

Antibody to Viruses Affecting Cattle in Commercial Tissue Culture Grade Fetal Calf Serum¹

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Commercial fetal calf serum (FCS) for tissue culture use was tested for neutralizing activity against several viruses which affect cattle. Certain lots of FCS contained no neutralizing activity, whereas other lots contained neutralizing activity to several viruses. It was concluded that the neutralizing activity found in certain lots of sera was due to specific antibody and that its presence could be most easily explained by the contamination of the FCS with serum from postcolostral bovine serum. A nonantibody inhibitor to vesicular stomatitis virus was also found at low levels in most lots of serum. Because those sera which had antibody had antibody to several viruses, it was suggested that the use of the micro-serum neutralization test with a few bovine viruses which are widespread in the bovine population should be satisfactory to detect FCS which was contaminated with postcolostral bovine serum.

Under normal conditions immunoglobulins are transferred from a cow to its calf solely via colostrum during the 1st day of a calf's life. There appears to be no evidence for intrauterine transfer as occurs in many other species (4). As the bovine fetus matures, it gradually develops immunocompetence so that it is able to respond to certain antigenic stimuli beginning at about the 118th day of gestation (16). Bovine viral diarrhea (BVD) (1, 10), bluetongue (3, 12), and infectious bovine rhinotracheitis (IBR) (5, 9) viruses have been reported to infect the bovine fetus by traversing the placental barrier. Antibody to these viruses has been found in infected fetuses (10, 13, 15). Recently, Hubbert et al. (8) tested the sera of apparently normal fetuses for antibody to the bovine strain of parainfluenza 3 (PI3-SF4), BVD, and IBR viruses. Antibody to these viruses is found in the majority of adult cattle; however, they only found antibody to BVD virus in 3 of 147 fetal sera and no antibody to either PI3-SF4 or IBR virus in about 100 fetal sera. Horner et al. (7) also tested the sera of individual fetuses. By using a variety of infectious agents as antigens, they found antibody only to BVD virus in a small percentage of the sera.

Because of its availability, its growth promoting capacity, and its absence of immunoglobu-

lins, fetal calf serum (FCS) has been widely used in culturing cells of many species. However, Kniazeff et al. (11), noting that many investigators found that pooled FCS neutralized certain viruses, tested individual sera from bovine fetuses in their 4th to 6th month of gestation. They found that a few fetuses had antibody to BVD, but not to IBR or PI3-SF4 virus. The antibody detected in the fetus was attributed to either fetal synthesis or to transplacental transfer. Considering present information, transplacental transfer seems to be a remote possibility for explaining the presence of antibodies in the serum of bovine fetuses.

Investigations of Boone et al. (2) showed that different lots of commercial FCS for use in tissue culture varied considerably in chemical composition. They suggested that high gamma globulin levels may be associated with adulteration with postcolostral serum.

In our work with bovine viruses, we have used the micro-serum neutralization (micro-SN) test in which virus and test serum are mixed and incubated together. Cells are seeded in this mixture so that this procedure requires a concentration of about 10% serum for suitable cell growth. Because of the requirement of serum for cell growth, antibody in the culture medium is a significant factor as compared with neutralization tests conducted on confluent cultures in which the concentration of serum in the medium is minimal. This report concerns the

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examination of commercial FCS for antiviral inhibitors to viruses affecting cattle to determine the suitability of different types and lots of FCS for use in the micro-SN system.

MATERIALS AND METHODS

Test serum. All tissue culture sera tested were from different serially numbered lots purchased from Grand Island Biological Company, Grand Island, N.Y., during the interval from the autumn of 1970 to the spring of 1973. The sera were heat inactivated and stored at -20°C until tested. The sera were identified by the manufacturer as (i) FCS, (ii) virus-screened FCS (VS-FCS), (iii) immunoprecipitin-tested FCS (IPT-FCS), or (iv) gamma globulin-free newborn calf serum (GG-free NCS). The latter two sera were subjected to a modified Cohn fractionation (6) procedure by the manufacturer to remove gamma globulins.

Viruses. The following viruses were used in the micro-SN test: IBR, PI3-SF4, bovine adenovirus type 1, bluetongue strains 8 and OX 183 (BT 8 and BT OX 183), the New Jersey serotype of vesicular stomatitis (VS), and two bovine enterovirus strains identified as ED-1244 and 66-P-188 (supplied through the courtesy of J. Storz, Colorado State University, Fort Collins, Colo.).

Micro-SN test. The micro-SN test was conducted as previously described (14) by using 50 to 100 mean tissue culture infective dose of virus, except for the following modifications. Medium for cells in microtiter consisted of Eagle minimal essential medium with modified Earle salts (Grand Island Biological Co.) and 10% IPT-FCS. The medium was buffered with 0.2% NaHCO_3 , 7.5 mM *N*-2-hydroxyethylpiperazine-*N'*-2'-ethane-sulfonic acid, and 5 mM each of TES and MOPS. Each serum was tested in triplicate by using serial twofold dilutions and starting with undiluted serum. A serum toxicity control was included for each dilution of serum.

Complement enhancement test. Several sera which showed some inhibition of IBR virus, a herpesvirus, were tested with and without complement diluted 1:3 as described by Yoshino and Taniguchi (17), except that serum-virus-complement mixtures were assayed in microtiter by using six replicates per dilution. Complement has been found to enhance detection of antibody to IBR virus in certain bovine sera (C. R. Rossi and G. K. Kiesel, unpublished data). Cells consisted of a strain of bovine embryonic lung cells for assay of VS, IBR, BT 8, BT OX 183, and PI3-SF4 viruses and the AU-BEK cell line, established in this laboratory (C. R. Rossi and G. K. Kiesel, *In Vitro*, in press), for assay of bovine adenovirus type 1 and bovine enteroviruses 66-P-188 and ED-1244.

RESULTS AND DISCUSSION

Results with the micro-SN test for detecting neutralizing substances in commercial sera against viruses which affect cattle are shown in Table 1. Low neutralizing titers against VS

virus were found in most of the sera tested. The presence of neutralizing substances in IPT sera indicates the activity was not associated with the gamma globulin fraction of serum and suggests its non-antibody, nonspecific nature. Excluding VS virus, the five IPT-FCS tested did not inhibit replication of any other virus. At least two lots of IPT-FCS were toxic enough to prevent attachment and growth of bovine embryonic lung and AU-BEK cells when used at normal concentrations. This toxicity could be removed to allow satisfactory cell growth in the microtiter system by heating the serum at 56°C for 0.5 h. The GG-free NCS, one lot of FCS, and two lots of VS-FCS had inhibitory activity to several of the viruses, whereas most lots were completely free of any viral inhibitory activity. Explanations which can be offered for the presence of viral inhibitory activity in the commercial sera tested include the presence of (i) nonspecific inhibitors, (ii) fetal antibodies, (iii) natural antibodies, or (iv) antibodies due to contamination with post-colostral bovine serum or, in the case of GG-free NCS, ingestion of colostrum and incomplete removal of immunoglobulins. Serum lots 1, 3, 10, and 12 had inhibitory activity to viruses from several taxonomic groups, whereas most other lots of serum had no neutralizing activity against any virus, except for that against VS virus as previously mentioned. The distribution of neutralizing activity to several lots and types of sera tends to implicate antibody as the neutralizing substance detected against PI3-SF4, adenovirus type 1, and the two enteroviruses. Enhancement of neutralization to IBR virus by complement further indicates the specificity and antibody character of the neutralizing substances. The source of the antibody in these sera is probably contamination with serum from post-colostral bovine serum, in which these antibodies are common. The probability of fetal synthesis or natural antibody being distributed in the sera, as evidenced by the distribution of neutralizing activity in the sera, is remote.

Because antibody was found to most of the test viruses in those lots of serum which contained antibody, it is likely that antibodies to many other viruses were also present in these same lots. Therefore, the micro-SN test would appear to be a useful technique for screening lots of commercial serum for antibody to bovine viruses and as an aid in identifying serum contaminated with post-colostral bovine serum. The use of a few widespread bovine viruses should be appropriate in detecting such contamination.

TABLE 1. Serum neutralizing titers of commercial bovine sera for viruses which affect cattle^a

Serum code no.	Type of serum	Micro-SN test								Complement enhancement test	
		Bluetongue strains		Bovine adenovirus type 1	SF4 strain of para influenza type 3	Bovine enterovirus strains		Vesicular stomatitis	Infectious bovine rhinotracheitis	Infectious bovine rhinotracheitis	
		8	OX 183			66-P-188	ED-1244			Complement	Inactivated complement
1	Gamma G free newborn calf	0	0	16	64	2	16	NT	± ^b	0	0
2	Fetal calf	0	0	0	0	0	0	2	0	NT	NT
3		0	0	4	8	8	16	0	±	0	0
4		0	0	0	0	0	0	1	0	NT	NT
5		0	0	0	0	0	0	NT	0	NT	NT
6		0	0	0	0	0	0	NT	0	NT	NT
7		Virus-screened fetal calf	0	0	0	0	0	NT ^a	NT	0	NT
8	0		0	0	0	0	0	1	0	NT	NT
9	0		0	0	0	0	0	NT	0	NT	NT
10	0		0	16	2	2	4	NT	±	2	0
11	0		0	0	0	0	0	1	0	NT	NT
12	0		0	8	2	1	4	1	±	0	0
13	0		0	0	0	0	0	NT	0	NT	NT
14	0		0	0	0	1	0	1	0	NT	NT
15	0		0	0	0	0	0	1	0	NT	NT
16	0		0	0	0	0	0	1	0	NT	NT
17	0	0	0	0	0	0	1	0	NT	NT	
18	Immuno-precipitin-tested fetal calf	0	0	0	0	0	0	NT	0	NT	NT
19		0	0	0	0	0	0	2	0	NT	NT
20		0	0	0	0	0	0	NT	0	NT	NT
21		0	0	0	0	0	0	2	0	NT	NT
22		0	0	0	0	0	0	0	0	NT	NT

^a NT, Not tested.^b ±, Partial inhibition of CPE by undiluted serum.

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