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VALIDITY OF SPA IN QUANTAL ASSAY OF ANTIBODIES TO BVD VIRUS IN FIELD SERUM SAMPLES

BY

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INTRODUCTION

Bovine viral diarrhea (BVD) in cattle was first described by Olafson et al. (1946). The disease has been found to be of economical importance specially in breeding programs. Although the serum neutralization test (SNT) was currently the serological test of choice for detection of BVD virus antibodies in bovine serum (Hafez and Frey, 1973; Tsvetkov et al., 1980; Seoket et al., 1985; Howard et al., 1986; Frey et al. 1987 and Dedsk et al., 1988), yet it has several undesirable features including the prolonged time it needs to complete the test, the requirments for cell culture expertise and use of aseptic devices for handling the samples and other reagents used.

Utilization of immunodiffusion (ID) test with cell culture antigens for detecting BVDV antibodies in cattle has been reported (Lohr and Reisshauer, 1981; Petkova et al., 1982 and Bolin et al., 1985). In spite of its specificity, the ID test has been found to be of relative lower sensitivity for detecting BVD antibodies if compared with the SNT.

For the above mentioned reasons, a need exists for a rapid accurate, economical and sensitive diagnostic test for detection of BVDV antibodied. The successful application of Staphylococcus aureus protein-A agglutination (SPA) test for detection of antibodied and antigen (S) of other viruses (Zalan and Wilson, 1976 and 1978; El-Sanousi, 1985 and Madboly et al., 1987) forced us to apply this test for determination of BVDV antibodies in field serum samples in comparative trials with the SNT and ID test.

MATERIALS AND METHODS

Virus and cells: A reference cytopathic strain of BVD virus (Singer strain), was kindly supplied by Ames Iowa Laboratories, U.S.A. The virus has been propagated in primary bovine kidney (BK) cells till it reached an end titer of 10^4 $TCID_{50}$ul. Primary bovine kidney (BK) cells were prepared according to the procedures adopted by crust et al. (1979) using kidneys obtained from freshly slaughtered calf. The cultured cells have been grown in Eagle's Minimum Essential Medium (MEM) supplied with 10% newborn calf serum (NCS) and 0.5% Lactalbumin Hydrolysate (LAH).

Sera and antisera: 76 bovine serum samples have been collected aseptically as possible from different farms, heat inactivated at 56°C for 30 min. and stored at - 20°C until used. These serum samples were tested for the presence of antibodies to BVD virus in the SNT, SPA and ID tests. A reference bovine anti- BVD hyperimmune serum was gifted to us from same National Veterinary Laboratories, Ames Iowa, U.S.A., with a reference negative control bovine serum, screened to be free from anti-BVD antibodies.

Serum nutetralization test: The micro method of SNT described by Frey and Liess (1971), using the 96-well flat bottomed microtiter plate, has been followed in the present work (see El-Bagoury, 1990).

Staphylococcus aureus protein A (SPA) agglutination test: The SPA test was carried out according to the method described by El-Sanousi (1985) as modified later by Madboly et al. (1987), using the micro-plate SPA test. The SPA suspension has been prepared following the procedured described by Kessler (1975).

Immuno-diffusion (ID) test: The Agar Gel Precipitation Test (AGPT) was performed for surveying bovine sera for the presence of anti-BVD precipitating antibodies, using the technique adopted by Harkness et al. (1978). The precipitating antigen has been prepared from BVD-infected BK cells, where the cell monolayer was scraped from the glass surface 48 hours after infection using a rubber policemann. The scrapped cells have been pelleted down and resuspended in few mls of phosphate buffered saline (PBS) (1/250 of the original volume), then exposed to 3 cycles of freezing and thawing. The prepared antigen was preserved at -20°C till used. The AGPT was carried out at 37°C in a humid chamber and presence of specific line of precipitation has been checked up at 24, 48 and 72 hours.

RESULTS

Because of its simplicity and rapidity, the SPA technique has been introduced in this work for detecting anti-BVD virus antibodies, and compared with the conventionally used SNT and ID tests. The validity of SPA test for surveying anti-BVD antibodies in collected serum samples has been proved when the 4 following categories of samples were tested: (see tables 1 to 5).

Group I (samples $ 1 to 17), positive (+ve) with high SN antibody titers (32 to 64).

Group II (samples $ 18 to 41), + ve with medium SN antibody titers (8 to 16).

Table 1 : Comparative determination of BVD-antibodies in field serum samples using different serological techniques Group I of high positive titer by SNT (Over 32) .

Serial No.	Code No.	Species	Serological test		
			SNT	AGPT	SPA
1	16	Cattle	64	-	128
2	26	Cattle	64	-	128
3	30	cattle	64	-	128
4	47	cattle	64	-	32
5	53	cattle	64	-	64
6	29	cattle	32	-	128
7	42	cattle	32	-	64
8	48	cattle	32	-	32
9	51	cattle	32	-	16
10	61	cattle	32	-	32
11	74	cattle	32	-	64
12	78	cattle	32	-	32
13	83	cattle	32	-	64
14	91	cattle	32	-	64
15	104	cattle	32	-	32
16	115	cattle	32	-	32
17	144	cattle	32	-	16

S.N.T : Serum neutralization test
AGPT : Agar gel precipitation test
SPA : Staphylococcus protein A-agglutination test .

Table 2 : Group II of moderate titer (8-16) .

Serial No.	Code No.	Species	Serological test		
			SNT	AGPT	SPA
18	10	Cattle	16	-	64
19	49	Cattle	16	-	32
20	56	cattle	16	-	32
21	96	cattle	16	-	64
22	103	cattle	16	-	32
23	110	cattle	16	-	8
24	129	buffalo	16	-	64
25	167	buffalo	16	-	16
26	183	buffalo	16	-	64
27	188	buffalo	16	-	64
28	206	sheep	16	-	128
29	156	sheep	16	-	16
30	100	cattle	8	-	128
31	116	cattle	8	-	64
32	123	cattle	8	-	32
33	133	cattle	8	-	16
34	142	cattle	8	-	8
35	168	buffalo	8	-	32
36	169	buffalo	8	-	16
37	178	buffalo	8	-	32
38	184	buffalo	8	-	16
39	200	buffalo	8	-	32
40	209	sheep	8	-	16
41	215	sheep	8	-	64

Table 3 : Group III of low titer (4)

Serial No.	Code No.	Species	Serological test		
			SNT	AGPT	SPA
42	9	cattle	4	-	8
43	18	cattle	4	-	16
44	28	cattle	4	-	4
45	67	buffalo	4	-	8
46	97	buffalo	4	-	4
47	134	sheep	4	-	16
48	145	sheep	4	-	4
49	160	sheep	4	-	8
50	199	sheep	4	-	4
51	202	sheep	4	-	4
52	207	sheep	4	-	16
53	222	sheep	4	-	32

Table 4 : Group IV "toxic sera"

Serial No.	Code No.	Species	Serological test		
			SNT	AGPT	SPA
54	146	Cattle	Toxic	-	-
55	24	Cattle	Toxic	-	4
56	39	Cattle	Toxic	-	16
57	45	Cattle	Toxic	-	-
58	52	Cattle	Toxic	-	-
59	57	Sheep	Toxic	-	-
60	157	Sheep	Toxic	-	8
61	62	Sheep	Toxic	-	-
62	219	Sheep	Toxic	-	8
63	77	Sheep	Toxic	-	-
64	101	Sheep	Toxic	-	-
65	130	Sheep	Toxic	-	4

Table 5 : Group V "SNT negative sera"

Serial No.	Code No.	Species	Serological test		
			SNT	AGPT	SPA
66	37	Cattle	negative	-	-
67	35	Cattle	negative	-	16
68	75	Cattle	negative	-	4
69	41	Cattle	negative	-	-
70	4	buffalo	negative	-	-
71	76	buffalo	negative	-	-
72	210	buffalo	negative	-	8
73	94	sheep	negative	-	4
74	21	sheep	negative	-	-
75	186	sheep	negative	-	-
76	159	sheep	negative	-	-

Group III (samples $ 42 to 53), + ve with low SN antibody titer (4).
Group IV (sample $ 54 to 65), including only cytotoxic serum samples.
Group V (samples $ 66 to 76), negative (-ve) without anti-BVD SN antibodies.
Looking to the data presented in tables 1 to 5, one can notice the total negativity given by the AGPT when used for screening specific anti-BVD antibodies. Unlike, the SPA showing in this communication a clear positivity with end titers ranging from 16 to 128 (for the groups I and II), 8 to 128 (for group III), 4 to 32 (for group IV). It could also be observed from the group IV (Table 4), that 5 out of 12 cytotoxic serum samples giving a noticeable end titers in the SPA test which ranged from 4 to 16. Again, out of 12 serum samples, which were negative in the SNT, only 4 samples showed positive reactivity in the SPA test with end titers ranged from 4 to 16.

DISCUSSION

Currently, the SNT is the serological test of choice for detection of anti-BVD virus antibodies in bovine sera. Although this test is accurate for diagnosis, it has several undesirable features, including the length of time required to complete the test, requirments for cell culture expertise and the need of aseptic devices during performance of the technique. The data reported by other investigators showed in most cases the superiority of the SNT over most of the other serological assays as an indicator of immunity to BVD virus (Robinson, 1970).

For the detection of BVD virus antibodies in the sera collected from field animals, we introduced the SPA test as a simple technique together with the SNT and AGPT for detection of such antibodies. From the figures presented in Tables 1 to 5, it could be concluded that the SPA-agglutination test is relatively

more sensitive than the SNT in detecting antibodies to BVD virus giving end titers that ranged from 16 to 128. Surprising is the capability of the SPA test as a binding assay to catch specific anti-BVD antibodies in field sera which have shown cytotoxicity in the SNT (see Table 4), which might be attributed to the fact that the SPA test is capable to screen neutralizing and other non-neutralizing antibodies because of the binging affinities of protein A to different types and classes of immunoglobulins (Schmidt and Klein, 1980; Grangeot-Keros et al., 1982; and Mayers and Klostergaard, 1983). The SPA test was also interesting in being capable of detecting anti-BVD antibodies is serum samples which were originally negative in the SNT (see Table 5).

Although the ID test was found by some workers as a specific, simple technique for the detection of antibodies, but it is of relatively lower sensitivity than the SNT as reported by Harkness et al., (1978). In the present work, the ID test showed itself non-suitable and relatively non-sensitive for screening of antibodies to BVD virus in bovine sera; these findings have been shown to be in consistency with the data reported by Bolin et al. (1985), where he proved that the AGPT could give positive reactions only in cases when the SNT titers were 256 or higher.

SUMMARY

76 bovine serum samples were collected from different farms and tested for the presence of antibodies to BVD virus. For this purpose, the SPA-agllutination test has been introduced as a new approach in comparison with the serum neutralization test (SNT) and the immunodiffusion (ID) technique. Therefore, the samples have been categorized in four groups according to their titers in the SNI: 1st group included positive sera with high antibody titer (32 to 64); 2nd group included positive sera with medium antibody titer (8 to 16); 3rd group contained cytotoxic samples and the 4th group comprised all negative samples. The obtained

data could prove that the SPA test as a binding assay is relatively more sensitive than the SNT in detecting antibodies to BBVD virus giving end titers ranged from 16 to 128. The SPA test could also detect anti-BVD antibodies in cytotoxic serum samples.

Although the ID test is a simple technique for detection of antibodies yet it was of relative lower sensitivity than the SNT and SPA-agglutination tests, and showed itself in this work non-suitable for screening of antibodies to BVD virus.

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