DIAGNOSIS OF CANINE TRYPANOSOMOSIS BY POLYMERASE CHAIN REACTION

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Received - 19.10.12
Accepted - 16.02.13

Abstract

A clinical case of trypanosomosis in a German Shepherd dog and its diagnosis by polymerase chain reaction(PCR) was described.The dog was presented with clinical signs such as pyrexia, bilateral corneal opacity, pale mucous membranes, emaciation and lymphadenopathy. Wet film and stained smear examination of peripheral blood revealed Trypanosoma evansi. Blood sample was also subjected to PCR for detection of parasitic DNA using the forward and reverse Kin primers, Kin-1: 5'-GCG TTC AAA GAT TGG GCA ATG-3' and KIN-2: 5'-CGC CCG AAA GTT CAC C-3', which bind to an internal transcribed spacer region (ITS1). Polymerase chain reaction showed positive result and the amplicon size for T.evansi was 540bp. The case was successfully treated with a single dose of diminazine aceturate @10 mg/kg BW.

Keywords: *Trypanosoma evansi, PCR, Kin Primers, Diminazine aceturate.*

In India *Trypanosoma evansi* causes acute and chronic form of canine trypanosomosis. Biting flies are primarily associated with the spread of the disease. In dogs the disease is characterised by intermittent fever, progressive emaciation, oedema of head, pharyngeal region and other dependant parts, corneal opacity, anaemia and death (Gill,1991). Diagnosis of trypanos omosis can be done by conventional wet film and blood smear examination and by using different serological tests. Polymerase chain reaction (PCR) was reported to be more sensitive than the conventional methods and is useful for detection of low levels of parasitaemia. A case of trypanosomosis in a German Shepherd dog and its confirmation using PCR is reported.

Materials and Methods

A female German Shepherd dog of three years of age was presented at the University Veterinary Hospital, Mannuthy with a complaint of clouding of eyes since a week. The dog was reported to be weak and off feed. Detailed clinical examination of the dog was performed and clinical data were recorded. The peripheral blood was collected from the ear tip and subjected to wet film and blood smear examination. The whole blood samples were collected and analyzed for various haemato-biochemical parameters. Blood sample was also subjected to PCR for detection of parasitic DNA (Desquesnes and Davila, 2002).

Blood sample was collected using EDTA (1mg/ml of blood) as anticoagulant and stored at 4^{0}C till processed. Extraction of genomic DNA was done using phenol-chloroform method (Sambrooke *et al*., 1989) Polymerase chain reaction was carried out using the forward and reverse Kinprimers, Kin-1:5'-GCG TTC AAA GAT TGG GCA ATG-3' and KIN-2:5'-CGC CCG AAA GTT CAC C-3' (McLaughlin *et al*., 1996). These primers bind to an internal transcribed spacer region (ITS1) situated between 18S and 5.8S ribosomal subunit genes on nuclear DNA. The primers

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were custom synthesized and reconstituted in sterile water for assay. A 540 bp fragment of the ITS1 gene was amplified using PCR.

The PCR reaction was carried out in a total reaction volume of 25 µl, using 200 µl thin walled reaction tubes. The reagent concentrations were : 20pM of each primer, 1 unit of DyNAzmeII DNA polymerase (finnzymesoy), 1X PCR buffer (10mMtris-HCl (pH 9.0), 1.5mMMgCl$_2$, 50mMKCl and 0.01 per cent gelatin), 200 µM of each dNTPs, approximately 50ng of genomic DNA.

Table showing the touch down PCR thermocycling protocol used.

Temperature(^{0}C)	Duration	Cycles
94	5min.	1 cycle
94	30 s	4 cycles
58	30 s	
72	1 min	
94	30 s	8 cycles
56	30 s	
72	1 min.	
94	30 s	30 cycles
54	30 s	
72	1 min.	

The PCR products were loaded on 2% agarose gel and electrophoresis was performed at 120v for one hour and visualized on a gel documentation system.

Results and Discussion

Clinical examination of the dog revealed pyrexia (104.4^{o}F), bilateral corneal opacity, pale mucous membranes, emaciation and lymphadenopathy (Fig.1). Wet film and stained smear examination of peripheral blood revealed *Trypanosoma evansi* (Fig.2). The results of haemato-biochemical estimations are given in the table. There was severe anaemia with reduced haematocrit values and thrombocytopaenia. Blood glucose level was greatly reduced indicating hypoglycaemia which is consistent with the condition. Biochemical estimations revealed hypoprote inaemia with hypoalbuminaenia. Clinical signs and haemato-biochemical findings observed in this case were in concordant with the findings of many workers (Balakrishnan *et al*., 1994., Arora and Pathak, 1995 and Varshney *et al*., 2003).

Polymerase chain reaction showed positive result and the amplicon size for *T.evansi* was 540bp (Fig.3). Several diagnostic assays based on detection of trypanosomal DNA by PCR have been developed and is reported to be more sensitive than conventional diagnostic techniques in different hosts and has the advantage that it can identify parasites at the species level (Wuyts *et al*., 1995., Desquesnes and Davila, 2002., Ravindran *et al*., 2008). The Kin primer based PCR was reported as an effective tool for detecting trypanosomes in naturally infected cattle (Solano *et al*., 1999).

The dog was treated with single dose of Berenil (diminazine aceturate) (10 mg/Kg BW) by deep intramuscular route. Supportive therapy was also given with 50 ml of 25% dextrose solution intravenously and one ml

Table. Haemato-biochemical parameters

Parameter	Value	Parameter	Value
RBC	1.53*106/µl	Platelet count	1 ,23,000/ µl
Haemoglobin	2.4g/dl	Blood Glucose(RBS)	10mg%
VPRC	10%	Total Protein	6.2g%
Total WBC	12,500/ µl	Albumin	1.3g%
Neutrophils	76%	Globulin	4.9g%
Eosinophils	1%	A/G ratio	1:3.7
Lymphocytes	23%	SGPT	12 units

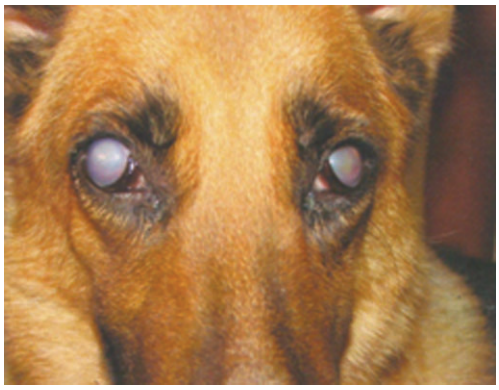

Fig.1: Bilateral Corneal Opacity

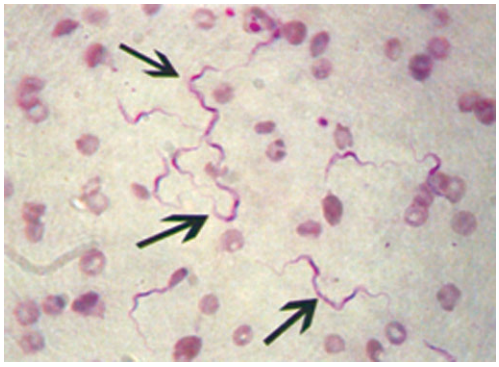

Fig.2: T. *evansi* in blood smear

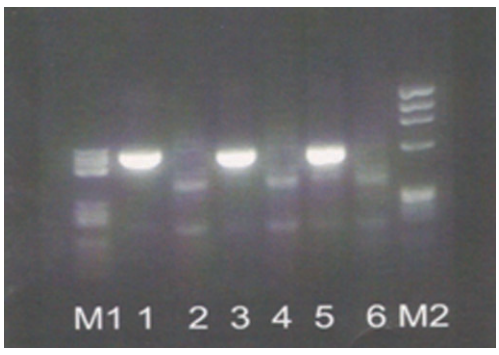

Fig.3: PCR 1,3,5 positive bands at 540bp

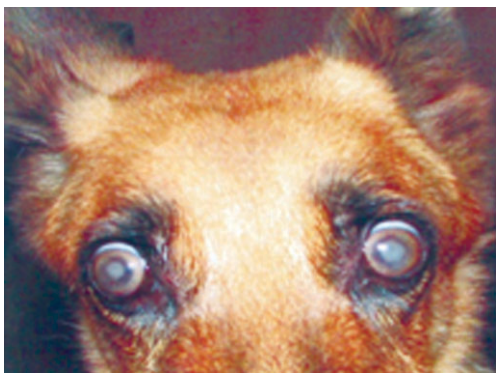

Fig.4: After Treatment

each of iron dextran (Inferon) and B complex vitamins intramuscularly. On day two, the body temperature came down to 102.4^{0}F and there was slight improvement in the appetite and general activity. Wet film and blood smears were negative for trypanosomes. Blood sample was also subjected to PCR which showed a negative result (Fig.3). Corneal opacity was reduced by 5th day (Fig. 4) and the dog resumed its normal appetite and general activity by one week post treatment. Treatment with diminazine aceturate was very effective in eliminating trypanosomes in dogs as reported by Varshney *et al*. (2003).

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