Papers and Articles

Protection of the bovine fetus from bovine viral diarrhoea virus by means of a new inactivated vaccine

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A model is described for the validation of vaccines designed to protect the bovine fetus from bovine viral diarrhoea virus (BVDV). The fetopathic nature of the challenge strain of virus was confirmed and the method used to test a commercial vaccine (Bovidec) developed from a Compton prototype. Heifers were vaccinated two or three times about the time of impregnation and challenged when they were between 25 and 80 days of gestation. There was no evidence of a viraemia in the heifers after the challenge, or of infection with BVDV of 13 live-born calves or two aborted fetuses.

BOVINE viral diarrhoea virus (BVDV) is an important cattle pathogen with a worldwide distribution. It can cause severe, if not fatal, infections and create devastating economic losses. The pathogenesis of bovine viral diarrhoea is complex and central to it is the tropism of the virus for the reproductive system (Brownlie 1991). Although BVDV has two biotypes that occur naturally, namely the non-cytopathogenic (BVDVnc) and the cytopathogenic (BVDVc), only the BVDVnc biotype crosses the placentome to establish an infection in the fetus (Brownlie and others 1989). This virus may infect the fetus at any time during pregnancy but only when infection occurs within the first 110 to 120 days of development (Liess and others 1984, Brownlie and others 1986) does the calf become persistently infected. The birth of these persistently infected animals provides a pathway for the virus to infect successive generations of cattle and blocking this pathway is imperative for any control programme. In a country such as the United Kingdom, where the virus is widely endemic, such control requires an effective vaccine.

It is known that an experimental infection of heifers, at least six weeks before they are inseminated, with an inoculum containing nine strains of BVDV will protect the fetus from teratogenic effects (Duffell and others 1984). Furthermore, commercially available live vaccines against BVDV have been used extensively in some countries, notably the USA, for up to 30 years (Coggins and others 1961). Nevertheless, disease associated with BVDV remains widespread and a major problem to cattle farmers throughout the world. Not only have live BVDV vaccines failed to control the disease but such conventionally attenuated live preparations have been associated with many adverse reactions; fetal infection after the vaccination of pregnant animals (Liess and others 1984), the induction of mucosal disease (Peter and others 1967, Donis and Krejci 1993) and increased mortality rates in beef calves possibly associated with vaccine virus-induced immunosuppression (Martin 1983). Recent assessments of BVDV vaccines have emphasised the uncertainty surrounding their use and the need to review critically the safety and efficacy of those available (Harkness and Roeder 1988, Bolin 1990). Nevertheless, there is widespread recognition that they need to be developed urgently, preferably in an inactivated form, to immunise female cattle before they are bred, to prevent the infection of the fetus in early gestation (Nettleton and others 1985, Roeder and Harkness 1986, Moennig and Plagemann 1992, Liess 1993, Brownlie 1994).

Although vaccines purporting to protect the unborn calf are available in countries other than the UK, there is limited documented evidence of their efficacy after an experimental challenge with BVDV. It is for this reason that a critical review of the efficacy and safety of the BVDV vaccines available in the USA was suggested (Bolin 1990). This lack of information makes it difficult to compare new vaccines directly with those commercially available.

Two experimental studies of inactivated vaccines have revealed their potential benefit but they failed to give complete protection. In Denmark, Meyling and others (1987) used three injections of an inactivated detergent-split vaccine, plus Quil-A adjuvant, to vaccinate eight cattle in early pregnancy. They were then challenged intranasally/orally with a mixture of four strains (including the vaccine strain) of BVDV between 37 and 97 days of pregnancy. Fetuses from only two of the heifers were protected and the remaining six vaccinated and four unvaccinated control animals gave birth to BVDV-infected offspring. At about the same time, Harkness and others (1987) prepared an inactivated vaccine of four field isolates and vaccinated cattle before they were inseminated. They were then challenged intranasally, at about 80 days gestation, with nine field strains. Seven of 11 fetuses (64 per cent) were protected compared with none of the 10 fetuses from unvaccinated controls.

Sheep have also been used for the initial testing of the fetoprotective efficacy of one BVDV vaccine. In Sweden, Carlsson and others (1991) achieved encouraging results with an experimental immunostimulating complex (ISCOM) subunit vaccine incorporating a Danish isolate of BVDV. After natural service, 15 ewes received two doses of vaccine three weeks apart. Three weeks after the second dose, at 47 to 64 days gestation, the vaccinated ewes and 14 unvaccinated ewes were challenged intramuscularly and subcutaneously with a heterologous Swedish BVDV isolate. The 15 vaccinated ewes delivered 26 live lambs none of which showed evidence of in utero infection, whereas only six live lambs were born to the unvaccinated ewes.

It is axiomatic that any new vaccine should attempt to protect the host against all fields strains of the virus. It is now clear that genomic variants are frequently generated during RNA virus replication and that these variants, sometimes termed quasispecies

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TABLE 1: Clinical and virological results observed in unvaccinated heifers in early pregnancy after an intranasal challenge with BVDV isolate Pe515ncCl

Dam	Days gestation at challenge	Abortion (BVDV status of fetus)	Calf with persistent viraemia	Calf with active immunity
D10	98	–	–	+
D12	95	–	–	+
D14	82	–	+	NA
D558	95	–	–	+
D559	66	–	+	NA
D563	85	+ (+)	NA	NA
D571	81	–	–	+
D577	90	–	–	+
D581	66	–	+	NA

NA Not applicable

(Eigen and Biebricher 1988), can display antigenic variability. However, the dilemma for designing pestivirus vaccines has always been the lack of any clear typing system or definition of separate serotypes, and this is indicated by the current uncertainty about the need to incorporate more than one virus in the vaccine to achieve broad protection. The available evidence suggests that although BVDV isolates can be separated into groups by polyclonal sera (Fernelius and others 1972), and monoclonal antibodies (Bolin and others 1988, Corapi and others 1990) and their genomic profile at the 5′ UTR region (Pellerin and others 1994, Vilček and others 1994), the polyclonal serum produced by infected cattle efficiently neutralises both the infecting virus and many, if not all, other BVDV isolates (Castrucci and others 1975, Hafez and others 1976). This was demonstrated very clearly when three inactivated vaccines were tested against a range of BVDV isolates in the USA (Bolin and Ridpath 1990).

In the UK, data on the antigenicity of recent BVDV isolates has come from three studies. By infecting gnotobiotic calves intranasally, Howard and others (1987) demonstrated extensive cross-neutralisation between 14 different BVDV isolates. A separate study showed that six Scottish isolates were also closely related serologically, although they could be distinguished from three isolates of ovine border disease virus (Nettleton 1987). Using a panel of monoclonal antibodies, Edwards and others (1988) examined 101 field isolates of ruminant pestiviruses which included 84 bovine, one cervine and 16 ovine isolates. The results indicated the presence of at least two antigenic groups of pestiviruses one of which predominated in cattle and one in sheep. Approximately 90 per cent of all the cattle isolates belonged to one antigenic group, suggesting that a vaccine virus from this group would protect against other members of the group.

Recently, an inactivated vaccine has been developed at Compton that protects calves from respiratory infection after an intranasal challenge with homologous and heterologous strains of BVDV (Howard and others 1994). This paper reports on the efficacy of a commercially developed form (Bovidec) of this vaccine that protects the unborn fetus.

Materials and methods

Experimental design

An initial experiment was conducted in pregnant heifers, seronegative to BVDV, to confirm that the challenge virus could cross the placentome and establish persistent infections in the fetus. Nine heifers, pregnant between 66 and 98 days, were infected intranasally with the challenge virus (Table 1). They were monitored for viraemia for two weeks after the challenge and for antibody throughout pregnancy.

The calves were bled at birth and tested for the presence of virus and antibody; further samples were collected to confirm the persistence of viraemia and to monitor the antibody status of the calves.

In the second experiment, 30 heifers, seronegative to BVDV, were selected from one of the institute's herds. They were kept in strict isolation to avoid adventitious infection and subsequent seroconversion to the virus. They were randomly allocated to three groups of 10, but maintained together in order to represent normal farm conditions. Groups A and B were vaccinated while group C remained unvaccinated as controls. The oestrus of the heifers was synchronised so that they could be inseminated as a group. The heifers that failed to hold to the first service were retained within their group only if they held successfully to either of the two subsequent inseminations. All the pregnant heifers were challenged at the end of the first trimester of pregnancy and monitored as before.

The occurrence of abortions was recorded and fetal tissue and a blood sample, if possible, were collected for virus isolation. The calving dates were recorded and blood samples collected as before.

Vaccination

The vaccine, Bovidec (C-Vet), based on the experimental protocol of Howard and others (1994), was used. The animals in group A were vaccinated twice subcutaneously three weeks apart with 4 ml. Group B received a third dose after a further three weeks.

Intranasal challenge

The strain of BVDV Pe515ncCl (Brownlie and others 1984) was used for the intranasal challenge. A dose of 5×10^6 TCID50 in 5 ml was administered by intranasal aerosol to all three groups.

Assay of samples for BVDV

Samples of blood and tissues were stored at –70°C. On thawing, these samples were seeded on to cultures of calf testis cells and the presence of BVDV was detected by indirect immune fluorescence.

Assay for antibodies to BVDV

A serum neutralisation test (to strain NADL) was used for the initial selection of seronegative heifers and to determine the antibody status of the calves.

To monitor immunity in the dams, sera were screened by ELISA, using a modification of the single dilution assay (to strain NADL) of Howard and others (1985). The results are expressed as units/ml by comparison with a reference hyperimmune serum.

Results

Timing of pregnancy and vaccinations

Owing to the difficulties of detecting oestrus and of mass insemination, not all the heifers held successfully to the first insemination. The range of pregnancy dates and the reduced numbers within the groups are a result of this difficulty. In the second experiment, the heifers that failed to hold either to the first or to two repeat services, were excluded from the trial (Table 2).

All the heifers in group A received the first vaccination and two of them also received the second vaccination before they were impregnated. One animal, 0098, had the second vaccination and was impregnated on the same day while the remaining three received the second vaccination after they were impregnated. In group B, all the animals received their first vaccination before they were impregnated; three received their second vaccination before being impregnated; seven received their third vaccination after being impregnated and the remaining animal, 0028, was impregnated and vaccinated for the third time on the same day.

TABLE 2: Clinical and virological results observed in unvaccinated and vaccinated heifers in early pregnancy after an intranasal challenge with BVDV isolate Pe515ncCl

Group	Dam	Days gestation at challenge	Viraemia after challenge	Abortion* (BVDV status of fetus)	Calf with persistent viraemia	Calf with active immunity
A	0098	70	–	+ (–ve)	NA	NA
(vaccinated	0112	25	–	–	–	–
twice)	0133	31	–	–	–	†
	0153	80	–	+ (–ve)	NA	NA
	0244	68	–	–	–	–
	0273	75	–	–	–	–
B	0028	49	–	–	–	†
(vaccinated	0079	58	–	–	–	–
three	0114	73	–	–	–	–
times)	0137	71	–	–	–	–
	0155	76	–	–	–	–
	0242	77	–	–	–	†
	0249	68	–	–	–	–
	0266	71	–	–	–	–
	0278	25	–	–	–	–
C	0063	73	–	+ (+ve)	NA	NA
(controls)	0110	68	–	–	–	–
	0128	72	–	–	+	NA
	0144	31	+	–	+	NA
	0170	68	+	–	+	NA
	0251	30	+	–	+	NA

* The following tissues from the aborted fetuses were examined for BVDV: gut from ileo-caeco-colon junction, mesenteric lymph node, liver, spleen, thymus, prescapular lymph node, tonsil, cerebellum, brain cortex, spinal cord and placenta

† Pre-colostral samples not obtained. Antibody decline consistent with maternal antibody

NA Not applicable

Detection of viraemia after BVDV challenge of vaccinated and unvaccinated heifers in early pregnancy

In the first experiment a viraemia was detected in each of the nine heifers between five and seven days after challenge. However, in the second experiment, viraemia was not detected in any of the 15 heifers in the vaccinated groups A and B (Table 2), but was detected in three of the six heifers in group C. Evidence of viraemia in two of the three remaining controls was provided by fetal infections in two of the heifers (see below).

There were no reactions to vaccination and there was no apparent clinical disease in any of the heifers during the two weeks after challenge.

Abortions during the experimental study

One animal (D563) in the first experiment aborted (Table 1) and BVDV was detected in fetal samples. In the second experiment an unvaccinated control animal (0063) aborted and again virus was recovered from the fetal tissues. However, two of the vaccinated animals of group A (Table 2) aborted, but the abortions were considered to be unrelated to BVDV infection. None of the heifers in this group showed evidence of viraemia after challenge and no virus was isolated from a range of tissues taken from the aborted fetuses, including gut (ileo-caeco-colonic junction), mesenteric and prescapular lymph nodes, liver, spleen, thymus, tonsil, cerebellum, brain cortex, spinal cord and placenta. Tests for *Salmonella* species and *Brucella abortus* were negative. In addition, heifer 0098 was reported to have been bullied by other cattle on the trial two to three days before she aborted.

No abortions occurred in group B.

Efficacy of the challenge strain

Eight live calves were born to the nine heifers in the experiment to test the efficacy of the challenge strain. Three of the calves had a persistent viraemia (Table 1), because BVDV was isolated on two occasions at least three weeks apart. The assay of neutralising antibody in sera from the remaining five calves showed that they all had an active immunity to BVDV and were therefore infected in utero; the calf born to dam D571 was weak at birth, unable to suckle and, without intensive care, would have died.

These results, together with the abortion referred to above, provided evidence that the strain Pe515ncCl infected each of the nine fetuses in this group of heifers.

The aborted fetus and the three persistently viraemic animals were between 66 and about 85 days gestation at challenge. The age of the fetuses that seroconverted were 81 to 98 days of age (Table 1).

Evidence for in utero protection of the early fetus in vaccinated heifers

The heifers were challenged with virus when they were between 25 and 80 days of gestation (Table 2).

None of the 13 live-born calves in the two vaccinated groups (A and B) were viraemic and BVDV was not recovered from the two aborted fetuses from group A. Precolostral serum samples were obtained from 10 of these 13 calves and they were all seronegative. However, the calves born to dam 0133 in group A and dams 0028 and 0242 in group B (Table 2) did suck from their dams before blood samples could be taken. The serum samples from these calves contained neutralising antibody to BVDV but assays on sera taken over the following three to four months demonstrated that it was maternal in origin and the calves were confirmed as non-viraemic (Table 2).

In contrast to the results from the vaccinated heifers, 14 of the 15 calves born to the control animals in the first experiment and in group C of the second experiment, were born with evidence of fetal infection. In the first experiment there was one infected fetus, three of the eight live-born calves were born persistently infected and the remaining five calves had an active immune response at birth, clear evidence of in utero infection. In the second experiment, four of the six calves in group C had a persistent viraemia, one fetus was aborted and infected with BVDV (see above) but the remaining heifer, 0110, and its fetus apparently did not become infected; the antibody status of 0110 was negative from days 0 to 91 and it had the lowest ELISA value of any heifer at day 270.

Antibody responses to vaccination and challenge

Responses to vaccination. – The animals in groups A and B remained seronegative by ELISA until three weeks after the second vaccination (Table 3). At this time all of the animals showed an increase in units of antibody which ranged from 318 to 5307. This increase appeared to be transient in group A and the titres of subsequent samples were lower. However, when sera in group A were tested for neutralising antibody, a low titre was detected in four heifers three weeks after the first vaccination.

After the third vaccination, the heifers in group B had higher ELISA units of antibody (Table 3) and these persisted until the challenge 90 days after the first vaccination.

No antibody was detected in the control group either by ELISA or neutralisation test until after the challenge.

Responses after challenge. – Fourteen days after challenge, five of the six animals in group A showed a marked rise in their ELISA titres. The mean values for the group rose from 304 at the time of challenge to a maximum of 16,181 three weeks later; thereafter, they declined although they remained well above the pre-challenge levels. One animal, 0153, showed no serological response to challenge.

There was a clear difference in the pattern of serological response after challenge between the two vaccinated groups. In group B, the titres increased only moderately after challenge, with the mean value rising from 1196 at challenge to a peak of 4682 units of antibody three weeks later.

The control animals in both the first and second experiments were slower to respond; by three weeks after challenge, the mean

TABLE 3: Mean antibody levels (ELISA) in heifers after vaccination and challenge with BVDV isolate Pe515ncCl

Day after vaccination	Group A* (vaccinated twice)	Group B (vaccinated three times)	Group C (controls) Experiment 1†	Experiment 2
0 (1st vaccination)	–	–		–
7	–	–		ND
21 (2nd vaccination)	–	–		–
42 (3rd vaccination B only)	797	1353		–
55	425	2101		ND
70	363	2487		–
74			–	
83	361	1814		ND
91 (challenge)	345	1065		–
97	286	917		–
104	16,020	3647		170
111	23,399	6431		337
146	13,910	4359		1636
174	6713	2866		2535
198			9305	
207	5167	3797		3835
237	4213	2706		8671
254			12,363	
270	ND	2313		11,165
293			12,179	

* Mean value for 0112, 0133, 0244 and 0273 (excluding heifers 0098 and 0153 which aborted)
† Mean value for D14, D559, D571 and D581; the heifers in group C were challenged on day 0
– Negative ELISA values
ND Not determined

titre of the animals in the second experiment was only 337 units of ELISA antibody.

However, as pregnancy proceeded, all the animals showed a substantial increase in circulating antibody levels and, in most cases, these continued to rise until the last observation before calving. At the time of parturition, the mean antibody values for the control heifers carrying viraemic calves was 12,179 in the first experiment and 11,165 in the second experiment.

Discussion

The central pathway taken by BVDV to establish persistent infection is by the infection in utero of the early fetus. About 66 per cent of infected fetuses survive to full term (Brownlie and others 1986) and they constitute about 0·5 to 1 per cent of the National Herd (Meyling 1985, Howard and others 1986). It is these persistently infected animals that act as the main reservoir of BVDV for other cattle and, as a result any method of protecting the fetus from early infection should effectively diminish this source of virus. This paper describes the development and experimental assessment of an inactivated vaccine that appears to be efficacious and safe for the prevention of fetal infection in heifers.

Although the experimental design had planned for three groups of 10 animals, the practical difficulties of impregnating 30 maiden heifers within two oestrous cycles reduced the final group sizes. Since the design required all the animals to be challenged on the same day, it was not possible to exercise any late allocation to the groups because the animals had already been selected by their vaccination regimen. Thus, although 21 of 30 (70 per cent) were successfully impregnated, they were unevenly distributed, with six in groups A and C and nine in group B. However, the nine control animals from the first experiment were within the same interval of gestation and were challenged with the same strain of virus.

In the first experiment all the dams were shown to have a viraemia after challenge. In the second experiment the evidence showed that five of the six control heifers became viraemic, indicating that 14 of the 15 control animals must have had a transient viraemia.

A critical sign of the BVDV infection of pregnant cattle is the birth of congenitally damaged calves, in addition to those persistently infected. The teratogenic effects of BVDV infection appear to be the result of fetal infection at about the time of immune recognition or later (Brown and others 1974, 1975). The calf of dam D571 (Table 1) was weak at birth and may have been congenitally damaged by the BVDV challenge but, as shown by its active immunity at birth, it had been able to mount an immune response and eliminate the virus.

The first noticeable difference between the vaccinated and control animals after challenge with BVDV, was the prevention of any detectable viraemia in both the vaccinated groups. Thus, the vaccine appeared to provide protection whether it was given on two or three occasions. Two of the six heifers in group A aborted, and although it is often difficult to identify the cause of abortion even when BVDV is suspected, there was no evidence for the involvement of a viral infection in these cases. One abortion appeared to be the result of trauma.

The implications for protection that can be derived from the antibody responses observed after vaccination with this inactivated vaccine and challenge with live non-cytopathogenic BVDV are interesting. The antibody responses to the first vaccination appeared to be weak, although there was evidence of priming in the improved antibody responses to the second dose (Table 3). The responses after the second vaccination were typical of an anamnestic response, with antibody titres rising more rapidly and to higher levels than after the primary inoculation. However, the response to the third vaccination in group B was greater and the titres remained high until the point of challenge. An inference drawn from the antibody response to vaccination was that two doses might be insufficient to give protection whereas three doses might be adequate. Previous work (Howard and others 1989) showed that the colostral transfer of antibody to young calves protected them against a respiratory challenge but only when there were adequate levels of antibody. This result concurred with the protection given by the prototype vaccine (Howard and others 1994) to calves, which resisted the establishment of viraemia or respiratory infection; some animals shed virus from the nose but did not develop viraemia when the vaccine was diluted and the antibody response was reduced. In this study either two or three doses of vaccine prevented viraemia in the dam and protected the fetus; thus in utero protection may be provided by lower titres of circulating antibody in the dam than are required to prevent a respiratory infection.

Proliferative lymphocyte responses to both live and killed BVDV have been reported by Larsson and Fossum (1992) and Hooper and others (1993). In the latter study, seven of eight animals infected experimentally with the Pe515ncCl isolate of BVDV, had sufficient memory 11 months later to respond in vitro to a secondary stimulation with BVDV. These observations and the present results suggest that the Bovidec vaccine can stimulate protective cell-mediated immune mechanisms in addition to humoral antibody.

This study has provided a stringent model of in utero infection to validate the efficacy of vaccines that are designed to protect the early fetus. The model has been used to test an inactivated BVDV vaccine, and its ability to protect the fetus more precisely than in previous work. Bovidec gave 100 per cent protection to the 15 fetuses of 15 vaccinated cattle whereas there was evidence of in utero infection of 14 of 15 fetuses in the unvaccinated control cattle.

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