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RESEARCH COMMUNICATION

AN INVESTIGATION INTO ALTERNATIVE METHODS FOR THE SERODIAGNOSIS OF DOURINE

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ABSTRACT

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The complement fixation test (CFT), indirect fluorescent antibody test (IFAT), card agglutination test for trypanosomiasis (CATT) and enzyme-linked immunosorbent assay (ELISA) were compared in their application to the serological diagnosis of *Trypanosoma equiperdum* infection in 43 horses. The CFT remains a reliable test for dourine, especially in countries where other members of the subgenus *Trypanozoon* do not occur. The IFAT is a good 'back-up' test, but, requiring skilled operators it has the disadvantage of making it labour intensive, and interpretation of results subjective. This makes it more suited to small numbers of samples. The ELISA is suitable for large numbers of samples and could readily be used in routine diagnostic procedures. The CATT could be of value in field situations, although it does not appear to be as sensitive as the CFT. Its possible application under these conditions should be further investigated.

INTRODUCTION

The complement fixation test (CFT) for dourine was introduced by Watson (1915). Shortly after 1920 the CFT came into use at the Veterinary Research Institute, Onderstepoort, and it was later adapted to microtitre plates (Herr, Huchzermeyer, Te Brugge, Williamson, Roos & Schiele, 1985).

The CFT is routinely used when equine sera are tested for antibodies to *Trypanosoma equiperdum*. However, non-specific or anticomplementary reactions are often encountered when testing mule or donkey sera (Meyer, 1910; Williamson & Herr, 1986). This problem is not unique to mules and donkeys, but also occurs with horse serum, particularly if the serum has been in transit for some time. This is of importance in southern Africa where animals are frequently far from laboratories.

Parkin (1948) reported "low antibody" and "high antibody" cases of dourine in South Africa. He conducted various challenge experiments and concluded that the "low antibody" cases were not true cases of dourine.

For the above reasons, as well as for the purpose of having a 'back-up' test where animals need to be certified for export purposes, we investigated alternative methods for the serodiagnosis of dourine.

MATERIALS AND METHODS

Sera

Sera positive to the CFT were taken from those submitted to this laboratory for routine diagnostic testing and, whenever possible, a brief history of the animal was obtained. This included clinical signs, such as poor condition, anaemia, enlarged lymphnodes, locomotory disturbances, abortion, eye infections, skin plaques and oedema of the abdomen, legs or genitals. Negative sera were obtained from a closed herd of horses stationed at the Veterinary Research Institute, Onderstepoort, with no clinical signs or history of dourine.

Complement fixation test

Antigen

Rats were infected by intraperitoneal inoculation with a laboratory-maintained strain of *T. equiperdum* and bled at the peak of parasitaemia, using sodium citrate as anticoagulant. The blood was filtered through a muslin filter and centrifuged at 2 500 *g* for 20 min at 4 °C. The parasite-rich buffy coat layer was then collected, diluted 1:10 in normal saline and washed at least once by certrifugation at 2 500 *g* for 20 min at 4 °C. Distilled water was then added to the concentrated antigen to lyse the remaining erythrocytes, centrifuged at 1 800 *g* for 20 min at 4 °C and the supernatant discarded. The remaining pellet was then diluted with an equal volume of normal saline and stored in 0,1 mℓ aliquots at −196 °C. Antigen was titrated against a positive serum of known titre and the optimal dilution giving that end-point was selected.

Test procedure

The test was carried out in microtitre plates and interpreted as described by Herr *et al.* (1985).

Indirect fluorescent antibody test

IFAT antigen

Antigen harvested as above was diluted in phosphate buffered saline (PBS) (pH 7,2) and applied to 12-well teflonized glass slides to have approximately 30 parasites per microscopic field at a magnification of ×400, using an ordinary light microscope. The glass slides, fixed for 10 min in acetone at −20 °C, were air dried, wrapped in tissue paper, sealed in plastic bags and stored at −20 °C.

Conjugated antisera

Commercial rabbit anti-horse IgG (heavy and light chain) conjugate with fluorescein isothiocyanate[1], was used for the IFAT.

Sera

Test sera and known positive and negative control sera were diluted in PBS and used at a dilution of 1/80 for screening purposes.

Test technique

The technique used was essentially a modification of that used by Joyner, Donelly, Payne & Brocklesby (1972). Prior to use, antigen slides were taken from storage at −20 °C and incubated at 37 °C for 10 min. Slides were then removed from their protective covering, test sera were applied and incubated in a humid chamber for 30 min at 37 °C. One well on each slide was used for the negative control and the opposing well for the positive control.

After incubation the slides were rinsed in phosphate buffered saline (PBS), washed in fresh PBS for 10 min

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[1] Bio-Yeda Ltd, Kiryat Weizmann, Rehovot 76326, Israel

TABLE 1 Results of the different tests

No.	Previous test history	Clincal signs	CFT SAU/mℓ	IFAT 1/80	CATT	ELISA reading	ELISA P/N
1	U	N	—	—	—	0,036	0,032
2	U	N	—	—	—	0,138	1,227
3	U	N	—	—	—	0,100	0,889
4	U	N	—	—	—	0,088	0,782
5	U	N	—	—	—	0,088	0,782
6	U	N	—	—	—	0,086	0,764
7	U	N	—	—	—	0,070	0,622
8	U	N	—	—	—	0,065	0,577
9	U	N	—	—	—	0,043	0,382
10	U	N	—	—	—	0,104	0,924
11	U	N	—	—	—	0,064	0,568
12	U	N	—	—	—	0,089	0,791
13	U	N	—	—	—	0,090	0,8
14	U	N	—	—	—	0,080	0,711
15	U	N	—	—	—	0,062	0,551
16	U	N	—	—	—	0,049	0,436
17	U	N	—	—	—	0,080	0,711
18	U	N	86	—	—	0,130	1,155
19	U	N	24	—	++	0,202	1,796
20	P	N	688	P	+	0,407	3,618
21	P	N	784	P	++	0,270	2,4
22	P	N	344	P	+	0,282	2,507
23	P	N	784	P	—	0,302	2,684
24	P	N	480	P	+++	0,243	2,16
25	S	N	24	P	+++	0,570	5,067
26	P	N	120	P	+++	0,353	3,137
27	P	N	480	P	++	0,480	3,644
28	U	U	784	P	++	1,209	10,747
29	P	U	480	P	+	0,227	2,018
30	U	U	392	P	−	0,109	0,968
31	P	Y	784	P	+++	0,693	5,681
32	P	Y	784	P	++	1,115	9,911
33	P	Y	784	P	++	1,136	10,098
34	P	Y	784	P	+	0,468	4,16
35	P	Y	784	P	+	1,409	12,524
36	P	Y	784	P	±	0,936	8,32
37	P	Y	784	P	++	0,352	3,129
38	U	Y	784	P	+++	1,543	13,716
39	U	Y	21	P	++	1,019	9,058
40	U	Y	784	P	++	1,004	8,924
41	P	Y	240	P	++	1,447	12,862
42	P	Y	290	P	+	1,910	16,978
43	P	Y	240	P	+	1,420	12,444

SAU/mℓ = South African Units/mℓ
P = Positive
N = No clinical signs present
\+ = Degree of agglutination
P/N = Positive/Negative ratio
Y = Clinical signs present
U = Unknown
S = Suspect

on a magnetic stirrer and in distilled water for 5 min. Without drying, the slides were then flooded with approximately 1 mℓ of diluted conjugate, incubated for 30 min and washed for 5 min in PBS as before. After being washed, a glass coverslip was mounted on the slide, using a 1:1 mixture of PBS and glycerol.

Microscopy

Fluorescence was observed under a Leitz Orthoplan microscope with an Osram HBO 50 W high pressure mercury lamp, using incident light excitation with a Ploemopak-2 fluorescence vertical illuminator. The microscope was fitted with a water immersion objective (× 50) and wide-field eye pieces (× 10).

Card agglutination test for trypanosomiasis

The CATT is a 5 min agglutination test. The antigen was of *T. gambiense* origin[2].

Enzyme-linked immunosorbent assay

This test was performed essentially as described by Williamson, Oberem, Poerstamper, De Waal, Matthee & Brett (1988). The antigen was harvested as for the CFT and, after being washed 3 times in 0,1 M phosphate buffered saline, the resulting suspension was sonicated for 30 s in an ice bath. A continuous cycle at maximum amplitude with a microtip on a B-30 Sonifier cell disruptor [3], was used. The plates were coated with antigen by incubation for 18 h at 4 °C. A blocking step with 1 % ovalbumen[4] for 60 min at 37 °C was included after coating with antigen. The conjugate used was anti-horse IgG (whole molecule) peroxidase conjugate[5].

RESULTS

The results of the 4 different tests together with the clinical signs are summarized in Table 1. The horses with no clinical signs of dourine are listed first, followed by those of whom no history was supplied. Lastly, those with clinical signs of dourine are shown.

DISCUSSION

There were no ELISA ratios of positive serum over negative serum (P/N) of higher than 1,796 amongst the first 19 horses which were stationed at the Veterinary Research Institute, Onderstepoort. There were, however, 2 horses (No. 18 and 19) that showed reactions on

[2] SmithKline - RIT, Rue de l'Institut, 89; 1330, Rixensart, Belgium

[3] Branson Sonic Power Co.

[4] Sigma Albumen, Turkey egg A-7269
Sigma Chemical Company, P.O. Box 14508, St Louis, MO 63178, USA

[5] Sigma Anti-horse IgG (whole molecule) A-6917
Sigma Chemical Company, P.O. Box 14508, St Louis, MO 63178, USA

the CFT (Table 1). No. 19 also reacted to the CATT, as well as giving the highest reaction on the ELISA in this group. Both, however, were negative on the IFAT. The CFT titres of 86 South African Units per mℓ (SAU/mℓ) and 24 SAU/mℓ which are equivalent to a 3^+ reaction in a 1/16 serum dilution and a 4^+ reaction in a 1/4 serum dilution, respectively, would be regarded as merely suspicious, using our present criteria (Herr *et al.*, 1985). The tests would then be repeated at a later date. In our experience, some of these suspicious reactions disappeared after a month, and it is not known if they were due to antigenic cross-reactions to other organisms.

Amongst the remainder of the horses tested, the ELISA and the CATT failed to detect Horse No. 30, which reacted to both the CFT and the IFAT. It was not known whether there were clinical signs in this case. The CATT also failed to detect Horse No. 23, which reacted positively to the other 3 tests. There were no clinical signs in this case.

It is interesting to note that where horses showed clinical signs of dourine the CATT, as well as the ELISA was positive, the P/N ratio being higher than 3.

The CFT remains a reliable test for dourine, especially in countries where other members of the subgenus *Trypanozoon* do not occur. The IFAT can reliably be used as a 'back-up' test, but its interpretation is both subjective and labour intensive. It is therefore more suited to the testing of small numbers of sera. The ELISA is a very useful test, suitable for large numbers of samples and can readily be used to supplement the CFT in routine diagnostic procedures. The CATT has its advantage in its simplicity, although the interpretation is subjective. It is not quite as sensitive as the other 3 tests, but did detect all animals with clinical signs of dourine. It could be usefully employed especially as a field test in outlying districts, and should be further investigated.

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