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Research Article

Molecular detection of canine ehrlichiosis by nested PCR

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Abstract

***Ehrlichia canis* is primarily implicated in canine ehrlichiosis. *E. canis* is a small, gram-negative, obligatory intracellular, pleomorphic coccoid bacteria belonging to the family *Ehrlichiaceae*. A molecular technique like polymerase chain reaction (PCR) using parasite-specific primers provides a better diagnostic tool in terms of both sensitivity as well as specificity. A total number of 52 clinically suspected cases of ehrlichiosis from dogs of different breeds, ages and genders were presented. Dogs were tentatively selected based on clinical examination including history of ticks or presence of tick infestation, physical examination (temperature, respiration rate and pulse rate) and clinical signs (fever, anorexia, melena, epistaxis, anaemia, petechial or ecchymotic haemorrhages, lymphadenopathy and corneal opacity). Nested PCR technique was adopted as a confirmatory diagnostic technique, which revealed eleven positive cases of *E. canis*.**

Key words: Canine ehrlichiosis, *Ehrlichia canis*, Nested polymerase chain reaction, Parasite specific primers

Highlights

- The blood samples from the suspected dogs were screened for ehrlichiosis by using nested polymerase chain reaction (nPCR).
- The 16S rRNA gene was targeted for nPCR amplification.
- Eleven (21.15%) out of fifty two cases were positive for *E. canis*.

INTRODUCTION

Canine ehrlichiosis is an important tick-borne disease of dogs worldwide. The usual diagnostic techniques include the portraying of intra-cytoplasmic *Ehrlichia* spp. in blood or buffy coat smears as well as inclusions of corpuscles or morulae within monocytes (Borin *et al*., 2009), immunological techniques viz. Dot-enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence assay (IFA) (Nakaghi *et al*., 2008), demonstration of organism *in-vitro* cell cultures (Iqbal *et al*., 1994) and molecular techniques such as nested polymerase chain reaction (nPCR) (Nakaghi *et al*., 2008; Souza *et al*., 2010). Serology (ELISA and IFA) is the most commonly used diagnostic method in canine ehrlichiosis (Belanger *et al*., 2002). However, cross-reactivity and an inability to differentiate between current and past infections are disadvantages of these methods (Waner *et al*., 2001). Therefore, molecular techniques like polymerase chain reaction (PCR) using parasite-specific primers provide a better diagnostic tool in terms of both sensitivity as well as specificity and have been widely used in the laboratory diagnosis of canine ehrlichiosis (Carlos *et al*., 2007). PCR is a sensitive method for the prompt detection of ehrlichiosis in dogs even before the onset of clinical signs (McBride *et al*., 1996). Therefore, the advantages of molecular detection of *Ehrlichia* include diagnosis before the development of antibodies in the early stages of the disease and identifying new and also closely related species of *Ehrlichia* using

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species-specific primers and sequencing (Chen *et al*., 1994).

MATERIALS AND METHODS

A total number of 52 suspected cases of ehrlichiosis from dogs of different breeds, ages and genders were presented in the Veterinary Clinical Complex of Faculty of Veterinary & Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, U.P., where ten apparently healthy dogs brought for general health check-up were also taken as a control group**.** Dogs were tentatively selected based on 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, lymphadeno-pathy and corneal opacity.

For nested polymerase chain reaction (nPCR), blood sample was collected in EDTA vial and was stored at -20°C until DNA extraction. To conduct the PCR assays, genomic DNA was isolated from whole blood using a QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per the manufacturer's protocol. The 16S rRNA gene was targeted for PCR amplification. The previously described genus (*Ehrlichia*)-specific primers [ECC (5′ -AGAACGAACGCTGGCG GCAAGC-3′) and ECB (5′-CGTATTACCG CGGCTGCTGGCA-3′)] and *E. canis*-specific primers [ECAN5 (5′-CAATTATTTATAGCC TCTGGCTATAGGA-3′) and HE3 (5′-TATA GGTACCGTCATTATCTTCCCTAT-3′)] (Anderson *et al*.,1992; Dawson *et al*., 1994; Dawson *et al*., 1996; Murph y *et al*., 1998) were used in current study.

RESULTS

Nested PCR technique was adopted as the confirmatory diagnostic technique, which revealed eleven positive cases of *E. canis* out of fifty two suspected dogs and the healthy controls. The extracted DNA from blood samples were of satisfactory quality as presented in Fig.1. Genus-specific PCR products were obtained from the isolated genomic DNA samples using specific primers ECC and ECB, which were subsequently employed as templates in nested PCR to produce a 389 bp amplicon, specific for *E. canis* in case positive samples using species-specific primers ECAN5 and HE3; while no amplification as observed in the samples (Fig. 2).

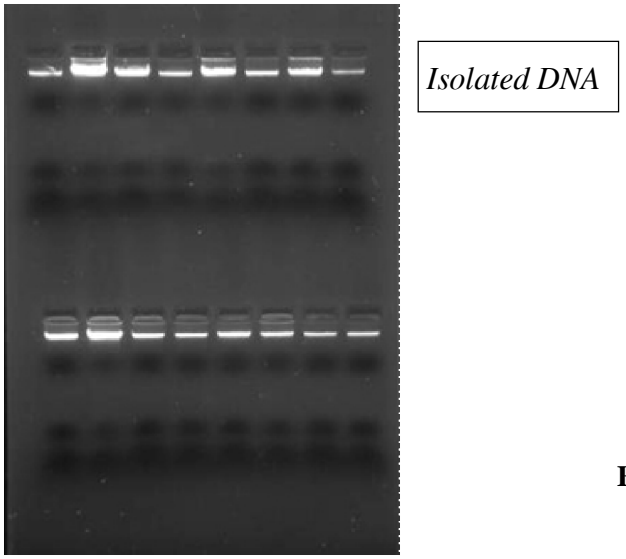

Fig. 1. Screening of isolated DNA on 1.5% agarose gel electrophoresis using UV trans-illuminator imaging system

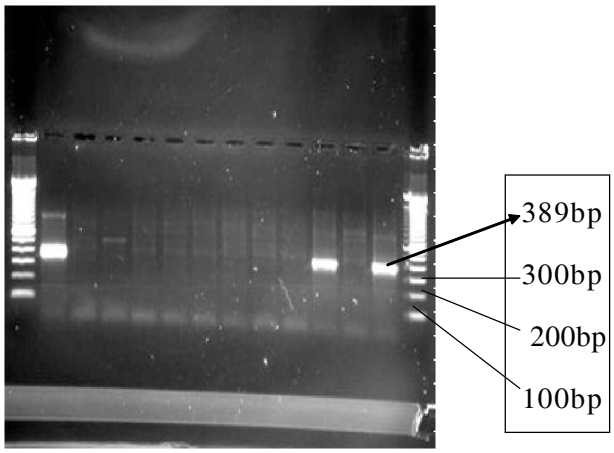

Fig. 2. Amplicon (389bp) of *E. canis* 16S rRNA amplified by nPCR on 1.5% agarose gel electrophoresis, Lane M = Gene-ruler TM 100 bp ladder, lane 1–12 = clinically suspected samples and 1, 10 and 12 - positive samples of canine ehrlichiosis

DISCUSSION

In the present study, *E. canis* was successfully detected using nested-PCR. However, it is important to bear in mind that sub-clinical and chronic ehrlichial infections are not as readily diagnosed as acute infections when canine blood is used to detect *E. canis*. Therefore, ideally, PCR using both blood and spleenic aspirates should be considered to overcome this limitation (Harrus *et al*., 2004). Although PCR makes it possible to detect all the sequenced *Ehrlichia* spp. and can be used to identify the individual species of *Ehrlichia* by designing species-specific primers, sensitivity is influenced by the sort of samples used (Harrus *et al*., 1998). During the chronic phase of the disease, the sensitivity of PCR in blood samples may decrease because of low number of organisms present in the sample. Additionally, there is no standardization among the laboratories, and insufficient quality controls can result in both false-positive and false-negative results (Neer *et al*., 2002).

In conclusion, *E. canis* DNA was detected in the suspected dogs, and the overall detection rate of *E. canis* was 21.15%. The detection of *E. canis* DNA by nPCR in the present study confirmed the presence of substantial natural infection among the dog population.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SB: Preparation of diagnostic technique; PRA: Correction of language of research paper.

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