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CHARACTERIZATION OF TRYPACIDE DRUG -RESISTANT T.EVANSI

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SUMMARY

Molecular karyotyping was used for comparing the isolates by pulsed field gel electrophoresis. Sixteen isolates T.evansi from Eastern, Central, and Western Sudan were collected. Isolates from Eastern Sudan that were kept under continuous prophylactic treatment with Trypacide^{R} (Quinapyramine sulphate), this isolates bear one pattern and belonged to one karyotype group. From Western and Central Sudan, in which trypanosomosis management was done by individual treatment of proven parasitaemic cases, isolates with diverse karyotype patterns were obtained.

Keywords: Trypanosoma evansi, drug resistance, quinapyramine sulphate (Trypacide^{R}), PFG karyotype

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 parasites has been reported in different region since 1908, affecting camels and to lesser extent horses. The extensive use of commercially available trypanocides such as suramin and quinapyramine has resulted in the appearance of strains resistant to these drugs (Boid *et al.*, 1989). Such drug-resistant has been an important subject in the study of these parasites (Zhang *et al.*, 1992, 1993). 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 recurring in isolates from herds kept under chemoprophylaxis could help in the identification of drug resistant parasites. The aim of this study to characterize all isolates by molecular karyotyping to see if there are any differences between sensitive and resistant isolates.

MATERIALS AND METHODS

Trypanosomes

16 T.evansi isolates from Central, Eastern and Western Sudan and one isolate from Kenya were used. Their origins, designation, date of isolation, and drug sensitivity are given in (El Rayah *et al.*, 1999).

Mice

Female ICR albino mice weighing 20 to 25 g were used. Mice were housed in standard cages, five per cage, and received pelleted food and water ad libitum.

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 that rising parasitaemia they were bled by cardiac puncture while under anesthesia, using a heparinized syringe. Lanham and Godfrey (1970) separated the trypanosomes from the blood by anion-exchange chromatography on DE53 cellulose (Whatman Biosystems Ltd. UK.) as previously described with minor modifications. Trypanosomes were counted in CASY1 (Scharfe system; Reutlingen, FRG) cell-analyzing systems.

Preparation of chromosomal DNA

Chromsomal DNA agarose blocks were prepared as described (Pleog *et al.*, 1984) and stored at $4C^{0}$ in 50 mM until required. PFG electrophoresis was carried out using a Pulsephore apparatus(Phamacia) with hexagonal electrode array, 1% agarose and a 6-phase programme of 6 days (1200s, 1000s, 900s,

800s, 700s, and 600s; 86 V) for large chromosomes (1-3MB), and 4 phase programme of 72 hours (300s, 200s, 150s, 100s; 110V). Gels were stained and photographed.

RESULTS

Chromosomes DNA patterns of large and intermediate chromosomes of *T.evansi* stocks are shown in Fig 1,2,3,4. The patterns were allocated A, B, C, and etc F, arbitrary. 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 *S.cerevisiae* chromosome size-marker. *T.evansi* stocks isolated from Eastern Sudan in July 1994 showed the same pattern for large and intermediate-sized chromosomes (Fig. 1, 2, tracks 1-5, pattern A). Eastry 6 that was isolated in Feb. 1995 showed a minor difference. IT had a more extra band of large-sized chromosomes (> 2200 kb) (Fig. 1, track 6 pattern B). The Central and Western isolates have individual karyotype (Fig 2, 4. Tracks, 7,8,9,10,11,12,13,14,15,16,17. Patterns, C, D, E, F, H, J, I.). Two isolate from Western Sudan showed the same pattern (Fig 1, 2 , Track, 9, 10, Pattern, D.) and closely related to Westry 6 (Track 12 pattern E), they have more extra band of intermediate-sized chromosomes. Westry 2 which isolated from Western Sudan in May 1995 showed the same pattern of Eastern isolates (Fig 1 and 2 , track,8 , pattern A.). The Central *T.evansi* stocks were all isolated during August 1996. They had showed different patterns except two which had the same pattern (Fig 3 and 2; tracks,14, 15, Pattern; H, I, J). *T.evansi* STIB 779 clone 1 originally isolated from Somalia and cloned at Swiss Tropical institute was used in this study for comparison with our isolates. It had different pattern, (Fig 1.a tracks 13, pattern K), which is used as African isolate marker.

DISCUSSION

The approach used here is studying the phenotype of the parasite by studying the chromosomal arrangement and to see if all isolates were derived from the same progenitors or there are differences between them.

Separation of chromosomes-sized DNA molecules can produce finer distinction between *T.evansi* isolates than analysis of kDNA (Lun *et al.*, 1992; Waitumbi and Young, 1994). Selection pressure and population bottleneck would act to restrict the divergence between *T.evansi* population. Trypanosome chromosomal DNA molecules are subject to length alterations for a number of reasons, e.g. telomere growth and contraction (Bernards *et al.*, 1983), rearrangements associated with antigenic variation (Ploeg *et al.*, 1984), deletions/ insertion. Therefore stocks with an identical karyotype may be assumed to be very closely. Five of the Eastern Sudanese *T.evansi* stocks were indistinguishable by PFG and another stock shared 7 of 8 resolved bands comprising this karyotyping. These stocks are therefore closely related, even though they were of different geographical origin in Eastern Sudan. It worth mentioning that nomads in Eastern Sudan have good financial status which enables them to keep their animals under continuous chemoprophylactic treatment, this lead to appearance of highly resistant *T.evansi* isolates to Trypacide by selection pressure, which is probably expressed by the presence of a single karyotype pattern. The findings confirms 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 and Central Sudan had different molecular karyotype if compared with Eastern T.evansi. This may indicate that *T.evansi* was introduced more than once into Sudan and also it may be due to different drug regime 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) diverges faster than the same parasite in China .One isolate of *T.evansi* in Western Sudan has karyotype pattern (pattern A) which was observed previously 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 herds movements explain the divergence in the karyotype patterns in this study.

This study explain partially the persistence of drug-resistance phenomenon in the field by application of one trypanocide drug in the field for long periods which lead to selection pressure of parasite which survive and resist this drug.

Large-sized chromosomes of Western and Eastern *T. evansi* isolates

Location	K W W W W W W E E E E E E
Type	G F E D D A C B A A A A A
Track	M 13 12 11 10 9 8 7 6 5 4 3 2 1 M

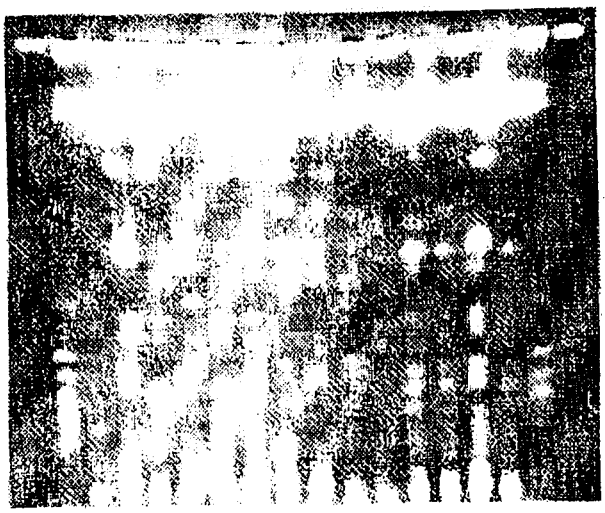

Figure 1:

Location : (E) Isolate from Eastern Sudan (W) Isolate from Western Sudan
(K) Isolate originally from Kenya

Type: A,B,C ... etc Karyotype patterns

Tracks:

1) Eastry 1	4) Eastry 4	7) Westry 1	10) Westry 4
2) Eastry 2	5) Eastry 5	8) Westry 2	11) Westry 5
3) Eastry 3	6) Eastry 6	9) Westry 3	12) Westry 6

13) STIB 779 clone 1 M) *S. cervisiae*

Intermediate-sized chromosomes of Western and Eastern *T. evansi* isolates

Location	K W W W W W W E E E E E E
Type	G F E D D A C B A A A A A
Track	M 13 12 11 10 9 8 7 6 5 4 3 2 1 M

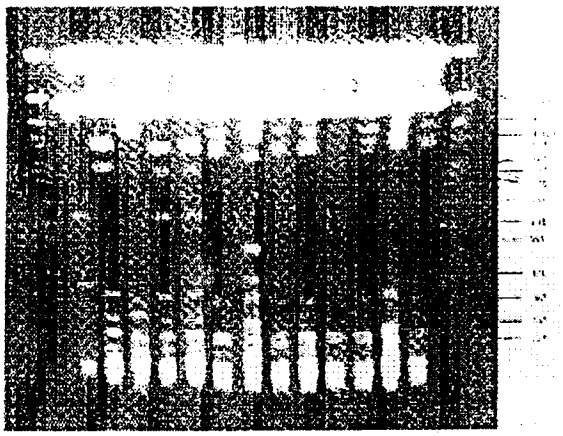

Figure 2:

Location : (E) Isolate from Eastern Sudan (W) Isolate from Western Sudan
(K) Isolate originally from Kenya

Type: A,B,C ... etc Karyotype patterns

Tracks:

1) Eastry 1	4) Eastry 4	7) Westry 1	10) Westry 4
2) Eastry 2	5) Eastry 5	8) Westry 2	11) Westry 5
3) Eastry 3	6) Eastry 6	9) Westry 3	12) Westry 6

13) STIB 779 clone 1 M) *S. cervisiae*

Large-sized chromosomes of Western, Central and Eastern *T. evansi* isolates

Location	K W W WW W W C C C C E E E
Type	G F E D D A C B A A A A A A
Track	M 13 8 12 11 10 9 2 15 17 16 14 6 2 1 M

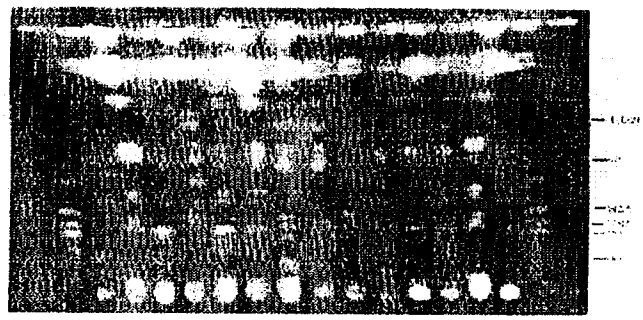

Figure 3:

Location : (E) Isolate from Eastern Sudan (C) Isolate from Central Sudan
(W) Isolates from Western Sudan (K) Isolate originally from Kenya

Type: A,B,C ... etc Karyotype patterns

Tracks:

1) Eastry 1	2) Eastry 2	6) Eastry 6	7) Westry 1
8) Westry 2	9) Westry 5	10) Westry 2	11) Westry 3
12) Eastry 3	14) Cestry 1	15) Cestry 4	16) Cestry 5
17) Cestry 6	M) *S. cervisiae*		

Intermediate-sized chromosomes of Western, Central and Eastern *T. evansi* isolates

Location	K W W W W W W C C C C E E E
Type	G F E D D A C B A A A A A A
Track	M 13 8 12 11 10 9 2 15 17 16 14 6 2 1 M

Figure 4:

Location : (E) Isolate from Eastern Sudan (C) Isolate from Central Sudan
(W) Isolates from Western Sudan (K) Isolate originally from Kenya

Type: A,B,C ... etc Karyotype patterns

Tracks:

1) Eastry 1	2) Eastry 2	6) Eastry 6	7) Westry 1
8) Westry 2	9) Westry 5	10) Westry 2	11) Westry 3
12) Eastry 3	14) Cestry 1	15) Cestry 4	16) Cestry 5
17) Cestry 6	M) *S. cervisiae*		

The above observations have important epidemiological implications in the control of *T.evansi* infections in camels. Firstly, the data indicate rigorous application of trypanocides may lead to the homogeneity of karyotype patterns in camel herds, since karyotype heterogeneity has been observed in herds where trypanocides is less intense. Thus, the occurrence of karyotype homogeneity amongst *T.evansi* isolates from field situation where anti-trypanosomal compounds have been used may infer the existence of drug-resistance trypanosomes. Finally because *T.evansi* karyotype appear 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.

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