

Detection of Antibody to Bovine Syncytial Virus and Respiratory Syncytial Virus in Bovine Fetal Serum

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Batches of commercial fetal bovine serum, described by the suppliers as antibody-free, all contained antibody to bovine syncytial virus (BSV) when tested by indirect immunofluorescence. Antibody to bovine respiratory syncytial virus (RSV) was not detected in these sera. Twenty-four percent of individual fetal bovine sera contained antibody to BSV, and 14% contained antibody to RSV when tested by indirect immunofluorescence. BSV antibody titers in fetal sera from dams with high BSV antibody levels were variable but always higher than RSV antibody titers. Radial immunodiffusion studies with BSV-positive sera revealed the presence of immunoglobulin M (IgM), IgG, and IgA, but the quantity of these immunoglobulins was not directly related to the BSV antibody titers. The evidence suggests that the antibody present in fetal sera arose as the result of infection rather than from maternal transfer across the placenta.

At least 14 different viruses have been isolated from bovine fetal tissue (10), including bovine syncytial virus (BSV) (17), but the isolation of bovine respiratory syncytial virus (RSV) has not been reported. In most cases, it was not known how or when the fetus became infected. The identification of immunoglobulins in the bovine fetus (5) and the evidence that maternal antibody was not transferred to the fetus (1) demonstrated the immunological competence of the fetus. Thus, the presence in fetal bovine sera (FBS) of precolostral antibody against bovine diarrhea virus (8), parainfluenza virus type 3 (16, 18), and blue tongue virus (9) suggested that virus spread could occur from the dam to the fetus.

On several occasions we have isolated BSV from fetal calf lung cells (FCL) which were being routinely subcultured. The BSV could have arisen from either of two possible sources. First, cells may have become infected in utero, the virus then being carried as a latent infection, or second, the commercial fetal serum which was used in the growth medium may have contained BSV as a contaminant. Since cells from the same frozen stocks did not always yield BSV, we considered the additional possibility that the commercial fetal serum might contain BSV antibody. In this paper we show by indirect immunofluorescence that all of the commercial sera examined and 24% of individual fetal sera from an abattoir did contain BSV antibody. We also

show that although RSV antibody was not detected in commercial sera, 14% of individually tested fetal sera contained low but significant levels of RSV antibody.

MATERIALS AND METHODS

Media. FCL were cultured in Eagle growth medium (Glasgow modification; 11) containing 10% FBS or 10% newborn lamb serum, where indicated, and maintained in Eagle medium containing 1% FBS. Although no direct examinations were made to see if the FBS contained BSV, we have never isolated this virus from other cells with the same batches of serum.

FCL. FCL were obtained from the lungs of healthy bovine fetuses by dispersion in 0.25% trypsin and cultivation in growth medium. Primary monolayers were subcultured and examined for BSV and RSV antigen by indirect immunofluorescence (see below). These cells were subcultured and used for all subsequent work.

Viruses and immunofluorescence. BSV was isolated and characterized as described in Results. RSV was obtained from J. Stott (Agricultural Research Council, Compton, England). For immunofluorescence, BSV-infected FCL were mixed in equal proportions with uninfected FCL and dispensed on cover slips in 5-cm dishes (10^5 /ml). The dishes were incubated in growth medium at 37°C for 2 days in 5% CO₂. For RSV, semiconfluent FCL cultures were infected with RSV at an estimated input multiplicity of 20 plaque-forming units per cover slip. After adsorption at room temperature for 2 h, 5 ml of maintenance medium was added, and the dishes were incubated at 37°C for 3 days in 5% CO₂. Infected and control noninfected cells were then washed three times in phosphate-buffered saline and fixed for 5 min with acetone. Indirect immunofluorescence was performed by standard procedures with fluorescein isothiocyanate.

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nate-labeled bovine antiglobulin (Nordic). For direct immunofluorescence, the fluorescein-labeled BSV antiserum was that used by Clarke et al. (2). This serum stained BSV-infected FCL but not uninfected or RSV-infected FCL. Fluorescein-isothiocyanate labeled antiserum prepared against simian foamy virus (strain MK5) was that used by Fleming and Clarke (7).

Specificity of staining. The specificity of the antiviral staining by fetal sera was established by the fact that the pattern of positive staining was identical to that obtained with the appropriate hyperimmune rabbit anti-BSV or anti-RSV serum and also by the loss of specific staining after the absorption of positive sera with acetone-fixed cells infected with either BSV or RSV, respectively.

Radial immunodiffusion. Immunoglobulin (immunoglobulin G [IgG], IgM, IgA) in fetal serum was estimated by the procedure of Mancini et al. (13) as modified by Fahey and McKelvey (5); specific antisera were prepared in New Zealand rabbits with the appropriate purified bovine immunoglobulins. Prior to use, each antiserum was selectively adsorbed with immunoglobulin-free FBS and the appropriate classes of immunoglobulin. Specificity was checked by immunoelectrophoresis and radial immunodiffusion.

Electron microscopy. For the preparation of thin sections, infected cells were fixed with glutaraldehyde followed by osmium and embedded in Spurr resin. Sections were stained with uranyl acetate followed by lead citrate.

Source of sera. Pooled FBS were obtained commercially (Biocult, Flow Laboratories, and Grand Island Biological Co.) and were tested with and without

heat inactivation at 56°C for 30 min. These sera were described as immunoglobulin-free when tested by immunoelectrophoresis and free of cytopathic and non-cytopathic viruses. Individual fetal sera were obtained from an abattoir.

RESULTS

Identification of BSV in FCL. FCL, which were subcultured routinely, occasionally showed spontaneous progressive degeneration, producing a characteristic syncytial appearance, identical to that previously described by Clarke et al. (2), who identified the causative agent as BSV. Passage of these infected cells was only possible when uninfected FCL were added at the time of subculture. Within 2 to 3 days these mixed cultures also showed characteristic syncytia.

Electron microscopic examination of thin sections of infected cells showed particles (Fig. 1) which were typical of the viral agent BSV (4). By direct immunofluorescence, infected cultures stained brightly with fluorescein-labeled BSV antiserum (Fig. 2), and the distribution of viral antigen was as described for BSV (12). Controls, which were stained with fluorescein-labeled antiserum against simian foamy virus strain MK5, showed no fluorescence.

Detection of BSV and RSV antibody in FBS. Individual FBS and batches of commercial

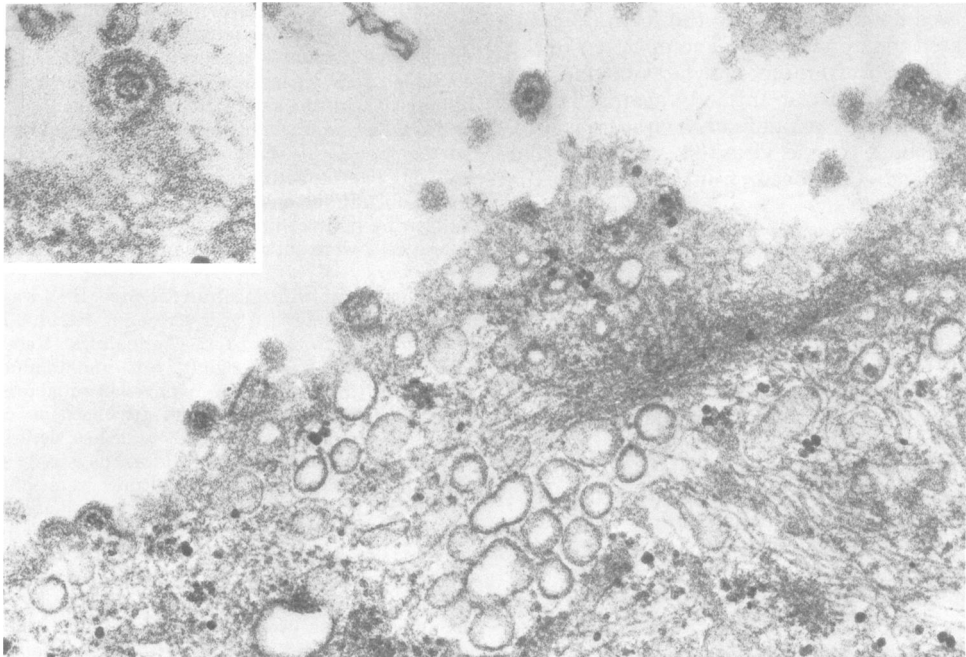


FIG. 1. Thin-section electron micrograph of FCL infected with BSV; $\times 85,000$. Inset shows typical BSV particle; $\times 112,500$.

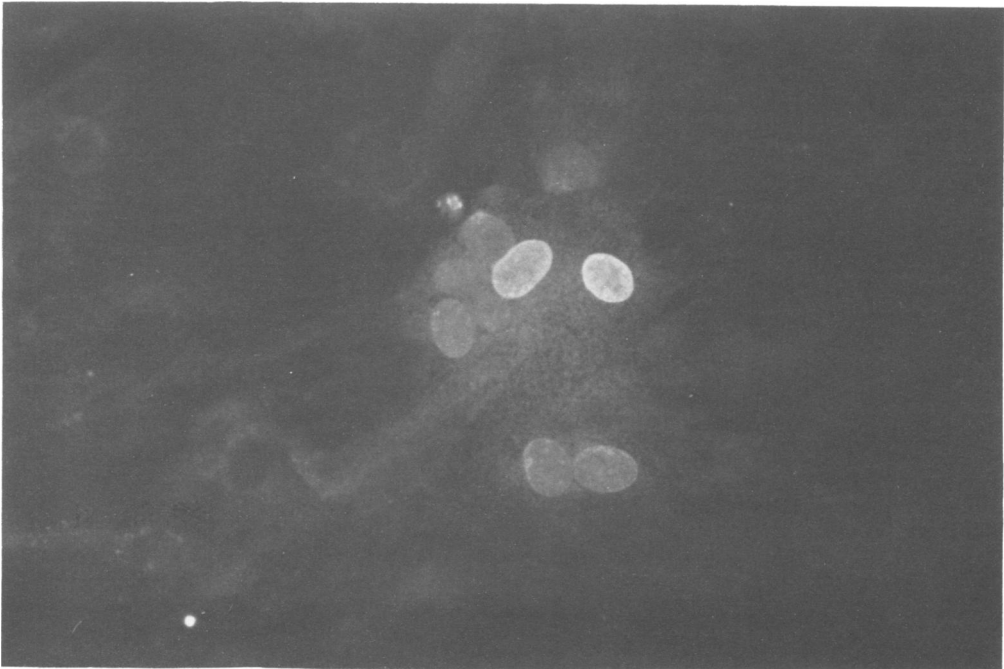


FIG. 2. Direct immunofluorescence of FCL infected with BSV showing a typical syncytium with nuclear fluorescence.

FBS were screened for BSV or RSV antibody by indirect immunofluorescence, using FCL monolayers infected with the appropriate virus. The sera were tested at dilutions up to 1:20. In addition, sera from calves and adult cows were also included at the above dilutions. All of the commercial sera contained BSV antibody, but no RSV antibody was detected (Table 1). In individual FBS there was a higher proportion of BSV-positive fetal sera (24%) than RSV-positive sera (14%). Of these RSV-positive sera only 50% were positive at a 1:20 dilution, the others being positive at a 1:5 dilution. The higher proportion of BSV-positive sera was also reflected in the adult sera (BSV, 82%; RSV, 64%). Six of the 12 RSV-positive sera were also positive for BSV. Only seven of the fetal sera analyzed were from fetuses younger than 150 days, and none of these contained detectable antibody.

BSV antibody titers in fetal sera with known high-titer parent. Twelve dam sera with BSV antibody titers of at least 1:10,000 were available with the corresponding positive fetal serum. These fetal sera were therefore titrated for BSV antibody. Despite the very high levels of antibody in the dam sera, the fetal fluorescent-antibody titers varied (Table 2). In addition, 18 dams which were negative for BSV antibody produced corresponding negative fetal sera.

Radial immunodiffusion of BSV-positive

TABLE 1. BSV and RSV antibody in commercial FBS and adult and individual FBS

Source	No. positive/no. tested (% positive)	
	BSV ^a	RSV ^a
Commercial pooled fetal sera	15/15 (100)	0/15 (0)
Individual fetal sera	39/159 (24)	12/83 (14)
Adult sera	82/100 (82)	76/119 (64)

^a Indicator virus.

fetal sera. The 12 positive fetal sera referred to (Table 2) were also examined quantitatively by radial immunodiffusion for the presence of IgG, IgM, and IgA. IgM was present in all of those sera which contained IgG. This suggests that antigenic stimulation had occurred in the fetus. However, there was no correlation between the level of IgG or IgM and the corresponding BSV antibody titer. It is also worth noting that serum 4, which was negative by radial immunodiffusion, nevertheless contained both BSV and RSV antibody when tested by immunofluorescence. Similarly, commercial fetal serum, which contained BSV antibody by immunofluorescence, contained no detectable immunoglobulin by radial immunodiffusion tests.

Neutralizing ability of commercial FBS. Clarke et al. (2) showed that BSV remained cell associated in bovine fetal cell culture and stated that it was not possible to obtain infectious cell-

TABLE 2. BSV antibody titers and immunoglobulin levels from fetuses borne of dams with high anti-BSV titers^a

Serum no.	Fluorescent-antibody titer ^b	Immunoglobulin (mg/ml)		
		IgG	IgM	IgA
1	640	0.05	0.03	— ^c
2	10	1.5	0.2	ND ^d
3	20	0.07	0.04	0.3
4	640	—	—	—
5	640	0.4	0.1	0.3
6	640	0.01	0.05	ND
7	10	0.01	0.06	ND
8	10	—	—	—
9	40	—	—	—
10	320	0.07	0.08	ND
11	20	—	—	—
12	1,280	0.05	0.03	0.1

^a Parent titer \geq 1:10,000.

^b Reciprocal of dilution beyond which fluorescence was not detected.

^c —, Immunoglobulin not detected.

^d ND, not done.

free virus. At first this was also our experience. However, this problem was overcome by the use of growth medium containing BSV-antibody-free newborn lamb serum instead of FBS. BSV-infected FCL were scraped into the growth medium and then centrifuged at $1,000 \times g$ for 10 min. The cell pellet was resuspended in Eagle medium containing 10% newborn lamb serum and sonically treated for 20 s. After centrifugation, the clear supernatant fluid was either frozen at -70°C or added to uninfected FCL and incubated for 3 days at 37°C . These monolayers were then subcultured again in medium containing newborn lamb serum. Usually, syncytia appeared within a further 3 days. BSV infection was confirmed by immunofluorescence. When FCL, infected with BSV in the presence of lamb serum, were then transferred to medium containing FBS, the extent of cytopathic effect became noticeably diminished during subsequent subculture. The effect was reversed when the infected cells were again cultured in medium with lamb serum, and because of the cytotoxic effect of the BSV it was difficult to maintain these cultures for more than 2 days.

DISCUSSION

There is only one report of the presence of BSV antibody in FBS, and this was demonstrated only after experimental in utero inoculation (19). There have been no reports of RSV antibody in FBS. Whereas it is generally accepted that maternal antibody transfer across the placenta does not occur in cattle, there is evidence that antibody to other viruses is present in fetal sera (10).

The direct isolation of BSV from cultured bovine fetal cells previously free of BSV antigen and the presence of antibody to BSV and RSV in FBS provide strong evidence that congenital virus infections do occur. The spontaneous appearance of BSV from apparently uninfected cells is likely to result from the integration of BSV into the cellular genome, utilizing reverse transcriptase (15). The experiments described in this report, however, do not entirely rule out the possibility of virus in the serum, although the presence of BSV antibody in the fetal serum would almost certainly neutralize free infectious virus. The failure to detect RSV antibody in batches of commercial serum was probably because the very low RSV antibody levels in individual samples would be diluted out in pooled commercial preparations. It seems unlikely that the BSV antibody in fetal sera arises from maternal antibody transfer across the placenta, because it is generally accepted that this does not happen in cattle (1) and BSV was isolated from FCL only after they had been subcultured several times. Thus, these cells presumably had been infected with BSV in utero. The variability in BSV fetal antibody levels from high-titer parents may also argue against maternal transfer; on the other hand, variability might be due to the time of collection of the fetal sera or even due to placental leakage. The detection of IgM-specific antibody in fetal sera will provide better evidence of antigenic stimulation by viruses. Quantitative analysis of immunoglobulin levels by radial immunodiffusion showed that the BSV antibody titers did not correlate with IgG, IgM, or IgA levels; thus, antigenic stimulation by other microorganisms may have occurred, and this is further borne out by the observation that some fetal sera had antibodies to both BSV and RSV. In addition, it is known that some FBS possess antibodies to other viruses such as bovine diarrhea virus (3), and unpublished observations in this laboratory showed that fetal sera have antibodies to parainfluenza virus type 3, infectious bovine rhinotracheitis, and rotavirus. That some fetal sera were negative by radial immunodiffusion but positive by indirect immunofluorescence probably reflects the higher sensitivity of the immunofluorescence technique.

It therefore seems that congenital infection with various unrelated viruses may occur, which could explain the presence of immunoglobulin in FBS (14). These observations have important implications for vaccine manufacturers, since the presence of virus-specific neutralizing antibody in fetal serum could participate in the production of chronic infections and may also mask the presence of latent infections already

present in the bovine cells. Finally, the presence of virus antibody in FBS could account for the difficulty sometimes encountered in the isolation of virus from infected animal specimens.

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