Journal of Advances in Biology & Biotechnology

Volume 27, Issue 8, Page 471-479, 2024; Article no.JABB.119520
ISSN: 2394-1081

Epidemiology of Canine Ehrlichiosis in Jabalpur Region of Madhya Pradesh, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i81159

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119520

Original Research Article

Received: 16/05/2024
Accepted: 18/07/2024
Published: 23/07/2024

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Cite as: *Gupta, Rohini, Devendra Kumar Gupta, Brejesh Singh, Aditya Agrawal, Pratibha Sharma, Salil Kumar Pathak, Vikram Punia, Apra Shahi, Suman Kumar, Kshemankar Shrman, Amita Tiwari, and Bhavana Gupta. 2024. "Epidemiology of Canine Ehrlichiosis in Jabalpur Region of Madhya Pradesh, India". Journal of Advances in Biology & Biotechnology 27 (8):471-79. https://doi.org/10.9734/jabb/2024/v27i81159.*

ABSTRACT

Numerous plant and animal species that are either directly or indirectly linked to tick-borne haemo-protozoan infections thrive in the tropical and subtropical climates of the Indian subcontinents. The climate in India is home to the brown dog tick or *Rhipicephalus sanguineus*, which is a carrier of the canine disease *Ehrlichia*. The main purpose of the study is to explore the prevalence and their risk factors associated with Canine *Ehrlichiosis*. For this 422 canines suspected for positive ehrlichiosis was examined through Giemsa stained thin blood smear and out of them 25 canine samples were found positive showed incidence rate of 5.92%. Prevalence of ehrlichiosis was more in summer season when compare in to winter and monsoon which might be due to high activity of tick vectors. Younger and male dogs showed higher prevalence in comparison to older and female canines. The clinical signs were tick infestation (96%), lymphadenopathy (80%), anorexia (76%), pyrexia (72%), anaemia (56%) and pale conjunctival mucous membrane (56%) along with epistaxis, lameness and ocular problem. This study identifies the present scenario and associated risk factors of the disease, which may help to reduce the infection of canine ehrlichiosis.

Keywords: Canine ehrlichiosis; prevalence; CME; canine.

1. INTRODUCTION

Canine population is getting increasing thus also increasing the rate of parasitic infestation leading to major health concern especially in tropical regions. Indian sub continental tropical climate provides a great platform for flourishing of the ticks especially the brown dog tick, (*R. sanguineus*) which is a vector for various canine diseases [1]. Canine ehrlichiosis is an important tick-borne disease of canine caused by an obligate intracellular, coccal or pleomorphic, gram-negative bacterium Ehrlichia canis and transmitted by tick vectors i.e. brown dog tick *Rhipicephalus sanguineus* [2]. The most important species of Ehrlichia in dogs is *Ehrlichia canis*, which parasitizes circulating monocytes intracytoplasmically in the form of clusters called morulae, which can result in canine monocytic ehrlichiosis, a potentially fatal illness [3]. The disease occurs in acute, subclinical and chronic form that leads to multisystemic dysfunction involving the liver, kidney, heart and nervous system resulting in death in some untreated dogs [4] In India, the prevalence rate of canine ehrlichiosis is high in tropical and sub-tropical regions viz. Mumbai (27.2%) and Delhi (39.5%), as compared to the temperate and dry-arid regions such as Sikkim (0%) and Ladakh (0%) [5].

Clinically canine ehrlichiosis characterized with fever, anorexia, thrombocytopenia, weakness, lethargy, lymphadenomegaly, hemorrhagic disorder and neurologic abnormalities [6-8]. There is evidence linking a number of risk variables to the disease's incidence, however, there are not many comparable studies from this region of the nation. Consequently, the current study was conducted to look at the frequency and relationship between Canine Monocytic Ehrlichiosis (CME) and different risk factors.

2. MATERIALS AND METHODS

For this study, a total of 5688 dogs were screened for ehrlichiosis during the period of one year i.e. from May, 2022 to April, 2023 that were brought to Veterinary Clinical Complex (V.C.C.), College of Veterinary Science & A.H., Jabalpur (M.P.). The dogs were examined for the presence of clinical symptoms viz. bleeding tendencies/epistaxis, intermittent fever, arthritis (swelling of legs), laboured breathing and lethargy for at least one to two weeks or longer. Blood sample was collected aseptically from cephalic vein or tip of ear of each dog for preparing the thin blood smear. The blood smear was stained with Giemsa stain and observed under oil immersion objective (100X). Presence of *E. canis* morulae was confirmed as per characteristics described by Soulsby, 1982 (Fig. 1).

2.1 Statistical Analysis

The prevalence of the disease was determined using descriptive statistics and the associated risk variables influencing the prevalence of CME were studied using Chi-square test of significance. When $P<0.05$, all results were deemed statistically significant.

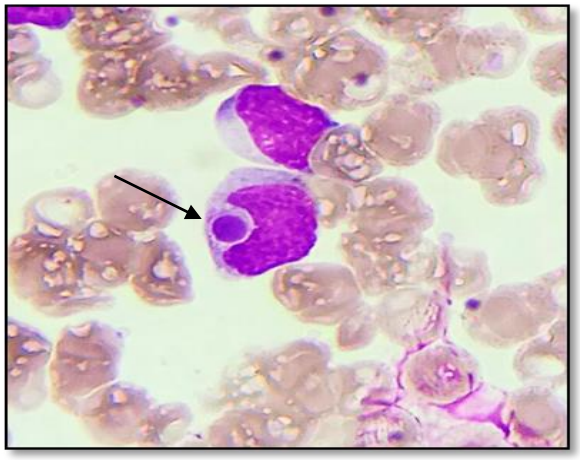

Fig. 1. Microscopic examination of blood smear showing *E. canis* in monocytes under 100X

3. RESULTS AND DISCUSSION

3.1 Overall Prevalence of Ehrlichiosis in Dogs

On the basis of clinical examination and symptoms 422 dogs were suspected for canine ehrlichiosis among the total 5688 dogs that were brought to the VCC, Jabalpur. On blood smear examination, 25 dogs were positive for canine ehrlichiosis with a prevalence of 5.92% (Fig. 1). However, an overall prevalence rate of 0.43% (25/5688) was observed in dogs (Table 1).

In the contrast a higher prevalence of *E. canis* on blood smear examination using the Giemsa staining i.e. 41.59%, 8.4%, 62.7% and 13.58% in Ludhiana, northern India, Gujarat and in chittoor district of Andhra Pradesh, India was reported by Singh et al. [9], Behera et al. [10], Bhadesiya and Raval [11] and Prameela et al. [12] respectively. Padmaja et al. [13] showed that the prevalence of *E. canis* in Punjab was 1.5% whereas Ratnalikar et al. [7] and Yadav et al. [14] revealed that 4.03% (5/124) and 13.04% (27/207) respectively.

The prevalence of ticks, which are the vectors for E. canis, can vary depending on climatic conditions and geographical factors which might be due to different environmental conditions as compared to other parts of India, leading to variations in tick populations and subsequently, the prevalence of ehrlichiosis. Climate change can influence the distribution and abundance of ticks. Variations in temperature and precipitation patterns may establish suitable conditions for tick proliferation in certain regions. However, the low population density of dogs in the study area may also be the contributing factor for the lower prevalence of canine ehrlichiosis in present study.

3.2 Age Wise Prevalence of Ehrlichiosis in Dogs

Canine ehrlichiosis positive dogs were classified into three age groups to assess the age-specific prevalence of ehrlichiosis. The dog with age group <1 year had the highest prevalence i.e. 40% (10/25), followed by the age group 1-3 years, 36% (09/25), while dogs with age group >3 years had the lowest prevalence i.e. 24% (6/25). The results are summarized in Table 2.

The present findings are in concurrence with the results of Thirunavukkrasu et al. [15], Chandrasekar et al. [16], Milanjeet et al. [17], Bhadesiya and Raval [11] and Kalaivanan et al. [18] who reported higher prevalence of ehrlichiosis in younger dogs <1 year of age. However, Behera et al. [10], Kottadamane et al. [19], Senthil et al. [20] and Dhavalagi et al. [21] reported highest prevalence of ehrlichiosis in dogs of 1-3 years age group, followed by 3-6 years age group while infection levels were lower in <1 year of age group. *E. canis* parasitizes the host monocytes. In the growing host, high bone marrow activity with active precursor cells i.e. monoblasts gives an opportunity for the organism to parasitize more number of cells and rapid multiplication, resulting in higher occurance in younger age group. Furthermore, the immune system in young animals is not fully developed to combat the invading organisms. However, *E. canis* has infected in all the age groups which depends on the transmitting vector and the immune status of the host.

Table 1. Overall prevalence of ehrlichiosis in dogs

Particulars	No. of dogs Screened	No. of dogs Affected	Prevalence (%)
Total dog population	5688	25	0.43
Suspected dog	422	25	5.92

Table 2. Age wise prevalence of ehrlichiosis in dogs

Age groups (years)	No. of dogs Affected (n=25)	Prevalence (%)
<1	10	40
1-3	09	36
>3	06	24
$\chi^2=1.04^{NS}$	Non Significant p> 0.05	

3.3 Breed Wise Prevalence of Ehrlichiosis in Dogs

The prevalence of canine ehrlichiosis in different breeds was observed and it was concluded that Labrador retrievers accounted for the highest percentage of ehrlichiosis i.e. 36% (09/25), followed by Non-descript 20% (5/25), German Shepherd 12% (3/25), Pug 4% (1/25), Saint Bernard 4% (1/25), Great Dane 4% (1/25), Golden Retriever 4% (1/25), Dobermann 4% (1/25), American Bully 4% (1/25), Beagle 4% (1/25), Belgian Malinois 4% (1/25) (Fig. 2).

Labrador retriever was most affected breed with *E. canis* was reported by Bhadesiya et al. [22], Bai et al. [23], Dhavalagi et al. [21] whereas, Milanjeet et al. [16] and Yadav et al. [24] reported higher prevalence in German shepherd as compared to other breeds of dogs. However, Senthil et al. [20] reported highest prevalence of canine ehrlichiosis in non-descript dogs, followed by Labrador retriever, Spitz, German shepherd, Golden retrievers, Pug, Doberman and others. Some breeds may be genetically predisposed to certain diseases or infections including ehrlichiosis. Labrador retriever's susceptibility to contracting the diseases might be acquired by different genetic factors. Higher occurrence of ehrlichiosis in Labrador breed might be due to higher risk of unnoticed ticks, attached under their hair coat. Ehrlichiosis may be more prevalent in certain regions where ticks carrying the Ehrlichia organism are abundant. Labrador retrievers living in these areas are naturally at a higher risk of exposure. Moreover, it must also be kept in consideration that a particular breed might be more kept by the owners in area under study as compared to other breeds resulting in different breed wise prevalence. However, secondly non-descript breeds were more affected with tick infestation might be because of poor grooming management practices as well as higher population density of this breed presented in the clinics during the study period.

3.4 Gender Wise Prevalence of Ehrlichiosis in Dogs

Out of total 25 dogs positive for ehrlichiosis, 16 were males and 9 were females indicating a prevalence of 64% and 36%, respectively (Table 3).

A higher prevalence of ehrlichiosis in male dogs (64%) in comparison to females (36%) are in concordance with the earlier report of Bai et al. [23] and Singh et al. [25] who reported more number of cases in male than female dogs. However, in contrast Kalaivanan et al. [18], Dhavalagi et al. [21] and Sarawade et al. [26] reported higher occurrence of canine ehrlichiosis in female dogs as compared to males. Meanwhile, Rodriguez et al. [27] reported no significant difference in males and female dogs in canine ehrlichiosis. The results of this study could be explained by the owners' inclination to retain male dogs as pets rather than female ones. The fact that more male dogs than female canines are brought to the clinics may potentially be the cause of this.

3.5 Season Wise Prevalence of Ehrlichiosis in Dogs

The prevalence of ehrlichiosis based on the season was recorded and the results indicated highest (48%) prevalence in summer season, followed by winter (28%) and monsoon (24%) (Table 4).

Season wise prevalence of ehrlichiosis in the present study was in agreement with Milanjeet et al. [16] and Behera et al. [10] who reported

highest prevalence of canine ehrlichiosis in summer. Contrarily, Kalaivanan et al. [18] and Sarawade et al. [26] reported highest prevalence in winter whereas Kottadamane et al. [19] reported highest prevalence of ehrlichiosis in rainy and summer season. The reason behind seasonal variation is due to the increased activity of the *Rhipicephalus sanguineus* (brown dog tick) or *Amblyomma americanum* (lone star ticks) during warmer months, particularly in spring and summer. Therefore, dogs are possibly to be exposed to infected ticks during these seasons, leading to a higher prevalence of canine ehrlichiosis which might be more abundant in hot and humid period of the year [28].

3.6 Clinical Sign Observed in Ehrlichiosis in Dogs

In the canines, clinical signs such as bleeding tendencies/epistaxis, intermittent fever, arthritis (leg swelling), labored breathing, ecchymotic hemorrhages, congested mucous membrane, corneal opacity, Petechial haemorrhages, enlarged lymph node, presence of ticks, pale mucous membrane and lethargy lasting at least one to two weeks were observed and examined (Fig. 3). The clinical findings in the canine ehrlichiosis positive canines were tick infestation 96% (24/25), lymphadenopathy 80% (20/25), anorexia 76% (19/25), pyrexia 72% (18/25), anaemia 56% (14/25), pale conjunctival mucous membrane 56% (14/25) along with epistaxis, lameness and ocular problem etc. The results are summarized in Fig. 4.

Lymphadenopathy was the most predominant clinical sign followed by pyrexia, depression, bleeding tendencies (epistaxis), icteric mucous membrane, ascites and lameness in the dogs infected with canine ehrlichiosis was observed by Elitok and Ungur [29] and Roopali et al. [30]. Dhavalagi et al. (2021c) and Senthil et al. [20] reported clinical signs like anorexia, lethargy, fever, pale and congested mucous membrane along with dyspnoea in dogs. Singh et al. [25], Ratnalikar et al. [7] and Sharma et al. [8] revealed clinical signs of tick infestation, melena, pale mucous membrane, lymphadenopathy and epistaxis in dogs affected with canine

Table 3. Gender wise prevalence of ehrlichiosis in dogs

Gender	No. of dogs affected (n=25)	Prevalence (%)
Male	16	64
Female	09	36
$\chi^2=1.96^{NS}$	Non Significant p> 0.05	

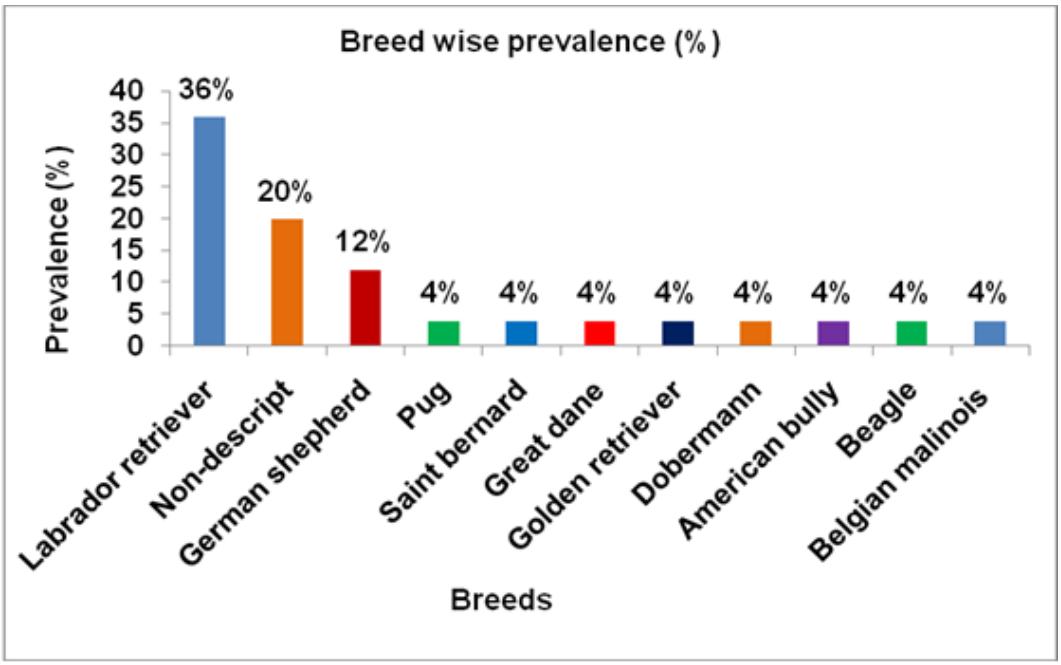

Fig. 2. Breed wise prevalence of ehrlichiosis in dogs

Fig. 3 Clinical signs observed in ehrlichiosis in dogs: A) Presence of ticks B) Enlarged lymph node C) Pale mucous membrane D) Pale gums E) Hematemesis F) Corneal opacity G) Petechial haemorrhages H) Icterus mucous membrane I) Congested mucous membrane J) Epistaxis K) Ecchymotic hemorrhages L) Emaciated condition

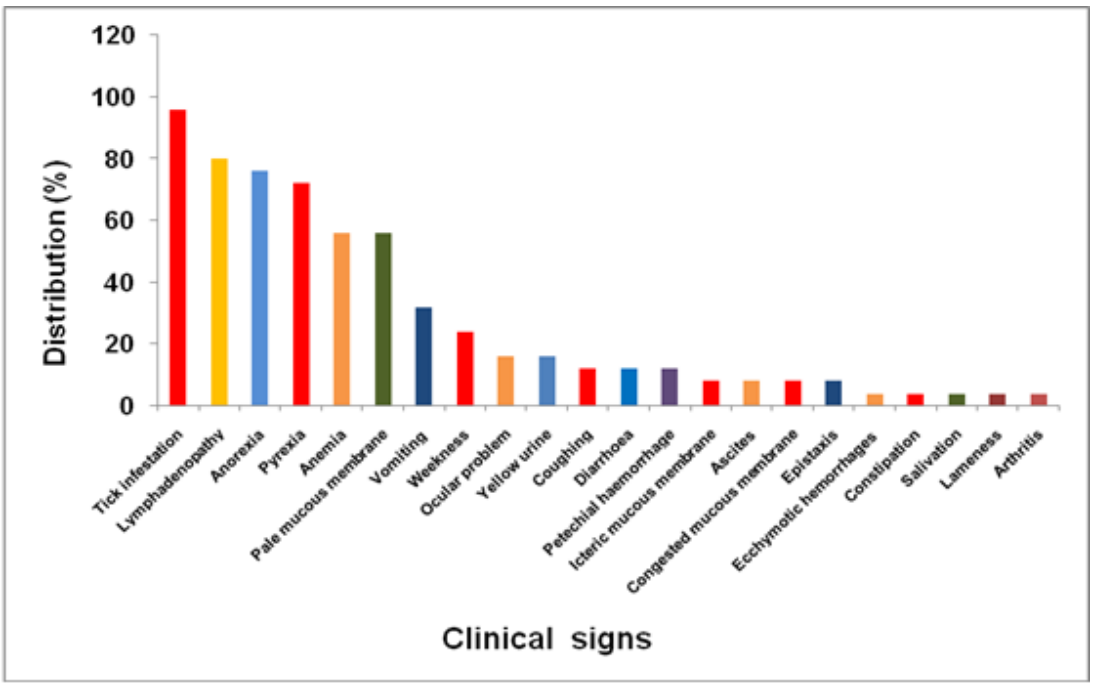

Fig. 4. Distribution of different clinical parameters observed in ehrlichiosis in dogs

Table 4. Season wise prevalence of ehrlichiosis in dogs

Season	No. of dogs affected (n=25)	Prevalence (%)
Summer {May, June (2022); March- April (2023)}	12	48
Monsoon (July 2022- October 2022)	06	24
Winter (November 2022- February 2023)	07	28
χ^2=2.48	Non Significant p> 0.05	

ehrlichiosis. Kumar et al. [31] and Dhliwayo et al. [32] reported hypoalbuminaemia, anemia and thrombocytopenia as the most common laboratory findings in dogs infected with canine ehrlichiosis. Less common clinical indicators include vomiting (32%) and ocular lesions (16%) which corroborated with the reports of Oria et al. [33] and Gaunt et al. [34]. Kottadamane et al.

[19] and Roopali et al. [30] observed lameness similar to the findings of the present study which might be due to edema and arthritis in the rear limb.

Variations in the clinical signs could be attributed to a number of factors including difference in various strains of *Ehrlichia* pathogenicity in different breeds, dog's immune response, any underlying health conditions, co-occurrence of other diseases, etc. The most important clinical findings of ehrlichiosis includes pyrexia, pale mucosa, bleeding tendencies in form of epistaxis and melena which leads to thrombocytopenia and the deposition of immune complexes on the vascular wall [35]. Ehrlichia infection can lead to inflammation of lymph nodes, resulting in lymphadenopathy. The reticulo-endothelial system is the site for replication of canine ehrlichiosis, where they cause the proliferation of lymphocytes leading to reactive histiocyte in lymph node resulting into development of lymphadenopathy. The epistaxis is associated with thrombocytopenia, mild vasculitis and thrombocytopathy. The systemic inflammation can lead to decreased appetite and anorexia. Additionally, the effects of the infection on the gastrointestinal tract contribute to development of gastrointestinal symptoms.

Fever is a common response to infection and inflammation. The body's immune response to *Ehrlichia* leads to the release of inflammatory mediators, resulting in fever. It may be also due to overproduction of interleukin-1 by the antigen-presenting cells and B cells and due to exogenous pyrogenic products of the causative agent [36]. Ehrlichia organisms can get entry in red blood cells, leading to destruction of these cells and subsequent anemia. The non regenerative anaemia involves bone marrow hypoplasia leading to impaired production of cellular components of blood leading to pale mucous membranes due to decreased red blood cell count.

Clinical signs such as vomiting, weakness, ocular problems, yellow urine and diarrhea develop as a result of systemic inflammation affecting various organ systems, including the gastrointestinal tract, muscles, and eyes. The ophthalmic abnormalities such as ocular discharge and corneal opacity might be attributed due to cuffing of blood vessels in sclera and association with plasma cell cuffing with the veins of ganglion cells. Less common clinical signs like petechial hemorrhage, icteric mucous membrane, ascites, congested mucous membrane, epistaxis, ecchymotic hemorrhages, constipation, salivation, lameness and arthritis can be related to the systemic effects of the infection on different organs and tissues. Overall, the clinical signs of ehrlichiosis develop as a result of the body's immune response as well as the direct effects of Ehrlichia organism on various organ systems.

4. CONCLUSION

In the present study the overall prevalence of ehrlichiosis in dogs at Jabalpur region was 5.92% when examined by blood smear method. Younger canine are more at risk of ehrlichiosis in comparison to older one and enhances in the rate of infection was more in summer in comparison to winter and monsoon seasons. This study summarizes the current status of CME as well as different risk factors that helps the disease to persist and enhance. Subside of these factors might be helpful to control or reduce the rate of infection of ehrlichiosis in canines.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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