MOLECULAR PREVALENCE OF CANINE MONOCYTIC EHRLICHIOSISIS[#]

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ABSTRACT

Evaluation of 104 blood samples collected from clinically suspected canine ehrlichiosis dogs from canine outdoor of Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan (India) was performed for the presence of *Ehrlichia canis* using PCR-based assay targeting a portion of the 16S rRNA gene. Nested PCR assay produced amplicons of expected size (389 bp) specific for *E. canis* in 20.19% (21/104) of samples. The molecular prevalence of canine ehrlichiosis was 20.19 per cent (21/104). Breed-wise, sex-wise and age-wise highest prevalence of canine ehrlichiosis was recorded in German Shepherd breed of dogs (26.31%), males (23.53%) and dogs with age greater than 1-3 years (32.14%), respectively.

Key words: *Ehrlichia canis,* 16S rRNA gene, dogs

Introduction

Ehrlichiosis is an infectious disease that is caused by a gram-negative bacterium of the genus *Ehrlichia*, which includes species such as *E. canis, E. chaffeensis*, and *E. ewingii* and infects several animal species including humans (Dumler *et al*., 2001; Perez *et al*., 2006). With global warming, expanding tick habitats and increasing international travel the spread of disease to former non-endemic areas is of great concern. Canine monocytic ehrlichiosis is caused by *E. canis* and is transmitted by the tick vector *Rhipicephalus sanguineus* during its blood meal (Azevedo *et al*., 2011; Dumler *et al*., 2001). This bacterium mainly parasitizes the cells of the mono0nuclear phagocyte system and causes clinical and hematological abnormalities such as fever, anorexia, vomiting, diarrhoea, petechial haemorrhages, anaemia, and thrombocytopenia (Moreira *et al*., 2003).

The disease was first described in Algeria in 1935 by Donatien and Lestoquard. Mudaliar (1944) reported *E.canis* infection for the first time in India from Chennai. In the present paper prevalence of canine monocytic ehrlichiosis has been reported according to age, gender and breed.

Materials and Methods

Animals

A total number of 104 clinically suspected cases of dogs of different breeds, ages and genders presented to the canine outdoor of Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, RAJUVAS, Bikaner during the period of September 2016 to December 2016. Dogs were tentatively selected on the basis of the clinical examination including history of ticks or presence of tick infestation, physical examination (temp, respiration rate and pulse rate) and clinical signs such as fever, anorexia, melena, epistaxis, anaemia, petechial and ecchymotic haemorrhages, lymphadenopathy, splenomegaly, and corneal opacity. Diagnosis was made by whole blood and buffy coat smear examination using Geimsa's stain (Coles, 1986) to demonstrate intracytoplasmic inclusion bodies or morulae of *E. canis* in leucocytes (circulating monocytes and lymphocytes) under microscope and confirmation was done by using nested polymerase chain reaction.Molecular evaluation -Nested Polymerase Chain Reaction (nPCR)

Genomic DNA isolation

For nested Polymerase Chain Reaction (n PCR), blood sample was collected in EDTA vial and was stored at –20 °C until DNA extraction. For conducting the PCR assays, genomic DNA was isolated from whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per spin protocol.

Nested Polymerase Chain Reaction (nPCR) assays protocols

The PCR assays (primary as well as nested) were designed to target a portion of the 16S rRNA gene so as to amplify all *Ehrlichia* spp. (primary) and *E. canis* (nested PCR) as described by Murphy *et al*. (1998). The sequences of the primers were as follows:

Genus specific primers

ECC: 5' AGA ACG AAC GCT GGC GGC AAG C 3'
ECB: 5' CGT ATT ACC GCG GCT GCT GGC A 3'

Species specific primers

ECAN5: 5' CAA TAA TTT ATA GCC TCT GGC TAT AGG A 3'
HE3: 5' TAT AGG TAC CGT CAT TAT CTT CCC TAT 3'

Two rounds of PCR in a final volume of 25 µl were carried out in a PCR thermal cycler (Applied Biosystems, USA). The PCR products (primary as well as nested) were checked for amplification by electrophoresis on a 1.5% agarose gel and visualized using a gel documentation system (Syngene, UK).

Results and Discussion

Nested PCR technique was adopted as confirmatory diagnostic technique, which revealed twenty one positive cases for *E. canis* out of one hundred four dogs examined. Genus-specific PCR products were obtained by using genus specific primers ECC and ECB, when employed as templates in nested PCR produced a 389 bp amplicon species-specific of *E.canis* in the positive samples using species specific primers ECAN5

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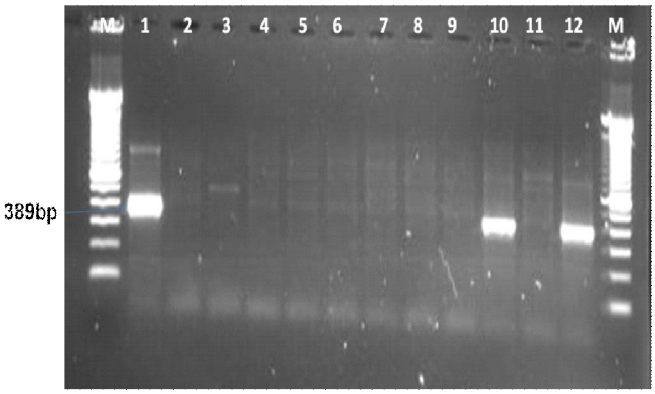
and HE3 (Fig. 1).

The molecular prevalence of canine ehrlichiosis was 20.19 per cent (21/104) by using nPCR. This finding is in agreement with Abd Rani *et al.* (2011), Parmar *et al*. (2013) and Guedes *et al*. (2015). In the past, various studies had been carried out regarding the molecular prevalence of *E. canis* worldwide and the prevalence rate had been reported to range from 3.1% to 88.0% (Murphy *et al.,* 1998; Dagnone *et al.,* 2003; Bulla *et al.,* 2004; Macieira *et al.,* 2005; Diniz *et al.,* 2007; Carvalho *et al.,* 2008; Alexandre *et al.,* 2009; Dagnone *et al.,* 2009; Faria *et al.,* 2010 and Silva *et al.,* 2012).

Fig. 1: Amplicon (389bp) of *E. canis* 16S rRNA amplified by nPCR on 1.5% agarose gel electrophoresis, Lane M = Generuler TM 100 bp ladder, lane 1–12 = clinically suspected samples and 1, 10 & 12 - positive samples of canine ehrlichiosis



As regards the Indian scenario, a small number of published reports are available: the prevalence of *E. canis* was reported to be 50% (49/98) in Chennai (Lakshmanan *et al.,* 2007) and 20.6% from four different regions of India (Abd Rani *et al.,* 2011), 41.59% (89/214) in Ludhiana, Punjab (Singh *et al.,* 2014) and 44.44% (40/90) in Bangalore, Karnataka (Choudhary *et al*., 2015). In the present study nested PCR assay targeting a portion of the 16S rRNA gene detected 20.19% (20/104) of samples to be positive for *E. canis.* The variation in the prevalence may be attributed to sample size, geographical area, climatic conditions which directly influence the tick population and time of sample collection.

Age-wise prevalence of canine ehrlichiosis (based on nPCR)

In the present study, maximum prevalence of canine ehrlichiosis was recorded in above 1-3 years age group (32.14%), followed by 23.81% in above 3-5 year age group, 18.75% in above 5-7 year age group, 16.67% in above 7-9 year age group, 12.50% in above 9-11 year age group and 5.26% in below 1 year age group. Age wise prevalence is given in Table 1. Moreira *et al*. (2003), Bindu *et al*. (2006) and Choudhary *et al.* (2012) reported dogs aged above 1-3 years were affected highest with canine ehrlichiosis. However, Rahman *et al*. (2010) and Bhadesiya and Raval (2015) reported no significant difference between age group. Ezekoli *et al*. (1983), Abdullahi *et al*. (1990), Samaradni *et al*. (2005) and Lakshmanan *et al.* (2006) observed that the prevalence of haemoprotozoan infections to be highest in young dogs.

Table 1: Age wise prevalence of canine ehrlichiosis

Age (years)	No. of dogs examined	Positive dogs	Prevalence (%)
< 1	19	1	5.26
>1-3	28	9	32.14
>3-5	21	5	23.81
>5-7	16	3	18.75
>7-9	12	2	16.67
>9-11	8	1	12.5

Table 2: Sex-wise prevalence of canine ehrlichiosis

Gender	No. of dogs examined	Positive dogs	Prevalence (%)
Male	68	16	23.53
Female	36	5	13.89

Table 3: Breed-wise prevalence of canine ehrlichiosis

Dog Breeds	No. of dogs examined	Positive dogs	Prevalence (%)
German Shepherd	38	10	26.31
Labrador	29	5	17.24
Saint Bernard	10	2	20
Non descriptive*	13	2	15.38
Others	14	2	14.28

* Nondescript dogs: dogs whose breed could not be ascertained

Sex-wise prevalence of canine ehrlichiosis (based on nPCR)

In the present study, a higher prevalence was recorded in males (23.53%) in comparison to females (13.89%). Similar findings were reported by Costa *et al.* (2007), Choudhary *et al*. (2012), Kachhawaha *et al*. (2013), Parmar *et al*. (2013) and Kitaa *et al.* (2014). Sex wise prevalence of canine ehrlichiosis is given in Table 2.

This over-representation by males could be due to bias by most dog owners for males as compared to females based on reproductive behavior and for security purpose. It may be the probable reason of occurrence of high prevalence in males as compared to females.

Breed-wise prevalence of canine ehrlichiosis (based on nPCR)

The present study reported maximum prevalence in German Shepherd dogs (26.31%) followed by 20% in Saint Bernard, 17.24% in Labrador, 15.38% in Non descriptive and 14.28% in others. The breed wise prevalence is presented in Table 3.

This study revealed that German shepherd dogs were more susceptible for canine ehrlichiosis which is in agreement with the observations of Bindu *et al*. (2006), Choudhary *et al*. (2012), Singh *et al*. (2014), Kitaa *et al.* (2014) and Guedes *et al.* (2015). The finding supports the hypothesis that GSD are more susceptible to the disease due to weaker cellular immune response against *E.canis* (Nyindo *et al*., 1980 and Harrus *et al*., 1997). However, Liddell *et al.* (2003) did not observe any differences among dogs with or without confirmed ehrlichiosis by sex, age, and breed or fertility status. The higher numbers may be explained by the popularity of this breed as guard dogs.

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