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Systemic vaccination with inactivated bovine virus diarrhoea virus protects against respiratory challenge

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Abstract

Inactivated bovine virus diarrhoea virus, strain 11249nc, inoculated subcutaneously three times with Quil-A into calves protected against intranasal challenge with the same strain. Virus was isolated from nasopharyngeal swabs taken 4 to 8 days post challenge and blood taken 4 to 6 days post challenge from control calves but not from vaccinated calves. A second strain of virus, Ky1203nc, was selected on the basis of previously established data on its antigenicity and the amount of viral antigen produced by five cell cultures compared using an ELISA. Cultures of one cell line, MDBK, yielded a greater amount of viral antigen than the others. Strain Ky1203nc grown in MDBK cells was inactivated with β-propiolactone, mixed with adjuvant and used as a vaccine inoculated into calves subcutaneously three times. All of 5 calves were protected against intranasal challenge with a heterologous strain. In contrast virus was isolated from nasopharyngeal swabs taken from 5 control calves and from the blood of 4 controls. All 5 control calves, but none of the vaccinates, had a leukopenia after challenge. We conclude that the selected strain and system of vaccine preparation provide an effective means of protecting calves against respiratory infection and that live vaccines are not required to protect against challenge via the respiratory tract.

Keywords: Bovine virus diarrhoea virus; Cattle, bovine virus diarrhoea virus; Vaccination

1. Introduction

Bovine virus diarrhoea virus (BVDV) is a non-arthropod-borne member of the Flaviviridae in the genus *Pestivirus*. Two biotypes have been isolated, one cytopathogenic (cy) and the other non-cytopathogenic (nc). Serological evidence indicates that the virus is endemic in cattle worldwide (Harkness et al., 1978; Stott et al., 1980; Meyling, 1984; Bolin et al., 1985) and it is the nc biotype that is evident in animals that are not suffering fatal

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mucosal disease (Malmquist, 1968; Brownlie et al., 1984; Brownlie et al., 1991; Brownlie 1991). Experimental infection of normal cattle that have not previously been infected with nc-BVDV results in a transient self-limiting infection that is typically characterised by a period of 2–10 days, beginning 3–5 days after infection, when viraemia and nasal shedding of virus is evident. A leukopenia occurs that is maximal between days 3–7 but the infection is frequently clinically unapparent (Baker 1987; Brownlie 1991). Evidence has been presented that infection with BVDV predisposes calves to secondary bacterial infection and combined with other viruses gives a more severe disease than either virus alone (Greig et al., 1981; Potgeiter et al., 1984a; Potgeiter et al 1984b; Wray and Roeder 1987).

The nc-virus has been associated with outbreaks of respiratory disease in calves, often in association with other microorganisms (Stott et al., 1980; Baker 1987) and may contribute to the respiratory disease complex in cattle. One mode of spread of virus through a herd thus appears to be laterally from animals infected in their respiratory tract.

Infection of seronegative pregnant cows with nc-BVDV, but not cy-BVDV, also results in a transient infection in the cow but the virus can cross the placenta and infect the foetus (Baker 1987; Brownlie 1991; Brownlie et al., 1991). A number of sequelae are possible. Infection may result in abortion or the birth of a congenitally damaged calf. If post 100–110 days gestation, by which time the calf is immunologically competent, a specific antibody response is produced *in utero* by the calf. If the foetus is in the first trimester foetal infection results in a specific tolerance to the virus and the birth of a calf that is persistently viraemic (Baker 1987; Brownlie 1991). These animals may subsequently die of mucosal disease if superinfected with the cytopathic biotype of the virus (Brownlie et al., 1984; Bolin et al., 1985). The cattle that are persistently viraemic with nc-BVDV also provide a reservoir of infection within a herd, they shed high titres of virus from the nasopharynx providing virus that can circulate laterally through a population by the respiratory route.

Previous studies have shown that antibody given passively protects against respiratory challenge with BVDV (Howard et al., 1989) and that inactivated vaccines protect against respiratory disease caused in calves by respiratory syncytial virus and mycoplasma (Stott et al., 1987; Howard et al., 1987b). It therefore seemed reasonable to assume that an inactivated BVDV vaccine that produced an effective response as judged by antibody titre should produce protection against respiratory challenge with this virus. The purpose of this study was to select a strain of virus that was antigenically sufficiently representative that it would protect against most strains, to identify a system for effective production of viral antigen and to determine whether effective immunity could be induced with inactivated virus.

2. Materials and methods

2.1. Virus strains

BVDV strain 11249nc, isolated from calves during an outbreak of respiratory disease (Stott et al., 1987), was used as the challenge virus. This strain was used previously to assess the effect of passive antibody on respiratory infection and was chosen as it gave more extensive nasal shedding than many strains (Howard et al., 1989). Strain Ky1203nc was

isolated from an outbreak of mucosal disease (Clarke et al., 1987) and was selected as a potentially effective vaccine strain from previous investigations showing it to cross react extensively with other BVDV isolates (Howard et al., 1987a). It grew to a relatively high titre in cell culture (Clarke, unpublished observations) and allowed a heterologous challenge to be assessed.

For challenge and isolation from bovine samples, strain 11249nc was grown in calf testis (CT) cells which were free of adventitious virus and maintained in Eagles basal medium (Gibco Ltd.) containing heated (56°C, 30 min) foetal calf serum and lactalbumin hydrolysate. Titrations of virus were made in CT cell cultures and stained by an immunofluorescent method. The technique is such that a positive isolation represents a virus titre of $> 10^{0.5}$ TCD_{50} per ml.

2.2. *Antibody assays*

Neutralising antibody and ELISA titres were measured as described previously (Howard et al., 1987a). Neutralising assays were with strain 11249nc and ELISA with antigen from strain NADL.

2.3. *Vaccine preparation*

Strain 11249nc was grown at 36°C for 7 days in 11 roller containers seeded with CT cells in 50 ml of medium. Virus was inactivated by addition of 50 μl of β-propiolactone (Sigma) and 0.5 ml N NaOH followed by rolling for 3 h at 36°C. The suspension was assayed for infectious virus and stored at −20°C. Strain Ky1203nc was grown in rollers seeded with MDBK, free of adventitious virus, (European Animal Cell Culture Collection, Salisbury, UK) in 125 ml of medium at 36° for 7 days and virus inactivated in the same manner. The virus titre in non-inactivated cultures was $10^{6.5}$ TCD_{50} per ml. A standard dose of vaccine was 4 ml of culture fluid containing 1 mg Quil-A (Superfos A/S, Denmark) (Howard et al., 1987b).

To compare the yield of BVDV antigen in different cells strain Ky1203nc was inoculated into rollers seeded with; CT, bovine Nasal Mucosa line 5 (NM5, E.J. Stott unpublished), Embryo bovine lung cells (EBL, Chanter et al., 1986), a bovine Lung-wash cell line (LWC) or MDBK cell cultures. After 6 days the cells were washed with phosphate buffered saline (PBS), scraped from the glass and extracted in 2 ml of 1% Nonidet P40 incubated at 37°c for 60 min. Extracts performed in duplicate were titrated for antigen content using the ELISA described previously (Howard et al., 1985). Antigen bound to microtitre plates was detected with gnotobiotic porcine-anti-BVDV serum diluted 1/100 in PBS-2%foetal calf serum followed by horse radish peroxidase coupled rabbit-anti-pig serum (Howard et al., 1986).

2.4. *Calves*

Normal, healthy, conventionally reared Friesian or Friesian-cross calves were held in isolation until maternally derived antibody had declined to undetectable levels and were aged about 6 months. None were persistently viraemic. In the first experiment five calves

were inoculated subcutaneously with 4 ml of inactivated 11249nc vaccine with adjuvant on weeks 0, 3 and 6. Four calves were inoculated with adjuvant alone. All 9 calves were challenged intranasally, on week 9, with 5 ml of strain 11249nc containing 10^6 TCD_{50} per ml. Blood was taken into EDTA and nasopharyngeal swabs were taken into transport broth for virus isolations (Howard et al., 1989) prior to challenge and on days 4, 6, 8, and 11 post challenge. Blood for serology was taken on weeks 0, 3, 6, 9 and 12.

In the second experiment five calves were inoculated with 4 ml of inactivated Ky1203nc vaccine containing Quil-A, five were inoculated with 0.4 ml of vaccine diluted in 3.6 ml of medium prior to addition of Quil-A and five calves were inoculated with adjuvant alone. These inoculations were on weeks 0, 3, and 6. All 15 calves were challenged intranasally on week 8 with strain 11249nc. Blood and nasopharyngeal swabs for virus isolation and blood for leukocyte counts were taken on the day of challenge and on days 0, 3, 6, 8, 10 and 12 post challenge. Blood for leukocyte counts was also taken 3 and 1 days prior to challenge.

3. Results

3.1. Inactivated strain 11249nc protected against homologous challenge

The mean neutralising antibody titre against strain 11249nc in a group of 5 calves increased from $<10^1$ to $>10^3$ after inactivated BVDV strain 11249nc was inoculated subcutaneously (Table 1). After intranasal challenge virus was isolated from nasopharyngeal swabs taken from all four control calves and from the blood of three of the calves but virus was not isolated from nasopharyngeal swabs or blood taken from any of the vaccinated animals (Table 2). These results established that it was possible to protect against a homologous challenge given by the respiratory route following systemic vaccination.

3.2. Comparison of cells for production of viral antigen

Extracts of cells infected with strain Ky1203nc were titrated for viral antigen. Four of the cell cultures yielded similar amounts of antigen while MDBK cells gave a titre of antigen that was about 16 fold higher than the other four (Fig. 1).

Table 1
Neutralising antibody[a] response produced by killed BVDV vaccine

Group	No. of calves	Week[b]				
		0	3	6	9	12
Non-vaccinated	4	<0.7	ND	ND	0.93±0.45	2.94±0.09
Vaccine 11249nc	5	<0.7	1.17±0.56	2.88±0.27	3.12±0.22	3.18±0.14

[a] Tested against strain 11249nc. Mean titre ($10^n \pm SD$)
[b] Calves vaccinated on weeks 0, 3 and 6; challenged on week 9 with strain 11249nc

Table 2
Protection from respiratory infection following vaccination with BVDV 11249nc

Gro up	No. of calves	Sa mpl e	Day				
			0	4	6	8	11
Co ntro l	4	N. Ph.	0[a]	2	4	3	0
		Blo od	0	3	3	0	0
Vac cin e	5	N. Ph.	0	0	0	0	0
112 49n c		Blo od	0	0	0	0	0

[a]Number of calves with infection detected by sampling on indicated day. N. Ph. = nasopharyngeal swab

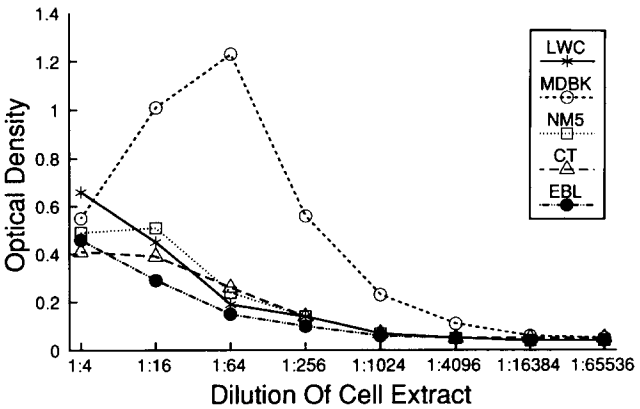

Fig. 1. Relative yield of antigen from BVDV strain Ky1203nc in five cell cultures. △, calf testis cells; □, nasal mucosa line 5; ●, embryo lung cells; ○, MDBK cells; ×, lung-wash cell line.

Table 3
Antibody responses by ELISA[a] in calves vaccinated with strain Ky1203nc

Group	No. of cal ves	Week[b]					
		0	3	6	8	10	12
Non-vaccinate d	5	<1. 4	N D	ND	1.4	2.09 ±0.6 0	2.50 ±0.1 2
Vaccine standard dose	5	<1. 4	<1 .4	3.07 ±0.3 0	3.52 ±0.1 3	4.38 ±0.2 5	4.09 ±0.2 9
Vaccine 1/10 dose	5	<1. 4	<1 .4	2.32 ±0.3 6	2.82 ±0.4 6	3.88 ±0.3 6	3.79 ±0.2 3

[a]Mean number of units of antibody (10^n) ± SD
[b]Calves vaccinated on weeks 0, 3 and 6; challenged on week 8 with strain 11249nc intranasally

3.3. *Inactivated Ky1203nc protects against heterologous challenge*

The serological response of calves to three standard doses of vaccine and a 1/10 dilution of vaccine is shown in Table 3. Two doses of standard vaccine resulted in an antibody titre of $>10^3$. Calves that received the diluted vaccine all responded serologically but the antibody titre obtained was lower. Efficacy of vaccination against respiratory challenge with strain 11249nc is shown in Table 4. Virus was isolated from nasopharyngeal swabs

Table 4
Relationship between antibody at time of challenge and susceptibility of individual animals to challenge with strain 11249nc

Animal	Vaccine[a]	Antibody[b]	Virus isolation[c]		Leucopenia %[d]
			N. Ph. swab	Blood	
X502	S	3.71	−	−	5
A21	S	3.57	−	−	0
X694	S	3.51	−	−	0
X657	S	3.42	−	−	0
X684	S	3.38	−	−	5
X650	1/10	3.38	−	−	0
A34	1/10	3.06	+ (3)	−	31
X654	1/10	2.76	−	−	2
X677	1/10	2.75	+ (3,6)	−	37
X679	1/10	2.14	−	−	46
A10	−	<1.4	+ (6)	−	46
A407	−	<1.4	+ (6)	+	48
X192	−	<1.4	+ (6,8)	+	53
X658	−	<1.4	+ (6)	+	52
X659	−	<1.4	+ (3,6)	+	54

[a]Animals given standard dose (S) of Ky1203nc vaccine, diluted dose (1/10), or no vaccine (−)
[b]Units of antibody (10^n) by ELISA on day of challenge, animals arranged in decreasing order
[c]Isolations from nasopharyngeal swab taken on days 0, 3, 6, 8, 10. Figures in parenthesis indicate days on which virus was isolated. Isolations from blood on day 6
[d]Percentage reduction in cell count, compared to average of 3 preinoculation values, on day 3 or 6 post challenge.

taken from 5/5 and blood from 4/5 non-vaccinated controls. In marked contrast, virus was not isolated from nasopharyngeal swabs or blood taken from any of the calves given the standard vaccine. Virus was not isolated from the blood of the calves given the diluted vaccine and only isolated from the respiratory tract in 2/5 of these animals. Leukopenia, a decrease of >30% of preinoculation values, was evident in 5/5 non-vaccinated controls, in 0/5 calves given the standard vaccine and 3/5 animals given the diluted vaccine. No overt clinical signs of infection were seen in any of the animals.

The relation between antibody titre at the time of challenge and immunity is shown in Table 4 for individual calves. An ELISA titre ($\log_{10}$) of >3.06 can be expected to indicate animals that are totally refractile to infection, a value of >2.14 to 3.06 indicates partial immunity.

4. Discussion

The results show that parenteral inoculation of inactivated BVDV protects against respiratory infection as judged by an absence of nasal shedding, viraemia and leukopenia in vaccinated calves. Protection was evident following challenge with heterologous virus as well as when the challenge and vaccine strain were the same. It appeared that an antibody titre of $>10^{3.06}$ at the time of challenge equated to immunity from infection. This was

achieved in one calf given three doses of the 1/10 Ky1203nc vaccine. It was also achieved by two doses of the standard vaccine. Thus two doses may give effective protection. The duration of immunity is clearly of practical relevance for a vaccine, we have not assessed this but the time when calves are at risk is also limited to a few months. We selected as the vaccine strain an isolate previously shown to induce an antibody response in gnotobiotic calves that cross reacted widely with other isolates (Howard et al., 1987a). A cell culture system was chosen that produced high levels of viral antigen. Previous inactivated BVDV vaccines have not been universally acclaimed as successful. This has been as a result of doubts as to efficacy and generation of a poor antibody response compared to modified live vaccines (Baker 1987; Neaton 1986). The high antibody titres generated by the reported vaccine may result from the choice of adjuvant. Previous experience indicated Quil-A to be a highly effective adjuvant for an inactivated respiratory syncytial virus vaccine in calves enabling priming in the face of maternal antibody (Howard et al., 1987b). Relatively poor growth in cell cultures makes production of antigen inefficient and could explain the poor antigenicity of some preparations. For the culture of virus particular attention must be paid to identifying batches of sera that do not contain adventitious BVDV or viral antibody. The frequency of viraemic cattle has been reported to be 0.4–0.8% (Meyling 1984: Howard et al., 1986) thus pooled foetal calf sera are often likely to contain virus and BVDV infection of cell lines as a result of the use of infected FCS is notoriously common (Nuttall et al., 1977). Foetal infection after immunocompetence will result in a specific antibody response being generated in the foetus. Thus antibody positive sera will be at least as common as virus contamination and low titres of antibody will reduce virus growth.

Natural infection with BVDV is likely to be by the respiratory route and the challenge was selected to mimic this. It has often been proposed that effective protection against respiratory infection would require a local immunity and probably require a live vaccine. This is clearly not the case for BVDV. But it could be argued that although the natural route of infection is via the respiratory tract BVDV should not be regarded as a localised respiratory pathogen as infection often results in a viraemia and leukopenia (Baker 1987; Brownlie 1991). However, previously it has been established that systemic vaccination with an inactivated respiratory syncytial virus vaccine protects against an undoubtable respiratory pathogen (Stott et al., 1987; Howard et al., 1987b). We conclude from the published observations and those reported here that it is not necessary to use live avirulent virus to achieve protection in the respiratory tract and immunity is not dependent on a local immune response. Indeed the antibody titres achieved in this report were as high as those reported following natural infection (Brownlie 1991) and the opinion that modified live vaccines necessarily induce higher antibody levels is not justified (Baker 1987). It should be emphasised that for BVDV use of live vaccines is potentially hazardous. A live nc virus could result in foetal infection or damage if pregnant cattle were exposed to it, whilst a live cytopathogenic vaccine could cause mucosal disease if given to animals that were immunotolerant and viraemic as a result of foetal infection (Brownlie et al., 1984; Bolin et al., 1985).

The mechanism by which the BVDV vaccine affects immunity has not been established. Previous studies have shown that passive antibody can mediate protection against respiratory or systemic challenge with BVDV (Howard et al., 1989). Thus antibody produced by vaccination may mediate immunity. Other studies of BVDV infection using monoclonal

antibodies to deplete CD4 or CD8 T cells in cattle (Howard et al., 1993) showed CD4 cells were important in recovery from primary infection but did not establish a central role for CD8 cells. Vaccination may prime the CD4 cells allowing more rapid help for antibody production or generation of cytokines effective in potentiating effector cells following challenge.

Respiratory infection is the major route of lateral spread within a herd. Protection against infection by this route would therefore be expected to have a marked effect in reducing respiratory disease that involved BVDV that was due to lateral infection either from transiently infected calves or persistently viraemic cattle. A reduced level of BVDV in a herd as a result of vaccination would also reduce the likelihood of a pregnant cow coming into contact with the virus and thus reduce the incidence of viraemic cattle. Since the vaccine reduces viraemia and leukopenia it may protect against foetal infection by reducing systemic spread in the pregnant dam. This remains to be established.

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References

Baker, J.C. 1987. Bovine viral diarrhoea virus: a review. J. Am. Vet. Med. Assoc., 190: 1449–1458.

Bolin, S.R., McClurkin, A.W., Cutlip, R.C. and Coria, M.F., 1985. Severe clincal disease induced in cattle persistently infected with noncytopathogenic bovine viral diarrhoea virus. Am. J. Vet. Res., 46: 573–576.

Brownlie, J. 1991. The pathways for bovine virus diarrhoea virus biotypes in the pathogenesis of mucosal disease. Arch Virol., Suppl 3, 79–96.

Brownlie, J., Clarke, M.C. and Howard, C.J., 1984. Experimental production of fatal mucosal disease in cattle. Vet. Rec., 114: 535–536.

Brownlie, J., Clarke, M.C. and Howard, C.J., 1991. Experimental infection of cattle in early pregnancy with a cytopathic strain of bovine virus diarrhoea virus. Res. Vet. Sci., 46: 307–311.

Chanter, N., Rutter, J.M. and Luther, P.D., 1986. Rapid detection of toxigenic Pasteurella multocida by an agar overlay method. Vet. Rec., 119: 629–630.

Clarke, M.C., Brownlie, J. and Howard, C.J., 1987. Isolation of cytopathic and non-cytopathic bovine viral diarrhoea virus from tissues of infected animals. In Pestvirus infections of ruminants, p. 43–51, Ed. J.W. Harkness, Pub. CEC, Luxembourg.

Greig, A., Gibson, I.R., Nettleton, P.F. and Herring, J.A., 1981. Disease outbreak in calves caused by a mixed infection with infectious bovine rhintracheitis virus and bovine virus diarrhoea virus. Vet. Rec., 108: 480.

Harkness, J.W., Sands, J.J. and Richards, M.S., 1978. Serological studies of mucosal disease in England and Wales. Res. Vet. Sci., 24: 98–103.

Howard, C.J., Brownlie, J. and Clarke, M.C., 1987a. Comparison by the neutralisation assay of pairs of non-cytopathogenic and cytopathogenic stains of bovine virus diarrhoea virus isolated from cases of mucosal disease. Vet. Microbiol., 13: 361–369.

Howard, C.J., Brownlie, J. and Thomas, L.H., 1986. Prevalence of bovine virus diarrhoea viraemia in cattle in the UK. Vet. Rec., 119: 628–629.

Howard, C.J., Clarke, M.C. and Brownlie, J. 1985. An ELISA for the detection of antibodies to bovine virus diarrhoea virus in cattle sera. Vet. Microbiol., 10: 359–369.

Howard, C.J., Clarke, M.C. and Brownlie, J., 1989. Protection against respiratory infection with bovine virus diarrhoea virus by passivly aquired antibody. Vet. Microbiol., 19: 195–203.

Howard, C.J., Clarke, M.C., Sopp, P. and Brownlie, J., 1993. Immunity to bovine virus diarrhoea virus in calves: the role of different T cell populations analysed by specific depletion in vivo with monoclonal antibodies. Vet. Immunol. Immunopathol., 32: 303–314.

Howard, C.J., Stott, E.J., Thomas, L.H. Gourlay, R.N. and Taylor, G., 1987b. Protection against respiratory disease in calves induced by vaccines containing respiratory syncytial virus, parainfluenza type 3 virus, Mycoplasma bovis and M. Dispar. Vet. Rec., 121: 372–376.

Malmquist, W.A. 1968 Bovine viral diarrhoea - mucosal disease: etiology, pathogenesis and applied immunity. J. Am. Vet. Med. Assoc., 152: 763-770.

Meyling, A., 1984. Detection of BVD virus in viraemic cattle by an indirect immunoperoxidase technique. Current Topics in Veterinary Medicine and Animal Science, 29: 37–46.

Neaton, H.J., 1986. Which BVD vaccine should I use? Vet. Med., 81: 876–881.

Nuttall, P.A., Luther, P.D. and Stott, E.J., 1977. Viral contamination of bovine foetal serum and cell cultures. Nature, 266, 835–837.

Potgeiter, L.N.D., McCracken, M.D., Hopkins, F.M. and Walker, R.D. 1984a Effect of bovine viral diarrhoea virus infection on the distribution of infectious bovine rhinotracheitis virus in calves. Am. J. Vet. Res., 45: 687–690.

Potgeiter, L.N.D., McCracken, M.D., Hopkins, F.M., Walker, R.D. and Guy, J.S. 1984b Experimental production of bovine respiratory disease with bovine diarrhoea virus. Am. J. Vet. Res., 45: 1582–1585.

Stott, E.J., Thomas, L.H., Collins, A.P., Jebett, N.J., Smith, G.S. Luther. P.D. and Caswell, R., 1980. A survey of virus infections of the respiratory tract of cattle and their association with disease. J. Hyg., 85: 257–270.

Stott, E.J., Thomas, L.H. Howard, C.J. and Gourlay, R.N., 1987. Field trials of a quadrivalent vaccine against respiratory disease. Vet. Rec., 121: 342–347.

Wray, C. and Roeder, P.L. 1987. Effect of bovine virus diarrhoea-mucosal disease infection on salmonella infection in calves. Res. Vet. Sci., 42: 213–218.