Proline racemase gene based PCR: an alternative *T. vivax* specific diagnosis

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Introduction

• Animal trypanosomosis caused by *T. vivax* is a widespread disease, not only restricted to Africa.

• Could be considered as trypanosome species of global concern.

• PCR allows the detection of trypanosome infections even with low parasitemia.

• In case of *T. vivax*, most PCR primers usually are based on West African genotypes.

• In East Africa, other *T. vivax* genotypes circulate.
Introduction

• We observed that results obtained with 18S PCR RFLP and TVM PCR were not consistent with ITS1 PCR.

• The proline racemase gene (PRAC), first observed in *T. cruzi*, was also found in the genome of West African *T. vivax* ILRAD 1392.

• PRAC is absent in other African trypanosomes.

• We developed a PCR targeting the PRAC gene.
Material and Methods

• The sequence of the *Tv*PRAC gene (1038bp) was obtained from GenBank using accession number EF175213.1

• **Primers**
  – Complementary to bp 764-783 and 983-1002
  – *Tv*PRAC-F: 5’CGCAAGTGGGACCCTCAGCT3’
  – *Tv*PRAC-R: 5’ACGCGGGGCGCAACAGAAGT3’

• Expected amplicon length: 239 bp
Specificity of *Tv*PRAC PCR

- Trypanosome species
  - West African *T. vivax* (ILRAD700)
  - *T. vivax* (non-tsetse and tsetse from Ethiopia)
  - *T. vivax* from Venezuela
  - *T. congoense* Savannah type
  - *T. brucei brucei* (AnTat 1.1)
  - *T. evansi* RoTat 1.2
  - *T. equiperdum* OVI
  - *T. theileri*
- Bovine, goat, camel, mouse and human
- Other hemoparasites
  - *Babesia bovis*
  - *B. bigemina*
  - *Anaplasma marginale*
  - *Theileria parva.*
ITS-1 PCR on different trypanosomes

Lane 1 = 100 bp marker
2 = T. vivax (ILRAD700)
3 & 4 = T. vivax (non-tsetse Ethiopia)
5, 6 & 7 = T. vivax (tsetse infested area Ethiopia)
8 & 9 = T. vivax Venezuela
10 = T. congoense Savannah
11 = T. brucei brucei
12 = T. evansi
13 = T. equiperdum
14 = T. theileri
15 = negative control
16 = 100 bp marker
TvPRAC PCR on trypanosomes species

Lane 1 = 100 bp marker
2 = T. vivax (ILRAD700)
3 & 4 = T. vivax (non-tsetse Ethiopia)
5, 6 & 7 = T. vivax (tsetse infested area Ethiopia)
8 & 9 = T. vivax Venezuela
10 = T. congoense Savannah
11 = T. brucei brucei
12 = T. evansi
13 = T. equiperdum
14 = T. theileri
15 = negative control
16 = 100 bp marker
TvPRAC PCR on other species

Lane
1= 100 bp marker 100
2. *T. vivax* ILRAD 700
3= *Theileria parva*
4= *Anaplasma marginale*
5= *Babesia bovis*
6= *Babesia bigemina*
7= bovine
8= goat
9= camel
10 = mouse
11= human
12 = negative
13= 100 bp marker

PCR is negative with DNA extracted from bovine, goat, mouse and human blood and with other hemoparasites of cattle
Comparison with ribosomal RNA gene based PCRs

– On isolates from experimentally infected calves
– 411 bovine blood samples collected in Ethiopia

– Comparison was made with ITS1 and 18s PCR-RFLP
Analytical sensitivity

- A five-fold dilution series of *T. vivax* DNA in water ranging from 1ng/µl down to 0.064 pg/µl

- The isolates are from experimentally infected calves purified by MAECT
## Analytical sensitivity

<table>
<thead>
<tr>
<th>T. vivax isolates</th>
<th>TvPRAC PCR</th>
<th>ITS-1 PCR</th>
<th>18S PCR-RFLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>4337</td>
<td>8</td>
<td>1.6</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>4338</td>
<td>40</td>
<td>1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Di</td>
<td>8</td>
<td>40</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Fc</td>
<td>1.6</td>
<td>0.32</td>
<td>8</td>
</tr>
</tbody>
</table>

- Variation in lowest concentration detection limit among strains tested and among the PCRs
Analytical sensitivity

Lane 1 = 100 bp marker, lanes 2 to 6: *T. vivax* 4337 DNA at 1000, 200, 40, 8, 1.6 pg/µl,

lanes 7 to 11: *T. vivax* Fc DNA at 1000, 200, 40, 8, 1.6 pg/µl, 12 = negative extraction control.

The difference in sensitivity = is it because of genetic heterogeneity???
# Result on field

<table>
<thead>
<tr>
<th>Test</th>
<th>TvPRAC PCR</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>18S PCR-RFLP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive*</td>
<td>13</td>
<td>3.2</td>
<td>12</td>
<td>2.9</td>
<td>25</td>
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<tr>
<td>Negative</td>
<td>21</td>
<td>5.1</td>
<td>365</td>
<td>88.8</td>
<td>386</td>
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<tr>
<td>ITS-1 PCR</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive*</td>
<td>25</td>
<td>6.1</td>
<td>68</td>
<td>16.5</td>
<td>93</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>2.2</td>
<td>309</td>
<td>75.2</td>
<td>318</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td><strong>8.3</strong></td>
<td>377</td>
<td>91.7</td>
<td>411</td>
</tr>
</tbody>
</table>
Conclusion

- *TvPRAC* PCR can detect the presence of both west African, and east African *T. vivax* strains (from tsetse and non-tsetse regions) as well as Latin American strains

- *TvPRAC* PCR can be used as species specific molecular tool for identification of *T. vivax* infection and diagnosis of disease caused by this trypanosome

- Specificity of *TvPRAC* in insect vector remains to be assessed
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