A Comparison of Some Serological Tests for Bluetongue Virus Infection

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ABSTRACT

The plaque neutralization, complement fixation, and agar gel precipitin tests were compared by measuring bluetongue virus antibody in 137 serums from experimental animals (cattle and sheep) and suspected field reactors (cattle and deer).

In general, the tests agreed well with each other. Plaque neutralization titers began earlier than the other two and went much higher than the complement fixation titers. Plaque neutralization titers usually peaked between two and three weeks after exposure and complement fixation titers from four to six weeks. The greater sensitivity of the plaque neutralization test allowed the detection of all complement fixation and agar gel precipitin reactors whereas occasionally the latter two tests failed to detect plaque neutralization reactors.

RÉSUMÉ

Cette expérience visait à comparer la valeur respective des épreuves de la réduction des plages, de la déviation du complément et de l'immunodiffusion en gélose, pour la recherche des anticorps contre la fièvre catarrhale du mouton. À cette fin, les auteurs utilisèrent 137 échantillons de sérum provenant de bovins et de moutons infectés expérimentalement, ainsi que de bovins et de chevreuils soupçonnés d'être atteints d'une infection naturelle. En général, les résultats des trois épreuves s'avérèrent concordants. L'épreuve de la réduction des plages permit cependant de déceler des anticorps plus tôt qu'à l'aide des deux autres épreuves, et à des titres beaucoup plus élevés que par celle de la déviation du complément. Les titres obtenus par l'épreuve de la réduction des plages atteignirent un sommet, de deux à trois semaines après l'infection, comparativement à quatre à six semaines pour l'épreuve de la déviation du complément. Une plus grande sensibilité de la part de l'épreuve de la réduction des plages permit de déceler tous les réacteurs aux deux autres épreuves, lesquelles s'avérèrent parfois impuissantes à déceler des réacteurs à l'épreuve de la réduction des plages.

INTRODUCTION

The serodiagnosis of bluetongue virus (BTV) infection, often not a clinically noticeable event, is of importance for epidemiological studies in endemic areas and for the exclusion of possible carrier animals from BTV-free areas. Consequently, the relative efficacy of available serological tests assumes importance. In the present report, three tests which have been commonly used in North America are compared quantitatively and qualitatively, namely the plaque neutralization (PN) test, the modified direct complement fixation (MDCF) test, and the agar gel precipitin (AGP) test (1-5, 7, 10, 16, 17).

MATERIALS AND METHODS

Serums and Test Procedures

A total of 137 serums from cattle, sheep and deer were coded, randomized and tested

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in the three systems as unknowns. Tests were repeated as felt necessary by the scientist involved for reasons of peculiar test results, failure to find the end point, contamination, etc. Only after testing was completed and results compiled was the code used to identify the serums.

The serums comprised 105 serial or individual samples from experimental animals (17 cattle and six sheep) and 32 samples from field suspects (12 cattle, 19 deer) done as courtesy tests for other countries. The serums represented different isolates or strains including BT8, BT-Cyprus, BT-Texas, and BT-OX183 (1, 17). Pertinent histories to the extent of their availability are given in Tables I and II.

The tests were done as previously described (2-5, 10, 17) with the following changes.

Plaque Neutralization Test — BT8 virus only was used. The plaquing was done under agar[1] in L-929 cells[2] grown in plastic plates[3] using four or six serum wells (two-fold dilutions) and paired controls for each serum. Each serum well with its paired control yielded one datum point of log serum dilution on probit virus survival. The regression line was located on log probit paper and the 50% effective dose (ED_{50}) graphically calculated (6, 13, 17).

Complement Fixation Test — The MDCF method described previously (2-5, 12) was used. In our laboratory the test serum was inactivated at 60°C for 30 minutes. The bluetongue viral antigen was an acetone-ether extract of BT-8 infected mouse brain. Three extractions and elutions were performed from each batch of mouse brains (5). Uninfected tissue was prepared in the same manner and used as the control in all tests. Three 50% corrected hemolytic units of guinea pig complement, i.e. three units multiplied by the deterioration correction factor of 1.8 (= 5.4 units after correction), were used in the test. The complement was diluted in pH 7.2 veronal buffered saline containing 5% selected, unheated, pretested, normal calf serum as supplementary factor. The tests were held overnight at 4-9°C to allow for fixation. After addition of equal parts of 5% suspension of sheep red blood cells and diluted amboceptor, the tests were incubated for 30 min at 37°C. The percent hemolysis was read with the aid of a colour standard and the titers of the serums were expressed in terms of the highest dilution with which 50% hemolysis or less occurred with antigen.

Agar Gel Precipitin Test — The micro AGP test has been described previously (4, 10). It consisted of a plastic template containing the diffusion wells which was laid over a thin layer of agar on a microscope slide. The antigen consisted of a concentrated, pressure dialysed, bovine foetal kidney tissue culture inoculated with BT-8 virus. Noninoculated tissue culture processed similarly was used as normal antigen control. The test was incubated at 20°C in a humid chamber for four days. At the end of incubation the templates were removed, the slides washed in distilled water and stained with thiazine red R.

RESULTS

In general there was good agreement amongst the three tests (Table I, II). The PN test was the most sensitive, titers being detected earlier than with the MDCF or AGP tests and going much higher than the MDCF test (AGP being qualitative only). The magnitude of the difference (PN vs. MDCF) varied, however, from several-fold to more than 1000-fold.

PN reactivity began at one to two weeks postexposure and usually peaked between two and three weeks while MDCF titers became positive at about two to three weeks and usually peaked at four to six weeks. AGP conversions occurred at about the same time as MDCF or slightly earlier. In some cases the PN profiles had a marked peak in the two to three weeks area.

In most cases, reactivity in all three tests remained substantial for the duration of the bleeding period, often several weeks or months. In a notable exception (animal 2) the PN titer appeared to be transitory, peaking at about 16 days but becoming very low by postinoculation day (PID) 128, the MDCF and AGP tests remaining negative.

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[1]Purified Agar (batch no. 107 9748), Oxoid Ltd., London, England.

[2]L-929 Mouse Fibroblast Cell Line, Microbiological Associates Inc., Bethesda, Maryland.

[3]FB6 Tissue Culture Plate, Linbro Chemical Co. Ltd., New Haven, Connecticut.

TABLE I. Serum from Experimental Animals used to Compare Plaque Neutralization, Complement Fixation and Agar Gel Precipitin Tests for Bluetongue Virus Exposure

	Animal	History	PID	PN[a] Titer	CF Titer 1[b]	CF Titer 2	AGP Test
I (a)	Cattle						
1.	C-8-71	BT8-10^3 p.f.u. cell culture virus IV	0	<20	–	–	–
			2	<20	–	–	–
			4	<20	–	–	–
			8	50	–	NS5	–
			16	500	–	5NS10	–
			32	>10,000	40	80NS5	+
			64	>10,000	40	40	+
			128	5,000	40NS5	40	+
2.	C-7-71	BT-10^3 p.f.u. cell culture virus IV	0	<20	–	–	–
			2	<20	–	–	–
			4	<20	–	–	–
			8	100	–	–	–
			16	1,200	–	–	–
			32	1,000	–	AC5[d]	–
			64	300	–	–	–
			128	50	5	–	–
3.	C-16-72	BT8-10^3 p.f.u. IV and 10^3 p.f. SC	0	<20	–	–	–
			17	⩾10,000	–	–	–
			64	>10,000	80	80	+
4.	C-5-71	BT8-10^7 p.f.u. IV cell culture virus	0	<20	–	–	–
			11	⩾10,000	–	–	–
			18	>10,000	10	10	+
			31	>10,000	>160	80	+
			69	9,800	80	80	+
5.	C-8-70	BT8-cell culture virus IM and SC	0	<20	–	–	–
			8	2,000	–	–	–
			18	5,000	10NS5	10NS5	+
			20	5,900	10NS5	10	+
			24	>10,000	40	20	+
			25	>10,000	20	10	+
			27	>10,000	80	40	+
			29	>10,000	10NS5	40	+
			33	>10,000	20	20	+
			35	>10,000	40NS5	20	+
6.	C-1-71	BT8-infectious deer blood SC	0	<20	–	–	–
			7	200	–	–	–
			13	>10,000	–	–	+
			20	>10,000	–	–	+
			24	>10,000	5	5	+
			26	>10,000	20	20	+
			28	>10,000	80	80	+
			30	>10,000	20	20	+
			32	>10,000	160NS5	⩾160NS5	+
			33	>10,000	80	40	+
7.	C-9-70	BT8 cell culture virus IV and IM	0	<20	–	–	–
			5	<20	–	–	–
			10	800	–	–	–
			16	7,800	–	–	–

TABLE I. Serum from Experimental Animals used to Compare Plaque Neutralization, Complement Fixation and Agar Gel Precipitin Tests for Bluetongue Virus Exposure (continued)

	Animal	History	PID	PN[a] Titer	CF Titer 1[b]	CF Titer 2	AGP Test
			21	>10,000	40	10	+
			26	>10,000	20	20	+
			35	>10,000	80	80	+
			45	>10,000	>160	80	+
			57	>10,000	80	80	+
			71	>10,000	⩾160	⩾160	+
8.	C-2-74	BT-Texas "Station" Sheep blood IV and SC	0	35	–	–	–
			2	<20	–	–	–
			4	<20	–	–	–
			8	120	–	–	–
			16	4,000	–	–	±
			32	800	20	20	+
			63	2,000	40	40	+
9.	C-506605	BT-Texas "Station"	91	1,900	40	20	+
10.	C-12-71	BT-Cyprus cell culture virus, 6 x 10^4 p.f.u. IV and SC	0	<20	–	–	–
			4	<20	–	–	–
			16	160	–	–	–
			64	200	10AC5	–	–
11.	C-17-71	BT-Cyprus cell culture virus, 6 x 10^4 p.f.u. IV and SC	0	20	–	–	–
			16	300	–	–	–
			36	400	–	–	–
12.	C-1-72	BT-Cyprus cell culture virus, 10^3 p.f.u. IV and SC	0	110	–	–	–
			16	1,600	–	–	–
			32	200	–	–	–
13.	C-9-73	BT-OX183 bovine blood IV and SC	0	<20	–	–	–
			25	6,200	–	–	–
14.	C-1272	EHDV[c] (American Type Culture Collection) - cell culture virus IV and SC	64	<20	–	–	–
15.	C117Y	normal calf		<20		5NS5	–
16.	C113Y	normal calf		<20	–	–	–
17.	C-506-113	BT8	0	<20	–	5	–
			20	940	20	20	+
			32	250	20	40	+
			96	500	40	20	+

TABLE I. Serum from Experimental Animals used to Compare Plaque Neutralization, Complement Fixation and Agar Gel Precipitin Tests for Bluetongue Virus Exposure (continued)

	Animal	History	PID	PN[a] Titer	CF Titer 1[b]	CF Titer 2	AGP Test
I (b) Sheep							
18.	S-8-70	BT8	0	<20	–	–	–
			5	<20	–	–	–
			8	7,600	–	–	–
			11	>10,000	5	5	–
			14	>10,000	10	10	±
			17	10,000	10	10	+
			22	9,200	40	20	+
			27	>10,000	80NS5	80	+
			28	9,000	40	80	+
			34	>10,000	80	40	+
19.	S-11-70	BT8 cell culture virus IM and SC	1	<20	–	–	–
			11	5,000	–	–	±
			23	>10,000	20	10	+
			29	10,000	40	40	+
			33	>10,000	20	40	+
			34	>10,000	40	40	+
20.	S161-66	BT-Texas "Station"	45	7,000	80NS10	80NS5	+
21.	S24739-61	BT-Cyprus	21	2,200	⩾160	⩾160	+
			98	3,000	⩾160NS10	80	+
			123	3,600	160NS5	160NS5	+
			157	2,100	80NS5	80NS5	+
			166	1,300	160NS5	40	+
22.	S-2-69	BT-Cyprus	63	600	⩾160	80	+
23.	Normal sheep No. 8			30	–	–	–

[a]Reciprocal ED_{50}
[b]1st and 2nd test
[c]Titer 1:40, Non-specific reaction at 1:5 dilution
[d]Anti-complementary
[e]Epizootic hemorrhagic disease virus

In no case did the PN test miss CF or AGP reactors, although PN titers were often substantial in the presence of negative MDCF or AGP results. This was the case with homologous (BT8) as well as heterologous sera. Obviously this is often explainable by the time of bleeding, the MDCF and AGP reactions becoming positive some time after the PN reaction.

DISCUSSION

As might be expected from other virus antibody systems (11, 13-15) the PN test was more sensitive than the MDCF or AGP test. As with any serological methods, the three tests in the present study yield some results in the "questionable" area. With several sera, use of the other two tests clarified results when this happened (Table I, II). To reduce the percentage of serums in this category, further study on the nature of nonspecific reactivity is necessary.

When selecting the most suitable serological test for any particular application, considerations must include efficiency, practicability, reliability and sensitivity. Depending on the application, the relative importance of these factors varies and rarely would any one test be better than all others for all of these criteria.

The question of efficiency will vary with the purpose, for instance a serological survey versus quantification of individual serums. The present data do not bear directly on this subject.

TABLE II. Serum from Field Suspect Animals Used to Compare Plaque Neutralization, Complement Fixation and Agar Gel Precipitin Tests for Bluetongue Virus Exposure

	Bovine	PN Titer	CF Titer 1	CF Titer 2	AGP Test
II(a) Cattle					
23	T-1	600	20	20NS5	+
	T-2[a]	900	20	20	+
24	E-4	>10,000	80	40	+
25	E-9	1,100	20	10	+
26	E-12	700	–	–	–
27	E-17	2,200	10	5	+
28	E-26	600	40	20	+
29	E-34	800	–	5	–
30	B-5	1,000	20	20	+
31	B-20	1,700	40	20	+
32	B-32	1,500	20	10	+
33	J-49	<20	NS10	NS10	–
34	J-50	>10,000	80NS10	40AC10	+
II (b) Deer					
35	D-304	45	–	nd[b]	–
36	D-303	60	5	nd	–
37	D-636	4,500	20	10	+
38	D-575	250	–	–	–
39	D-50	<20	–	–	–
40	D-335	<20	–	nd	–
41	D-727	1,600	80	40	+
42	D-641	<20	5	5	–
43	D-302	550	20NS10	nd	+
44	D-582	110	10	10	±
45	D-317	150	–	nd	+
46	D-299	125	20NS10	20NS10	–
47	D-578	600	40	20	+
48	D-639	400	20AC5	nd	+
49	D-574	250	40	10	+
50	D-489	<20	–	–	–
51	D-307	500	10	Tr 5	+
52	D-54	<20	–	–	–
53	D-301	250	40NS5	nd	+

[a]T-2 = Bovine T-1 30 days later
[b]Not done

In some situations the PN test is less practical than the other tests because of the necessity for a cell culture system, large numbers of experimental units, etc., although these factors have diminished in recent years with technical advances in cell culture and inoculation equipment.

Reliability is demonstrated to some extent for all tests inasmuch as samples from inoculated animals taken at known times postexposure were included. Since these were coded and tested "blind", their subsequent agreement with "expected" profiles becomes a measure of reliability. All of these tests have been used in other circumstances and have demonstrated to a greater or lesser extent their reliability (1, 4, 5, 10, 17). Based on the present data only, the PN test would appear to be the most reliable for the detection of exposed animals. This in turn is probably a reflection of the inherent sensitivity of the PN test, which has been appreciated in the field of viral immunology for some years (11, 13-15). The variation in the size of the differences between PN and MDCF titers may be in part due to the former's ability to measure all classes of antibody (14). This could result in a summation of complement fixing antibody and noncomplement fixing antibody in the PN reaction.

A major advantage of either the MDCF or AGP test over the PN test would involve the group reactivity of the former two. Neutralization tests are said to be serotype specific for the BT group of viruses (8, 9) although this was based on the rather insensitive "alpha" procedure (6, 8, 9). The ability of the PN test to detect animals exposed to BT-Cyprus, BT-Texas and BT-

OX183 more efficiently that either the MDCF or AGP test in the present data would not corroborate this. Only by comparing tests on all the serotypes within the BT group could one definitively answer this question. It may be that cross reaction antibody levels are much lower than the homologous titer and not detectable by neutralization tests other than PN (i.e. a quantitative rather than a qualitative difference).

Inferences about "strains" are sometimes based on titer magnitude of field serums. That is, conclusions drawn from quantitative data are of a qualitative nature. Obviously one cannot make this assumption with any of these tests in as much as heterologous titers are often higher than homologous reactions in different animals. Some low PN reactions may occur after antigen exposure as compared to successful infection. Animal 2 had the same history as Animal 1, yet gave a profile consistent with a low (noninfectious?) antigenic dose (15).

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