Evaluation of Card Agglutination Test for *T.evansi* in Experimentally Infected Rabbits

By

Mohamed ELsiddig Mohamed Ahmed Alarabi  

Supervisor  
Dr. Khitma Hassan Elmalik  
(B.v.sc., M.v.sc., Ph.D.)

A dissertation submitted to University of Khartoum  
in partial fulfillment for Requirement of the Degree  
of Master of Tropical Animal Health (M.T.A.H)

Department of Preventive Medicine and Public  
Health  
Faculty of Veterinary Medicine  
University of Khartoum  
March 2008
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>IV</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>V</td>
</tr>
<tr>
<td>ENGLISH ABSTRACT</td>
<td>VI</td>
</tr>
<tr>
<td>ARABIC ABSTRACT</td>
<td>VII</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Objectives of the study</td>
<td>2</td>
</tr>
<tr>
<td><strong>CHAPTER ONE. LITERATURE REVIEW</strong></td>
<td>3</td>
</tr>
<tr>
<td>1-1 Historical note</td>
<td>3</td>
</tr>
<tr>
<td>1-2 Definition</td>
<td>3</td>
</tr>
<tr>
<td>1-3 Classification</td>
<td>3</td>
</tr>
<tr>
<td>1-4 Morphology</td>
<td>4</td>
</tr>
<tr>
<td>1-5 Host</td>
<td>4</td>
</tr>
<tr>
<td>1-5-1 Domestic host</td>
<td>4</td>
</tr>
<tr>
<td>1-5-2 Wild hosts</td>
<td>5</td>
</tr>
<tr>
<td>1-6 Epidemiology</td>
<td>5</td>
</tr>
<tr>
<td>1-6-1 Transmission</td>
<td>5</td>
</tr>
<tr>
<td>1-6-1-1 Mechanical transmission</td>
<td>5</td>
</tr>
<tr>
<td>1-6-1-1-A Cyclical transmission</td>
<td>7</td>
</tr>
<tr>
<td>1-6-1-2 Incubation period</td>
<td>8</td>
</tr>
<tr>
<td>1-7 Clinical signs</td>
<td>8</td>
</tr>
<tr>
<td>1-7-1 Clinical Pathology</td>
<td>10</td>
</tr>
<tr>
<td>1-8 Diagnosis</td>
<td>11</td>
</tr>
<tr>
<td>1-8-1 Parasitological Diagnosis</td>
<td>11</td>
</tr>
<tr>
<td>1-8-2 serological Diagnosis</td>
<td>12</td>
</tr>
<tr>
<td>1-8-2-1 Complement fixation test (CFT)</td>
<td>13</td>
</tr>
<tr>
<td>1-8-2-2 Capillary agglutination test (CAT)</td>
<td>13</td>
</tr>
<tr>
<td>1-8-2-3 The indirect fluorescent antibody test (IFAT)</td>
<td>13</td>
</tr>
<tr>
<td>1-8-2-4 Enzyme–linked immunosorbent assay (ELISA)</td>
<td>14</td>
</tr>
<tr>
<td>1-8-2-5 Species–specific monoclonal antibody</td>
<td>14</td>
</tr>
<tr>
<td>1-8-2-6 Sandwich ELISA</td>
<td>14</td>
</tr>
<tr>
<td>1-8-2-7 The Card Agglutination Test (CATT)</td>
<td>15</td>
</tr>
<tr>
<td>1-9 Control</td>
<td>15</td>
</tr>
<tr>
<td>1-9-1 Chemotherapy</td>
<td>16</td>
</tr>
<tr>
<td>1-9-2 Chemotherapy and chemophylaxis</td>
<td>17</td>
</tr>
<tr>
<td>1-9-2-1 Berenil (Diminazene aceturate) (Hoechst, Germany)</td>
<td>17</td>
</tr>
<tr>
<td>1-9-2-3 Antrycide pro-salt (Quinapramine sulphate and</td>
<td>19</td>
</tr>
<tr>
<td>Quinapramine chloride) Wockhart Ltd, Mumbai, India</td>
<td>19</td>
</tr>
<tr>
<td>1-9-2-4 Ethidum (Homidium bromide) laprovet France</td>
<td>19</td>
</tr>
</tbody>
</table>
CHAPTER TWO. MATERIALS AND METHODS ..................25
2-1 Experimental animals ........................................25
2-2 Experimental design ........................................26
2-2-1 Parasite ......................................................26
2-2-2 Inoculation ..................................................26
2-2-3 Trypanosomes counting ......................................26
2-2-4 Parasite detection methods ..................................26
2-2-4-i Wet blood smears preparation ..............................27
2-2-4-ii Dry blood smears preparation .............................27
2-2-4-iii Packed cell volume .......................................27
2-2-4-iv Haemoglobin concentration (Hb) .........................28
2-2-5 Card Agglutination Test for Trypanosomes (CATT) ..........28
2-2-5-a Test Kit ......................................................29
2-2-5-b Test Procedure .............................................29
2-2-6 Body Temperature ............................................30
2-2-7 Body weight ..................................................30
2-3 Treatment .......................................................30
2-4 Statistical analysis .............................................30

CHAPTER THREE. RESULTS ....................................31
3-1 Detection of parasitaemia ......................................31
3-2 Haematological changes .......................................31
3-2-1 Average haemoglobin values for rabbits before, during infection and after treatment from T.evansi infection .......................31
3-2-2 Average packed cell volume values for rabbits before, during infection and after treatment from T.evansi infection .......................32
3-2-3 Average body weight values for rabbits before, during infection and after treatment from T.evansi infection .......................33
3-3 Card Agglutination Test (CATT-T.evansi) ...............36

CHAPTER FOUR DISCUSSION ....................................39
Conclusions and recommendations .................................42

REFERENCES ....................................................43
DEDICATION

To my parents and my grandmother Alia, to my wife, teachers, brothers, sisters and friends with love and gratitude.
ACKNOWLEDGEMENTS

My special praise and unlimited thanks are to the Almighty, Allah, the Merciful and the Great, who gave me strength, patience and helped me to complete this study.

My deep sense of gratitude and appreciation are extended to my supervisor Dr. Khitma Hassan Elmalik, Department of Preventive Medicine& Public Health, Faculty of Veterinary Medicine, University of Khartoum for her guidance, advice and encouragement.

I would also extended my special thanks to Dr. Intisar Elhaj Elrayah Director of Tropical Medicine Research institute (TMRI), Khartoum, Sudan for help to obtain Test reagents.

Special thanks to Dr. Atif Alamin Department of Preventive Medicine& Public Health, Faculty of Veterinary Medicine, University of Khartoum for his valuable help.

I would also like to express my deepest thanks to ustaz ahmed abdelwahid Department of Preventive Medicine& Public Health, Faculty of Veterinary Medicine, University of Khartoum for his support and help during the experimental work.

My special thanks and appreciation also extended to my friends for continuous support, encouragement and patience.
Abstract

Improved diagnostic techniques for *T. evansi* are needed for diagnosis of cases and evaluation of chemotherapeutic efficacy of drug. This study was planned to evaluate the Card Agglutination Test for Trypanosomosis (CATT) particularly developed for *T.evansi* to correlate antibody to parasite detection in experimentally infected animals.

Eight locally bred rabbits were purchased from home-raised flocks. Six rabbits were inoculated on day 16 from purchase, by *Trypanosome evansi* isolate inter peritoneal (I/P) and 2 rabbit were left uninoculated as control. A direct card agglutination test for detection of anti-trypanosome antibodies in serum or plasma of infected animal was used, the antigen consists of cloned bloodstream form trypanosomes of RoTat 1.2; a predominant variable antigen type (VAT) of *T.evansi*.

The test was conducted in sera collected from eight different rabbits, taken during different stages of pre, post infection and after treatment. In pre infection stage all rabbits were negative. Sera collected during infection stage were positive in majority of inoculated rabbits, negative in uninoculated rabbit. Sera collected for 30 days after treatment showed variable results as some rabbits were positive for some time while others became negative. Parasites were not detected after treatment with Cymelarsan.

Death due to trypanosomosis is usually a result of severe anaemia, In this study the PCV, Hb & body weight of the infected Rabbits were lower than that of the negative uninfected Rabbits. It was concluded that CATT is highly sensitive in antibody detection but the results should be carefully evaluated because antibodies can persist after treatment. The possible way is to consider the low PCV, Hb and antibody titre for evaluation of the infection situation.
الخلاصة

العوامل التشخيصية المطورة لـ*Trypanosoma evansi*.

وضعت هذه الدراسة لتقييم اختبار تلازن الكرت للتراباسوما ايفنزاي في تطور تحديد لـ*Trypanosoma evansi*.

حيوانات التجارب المصابة.

ثمانية أرانب من سلالة محلية مربعة منزلية تم شراؤها، ستة أرانب تم حقنها في اليوم السادس عشر من شراؤها بعطرة*Trypanosoma evansi* حقن داخل الغشاء البروتوني بجرعة 1 مل واثنين من الأرانب ترك دون حقن كقياس، الاختبار المباشر لـ CATT للكشف عن الأجسام المضادة للتراباسوما*Trypanosoma evansi* في مصل أو بلازما للحيوانات المصابة استخدمت محتويات الانتجين من جزيء الدم المستنسخ من التراباسوما* Trypanosoma evansi*.

الاختبار اجري في السيرم الذي تم جمعه من 8 أرانب مختلفة وفي مراحل مختلفة من البحث قبل، بعد العدوى وبعد العلاج، سيرم كل الأرانب اظهر نتيجة سلبية في فترة ما قبل الاصابة، السيرم الذي تم جمعه اثناء فترة الاصابة معظمهم اظهر نتيجة موجبة في الأرانب المصابة واظهر نتيجة سلبية في سيرم كل الأرانب الغيرمصابة.

السيرم جمع في 30 يوم بعد العلاج اظهر نتائج مختلفة في بعض الاحيان اظهر نتيجة موجبة.

وفي أخرى سالبة، الطفيلييات لم تظهر بعد العلاج بالسمرثران.

الموت نتيجة للإصابة بمرض التراباسوما دائما نتيجة للانثيما الحادة، وفي هذه الدراسة كبوس الدم (PCV) والهيموغلوبين (HB) وزن الجسم في الأرانب المصابة كان أقل من الغير مصابا، واستنتج من ذلك ان اختبار CATT مصابة، والنتائج لابد ان تكون أكثر دقة للتقييم لأن الأجسام المضادة تستطيل البقاء بعد العلاج، الطريقة الممكنة للوصول في الاعتبار انخفاض كبوس الدم، الهيموغلوبين ومقدار الأجسام المضادة لتحديد مدى الاصابة.
Introduction

Trypanosomosis, caused by *Trypanosoma evansi* is one of the major and most important diseases of camels in the arid and semiarid zone of the world. Camels managed under nomadic pastoralism have higher risk of being exposed to *T. evansi* infection than camels under a ranching system of management (Ngaira, *et al* 2003).

*T. evansi* belongs to the genus *Trypanosoma* which are haemoflagellate protozoa that belong to the order: Kinetoplastida. They parasitize man, domestic and wild animals causing trypanosomosis. Unlike other parts of the world, in the Sudan, *T. evansi* is primarily a parasite of camels causing a disease locally known as `Guffar`. The disease is common all over the country as it has been reported in herds in Kordofan, Darfur States in the west, Kassala, Gadaref, Red Sea States in the east, in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum States. Guffar is believed to be transmitted mechanically by biting flies, particularly, horseflies (Diptera: Tabanidae) (Losos, 1986). Generally, the disease assumes a chronic course resulting in serious morbidity and moderate mortality. Diagnosis is largely based on demonstration of the causative agent by the standard trypanosome detection methods as parasitological examination. Serological tests have also been developed to detect *Trypanosoma* antigen or antibodies against them (Luckins, 1979).

Since control of the vector flies is not feasible at present, control of Guffar is basically through chemotherapy and chemoprophylaxis. The most common drugs used in chemotherapy are still Naganol and
Quinapyramine salts (e. g., Antrycide). Due to the development of resistance to the former, Quinapyramine salts remain the drugs of choice in the treatment of *T. evansi* in camels and experimentally infected laboratory animals. They are also considered as the standard chemotherapeutic agents against which other drugs are evaluated (Harone, *et al.*, 2003). Improved diagnostic techniques are needed for diagnosis of cases and evaluation of chemotherapeutic efficacy of drugs this study was planned to evaluate the Card Agglutination Test for Trypanosomosis (CATT) particularly developed for *T. evansi* to correlate antibody to parasite detection in experimentally infected animals. The objectives of the study are:-

1- To observe the pathogenicity of *T. evansi* in experimentally infected rabbits as measured by temperature reactions, body weight changes, haemoglobin and PCV.
2- To evaluate the efficiency of CATT technique for monitoring the antibodies response in infected rabbits, during infection and after treatment.
3- To evaluate the restoration to normal values after Cymelarsan therapy as indicated by clinical and immunological changes.
CHAPTER ONE
LITERATURE REVIEW

1-1 Historical note:

*T.evansi* was the first pathogenic trypanosome discovered by Griffith Evans (1880) in the blood of equines and camels affected by this disease in Punjab State, India (Hoare, 1972). In the Sudan, *T.evansi* in camels was identified by Balfour in camels as early as 1904 (Karib, 1961), being the first of the *Trypanosoma* species reported in the country.

1-2 Definition

*Trypanosoma* species

Trypanosomes are microscopic elongated unicellular flagellates. They occur in vertebrates, principally in the blood and tissue fluids. They are transmitted by blood-sucking arthropods.

1-3 Morphology:

In stained blood Preparations *T. evansi* appears as an slender form of *T.brucei*. The cytoplasm stains blue or purple and the nucleus usually lie near the center or in the anterior half (Soulsby, 1982). The kinetoplast is small and typically occupies a sub-terminal or marginal position in the body at the tapering posterior end. Both kinetoplast and nucleus stain red (Losos, 1980). The organism bears a single flagellum (Karina and Amanda, 2001).
1-4 Host:

1-4-1 Domestic host:

In Africa and South America there is very little evidence to suggest that domesticated livestock other than camels and horses are clinically affected by *T.evansi*. There are, however, reports from the Sudan of serological evidence of infection in goats and sheep (Boid *et al*, 1981) and in cattle from Brazil (Franke *et al* 1994). Sheep and goats are known to carry symptoms of chronic infection of *T. evansi* for up to a year or longer (Malik and Mahmoud, 1978), and since camels may often be herded in close proximity to sheep and goats, these animals have been suggested as natural reservoirs of *T. evansi* infection (Boid *et al*, 1981). In Asia, a much wider range of hosts is involved including Bactrian and dromedary camels, cattle, buffalo, horses and pigs (Silva *et al* 1995; Pathak *et al*, 1993; Partoutomo *et al*, 1994; Balakrishnan, 1994) cases of *T.evansi* in dogs in Asia were reported by Losos & Ikede (1972).
1-4-2 Wild hosts

Numerous species of wild animals have been shown to be susceptible to *T. evansi* infections which caused severe disease and death (Hoare, 1972; Morales *et al*, 1976; Losos, 1986). Such infections were found in captive tigers and other large felines from India and Sumatra (Raju and Swaminath 1947), in deer from Mauritius (Adams & Lionnet, 1933), and in wild dogs from South America (Curasson, 1943; Rodriguez, 1956).
1-5 Epidemiology:

1-5-1 Transmission

1-5-1-1 Mechanical transmission:

Camel trypanosomosis has been reported to be transmitted mechanically from camel to camel by a number of species of haematophagous biting flies including the following genera *Tabanus*, *Stomoxys*, *Lyprosia* and *Haematobia* (*Diptera*). (Rutter, 1967.; Scott, 1973). The role of tabanid flies in mechanical transmission of the disease was first described by Rogers (1901). In the Sudan they play an important role in the mechanical transmission of animal trypanosomosis (Karib, 1961). They were also considered as major cause of the seasonal migration of cattle from the south to north during the rainy season (Kheir, *et al*, 1995). Lewis (1954) reported seventy species of Tabanid flies in the Sudan and Yagi (1968) added four more species to those recorded by Lewis. The main tabanid species found throughout the country and involved in animal irritation together with mechanical transmission of pathogens are *Atylotus agrestis*, *A. fuscipes*, *Tabanus taeniola*, *T. sufin*, *T. biguttatus*, *T. gratus*, *Philoliche*, *magretti*, *Ancala latipes*, and *Africana* (Razig and Yagi, 1975).
Salivarian trypanosomes, which include the important agents of African trypanosomosis, are spread mainly by at least 30 species and subspecies and races of tsetse flies which infest vast areas of tropical Africa (Losos, 1986). Parasites in the blood stream of the infected mammal are taken by the fly as it feeds and they undergo a cycle of development and division in the gut until the infective or metacyclic trypanosomes are produced (Uilenberg, 1998). The incidence of trypanosomosis in animals is high during or after the rainy season due to the growth and proliferation of tsetse flies during this favorable season. However, a high transmission index was not necessarily linked to a high fly infection rate and vice versa (Wilson et al., 1972). The tsetse infested area in the Sudan is estimated at 300,000 Km² in the Sudan (Razig and Yagi, 1973). Lewis (1949) recognized seven Glossina spp in the Sudan. These are G. morsitans submorsitans, G. fuscipes, G. fusca, G. fuscipleuris, G. longipenis, G. pallidipes and G. tachenoides. So far there has been no evidence to suggest the biological transmission of T. evansi by any organism (Uilenberg, 1998)
1-5-1-2 Incubation period: -

In experimentally infected animals The incubation period before appearance of the parasites depends on the strain of trypanosomes, and their virulence their concentration in the inoculum, and the strain of laboratory animal used. Sensitivity of this in-vivo culture system may perhaps be increased by the use of immunosuppressed laboratory animals.

1-6 Clinical signs:

Trypanosomosis due to *T.evansi* is a chronic wasting disease characterized by intermittent fever (38.5 – 40.1°C), anemia, fluctuating parasitaemia, emaciation, weakness with paler mucous membrane and dry scruffy coat (Syakalima, 1992). The animal stands with its nose somewhat depressed and head hanging forward. The eyes turn dull and half closed with considerable amount of tears (Karram *et al*., 1991). In addition it was observed that 100% of infected camels stared at the sun (Abo-shehada *et al*., 1999). Hematological studies revealed that in trypanosomosis-affected animals there was severe oligocythemia with significant decrease in both hemoglobin and packed cell volume (Karram *et al*., 1991).

*T. evansi* infection can be manifested in both acute and chronic forms; the acute form of the disease is characterized by progressive anaemia, high fever, anorexia, loss of condition and often rapid death.
The chronic form which is more common shows relapsing parasitaemia with or without pyrexia, emaciation, oedema of the abdomen and legs, abortion and death in some animals (Haroun et al., 2000). Production losses occur due to lower milk and meat yields in adults (Richard, 1979). Abortion, premature birth and an inability to feed young, all greatly reduce the reproductive potential in affected herds (Yagil, 1982). Chronically-infected animals may survive for three to four years; the disease in this form is characterized by anemia, emaciation, recurrent fever, disappearance of the hump, atrophy of the thigh muscles, oedema of the dependant parts, corneal opacity, diarrhea and sexual excitement (Singh et al., 1980). Other clinical changes recorded in experimentally-induced chronic infections include debility, alopecia, keratinization, depletion of sub-cutaneous fat and facial oedema (Raisinghani et al., 1980). Also an infected animal suffers from hypoglycaemia (Igbokwe, 1994).

Animals which recover from the acute disease are often clinically normal with low numbers of circulating trypanosomes. In chronic infections too, low numbers of trypanosomes are present but parasitaemia often increases with physiological stress. Localization of organisms in the tissues results often in low grade parasitaemia (Losos, 1980). The low and intermittent parasitaemia as a result of antigenic variation in
trypanosome population (Jones and Mckinnell, 1984) will interfere with
detection of parasites even in acute infections (Murray et al, 1977).

1-6-1  Clinical Pathology:

The main pathological features include degenerative and necrotic
changes involving various organs (Raisinghani et al., 1980; Haroun et al,
2000) and in camels, anemia is described cytologically as macrocytic
(Jatkar & Purohit. 1971) and etiologically as hemolytic (Raisinghani et al.
experimental by infected in India over a period of one year. He showed
reductions in haemoglobin, packed cell volume and serum levels of
calcium, potassium and sodium. Increases in reticulocytes, eosinophils
and organic phosphate, fluctuated reciprocally with bouts of parasitic and
non parasitic phases. The level of blood glucose also falls with increasing
parasitaemia (Jatkar & Singh, 1971).

Naturally–infected camels were found to have higher serum protein
and gamma–globulin levels but lower albumin and Beta-globulin levels
than uninfected animals (Boid et al., 1980). The level of IgM in both
naturally and experimentally–infected animals increased by as much as
five times the pre– infection level. These values may remain high despite
drug treatment.
1-7 Diagnosis:

Many diagnostic techniques including parasitological and serological were devised. The techniques differ in their reproducibility, specificity and sensitivity and each can be applied according to the prevailing situation (Abdel Rahman et al, 2001). However, characteristic clinical symptoms of emaciation and anaemia are still used for the provisional diagnosis of the disease (Luckins et al, 1979).

1-7-1 Parasitological diagnosis:

Diagnosis of infection by examination of peripheral blood is satisfactory in animals with acute infections but more difficult in a chronic disease (Luckins, 1979). Examination of the blood by light microscopy, either by the wet film method or as Gimsa-stained thin or thick smears is quite common. Although examination of wet films is rapid, it is comparatively less sensitive and many sub-patent infections go undetected (Rae et al, 1989).

The microhaematocrit centrifugation technique (MHCT) prepared by Woo (1969) is more sensitive than the wet film or the stained preparation examination and can also indicate the degree of anemia by the percentage of packed cell volume (PCV). However, the method
requires centrifugation. To increase the sensitivity of this method the micro-capillary tube is broken and the buffy zone expressed on a microscopic slide. The preparation is then examined in a similar way to dry and wet smears. Wet preparations made of the buffy zone are examined under phase contrast or dark-field illumination microscopes (Murray et al, 1977). The technique has increasing application in the field (Kelley and Schillinger, 1983). To conclude no single direct parasitological method alone was totally effective. The Microhaemtocrit centrifuge technique, mouse inoculation of blood and Gimsa–stained smears are proposed as the most effective diagnostic combination (Monzon, 1990). The technique of inoculation of camel blood samples into laboratory rodents is of some diagnostic value as above (Godfrey and Killick – Kendrick, 1962) but is not used routinely in the field (Boid et al., 1985).
1-7-2  **Serological diagnosis:**

Recently much attention has been given to the development of specific serological tests for *T. evansi* infections in camels.

1-7-2-1  **The complement fixation test (CFT):**

The complement fixation test was one of the first techniques used to diagnose *T.evansi* infection in camels (Schoening, 1924). However, because the test is difficult to perform and standardization is of paramount importance, it has not been used routinely as a diagnostic assay (Rae. and Luckins, 1984).

1-7-2-2  **Capillary agglutination test (CAT):**

The test was described by Ross (1971) it is similar to that which has been widely used in the diagnosis of anaplasmosis, and trypanosomosis in man and animals using a particulate antigen prepared from *T. brucei*. The results appeared promising and the test was used by Clarkson *et al* (1973).

1-7-2-3  **The indirect fluorescent antibody test (IFAT):**

The indirect fluorescent antibody test has been used extensively in the detection of trypanosomal antibodies in animals and humans. Antigens are usually prepared from blood smears which are fixed in acetone and then stored at a low temperature. The IFAT has proved to be both specific and sensitive in detecting trypanosomal antibodies in
infected cattle (Wilson, 1969.; Luckins and Mchlitz, 1978) and camels (Luckins et al., 1979).

1-7-2-4 **Enzyme–linked immunosorbent assay (ELISA):**

Enzyme-linked immunosorbent assay was a major breakthrough in the diagnosis of animal trypanosomosis. This test is specific and sensitive, and can be used readily for large scale screening of many serum samples Rae et al, 1989. The ELISA has been used successfully for serodiagnosis of camel trypanosomosis. Luckins et al, 1979; Boid et al, 1980; Rae et al, 1989.

1-7-2-5 **Species–specific monoclonal antibody:**

The introduction of this technology has also been a major breakthrough in the diagnosis of trypanosomosis due to *T.brucei*. No cross reactions occur between other species of trypanosome or other haemoproteozoan parasites Nantulya et al, 1989

1-7-2-6 **Sandwich ELISA.**

*T.brucei* group specific monoclonal-antibody assay has been used as sandwich ELISA test to diagnose *T. evansi* infections in different animal species. In camels from an endemic area, the test detected circulating antigens in many *T. evansi*-parasite positive animals Nantulya et al, 1989.
The techniques of PCR or ELISA are not only beneficial for diagnosis of the parasite but may also be useful for trypanosomosis control programmes (Chansiri. et al, 2002; Monzon et al, 2003).

1-7-2-7  The Card Agglutination Test (CATT):

A card agglutination test set has been introduced into the market for the diagnosis of Gambian sleeping sickness. After that the test was found suitable for diagnosis of trypanosomosis in camels (Zweygarth, et al., 1984). The test uses the formalin fixed variable antigen types of T. evansi that are used in the agglutination test. The test, which is simple to perform, has been used for diagnosis of T. evansi (Bajyanasonga et al., 1987), and also used to detect antibodies against T.evansi (Hilali et al, 2004). The sensitivity of CATT/T.evansi was higher than that of latex agglutination test (Suratex), but not significantly so. Both tests had equally high specificity (Ngaira et al, 2003). CATT/T.evansi can detect aparsitaemic infection rapidly and it is more sensitive than parasitological methods in revealing the true extent of trypanosomosis in a herd. The test effectively complemented parasitological methods in the detection of T.evansi infection in camels.
1- 8 Control:

*T. evansi* infection is one of the major constraints for raising camels. Control of vector insects is not feasible now; and despite the encouraging reports of vaccination experiments against *T. evansi* in laboratory rodents (Ryu, 1975; Hertzkorn, 1980, and Bremer, 1982; cited by Schillinger and Rottcher, 1984), the production of a vaccine is still a remote possibility due to the problem of antigenic variation (Gray & Luckins, 1976). The only method available for controlling trypanosomosis infections in camels is therefore chemotherapy and chemoprophylaxis. The efficacy of existing trypanocidal drugs has been reviewed by a number of authors (Finelle, 1973; Borst, 1977; Losos, 1980; and Mahmoud and Gray, 1980).

1-8-1 Chemotherapy:

Treatment of camels infected with *T. evansi* with the current available trypanocides requires greater care. The reasons being their low trypanocidal effect against *T. evansi* on one hand, and their specific toxicity for camels on the other hand. For example Berenil, which is well tolerated in cattle, produces systemic toxicity, in some cases ending in death, of camels, when administered in the recommended therapeutic dosage of 3.5mg/kg, (Leach, 1961; Fazil, 1977; Homeida *et al*, 1981). Four compounds, namely suramin, Berenil, isometamidium and
Quinapyramine have been used for many years to treat trypanosomosis in camels, cattle, buffalo, horses and pigs. Treatment of camels was and, has until recently depended upon two drugs: suramin and quinapyramine drugs. However, resistance, poor tolerance and/or complicated administration highlighted the need for a novel trypanocidal drug (Van Gool et al, 1992). The drug resistance in *T.evansi* might be an underestimated problem with potentially serious implications for the future control of camel trypanosomosis owing to the withdrawal of drugs from the world market. (Mutugi & Boid et al, 1995).

Trypanosomosis is endemic mostly in the developing countries where the resources are very meager. In such countries, purchase of drugs is not usually possible due to economic constraints.

The major problem in the control of camel trypanosomosis is still that of drug resistance. Drug resistance may be caused by under-dosing, using the wrong intervals between chemoprophylactic doses, discontinuing prophylaxis despite continued risk of infection, applying preventive instead of curative doses and injecting the drug inaccurately.

Drug resistance of trypanosomes is now a major problem, but its underlying mechanisms are still not fully understood. (Witola et al, 2004).
Chemotherapy and chemoprophylaxis:

Trypanocides used in treatment and protection are the most common single method employed for the control of camel trypanosomosis, both curative and preventive as in the case of the quinapyramine compounds (Losos, 1986). The difference between cure and prevention depends upon the drug being used and in some cases upon the dosage rate at which it has been administered. Effective application of chemotherapy and chemoprophylaxis in the field depends on several factors which include the strain of trypanosome causing infection, severity of the challenge, species of animal, and lastly the occurrence of resistant strains. (Losos, 1986).

Berenil (Diminazene aceturate) (Hoechst, Germany)

This drug qualifies as a wide spectrum trypanocidal drug (Losos, 1986). The drug is notable among anti-trypanosomal drugs in being rapidly excreted from the body, mainly through urine (Kellner et al, 1985) and is therefore not considered to have pronounced prophylactic activity (Fairclough, 1963; Williamson, 1970). Yet, biologically active berenil, which is presumably retained in the tissues, has been shown by others to have a prophylactic effect that may last for several weeks against natural infections with *T. vivax*, *T. congolense* and *T. brucei* (Van Hove and Cunningham, 1964; Lumsden et al 1972; Zahalsky and
Weinberg, 1976; Williamson, 1976) and against experimental infection with *T. brucei* in mice (Whitelow and Urguhart, 1985). A curative dose of Berenil against *T. evansi* at 3.5 mg/kg weight could confer protection for a period of 2 days (El-Amin and El Amin, 1992). However, at a dose rate of 10mg/kg the drug was very effective in eliminating *T.evansi* (ALAmīn et al, 1982) in experimentally infected Asian buffalo calves (Verma et al, 1973).

1-8-2-3 Antrycide pro-salt (Quinapyramine sulphate and Quinapyramine chloride) Wockhart Ltd, Mumbai, India:

Antrycide pro-salt is the principal drug in the quinapyramine compounds group, which is curative against *T.evansi* in all species of hosts (Finelle, 1973) and additionally gives camels a prophylactic protection for three to four months (Njogu, 1986). It is administrated subcutaneously as a 10% aqueous solution using cold water at a dose rate of 5.0mg/kg, (Finelle, 1973). The main disadvantage of quinapyramine compounds is cost, as it is more expensive than suramin. The drug was originally supplied for prophylactic use as a mixture of quinapyramine sulphate (3parts) and quinapyramine chloride (2parts) which was made up in water and administrated at a dose of 7.4mg/kg.s.c. This gave two months protection for camels against *T.evansi* and relapses were treated with suramin (Finelle, 1973).
Ethidium (Homidium bromide) laprovet France:

Homidium was extensively used in the 1960s and 1970s but its usefulness has been greatly reduced due to widespread resistance over the years (Scott and Pegram, 1974), it has remained essentially a curative drug in the field despite claims that the drug has some prophylactic properties (Dolan et al., 1990).

More recently, De Dekan et al. (1989) have reported success in protecting rabbits for more than 300 days against seven challenges of T.congolense using slow release device implanted sub-cutaneously. This finding suggests that the commonly used drug products, Novidium or Ethidium lake prolonged prophylactic activity because the active principle, Homidium is readily eliminated from the body, should this slow release device technology prove cost-effective in the field it will undoubtedly be widely accepted in many areas with a yearlong high incidence of Trypanosomosis (Kinabo, 1993) in a field trial carried out in South West Kenya over a twelve month period, which included several months of high trypanosome challenge, the average period of protection recorded for Ethidium was 4–6 weeks (Steven, et al., 1995).

Severe general reaction have only occasionally been seen in horses. The drug may cause local reaction and should be administered by deep intramuscular injection taking care to avoid leakage into subcutaneous
tissues. Dividing the dose between two sites may reduce local reactions in the horse. After some years of mass field therapy of cattle infection with *T.congolense* resistant to the normal curative dose of 1 mg/kg may become widespread, but resistant strains *T.vivax*, through they may occur (Strephen, 1963) they are evidently much less frequent in northern Nigeria (Jones–Davies & Folkers, 1966; Williamson & Stephen, 1960).

1-8-2-5 **Cymelarsan (Melarsan oxide) Rhone Merieux, France:**

Cymelarsan is an injectable trivalent arsenical. It is a derivative of melarsanoxide, the cysteamine derivative, and is a white powder highly soluble in water. Preliminary work carried out, mainly using mice, indicated that cymelarsan is very active against trypanosomes of *T. brucei* group, the animal pathogens *T. brucei* and *T. evansi* and the human pathogens *T. gambiense* and *T. rhodesiense*. (Raynaud et al., 1989). No activity was demonstrated against the important cattle pathogens *T. congolense* and *T. vivax*.

Cymelarsan has been shown not to be mutagenic or embryo-toxic by standard laboratory procedures. It has also been administered to a number of pregnant camels without ill-effects (Raynaud et al., 1989). The drug showed good efficacy against *T. evansi* infection in camels at dose rate of 1.25, 0.625, 0.6, 0.5 and 0.25mg/kg bwt. It eliminated the parasites
rapidly and completely. After treatment all animals recovered quickly and no relapse was observed. Animals which received cymelarsan subcutaneously showed localized swelling and oedema around the injection site during the first 36h post treatment (VanGool et al, 1992).

1-8-3 Drug Resistance

Drug resistance is known to occur amongst *T. evansi* isolates and there have been reports of its occurrence in several different countries in Africa and Asia. It is not known how extensive its occurrence is, or whether it is, or likely to be a serious problem for the control of the disease. A number of different approaches have been used to detect drug resistant trypanosomes including the presence of specific genetic markers, in vivo tests in rodents or in vitro assays. These latter are particularly useful since *T. evansi* can be cultured in the absence of feeder cell monolayers that could affect the metabolism of the drugs used.
Immunity:

Host specific immunity eventually clears the dominant antigenic types of infecting trypanosome; and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. Sequences of dominant antigenic variants tend to follow specific order, although the particular sequence can be influenced by the host immune response and other factors (Gray, 1965; Capbem et al, 1977; Miller & Turner, 1981; Barry & Turner, 1991). Variation in switch rates may also play an important role in the wide variety of pathogenic microorganisms that undergo programmed antigenic variation (Deitsch, et al 1997; Fussenegger, 1997; Nash, 1997; O’Connor et al, 1997; Serkin & Seifert, 1998; Zhang, et al, 1998). The parasite has an ability to change its antigenic surface properties and consequently escape the immune surveillance (Vickerman, 1989). Each parasite cell is covered with a nearly uniform and strongly antigenic glycoprotein coat. The parasites genome contains several hundred alternatives and highly diverse surface antigens, of which only a single one is expressed in any individual. A parasite switches its antigenic expression and entire coat in each cell generation at a rate of one per thousand to one per hundred (Barry & Turner, 1991; Barry, 1997; Turner, 1997). These switches appear to occur in an apparently random way, creating a diverse set of antigenic variants (Turner & Barry, 1989). In spite of this diversity, the parasitaemia
develops as a series of out breaks, each one dominated by relatively few antigenic types (Barry and Turner, 1991).

1-9-1 Trypanotolerance:

Several indigenous West African taurine (Bos Taurus) breeds, such as the longhorn (N`Dama) cattle are well known to control trypanosome infection. (Berthier et al, 2003). Range trypanotolernt cattle are now widely accepted as an important means of exploiting many tsetse infested areas (Ilard, 1985). All workers agreed that N`Dama and Matura breeds of cattle tolerate infection with pathogenic African trypanosomes better than Zebu or European breeds (Nyindo, 1992). Rahman et al, (1993) reported the existence, of a zebu breed, the western baggara cattle in Sudan which survived a natural tsetse challenge and showed a better ability to control parasitaemia compared to other indigenous zebu, e.g. the Butana and Kinana, which succumbed to trypanosomosis. This probably confirms the historical record noted by Archibald in 1927 (cited by Njogu, 1986) which made the first report of trypanotolerance in cattle from an East African zebu in the Sudan. The gene-based ability called trypanotolerance results from various biological mechanisms under multigenic control (Berther et al, 2003).
CHAPTER TWO
MATERIALS AND METHODS

2-1 Experimental animals

Eight locally bred rabbits were purchased from home-raised flocks. They were selected according to their age, being less than six months and in an acceptable health state. The animals were kept for 15 days adaptation period during which they were initially serially marked using picric acid. During the pre infection period temperature, PCV, hemoglobin and body weight were recorded and a daily wet smear preparation was examined for freedom of trypanosomes. The examinations done were according to the schedule below:-

<table>
<thead>
<tr>
<th>Examination</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet blood smear and temperature</td>
<td>daily</td>
</tr>
<tr>
<td>PCV and hemoglobin</td>
<td>Twice weekly</td>
</tr>
<tr>
<td>Body weight</td>
<td>Once a week</td>
</tr>
</tbody>
</table>
2-2 Experimental design:-

2-2-1 Parasite:-

A *Trypanosoma evansi* isolate, originating from a naturally infected camel in north kordofan state, Sudan was donated by Dr. Atif Alamin. The isolate was propagated in mice which infected blood was used to infect rabbits.

2-2-2 Inoculation
Six rabbits were inculcated on day 16 from purchase, by *Trypanosoma evansi* isolate inter peritoneal (I/P) dose 1ml hall blood contain range 450 parasites and 2 rabbit were left uninoculated as control.

2-2-3 Trypanosomes counting:-

The rapid matching wet – count technique described by Herbert and Lumsden (1976) was used. This entailed examining a drop of mouse blood under the x 40 magnification of a microscope and counting the number of trypanosomes in each field. Each count per field was matched with the log figures obtained from the references table (Herbert and Lumsden, 1976). The log figures were converted to absolute number of trypanosomes per ml of blood.
2-2-4 Parasite detection methods:-

Daily collected blood was examined by the following methods:-

(i) **Wet blood smears preparation:**

   Wet blood films were prepared by aseptic puncture of peripheral ear veins of rabbits using sterile needles. A drop of blood was taken on to a clean glass slide and covered with cover slip and examination for *T.evansi* was done under compound light microscope at X40 magnification.

(ii) **Dry blood smears preparation:**

   Simultaneous dry blood films were taken from the ear vein. These were stained in 10% Giemsa’s stain and examined under a compound light microscope using oil immersion lens (X100).

(iii) **Packed cell volume:**

   The per cent packet volume (PCV %) was determined by the PCV reader. Each centrifuged capillary tube was read and recorded for each rabbit.
(iv) **Haemoglobin concentration (Hb):**

Dry clean test tubes were prepared for sample and standard. To each tube, 4 ml cyanide reagent was added. Then 0.2 ml of blood sample and Hb standard solution were added to the samples and standard tubes, respectively. The tubes were allowed to stand for 15 min, and then the optical density (O.D.) was read at 540 nm in the colorimeter using cyanide reagent as blank.

**Calculation**

\[
\text{Hb concentration (g/dL)} = \frac{\text{OD of sample} \times 32}{\text{OD of standard}}
\]

(vi) **Serum collection :-**

Serum collected by aseptic puncture of peripheral ear veins of rabbits using sterile syringe capacity 1 ml, then to be in bottle with cover.

2-2-5 **Card Agglutination Test for Trypanosomes (CATT):**

A direct card agglutination test for detection of anti-trypanosome antibodies in serum or plasma of infected animals was used The antigen consists of cloned bloodstream form trypanosomes of RoTat 1.2; a predominant variable antigen type (VAT) of *T.evansi*. The antigen was obtained from the Tropical Medicine Institute, Antwerp-Belgium, (Magnus, 1988). The organisms have been fixed, stained and freeze-dried in order to obtain maximal stability. They are agglutinated by antibodies directed against the RoTat 1.2 variable antigen epitopes and also by antibodies against invariable surface antigen components.
(a) Test Kit

Reagents and accessory materials were obtained from the Institute of Tropical Medicine (Antwerp, Belgium). A complete test kit for 250 screening tests contains the following: 5 vials CATT-antigen, a vial positive control, a vial negative control, and a vial CATT-buffer. The reagents for the test are mixed as follows:

(b) Test Procedure:

A 2.5ml of CATT buffer was added to a vial of freeze dried CATT antigen using a sterile syringe. The vial was then shaken for a few seconds so as to obtain a homogeneous suspension. 0.5ml of CATT buffer was added to the vials of positive and negative controls respectively using sterile syringe. On a test area of the card, 25µl of the non diluted serum was added to the well containing the homogenized CATT antigen (approximately 45µl). After rotating the card gently, for 5 minutes or a horizontal rotator agglutination was observed and the degree of agglutination was determined as follows:

1- Very strong agglutination (+++).
2- Strong agglutination (++).
3- Moderate agglutination (+).
4- Weak agglutination (+).
5- Absence of agglutination (-).
In this experiment the results were taken as absolute positive and negative.

2-2-6 Body Temperature:-

The measurements of temperature of experimental animals were made to the nearest ± 0.1°C using digital thermometer (ACON). The thermometer was inserted into the rectum to a depth of approximately 2cm.

2-2-7 body weight:-

Once weekly done by electric sensitive scales for each rabbit.

2-3 Treatment:-

Experimental Animals were treated after 30 days by Cymelarsan at a dose of 0.3 ml/Kg given I/M.

2-4 Statistical analysis:-

The data has been analyzed using various statistical packages, means and standard errors were obtained using a computer software statistical package for social scientists (SPSS) for short. The experimental designs, ANOVA procedure and mean separations were obtained using another computer package known as SAS software.
CHAPTER THREE
RESULT

3-1 Detection of parasitaemia: -

Following inoculation of rabbits with *T.evansi*, the infected rabbit No (3) became parasitaemic on day 5 and parasitaemia was detected subsequently in other rabbits till day 10 when all rabbits became parasiteamic, making the incubation period to be 5 – 10 days. Parasites disappeared on day 13 from rabbit No (4) and progressively in other rabbits. And daily check using wet blood smear showed intermitted parasitaemia. Rabbit No (2) died after 3 days from inoculation, rabbit No (6) died after 24 days from inoculation the rest persisted to the end of the experiment of 75 days. After treatment all infected rabbits became a parasitaemic.

3-2 Haematological changes:-

3-2-1 Average haemoglobin values for rabbits before, during infection and after treatment from *T.evansi* infection.

In pre infection haemoglobin in infected rabbits ranged between 10.01 - 11.27 g/dl, after infection it was 9.57 – 10.64 g/ dl after treatment it became 9.17 - 10.64 g/dl. In the control group the range was between 10.01±0.00 - 10.92±1.00 g/dl. Throughout the experimental period. Table (1). The decrease in haemoglobin values during infection was significantly different from pre-infection and post treatment values as shown in table (4).
Table (1):- Average haemoglobin values for rabbits infected with *T.evansi* and control groups during pre-infection, infection and treatment stages.

<table>
<thead>
<tr>
<th>Pre infection</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) week</td>
<td>10.99±0.61</td>
<td>10.69±0.38</td>
</tr>
<tr>
<td>2(^{nd}) week</td>
<td>10.85±1.31</td>
<td>10.67±1.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infection</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) week</td>
<td>10.66±1.59</td>
<td>10.92±1.00</td>
</tr>
<tr>
<td>2(^{nd}) week</td>
<td>10.57±1.41</td>
<td>10.78±1.50</td>
</tr>
<tr>
<td>3(^{rd}) week</td>
<td>10.02±0.93</td>
<td>10.64±0.50</td>
</tr>
<tr>
<td>4(^{th}) week</td>
<td>9.47±1.74</td>
<td>10.88±0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treated</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) week</td>
<td>9.69±1.75</td>
<td>10.85±0.50</td>
</tr>
<tr>
<td>2(^{nd}) week</td>
<td>10.01±2.19</td>
<td>10.74±0.25</td>
</tr>
<tr>
<td>3(^{rd}) week</td>
<td>10.24±1.64</td>
<td>10.5±0.00</td>
</tr>
<tr>
<td>4(^{th}) week</td>
<td>10.08±2.00</td>
<td>10.01±0.00</td>
</tr>
</tbody>
</table>

3-2-2 Average packed cell volume values for rabbits before, during infection and after treatment from *T.evansi* infection.

In pre infection the PCV range was 34 – 40, in infection it ranged between 27.5 – 37.5, In treated it's ranged between 25.5 – 36.8. In the control group the range was between 30.00±0.00 - 38.50±1.50 (Table 2). The PCV decreased significantly during infection and remain decreased even after treatment as observed for 30 days after treatment (table 4).
Table (2):- Average packet cell volume values for rabbits infected with *T.evansi* and control groups during pre-infection, infection and treatment stages.

<table>
<thead>
<tr>
<th>Average per week</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; week</td>
<td>37.8±0.51</td>
<td>36.50±0.50</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>38±1.11</td>
<td>38.25±1.75</td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; week</td>
<td>37.00±1.00</td>
<td>38.50±1.50</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>35.10±0.91</td>
<td>37.75±1.25</td>
</tr>
</tbody>
</table>
3rd  30.30±1.41  35.00±1.00  week
3-2-3 Average body weight values for rabbits before, during infection and after treatment from *T.evansi* infection.

In pre infection body weight ranged between 0.783 – 1.845 kg, during infection it was between 0.788 – 1.69 kg, after treatment the range was 0.79 – 1.4 kg. In the control group the range was 0.77±0.00 - 0.88±0.08 kg. (Table 3). The two groups started with different body weights, the infected were heavier. The infected group lost weight which was not restored after treatment. Even though the changes in weight were not significant (table 4).

**Table (3):- Average body weight values for rabbits infected with *T.evansi* and control groups during pre-infection, infection and treatment stages.**

<table>
<thead>
<tr>
<th>Average per week</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1\textsuperscript{st} week</td>
<td>1.381±0.14</td>
<td>0.83±0.04</td>
</tr>
<tr>
<td>2\textsuperscript{nd} week</td>
<td>1.37±0.11</td>
<td>0.83±0.04</td>
</tr>
</tbody>
</table>
(Table 4):-Comparison between Pre infection, Infection and Treated stages for hemoglobin, packet cell and Body weight:

<table>
<thead>
<tr>
<th></th>
<th>stage</th>
<th>Infected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb</strong></td>
<td>Pre-Infection</td>
<td>10.85±0.18(^a^)A</td>
<td>10.66±0.16(^a^)A</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>10.17±0.18(^a^)B</td>
<td>10.77±0.13(^a^)A</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>9.85±0.27(^a^)B</td>
<td>10.68±0.04(^a^)A</td>
</tr>
<tr>
<td><strong>PCV</strong></td>
<td>Pre-Infection</td>
<td>37.58±0.95(^a^)A</td>
<td>37.15±0.95(^a^)A</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>31.46±1.39(^a^)B</td>
<td>36.75±0.75(^a^)A</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>29.94±1.76(^a^)B</td>
<td>35.53±1.28(^a^)A</td>
</tr>
<tr>
<td><strong>Body</strong></td>
<td>Pre-Infection</td>
<td>1.37±0.11(^a^)A</td>
<td>0.83±0.04(^b^)A</td>
</tr>
</tbody>
</table>
### Table key:

- \(X_{\text{capital litter}}\) = compare between the same rabbit in each phase.
- \(X_{\text{small litter}}\) = compare between rabbits (1-8)

<table>
<thead>
<tr>
<th>Weight</th>
<th>Infection</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.24±0.12(^a)A</td>
<td>0.86±0.07(^a)A</td>
</tr>
<tr>
<td></td>
<td>1.11±0.10(^a)A</td>
<td>0.83±0.04(^a)A</td>
</tr>
</tbody>
</table>

**Graph No (1)** Average temperature records for rabbits in pre-infection, infection and treatment stages
Temperature for infected & control rabbits in pre infection stage ranged between 37.7 – 37.9 °C, in infection stage temperature became more highly than control it retched 39.3 and after treatment became lower average 38.9 °C.

3-3  **Card Agglutination Test (CATT-*T.evansi)*:**

The test was conducted on serum collected from eight different rabbits, taken during different stages at pre-infection, infection and
treatment. Results for each rabbit of 32 serum specimens tested in pre infection stage and collected twice weekly were all negative. Results for all, serum collected during infection stage were 56 samples of serum collected in 30 days and result were positive for infected rabbit No (1,3& 4), negative in rabbit No (7&8) uninoculated rabbit, rabbit No (5) result were 89% positive, 11% negative, rabbit (6) result were 83% positive, 17% negative and rabbit No (2 ) died after inoculation.

Serum collected after treatment were 48 samples collected in 30 days and result were positive for infected rabbit No (1&4 ), negative in rabbit No (7&8) uninoculated rabbits, rabbit No (3) result were 67% positive, 33% negative, rabbit No (5) result were 67% positive, 33% negative and rabbit No (2&6) died before treatment. Negative result of rabbit No (3) appeared on 7th and 10th weeks, on day 30 and 57 from inoculation. Negative result of rabbit No (5) appeared on 3rd, 9th and 10th week on day 7, 57 and 60 from inoculation (table 5).

Table No (5): - result of Card Agglutination Test for each rabbit
<table>
<thead>
<tr>
<th>Rabbit No</th>
<th>Pre infection</th>
<th>Infection</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>- ve</td>
<td>- ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>- ve</td>
<td>- ve</td>
<td>D</td>
</tr>
<tr>
<td>3</td>
<td>- ve</td>
<td>- ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>- ve</td>
<td>- ve</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>- ve</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>6</td>
<td>- ve</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>7</td>
<td>- ve</td>
<td>- ve</td>
<td>- ve</td>
</tr>
<tr>
<td>8</td>
<td>- ve</td>
<td>- ve</td>
<td>- ve</td>
</tr>
</tbody>
</table>

CHAPTER FOUR
DISCUSSION

47
Camel trypanosomosis present special problems with regard to diagnosis. The clinical signs are not pathognomonic and the standard techniques for the detection of trypanosomes are not sufficiently sensitive (Boid et al., 1985). Although significant improvements have been made recently in diagnosis, a high proportion of infections still remain undetected as the chronic, more common form of the disease is often aparasitaemic (Luckins et al., 1979). In the face of these constraints, alternative methods of diagnosis have been developed, most of which are for the detection of antibody response to the antigens of the circulating trypanosomes (Allen et al., 1992).

The results obtained in this study using temperature measurement, haemoglobin values, packed cell volume and body weight for rabbits before, during infection and after treatment from *T.evansi* infection. The study showed that normal temperature before infection, increased after inoculation of *Trypanosoma evansi* compared to no change in control rabbits. Haemoglobin in infected group showed significant decrease post infection and this finding agrees with several workers (Roberts and Gray, (1973); Onaha, et al., (1996)), average of haemoglobin before infection was 10.85±0.18 g/dl, after infection was 10.17±0.18 g/dl and there was no significant different in control group, the level of haemoglobin continued to be low post treatment and this finding disagree with (Roberts and Gray, (1973). but the observation period in this work was short which may
have not allowed for complete recovery of haemoglobin values. The study showed that there was a drop of the packed cell volume (PCV) value post infection in all rabbits this agrees with woodruff, (1996); Losos and Tabel, (1976); Igbokwe and Mahmoud, (1992); Ngeranwa, et al., (1993); Onah, et al., (1996) who found that Trypanosomosis is a major cause of anemia and PCV drop in different kinds of animals. In this study average of PCV before infection was 37.58±0.95, after infection was 31.46±1.39 and after treatment it became 29.94±1.76. Death due to trypanosomosis is usually a result of sever anaemia, and animals that are capable of compensating the reduction in PCV and erythrocytes indices during the course of infection often survive (Onah et al, 1996). In this study the PCV of the infected rabbits was lower than that of the negative uninfected animals. This low PCV level can be attributed to parasitaemia and subsequently the destruction of erythrocytes by T. evansi haemoflagellates. However, this may not be a universal proposition, since Boid et al (1981) reported that T. evansi appeared to have little effect on the haematological picture and PCV of infected sheep, though there was a progressive fall in the PCV of similarly infected goats and camels.

The study showed that there was a dropped in the body weight after infection and treatment but also there was an observed drop in control rabbits which may indicate the effect of other factors. This finding is important as body weight changes may not indicate infection all the
time. Clearance of parasitaemia in rabbits treated with Cymelarsan indicates the high efficacy of this drug in parasites clearance. This result agrees with Partoutomo, et al (1994) who found all experimental animal (Friesian Holstein cattle) treated with Cymelarsan I/M remained parasitologically negative up to 80 days after treatment. The rabbits treated in this study remained negative for 30 days. Results obtained by CATT for 136 serum sample collected during, before and after infection were 100% negative in pre-infection for all sera collected before infection, Antibodies were detected in all infected rabbits at various stages. After treatment serum collected from some rabbits were 100% positive, some Rabbits were 67% positive and no agglutination reaction for control rabbits. These results agree with Magnus (1988) who suggested that CATT test was highly sensitive but was not strictly species-specific. In conclusion these results show that CATT-\textit{T. evansi} was reliable enough to detect aparasitaemic infection rapidly and was more sensitive than parasitological methods in revealing the true extent of trypanosomosis in a herd (Ngaira \textit{et al}, 2003; Delafosse and Doutoum, 2004; Hilali \textit{et al}, 2004).

**Conclusions and recommendations**

1. The card agglutination test for trypanosomosis (CATT-\textit{T. evansi}) was
Found to be a good serological test in the field because it's a rabid test and also easy to use.

2. The card agglutination test for trypanosomosis (CATT-\textit{T. evansi}) detected anti bodies of \textit{T.evansi} for 30 days after treatment but cant not detected anti bodies of non- infected rabbits.

3. The card agglutination test for trypanosomosis (CATT-\textit{T. evansi}) should be used with haemoglobin values, packed cell volume and body weight.

References


Anon (1907) Annual Reports of the Sudan veterinary service. vol. 1 Mc Corquodiale & co. (Sudan) Ltd.


Bajyana-Songa, E., Hamers-Casterman, C., Hamers, R., Pholpark, M., Pholpark, S., Leidl, K., Tangchaitrong, S., Chaichanopoonpol, I.,


Protozoologica. **33**: 1-51.


pulp International laboratory for Research on Animal Disease, Nairobi Kenya.


**Levine, N. D; Corliss, J. O; Cox, F. E. G; Deroux, G; Grain, J; Honigberg, B. M; Leedale, G. F; Loeblich, A. R; Lom, J; Lynn, D; Merinfeld, E. G; Page, F. C; Poljansky, G; Sprague, V; Vavra, J; and Wallace, F. G.** (1980). A newly revised classification of the Protozoa. J. Pro. 27: 37-58.


Stevens, J. R; and Sylvain, B. (2003). Systematics of trypanosomes of medical and veterinary importance CABL Publishing is a division of CABInternational pp (xix).


Turner, C. M. R (1997). The rate of antigenic variation in fly transmitted and syringes pass aged infection of trypanosoma brucei. FFMS


Parasitology. 99, 67-75.


