J. Protozool. Res., 8, 190-193 (1998)
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Performance of Ecological Tests for *Trypanosoma evansi* Infections in Camels from Niger

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Received 20 August 1998

1. INTRODUCTION

Surra is a widespread infectious disease responsible for important economical losses. The causing agent is the protozoan parasite *Trypanosoma* (*T.*) *evansi*which is mechanically transmitted by different vectors such as flies (*Tabanidae, Stomoxys,* ....) and vampire bats (*Desmodus rotondus*) (Hoare, 1972).

The parasite can infect a variety of hosts and causes a species-specific pathology. Camels and horses are very sensitive to *T. evansi*and death can occur within three months. In water buffaloes and cattle, the infection rather seems to induce immunosuppression which may be the cause of vaccination failure (e.g. rinderpest, pasteurellosis) and increased susceptibility to other diseases (e.g. clostridial infections, anthrax, haemorrhagic septicemia etc.) (Stephen, 1986).

The diagnosis of surra can be achieved in different ways. Trypanosome detection tests lack sufficient sensitivity and have to be conducted immediately after sampling.

Serological tests are much more sensitive and can be executed groupwise on samples which can be stored for a long time. Serological tests combined with parasitological examination are useful tools for epidemiological surveys. However the present antibody or antigen detection tests remain to be improved.

In our laboratory (ITMAS), several antibody detection assays, based on the predominant variable antigen type (VAT) RoTat 1.2, have been developed. These include direct and indirect agglutination tests (CATT/*T. evansi*and LATEX/*T. evansi*), which can be conducted under field conditions as well as variant specific immune trypanolysis and ELISA, which need rather sophisticated laboratory equipment and reagents.

In a previous study (Verloo et al. 1997), CATT/*T. evansi*, LATEX/*T. evens*, ELISA/*T. evansi*and immune trypanolysis have been evaluated with sera from rabbits, experimentally infected with *T. evansi* populations originating from The Philippines, Colombia, South-America, China, Kenya, Kazakstan, Indonesia and isolated from different host species (water buffalo, dog, capybara, camel, horse).

Overall sensitivity of all these antibody detection tests was 100% from day 30 on. Pre-infection sera yielded negative results. Only direct agglutination CATTy/*T. evansi*has been evaluated in the field (Drill et al. 1994; Dial et al. 1997).

In the present study CATT/*T. evansi*, LATEX/*T. evansi*and immune trypanolysis tests were performed on a random series of dromedary camel serum samples from 24 parasitologically confirmed and 76 putatively non-infected animals originating from Niger.

2. MATERIALS AND METHODS

2.1. Field sampling:

Samples originate from camels in the Tahoua district in Niger. Parasitological examination by blood smears and mini hematocrit centrifugation technique (Woo 1969),

followed by serum sampling and screening with CATT/*T. evansi* at 1/4 dilution were conducted in the field. Sera were kept frozen until expedition on dry ice at ITMAS where further storage was at -70℃. Twenty four parasitologically positive and 76 parasitologically negative animals (single examination) were randomly selected without taking into account the CATT/*T. evansi* results obtained in Niger.

2.2. CATT/*T. evansi*:

The CATT/*T. evansi* was a rapid direct agglutination test which used Coomassie stained, freeze-dried trypanosomes of *T. evansi* variable antigen type (VAT) RoTat 1.2 (Bajyan and Hamer 1988). Twofold serum dilutions in PBS (phosphate buffered saline 0.01M, pH 7.4) were tested. Twenty five μl was mixed with 45 μl of reagent on a reaction zone of the agglutination card. The card was rocked for 5 minutes on a CATT rotator at 70 rpm. A reaction was scored positive when macroscopic agglutination was visible. The end titre is defined as the highest dilution of the test serum still showing a positive result.

2.3. LATEX/*T. evansi*:

LATEX/*T. evansi* is a rapid indirect agglutination test in which the antigen consists of purified variable surface glycoprotein (VEG) (Lejon et al. 1998) of *T. evansi* VAT RoTat 1.2 covalently coupled to latex particles according to Büscher et al. (1991). The reagent is stabilized by lyophylisation and rehydrated with purified H_20 before use. Twenty μl of twofold dilutions in PBS is mixed with 20 μl of reagent on a test card. Further manipulations are the same as for CATT/*T. evansi*.

2.4. Trypanolysis:

Immune trypanolysis was performed according to van Meirvenne et al. (1995) with *T. evansi* VAT RoTat 1.2. The sera were tested at a 1/4 dilution. Live trypanosomes of well defined variable antigen type (in this case RoTat 1.2) were incubated for (60 minutes with test serum and guinea pig serum as the source of complement. If variant specific antibodies were present in the serum, lysed trypanosomes were observed under the phase contrast microscope.

3. RESULTS

3.1. CATT/*T. evansi* and LATEX/*T. evansi*:

The evaluation parameters used were defined as follows (Dukes et al. 1984), where TP=true positive, TN=true negative, FP=false positive and FN=false negative. In this case the number of parasitologically confirmed (TP+FN) cases was 24 and the number of parasitologically non confirmed cases which are considered non infected (TN+FP) was 76. Sensitivity (%)=TP x 100/(TP+FN) and specificity (%)=TNx100/ (TN+FP). Sensitivity and specificity of CATT/*T. evansi* and LATEX/*T. evansi* were calculated in function of serum dilution and results are given in Tables 1and 2.

Table 1: Sensitivity and speciicily the CATT/*T.* evansi at different serum dilution.

	Serum dilutions used in the CATT/*T. evansi*					
	1/1	1/4	1/8	1/16	1/32	*Niger 1/4
Sensitivity (%)	100	92	88	76	28	96
Specificity (%)	49	69	79	84	92	72

*Niger 1 14 = CATT/*T. evansi* screening test conducted in Niger by Robert Tibayrenc at a 1/4 dilution.

Table 2: Sensitivity and specificity of the LATEX/*T. evansi* at different serum dilutions.

	Serum dilutions used in the LATEX/*T. evansi*					
	1/4	1/8	1/16	1/32	1/64	1/128
Sensitivity (%)	96	96	84	68	32	4
Specificity (%)	63	73	76	81	89	98

3.2. Immune trypanolysis:

All 24 sera from parasitologically confirmed camels were positive in immune trypanolysis. Of the 76 supposedly non-infected camels 23 were also tested positive. giving a total of 47 positive and 53 negative cases. Taking the results of the immune trypanolysis as reference, sensitivity and specificity of CATT/*T. evansi* and LATEX/*T. evansi* was then calculated as follows. Sensitivity (%)=TPxIOO/(all immune trypanolysis positives). with "all immune trypanolysis positives"=47. specificity (%)=Tex 100/(all immune trypanolysis negatives) with all "immune trypanolysis negatives"=53. Results in function of serum dilution are summarized in Tables 3 and 4.

Table 3: Sensitivity and specieÅäicily of the CATT/*T. evansi* at different dilution as compared to the results of the immune trypanolysis test.

	Serum dilutions used in the CATT/*T. evansi*					
	1/1	1/4	1/8	1/16	1/32	*Niger 1/4
Sensitivity (%)	100	96	81	66	28	96
Specificity (%)	70	98	100	100	100	100

*Niger l/4 = CATT/*T. evansi* screening test conducted in Niger by Robert Tibayrenc at 1/4 dilution.

Table 4: Sensitivity and specificily of the LATEX/*T. evansi* at different serum dilutions as compared to the results of the immune trypanolysis test.

	Serum dilutions used in the LATEX/*T. evansi*					
	1/4	1/8	1/16	1/32	1/64	1/128
Sensitivity (%)	98	93	83	66	34	4
Speciecficily (%)	89	100	100	100	100	100

4. DISCUSSION

The diagnostic performance of CATT/*T. evansi* test in natural camel infections has been investigated and published by Diall et al. (1994) and Dia et al. (1997). Diall et al. obtained a sensitivity of 89% and a specificity of 73% at a l/5 serum dilution. Dial et al. obtained a sensitivity of 80% and a specificity of 77% at a 1/4 serum dilution. Results for CATT/*T. evansi* and LATEX/*T. evansi* obtained within the present study are similar.

CATT/*T. evansi* results obtained in Niger and at ITMAS on the same batch of serum samples at 1/4 dilution show minor differences. The slightly lower sensitivity and specificity observed at ITMAS can be due to various technical factors.

Positive immune trypanolysis results are indicative for the presence of RoTat 1.2 variant

specific antibodies. Earlier, in rabbits experimentally infected with *T. evansi* originating from different parts of the world (Verloo et al. 1997), we observed that RoTat 1.2 specific antibodies appeared in all rabbits within one month of infection. Both results strengthen the statement of Bajyana and Hamer (1988) that RoTat 1.2 is a wide-spread predominant VAT, i.e. a VAT that appears early in the infection and that is expressed by many if not all *T. evansi* populations.

All serum samples from parasitologically confirmed animals were positive in immune trypanolysis, whereas some were negative in CATT/*T. evansi* and LATEX/*T. evansi* even at a 1/4 dilution. This difference between the immune trypanolysis and agglutination test results suggest that different antibodies are involved.

Twenty three out of 76 (30%) sera from supposedly parasitologically negative animals were positive in immune trypanolysis. At 1/8 dilutions both agglutination assays were 100% specific as compared to the results of the immune trypanolysis tests. The lower specificity of the agglutination tests with reference to the parasitological results can be explained by the low sensitivity of a single parasitological examination and/or by persistence of trypanolytic antibodies still circulating in cured host. An other explanation might be the presence of specific trypanolytic activity in some camel sera. This remains to be checked with other VATs. At (1/8 dilutions the agglutination assays give some false positive results when compared to immune trypanolysis. Cryptic VSG epitome that become exposed on the surface in CATT/*T. evansi* and LATEX/*T. evansi* reagents or even non VSG antigens which are not exposed at the cell surface of living trypanosomes can be responsible for this.

Both CATT/*T. evansi* and LATEX/*T. evansi* tests are currently being evaluated in experimental and field infections in different countries and with different animal species.

5. ACKNOWLEDGEMENTS

This investigation received financial support from ILRI, Nairobi .

6. REFERENCES

Bajyana , S.E. & Hamer, R. 1988. *Ann. Soc. Belg. Med. Trop. 68: 233.*

Büscher, P., Draelants, E., Magnus, E., Vervoort, T. & Van Meirvenne, N. 1991. *Ann. Soc. Belg. Med. Trop. 71: 267.*

Dial, M.L., Van Meirvenne, N., Magnus, E., Lackins, A.G., Diop, C., Thiam, A., Jacquiet, P. & Hamer, R. 1997. *Revue Elve. Med. Vet. Pays trop 50: 29.*

Diall, O., Banyana, S.E., Magnus, E., Kouyate, B.. Dialog, B., Van Meirvenne, N. & Hamer, R. 1994. *Rev. sci. tech. Off. int. Epiz, 13: 793.*

Dukes, P., Rickman, L.R., Killick-Kendrick, R., Kakoma, I., Wurapa, F.K., De Raadt, P. & Morrow, R. 1984. *Tropenmed. Parasite. 35: 141.*

Hoare, C.A. 1972. The trypanosomes of mammals. *Oxford: Blackwell Sci. Publications 1 - 749.*

Lejon, V., Büsheer, P., Magnus, E., Moons, A., Outers, I. & Van Meirvenne, N. 1998. *Acta Trop. 69: 151.*

Stephen, L.E. 1986. Trypanosomiasis. *A veterinary perspective. Oxford 1-539.*

Van Meirvenne, N., Magnus, E. & Büsheer, P. 1995. *Acta Trop. 60: 189.*

Verloo, D., Van Meirvenne, N., Lejon, V., Magnus, E. & Büscher, P. 1997. *Abstracts 24th Meet. of the Intl. Sci. Council for Trypanosomosis Research and Control, Maputo, Mozambique 74.*

Woo, P.T.K. 1969. *Can. J. Zoom. 47: 921.*