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# Prevalence of canine monocytic ehrlichiosis in dogs in and around Meerut district of Uttar Pradesh, India

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#### Abstract

The present study was conducted to explore the prevalence of canine monocytic ehrlichiosis (CME) in dogs in and around Meerut district for its better clinical management. A total of 366 dog's blood samples were collected from Department of Veterinary Clinical Complex, SVPUAT, Meerut and nearby private Veterinary Clinics with the history of tick infestation and characteristic clinical findings with CME and were screened on the basis of blood smear examination, followed by molecular detection by polymerase chain reaction during the period from January 2022 to June 2022. The blood smear examination with Giemsa stain detected morulae of *E. canis* and it showed as intracytoplasmic inclusion bodies of varying sizes and shapes in monocytes. Thirteen dogs were found positive for canine ehrlichiosis resulting in a prevalence of 3.55%. The highest affection of *E. canis* was found within the age group of 1-3 years (38.4%), followed by the 4-5 years age group (30.76%), then 6-7 year of age group (23.07%) and lowest infection levels (7.69%) were found in the age group of 4 month -1 year. The maximum prevalence of canine ehrlichiosis was found in Labrador (6.15%) followed by Rottweiler (5.55%), Bull mastiff (4.16%), Golden retriever (3.22%), German shepherd (2.5%), Pitbull (2.40%), and non-descriptive (1.40%) breed of dog. Higher prevalence was recorded in males (4.10%) in comparison to females (2.92%).

Keywords: Ehrlichiosis, prevalence, Dogs and PCR

#### 1. Introduction

Canine monocytic ehrlichiosis (CME) is an important tick-borne disease with a worldwide distribution (Harrus and Waner, 2010)<sup>[1]</sup> and caused by the bacteria *Ehrlichia canis* (Dumler *et al.*, 2001)<sup>[2]</sup>. CME is a multi-systemic ailment manifesting in acute, subclinical or chronic form (Harrus and Waner, 2010)<sup>[1]</sup>. *Ehrlichia* spp. is a gram negative obligate intracellular bacteria with tropism for hematopoietic cells. *Ehrlichia* is an alpha proteobacterium belonging to the family Anaplasmataceae (Aiello *et al.*, 2016)<sup>[3]</sup>. Canine monocytic ehrlichiosis was initially identified by Donatien and Lestoquard in Algeria in 1935 (Donatien and Lestoquard, 1937)<sup>[4]</sup>. Mudaliar (1944)<sup>[5]</sup> reported *E. canis* infection for the first time in India from Chennai.

The prevalence of *E. canis* majorly depends on the distribution of tick vector, *Rhipicephalus* sanguineus that is found widespread across tropical and sub-tropical regions (Shaw *et al.*, 2001)<sup>[6]</sup>. *Dermacentor variabilis* is also known to transmit *E. canis* experimentally (Johnson *et al.*, 1998)<sup>[7]</sup>. The frequency, distribution, and suitability of habitats, as well as the intensity and temporal pattern of vector activity throughout the year, as well as the survival, growth, and spread of infections inside arthropod vectors, all depend on the weather (Confalonieri *et al.*, 2007; Rogers and Randolph, 2006)<sup>[8, 9]</sup>. The Indian subcontinent's tropical environment not only supports a wide variety of flora and fauna, but it also promotes the growth of harmful microbes. The dog population in India was 25 million and, divided into four categories like pets, family, community and feral dogs (Abd Rani *et al.*, 2011)<sup>[10]</sup>. In canines, the major species that are capable of producing infection includes *E. canis* (causes canine monocytotropic ehrlichiosis), *E. ewingii* (causes canine granulocytic ehrlichiosis) and *E. chaffeensis* causing human monocytic ehrlichiosis (Anderson *et al.*, 1992, Irwin *et al.*, 2004) <sup>[11, 12]</sup>.

*E. canis* can infect all breeds of dogs but the German shepherd dog appears to be more susceptible, showing a more severe form of the disease with higher morbidity and mortality compared to other breeds (Kuehn and Gaunt, 1985)<sup>[13]</sup>. Keeping in view, the present study was conducted for the study of prevalence of CME in dogs in and around Meerut district.

# 2. Materials and Methods

## 2.1 Ethical Approval

The permission for sampling and other procedures was duly approved by the institutional Animal Ethics Committee (IAEC), SVPUAT, Meerut vide no.

IAEC/SVPUAT/2022/105 dated 24/05/2022.

#### 2.2 Study area

Blood samples (3-4 ml) were collected at Veterinary Clinical Complex (VCC) of College of veterinary science, SVPUAT Meerut and private pet clinic from Meerut from January, 2022 to June, 2022 of 366 dogs. The samples were screened for presence of *Ehrlichia canis*. On the basis of history of tick infestation, physical examination and clinical signs like fever, anorexia, melena, epistaxis, anaemia, petechial and ecchymotic haemorrhages, lymphadenopathy, splenomegaly, and corneal opacity, dogs were tentatively diagnosed as ehrlichiosis.

Table 1: Month wise blood collected from dogs

Month	Dogs cases sample collected in VCC
January	54
February	63
March	50
April	43
May	72
June	84
Total	366

#### 2.3 Screening by blood smear examinations

Thin blood smear was prepared, air dried, fixed with methanol, stained with Giemsa and examined under microscope.

# **2.4 Detection of** *Ehrlichia canis* by Polymerase Chain Reaction (PCR)

The genomic DNA from the blood samples were extracted by QIA amp Mini Kit (Harrus *et al.*, 1998) <sup>[14]</sup>. The PCR was standardized for the primer set of *Ehrlichia* spp. The details of primers are given in Table 2. 16S rRNA gene was screened for positive samples. Extracted DNA ( $4\mu$ L) was used as a template to amplify a portion of *E. canis* 16S rRNA. Qualitative and Quantitative analysis of genomic DNA was performed by agarose gel electrophoresis method.

Table 2: Sequence of primers used for Genus specific and nested PCR (Species specific)

Ehrlichia canis	Primer sequence Primary PCR –Nested PCR	Target gene	Product size	References
Genus specific primers	EC9:F 5'-AAGGATCCTACCTTGTTACGACTT-3'	16SrRNA	15000bp	(Kawahara <i>et al.</i> , 1999) <sup>[15]</sup>
Senius specific primers	EC12:R 5'- AATCTAGAGTTTGATCMTGG-3'	robiidair	1900000	(1147) analu er en (, 1999)
Species specific primers	HE3:F 5'-TATAGGTACCGTCATTATCTTCCCTAT-3'	16SrRNA	389hn	(Wen et al. 1997) $[16]$
	ECA:R5'-ATTATTATAGCCTCTGGCTATAGGAA-3'	IOSIKIVA	3070p	(Well et al., 1997) = -

#### 3. Results and Discussion

The Giemsa blood smear examination detected morulae of E.

*canis* as intracytoplasmic inclusion bodies of varying sizes and shapes in monocytes (Fig.1).



Fig 1: Giemsa stained thin blood smear showing morulae in monocyte of dog blood

Out of 366 dogs, 13 dogs were found positive for canine ehrlichiosis by blood smear examinations resulting in a prevalence of 3.55% (table 3).

**Table 3:** Clinical cases presented under study

Month & Year	No. of sample collected	Result
January 2022	54	2 Positive
February 2022	63	1 Positive
March 2022	50	2 Positive
April 2022	43	1 Positive
May 2022	72	2 Positive
June 2022	84	5 Positive
Total	366	13 Positive

The study result was corroborated with the findings of Milanjeet *et al.* (2014) <sup>[17]</sup> who also found 2.34% of cases to be positive for *E. canis* morulae in the region of Punjab. There was 8.33% positive of *E. canis* in blood smear examination in the region of Hisar and Haryana (Bai *et al.*, 2017) <sup>[18]</sup>. The age groups of dogs with canine ehrlichiosis were from 4 months to 7 years (Table 4). The majority dogs were affected from 1 to 3 years. The highest affection (38.4%) was found within the age group of 1-3 years, followed by the 3-5 years age group (30.76%), then 4 month -1 year of age group (23.07%).The lowest infection levels (7.69%) were found in the age group of 5-7 year.

Table 4: Age wise prevalence of canine ehrlichiosis

Age group	Positive dogs	Prevalence (%)
4 month to 1 years	3	23.07%
1 to 3 years	5	38.4%
3 to 5 years	4	30.76%
5 to 7 years	1	07.69%

These study is correlated with Kottadamnae *et al.* (2017) <sup>[19]</sup> where the prevalence of canine ehrlichiosis was highest (41.09%) in the age group of 1-3 years, followed by the 3-6 years age group (32.87%), then < 1 year of age group dogs (13.69%) and > 6 years age group dogs (12.32%). Moreira *et al.* (2003) <sup>[20]</sup> and Choudhary *et al.* (2012) <sup>[21]</sup> reported dogs of age group 1-3 years were affected highest with canine ehrlichiosis. The present study is also coincided with these findings with the opinnion that dogs between 1-3 years of age are more susceptible to canine ehrlichiosis. The present study reported that maximum prevalence of canine ehrlichiosis was found in Labrador (6.15%) followed by Rottweilers (5.55%), Bull mastiff (4.16%), Golden retriever (3.22%), German Shepherd (2.5%), Pitbull (2.40%), and non-descriptive (1.40%) breed of dog (Table 5).

Table 5: Breed-wise	prevalence of	canine e	ehrlichiosis	in dogs
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Breed	Total cases studied	Positive cases	Positive (%)
Labrador	65	4	6.15%
Rottweiler	54	3	5.55%
German Shepherd	80	2	2.5%
Bull Mastiff	24	1	4.16%
Pit bull	41	1	2.40%
Golden Retriever	31	1	3.22%
Non-descript	71	1	1.40%

It indicates that the Labrador breed of dog is more prone to canine ehrlichia infection. Bhadesiya and Modi (2015) <sup>[22]</sup>, Mondal *et al.* (2019) <sup>[23]</sup> also reported that Labrador breed of dogs was most susceptible. Dhankar *et al.* (2011) <sup>[24]</sup> reported higher prevalence of ehrlichiosis amongst exotic breed of dogs than Indian non-descript dogs. Milanjeet *et al.* (2014) <sup>[17]</sup> reported that highest prevalence of canine monocytic ehrlichiosis in cross breeds (47.05%) than non-descript breeds of dogs (34%). In the present study the higher prevalence was recorded (Table 6) in males (4.10%) in comparison to females (2.92%).

Table 6: Sex-wise prevalence of canine ehrlichiosis

Sex	Total	Positive dogs	Prevalence
Male	195	8	4.10%
Female	171	5	2.92%

This finding corroborate with the finding of Risheen *et al.*  $(2022)^{[25]}$  where the higher prevalence was recorded in males as compared with females. Similar findings were also reported by Kitaa *et al.*  $(2014)^{[26]}$  and Choudhary *et al.*  $(2012)^{[21]}$ . Thirunavukkarasu *et al.*  $(1994)^{[27]}$  noticed that there was higher incidence of canine ehrlichiosis in males than in females in a survey of Madras city. Higher prevalence of canine ehrlichiosis was observed in males as compared with females (Himalini *et al.* 2018)<sup>[28]</sup>.

Out of 366 samples, only 8 blood smear samples (2.18%) were detected positive for presence of morula within monocytes. Same samples when processed by PCR with the species specific Nested PCR targeting 16S rRNA gene and detected 13 (3.55%) samples positive for *E. canis* (Fig. 2).





Fig 2: Detection of Ehrlichia canis by Polymerase Chain Reaction

L- Ladder (50 bp), L1 - Primary PCR product (1500 bp), L2 - Nested PCR product (389 bp), L3- Negative control.

#### 4. Conclusions

The prevalence of *E. canis* in and around Meerut district was 3.55%. PCR is more sensitive than thin blood smear examinations. The highest affection of *E. canis* was found within the age group of 1-3 years (38.4%), followed by the 4-5 years age group, then 6-7 year of age group. *E. canis* infection was highest in Labrador (6.15%) followed by Rottweiler (5.55%), Bull mastiff (4.16%), Golden retriever (3.22%), German shepherd (2.5%), Pitbull (2.40%), and non-descriptive (1.40%) breed of dogs. Higher prevalence was recorded in males (4.10%) in comparison to females (2.92%).

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