Polymerase chain reaction based detection of *Toxoplasma gondii* from lymph node aspirates of dogs

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Abstract The purpose of the study was to detect DNA of *Toxoplasma gondii* in lymph node aspirates of dogs using amplification of SAG-2 gene fragment. Blood and lymph node aspirates were collected from 20 clinically ill dogs of different age groups. Lymph node aspirates were analysed with polymerase chain reaction (PCR). Two numbers of dogs were found to be positive by PCR. The PCR positive dogs showed modified direct agglutination test (MDAT) titre of 1:512 and 1:4,096. The remaining sera samples showed the titre value of less than 1:64 by MDAT and were also negative by PCR. This study indicates that lymph node aspirates could be an alternative ideal specimen for the detection of *T. gondii*.

Keywords *Toxoplasma gondii* · Dogs ·
Lymph node aspirates · PCR · MDAT

Introduction

Toxoplasmosis, a zoonotic disease, is caused by *Toxoplasma gondii*, an obligate intracellular coccidian parasite. The infection is common throughout the world and it is

estimated that over 500 million humans are infected with *T. gondii* (Singh 1999). Antibody to *T. gondii* has been detected in animals and humans around the world using various diagnostic tests. More recently, the polymerase chain reaction (PCR) technique is being used to confirm human and animal toxoplasmosis. In the present investigation, the lymph node aspirates collected from ill dogs, were tested by PCR for the presence of *T. gondii* DNA.

Materials and methods

Dogs that attended the out-patient units of Madras Veterinary College teaching hospital, Chennai, India with clinical signs of dermatitis, lymphadenopathy, tumor, corneal opacity and canine distemper were selected for the study. From among 20 such dogs, lymph node aspirates were collected from prescapular lymph node in PBS (pH 7.4) as per the standard procedure and the material was used for PCR assay (Sambrook and Russell 2001). The animals were grouped according to age as 2–4 years (4 nos), 4–6 years (4 nos), 6–8 years (6 nos) and above 8 years (6 nos). Blood samples (2 ml) were collected aseptically from the same dogs and serum was separated. These serum samples were stored at –20 °C until further use. Reference positive and negative control sera were provided by Dr. David Buxton, Moredun Research Institute, International Research Centre, UK and stored at –20 °C until use.

DNA extraction was done by standard protocol (Sambrook and Russell 2001). The lymph node aspirates (~50 µl) collected from clinically ill dogs were added to 1 ml of PBS, centrifuged and the pellet resuspended in 10 µl of distilled water. To this, 1 ml of extraction buffer with Proteinase K (200 µg/ml) was added and incubated at 56 °C for 3 h. The tubes were then centrifuged at 2,000 rpm for

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10 min and supernatant was collected. To this (~1 ml), 0.2 ml of ammonium acetate, followed by 2 ml of absolute ethanol were added and gently mixed. The tubes were centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and 1 ml of 70 % ethanol was added and centrifuged at 10,000 rpm for 1 min. The washing step was repeated twice. After the final wash, the supernatant was discarded and the tubes were air dried to remove traces of 70 % ethanol. The sediment containing DNA was resuspended in 10 µl of distilled water and stored at –20 °C. The nucleotide sequences of the primers for amplification of the 5' region of the *SAG2* gene (Howe et al. 1997) were as follows. The primer sequences are 5'-GAAATGTTTCAGGT TGCTGC-3' (forward) and 5'-GCAAGAGCTAAGTTG AACAC-3' (reverse). Twenty-five µl reactions were set up for each sample. The reaction mix consisted of 2 µl of template DNA (sample), 5 µl of 10 × PCR buffer (Genei, Bangalore), 0.2 µl (1 U) of *Taq* DNA polymerase (Genei, Bangalore), 0.2 µM of each dNTP (Genei, Bangalore) and 2 µl each of forward and reverse primer (15 pmol or 100 ng of each primer) made up to 25 µl using distilled water. Routinely, master mix was set up and distributed to the PCR tubes, to which 2 µl of template DNA of each sample was added. A positive control (*T. gondii*, VEG strain DNA was provided by Dr. Sreekumar, TANUVAS, Chennai) and negative control (water) were run along with the samples. The cycling conditions used were as follows. After an initial denaturation of 5 min at 94 °C, 35 cycles of 45 s denaturation at 94 °C, 1 min annealing at 60 °C and 1 min extension at 72 °C was carried out which was followed by a final extension for 10 min at 72 °C (Sambrook and Russell 2001). The PCR products were analysed by agarose gel electrophoresis in a submarine horizontal electrophoresis unit (Genei, Bangalore). After sufficient migration, the gel was examined under UV transilluminator in a gel documentation system and photographed.

Modified direct agglutination test reference antigens were kindly supplied by Department of Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India. Mouse adapted RH strain of *T. gondii* was obtained from Department of Veterinary Parasitology, Veterinary College, Tirupathi, India. Modified direct agglutination test antigen: The modified direct agglutination test antigen was prepared as per the procedure of Vijaya Bharathi et al. (2003) and stored in sterile vials at 4 °C. A drop of the suspension was examined under high power to ascertain whether the tachyzoites retain their normal crescentic shape and sufficient concentration (10^6 /ml). Modified direct agglutination test was done as per the procedure described by Desmonts and Remington (1980). Double-fold dilutions of serum samples from 1:2 to 1: 4,096 were tested. To avoid interpreting non-specific reactions as positive results, only titres of 1:64 or high were

considered as positive for modified direct agglutination test (MDAT).

Results

Of the 20 lymph node aspirates from cases of clinically ill dogs, two were found to contain DNA of *T. gondii*. Amplification of fragments in the size range of 240 bp was achieved in two samples (Fig. 1). The target fragment was also amplified from the positive control DNA. No amplification was noticed in any of the other samples as well as negative control DNA. In the present study the *T. gondii* positive samples were obtained from an 8-year-old dog with a malignant tumor in the chest region, and a 5-year-old dog suspected to be suffering from canine distemper infection. The serum from the first dog was positive by MDAT with the titre of 1:512. The second positive dog had a titre of 1:4,096 for *Toxoplasma* antibodies using MDAT. The other PCR negative dogs showed negative results against *T. gondii* antibodies by MDAT with the titre value of less than 1:64.

Discussion

In the present investigation, among 20 lymph node aspirates from clinically ill dogs, two were positive by PCR. Sera samples from the positive dogs were also positive by MDAT. Dubey and Beattie (1988) have indicated that *T. gondii* multiply in the lymph node of animals. Lymph

Fig. 1 Agarose gel electrophoresis showing amplification of *Toxoplasma gondii* specific 240 bp *SAG2* gene fragment from the lymph node aspirate (LN) from dogs. *M* molecular weight marker (50 bp DNA ladder), *C* control *T.gondii* DNA (VEG strain), *LN* lymph node aspirate

node has been found to be an ideal tissue for diagnosis of *T. gondii* using PCR (Sreekumar et al. 2004). This study indicated that *T. gondii* multiply in the lymph node of animals. It is concluded that lymph node has been found to be an ideal tissue for the collection of specimen for diagnosis of *T. gondii* using PCR. Both the positive cases suffered from immunosuppression due to malignancy and canine distemper. Thus, it is possible that the immunosuppression due to malignancy and canine distemper virus had caused reactivation of a pre-existing *T. gondii* infection in these dogs. It has already been authenticated that *T. gondii* is an important opportunistic pathogen of the immunocompromised persons (Belanger et al. 1999).

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