Ciência Rural, Santa Maria, v.54:02, e20220364, 2024 http://doi.org/10.1590/0103-8478cr20220364
ISSNe 1678-4596
MICROBIOLOGY

Anamnestic response against bovine viral diarrhea virus and bovine herpesvirus type 1 in young Holstein heifers vaccinated with four different commercial formulations

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ABSTRACT: This study evaluated the vaccine-induced serological response after administering four commercial formulations containing Bovine Viral Diarrhea Virus (BVDV) type-1, BVDV-2, and Bovine Herpesvirus type 1 (BoHV-1) to young heifers with circulating maternal antibody titers. The study also determined the anamnestic response to vaccinations after the complete metabolization of maternal antibodies when the calves reached six months. Eighty-seven Holstein heifers were selected and randomly distributed into four experimental groups based on the vaccine administered. The four vaccine-based experimental groups were as follows: vaccine A (n = 22), vaccine B (n = 22), vaccine C (n = 24), and vaccine D (n = 19), given on the 60th, 90th, and 180th day of life, respectively. Virus-neutralization (VN) tests were performed at baseline (day 60) and 30 days after administering the second (day 120) and third doses (day 210). We analyzed the effect of vaccine group ($P \leq 0.001$), day of vaccination ($P \leq 0.001$), and group versus vaccine day interaction ($P \leq 0.001$) for antibody titers produced against BVDV-1, BVDV-2, and BoHV-1 using the PROC MIXED method (Statistical Analysis System –SAS 9.4). Antibody titers against BVDV-1, BVDV-2, and BoHV-1 were similar at baseline and on day 60 of life. The mean antibody titers were constant and persisted against BVDV-1 in heifers immunized with vaccines A and C. Heifers immunized with vaccine A alone had a similar effect against BVDV-2. Regarding BoHV-1, the antibody titers decreased between days 60 and 210 in groups B, C, and D. The antibody titer for heifers in group A also decreased between days 60 and day 120, and an intense increase in titers was observed on day 210. After being immunized with formulations B, C, and D, the frequency of animals with titers above protective levels for BVDV-1, BVDV-2, and BoHV-1 was very low or null. Indicators of anamnestic response were observed in heifers vaccinated with formulation A only. Therefore, it can be concluded that passive immunity negatively interferes with antibody production after vaccination.
Key words: passive immunity, serum neutralization and vaccines.

Resposta anamnéstica contra o vírus da diarreia viral bovina e herpesvírus bovino tipo 1 em novilhas Holandesas jovens vacinadas com quatro diferentes formulações comerciais

RESUMO: O objetivo desse estudo foi avaliar a resposta sorológica induzida após vacinação com quatro formulações comerciais contendo o BVDV-1, BVDV-2 e BoHV-1 em novilhas jovens com títulos de anticorpos maternos circulantes, assim como analisar uma possível resposta anamnéstica em vacinações, após a completa metabolização dos anticorpos maternais aos seis meses de idade. Foram selecionadas 87 novilhas Holandesas, distribuídas aleatoriamente em quatro grupos experimentais de acordo com as vacinas: vacina A (n = 22), vacina B (n = 22), vacina C (n = 24) e vacina D (n = 19), aplicadas aos dias 60, 90 e 180 de vida. Testes de vírus-neutralização (VN) foram realizados no momento basal (D60) e 30 dias após a aplicação da 2ª dose (D120) e 3ª dose (D210). Observou-se efeito do grupo ($P \leq 0,001$), dia da vacinação ($P \leq 0,001$), e interação grupo *versus* dia ($P \leq 0,001$) para os títulos de anticorpos produzidos contra o BVDV-1, BVDV-2 e BoHV-1, por meio do comando PROC MIXED (Statistical Analysis System, versão 9,4). Os títulos de anticorpos contra o BVDV-1, BVDV-2 e BoHV-1 eram semelhantes no momento basal aos 60 dias de vida. Os títulos médios de anticorpos foram constantes e persistentes contra o BVDV-1 nas novilhas imunizadas com as vacinas A e C, porém apenas a vacina A teve este mesmo perfil contra o BVDV-2. Em relação ao BoHV-1, os grupos B, C e D apresentaram queda nos títulos de anticorpos do D60 ao D210, enquanto as novilhas do grupo A apresentaram queda do D60 ao D120, com aumento intenso dos títulos no D210. A frequência de animais com títulos acima dos protetores contra o BVDV-1, BVDV-2 e BoHV-1 foram muito baixos ou nulos após as vacinações nos animais imunizados com as formulações B, C e D. Sugere-se que a imunidade passiva interferiu negativamente na indução de anticorpos pelas vacinas, observando-se indicadores de resposta anamnéstica apenas para as novilhas vacinadas com a formulação A.
Palavras-chave: imunidade passiva, soroneutralização, vacinas.

INTRODUCTION

Calves are born stressed and immunosuppressed, with agammaglobulinemia and an immature adaptive immune system secondary to absent antigenic stimulation during the fetal period. The calves are; therefore, wholly dependent upon passive immunity acquired through colostrum ingestion during the postnatal phase (CHASE et al., 2008). The acquisition of passive immunity depends upon the intake, absorption, and quality of the colostrum produced by the cow (GODDEN et al., 2019).

Received 06.27.22 Approved 04.10.23 Returned by the author 06.21.23
CR-2022-0364.R2
Editor: Rudi Weiblen

The colostrum's quality reflects the calf's immune response capability, stimulated by natural infection or vaccination (BACCILI et al., 2018).

The duration of passive immunity provided by the colostrum depends on the number of immunoglobulins ingested and the half-life of the antibodies;the half-lives of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) are reported to be 21–23 days (FULTON et al., 2004). Antibody concentration tends to be stable in the first weeks of life and gradually declines until it reaches zero around six months of life (MENANTEAU-HORTA et al., 1985; KIMMAN et al., 1989; BACCILI et al., 2018). The exact timing of immunity loss is imprecise. In addition, field tests capable of detecting this possible window of susceptibility and determining the opportune time for initiating vaccination protocols in calves are unavailable (CHASE et al., 2008; WINDEYER& GAMSJÄGER, 2019).

Although, passive immunity benefits the heifer's health, growth, and adult life, high maternal antibody titers inhibit neonatal immunoglobulin production (TIZARD, 2013). Research investigating the response to vaccination in the presence of maternal antibodies generally shows varying results. Even so, most studies fail to prove seroconversion, characterized by at least a fourfold increase in antibody titers, after vaccination (BRAR et al., 1978; ELLIS et al., 2014; SILVA et al., 2020; GOMES et al., 2021). Previous studies have also shown that calves vaccinated with parenteral formulations in the presence of maternal antibodies have persistent specific antibody titers, generate immunological memory, and show fewer clinical signs when challenged with agents of bovine respiratory disease complex (BRDC) (CHAMORRO et al., 2016; WINDEYER& GAMSJÄGER, 2019).

Immunological memory enables the formation of the anamnestic immune response, which is critical against future infectious challenges. Evidence showed that vaccination of calves that possess maternal antibodies are primed to develop responses in future vaccination programs or in the presence of natural exposure to infectious agents, even if they do not seroconvert (BRAR et al., 1978; ENDSLEY et al., 2003; RIDPATH et al., 2003).

In Brazil, few studies support technicians and producers in expanding vaccination protocols for dairy calves to prevent BRDC. Nonetheless, the incidence of BRDC has been noted at increasingly younger ages at around 30–45 days of age in intensive breeding systems (GOMES et al., 2021). In addition, formulations containing live and modified viruses and products with an intranasal application, widely used in international studies, are restricted in Brazil. Even so, vaccines against BVDV marketed in Brazil contain BVDV-1 alone; although, a few formulations indicate the presence of BVDV-2on the package insert.

Consequently, a large dairy production system, including a farm that had conducted research and discarded animals that were being persistently infected with BVDV in the state of São Paulo, requested assistance to update its vaccination protocol for animals in the young phase. Thus, a partnership was established associating the interest of the farm with the research on this scientific gap. Four commercial vaccines for parenteral use with BVDV-1, BVDV-2, and BoHV-1 in their formulations were selected and tested in vaccinated animals on days 60, 90, and 180 of life. Therefore, this study reported the vaccine-induced serological response of four commercial vaccines containing BVDV-1, BVDV-2, and BoHV-1 in young heifers with titers of circulating maternal antibodies. In addition, the study analyzed the anamnestic response to subsequent vaccinations at six months of age when heifers have low or null titers of circulating maternal antibodies.

MATERIALS AND METHODS

The study was conducted in a commercial herd in São Paulo. The cows were vaccinated prepartum with parenteral vaccine A. The 3 Q's of colostrum management is adopted by the farm, which include quality (colostrum IgG concentration > 50 g/L), quantity (colostrum intake >10% of body weight), and quicky (feed calf and milk dam within 2 hours of calving). Eighty-seven healthy Holstein heifers were selected on approximately the 60th ± 7 days of life. The exclusion criteria included a history of failure in transferring passive immunity, low body condition score, presence of ectoparasites, and health alterations. The calves did not receive drug treatment during the study period and were kept in separate lots in the rearing system of their farms. The animals were randomly assigned to four experimental groups: vaccine A (n = 22), vaccine B (n = 22), vaccine C (n = 24), and vaccine D (n = 19). They were immunized with three doses of vaccines A to D (Table 1), administered on days 60± 7, 90± 7, and 180± 7 of life according to the manufacturer's instructions on dose and route of administration.

The animals' serological response was evaluated on days 60 (July 28, 2021), 120 (September 29, 2021), and 210 (November 29, 2021)

Table 1 - Commercial polyvalent vaccines indicated for preventing BVDV and BoHV-1.

Vaccines	----------Formulations*----------
Vaccine A (n = 22)	BVDV 1 and 2
	Bovine herpes virus (IBR) type 1, PI3, and modified live BRSV
	Inactivated *Leptospira* bacterins
	Adjuvant: Amphigen®
Vaccine B (n = 22)	BVDV 1 and 2
	Bovine herpes virus (IBR) type 1, PI3, and modified live BRSV
	Inactivated *Pasteurella haemolytica*, *Leptospira* bacterins
	Adjuvant: Aluminum hydroxide
Vaccine C (n = 24)	BVDV (type not informed)
	Bovine herpes virus (IBR) type 1 and PI3
	Inactivated *Pasteurella haemolytica* and *multocida* bacterins, and *Histophilussomni*
	Adjuvant: Aluminum hydroxide
Vaccine D (n = 19)	BVDV 1 and 2
	Bovine herpes virus (IBR) type 1 and type 5
	Leptospira bacterins
	Campylobacter bacterins
	Adjuvant: 10% aluminum hydroxide
	Additions: Selenium (as sodium selenite) 10 mg/dose

*Compositions extracted from commercial product package inserts.

of life (Figure 1). Blood samples were collected in a vacuum system using siliconized tubes without anticoagulant (BD Vacutainer®). Samples were centrifuged at 1,080xg for 15 mins to obtain serum stored in 1mL triplicates. Animal samples were identified with labels and stored in a freezer at -20 ºC for subsequent transportation to the Virology sector of the Universidade Federal de Santa Maria, Rio Grande do Sul (UFSM).

The Singer (BVDV-1) and VS-253 (BVDV-2) strains used in the virus-neutralization (VN) tests were provided by Dr. Ruben Donis (University of Nebraska in Lincoln, Lincoln, NE, USA). The Cooper strain of BoHV-1 was used in the VN tests. Multiplication, titration, and VN tests were performed on pestivirus-free Madin-Darby bovine kidney (MDBK) cells. The cells were maintained in minimum essential medium (MEM) (Vitrocell®, Nova Campinas, São Paulo, Brazil), supplemented with penicillin (10,000IU/mL), streptomycin (10mg/mL), ciprofloxacin (10 mg/mL), and amphotericin B (250µg/mL). For the MDBK cell culture, 10% equine serum was used. VN assays were performed in 96-well plates containing increasing serum dilutions, starting with 1:4. Samples were incubated with 100–200 $TCID_{50}$ of the respective virus for two hours. Subsequently, we added a suspension of MDBK cells, and the plates were incubated at 37 °C with 5% CO_2. Neutralizing titers were determined by the presence or absence of cytopathic effect on the infected cells after 96 hours of incubation. Antibody titers were taken as the reciprocal of the highest dilution of serum that prevented the production of cytopathic effects (CPE).

Statistical analysis was performed using the SAS statistical program (SAS 9.4, Institute Inc., Cary, NC). All variables were evaluated for distribution relative to the Gaussian curve using the Guided Data Analyses function. Variables were tested for the fixed effects of treatments (vaccines A–D) and days (0, 60, and 210), as well as the interaction of treatment versus day effects, using the PROC MIXED model. This model is the standard SAS procedure for analyzing unbalanced mixed models since it distinguishes between fixed and random effects (LITTELL et al., 1996).

Variables were tested for the fixed effects of treatments (vaccines A–D) and days (0, 60, and 210). In addition, the interaction of treatment versus day effects using the PROC MIXED method (PROC MIXED, SAS) was analyzed with the post hoc Least Significant Difference (LSD) test. Models were tested for covariance structures using the Akaike Information Criterion (AIC).

The frequencies of titers considered above the protective levels were as follows: (1) ≥ 60 for BVDV (HOWARD et al., 1989); and (2) ≥

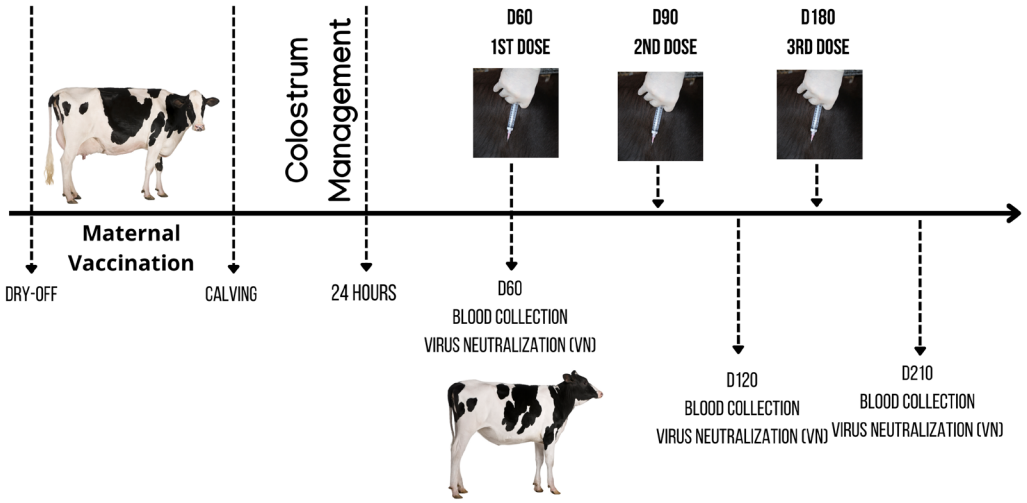

Figure 1 - Vaccination scheme and times of serological tests conducted to evaluate the effect of colostrum antibodies and anamnestic response on the action of four commercial formulations against BVDV-1, BVDV-2, and BoHV-1.

32 for BoHV-1 (POSPISIL et al., 1996). Qualitative data were presented using frequency values, and the Chi-square test was used to perform comparisons between groups. Analyses were considered significant when $P \leq 0.05$ (*).

RESULTS AND DISCUSSION

This study evaluated the influence of maternal antibodies on the serological response in young Holstein heifers vaccinated with four vaccine formulations, including BVDV-1, BVDV-2, and BoHV-1, produced from heifers available in the Brazilian market. In addition, the study evaluated the anamnestic response to further vaccination at six months of life. This study was developed in partnership with a commercial dairy herd during a review period of the vaccination schedule in a herd free of animals persistently infected with BVDV to choose the optimal vaccine formulation among the diversity of products registered at the Ministry of Agriculture, Livestock and Food Supply (MAPA). Therefore, the study's results should be disseminated to inform and assist technicians and producers in developing health calendars for young cattle.

The data were analyzed using the MIXED method to determine the effect of the vaccine formulation group ($P \leq 0.001$), day of vaccination ($P \leq 0.001$), and group *versus* day of vaccination interaction ($P \leq 0.001$) for antibody titers produced against BVDV-1, BVDV-2, and BoHV-1.

Tables 2 and 3 show the mean titers of specific antibodies (log_2) for BVDV-1, BVDV-2, and BoHV-1 and the rates (%) of animals presenting titers above the protective ones after the application of the vaccination protocols. The baseline antibody titers against BVDV-1 ($Log_2 = 6$), BVDV-2 ($Log_2 = 6$), and BoHV-1 ($Log_2 = 4$) were similar among the groups that received the A, B, C, and D vaccines at the beginning of the study when the calves were 60 days of age. The results of the comparative analysis of the antibody titers between the groups indicate that the experimental groups and different vaccine formulations were adequate. The frequency of animals with titers above those considered protective against BVDV-1 (≥ 60), BVDV-2 (≥ 60), and BoHV-1 (≥ 32) were similar between the experimental groups, and they were congruent with cutoff points used in previous studies by HOWARD et al. (1989) and POSPISIL et al. (1996). A considerable frequency of animals had titers below protective levels on the 60th day of life. The percentage of animal antibody titers that were below protective levels were as follows: (1) 45–58% of the animals vaccinated with BVDV-1; (2) 53–59% of the animals vaccinated with BVDV-2; and (3) 23–45% of the animals vaccinated with BoHV-1. According to anamnesis performed on the farm, cows were vaccinated in the prepartum period,

Table 2 - Mean specific antibody titers in Log_2 (antilog – actual titers) for BVDV-1, BVDV 2, and BoHV-1 in serum from Holstein heifers vaccinated with different commercial vaccine formulations.

Virus		----------Neutralizing antibodies (Log_2)----------			
		Vaccine A	Vaccine B (n=22)	Vaccine C	Vaccine D
		(n = 22)		(n = 24)	(n = 19)
BVDV-1	D60	6 (64)^{Aa}	6 (64)^{Aa}	6 (64)^{Aa}	6 (64)^{Aa}
	D120	5 (32)^{Aa}	3 (8)^{Bb}	5 (32)^{Ab}	3 (8)^{Bb}
	D210	5 (32)^{Aa}	2 (4)^{Bc}	5 (32)^{Ab}	3 (8)^{Bb}
BVDV-2	D60	6 (64)^{Aa}	6 (64)^{Aa}	6 (64)^{Aa}	6 (64)^{Aa}
	D120	5 (32)^{Ab}	3 (8)^{Bb}	3 (8)^{Bb}	3 (8)^{Bb}
	D210	6 (64)^{Aa}	2 (4)^{Cc}	3 (8)^{Bb}	2 (4)^{Cc}
BoHV-1	D60	4 (16)^{Ab}	4 (16)^{Aa}	4 (16)^{Aa}	4 (16)^{Aa}
	D120	3 (8) (4)^{Ac}	2 (4)^{Bb}	2 (4)^{Bb}	1 (2)^{Cb}
	D210	7 (128)^{Aa}	1 (2)^{Cb}	3 (8)^{Bb}	1 (2)^{Cb}

(D) day; capital letters in the same row show difference between treatments; lower case letters in the same column show difference between times of the same treatment. The analyses were considered significant when $P \leq 0.05$.

and calves received colostrum according to the gold standard of their care (GODDEN et al., 2019). The BVDV-free status of the herd (BASQUEIRA et al., 2020) might account for lower concentrations of antibodies in the cows' colostrum. In addition, vaccine A used in prepartum vaccination protocols might not have elicited an adequate serological response in the animals, as reported by BACCILI et al. (2019). This study was limited due to a lack of access to data on transferring passive immunity in the calves.

These results should lead to new studies on the efficacy of various vaccine formulations and strategies aimed at preventing infections in the first year of the lives of cattle. Additionally, maternal antibodies, even those below protective titers, seem to influence the immune response induced by vaccinations on days 60 and 90 of life. These results confirmed the interpretation of serologies on days 120 and210 by previous authors (VAN DONKERSGOED et al., 2001; KIRKPATRICK et al., 2001; ELLIS et al., 2014; EARLEY et al., 2018; SILVA et al., 2020).

Antibody evaluation in heifers on the 120th day of life, which corresponds to 30 days after the first booster, revealed varying serological responses to BVDV-1, BVDV-2, and BoHV-1; commercial vaccines used in the vaccination protocols. Analysis of the antibody response to BVDV-1 showed that the highest titers were present in heifers vaccinated with

Table 3 - Frequency (%) of Holstein females with titers above the protective ones (≥ 60) for BVDV-1, BVDV 2, and BoHV-1 (≥ 32) after vaccination with different types of commercial formulations.

Virus		----------Protective titers (n/%)----------				
		Vaccine A (n = 22)	Vaccine B (n = 22)	Vaccine C (n = 24)	Vaccine D (n = 19)	X^2
BVDV-1 (≥ 60)	D60	45% (10/22)	55% (12/22)	54% (13/24)	58% (11/19)	0.942
	D120	45% (10/22)	0% (0/22)	17% (4/24)	0% (0/19)	0.001
	D210	45% (10/22)	0% (0/22)	42% (10/24)	0% (0/19)	0.001
BVDV-2 (≥ 60)	D60	55% (12/22)	59% (13/22)	54% (13/24)	53% (10/19)	0.354
	D120	23% (5/22)	0% (0/22)	0% (0/24)	0% (0/19)	0.001
	D210	59% (13/22)	0% (0/22)	0% (0/24)	0% (0/19)	0.001
BoHV-1 (≥ 32)	D60	23% (5/22)	45% (10/22)	42% (10/24)	42% (8/19)	0.621
	D120	9% (2/22)	0% (0/22)	4% (1/24)	0% (0/19)	0.001
	D210	91% (20/22)	0% (0/22)	8% (2/24)	0% (0/19)	0.001

(D) day; X^2 = Chi-square test. Capital letters in the same row demonstrate the difference between treatments. Analyses were considered significant when $P \leq 0.05$.

formulations A and C. At the same time, the lowest titers were observed in animals vaccinated with formulations B and D. This difference was also noted in animal serologies after the second booster on day 210. Analysis of vaccine timing revealed the persistence and stability of mean antibody titers between days 60–210 in animals immunized with formulation A, while a decrease in values was noted in heifers vaccinated with formulations B, C, and D. The frequencies of animals presenting antibody titers above the protective levels also differed between the experimental groups on day 120 (P = 0.001) and day 210 (P = 0.001). Improved responses were observed in animals vaccinated with formulation A, followed by formulation B. Heifers vaccinated with formulation B or D did not have titers above the protective levels on days 120 and 210 of life.

Serological analysis of the response to BVDV-1 showed that antibody titers persisted after vaccination in groups A and C. These results contrasted the drop in antibodies in groups B and D heifers, even when the animals received three doses of the formulations. The age of the animals was standardized. The concentration of maternal antibodies at baseline was similar in the experimental groups. The vaccine formulations were administered parenterally, and immune responses were attributed to vaccine composition. Divergences between BVDV isolates used in the VN, and those present in the vaccine compositions might have been another contributory factor to the serological results (MÓSENA et al., 2022). Vaccines A and B are polyvalent and contain inactivated BVDV-1 diluted with different adjuvants: aluminum hydroxide (vaccine B) and ISCOM-type adjuvant (vaccine A). Immunostimulant complexes (ISCOMs) are structures composed of cholesterol, phospholipids, and saponins (QuilA), sized between 30 and 40 nm. Saponin-based adjuvants increase the penetration of antigen-presenting cells when the vaccine is injected. Therefore, increased uptake of deposited antigens ensues, followed by antigen-specific stimulation in regional lymph nodes and the development of cellular (Th1) and humoral (Th2) immune responses (SJÖLANDER et al., 1998; SALIBA et al., 2017).

The results of BVDV-2 are of great concern since adequate magnitude and maintenance of antibody titers between days 60 and 210 were observed only in animals immunized with vaccine A. The circulating antibody titers decreased in the other groups between days 120 and 210. Thirty days after the administration of the second dose of vaccine (day 120), animals in group A had higher titers; similar results were seen in groups B, C, and D. Thirty days after administering the third booster (day 210), the best responses were observed in animals belonging to group A, followed by group C, and groups B and D, which had similar results. The frequency of animals with titers above the protective level was also higher in group A. Worrisome results were observed in groups B, C, and D since heifers in these groups did not have titers above the protective ones for BVDV-2. The reduced efficacy of the formulations for BVDV-2 is concerning since many vaccines still do not have this antigen in their formulation, despite the prevalence of BVDV-2 in Brazil. The national incidence of BVDV-2 is 25.7%, with regional differences. However, a prevalence of 48% was observed in states in the southern region of Brazil (FLORES et al., 2018). Research has shown the existence of cross-reactivity between BVDV-1 and BVDV-2.

The cross-reactivity is low, and it is insufficient to warrant immunoprotection. Previous joint research published by our research team identified circulating BVDV-2 on the farm where this study was conducted. The presence of BVDV-2 on this farm served as the basis for selecting formulations that contained BVDV-1 in addition to BVDV-2 (BASQUEIRA et al., 2020). Despite the product description provided on their inserts, the response against BVDV-2 was unsatisfactory after immunization with formulations B, C, and D. These unsatisfactory responses are secondary to the presence of maternal antibodies and the compositions of the products used in the heifers' immunization protocols. The differential ISCOM adjuvant of vaccine A was previously highlighted.

Serological response against BoHV-1 was similar to BVDV-1 and BVDV-2, with better results for vaccine A, followed by vaccines C, B, and D, respectively. Between days 60 and 120, a decrease in antibody titers for all experimental groups was observed. However, a comparative analysis of days 120 and 210 showed increased antibody titers in animals immunized with formulation A only. The frequencies of protected animals slowly declined to minimal and reached zero protection for animals vaccinated with C, B, and D formulations on the 120th and 210th day. Heifers in group A alone showed possible anamnestic responses after receiving the third booster on day 210. Vaccine A contains attenuated and thermosensitive BoHV-1 in its formulation, in addition to having varying adjuvants. Even though vaccinating young calves in the presence of maternal antibodies did not result in seroconversion, an anamnestic response was noted in group A, thus confirming the results of previous studies (BRAR et al., 1978; MENANTEAU-HORTA et al., 1985).

The absence of a control group that did not receive the vaccination is a limitation of this study. However, the study's results are practical and applicable to dairy herds and demonstrated that adequate colostrum management will protect heifers in the first months of life. The importance of the study can also be associated with its finding that choosing vaccines based on their composition, as described in package inserts, will not correlate with serological responses.

The results of this study reinforce the need to guarantee passive immunity through maternal vaccination protocols and colostrum management since the presence of maternal antibodies limits active immune responses to parenteral vaccination protocols. Future research should focus on the use of protocols with intranasal vaccines for respiratory viruses, which are common in the first month of life, assessing the need for booster doses associated with modified live vaccines starting at 90 days of life, as recommended by the manufacturers. A limitation of this protocol is the absence of BVDV in the formulation of intranasal vaccines and the biological risks related to using live and modified vaccines in animals younger than three months of age.

CONCLUSION

This study showed that passive maternal immunity interferes with early vaccination of heifers using the tested formulations. In particular, the results showed that this interference is identifiable by observing indicators of the anamnestic response in heifers vaccinated with formulation A.

ACKNOWLEDGMENTS

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Cnpq) for the fellowship of research productivity (Process number 312244/2021-5).

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they do not have financial or personal conflicts of interest that might affect their objectivity in this study.

AUTHORS' CONTRIBUTIONS

VG and SS conceptualized the study. SS provided animal reviews and sample collection. NHP and EFF conducted serological analyses. CCB tabulated the data and performed statistical analyses. CCB, VG, and RSM prepared the manuscript. VG and EFF reviewed the manuscript. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The Ethics Committee approved this study on Animal Use at the School of Veterinary Medicine and Zootechnics at the Universidade de São Paulo (USP) (Certificate No. 7782291020).

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