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EVALUATION OF CARD AGGLUTINATION TEST/ *Trypanosoma evansi* IN EXPERIMENTALLY INDUCED INFECTION IN RABBIT

تقييم إختبارالتراص الورقى لمثقبية الجمال *Trypanosoma evansi* في الأرانب المصابة تجريبياً

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المستخلص

صممت هذه الدراسة لتقييم اختبار التراص الورقي لداء المثقبيات . هذه التقنية طورت خصيصا لمثقبية الجمال . تم شراء ثمانية من سلالة الارانب المحلية المربية في المنازل .تم حقن ست ارانب في اليوم السادس عشر من الشراء بواسطة مثقبية الجمال في البريتون وتركت اثنتان بدون حقن كمجموعة تحكم .تم استخدام اختبار التراص الورقي لفحص الاجسام المضادة في السيرم او البلازما ، يحتوي المستضد على (RO Tat 1.2) من نوع (VAT) لمثقبية الجمال . تم استخدام الاختبار في السيرم الذي جمع من ثمانية ارانب من مختلف المراحل قبل العدوى وبعد العدوى وبعد العلاج بواسطة الساملرسان . كانت كل الارانب سالبة في مرحلة قبل العدوى . السيرم الذي تم جمعه اثناء مرحلة العدوى كان موجبا في كل الارانب المحقونة وسالب في غير المحقونة . اظهر السيرم الذي تم جمعه بعد 30 يوم بعد العلاج نتائج مختلفة فكانت بعض الارانب موجبة لبعض الوقت و الاخرى كانت سالبة .يكون الموت الناتج من داء المثقبيات نتيجة لفقر الدم عادة . في هذه الدراسة حجم الدم المكبوس والهيمقلوبين ووزن الجسم للارانب المصابة اقل من مجموعة التحكم السالبة .الخلاصة كانت ان اختبار التراص الورقي لداء المثقبيا ت ذوحساسية عالية في فحص الاجسام المضادة ولكن النتيجة يجب تقييمها بحرص لان الاجسام المضادة يمكن ان تبقى بعد العلاج.الطريق الامثل هو اعتبار حجم الدم المكبوس والهيمقلوبين والاجسام المضادة معيار لتقييم وضع العدوى

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Abstract

This study was planned to evaluate the Card Agglutination Test for Trypanosomiasis (CATT). This technique was particularly developed for *T. evansi*. Eight locally bred rabbits were purchased from home-raised flocks. Six rabbits were inoculated on day 16 after purchase, by *Trypanosome evansi* isolate inter peritoneal (I/P) and 2 rabbits were left un inoculated as control. A direct Card Agglutination Test for detection of anti-trypanosome antibodies in serum or plasma of infected animals was used. The antigen consisted of cloned bloodstream form trypanosomes of RoTat 1.2 and 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-infection, post-infection and after treatment with Cymelarsan. In pre-infection stage all rabbits were negative. Sera collected during infection stage was positive in all of inoculated rabbits, negative in un inoculated ones. Sera collected at 30 days after treatment showed variable results as some rabbits were positive for some time while others became negative. Death due to trypanosomiasis is usually a result of sever anaemia. In this study the Packed Cell Volume (PCV), HB & body weight of the infected rabbits were lower than that of the negative control. 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, haemoglobin (Hb) and antibody titer for evaluation of the infection situation.

Key words: Camel, CATT/ *T. evansi*, Sudan

Introduction

Trypanosomiasis, caused by *Trypanosoma evansi* is one of the major and most important diseases of camels in the arid and semi-arid 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). 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 *et al*. 1979). Improved diagnostic techniques are needed for diagnosis of cases and evaluation of chemotherapeutic efficacy of drugs. This study was planned to:

1- observe the pathogencity of *T. evansi* in experimentally infected rabbits as measured by temperature changes, body weigh changes, haemoglobin (Hb) and Packed Cell Volume (PCV).

2- evaluate the efficiency of CATT/ *T. evansi* 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.

Materials and Methods

Study animals

Eight locally bred healthy rabbits less than six months old were purchased from home-raised flocks. The animals were kept for 15 days as 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 to ensure freedom of trypanosomes. The examinations which were carried out included: wet blood smear and temperature on daily basis; PCV and hemoglobin twice a week and body weight weekly.

Experimental design:-

Parasite:-

Trypanosoma evansi isolate was obtained from a naturally infected camel in north kordofan state. The isolate was propagated in mice in our laboratory as a source of rabbit's infection.

Inoculation

Six rabbits were inoculated interperitoneally (IP) on day 16 after purchase, by *Trypanosoma evansi* isolate using a dose of 1ml whole blood containing

about 450 parasites. The remaining two rabbits were left as uninoculated.

Trypanosomes counting:-

The rapid matching wet – count technique was used. This entailed examining a drop of mouse blood under high magnification (x 40) of a microscope and counting the number of trypanosomes in each field. The log figures were converted to absolute number of trypanosomes per ml of blood.

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 then covered with a cover slip. A compound light microscope with objective lens 40 was used to examine *T.evansi*.

(ii) Dry blood smears preparation:

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

(iii) Packed Cell Volume (PCV):

The Packed Cell Volume (PCV %) was determined using the haematocrit. Each centrifuged capillary tube was read and recorded for each rabbit (Maxwell, 1957).

(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 (Maxwell, 1957).

Calculation

$$\text{Hb concentration (g/dL)} = \frac{\text{OD of sample X 32}}{\text{OD of standard}}$$

Card Agglutination Test for Trypanosomiasis (CATT/ *T. evansi***):**

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 were agglutinated by antibodies directed against the RoTat 1.2 variable antigen epitopes and also by antibodies against invariable surface antigen components. Reagents and accessory materials were obtained from the Institute of Tropical Medicine (Antwerp, Belgium). A complete test kit for 250 screening tests contained the following: 5 vials CATT-antigen, a vial positive control, a vial negative control, and a vial CATT-buffer. The reagents for the test were mixed as follows: A 2.5ml of CATT buffer was added to a vial of freeze dried CATT antigen using a sterile syringes. The vial was then shaken for 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 (-)

Cut-off point: In this experiment the results were taken as absolute positive and negative

Treatment:-

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

Statistical analysis:-

The data was analyzed using two statistical packages, means and standard errors were obtained using a computer software statistical package for social scientists (SPSS). The experimental designs, ANOVA procedure and mean separations were obtained using another computer package known as SAS software.

Results

Following inoculation of rabbits with *T. evansi*, the infected rabbit No (3) was positive on day 5 and parasitaemia was detected subsequently in other infected rabbits till day 10. The parasites disappeared on day 13 from rabbit No (4) and progressively in other rabbits. The daily checking of rabbits by using wet blood smear showed evidence for intermitted parasitaemia. Rabbit No (2) died after 3 days from inoculation, rabbit No (6) died after 24 days from inoculation. The rest survived till the end of the experiment, another 75 days. After treatment all infected rabbits showed negative results.

Haematological changes:-

Haemoglobin in infected rabbits ranged between 10.01 - 11.27 g/dl during pre- infection , 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. The decrease in haemoglobin values during infection was different from pre-infection and post treatment.

Packed Cell Volume (PCV)

In pre- infection the PCV range was between 34 – 40, during infection it ranged between 27.5 – 37.5. After treatment it ranged between 25.5 – 36.8. In the control group the range was between 30.00 ± 0.00 - 38.50 ± 1.50 . The

PCV decreased significantly ($P < 0.05$) during infection and remain decreased even after treatment as observed for 30 days after treatment (table 1).

Body weight

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. The two groups started with different body weights, the infected were heavier. The infected group lost weight which was not restored after treatment (Table 2).

Table(1):- Average of PCV values for rabbits infected with *T. evansi* at different stages

stage		Rabbits	
		Infected	**Control**
Pre- infection	1st week	37.8±0.51	36.50±0.50
	2nd week	38±1.11	38.25±1.75
Post-infection	1st week	37.00±1.00	38.50±1.50
	2nd week	35.10±0.91	37.75±1.25
	3rd week	30.30±1.41	35.00±1.00
	4th week	28.20±2.50	37.50±0.00
Post-treatment	1st week	30.25±2.50	37.00±0.00
	2nd week	31.25±2.29	37.00±0.00

	3rd week	31.67±1.01	34.75±1.25
	4th week	30.50±0.50	30.00±0.00

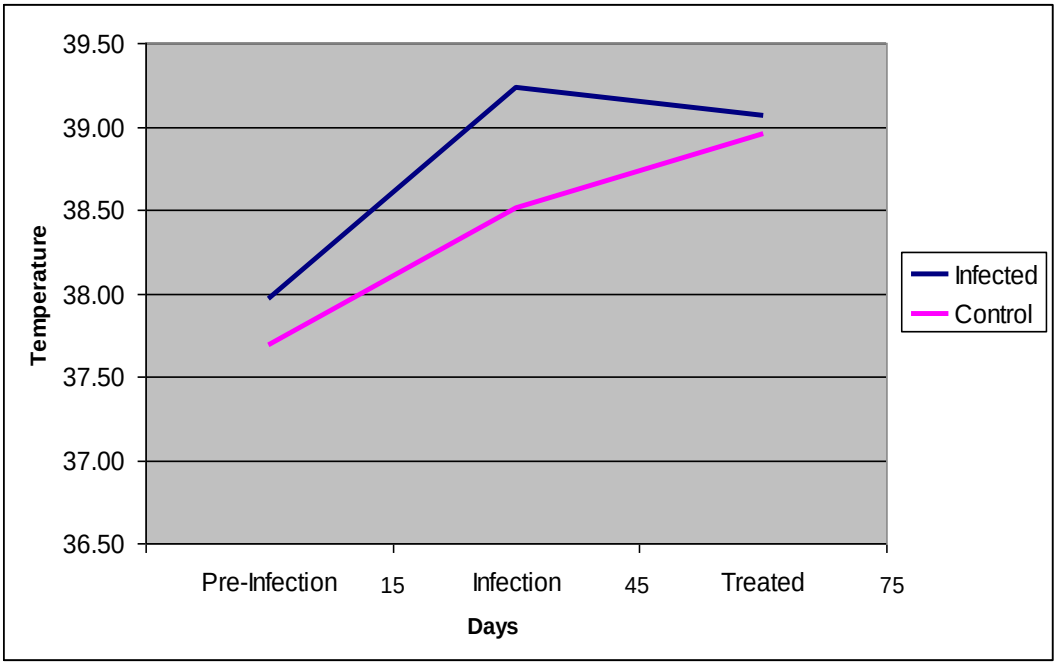
Table (2):- Average body weight values for rabbits infected with *T. evansi*

stage		Rabbits	
		Infected	**Control**
Pre infection	1st week	1.381±0.14	0.83±0.04
	2nd week	1.37±0.11	0.83±0.04
Post-infection	1st week	1.33±0.12	0.78±0.00[b]A
	2nd week	1.33±0.12	0.84±0.06[a]
	3rd week	1.23±0.12	0.85±0.07
	4th week	1.19±0.13	0.88±0.08
Post-treatment	1st week	1.22±0.16	0.95±0.14
	2nd week	1.15±0.13	0.90±0.10
	3rd week	1.11±0.11	0.78±0.02
	4th week	1.12±0.13	0.77±0.00

Temperature

Temperature for infected & control rabbits in pre- infection stage ranged between 37.7 – 37.9 c^0 , in infection stage temperature became more highly than control it retched 39.3 and after treatment became lower average 38.9 c^0 (Figure).

Figure 1: Average temperature records for rabbits in pre- infection, infection and treatment stages



Card Agglutination Test for Trypanosomiasis (CATT/*T. evansi)*:

The test was conducted on serum collected from eight different rabbits, taken during different stages at pre-infection, post-infection and post-treatment. Results for all serum specimens tested in pre infection stage were negative. Results for all , serum collected during infection stage were **56** samples of serum collected in 30 days and result were positive for infected rabbits (No 1,3& 4), negative in rabbits (No 7&8) un inoculated rabbit. However rabbit No (5) results were 89% positive,11% negative, rabbit (6) result were 83% positive, 17% negative. Serum collected after treatment were **48** samples collected in 30 days and result were positive in rabbit No (1&4), negative in rabbit No (7&8), rabbit No (3) result were 67% positive , 33% negative, rabbit No (5) result were 67% positive , 33% negative and rabbit No (6) died before treatment. Negative result of rabbit No (3) appeared on **7 th and** 10 th weeks, on day **30** and **57** of inoculation. Negative result of rabbit No (5) appeared on **3rd, 9 th** and **10th** week on day **7, 57** and **60** from inoculation.

Discussion

Camel trypanosomiasis presents 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 an additional common chronic 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 were for the detection of antibody response to the antigens of the circulating trypanosomes (Allen *et al*. 1992).

The results obtained in this study were based on monitoring temperature changes, recording of haemoglobin, packed cell volume, and body weight for rabbits before infection, during infection and after treatment. The results showed a normal temperature before infection, which increased

after inoculation with *T. evansi*. Haemoglobin concentration in infected group showed a decrease after infection. This finding agrees with several workers (Onaha, *et al*. 1996). The haemoglobin before and after infection were 10.85±0.18 g/dl and 10.17±0.18 g/dl respectively and there was no significant difference in control group. The level of haemoglobin continued to be low after treatment. However, the observation period in this work was shorter which may 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 which agrees with Ngeranwa, *et al*. (1993); Onah *et al*. (1996) who found that Trypanosomosis is a major cause of anaemia and PCV drop in different kinds of animals. In this study average of PCV before infection was 37.58±0.95, before infection was 37.58±0.95 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 drop in the body weight after infection and treatment but also there was an observed drop in control rabbits which may indicate that the effect was due to 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 efficiency 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 samples collected from 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/*T. evansi* was reliable enough to detect aparasitaemic infection rapidly and was more sensitive than parasitological methods in revealing the true extent of trypanosomiasis in a herd (Ngaira *et al*, 2003; Delafosse and Doutoum, 2004; Hilali *et al*. 2004).

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