

Introduction

With 4,623,000 camels (*Camelus dromedaries*), Sudan has the largest camel population in the world after Somalia (FAO and the Annual Report of the Ministry of Animal Resources, Fisheries and Ranges, 2010). Camels are important source of meat, milk and hair. They are used for transportation and drought power; they are also exported to North Africa and the Middle East thus contributing a significant proportion to the gross national product (Abo Sin, 1988). Camel trypanosomosis is a serious protozoan disease which mainly caused by *Trypanosoma evansi*, a parasite that infects livestock and a potential human pathogen, is a major threat to these valuable animals (Joshi *et al.*, 2005; Van Vinh Chau *et al.*, 2016). Recently, it has been reported that the camel trypanosomosis is caused not only by *T. evansi* but also by *T. vivax* (Mossaad *et al.*, 2017).

In Sudan, the disease caused by *T. evansi* in camels is known as Guffar. which may be acute with high fever, anaemia, weakness and death. Generally, the disease assumes a chronic course resulting in serious morbidity and moderate mortality (Parsani *et al.*, 2006).

The subgenus trypanozoon which include *T. evansi*, has the potential for the mechanical transmission through contamination by sucking flies and through serial biting action by biting insects such as *tabanids* and *stomoxys* including *tsetse* as mechanical vectors (Gingrich *et al.*, 1983; Mihok *et al.*, 1995). In the

particular case of *T. evansi*, due to a loss of genetic material (maxicircles DNA), the parasite can no longer undergo its cycle in tsetse flies, thus it is mainly mechanically transmitted by biting insects, for this reason, *T. evansi* spread outside the tsetse belt in Africa, towards the Middle East and Southern Asia. (Desquesnes *et al.*, 2013).

T. evansi can infect cattle, camels, dogs, equines, sheep and goats. With this wide hosts range and vectors diversity, the control of the disease through vector flies seems to be not feasible, therefore, the control of Guffar is by chemotherapy and chemoprophylaxis. Field veterinarians use an array of trypanocidal drugs against *T. evansi* infection such as cymelarsan, ethidium bromide, isometamedium chloride and quinapyramine compounds. Drug resistance is known to occur amongst the *T. evansi* isolates and has been reported in different countries in Africa and Asia. In Sudan an isolate of *T. evansi* from Kassala was found to be resistant to sumarin at its maximum tolerated dose (Abebe *et al.*, 1993). More recently, Babiker and Hassab Alrasoul (2014) reported that *T. evansi* could no longer be treated with quinapyramine in West Omdurman.

In this study we investigated the susceptibility of *T. evansi* isolated previously in Kassala state to Two different trypanocidal drugs (quinapyramine sulphate and diminazene diaceturate).

Objectives of the study

The objective of this study was to investigate the susceptibility of *T. evansi* isolated from a camel in Kassala state, Eastern Sudan to different available trypanocidal drugs in the market. This includes quinapyramine sulphate and diminazene diacetate using *in-vivo* rodent model.

Chapter One

Literature Review

1.1. Origin, history, and geographical distribution

Trypanosoma (Trypanozoon) evansi is the first pathogenic mammalian trypanosome to be described in the world, in 1880, by Griffith Evans, in the blood of Indian equines and dromedaries (Desquesnes *et al.*, 2013). It is thought to be derived from *T. brucei brucei* (cyclically transmitted by tsetse flies), but it is no longer able to undergo its cycle in *Glossina* due to the loss of the maxcircles of kinetoplastic mitochondrial DNA (Borst *et al.*, 1987; Lai *et al.*, 2008). For this reason, *T. evansi* spread outside the tsetse belt in Africa, towards the Middle East and Southern Asia, and was exported with livestock to Latin America, and even to Europe (Desquesnes *et al.*, 2013).

1.2. Disease synonyms and parasite taxonomy

T. evansi belongs to phylum Sarcomastigophora, order Kinetoplastida; family Trypanosomatidae; Genus *Trypanosoma*; Subgenus *Trypanozoon*, Species *Trypanosoma evansi* According to (Levine *et al.*, 1980 and Lan *et al.*, 2004), the disease has many vernacular names for instance in Sudan the disease is known as Guffar (Shommein *et al.*, 1987), and Surra (rotten) in India whereas it is known as Salef in Somali (Desquesnes *et al.*, 2013).

1.3. Morphological features of *T. evansi*

T. evansi is 15 -33 μm in length, a strictly blood stream slender form protozoan with small subterminal kinetoplast, thin posterior extremity, large undulating membrane, free flagellum, thin parasite with central nucleus, (Lai *et al.*, 2008) truncated forms whose posterior extremities are truncated just below the kinetoplastida location is also observed (Desquesnes *et al.*, 2013)

1.4. Host range of *T. evansi*

T. evansi has wide range of domestic and wild hosts worldwide and almost all mammalian species are receptive to it but their susceptibility depends on host species and geographical distribution (Desquesnes *et al.*, 2013).

In Africa and the Middle East, *T. evansi* is mainly a parasite of camels and highly pathogenic in Equidae, especially in horses, it can infect cattle; however, they are sometimes refractory to the infection (Dia and Desquesnes, 2007). *T. evansi* is occasionally found in domestic cats (Reduth *et al.*, 1994) and regularly in dogs. sheep and goats are known to carry symptoms of chronic infection of *T. evansi* for up to a year or longer (Kheir and Majid, 1999).

In Asia, a much wider range of host is involved including water buffaloes, elephants, horses but also cattle, pigs, and goats (Dargantes *et al.*, 2009).

In Asia, cattle are more susceptible than in Africa or Latin America, and they can exhibit strong clinical signs (Tuntasuvan and Luckins, 1998)

1.5. Clinical signs

Generally, trypanosomes including *T. evansi* have various clinical signs, including fever, anaemia, loss of appetite and weight, loss of condition and productivity, nervous signs and/or abortion, cachexia, and death, with or without more peculiar signs related to the host species (Desquesnes *et al.*, 2013) furthermore their intensity is quite variable within a host species, depending on the geographical area or the epidemiological situation.

In camels, (*Camelus dromedarius* and *C. bactrianus*), may be acute with high fever, anaemia, weakness, and death; it is also frequently fatal sometimes within a few months; however, it is more often chronic than in horses and can frequently last 2-3 years (Parsani *et al.*, 2008). Signs of illness appear with intermittent fever (41°C), approximately about a week; the animals appear dull and lustreless and become progressively weaker with staring hair, loss of appetite and weight, abortion, oedema (ventral parts, udder or scrotum, and sheath), anaemia with pale mucous membrane, and petechial or ecchymotic haemorrhages. A specific odour of the urine is detected by camel owners, which is efficient for diagnosing the disease (Stephen, 1986).

In horses, the acute disease is with an incubation period of 1–4 weeks, and sometimes up to 8 weeks, after which the following symptoms appear: fluctuating fever with high peaks with parasitemia (41.5°C up to 44°C), weakness, lethargy, anaemia, severe weight loss, transient local or general cutaneous eruption, petechial haemorrhages on the eyelids, especially the

nictitating membrane, vulvar and vaginal mucosa, haemorrhages into the anterior chamber of the eye (where trypanosomes can be also found in gelatinous material from the inner canthus), abortion, and alteration of locomotion, with nervous signs (Stephen, 1986) and oedema appears after sometime. In the chronic form there is emaciation often accompanied by jaundice and highly coloured urine (Stephen, 1986).

In cattle: Trypanosomosis due to *T. evansi* has long been considered as a mild, chronic, or asymptomatic disease in cattle especially in Africa and Latin America (Desquesnes *et al.*, 2013). Where as in India, the disease is characterized by very high mortality rates (Gill, 1977).

In sheep and goats, natural infection is generally considered as mild or asymptomatic in sheep. Goats are also most often of low susceptibility (Desquesnes *et al.*, 2013).

In Dogs: Dogs are highly susceptible to *T. evansi*, and they often exhibit strong clinical signs leading to death, sometimes within a week and most often within a month in acute cases (Gill, 1977).

Clinical signs are intermittent fever (39°C–41°C), oedema of the head, including larynx, oedema of the abdominal wall and legs, anaemia, weakness, lack of appetite leading to emaciation and, sometime, paresis of the hindquarters; myocarditis has been described and can be fatal (Desquesnes, 2004); sexual excitement has also been mentioned. Ocular signs are most often observed in dogs, with conjunctivitis, lachrymation, keratitis, corneal opacity, and/or

haemorrhagic signs, which can lead to fibrin deposits in the anterior chamber of the eye, the parasites have sometimes been observed in ocular aqueous fluid.

1.6. *T. evansi* in Sudan

According to the 2010 annual report of FAO and the ministry of animal resources, fisheries, Sudan has the largest camel population in the world after Somalia.

The most important protozoan disease is camel trypanosomosis primarily caused by *T. evansi*. The disease was first reported in the country by Balfour in 1904 (Karib, 1961). Locally known as “Guffar” and it is a serious protozoan disease of camels. The disease is common in Kordofan and Darfur States in the west, Kassala, Gadaref and Red Sea States in the east and to a lesser extent in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum States (Karib, 1961). Recently, it has been reported that the camel trypanosomosis is caused not only by *T. evansi* but also by *T. vivax* (Mossaad *et al.*, 2017)

1.7 Diminazene aceturate

Diminazene aceturate was introduced for the treatment of babesiosis and African trypanosomosis in livestock in 1955. It belongs to the diamidine class of compounds. Diminazene is substantially less potent against *T. congolense* than it is against *T. brucei* group trypanosomes. This feature is attributable to the fact that its uptake into the latter parasites via the P2/TbAT1 transporter allows concentrative and rapid uptake. Diminazene today is the most commonly used trypanocide in cattle, sheep and goats, due to its activity against both *T. congolense* and *T. vivax* and its relatively low toxic side effects. The compound

also effectively cures surra and is, for example, the mainstay of treatment of *T. evansi* in the Philippines. The recommended therapeutic dose is 3.5 mg kg⁻¹ body weight for AAT due to *T. congolense* and *T. vivax* (7 mg kg⁻¹ may be recommended against resistant isolates) and 7 mg kg⁻¹ is indicated for AAT due to *T. brucei* and for surra, administered by intramuscular or subcutaneous injection. The common practice of administering 3.5 mg kg⁻¹ of the drug to treat *T. evansi* infections is considered an underdosing, and this misuse may have contributed to the emergence of resistant strains in South-East Asia.

Diminazene is only applied as a curative agent and is not used for prophylaxis, as it is rapidly metabolized and excreted. In trypanosomes, the kDNA is a known target of the drug, and kDNA binding can cause inhibition of replication and kDNA loss. the drug is not active on CNS infections as it cannot cross the blood–brain barrier; the compound is selectively toxic to trypanosomes, as they express transporters that specifically accumulate diminazene; and trypanosomes may become resistant to the drug by losing these transporters or their activity. As mentioned above, diminazene uptake in *T. brucei* mainly occurs via an aminopurine transporter called P2 or TbAT1, Loss of P2/TbAT1 activity was shown to cause diminazene resistance in *T. brucei* and *T.equiperdum* and *T.evansi*. (Giordani *et al.*, 2016)

1.8. Quinapyramine sulphate

Quinapyramine was developed from the early trypanocide Surfen C, The compound was applied to treat cattle infected with trypanosomes until 1976, when it was withdrawn from many areas due to emergence of widespread resistance The drug was subsequently reintroduced in 1984 to treat *T. evansi* in camels and horses. It is considered the most effective treatment (5 mg kg⁻¹ via subcutaneous injection), although the drug induces severe but transient side effects in these animals. The prosalt form of quinapyramine (a mixture of the soluble sulphate and the insoluble chloride salts) was the first prophylactic

Drug available for animal infections. A 7.4 mg kg⁻¹ dose of this prosalt suspension has both a curative and a prophylactic (up to 4 months) effect on

T. evansi infections in horses and camel. Quinapyramine is a quinoline pyrimidine and Quinapyramine's mode of action remains unknown. Hypotheses include the interference with nucleic acid synthesis and inhibition of cytoplasmic ribosomes. Quinapyramine accumulates in the liver and kidneys, where its concentration remains high for weeks and can cause organ-specific toxicity. Excretion occurs mainly via urine (Giordani *et al.*, 2016)

Chapter Two

Materials and Methods

2.1. *Parasite*

T. evansi isolated from camels in Eastern Sudan in 2016. The parasite was confirmed as *T.evansi* using parasitological, molecular techniques and sequencing analysis (Mossaad *et al.*, 2017). The parasite was kept in liquid nitrogen and maintained in mice.

2.2. Experimental animals

Twenty Wistar albino rats were obtained from the experimental animal unit, Faculty of Veterinary Medicine, University of Khartoum. Rats were divided equally into three groups (5 rats/ group). Rats were kept in plastic cages that provide good ventilation with free access to water and feed throughout the experiment.

2.3. *In vivo* drug susceptibility test

The *in vivo* drug susceptibility test was performed as described previously (Alaa, 2005) with some modifications. Briefly, an infected rat with *T.evansi* was heart-bled after being anaesthetized. Harvested blood was diluted in normal saline to give a final concentration of 8.1 antilog parasitaemia. Three groups of rats were infected with *T.evansi* via intraperitoneal injection of 0.3 ml of diluted infected blood in normal saline (8.1 antilog parasitaemia) while one additional group was kept as uninfected non-treated as negative control. To evaluate the drug susceptibility of the chosen trypanocidal drugs, group one was treated at

the peak of the acute phase of infection (day 4) via subcutaneous injection of quinapyramine sulphate (Interquin) (Interchemie werken Co., Holland) at 3 mg/kg (one dose) as recommended by the manufacturer. Group two was treated at the peak of the acute phase of the infection (day 4) via subcutaneous injection of diminazene diacetate (DIMINAVETO) (V.M. D Arendonk Co., Belgium) at 3.5 mg/kg (one dose) as recommended by the manufacturer, while group 3 was left infected untreated. Antilog parasitaemia was monitored and recorded daily by wet blood smears prepared from the rat tail from day 1-18.

2.4. Wet blood films

Wet blood films were used for the daily monitoring of the parasitaemia. A drop of blood obtained from clipped tail vein was put on a clean glass microscopic slide, covered with cover slip and examined under light microscope using $\times 40$. Parasitaemia was estimated according to Herbert and Lumsden matching method, when the parasites were not seen in 5, 10 and 20 microscopic fields, parasitaemia was recorded as less than antilog 5.4 organisms/ml (Fig.1) (Herbert and Lumsden, 1976).

2.5. Giemsa stained thin smear

A drop of blood was placed at one end of a clean microscopic slide and a thin film was spread out. The smear was then air-dried, fixed in methanol for 1 min and allowed to dry. This was followed by Giemsa staining at 10% for 25 min.

After drying, the smears were examined at a magnification of 40×light microscope with oil immersion (OIE. 2012)

2.6. Packed cell volume

A plain capillary tube was filled about $\frac{3}{4}$ its length with anti-coagulated blood by the capillary action, the dry end was closed with crystaseal and centrifuged at rate 12000 RPM for 5 minutes. The Percentage packed cell volume was estimated using the haematocrit reader (OIE. 2012).

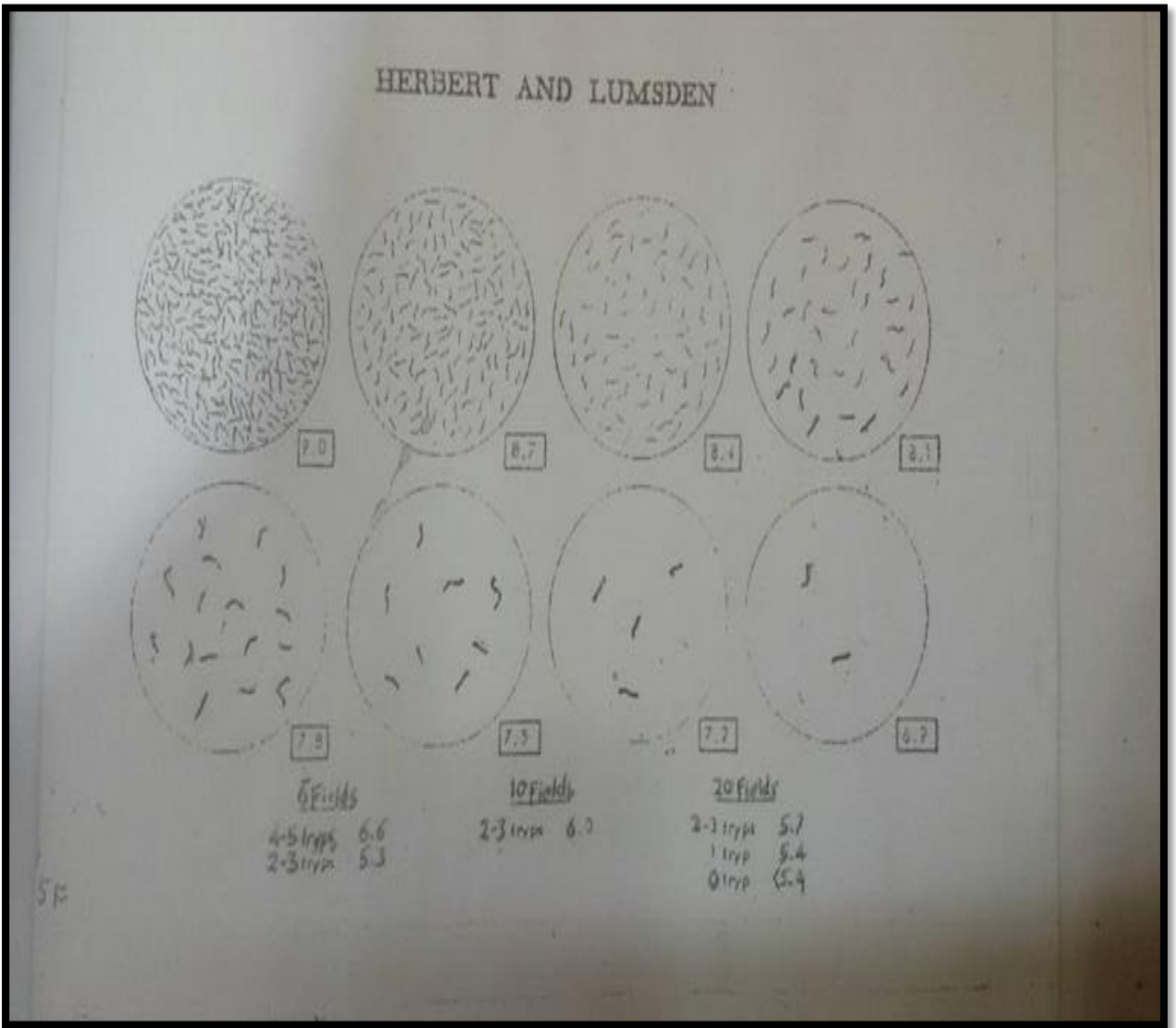


Figure 1. A rapid matching method for estimating the host's parasitaemia in wet blood films as described by (Herbert and Lumsden, 1976)

Chapter Three

Results

Infected rats at day 0 were monitored for parasitemia using wet smears, until it has reached the peak at day 4 (Antilog parasitaemia 7.9).

Group one was left infected and untreated. Group 2 was treated with diminazene diaceturate (DIMINAVETO) (V.M. D Arendonk Co. Belgium) at Day 4.

Group 3 was treated with Quinapyramine Sulphate (Interquin), (Interchemie werken Co. Holland) at the same day (day 4).

Monitoring of parasitaemia from day 4 (Treatment) until day 18 the end of the experiment showed that the parasite has disappeared immediately in group 2 which has been treated with diminazene diaceturate (Diminaveto).

The disappearance has continued throughout the experiment. However, the parasite appeared separately in two different rats at day 8 and day 15 while the other three rats did not show any relapse of parasitemia (Fig. 2).

Moreover, it has been recorded that the parasitemia has increased in group 3 which has been treated with Interquin until day 5 up to (Antilog 8) followed by a sharp drop in parasitemia at day 6 until it has disappeared completely at day 7. Then parasitemia has increased irregularly at days 8, day 11 and then continued higher than Antilog 6 up to the end of the experiment (Fig.2).

Group 1 which was infected and non-treated showed the normal growth curve of the parasite with peaks of parasitaemia every 5 -7 days (Fig 2).

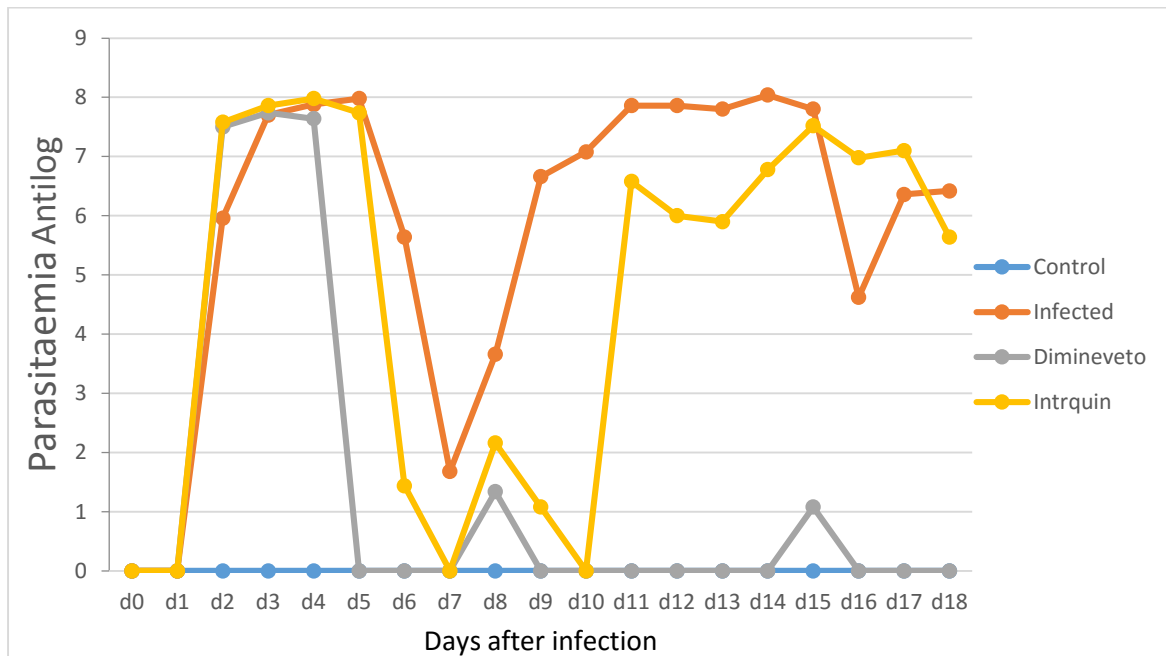


Figure 2. Parasitemia Antilog in the rats. Blue line indicates the control group. Brown line indicates the infected non-treated group. Gray line indicates diminazine-treated group. Yellow line indicates quinapyramine-treated group. Each group composed of five rats. Values are the mean of antilog of parasitemia using wet blood smears.

The association between a reduced PCV and anemia

Because anemia is one of the consequences of trypanosome infections (Van den Bossche 2001) and low PCV is an indicator of anemia in animals, the mean PCVs in both treated and non-treated animals were assessed. PCV was significantly low in the infected-non-treated and quinapyramine-treated groups as compared to the control group (Student's *t*-test: $P < 0.05$). Surprisingly the group that has been treated with diminazene diaceturate showed normal PCV equivalent to the Control group with no statistical difference (Student's *t*-test: $P > 0.05$) (Fig. 3).

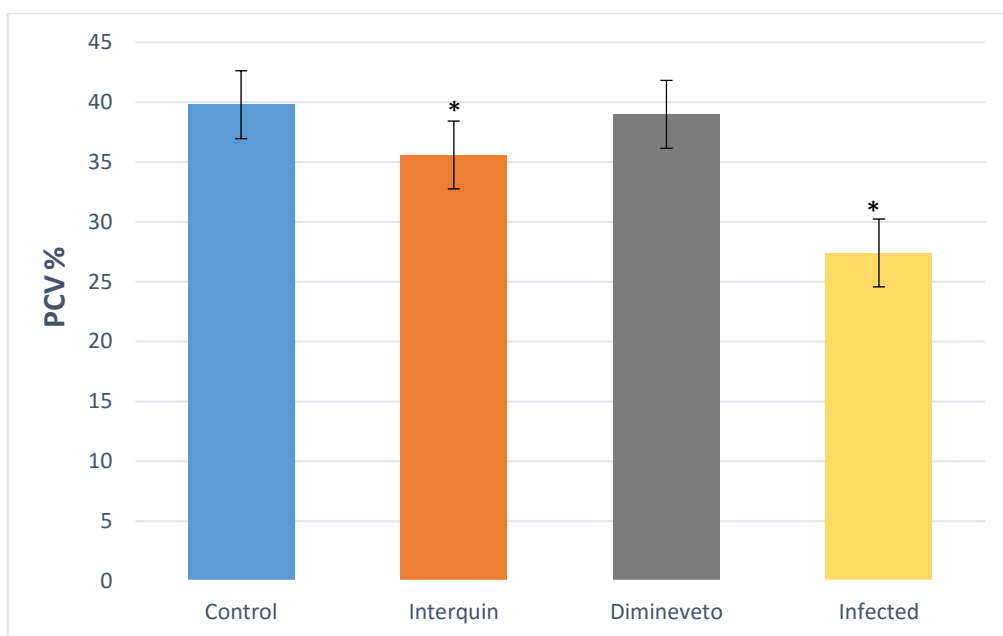


Figure 3. Comparison of PCV values between treated and non-treated rats. Values are mean \pm SD. The significant difference by Student's *t*-test (* $P < 0.05$)

Chapter Four

Discussion

Treatment of *T. evansi* infection is known to be with diminazene aceturate, isometamedium chloride, quinapyramine sulphate, suramin, melarsomine (cymelarsan). suramin and quinapyramine are used for the treatment of *T. evansi* infection in camels. Most of the drugs are either not curative such as isometamedium chloride or are too toxic for camel such as diminazene aceturate (Ian *et al.*, 2004).

In this study the treatment of rats experimentally infected with *T. evansi* with available trypanocidal drugs was assessed. We found that diminazene diacetate (Diminaveto) has completely cleared the parasitaemia immediately after the treatment (day 4). The parasitemia remained undetectable until day 8 in which we have recorded Antilog 6.7 in only one rat which has again disappeared at day 9. The same phenomenon was observed in another rat at day 15 and the parasitemia again has disappeared in that rat at day 16 (figure1). However, at the end of the experiment (day18), rats were sacrificed, blood was collected and genomic DNA was extracted. To confirm that the two relapses of parasitemia at day 8 and 15 were not a failure of treatment, PCR was performed to amplify ITS-1 of *T. evansi*. Surprisingly, none of the rats in the group showed positive bands for *T. evansi* (Data not shown). These results suggested that *T. evansi* is susceptible to diminazene diacetate.

In contrast, we have reported failure of treatment of *T. evansi* infection in rats when treated with quinapyramine sulphate (Interquin). After rats were treated at day 4, monitoring of parasitemia every day showed that parasitemia remained comparable to that of non-treated rats group. Similar results were published by Babiker and Hassab Alrasoul (2014) who reported failure of treatment of experimentally infected rats with Biquin and antrycide pro-salt (quinapyramine sulphate and quinapyramine chloride). These results should alarm the veterinary authorities that quinapyramine sulphate available in the market is no longer effective in the treatment of camels infected with *T. evansi*. More studies are needed to investigate whether the failure of treatment is due to emergence of drug resistance, which has previously been reported (Mekonnen *et al.*, 2018), or it is just due to a variation in the quality of drug produced by different companies. The challenge is that quinapyramine sulphate is the only available drug against *T. evansi* infection in camels since diminazine diacetate is highly toxic for camels.

All rat groups were subjected to PCV assessment at the end of the experiment (day 18). Because anemia is one of the consequences of trypanosome infections (Van den Bossche, 2001 and Mossaad *et al.*, 2017) and low a PCV is an indicator of anemia in animals. Normal PCV compared to that of non-treated non-infected group, was recorded in diminazine-treated group. This may confirm the complete recovery of rats. In contrast, significantly lower PCV

comparable to that of infected-non-treated group was recorded in quinapyramine-treated group confirming the failure of treatment.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the treatment of *T.evansi* experimentally infected rats with diminazene diaceturate (Diminaveto) has completely cleared the parasitaemia while the treatment of *T.evansi* infected rats with quinapyramine sulphate (Interquin) has failed to clear the parasitaemia in infected rats this might be considered as an alarm to the veterinary authorities that the failure of treatment could be due to emergence of drug resistance which requires further investigation.

We recommend that treatment of infected animals should be under observation of the authority after confirming the infection with the appropriate laboratory tools.

APPENDIX



The test drug quinapyramine sulphate (Interquin)



The Standard drug Diminazine diacteurate (DIMINAVETO)



Wistar albino rats used as experimental animals

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