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Research Article

# Comparison between Trypanosoma vivax Strains Isolated from Central and Western of Africa Following RAPD Assay

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## Abstract

*Trypanosoma vivax* was isolated from cattle with natural infection in Raga city South of Sudan. It is denoted as DRSS06 (Deleba Raga South Sudan 2006). It was then used in the present study. The parasite was identified in the laboratory of Professor Ahmed Ali Ismail at College of Veterinary Medicine /Sudan University of Science and Technology based on its morphological character. A single polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay was used to confirm the *T. vivax* species.

The comparative characterization of *T. vivax* DRSS06 from Southern Sudan and T. vivax IRALD700 from Nigeria was done by using of Short oligodeoxyribonucleosite primers (10-mers) of arbitrary sequence in polymerase chain reaction (PCR) to generate genomic fingerprints (RAPD).

There was a similarity in the pathogenicity between the two strains. They had caused severe disease in goats and sheep with clinical signs as anaemia, loss of weight, poor condition, recumbency which culminating in death. However, there was no genetic similarity between the two strains.

**Keywords:** Characterization; PCR–RFLP; RAPD; *Trypanosoma vivax*

## Introduction

Despite the high economic relevance of the Trypanosomosis caused by *T. vivax*, little work had been done on its molecular characterization. However [1], reported that a causal search of the Entrez database returns only 22 *T. vivax* and 77 *T. congolense* DNA sequences, demonstrating the stark under-representation of these important species in the genomics age. The same author reported that *T. vivax* and *T. congolense* genomics could affect important research areas such as the identification of new targets for rational drug design and development new molecular markers for diagnosis and studies on population genetics.

A single polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay was used to characterize all important bovine trypanosome species. This is a sensitive pan-trypanosome PCR assay amplifying all species including *T. vivax* to

a comparable extent using a single primer pair [2,3]. Compared to standard parasitological techniques, molecular diagnostic tools, and in particular the polymerase chain reaction, allow the detection of trypanosome infections with much lower parasite numbers [2]. A single polymerase chain reaction amplification of the spliced-leader intergenic region also used to characterized *T. cruzi* [4].

For *T. vivax*, also other target sequences have been used such as satellite and microsatellite sequences, spliced-leader sequences and cathepsin L-like genes [5,6]. A proline racemase based PCR could be used, preferably in combination with ITS-1 PCR, as a species-specific diagnostic test for *T. vivax* infections worldwide [7]. This method is genome- wide profiling technique that does not require prior availability of any sequenced data. Therefore it is applicable to any organism.

Random Amplified Polymorphism (RAPD) method also relies on the use of a single primer for PCR at low stringency. It is simple, fast and require very little DNA template [8-10].

The objective of this study is to compare two strains of *T. vivax* from Sudan and Nigeria by using of Short oligodeoxyribonucleosite primers (10-mers) of arbitrary sequence in polymerase chain reaction (PCR) to generate genomic fingerprints (RAPD).

## Material and Methods

Molecular characterization was performed at The Institute of Tropical Medicine, Antwerp, Belgium**.**

### PCR-Restriction fragment length polymorphism: (PCR-RFLP)

### Filter paper samples

The parasite *T. vivax* DRSS06 stock was isolated from goat blood with high parasitaemia. It is spotted onto filter paper (Whatman No 3) and allowed to dry at room temperature. Then put into plastic bags and stored at -20 ºC until transported to Belgium. The strain from Nigeria *T. vivax* ILRAD700 was obtained from Institute of Tropical Medicine (ITM). Antwerp, Belgium.

### Extraction of DNA from filter papers

Using saponine-PBS extraction as following steps:

Filter paper discs containing the blood spot were cut using a cutter, transferred to a 1.5 ml Eppendorf tube and 1 ml of 0.5% PBS-saponine was added. Then the tube was mixed well and left overnight in refrigerator. The sample was centrifuged at 15000 rpm for 5 minutes then the supernatant was discarded. 1ml PBS was added mixed by hand and put the tubes in refrigerator for 1-2 h. Then the sample was centrifuged at 15000 rpm for 5 minutes. The supernatant was discarded as much as possible. Hundred µl Chelex 10% (Bio- Rad, Hercules, CA) was added then incubated for 10 min at 95°C in the thermomixer.

Thereafter, the sample was centrifuged at 14000 rpm for 5 minutes. One hundred and twenty five µl supernatant was transferred into a new 1.5 ml Eppendorf tube and stored at – 20 °C until used.

### PCR amplification

Ten µl of extracted DNA were mixed with 40 µl of PCR- mix. The samples were placed in thermocycler. Amplifications were performed in a Biometra® Tri- block programmable thermocycler (PTC- 100 TM. M.J. Research Inc.). Cycling conditions were as follows: first denaturation at 95°C for 5 minutes, second denaturation at 94°C for one minute. Primer- template was annealing at 40 °C for one minute and 30 second. Polymerization was at 72°C for 2 minutes (35 cycles). The final elongation step was at 72°C for 5 minutes.

### Post PCR

Three to 5 µl of 5% loading buffer was mixed with 20 µl PCR from each sample. 100bp DNA ladder (MB1 Fermentas, Lithuania) was included. Then subjected to electrophoresis in 2% agarose gel (Muped 11, Eurogentec, Belgium) for 40-60 minutes at 100V. The gel was stained by ethidium bromide (0.5 µg/ml) (Sigma, USA) for 30 minutes. Then the gel was washed twice with tab water, photographed using an ultra violet illumination at 254 nm and analyzed on an Imagemaster Video Detection System (Pharmacia, UK).

### Primers used

The primers were designed using a DNA computer software programme GeneJockeyII on aligned small sub-unit ribosomal gene (Ssu-rDNA) sequences available in the GeneBankTM and the expected amplicon lengths were estimated. The first amplification was done on the 18S gene using the forward primer 18STn F2/ (CAACGATGACACCCATGAATTGGGGA) and 18STn R3 (TGGGGGAC-CAATAATTGCAATAC) as reverse primer. A semi-nested second amplification was done using the forward primer 18ST nF2 of the first amplification with the reverse primer 18STn R2 (GTGTCTTGTTCT-CACTGACATTGTAGTG).

### Random amplified polymorphism RAPD

DNAs were extracted according to the method described by Oury *et al.* (1997). RAPD amplifications were performed according to the protocol described by (8, 9, 11). Genomic DNA samples (20 ng) were amplified in 60 µl of specific buffer (10 mM 96 Afr. J. Biotechnol. Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), supplied by Roche Diagnostics (Mannheim, Germany), in presence of 0.2 µM of primer, 4 x 100 µM dNTP and 0.9 U of Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany). The characterization was based on the use of ten base-long primers from Operon Technologies (USA).

## Results and Discussion

The diagnosis of animal trypanosomiasis in field samples is currently based on clinical signs and less frequently on traditional parasitological techniques characterized by their very low sensitivity, especially in the subacute and chronic phase of the infection [3].

The PCR amplification using the second run primer pair gave for all trypanosomes DNA tested a major product between 600 and 700 bp as predicated from the GenBankTM sequences (Figure 1). RFLP using Msp I digestion gave very distinct profiles of *T. vivax* (Figure 2).

PCR-RFLP, Ssu-rDNA amplification was uses to characterize the species- specific [2]. Compared to standard parasitological techniques, molecular diagnostic tools, and in particular the PCR, allow the detection of trypanosome infections with much lower parasite numbers [2]. So, this technique was used to confirm traditional parasitological techniques.

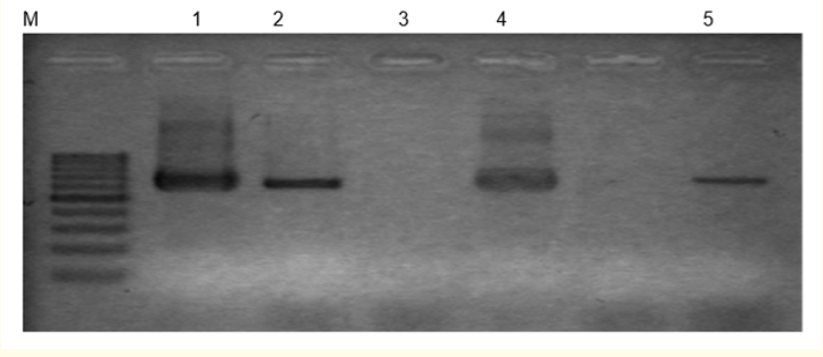


**Figure 1:** The first run amplification (using primers 18ST nF2 and 18ST nR3) lane (1,2) *T. vivax*, lane (3) negative control, lane (4, 5) *T. congolense* positive control and M 100 bp DNA ladder.

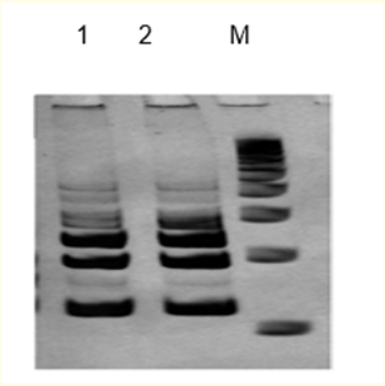


**Figure 2:** RFLP restriction enzyme analysis using Ddel enzyme on 18S-rDNA from samples and fractionated on 10% PAGE gel, stained with silver. (Lanes 1, 2) *T. vivax* and (lane 3) 100bp DNA ladder.

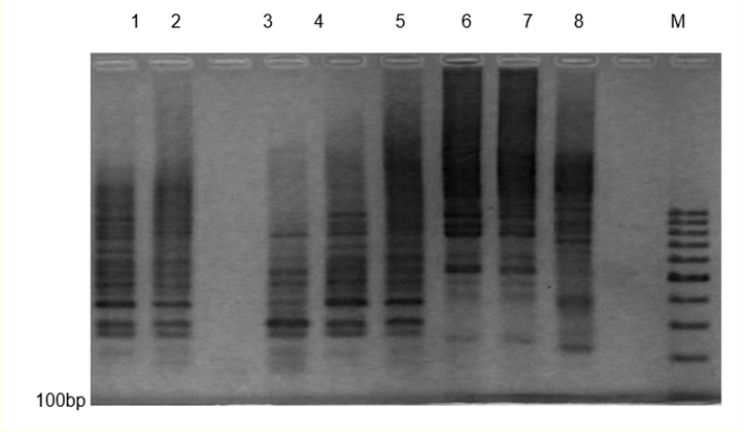


**Figure 3:** Random amplified polymorphic DNA fragment patterns generated using RAPD606. Lanes 1-5 *T. vivax* DRSS06. 6-7 T. vivax ILRAD700, 8 *T. congolense* TRT57 and M was a 100 bp DNA ladder.

The random amplified polymorphism DNA (RAPD) profile in the figure (3) consisted of several bands with *T. vivax* DRSS06 three well defined bands between 200 and 400 bp (except for sample 3 where the amplification was not as strong). While in Nigerian isolate the RAPD profile consisted of several bands between 500 and 900 bp. There were no RAPD bands shared by the 2 strains. There was no genetic similarity between the two isolates.

RAPD markers have been found to have a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and animals breeding [12].

In the present study the isolate *T. vivax* DRSS06 showed a similar pathogenesis potential with *T. vivax* (isolate from Nigeria) [13-15]. This similarity is not reflecting genotypically as the two strains were not identical as based on the results of the performed RAPD. In contrast [16], in this phylogenic analysis of Cathepsin L-Like gene data in *T. vivax* strains from Africa and *T. vivax* strains, from South America, reported clone similarity between the two strains. Further, similarity finding has been reported by [17] who noticed similarity between a Brazilian *T. vivax* stock from cattle and a West African *T. vivax* strain on their spliced- leader gene repeat sequence.

In conclusion, although there are similarity in the pathogenisis between *T. vivax* DRSS06 from Southern Sudan and *T. vivax* IRALD700 from Nigeria, there was no genetic similarity between them by using Random Amplified Polymorfis DNA (RAPD).

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## Bibliography

1. Davila AMR., *et al*. "Comparative genomics to uncover the secrets of tsetse and livestock-infective trypanosomes". *TRENDS in Parasitology* 19 (2003): 436-439.

2. Geysen D., *et al*. "PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle". *Veterinary Parasitology* 110 (2003): 171-180.

3. Liliana TG., *et al*. "A new PCR-RFLP for species-specific diagnosis of South American". *American Journal of Animal and Veterinary Sciences* 9 (2014): 128-136.

4. Lina MV., *et al*. "The identification of two Trypanosoma cruzi I genotypes from domestic and sylvatic transmission cycles in Colombia based on a single polymerase chain reaction amplification of the spliced-leader intergenic region". Mem Inst Oswaldo Cruz, Rio de Janeiro 108 (2013): 932-935.

5. Ventura RM., *et al*. "Trypanosoma vivax: characterization of the spliced-leader gene of a Brazilian stock and species-specific detection by PCR amplification of an intergenic spacer sequence". *Express Parasitology* 99 (2001): 37-48.

6. Nakayima J., *et al*. "Molecular epidemiological studies on animal trypanosomiases in Ghana". *Parasite Vectors* 5 (2012): 217.

7. Regassa F., *et al*. "A Proline Racemase Based PCR for Identification of Trypanosoma vivax in Cattle Blood". *PLoS ONE* 9 (2014): 84819.

8. Welsh J and McClelland M. "Fingerprinting with arbitrary primed PCR". *Veterinary Parasitology* 78 (1990): 259-264.

9. Williams JGK., *et al*. "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers". *Nucleic Acid Research* 18 (1990): 6531-6535.

10. Masake R A., *et al*. "Sensitive and specific detection of T. vivax using the PCR". *Express Parasitology* 85 (1997): 193-205.

11. de Almeida PJ., *et al*. "Diagnostic evaluation of PCR in goats experimentally infected with Trypanosoma vivax". *Acta Tropica* 66 (1997): 45-50.

12. Tibayrenc M., *et al*. "Genetic characterization of six parasitic protozoa: Parity between random-primer DNA typing and multilocus enzyme electrophoresis". *Proceedings of the National Academy of Sciences of the United States of America* 90 (1993): 1335-1339.

13. Bardakci F and Skibinski D O F. "Application of the RAPD technique in tilapia fish: species and sub species identification". *Heredity* 73 (1994): 117-123.

14. Shadia AM Lazim. "The pathogenicity and molecular characterization of Trypanosoma vivax isolated from tsetse belt, Southern Sudan, in goats and sheep, PhD, Thesis, University of Baher Elghazal, Sudan". (2017): 217.

15. Anosa VO. "Haematological and biochemical changes in human and animal Trypanosomosis. Part 1". *Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux* 41.1 (1988A): 65-78.

16. Anosa VO. "Haematological and biochemical changes in human and animal Trypanosomosis. Part 11". *Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux* 41.2 (1988B): 151-164.

17. Cortez AP., *et al*. "Cathepsin L-like genes of Trypanosoma vivax from Africa and South America--characterization, relationships and diagnostic implications". *Molecular and Cellular Probes* 23 (2009): 44-51.