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Short communication

Detection of *Babesia bigemina* DNA in ticks by DNA hybridization using a nonradioactive probe generated by arbitrary PCR

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Abstract

The Dig-labeled probe specific to *Babesia bigemina* generated from monomorphic RAPD fragment of ~873 bp size amplified by a 10 mer CGGTGGCGAA, detected up to 100 ng of template DNA. This nonradioactive probe also detected *B. bigemina* in preparations of larval tick DNA from two of the five samples on dot-blot hybridization.

Keywords: DNA probe; *Babesia bigemina*; Ticks; RAPD-PCR

1. Introduction

Babesiosis is one of the economically important tick-borne protozoan diseases of cattle in tropical and subtropical regions of the world. With the increased reports of replacement of multihost tick by one host tick (Khan, 1990, 1994; Sangwan et al., 2000), there is great concern over the diseases transmitted by the common one host tick namely, *Boophilus microplus*. Hence, there is an urgent need to monitor the epidemiology of the diseases transmitted by them. Diagnosis of babesiosis by various methods has been a subject of several reviews (Bose et al., 1995; Figueroa and Buening, 1995; Montenegro-James and James, 1998; OIE, 2000; Sparagano, 1999).

As there are serious limitations with ELISAs for diagnosis of *Babesia bigemina* infection due to the poor quality of the parasite antigen prepared from infected blood, molecular or DNA based methods are preferred for unequivocal detection of the parasite. DNA hybridization, the formation of duplex between two complementary nucleotide sequences is of great value in epidemiology and clinical diagnosis of infectious diseases.

The RAPD technique, widely used in phylogenetic studies and assessment of genetic variation, has shown potential for detecting species-specific or strain-specific fragments, which in turn may be used as probes (Hardrys et al., 1992). The present communication reports the development of a highly specific

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and sensitive nonradioactive probe generated from monomorphic fragment of RAPD-PCR.

2. Materials and methods

2.1. Parasite isolates and isolation of genomic DNA

B. bigemina isolates from Izatnagar (Uttar Pradesh, North India) and Wayanad (Kerala, South India) were used in the present study. Izatnagar isolates were isolated from naturally infected bovine calves procured from the Division of Livestock Production and Management, Indian Veterinary Research Institute, Izatnagar. Wayanad (Kerala) isolates of *B. bigemina* were isolated by experimental feeding of the larvae of engorged adult *Boophilus microplus* collected from Wayanad (Kerala), a *Babesia* endemic area on naive bovine calves.

After confirming the infection status by the presence of paired or single piroplasms, the calves were splenectomised. On 6th day post-splenectomy, the animals were administered dexamethasone (Dexona at 3 ml/50 kg) intramuscularly, daily for 5 days for achieving immunosuppression. At the peak parasitaemia, blood was collected in the presence of an anticoagulant. Purification of *B. bigemina* rich erythrocytes free of leucocytes from infected bovine blood was made according to the method of Ray et al. (1998). Genomic DNA was isolated from leucocyte-free *B. bigemina* rich erythrocytes following the technique outlined by Sambrook et al. (1989).

Larval ticks from adult *Boophilus microplus* engorged on five different bovine animals of *Babesia* endemic area of Wayanad were washed in absolute ethanol prior to trituration in a sterile mortar. Genomic DNA of larval ticks was also isolated using the phenol–chloroform–isoamyl alcohol method of Sambrook et al. (1989).

2.2. RAPD-PCR and identification of monomorphic fragment

The RAPD-PCR reaction was set up in 25 μl reaction volume in 0.2 ml thin walled PCR tubes (Axygen). The reaction mixture was as follows: 1 μl of the template DNA (10 ng/μl), 2.5 μl of 10× PCR buffer (Bangalore Genei with 15 mM $MgCl_2$), 1 Unit of Taq DNA polymerase (Bangalore Genei) and 1 μl of primer (15 pmol or 50 ng). The random 10-mer BG27 (CGGTGGCGAA) with 70% GC content was used for the assay. A control sample containing bovine leucocyte DNA was run along with reaction.

The cycling conditions used were initial denaturation at 94 °C for 5 min, followed by 45 cycles each of 1 min denaturation at 94 °C, 45 s annealing at 36 °C and 1 min elongation at 72 °C. This was followed by a final extension for 5 min at 72 °C. After the reaction, the products were analyzed on an agarose (1.5%) gel. Highly intense monomorphic fragment was identified and this was eluted from agarose gel using Clean Genei Kit (Bangalore Genei, India).

Reamplification of the selected RAPD band of interest was achieved with the same primer and the fragment was eluted after electrophoresis on a 2% agarose gel.

2.3. Southern transfer

To confirm specificity, agarose gel after electrophoresis of the RAPD-PCR products of four *B. bigemina* isolates and leucocyte DNA, was Southern transferred on to nitrocellulose membrane. The Southern transferred membrane was then subjected to UV cross-linking and later used for hybridization with the newly developed Dig-labeled probe.

2.4. Dot-blotting of genomic DNA

Dot-blotting of the genomic DNA of *B. bigemina*, *Theileria annulata*, *Trypanosoma evansi*, bovine leucocyte DNA, bubaline leucocyte DNA and *Toxoplasma gondii* DNA on nitrocellulose membrane (Sigma) was carried out using minifold dot-blotting apparatus (Bio-Rad). The DNA isolated from larval ticks was also used for dot-blotting on to nitrocellulose membrane. The blot was dried and soaked in 0.4N NaOH for 10 min. It was further rinsed in 2× SSC and air-dried. The blot was subjected to UV cross-linking (120 mJ/cm^2 energy) for 5 min using UV cross-linker (UVP).

2.5. Digoxigenin DNA labeling, dot-blot hybridization and detection

The process of labeling, dot-blot hybridization and detection by colorimetric assay were carried out