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A CARD AGGLUTINATION TEST (CATT) FOR VETERINARY USE BASED ON AN EARLY VAT RoTat 1/2 OF *TRYPANOSOMA EVANSI*

by

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Summary — A variable antigen type RoTat 1/2 of *T. evansi* has been assayed in the CATT test and compared with the Testryp® CATT. It was found that a more sensitive CATT test can be developed based on another early expressed VAT RoTat 1/2 of *T. evansi* in the sero-diagnosis of animal trypanosomiasis.

KEYWORDS: Trypanosomiasis, Animal; *Trypanosoma evansi*; Serodiagnosis; Card Agglutination Test (CATT)

Introduction

It is well known that certain variable antigens are expressed in common in different strains of trypanosomes (3,5) and a field test based on a well-defined selected serotype LiTat 1/3 is marketed by Smith Kline-RIT (Belgium) for the diagnosis of human sleeping sickness.

More recently, the usefulness of Testryp® CATT had been assayed for animal trypanosomiasis (1,4,6). It was found that such tests lack sufficient sensitivity to be of field value for domestic animals trypanosmiasis, especially for camels and buffaloes. For buffaloes, however, this lack of sensitivity can be overcome by the addition of specific anti IgG reagents to the agglutination test (2).

In the present work, the feasability of the CATT test based on an early *Trypanosoma evansi* serotype RoTat 1/2 common to different strains has been examined for the diagnosis of domestic animals trypanosomiasis and results are discussed.

Material and methods

Sera

The following sera were examined:

Monospecific antisera against 16 cloned VATs of *T. evansi* were obtained from rabbits by inoculation of cloned trypanosomes and bleeding on 6th day of infection. Multivalent antisera raised against different repertoires of *T. evansi* were examined. Moreover, antisera against *T. b. brucei* and *T. congolense* repertoires were provided by Prof. Van Meirvenne (Antwerpen).

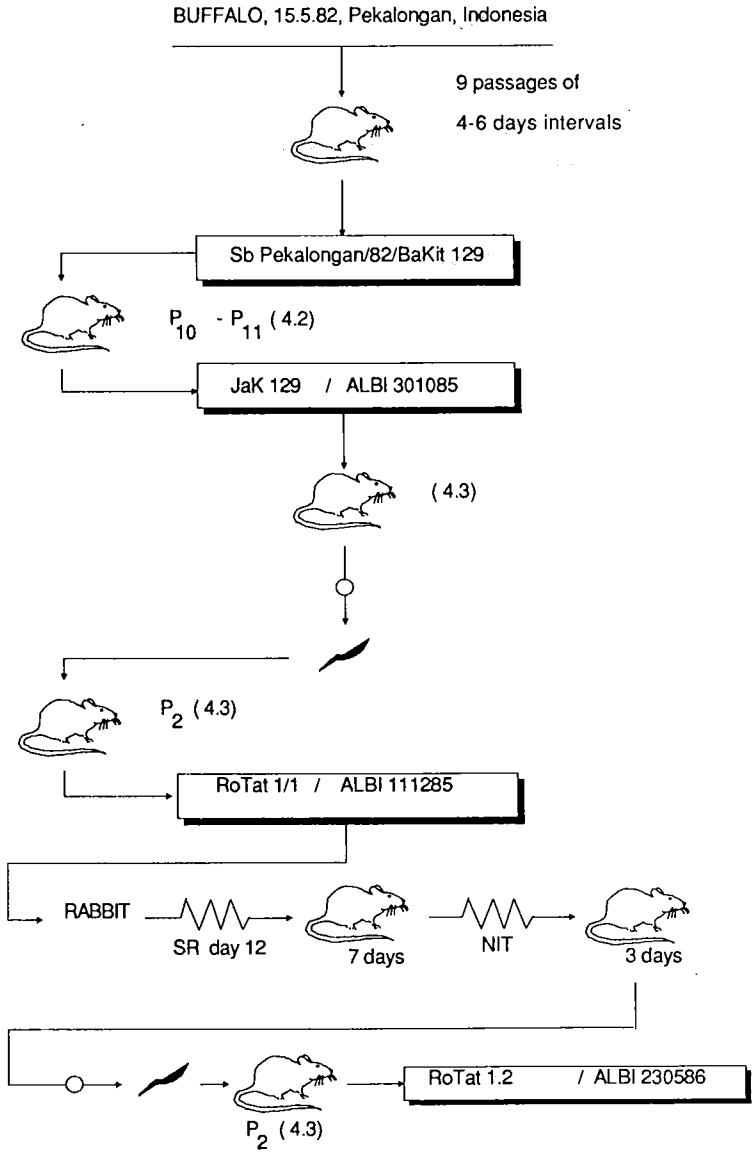

Figure 1.

Pedigree of the VAT RoTat 1.2.

°: Population
ALBI: Algemene Biologie
Sb: Stabilate
P(x,y): Passage of x, y days interval
NIT: neutralization of infectivity test
SR: spontaneous relapse population obtained in the rabbit infected for 12 days
: VAT clone set up in mice

The second group consisted in pooled sera collected on bovine and water-buffalo experimentally infected with a strain of *T. evansi* at RIAD (Research Institute for Animal Disease) in Indonesia. A pooled serum from a buffalo free of tryapanosomes in Italy was used as a control (2).

The third group consisted of sera obtained from 4 infected and 2 non infected camels from Northern region of Kenya.

Another group included 139 samples collected on swine, cattle and buffaloes from different regions of Thailand (2).

In addition documented sera from 7 Africans patients (Daloa) with *T.b. gambiense* infection and control sera from healthy Europeans were tested.

Antigen

The antigen was prepared as described previously by Magnus et al., (2). The clone population used was the VAT RoTat 1/2 (*Rode* Trypanozoon antigen type) derived from a stock of *T. evansi* isolated from a buffalo in 1982 in Pekalongan locality in Indonesia (Fig. 1). The predominant VAT character of RoTat 1/2 was demonstrated in spontaneous relapse populations of different strains of *T. evansi* by trypanolysis and immunofluorescence tests (Bajyana Songa et al., unpublished).

The Testryp® CATT reagents used were obtained from Smith Kline-RIT (Belgium).

Card Agglutination test

The test was performed as described previously (1, 2). Specific antisera to buffalo, camel and human IgG were produced in the rabbit and used as enhancing antibodies.

Results

VAT-specific character of the RoTat 1/2 CATT

Monospecific rabbit sera, each reacting with a single VAT were examined in the CATT test with RoTat 1/2 and the Testryp® antigens. Only sera specific for RoTat 1/2 and an iso VAT RoTat 2/3 gave a high titer in the RoTat 1/2 CATT test (Table 1).

The fact that the RoTat 1/2 antigen barely reacts with the heterologous antisera demonstrates the VAT specific character of the antigen preparation.

TABLE 1

VAT-specific character of the CATT: end-titres of monospecific rabbit antisera against 16 cloned VATs of *T. evansi*

Sera	End-titres (1/n)	
VAT-specificity	RoTat 1/2	Testryp(R)
RoTat 1/1	0	0
1/2*	64	0
1/3	0	0
1/4	0	0
1/5	0	8
1/6	2	0
1/7	0	0
1/8	0	0
2/1	0	0
2/2	2	0
2/3*	64	0
3/1	0	2
4/1	0	0
4/2	0	0
7/1	0	0
8/1	0	0

*: homologous combination; VAT 1/2 = 2/3
0: negative at dilution 1/2.

Sensitivity of the test

The sensitivity of the test was demontrated by the titration of repertoire-specific rabbit antisera (Table 2, 3), pooled sera from bovine and buffalo sera experimentally infected with *T. evansi*, sera from infected camels and from patients with *T.b. gambiense* infection (Table 4, 6, 7). In all sera, the end-titres ranged from 1/5 to 1/40 with Tertryp® and up to 1/256 with RoTat 1/2 antigen. The serum (No 1866) of a European from Montpellier has been found

TABLE 2

End-titres of repertoire-specific rabbit antisera with *T. evansi* or *T. congolense* infection

Trypanosomes	Antisera		Results of the CATT (1/n)	
species	Repertoire	code	Testryp(R)	RoTat 1/2
E. evansi	RoTAR 1	K6135/28	20	80
		K6170/30	10	40
		K6172/29	5	20
		K6173/29	< 10	10
	RoTAR 2	K6127/35	10	20
		K6148/42	20	80
	RoTAR 3	K6129/35	10	40
		K6143/20	10	20
	RoTAR 4	K6124/30	< 5	5-10
		K6134/35	10	40
		K6144/20	10	20
		K6154/29	< 10	10
		K6171/29	< 10	10
T. congolese				
	AnNAR 2	K549/31	5	40
	AnNAR 3	K598/35	5	40
		K599/29	5-10	80
	AnNAR 4	K700/34	-	-
	AnNAR 5	K550/34	5	20
	AnNAR 6	K597/35	5	20

TABLE 3

Results of the CATT test with repertoire-specific rabbit antisera against 3 repertoires of *T.b. brucei*

Antisera		Results of the CATT test (dil.: 1/10)	
Repertoire	code	Testryp(R)	RoTat 1/2
AnTAR 1	K263/30	+	+
	K265/30	(+/−)	(+/−)
	K242/30	++	++
	K346/30	(+/−)	(+/−)
AnTAR 2	K272/30	+/++	++
	K558/29	++	++
AnTAR 5	K507/30	(+/−)/+	(+/−)/+
	K534/29	+	+

(+/−): weakly positive
\+ : positive
++ : strongly positive

TABLE 4

CATT titres of pooled sera collected on cattle and water-buffalo experimentally infected with *T. evansi*

Origin of sera		End-titres (1/n)			
		Testryp(R)		RoTat 1/2	
		a	b	a	b
bovine	uninfected	2	2	2	2
	infected	40	1024	128	1024
buffalo	uninfected (Indonesia)	4	4	4	4
	(Italy)	2	2	2	2
	infected	20	512	256	1024

a: without enhancing antibodies.
b: with an additional rabbit ant-buffalo IgG.

TABLE 5

Results of the CATT test with 139 sera collected on swine, cattle and buffaloes from different regions of Thailand

Sera			Results of the CATT			
Province	animal	No	A+B+	A−B−	A+B−	A−B+
Lampang	swine	33	17	13	2	1
	cattle	31	16	6	8	1
Mahasara-kam	cattle	23	0	18	3	2
Udorn	cattle	5	3	1	0	1
	buffalo	23	20	1	2	0
Khon Kaen	buffalo	24	23	1	0	0
Total		139	79	40	15	5
%		100	56,8	28,8	10,8	3,6

A: antigen RoTat 1/2 for all sera diluted 1/10
B: antigen Testryp(R) for swine-sera diluted 1/5 and anti-buffalo IgG added at the dilution 1/10 and 1/80.

to be positive in the CATT test. The high titer of antibodies indicates a probable contact with the trypanosomes.

With camel and buffalo sera, the CATT titres were consistently higher with RoTat 1/2 than with Testryp® antigen. The addition of enhancing anti-IgG, raised the titres of infected sera by one to two orders of magnitude.

The CATT test with repertoire-specific rabbit antisera against 3 repertoires of *T.b. brucei* gave a comparable result at the dilution 1/10 by using both antigens (Table 3).

TABLE 6

CATT titres of sera collected in Kenya on camels with *T. evansi* infection

sera		End titres (1/n)			
locality	code	RoTat 1/2		Testtry(R)	
		a	b	a	b
Ngare Ndare	87	0	0	0	0
	92	0	0	0	0
Galana	50	32	512	2	16
	83	16	128	2	8
	173	128	1024	16	512
Ngurunit	509	16	128	4	32

a: without enhancing antibodies
b: with an additional rabbit anti-camel IgG
0: negative at dilution 1/2

TABLE 7

End-titre ranges of sera collected on 7 Africans with *T.b. gambiense* infection and from 12 Europeans by using Testryp® antigen or RoTat 1/2

Origin	code	End-titres (1/n)			
		Testryp(R)		RoTat 1/2	
		a	b	a	b
Montpellier	4048	0	0	0	0
	4046	0	0	0	0
	4317	0	0	0	0
	3392	0	0	0	0
	3429	0	0	0	0
	2714	0	0	0	0
	9145	0	0	0	0
	8773	0	0	0	0
	1866	32	1024	32	1024
Antwerpen	NHS*	0	0	0	0
Tournai	A/EB	0	0	0	0
Daloa	1003/1	8	512	8	512
	1004/1	8	1024	16	512
	1006/1	16	512	32	1024
	1007/1	16	1024	64	1024
	1014/1	16	1024	64	1024
	1015/1	16	1024	64	1024
	1017/1	32	1024	128	1024

a: without enhancing antibodies
b: with anti-human IgG
0: negative at dilution 1/2
NHS: Normal human serum

The results of CATT with the samples collected on swine, cattle and buffaloes in Thailand are summarized in table 5. A non negligible discrepancy in the detection of infection, however, seems to be apparent between the two antigens. 10.8% of CATT positive with RoTat 1/2 were negative with Testryp® antigen and 3.6% of Testryp® CATT positive were negative with RoTat 1/2. The cloned VAT RoTat 1/2 is probably more ubiquitous in *T. evansi* repertoires than LiTat 1/3 used in Testryp® CATT.

In all cases, no changes of results were observed after 6 months of storage at $-80\,^{\circ}C$ of RoTat 1/2 antigen.

Discussion

The CATT test, being based on the variable antigen type of trypanosomes is very specific and does not suffer from interference by other infections. However, its usefulness is limited by a relative lack of sensitivity attributable to the presence of other repertoires lacking the early VAT of the CATT test. Some infections will remain undetectable also if the VAT type used in the test is only expressed late in infection or is not very immunogenic in the species considered. At present, the Testryp® CATT seems satisfactory for *T.b. gambiense* in human but only seems to detect *T.b. rhodesiense* in some foci and not in others (Van Meirvenne, personnal communication). The Testryp® CATT test will also detect *T. congolense* infections in swine and *T. evansi* infection in swine, water-buffalo, cattle and camels. In the three latter species, however, the test is insufficiently sensitive to be used as a field diagnostic unless enhancing anti IgG antibodies are added to the reagents (2).

In the present work, the results show that a more sensitive CATT test can be developed based on another early expressed VAT of *T. evansi*. This results in 2 to 16 fold increase in the sensitivity and allows the CATT test to be used in the field without further addition of enhancing antibodies.

The replacement of fixed trypanosomes by an antigen produced by recombinant DNA technology might offer some advantages in terms of stability of reagent and batch to batch reproducibility.

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Un test d'agglutination sur carte (CATT) en usage vétérinaire basé sur un VAT RoTat 1/2 de *Trypanosoma evansi*

Résumé — Un antigène variable RoTat 1/2 de *T. evansi* a été évalué en test CATT en comparaison avec l'antigène Testryp® CATT. Le test pratiqué sur des sérums d'animaux infectés par *T. evansi* s'est révélé beaucoup plus sensible avec l'antigène RoTat 1/2 qu'avec l'antigène original du Testryp® CATT.

Een kaartagglutinatie test (CATT) voor veterinair gebruik gebaseerd op een VAT RoTat 1/2 van *Trypanosoma evansi.*

Samenvatting — Een variabel antigeen type RoTat 1/2 van *T. evansi* werd vergeleken in de CATT test met de Testryp® CATT die gebruikt wordt voor menselijke *T.b. gambiense* infecties. Hieruit bleek dat een gevoelige CATT test ontwikkeld kan worden in de serodiagnose van dierlijke trypanosomiasis met RoTat 1/2 van *T. evansi* als antigen.

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