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THE USE OF THE CARD AGGLUTINATION TEST (TESTRYP® CATT) FOR THE SERODIAGNOSIS OF *T.EVANSI* INFECTION

by

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Summary — The Testryp® CATT originally developed for diagnosis of human *T. gambiense* infection was evaluated for the serodiagnosis of animal *T. evansi* (Surra) infection. Sera were obtained from dogs, goats, rabbits, water-buffaloes and cattle experimentally infected with cloned and uncloned populations of *T. evansi* strains from different countries. Comparison between sera of infected and uninfected animals showed that the threshold of positivity lay around serum dilution of 1/2 with the exception of water-buffaloes where this threshold must be considered at a 1/8 serum dilution.

KEYWORDS: Surra, *Trypanosoma evansi*, Serodiagnosis; Testryp® CATT

Introduction

Trypanosoma (Trypanozoon) brucei evansi infects various domestic animals such as water-buffaloes, camels, horses and pigs. The course of the disease is often chronic and the infection may remain undetected. Nevertheless it causes major economic losses due to abortion, reduced working capacity of draft animals and high morbidity and mortality due to other concomitant infections.

Draft animals are of high value in the developing countries and timely treatment of the disease in them is economically justified. Moreover, it is the health of the individual animal which is at risk. The development of a simple spot field test for the detection of *Trypanosoma (Trypanozoon) brucei evansi* infection which can be accompanied by immediate treatment of infected animals is most desirable.

This is quite relevant for regions where the veterinary laboratories are located far from the sick animals.

Recent studies on the immunogenic variable antigen have demonstrated the presence of isoVATs in different repertoires of trypanosomes. This discovery was successfully exploited in the development of the Card agglutination test (CATT) for the diagnosis of human sleeping sickness caused by *Trypanosoma (Trypanozoon) brucei gambiense* (5).

The Testryp® CATT (Smith Kline RIT, Belgium), a kit available commercially, was used in the present work and is based on a well-defined selected serotype LiTat 1.3. This test is easy to perform in field conditions, and is adapted for mass screening and surveillance for Gambian sleeping sickness (4).

The card agglutination test was recently used for serodiagnosis of animal trypanosomiasis. When applied for the diagnosis of camel trypanosomiasis, the test showed low sensitivity (8), though subsequently it was successfully used for antibody detection in pigs experimentally and naturally infected with *Trypanosoma congolense* (6).

In the present work the feasability of the Testryp® CATT has been examined for the serodiagnosis of *Trypanosoma (Trypanozoon) brucei evansi* infection of domestic animals and the results are reported.

Materials and methods

Trypanosomes

Cloned population of LiTat 1.3 (LiTAR 1) derived from a *T.b. gambiense* stock (1) and AnTat 3.3 (AnTAR 3), derived from a *T. evansi* stock (3) were used as reference clones. AnTat 3.3. is the IsoVAT of AnTat 1.8 derived from *Trypanosoma (Trypanozoon) brucei brucei* initially used in the Card agglutination test (2, 7). The following repertoires of *T. evansi* were obtained (Bajyana Songa, unpublished) from various geographical regions; Indonesia (RoTAR 1 and 2 = *R*ode *T*rypanozoon *A*ntigen *R*epertoire 1 and 2), South America (RoTAR 3), Sudan (RoTAR 4 and 5 and a clone derived from the TREU 1923 stock) and Thailand (RoTAR 6, 7, 8 and 9). In addition, infection was induced with uncloned stock from which the RoTAR 1 and 2 were isolated.

Sera

In a first group 7 dogs and 2 goats were experimentally infected with AnTat 3.3 and their serum samples were collected at weekly intervals during two months post-inoculation. A second group of animals consisted in 25 rabbits which were infected with various clones belonging to the repertoires as cited above. Their sera were collected at different intervals during the first two months of infection. Pooled sera from cow and water-buffalo experimentally infected with uncloned populations of *T. evansi* were obtained from Indonesia. Pre-infection sera were used as control. Moreover sera from uninfected water-buffaloes were obtained from Italy.

Tests

The sera from infected and uninfected animals were tested for the presence of antibodies against AnTat 3.3 and LiTat 1.3 by specific trypanolysis and direct agglutination (Testryp® CATT) tests. The trypanolytic test was carried out as previously described (2). Using 1/10 dilution of sera, agglutination antibodies were detected with the Testryp® CATT (Smith-Kline RIT, Belgium) according to the manufacturer's instructions.

Results

The following results deal with sero-epidemiological aspects by assessing the presence of IsoVATs of AnTat 3.3 of *T. evansi* and LiTat 1.3 of *T. gambiense* in the repertoires of isolates of *T. evansi* from Asia, Africa, South America with the use of the direct agglutination test (Testryp® CATT) in serodiagnosis of surra.

1. *IsoVATs of the AnTat 3.3*

The existance of IsoVATs of the AnTat 3.3 can be shown by infecting laboratory animals with different stock isolates of *T. evansi* and testing the sera at different intervals for trypanolytic antibodies. Sera from 3 out of 10 cloned isolates gave rise to lytic antibodies lysing over 90 % of AnTat 3.3, whereas 3 gave partial lytic activity under 90 % and 4 were negative. These results indicate that IsoVATs of AnTat 3.3 are present in strains of *T. evansi* coming from Indonesia (RoTAR 1 and 2), Latin America (RoTAR 3), Soudan (TREU 1923) and Thailand (RoTAR 6 and 7) (Table 1).

2. *IsoVATs of LiTat 1.3.*

Sera from infected animals were tested by Testryp® CATT for direct agglutination and by the trypanolytic test with LiTat 1.3 *T. gambiense* trypanosomes. Most of *T. evansi* repertoires gave rise to agglutinating antibodies indicating that the IsoVATs of LiTat 1.3 are expressed early in *T. evansi* infection.

Nevertheless, sera from 3 of 36 of the infected animals showed negative reactions. Partial trypanolytic activity was found in the sera of animals infected with 7 repertoires (Table 1).

TABLE 1

AnTat 3.3 and LiTat 1.3 reactivity: Trypanolytic and direct agglutination test of repertoire specific anti-sera obtained from different strains of *T. evansi*

Infecting Populations	Origin of sera	No infected animals	Trypanolysis		CATT test
			LiTat 1/3	AnTat 3/3	No of positive animal
AnTAR 3	dog	7	± (6)	+ (7)	7
AnTAR 3	goat	2	± (2)	+ (2)	2
RoTAR 1	rabbit	7	± (2)	+ (7)	6
RoTAR 2	" rabbit	3	± (3)	± (3)	3
RoTAR 3	" rabbit	2	± (1)	+ (2)	2
RoTAR 4	" rabbit	4	± (2)	—	4
RoTAR 5	" rabbit	1	—	—	1
RoTAR 6	" rabbit	1	±	±	0
RoTAR 7	" rabbit	1	—	±	1
RoTAR 8	" rabbit	3	± (2)	—	2
RoTAR 9	" rabbit	1	—	—	1
TREU 1923*	" rabbit	2	± (2)	+ (2)	2
Indonesia stock	cow	1	±	+	1
	water-buffalo	1	+	+	1

– : lysis < 10 % in all sera
± : lysis < 90 % and > 10 %
+ : lysis > 90 %
* clone derived from stock
( ): number of sera

3. *Early detection of antibodies against T. evansi by Testryp® CATT*

The dogs and goats were experimentally inoculated with AnTat 3.3 clone. All 7 dogs displayed a positive reaction 2 weeks post-inoculation and only 1 out of 7 animals became negative 8 weeks post-infection (Fig. 1 and Table 1). Comparable results were obtained in the 2 infected goats (Table 2) where positive reaction appeared after 13 days and persisted for 38 days post-infection.

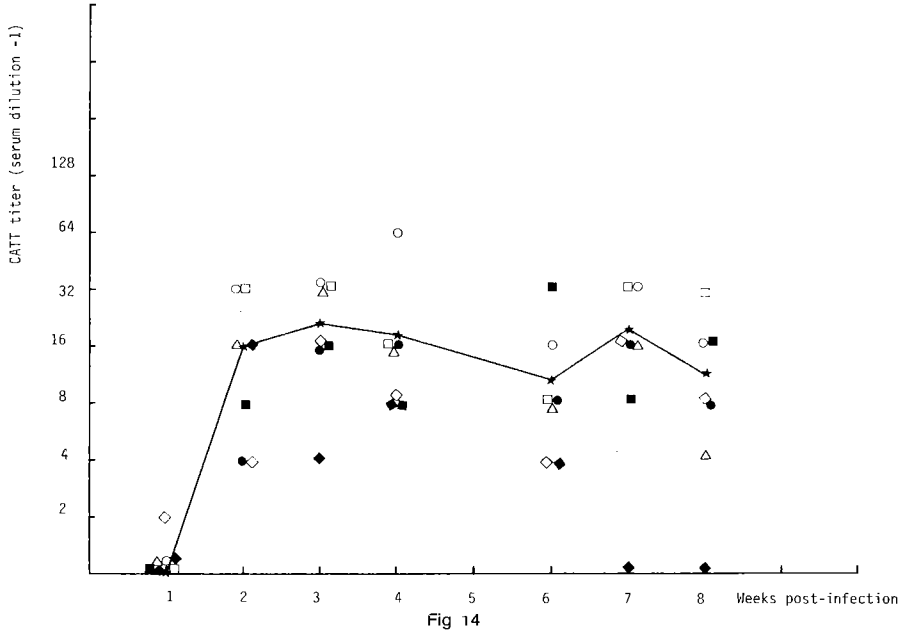

Fig 14

Evolution of the CATT titre in 7 dogs inoculated with *T. evansi* of the AnTat 3.3 repertoire. The star symbol corresponds to the average titer whereas each of the other symbol corresponds to an individual dog.

TABLE 2

Evolution of CATT titers of goat sera experimentally infected with AnTat 3.3 *T. evansi*

days post-infection	CATT titre goat 1	goat 2	goat 3
0	NT	NT	NT
7	NT	NT	NT
13	1/8	1/16	–
17	1/32	1/32	–
24	1/16	1/32	–
27	1/16	1/16	–
38	1/8	1/16	–

goat 3 was not infected

– : negative results at dilution 1/2

NT: not tested

These results show that the CATT will detect the infection during the first two weeks following infectious inoculation.

Testryp® CATT test has also been used to detect antibodies from the sera of 25 rabbits infected with cloned populations from the 9 RoTAR

repertoires and TREU 1923 clone. All but 3 rabbits developed a positive reaction during the first month after infection. From 21 rabbits which were tested on days 6, 12 and 30 post-inoculation a majority (80,1 %) of infected animals became positive on day 12 (Table 3). The positive reactions correspond to 8 out of the 9 RoTAR repertoires suggesting that the CATT test detects an early predominant VAT common to most repertoires.

TABLE 3

End-titre range of CATT test of sera from 21 rabbits with *T. evansi* infection

sera		end-titres					% positives
days p.i.	non-reactive	1/2	1/4	1/8	1/16	1/32	
0	21	0	0	0	0	0	0
6	19	1	0	1	0	0	9.5
12	4	1	9	8	1	0	80.1
30	3	2	5	5	4	2	85.7

Pooled sera of experimentally infected cattle and water-buffaloes from Indonesia were tested. The sera from the infected showed a CATT titer of 1/20 (buffalo) and 1/40 (cattle). Samples from the uninfected cattle showed negative reaction at 1/2 dilution. In contrast, the sera of uninfected water-buffaloes from Italy and Indonesia showed a positive titer of 1/2 and 1/4 respectively.

TABLE 4

CATT titres of sera of experimentally infected cattle and water-buffalo

origin		end-titres of sera
bovine	– uninfected	1/2
	+ infected	1/40
buffalo	– (a) uninfected (Indonesia)	1/4
	(b) infected (Italy)	1/2
	+ infected	1/20

The serum of uninfected water-buffalo seems to contain a factor capable of agglutinating trypanosomes. This implies that the sera or blood samples of water-buffaloes have to be diluted to 1/10 before testing.

Discussion and conclusions

Our results show that the CATT test developed for the human chronic sleeping sickness and based on an early VAT Litat 1.3 of *T. gambiense* can be used for the detection of *T. evansi* infection in dogs, rabbits, cattle, water buffaloes and probably goats. Nine out of 10 *T. evansi* repertoires induced CATT positive reactions. In these repertoires, as in the AnTAR 3, the IsoVATs of LiTat 1.3 are presumed also predominant VATs.

The advantage of the CATT is its specificity due to the uniqueness of the variable antigen which is present only in Salivarian trypanosomes. It detects antibodies against an immunogenic predominant VAT in the majority of infected animals within two weeks of infection. The CATT test will detect more

cases of infection than the trypanolytic test. The agglutination of a small proportion of trypanosomes is easier to detect than the lysis of a similar proportion. Using those repertoires for infection which we know will give rise to CATT positive sera, 34 out of 36 animals developed CATT positive sera whereas only 21 out of 36 developed trypanolytic antibodies against LiTat 1.3 population.

However it must be kept in mind that some repertoires might not contain the LiTat 1.3 VAT utilized in the CATT test or that in some repertoires (or in some animals) the variant might appear only late in infection. This might be the case with the RoTAR 6 repertoire. Tests designed to detect all infections might have to contain several different VATs or be constructed around common antigens.

The duration of the time the agglutination antibodies persist has not been extensively studied but in one dog out of seven, the CATT test was negative by week 8 post-infection. This could be due to the relatively low sensitivity of the CATT test. In the detection of camel trypanosomiasis, Zweygarth *et al.* (8) considered this lack of sensitivity as a major handicap.

In our experiments using the sera from buffaloes, the control animals showed positive reactions at a titre of 1/2 and 1/4 whereas the infected animal showed a titre of 1/20. This means that for the water-buffaloes the CATT test cannot be used on undiluted heparinized blood in the field as it is now for human trypanosomiasis.

In conclusion, the CATT developed for the detection of anti-*T. gambiense* antibodies in human sleeping sickness can be utilized for the early detection of *T. evansi* infection in goats, dogs, rabbits, cattle and with a lower sensitivity in water-buffaloes. Methods are being developed to increase the sensitivity of the test for water-buffaloes and camels.

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Serodiagnose van *T. evansi* infectie met de kaart agglutinatie test (Testryp® CATT)

Samenvatting — Een evaluatie van de serodiagnose van dierlijke trypanosomiasis, veroorzaakt door *T. evansi* (Surra), werd uitgevoerd met de Testryp® CATT, die ontwikkeld werd voor de diagnose van humane trypanosomiasis, veroorzaakt door *T. gambiense*. Sera waren afkomstig van honden, geiten, konijnen, waterbuffels en een runddie experimenteel geïnfekteerd werden met uit verschillende landen afkomstige gekloonde en ongekloonde populaties van *T. evansi*. Vergelijking tussen sera van geïnfekteerde en niet-geïnfekteerde dieren toont aan dat de drempelwaarde van een positieve uitslag ongeveer ligt bij de serumverdunning 1/2, met uitzondering van de waterbuffels waar de drempelwaarde van de serumverdunning 1/8 is.

Serodiagnosis of *T. evansi* infection with the card agglutination test (Testryp® CATT)

Summary — Le Testryp® CATT developpé pour diagnostiquer la trypanosomiase humaine due à *T. gambiense* a été évalué dans le sérodiagnostic de la trypanosomiase animale (Surra) dont *T. evansi* est responsable. Les serums proviennent de chiens, de chèvres, de lapins, de buffles d'eau et de vache expérimentalement infectés avec des clônes et de populations stocks de *T. evansi* isolées de plusieurs pays. La comparaison de réactivité entre les sera provenant des animaux infectés et non infectés montre que le seuil de positivité se situe aux alentours de la dilution 1/2 pour la plupart des animaux exceptés les buffles d'eau où elle se situe à la dilution 1/8.

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