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MOLECULAR CHARACTERISATION OF DRUG-RESISTANT *Trypanosoma evansi* ISOLATED FROM NATURALLY INFECTED CAMELS IN SUDAN

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ABSTRACT

Polymerase Chain Reaction (PCR) was used on 13 isolates of *T. evansi* from Butana, Gadarif and Southern Kordofan state. The result showed that PCR was very sensitive in detecting differences in Butana and Gadarif states. The isolates which tested by PCR in Butana and Gadarif states, 9 of them were resistant to Quinapyramine sulphate and 7 isolates were sensitive to Cymelarsan. One isolate out of 4 isolates tested by PCR in southern Kordofan state was sensitive to both Quinapyramine sulphate and Cymelarsan.

Key words: Camel, polymerase chain reaction (PCR), Sudan, trypanosomosis

Trypanosomosis is one of the major disease affecting camels, caused mainly by *Trypanosoma evansi* and transmitted mechanically, predominantly by *Tabanus* and other biting flies. The disease generally takes a chronic form where huge production losses occur due to lowered milk and meat production in adults, abortion, and mortalities in young camel calves (Schwartz and Dioli, 1992).

PCR is based on the use of an enzyme, DNA polymerase, which amplifies sequences of DNA bases, until sufficient material is produced to be detected. It does so by polymerisation of nucleic acids (FAO, 1998).

The management of African trypanosomosis at the farmer level has been predominantly dependent on the use of trypanocidal drugs; it was estimated that about 35 million doses per year were used in Africa to cure the disease (Geerts and Holmes, 1997).

Drug resistance emerged as one of the major obstacles for the control of trypanosomosis. It can be defined as the ability of a trypanosome strain to survive, despite the administration of a trypanocide given in doses equal to or higher tshan those usually recommended. Therefore, this study is planned to:

1. Determine the extent of drug resistance of some Sudanese isolates of *Trypanosoma evansi* from Butana, Gadarif and Kordofan states against Quinapyramine sulphate and Cymelarsan drugs using an in-vivo method.
2. Study the DNA profile of some isolates to demonstrate possible differences between resistant and sensitive isolates.

Materials and Methods

The study was conducted in 3 districts namely Butana, Gadarif and southern Kordofan from herds of traditionally managed dromedary camels.

Camels with trypanosomosis were sampled, regardless of the age, sex, breed, and season. The history of each case was taken and the clinical manifestations recorded. A total of 13 isolates of *T. evansi* (7 from Butana, 2 from Gadarif and 4 from Southern Kordfan) were collected.

One drop of blood from ear vein of sick or suspected camel with trypanosomosis was placed on clean slide and covered with a cover slip, examined under microscope at a magnification 40x10. Positive samples were taken for further use.

Propagation of *T. evansi* isolates in laboratory rodents and cryopreservation of *T. evansi*

About 3 ml of blood was collected from the jugular vein of camels with trypanosomosis using vacutainer with EDTA after confirmation by wet mount. Initial propagation of trypanosomes was done in albino white mice. Each mouse was injected intraperitoneally with 0.5 ml of the trypanosome positive camel blood.

Tail blood of the inoculated mice was monitored for parasitaemia daily by using wet mount. At peak

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parasitaemia trypanosomes were harvested by cardiac puncture. About 0.85 ml of blood with trypanosomes was added to the 0.85 ml of phosphate-buffered saline glucose (PSG) containing 20% glycerol into cryo-tube (2 ml) and then transferred through gradual cooling to liquid nitrogen (-196°C) (in the phase of liquid nitrogen).

Experimental design

Each isolates of *T. evansi* were tested for drug resistance against Quinapyramine and Cymelarsan. For that purpose, albino mice were used as described by Eisler (2001).

Control group

Positive control: Each isolates of *T. evansi* from each district was inoculated intraperitoneally in 6 albino mice and observed for 2 months without drug administration.

Experimental group

Each isolates of *T. evansi* from each district was inoculated intraperitoneally in 6 albino mice and then tested for drug resistance against Quinapyramine and Cymelarsan, the drugs were given subcutaneously. The mice were monitored over 2 months. A trypanosome isolate was considered as drug-sensitive if at least 5 out of the 6 treated mice were cured. If fewer than 5 mice were cured, the isolate was considered resistant to the dosage used (Eisler, 2001).

Polymerase Chain Reaction (PCR)

Extraction of DNA from blood samples

Whole blood was used for extraction of total genomic DNA using commercially available QIAamp blood kit according to the manufacturer's instruction.

The DNA (5 µl) was added to 17.5 µl mixture, 2 µl primers and 0.5 µl polymerase (Taq). Then the mixture was centrifuged for 1 minute. Then the PCR was running for 2 hours at 56°C. PCR containing amplified products were loaded on to gels of Seakem agarose and electrophoresed gels were stained with ethidium bromide and *T. evansi* primary PCR products were easily identified following visualisation under UV light.

Results

The results of all states showed that out 36 mice tested (18 mice tested group and 18 mice control group) only 6 mice were cured with Quinapyramine sulphate. Out of 36 mice tested (18 mice tested group and 18 mice control group) 16 mice were cured with Cymelarsan (Table 1).

Polymerase Chain Reaction (PCR) was used on 13 isolates of *T. evansi* from Butana, Gadarif and southern Kordofan states. The result showed that PCR was very sensitive in detecting differences in Butana and Gadarif state. All isolates were similar. As to the isolates which were tested by PCR in Butana and Gadarif states, 9 of them were resistant to Quinapyramine sulphate and 7 isolates were sensitive to Cymelarsan. One isolate out of 4 isolates tested by PCR in southern Kordofan state was different sensitive to both Quinapyramine sulphate and Cymelarsan (Figs 1, 2).

Discussion

In this study, Quinapyramine sulphate has shown resistance in camels of Butana and Gadarif states. Quinapyramine sulphate is the drug of choice used nowadays, for the treatment of camel trypanosomosis in the Sudan. Expired preparations and sub-curative doses as well as bad administration of the drug were used by camel owners who have special preference to this preparation. Consequently, over this long period of extensive usage, various population of *T. evansi* had been exposed to this drug. This situation provided ideal condition for the development of acquired trypanocidal drug resistance. Quinapyramine sulphate has been reported to be a drug of choice for the treatment of Suramin-resistant *T. evansi* in camels (Finelle, 1973; Curd and Davey, 1950; Leach, 1961; Gill and Malhotra, 1971; Luckins *et al*, 1979). Our results confirm the previous observation of emergence of strains resistant to routine treatment with Quinapyramine sulphate (Luckins *et al*, 1979;

Table 1. Testing *Trypanosoma evansi* in mice for drug resistance in Butana, Gadarif and southern Kordofan states.

Drug	No. of isolates tested	No. of mice in control group	No. of mice in tested group	No. of mice cured	Interpretation
Quinapyramine sulphate	13	18	18	6 33.33%	Drug-resistance
Cymelarsan	13	18	18	16 88.89%	Drug-sensitive

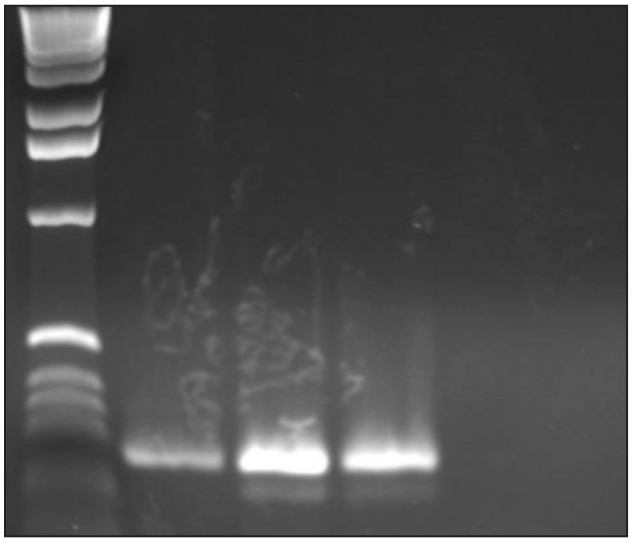

Fig 1. PCR of *Trypanosoma evansi* from Gadarif state

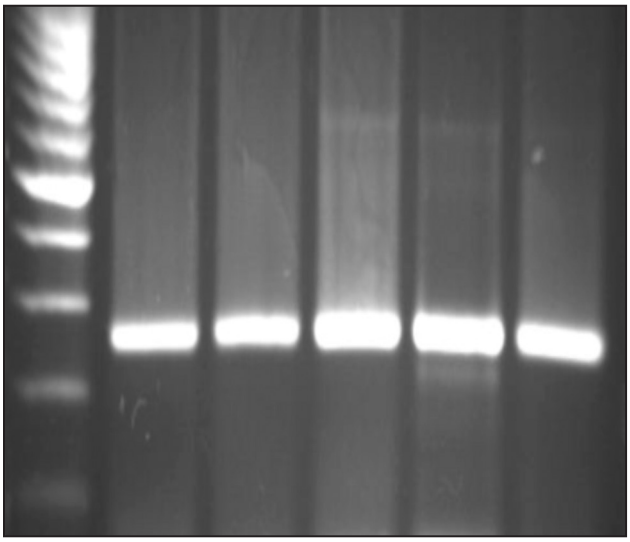

Fig 2. PCR of *Trypanosoma evansi* from Butana state

Mahmoud and Osman, 1979; Schillinger *et al,* 1984; El Rayah and El Malik, 1990; El Rayah, 1997). Another study by Khier and Abdalla (1983) showed that stocks were only sensitive to Quinapyramine sulphate at doses higher than the normal prescribed doses.

Cymelarsan was developed in 1980s for use against Surra (*T. evansi* infection) in camels, as the other compounds in use against this disease are either frequently associated with resistance problems to Suramin and Quinapyramine (Luckins, 1998; Bourdichon, 1998). It has also been tested against *T. evansi* in domestic buffaloes and *T. evansi* and *T. brucei* in other animals (FAO, 1998). Treatment of *T. evansi* infected camel in Morocco with Cymelarsan reduced sero-prevalence level from 58 to 19% within a year (Rami *et al,* 2003).

In this study Polymerase Chain Reaction (PCR) was very sensitive in detection of *T. evansi* infection in camels. Several methods had been developed for the detection of *T. evansi* infection including microscopy, card agglutination test (CAT), microhaematocrit centrifugation technique (MHCT) (Woo, 1970), enzyme-linked immunosorbent assay (ELISA) (Indrakamhang *et al,* 1982), DNA hybridisation (Viseshakul and Panyim, 1990) and polymerase chain reaction (PCR) (Wuyts *et al,* 1994; 1995; Omawa *et al,* 1999). The PCR provided high sensitivity among these methods and it had been reported that it could detect *T. evansi* 3 days earlier than by microscopy (Ijaz *et al,* 1998). A study by Wasana *et al* (2000) had shown that PCR-based assay is one of the most powerful tools for the detection of *T. evansi* in several animals and vectors and is beneficial for epidemiological studies of this parasite and for the control programme. In Sudan, Aradaib and Majid (2006) indicated that nested polymerase chain reaction (nPCR) based assay, using well characterised *T. evansi* primers, provides a simple, rapid, sensitive and specific detection of *T. evansi* in naturally infected camels (*Camelus dromedarius*) and can be as a valuable tool during epidemiological surveys and control programme.

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