

EVALUATION OF THE CARD AGGLUTINATION TEST (CATT/*T. EVANSI*) IN COMPARISON WITH PCR FOR DETECTION OF *TRYPANOSOMA EVANSI* INFECTION IN DONKEYS IN EGYPT

By

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Abstract

This study was initiated to evaluate CATT test in comparison with PCR for detection of *Trypanosoma evansi* infection in donkeys in Egypt. Six donkeys free from *T. evansi* were inoculated with *T. evansi* strain (Egypt CD2) which had been isolated from naturally infected horse. Parasitological examination of the donkeys revealed a prepatent period of *T. evansi* 1-4 DPI. Two blood samples (heparinized and plain) were taken from each donkey every 4 days starting from day 0 until the end of the experiment for serological examination (CATT/*T. evansi*). Body temperature was also estimated. Serological examination of the experimentally inoculated donkeys' no.1, 2, 4 and 5 showed that seroconversion occurred on 12 DPI and remained until death of animals. Other experiment, blood samples from 416 donkeys collected randomly from different governorates of Egypt, they were tested parasitologically (stained blood smear, mice inoculation (MI) and microhaematocrit centrifugation technique (MHCT) and serologically by using CATT/*T. evansi* for diagnosis *T. evansi* infection. From the previous animals, 26 parasitologically negative donkeys were used for comparison between the results obtained by CATT/*T. evansi* & PCR techniques. Of 26 parasitologically negative samples, 9 (34.6%) & 14(53.85%) samples were found positive by using CATT and PCR techniques respectively. Despite of the simplicity and low-cost of CATT test, it is recommended to use it in combination with PCR technique for accurate and reliable results.

Key words: CATT, donkeys, experimental infection, PCR.

Introduction

Trypanosoma evansi causes a fatal animal loss mainly among camel, cattle, horses, donkeys, zebra, mules, dogs and pigs can be infected (Dia *et al*, 1997; O.I.E., 2004). Trypanosomiasis is widely distributed sickness outside the Tse Tse belt in Africa. *T. evansi* in horse induces acute and chronic clinical symptoms in the diseased animals which last up to 3 months to 3 years. However, some infected horses did not show symptoms (Desquesnes *et al*, 2013).

Serological techniques like direct agglutination test (CATT/*T. evansi*) based on Ro-Tat 1.2 VSG and ELISA/*T. evansi* and molecular tests like PCR are used as specific diagnostic tools for surra and have been underwent in various studies in different hosts and countries (Gutierrez *et al*, 2000; Verloo *et al*, 2000). CATT/*T. evansi* is considered to be a well-validated field test (Claes *et al*, 2004).

This study aimed to evaluate card aggluti-

nation test (CATT/*T. evansi*) for detection of antibodies against *Trypanosoma evansi* (*T. evansi*) in experimentally and naturally infected donkeys in comparison with molecular study.

Material and methods:

Experimental infection: Six donkeys (one year old) free from *T. evansi* after parasitological (buffy coat technique (BCT), mouse inoculation and serological examination (CATT/*T. evansi*) were assigned into experimental (no. 1, 2, 3, 4 & 5) and control (no.6) groups. All were parasite-free ones.

Trypanosoma evansi strain (Egypt CD2) was isolated from Egyptian naturally infected horse. Donkeys (1, 2, 3, 4 & 5) were each intravenously inoculated with 350.000, 500.000, 250.000, 50.000 & 100.000 trypanosomes/donkey respectively. Two blood samples (heparinized & plain, 5ml each) were taken from each donkey every 4 days starting from day 0 until 50 days post-inoculation (DPI). Body temperature and clinical

symptoms were recorded daily for each one.

Sera were tested by card agglutination test (Fig. 1, CATT / *T. evansi*). Blood samples of 416 donkeys obtained from different Egyptian governorates from March 2016 to February 2017. Samples were tested parasitologically (stained blood smears, buffy coat technique (BCT), mouse inoculation (MI) or serologically by CATT/*T. evansi*.

Serological examination by CATT/ *T. evansi*: Sera of the animals were prepared by two fold serial dilutions (1:4-1:128) with the supplied buffer for testing in CATT /*T. evansi* (Bajyana Songa and Hamers, 1988). Sample was considered positive when blue granular agglutination was appeared at dilution 1:4. End point titer was determined for the sera of infected animals by testing higher dilution (Verloo *et al*, 2000).

Molecular technique (PCR): Twenty six parasitologically negative donkey samples were examined by molecular technique (PCR) in comparison with CATT / *T. evansi* which was performed on the same samples. Genomic DNA was extracted from these 26 donkeys frozen whole blood samples. Samples were thawed then centrifuged at 3000rpm/5 min at 4°C. Sediment was collected. The DNA extraction was done (Ijaz *et al*, 1998). The mixture was subjected to 40 cycles of amplification in the thermocycler (PTC 100mj Research USA). Amplified products were electrophoresis analyzed in 1.5% agarose gel and UV illumination after ethidium bromide staining of tDNA. Specific DNA fragment of *T. evansi* was amplified of size 164 base pair (bp) (Ijaz *et al*, 1998).

Ethics: All investigations reported in the study were approved by the institutional animal use protocol committee of Cairo University, Egypt.

Results

Parasitological examination of the infected donkeys with *T. evansi* revealed a prepatent period of *T. evansi* 1-4 DPI with a patent period varied from 7 to 50 DPI (Tab. 1). The body temperature of five experimentally infected donkeys was normal (37.5-38.5°C)

during the first two days (donkey no. 1, 4 & 5) or 3 days (donkey no. 2). The first peak of pyrexia (40°C) was continued 1-2 days PI, and a second one (39- 40.1°C) was estimated for 2-5days. Body temperature showed no significant increase until the end of experiment on 50th DPI. Body temperature of donkey no.3 was normal from 1st DPI until its death 8th DPI. Body temperature of negative control donkey (no.6) remained normal and it didn't show any clinical symptoms throughout the experimental period (50DPI).

Lacrimation from both eyes appeared in all inoculated donkeys on 3rd DPI and continued until the experimental end (50 DPI). This eye discharge was watery in nature then it changed into mucoid one and it collected at internal eye canthus. Alopecia recorded after 15th DPI and continued until experimental end. Vulver discharge was seen in donkeys' no.2 and 4 on days 32 PI & 4PI respectively. This discharge continued till death of animals. Emaciation was began in appearance on the 10th DPI and continued till death of animals. All experimentally inoculated animal were reluctant to walk, dull and depressed, paresis of hind limbs then complete paralysis were in donkeys no. 2, 4 & 5 one or two days prior to animal death.

T. evansi was diagnosed from donkey no. 1, 2, 3, 4 & 5 until 50, 28, 7, 40 & 36 DPI respectively. Donkeys nos.1 & 6 survived until experimental end while the other donkeys died on 28, 8, 40 & 36 DPI (Tab. 2).

Serological examination of the experimentally inoculated donkeys nos.1, 2, 4 & 5 showed that seroconversion occurred on 12 DPI (end-titer 1:32), 8(1:8), 4(1:8), 16(1:4) respectively and remained until death of the inoculated ones. Maximum end point titer recorded in donkey no.1 was 1:32. Donkey no. 3 died on 8th DPI before seroconversion report. Donkey (control) remained normal.

Natural infection: The 416 donkeys from different governorates of Egypt which were found negative parasitologically revealed positive results by using CATT/*T. evansi* revealed 63 (15.12%) donkeys positive at

dilution of 1:4.

Comparison between CATT and PCR: Out of 26 parasitologically negative samples, 9

(34.6%) and 14 (53.85%) samples were positive by using CATT and PCR technique respectively (Tab. 3).

Table 1: Details PCR mixture of 50µl volume, primers and amplification conditions used in PCR technique.

PCR mixture(50 µl)	Primers (TBR1 &TBR2) (5'-3')	PCR condition for each cycle
DNA template (1µl) Primer 1 (TBR1; 0.5µl) Primer 2 (TBR2; 0.5µl) PCR buffer (5µl) DNTP (2µl) DNA polymerase (1µl) Dist. Water (40µl), 30µl mineral oil added to each sample.	F:5'-GAATAT TAA ACA ATG CGC AG-3' R: 5'-CCA TTT ATT AGC TTT GTT GC-3'	Denaturation at 94°C for 1 min Annealing at 55°C for 75 sec. Extension at 72°C for 1.5 min. Prior to cycling mixture subjected to 94°C incubation for 4min. Extension of last cycle at 72°C for 10 min.

Table 2: Donkeys experimentally infected with *T. evansi*.

Donkey No.	Infective dose	Prepatent period (days)	Duration of parasitaemia (days)	seroconversion DPI	Death after (days) inoculation (DPI)
1	350.000	One	50	12	50
2	500.000	3	26	8	28
3	250.000	3	7	none	8
4	50.000	2	40	4	40
5	100.000	4	36	16	36

Table 3: Comparison of 26 parasitologically negative donkeys by PCR and CATT/ *T. evansi*

No. of Donkeys	Serological using CATT	Parasitological (mice inoculation)	Positive by PCR
9	+ve	-ve	9
9	±ve	-ve	3
8	-ve	-ve	2

Discussion

The experimental infection proved that *T. evansi* strain from naturally infected horse was infectious for donkeys. A prepatent period was 1-4 DPI in experimentally infected donkeys. *T. evansi* specific antibodies were estimated with high titer using CATT on 12th DPI (end-titer 1:32), 8th (1:8), 4th (1:8) & 16th (1:4) in inoculated donkeys no.1, 2, 4 & 5 respectively.

CATT/ *T. evansi* was compared with PCR for diagnosis of *T. evansi* among 26 parasitologically negative donkeys. Nine donkeys were positive and 9 doubtful but, PCR was 14 positive (9 CATT+ve, 3 CATT±ve, & 2 CATT-ve). Five PCR positive samples were 3 CATT±ve, 2 CATT-ve. Serological test was unable to distinguish between active infections and cured one, but PCR provided a definite tool to assess therapy effectiveness and disease progression (Clausen *et al*, 1999; Tehseen *et al*, 2017).

The seropositive but parasitologically negative animals must be confirmed by CATT/ *T. evansi* performances by PCR test (TBR primers) which proved to be accurate test

(Desquesnes and Davila, 2002). This agreed with Magnus (1988) who found that CATT test was highly sensitive but not strictly species-specific, and recommended to use CATT & PCR for reliable diagnosis.

The 416 donkeys were negative parasitologically while serological examination revealed 63 (15.12%) donkeys positive at dilution of 1:4. The value obtained in the present study disagreed with Hilali *et al*. (1998) who reported 41(82%) donkeys had antibodies against *T. evansi* using CATT. This difference may be due to low of examined animals (50 donkeys) by these authors.

The absence of parasitologically positive donkeys might be attributed to parasite occurrence in early period after infection but persistence of antibodies against *Trypanosoma* sp. for long time even after treatment increased probability of serological techniques detection (Luckins *et al*, 1979). In routine Surra surveys, CATT/*T. evansi* proved validated test, and overestimated antibodies persisted for several months (Desquesnes and Davila, 2002).

Dedication

This paper is dedicated to the spirit of the late Professor Dr. Mossad Hilali.

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Explanation of figure

Fig. 1: Positive and negative sera examined by CATT. Grades 1-3: +++ve, Grades 4-5: ++ve, Grades 6-7: +ve, Grade 8: ±ve & Grades 9-10: -ve

