Eosinophilia	0	7 (21.21)	4 (11.11)
↑AST	10 (83.33)	31 (93.93)	31 (86.11)
Hypoalbuminaemia	10 (83.33)	21 (63.63)	22 (61.11)
↓Albumin:Globulin	9 (75)	24 (72.72)	27 (75)
Hypercreatinaemia	9 (75)	25(75.75)	26 (72.22)
↑ALT	8 (66.66)	22 (66.66)	23 (63.88)
↑ALKP	8 (66.66)	25 (75.75)	26 (72.22)
Hyperglobulinaemia	7 (58.33)	24 (72.72)	24 (66.66)
↑BUN	8 (66.66)	16 (48.48)	19 (52.77)
Hyperproteinaemia	6 (50)	22 (66.66)	21 (58.33)
Hyperbilirubinaemia	5 (41.66)	15 (45.45)	16 (44.44)
Hypoproteinaemia	2 (16.66)	7 (21.21)	7 (19.44)

Figures in parentheses indicate percentage & (n) indicates number of dogs

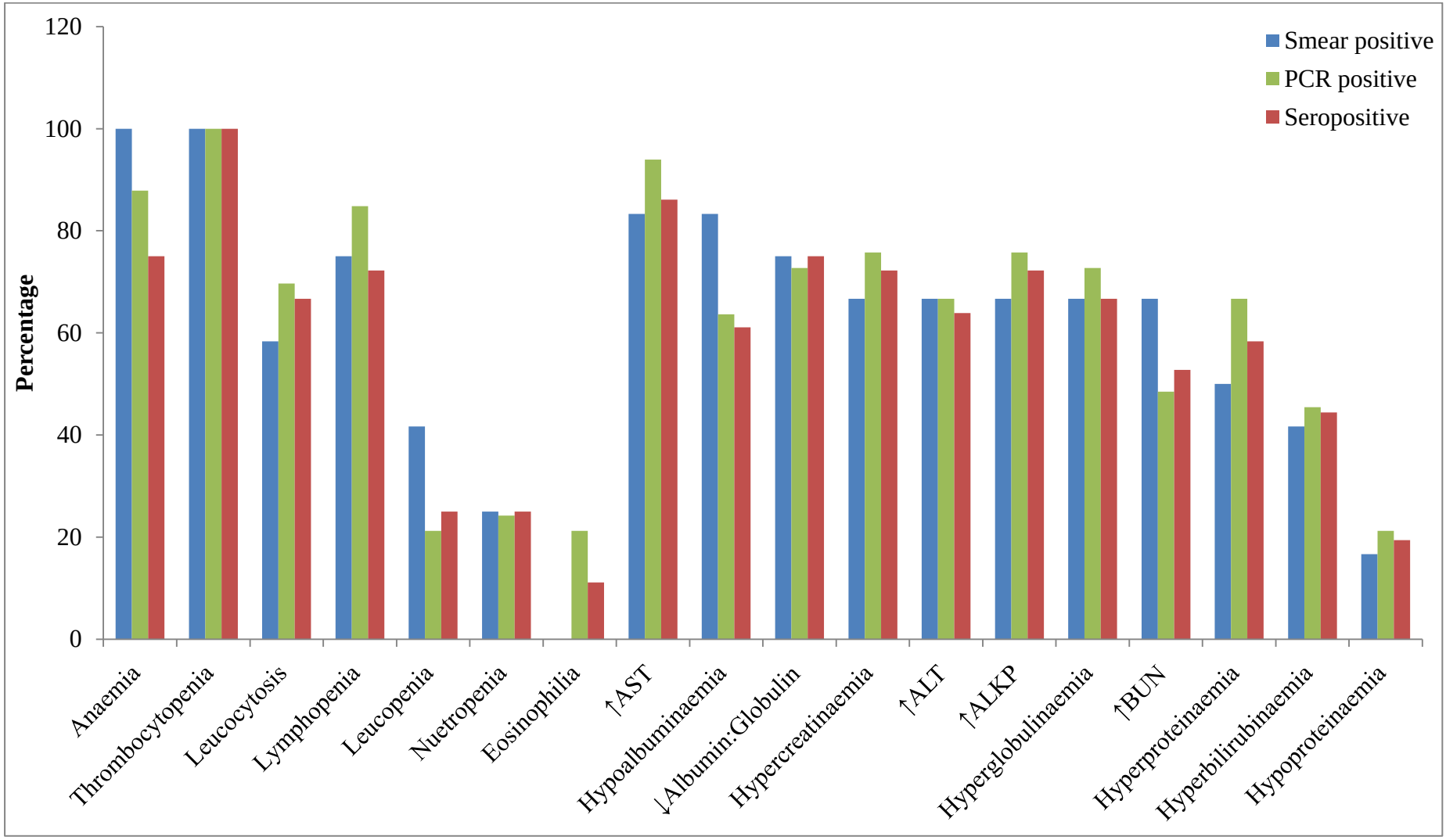


Figure 21: Hemato-biochemical findings in dogs with ehrlichiosis

4.5. Hemato-biochemical parameters in dogs with ehrlichiosis diagnosed by blood smear examination

4.5.1. Hematological parameters

Mean value of haemoglobin in smear positive dogs infected with *E. canis* were 7.97±1.97g/dl whereas mean value of haemoglobin in apparently healthy dogs were 13.19±0.76g/dl (Table 12, Figure 22). The Hb values were significantly lower in *E. canis* ($P<0.05$) infected dogs as compared to the control group. This reduction in the haemoglobin due to the effect of *E. canis* on circulating blood accompanied by bone marrow suppression or detruction and malfunction of erythropoiesis, in that bone marrow unable to compensate for the peripheral destruction of erythrocyte (Waner *et al* 1995).

Mean TLC value in smear positive dogs infected with *E. canis* were 11.034±7.15 (10^3/µL) whereas mean TLC value of apparently healthy dogs were 8.56±1.106 (10^3/µL). Non-significant variation was observed in TLC values of *E. canis* infected dogs as compared to that of control group. (Table 12, Figure 22).

Mean TEC values in smear positive dogs infected with *E. canis* were 3.42±0.77 (10^6/µL) whereas TEC of apparently healthy dogs were 6.3±0.55 (10^6/µL).The TEC values were significantly lower in *E. canis* ($P<0.05$) infected dogs as compared to that of control group. Similar finding observed by De Castro *et al* (2004) with mean values of RBC count 3.91±1.01 ($\times 10^6$/µl). (Table 12, Figure 22).

Mean PCV value in smear positive dogs infected with *E. canis* were 22.74±5.076 per cent and whereas PCV value in apparently healthy dogs were 37.94±3.710 per cent (Table 12, Figure 22). The PCV values were significantly lower ($P<0.05$) in *E. canis* infected dogs as compared to the control group. Castro *et al* (2004) noted more severe anemia with mean PCV 21 percent by experimental inoculation of *E. canis* strain in four dogs. Differences in prevalence are likely a result of differences in strain pathogenicity and definition of anemia. Similar values are found with experimental study around 27.75±6.32 (%) PCV in positive dogs (De Castro 2004).

Mean value of platelets (10^5/ µL) in smear positive dogs infected with *E. canis* were 0.63±0.410 whereas in apparently healthy dogs platelets (10^5/ µL) value were 3.23±0.45. The platelets values were significantly lower ($P<0.05$) in *E. canis* infected dogs as compared to the control group. Qualitative observation of stained slides

showed 28.57% (20/70) cases with severe thrombocytopenia. Thirteen cases (18.75%) showed thrombocytosis. Mild to severe thrombocytopenia in *Ehrlichia* infected dogs has been reported in literature (Codner and Farris-Smith 1986, Waner *et al* 1997 and Niwetpathomwat *et al* 2006) (Table 12, Figure 22).

Mean neutrophils count (%) in smear positive dogs infected with *E. canis* were 79.33±13.41 whereas apparently healthy dogs were having 72.5±2.66 and no significant difference between *E. canis* infected dogs as compared to the control group (Table 12, Figure 22).

Mean lymphocytes count (%) in smear positive dogs infected with *E. canis* were 19.08±13.310 whereas apparently healthy dogs were having 26.5±2.89. no varied significant difference between *E. canis* infected dogs as compared to the control group (Table 12, Figure 22).

Mean eosinophils count (%) in smear positive dogs infected with *E. canis* were 1.5±1.0 whereas in apparently healthy dogs it was 2.3±1.16. No varied significant difference between *E. canis* infected dogs as compared to the control group (Table 12, Figure 22).

4.5.2. Biochemical parameters

Mean value of total bilirubin (mg/dL) in smear positive dogs infected with *E. canis* were 1.56±2.910 whereas the mean value of total bilirubin (mg/dL) in apparently healthy dogs was 0.38±0.16. No significant difference observed between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of AST (U/L) in smear positive dogs infected with *E. canis* were 26.58±10.98 whereas the mean value of AST (U/L) in apparently healthy dogs was 13.8±1.55. The AST values were significantly higher in *E. canis* ($P<0.05$) infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of ALT (U/L) in smear positive dogs infected with *E. canis* were 59.17±28.012 whereas the mean value of ALT (U/L) in apparently healthy dogs were 27.2±9.65. Significant difference found between the *E. canis* ($P<0.05$) infected dogs to the control group. (Table 13, Figure 23).

Mean value of ALKP (U/L) in smear positive dogs infected with *E. canis* were 132.5±52.05 whereas the mean value of ALKP (U/L) in apparently healthy dogs was 80.2±15.51. Significant difference found between the *E. canis* infected dogs and

control group (Table 13, Figure 23).

Mean value of total protein (g/dL) in smear positive dogs infected with *E. canis* were 6.58±1.47 whereas the mean value of total protein (g/dL) in apparently healthy dogs was 6.1±0.61. No varied significant difference between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of albumin (g/dL) in smear positive dogs infected with *E. canis* were 1.98±0.58 whereas the mean value of albumin (g/dL) in apparently healthy dogs was 3.00±0.21. There was a significant reduction in the albumin value in dogs infected with *E. canis* as compared to control group ($P<0.05$) (Table 13, Figure 23).

Mean value of globulin (g/dL) in smear positive dogs infected with *E. canis* were 4.60±1.56 whereas the mean value of globulin (g/dL) in apparently healthy dogs was 3.1±0.58. Increase in the globulin value in smear positive dogs infected with *E. canis* ($P<0.05$) as compared to control group ($P<0.05$) (Table 13, Figure 23).

Mean value of albumin: globulin ratios in smear positive dogs infected with *E. canis* were 0.52±0.32 whereas the mean values of albumin: globulin ratio in apparently healthy dogs was 0.910±0.23. The albumin: globulin ratio was significantly lower in smear positive dogs infected with *E. canis* ($P<0.05$) as compare to control group (Table 13, Figure 23).

Mean value of BUN (mg/dL) in smear positive dogs infected with *E. canis* were 22.83±10.54 whereas the mean value of BUN (mg/dL) in apparently healthy dogs was15.5±5.21. Not much significant significance found between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of creatinine (mg/dL) in smear positive dogs infected with *E. canis* were 1.21±0.52 whereas the mean value of creatinine (mg/dL) in apparently healthy dogs was 0.83±0.03. Not much significant significance found between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

4.6. Hemato-biochemical parameters in dogs with ehrlichiosis diagnosed by Polymerase chain reaction

4.6.1. Hematological parameters

Mean value of haemoglobin in PCR positive dogs infected with *E. canis* were 10.15±3.06g/dl whereas mean value of haemoglobin in apparently healthy dogs was 13.19±0.76g/dl. The Hb value were significantly lower in *E. canis* ($P<0.05$) infected

dogs as compared to the control group (Table 12, Figure 22).

Mean TLC value in PCR positive dogs infected with *E. canis* 13.27±7.18 (10^3/µL) whereas mean TLC of apparently healthy dogs was 8.56±1.106 (10^3/µL). Non-significant variation was observed in TLC values of *E. canis* infected dogs as compared to that of control group (Table 12, Figure 22).

Mean TEC value in PCR positive dogs infected with *E. canis* were 4.82±2.145 (10^6/µL) whereas TEC of apparently healthy dogs was 6.3±0.55 (10^6/µL).The TEC values were significantly lower in *E. canis* ($P<0.05$) infected dogs as compared to that of control group indicating significant difference between the groups (Table 12, Figure 22).

Mean PCV value in PCR positive dogs infected with *E. canis* were 29.06±10.94 and whereas PCV value in apparently healthy dogs was 37.94±3.710 (Table 12, Figure 22). The PCV values were significantly lower ($P<0.05$) in *E. canis* infected PCR positive dogs as compared to the control group.

Mean value of platelets (10^5/ µL) in PCR positive dogs infected with *E. canis* were 0.75±0.57 whereas in apparently healthy dogs platelets (10^5/ µL) value was 3.23±0.45. The platelets values were significantly lower ($P<0.05$) in *E. canis* infected dogs as compared to the control group. De Castro *et al* (2004) observed significant decrease in mean values of platelets 174.00±29.00 ($\times10^3$/µl) indicating thrombocytopenia. Bulla *et al* (2004) found dogs positive for *E. canis* by nested PCR were having platelet count between 1 to 2 lakh/µl in 21% dogs and less than 1 lakh/µl was seen in 63.1% dogs. Anaemia with neutrophilic leucocytosis and thrombocytopenia has been reported by Lakkawar *et al* (2003) in monocytic ehrlichiosis in a dog. (Table 12, Figure 22).

Mean neutrophils count (%) in PCR positive dogs infected with *E. canis* were 78.65±14.88 whereas in apparently healthy dogs it was 72.5±2.66 and No significance difference between *E. canis* infected dogs as compared to the control group (Table 11, Figure 22).

Mean lymphocytes count (%) in PCR positive dogs infected with *E. canis* were 19.16±13.81 whereas in apparently healthy dogs it was 26.5±2.89. No varied significant difference between *E. canis* infected dogs as compared to the control group (Table 12, Figure 22).

Mean eosinophils count (%) in PCR positive dogs infected with *E. canis* was 1.89±2.55 whereas in apparently healthy dogs it was 2.3±1.16. No varied significant difference between *E. canis* infected dogs as compared to the control group (Table 12, Figure 22).

4.6.2. Biochemical parameters

Mean value of total bilirubin (mg/dL) in PCR positive dogs infected with *E. canis* were 0.85±1.75 whereas the mean value of total bilirubin (mg/dL) in apparently healthy dogs was 0.38±0.16. Not much significance difference observed between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of AST (U/L) in PCR positive dogs infected with *E. canis* were 29.49±13.510 whereas the mean value of AST (U/L) in apparently healthy dogs was 13.8±1.55. The AST values were significantly higher in *E. canis* ($P<0.05$) infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of ALT (U/L) in PCR positive dogs infected with *E. canis* were 52.49±26.35 whereas the mean values of ALT (U/L) in apparently healthy dogs were 27.2±9.65. The mean value of PCR positive dogs is apparently higher when compared to the control group. Significant difference found between the *E. canis* ($P<0.05$) infected dogs to the control group. The increase in SGPT activity and the decrease in serum albumin occurred simultaneously with the development of many expanding foci of reticulo-endothelial cells in the hepatic sinusoids, which compressed and caused necrosis of adjacent hepatocytes (Neitz et al 1938 and Walker *et al* 1970). (Table 13, Figure 23).

Mean value of ALKP (U/L) in PCR positive dogs infected with *E. canis* were 125.79±51.25. Whereas the mean value of ALKP (U/L) in apparently healthy dogs was 80.2±15.51. Mean value of ALKP in PCR positive *E. canis* affected dogs are higher ($P<0.05$) as compared to control group. Significant difference found between the groups (Table 13, Figure 23).

Mean value of total protein (g/dL) in PCR positive dogs infected with *E. canis* were 7.09±1.64 whereas the mean value of total protein (g/dL) in apparently healthy dogs was 6.1±0.61. Considerable increase in the mean values of total protein in PCR positive dogs infected with *E. canis* ($P<0.05$) as compared to control group. Harrus *et al* (1996) reported significant hyperproteinaemia in the infected dogs. Gammaglobulin concentrations increase during the febrile phase of canine ehrlichiosis and persist

during the subclinical and chronic phase of the disease (Ristic and Holland 1993). (Table 13, Figure 23).

Mean value of albumin (g/dL) in PCR positive dogs infected with *E. canis* were 2.54±0.80 whereas the mean value of albumin (g/dL) in apparently healthy dogs was 3.00±0.21. Non-significant reduction in the albumin value in dogs infected with *E. canis* as compared to control group (P<0.05) (Table 13, Figure 23).

Mean value of globulin (g/dL) in PCR positive dogs infected with *E. canis* were 4.55±1.53 whereas the mean value of globulin (g/dL) in apparently healthy dogs was 3.1±0.58. Significant increase in mean values of globulin in PCR positive dogs infected with *E. canis* (P<0.05) as compared to control group (Table 13, Figure 23).

Mean value of albumin: globulin ratio in PCR positive dogs infected with *E. canis* were 0.63±0.21 whereas the mean albumin: globulin ratio in apparently healthy dogs was 0.91±0.23. Mean value of albumin: Globulin ratios were significantly lower in dogs infected with *E. canis* (P<0.05) as compared to control group. (Table 13, Figure 23).

Mean value of BUN (mg/dL) in PCR positive dogs infected with *E. canis* were 20.65±11.043 whereas the mean value of BUN (mg/dL) in apparently healthy dogs was 15.5±5.21. Not much significant significance found between *E. canis* infected dogs as compared to the control group (Table 13, Figure 23).

Mean value of creatinine (mg/dL) in PCR positive dogs infected with *E. canis* were 1.38±0.65 whereas the mean value of creatinine (mg/dL) in apparently healthy dogs was 0.83±0.03. Significance difference found between the groups (Table 13, Figure 23)

4.7. Hemato-biochemical parameters in dogs with ehrlichiosis diagnosed by serological examination

4.7.1. Hematological parameters

Mean value of haemoglobin in seropositive dogs infected with *E. canis* were 8.98±2.48g/dl whereas mean value of haemoglobin in apparently healthy dogs was 13.19±0.76g/dl. The mean Hb value were significantly lower in *E. canis* (P<0.05) infected dogs as compared to the control group (Table 12, Figure 22). This finding is agreeing with finding of De castro *et al* (2004) they got mean values of Hb about 9.02±2.24g/dl in experimentally infected dogs with *E. canis*. Kahn *et al* (2005) found

that a total of 54.67% (41/75) cases were having haemoglobin value less than 10 g/dl. Eljadar (2010) observed mean values of Hb was 9.29±0.36 g/dl in his study on monocytic ehrlichiosis.

Mean TLC value in seropositive dogs infected with *E. canis* were 12.73±7.05 (10^3/µL) whereas mean TLC of apparently healthy dogs was 8.56±1.106 (10^3/µL). Non-significant variation was observed in TLC value of *E. canis* infected dogs as compared to that of control group (Table 12, Figure 22).

Mean TEC value in seropositive dogs infected with *E. canis* were 4.54±2.56 (10^6/µL) whereas TEC of apparently healthy dogs was 6.3±0.55 (10^6/µL). Non significant decrease in the mean values of *E. canis* ($P<0.05$) infected dogs as compared to that of control group (Table 12, Figure 22).

Mean PCV value in dogs infected with *E. canis* were 26.98±7.61per cent and whereas PCV value in apparently healthy dogs was 37.94±3.71 per cent. The PCV values were significantly lower ($P<0.05$) in *E. canis* infected seropositive dogs as compared to the control group (Table 12, Figure 22).

Mean value of platelets (10^5/ µL) in seropositive dogs infected with *E. canis* were 0.56±0.40 whereas in apparently healthy dogs platelets (10^5/ µL) value was 3.23±0.45. The mean platelets value were significantly lower ($P<0.05$) in *E. canis* infected sero positive dogs as compared to the control group. Shipov *et al* (2008) diagnosed the case of CME by using a commercial kit (Immunocomb, Biogal, Kibbutz, Galed, Isreal). Dogs were showing anaemia (80%), leucopaenia (57.5%), thrombocytopenia (100%) and pancytopenia. Variable leucopaenia, anaemia, thrombocytopenia, and hypergamma-globulinaemia are the most common signs of subacute phase of ehrlichiosis (Lakkawar *et al* 2003) (Table 12, Figure 22).

Mean neutrophils count (%) in seropositive dogs infected with *E. canis* were 77.53±15.55 whereas in apparently healthy dogs it was 72.5±2.66. Not much significance difference found between *E. canis* infected dogs as compared to the control group ((Table 12, Figure 22).

Mean lymphocytes count (%) in seropositive dogs infected with *E. canis* was 21.08±15.17 whereas in apparently healthy dogs it was 26.5±2.89. No varied statistical significant difference found between *E. canis* infected dogs as compared to the control group and other two groups. Mean values of lymphocytes was 21.74±1.38 per cent observed in CME (Eljadar 2010) (Table 12, Figure 22).

Table 12. Hematological findings in dogs with ehrlichiosis

Parameters (0^{th} day)	Hb (g/dL)	TLC (10^3/µL)	TEC (10^6/µL)	PCV (%)	Platelet (10^5/µL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
Smear positive (n=12)	7.97±1.97[a]	11.034±7.15[a]	3.42±0.77[a]	22.74±5.076[a]	0.63±0.410[a]	79.33±13.41[a]	19.08±13.310[a]	1.5±1[a]
PCR Positive (n=33)	10.15±3.06[a]	13.27±7.18[a]	4.82±2.145[ab]	29.91±8.86[b]	0.75±0.57[a]	78.65±14.88[a]	19.16±13.81[a]	1.89±2.55[a]
Sero positive (n=36)	8.98±2.48[a]	12.73±7.05[a]	4.54±2.56[ab]	26.98±7.61[ab]	0.56±0.40 [a]	77.53±15.55[a]	21.08±15.17[a]	1.36±2.06[a]
Control (n=10)	13.19±0.76[b]	8.56±1.106[a]	6.3±0.55[b]	37.94±3.710[c]	3.23±0.45[b]	72.5±2.66[a]	26.5±2.89[a]	2.3±1.16[a]

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

Table 13. Biochemical findings in dogs with ehrlichiosis

Parameters (0 day)	Total bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALKP (U/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A: G ratio	BUN (mg/dL)	Creatinine (mg/dL)
Smear positive (n=12)	1.56±2.910^{a}	26.58±10.98^{b}	59.17±28.012^{b}	132.5±52.05^{b}	6.58±1.47^{a}	1.98±0.58^{a}	4.60±1.56^{b}	0.52±0.32^{a}	22.83±10.54^{a}	1.21±0.52^{ab}
PCR Positive (n=33)	0.85±1.75^{a}	29.49±13.510^{b}	52.49±26.35^{b}	125.79±51.25^{b}	7.09±1.64^{a}	2.54±0.80^{ab}	4.55±1.53^{b}	0.63±0.210^{a}	20.65±11.043^{a}	1.38±0.65^{b}
Serology positive (n=36)	0.91±1.77^{a}	28.45±14.00^{b}	58.61±28.05^{b}	128.11±49.76^{b}	7.18±1.83^{a}	2.45±0.75^{ab}	4.74±1.68^{b}	0.59±0.28^{a}	22±10.66^{a}	1.37±0.63^{b}
Control (n=10)	0.38±0.16^{a}	13.8±1.55^{a}	27.2±9.65^{a}	80.2±15.51^{a}	6.1±0.61^{a}	3.00±0.21^{b}	3.1±0.58^{a}	0.910±0.23^{b}	15.5±5.21^{a}	0.81±0.12^{a}

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

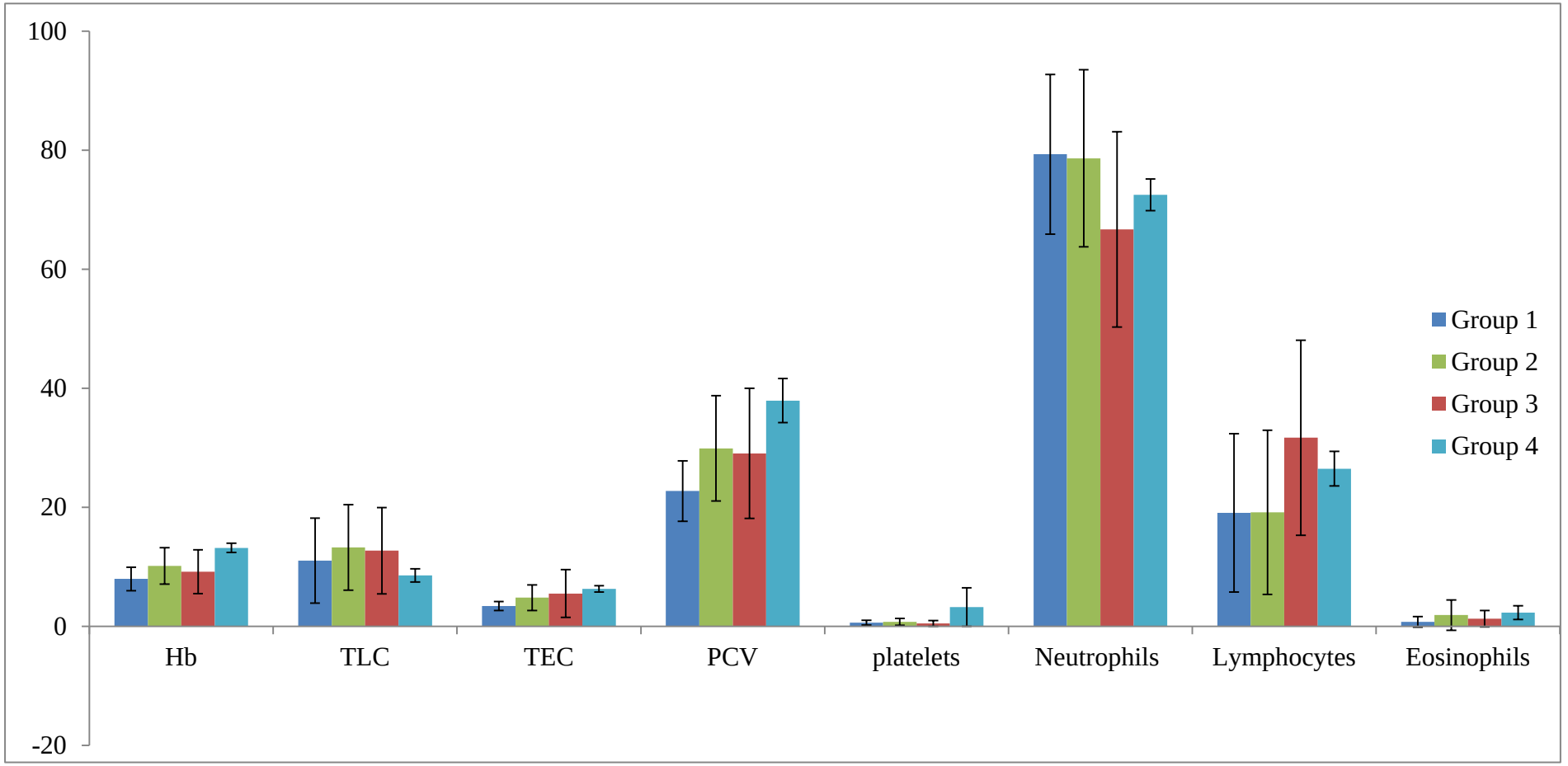


Figure 22: Hematological findings in dogs with ehrlichiosis

(Group 1- Smear positive cases, Group 2-PCR positive cases, Group 3-Seropositive cases, Group 4- Control)

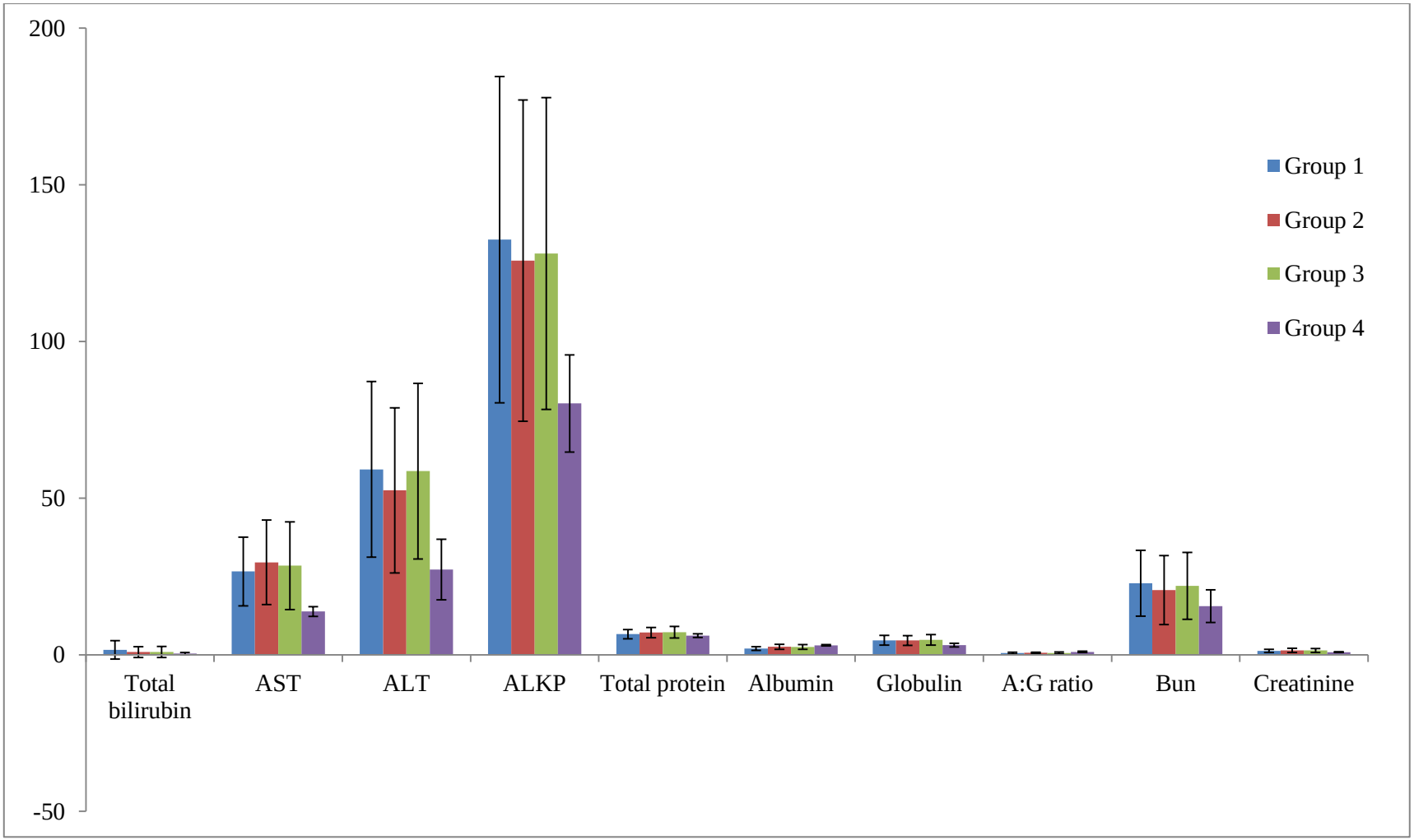


Figure 23: Biochemical findings in dogs with ehrlichiosis

Group 1 - Smear positive cases, Group 2 - PCR positive cases, Group 3 - Seropositive cases, Group 4 - Control

Mean eosinophils count (%) in seropositive dogs infected with *E. canis* was 1.36±2.06 whereas in apparently healthy dogs it was 2.3±1.16. No varied significant difference between *E. canis* infected dogs as compared to the control group and positive group. Earlier mean values of eosinophils obsereved were 1.64±0.36 per cent in CME (Eljadar 2010) (Table 12, Figure 22).

4.7.2. Biochemical parameters

Mean value of total bilirubin (mg/dL) in seropositive dogs infected with *E. canis* were 0.91±1.77 whereas the mean value of total bilirubin (mg/dL) in apparently healthy dogs was 0.38±0.16. No significance difference observed between *E. canis* infected seropositive dogs as compared to the control group (Table 13, Figure 23).

Mean value of AST (U/L) in seropositive dogs infected with *E. canis* were 28.45±14.00 whereas the mean value of AST (U/L) in apparently healthy dogs was 13.8±1.55. The AST values were significantly higher in *E. canis* ($P<0.05$) infected sero positive dogs as compared to the control group ($P<0.05$). (Table 13, Figure 23).

Mean value of ALT (U/L) in seropositive dogs infected with *E. canis* were 58.61±28.05 whereas the mean value of ALT (U/L) in apparently healthy dogs was 27.2±9.65. Mean value of ALT in seropositive dogs are significantly higher when compared to the control group. Previously mean values of ALT observed were 60.0±7.99 IU/L in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of ALKP (U/L) in seropositive dogs infected with *E. canis* were 128.11±49.76 whereas the mean value of ALKP (U/L) in apparently healthy dogs was 80.2±15.51. Mean value of ALKP in seropositive dogs are significantly higher when compared to the control group. Previous workers have seen the mean values of ALKP as 196.4±19.22 IU/L in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of total protein (g/dL) in seropositive dogs infected with *E. canis* were 7.18±1.83 whereas the mean value of total protein (g/dL) in apparently healthy dogs was 6.1±0.61. Slight increase in the mean values of total protein in seropositive dogs infected with *E. canis* ($P<0.05$) as compared to control group. (Table 13, Figure 23).

Mean value of albumin (g/dL) in seropositive dogs infected with *E. canis* were 2.45±0.75 whereas the mean value of albumin (g/dL) in apparently healthy dogs was 3.00±0.21. Non - significant reduction in the albumin value in dogs infected with *E. canis* as compared to control group ($P<0.05$). Previously observed mean value of albumin was 2.55±0.08 g/dl in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of globulin (g/dL) in seropositive dogs infected with *E. canis* were 4.74±1.68 whereas the mean value of globulin (g/dL) in apparently healthy dogs was 3.1±0.58. Significant increase in the mean value of globulin in seropositive dogs infected with *E. canis* ($P<0.05$) as compared to control group. Previously observed mean value of globulin was 4.11±0.17g/dl in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of albumin & globulin ratio in seropositive dogs infected with *E. canis* were 0.59±0.28 whereas the mean value of albumin: globulin ratio in apparently healthy dogs was 0.91±0.23. Mean value of albumin: globulin ratios were significantly lower in dogs infected with *E. canis* ($P<0.05$) as compared to control groups. Previously observed mean value of albumin & globulin ratio was 0.69±0.04g/dl in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of BUN (mg/dL) in seropositive dogs infected with *E. canis* were 22±10.66 whereas the mean value of BUN (mg/dL) in apparently healthy dogs was 15.50±5.21. Not much significant difference found between *E. canis* infected dogs as compared to the control group. Previous workers have seen the mean values of BUN as 25.5±4.46mg/dl in CME (Eljadar 2010) (Table 13, Figure 23).

Mean value of creatinine (mg/dL) in seropositive dogs infected with *E. canis* were 1.37±0.63 whereas the mean value of creatinine (mg/dL) in apparently healthy dogs was 0.81±0.12. Mean value of creatinine in seropositive dogs are significantly higher when compared to the control group. Previously observed mean value of creatinine was 1.2±0.14mg/dl in CME (Eljadar 2010) (Table 13, Figure 23).

Above findings were agreeing with findings of Varela *et al* (1997) they reported that the dog was mildly depressed and had pale mucous membranes and bilateral epistaxis. An indirect fluorescent antibody titer against *Ehrlichia canis* was positive at a dilution of 1:40. Initial treatment with tetracycline (15 mg/kg PO qid for 14 days), melphalan (0.1 mglkg PO sid), and prednisone (0.5 mg/kg PO sid for 14 days) was done. After 12 days of treatment, the physical condition of the dog had improved and no more episodes of epistaxis were noted. The CBC had also improved. High levels of ALT and AST activities. The prednisone dose was decreased to 0.5 mg/kg every other day for I5 additional days.

4.8 Therapeutic manangement of ehrlichiosis in dogs

The primary goal of present study was to evaluate the efficacy of oral doxycycline (@10mg/kg body weight) for a period of 21days and injectable

tetracycline (@20-22mg/kg body weight) for a period of five days followed by oral doxycycline in management of ehrlichiosis in dogs. The response to the treatment was evaluated on the basis of hematological and biochemical parameters before and after treatment and on the basis of blood smear and PCR analysis.

4.8.1. Efficacy of oral doxycycline and injectable tetracycline treatment in dogs with ehrlichiosis

4.8.1.1. Effect of oral doxycycline and injectable tetracycline on vital body parameters of dogs with ehrlichiosis.

Five out of twelve dogs positive by blood smear examination suffering from ehrlichiosis were treated with oral doxycycline. Seven out of twelve dogs positive by blood smear examination suffering from ehrlichiosis were treated with injectable tetracycline. All doxycycline treated five dogs and tetracycline treated seven showed apparent clinical recovery with improvement in appetite and physical activity. During the course of treatment, there was significant decrease ($P<0.05$) in mean value of rectal temperature were observed on 21st day as compared to day 0, whereas non-significant decrease ($P<0.05$) observed in mean values of heart rate, respiration rate and pulse rate on 15th and 21st day as compared to the day 0 (Table 14).

Waner and Harrus (2000a) stated that more than 10 days of doxycycline therapy is required for ehrlichiosis and shown that doxycycline has ability to restore phagosome-lysosome fusion in infected cells, because usually ehrlichiae survive and multiply in the infected cells by inhibiting this fusion. They said doxycycline, tetracycline, minocycline and chloramphinicol drugs have known efficacy against the *E. canis*. Shipov *et al* (2008) in their study have seen that 37.5% of cases were having increased body temperature (>107.25°F) with other related signs of CME and they treated dogs with oral doxycycline @ 10 mg/kg PO every 24 h. in combination with the steroids. The treatment of choice in the acute phase of CME is doxycycline for a period of 2 to 3 weeks, however, in the subclinical phase dogs may need a prolonged treatment regime (Harrus *et al* 2004, and Waner *et al* 1997). Whereas in chronic phase of the disease might be cost prohibitive like blood transfusion and use of hematopoietic growth factors, but still outcome is usually unrewarding (Aroch and Harrus 2001, Mylonakis *et al* 2004). Tetracycline were commonly used in the treatment of CME, with doxycycline in particular being the most acceptable and widely used (Bartsch and Greene 1996, Breitschwerdt *et al* 1998).

Table 14: Comparative therapeutic study of vital body parameters of dogs with ehrlichiosis diagnosed by blood smear examination

Parameters	Rectal Temperature (°F)	Heart rate (per minute)	Respiration rate (per minute)	Pulse rate (per minute)
Smear positive cases (0 day)	103.96±1.53^{a}	84.83±6.48^{a}	25.33±5.25^{a}	83±6.49^{a}
Doxycycline treated (15 day)	102.23±0.74^{ab}	86.75±8.54^{a}	30±3.65^{a}	84.5±8.39^{a}
Doxycycline treated (21st day)	101.85±0.72^{b}	79.5±5.5^{a}	29.5±2.52^{a}	77.5±5.51^{a}
Tetracycline treated (15 day)	102.1±1.02^{ab}	86.86±6.84^{a}	30.14±3.024^{a}	84.57±6.78^{a}
Tetracycline treated (21st day)	101.61±0.93^{b}	78±5.32^{a}	29.43±2.610^{a}	76±5.32^{a}

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

Among 155 screened samples 42 dogs were having clear signs of ehrlichiosis were selected for molecular and serological study. Out of 42, 33 dogs were positive by PCR. One dog died before treatment and rest were 32. 17 out of 32 dogs positive by PCR were treated with oral doxycycline. 15 out of 32 dogs positive by PCR were treated with injectable tetracycline. All 17 dogs showed clinical recovery with improvement in appetite and physical activity and tetracycline treated 15 dogs showed apparent clinical recovery with improvement in appetite and physical activity.

During the course of treatment, significant decrease ($P<0.05$) in mean value of rectal temperature were observed on 15th day and 21st day as compared to day 0, whereas significant decrease ($P<0.05$) was observed in mean values of heart rate, pulse rate on 15th and 21st day as compared to the day 0 and non- significant decrease in respiration rate in both treatment groups and mean values were almost similar between the two treatment groups ($P<0.05$) (Table 15). Mylonakis *et al* (2003) mentioned 50 dogs positive by IFA and PCR were experienced full recovery soon after the completion of doxycycline treatment.

The best drug to treat ehrlichiosis, regardless of the species of *Ehrlichia* or the form of the disease, is tetracyclines (Sainz *et al* 2000). Most effective antibiotic to

fight *E. canis* is doxycycline administerd per os for more than 20 days. (Sainz *et al* 2000, McClure *et al* 2010). Instead of doxycycline other tetracycline can be used – tetracycline in the dose of 22 mg/kg body weight every 8 hours. As adjunctive therapy liquids, corticosteroids and vitamins were also used (Mylonakis *et al* 2004).

Table 15: Comparative therapeutic study of vital body parameters of dogs with ehrlichiosis diagnosed by PCR

Parameters	Rectal Temperature (°F)	Heart rate (per minute)	Respiration rate (per minute)	Pulse rate (per minute)
PCR positive cases (0 day)	104.15±1.42^{a}	90.78±11.14^{a}	28.19±6.49^{a}	88.78±10.710^{a}
Doxycycline treated (15 day)	102±0.85^{b}	85.35±10.87^{ab}	29.59±3.52^{a}	83.71±10.53^{ab}
Doxycycline treated (21st day)	101.940.69^{b}	77.85±4.510^{b}	29.62±2.310^{a}	75.92±4.67^{b}
Tetracycline treated (15 day)	102.05±0.98^{b}	83.33±8.35^{ab}	30.07±2.55^{a}	82±8.32^{ab}
Tetracycline treated (21st day)	101.58±0.88^{b}	77.91±4.17^{b}	29.92±2.410^{a}	75.67±4.14^{b}

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

Serology done on total of 84 suspected serum samples among them 73 dogs were seropositive (Table 4), among 73, 36 dogs were treated. 3 dogs were positive only by serology and were treated with oral doxycycline. All three dogs showed apparent clinical recovery and improvement in physical activity ($P<0.05$). During the course of treatment significant difference seen in the mean values of rectal temperature in both treated groups on day 15 and day 21. Not much significant difference seen in the mean value of heart rate and pulse rate on day 15, but slight significant difference seen on day 21 in both treatment groups. Iqbal *et al* (1994b) and Greene (1995) proposed that minimum of 21 days of doxycycline therapy is required for treatment in CME. In some cases with ocular disease, prednisolone limited to first few days, short term anti-inflammatory or even immunosuppressive glucocorticoid therapy has been reported to be effective by Greene (1995) (Table 16).

Table 16: Comparative therapeutic study of vital body parameters of dogs with ehrlichiosis by serological examination

Parameters	Rectal Temperature (°F)	Heart rate (per minute)	Respiration rate (per minute)	Pulse rate (per minute)
Seropositive cases (0 day)	104.13±1.52[a]	89.17±11.01[a]	29.36±7.132[a]	87.58±10.97[a]
Doxycycline treated (15 day)	102.01±0.78[b]	84.5±10.50[ab]	29.55±3.33[a]	82.86±10.08[ab]
Doxycycline treated (21st day)	101.77±0.72[b]	79.33±4.77[b]	29.94±2.69[a]	77.44±4.810[b]
Tetracycline treated (15 day)	102.11±0.943[b]	84.41±8.47[ab]	30±2.87[a]	83±8.37[ab]
Tetracycline treated (21st day)	101.62±0.86[b]	78±4.06[b]	30.29±2.64[a]	75.79±4.04[b]

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

4.8.1.2. Hematobiochemical changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by blood smear examination

During the course of treatment, there was significant increase in the mean values of haemoglobin in both the treatment groups when observed on day 21 as compared to day 0 ($P<0.05$). Significant increase was seen in the mean values of Hb, TEC, PCV, platelets and eosinophils in both the treatment groups on day 15 and 21. Significant decrease in the mean value of eosinophils was also found in treated group as compared to affected group (Table 17, Figure 24). Even though decrease in the mean values of TLC, total bilirubin, AST, ALT, ALKP, total protein, albumin-globulin ratio, BUN, creatinine and increase in albumin were observed on Day 15 and 21 as compared to day 0, no significant difference found between the treatment groups and within the treatment groups with the positive cases. Symptoms of acute ehrlichiosis usually subside within 48-72 hours from the administration of the antibiotic. Hematological parameters were also improved (Kuehn and Gaunt 1985, Sainz *et al* 2000) ($P<0.05$) (Table 18, Figure 25).

4.8.1.3. Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by PCR.

During the course of treatment, there was significant increase in the mean values of PCV and platelets in both the treatment groups were observed on day 15 and 21 as compared to day 0 ($P<0.05$). Significant decrease in the mean value of eosinophils, ALKP and creatinine were observed in treated groups as compared to affected group. Even though there were decrease in the mean values of TLC, total bilirubin, AST, ALT, total protein, albumin-globulin ratio, BUN and increase in Hb, TEC and albumin were observed on Day 15 and 21 as compared to day 0, not much statistical significant difference found between the treatment groups and within the treatment groups with the positive cases ($P<0.05$). (Table 19 & 20, Figure 26 & 27 respectively).

4.8.1.4. Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by serology.

During the course of treatment, significant difference were observed in mean values of Hb, PCV, platelets, eosinophils (Table 21, Figure 28) ALT, ALKP and creatinine (Table 22, Figure 29) after treatment in both the treatment groups as compared to mean values of day 0 ($P<0.05$). Huxsoll *et al* (1976) characterized *E. canis* infection by rising antibody titers and hypergammaglobulinemia and dogs cleared of infection with tetracycline are fully susceptible to reinfection with homologous strain of *E. canis* and develop severe disease. Differences in response to infection may reflect underlying immunological defects, particularly in cell mediated system. Sasanelli et al (2009) reported a case of *E.canis* infected dog with an antibody titer of 1:160. Animal was treated with oral doxycycline (@10mg/kg/day) daily for six weeks. Clinical and parasitological recovery and normalisation of the blood values were achieved in two weeks after treatment.

Shipov *et al* (2008) diagnosed the case of CME by using a commercial kit (Immunocomb, Biogal, Kibbutz, Galed, Isreal). Dogs were showing anaemia (80%), leucopaenia (57.5%), thrombocytopenia (100%) and pancytopenia. Increased activities of ALP (70%), LDH (66.6%), hyperglobulinemia (65%), AST (60%), hyperprotienemia (35%). All the dogs were treated with doxycycline over period of time and all survived dogs were recovered and improvement in the blood picture was noted. Buhles *et al* (1974) studied pathogenesis of tropical canine pancytopaenia.

Table 17. Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by blood smear examination

Parameters	Hb (g/dL)	TLC (10^3/µL)	TEC (10^6/µL)	PCV (%)	Platelet (10^5/µL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
Smear positive cases	7.97 ± 1.97^{a}	11.034 ± 7.15^{a}	3.42 ± 0.77^{a}	22.74 ± 5.076^{a}	0.63 ± 0.410^{a}	79.33 ± 13.41^{a}	19.08 ± 13.310^{a}	1.5 ± 1^{a}
Doxycycline treated (15 day)	9.5 ± 1.38^{ab}	12.36 ± 5.87^{a}	4.10 ± 0.14^{ab}	31.8 ± 3.07^{b}	2.74 ± 2.09^{b}	82.5 ± 10.38^{a}	13 ± 5.83^{a}	0.75 ± 0.87^{b}
Doxycycline treated (21^{st} day)	11.9 ± 1.04^{b}	10.53 ± 2.00^{a}	5.62 ± 0.48^{d}	37.08 ± 5.074^{b}	2.1 ± 0.25^{ab}	79 ± 4.24^{a}	13.5 ± 3.42^{a}	0^{b}
Tetracycline treated (15 day)	10.33 ± 2.06^{ab}	10.45 ± 5.72^{a}	4.57 ± 0.42^{bc}	30.35 ± 5.67^{ab}	2.85 ± 0.69^{b}	73 ± 12.26^{a}	15.71 ± 11.16^{a}	0.43 ± 0.53^{b}
Tetracycline treated (21^{st} day)	12.26 ± 0.63^{b}	8.35 ± 2.58^{a}	5.54 ± 0.58^{cd}	36.94 ± 1.98^{b}	2.45 ± 0.31^{b}	69.14 ± 3.67^{a}	15 ± 2.89^{a}	0^{b}

Figures with same superscripts in a column do not differ significantly at ($p<0.05$)
Figures with different superscripts in a column differ significantly at ($p<0.05$)

Table 18. Biochemical changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by blood smear examination.

Parameters	Total bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALKP (U/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A:G ratio	BUN (mg/dL)	Creatinine (mg/dL)
Smear positive cases	1.56±2.910[a]	26.58±10.98[a]	59.17±28.012[a]	132.5±52.05[a]	6.58±1.47[a]	1.98±0.58[a]	4.60±1.56[a]	0.52±0.32[a]	22.83±10.54[a]	1.21±0.52[a]
Doxycycline treated (15 day)	0.63±0.44[a]	20.7±12.84[a]	55±18.87[a]	109.25±10.28[a]	7.15±0.24[a]	2.1±0.48[a]	5.05±0.49 [a]	0.42±0.13[a]	17±6.3[a]	0.83±0.26[a]
Doxycycline treated (21st day)	0.3±0.08[a]	21.25±3.510[a]	35±5.29[a]	96±5.89[a]	6.75±0.37[a]	2.38±0.17[a]	4.45±0.42[a]	0.54±0.09[a]	16.25±6.02[a]	0.75±0.26[a]
Tetracycline treated (15 day)	0.71±0.32[a]	26.14±11.48[a]	45±24.87[a]	107.86±52.97[a]	6.43±0.81[a]	2.37±0.59[a]	4.057±0.91[a]	0.62±0.24[a]	16.14±5.84[a]	0.83±0.39[a]
Tetracycline treated (21st day)	0.53±0.21[a]	18±4.9[a]	31.29±9.53[a]	58.86±39.28[a]	6.4±0.22[a]	2.69±0.24[a]	3.71±0.27[a]	0.76±0.11[a]	17±6.48[a]	0.8±0.37[a]

Figures with same superscripts in a column do not differ significantly at ($p<0.05$)
Figures with different superscripts in a column differ significantly at ($p<0.05$)

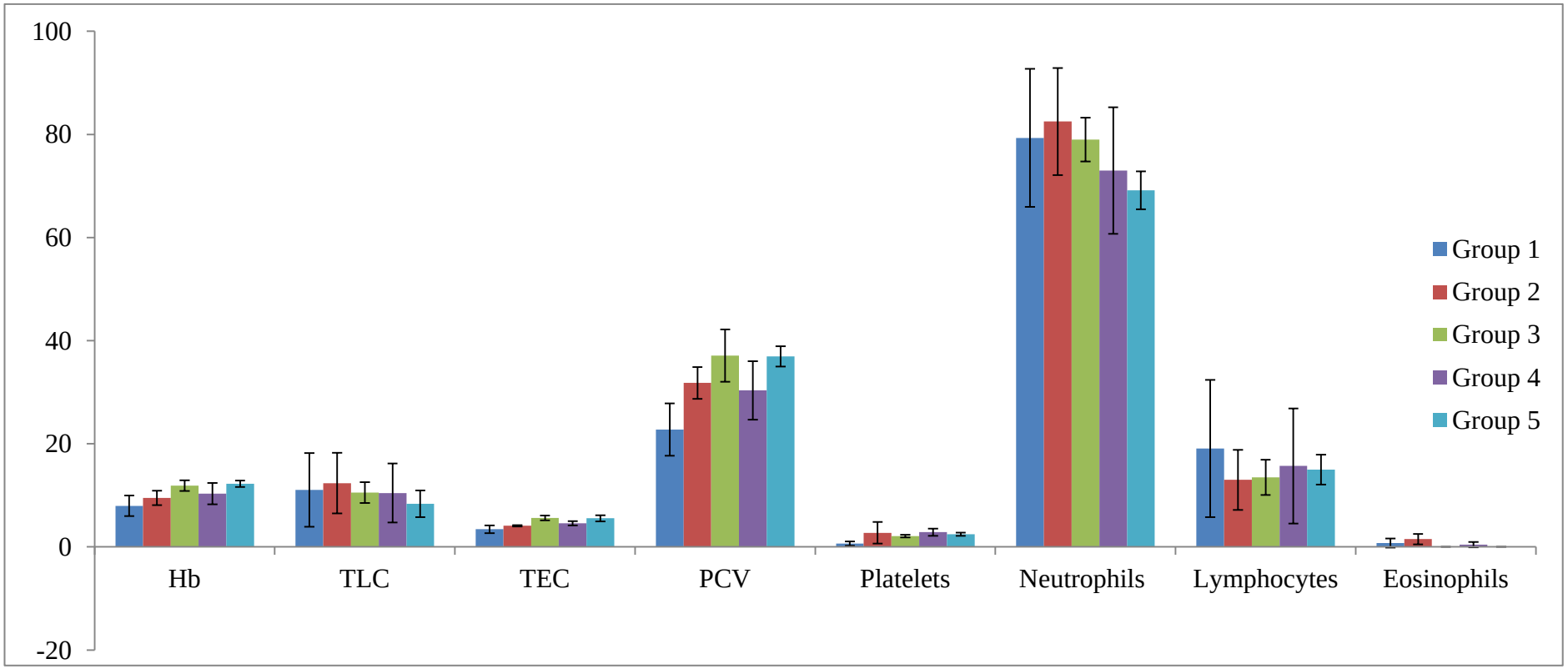


Figure 24: Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by blood smear examination

Group 1- Smear positive cases (0 day), Group 2- Doxycycline treated (15 day), Group 3- Doxycycline treated (21st day), Group 4- Tetracycline treated (15 day), Group 5- Tetracycline treated (21st day)

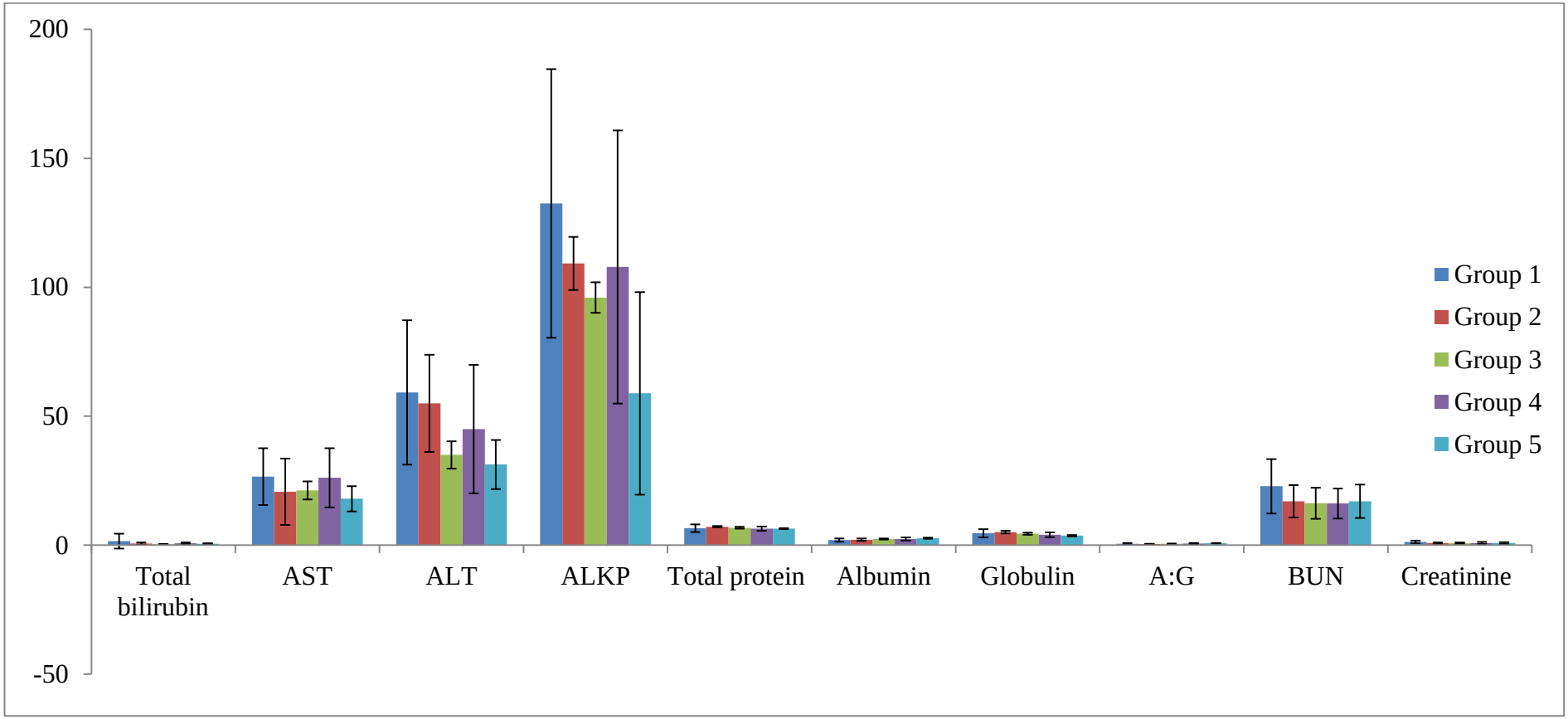


Figure 25: Biochemical changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by blood smear examination

Group 1 - Smear positive cases (0 day), Group 2 - Doxycycline treated (15 day), Group 3 - Doxycycline treated (21st day), Group 4 - Tetracycline treated (15 day), Group 5 - Tetracycline treated (21st day)

Table 19. Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by PCR

Parameters	Hb (g/dL)	TLC (10^3/µL)	TEC (10^6/µL)	PCV (%)	Platelet (10^5/µL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
PCR positive cases (0 day)	10.15±3.06[a]	13.27±7.18[a]	4.82±2.145[a]	29.91±8.86[a]	0.75±0.57[a]	78.65±14.88[a]	19.16±13.81[a]	1.89±2.55[a]
Doxycycline treated (15 day)	11.57±2.63[a]	14.210±5.59[a]	5.29±1.17[a]	35.69±7.13[ab]	2.00±1.15[b]	76.59±11.81[a]	16.76±8.70[a]	1±1.06[ab]
Doxycycline treated (21st day)	12.17±1.48[a]	12.12±3.95[a]	5.87±0.79[a]	37.61±3.38[b]	2.53±0.61[b]	74.46±7.87[a]	14.85±3.21[a]	0.15±0.38[b]
Tetracycline treated (15 day)	10.94±2.06[a]	12.08±7.26[a]	4.910±1.07[a]	32.41±7.29[ab]	2.74±0.74[b]	74.6±8.44[a]	15.8±8.48[a]	0.73±1.038[ab]
Tetracycline treated (21st day)	12.22±1.75[a]	10.36±3.23[a]	5.40±0.73[a]	36.64±5.41[ab]	2.56±0.36[b]	72.67±5.056[a]	16.08±5.41[a]	0[b]

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

Table 20. Biochemical changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by PCR.

Parameters	Total bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALKP (U/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A:G ratio	BUN (mg/dL)	Creatinine (mg/dL)
PCR positive cases (0 day)	0.85±1.75[a]	29.49±13.510[a]	52.49±26.35[a]	125.79±51.25[a]	7.088±1.64[a]	2.54±0.80[a]	4.55±1.53[a]	0.63±0.21[a]	20.65±11.04[a]	1.38±0.65[a]
Doxycycline treated (15 day)	0.48±0.28[a]	27.29±10.56[a]	47.65±18.810[a]	102.47±40.14[ab]	7.065±0.82[a]	2.53±0.45[a]	4.54±0.89[a]	0.59±0.19[a]	16.35±4.99[a]	1.082±0.43[ab]
Doxycycline treated (21st day)	0.48±0.23[a]	21.85±4.69[a]	36.46±8.09[a]	92.85±22.97[b]	6.69±0.310[a]	2.34±0.23[a]	4.38±0.46[a]	0.54±0.01[a]	16.15±4.83[a]	0.81±0.21[b]
Tetracycline treated (15 day)	0.58±0.27[a]	27.87±11.48[a]	44.93±19.37[a]	102.53±44.22[ab]	6.97±0.95[a]	2.43±0.41[a]	4.55±0.95[a]	0.56±0.19[a]	17.47±6.67[a]	1.09±0.49[ab]
Tetracycline treated (21st day)	0.46±0.19[a]	19.58±4.19[a]	33±10.98[a]	65±22.24[b]	6.59±0.34[a]	2.58±0.29[a]	4.00±0.51[a]	0.68±0.16[a]	16.28±5.52[a]	0.83±0.310[b]

Figures with different superscripts in a column differ significantly at ($p<0.05$)
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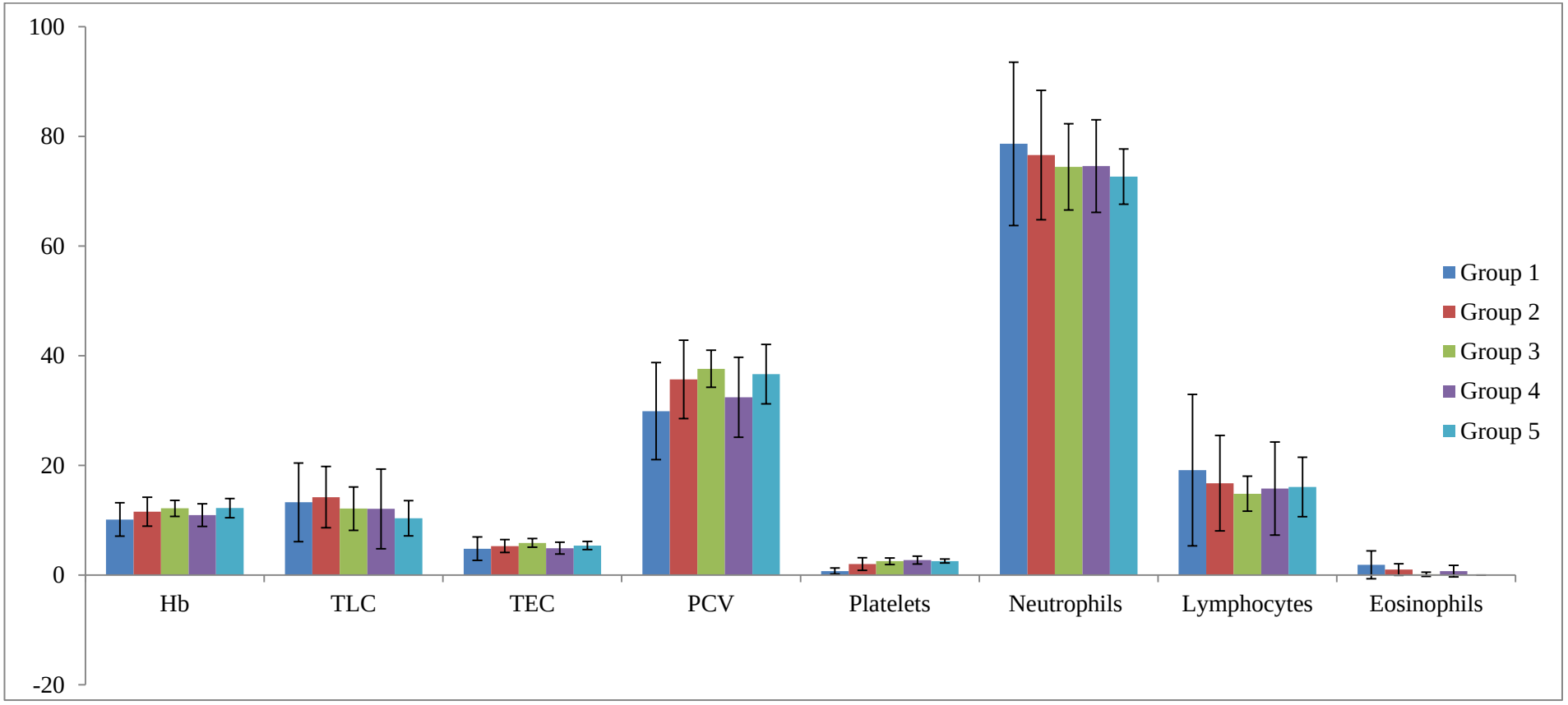

Figure 26: Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by PCR

Group 1 - Smear positive cases (0 day), Group 2 - Doxycycline treated (15 day), Group 3 - Doxycycline treated (21st day), Group 4 - Tetracycline treated (15 day), Group 5 - Tetracycline treated (21st day)

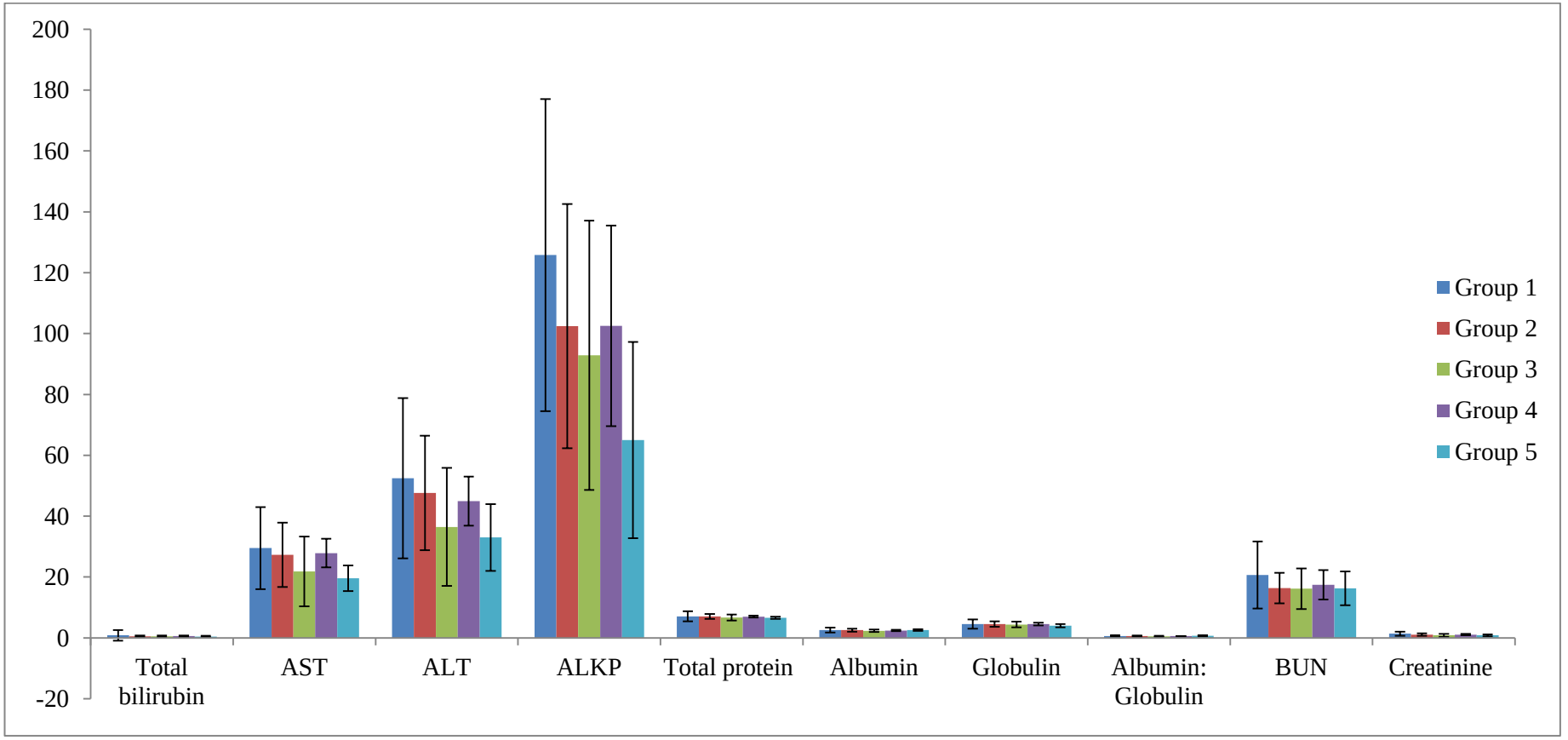

Figure 27: Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by PCR

Group 1 - Smear positive cases (0 day), Group 2 - Doxycycline treated (15 day), Group 3 - Doxycycline treated (21st day), Group 4 - Tetracycline treated (15 day), Group 5 - Tetracycline treated (21st day)

Table 21. Hematological changes after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by serology

Parameters	Hb (g/dL)	TLC (10^3/µL)	TEC (10^6/µL)	PCV (%)	Platelet (10^5/µL)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
Sero positive cases (0 day)	8.98±2.49^{a}	12.73±7.05^{a}	4.54±2.56^{a}	26.95±7.61^{a}	0.56±0.40^{a}	77.53±15.55^{a}	21.083±15.16^{a}	1.36±2.06b
Doxycycline treated (15 day)	11.45±2.45^{b}	13.11±5.40^{a}	5.20±1.10^{a}	35.35±6.51^{bc}	1.85±1.064^{b}	77.32±10.86^{a}	16.81±7.93^{a}	1.05±0.910^{ab}
Doxycycline treated (21st day)	12.26±1.26^{b}	11.83±3.45^{a}	5.98±0.69^{a}	38.16±3.55^{c}	2.43±0.56^{c}	73.27±7.94^{a}	15.44±3.18^{a}	0.11±0.32^{a}
Tetracycline treated (15 day)	10.65±2.10^{ab}	11.610±6.89^{a}	4.96±0.97^{a}	31.53±7.33^{ab}	2.74±0.69^{c}	74.29±8.07^{a}	15.94±7.95^{a}	0.650.99^{ab}
Tetracycline treated (21st day)	12.25±1.83^{b}	10.96±3.58^{a}	5.4±0.81^{a}	36.74±5.63^{bc}	2.55±0.35^{c}	73.57±5.39^{a}	15.36±5.53^{a}	0^{b}

Figures with different superscripts in a column differ significantly at ($p<0.05$)
Figures with same superscripts in a column do not differ significantly at ($p<0.05$)

Table 22. Biochemical changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by serology

Parameters	Total bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALKP (U/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A:G ratio	BUN (mg/dL)	Creatinine (mg/dL)
Sero positive cases (0 day)	0.91±1.77[a]	28.42±14.0[a]	58.61±28.05[b]	128.11±49.76[b]	7.19±1.82[a]	2.45±0.75[a]	4.74±1.68[a]	0.59±0.28[a]	22±10.66[a]	1.37±0.63[b]
Doxycycline treated (15 day)	0.49±0.29[a]	27.77±9.70[a]	48.59±17.86[ab]	106.27±36.71[ab]	7.00±0.76[a]	2.49±0.49[a]	4.51±0.810[a]	0.58±0.20[a]	16.09±5.04[a]	1.00±0.42[ab]
Doxycycline treated (21st day)	0.44±0.23[a]	22.06±4.95[a]	35.56±7.01[a]	93.22±10.31[a]	6.67±0.38[a]	2.32±0.20[a]	4.37±0.43[a]	0.54±0.087[a]	15.89±4.44[a]	0.85±0.20[a]
Tetracycline treated (15 day)	0.55±0.26[a]	27.59±10.94[a]	45.47±20.46[ab]	103.71±45.810[ab]	7.02±0.810[a]	2.44±0.51[a]	4.58±0.92[a]	0.56±0.110[a]	17.12±6.44[a]	1.059±0.47[ab]
Tetracycline treated (21st day)	0.46±0.19[a]	19.93±4.110[a]	34.5±11.21[a]	66.5±32.810[a]	6.58±0.33[a]	2.54±0.30[a]	4.04±0.410[a]	0.66±0.16[a]	16.27±5.77[a]	0.84±0.310[a]

Figures with different superscripts in a column differ significantly at ($p<0.05$)
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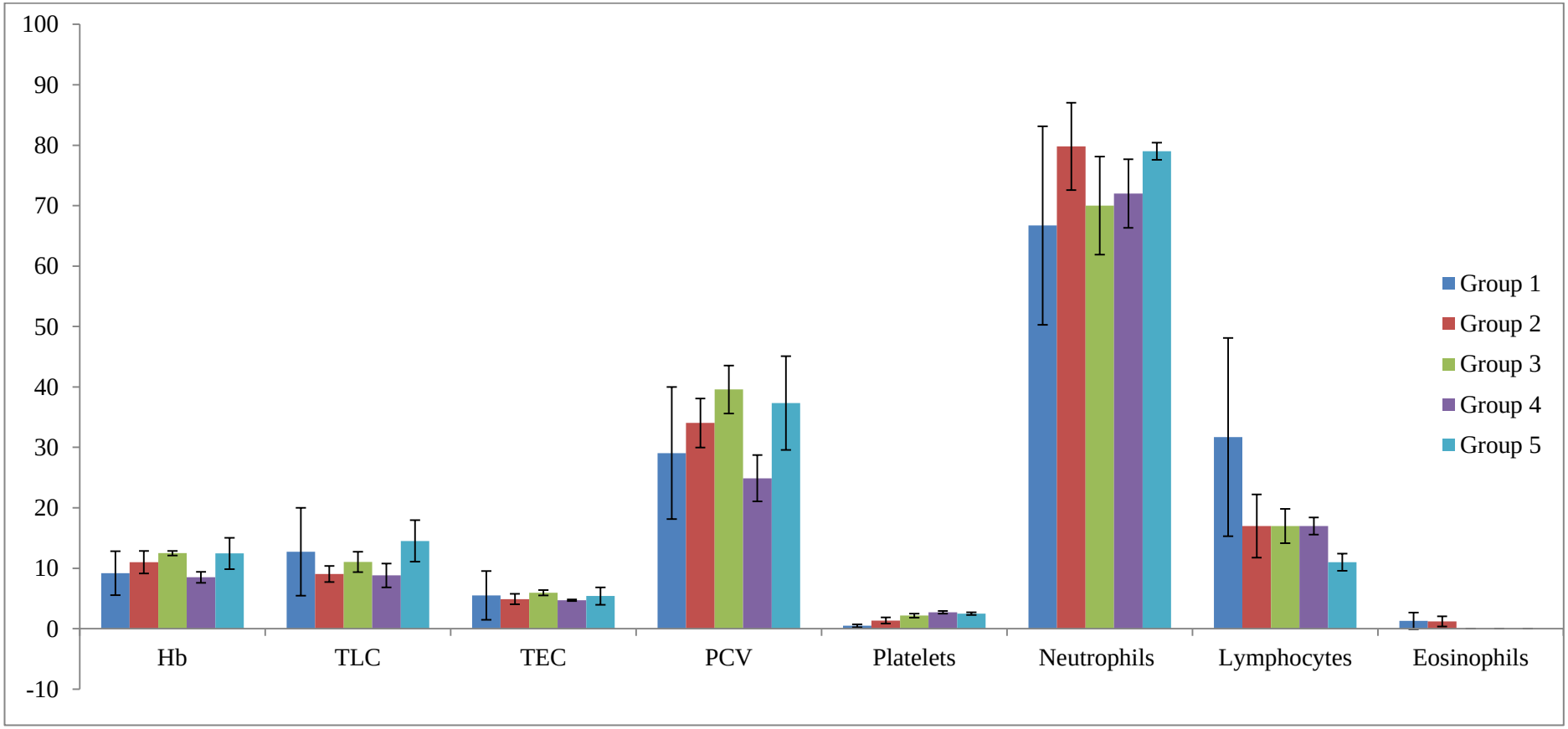


Figure 28: Hematological changes before and after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by serology

Group 1 - Seropositive cases (0 day), Group 2 - Doxycycline treated (15 day), Group 3 - Tetracycline treated (15 day), Group 4 - Doxycycline treated (21st day), Group 5 - Tetracycline treated (21st day).

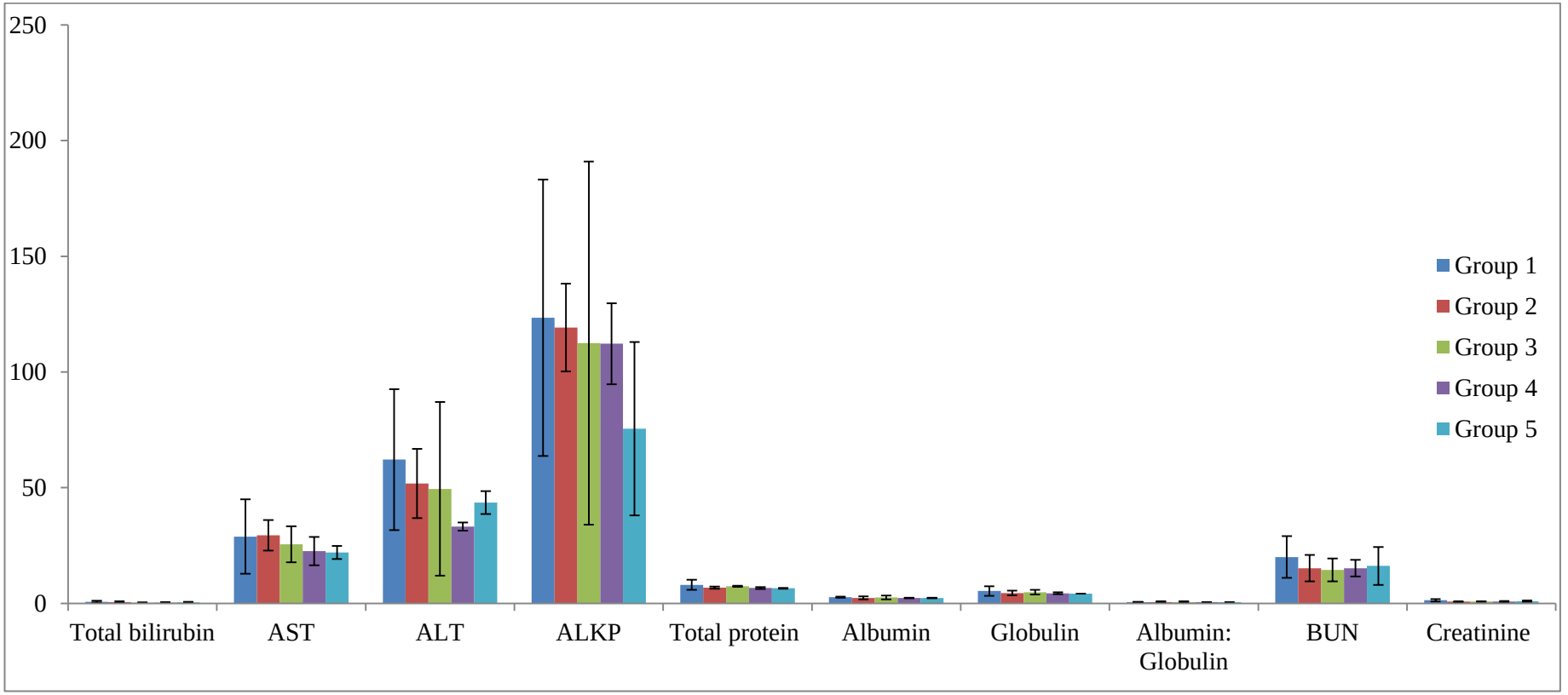


Figure 29: Biochemical changes after treatment with oral doxycycline and injectable tetracycline in dogs with ehrlichiosis diagnosed by serology

(Group 1 - Seropositive cases (0 day), Group 2 - Doxycycline treated (15 day), Group 3 - Doxycycline treated (21st day), Group 4 - Tetracycline treated (15 day), Group 5 - Tetracycline treated (21st day)

Challenged inoculation done for 14 German shepherd dogs with *E. canis*. Nine dogs developed pancytopenia, hemorrhage and after 80 days antibody titres were increased and all dogs developed hypergammaglobulinemia. Dogs were treated with tetracycline and after treatment levels of serum albumin-globulin ratio returned to normal.

4.8.1.3. Blood smear, PCR examination, serological examination and survivability of the affected dogs

All 12 cases positive by blood smear examination for *E. canis* morulae were positive by PCR and serology on day 0. Out of 12, 5 cases were treated with oral doxycycline and rest 7 cases were treated by injectable tetracycline. All 12 cases were negative by blood smear examination on day 15. On day 15, out of 5 doxycycline cases only 3 cases were negative by PCR, whereas on day 21 all 5 cases were negative by PCR. Out of 7 tetracycline treated cases only one case was negative by PCR on day 15, whereas 4 cases were negative by PCR on day 21, rest 3 were still positive on day 21.

The PCR positive dogs affected with *E. canis* were also treated with same drugs, out of 33 PCR positive cases, which includes both smear positive and seropositve cases, 17 cases were treated with oral doxycycline, rest 15 cases were treated with injectable tetracycline, among 17 cases, five cases were negative on day 15 and all were negative on day 21. Among 15 injectable tetracycline treated cases, only three cases were negative by PCR on day 15, whereas 10 cases were negative on 21. All the dogs survived except one dog which died due to acute blood loss before treatment.

Dogs positive by only serology were 3, means were negative by blood smear and PCR. Among 3 dogs, 3 dogs were treated with oral doxycyline. As these dogs were clinically and hemato-biochemically apparently normal, so good improvement was noticed in all 3 dogs within a week.

Komnenou *et al* (2007) tested 90 dogs for *E. canis* antibodies and all dogs were positive by Immunocomb®, Biogal test kit. Dogs were having thrombocytopenia, anaemia, leucopaenia, leucocytosis, hyperproteinemia, increased ALT and ALKP activities, hypoproteinemia and hypercreatinemia were the main hematological and biochemical abnormalities. In every dog, oral doxycycline was prescribed at the dosage of 5 mg/kg body weight, q12 h, for 4 weeks. Twenty out of 90 (22.2%) dogs were also treated with prednisolone at 1 mg/kg body weight, q 24 h,

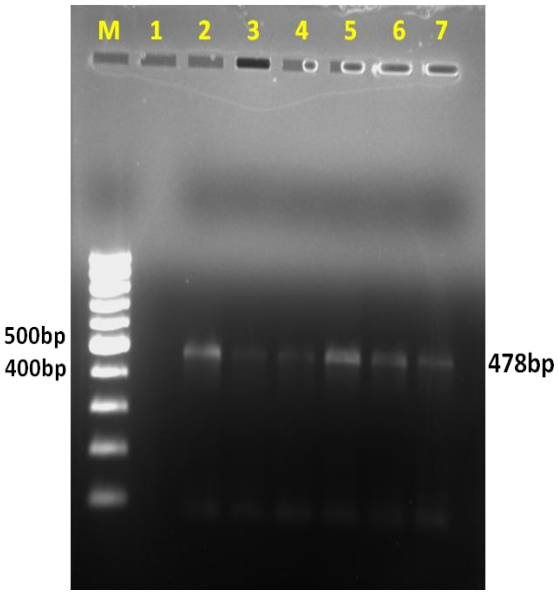


Figure 30. Standardization of primary PCR (E-PCR) assay

Lane M: Generuler™ 100bp Ladder

Lane 1: Negative control

Lane 2: Positive control

Lane 3 to 7: Samples positive for *E. canis* by blood smear Examination

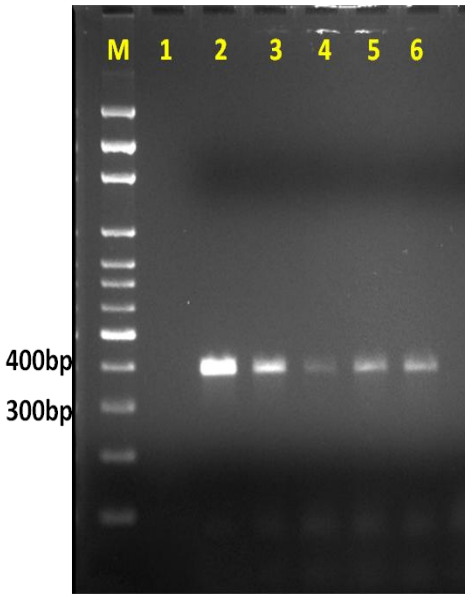


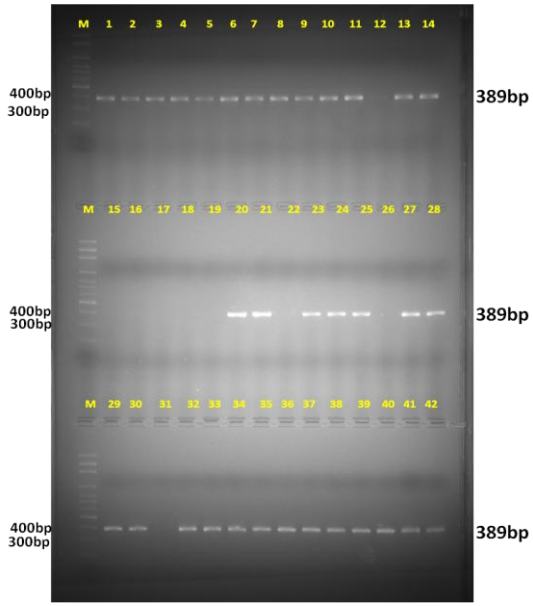
Figure 31. Standardization of nested PCR (Ec-PCR) assay

Lane M: Generuler™ 100bp Ladder

Lane 1: Negative control Lane 2: Positive control

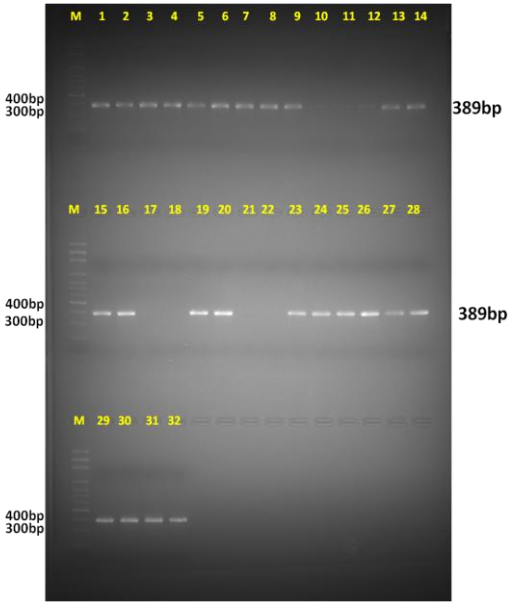
Lane 3 to 6 samples positive for *E. canis* by blood smear examination

Figure 32. Screening *of E. canis* suspected samples on day 0 by n-PCR



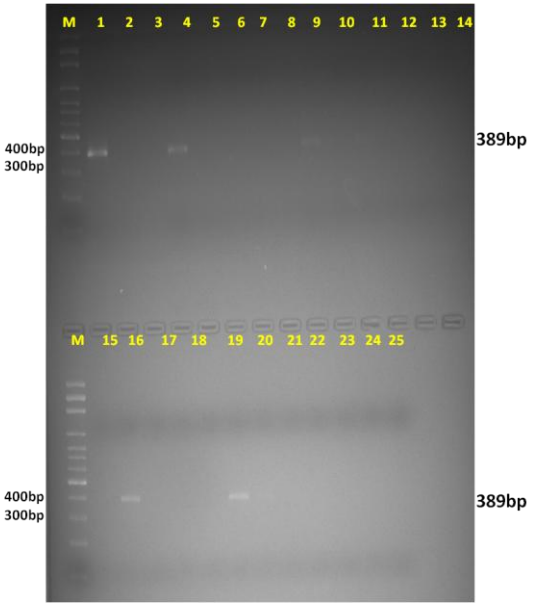
Lane M:Generuler^{TM} 100bp Ladder
Lane 1-42: Suspected samples
Lane 1-11,13,14,20, 21,23-25,27-30, 32-42: Samples Positive for *E. canis* on day 0
Lane 12, 15 to 19, 21 26, 31: Suspected samples negative for *E. canis*
Lane 8: Positive control

Figure 33. Screening of samples after 15 day of therapy by n-PCR



Lane M:Generuler^{TM} 100bp Ladder
Lane 1-32: day 0 positive samples for *E. canis*
Lane 10-12, 17, 18, 21, 22: 7 Samples become negative for *E. canis* on day 15
Lane 1-9, 13-16, 19, 20, 23-32: samples positive for *E. canis* on day 15
Lane 8: Positive control

Figure 34. Screening of samples on 21 day of therapy by n-PCR



Lane M: Generuler^{TM} 100bp Ladder
Lane 1, 3, 9, 16, 19: Samples positive for *E. canis* on day 21 were treated with injectable tetracycline

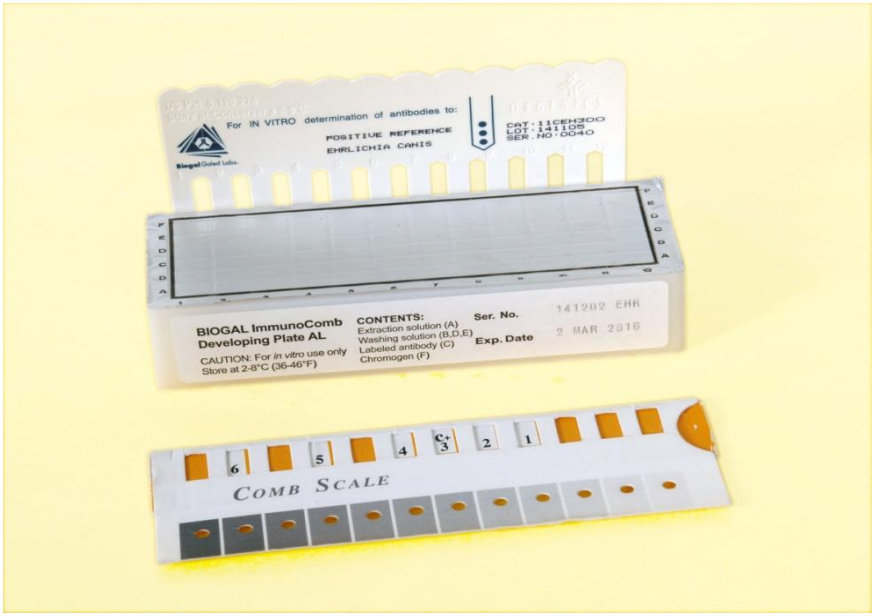


Figure 35. ImmunoComb® canine *Ehrlichia* antibody test kit assemble

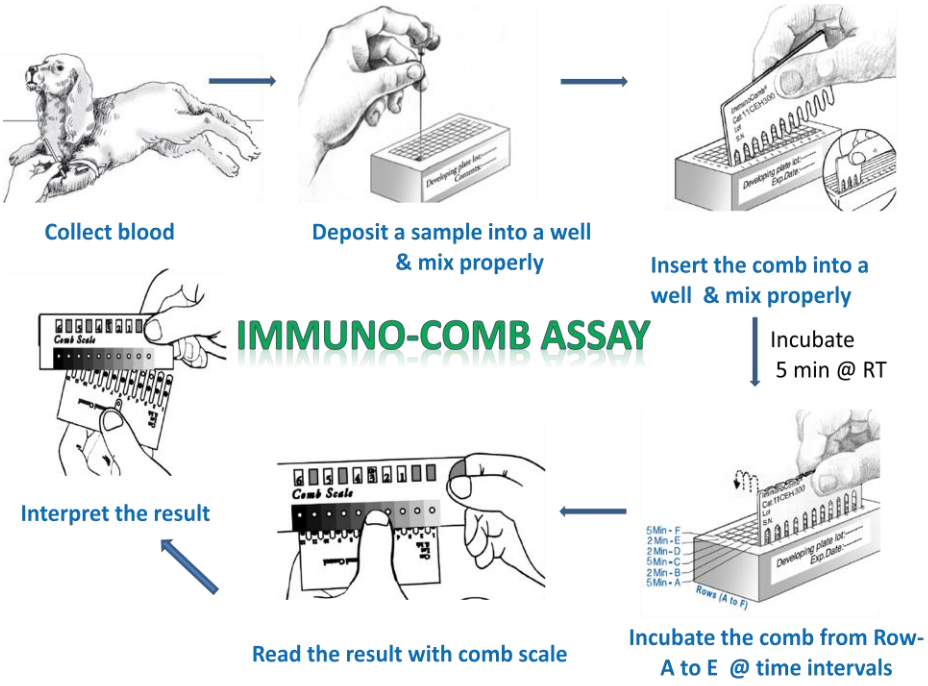


Figure 36. Protocol for ImmunoComb® canine *Ehrlichia* antibody detection

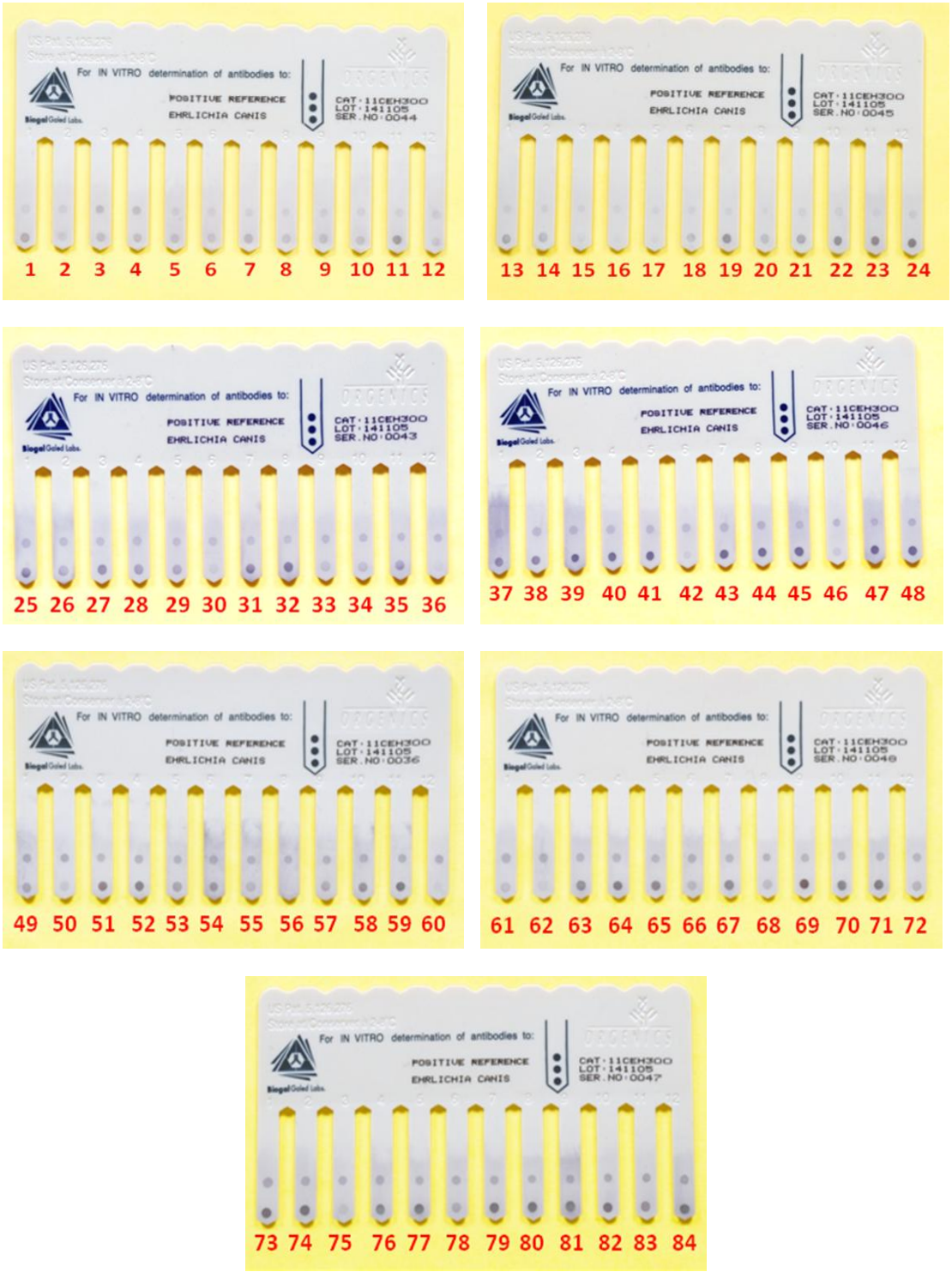


Figure 37. Screening of 84 *E. canis* suspected samples by ImmunoComb® canine ehrlichia antibody test. The developed spots represent different concentrations of serum antibodies and the upper spot represents a known positive control indirect immunofluorescent antibody test (IFA) pretititrated serum sample of 1:80 (Table 23).

for 1 to 2 weeks. 45 (50%) dogs, responsed to the above mentioned treatments, Twenty-five out of 45 dogs (55.5%) responded completely, 11/45 dogs (24.5%) partially, and 9/45 dogs (20%) poorly to the systemic and topical treatment.

Table 23. Screening of *E.canis* suspected cases by using ImmunoComb® canine ehrlichia antibody test kit (Biogal, Galed Labs.)

Score	Titre	Tooth number and Results (n = 84)
≥S 5	1:320- 1280	High positive reaction to *E. canis*
		1, 11, 13, 14, 19, 21, 22, 23, 24, 25, 27, 28, 31, 32, 37, 38, 39, 40, 41, 43, 44, 45, 47, 48, 51, 52, 58, 59, 63, 64, 65, 67, 69, 70, 71, 73, 74, 75, 76, 77, 79, 80, 81, 82, 84
S 3-S 4	1:80- 1: 160	Medium positive reaction to *E. canis*
		3, 4, 5, 6, 7, 8, 9, 10, 18, 20, 26, 29, 35, 49, 53, 54, 57, 78, 83
S 1-2	1:20- 1:40	Low positive reaction to *E. canis*
		2, 12, 33, 34, 55, 61, 66, 68, 72
S 0	Nil	Negative reaction
		15, 16, 17, 30, 36, 42

(n) Number of dogs screened for ehrlichiosis

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study was conducted to know the prevalence, clinico-hemato-biochemical changes and therapeutic management of ehrlichiosis in dogs in clinical cases of dogs presented at Small Animal Clinics of GADVASU, Ludhiana from January 2014 to December 2014.

Overall prevalence from suspected cases of canine ehrlichiosis based on clinical findings recorded by blood smear examination was 7.74 per cent (12/155), serology was 86.90% (73/84) and PCR was 78.57 per cent (33/42).

Month wise prevalence of *Ehrlichia canis* infection was seen in the months of June, July, August, September, October and November. In seasonwise prevalence of *Ehrlichia canis* infection was seen in autumn season followed by rainy and summer season and lowest in spring season. The seasonal activity of brown dog tick (*R. sanguineus*) might have led to the higher incidence of ehrlichiosis in warm months.

Different clinical signs viz. congested mucus membrane, fever, inappetance, anorexia, vomiting, melena, epistaxis, weakness, lethargy, depression and anaemia presented by pale mucous membrane, tick infestation, hind limb weakness respiratory distress and other rare findings were corneal opacity with seizures, oedema of legs, bleeding tendencies, oocular discharge and petechieal haemorrhages were found in ehrlichiosis.

Hemato-biochemical findings viz. leucocytosis, anaemia, thrombocytopenia, leucocytosis, mild leucopaenia, neutropenia, lymphopenia, eosinophilia, hyperbilirunemia, hyperproteinemia, hypoalbuminemia, hypercreatinemia, hyperglobulinemia, decrease in albumin and globulin ratio and increase in aspertate transaminase, alanine transaminase and alkaline phosphatase activity were most common findings in *E. canis* infection.

Among the two drugs used for the treatment of ehrlichiosis in dogs, oral doxycycline were showed higher efficacy as compared to injectable tetracycline. All the cases treated with doxycycline were negative on day 21 whereas 58.33 percent cases treated with injectable tetracycline were negative on day 21. Other changes

observed before and after treatment in different groups are as under:

Dogs positive by blood smear examination (n=12)

In group A treated with oral doxycycline showed significant (<0.05) increase in mean value of Hb (11.9±1.04g/dL), TEC (5.62±0.48×10^6/µL), PCV (37.08±5.074%) and platelets (2.1±0.25×10^5/µL) were observed on day 21 as compared to day 0.

In group B treated with injectable tetracycline showed significant (<0.05) increase in the mean value of Hb (12.26±0.63g/dL), TEC (5.54±0.58×10^6/µL), PCV (36.94±1.98%) and platelets (2.45±0.31×10^5/µL) were observed on day 21 as compared to day 0. Not much varied statistical significant difference found in the biochemical parameters of smear positive dogs.

Dogs positive by polymerase chain reaction (n=33)

In group A treated with oral doxycycline showed significant (<0.05) increase in mean value PCV (37.61±3.38%), platelets (2.53±0.61×10^5/µL) and eosinophils (0.15±0.38%), decrease in mean values of ALT (36.46±8.09U/L), ALKP (92.85±32.97U/L) and creatinine (0.86±0.21mg/dL) were observed on day 21 as compared to day 0.

In group B treated with injectable tetracycline showed significant (<0.05) increase in the mean value of PCV (36.64±5.41%), platelets (2.56±0.36×10^5/µL) and decrease in the mean values of ALT (33±10.98U/L), ALKP (65±32.24U/L) and creatinine (0.83±0.310mg/dL) were observed on day 21 as compared to day 0.

Dogs positive by serological examination (n=36)

In group A treated with oral doxycycline showed significant (<0.05) increase in mean value Hb (12.26±1.26g/dL), PCV (38.16±3.55%), platelets (2.43±0.56 ×10^5/µL) and eosinophils (0.11±0.32%), decrease in mean values of ALT (35.56± 7.01U/L), ALKP (93.22±10.31U/L) and creatinine (0.85±0.20mg/dL) were observed on day 21 as compared to day 0.

In group B treated with injectable tetracycline showed significant (<0.05) increase in the mean value of Hb (12.25±1.83g/dL), PCV (36.74±5.63%), platelets (2.55±0.35×10^5/µL) and decrease in the mean values of ALT (35.56±7.01U/L), ALKP (66.5±32.810U/L) and creatinine (0.84±0.310mg/dL) were observed on day 21 as compared to day 0.

Based on above studies following points of conclusions emerge:

- Hematolgical studies depicted decreased levels of Hb, TEC, PCV and platelets in dog infected by ehrlichiosis.
- Biochemical studies showed decreased levels of albumin, albumin & globulin ratio and increased level of AST, ALT, ALKP, globulin and creatinine.
- Thrombocytopenia is common hematological finding.
- Pancytopenia a common finding in *Ehrlichia canis* positive dogs
- Blood smear examination is less sensitive technique when compared to PCR and serology.
- Most of the cases were still positive in 15 day but negative after 21 day post treatment by PCR. Hence affected dogs should be treated up to 21 days.
- Oral doxycycline showed highest therapeutic efficacy as compared to injectable tetracycline.
- As the results of serology were compareable with the PCR based results, serology (ImmunoComb assay) can be used as a penside diagnostic test.

REFERENCES

Abeygunawardena I S, Kakoma I, and Smith R D. 1990. Pathophysiology of canine ehrlichiosis, Ehrlichiosis: A vector-borne disease of animals and humans. Kluwer, Dordrecht.The Netherlands. pp. 78-92.

Aguiar D M, Mitika K, Hagiwara, Marcelo B. Labruna.2008. In vitro isolation and molecular characterization of an *Ehrlichia canis* strain from são paulo, brazil. *Brazilian Journal of Microbiology* **39**: 489-493.

Aguirre E, Sainz A, Dunner S, Amusategui I, Lopez L, Franco F R, Luaces I, Cortes O and Tesouro M. A. 2004. First isolation and molecular characterization of *Ehrlichia canis* in Spain.*Veterinary Parasitology* **125(3/4)**: 365-372.

Ajay K and Varshney J. P. 2006. Studies on clinical variants in naturally occurring cases of Canine Ehrlichiosis. *Intas Polivet* **7(1)**: 94-101.

Akhtardanesh B, Ghanbarpour R and Blourizadeh H. 2010. Serological evindence of canine monocytic ehrlichiosis in Iran. *Comparative Clinical Pathology* **19**: 469-74.

Alexandor N, Santos A S, Nucio M S, de Sousa R, Boinas F and Bacellar F. 2008. Detection of *Ehrlichia canis* by polymerase chain reaction in dogs from Portugal. *The Veterinary Journal* **181**: 343-44.

Amusategui I, Tesouro M A, Kakoma I and Sainz A. 2008. Serological reactivity to *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Neorickettsiaristicii*, *Borrelia burgdorferi* and *Rickettsia conorii* in dogs from Northwestern Spain. *Vector Borne and Zoonotic Diseases* **8(6)**: 797-804.

Aroch I, Harrus S. 2001. The use of hematopoietic growth factors, recombinant human granulocyte colony stimulating factor and recombinant human erythropoietin in severe pancytopenia due to canine monocytic ehrlichiosis. *Israel Journal of Veterinary Medicine* **56**: 65-69.

Asgarali Z, Pargass I, Adam J, Mutani A and Ezeokoli C. 2012. Hematological parameters in stray dogs seropositive and seronegative to *Ehrlichia canis* in north Trinidad. *Ticks and tick borne diseases* **3**: 207-211.

Banerjee P S, Mylonakis M E, Garg R, Vatsya S, Yadav C L. 2008. Concurrent hepatozoonosis and granulocytic ehrlichiosis in a dog. *Journal of Veterinary Parasitology* **22**: 9-11.

Baneth G, Harrus S, Ohnona F S, Schlesinger Y. 2009. Longitudinal Quantification of *Ehrlichia canis* in Experimental Infection with Comparison to natural infection. *Vetrinary Microbiology* **136(3/4)**: 321-25.

Baneth G, Waner T, Koplah A, Weinstein S and Keysary A. 1996. Survey of *Ehrlichia canis* antibodies among dogs in Israel. *Veterinary Record* **138**: 275-95.

Bartsch R C and Greene R T. 1996. Post-therapy antibody titers in dogs with

ehrlichiosis: follow-up study on 68 patients treated primarily with tetracycline and or doxycycline. *Journal of Veterinary Internal Medicine* **10**: 271-74.

Baticados A M and Baticados W N. 2011 Serological Evidence for Ehrlichia canis Exposure in Military Dogs and Other Canines in Metropolitan Manila, Philippines. *Israel Journal of Veterinary Medicine* **66(4)**: 151-56.

Batmaz H, Nevo E, Waner T, Senturk S, Yilmaz Z and Harrus. 2001. Seroprevalence of *Ehrlichia canis* antibodies among dogs in Turkey. *Veterinary Record* **148**: 665-66.

Beugnet F, Latour S, Chenal L, Malivert B and Viallard J. 2002. Seroprevelence of Canine Monocytic Ehrlichiosis on Reunion. *Veterinary Record* **150(20)**: 636-37.

Botros B A, Elmolla M S, Salib A W, Calamaio C A, Dasch G A, Arthur R R. 1995. Canine ehrlichiosis in Egypt: Seroepidemiological survey. *Onderstepoort Journal of Veterinary Research* **62**: 41-43.

Breitschwerdt E B, Hegarty B C and Hancock S I. 1998. Doxycycline hyclate treatment of experimental canine ehrlichiosis followed by challenge inoculation with two *Ehrlichia canis* strains. *Antimicrobiology Agents Chemotherapy* **42**: 362-68.

Bremer W G, Schaefer J J, Wagner E R, Ewing S A, Rikihisa Y, Needham G R, Jittapalapong S, Moore D L, Stich R W. 2005. Transstadial and intrastadial experimental transmission of *Ehrlichia canis* by male *Rhipicephalus sanguineus*. *Veterinary Parasitology* **131**: 95-105.

Bressler C, Himes L C and Moreau R E. 2003. Portel vein and aortic thromboses in a Siberian Husky with Ehrlichiosis and Hypothyroidism. *Journal of Small Animal Practice* **44(9)**: 408-10.

Buhles W C, Huxsoll D L and Ristic M.1974. Tropical Canine Pancytopaenia: Clinical, Hematological and Serological Response of dogs to *Ehrlichia canis* infection, Tetracycline therapy and challenge inoculation. *Journal of Infectious Diseases* **130**: 357-67.

Bulla C, Takahira R K, Araujo-junior J P, Trinca L A and Lopes R S. 2004. The relationship between the degree of thrombocytopenia and infection with *Ehrlichia canis* in an endemic area. *Veterinary Research* **35(1)**: 141-46.

Burghen G A, Beisel W R, Walker J S, Nims R M, Huxsoll D L, and Hildbrandt P K. 1971. Development of hypergammaglobulinemia in tropical canine pancytopenia. *American Journal of Veterinary Research* **32**: 749-56.

Cadman H F, Kelly P J, Matthewman L A, Zhou R and Mason P R. 1994. Comparison of the dot-blot enzyme linked immunoassay with immunofluorescence for detecting antibodies to *Ehrlichia canis*. *Veterinary Record* **135**: 362.

Cardoso L, Tuna J, Vieira L, Yisachar-mekuzas Y and Baneth G. 2010. Molecular

detection of Anaplasma platys and Erhlichia canis in dogs from the North of Portugal. *The Veterinary Journal* **183**: 232-33.

Carlos R S A, Carvalho F S, Wenceslau A A, Almosny N R P, Albuquerque G R 2011. Risk factors and clinical disorders of canine ehrlichiosis in the South of Bahia, Brazil. *Brazilian Journal of Veterinary Parasitology* **20(3)**: 210-14.

Carvalho F S, Wenceslau A A, Carlos R S and Albuquerque G R. 2008. Epidemiological and molecular study of *Ehrlichia canis* in dogs in Bahia, Brazil. *Genetics and Molecular Research* **7(3)**: 657-62.

Castro M B, Machado R Z, Alessi A C, Aquino L P C T. 1997. *Ehrlichia canis* (Donatien and Lestoquard, 1935): Experimental infection in dogs. In: Proceedings of the IVth Biennial Meeting Society for Tropical Veterinary Medicine, Montpellier, French.

Castro M B DE, Machado R Z, Aquino L P C T, Alessi A C and Costa M T. 2004. Experimental Acute Canine Monocytic Ehrlichiosis: Clinicopathological and Immunopathological findings. *Veterinary Parasitology* **119(1)**: 73-86.

Chandrasekar M, Nambi A P, Ramprabhu R and Dhanapalan P. 2002. Epizootiological studies on Canine Ehrlichiosis. *Indian veterinary Journal* **79(12)**: 85-86.

Chipde V D, Rode A M, Dakshinkar N P, Pradhan M S, Sarode D B and Shrikhande G B. 2008. Therapeutic management of canine ehrlichiosis. *Indian Veterinary Medical Journal* **28**: 146-48.

Chipde V S, Rode A M, Pradhan M S, Dakshinkar N P and Sarode D B. 2007. Prevenlence of Canine Ehrlichiosis in Nagpur city. *Royal Veterinary Journal of India* **3(2)**: 121-23.

Choudhary 2009. 'Studies on ehrlichiosis in dogs'. M.V.Sc. Thesis. Karnataka Veterinary and Animal Fishries Science University Bidar.

Cockwill K R, Taylor S M, Snead E C, Dickinson R, Cosford K, Malek S, Lindsay L R and Diniz P P 2009. Granulocytic anaplasmosis in three dogs from Saskatoon, Saskatchewan. *Canadian Veterinary Journal* **50**: 835-40.

Codner E C, Caceci T, Saunders G K, Smith C A, Robertson J L, Martin R A, and Troy G C. 1992. Investigation of glomerular lesions in dogs with acute experimentally induced *Ehrlichia canis* infection. *American Journal of Veterinary Research* **53**: 2286-91.

Codner E C, Farris-Smith L L 1986. Characterization of the subclinical phase of ehrlichiosis in dogs. *Journal of American Veterinary Medical Association* **189**: 47-50.

Cowell B L, Tyler R D, Clinkenbeard K D C and Meinkoth J H. 1988. Ehrlichiosis and Polyarthritis in three dogs. *Journal of American Veterinary Medical Association* **192**: 1093-95.

Dagnone A S, De Morais H S, Vidotto M C, Jojima F S and Vidotto O 2003. Ehrlichiosis in anemic, thrombocytopenic, or tickinfested dogs from a hospital population in South Brazil. *Veterinary Parasitology* **117**: 285-90.

Dantas-Torres F. 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. *Veterinary Parasitology* **152**: 173-85.

Das and Konar 2013. Clinical and hematological study of canine ehrlichiosis with other hemoprotozoan parasites in Kolkata, West Bengal, India. *Asian Specific Journal of Tropical biomedicine* **3(11)**: 913-15.

Davoust B, Keundjian A, Rous V, Maurizi L and Parzy D. 2005. Validation of chemoprevention of canine monocytic ehrlichiosis with doxycycline. *Veterinary microbiology* **107**: 279-83.

Davoust B, Mediannikov O, Chene J, Massot R, Tine R, Diarra M, Demoncheaux J P, Scandola P, Beugnet F and Chabanne L. 2013. Study of ehrlichiosis in kennel dogs under treatment and prevention during seven months in Darkar (Senegal). *Comparative Immunology, Microbiology and Infectious Disaeses* **36**: 613-17.

De Castro M B, Machado R Z, De Aquino L P C T, Alessi A C and Costa M T. 2004. Experimental acute canine monocytic ehrlichiosis: Clinicopathological and immunopathological findings. *Veterinary Parasitology* **119**: 73-86.

Dhankar S, Sharma R D and Jindal N. 2011. Epidemiological observations on canine ehrlichiosis in Haryana and Delhi states. *Haryana Veterinarian* **50**: 9-14.

Donatien A and Lestoquard F.1935. Existence en Algerie dune *Rickettsia* duchien, Bulletin de la Societe de Pathologie Exotique **28**: 418-19 (Quoted in *Advances in Veterinary Science and Comparative Medicine* **13**: 340, 1969).

Donatien A and Lestoquard F. 1940. Rickettsiose bovine Algerienne a *R. bovis*. *Bulletin de la Societe de pathologie exotique et de ses filiales* **33**: 245-48.

Dos Santos L G F, Melo A L T, Filho J M, Witter R, Labruna M B and de Aguiar D M. 2013. Molecular detection of Ehrlichia canis in dogs from the Pantanal of Mato Grosso State, Brazil. *Brazilian Journal of Veterinary Parasitology* **22(1)**: 114-118.

Doyle C K, Labruna M B, Breitschwerdt E B, Tang Y W, Corstvet R E, Hegarty B C, Bloch K C, Li P, Walker D H and Mcbride J W. 2005. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the dsb gene. *The Journal of Molecular Diagnostics* **7**: 504-10.

Dubie T, Mohammed Y, Terefe G, Muktar Y and Tesfaye J. 2014. An insight review on canine ehrlichiosis with emphasis on its epidemiology and pathogenesity importance. *Global Journal of Veterinary Medicine and Research* **2(4)**: 059-067.

Dumler J S, Barbet A F, Bekker C P J, Dasch G A, Palmer G H, Ray S C, Rikihisa Y

and Rurangirwa F R. 2001. Reorganisation of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: Unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia,* descriptions of six new species combinations and designarion of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of Ehrlichia Phagocytophila. *International Journal of Systematic and Evolutionary Microbiology* **51**: 2145-65.

Dutta P R, Hafiz A, Bora S, Phukan A, Baishya B C and Kalita D N. 2013. Prevalence of canine diseases in Guwahati city. *Indian Veterinary Journal* **90**: 103-04.

Eiras D F, Craviotto M B, Vezzani D, Eyal O and Baneth G. 2013. First description of natural *Ehrlichial canis* and *Anaplasma platys* Infections in dogs from Argentina. *Comparative Immulogy, Microbiology and Infectious Diseaases*. **36**: 169-73.

Eljadar M S M. 2010. 'Clinico - diagnostic studies on vector transmitted haemoprotozoan diseases in dog.' M. V. Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Eshoo M W, Crowder C D, Li H, Matthews H E, Meng S, Sefers S E, Sampath R, Stratton C W, Blyn L B, Ecker D J and Tang Y W. 2010. Detection and Identification of *Ehrlichia* Species in Blood by Use of PCR and Electrospray Ionization Mass Spectrometry. *Journal of Clinical Microbiology* **48(2)**: 472-78.

Ettinger S J and Feeldman E C. 2005. *Text book of Veterinary Internal Medicine.* 6th Edn. pp 643-44. W.B. Saunders Company, Missouri.

Ewing S A. 1969. Canine ehrlichiosis. In: Brandly CA, Cornelius CE (Eds): Advances in Veterinary Science and Comparative Medicine, New York Academic Press. pp: 331-53.

Flacke G, Spiering P, Cooper D, Gunther M S, Robertson I, Palmer C and Warren K. 2010. A survey of internal parasites in free-ranging african wild dogs (*Lycaon pictus*) from KwaZulu-Natal, South Africa. *South African Journal of wildlife Research* **40**: 176-80.

Fourie J J, Stanneck D and Jongejan F. 2013. Prevention of transmission of Babesia canis by Dermacentor reticulatus ticks to dogs treated with an imidacloprid/ flumethrin collar. *Veterinary Parasitology* **192**: 273-78.

Frank J R and Breitschwerdt E B. 1999. A retrospective study of ehrlichiosis in 62 dogs from North Carolina and Virginia. *Journal of Veterinary Internal Medicine* **13:** 194-201.

Gal A, Loebe E, Mekuzas Y Y and Baneth G. 2008. Detection of *Ehrlichia canis* by PCR in different tissue obtained during necropsy from dogs surveyed for naturally occurring canine monocytic ehrlichosis. *The Veterinary Journal* **175**: 212-17.

Gallego L S, Llull J, Osso M, Hegarty B and Breitschwerdt E. 2006. A serological study of Exposure to arthropod borne pathogens in dogs from northeastern spain. *Veterinary Research* **37**: 231-44.

Gaunt S D, Beall M J, Stillman B A, Lorentzen L, Diniz P, Chandrashekar R and Breitschwerdt E B. 2010. Experimental infection and co-infection of dogs with Anaplasma platys and *Ehrlichia canis*: hematologic, serologic and molecular findings. *Parasites and Vectors* **3**: 33.

Gershwin L J, Krakowka, S and Olsen R G 1995. Cytokines. In: *Immunology and Immunopathology of Domestic Animals*, 2nd Edn. Mosby, St. Louis: pp. 40-46.

Greene R T. 1995. Canine ehrlichiosis: clinical implications for humoral factors. In: Current Veterinary Therapy XII. (eds Bonagura JD, Kirk RW) W.B Saunders, Philadelphia: 290-293.

Harikrishnan T J, Chellapa D J, Pazhanivel N and Rajavelu G. 2009. Serodiagnosis of canine ehrlichiosis by enzyme linked immunosorbent assaya. *Indian Veterinary Journal* **86**: 668-70.

Harrus S, Alleman A R, Bark H, Mahan S M and Waner T. 2002. Comparison of three enzyme-linked immunosorbant assays with the indirect immunofluorescent antibody test for the diagnosis of canine infection with *Ehrlichia canis*. *Veterinary Microbiology* **86:** 361-68.

Harrus S, Aroch I, Lavy E and Bark H 1997a. Clinical manifestations of infectious canine cyclic thrombocytopenia. *Veterinary Record* **141**: 247-50.

Harrus S, Kass P H, Klement E and Waner T 1997b. Canine monocytic ehrlichiosis: a retrospective study of 100 cases and an epidemiological investigation of prognostic indicators for the disease. Veterinary Record **141**: 360-63.

Harrus S, Kenny M, Miara L, Aizenberg I, Waner T and Shaw S. 2004. Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection. *Antimicrobial Agents and Chemotherapy* **48**: 4488-90.

Harrus S, Ofri R, Aizenberg I and Waner T 1998a. Acute blindness associated with monoclonal gammopathy induced by *Ehrlichia canis* infection. *Veterinary Parasitology* **78(31)**: 155-60.

Harrus S, Waner T, Aizenberg I and Bark H. 1998b.Therapeutic effect of doxycycline in experimental subclinical canine monocytic ehrlichiosis: Evaluation of a 6-week course. *Journal of Clinical Microbiology* **36(7)**: 2140-42.

Harrus S, Waner T, Aizenberg I, Foley J E, Poland A M and Bark H. 1998c. Amplification of ehrlichial DNA from dogs, thirty four months after infection with *Ehrlichia canis*. *Journal of Clincal Microbiology* **36**: 73-76.

Harrus S, Waner T, Avidar Y, Bogin E, Huo-Cheng P and Bark H 1996a. Serum protein alterations in canine ehrlichiosis. *Veterinary Parasitology* **66**: 241-49.

Harrus S, Waner T and Bark H. 1997c. Canine monocytic ehrlichiosis update *Compendium for Continuing Education for the Practicing Veterinarian* **19**: 431-44.

Harrus S, Waner T, Eldor A, Zwang E and Bark H. 1996b. Platelet dysfunction associated with experimental acute canine ehrlichiosis.*Veterinary Record* **139**: 290-93.

Harrus S, Waner T, Weiss D J, Keysary A and Bark H. 1996c. Kinetics of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. *Veterinary Immunology and Immunopathology* **51**: 13-20.

Hasegawa M Y, Kohayagawa A, Brandao L P, Morgulis M S and Hagiwara M K. 2005. Evaluation of neutrophil oxidative metabolism in canine monocytic erhlichiosis. *Veterinary Clinical Pathology* **34**: 213-14.

Headly S A, Scorpio D G, Barat N C, Vidotto O and Dumler J S 2006. Neorickettsia helminthoeca in dog, Brazil, *Emerging Infectious Diseases* **12**: 1303-04.

Hernandez G V, Andre M R, Faria J L M, Munhoz T D, Rodriguez M H, Machado R Z, Costa M T. 2012. Molecular and serological detection of *Ehrlichia canis* and *Babesia vogeli* in dogs in Columbia. *Veterinary Parasitology* **186**: 254-60.

Hildebrandt P K, Huxsoll D L, Walker J S, Nims R M, Taylor R and Andrews M 1973. Pathology of canine ehrlichiosis (Tropical canine pancytopenia). *American Journal of Veterinary Research* **34**: 1309-20.

Hsieh Y C, Lee C C, Tsang C L and Chung Y T. 2010. Detection and characterization of four novel genotypes of *Ehrlichia canis* from dogs. *Veterinary Microbiology* **146**: 70-75.

Huxsoll D L 1976. Canine Ehrlichiosis (tropical canine pancytopaenia): A review. *Veterinary Parasitology* **2(1)**: 49-60.

Huxsoll D L, Hildebrant P K, Nims R M and Waler J S. 1970. Tropical Canine pancytoaenia. *Journal of American Veterinary Medicine Association.* **157**: 1627-32.

Inokuma H, Beppua T, Okudaa M, Shimada B Y and Sakatac Y. 2003. Epidemiological survey of *Anaplasma platys* and *Ehrlichia canis* using ticks collected from dogs in Japan. *Veterinary Parasitology* **115**: 343-48.

Inokuma H, Ohno K, Onishi T, Raoult D and Brouqui P. 2001. Detection of ehrlichia infection by PCR in dogs from Yamaguchi and Okinawa prefectures, Japan. *The Journal of Veterinary Medical Science* **63**: 815-17.

Inokuma H, Oyamada M, Kelly P J, Jacobson L A, Fournier P E, Itamoto K, Okuda M, Brouqui P 2005. Molecular detection of a new Anaplasma species closely related to *Anaplasma phagocytophilum* in canine blood from South Africa. *Journal of Clinical Microbiology* **43**: 2934-2937.

Iqbal Z, Chaichanasiriwithaya W and Rikihisa Y. 1994a. Comparison of PCR with

other tests for early diagnosis of Canine ehrlichiosis. *Journal of Clinical Microbiology* **32**: 1658-62.

Iqbal Z and Rikihisa Y. 1994b. Reisolation of *Ehrlichia canis* from blood and tissues of dogs after doxycycline treatment. *Journal of Clinical Microbiology* **32**: 1644-49.

Jain N C. 1986. *Schalm's Veterinary Haematology*. Lea and Febiger, Philadelphia. pp. 41-43, 71-72, 993-996.

Johnson E M, Ewing S A, Barker R W, Fox J C, Crow D W and Kocan K M. 1998. Experimental transmission of *Ehrlichia canis* (Rickettsials: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). *Veterinary Parasitology* **74**: 277-88.

Juyal P D, Kalra I S and Singla L D. 1994. Prevalence of haemoprotozoans in domestic animals in Punjab. In: *Proceedings of the 6th national congress of Veterinary Parasitology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur*. pp. 22-24.

Juyal P D, Sandhu B S, Kalra I S and Sood N. 1992. *Erhlichia canis* and *Heaptozoon canis* in naturally infected dogs in Punjab. *Journal of Veterinary Parasitology* **6**: 21-26.

Kahn C M, Line S and Aiello S E (Ed). 2005. *Reference guide Table 6 and 7 in Merck Manual*. 9th Edition Merck & Co., Inc. White House Station, NJ, USA. pp 2584-87.

Kakoma I, Carson C A, Ristic M, Stephenson E M, Hildebrandt P K and Huxsoll D L. 1978. Platelet migration inhibition as an indicator of immunologically mediated target cell injury in canine ehrlichioisis. *Infection and Immunity* **20**: 242-47.

Keefe T J, Holland C J, Salyer P E and Ristic M. 1982. Distribution of Ehrlichia canis among military working dogs in the world and selected civilian dogs in the United States. *Journal of American Veterinary Medical Association* **181**: 236-38.

Komnenou A A, Mylonakis M E, Kouti V, Tendoma L, Leontides L, Skountzou E, Dessiris A, Koutinas A F and Ofri R. 2007. Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichia canis*): A retrospective study of 90 cases. *Veterinary Ophthalmology* **10**: 137-42.

Kordick S K, Breitschwerdt E B, Hegarty B C, Southwick K L, Colitz C M, Hancock S I, Bradley J M, Rumbough R, Mcpherson J T and Maccormack J N. 1999. Coinfection with Multiple Tick-Borne Pathogens in a Walker Hound Kennel in North Carolina. *Journal of Clinical Microbiology* **37(8)**: 2631-38.

Kruss H, A Weber, M Appel, B Enders, HD Isenberg, HG Schiefer, W Slenczka, A Graevenitz and H Zahner 2003. Zoonoses: Infectious Diseases Transmissible

from Animals to Humans. 3rd edition. Washington. D.C ASM Press. pp. 456.

Kuehn N F and Gaunt S D. 1985. Clinical and haematologic findings in Canine Ehrlichiosis. *Journal of American Veterinary Medicine Association* **186**: 355-58.

Kumar A, Bhar A and Haque S. 2010. Occurrence of canine monocytic ehrlichiosis. *Indian Veterinary Journal* **87**: 183-87.

Kumar S K, Vairamuthu S and Kathiresan D. 2009. Prevalence of Haemoprotozoans in canines in Chennai city. *Tamilnadu Journal of Veterinary & Animal Sciences* **5**: 104-08.

Lakkawar A W, Nair M G, Varshney K C, Sreekrishnan R and Rao V N. 2003. Pathology of canine monocytic ehrlichiosis in a German Shepherd dog. *Slovenian Veterinary Research* **40**: 123-32.

Lakshmanan B, John L, Gomathinayagam S and Dhinakarraj G. 2006. Prevalence of *Ehrlichia canis* in Chennai. *Indian Veterinary Journal* **83**: 353-54.

Lakshmanan B, John L, Gomathinayagam S and Dhinakarraj G. 2007. Molecular detection of *Ehrlichia canis* from blood of naturally infected dogs in India. *Veterinarski Arhiv* **77**: 307-12.

Leiva M, Naranjo C and Pena M T. 2005. Ocular signs of Canine Monocytic Ehrlichiosis: A retrospective study in dogs from Barcelona, Spain. *Veterinary Ophthalmogy* **8(6)**: 387-93.

Lim S, Irwin P J, Lee S, Ahn K, Myung B and Shin S. 2010. Comparison of selected canine vector-borne diseases between urban animal shelter and rural hunting dogs in Korea. *Parasites and Vectors* **3**: 32-36.

Louly C C B, Fonseca I N, de Oliveira V F, Linhares G F C, de Menezes L B and Borges L M F. 2007. Seasonal dynamics of Rhipicephalus sanguineus (Acari: Ixodidae) in dogs from a police unit in Goiânia, Goiás, Brazil. *Ciência Rural* **37(2)**: 464-69.

Lovering S L, Pierce K R and Adams L.G 1980. Serum complement and blood platelet adhesiveness in acute canine ehrlichiosis. *American Journal of Veterinary Research* **41**: 1266-71.

Luckschander N, Kleiter M and Willmann M 2003. Renal amyloidosis in a dog with chronic Ehrlichiosis. SAT, *Schweizer Archiv for Tierheilkunde* **145(10)**: 482-85.

Mallapur S S. 2002. 'Studies of erhlichiosis in dogs of Mumbai.' M. V. Sc. thesis konkan krishi Vidyapeeth, Dapoli, India.

Manohar B M and Ramakrishnan R. 1982. Experimental Ehrlichiosis in dogs. *Cheiron* **13**: 144-150.

Mathewman L A, Kelly P J, Bobade P A, Tagwira M, Mason P R, Majok A, Brouqui

P and Raoult D. 1993. Infections with *Babesia canis* and *Ehrlichia canis* in dogs in Zimbabwe. *Veterinary Record* **133**: 344-46.

Matjila P T, Leisewitz A L, Jongejan F and Penzhorn B L. 2008. Molecular detection of tick borne protozooal and ehrlichial infections in domestic dogs in South Africa. *Veterinary Parasitology* **155**: 152-57.

Mavromatis K, Doyle C K, Lykidis A, Ivanova N, Francino M P, Chain P, Shin M, Malfatti S, Larimar F, Copeland A, deter J C, Land M, Richardson P M, Yu X J, Walker D H, McBride J W and Kypides N C. 2006. The genome of the obligately intracellular bacterium *Ehrlichia canis* reveals themes of complex membrane structure and immune evasion strategies. *Journal of Bacteriology* **188**: 4015-23.

McBride J W, Corstvet R E, Gaunt S D, Chinsangaram J, Akita G Y, Osburn B I. 1996. PCR detection of acute *Ehrlichia canis* infection in dogs. *Journal of Veterinry Diagnostic Investigations* **8(4)**: 441-47.

McClure J C, Crothers M L, Schaefer J J, Stanley P D, Needham G R, Ewing S A, Stich RW. 2010. Efficacy of a doxycycline treatment regimen initiated during three different phases of experimental ehrlichiosis. *Antimicrobial Agents Chemotherapy* **54**: 5012-20.

Meinkoth J H, Hoover J P, Cowell R L, Tyler R D and Lims J 1989. Ehrlichiosis in dog with seizure and non regenerative anemia. *Jounal of American Veterinary Medical Association* **195**: 1754-55.

Melo A L T, Martins T F, Horta M C, Moraes-Filho J, Pacheco R C and Labruna M B 2011. Seroprevalence and risk factors to Ehrlichia spp. and Rickettsia spp. in dogs from the Pantanal Region of Mato Grosso State, Brazil. *Ticks Tick Borne Diseases* **2(4)**: 213-18.

Mendonca C DE S, Mundim A V, Costa A S and Moro T V 2005. Canine Ehrlichiosis: Haematalogical Alterations in Naturally infected domestic dogs. *Bioscience Journal* **21(1)**: 167-174.

Milanjeet. 2013.'Prevalence and Molecular Detection of Canine Monocytic Ehrlichiosis.' M. V. Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Milanjeet, Singh H, Singh N K, Singh N D, Singh C and Rath S S. 2014. Molecular prevalence and risk factors for the occurrence of canine monocytic ehrlichiosis. *Veterinarni Medicina* **59**(3): 129-36.

Moreira S M, Bastos C V, Araujo R B, Santos M and Passos L M F. 2003. Retrospective study (1998 -2001) on Canine Ehrlichiosis in Belo Horizonte, MG, Brazil. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* **55(2)**: 141-47.

Moreira S M, Filho O De A, Ribeiro M F B, Bastos C De V and Passos L M F. 2009. Evaluation of clinical, hematological and immunologi-cal parameters of dogs with acute monocytic ehrlichiosis before and after treatment with tetracycline.

Veterinary Immunology and Immunopathology **128**: 211-347.

Mudaliar S. V. 1944. Canine Rickettsioses in South India-A prelimnary note. *Indian Veterinary Journal* **20**: 163-64.

Muhairwa A P, Mwakijungu E O, Msoffe P L M and Mtambo M M A. 2012. Seroprevalence and factors affecting canine monocytic ehrlichiosis and canine brucellosis in Tanzania. *Research opinions in animal and veterinary sciences* **2(3)**: 181-88.

Mundim A V, Morais I A, Tavares M, Cury M C and Mundim M J S. 2008. Clinical and hematological signs associated with dogs naturally infected by Hepatozoon spp. and with other hematozoa. A retrospective study in Uberandia, Minas, Gerais, Brazil. *Veterinary Parasitology* **153**: 3-8.

Murphy G L, Ewing S A, White L C, Fox J C and Kocan A A. 1998. A molecular and serological survey of *E. canis, E. chaffensis* and *E. ewingii* in dogs and tick from Oklahoma. *Veterinary Parasitology* **79**: 325-39.

Mylonakis M E, Koutinas A F, Billinis C, Leontides L S, Kontos V, Papadopoulos, O, Rallis T and Fytianou A. 2003. Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (*Ehrlichia canis*): A comparison between five methods. *Veterinary Microbiology* **91**: 197-204.

Mylonakis M E, Koutinas A F, Breitschwerdt E B, Hegarty B C, Billinis C D, Leontides L S and Kontos V S. 2004. Chronic canine Ehrlichiosis (*Ehrlichia canis*): A retrospective study of 19 natural cases. *Journal of American Veterinary Medical Association* **40(3)**: 174-84.

Mylonakis M E, Siarkou V I, Leoutides L, Bourtri - Hatzopoulou E, Kontos V I, Koutinas. 2009. Evaluation of a serum based PCR assay for the diagnosis of canine monocytic ehrlichiosis. *Veterinary Microbiology* **138**: 390-93.

Nakaghi A C H, Machado R Z, Costa M T, Andre M R and Baldani M T. 2008. Canine Ehrlichiosis: Clinical, haematological, serological and molecular aspects. *Ciencia Rural, Santa Maria* **38**: 766-70.

Nazari M, Lim S Y, Watanabe M, Sharma R S K, Cheng N A B Y, Watanabe M. 2013. Molecular Detection of Ehrlichia canis in dogs in Malaysia. *PLOS Negleted Tropical Diseases* **7(1)**: e1982.

Neer T M. 1998. Canine monocytic and granulocytic ehrlichiosis. In: Greene C E. (ed) *Infectious Diseases of the Dog and Cat*. Philadelphia: Saunders Cap.**28**: 139-47.

Neer T M, Edward B B, Russell T G and Michael R L. 2002. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *Journal of Veterinary Internal Medicine* **16**: 309-15.

Neitz W O and Thomas A D. 1938. Rickettsiosis in the dog. *Journal of South African Veterinary Association* **9**: 166-74.

Nicolson W L, Allen K E, McQuiston J H, Breitschwerdt E B, Little S E. 2010. The increasing recognition of rickettsial pathogens in dogs and people. *Trends in Parasitology*. **26(4)**: 205-12.

Niwetpathomwat A, Assarasakorn S, Techangamsuwan S, Suvarnavibhaj S and Kaewthamasorn M. 2006. Canine dirofilariasis and concurrent tickborne transmitted diseases in Bangkok, Thailand. *Comparative Clinical Pathology* **15:** 249-53.

Nyindo M, Huxsoll D L, Ristic M, Kakoma I, Brown J L, Carson C A and Stephenson E H. 1980. Cell-mediated and humoral immune response of German shepherd dogs and beagles to experimental infection with *Ehrlichia canis*. American Journal of Veterinary Research **41**: 250-54.

Nyindo M B A, Ristic M, Huxsoll D L and Smith A R. 1971. Tropical Canine Pancytopaenia *in vitro* cultivation of the causative agent *Ehrlichia canis*. *American Journal of Veterinary Research* **32**: 1651-57.

Okewole E A and Adejinmi J O. 2009. Comparison of two clinic-based immunoassays with the immunofluorescence antibody test for the field diagnosis of canine monocytic ehrlichiosis. *Acta Microbiologica et Immunologica Hungarica* **56**: 145-55.

Oliveira D, Tie-Nishmori C, Costa M T, Machado R Z and Castro M B. 2000. Anti-*Ehrlichia canis* antibodies detection by "dot-ELISA" in naturally infected dogs. *Revista Bresileira de Parasitologia Veterinaria* **9(1)**: 1-5.

Oriá A P. 2001. Correlação entre uveítes, achados de patologia clínica, sorológicos (Reação de imunofluorescência indireta e Dot-blot ELISA) e de anatomopatologia do bulbo do olho, em animais da espécie canina, natural e experimentalmente infectados pela Ehrlichia canis. Dissertação (Mestrado em Cirurgia Veterinária) - Curso de Pós-graduação em Cirurgia Veterinária, Universidade Estadual Paulista, Campus de Jaboticabal. 69f.

Pasa S and Azizoglu A. 2003. Clinical and some haematologic findings in dogs with Ehrlichiosis: 4 cases, *Indian Veterinary Journal* **80**: 33-35.

Perea M L, Kumthekar S M, Sabarinath A, Karpathy S E, Sharma R N and Stone D M. 2009. Doxycycline treatment of asymptamatic dogs seropositive for *Ehrlichia canis. West Indian Veterinary Journal* **9(2)**: 11-13.

Pierce K R, Marrs G E, and Hightower D. 1977. Acute canine ehrlichiosis: platelet survival and factor 3 assay. *American Journal of Veterinary Research* **38**: 1821-25.

Pretorius A M and Kelly P J. 1998. Serological survey for antibodies reactive with Ehrlichia canis and E. Chaffeensis in dogs from the Bloemfontein area, South Africa. *Journal of South African Veterinary Association* **69**: 126-28.

Price J E and Sayer P D. 1983. Canine Ehrlichiosis. In: Kick R W (ed) *Current Veterinary Therapy VIII*. WB Saunders Co. Philadelphia, pp. 1197-1202.

Procajło A, Skupień E M, Bladowski M and Lew S 2011. Monocytic Ehrlichiosis in dogs. *Polish Journal of Veterinary Sciences* **14(3)**: 515-20.

Pusterla N, Chang C C, Chomel B B, Chae J S, Foley J E, DeRock E, Kramer V L, Lutz H and Madigan J E 2000. Serologic and molecular evidence of *Ehrlichia* spp.in coyotes in California. *Journal of Wildlife Diseases* **36**: 494-99.

Pusterla N, Huder J, Wolfensberger C, Litschi B, Parvis A and Lutz H. 1997. Granulocytic ehrlichiosis in two dogs in Switzerland. *Journal of Clinical Microbiology* **35**: 2307-09.

Pyle R L 1980. Canine ehrlichiosis. *Journal of American Veterinary Medical Association* **177**: 1197-99.

Rajagopal A S, Basith S A, Gomathinayagam G and Dhinakarraj 2009. Evaluation of PCR and IFAT in the Diagnosis of Canine Ehrlichiosis. *Journal of Applied Animal Research* **35(2)**: 189-91.

Ramprabhu R, Prathaban S, Nambi A and Dhanapalan P. 2001. Concurrent trypanosomiasis and ehrlichiosis in a a dog - a case report. *Veterinarski Arhiv* **71**: 105-08.

Rani P A M Abd, Irwin P J, Coleman G T, Gatne M and Traub R J. 2011. A survey of canine tick-borne diseases in India. *Parasites and Vectors* **4**: 141.

Reardon M J and Pierce K R. 1981. Acute experimental canine ehrlichiosis. I. Sequential reaction of the hemic and lymphoreticular systems.*Veterinary Pathology* **18**: 48-61.

Rikihisa Y. 1991. The tribe Ehrlichiae and Ehrlichial disease. *Journal of Clinical Microbiology Reviews* **4**: 286-308.

Ristic M and Holland C J. 1993. Canine ehrlichiosis. In Z. Woldehiwet and M. Ristic (ed.), Rickettsial and chlamydial diseases of domestic animals. Pergamon Press, Oxford, United Kingdom, pp. 169-86

Rodriguez-Vivas R L, Albornoz R E F and Bolio G M E. 2005. *Ehrlichia canis* in dogs in Yucantan, Mexico: Seroprevelence, prevalence of infection and associated factors. *Veterinary Parasitology* **127(1)**: 75-79.

Rothschild M A, Oratz M and Schreiber S S. 1984. Pathophysiology of plasma protein metabolism. Macmillan, London, United Kingdom : 121-140.

Sacchini F, Cessford R J and Robinson B M. 2007. Outbreak of Canine Monocytic Ehrlichiosis in Saudi Arabia. *Veterinary Clinical Pathology* **36(4)**: 331-35.

Sainz A, Tesouro M A, Amusategui I, Rodriguez F, Mazzucchelli F and Rodriguez M. 2000. Prospective comparative study of 3 treatment protocols using doxycycline or imidocarb diproprionate in dogs with naturally occurring ehrlichiosis. Journal of Veterinary Internal Medicine **14**: 134-39.

Samaradni D, Maske D K, Shobha R and Shinde P N. 2005. Bionomics and

haemodynamics in blood protozoal infections in dogs from Nagpur [M.S.]. *Indian Journal Animal Health* **44**: 57-66.

Santos F, Coppede J S, Pereira A L A, Oliveira L P, Roberto P G, Benedetti R B R, Zoculoto L B, Lucas F, Sobreira L, Marins M. 2009. Molecular evaluation of the incidence of *Ehrlichia canis*, *Anaplasma platys* and *Babesia* spp. in dogs from Ribeirao. Preto, Brazil. *The Veterinary Journal* **179**: 145-48.

Sasanelli M, Paradies P, Lubas G, Otranto D and de Caprariis D. 2009. Atypical clinical presentation of coinfection with *Ehrlichia, Babesia and Hepatozoon* species in a dog.*Veterinary Record* **164**: 22-23.

Shah S A, Sood N K, Uppal S K and Gupta Kuldip. 2010. Ehrlichiosis in anemic and thrombocytopenic dogs in Punjab, India. *Indian Journal of Veterinary Pathology* **34**: 36-37.

Shimada T, Ishida Y, Shimizu M, Nomura M, Kawato K, Iguchi K and Jinbo T. 2002. Monitoring C-reactive protein in beagle dogs, experimentally inoculated with *Ehrlichia canis*. *Veterinary Research Communications* **26**: 171-77.

Shipov A, Klement E, Tager L R, Waner T and Harrus S. 2008. Prognostic indicators for canine monocytic ehrlichiosis. *Veterinary Parasitology* **153**: 131-38.

Siarkou V I, Mylonakis M E, Bourtzi-Hatzopoulou E 2007. Sequence and phylogenetic analysis of the 16S rRNA gene of *Ehrlichia canis* strain in dogs with clinical monocytic ehrlichiosis. Veterinary Microbiology **125**: 304-12.

Simpson C F. 1972. Structure of *Ehrlichia canis* in Blood monocytes of a dog. *American Journal of Veterinary Research* **33**: 2451-54.

Singh H, Jyoti, Haque M, Singh N K and Rath S S. 2011a. Prevalence of canine parasitic infections in and around Ludhiana, Punjab. *Journal of Veterinary Parasitology* **25(2)**: 179-80.

Singh H, Jyoti, Haque M, Singh N K and Rath S S. 2011b. Prevalence of canine parasitic infections. *Indian Veterinary Journal* **88**: 76-77.

Singla L D, Singh H, Kaur P, Singh N D, Singh N K and Juyal P D. 2011. Serodetection of *Ehrlichia canis* infection in dogs from Ludhiana district of Punjab, India. *Journal of Parasitic Diseases* **35**: 195-98.

Smith R D, Ristic M, Huxsoll D L and Baylor R A. 1975. Platelet kinetics in canine ehrlichiosis: evidence for increased platelet destruction as the cause of thrombocytopenia. Infection and Immunity **11**: 1216-21.

Soulsby E J L. 1982. Helminths, Arthropods and Protozoa of domesticated animals. 7th edn, ELBS and Bailliere Tindall, London, pp. 809.

Sousa M G, Higa A G, Gerard D G, Costa M T and Machado R Z. 2004. Treatment of Naturally Occurring Canine Ehrlichiosis with Doxycycline, Preceded or not by Imidocarb Dipropionate. *Revista de Ciências Agroveterinárias Lages* **3(2)**: 126-30.

Stich R W, Rikihisa Y and Ewing S A 2002. Detection of *Ehrlichia canis* in canine carrier blood and individual experimentally infected ticks with p30-based PCR assay. *Journal of Clinical Microbiology* **40**: 540-46.

Stiles J. 2000. Canine rickettsial infections. *Veterinary Clinics of North America. Small Animal Practice*. **30**: 1135-49.

Suksawat J, Pitulic C, Arraga- Alvarado C, Madrigal K, Hancock S I and Breitschwerdt E B 2001. Coinfection with three *Ehrlichia* species in dogs from Thailand and Venezuela with emphasis on consideration of the 16S ribosomal DNA structure. *Journal of Clinical Microbiology* **39**: 90-93.

Suto Y, Suto A, Inokuma H, Obayashi H and Hayashi T 2001. First confirmed canine case of Ehrlichia canis infection in Japan. *Veterinary Record* **148**: 809-11.

Troy G C. 1980. Canine ehrlichiosis: A retrospective study of 30 naturally occurring cases. *Journal of American Animal Hospital Association* **16**: 181-87.

Troy G C and Forrester S D 1990. Canine Ehrlichiosis. In: Greene C. E., Infectious Diseases of the Dog and Cat. W. B. Saunders, Philadelphia, pp. 48-59.

Van der Krogt J S. 2010. *Ehrlichia canis* infections on the island of Curacao., An overview of the clinical picture and current diagnostics and therapies, Utrecht University.

Varela A S. 2003. Tick-borne *Ehrlichiae* and *Rickettsiae* of Dogs. International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Varela F, Font X, Valladares J E and Alberola J. 1997. Thrombocytopathia and light-chain proteinuria in dog naturally infected with *Ehrlichia canis*. *Journal of Veterinary Internal Medicine* **11**: 309-11.

Villaescusa A, Tesouro M A, Sancho M G, Ayllon T, Franco F R and Sainz A. 2012. Evaluation of peripheral blood lymphocyte subsets in family-owned dogs naturally infected by *Ehrlichia canis*. Comparative Immunology, Microbiology and Infectious Diseases **35(4)**: 391-96.

Walker J S, Rundquist J D, Taylor R, Wilson B L, Andrews M R, Barck J, Hogge Jr A L, Huxsoll D L, Hildebrant P K and Nims R M 1970. Clinical and clinicopathologic findings in Tropical Canine Pancytopenia. *Journal of American Veterinary Medicine Association* **157**: 43-55.

Waner T and Harrus S. 2000a. Canine Monocytic Ehrlichiosis. In: Carmichael L E (Ed.) *Recent Advances in Canine Infectious Diseases.*, Publisher: International Veterinary Information Service (www.ivis.org).

Waner T, Harrus S, Bark H, Bogin E, Avindar Y and Keysary A. 1997. Characterization of the subclinical phase of Canine Ehrlichiosis in experimentally infected beagle dogs. *Veterinary Parasitology* **69**: 307-17.

Waner T, Harrus S, Jongejan F, Bark H, Keysary A and Cornelissen A W C A. 2001. Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by *Ehrlichia canis. Veterinary Parasitology* **95(1)**: 1-15.

Waner T, Harrus S, Weiss D J, Bark H and Keysary A. 1995. Demonstration of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. Veterinary Immunology and Immunopathology **48**: 177-82.

Waner T, Keysary A, Bark H, Sharabani E and Harrus S. 1999. Canine monocytic ehrlichiosis - an overview. *Journal of Israel Veterinary Medical Association* **54(4)**: 1-8.

Waner T, Navesh A, Wudovsky I and Carmichael L E. 1996. Assessment of maternal antibody decay and response to canine parvovirus vaccination using an enzyme-linked immunosorbent assay. *Journal of Veterinary Diagnostics Investigation* **8**: 427-32.

Waner T, Strenger C and Keysary A. 2000b. Comparison of a clinic-based ELISA test kit with the immunofluorescence test for the assay of Ehrlichia canis antibodies in dogs. *Journal of Veterinary Diagnostic Investigation* **12**: 240-44.

Wen B, Rikihisa Y, Mott J M, Greene R, Kim H Y, Zhi N, Couto G C, Unver A and Bartsch R. 1997. Comparison of Nested PCR with Immunofluorescent-Antibody Assay for Detection of *Ehrlichia canis* Infection in Dogs Treated with Doxycycline. *Journal of Clinical Microbiology* **35(7)**: 1852-55.

Woody B J and Hoskins J D. 1991. Ehrlichial diseases of dogs. Veterinary Clinics of North America: Small Animal Practice **21**: 75-98.

Yabsley M J, Mckibben J, Macpherson C N, Cattan P F, Cherry N A, Hegarty B C, Breitschwerdt E B, O'Conner T, Chandrashekar R, Paterson T, Perea M L, Ball G, Friesen S, Goedde J, Henderson B and Syvester W. 2008. Prevalence of *Ehrlichia canis, Anaplasma platys, Babesia canis vogeli, Hepatozoon canis, Bartonella vinsonii berkhoffii and Rickettsia* spp. in dogs from Grenada. *Veterinary Parasitology* **151(2/4)**: 279-85.

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