Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 3, 359-363

Patterns of relapse in experimental *Trypanosoma evansi* infections: Evidence of resistance to widely used trypanocides in Egypt

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ARTICLE INFO

Recieved: 30 May 2025

Accepted: 1 July 2025

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Keywords:

Diminazene aceturate, Drug resistance, Egypt, Quinapyramine, Trypanocides, *Trypanosoma evansi*.

ABSTRACT

The emergence of drug resistance and treatment relapse in *Trypanosoma evansi* infections poses a growing threat to animal health and productivity in endemic regions. Using a murine model, this study evaluated the *In vivo* efficacy of commonly used trypanocidal agents quinapyramine, diminazene aceturate, and melarsomine against Egyptian isolates of *T. evansi*. Eight experimental groups of Swiss albino mice were monitored over 60 days for parasitemia clearance and relapse following treatment at standard and double doses. The results revealed marked resistance to both quinapyramine and diminazene aceturate, as evidenced by early relapse and high mortality, even at elevated doses. In contrast, melarsomine (Cymelarsan) demonstrated superior efficacy, completely clearing parasitemia at a dose of 0.5 mg/kg with no relapse observed. Melarsomine is still a viable option for treating resistant *T. evansi* strains, according to the study, which also emphasizes relapse as a crucial sign of developing resistance. These findings underscore the urgent need for regular drug efficacy monitoring and reevaluation of treatment protocols in endemic settings.

Introduction

The most common pathogenic trypanosome that affects animals is *Trypanosoma evansi*. Cattle, buffaloes, horses, donkeys, and camels are among the animals it infects (Chandu *et al*., 2021). The parasite's negative effects on the health of infected animals result in significant financial losses. These include infertility, weakness, abortion, decreased milk production, weight loss, and a decreased capacity for strenuous work. Anemia, compromised immunity, nerve damage, and ultimately death may result from the illness if left untreated (Fesseha *et al*., 2022). There have been reports of a high prevalence of *T. evansi* infections in Egypt. Buffaloes and donkeys in the Giza area were discovered to be highly afflicted by researchers (Zayed *et al*., 2010). In a same vein, several instances of the parasite in sheep and goats were documented in Cairo (Ashour *et al*., 2013). A significant rate of infection in cattle was also found in later studies conducted in Southern and Lower Egypt (Elhaig *et al*., 2016; Fereig *et al*., 2017).

T. evansi can be controlled using various trypanocidal drugs, including polysulfonated naphthylamine, quinapyramine sulfate and chloride, melarsomine, homidium salts, and diminazene aceturate (Desquesnes *et al*., 2013). Although these medications have been in use for more than 50 years, the parasite has gradually developed increasing resistance to them over time (Geerts and Holmes, 1998).

Melarsomine has shown the ability to overcome resistance to polysulfonated naphthylamine and quinapyramine when given as a deep intramuscular injection at a dose of 0.25 mg/kg (Luckins, 1998). However, its high cost compared to other treatments limits its widespread use (Giordani *et al*., 2016). Studies have also shown that even with this dose, relapses can still occur in buffaloes and cattle (Desquesnes *et al*., 2011).

Quinapyramine was used in the past (Indrakamhang, 1998), but today, diminazene aceturate is the most used drug for treating surra in various animals, including horses, buffaloes, cattle, pigs, deer, elephants, and dogs (Tuntasuvan and Luckins, 1998). However, after more than 40 years of use, treatment failures have been frequently reported, likely due to drug resistance (Tuntasuvan *et al*., 2003a; Tuntasuvan *et al*., 2003b; Hin-On *et al*., 2004).

T. evansi rapidly becomes resistant to medications such as isometamidium chloride and diminazene aceturate. Poor living conditions, large parasite burdens, compromised immune systems in infected animals, or improper dosage during therapy are frequently cited as causes of this resistance (Osman *et al*., 1992; Mutugi *et al*., 1994). Populations that exhibit drug resistance can be made responsive to treatment by either increasing the dosage or employing a combination of treatment approaches (Joshi and Singh, 2000; Ndung *et al*., 2020).

The present study was conducted to evaluate and compare the efficacy of different dose levels of quinapyramine, melarsomine, and diminazene aceturate in the treatment of experimental *Trypanosoma evansi* infections in a murine model. The study further aimed to investigate the relationship between drug dosage and treatment failure, with a particular focus on relapse as an indicator of resistance. By analyzing relapse patterns and therapeutic outcomes over a 60-day observation period, the study sought to identify the most effective dosing strategies for potential field application and to provide deeper insights into long-term treatment outcomes in *T. evansi* infections.

Materials and methods

Ethical approval

The study was conducted by the ethical guidelines outlined in Alexandria University's Declaration. Ethical approval was obtained from the Research Ethics Review Committee of the Faculty of Veterinary Medicine (Protocol No. 161). Additionally, the *In vivo* experiments were carried out following approval from the Institutional Animal Care and Use Committee

ISSN: 2090-6277/2090-6269/

(IACUC) under Protocol No. 194.

Experimental animals

A total of 56 female Swiss albino mice, weighing 20-25 g, aged 8-9 weeks, obtained from the Medical Technology Center, Medical Research Institute, Alexandria University, were used for the *In vivo* experiment. Mice were maintained in cages and housed at a temperature of 20 ± 2˚C and relative humidity of 50-60% with artificial light. Mice were provided with pelleted feed and water ad libitum. Mice were allowed for adaptation for ten days before the beginning of the experiment.

Trypanosoma evansi strain

The *T. evansi* strain was initially isolated from a naturally infected camel provided kindly by Dr. Tahani S. Behour from the Biotechnology Research Unit of the Animal Reproduction Research Institute in Giza, Egypt, and subsequently maintained on Swiss albino mice.

Tested drugs

The effectiveness of three compounds with trypanocidal activity against *T. evansi* infection was evaluated using murine models. The following drugs were tested: Diminazene aceturate (Batrynil®, Arab Company for Medical Products, Egypt), Bis (aminoethylthio)-4 melamino-phenylarsine dihydrochloride (Cymelarsan®, Merial, France), and Quinapyramine sulfate combined with quinapyramine chloride in a ratio of 3:2 (Triquin®, Vétoquinol, Mumbai). All stock solutions were freshly prepared on the day of the experiment using double-distilled water and stored at −20 °C until use.

Donor mice infectivity

Donor mice were experimentally infected with *T. evansi* by intraperitoneal injection of 1×10^5 trypanosomes suspended in phosphate-buffered saline. Parasitemia was monitored daily using wet blood films obtained from the tail vein. Once a high level of parasitemia was detected, Blood was collected from infected donor mice, then appropriately diluted in EDTA buffer before being used for inoculation in the test animals in different experimental groups as described by Mdachi *et al.* (2023).

In vivo experiments

Eight groups (Groups 1 through 8) of seven mice each were created from the mice. As the negative control, Group 1 was made up of mice that were healthy and not affected. On Day 0, mice in Groups 2 through 8 received an intraperitoneal inoculation of 1×10^5 *T. evansi*. After the inoculation, the parasitemia was tracked for three days. As the positive control, Group 2 was not given any therapeutic intervention. The medication was injected intraperitoneally for three days in a row, starting 72 hours after the infection.

Quinapyramine was administered in a dosage of 3 mg/kg to Group 3. Group 4 received Quinapyramine at a dosage of 6 mg/kg. Melarsomine was provided to Group 5 at a dosage of 0. 25 mg/kg. For Group 6, Melarsomine was given at a dosage of 0. 5 mg/kg. Diminazene aceturate was supplied to Group 7 at a dose of 3.5 mg/kg, and Diminazene aceturate was given at a dose of 7 mg/kg to Group 8. Wet blood samples from the tail vein were used every day to measure parasitemia levels using Herbert and Lumsden's rapid matching method (Herbert and Lumsden, 1976). For every sample, 20 microscopic areas were scanned at a magnification of ×400 (Da Silva *et al*., 2009; Obi *et al*., 2020). After seven days of daily assessments, there were three observations every week until sixty days after the infection.

Data evaluation

IBM SPSS software version 20.0 (Armonk, NY: IBM Corp.) was used to analyze the data after it was entered into a computer program. The data distribution's normality was evaluated using the Shapiro-Wilk test. The quantitative data was summarized using descriptive statistics such as mean, standard deviation, median, and range (minimum and maximum values). The 5% level of significance was used to establish statistical significance.

Results

Effect of trypanocidal drugs on T. evansi

The dose had an impact on each drug's response. The findings demonstrated that at a level of 0.5 mg/kg, *T. evansi* exhibited great sensitivity to Cymelarsan. All seven infected mice had their parasite totally eradicated by this dosage, and there was no subsequent infection recurrence. Six of the seven mice were treated by Cymelarsan at the lower dosage of 0.25 mg/kg. The mortality rate was 14.28%, with one mouse passing away on day 24 after infection. On the second day following infection, parasitemia was initially found; on the third day, all mice had parasitemia, at which point treatment started (Figs. 1 and 2). Throughout the trial, Group 6 (treated with 0.5 mg/kg Cymelarsan) did not have any parasites. On the other hand, all the mice in the positive control group (Group 2), which was left untreated, perished by day 7 due to rapidly rising parasitemia.

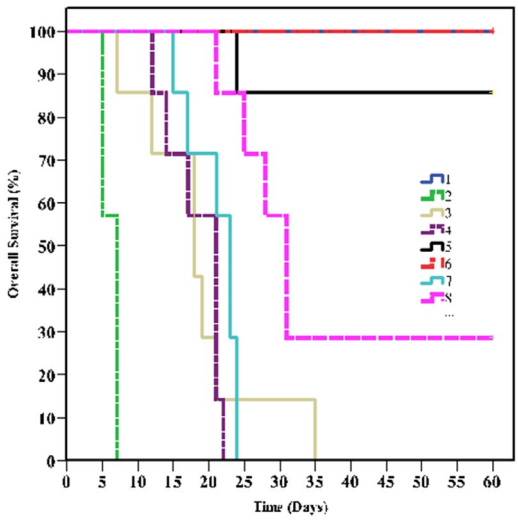

Fig. 1. Kaplan-Meier survival curve for groups and overall survival.

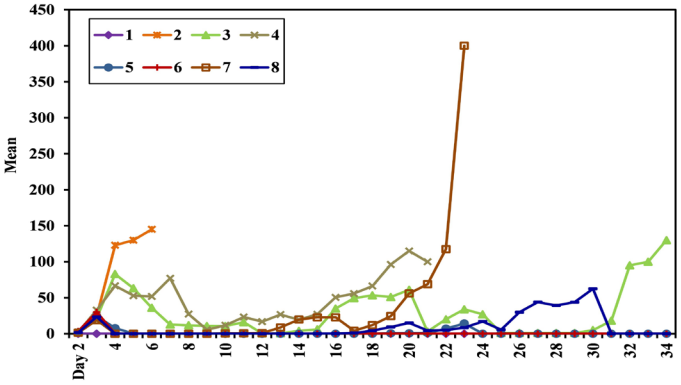

Fig. 2. Comparison of groups under study based on parasitemia.

Treatment with 3 and 6 mg/kg of quinapyramine sulfate and chloride did not work. A high level of resistance to these medications was demonstrated by the deaths of every mouse in these groups by days 7 and 12,

respectively (Figs. 3 and 4).

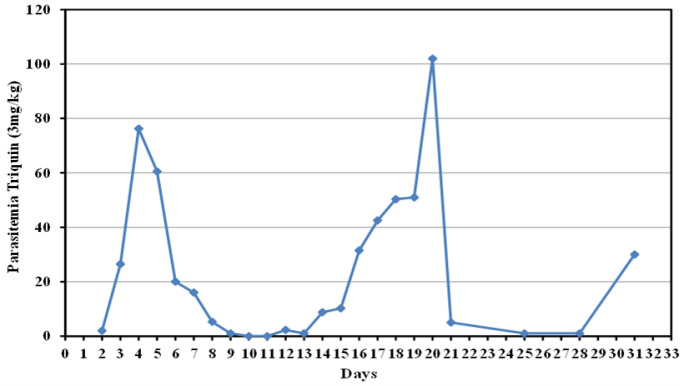

Fig. 3. Swiss albino mice infected with *T. evansi* are observed daily following treatment with 3 mg/kg of Triquin.

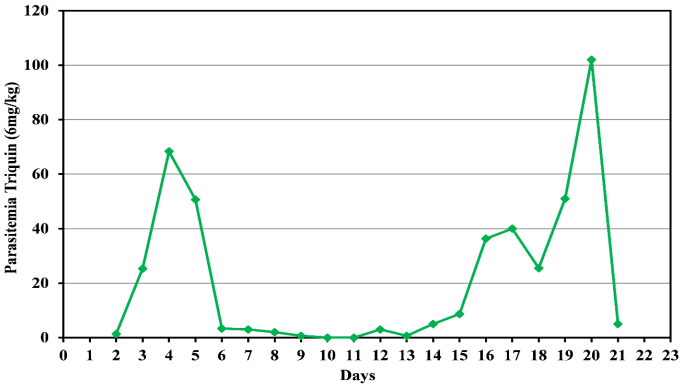

Fig. 4. Swiss albino mice infected with *T. evansi* on a daily basis following treatment with 6 mg/kg of Triquin.

At first, mice given 3.5 mg/kg diminazene aceturate showed signs of improvement; nevertheless, by day 10, parasitemia had returned, and all the mice perished. Only two mice made it to the end of the study when the dose was raised to 7 mg/kg, and most animals continued to relapse. Although it might be a little less than the resistance seen to quinapyramine, the high death rates (100% and 71.43%) indicate considerable resistance to diminazene aceturate (Figs. 5 and 6).

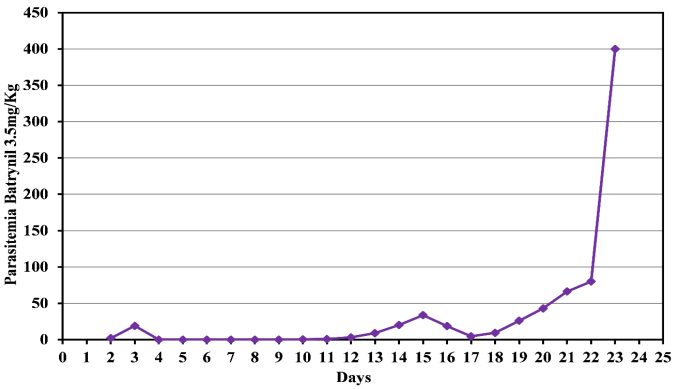

Fig. 5. Swiss albino mice infected with *T. evansi* are observed daily following treatment with 3.5 mg/kg of batrynil.

The most successful treatment was 0.5 mg/kg of Cymelarsan, which completely eradicated the illness without causing any recurrence. With only one recorded death, it was largely successful even at the lower dose of 0.25 mg/kg. This suggests that although the Egyptian isolate of *T. evansi* exhibited strong resistance to both, it is extremely susceptible to Cymelarsan.

Patterns of relapse and resistance

In four and three mice, respectively, Triquin at doses of 3 and 6 mg/kg momentarily decreased parasitemia. But by day 14, there were relapses, and by day 20, parasitemia reached its climax, which killed all the mice (Figs. 3 and 4). In mice given 3.5 mg/kg diminazene aceturate, parasitemia returned between days 13 and 15, and in every instance, the animals died (Fig. 5). Only two mice were healed at the higher dose of 7 mg/kg, while five mice relapsed on day 16 and died subsequently (Fig. 6). After receiving 0.25 mg/kg Cymelarsan, one mouse experienced a relapse on day 19 and passed away on day 24 (Fig. 7). Nevertheless, no relapse was seen in any of the mice when the dosage was raised to 0.5 mg/kg.

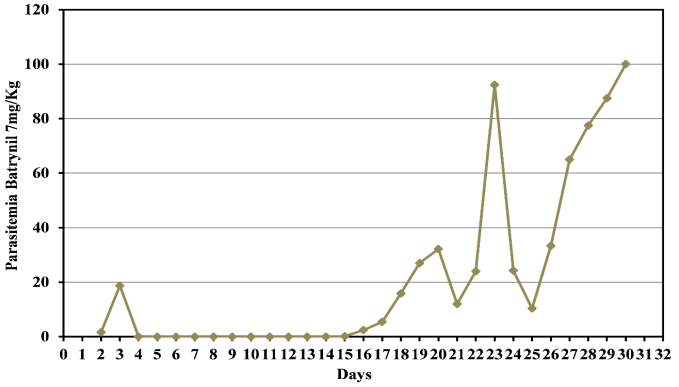

Fig. 6. Swiss albino mice infected with *T. evansi* are observed daily following treatment with 7 mg/kg of Batrynil.

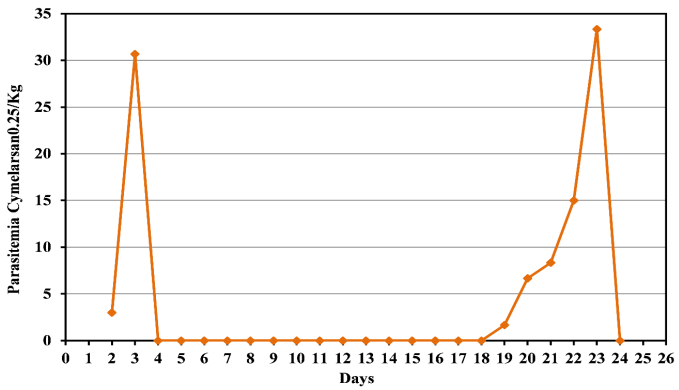

Fig. 7. Swiss albino mice infected with *T. evansi* on a daily basis following treatment with 0.25 mg/kg of Cymelarsan.

Discussion

The purpose of this investigation was to determine how sensitive Egyptian isolates of *Trypanosoma evansi* were to widely accessible trypanocidal medications. A single daily dose was given for three days in a row as part of the treatment plan; the dosage was increased to improve drug efficacy and lower the chance of relapse. For sixty days, the entire experiment was observed.

Our findings revealed a concerning level of drug resistance in the Egyptian *T. evansi* isolates. Specifically, the parasite showed complete resistance to the combination of quinapyramine sulfate and chloride (Triquin), as neither tested dose produced a curative effect. Resistance to diminazene aceturate (Batrynil) was also observed to be moderate at the standard dose and high at the double dose. In contrast, *T. evansi* was found to be sensitive to Cymelarsan (melarsomine hydrochloride), particularly at 0.5 mg/kg, which eliminated the infection completely without relapse. Even the lower dose (0.25 mg/kg) showed high effectiveness, curing most cases.

Our findings were supported by Mekonnen *et al.* (2018) who reported that treatment of 2 out of 6 mice infected with *T. evansi* (type A), using MelCy (Cymelarsan) at a dose of 0.125 mg/kg BW for 4 consecutive days,

while none was cured of *T. evansi* (Type B) infected mice, but complete cure was achieved only at a much higher dose (2 mg/kg) combined with diminazene diaceturate (DIM; Veriben) at a dose of 20 mg/kg BW. Aregawi *et al*. (2020) observed that a dose of 3.5 mg/kg diminazene in mice failed to prevent relapses, while a dose of 7 mg/kg administered 27 days after the standard treatment to all 10 mice cleared the parasites with no parasitemia detected for three months, indicating that higher doses may be necessary.

Previous research also highlights inconsistencies in drug effectiveness across species and geographical strains. For example, Lun *et al*. (1991); Tuntasuvan *et al*. (2003a); Colpo *et al*. (2005); Da Silva *et al*. (2009) and Howes *et al*. (2011) all reported diminazene failure in treating trypanosomosis in horses, mules, dogs, cats, and buffaloes. Eke *et al*. (2020) observed early relapse and death in animals treated with diminazene, emphasizing its limited efficacy in certain *T. evansi* infections. In contrast, Musa *et al*. (1994) demonstrated that Cymelarsan at both 0.25 and 0.5 mg/kg BW was highly effective and safe in dromedary camels, with no observed relapses throughout a 90-day PI. Similarly, Desquesnes *et al*. (2011) recommended increasing the Cymelarsan dose to 0.5 mg/kg for cattle due to relapses observed at the lower dose (0.25 mg/kg).

Recent findings by Mdachi *et al*. (2023) revealed that 75% of *T. evansi* isolates from camels showed multidrug resistance, highlighting the urgency for integrated control strategies, including better vector management and more cautious drug use. Further, Abdel-Rady *et al*. (2024) compared Cymelarsan and quinapyramine (Aquin-1.5) in dromedaries and found Cymelarsan to be significantly more effective (100% vs. 83.3% cure rates). Jatau *et al*. (2010) reported similar success with a 7 mg/kg dose of diminazene in rats infected with a Nigerian strain *T. evansi*, though this dose is higher than standard recommendations. Additionally, administering both the standard and double doses of diminazene to rats and cats on alternate days over a five-day period was found to be effective and non-toxic (Da Silva *et al*., 2008; Da Silva *et al*., 2009).

In the study by Al-Rawashdeh *et al*. (1999), 82% of camels were treated with Melarsomine (Cymelarsan) at a dose of 0.25 mg/kg. Although the initial treatment was effective, symptoms of trypanosomiasis reappeared in some animals, necessitating a second round of therapy. Despite retreatment, 18% of the camels experienced relapse within the Jordan Valley. Sutcliffe *et al*. (2014) attributed such treatment failures-even at higher doses-to the prevalence of low-quality, or substandard, trypanocidal drugs commonly found in many African markets. They emphasized the importance of verifying the source and authenticity of these drugs to ensure therapeutic effectiveness. Conversely, Van den Bossche and Rowlands (2001) suggested that parasitemia may sometimes drop to levels undetectable by microscopy, potentially leading to underdiagnosis or false assumptions of cure.

Several studies have documented both relapse and mortality following treatment of *T. evansi* infections. Dargantes (2010) reported that diminazene was ineffective in rats and goats, with relapses occurring as early as 27 days post-treatment. Similarly, Divina (2004) observed a reappearance of parasitemia in mules and horses after a five-day treatment course in cases of severe infection. The inefficacy of diminazene has also been highlighted by Tuntasuvan and Luckins (1998); Tuntasuvan *et al*. (2003b), and Kongkaew *et al*. (2012), who found frequent relapses in cattle, horses, pigs, and elephants despite treatment. Notably, equines showed persistent infections even after receiving repeated doses of diminazene at 3.5 mg/kg body weight. These findings collectively suggest that, at standard dosages, diminazene may be insufficient to fully eliminate *T. evansi* infections (Tuntasuvan *et al*., 2003b).

Two main theories may explain the observed treatment failures and relapse patterns; the first one suggests that some trypanocides act on specific structures within the parasite. For instance, diminazene interferes with kinetoplast DNA synthesis, while quinapyramine disrupts kDNA condensation, affecting protein synthesis (Mamman *et al*., 1994; Mehlhorn, 2008). However, certain strains of *T. evansi* have lost their kinetoplasts known as dyskinetoplastic variants rendering these drugs less effective (Zweygarth *et al*., 1990; Brun *et al*., 1998). This may explain why even after temporary parasite clearance, relapses and eventual death occurred. According to the second theory, *T. evansi* may hide in protected tissues such as the brain, testes, or other organs (Sudarto *et al*., 1990; Boid *et al*., 1996; Tuntasuvan *et al*., 2003a). Most drugs are more effective against parasites in the bloodstream but have limited access to these tissue sites (Desquesnes *et al*., 2013). This creates a reservoir from which the parasite can re-emerge after treatment, causing a relapse.

Conclusion

The problem of drug-resistant strains of *Trypanosoma evansi* in Egypt is highlighted by this study. Our results show that Cymelarsan (melarsomine hydrochloride) performs better than conventional trypanocides such as diminazene aceturate and quinapyramine, which have limited effectiveness. The most successful treatment for *T. evansi* was Cymelarsan at 0.5 mg/kg, which eradicated the parasite entirely and showed no signs of recurrence. This implies that Cymelarsan sensitivity is quite high in the Egyptian isolate. On the other hand, even at greater dosages, there was significant resistance to diminazene aceturate and quinapyramine (sulfate and chloride).

Acknowledgments

This research was supported by the Science and Technology Development Fund (STDF), Egypt, through a collaborative grant between Alexandria University and Keele University, UK, under the Newton Fund Institutional Links Programme. The project, titled "Investigating Drug Resistance and New Treatments for *Trypanosoma evansi* in Egyptian Camels" (Grant Code: 27730), provided essential funding and resources for the successful completion of this study.

Conflict of interest

The authors have no conflict of interest to declare.

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