

THE USE OF THE CARD AGGLUTINATION TEST (TESTRYP® CATT)
FOR THE DETECTION OF *T. EVANSI* INFECTION:
A COMPARISON
WITH OTHER TRYPANOSOMIASIS DIAGNOSTIC TESTS
UNDER FIELD CONDITIONS IN THAILAND

by

E. BAJYANA SONGA¹, C. HAMERS-CASTERMAN¹, R. HAMERS¹, M. PHOLPARK²,
S. PHOLPARK², K. LEIDL², S. TANGCHAITRONG³, I. CHAICHANOPOONPOL³,
C. VITOORAKOOL³ & T. THIRAPATSAKUM⁴

¹Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie,
Paardenstraat 65, B-1640 St.-Genesius-Rode, Belgium

²North Eastern Regional Veterinary Laboratory, Tha Pra, Khon Kaen, Thailand

³Northern Veterinary Research and Diagnostic Center, Hang-Chat, Lampang, Thailand

⁴Chulalongkorn University Large Animal Hospital, Nakhon Pathom, Thailand

Summary — Card agglutination (CATT), complement fixation (CF) and indirect haemagglutination (IHA) tests were used in the diagnosis of animal trypanosomiasis due to *T. evansi* in Thailand. In swine, cattle and sheep, the CATT test could be used without modification whereas in water buffalo, it was necessary to add anti immunoglobulin to avoid false positive reactions.

Comparable results were obtained in water buffalo with the CF test and with the CATT test. In contrast, in swine, a low correlation was found between the IHA and the CATT test. The proportion of seropositive animals detected by the CATT test is higher in imported than in native stock.

The incidence of brucellosis is very low but is higher in trypanosome-infected animals than in healthy animals.

KEYWORDS: *Trypanosoma evansi*; Animal Trypanosomiasis, Serodiagnosis; Card Agglutination Test; Thailand.

Introduction

Recently, the use of Testryp® CATT developed for the diagnosis of human sleeping sickness (8, 11) has been extended to the detection of *T. congolense* infections in pigs in République Populaire du Congo (13) and *T. evansi* in camels in Kenya (18). Our preliminary studies with different strains of *T. evansi* coming from different geographic regions (Latin America, Sudan, Nigeria, Indonesia) had shown that the experimentally infected animals (buffaloes, dogs, swine, cattle and rabbits) possessed antibodies agglutinating LiTat 1/3 variant used in Testryp® CATT. In goats, dogs and rabbits, these agglutinating antibodies appeared within the first two weeks of infection (3).

These encouraging laboratory results led us to evaluate the CATT test in the field in Thailand where trypanosomiasis due to *T. evansi* had been detected in cattle, buffaloes and swine.

Materials and Methods

Sera

Sera from 392 different animals were collected in different regions of Thailand (table 1). Group 1 consisted of 175 samples collected on swamp buffaloes in the provinces of Khon Kaen, Udorn and Nakhon Pathom. Group 2 were collected from swine in the Lampang and Nakhon Pathom regions. Group 3 were collected from cattle in the Nakhon Pathom, Khon Kaen and Mahasarakam region.

TABLE 1
Regional origin of sera tested

Province	Locality	Species and breed	Code serum	Number of sera
Khon Kaen	Chiang Yuen	buffalo (swamp)	1,683/28	24
	Pon Ngan/ Pon Swang	buffalo (swamp)	2,123/1	12
Mahasarakam	Mahasarakam	cattle (Brahman & dairy)	1,815/28	29
Udorn	Kaset Samboon Nangwaeng Ded	buffalo (swamp)	1,594/28	50
	Kaset Ban Kao Sarn	buffalo (swamp)	1,562/28	25
	Kaset Ban Nong Gae	buffalo (swamp)	1,563/28	5
	Kaset Ban Pran Muyen	buffalo (swamp)	1,564/28	8
	Kasatrakorn ban Rob Ampor Muang	buffalo (swamp)	1,428/28	11
Lampang	Ampor-Muang/ Chiangrai	cattle (American brahman)	149/29	145
	Ampor-Muang	pigs	561/1	56
	Lampang	(Landrace & Duroc)	146/28	11
Nakhom Pathom	Mr. Wichai Farm	pigs (Landrace)		3
		cattle (Brahman)		2
	Large Animal hospital	sheep (Suffolk)		2
	Chulalongkorn University Nakhon Pathom	buffalo (swamp)		1
	Farm Thai Rung Kit	pigs (landrace)		4

Finally group 4 consisted of sera from 2 Suffolk sheep from Nakhon Pathom.

Antisera

Specific antiserum to buffalo IgG was obtained by immunization of rabbit by IgG isolated from pooled normal buffalo serum following described method (6). The specificity was tested by immunodiffusion.

For pigs, a commercial anti-porcine IgG (Bio-Yeda, lot N R 771, code 1168, Israël) produced in rabbit was used.

Card agglutination test (CATT)

The serum samples were analysed on plastic cards with the reagents used for detection of human *T.b. gambiense* infection (Testryp® CATT, Smith Kline-RIT Belgium) using the manufacturers' instructions without modification in the case of swinesera. In a field assay, the test was also performed on undiluted heparinized swineblood. For cattle and sheep the test was performed at a serum dilution of 1/10 in the CATT agglutination buffer instead of one in five.

For assaying the waterbuffalo sera, rabbit antibuffalo IgG sera were always mixed at a dilution of 1/10 with the antigen and the sera of the buffalo were diluted at 1/80 and 1/10.

The reactions were scored as + + + (very strongly positive), + + (strongly positive), + (positive), ± (weakly positive) and - (negative).

The titer of serum is defined by the last positive dilution. The addition of anti IgG sera raises the agglutination titer by as much as two orders of magnitude for positive sera and allows a more accurate titration. This procedure was used to evaluate the titer in positive water buffalo and swine sera.

The test used was significant at the following serum dilutions with or without the use of enhancing anti IgG.

Water buffalo	> 1/4
Cattle	1/2
Swine	1/2

The use of enhancing antibodies, however, induces a prozone phenomenon and hence are not reliable at low serum dilutions.

Complement Fixation test (CFT) for trypanosomiases

The CF test was done as described by Staak and Lohding (15). Trypanosomes from an uncloned strain of *T. evansi* isolated in Khon Kaen were used as antigen after ultrasonic treatment. A titre of 1/5 more of serially diluted sera was regarded as positive.

Indirect Hemagglutination test (IHA) for trypanosomiases

Soluble antigen was prepared from an uncloned strain of trypanosomes isolated in Khon Kaen using the technique of Pholpak and Kornkovit (14). The test was performed on sheep red blood cells sensitized with *T. evansi* antigen after glutaraldehyde treatment (1).

Complement Fixation test for brucellosis

The test was performed as described by Herbert (7). *Brucella abortus* antigen was obtained from the department of Livestock Development, Bangkok, Thailand.

Parasitological examination

The parasitological tests performed to detect active infection were: microscopical observation of fresh heparinized blood, of giemsa stained smears or buffy coat, and subinoculation into mice.

Results

1. The serological findings in buffaloes

A first series of observations were made on sera coming from the North Eastern region where a high abortion rate seemed correlated with trypanosome infection (10). A sample of sera from 24 buffaloes found to be positive for trypanosomiasis by the complement fixation test were examined by the CATT test. 23 out of the 24 sera were also positive in the CATT test (table 2). The twenty animals which had shown a detectable parasitaemia were positive both for the CATT test and the CFT test. An additional sample of sera coming from the Khon Kaen and Udon provinces from 99 randomly selected animals were assayed with both the CATT and CFT. The results are summarized in figure 1B.

TABLE 2
Catt titres and parasitaemia in Swamp Buffalo showing positive CFT

Sera	T. evansi	Parasitaemia T. theileri	microfilaria	Serological titres	
				CATT	CFT
1683/28/ 1	+	—	—	320	40
2	+++	—	—	160	40
3	++	—	+	320	80
4	++	++	—	320	160
5	+	+	—	640	80
6	++	++	—	> 640	160
7	?	+?	+	640	80
8	+	—	—	320	80
9	+++	—	—	640	80
10	+++	—	—	320	80
11	+	—	—	320	160
12	++	—	+	160	160
13	—	—	+	—	120
14	+	—	+	320	20
15	+	+	—	320	20
16	+	+	+	640	80
17	—	—	—	640	160
18	++	—	—	80	80
19	+++	—	—	320	20
20	+	—	—	10	80
21	+++	—	—	320	5
22	+++	—	—	320	20
23	—	—	—	640	20
24	+++	—	—	320	20

On these two sets of data the following observation can be made:

- both tests seem equally efficient in detecting trypanosome infection;
- low but not negligible discrepancy in the detection of infection is apparent in the second set of experiments.

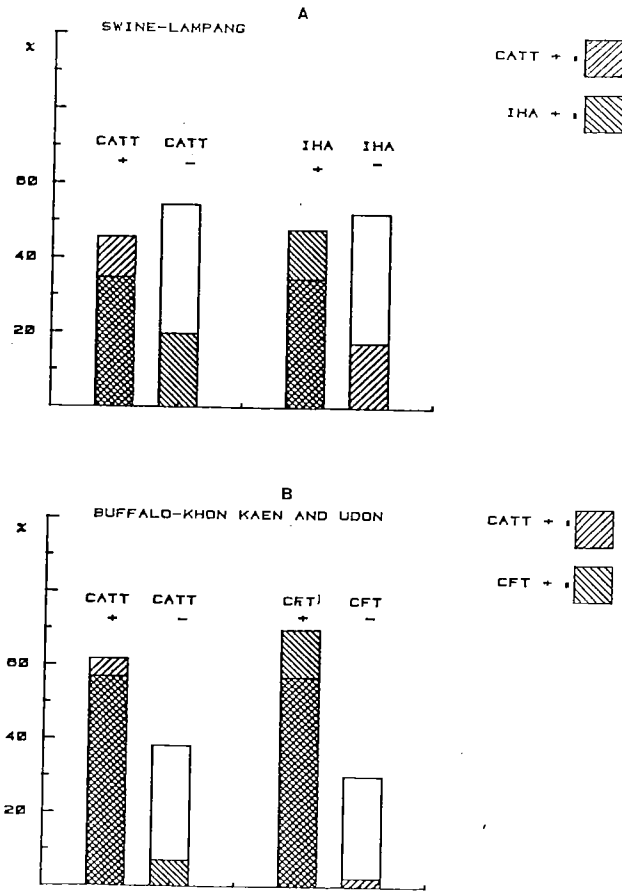


Figure 1.

A. Correlation between positive reactions in the CATT test and in the IHA assay in swine sera.
B. Correlation between positive reactions in the CATT test and in the CF assay in buffalo sera.

7.9% of the CATT⁺ were CFT⁻, whereas 18.6% CFT⁺ were CATT⁻. This seemingly large proportion of CATT⁻ CFT⁺ sera is mainly due to low titer (< 1/10) CFT. Notwithstanding the general conclusion that a very good correlation is found between a positive CATT test and a positive CFT test, no useful correlation seems to exist between the actual CATT titres and the CFT titres (Fig. 2). This is apparently due to the fact that infection in different animals gives rise to antibodies of different classes and in different amounts. The CATT test detects essentially antibodies against early variable surface

glycoprotein whereas in the CFT, the common antigens play an important role.

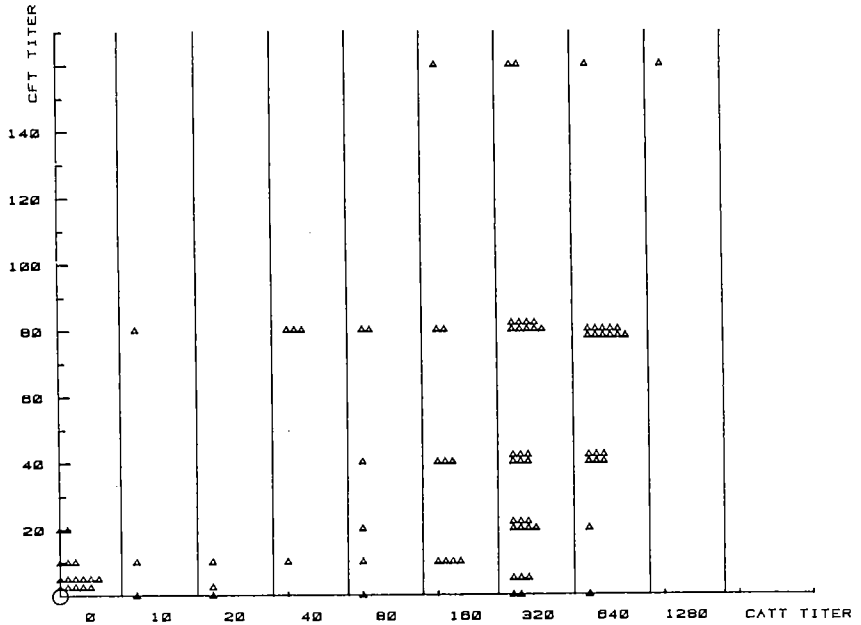


Figure 2.

Correlation between CATT titres and CFT titres in buffalo sera. The CATT titres were obtained by using the corresponding enhancing anti Ig reagent as described under methods.

A third series of sera included 23 sera collected in various villages in the Khon Kaen region and one serum collected in Nakom Pathom during the dry season. Only the serum from Nakom Pathom and one serum from Pon Ngam were positive. This might correlate with the low incidence of the disease during the dry season.

II. The serological findings in swine

The specific character of the test was shown using a series of serum collected from experimentally infected animals (17). Pigs were experimentally infected with a *T. evansi* strain isolated from buffalo. The results are shown in table 3. As can be seen, no animal presented a positive reaction at the time of infection. The infected animals showed a positive reaction at the dilution of 1/5 after 3 months. A second series of observations were carried out in 46 samples collected on a farm which had a history of abortions in the Lampang region. These sera had already been screened using an indirect haemagglutination assay (IHA). From the CATT and IHA tests the following observations can be made (Fig. 1A & 3).

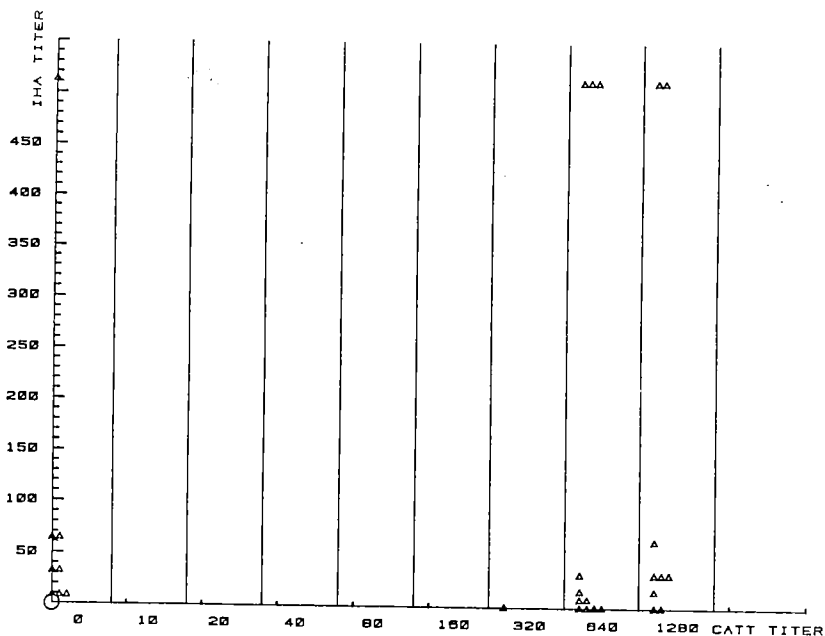


Figure 3.

Correlation between CATT titres and IHA titres in swine sera.

The CATT titres were obtained by using the corresponding enhancing anti Ig reagent as described under methods.

TABLE 3
CATT test results in experimentally infected swine of European origin

Animal number	Days post infection	CATT test
3,870	0	—
	101	++
	116	+/+++
4,253	0	—
	116	++
4,506	0	—
	74	—
	101	++/++++
	116	++

- Both tests detect a high proportion of positive animals (45.6% for the CATT and 47.8% for the IHA, fig. 1A).
- However one third of the sera are positive with one test and negative with the other.
- The analysis of the titer (using enhancing anti swine antibodies) reveal three distinct patterns of reaction: high IHA/high CATT, low IHA/low CATT and/or negative, low IHA/high CATT. No intermediate titres are found and a single serum showed a high IHA/negative CATT.

From the results of this study, it can be concluded that both tests must be considered as complementary. In the Nakhom Pathom area, the CATT used alone, allowed the serological detection of trypanosomiasis in two farms where an abortion problem had been encountered and where trypanosomes had been isolated from infected animals (Table 4).

TABLE 4
CATT titres in swine (without enhancing anti IgG) from two farms with abortion problems

Locality	Animal numbers	Sex	1:2	1:4	CATT 1:8	1:16	1:32
Mr. Wichai farm	1	M	—	—	—	—	—
	2	M	—	—	—	—	—
	3	F	+++	++	±	—	—
Thai Rung	4	M	++	++	—	—	—
	5	M	+++	+/++	+	±	—
	6	M	—	—	—	—	—
	7	M	++	+	—	—	—

TABLE 5
Brucellosis negative sera in CATT positive and CATT negative cattle sera

	Brucellosis +	Brucellosis —
CATT + 37	8	29
± 5	0	2
- 103	2	101

The CATT was also performed on a small sample of swine under condition known to be useful in diagnosis of human sleeping sickness: the agglutination test is performed directly on heparinized blood and can be read ten minutes after the animal has been bled. Table 6 shows that this test which can be performed on the farm is efficient if tested on serum and will detect strongly agglutinating samples quite readily.

TABLE 6
Comparison of the CATT test performed on whole heparinized swine blood under farm conditions and on serum diluted 1/5 under laboratory conditions

Animal #	breed	Sex	CATT test	
			Heparinized blood	Serum diluted 1/5
388	Landrace	M	++	++
138	Landrace	M	±/+	++
3	Duroc	M	—	—
4	Landrace	F	++	++
5	Landrace	F	+++	+++
6	Landrace	F	—	—
7	Landrace	M	±/+	+/++
8	Landrace	F	—	—
9	Landrace	M	—	—
10	Duroc	M	—	—

III. Serological findings in cattle

A first series of observations were performed in the region of Khon Kaen and Mahasarakam on a small sample of 29 animals (15 native Brahman and 14 dairy cows). Only one out of the native Brahman breed and 2 out of the dairy cows were infected. This supports the previous observation that the incidence of the disease in this region is lower in native cattle than in water buffalo. In native cattle the disease however can still be quite dramatic.

Two cases of cattle trypanosomiasis were examined in Nakom Pathom. One was a gestating cow (8 months) in a very characteristic «decubitus» position and which was found dead the day after we examined it. This cow which displayed the classical symptoms of clinical trypanosomes and for the CATT test. The one year-old calf which was kept in the same enclosure as the cow was positive for the CATT test without however showing a parasitaemia after either blood subinoculation or microscopical observation.

A survey was also undertaken of a herd of American Brahman cattle in the Chiang Rai (Lampang) region. These animals were being monitored for different parasites within a project aiming at establishing cattle of high economic value in the region. The CATT test performed on these animals detected 25% which were positive (Fig. 4), indicating a higher susceptibility of these imported breeds to the disease: an observation well known for other regions where trypanosomiasis is endemic. Moreover out of 145 animals, ten only were positive for brucellosis but of these, 8 were found within the CATT positive group and only 2 among the CATT negative group (Table 6).

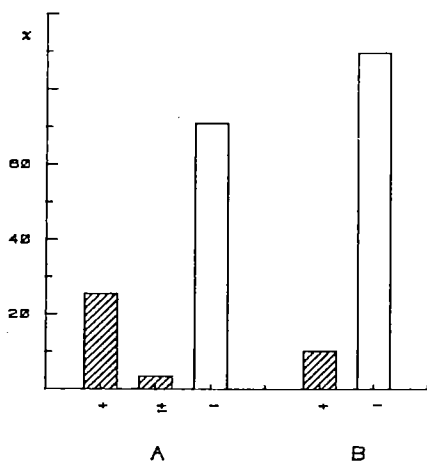


Figure 4.

Distribution of CATT test positive cattle in a sample from Northern Thailand Lampang (A) and North East Thailand (Khon Kaen and Mahasarakam) (B).

IV. Serological findings in sheep

Two Suffolk sheep, imported from Scotland less than two months ago were brought to the Chulalongkorn Large Animal Hospital and were found

to be positive for the CATT test. No differential diagnosis could be made for the neurological symptoms these animals exhibited. One animal with abundant nasal discharge showed paralysis of the hind limbs, cervical adenopathies and a tendency to circle, the other animals was very thin and showed paralysis of the fore and hind limbs. These symptoms are suggestive of Louping illness or of trypanosomiasis with CNS involvement. Active trypanosomiasis was, however, not confirmed by subinoculation.

Discussion and conclusions

In the preliminary studies prior to initiating the field test, it has been found that non infected control buffalo sera (from Italy) contained an agglutinating factor which at a serum dilution of 1/4 agglutinated the stained trypanosomes of the CATT. This difficulty could be overcome by adding anti-buffalo IgG to the test. This does not affect the negative control sera but raises the titer of infected sera by one to two orders of magnitude. Under these conditions, however, the reading can be somewhat more difficult apparently because of smaller aggregate size.

In water buffalo, the correlation between the complement fixation test and the CATT test and parasitaemia was very satisfactory. 23 out of 24 CFT positive animals were CATT positive and the 20 animals positive in parasitaemia were all simultaneously CFT and CATT positive. Little correlation, however, is found in the actual titres but this is probably a result of the fact that the CATT test is essentially an assay for antibodies against an early appearing variant whereas in the complement fixation test the common antigens play an important role.

In swine, it was shown that the experimentally infected animals were found to become positive in the CATT test. However, the correlation between the CATT test and an indirect haemagglutination test was rather poor: approximately one third of animals which were positive in one test were negative in the other test. Several interpretations for this observation can be put forward. First of all, the difference is due to the antigen. The indirect haemagglutination test is based upon those antigens which can be easily coupled to red cells, and includes the common antigens of trypanosomes. Pending further investigations both tests must be considered as complementary. The CATT test detected a number of positive cases including the breeding boars which apparently, the test performed satisfactorily on whole blood and the feasibility of doing the test on site was demonstrated. The on site testing is extremely useful, as treatment can be immediately initiated following positive diagnosis, especially when long distances and bad roads separate the farms from the diagnostic services.

In cattle, within the North East region, the incidence of trypanosomiasis is much lower than in water buffaloes, 2.7% against 20.0% (9), and the CATT test correlates quite well with the CF test. Most of the cattle in this region are trypanotolerant native breeds. These animals, however, can also develop a characteristic disease and mention must be made here of a pregnant cow seen in Nakhon Pathom in the terminal stage of trypanosomiasis. A completely different picture was found in imported cattle. In the North, a herd mainly

of American Brahman stock showed an incidence of 30% positive by the CATT test. An interesting finding in these cattle has been that although the incidence of brucellosis positive animals is quite low (10/140), they are found mainly (8/10) among the trypanosome positive animals. This would suggest that as has been found in *T. brucei* infection in mice and cattle, the infection leads to a state of immune suppression in which the animals are more susceptible to disease, opportunistic or other. Recently using the Testryp® CATT for the detection of *T. congolense* in pigs in République Populaire du Congo (13) and *T. evansi* in camels in Kenya (18) the sensitivity reported for human trypanosomiasis itself was not found. This relative lack of sensitivity in contrast with our own results from Thailand might be attributable to the presence of other repertoires lacking the LiTat 1/3 antigen of the CATT test. A recent report (2) from Cameroun discusses the lack of reliability of the CATT test. Further studies are needed on the repertoires of *T. evansi* to find out whether the VAT type LiTat 1/3 at present used in the test (12, 16) is ubiquitous or whether some parasites which lack the gene exist and will escape detection.

In conclusion, the Testryp® CATT developed for human trypanosomiasis is a useful assay for identifying *T. evansi* infection in cattle and pigs and with the appropriate modification in water buffaloes.

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Le test d'agglutination sur carte (Testryp® CATT) dans la détection de l'infection à *T. evansi*: comparaison avec d'autres tests pour le diagnostic de la trypanosomiase pratiqués sur le terrain en Thaïlande.

Résumé — Les tests immunologiques d'agglutination sur carte (CATT), de fixation du complément (CF) et d'hémagglutination indirecte (IHA) ont été utilisés dans le diagnostic de la trypanosomiase animale à *T. evansi* en Thaïlande. Chez le porc, les bovins et les ovins, le test CATT peut se pratiquer sans modification. Chez le buffle, le test doit être modifié par addition d'anti-immunoglobuline afin d'éviter de fausses réactions positives.

Des résultats comparables ont été obtenus avec le test CATT et le test CF chez le buffle. Une faible corrélation entre le test CATT et IHA a été observée par contre chez le porc. Avec le test CATT, comparativement au bétail local, la proportion de bovins séropositifs était plus élevée chez les animaux importés. L'incidence de la brucellose, quoique très faible en général, s'est avérée plus élevée chez le bétail trypanosé comparativement aux animaux sains.

De kaartagglutinatietest (Testryp® CATT) voor opsporing van *T. evansi* infecties: vergelijking met andere diagnostische tests voor trypanosomiasis uitgevoerd op het terrein in Thailand.

Samenvatting — Kaartagglutinatietest (CATT), complement fixatie (CF) en indirecte haemagglutinatie (IHA) tests werden gebruikt in Thailand voor de diagnose van dierlijke trypanosomiasis te wijten aan *T. evansi*. De CATT test voor trypanosomiasis kan gebruikt worden zonder wijzigingen voor varkens, runderen en schapen. Voor waterbuffels is het noodzakelijk de test te wijzigen door toevoeging van een anti-Ig reagens teneinde vals positieve reacties te vermijden.

In waterbuffels werden gelijkaardige resultaten bekomen met de CF test en de CATT test. In varkens daarentegen was de correlatie tussen de IHA test en de CATT test minder goed. Aan de hand van de CATT test werden er meer seropositieve dieren gevonden in ingevoerde dieren dan in inheems vee. Brucellose komt heel weinig voor maar schijnt aanzienlijk frequenter te zijn, bij trypanosoom-geïnfecteerde dieren.

REFERENCES

1. Anon: A procedural guide to performance of the serology of toxoplasmosis (Revised ed.). Center for Disease Control, Atlanta, Georgia, USA, 1976.
2. Asonganyi T: Performance of Card Agglutination test for Trypanosomiasis (C.A.T.T.): trends and perspectives. Ann. Univ. Sci. Santé, 1985, 2, (1), 81-86.
3. Bajyana Songa E, Kageruka P, Hamers R: The use of the card agglutination Test (Testryp® CATT) for the serodiagnosis of *T. evansi* infection. Ann. Soc. belge. Méd. Trop., 1987, 67, 51-57.
4. Boid R: Isoenzyme analysis of stocks of trypanosomes isolated from cattle in Indonesia. Research in Veterinary Science, 1985, 39, 388-389.
5. Chang LC, Lee WH, Ma CH: Preliminary Study on the first occurrence of Surra in Taiwan pigs. Vet. Med. Reviews 1976, 1, 112-114.
6. Goding JW: Conjugation of antibodies with fluorochromes: modifications to the standard methods. Journ. Imm. Meth., 1976, 13, 215-226.
7. Herbert WJ: Veterinary Immunology; Chapter 17, p. 177, University of Glasgow, Glasgow, U.K., 1977.
8. Liveyns R, Crooy P: Historique du développement et caractéristiques d'un test de dépistage de la maladie du sommeil. Proceedings of Symposium in diagnosis of African sleeping sickness due to *T. gambiense*. Antwerp 16-17 nov. 1983, pp. 51.
9. Löhr KF, Pholpark S, Srikitjakarn L, Thaboran P, Betterman G, Staak C: *T. Evansi* infection in buffaloes in North-East Thailand. I. Field investigation, Trop. Anim. Hlth Prod., 1985, 17, 121-125.
10. Löhr KF, Pholpark S, Siriwan P, Loesirikul N, Srikitjakarn L, Staak C: *T. evansi* infection in Buffaloes in North-East Thailand. II. Abortions Trop. Med. Animal. Hlth Prod., 1986, 18, 103-108.
11. Magnus E, Vervoort T, Van Meirvenne N: A card Agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of T.b. gambiense trypanosomiasis. Ann. Soc. Belge Méd. Trop., 1978, 58, 169-176.
12. Magnus E, Le Ray D, Van Meirvenne N: Variable antigen repertoires of *Trypanosoma (T.) brucei*. Proceedings of Symposium on diagnosis of African sleeping sickness due to *T. gambiense*. Antwerp, 16-17 nov. 83, p. 39-44.
13. Noireau F, Gouteux JP, Frézil JL: Sensibilité du Test d'Agglutination sur carte (Testryp) dans les infections porcines à *Trypanosoma (N.) congolense* en République Populaire du Congo. Ann. Soc. Belge Méd. Trop, 1986, 66, 63-68.
14. Pholpak S, Kornkovit M: Trypanosome Antigen production. Proceedings of the 9th Annual Veterinary conference of the Thai Veterinary Medicine Association, Bangkok, December 1982, p. 229-236.
15. Staak C, Lohding A: The complement Fixation test and African Trypanosomiasis: I. Experimental Infection and re-infection on cattle before and after treatment. Tropenmedizin und Parasitology, 1979, 30, 13-18.
16. Vervoort T, Magnus E, Van Meirvenne N: Serological tests for sleeping sickness: importance of antigen selection. Proceedings of Symposium on diagnosis of African sleeping sickness due to *T. gambiense*. Antwerp, 16-17 nov. 1983, p. 47-50.
17. Vitoorakool C, Udompan S, Pornsuksawang W, Singhasanti N: Study on the trypanosome antibody in the infected swine. Paper presented to the 12th Annual Veterinary Medicine association. Bangkok, Dec. 1985.
18. Zweygarth E, Sabwa C, Röttcher D: Serodiagnosis of trypanosomiasis in dromadary camels using a card agglutination test set (Testryp. CATT). Ann. Soc. Belge Méd. Trop., 1984, 64, 309-313.