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Amplification of Microsatellite Loci Using Genomic DNA of *Trypanosoma evansi* Isolates from Different Geographical Areas of India

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ABSTRACT

Saini VK, Kaushik J, Singh J, Sarkhel SP, Kumar S, Gupta AK, Yadav SC and Kumar R. (2012). Amplification of microsatellite loci using genomic DNA of *Trypanosoma evansi* isolates from different geographical areas of India. *J Immunol Immunopathol*. 14(2): 110-114.

Surra is an economically important disease of livestock caused by an extracellular haemoprotozoan parasite, *Trypanosoma evansi.* In this study, genomic DNA was extracted from six *Trypanosoma* isolates collected from different geographical areas, and twelve microsatellite loci were successfully amplified in polymerase chain reaction (PCR) with selected microsatellite primers. For most of the microsatellite loci, the PCR amplicon size in Indian isolates was found similar to that of *Trypanosoma brucei* TREU927, except for three microsatellite loci (TB11/1, TB10/1 and TB7/12). For the microsatellite loci TB11/1, TB10/1 and TB7/12, the PCR amplicon size in *T. evansi* isolates was found to be 160, 210 and 200 (bp), respectively. Similar amplicon size patterns using twelve microsatellite primers were observed in all six Indian *T. evansi* isolates used in this study.

Keywords: Surra, Microsatellite loci, *Trypanosoma evansi*, Polymerase chain reaction

INTRODUCTION

Infection with *Trypanosoma evansi* causes a disease named surra. It is transmitted mechanically by biting insects such as tabanids and *Stomoxys* spp (Hoare, 1972). It is known to infect a variety of domestic (horses, camels, cattle, buffaloes and dogs) and wild (tigers, elephants, deer, coatis, capybaras and marsupials) animals in Asia, Africa, Middle East and South America (Lun *et al*., 1993; Omanwar *et al*., 1999; Njiru *et al.,* 2004; Herrera *et al*., 2004). Surra may occur in acute, sub-acute, chronic and inapparent forms. Both acute and sub-acute forms of the disease are usually fatal. Variations in parasite strain virulence, variation in host susceptibility and variation in disease manifestation (acute, sub-acute and chronic) are commonly observed conditions in the field in this disease.

With the advent of PCR, it was realised in the late 1980s that microsatellites may be the most powerful mendelian markers ever found. They have since been widely studied in conjunction with some genetic diseases. They have also been used in mapping programmes, and by population biologists, for kinship investigations and for more classical studies of the population genetic structure. Several approaches were used to study genetic diversity *viz.,* isoenzyme electrophoresis, restriction fragment-length polymorphism, amplified-fragment length polymorphism, random amplified polymorphic DNA and microsatellite genotyping. Microsatellites are short tandem repeats (2–6 bp) occurring abundantly and randomly distributed over eukaryotic genomes. Microsatellite profiling of different isolates may be a useful alternative for genetic diversity studies among

different isolates due to their random and abundant availability in the eukaryotic genome (Hamada *et al.,* 1982). Microsatellites are very useful because they detect high level of polymorphism, high reproducibility and require low quantity of template DNA. The high variability in microsatellites makes them suitable for identifying genetic variability in closely related species, and therefore they are widely used in genetics and in phylogenetic studies (Oliveira *et al.,* 1997; Schwenkenbecher *et al.,* 2004). This study was undertaken to study PCR-based amplification of microsatellite loci in *T. evansi* isolates of different geographical areas of India.

MATERIALS AND METHODS

Source of isolates used in this study

Six isolates of *T. evansi* were used in this study, collected from different geographical regions *viz.,* Hisar (Haryana), Hardoi (UP), Bikaner (Rajasthan), Junagarh (Gujarat) and Karnal (Haryana) and propagated *in vivo* in mice. The details of isolates are described in Table 1.

Table 1: Details of *T. evansi* isolates used in this study

Identity of *T. evansi* isolates	Host
T.ev-India-NRCE-Horse1 /Hisar/Haryana	Horse
T.ev-India-NRCE-Camel 1/Bikaner/ Rajasthan	Camel
T.ev-India-NRCE-Donkey 2/Junagarh/Gujarat	Donkey
T.ev-India-NRCE-Donkey1/Hardoi/Uttar Pradesh	Donkey
T.ev-India- NRCE-Cattle1/ Karnal/Haryana	Cattle
T.ev-India-NRCE-Horse 2 /Karnal/Haryana	Horse

Propagation of *T. evansi* isolates in mice

Parasites were maintained and propagated *in vivo* in mice model. Initial inoculation to mice was done with 1×10^5 trypanosomes by intra-peritoneal route. Thereafter, the parasitaemia was monitored regularly. At the peak of peripheral parasitaemia, mice were bled by cardiac puncture for isolation and purification of parasites. The trypanosomes from *T. evansi*-infected mice blood (first parasitaemic peak) were purified by DEAE-cellulose chromatography, followed by centrifugation (Lanham and Godfrey, 1970).

Extraction of *T. evansi* DNA

Genomic DNA was extracted from purified *T. evansi* pellets with some modification of the method used by Sambrook and Russell (2001). In brief, 500 µl of purified *T. evansi* pellets was treated with 5.0 ml cell lysis buffer mixed well and incubated for 1 hr at 37°C. After incubation, 50 µl of proteinase K was added to a final concentration of 100 µg/ml and mixed well. Later, it was incubated at 50°C for 3 hrs in a water bath with intermittent shaking. An equal volume of phenol:chloroform:isoamyl alcohol (PCI) in 25:24:1 ratio was added to the above solution and mixed for 1 min. The tube was centrifuged at 3000 x g for 10 min at room temperature (RT), and the upper aqueous phase was collected in a clean centrifuged tube. The aqueous phase was once more treated with an equal volume of PCI and then once with an equal volume of chloroform:isoamyl alcohol (24:1). The upper aqueous phase was finally collected after centrifugation at 3000 x g for 10 min at RT. To the aqueous phase, one-tenth volume of 3M sodium acetate, pH 5.2 was added, and DNA was precipitated by adding an equal volume of ice-cold isopropanol and incubating it at 4°C overnight. The DNA pellet was obtained by centrifugation at 4000 x g for 15 min at RT. The pellet was washed once in 70% ethanol and centrifuged at 12000 rpm for 8 min. The DNA pellet settled at the bottom of the tube and was air dried by placing the tube open for 2-3 min. Finally, the DNA pellet was resuspended in 30-50 µl of nuclease-free water and stored at -20°C or at lesser temperature. The concentration and purity of isolated DNA was determined using Nanodrop spectrophotometer (Bio Photometer plus, Eppendorf).

Selection of microsatellite primers

In this study, a total of twelve microsatellite loci were selected. The primer sequences for four microsatellite loci (TB11/13, TB2/19, TB8/11 and TB1/8) were obtained from the published data reported by Balmer *et al.* (2006). Primer sequences for eight microsatellite loci (TB11/1, TB8/1, TB11/29, TB7/12, TB10/19, TB10/1, TB3/3 and TB4/2) were used as described by Salim *et al.* (2011). Selections of these markers used in this study were obtained from *Trypanosoma brucei* genome project release 4.

Standardisation of PCR conditions

PCR conditions were standardised using twelve microsatellite primers (Table 2) on DNA extracted from purified trypanosomes of various *T. evansi* isolates of different agro-ecological zones of India. All PCRs were carried out in 25 µl reaction mixture using Fermentas Master Mixture containing 0.05 unit/µl Taq DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 0.4 µM of each primer and 1.0 µl templates DNA (200 ng/µl concentration).

Analysis of PCR products

PCR products were electrophoresed in 1.5% agarose in 1x TAE buffer and visualised under UV transilluminator (Gel documentation system, Aplegen, CA) using staining dye Ethidium bromide. A 100-bp gene ruler (Fermentas) was used as a marker.

RESULTS AND DISCUSSION

In this study fifteen microsatellite markers were initially selected, but only twelve microsatellite loci were successfully amplified with the selected primers. Three microsatellite markers were rejected due to poor amplification. The PCR conditions for twelve microsatellite markers were standardised using different annealing temperatures and numbers of cycles in the thermocycler (peqSTAR 96 universal gradient cycler, peqlab, USA).

Table 2: Standardisation of PCR conditions and PCR amplicon size

Locus	Primer sequence (5`-3`)	PCR conditions	PCR amplicon size(bp)
TB11/13	F:CAAGAACTCTGCATTGAGC R:ATCTGTTGGCGATGGTGA	94°C (5 min) x 1 94°C (30 s) 54°C (45 s) 72°C (1 min) x 35	157
TB2/19	F:CTGGTGCGTGTAACTGTG R:GAAGTGAGGACATGCACG		97
TB11/1	F:AGTATGTGTGGACAGTTAAGA R: ACCTTTGAGTCTTTCCTGTT	72°C (10 m) x1	160
TB8/1	F:CCAAATATGCGATTAGTTTCC R:TGTTTATGTGGAAGGAAATGAA		153
TB8/11	F:TGTAGCAGTGGTACGCAC R:CACCCAACGCATGTAAGC	94°C (5 min) x 1 94°C (30 s) 60°C (50 s) 72°C (1 min) x 40	115
TB11/29	F:AATGAGTGATACTATGAAAGTGT R: CACCATCACTGCTCTTATCA		152
TB7/12	F:CATGGCGTACGTTGCTTCGGTTTC R:GGTCGGTGTTGGCAGTGTGCATAG	72°C (10 min) x 1	200
TB10/19	F:CTGTTCGTTCTGAATTGTGTGCG R:GTGCACTTCCTTCTCTCATCCTTTTC		163
TB10/1	F:GCTCTACGCACCCACACAATCCGT R:CTCACTTGAGTAACCTCTCATTGC		210
TB1/8	F: AGGTTTAGTGCATGTCGGA R: CCTGTTGTACGGAGGTCA		120
TB3/3	F:CATTCGAAGTAAATGCGCGTATAAC R: GGTTGGAGCTTTCGACACAAGCG	94°C (5 min) x 1 94°C (30 s) 64°C (50 s) 72°C (1 min) x 40 72°C (10 min) x 1	102
TB4/2	F:GCCGCTTGATCATTAGGTAACCAC R:CCGCCTCACTTTAAGGATGGTGCC		92

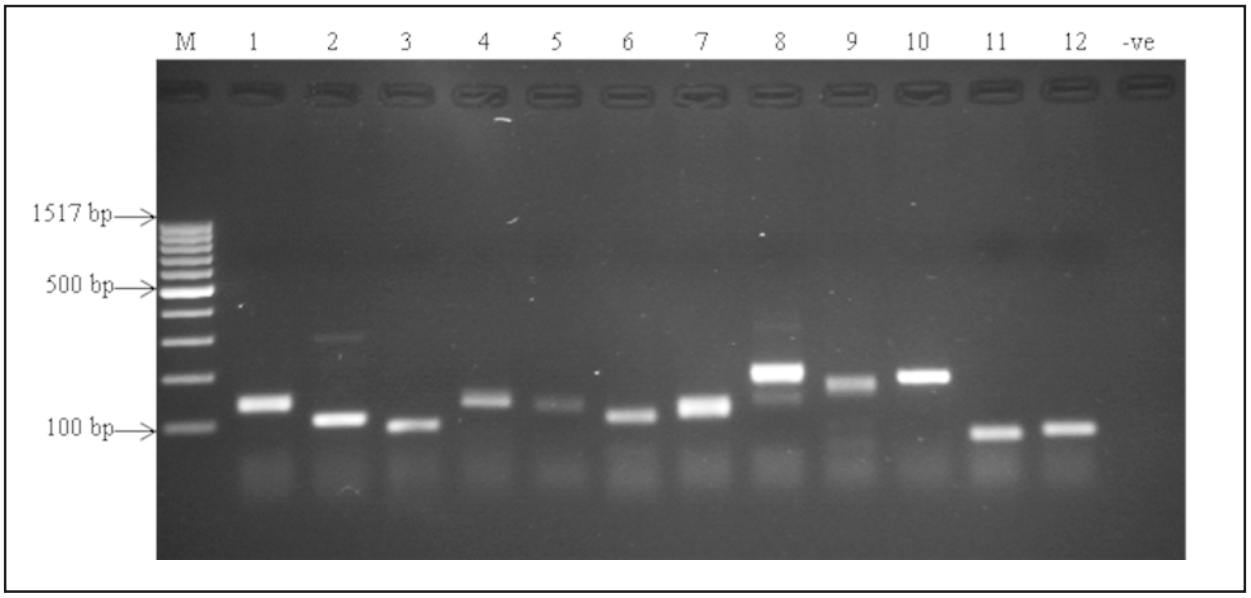

Figure 1: PCR of all the twelve microsatellite primers with *T. evansi* DNA of Junagarh donkey origin (Lane M: 100 bp DNA ladder; Lane 1: TB11/13 primer; Lane 2: TB1/8 primer; Lane 3: TB2/19 primer; Lane 4: TB8/1 primer; Lane 5: TB11/1 primer; Lane 6: TB8/11 primer; Lane 7: TB10/19 primer; Lane 8: TB10/1 primer; Lane 9: TB11/29 primer; Lane 10: TB7/12 primer; Lane 11: TB3/3 primer; Lane 12: TB4/2 primer; -ve : negative control).

The standardised PCR conditions and the respective amplicon size for all the twelve microsatellites are summarised in Table 2. The PCR product size of *T. brucei* TREU927 is taken as reference (MacLeod *et al.,* 2005) and compared with PCR amplicon size of *T. evansi* microsatellite loci of Indian *T. evansi* isolates. For most of the microsatellite loci of *T. evansi* studied, the PCR amplicon size is similar to that of the PCR product size of *T. brucei* TREU927, but for three microsatellite loci (TB11/1, TB10/1 and TB7/12), it was found to be different. For the microsatellite loci TB11/1, TB10/1 and TB7/12, the PCR amplicon size in this study in *T. evansi* was found to be 160, 210 and 200 (bp), respectively. The reported PCR amplicon size for the microsatellite loci TB11/1, TB10/1 and TB7/12 in *T. brucei* TREU927 was 140, 146 and 236 (bp), respectively (MacLeod *et al.,* 2005). McInnes *et al.* (2012) also observed unscorable results on eleven microsatellite loci due to monomorphism and poor PCR amplification. These results suggest genetic variability among *T. brucei* TREU927 and *T. evansi* isolates from India based on microsatellite study. To know genetic diversity among *T. evansi* isolates from different geographical areas and hosts, and population genetics, microsatellite markers genotyping are required, which may be useful in understanding the molecular epidemiology of disease, clinical and evolutionary research, vaccine and drug design, as well as development of effective strategies for containment of disease.

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