Characterization of quinapyramine (Trypacide®) drug-resistant *Trypanosoma evansi*

Intisar E. El Rayah\(^1\)* and Khitma H. El Malik\(^2\)

Trypanosomosis Unit, Tropical Medicine Research Institute (TMRI), P.O.BOX 1304, Khartoum 11111, Sudan.

\(^2\)Faculty of Veterinary Science, University of Khartoum, P.O Box 32, Khartoum North, Sudan.

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Molecular karyotyping by pulsed field gel electrophoresis was used to characterize *Trypanosoma evansi* isolates. Ten *T. evansi* isolates from camels were collected in Eastern and Western Sudan. Isolates from Eastern Sudan which were kept under continuous prophylactic treatment with quinapyramine (Trypacide®), were found to bear a single pattern and belonged to one karyotype group. From Western Sudan where trypanosomosis management was done by individual treatment of proven parasitaemic cases, isolates with diverse karyotype patterns were obtained. This study concluded that the occurrence of karyotype homogeneity amongst *T. evansi* isolates from field situations where anti-trypanosomal compounds have been used may infer the existence of drug-resistance.

**Key words:** *Trypanosoma evansi*, drug resistance, Trypacide®, karyotyping, Sudan.

INTRODUCTION

Trypanosomosis caused by *Trypanosoma evansi* (Surra) has been recorded in Africa, South America and the Middle East (Luckins et al., 1979). In Sudan, this parasite has been reported in different regions since 1908, affecting camels and to lesser extent horses. The extensive use of commercially available trypanocides such as suramin and quinapyramine (Trypacide®) has resulted in the appearance of strains resistant to these drugs (Boid et al., 1989). Such drug-resistance has been an important subject in the study of these parasites (Zhang et al., 1992, 1993; El Rayah et al., 1999; Luckins, 2000). Waitumbi and Young (1994) observed that distinguishing *T. evansi* isolates by molecular electrophoretic karyotype is more discriminating than kDNA analysis. Hence, the observation of karyotype patterns reoccurring in isolates from herds kept under chemoprophylaxis could help in the identification of drug-resistant parasites.

The aim of this study was to characterize the isolates by molecular karyotyping to see whether there are any differences between sensitive and resistant isolates.

MATERIALS AND METHODS

**Trypanosomes**

Twelve *T. evansi* isolates from Eastern and Western Sudan and one from Kenya were used. Their origins, designation, date of isolation, and drug sensitivities were previously described (El Rayah et al., 1999) and are summarized in Table 1.

**Preparation of trypanosome suspensions**

Female Swiss ICR mice were infected by intraperitoneal inoculation of thawed cryopreserved stabilates of *T. evansi*. After 2-4 days, when the mice showed rising parasitaemia, they were bled by cardiac puncture while under anesthesia, using a heparinized syringe. The trypanosomes were separated from the blood by anion-exchange chromatography on DE53 cellulose (Whatman Biosystems Ltd. UK.) as previously described by Lanham and Godfrey (1970). Trypanosomes were counted in CASY1 (Schaerfe system; Reutlingen, FRG) cell-analyzing systems. Cleaned trypanosomes suspension were centrifuged for 15 min at 2500 rpm at 60°C; then the pellet was resuspended in 10 ml PSG (phosphate buffer saline...
Table 1: Origin & in vivo drug sensitivities of T.evansi isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Year</th>
<th>Quinapyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastry 1a</td>
<td>Kassia</td>
<td>1994</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Eastry 2</td>
<td>Kassia</td>
<td>1994</td>
<td>4</td>
</tr>
<tr>
<td>Eastry 4</td>
<td>Toker</td>
<td>1994</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Eastry 5</td>
<td>Elhabsha</td>
<td>1994</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Eastry 6</td>
<td>Elgadri</td>
<td>1995</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Westry 1b</td>
<td>Elobied</td>
<td>1993</td>
<td>4</td>
</tr>
<tr>
<td>Westry 2</td>
<td>Ryash</td>
<td>1995</td>
<td>3</td>
</tr>
<tr>
<td>Westry 3</td>
<td>BurBur</td>
<td>1996</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Westry 4</td>
<td>Elkhorelbied</td>
<td>1996</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Westry 5</td>
<td>BurBur</td>
<td>1996</td>
<td>315 (IC50)</td>
</tr>
<tr>
<td>Westry 7</td>
<td>Elobied</td>
<td>1996</td>
<td>103 (IC50)</td>
</tr>
<tr>
<td>STIB 779(1)</td>
<td>Kenya</td>
<td>1979</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

*MCD100 = Minimum Curative Dosage in 100% infected mice.*

Figure 1. Large sized chromosomes of sudanese T. evansi.  
Location: [E]: Isolates from Eastern Sudan, [W]: Isolates from Western Sudan, [K]: Isolate originally from Kenya.  
Type: A, B, C to F (Karyotype patterns).  
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Figure 2. Intermediate-sized chromosomes of Sudanese *T. evans*.

**Location:** [E]: Isolates from Eastern Sudan, [W]: Isolates from Western Sudan, [K]: Isolate originally from Kenya.

**Type:** A, B, C to F (Karyotype patterns).


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**RESULTS**

Patterns of large and intermediate chromosomes DNA of *T. evansi* stocks are shown in Figures 1 and 2. The patterns were allocated A, B, C to F arbitrarily. The Sudanese *T. evansi* patterns were characterized by large numbers of large-sized chromosomes (3000-750 kb) and several discrete intermediate-sized chromosomes (825-200 kb) which tended to be larger than those of *Sacharomyces cerevisiae* chromosome size-marker. All *T. evansi* stocks (Eastry 1-6) isolated from Eastern Sudan in 1994 showed the same pattern for large and intermediate-sized chromosomes (Figure 1, 2, tracks 1-5, pattern A). Eastry 6 which was isolated in 1995 showed a minor difference. It had an extra band of large-sized chromosomes (> 2200 kb) (Figure 1, track 6, pattern B).

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**Pulsed field gel electrophoresis**

Chromosomal DNA was prepared in agarose blocks as previously described (Van der Pleog et al., 1984a, 1984b). Pulsed field gel electrophoresis (PFG) was carried out using a Pulsaphore apparatus (Pharmacia LKB) with hexagonal electrode array. Agarose gel (1%) was applied. For large chromosomes (1-3 MB) 6-phase programme of 6 days [1200 s, 1000 s, 900 s, 800 s, 700 s, and 600 s (pulse frequency/second; 86 V)] was used. For intermediate size (825-200 kb) 4-phase programme of 72 h (300 s, 200 s, 150 s, 100 s; 110 V) was used. Gels were stained using ethidium bromide, 2 µg/ml and photographed.
The Western isolates (Westry 1, 2, 3, 4, 5 and 7) had individual karyotypes (Figure 2, tracks, 7, 8, 9, 10, 11, 12, 13, patterns C, D, E, F). Two isolates from Western Sudan (Westry 5 and 7) showed the same pattern (Figure 1, 2, track 9, 10, pattern D), they were closely related to Eastry 6 (Track 12, pattern E), and they had more extra bands of intermediate-sized chromosomes. Westry 2 which was isolated in Western Sudan in 1995 showed the same pattern of all Eastern isolates (Figure 1 and 2, track 8, pattern A). T. evansi STIB 779 clone 1 originally isolated from Somalia and cloned at Swiss Tropical Institute, was used in this study for comparison with the sudanese isolates. It showed a different pattern (Figure 1, track 13, pattern K), which is used as African isolate marker.

DISCUSSION

Five of the Eastern Sudanese T. evansi isolates (Easty 1 – 5) were indistinguishable by PFG and another isolate (Easty 6) shared 7 of 8 resolved bands comprising this karyotype. These isolates are therefore closely related, even though they were of different geographical origins in Eastern Sudan. It is worth mentioning that nomads in Eastern Sudan have good financial status that enables them to keep their animals under continuous chemoprophylactic treatment. However, this probably lead to the appearance of highly resistant T. evansi isolates to Quinayramine (Trypacide®) by selection pressure, which may be expressed by the presence of a single karyotype pattern. The findings confirm the results of Waitumbi et al. (1994) who found that rigorous application of trypanocide may lead to homogeneity of karyotype patterns in camel herds, since karyotype heterogeneity has been observed in herds where trypanocide usage is less intense. In contrast, T. evansi isolates from Western Sudan showed different molecular karyotypes compared to Eastern T. evansi isolates. This may indicate that T. evansi was introduced more than once into Sudan and also it may be due to different drug regimes used in the Western Sudan. Lun et al. (1992) found indistinguishable karyotypes in remote isolates of T. evansi from China. It is unlikely that our T. evansi isolates and African T. evansi (Waitumbi and Young, 1994) diverged faster than the same parasites in China. One isolate of T. evansi in Western Sudan has a karyotype pattern (pattern A) which was previously observed in the Eastern isolates. It is therefore possible that the parasites with the karyotype pattern "A" infected camels in Eastern Sudan was introduced into Western Sudan. Different times of the isolation, regions and camel herd movements may explain the divergence in the karyotype patterns in this study.

This study explains partially the persistence of drug-resistance phenomenon in the field by application of a single trypanocidal drug for prolonged periods that may lead to selection pressure to the parasites which survive and resist this drug (Eirayah et al 1999). The above observations have important epidemiological implications in the control of T. evansi infections in camels. First, the data indicate that rigorous application of trypanocides may lead to a homogeneity of karyotype patterns in camel herds, since karyotype heterogeneity has been observed in herds where trypanocidal usage is less intense. Thus, the occurrence of karyotype homogeneity amongst T. evansi isolates from field situations where anti-trypanosomal compounds have been used may infer the existence of drug-resistant trypanosomes (Eirayah et al., 1999; Luckins, 2000). Secondly, because T. evansi karyotype appears to remain stable over long periods of time in vivo, molecular karyotyping is a useful epidemiological tool since it allows the dynamics of different trypanosomes populations to be studied in field situations. Nevertheless, more studies is needed to investigate such a phenotype as observed in other different trypanosomes (e.g. T. b. brucie), in which some authors speculated that resistance is the result of loss of a particular purine transporter, P2/TbAT1 (Harry et al., 2004).

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REFERENCES