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PREVALENCE OF *Trypanosoma evansi* IN CAMELS THROUGH POLYMERASE CHAIN REACTION AND HAEMATOCRIT CENTRIFUGATION TECHNIQUE IN PUNJAB (PAKISTAN)

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ABSTRACT

Prevalence of *Trypanosoma evansi* infection among camels in Punjab (Pakistan) was studied using haematocrit centrifugation technique (HCT) and polymerase chain reaction (PCR). Two different primer pairs (one specific for *T. evansi* and the other for *T. brucei*) were used. Among 100 camels of either sex and all ages from different localities of Punjab, the prevalence was 4% by haematocrit centrifugation technique, 9% with PCR primer-1 and 13% with primer-2. The observations establishes evidence of the usefulness of PCR as a diagnostic tool for epidemiological studies.

Key words: Camel, PCR, prevalence, *Trypanosoma evansi*

Trypanosomosis (Surra) caused by *Trypanosoma evansi* is one of the major protozoan disease affecting camels and equines in many parts of the world. It is widely distributed in tropical and subtropical countries (Soulsby, 1982). Prevalence of Surra is very important from working animals point of view as it causes remarkable economic loss to the owners. The Infection is manifested in acute and chronic forms. In camel, it generally runs a chronic course which can also be characterised by sub-patent or low parasitaemia and an increase in parasitaemia can take place following stress induced immuno-suppression. Current parasitological and serological diagnostic methods for diagnosis of trypanosomosis have important limitations either in their sensitivity or specificity. These methods are useful only when there is high parasitaemia. Recently, the sensitivity and specificity of PCR has been utilised even to detect the presence of parasites in specific vectors, and reportedly find a single trypanosome (Masiga *et al,* 1992). In Pakistan, prevalence of Surra in camels has been reported by using various parasitological and serological tests (Butt *et al,* 1996; Hasan *et al,* 2006). The current project was designed to study the prevalence of Surra in camels in Punjab by using a highly sensitive and specific DNA amplification based technique. It is hypothesised that *T. evansi* has been originated from *T. brucei* (Luckins, 1998), and therefore two different sets of primers were used to detect trypanosomosis. One was specific for *T. evansi* and other for *T. brucei*.

Materials and Methods

A total of 100 camels of either sex and different ages were randomly selected from different localities of Punjab and 6 ml of blood collected aseptically from the jugular vein with the help of a disposable syringe was transferred to a heparinised vacutainer (Coles, 1986). The samples were stored in capillary tubes at -20°C until further processing.

Blood Examination through Haematocrit Centrifugation Technique (HCT)

HCT was performed according to the method described by Coles (1986).

Blood Examination by Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from blood samples using a commercially available DNA extraction kit GENTRA PUREGENE®, USA. Two different primer pairs were used in the study and their relative specificity to detect *T. evansi* was evaluated. The primer-1 specific for *T. evansi* (RoTat 1.2 forward and RoTat 1.2 reverse) (Claes *et al,* 2004) and primer-2 (TBR1 and TBR2 of *T. brucei*) which has been used to detect *T. evansi* (Ijaz *et al,* 1998) are listed in table 1. The PCR amplification was performed in a 50 µl reaction mixture containing 1X

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Taq Buffer, 0.2 mM dNTP mixture, 1.5 mM $MgCl_2$, 2.5U/µl Taq Polymerase, 4 µM of each primer, 2 µl of DNA extracted from the blood sample and 31.5 µl of DNase free deionised water. The tubes containing the reaction mixture were subjected to 30 cycles of amplification in a thermocycler. During each cycle the DNA sample was denatured at 93°C for 30 seconds, annealed at 45°C for 30 seconds and extended at 72°C for 1 minute. Prior to cycling and at the end of cycling the mixture was subjected to incubation at 93°C for a period of 3 minutes and final extension at 72°C for a period of 5 minutes, respectively. PCR product was then analysed by 2.5% agarose gel electrophoresis.

Results and Discussion

Haematocrit centrifugation technique demonstrated *T. evansi* presence in only 4 samples (4% prevalence) while 9 (9%) and 13 (13%) animals were positive by PCR with primer-1 and primer-2, respectively. Primer-1 was targeted against variant surface glycoprotein (VSG) of *T. evansi* Rode Trypanozoon antigen type (RoTat) 1.2, which is an antigen used for the diagnosis of *T. evansi* in different parts of the world (Verloo *et al,* 2000; Bajyana and Hamers, 1988). However, Ngaira *et al* (2005) has reported the detection of non-RoTat 1.2 *T. evansi* in Kenya. Hence, the 4 samples which were negative with primer-1 may be non-RoTat 1.2 trypanosomes or the animals may be carriers of *T. brucei*. Ijaz *et al* (1998) have reported close homology between *evansi* and *brucei* by using *T. brucei* primers (TBR1 and TBR2) to detect *T. evansi* infection in experimentally inoculated mice but their study was unable to differentiate between *T. brucei* and *T. evansi* infections as till that time nucleotide sequence of *T. evansi* was not available. In a previous study conducted in Punjab (Pakistan) Hasan *et al* (2006) had reported 3.3% and 4% prevalence of *T. evansi* by using HCT & Suratex®. Butt *et al* (1996) had reported 3% prevalence of *T. evansi* by using HCT in camels in and around Faisalabad. Results indicate a significant difference in the sensitivity of the HCT and PCR. A high prevalence of infection with *T. evansi* (9%) observed in the present study with PCR is due to the high sensitivity of the technique. The difference in the sensitivity of PCR and HCT has also been reported by Njiru *et al* (2004) who reported 5.3% prevalence by using HCT and 26.6% by PCR in Kenya. Prevalence of *T. evansi* in camels in Punjab (Pakistan) is low compared with 33% in Jordan (Al-Rawashdeh *et al,* 2000), 17.05% in Rajasthan, India (Singh *et al,* 2004), 10% in Iran (Zarif-Fard and Hashemi-Fesharki, 2001), 33% in Sudan (Elamin *et al,* 1999) and 56% in Somalia (Baumann and Zessin, 1992). The low prevalence

Table 1. Primer sequences and predicted amplicon size.

Primers	Sequences	Amplicon Size
Primer-1		
RoTat 1.2		
FORWARD	GCGGGGTGTTTAAAGCAATA	205
RoTat 1.2		
REVERSE	ATTAGTGCTGCGTGTGTTCG	
Primer-2		
TBR1	GAATATTAAACAATGCGCAG	164bp
TBR2	CCATTTATTAGCTTTGTTGC	

*Primer sequences are shown in the 5´-3´ orientation.

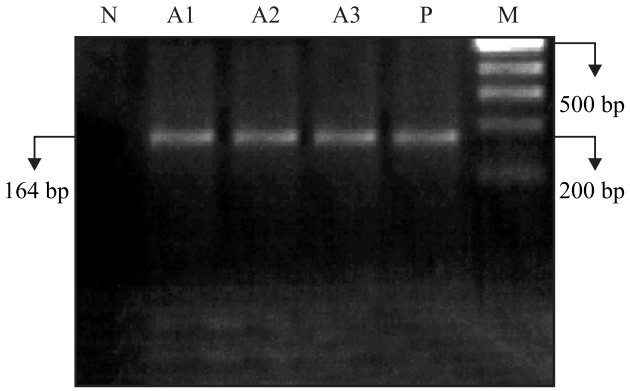

Fig 1. Amplicon of 164 bp with Primers specific for *T. brucei.*
N = Negative control. P=Positive control
A1, A2, A3 = Experimental samples.
M = Marker (1 Kb ladder)

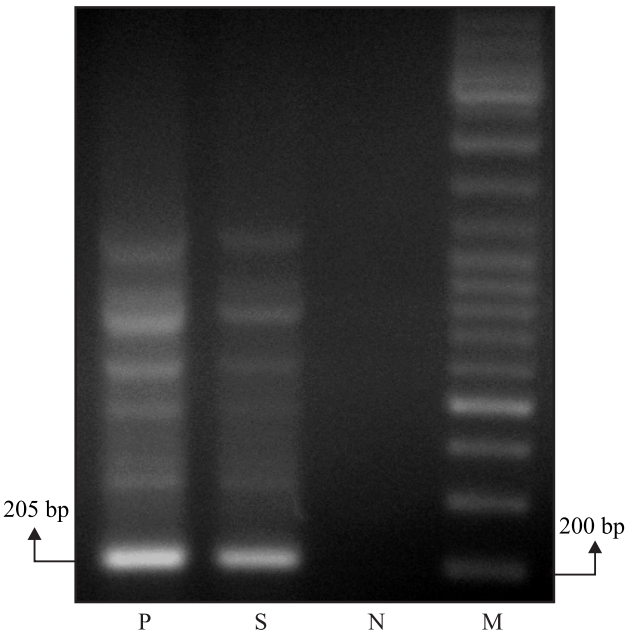

Fig 2. Amplicon of 205bp with primers specific for *T. evansi.*
N = Negative control. P = Positive control
A2 = Experimental samples. M = Marker (1 Kb ladder).

in this study may be due to the awareness of the animal owners about the disease, variation in the geographical conditions, adoption of better control measures, greater tendency to treatment in this area and seasonal differences. Further studies need to be conducted for the detection of reservoirs, prevalence in different geographical regions of the country for establishing control measures for the disease.

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