A Bovine Virus Diarrhea Calfhood Vaccination Trial in a Persistently Infected Herd: Effects on Titres, Health and Growth

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ABSTRACT

A controlled calfhood vaccination trial to prevent bovine virus diarrhea was conducted in a 100 head cow-calf operation with a three year history of annual calf losses due to enteric bovine virus diarrhea (persistently infected herd).

Approximately 50% of the calves were vaccinated at six, 12 and 24 weeks of age. Paired serum samples and growth data were collected on three occasions for comparison between vaccinates and controls.

Three vaccinated calves died of enteric bovine virus diarrhea in the first year of the trial and one nonvaccinated calf died in the second year. Two of the three vaccinated calves had developed bovine virus diarrhea virus neutralization antibody titres of 2048 or greater before developing clinical signs. The control and third vaccinated calf failed to seroconvert before dying of enteric bovine virus diarrhea. Approximately 90% of the vaccinated calves seroconverted compared to approximately 40% of the controls. Paired serum samples collected from 75% of the cows in the spring, summer and fall of each year of the trial, showed persistent high bovine virus diarrhea virus neutralization titres in all samples.

Calf vaccination before 12 weeks of age had little effect on seroconversion due to high levels of passive antibody to bovine virus diarrhea. Growth data showed that there was no improvement in weight gain or rate of growth in the vaccinated calves.

Key words: Bovine virus diarrhea vaccination, calf vaccination for bovine virus diarrhea, bovine virus diarrhea vaccination effects on growth and immunity, NADL strain of bovine virus diarrhea vaccine.

RÉSUMÉ

Cette expérience consistait à procéder à une vaccination contrôlée des veaux, afin de prévenir la diarrhée à virus bovine dans une exploitation vaches-veaux qui comptait 100 têtes et qui, depuis trois ans, subissait des pertes annuelles imputables à l'infection des veaux par la forme entérique de la diarrhée à virus bovine.

Les auteurs vaccinèrent environ 50% des veaux dont l'âge atteignait six, 12 et 24 semaines. À trois reprises, ils prélevèrent des échantillons appariés de sérum et des données relatives à la croissance, dans le but d'établir une comparaison entre les veaux vaccinés et les témoins.

Trois des veaux vaccinés moururent de la forme entérique de la diarrhée à virus bovine, au cours de la première année de l'expérience, tandis qu'un témoin y succomba, au cours de la deuxième. Deux des trois veaux vaccinés avaient déjà développé des titres d'anticorps neutralisants de 1:2048 ou plus, avant de manifester des signes cliniques de la maladie. Le troisième veau vacciné et les témoins ne développèrent toutefois pas d'anticorps avant de succomber à la forme entérique de la maladie. Environ 90% des veaux vaccinés développèrent des anticorps, comparativement à environ 40% des témoins. Tous les échantillons appariés de sérum, prélevés chez environ 75% des vaches, au printemps, à l'été et à l'automne de chaque année de l'expérience, recelaient des titres élevés et persistants d'anticorps neutralisants.

Le fait de vacciner les veaux avant qu'ils aient atteint l'âge de 12 semaines, exerça peu d'influence sur le développement d'anticorps, parce qu'ils possédaient une solide immunité passive. Les données relatives à la croissance révélèrent que la vaccination des veaux n'améliora pas le gain de poids ou le taux de croissance.

Mots clés: vaccination contre la diarrhée à virus bovine; vaccina-

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tion des veaux contre la diarrhée à virus bovine; effets de la vaccination contre la diarrhée à virus bovine sur la croissance et l'immunité; souche NADL de vaccin contre la diarrhée à virus bovine.

INTRODUCTION

Losses in cow-calf, feedlot and dairy operations attributed to infection with bovine virus diarrhea (BVD) virus include abortion, the birth of stillborn or deformed calves as well as death due to diarrhea (2, 8, 13, 20, 25). In addition, unsubstantiated reports claim that subclinical infection with BVD virus may reduce the growth rate of calves (16, 34). This study was conducted in a purebred Hereford, cow-calf operation, in which one to three calves had died annually for the preceding three years with the enteric form of BVD.

The purposes of the study were: to evaluate the efficacy of calfhood vaccination in the prevention of enteric BVD; to compare the incidence and changes in BVD virus neutralization (VN) antibody titres in vaccinated and control calves; and to assess the effect of BVD vaccination on the growth and health status of nursing calves.

MATERIALS AND METHODS

ANIMALS

The herd of approximately 90 cows, calved on pasture between the months of April and July where it remained until late fall. Following disinfection of the navel at birth, each calf was identified with an ear tag and injected with a vitamin E and selenium preparation. No other prophylactic medications or vaccinations were administered to the calves. The farm manager recorded any health problems and treatments administered to either cows or calves as did the researchers at the time of the farm visits. Disposable gloves were worn whenever calves were examined orally and balling guns were routinely disinfected between calves.

Creep feed was provided starting in June and the calves were weaned in the fall and either kept as replacements or fattened. Through the winter, the cows were housed in modified bank barns with access to dry lots. During the three years prior to this study there had been no major health problems in the cow herd nor had infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3) or BVD vaccines been used. Most cows were bred artificially and newly acquired animals were quarantined for one month before being mixed with the herd. All calves that developed BVD were admitted to the veterinary teaching hospital (Ontario Veterinary College, University of Guelph) for confirmation of the clinical diagnosis, and postmortem examination.

EXPERIMENTAL DESIGN

The calves born in 1978 and 1979 although allocated into six and seven blocks respectively according to date of birth remained with the cow herd and were managed as previously described. Within each block, males and females were randomly designated as being either vaccinates or controls. At six, nine, 12, 15, 24 and 27 weeks of age, blood was collected from the jugular vein for the determination of BVD VN antibody titres. Calves were restrained in a chute that facilitated individual clinical examination including inspection of the mouth. Abnormalities of the oral mucosa and muzzle were sampled and the scrapings examined by direct electron microscopy for the detection of virus particles (Veterinary Services Branch — Virology, Ontario Ministry of Agriculture and Food). At six, 12 and 24 weeks of age, test calves were vaccinated with a commercial modified live virus vaccine[1] containing the NADL strain of BVD virus, administered intramuscularly according to the manufacturer's directions. Growth data (weight, height at the withers and girth size) were collected on all calves at the time of vaccination. The BVD VN antibody titres, growth and health data from vaccinates and controls were compared within each year. The growth data including change in weight (kg/day) and change in height and girth (cm/day) were analysed using analysis of variance (ANOVA) to assess the effects of vaccination, sex, block and their respective interactions on the growth rate of calves.

Paired serum samples collected in the spring, summer and fall of 1978 and 1979 from 75 cows were analysed to determine the incidence of and changes in BVD VN antibody titres, as an indicator of the immune status of the herd, relative to BVD.

SEROLOGY

Endpoint titres were determined on samples previously stored at -20°C, thawed and heat inactivated at 56°C for 30 minutes using standard VN techniques (37). The mean value of the $\log_2$ of the titre at six, nine, 12, 15, 24 and 27 weeks of age was plotted to ascertain the differences in titre pattern between vaccinated and control calves. For the purposes of this study, seroconversion in an individual calf referred only to antibody production following natural or artificial exposure to BVD antigen as evidenced by one of the following patterns: a fourfold increase in the BVD VN antibody titre in any two consecutive serum samples; or persistence of a titre value greater than 32 in all serum samples from a calf in which the rate of antibody decay was zero or substantially slower than the expected rate of decay of passively acquired BVD antibody (6, 22). All other titre patterns were not considered indicative of seroconversion.

For purposes of plotting, titre patterns were expressed as the $\log_2$ of the dilution and a titre of <8 was given a $\log_2$ *value of 2 in order* to differentiate it from a titre of 8 which was given a $\log_2$ value of 3.

[1]Convac-BVD, Connaught Laboratories, Toronto, Ontario, Canada.

RESULTS

HEALTH

The calves born in 1978 and 1979 showed few health problems. Sporadic cases of mild pneumonia and indigestion of unknown etiology were observed and treated when necessary. In 1978 3% of selected calves examined at the time of vaccination had oral lesions of bovine papular stomatitis (BPS). Examination of scrapings from the hyperemic and proliferative lesions on the muzzle and/or gums and/or hard palate of affected calves by direct electron microscopy confirmed the presence of the BPS virus. In 1979, routine examination of the mouth of every calf at the time of vaccination, revealed BPS in 80% of the animals.

In 1978, three vaccinated calves (K251, K252 and K268) and in 1979 one control calf (L365) died of enteric BVD. The calves, healthy and in good body condition until the time they developed clinical signs of disease, became depressed, anorectic, diarrheic and subsequently dehydrated and died.

Gross and histopathological findings were typical of BVD and included ulceration of the oral mucosa, esophagus and digestive tract, with evidence of depletion of lymphoid elements in the Peyer's patches and mesenteric lymph nodes. Bovine virus diarrhea virus was isolated from one calf (K252).

Calves K251 and K252 were in the same block and had been vaccinated twice before clinical signs of BVD developed at approximately 16 weeks of age. The BVD VN titres for these two calves (Table I) show that the calves had responded immunologically long before they developed clinical signs of disease.

Calves K268 and L365 developed signs of BVD at 15 and 34 weeks of age respectively. Neither calf seroconverted to BVD virus before or after developing clinical signs of disease (Table II).

Table I. Bovine Virus Diarrhea VN Titres of the Calves that Died of Enteric BVD After Seroconversion

Calf Number	Age in Weeks					
	6	9	12	15	24	27
K251[a]	<8	1024	>2048	>2048	—	—
K252[a]	<8	2048	256	512	—	—

[a]Calves vaccinated at six and 12 weeks of age with Convac BVD vaccine (Connaught Laboratories)
(-)Indicates sample unavailable due to death of calf

TABLE II. Bovine Virus Diarrhea VN Titres of the Calves that Died of Enteric BVD Without Evidence of Seroconversion

Calf Number	Age in Weeks						
	6	9	12	15	24	27	34
K268[a]	64	<8	<8	<8	—	—	—
L365[b]	<8	<8	<8	<8	<8	<8	<8

[a]Calf vaccinated twice at six and 12 weeks of age with Convac BVD vaccine (Connaught Medical Laboratories)
[b]Nonvaccinated control calf
(-)Sample unavailable due to death of calf

GROWTH

Vaccinated calves gained 0.82 kg/day and 0.93 kg/day in 1978 and 1979 respectively compared to controls which gained 0.79 kg/day and 0.98 kg/day. However, vaccination in 1978 and 1979 had no effect on weight gain, ($p = 0.697$ and $p = 0.461$ respectively) change in height at the withers ($p = 0.256$ and $p = 0.461$ respectively), or change in girth size ($p = 0.112$ and $p = 0.469$ respectively).

TITRES

In 1978, 93% (44 out of 47) of the vaccinated calves seroconverted to BVD compared to 53% (16 out of 30) of the control calves. Similarly in 1979, 84% (41 of 49) of the vaccinated calves seroconverted compared to 31% (15 out of 46) of the control calves.

Figure 1 a and b illustrates the mean $\log_2$ *titre values for vaccinated* and control calves at six, nine, 12, 15, 24 and 27 weeks of age in 1978 and 1979 respectively. Included for comparison is a line which approximates the expected decline of passively acquired BVD antibody in a calf free from exposure to BVD vaccine or field virus (6, 22).

All cows sampled were seropositive for BVD VN antibody and there was no obvious change in individual cow titres between acute and convalescent samples regardless of season or year.

DISCUSSION

The most remarkable finding of this trial was that in 1978, two of the three vaccinated calves that died of BVD, had seroconverted prior to developing clinical signs. Calf K268 which had never seroconverted, died within four weeks of the second vaccination, whereas K251 and K252 died within seven to ten days of their second vaccination despite having seroconverted to BVDV. These latter calves had serum antibodies after the first vaccination at six weeks of age (Table I) and maintained high titres after being revaccinated at 12 weeks of age, before they developed enteric BVD. This finding contradicts the belief that serum antibodies to BVD protect against enteric disease (2) and the generalization that fatally infected cattle failed to produce BVD VN antibodies (1).

Shope *et al* (38) reported that passive antibody titres to BVD of 1/64 or greater were protective in calves previously made lymphopenic with corticosteroids and challenged intratracheally with virulent BVDV. Similarly, House and Manley (18) found that calves given immune serum subcutaneously to a serum titre of 1/18 or greater were protected from

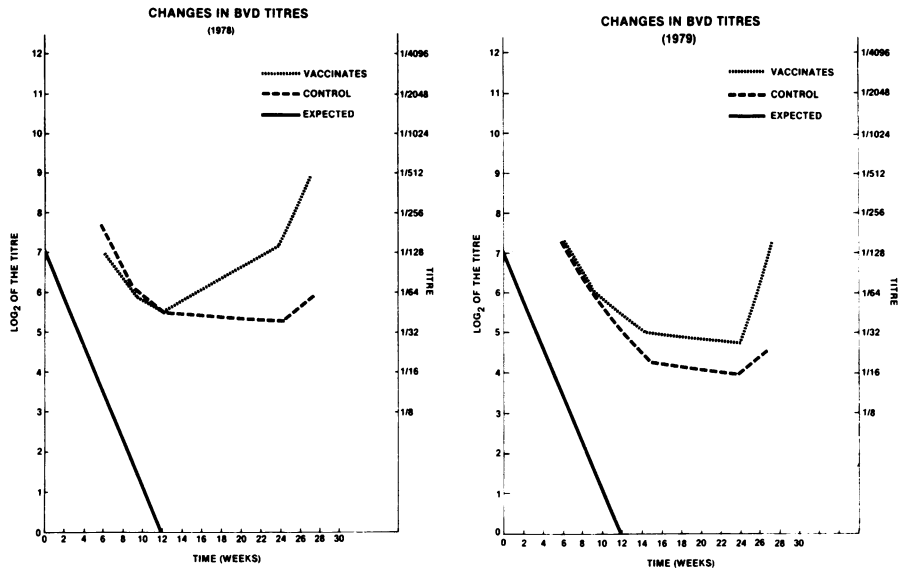

Fig. 1. The mean $\log_2$ titre values for vaccinated (——) and control (--------) calves at six, nine, 12, 15, 24 and 27 weeks of age in 1978 (Fig. 1a) and 1979 (Fig. 1b). The slope of the solid line approximates the expected decline of passively acquired BVD antibody in a calf unexposed to either vaccine or field virus (22).

experimental challenge with virulent BVDV. Also, field and presumably vaccine strains of BVDV do not infect, immunologically stimulate or cause malformations of the fetus of a seropositive cow, or cause abortion (4, 10, 13, 22). These latter reports provide evidence that VN antibody against BVDV prevents viremia. If VN antibody is effective in preventing viremia and enteric BVD, why did the seropositive calves die?

The obvious explanations are: 1) the diagnosis was incorrect, 2) the calves were infected with a heterologous strain of BVDV that was not neutralized by serum antibody, or 3) immunological factors, in addition to serum antibody, are at least partly responsible for protection against BVDV.

First of all, the clinical diagnoses of BVD in this study were confirmed by pathologists at the Ontario Veterinary College based on characteristic gross and histopathological changes.

Secondly, virus neutralizing antibody against BVDV is believed to cross react with all heterologous strains of BVDV (9, 15, 17). Recently however, Bohac *et al* (5) described clinical signs of BVD in cattle associated with the isolation of a Togavirus that did not cross react immunologically with BVDV. In addition, Steck *et al* (40) reported that four cattle which seroconverted to one strain of BVD vaccine virus were seronegative to another antigen strain of BVDV and subsequently these cattle died of enteric BVD following a challenge with this antigenically different strain.

The third explanation for the death of the seropositive calves involves more speculation. Bovine virus diarrhea virus has been reported to impair cellular, humoral and phagocytic immune responses (1, 19, 23, 29, 30, 36, 40) and this is believed to occur in at least some fatally infected seronegative cattle such as K268 and L365 (1, 16, 25). However, Lambert reported that some cattle that remained seronegative following repeated vaccination for BVD were immune to challenge with virulent BVDV, perhaps due to cellular factors (24). Humoral immunity in the seropositive calves that died in this trial was adequate but other protective factors may have been altered or lacking due to immunoincompetence or stresses on the immune system including BVDV itself (24).

In this study, it is impossible to rule out the presence of a heterologous strain of BVDV as the cause of death in seropositive calves. Similarly, it is not possible to rule out the need for immunological factors in addition to serum antibody that could have prevented the deaths of the seropositive calves.

The deaths of calves K251, K252 and K268 following vaccination may have occurred because they were immunoincompetent (24, 32); incubating a naturally acquired infection of BVD or some other pathogen (2, 28); stressed by weaning or other factors (24); or infected by a virulent, noncytopathic strain of BVD that was contaminating the vaccine (31).

The safety of BVD vaccines has been questioned before (24, 28, 32) and more recently, Martin *et al* (26), emphasized that the occurrence of clinical BVD in the feedlots they studied may have been due to vaccination for BVD. The number of clinical cases of enteric BVD decreased dramatically in association with the decreased use of BVD live virus vaccines (27). Although antibody levels have been shown to indicate protection in challenge trials, the increased stress of the field situation may interfere with the development of that protection.

The level of BPS (80%) as documented in 1979, was surprisingly high. Infection with BPS virus is believed to be benign, resulting only in a slight decrease in appetite (3). It is possible that BPS occurs more frequently and causes more severe clinical signs in cattle with a subclinical infection with BVDV which, according to Reggiardo (34) is a stressor of cattle and delays clearance of other pathogens (35). However, Brownlie *et al* (7) have reported that cattle infected with BVDV and challenged with *Brucella abortus* responded immunologically to the infection as quickly as controls. The occurrence of secondary infection following subclinical infection with BVDV and the prevention of this phenomenon by vaccination requires more research.

High levels of passive antibodies to BVD have been shown to interfere with active immunization (2,

6, 22, 39) whereas low levels (1/20-1/96) do not (6). These observations were considered in the design of this field trial and were the basis for the triple vaccination regime so that nonimmune calves were immunized early in life (six weeks of age) and calves were vaccinated at 12 and 24 weeks of age when specific passive antibody could no longer interfere with active immunization.

As illustrated by Fig. 1 vaccination before 12 weeks of age seldom induced an active immune response. This finding is consistent with recommendations in the literature on the optimum age to vaccinate calves with colostrally acquired serum antibody (2, 6, 10, 21). When data from those calves incapable of an active immune response to BVDV because of high passive BVD antibody titres were excluded from this study, over 90% of the calves seroconverted following vaccination indicating that the antigenicity of the vaccine was adequate.

In this study, 40% of control calves seroconverted by 27 weeks of age as a result of contact with field or vaccine virus. Since the vaccine was carefully handled so as to avoid contamination of control calves, the only source of vaccine virus was the vaccinated calves. Cattle vaccinated with a live BVD virus vaccine, NADL strain, grown on porcine kidney tissue culture similar to the vaccine used in this trial, did not become viremic or shed the virus under experimental conditions (14, 33). However, it is conceivable that the vaccine virus may be shed by vaccinated calves under field conditions. The more likely cause of seroconversion in the control calves however was the BVDV which was apparently enzootic in the herd.

This herd is described as being persistently infected with BVDV (enzootic) based on: a five year history of annual calf mortality due to enteric BVD; the high proportion of the cowherd that was seropositive for BVD VN antibody; the high proportion of calves with passively acquired BVD antibody; and the fact that 40% of the control calves seroconverted to BVDV by 27 weeks of age.

Enteric BVD tends to occur sporadically in herds with a large proportion of nonimmune cattle, subsequent to contact with BVDV (21). Characteristically, the herd experiences losses due to enteric BVD one year and is then free of clinical disease until herd immunity wanes. One possible explanation for the phenomenon of persistent infection in this herd could be the presence of an adult animal(s) intermittently or continuously shedding BVDV as described by Coria and McClurkin (11) and Cutlip *et al* (12). The animals described by these authors were seronegative for BVD VN antibody and although initially considered to be immunologically tolerant, were subsequently shown to produce some BVD VN antibodies as evidenced by immune complex induced glomerular nephritis (12). Waning passive antibody levels in the calves, concommittant with stress and exposure to BVDV shed by partially tolerant animals, could explain the annual occurrence of BVD in this herd. However, no cows seronegative for BVD VN antibodies were identified in the serological survey. An alternate explanation for persistence of the BVD problem in this herd is contact with purchased or neighbouring cattle infected with BVDV. Traditionally, additions to this herd had been quarantined, and as there was no contact with cattle from adjacent farms, periodic exposure to infected animals was unlikely. Accordingly, the most probable explanation for the persistence of enteric BVD in this herd is the presence of either seropositive or seronegative animals persistently shedding BVDV.

In this study, vaccination for BVD made no difference in the weight gain of the calves. Woods *et al* (42) reported that vaccination of steers against BVD was a stressor because the vaccinated calves gained less than the controls. This latter report and the results of the present trial are at variance with the unsubstantiated belief in the cattle industry that vaccination for BVD reduces the impact of subclinical and clinical disease on weight gain.

From the data presented in this report it was concluded:

1) that the use of this commercial, modified live virus BVD vaccine did not reduce mortality due to enteric BVD in this persistently infected herd,

2) that BVD VN antibodies which were induced in approximately 90% of the vaccinated calves, were not always protective as evidenced by the death of two calves that had seroconverted at least seven weeks prior to developing enteric BVD,

3) that based on the serological responses, vaccination before 12 weeks of age was of no benefit,

4) that BVDV may be enzootic in some herds and finally

5) that BVD vaccination had no significant effect on the growth rate of calves (weight gain, height at the withers or girth size) in this herd.

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