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Epidemiological pattern of canine ehrlichiosis in and around Bangalore

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Abstract

The epidemiological pattern of canine Ehrlichiosis revealed highest occurrence of canine Ehrlichiosis in the age group of > 2 years of dogs (65%), when compared to 6months to 2 year age group (30%) and 0-6 months age group (5%), Labrador breed of dog (40%) was most commonly affected breed when compared to other breeds of dogs our study revealed highest occurrence of canine ehrlichiosis in female dogs (55%) when compared to male dogs (45%). Most common clinical signs noticed were complete/partial anorexia (100%) followed by lethargy (75%) fever (60%), pale and congested mucous membrane followed by others signs.

Keywords: epidemiology, ehrlichiosis, age, breed, gender, clinical signs

Introduction

Canine Ehrlichiosis is caused by rickettsial agent belonging to genus *Ehrlichia*. Ehrlichia organisms are gram-negative obligate intracellular bacteria with tropism for hematopoietic cells. It is transmitted by the brown dog-tick, *Rhipicephalus sanguineus*, a worldwide distributed tick. The classic presentation is characterised by depression, lethargy, mild weight loss and anorexia with or without haemorrhagic tendencies. Generally all breeds of dogs are equally predisposed to Ehrlichiosis but German shepherd and Siberian Huskies develops more severe form of ehrlichiosis because of reduced cell-mediated immune response to *Ehrlichia canis* in these breeds, therefore; these breeds have worse prognosis [16, 13]. All age group of dogs seemed to be susceptible to Ehrlichiosis [30]. Canine ehrlichiosis was diagnosed in 26 different breeds amongst which German shepherd dogs showed the highest prevalence of chronic cases as well as the highest mortality rate. Ehrlichiosis can manifest in three phases. Signs of acute phase of the disease usually include anaemia, fever, depression, lethargy, loss of appetite, shortness of breath, joint pain and stiffness. In the subclinical phase the animal may appear normal or show only slight anaemia and can last for months or years. The chronic phase can be either mild or severe and characterized by weight loss, anaemia, neurological signs, bleeding tendencies, inflammation of the eye, oedema in the hind legs, and fever.

Materials and Methods

Dogs irrespective of age, breed and gender presented to veterinary college hospital, Hebbal, Bangalore with history and clinical signs suggestive of Ehrlichiosis were selected for the study purpose. The dogs having pyrexia and lymph node enlargement acted as a basis for selection based on the clinical signs. Further from these dogs blood samples were collected in EDTA and serum collection vials. These blood samples were subjected for haematological analysis. The blood samples with platelet count less than 1.5 lakh/ μ l were further subjected for blood smear examination, Lateral flow assay test and Polymerase chain reaction. The dogs which were healthy and came for vaccination or general check-up and found negative by blood smear examination and ehrlichia antibody test kit were taken as control group. Laboratory evaluation of samples was carried out on all 40 dogs. (20 healthy and 20 ehrlichia affected dogs). The evaluations included blood smear examination, Lateral flow assay and PCR.

Blood smear examination: Blood smear were prepared and stained with giemsa's stain and observed under the microscope.

Snap 4Dx plus test: Test procedure of Snap 4Dx plus test was performed as per the standard procedure given in the literature enclosed with kit of SNAP 4Dx Plus Test Kit of IDEXX Laboratories, Inc. (IDEXX LABORATORIES, One IDEXX Drive, Westbrook, Maine 04092 USA). An in-vitro diagnostic for detection of *Dirofilaria immitis* antigen, antibody to *Anaplasma phagocytophilum*, antibody to *Anaplasma platys*, antibody to *Borrelia burgdorferi*, antibody to *Ehrlichia canis* and antibody to *Ehrlichia ewingii* using serum, plasma or whole blood sample. The kit is provided with conjugate, pipette, sample tube and Snap device

Polymerase chain reaction: The extraction of DNA from the blood was done using MACHEREY-NAGAJ Genomic DNA extraction kit following the manufacturer's protocol and processed for detection of *Ehrlichia canis* by gradient PCR assay targeting a portion of 16S rRNA gene as described by Murphy *et al.*, 1998. The sequences of the primers were as follows:

Ehrlichia canis SEPas:5'-
CTTCTRTRGGTACCGTCATTATCTTCCCY-3'
Ehrlichia canis: SEPas^a 5'-
CAATTATTTATAGCCTCTGGCTATAGGAA-3' -

The extracted DNA from each sample was used as template in gradient PCR amplification of 393 base-pair fragment of the 16S rRNA gene in the thermocycler (Applied biosystem US). The reaction mixture of 50 µl comprised of 1 µl of template DNA, 12 µl of M/S Takara bio emerald Amp GP PCR master mix, 1 µl of each primers and 35 µl of nuclease free water. The thermocycle profile consisted of initial denaturation at 95 °C for 5 minutes by 35 cycles, annealing at 58 °C for 1 minute and Extension at 72 °C for 1 minute. This was followed by a final Extension at 72 °C for 5 minute. Amplified PCR products and molecular weight marker (100 base pair ladder) were electrophoresed through 1.2% agarose gel stained with ethidium bromide in tris-acetate EDTA (TAE) buffer for 20 minutes at 100V/hr and DNA fragments were visualized under UV fluorescence using a gel documentation system.

Results and Discussion

The study was conducted at Veterinary College Hebbal, Bangalore. The results of the present study obtained are presented herewith. In the present study 54 dogs were suspected for Canine ehrlichiosis based on the clinical signs. These 54 dogs were subjected for estimation of various hematological parameters. Out of 54 suspected dogs 32 dogs showed platelet count less than $150 \times 10^3/\mu\text{l}$. These 32 dogs were further subjected for various diagnostic tests like Blood smear examination, Lateral flow assay and Polymerase chain reaction. Out of 32 dogs only 2 samples (6.25%) were positive based on blood smear examination, 14 samples (43.75%) showed positive reaction in lateral flow assay (snap 4DX IDEXX) and 20 samples (62.5%) showed positive for Polymerase chain reaction. These 20 PCR positive cases were considered for epidemiological study of canine ehrlichiosis in and around Bangalore.

Epidemiological Pattern of Canine Ehrlichiosis

In the present study, 20 healthy dogs and 20 Ehrlichia affected dogs were selected. 20 ehrlichia affected dogs were selected by screening 32 dogs which showed platelet count less than 1.5 Lakh. Among these 32 dogs 20 dogs showed positive for canine ehrlichiosis based on Polymerase chain

reaction. These 20 ehrlichia affected dogs were further subjected for various epidemiological parameters like age wise, breed wise, gender wise and clinical signs wise occurrence of canine ehrlichiosis in our study.

Age wise Occurrence of canine Ehrlichiosis

The age wise occurrence of Canine ehrlichiosis based on PCR results is presented in Table 1 and fig 1. Out of 20 dogs positive for canine ehrlichiosis, one dog was in the age group of 0-6 months (5%), 6 dogs were in the age group of 6 months to 2 years of age (30%) and 13 dogs were in the age group more than 2 years (65%). In our study, more than 2years age group dogs were highly affected by canine Ehrlichiosis (65%) when compared to 6 months to 2 year age group (30%) and 0-6 months age group (5%). This is in agreement with several research workers [5, 1, 26, 12, 4]. On contrary dogs aged less than 2 years were more susceptible for Canine Ehrlichiosis as reported by some research workers [18, 3]. There is no correlation between different age groups and occurrence of canine ehrlichiosis in their study [30, 23]. Higher occurrence of canine ehrlichiosis in dogs more than 2 years of age in our study may be due to more risk of dogs to tick infestation probably due to their access to outdoor environment including exposure to other dogs.

Table 1: Age wise occurrence of canine Ehrlichiosis

Sl. No	Age of the dog	No. of Animals N=20	Percentage (%)
1	0-6 Months	1	5%
2	6 months – 2 years	6	30%
3	More than 2 years	13	65%

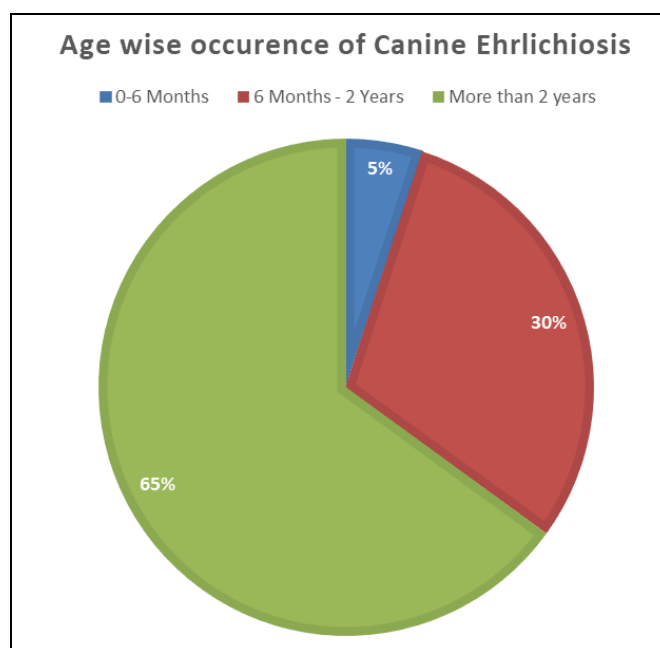


Fig 1: Pie chart depicting age wise occurrence of Canine Ehrlichiosis

Breed wise Occurrence of canine Ehrlichiosis

The Breed wise occurrence of Canine ehrlichiosis based on PCR results is presented in Table 2 and fig 2. Out of 20 dogs positive for canine ehrlichiosis, 8 dogs were Labrador (40%), 2 dogs were golden retriever (10%), 2 dogs were Pomeranian (10%), 2 dogs were Siberian husky (10%), one dog was Non-descript (5%), one dog was shitzu (5%), one was cocker spaniel (5%), one was German shepherd (5%), one was Doberman (5%) and one was Pug (5%). The results of the

present study showed highest positive cases of Canine Ehrlichiosis in Labrador (40%) followed by Pomeranian, golden retriever and Siberian husky (10% each) followed by shitzu, Doberman, Non-descript, cocker spaniel, pug and german shepherd (5% each). Higher susceptibility of Labrador breed of dog has been reported² whereas other research workers have reported highest incidence in german shepherd dogs [16, 1, 3]. Such variations in breed-wise occurrence of Ehrlichiosis in dogs might be due to variations in the population of different breeds, Variation in the dog breeds presented to Veterinary Hospital, variation in the number of dogs belonging to different breeds screened or the preference of owner to different breeds in different areas. In our study the Labrador breed of dogs were screened for ehrlichiosis in more number when compared to other breeds since they were over

presented when compared to other breeds of dogs.

Table 2: Breed wise occurrence of Canine Ehrlichiosis

Sl. No	Dog Breed	No. of Animals N=20	Percentage (%)
01	Labrador	8	40%
02	ND	1	5%
03	Shitzu	1	5%
04	Golden Retriever	2	10%
05	Pomeranian	2	10%
06	Cocker Spaniel	1	5%
07	Pug	1	5%
08	German Shepherd	1	5%
09	Doberman	1	5%
10	Siberian husky	2	10%

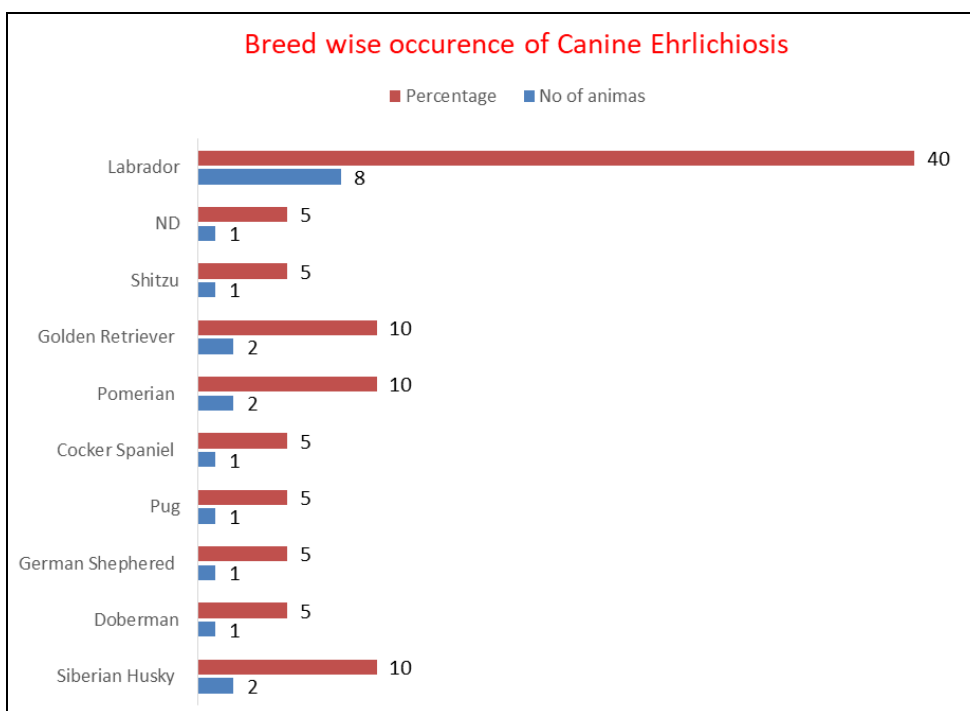


Fig 2: Bar chart representing Breed wise occurrence of Canine Ehrlichiosis

Gender wise occurrence of canine Ehrlichiosis

The gender wise occurrence of Canine ehrlichiosis based on PCR results is presented in Table 3 and fig 3. Out of 20 dogs positive for canine ehrlichiosis, 9 dogs were male (45%) and 11 dogs were female (55%). The results of our study revealed highest occurrence of canine ehrlichiosis in female dogs (55%) when compared to male dogs (45%). Higher occurrence of ehrlichiosis in female dogs has also been reported [29, 1, 3]. There is no breed correlation between occurrence of Ehrlichiosis and sex of animal [14, 5, 30]. Increase in occurrence of Ehrlichiosis in female dog may be seen because of interest among dog owners to keep female dog as companion and to opt for breeding in future. The number of female dogs screened for ehrlichiosis in our study was also more when compared to male dogs.

Table 3: Gender wise occurrence of canine Ehrlichiosis

Sl. No	Gender of Dog	No. of Animals N=20	Percentage (%)
01	Male	9	45%
02	Female	11	55%

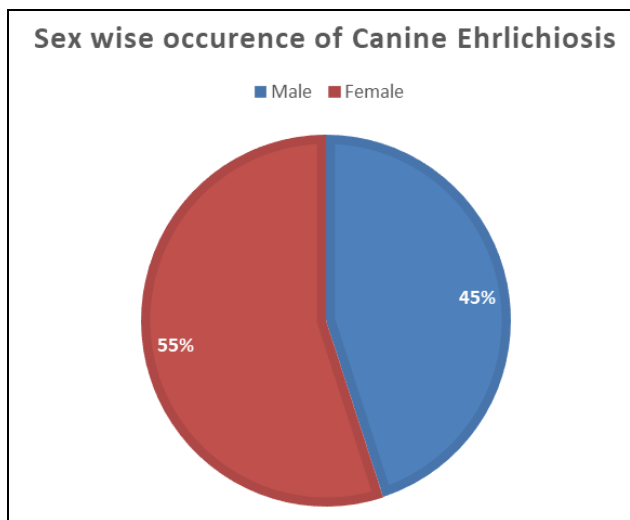


Fig 3: Pie chart depicting gender wise occurrence of Canine Ehrlichiosis

Clinical signs of canine Ehrlichiosis: The dogs which were positive by PCR test showed variety of clinical signs. The clinical signs showed by dogs in our study are depicted on

table 4 and figure 4. Out of 20 dogs examined, all 20 dogs(100%) have shown complete/partial anorexia, 12 dogs (60%) have shown fever, 8 dogs (40%) have shown lymphadenomegaly, 15 dogs (75%) have shown lethargy, 6 dogs (30%) have shown ocular discharge, 3 dogs (15%) have shown presence of ticks, 7 dogs (35%) have shown vomition, 5 dogs (25%) have shown diarrhea, 8 dogs (40%) have shown bleeding episodes, 5 dogs (25%) have shown panting, 11 dogs (55%) have shown pale mucous membrane, 6 dogs (30%) have shown congested mucus membrane, 2 dogs (10%) have shown weight loss. In our study most predominant signs of canine Ehrlichiosis recorded were Complete/partial anorexia (100%), fever (60%), lethargy (75%) and pale conjunctival mucus membrane (55%). The clinical findings which generated the highest index of suspicion for Canine Ehrlichiosis included lymphadeopathy, pyrexia, depression, anorexia, tick infestation, bleeding tendencies, pale and congested mucous membrane [14, 12, 13]. Corneal opacity was observed in two cases as a clinical sign in CME [15]. Lameness in canine ehrlichiosis was also reported [28]. Lymphadenomegaly noticed in canine erlichiosis may be due to replication of the organisms in the reticulo-endothelial system along with proliferation of medullary and paracortical lymphocytes and aggregation of reactive histiocytes in the lymph nodes resulting in generalized lymphadenopathy [16, 27]. Various workers have reported increase in temperature as major clinical sign in canine ehrlichiosis [29, 30]. Pyrexia may

be due to release of cytokine IL-1 in response to *E. canis* Pale mucous membrane in ehrlichia affected cases was reported [16, 4]. The reason behind pale mucous membrane is decreased Haemoglobin, decreased Packed cell volume and decreased RBC due to bone marrow suppression. Peticheal haemorrhages recorded in some cases of ehrlichiosis may be due to decrease in platelet count [16]. It was also opioned that besides thrombocytopenia platelet dysfunction also plays a role in bleeding tendencies in ehrlichiosis.

Table 4: Clinical signs in Canine Ehrlichiosis

Sl. No	Clinical signs	No. of Animals N=20	Percentage (%)
01	Complete /Partial anorexia	20	100%
02	Fever	12	60%
03	Lymphadenomegaly	8	40%
04	Lethargy	15	75%
05	Ocular discharge	6	30%
06	Presence of ticks	3	15%
07	Vomition	7	35%
08	Diarrhoea	5	25%
09	Bleeding episodes	8	40%
10	Panting	5	25%
11	CMM-pale	11	55%
12	CMM-congested	6	30%
13	Weight loss	2	10%

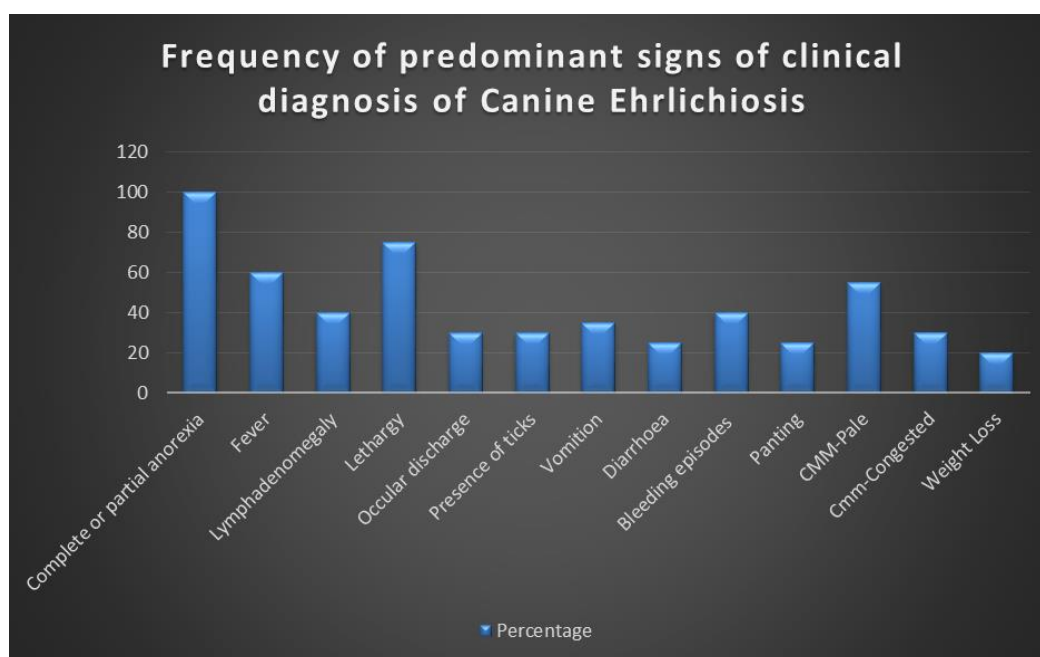


Fig 4: Bar Chart representing Clinical signs of Canine Ehrlichiosis

Conclusion

The epidemiological pattern of canine Ehrlichiosis revealed highest occurrence of canine Ehrlichiosis in the age group of > 2 years of dogs (65%), when compared to 6months to 2 year age group (30%) and 0-6 months age group (5%), Labrador breed of dog (40%) was most commonly affected breed when compared to other breeds of dogs our study revealed highest occurrence of canine ehrlichiosis in female dogs (55%) when compared to male dogs (45%). Most common clinical signs noticed were complete/ partial anorexia (100%) followed by lethargy (75%) fever (60%), pale and congested mucous membrane followed by others signs.

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