

Full Length Research Paper

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

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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 recurring 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 trypanosome suspension were centrifuged for 15 min at 2500 rpm at 60°C; then the pellet was resuspended in 10 ml PSG (phosphate buffer saline

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Table 1: Origin & in vivo drug sensitivities of *T.evansi* isolates

Isolate	Origin	Year	Quinapyramine
			MCD ₁₀₀
Eastry 1a	Kassla	1994	>10
Eastry 2	Kassla	1994	>10
Eastry 3	Toker	1994	4
Eastry 4	Kassala	1994	>10
Eastry 5	Elhabsha	1994	>10
Eastry 6	Elgadriif	1995	>10
Westry 1b	Elobied	1993	4
Westry 2	Ryash	1995	3
Westry 3	BurBur	1996	>10
Westry 4	Elkhorelbyied	1996	>10
Westry 5	BurBur	1996	315(IC ₅₀)
Westry 7	Elobied	1996	103(IC ₅₀)
Stib 779(1) ^c	Kenya	1979	>8

*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).

Tracks: [1] Eastry 1, [2] Eastry2, [3] Eastry 3, [4] Eastry 4, [5] Eastry 5, [6] Eastry 6, [7] Westry1, [8] Westry 2, [9] Westry 5, [10] Westry 4, [11] Westry 3, [12] Westry 6, [13] STIB 779 clone 1, [M] *S. cerevisiae*.

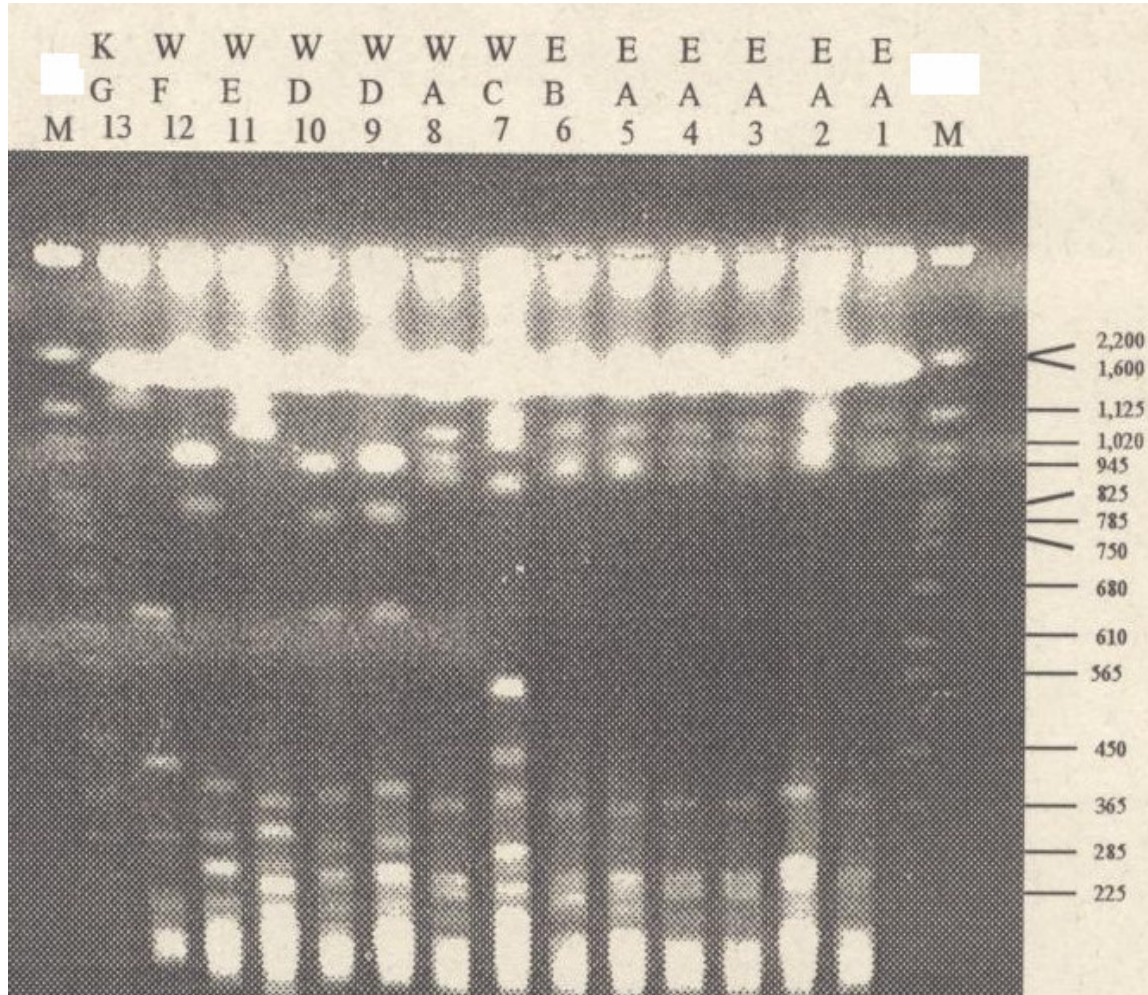


Figure 2. Intermediate- sized chromosomes of Sudanese *T. evansi*.

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

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

Tracks: [1] Eastry 1, [2] Eastry2, [3] Eastry 3, [4] Eastry 4, [5] Eastry 5, [6] Eastry 6, [7] Westry1, [8] Westry 2, [9] Westry 5, [10] Westry 4, [11] Westry 3, [12] Westry 6, [13] STIB 779 clone 1, [M] *S. cerevisiae*.

glucose) and further centrifuged at the same speed. Finally the pellet was resuspended in 750 μ l PSG, transferred into eppendorf tube, and centrifuged for 10 min at 1000 g. The supernatant was discarded and the pellet stored in ice.

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 (PFGE) 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 μ g/ml and photographed.

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).

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 (Eastry 1 – 5) were indistinguishable by PFG and another isolate (Eastry 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 (Elrayah 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 (Elrayah 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

- Boid R, Jones TW, Payne RC (1989). Malic Enzyme Type VII isoenzyme as an indicator of Suramin resistance in *Trypanosoma evansi*. *Exp. Parasitol.* 69: 317-323.
- El Rayah IE, Kaminsky R, Schmid C, El Malik KH (1999). Drug resistance in Sudanese *Trypanosoma evansi*. *Vet. Parasitol.* 80: 281-287.
- Harry P, de Koning, *Laura F, Anderson, Mhairi Stewart, Richard JS, Burchmore, Lynsey JM, Wallace, Michael P, Barrett (2004). The Trypanocide Diminazene Aceturate Is Accumulated Predominantly through the TbAT1 Purine Transporter: Additional Insights on Diamidine Resistance in African Trypanosomes. *Antimicrob. Agents Chemother.* Vol. 48(5): 1515-1519.
- Lanham SM, Godfrey DG (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol.* 28: 521-534.
- Luckins AG (2000). Control of Non Tsetse-Transmitted Animal Trypanosomiasis- Drugs and Drug Resistance in *Trypanosoma evansi*. Proceedings of the workshop on Drug Delivery and resistance in the context of integrated disease management, held 31 May-June 4 1999 in Nairobi Kenya, Newsletters on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV) Newsletter No. 2.
- Luckins, AG, Boid R, Rae P, Mahmoud MM, El Malik KH, Gray AR (1979). Serodiagnosis of infection with *Trypanosoma evansi* in camels in the Sudan. *Tropical Animal Health and Production.* 11(1): 1-12.

- Lun Z-R, Brun R, Gibson W (1992). Kinetoplast DNA and Molecular karyotypes of *Trypanosoma evansi* and *Trypanosoma equiperdium* from China. *Mol. Biochem. Parasitol.* 50: 189-196.
- Van der Ploeg LHT, Cornelissen AWCA, Barry JD, Borst P (1984a). Chromosomes of kinetoplastida. *Eur. Mol. Biol. Org. J.* 3: 3109-3115.
- Van der Ploeg, LHT, Cornelissen AWCA, Michels PAM, Borst P (1984b). Chromosomes rearrangements in *Trypanosoma brucei*. *Cell* 39: 213-221.
- Waitumbi JN, Young JR (1994). Electrophoretic Karyotyping is a sensitive epidemiological tool for studying *Trypanosoma evansi* infections. *Vet. Parasitol.* 52: 47-56.
- Waitumbi JN, Murphy IB, Peregrine AS (1994). Genotype and drug resistance phenotype of *Trypanosoma evansi* isolated from camels in northern Kenya. *Ann. Trop. Med. Parasitol.* 88 (6): 677-683.
- Zhang ZQ, Giroud C, Baltz T (1992). In vivo and in vitro sensitivity of *Trypanosoma evansi* and *T. equiperdium* to diminazene, suramin, Mel Cy, quinapyramine and isometamidium. *Acta Tropica* 50 : 101-110.
- Zhang ZQ, Giroud C, Baltz T (1993). *Trypanosoma evansi* . in vivo and in vitro determination of trypanocide resistance profile. *Exp. Parasitol.* 77: 387-394.