

Molecular Diagnosis of *Trypanosoma vivax* Infections (Review)

^{1,2,3}Shimelis Dagnachew, ²Melkamu Bezie and ³Burno Maria Goddeeris

¹Addis Ababa University, College of Veterinary Medicine and Agriculture, Debre Zeit, Ethiopia

²University of Gondar, Faculty of Veterinary Medicine, Gondar, Ethiopia

³KU Leuven, Faculty of Bioscience Engineering, 30 bus 2456, B-3001 Heverlee, Belgium

Abstract: Trypanosomosis is a worldwide vector-borne protozoan disease caused by the species of the genus *Trypanosoma*, which affects humans, as well as domestic and wild animals. It is best diagnosed by molecular methods particularly the polymerase chain reaction (PCR) both in the vertebrate and insect host. Molecular tests demonstrate sequences of nucleotides specific for a trypanosome subgenus, species or even type or strain. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated.

Key words: DNA • Molecular Diagnosis • PCR • *Trypanosoma vivax*

INTRODUCTION

Trypanosomosis is a worldwide disease caused by the species of the genus *Trypanosoma*, which affects humans, as well as domestic and wild animals. Trypanosomes are unicellular organisms (Phylum Protozoa) belonging to the family Trypanosomatidae and the order Kinetoplastida. Species of trypanosomes infecting mammals fall into two distinct groups (sections) [1]. The first group is the Stercoraria that includes species such as *T. cruzi*, *T. theileri* and *T. melophagium* in which trypanosomes are typically produced in the hindgut and are then passed on by contaminative transmission from the posterior. The second is the Salivaria that includes species such as *T. vivax*, *T. congolense* and *T. brucei*, in which transmission occurs by the anterior station and is inoculative. Many species of trypanosomes occur as parasites in a wide variety of animals and some of these parasites have been spread by humans from Africa to other continents. For example, *T. vivax* had been introduced to South America, by the importation of West African cattle in the eighteenth and nineteenth centuries [2]. Within the Salivarian trypanosomes, *T. vivax*, *T. congolense* and *T. brucei* are the three most important pathogenic species in livestock responsible for

considerable production losses and morbidity. Animal trypanosomosis caused by *T. vivax* is a widespread disease in Africa and South America, hindering livestock production and food self-sufficiency [3, 4].

In Africa, *T. vivax* is a heteroxenous parasite present in tsetse fly (*Glossina* spp.) infested regions and the parasite develops in the proboscis of this invertebrate host. *Glossina* spp. is the only vector in which *T. vivax* is able to multiply and remain in the infective phase throughout the insect's life. Outside tsetse fly areas, the parasite is carried by other haematophagous flies where transmission is non-cyclical. Thus, the parasites are mechanically transmitted across vertebrate hosts, with no growth or multiplication in the insects [1]. Of the three main species of tsetse-transmitted trypanosomes affecting ruminants in sub-Saharan Africa, only *T. vivax* has spread beyond the bounds imposed by its tsetse fly vector in Africa and established itself in South America.

The severity of trypanosome infection depends on the species and strain of the parasite involved. It has been stated that *T. vivax* infections predominate in cattle in West Africa and are rapidly fatal whilst *T. congolense* causes a chronic disease. In contrast, *T. vivax* may be commonly encountered in East and Central Africa but causes a mild disease in cattle in comparison to

T. congolense. There are exceptions to this rule: for example, the hemorrhagic *T. vivax* infections that occasionally break out in Kenya are rapidly fatal [5, 6]. Recent experimental works in Ethiopia also showed that *T. vivax* developed severe clinical diseases in young Zebu cattle [7, 8] which vary from previous reports. Typical features of these infections include high, persistent parasitemia, fever, very pronounced anemia and generalized visceral and mucosal hemorrhage, particularly in the gastrointestinal tract; however, the chronic form of the infection is usually undetected with the conventional techniques. In addition to the host parasite interaction; the epidemiology of non-tsetse transmitted trypanosomosis is influenced by many factors [2]. There may be seasonal outbreaks, where the populations of biting flies (tabanids, stable flies, etc.) are influenced by seasonal climatic differences. An appropriate diagnostic protocol is essential for the control of trypanosomosis using combined efforts such as restricted movement of diseased animals, treatment of *T. vivax* infected animals, epidemiological monitoring of the distribution and severity of the disease and vector control [9]. Therefore, the objective of this review focuses on molecular diagnosis of *T. vivax* infections in domestic animals.

Diagnosis of *Trypanosoma vivax* Infections:

Trypanosoma vivax infection can be diagnosed by clinical, parasitological, immunological and molecular methods. The clinical signs of animal trypanosomosis are indicative but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The classical direct parasitological methods for the diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and DNA methods may increase the sensitivity [10, 11]. Indirect methods rely on serological tests by detecting specific antibodies developed by the host against the infection or, inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks or months) after all trypanosomes have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. On the other hand, circulating trypanosomal

antigens are eliminated quickly after the disappearance of the trypanosomes and their presence therefore shows almost always that live trypanosomes are present in the animal [12].

Molecular Techniques for the Diagnosis of *Trypanosoma*

***vivax*:** Compared to standard parasitological techniques, molecular diagnostic tools and in particular the polymerase chain reaction (PCR), allow the detection of trypanosome infections with much lower parasite numbers, both in the vertebrate and in the insect host [13, 14, 15]. The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated. The diversity of PCR methods for identification of trypanosome taxa has also led to increased appreciation of trypanosome genetic diversity [16].

Targets for Molecular Diagnostic Techniques of

***Trypanosoma vivax*:** In most cases, PCR diagnosis aims to identify the parasites at the species level, which can be done using various targets. The preferred targets are those which are present in a high copy number in the genome of trypanosomatids; the more copies of the target, the greater the chances of amplifying it by PCR. Single copy genes are more difficult to amplify [17] and are rarely targeted since low parasitemia is a characteristic of trypanosome infection and the sensitivity would be too low. Mini-chromosomes of the nuclear DNA contain satellite DNA which has been the most favored target in the development of species-specific primers able to detect very small amounts of parasite DNA. Such primers were developed for the main pathogenic trypanosomes: *T. vivax* and *T. congolense* savannah, forest and Kilifi types [13]. Mini-exon genes of the nuclear DNA are also multi-copy genes and have been a favored target for the detection of *T. vivax* [18]. Other repetitive sequences have also been investigated, for example, in the detection of *T. vivax* DNA [19, 20, 21]. Internal transcribed spacers (ITSs) of ribosomal DNA are a suitable target for PCR-based trypanosome diagnosis [14]; the use of primers developed from the ITS region would provide a multi-species-specific diagnosis using a single PCR.

For identification of the trypanosome species in biological specimens from mammal or insect hosts, ribosomal genes and internal transcribed spacers are often chosen as target sequences [14, 15]. For *T. vivax*, also other target sequences have been used such as satellite and microsatellite sequences, spliced leader sequences, cathepsin L-like genes and proline racemase genes [13, 18, 22-24].

Types of Molecular Techniques Applied for the Diagnosis of *Trypanosoma vivax*: Based on the target genes of amplification, different types of PCR techniques have been developed for the molecular diagnosis of *T. vivax* infections in domestic animals.

***Trypanosoma vivax* gDNA PCR:** Several DNA sequence-based diagnostic methods have been developed for *T. vivax*, including: (a) *T. vivax*-specific probes based on satellite DNA or repetitive DNA sequence [25], (b) PCR assays using as targets cDNA sequences that code for the antigen used in Ag-ELISA test, which even though apparently present in all stocks possesses only about 40 copies in the genome of *T. vivax* [19] or satellite DNA sequence [13]. For the genomic DNA TV PCR oligonucleotide primers designed based on the nucleotide sequence of the 832-bp cDNA insert and used in amplifying DNA sequences from the blood of cattle infected with *T. vivax* isolates from West Africa, Kenya and South America have been developed. The PCR product of approximately 400 bp was obtained by amplification of DNA from all the isolates studied. Subsequently, this PCR was evaluated for its capacity to detect *T. vivax* DNA in the blood of three animals experimentally infected with the parasite. *T. vivax* DNA was detectable in the blood of infected animals as early as 5 days post-infection which couldn't be detected with Ag-ELISA. Analysis of the data obtained in the three animals during the course of infection revealed that the buffy coat technique, Ag-ELISA and PCR revealed infection in 42, 55 and 75% of the blood samples, respectively. PCR amplification of genomic DNA of *T. vivax* is thus superior to the Ag-ELISA in the detection of *T. vivax* [19].

Similar study using genomic DNA PCR assays for diagnosis of trypanosome infection in cattle evaluated their ability to detect trypanosome DNA in blood spot samples and the results compared with those obtained with standard parasitological techniques and serological methods (Ab-ELISAs for *T. vivax* and *T. congolense*). Kappa agreement analysis of these tests showed a significant agreement between PCR assays and results

from parasitological methods but there was no agreement when PCR was compared with serological assays. Some samples from *T. vivax* smear positive animals were negative by PCR, therefore modifications to the PCR assay conditions were recommended to improve agreement between PCR and parasitological assays. In both cases PCR proved consistently more sensitive than the parasitological and ELISA techniques [26].

***Trypanosoma vivax* ITS gene (rDNA) PCR:** Individual species-specific multi-copy trypanosome DNA sequences can be targeted to identify parasites. Highly conserved ribosomal RNA (rRNA) genes are also useful for comparisons between closely related species. The internal transcribed spacer regions (ITS) in particular are relatively small, show variability among related species and are flanked by highly conserved segments to which PCR primers can be designed. Individual variation in inter-species length makes the ITS region a useful marker for identification of multiple trypanosome species within a sample. They are made of transcriptional units (TU) separated by non-transcribed spacers (NTS). The TU is made of an 18S ribosomal subunit, internal transcribed spacer 1 (ITS-1), 5.8S ribosomal subunit, ITS-2, 28S ribosomal subunit, etc. [27]. The ITS1 is usually 300-800 bp in length and has a variable length depending on the *Kinetoplastida* species, but is presumed to be constant within a species (Figure 1). The following *Trypanosoma* taxa can be identified through a single PCR: *T. vivax*, *T. theileri*, *T. simiae*, *T. congolense* savannah, *T. congolense* forest and *T. congolense* Kilifi [14].

Using primers for ITS1 to make a diagnosis is three to five times cheaper than using the classical species-specific primers, as the number of reactions required per sample is reduced to a single one. The use of these primers can also lead to the identification of unexpected *Trypanosoma* species, especially in wild hosts, vectors and field stocks. They insure a permanent screening of any unexpected *Trypanosoma* species that could grow *in vivo* or *in vitro* as a mixed infection. However, the sensitivity of these primers is lower than that of satellite DNA primers, due to partial homology of the sequences and their limited repetitiveness. ITS1 sequences are only repeated 100-200 times in comparison with 10,000-20,000 times for satellite DNA. Consequently, the design of new primers is required to reach 100% homology with all *Trypanosoma* species of veterinary interest, especially for *T. vivax*. Nested PCR or primers with a better affinity could also improve the sensitivity of ITS for field diagnosis application [28].

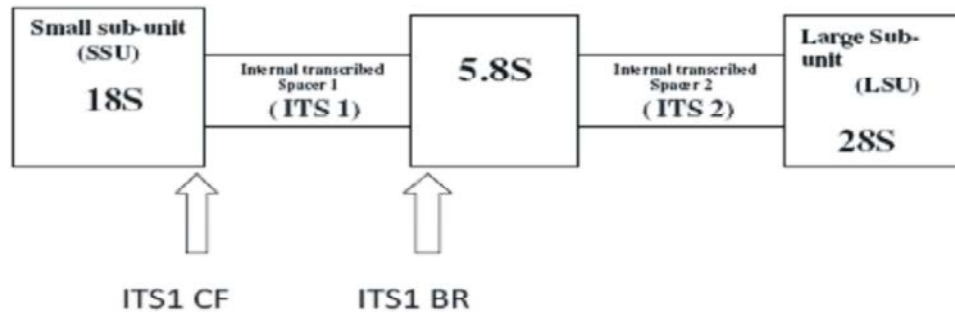


Fig. 1: Schematic diagram of rDNA showing ITS1 CF and ITS1 BR annealing positions
Source: Thumbi *et al.* [32].

Reports showed that the DNA sequences initially used as *T. vivax* specific target for hybridization or for PCR primers were only specific for the West African form of *T. vivax* that also occurs in South America [25, 29]. However, in East Africa, other *T. vivax* genotypes circulate [19]. Research findings in Ethiopia, on bovine trypanosomosis by Fikru *et al.* [30] showed that the results obtained with 18S PCR [31] and TVM PCR [21] were not consistent with ITS1 PCR with respect to *T. vivax* detection. Several PCR based diagnostic assays have been developed to improve the detection of pathogenic trypanosomes. These tests include use of species specific primers, single and nested PCRs' based on primers amplifying the (ITS) regions of ribosomal DNA. Studies conducted for the comparisons of three PCR based diagnostic assays and assessed for the agreement of these assays by screening 103 cattle blood samples randomly collected from trypanosome endemic areas in western Kenya showed that the nested ITS based PCR, the single ITS based PCR and the species specific based PCR detected 28.1%, 26.2% and 10.7% of the samples respectively as positive for trypanosome infection. Cohen kappa statistic used to compare agreements beyond chance between the assays showed highest degree of agreement (0.6) between the two ITS based tests and the lowest (0.2) between the nested PCR test and the species specific PCR. The single ITS and nested ITS based diagnostic assays detected higher numbers of positive cases and reduced the number of PCR reactions per sample to one and two respectively, compared to the five PCR reactions carried out using the species specific primers [32]. The single PCR ITS based diagnostic test is able to detect much more (17.5%) positives for *T. vivax*, compared to the TVW primers (3.9%) [14, 33]. These might be associated to the fact that TVW primers target certain DNA sequences that are not

conserved in all *T. vivax* isolates, resulting to false-negatives [20]. In addition, low sensitivity could be due to the TVW primers targeting molecules that are low in copy numbers as compared to ITS-PCR whose target gene could be higher in copy numbers [34, 35].

Recent studies with a “Touchdown” PCR targeting the ITS1 (ITS1 TD PCR) showed the possible detection and discrimination of different *Trypanosoma* taxa in a single run due to variations in PCR product sizes. The assay achieves analytical sensitivity of 10 parasites per ml of blood for detection of *T. congolense* savannah type and *T. brucei* and 100 parasites per ml of blood for detection of *T. vivax* in infected mouse blood. The ITS1 TD PCR was evaluated on cattle experimentally infected with *T. congolense* during an investigational new veterinary trypanocidal drug efficacy study. ITS1 TD PCR demonstrated comparable performance to microscopy in verifying trypanocide treatment success, in which parasite DNA became undetectable in cured animals within two days post-treatment. ITS1 TD PCR detected parasite recrudescence three days earlier than microscopy and had a higher positivity rate than microscopy (84.85% versus 57.58%) in 66 specimens of relapsing animals collected after treatments. This assay allows the potential for detection of mixed infections and may be applicable for drug efficacy studies and diagnostic discrimination of *T. vivax* and *T. congolense* against other pathogenic trypanosomes, including *T. brucei*, *T. evansi* and *T. equiperdum* [36].

The arrows show the annealing position for ITS1 CF at the 18S ribosomal small subunit and ITS1 BR annealing at the 5.8S ribosomal sub-unit, to amplify the region ITS1, known to vary in size within trypanosome species. The large boxes (SSU and LSU) represent conserved coding regions, while the smaller boxes represent the spacer regions.

PCR-RFLP Using Ssu-rDNA Amplification: The 'pan-trypanosome' test based on the ITS1 region of the ribosomal genes has been recently described by Desquesnes *et al.* [14] the test has limited application to the field situation as it lacks sensitivity, especially for *T. vivax*. Hence, the need for an easy universal and sensitive test to identify trypanosome infections in field samples coupled with species differentiation is still highly desirable. The association of PCR with RFLP provides an elegant, sensitive and specific tool to meet this demand. For this technique the 18S ribosomal sub-unit is an ideal target sequence as it is a mosaic of highly conserved and species-specific sequences, present as a multi-copy locus. Consequently a single PCR-RFLP assay was used to characterize all important bovine trypanosome species including *T. vivax* to a comparable extent using a single primer pair. Restriction enzyme analysis using MspI and Eco571 gave a clear distinction between *T. congolense*, *T. brucei*, *T. vivax* and *T. theileri* [37].

LAMP TV PCR: Loop-mediated isothermal amplification (LAMP) is a novel gene amplification method that amplifies DNA under isothermal conditions [38]. The technique uses four to six primers that recognize six to eight regions of the target DNA and relies on Bst DNA polymerase, an enzyme that synthesizes DNA through strand displacement activity. The LAMP method has several advantages over PCR in that: (i) LAMP amplification can be achieved using simple heating device that maintains temperature at isothermal (60-65°C), (ii) amplification can be achieved using partially or non-processed template therefore DNA extraction may not be necessary, (iii) reactions are rapid and require shorter time, (iv) sensitivity is equal or higher than that of PCR and (v) the technology allows the use of varied product detection formats [39]. These characteristics make LAMP ideal for *T. vivax* diagnosis in resource poor endemic regions. For this technique the nuclear satellite repeat sequence is a desired target because it is multicopy gene and widely conserved among *T. vivax* isolates in Africa and South America. The test was evaluated and compared with PCR tests using a panel of *T. vivax* isolates and archived field samples. The diagnosis of *T. vivax* with the LAMP assay is rapid with results obtained within 35 min. The analytical sensitivity is ~1 trypanosome/ml while that of the classical PCR tests ranged from 10 to 10³ trypanosomes/ml. The *T. vivax* LAMP test reported here is simple, robust and has future potential in diagnosis of animal trypanosomosis in the field [40]. On the analysis of

the *T. vivax* samples, the LAMP test detected 20/23, satellite repeat 15/23 and the diagnostic antigen PCR 7/23 (Table 1). The analysis of 357 archived bovine samples revealed a *T. vivax* prevalence of 7.6% through LAMP test, 5.3% by satellite repeat PCR and 1.7% with diagnostic antigen PCR (Table 1). Therefore, the *T. vivax* satellite DNA based LAMP assay is rapid and shows superior analytical sensitivity to classical PCR tests.

Spliced-Leader Gene (SL) TV PCR: PCR amplification of SL RNA gene has been demonstrated based on the location and sequences of the oligonucleotides primers for PCR. Primers TviSL1 and TviSL2 were designed for amplification of a *T. vivax*-specific 210-bp SL gene intergenic sequence (TviSL-PCR) (Figure 2).

The sequence of the spliced-leader gene repeat of a Brazilian *T. vivax* stock from cattle showed high similarity to sequences of West African *T. vivax* in both intron and intergenic sequences. A *T. vivax*-specific diagnostic PCR assay based on spliced leader gene intergenic sequences was able to amplify DNA from *T. vivax* stocks from South America (Brazil, Bolivia and Colombia) and West Africa. Species-specificity of this method was confirmed by results obtained by testing 15 other trypanosomes, including other species and subspecies that can also infect cattle. This PCR assay presented high sensitivity, detecting the DNA content of only one parasite and also revealing *T. vivax* infection in asymptomatic animals without detectable parasitemia by micro hematocrit or in Giemsa-stained blood smears. Use of crude preparations from field blood samples collected on both filter paper and glass slides as DNA template suggested that this method could be useful for the diagnosis of *T. vivax* in large epidemiological studies [18].

TvPRAC gene PCR: The proline racemase (PRAC) gene, originally described for *T. cruzi*, was also found in the genome of the West African *T. vivax* ILRAD 1392 strain isolated in Nigeria and was shown to be absent in other African trypanosomes. In *T. cruzi*, PRAC plays a crucial role in the inter conversion of free L- and D-proline enantiomers. The biochemical characterization of the corresponding protein revealed that *T. vivax* proline racemase (TvPRAC) exhibits characteristics and kinetic parameters comparable to *T. cruzi* PRAC [41]. Fikru *et al.* [24] developed a *T. vivax* specific PCR based on the *T. vivax* proline racemase (TvPRAC) gene based on the forward and reverse primers sets designed to bind at 764-783 bp and 983-1002 bp of the gene on DNA extracted

Table 1: The analysis of field samples from Kenya using *T. vivax* PCR and LAMP assay

Sample type	No of samples	PCR tests		
		DA-PCR	SA-PCR	SA-LAMP
<i>T. vivax</i> isolates	23	7 (30.3%)	15 (65.2%)	20 (90%)
Tsetse fly (<i>T. vivax</i> positive)	3	–	3	3
Buffy coat (Trypanosome positive)	16	3	7	8
Bovine samples	357	6 (1.7%)	19 (5.3%)	27 (7.6%)

DA = diagnostic antigen gene; SA = satellite repeat DNA. Source: Njiru *et al.* [40]

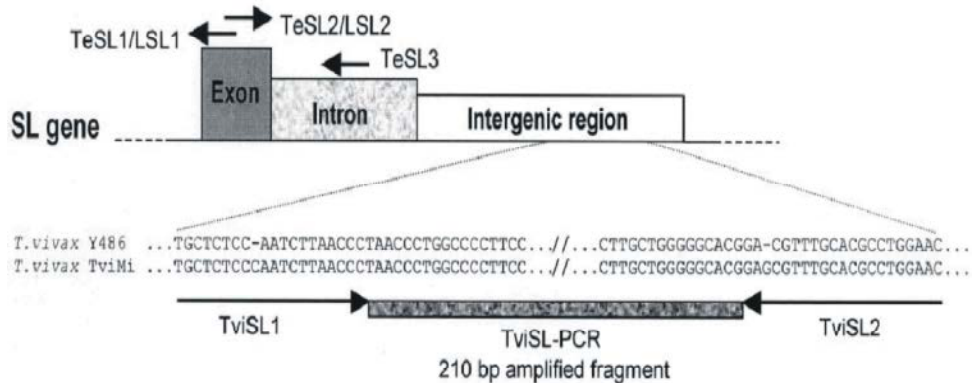


Fig. 2: Schematic representation of the SL gene repeat of *Trypanosoma* spp. showing amplification strategies and depicting localization of the primers used for amplification of different SL sequences
Source: Ventura *et al.* [18]

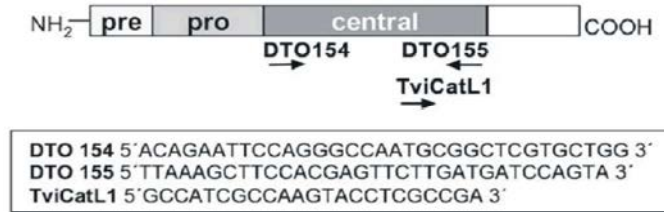


Fig. 3: Schematic representation of cathepsin L-like gene indicating the pre, pro and central (catalytic) domains and primers employed for PCR-amplification of sequences encoding the catalytic domains of trypanosomes or designed for the *T. vivax* specific assay TviCatL-PCR. Source: Cortez *et al.* [22]

from different haemotropic pathogens: *T. vivax* from Nigeria, Ethiopia and Venezuela, *T. congolense* savannah type, *T. b. brucei*, *T. evansi*, *T. equiperdum*, *T. theileri*, *Theileria parva*, *Anaplasma marginale*, *Babesia bovis* and *B. bigemina* and from bovine, goat, mouse, camel and human blood. The analytical sensitivity of the TvPRAC PCR was compared with that of the ITS-1 PCR and the 18S PCR-RFLP on a dilution series of *T. vivax* DNA in water. The diagnostic performance of the three PCRs compared on 411 Ethiopian bovine blood specimens showed that TvPRAC PCR proved to be fully specific for *T. vivax*, irrespective of its geographical origin. The sensitivity of the detection of *T. vivax* infections was 8.3%, 22.6% and 6.1% for TvPRAC PCR, ITS-1 PCR and 18S PCR-RFLP respectively. Hence 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 [24].

Cathepsin L-like genes TV PCR: Cathepsin L-like (CatL-like) enzymes are cysteine proteases that play a vital role in the metabolism, infectivity, cell differentiation, immunity and pathogenicity of trypanosomes and have been exploited as potential targets for the development of drugs, vaccines and diagnostic tests. An understanding of the evolutionary relationships among trypanosome cysteine proteases and the identification of species-specific molecules can assist in clarifying the potential role of these enzymes in the parasites' life cycles and pathogenesis. Cysteine proteases have been

characterized extensively in *T. cruzi*, *T. congolense* and *T. b. brucei* at the biochemical, molecular and immunological levels [22]. The characterization of genes encoding such proteases in *T. vivax* represent the first step toward their characterization, which might contribute to the definition of targets for chemotherapy and/or vaccines. The existence in the genomes of trypanosomes of multiple copies of CatL-like genes, which vary depending on species, suggested that sequences of these genes could provide markers for sensitive and specific diagnostic methods. An alignment of nucleotide sequences representing cdCatL-like proteases from *T. vivax* and *T. vivax*-like with sequences from several other trypanosome species was used to enable the design of the *T. vivax*-specific primer TviCatL1 (Figure 3). The sequence of this primer is identical among known *T. vivax* genotypes. The specific PCR (designated TviCatL-PCR) was developed for the amplification of a 177 bp region exclusively from the genomic DNA of *T. vivax* using the primer TviCatL1 (forward) together with primer DTO155 (reverse) (Figure 3) under the same conditions as for the amplification of cdCatL sequences.

The discovery of different CatL-like sequences for each genotype, defined previously by ribosomal DNA data, indicate that these sequences provide useful targets for epidemiological and population genetic studies. Regions in CatL-like sequences shared by all *T. vivax* genotypes but not by other trypanosomes allowed the establishment of a specific and sensitive diagnostic PCR for epidemiological studies in South America and Africa. Taken together, polymorphisms among CatL-like gene sequences corroborated substantial genetic complexity within and among *T. vivax* and *T. vivax*-like populations, despite the small number of isolates studied to date. CatL-like sequences proved useful for assessing the genetic structures of *T. vivax* and *T. vivax*-like populations and allowed the development of a specific and sensitive PCR method for the detection of these trypanosomes [22].

CONCLUSION

The development of good treatment and control strategies to protect livestock against trypanosomosis requires accurate data for the disease epidemiology. Furthermore reliable epidemiological studies are a prerequisite to design effective tsetse and trypanosomosis control programs. This in turn depends on accurate diagnosis and definitive identification of causative trypanosome species. Although microscopy

remains the most appropriate method for the clinical diagnosis in a field setting, it lacks sensitivity to be considered as a gold standard and the molecular methods provide an alternative sensitive method for the detection of trypanosomosis. Although PCR is expensive, especially when used as a diagnostic tool in hosts potentially infected with multiple species of trypanosome like cattle, the sensitivity and species-specificity of the technique justify its use in epidemiological studies. The ITS based assays definitely make PCR diagnosis more accurate, faster and less costly to carry out for large numbers of samples which enables to conduct large-epidemiological studies on African trypanosomosis in a simple and cost-effective way. The recent development of multi-species diagnosis through a single PCR might allow a more generalized use of PCR in field studies, which would improve the knowledge of parasite population dynamics in hosts and vectors considerably.

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