Investigation of Drug Susceptibility in Rats Experimentally Infected with *Trypanosoma evansi* Isolated from Camels in Sudan

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**ABSTRACT** - A number of 18 adult male outbred albino rats, weighing between 133-137g were used to investigate the drug susceptibility of *Trypanosoma evansi* strain isolated from naturally infected dromedary camels in Umbadir area, North Kordofan State, Sudan. The rats were divided into 3 groups (C, D and F) of 6 animals each. Group C and D were infected intraperitoneally with *T. evansi* (Umbadir stabilate) with 1×10⁴ Trypanosome for the inoculum. Group D rats were given quinapyramine sulphate (20 mg/Kg bwt) after parasitaemia was evident. Group F was left as a healthy, uninfected control for the stabilate. When parasite counts were one or more parasites per field, counting in haemocytometer was used for exact number of parasite per cubic millimeter using Neubaeur’s counter. Parasites from tail blood were first fixed, stained and diluted in trypomastigote diluting reagent. The parasites were diluted to the level that can be easily counted in WBC counting chamber in the haemocytometer. The total number of parasites was expressed as log₁₀ number of parasites per ml of blood. The presence and degree of parasitaemia were determined daily for each rat by examining tail blood. The identity of the local stabilates of *T. evansi* was confirmed through adopting PCR where primers that target the internal transcribed spacer one (ITS1) of the ribosomal DNA were used. There was significant reduction in serum glucose and potassium as well as a significant increase in total protein, urea, calcium, albumin and cholesterol in group C. The Umbadir stabilate showed a low mortality and high sensitivity to quinapyramine sulphate.

**Key-words**- Drug susceptibility, *T. evansi*, Dromedary camels, Sudan

**INTRODUCTION**

*Trypanosoma evansi* has a wide range of hosts and is pathogenic to most of the domestic and laboratory animals. Besides causing *Surra* disease in all the principal species of domestic animals [¹,²], *T. evansi* is also highly pathogenic to laboratory animals (rats, mice and rabbits) [³-⁶]. In New Zealand, about 24 white cross breed rabbits were challenged with strain of *T. congolense*. The infections were characterized by intermittent pyrexia, undulating parasitaemia, anorexia and emaciation [⁷]. The major plasma biochemical changes included hypoglycaemia, elevated total protein and plasma cholesterol. There were
significant elevation of alkaline phosphatase (ALP), asparate aminotransferase (AST), total bilirubin and fluctuating changes in the levels of plasma alanine aminotransferase (ALT) and urea [7]. The rats infected with T. evansi resulted in significant reduction in serum glucose and phosphorus; compared to significant increase in Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and total protein. Microscopically, the brain tissues of the infected rats revealed acute congestion of the meningeal capillaries, perivascular oedema, neuronecrosis (vaculation), gliosis and trypomastigotes in diluted capillaries. The lung revealed oedema, congestion, multifocal alveolar emphysema, hyperplasia of the peri-bronchial lymphoid tissues and haemorrhages. The spleen showed extensive haemorrhages, haemosiderosis and aggregation of histiocytes resulting in multinuclear giant cell formation. The kidneys showed acute congestion of the glomerular tufts [8].

MATERIALS AND METHODS

Ethics statement

The study protocol was approved by the Faculty of Veterinary Medicine, Sudan University of Science and Technology, according to their guidelines for sampling domestic animals in Sudan and in compliance with the animal welfare of Sudan.

Study area

The Trypanosome strain used in this study was isolated from a dromedary camel in Umbadir Area, North Kordofan State, Sudan, while the rest of the experiment events were carried out in the premises of the College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Sudan.

Preparation of the inocula

A strain of T. evansi originated from a naturally infected camel from Umbadir in North Kordofan State was used in this study. One albino rat was infected intraperitoneally with blood that was cryopreserved in liquid nitrogen, containing 1×10⁵ parasites/animal to obtain a large amount of the parasite for blood inoculation of experimental groups. Parasitemia in the inoculated rat was regularly monitored by collecting blood from the tail vein and analyzing it by light microscopy. Blood samples showing actively motile organisms with characteristic flagellar movement were considered as positive for the presence of T. evansi. At the peak of parasitemia, the rat was anesthetized with chloroform inhalation and with the help of a disposable syringe; blood was collected aseptically in EDTA anticoagulant by cardiac puncture. Using Neubauer’s counter the trypanosome titre was determined in order to be diluted to 1X10⁴ trypanosomes for the inoculums [9].

Experimental animals

Eighteen (18) adult male outbred Albino rats, weighing between 133 to 137 g were used in this study. The rats were divided into 3 groups (C, D and F) each containing 6 rats and were kept in a cage in the same environment with controlled temperature (25–30°C) and relative humidity around 60–70%.

Experimental design and grouping

The experimental rats were distributed into 3 groups of 6 rats each. Group C, the control group as infected with T. evansi (Umbadir stabilate) and left without treatment. Group D was infected with T. evansi (Umbadir stabilate) and was treated with the quinapyramine sulphate (20 mg/kg bwt), after the parasite was seen (at the latency). Group F was uninfected healthy control for Umbadir Stock.

Trypanosome sub-inoculation

Sub-inoculation of the experimental group C and group D was carried out intraperitoneally with the use of a sterile insulin syringe. Rat blood containing 1×10⁴ trypanosomes in 0.2 ml volume was inoculated in each rat individually at day zero. The numbers of inoculated flagellates were estimated by Neubauer Chamber and the dilutions to obtain the titre of the inoculum were made in sterile phosphate buffer saline with glucose (PSG).

Table 1: The experimental design of the Umbadir stabilate and protocol of treatment with Quinapramine sulphate

<table>
<thead>
<tr>
<th>Group</th>
<th>Stablate</th>
<th>Parasite</th>
<th>Treatment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Umbadir</td>
<td>T. evansi</td>
<td>Infected not treated</td>
</tr>
<tr>
<td>D</td>
<td>Umbadir</td>
<td>T. evansi</td>
<td>Infected and Treated with Q.S. (20mg/kg bwt)</td>
</tr>
<tr>
<td>F</td>
<td>Uninfected Healthy Control for Umbadir Stock</td>
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</table>

Estimation of parasitaemia

All infected rats were bled daily, as recommended by Eisler et al. [10] from the tip of the tail for trypanosomes detection using the following parasitological diagnostic methods:

Wet preparation

A drop of blood was mounted on a microscope slide and covered with 22x22 mm glass cover slip. Counts of parasite per field or per preparation were determined.

Haemocytometer count

The presence and degree of parasitaemia were determined daily for each rat by examining tail blood. A drop (5 µl) of blood was collected from the tail and mixed with the trypanosome counting reagent (45 µl). Parasitaemia was counted as for WBC count using Neubauer counter and the result was designated as a number of parasites per ml of blood. Parasitaemia was counted using 40 × magnifications.
during the 64 days of the experiment.

Drug Dosages
Quinapyramine sulphate was used at a dose rate of 20mg/kg bwt and dissolved in sterile water such that the required dose was contained in 0.2 ml of water for each rat and then inoculated intra-peritoneally.

Biochemical analysis
Blood for sera was collected in plain containers from the retro-orbital plexus. Serum samples were collected at four day intervals and were kept on -20°C until needed for biochemical analysis. All parameters were measured using commercial kits (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain), except the sodium and potassium where commercial kits (BioMed Schiffgraben 41, 30175 Hannover, Germany) were used. The values obtained were read with a spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, UK) at appropriated wavelengths and the values were calculated using standard formulae [11].

Polymerase Chain Reaction (PCR)
DNA extraction
For DNA extraction from blood using protein precipitation method, 5 ml of blood were collected in EDTA tube, 300 μl of blood were placed in a 1.5 Eppendorf tube, then 1000 μl of RCLB were added and mixed by inversion and centrifuged at 9000 rpm for 5 min. The supernatant was discarded and the pellet (white blood cells) was washed with 1000µl of RCLB, then 300 μl of WCLB were added, followed by 1 μl of proteinase k and the solution was incubated at 37°C overnight. 100 μl of protein precipitation solution (6M NaCl) were added on the next day and mixed by vortexing gently and 200 μl of cold chloroform were added, centrifuged at full speed for 6 min, the aqueous phase was then transferred into a clean Eppendorf tube. Double volume of cold Ethanol was then added to precipitate the DNA, centrifuged at 14000 rpm for 5 min, then the supernatant was poured off without disturbing the precipitate, washed with 70% Ethanol (600 μl), air dried at room temperature, resuspended in 100 μl of TE buffer or ddH₂O and then left to be dissolved. The DNA was stored in -20°C.

Amplification
The extracted genomic DNA was subjected to a PCR that amplifies the ITS1 region of the rDNA gene of *T. evansi* by using TeRoTat 920 F 5'-CTGAGAGGTTGGA ATGGAGAG-3' and TeRoTat 1070 R, 5'-GTTTCGGTGT TCTTGGTGTGA-3' primers set. To obtain the expected 151 bp PCR product, (maxime PCR premix kit) Master Mix, 2X in a 20 μl total volume was deployed. Each reaction includes, 1 μl of 10 mM of each primer, 4 μl extracted DNA and 14μl of ddH₂O. Thermocycling profile that starts with an initial hold for 2 min. at 95°C, followed by 35 cycles of 95°C for 30 sec., 64°C for 30 sec. and 72°C for 1 min and final extension step of 5 min at 72°C was adopted. PCR products were subjected to electrophoresis in 2% agarose (Vivantis Technologies, Malaysia) in Tris-borate EDTA buffer and was visualized under UV light. The ITS1-PCR detection method constituted a powerful molecular diagnostic tool for *T. evansi* detection as well as discrimination from other trypanosomes in one PCR.

Statistical Analysis
Data were presented as mean±standard error of mean (SE). The statistical analysis was performed using independent T-test and Statistical Package for the Social Science (SPSS) software. P-values less than 0.05 were considered statistically significant.

RESULTS
The overall mean of parasitaemia
The overall mean of parasitaemia in group C was 5.9±2.01 while in group D, it was 0.07±0.36 (Table 2).

Table 2: Overall means and Std. Deviation of parasitaemia levels in rats infected-not-treated (C group) Umbadir stabilates and rats infected-treated (D group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strains</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated</td>
<td>Umbadir</td>
<td>5.9</td>
<td>2.01</td>
<td>61</td>
</tr>
<tr>
<td>Treated</td>
<td>Umbadir</td>
<td>0.07</td>
<td>0.36</td>
<td>61</td>
</tr>
</tbody>
</table>

The response of Umbadir stabilate to Quinapyramine Sulphate in group (C)
Rats inoculated by 1X10⁷ of Umbadir stabilate of *Trypanosoma evansi* but were not treated with Quinapyramine Sulphate (group C) inflicted low mortalities during the experiment period where one died at day 30 post infection (pi), one at day 50, one at day 51 and one at day 54, with a mean survival period of 46.3±11 (Table 3).

Table 3: Comparison between rats infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate at a dose rate of 20mg/kg bwt (after patency Group D) and rats infected-not-treated control (Group C)

<table>
<thead>
<tr>
<th>Time to death</th>
<th>Control of 6 Rats</th>
<th>Infected Treated of 6 Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 30</td>
<td>1 rat n= 5 rats</td>
<td>All rats survived until the end of the study period</td>
</tr>
<tr>
<td>Day 50</td>
<td>1 rat n=4 rats</td>
<td></td>
</tr>
<tr>
<td>Day 51</td>
<td>1 rat n=3 rats</td>
<td></td>
</tr>
<tr>
<td>Day 54</td>
<td>1 rat n= 2 rats</td>
<td></td>
</tr>
<tr>
<td>X=46.3 ±11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The response of Umbadir stabilate to Quinapyramine Sulphate in group (D)
All rats survived until the end of the study period. Treatment of rats in group (D) was commenced on day 4 when the parasitaemia level was \( \log_{10} 2.2 \). By day 5, all treatment groups were negative. Treated rats remained negative until the end of the study period. Up to day 4, there was no significant difference between parasitaemia levels in both treated and control groups. By day 18, the treated group recorded a mean parasitaemia of \( \log_{10} 0 \) while that of the control was \( \log_{10} 6.8 \), which was significantly higher than the treatment group (p<0.05). In the control rats by day 31, the parasitaemia fluctuated between \( \log_{10} 7.3 \) to \( \log_{10} 8.0 \) until the end of study period (Fig. 1).

Fig. 1: Comparison of the means of parasitaemia levels (\( \log_{10} \)) between rats infected with \( T. evansi \) (Umbadir Stabilate) treated by Quinapyramine Sulphate at a dose rate of 20mg/kg bwt and rats infected-not-treated control

Serum biochemical changes

Serum total protein
The mean serum values of total proteins in group C were increased during the study. The statistical analysis showed a means of 8.2±1.9 g/dl while group D has shown no changes at all days of the experiment. The statistical analysis showed a means of 6.4±0.84 g/dl (Table 4). The normal ranges of some serum biochemical parameters of rats are shown in Table 5.

Table 4: Mean serum levels of biochemical changes in rats infected with \( T. evansi \) infected-not-treated control and infected-treated with Quinapyramine Sulphate at a dose rate of 20mg/kg bwt

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins</td>
<td>g/dL</td>
<td>8.2±1.9</td>
<td>6.4±0.84</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>45±18.4</td>
<td>74.9±19.8</td>
</tr>
<tr>
<td>Urea</td>
<td>mg/dL</td>
<td>29.8±8.3</td>
<td>19.6±1.7</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dL</td>
<td>4.7±0.88</td>
<td>4.3±0.54</td>
</tr>
</tbody>
</table>

Serum glucose
The mean serum values of glucose in group C were decreased. The statistical analysis showed a means of 45±18.4 mg/dl while group D was normal at all days of the experiment. The statistical analysis showed a means of 74.9±19.8 mg/dl (Table 4).

Serum Urea
The mean serum values of urea in group C were increased during the study. The statistical analysis showed a means of 29.8±8.3 mg/dl. While group D were showed no changes at all days of the experiment. The statistical analysis showed a means of 19.6±1.7 mg/dl (Table 4).

Serum Albumin
The mean serum values of albumin in group C were increased. The statistical analysis showed a means of 4.7±0.88 g/dl, while group D were normal at all days of the experiment. The statistical analysis showed a means of 4.3±0.54 mg/dl (Table 4).

Serum calcium
The mean serum values of calcium in group C were increased. The statistical analysis showed a means of 10.7±5.8 mg/dl while group D was normal at all days of the experiment. The statistical analysis showed a means of 8.7±3.3 mg/dl (Table 4).

Serum cholesterol
The mean serum values of cholesterol in group C were elevated during the study. The statistical analysis showed a means of 118.6±21.6 mg/dl while group D was normal at all days of the experiment. The statistical analysis showed a means of 98.8±24.8 mg/dl (Table 4).
Serum sodium
The mean serum values of sodium in group C and group D showed normal levels at all days of the experiment. The statistical analysis in group C showed a means of 147.6±4.7 mEq/l while group D showed a means of 148.8±3.5 mEq/l (Table 4).

Serum potassium
The mean serum values of potassium in group C were decreased. The statistical analysis showed a means of 4.4±1.3 mEq/l while group D was normal at all days of the experiment. The statistical analysis showed a means of 6±0.62 mEq/l (Table 4).

Confirmation of the identity of the test Trypanosoma by PCR
The stabilate of Trypanosomes used in this study were confirmed to be Trypanosoma evansi by PCR using specific primers that specifically target the ITS1 region of the rDNA gene of T. evansi. Using this specific technique, the DNA extract from whole blood of rat infected with the Trypanosome yielded an amplicon of the size 151 bp; a PCR product size expected for this species of the Trypanosome (Fig. 2).

DISCUSSION
In this study of Umbadir stabilated T. evansi which was isolated from a camel at Umbadir, North Kordofan, Sudan (named as Umbadir stock; sensitive to Quinapyramine Sulphate) was investigated and studied. During this study, the local isolate of T. evansi stock was compared in experimentally infected rats. Rats inoculated by 1X10^7 of the Umbadir stabilate of T. Evansi, but were not treated with Quinapyramine sulphate (group C), showed a pre-patent period of 3-4 days post infection, which was similar to the result reported by Da silva et al. [12]. The low mortalities recorded during the experiment in group C were in agreement with Faye et al. [13] while Desquesnes [14] reported that T. evansi (Sokoto isolate) was pathogenic to donkeys with low mortalities and high morbidity (100%). The result also was in agreement with those reported by Njiru et al. [15] and Tekle and Abebe [16] who encountered low mortality and high morbidity among camels infected with T. evansi in Ethiopia. In the rats infected-treated with Quinapyramine Sulphate after the patency at a dose of 20 mg/kg bw (D group), a prepatent period of 3-4 days post infection was recorded which is the similar to the result reported by Da silva et al. [12] where all rats remained negative and survived until the end of the study period which was attributed to the effect of the drug used.

The increase in serum urea in group C is in agreement with the result reported by Siwajothi et al. [17], Ajakaive et al. [18], Arora and Pathok [19] and Samia et al. [20] who found a similar increase in the concentration of urea in rats experimentaly infected with T. evansi. Megahed et al. [21] reported similar results when they found that the concentration of urea was increased in pregnant camels infected with T. evansi compared to healthy pregnant camels. More studies had similar results among which those conducted by T. b. brucei infected rabbits [12] and goats [23] and T. gambiense infected vervet monkeys [24]. The elevated serum urea levels had been associated with kidney diseases such as glomerulonephritis, urinary tract obstruction and excessive protein catabolism associated with severe toxicity and febrile conditions [25].

In the present study, the increase in the serum calcium in group C was similar to the result of the study conducted in sheep infected with T. congolense [26]. However, the levels of calcium were not changed in camels infected by the Trypanosome parasite as reported by Chaudhary and Iqbal [27] and Schenk et al. [28]. On the other hand, the result reported by Egbe-Nwiyi et al. [29] showed that the level of calcium was decreased in the rats infected with T. congolense.

In the present study, the serum sodium levels in the group C were found normal, unlike the result reported in sheep infected with T. congolense where it increased as reported by Tella [30]. The same also was found with the result reported by Arora and Pattok [19]; Samia et al. [20] and Wolker et al. [31], who found that the concentration of sodium was depressed in rats experimentally infected with T. evansi.

In the present study, the serum potassium levels in group C were decreased which is in line with the result reported in sheep infected with T. congolense where it decreased as reported by Tella [30] as well as in sheep infected with T. brucei [32]. Moreover, Arora and Pattok [19]; Samia et al. [31] also found that the concentration of potassium was depressed in rats experimentally infected with T. evansi, unlike the result obtained by Ikejian [33] who found that serum potassium levels increased in T. equiperdum and T. brucei infection of rats; and also with the result reported by Moon et al. [34] in T. rhodesiense infected mice, which had the normal level of potassium.
In the present study, the serum cholesterol in group C was increased, which was in agreement with the findings reported by Megahed et al. [21], who found that the concentration of cholesterol has increased in pregnant camels infected with *T. evansi* compared with healthy pregnant camels. Similar result was also reported by Sivajothi et al. [17], who found that cholesterol was increased in rats infected with *T. evansi*. However, the result reported by Egbe-Nwiyi et al. [29] in rats infected with *T. congolense* and that reported by Barghash [35] in rats infected with *T. evansi*, both showed that the level of cholesterol was decreased, which was not in line with our findings in this study.

The serum total proteins in group C were increased progressively during the study, which disagreed with the results reported by Hussain et al. [36], Sivajothi et al. [17], Biryumumaisho et al. [37], Katunguka-Rwakishaya [38], Allam et al. [39] and Megahed et al. [21]. Moreover, the result recorded in this study had contradicted the observations recorded in sheep infected with *T. brucei* studied by Taiwo et al. [40]. This increase of total protein was in agreement with the result reported by Arora and Pathok [19] and Samia et al. [20] who found that the concentration of total protein was increased in rats experimentally infected with *T. evansi*. Also, the increase in serum total proteins recorded in this study was in agreement with the result reported by Orhue et al. [41], Ekanem and Yusuf [42] and Sow et al. [43], who found that the concentration of total protein was increased in rats experimentally infected with *T. brucei* and *T. brucei*-infected rabbits. The increase in protein levels during the chronic phase of the infection is usually due to the increase in globulin levels, as a result of the immune response by the animals to the infection [44-46]. In the present study, the serum glucose in group C has decreased during the study, which is in line with the result reported by Sivajothi et al. [17], Sinha et al. [47], Arora and Pathok [19] and Samia et al. [20] who found that the concentration of glucose was decreased in rats experimentally infected with *T. evansi*. This situation could be explained by the parasites’ need for glucose for their cellular metabolism through their glycolytic pathway [48]. However, this finding was not in agreement with that reported by Youssif et al. [49], who found that goat infected by *T. evansi* had increased levels of glucose.

The increase of serum albumin reported in group C disagrees with the results reported by Arora and Pattok [19] and Samia et al. [20] who found that the concentration of albumin was depressed in rats experimentally infected with *T. evansi*. Also that result reported by Megahed et al. [21] found that the concentration of albumin was decreased in pregnant camels infected with *T. evansi* compared with healthy pregnant camels and, also, a decrease of albumin in camels infected by *T. evansi* was further reported by Hussain et al. [36].

The further confirmation of the identity of the candidate trypanosome by PCR through using primers that specifically targeted the ITS1 region of the rDNA gene of *T. evansi* that is performed in the present study, is similar to the result reported by Croof [50] who used molecular method (PCR) in his study of 40 camels which were tested parasitologically and serologically where 90% of them were found to be positive. PCR has been used in the detection of infection with *T. evansi* in buffaloes [51,52], in horses [53] and in camels [54]. There was no comprehensive data on the use of PCR for detection of infection in Sudanese breed of dromedary camels (*Camelus dromedarius*). Hunter [55] and Aradaib and Magid [56] suggested, the use of the reliable, easy to perform and less time-consuming PCR for accurate classification of trypanosome species in Sudan, where the morphological feature of the trypanosome is the main tool used for its classification.

**CONCLUSIONS**

18 adult male outbred albino rats were used to investigate the drug susceptibility of *T. evansi* strain isolated from naturally infected dromedary camels in Sudan. The rats were divided into 3 groups (C, D and F). Group C and D were infected intraperitoneally with *T. evansi* (Umbadir stabilate) with 1×10⁴ trypanosoma for the inoculum. Group D rats were given quinapyramine sulphate (20 mg/Kg bw) after parasitaemia was evident. Group F was left as healthy uninfected control for the stabilate. Parasites from tail blood were first fixed, stained and diluted in trypanosome diluting reagent to the level that can be easily counted in WBC counting chamber in the haemocytometer. The total number of parasites was expressed as log₁₀ number of parasites per ml of blood. The presence and degree of parasitaemia were determined daily for each rat by examining tail blood. The identity of the local stabilate of *T. evansi* was confirmed through adopting PCR where primers that target the internal transcribed spacer one (ITS1) of the ribosomal DNA were used. There was significant reduction in serum glucose and potassium as well as a significant increase in total protein, urea, calcium, albumin and cholesterol in group C. The strain used in the study (Umbadir stabilate) showed low mortality and high sensitivity to quinapyramine sulphate.

**REFERENCES**


