Development of multiplex PCR for the simultaneous detection of *Trypanosoma evansi, Babesia canis and Hepatozoan canis* in dogs

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ABSTRACT

Since co-infections with canine tick borne pathogens are common there is greater risk for multiple infections which may necessitate different clinical management strategies. In the present study a multiplex polymerase chain reaction (mPCR) by using the species-specific primers was developed for the simultaneous detection of the most pathogenic haemoparasites, *Trypanosoma evansi, Babesia canis and Hepatozoan canis* from the blood of naturally-infected dogs so as to enable early diagnosis and treatment.

Keywords: Multiplex PCR; haemoparasites; blood; dogs

INTRODUCTION

Haemoparasitism is a factor which poses serious health problems in domestic dogs such as retarded growth, generalized ill health, suppressed immunity and reduced work efficiency. Babesiosis followed by ehrlichiosis, hepatozoanosis and trypanosomosis are the most prevalent haemoparasitic infections of dogs in south India. Tick may also transmit multiple infections in dogs from endemic areas since the same tick species can be a common vector of several parasites (Kledmanee et al 2009) and this warrants awareness among veterinary professionals and practitioners. The detection of multiple haemoparasites in field cases by single PCR is time consuming and laborious which would delay the selection of treatment strategies for combination of parasites. This paper describes the development of a multiplex PCR targeting the simultaneous amplification of the specific genes of the important pathogenic haemoparasites of dogs.

MATERIAL and METHODS

Sample DNA was extracted from blood by using Dneasy blood kit (Qiagen) and PCR was standardized using species-specific primers targeting specific amplicons (as documented in Table 1) for the simultaneous detection of the haemoparasites from the blood samples of dogs. The blood samples which were previously positive for the above parasites by blood smear examination and single PCR were mixed and used as template samples for *Trypanosoma evansi, Babesia canis and Hepatozoan canis*. A PCR master mix of 2x (Ampliqon, Denmark) concentration was used in the reaction mixture and 100 bp DNA ladder (GeneDirex, USA) as a marker in gel electrophoresis. The forward and reverse primers targeting the beta tubulin mRNA gene of *T evansi* and 18S rRNA gene of *H canis* were designed using Fast PCR software whereas the primers specific to 18S rRNA gene of *B canis* (Laha et al 2014) were custom-synthesized.

The PCR was developed with following reaction mixture and cycling conditions:

DNA template: 4 µl, master mix: 13 µl, forward and reverse primer (10 pico moles each): 1 µl each (for each parasite), molecular grade water: 4 µl

The cycling conditions followed for the amplification were initial denaturation: 95°C for 8 min, denaturation: 94°C for 30 sec, annealing: 55°C for 30 sec and extension: 72°C for 45 sec with 30 cycles and final extension: 72°C for 10 min. The gel was visualized

Table 1. Sequences of the primers targeting specific genes of the haemoparasites

Haemoparasite	Nucleotide sequence (5'-3')	
	Forward	Reverse
B canis	AGGGAGCCTGAGAGACGGCTACC	TTAAATACGAATGCCCCCAAC
H canis	CTGACCTATCAGCTTTCGAC	CAGCAGAACTTCAACTACGAGC
T evansi	AATGGACTCCGTACGTGC	GTCCATACCCTCGCCAGTGTAC

under UV transilluminator and the images were documented using the gel documentation system (Vilber Lourmat, France).

RESULTS and DISCUSSION

Blood smear examination though has been the simplest and reasonably sensitive during acute infections, PCR is found to be more useful in the detection of subclinical and asymptomatic carriers and thus ideally suited for epidemiological investigations (Abd Rani et al 2011). In this study mPCR assays were developed for the simultaneous detection of *T evansi* and *B canis* targeting beta tubulin mRNA gene and 18S rRNA gene with amplicon size of 997 and 450 bp respectively and *T evansi* and *H canis* targeting beta tubulin mRNA gene and 18S rRNA gene with amplicon size of 997 and 346 bp respectively (Fig 1). Previously Kledmanee et al (2009) and Peleg et al (2010) also developed an mPCR for simultaneous detection of *E canis*, *Babesia* spp and *H canis* in dogs in Thailand and Israel respectively. Jain et al (2018) developed an mPCR for the detection of *Babesia canis vogeli*, *B gibsoni* and *E canis* in naturally-infected dogs in south India. However existing data on the application of mPCR in the simultaneous detection of these haemoparasitic infections in dogs are insufficient.

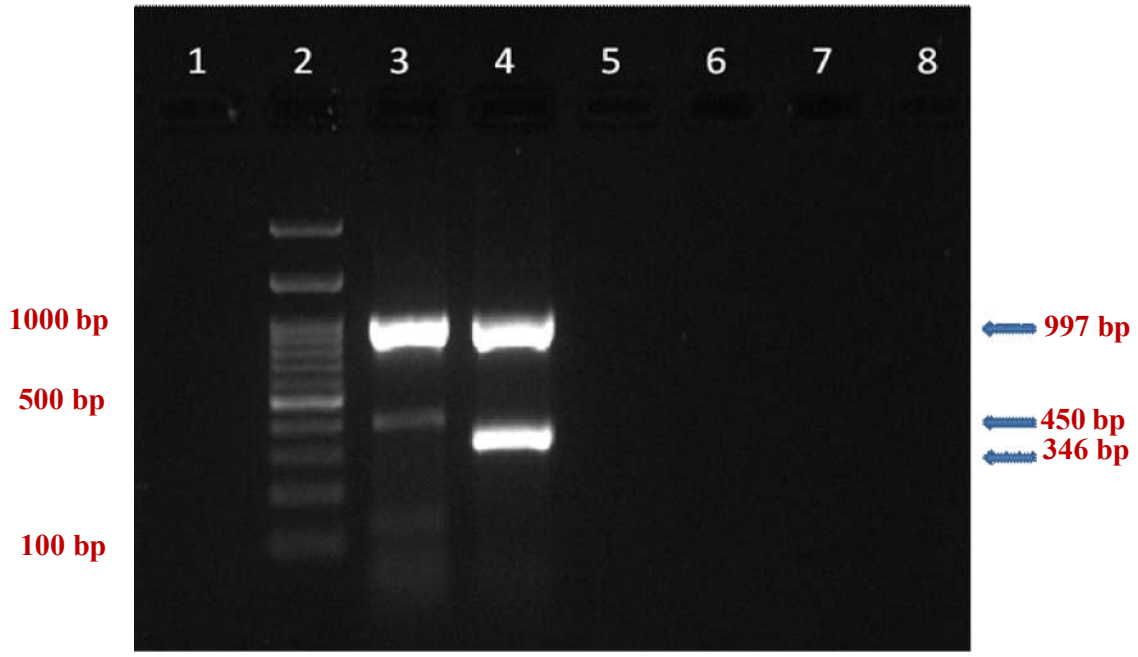

Fig 1. Multiplex PCR amplicons of *T evansi*, *B canis*, *T evansi* and *H canis* showing bands at 997, 450, 997 and 346 bp respectively

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