Comp. Immun. Microbiol. infect. Dis. Vol. 4, Nos. 3/4, pp. 243–246, 1981.
Printed in Great Britain.

0147-9571/81/030243-04$02.00/0

ORIGINAL EXPERIMENTAL WORKS

COMPARISON OF VARIOUS TESTS FOR THE SEROLOGICAL DIAGNOSIS OF *TRYPANOSOMA EQUIPERDUM* INFECTION IN THE HORSE*

V. P. CAPORALE*, F. BIANCIFIORI‡, F. FRESCURA‡, A. DI MATTEO*, D. NANNINI* and G. URBANI*

*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy; and ‡Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

Abstract—Comparative tests such as FAT, ELISA, RIA, IEO and CF in the diagnosis of dourine in the horse have proved a satisfactory concordance ratio of the ELISA with CF, which seems to be the most reliable test. Discrepancies have been observed as to the other tests which appear less sensitive than CF test.

Key words: Trypanosoma equiperdum, horse, complement fixation, immunoelectro-osmophoresis, immunofluorescence, enzyme-linked immunoassay, radio-immunoassay, dourine

COMPARAISON DE DIFFERENTS TESTS EN VUE DU DIAGNOSTIC SEROLOGIQUE DE LA DOURINE CHEZ LE CHEVAL

Résumé—Pour le diagnostic de la dourine la comparaison de différents tests parmi lesquels, l'IF, ELISA, le RIA, l'Immuno-électro-osmophorèse et la réaction de déviation du complément chez le cheval, a montré une concordance satisfaisante entre le test ELISA et la RFC, qui semble être la plus probante. Par contre, on a observé des discordances avec les autres épreuves qui semblent moins sensibles que la RFC.

Mots-clefs: Trypanosoma equiperdum, cheval, fixation du complément, immunoélectro-osmophorèse, immunofluorescence, ELISA, radio-immunoassay, dourine

INTRODUCTION

Serology has always played a major role in the diagnosis of *Trypanosoma equiperdum* infection in the horse. Complement fixation (CF) has been the most widely used technique [1]. Immunoelectro-osmophoresis (IEO) has also been employed for the serological diagnosis of the *Trypanosoma equiperdum* infection [1]. Immunofluorescence (IF) and the more recently enzyme-linked-immunoassay (ELISA) have been widely used for the serological diagnosis of many parasitic diseases [2, 3]. Radioimmunoassay (RIA) is also considered a very sensitive serological test for the diagnosis of parasitic diseases [4].

The present communication deals wth the comparison of the FAT, ELISA, RIA and IEO with CF test in their application to the serological diagnosis of *Trypanosoma equiperdum* infection.

* The present research has been carried out with the grant of the Ministero della Sanità, Direzione Generale Servizi Veterinari, Rome, Italy.

MATERIALS AND METHODS

Sera

Standard positive serum. Serum of *T. equiperdum* of a naturally infected horse containing 1000 complement fixing units [1].

Unknown sera. A total of 251 horse sera were examined. Sera were divided into four groups according to *Trypanosoma equiperdum* infection status.

Group 1. 171 sera of horses showing clinical symptoms of dourine positive to CF test.

Group 2. Twenty-eight sera of two horses experimentally infected with *Trypanosoma equiperdum* collected before infection and at various time intervals after infection.

Group 3. Twenty-seven sera of horses showing symptoms of dourine but negative to CF antibody assay.

Group 4. Twenty-five sera of horses belonging to a population where no evidence of dourine was recorded in the last 30 years.

Antigens. A rat adapted *T. equiperdum* strain [1] was used for the various antigen preparations utilized throughout this study.

CF and IEO antigens. These were prepared according to the technique described by Caporale *et al.* [1].

IF antigen. Albino rats inoculated intraperitoneally with 6×10^3 parasites per animal were bled at the peak of parasitaemia using sodium citrate as anti-coagulant. The blood, filtered through three layers of gauze, was centrifuged at $1650 \times g$ for 10 min at room temperature. The parasite, buffy coat rich was collected, then diluted 1:10 in normal saline and washed 3 times by centrifugation at $1650 \times g$ for 10 min. The resulting suspension, virtually red blood cell-free, was diluted in normal saline and spread on coverslips to have about 20 parasites per microscopic field at a magnification of $\times$ 400. The coverslips fixed for 10 min in acetone at $-20°C$ were air-dried and stored at $-20°C$ until use.

ELISA antigen. The blood cell free parasite suspension, prepared as described for the IF test, was freezed at $-20°C$ and thawed three times at room temperature. The resulting suspension was sonicated for 2 min in an ice-bath and centrifuged at $46{,}800 \times g$ for 20 min at 4°C. The supernatant was collected and stored at $-40°C$. At the time of use, the antigen was thawed and diluted in carbonate buffer 0.05 M, pH 9.6, to contain 16 g/ml of protein. Diluted antigen (0.2 ml) was placed in each well of a microtitre plate (Cooke A 129) and incubated for 18–24 hr at 4°C in a humid chamber. The plates were then stored until further use at 4°C for up to 3 months.

RIA antigen. This was prepared as the ELISA antigen but the assay plates used were polyvinylflexible microtitre plates (Cooke, M29 AR Dynatech Laboratories, Billingshurst, U.K.).

Conjugated antisera

Commercial horse radish peroxidase conjugated IgG fraction rabbit antihorse IgG serum (heavy and light chain) (Cappel Laboratories Inc., Cochranville, PA 19330, U.S.A.) was used. I^{131} conjugated anti-horse γ-globuline serum was prepared according to the technique described by Biancifiori *et al.*, [4]. Fluorescein isothiocyanate conjugated γ-globulins were prepared according to the technique described by Baldelli *et al.* [3].

Table 1. Results of antibody assay with five different serological tests performed with sera of horses with clinical symptoms of dourine

No of sera	CF (unit/ml)	ELISA +	ELISA −	ELISA ND	RIA +	RIA −	RIA ND	IEO +	IEO −	IEO ND	IF +	IF −	IF ND
19	62,5	17	2		4	15		5	13	1	4	15	
63	125	60	2	1	49	14		57	6		52	11	
22	250	19		3	18	4		22			19	3	
19	500	19			19			18	1		19		
36	1000	36			34	2		36			34	2	
12	2000	12			11	1		12			12		
TOTAL 171		163	4	4	135	36		150	20	1	140	31	

ND = not done.

Tests

CF and IEO tests were carried out according to the technique described by Caporale *et al.* An IF test was carried out according to the technique described by Baldelli *et al.* An ELISA test was carried out according to the technique described by Voller *et al.* Finally, and a RIA test was carried out according to the technique described by Biancifiori *et al.*

RESULTS AND DISCUSSION

Results of the antibody assay reported in Table 1 refer to animals with dourine symptoms whose sera were positive to CF (Group 1). ELISA gave a response which had 97.6% concordance ratio (CR) with CF results; RIA had a CR of 78.9%; IEO had a CR of 88.28%; IF had a CR of 81.9%. Concordance ratio appears to be satisfactory between CF and ELISA although the latter did not reveal positivity in four cases. Apparently, CF had a

Table 2. Results of antibody assay with five different serological tests performed with sera of horses experimentally infected with *Trypanosoma equiperdum*

No of sera	CF (unit/ml)	ELISA +	ELISA −	ELISA ND	RIA +	RIA −	RIA ND	IEO +	IEO −	IEO ND	IF +	IF −	IF ND
10	0		10			10			10			10	
6	125	6			5	1		5	1		6		
1	250	1			1			1			1		
4	500	4			2		2	2	2		4		
3	1000	3			2		1	2	1		2	1	
4	A.C.*	4			4			4			4		
TOTAL 28		18	10		14	11	3	14	14		17	11	

ND = not done.
* AC = anticomplementary.

Table 3. Results of antibody assay with five different serological tests performed with sera of horses free of *Trypanosoma equiperdum* infection

No of sera	CF (unit/ml)	ELISA +	ELISA −	ELISA ND	RIA +	RIA −	RIA ND	IEO +	IEO −	IEO ND	IF +	IF −	IF ND
25	0		17	3		24	1	3	20	2	1	24	

ND = not done.

Table 4. Results of antibody assay with five different serological tests performed with sera of horses with clinical symptoms of dourine

No of sera	CF (unit/ml)	ELISA +	ELISA −	ELISA ND	RIA +	RIA −	RIA ND	IEO +	IEO −	IEO ND	IF +	IF −	IF ND
27	0		19	8	2	25		1	26		2	25	

ND = not done.

sensitivity higher than ELISA although serum antibody titres with the latter were consistently higher. The other tests appeared less sensitive than CF test. This pattern was confirmed also in experimentally infected animals (Group 2), (Table 2); concordance rates, in this case, were ELISA: 100%; RIA: 93.3%; IEO: 77.8% and IF: 94.4%. In Table 3 the results referring to horses from a disease-free population in the last 30 years (Group 4) are reported. From these, specificity of the tests was 100% for CF, ELISA and RIA while it was 87.0% for IEO and 96.0% for IF. Finally, it is difficult to interpret the results reported in Table 4 which refer to horses with symptoms of the disease but negative to CF test (Group 3). Positivity encountered in RIA, IEO and IF tests could suggest a higher sensitivity compared to ELISA or CF. This seems in contrast with the results obtained in the other animal groups. From the results obtained in these experiments one could conclude that: (1) CF appears to be the most reliable test; (2) ELISA is the test which has a satisfactory concordance ratio with CF; (3) discrepancies encountered with other tests could be due to quantitative reasons, to the subjectivity of test interpretation, to non-optimal sensitivity of the antigen, to differences in the antibody population revealed by the different tests; (4) given the cumbersome procedure needed to prepare *T. equiperdum*, antigen ELISA is probably the test of choice because the antigen for this test is far more stable than CF antigen (unpublished observation) and the antigen quantity needed is much lower.

REFERENCES

1. Caporale, V. P., Nannini, D. and Semproni, G., Prove comparative tra deviazione del complemento in provetta, deviazione del complemento miniaturizzata ed immunoelettro-osmo-foresi nella diagnosi sierologica del Morbo Coitale Maligno (MCM), *Atti Soc. It. Sci. Vet.* 31, 787–789 (1977).
2. Voller, A., Bidwell, D. E. and Bartlett, A., Enzyme immunoassay in diagnostic medicine. Theory and practice, *Bull. WHO* 53, 55–65 (1976).
3. Baldelli, B., Frescura, T. Gialletti, L., L'immunofluorescenza nella diagnosi della Trichinosi sperimentale umana, *Atti Soc. It. Sci. Vet.* 22, 739–745 (1968).
4. Biancifiori, F., Frescura, T., Gialletti, L. and Morozzi, A., Solid-phase micro-radioimmunoassay for antibodies to *Trichinella spiralis*, *Arch. Vet. It.* 31, 44–47 (1980).