

## **Protection Against Respiratory Infection with Bovine Virus Diarrhoea Virus by Passively Acquired Antibody**

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### **ABSTRACT**

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Susceptibility to infection with bovine virus diarrhoea virus (BVDV) was compared for calves with varying amounts of specific antibody in their sera passively acquired from the ingestion of colostrum. Challenge consisted of intranasal exposure to a strain of BVDV isolated from an outbreak of respiratory disease. Resistance to infection, as judged by nasopharyngeal shedding of virus, was directly related to the titre of neutralizing antibodies in sera. Besides protecting against infection of the upper respiratory tract, passive antibody, which was mainly IgG1, also protected against viraemia and, to a lesser extent, leukopenia. In the presence of colostrum antibody, neutralizing and IgG1 antibody responses were apparently inhibited, but a specific IgG2 response occurred.

### **INTRODUCTION**

Infection of susceptible pregnant cows with non-cytopathogenic bovine virus diarrhoea virus (BVDV) results in a transient and often subclinical infection, but the virus can cross the placenta and replicate in the foetus. This may result in abortion, mummification and a variety of teratogenic defects (van Oirschot, 1983) or, if infection is during early gestation, replication in the immunologically immature foetus and the birth of a calf persistently infected with non-cytopathogenic BVDV and specifically immunotolerant to the virus (Malmquist, 1968; McClurkin et al., 1984; Brownlie et al., 1987). It is these animals that die from mucosal disease, an event triggered by superinfection with cytopathogenic BVDV (Brownlie et al., 1984; Bolin et al., 1985), the only proven mechanism by which this disease is produced. If infection occurs in the

second half of gestation, BVDV replicates in the foetal tissue, but the foetus is capable of mounting an immune response and the virus is controlled. Such calves are born free of virus, but with actively produced antibodies in their sera. Some show developmental defects (Braun et al., 1973; Brown et al., 1979; Done et al., 1980; Bielefeldt-Ohmann et al., 1982).

Healthy, normal calves or adult cattle experience a transient infection with either cytopathogenic or non-cytopathogenic strains (biotypes) of the virus and the neutralizing antibody responses with either biotype are similar (Nuttall et al., 1980; Howard et al., 1987a). Infection is characterized by shedding of the virus from the respiratory tract, a brief viraemia and leukopenia, sometimes an elevated temperature (Pritchard, 1983; Thomson and Savan, 1963; House and Manley, 1973; Nuttall et al., 1980) and may be associated with, and contribute to, outbreaks of respiratory disease and enteritis in young calves (Pritchard, 1963; Thomas et al., 1977; Stott et al., 1980; Baker, 1987). In recent studies (Stott et al., 1987; Howard et al., 1987b), the virus was implicated in a number of episodes of respiratory disease. The role of BVDV in these infections could be that of an immunosuppressive agent producing a period of increased susceptibility to other pathogenic microorganisms (Potgeiter et al., 1984a, b; Baker, 1987).

Following recovery from infection and production of an antibody response, cattle are considered to be immune to re-infection (Pritchard, 1963) and studies have shown that passive antibody provides protection against systemic BVDV (House and Manley, 1973; Shope et al., 1976). However, immunity to respiratory disease has not been addressed and experiments are described here which determine whether antibody passively acquired from colostrum, predominantly IgG1, protected against respiratory challenge and infection.

## MATERIALS AND METHODS

### *Calves*

Nine normal, healthy, conventionally reared, colostrum-fed Friesian or Friesian-cross calves were selected and placed in three groups based on the levels of specific antibody in their sera when aged ~ 12 days and moved into isolation facilities. They were fed on a mixed milk-replacer and solid diet during the experiment and challenged when 28–53 days old.

A further group of three conventionally reared Friesian or Friesian cross calves were held in isolation until aged ~ 6 months. By this time, none had detectable levels of antibody to BVDV in their sera.

Calves were challenged by inoculating them intranasally with 5 ml of cell culture fluid containing  $3 \times 10^8$  TCD<sub>50</sub> of virus. Serum samples were collected weekly for 12 weeks. Nasopharyngeal swabs (Thomas et al., 1977) were taken into cell culture medium prior to challenge and on Days 3, 5, 6, 7 and 10 post-challenge. Blood was sampled for virus on Day 6.

In order to detect leukopenia, cell counts were performed on five blood samples from each calf, taken in the week before challenge, to establish individual baselines. A significant leukopenia was considered to have occurred if the cell count was more than two standard deviations less than the pre-challenge mean in at least two consecutive samples assayed during the 14-day period post-challenge.

### *Virus strains*

A non-cytopathogenic strain of BVDV (11249nc) isolated from calves during an outbreak of respiratory disease (Stott et al., 1987) was used as the challenge virus. Cytopathogenic strain NADL was used as the source of antigen for some antibody determinations. Both BVDV strains were cultured in calf testis (CT) cells which were free of adventitious BVDV and maintained in Eagle's basal medium (Gibco Ltd.) containing irradiated or heated (56°C, 30 min) foetal calf serum and lactalbumin hydrolysate.

Titration of virus from calf tissues were made in CT cell cultures and stained by an immunofluorescent method (Clarke et al., 1987).

### *Antibody assays*

Titres of neutralizing antibody to the non-cytopathogenic strain 11249nc were determined as described previously (Howard et al., 1987a). Levels of specific IgG, IgG1 and IgG2 antibody to BVDV were measured by ELISA as described by Howard et al. (1985), but with tetramethylbenzidine (Sigma) as substrate and with strain NADL as the source of antigen.

## RESULTS

### *Relationship between serum antibody and susceptibility to infection*

The effect of passively acquired colostral antibody on respiratory infection with BVDV is given in Table 1. Three calves (Group C; A243, A245, A248) had no antibody detectable by the neutralization assay on the day of challenge to strain 11249nc. All shed virus from the respiratory tract for at least 8 days. A viraemia was detected in each, as was a leukopenia. Three calves (Group A; A675, A676, A679) had neutralizing antibody titres of 1280 on the day of challenge. Virus was not isolated from nasopharyngeal swabs taken from any calves, viraemia was not detected and a leukopenia was noted in only one. Three calves (Group B; A236, A242, A680) had intermediate amounts of colostrally acquired antibody. Viraemias were not detected, but a leukopenia was evident in two and one of these, A680 (the calf with the lowest titre), shed virus.

Virus was isolated from all three of the older antibody-negative calves (Group

TABLE 1

The effect of maternal antibody on susceptibility to respiratory infection with BVDV

Group	Calf	Age (days)	Antibody <sup>1</sup>	Leukopenia	Viraemia <sup>2</sup>	Isolations from nasopharynx on Day <sup>3</sup>					
						0	3	5	6	7	10
A	A675	29	1280	—	—	—	—	—	—	—	—
	A676	29	1280	—	—	—	—	—	—	—	—
	A679	41	1280	+	—	—	—	—	—	—	—
B	A236	35	480	—	—	—	—	—	—	—	—
	A242	28	240	+	—	—	—	—	—	—	—
	A680	34	60	+	—	—	1.2	—	2.7	2.2	1.7
C	A243	53	<10	+	2.7	—	—	1.2	2.2	2.2	1.2
	A245	52	<10	+	2.2	—	—	1.7	2.2	1.7	1.2
	A248	48	<10	+	3.7	—	1.7	2.2	2.7	2.2	2.2
D	X167	188	<10	+	—	—	0.7	ND	1.7	ND	0.7
	X484	177	<10	+	—	—	0.7	ND	2.2	ND	—
	X651	187	<10	+	0.7	—	0.7	ND	2.2	ND	—

<sup>1</sup>Neutralizing antibody titre on day of challenge.<sup>2</sup>Log<sub>10</sub> TCD<sub>50</sub> ml<sup>-1</sup> blood; — = < 0.5.<sup>3</sup>Log<sub>10</sub> TCD<sub>50</sub> ml<sup>-1</sup> suspension; — = < 0.2, ND = not done.

D; X167, X484, X651; Table 1), but there was an indication that the duration of shedding was reduced compared to the younger antibody-negative calves since virus was not isolated from two of these calves on Day 10 post-infection. All three older calves showed a transient leukopenia, but only one was shown to be viraemic.

#### *Antibody responses following infection*

The titres of neutralizing antibody in sera of nine calves (Groups A, B and C) on the day of challenge and 6 and 12 weeks later is compared in Table 2. The three calves (Group C) that had no antibody when exposed to BVDV produced a neutralizing antibody response with titres increasing by at least 1000-fold. Calves with intermediate levels of colostrum antibody mounted a neutralizing antibody response, but this was less marked. None of the three calves (Group A) that had neutralizing antibody titres of 1280 produced an increase in antibody titre following challenge. However, neither did the titre appear to decline over the 12-week period of the experiment, as would be expected for colostrally acquired antibody.

The mean IgG antibody titres measured by ELISA are compared for the

TABLE 2

Effect of passively acquired maternal antibody on neutralizing and specific IgG1 and IgG2 antibody responses in calves following BVDV infection

Assay	Calf <sup>1</sup> group	Antibody titre <sup>2</sup> on Week		
		0	6	12
Neutralization	A	3.1	3.1	3.1
	B	2.3 ± 0.46	3.2 ± 0.35	3.2 ± 0.10
	C	< 1.0	3.8 ± 0.17	4.2 ± 0.24
ELISA - IgG1	A	3.8 ± 0.28	3.6 ± 0.12	3.6 ± 0.15
	B	3.3 ± 0.09	3.6 ± 0.27	3.5 ± 0.23
	C	1.5 ± 0.74	4.2 ± 0.13	4.3 ± 0.04
ELISA - IgG2	A	2.0 ± 0.49	2.9 ± 0.32	3.2 ± 0.30
	B	2.1 ± 0.08	3.1 ± 0.32	3.3 ± 0.18
	C	< 1.4	3.4 ± 0.08	3.8 ± 0.34

<sup>1</sup>A, Calves A675, A676, A679; B, Calves A236, A242, A680; C, Calves A243, A245, A248.

<sup>2</sup>Titre ± SD. Geometric mean; log<sub>10</sub>.

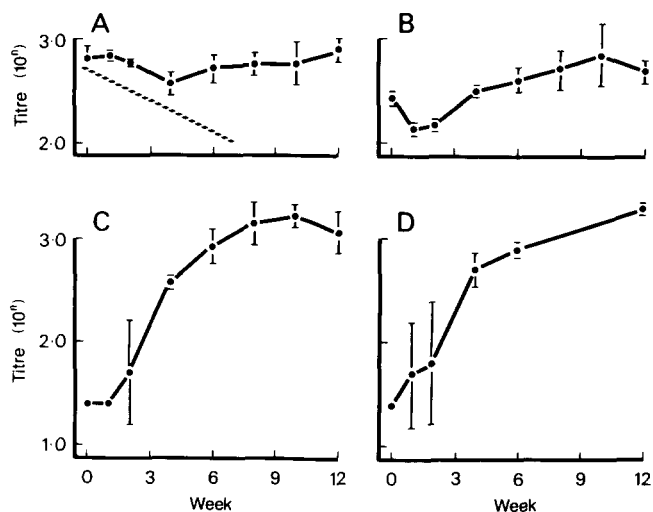


Fig. 1. Effect of passively acquired maternal antibody on the specific antibody response, detected by ELISA, following infection with BVDV. Group A = Calves A675, A676, A679; Group B = Calves A236, A242, A680; Group C = Calves A243, A245, A248; Group D = Calves X167, X484, X651. Geometric mean titres for groups and standard deviations are shown. Calves were infected on Week 0. The hatched line in A represents the expected rate of decline for antibody with a half-life of 21 days.

three groups of calves (A, B and C) and for the older antibody-negative animals (Group D) in Fig. 1. The response of the antibody-negative animals aged  $\sim 50$  days (Group C) and the calves aged  $\sim 6$  months (Group D) was very similar. Thus, no evidence for neonatal hyporesponsiveness to BVDV was noted. As with the neutralization assay, the calves with intermediate and high levels of colostral antibody responded less extensively. In both groups, there appeared to be an initial reduction in titre and this decline for Group A calves (Fig. 1) was similar to the expected half-life of IgG1.

In order to analyse further the antibody response following infection, the specific IgG1 and IgG2 antibody titres to BVDV were measured by ELISA on the day of challenge and after 6 and 12 weeks (Table 2). As was expected, the majority of the colostral antibody was IgG1. Little or no IgG1 response was detected in the six calves with colostral antibody (Groups A and B), but all nine calves produced a specific IgG2 antibody response.

## DISCUSSION

Passively acquired specific antibody to BVDV proved to be effective in protecting against respiratory infection, as judged by shedding from the nasopharynx. Six antibody-negative calves shed virus after challenge, but only one with antibody did so. Calves with a titre of  $\geq 240$  were protected, but those with a titre of  $\leq 60$  were not. This implies that immunity to respiratory infection can be forecast from serum titres.

The predominant immunoglobulin in bovine colostrum is IgG1 (reviewed by Butler, 1983). In adult cow sera, the source of the majority of immunoglobulin in colostrum, high titres of specific IgG1 and IgG2 antibody to BVDV have been detected, but not IgA or IgM (Howard et al., 1985). Thus, the passive transfer of specific IgA or IgM antibody is unlikely to occur in anything other than very small amounts. Furthermore, since the half-life of bovine IgA is  $\sim 3$  days (Butler, 1983) and the calves in Groups A and B had an average age of 33 days when challenged, any small amount of this immunoglobulin that was ingested would have been reduced by  $\sim 2000$ -fold by the time the calves were inoculated with virus. A similar argument applies to IgM which has a half-life of  $\sim 4$  days. Thus, these results can be used to infer that IgG derived from serum protects from respiratory challenge with BVDV and that high levels of local IgA are not necessary.

The decline in IgG antibody over the first 4 weeks in Group A (Fig. 1) is consistent with the usual half-life of maternally derived antibody. Also, the ratio of specific G1 to G2 in animals in Group A indicates that most antibody was IgG1, an observation consistent with its derivation from colostrum. In contrast, following infection, the IgG1 and IgG2 titres were similar, e.g. Group C in Table 2, an observation consistent with previous reports (Howard et al., 1985). These findings are all consistent with the view that the antibody present

at the time of challenge was maternally derived and not a result of prior infection.

Clear evidence that IgG antibodies given systemically protect against respiratory infection come from studies in mice in which monoclonal antibodies inoculated intravenously protected against intranasal challenge with respiratory syncytial virus (Taylor et al., 1984). Evidence for systemic immune responses protecting calves are implied from the reports that parenteral inoculation of inactivated vaccines induced immunity to respiratory syncytial virus (Stott et al., 1984) and parainfluenza type 3 virus (Probert et al., 1987). The relative contribution of cell-mediated and immunoglobulin-mediated mechanisms are difficult to assess from these observations; however, locally produced IgA is most unlikely to be involved.

Passively acquired colostrum antibody protected against viraemia and to a lesser extent leukopenia, but nasal shedding of virus occurred in Calf A680 despite an antibody titre sufficient to prevent a detectable viraemia. A previous report noted that passive antibody given subcutaneously alleviated the leukopenia and raised temperature that resulted from an intranasal challenge with BVDV (House and Manley, 1973). Another (Shope et al., 1976) noted that passively acquired colostrum antibody prevented a fatal viraemia in calves immunosuppressed with dexamethasone.

High levels of colostrum antibody suppressed antibody responses measured by both neutralization assays and ELISA as expected (House and Manley, 1973; Brar et al., 1978). However, after infection the decline in titre of maternal antibody appeared to be slowed and did not give the usual half-life of 21 days observed generally in cattle (Butler, 1983) and for BVDV specifically (Brar et al., 1978; Menanteau-Horta et al., 1985). Studies of the specific IgG2 antibody response indicated seroconversion, despite the presence of maternal IgG1 antibody. This observation indicates that inhibition of antibody responses may, to some extent, be isotype specific and that although passively acquired IgG antibody is highly effective in reducing the extent of respiratory infection, it does not make animals totally refractile to BVDV. Cattle appear to produce recurrent serological responses to natural infection with BVDV (Brownlie et al., 1987) which also indicates that immunity following natural infection is of limited duration. The calves that had no antibody to BVDV at the time of challenge produced a marked antibody response. The titres rose slowly to reach maxima some 8–9 weeks after exposure to virus. The long period taken for antibody titres to reach a plateau could be due to viral persistence in the calves for a period longer than indicated by nasopharyngeal shedding.

The main route of spread of BVDV within a group of calves is probably via respiratory infections. These may originate from both persistently viraemic animals or by lateral spread within a group of susceptible calves. From the results presented here, ingestion of colostrum containing antibody would clearly

be expected not only to reduce BVDV mediated disease directly, but also to contain the build up and spread of virus within a group of calves by controlling shedding of virus from infected animals and reducing lateral spread. It can also be argued from these findings that parenteral vaccination with inactivated BVDV should prevent respiratory infection with this virus providing an adequate titre of antibody in serum is achieved.

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