

## EVIDENCE FOR BIOTYPE-SPECIFIC EFFECTS OF BOVINE VIRAL DIARRHOEA VIRUS ON BIOLOGICAL RESPONSES IN ACUTELY INFECTED CALVES

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### **ABSTRACT**

The relationship between the two biotypes of bovine viral diarrhoea virus (BVDV) and the biological responses they induce was studied in 3- to 6-month-old calves inoculated intranasally with a homologous pair of non-cytopathic and cytopathic strains. Marked differences in virological and serological events occurred following exposure to a specific BVDV strain. The non-cytopathic biotype was frequently recovered from nasal secretions and blood cells during the first 28 days post-inoculation whereas the cytopathic counterpart was detected infrequently in nasopharyngeal swabs only. There was no correlation of the recovery of infectious virus *in vivo* with the biotype-specific neutralizing humoral immune response. Furthermore, seroconversion did not correlate with resistance to reinfection as judged by the transient viraemia and/or shedding of virus observed in a challenge experiment.

Bovine viral diarrhoea virus (BVDV) belongs to the genus *Pestivirus* in the family *Flaviviridae* (Wengler, 1991). The strains of BVDV are grouped into two biotypes, non-cytopathic (ncp) and cytopathic (cp), according to their behaviour in cell culture (Baker, 1987) and the rearrangements in the non-structural NS23/NS3 coding gene (reviewed in Meyers & Thiel, 1996). The ncp biotype is commonly isolated from cases of acute infection and is invariably present in animals born persistently viraemic because of infection in utero (Liess *et al.*, 1974 ; McClurkin *et al.*, 1984). In contrast, the cp biotype is usually found in association with a highly fatal form of BVD known as mucosal disease (MD). MD may be experimentally induced in persistently infected animals by superinfection with an antigenically homologous cp virus (for review see Brownlie, 1991).

Although our knowledge of the genomic and antigenic characteristics of the two biotypes derived from the same pair of BVDV strains has increased, only partial information is available to date on the relationship between these two biotypes and the effects they cause *in vivo*. This study was designed to examine the virological and serological events in calves subsequent to exposure to both biotypes from the same antigenic pair of BVDV. Moreover, the protective effect of biotype-specific responses was investigated in a challenge study. The inocula for the experiments were the homologous pair of ncp and cp biotypes of strain Pe515 originally isolated from a field case of MD (Brownlie *et al.*, 1984). The procedure for the preparation of BVDV strain Pe515 biotypes was described previously (Lambot *et al.*, 1997). Eighteen calves about 3 months old, free of both BVDV antigen and BVDV antibody, were randomly allotted into two groups of 7 (A and B) and one group of 4 (group C) animals in isolation units. Calves in group A were exposed to the cp biotype of Pe515 and calves in group B were inoculated with the homologous ncp biotype of Pe515. Animals in group C served as controls and were inoculated with culture medium from BVDV-free calf testis (CT) cells. Inoculation was by the intranasal route with 5 ml of culture medium containing  $10^8$  TCID<sub>50</sub> of the respective virus administered in each nostril. For assay of virus recovery, nasopharyngeal swabs and buffy-coat cells from blood samples were taken on a daily basis for 21 days post-inoculation and weekly for the next 3 weeks. All samples underwent one freeze–thawing cycle before being seeded on to CT cell cultures. Each sample was passaged three times at 7 day intervals and then tested for BVDV by the indirect immunofluorescent method (Mignon *et al.*, 1992).

Following inoculation of the ncp biotype, all calves shed virus from the respiratory tract for at least for 1 day during the subsequent 42 day observation period and six of them became transiently viraemic in the same period (group B in Fig. 1). In contrast, the overall frequency of infectious virus recovery was considerably less in group A: only two calves exposed to the homologous cp biotype shed virus for 1 day post-inoculation and none was viraemic. In order to determine if the higher efficiency of ncp virus recovery compared to that of the cp counterpart might be explained by differences in antibody levels between the two groups of animals, we examined the kinetics of the neutralizing antibody response of calves exposed to either ncp or cp virus. Neutralization tests were performed against the Pe515 cp biotype on bovine turbinate cell cultures in six-well plates as described previously (Lobmann *et al.*, 1984). Two patterns of antibody response were evident depending on the infecting virus biotype (Fig. 2). Calves inoculated with ncp virus started to respond 1 to 2 weeks earlier than those exposed to the cp biotype. Furthermore, the mean

neutralizing titre in these groups peaked at a significantly higher level ( $P < 0.05$ ; ANOVA) than that reached in calves given the cp virus. This clear difference between the groups of calves was detectable around 6 weeks post-inoculation and persisted up to the end of the experiment. Therefore, the high incidence of infectious particles in ncp-inoculated calves did not reflect a delay in establishing a humoral immune response to ncp virus. In order to gain further insight into the role of biotype in BVDV infection, the efficacy of biotype-specific responses in protecting animals against challenge was investigated. Ninety-one days after the first exposure, all calves were intranasally challenged with Pe515 ncp or cp according to the procedure described by Lambot *et al.* (1997). Based on their inoculation histories, calves were divided into six subgroups: two subgroups in primary infection (named C1 and C2) and four subgroups in challenge (A1-2 and B1-2). As shown in Table 1, the presence of high levels of neutralizing antibody in sera from infected calves did not prevent their re-infection with the same strain. We detected limited virus multiplication in some seropositive calves after challenge, irrespective of the biotype of Pe515 inoculated. It was noteworthy that five of the seven calves initially exposed to cp virus underwent a transient viraemia and/or virus shedding after the challenge, but only one calf first inoculated with ncp virus did so. This difference according to the initial exposure was not statistically significant but worthwhile mentioning. A possible explanation might be that the amounts of specific antibodies in calves at the time of challenge influence the amount of virus spread as previously observed (Howard *et al.*, 1989 ; Shope *et al.*, 1976). In our study, calves exposed initially to cp virus have lower amounts of BVDV-specific antibodies in their sera. Whether the biotype used in an acute infection can influence the level of protection to BVDV therefore deserves further investigation. Furthermore, the incidence of infectious virus observed in groups C1 and C2 was similar to that seen in the first infection (Table 1).

This study is, to our knowledge, the first comparison of biological responses induced by post-natal BVDV infection of calves with ncp and cp biotypes derived from the same strain. From our results, two interesting findings emerge. First, because significant differences appeared in the patterns of viraemia and shedding of virus between the ncp and cp biotypes, we speculate that the biotype could play a role in distribution of virus in host tissues during the course of infection. This hypothesis is strengthened by the clinical scores results from infected calves, which indicated no variation in virulence between the two biotypes of strain Pe515 as both compared to the control animals (data not shown). Furthermore, it was unlikely that cell culture passage attenuated the ability of cp virus to propagate *in vivo* compared to the ncp counterpart because both strains in the current study share the same passage histories. Our suggestion of tissue tropism differing between biotypes is also supported by the apparent restriction of the cp biotype to gut lymphoid tissue whereas the ncp virus is found in the respiratory tract, blood cells and blood-associated organs (Clarke *et al.*, 1987; Bielefeldt Ohmann, 1988). Interestingly, even among heterologous pairs of strains with different passage histories, inoculated into calves at various doses, patterns of virus shedding and/or viraemia similar to those observed in this study have been reported (Nuttall *et al.*, 1980; Bezek *et al.*, 1994). These results were, however, difficult to interpret. Our present comparative study of antigenically homologous strains with similar passage histories suggests that only the biotype used as inoculum may affect the outcome of an infection with regard to virus

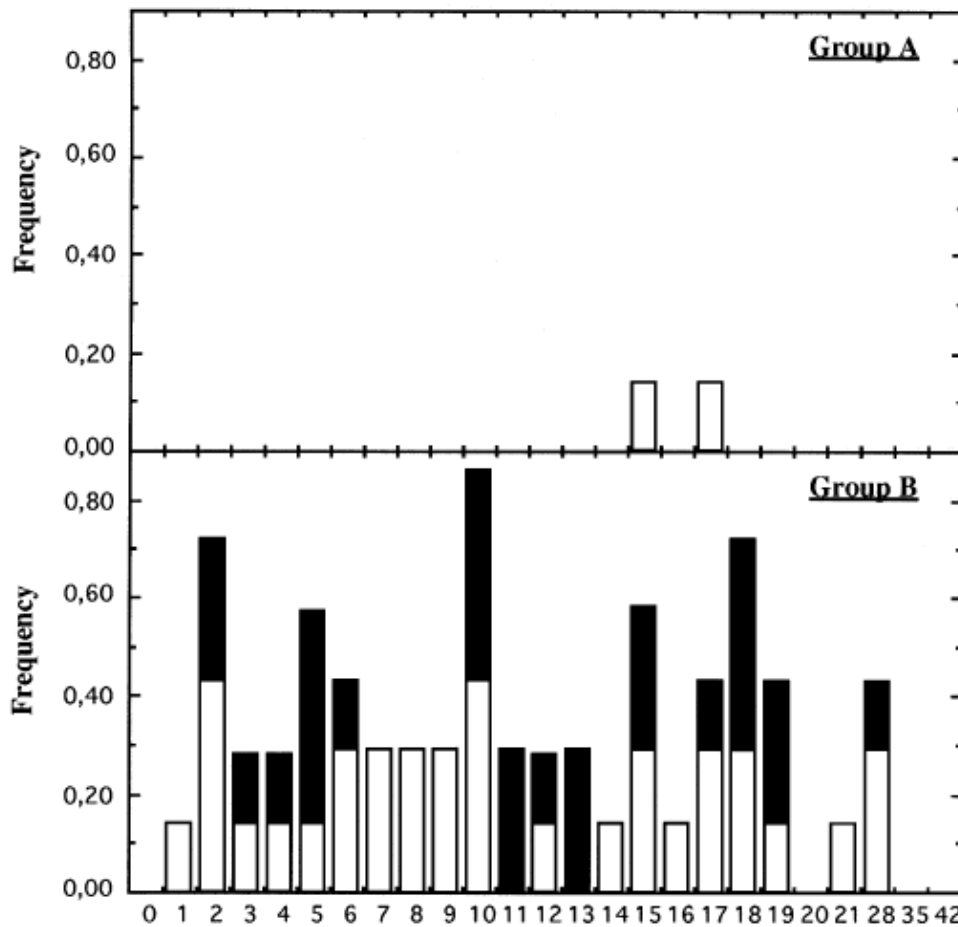
distribution *in vivo*. In other words, the hypothesis that the two different biotypes have a different distribution might be a general phenomenon of BVDV infections.

The second key finding of this report was that the unilateral recovery of infectious ncp virus in calves following exposure did not correlate with the induction of low levels of neutralizing antibody, because the neutralizing antibodies appeared earlier and were high-titre with the ncp strain compared to the homologous cp biotype. The neutralizing response against the ncp virus may merely reflect the greater antigenic exposure due to the greater multiplication of this biotype in animals. Previous work showed that the concentration of BVDV antigens greatly influences the titres of antibody to virus in vaccination experiments (Brownlie *et al.*, 1995). Alternatively, one might hypothesize that there is differential regulation of the BVDV-specific immune response according to the biotype. In a recent study, we observed a dissociation of the humoral and cellular components of the immune response induced by either biotype in cross-infection experiments (Lambot *et al.*, 1997). We are exploring the possibility that these differences in the immune response against either biotype may be due to a Th1/Th2-like regulatory mechanism.

One of the features of BVDV infection is the apparent difference in frequency of ncp or cp biotype isolation in cattle. The ncp biotype accounts for more than 90 % of infections in cattle originating either from persistently viraemic animals or through lateral spread within a group of susceptible calves (Dubovi, 1992). In contrast, the cp biotype is sparse and isolated mainly from fatal cases of MD (Clarke *et al.*, 1987; Moennig *et al.*, 1990). Our virological data offer an explanation to this phenomenon. Animals exposed to the ncp strain are viraemic and excrete more virus nasally than the cp counterpart. They are therefore more prone to transmit the virus in the herd. This would suggest that the cp biotype may be an epidemiological dead end because the main route of spread of BVDV within a group of calves is via respiratory infection (Bielefeldt Ohmann, 1983; Wentink *et al.*, 1991). In summary, our data highlight the biological effect of the BVDV biotype on virological and serological events during acute infections of calves. Further investigations into the mechanisms which underlie such effects need to be carried out.

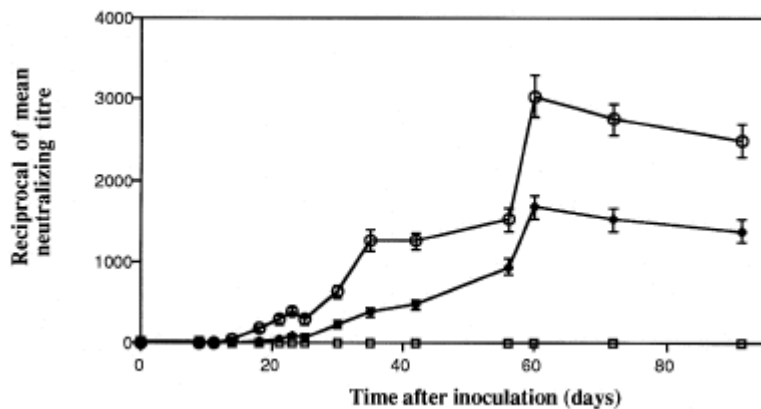
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**Figure 1.** Days after inoculation



Légende de la figure. Frequency of BVDV isolation from blood samples (■) and nasopharyngeal swabs (□) from calves after inoculation. No virus was recovered from control animals (not shown).

**Figure 2**



Légende de la figure. Neutralizing antibody response of calves inoculated with cp (◆) or ncp (○) biotypes of BVDV strain Pe515. Mock-infected animals served as control (□).

**Table 1.** Virus isolation from nasopharyngeal swabs and blood samples in the challenge experiment

Subgroup	Animal no.	Biotype used in :		Virus isolation*	
		Infection	Challenge	Blood cells	Nasal swab
A1	9118	CP	CP	10	17
	111			–	–
	9123			19	–
A2	7492	CP	NCP	+(11,13,14)	9
	257			9,14	–
	9275			–	–
	264			–	7,28
B1	277	NCP	NCP	–	–
	259			–	–
	265			–	–
	9120			–	–
B2	7491	NCP	CP	–	14
	9106			–	–
	272			–	–
C1	258	None	CP	–	–
	273			–	2
C2	267	None	NCP	+(4,5,6,7,8,9,16,19)	+(3,4,5,6,7,8,9,17)
	266			+(6,7,21,28)	+(2,5,6,7)

Légende de la figure. Number(s) in parentheses represent the day(s) when sampling was positive for BVDV.

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