



Retrospective Analysis of Clinical Presentation, Severity Grading, and Seasonal Dynamics of Canine Ehrlichiosis with Respect to Breed, Sex and Age in India

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Abstract: The present study tested 212 dogs for ehrlichiosis, based on the observation of two to three characteristic clinical symptoms of the disease. All assessed canines underwent blood smear screening to determine their disease status. The ehrlichia-positive dogs typically exhibited two notable clinical symptoms: elevated fever and lymphadenomegaly. Conversely, additional infrequent symptoms such as melena, mucosal pallor, depression, weight loss, tick infestation, ecchymotic and petechial haemorrhages, ascites, epistaxis, ocular abnormalities, vomiting, hind limb/scrotal oedema, and facial oedema were also noted. The essential metrics, including rectal temperature, heart rate, and respiration rate, exhibited a notable elevation. The incidence of positive ehrlichiosis cases was recorded in relation to age, sex, breed, and season. Among the age group of 0 to 6 months, 6 months to one year and more than a year the age group of more than one year showed highest positive percentage a positivity score of 68.07%. The gender with the highest positive rating was male, at 63.86%. The German Shepherd breed constitutes 38.65% of the total, whilst the monsoon season accounts for 40.34% of incidence.

Key words: Dog, clinical symptoms, ehrlichiosis, season, tick.

Canine ehrlichiosis is a severe and often lethal rickettsial disease transmitted by ticks, belonging to the Anaplasmataceae family, caused by Ehrlichia species, specifically *E. canis*, *E. chaffeensis*, *E. ewingii*, and potentially *E. ruminatum* (Kumar *et al.*, 2021). Canine monocytic ehrlichiosis is a tick-transmitted disease caused by *E. canis*. It impacts canines globally (Mittal *et al.*, 2017). Ehrlichia canis is a diminutive, gram-negative bacterium that is obligately intracellular and exists in two distinct forms. It flourishes within the monocytes and macrophages of mammalian hosts as a morula, which is a membrane-bound intracellular vacuole. The Rhipicephalus sanguineus tick transmits it to other canines. The propagation of this disease has been shown to involve Amblyomma and

Dermacentor ticks (Kukreti *et al.*, 2018). The acute stage is characterized by raised body temperature, depressive sensations, fatigue, anorexia, lymphadenopathy, splenomegaly, a propensity for haemorrhage, and ophthalmic manifestations (Harrus *et al.*, 2012). Canines that have not undergone appropriate treatment or have been inadequately treated advance to the subclinical phase. At this point, dogs may not display any discernible symptoms yet can persist as carriers of *E. canis*. In the chronic phase, symptoms intensify, and infected dogs may exhibit a diminished response to therapy (Neer and Harrus, 2006). Therefore, it is essential to swiftly and accurately evaluate suspected cases in dogs to alter the development of the condition (Neer, 1998). Canines possessing a robust immune system may eliminate the illness during either the first or latent phases. Nonetheless, certain dogs will ultimately progress to the enduring phase, characterized by diminished bone marrow function, dysfunction in all blood cell types, and an elevated mortality risk due to septicemia and/or bleeding (Mylonakis and Theodorou, 2017). Common clinical signs in naturally occurring disease include pyrexia, occasional hypothermia in severely pancytopenic dogs, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly, mucosal pallor, ocular abnormalities, and a tendency to bleed (Mylonakis and Theodorou, 2017). Tick infestation is frequently noted during the early phase of the disease. Ulcerative stomatitis, necrotic glossitis, hind leg and/or scrotal enlargement, bacterial skin infections, jaundice, and neurological manifestations such as seizures, coordination difficulties, balance issues, and neck pain are more frequently observed in chronic CME. The propensity for bleeding is more common and pronounced during the chronic phase of CME (Mylonakis *et al.*, 2019).

Canine ehrlichiosis can impact dogs of all breeds, genders, and ages; however, German shepherds and Siberian huskies often display more pronounced clinical symptoms. Numerous studies demonstrate a greater incidence of seropositivity in males particularly in older dogs. This can be ascribed to the increased susceptibility of male dogs to vectors that transmit diseases relative to

female dogs, together with specific behavioural characteristics. (Sainz *et al.*, 2015).

Based on the clinical presentation and severity grading of canine ehrlichiosis, the study was conducted to investigate the distribution patterns of the disease across different seasons, as well as among various age groups, breeds, and sexes of dogs.

Materials and Methods

Screening of dogs under research: Dogs suspected of Ehrlichia were screened based on the observation of classical indications of ehrlichiosis. According to findings from prior studies, the clinical indicator deemed pertinent for screening encompass mucosal pallor, elevated fever, vomiting, bleeding diathesis-manifested as dermal and mucosal lesions and ecchymoses, hind limb and/or scrotal edema, icterus, epistaxis, melena, hematemesis, hyphaema, neurological indications, and ascites. Weight reduction, visual impairments, enlargement of the spleen, mood disorder are some other clinical indicators.

Blood was collected from dogs in accordance with the ethical norms and guidelines established by the Institutional Animal Ethics Committee (IAEC), with prior approval obtained from the university's ethical committee, as documented in voucher no. IAEC/23-2/5 dated 25-02-2023. Microscopic inspection was conducted to identify morulae within monocytes and neutrophils in thin blood smears and buffy coat smears following Giemsa staining.

Different clinical signs and their frequency distribution in dogs: Frequency distribution of different clinical signs in ehrlichiosis were evaluated as % frequency of each and every clinical signs

$$\text{Frequency} = \frac{\text{No. of positive cases showing specific clinical signs (n)}}{\text{Total no. of positive cases (N)}} \times 100$$

Percentage positivity on the basis of sex: The formula for calculation is given below

$$\% \text{ Positivity} = \frac{\text{No. of positive cases by PCR in different sex (n)}}{\text{Total no. of positive cases by PCR (N)}} \times 100$$

Percentage positivity on the basis of age group: The formula for calculation is given below

$$\% \text{ Positivity} = \frac{\text{No. of positive cases by PCR in different age groups (n)}}{\text{Total no. of positive cases by PCR(N)}} \times 100$$

Percentage positivity on the basis of breed : This value was calculated as detailed below

$$\% \text{ Positivity} = \frac{\text{No. of positive cases by PCR in different breed (n)}}{\text{Total no. of positive cases by PCR(N)}} \times 100$$

Percentage positivity on the basis of season: This value was determined as described below.

$$\% \text{ Positivity} = \frac{\text{No. of positive cases by PCR in different season (n)}}{\text{Total no. of positive cases by PCR(N)}} \times 100$$

Results and Discussion

Clinical signs & symptoms and their frequency distribution in dogs: Observed clinical symptoms in ehrlichiosis affected dogs and their frequency distribution were presented in Table 1. Important clinical symptoms exhibited by the Ehrlichia positive dogs in order of decreasing frequency were fever (96.64%) and lymph adenomegaly (92.44%), followed by melena (91.60%), mucosal pallor (89.92%), depression (61.34%), weight loss (53.78%)

Table 1. Clinical signs and their frequency distribution in dogs

Parameters	No. of positive cases (n=119)	Frequency (%)
Fever	115	96.64
Lymph adenomegaly	110	92.44
Melena	109	91.60
Mucosal pallor	107	89.92
Depression	73	61.34
Weight loss	64	53.78
Tick infestation	62	52.10
Ecchymotic and Petechial hemorrhages	54	45.38
Ascites	27	22.69
Epistaxis	22	18.49
Ocular abnormalities	9	7.56
Vomiting	8	5.88
Hind limb/scrotal edema / facial edema	8	5.88
Icterus	6	5.04
Hematemesis	3	2.52
Hematuria	3	2.52
CNS signs	2	1.68

Table 2. Physiological parameters

Parameter	Control (n=6)	Dogs with ehrlichiosis (n=119) on day 0
Rectal temperature (°F)	101.68±0.51	103.84±1.12
Heart rate/(min)	83.02±0.34	99.85±1.23
Respiration rate/(min)	18.74±0.16	31.74±1.67
CRT (seconds)	1.84±0.19	2.82±1.45

tick infestation (52.10%) and ecchymotic and petechial hemorrhages (45.38%). Clinical symptoms with moderate frequency were ascites (22.69%) and epistaxis (18.49%), ocular abnormalities (7.56%), vomiting (5.88%), hind limb/scrotal oedema/facial oedema (5.88 %), icterus (5.04%). Symptoms with least frequency were haematuria (2.52%), hematemesis (2.52%) and CNS signs (1.68%).

Physiological parameters: Different vital physiological parameters were recorded by using standard method of examination and data is tabulated in the Table-2. Average value (mean ± SE) of rectal temperature (°F), heart rate (per min), respiration rate (per min) and CRT (seconds) values in the in ehrlichial dogs was higher.

Percentage positivity according to age, breed, sex and season was also analysed and the results are shown in Table 3.

Percent positivity according to age: Percentage positivity of ehrlichiosis was recorded on the basis of age. Dogs were divided into three categories, age group with highest positivity was category 3 (>1 year) with a positivity of (68.07 %) followed by category 2 (>6 months - 1 year) with a positivity of (18.49 %) and lowest positivity was recorded in category 1 (0-6 months) with a positivity of (13.44 %).

Percent positivity according to breed: In the present study percentage positivity of ehrlichiosis was recorded on the basis of breed. For that dog were categorized into 12 breeds. Breed with highest positivity was German shepherd with a positivity of 38.65 % followed by Labrador (18.49 %), Rottweiler (8.40%), non-descript 6.72%), Pitbull (5.88%), Spitz (4.20 %), Pug (2.38 %), Doberman (3.36%), Saint bernard (2.52%), Shih tzu (2.52%), Bull mastiff (1.68%) and Beagle (0.84 %). Most of the time, the breed wise positivity depends on popularity of breed in the region therefore it could neither

Table 3. Percentage positivity of CME according to age, breed, sex and season

Risk factors	Parameters	Positive cases (n=119)	Percentage (%) Positivity
Age	0-6 month	16	13.44
	6 month-1year	22	18.49
	More than 1 year	81	68.07
Breed	German Shepherd	46	38.65
	Labrador	22	18.49
	Rotweiler	10	8.40
	Non-descript	08	6.72
	Pitbull	07	5.88
	Sptiz	05	4.20
	Pug	04	3.36
	doberman	04	3.36
	Saint bernard	3	2.52
	Shih tzu	3	2.52
	Bull mastiff	2	1.68
	Beagle	1	0.84
Sex	Male	76	63.86
	Female	43	36.13
Season	Summer	36	30.25
	Monsoon	48	40.34
	Autumn	21	17.65
	Winter	14	11.76

be considered as breed-based prevalence nor the breed predisposition for the disease.

Percent positivity according to sex: In the present study percent positivity in dogs was recorded on the basis of sex. In present study none of the dog was either spayed/neutered, therefore, dogs were categorized in two categories male and female and positivity of male and female was (63.86%) and (36.13%), respectively.

Per cent positivity according to season: In the present study percent positivity in dogs was recorded on the basis of season. For that dog were categorized in four categories summer (March to June), monsoon (July to September), autumn (October to November), and winter (December to February), for which the positivity was recorded (30.25%), (40.34%), (17.65%), and (11.76 %), respectively. Season seems to play an important role in transmission of disease as proliferation of ticks occurs mostly in monsoon season.

The physiological findings of elevated body temperatures (pyrexia) in dogs infected with ehrlichiosis in this study align with several earlier findings of Mylonakis *et al.* (2019). The immunological response to Ehrlichia infection is facilitated by many humoral and cellular elements, particularly antibodies (Torina *et al.*, 2020). The exact cause of fever in dogs remains unexamined; nevertheless, it has been noted that specific intracellular bacteria can induce macrophages to produce pro-inflammatory mediators such as TNF- α , interleukin-1 (IL-1), and prostaglandins. These mediators may contribute to the fever associated with the condition (Mylonakis *et al.*, 2019). Qurollo *et al.* (2019) documented clinical evidence of lymphatic enlargement in dogs afflicted with ehrlichiosis (Petrov *et al.*, 2021). The hypertrophy of lymph nodes is largely attributed to heightened lymphocytic proliferation triggered by the antigenic response to Ehrlichia. Moreover, Waner and Harrus (2008) noted structural alterations in lymphopoietic tissue, characterized by a rise in plasma cells and a pervasive accumulation of lymphocytes and plasma cells surrounding blood arteries. Canines afflicted with vector-borne illnesses, including ehrlichiosis, have been reported to suffer from hemorrhagic complications including epistaxis, haematuria, and melena (Qurollo *et al.*, 2019). Thrombocytopenia is the principal alteration detected in laboratory assessments following acute CME, as evidenced by a prior study (Cockwill *et al.*, 2009). The exact mechanism is uncertain; however, autoimmune processes seem to be significantly involved (Aziz *et al.*, 2022). Platelet synthesis diminishes during the chronic period, whereas platelet consumption escalates. The presumed etiology of hemorrhagic diseases in canine ehrlichiosis appears to be multifaceted, perhaps encompassing thrombocytopenia, thrombocytopathia, and a reduction in the levels of coagulation components in afflicted dogs. The manifestation of splenic enlargement in dogs afflicted with ehrlichiosis aligns with the observations of Yadav *et al.* (2017). Splenomegaly may result from the excessive accumulation of platelets within the spleen. The migration inhibition factor (PMIF) has recently been identified as playing a vital role in the progression of CME by enhancing platelet accumulation and stasis, leading to a reduction in peripheral blood platelet count and potentially inducing

hemorrhagic diatheses. The spleen is a crucial reservoir for ehrlichial organisms, perhaps due to its abundance of macrophages that act as hosts for these pathogens. Evidence indicates that immunogenic pathways have a role in the pathogenesis of ehrlichiosis. Histological investigations reveal substantial infiltration of plasma cells in several organs and the occurrence of perivascular cuffing in the respiratory system, kidneys, spleen, meninges, and ophthalmic tissues (Harrus and Warner, 2012). The clinical observations of visual anomalies in dogs with ehrlichiosis in this study align with the results of Piso *et al.* (2021). Prior studies have thoroughly identified ocular disorders in dogs infected with *E. canis*, both naturally and experimentally (Komnenou *et al.*, 2007). A study revealed that visual abnormalities were present in all canines, predominantly during the acute phase, with a few cases noted in the subclinical stage (Panciera *et al.*, 2001). A study indicated that 37% of canines infected with *E. canis* exhibited a bilateral ocular condition, as documented by Leiva *et al.* (2005). The most significant pathologic change observed in the eye was linked to the aggregation of plasma cells around the veins in the ganglion cell layer. Hildebrandt *et al.* (1973) noted the narrowing of vessel walls in the sclera, particularly situated directly posterior to the limbus. A large percentage of canines affected with *E. canis* exhibit hypergammaglobulinemia, typically of a polyclonal nature. Nevertheless, a limited number of dogs exhibit paraproteinemia, marked by monoclonal gammopathy (Harrus *et al.*, 1998), potentially resulting in hyperviscosity that may manifest clinical signs. Instances of sudden blindness have been documented due to subretinal haemorrhage associated with hyperviscosity resulting from CME. The clinical observations of posterior limb edema, facial edema, and scrotal edema in dogs with ehrlichiosis in this study align with the results noted by Aziz *et al.* (2023). Hypoalbuminemia may contribute to hind limb edema by reducing plasma oncotic pressure and elevating plasma hydrostatic pressure. Nonetheless, the importance of these modifications in a therapeutic environment remains inadequately comprehended.

The percentage positive cases of ehrlichiosis in our investigation was documented according to age. The present investigation revealed that

the incidence of ehrlichiosis was higher in adult dogs over one year of age. The present results align with the conclusions of Parmar *et al.* (2013) who also reported a higher prevalence of ehrlichiosis in canines less than one year. The current investigation documented the percentage of positive cases of ehrlichiosis according to breed. The dogs were classified into eight breeds. The breed exhibiting the highest positivity was the German Shepherd at 38.65%, followed by the Labrador at 18.49%, Rottweiler at 8.40%, non-descript at 6.72%, Pitbull at 5.88%, Spitz at 4.20%, Doberman at 3.36%, Saint Bernard at 2.52%, Shih Tzu at 2.52%, Bull Mastiff at 1.68%, and Beagle at 0.84%. The positivity associated with a breed is predominantly influenced by its regional popularity; thus, it cannot be classified as either breed prevalence or breed predisposition to disease. The incidence of ehrlichiosis was predominantly higher in German Shepherds, aligning with the conclusions of Bhadesiya and Raval (2015). Conversely, Kottadamane *et al.* (2017) identified a significantly elevated prevalence of ehrlichiosis in Labrador dogs. The present investigation indicates that the German Shepherd dogs are highly susceptible to canine ehrlichiosis. This vulnerability may be ascribed to the compromised cell-mediated immune response noted in this breed (Lakshmanan *et al.*, 2006), resulting in an unfavourable prognosis. The German Shepherd breed exhibits a heightened susceptibility to ehrlichiosis due to diminished cellular immunological defence against *E. canis* and the presence of a white blood cell movement inhibiting factor (Nyindo *et al.*, 1980). The current investigation documented % positive in dogs according to sex. In the current study, none of the dogs were spayed or neutered; consequently, the dogs were classified into two categories: male and female, with positive rates of 63.86% and 36.13%, respectively. The total prevalence of ehrlichiosis in relation to the sex of the dog, specifically male and female, was observed to be higher in males than in females. The present findings of ehrlichiosis align with the research undertaken by Kottadamane *et al.* (2017). These investigations have demonstrated that it is still ambiguous whether tick infection is affected by gender or merely by exposure. Previous research indicates a markedly greater prevalence of ehrlichiosis in females than in males (Bhadesiya and Raval, 2015). The present

study gathered the proportion of confirmed cases in dogs according to the season. The dog was categorised into two groups: summer and winter, with positive frequencies of 78.57% and 21.42%, respectively. The season significantly influences disease transmission, as the rapid proliferation of ticks predominantly happens throughout the summer and rainy months. This analysis revealed that the prevalence of ehrlichiosis is higher in the summer season, aligning with the findings of Aziz *et al.* (2023). In contrast, Kottadamane *et al.* (2017) reported a higher prevalence of ehrlichiosis during the wet season. The heightened occurrence of the brown dog tick in warm and humid seasons may be the reason for this occurrence (Soulsby, 1982). The discrepancies in occurrence may be ascribed to the geographical location examined, the prevalence of the carrier - the brown dog tick (*Rhipicephalus* sp) in each study, the season in which the experiments were conducted, and potentially the exclusion of healthy animals.

Conclusion

The study observed that dogs with ehrlichiosis exhibited elevated body temperatures (pyrexia). Immunological responses involve antibodies and cellular elements, with pro-inflammatory mediators like TNF- α and IL-1 contributing to fever. Lymph node enlargement was noted, driven by lymphocytic proliferation due to antigenic stimulation. Structural changes in lymphoid tissues included increased plasma cells and lymphocyte accumulation around blood vessels. Hemorrhagic complications such as epistaxis and haematuria were common, often linked to thrombocytopenia caused by autoimmune mechanisms and platelet consumption. Splenomegaly was observed, likely due to platelet sequestration and pathogen reservoirs within the spleen. Visual abnormalities, including retinal haemorrhages and vessel narrowing, were documented, often during the acute phase, with hypergammaglobulinemia being prevalent. Edematous conditions affecting limbs and face correlated with hypoalbuminemia, though their clinical significance remains uncertain. The infection rate was higher in dogs over one year, with German Shepherds showing the greatest susceptibility, potentially due to impaired cell-mediated immunity. Males exhibited a higher prevalence than females, and seasonal variation

showed increased cases during summer, likely due to tick activity, with regional differences influencing these patterns.

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