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MICROBIOLOGY

A glycoprotein E gene-deleted bovine alphaherpesvirus 1 strain is attenuated and immunogenic for calves with passive immunity upon intranasal immunization

Ingryd Merchioratto[1] Isac Junior Roman[1] Natália Hettwer Pedroso[1] Pablo Sebastian Britto de Oliveira[1] José Valter Joaquim Silva Júnior[2,3,4] Rudi Weiblen[4] Eduardo Furtado Flores[4*]

[1]Programa de Pós-graduação em Medicina Veterinária, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[2]Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[3]Setor de Virologia, Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brasil.
[4]Setor de Virologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: eduardofurtadoflores@gmail.com. *Corresponding author

ABSTRACT: *Vaccination has been used to prevent the losses associated with Bovine alphaherpesvirus 1 (BoHV-1) infection but passively acquired antibodies may compromise vaccine efficacy. Intranasal immunization (IN) of calves with modified live viral BoHV-1 vaccines has proven to overcome the acquired passive antibodies and confer adequate protection. Herein, we evaluated the safety and immunogenicity of a glycoprotein E-deleted Brazilian BoHV-1 strain (BoHV-1gEΔ) for IN immunization of calves. Ten 1-to-2 months-old calves with virus-neutralizing titers (VN) ranging from 2-64 were immunized IN with viable BoHV-1gEΔ ($10^{7.1}$ $TCID_{50}$) and four remained as unvaccinated controls (VN titers 8-32). After IN immunization, calves presented a transient (2-6 days) mild nasal secretion and shed the vaccine virus in nasal secretions in low titers (<$10^{2.6}$$TCID_{50}$/mL) for 4-8 days. Interestingly, the vaccinated calves did not show an increase in VN titers after vaccination. Rather, they presented a gradual reduction in serum VN antibodies in the following weeks – similarly to unvaccinated controls. Upon IN challenge with a virulent heterologous BoHV-1 strain at day 55 post-immunization ($10^{7.63}$$TCID_{50}$), vaccinated calves shed significantly less virus from day 6 post-challenge onwards ($p < 0.07$) and for a shorter period of time than the controls ($p < 0.0024$). Importantly, both the duration and intensity of clinical signs were reduced in vaccinated animals. In addition, vaccinated calves showed an abrupt raise in VN titers post-challenge, indicating adequate immunological priming by vaccination. In summary, immunization of calves harboring passive antibodies with BoHV-1gEΔ by the IN route was able to prime the immunity to afford partial virological and clinical protection upon challenge.*
Key words: *bovine alphaherpesvirus, recombinant vaccine, intranasal immunization, passive immunity.*

Cepa de alfaherpesvírus bovino 1 com deleção do gene da glicoproteína E é atenuada e imunogênica para bezerros com imunidade passiva após imunização intranasal

RESUMO: *A vacinação tem sido usada para prevenir perdas associadas à infecção pelo alfaherpesvírus bovino 1 (BoHV-1), embora anticorpos adquiridos passivamente possam comprometer a eficácia das vacinas. A imunização intranasal (IN) de bezerros com vacinas de BoHV-1 vivas modificadas pode contornar o obstáculo relacionado à presença de anticorpos adquiridos passivamente, conferindo proteção aos animais vacinados. Nesse contexto, avaliou-se a segurança e imunogenicidade de uma cepa brasileira de BoHV-1 com deleção no gene da glicoproteína E (BoHV-1gEΔ) na imunização IN de bezerros. Dez bezerros, de um a dois meses de idade e com títulos neutralizantes (VN) variando de 2-64, foram inoculados IN com BoHV-1gEΔ ($10^{7.1}$$TCID_{50}$), e quatro permaneceram como controles não vacinados (títulos de VN 8-32). Após a instilação IN, os bezerros apresentaram secreção nasal transitória leve (2-6 dias) e excretaram o vírus vacinal nas secreções nasais em baixos títulos (<$10^{2.6}$$TCID_{50}$/mL) por 4-8 dias. Interessantemente, os bezerros vacinados não apresentaram aumento nos títulos de anticorpos neutralizantes após a vacinação. Em vez disso, eles apresentaram uma redução gradual nos anticorpos neutralizantes séricos nas semanas seguintes - semelhante aos controles não vacinados. Após o desafio IN com uma cepa BoHV-1 virulenta heteróloga no dia 55 pós-imunização ($10^{7.63}$$TCID_{50}$), os bezerros vacinados excretaram o vírus em títulos menores a partir do sexto dia pós-desafio ($p < 0,07$) e por um período de tempo menor do que o observado nos controles ($p < 0,0024$). É importante notar que tanto a duração quanto a intensidade dos sinais clínicos foram reduzidas nos animais vacinados. Além disso, os bezerros vacinados apresentaram um aumento abrupto nos títulos neutralizantes após o desafio, indicando uma imunização adequada por BoHV-1gEΔ. Em resumo, a imunização IN de bezerros com anticorpos passivos com a cepa BoHV-1gEΔ foi capaz de estimular a imunidade, proporcionando proteção virológica e clínica parciais após o desafio.*
Palavras-chave: *alfaherpesvírus bovino, vacina recombinante, imunização intranasal, imunidade passiva.*

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INTRODUCTION

Bovine alphaherpesvirus 1 (BoHV-1) – the agent of infectious bovine rhinotracheitis (IBR) - is associated with a variety of clinical manifestations in cattle, including respiratory and genital disease, transient infertility, and abortions (MUYLKENS et al., 2007). In addition, BoHV-1 is a major component of the bovine respiratory complex (BRC), a multifactorial condition with severe sanitary and economic impact in feedlot operations (JONES & CHOWDHURY, 2010). BoHV-1 is an enveloped DNA virus belonging to the family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Varicellovirus* (ICTV, 2019; MUYLKENS et al., 2007). After acute infection, BoHV-1 establishes lifelong latent infection in sensory nerve ganglia, from which it may be periodically reactivated and excreted (JONES & CHOWDHURY, 2010).

A number of attenuated and inactivated vaccines have been widely used to reduce the losses associated with BoHV-1 infection and disease, including some containing gene deletions for serological differentiation (reviewed by PETRINI et al., 2019). Most differential vaccines (differentiating infected from vaccinated animals, DIVA) are based on a single deletion of the glycoprotein E (gE) gene (BOSCH et al., 1996; EL-KHOLY et al., 2013; FRANCO et al., 2002; KAASHOEK et al., 1996; VAN ENGELENBURG et al., 1994;WEISS et al., 2015), whereas some contain deletion on the glycoprotein C (gC) gene and/or an additional deletion for further attenuation, usually the thymidine kinase (tk) gene (FLORES et al., 1993; KAASHOEK et al., 1996). In this line, a gE-negative BoHV-1 recombinant (BoHV-1gEΔ) has been constructed out of a Brazilian BoHV-1 strain and evaluated regarding to safety, immunogenicity and potential serological differentiation (WEISS et al., 2015, 2016).

Proper/adequate immunization with passively acquired antibodies has long represented a challenge for vaccine-based BoHV-1 control programs, particularly when administered intramuscularly (IM) or subcutaneously (SC) (PETRINI et al., 2019). In this sense, intranasal (IN) immunization with BoHV-1 modified live virus (MLV) vaccines arose as an alternative for early immunization of calves harboring passive antibodies. As demonstrated previously, passive antibodies apparently do not interfere with replication of the vaccine virus in the nasal cavity as to allow induction of an adequate immune response (PETRINI et al., 2019). In this article, we investigated the safety and immunogenicity of a BoHV-1gEΔ strain (WEISS et al., 2015) upon IN inoculation of calves with passive immunity.

MATERIALS AND METHODS

Experimental design

Colostrum-fed 1 to 2-months-old calves with passive virus neutralizing (VN) antibodies were immunized intranasally (IN) with the recombinant BoHV-1gEΔ and monitored thereafter. At day 55 post-immunization (pi), vaccinated and non-vaccinated controls were challenged by IN inoculation of a virulent BoHV-1 heterologous strain. Vaccinated and control calves were monitored in clinical, virological and serological aspects in the days following challenge.

Viruses and cells

The recombinant BoHV-1ΔgE was constructed of a Brazilian BoHV-1 strain (SV56/90) and was characterized *in vitro* and *in vivo* (WEISS et al., 2015, 2016). The experiments described herein used passage # 4 of the clone #3 of the recombinant BoHV-1gEΔ. The BoHV-1 strain EVI-123 (passage # 14) was used in the challenge experiment (virus kindly provided by Dr. Paulo Michel Roehe, Universidade Federal do Rio Grande do Sul, Brazil). Madin Darby bovine kidney cells (MDBK, ATCC – CCL-22) maintained in MEM (InLab, SP), supplemented with 10% inactivated fetal bovine serum (Vitrocell, Brazil), 100U/mL of penicillin and 100µg/mL of streptomycin (Invitrogen, USA) were used in all procedures.

Animal study

Colostrum-fed, one to two-months-old male Holstein calves from a large dairy herd were used in the experiment. Upon arrival at the Universidade Federal de Santa Maria (UFSM) animal facility, calves were submitted to a detailed clinical examination, vermifugated and submitted to blood collection for serology. Animals were maintained in facility rooms, either individually or in pairs, and were fed with milk replacement and, gradually, with pelleted food and alfalfa. Vaccinated and control groups of calves were housed in strict isolation from each other to prevent transmission of the vaccine virus. Ten calves with VN titers ranging from 4 to 64 were immunized IN with the recombinant BoHV-1gEΔ ($10^{7.1}$ $TCID_{50}$ divided in the two nostrils) and four remained as non-vaccinated controls (VN titers 8 to 32), receiving the same volume of culture medium intranasally.

Experimental calves were monitored in a daily basis (respiratory and systemic signs, and body temperature) and submitted to collection of nasal swabs for virus isolation up to day 14 post-immunization (pi) and blood for serology at days 0, 35 and 55 pi. Nasal swabs were submitted to virus isolation in MDBK cells as described previously (WEISS et al., 2010); positive samples were quantitated by limiting dilution and virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL. Serum samples obtained at different times after immunization were tested for virus neutralizing (VN) antibodies against BoHV-1 by VN assay, using the homologous virus as the challenge virus (WEISS et al., 2016). Titers were expressed as the reciprocal of the highest dilution that prevented virus replication.

Fifty-five days pi, vaccinated and controls were challenged by IN inoculation of a highly virulent BoHV-1 strain – EVI-123 in a dose of $10^{7.63}TCID_{50}$ per animal. Clinical signs and body temperature were recorded, and nasal swabs for virus isolation and quantitation were collected in a daily basis for 14 days. Blood for serology (VN) was obtained at the day of challenge and 14 days after challenge. The clinical monitoring consisted of daily clinical examination by two persons who attributed scores to the following parameters for each animal/day: nasal secretion (0 = absence to 4 = abundant, mucopurulent); ocular secretion (0 = absence or 1 = presence); dyspnea (0 = absence or 1 = presence); conjunctivitis (0 = absence or 1 = presence); nasal signs (0 = normal nasal mucosa to 6 = generalized pustules).

The daily clinical score of each group consisted of the mean of individual clinical scores. The clinical scoring was adapted from a previous study (ANZILIERO et al., 2011).

Statistical analysis

The difference between the time and amount of virus excreted in the nasal secretions by the vaccine group and the control group were analyzed by the paired T test, using the GraphPad Prism 6 program. Results were considered statistically different when $p < 0.05$ (95% confidence interval).

RESULTS

Safety of BoHV-1gEΔ in calves upon IN immunization

Intranasal immunization of calves with the recombinant BoHV-1gEΔ did not result in systemic or respiratory signs. Only a slight nasal secretion was transiently observed in the days following inoculation, yet it was also observed in control calves, possibly due to the inoculation of control medium. No increase in body temperature or change in behavior or food consumption was evidenced. Virus replication was detected in inoculated calves for up to eight days (4 to 8); nasal swabs contained virus titers usually < $10^{2.63}TCID_{50}$/mL. Virus shedding was not detected beyond day 8pi up to day 14 post- immunization (pi) when the collection was discontinued.

Serologic response after IN immunization and challenge

Intranasal BoHV-1gEΔ immunization was not followed by an increase in serum VN titers in vaccinated animals (Figure 1A). Rather, most vaccinated calves showed a decrease in VN titers, somewhat resembling that of the control calves, yet less pronounced and not reaching such low titers in all cases. Control calves presented a gradual decrease in VN titers from day 0 to day 55pi (day of challenge), reaching titers ≤ 2 (Figure 1B). This reduction is compatible with the waning of passively acquired colostrum antibodies.

However, following challenge, most vaccinated calves presented an abrupt and significant increase in VN titers, detected as early at day 7 post-challenge (pc) and confirmed at day 14pc (Figure 1A). In contrast, control calves presented a lower and slower increase in VN antibodies, reaching titers of 2 to 8 at day 14pc, resembling a primary serological response (Figure 1B).

Virological and clinical findings post-challenge

Upon challenge, vaccinated animals shed virus for 5 – 10 days (mean 7.25) whereas the controls shed virus from 7 to 11 days (mean = 10.25). The mean duration of virus shedding was significantly shorter in the vaccinated animals comparing to the controls ($p < 0.01$) (Table 1). In general, the mean virus titers in nasal secretions were also reduced in vaccinated animals comparing with the controls, reaching statistically significant values ($P < 0.01$) from day 6pi onwards (Figure 2).

Clinical findings after challenge

In general, control calves presented higher body temperatures than the vaccinated animals in the days following challenge, with differences reaching statistical significance ($p< 0.00012$ and $p < 0.0073$, respectively) from days 5 and 6 post-challenge (pc) (Figure 3). After challenge, animals from both groups presented some degree of nasal and respiratory signs yet both the magnitude and duration of these signs were noticeably reduced in the vaccinated animals compared to controls (Figure 4).

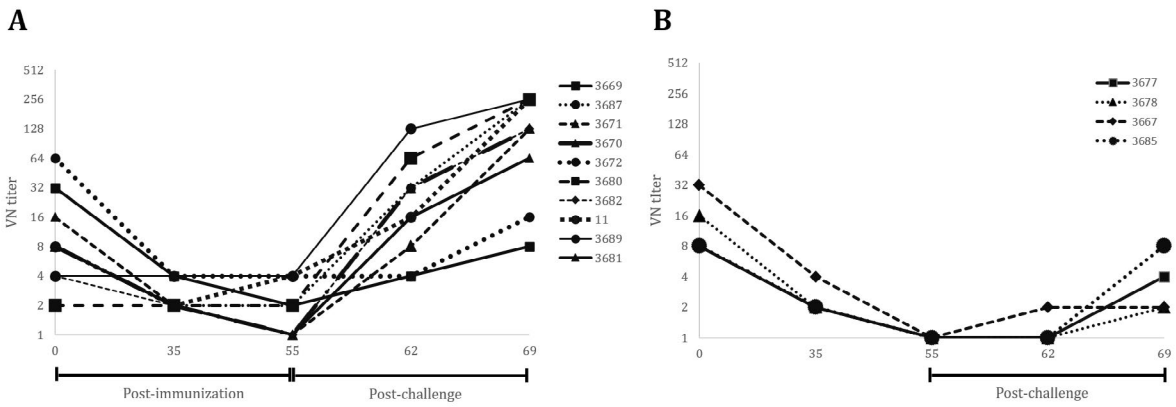

Figure 1 - Virus-neutralizing (VN) titers after intranasal immunization (IN) (day 0) and following challenge (day 55). A) Vaccinated; B) Controls.

The animals in the control group developed nasal and/or respiratory signs starting on day 1pc and lasting up to day 17pc in some animals. Nasal secretion was the most common sign, observed in all animals in this group. Nasal secretion increased in amount and progressed to the stages of serous, mucous to mucopurulent, reaching peak values in animals #78 and #85 between days 9-12. These animals presented alteration in pulmonary auscultation (coarse crackles) on day 10pc. Animal #85 had respiratory distress between days 9-11pc presenting laborious breath. Two of the control animals (2/4) developed lesions in the nasal mucosa, progressing to the stages of macules and papules. In animal #77, lesions progressed to vesicles and pustules, peaking between days 8 -12pc, extending to the lips and gums, and regressing by day 13pc.

The animals in the vaccinated group, conversely, developed nasal and respiratory signs

Table 1 - Virus isolation from nasal swabs of vaccinated (G1) and control calves (G2) following challenge with virulent *Bovine alphaherpesvirus* 1 (BoHV-1).

Group	ID	Day post-challenge										
		2	3	4	5	6	7	8	9	10	11	12
G1 (Vaccinated)	11	6.8 [a]	6.73	4.87	4.1	1.97	1.87	-	-	-	-	-
	3669	4.5	5.87	5.3	5.63	3.1	1.97	≤1.8	-	≤1.8	-	-
	3670	6.1	6.5	5.1	5.5	≤1.8	-	-	-	-	-	-
	3671	5.3	5.63	5.63	4.87	≤1.8	≤1.8	-	-	-	-	-
	3672	5.5	6.3	5.87	5.63	3.1	4.1	-	-	-	-	-
	3680	6.3	6.1	5.73	6.1	2.73	-	-	-	-	-	-
	3681	5.1	6.1	5.97	5.1	1.87	1.87	-	-	-	-	-
	3682	4.3	6.1	4.97	5.1	≤1.8	-	-	-	-	-	-
	3687	5.3	6.63	5.63	5.63	3.5	-	-	-	-	-	-
	3689	6.8	6.5	5.87	≤1.8	-*	-	-	-	-	-	-
G2 (Control)	3667	5.8	6.3	6.1	5.1	2.97	2.1	2.97	2.63	-	-	-
	3677	5.1	5.87	5.3	5.73	4.1	3.8	2.97	2.5	≤1.8	-	-
	3678	6.1	6.3	6.1	5.5	4.63	3.3	2.8	2.3	≤1.8	≤1.8	-
	3685	6.5	6.1	5.97	5.73	4.1	3.63	3.5	2.3	≤1.8	-	-

*Not detected; [a] Values expressed in median tissue culture infectious dose ($TCID_{50}$ / mL);

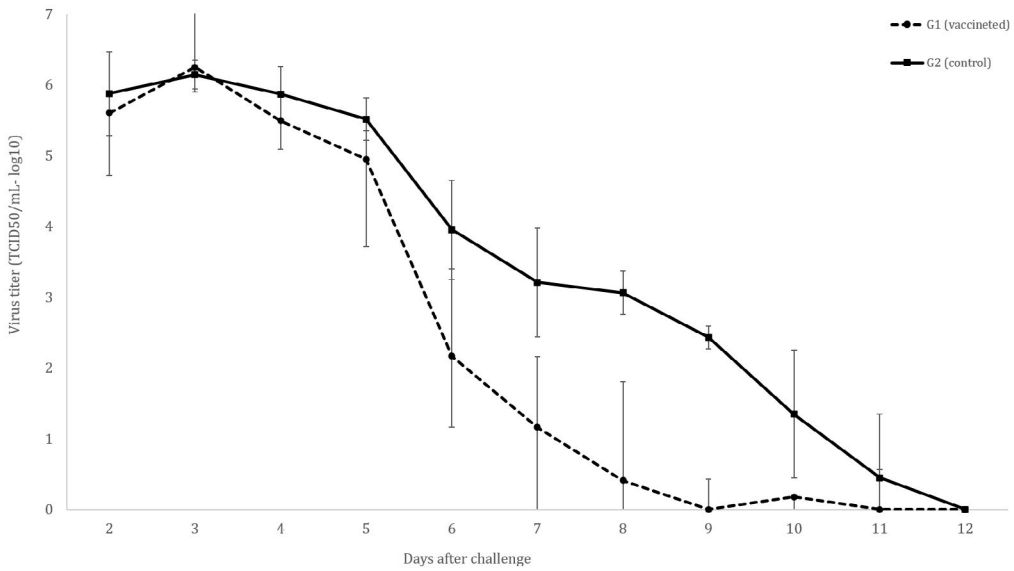

Figure 2 - Mean virus titers in nasal secretions of vaccinated (G1) and control calves (G2), after challenge with virulent *Bovine alphaherpesvirus* 1 (BoHV-1).

from day 2-16pc. Nasal secretion was also commonly observed in the animals of this group. Animal #11 presented mucopurulent discharge with peak at day 12pc. Three animals, #82, #87 and #89, experienced transient and mild respiratory distress between days 9 and 10pc. Eight out of 10 animals presented mild and transient lesions in the nasal mucosa. These lesions were observed between days 3 and 12pc, followed by regression and healing. Animal #80 showed the longest duration of lesions with evolution to multiple vesicles in the nasal mucosa. In general, the magnitude and duration of nasal and respiratory signs were reduced in the vaccinated animals, as demonstrated in the clinical scoring (Figure 4). The clinical scoring was lower in vaccinated animals than in the controls, yet this difference was more pronounced from day 9 up to day 12pc.

DISCUSSION

Results presented herein demonstrated that the recombinant BoHV-1gEΔ is attenuated for calves upon intranasal (IN) instillation and was able to confer partial virological and clinical protection upon heterologous challenge. Gene-deleted BoHV-1 recombinant strains, especially defective in glycoprotein (gE), have been widely used in efforts to control BoHV-1 infection, since they allow for serological differentiation between vaccinated and naturally infected animals (ACKERMANN; ENGELS, 2006; RAAPERI et al., 2014; CHASE et al., 2017). The recombinant BoHV-1gEΔ studied herein has been previously evaluated concerning safety and immunogenicity upon parenteral immunization using live virus or inactivated, adjuvanted virus antigens and serological differentiation was possible by using a gE-specific commercial ELISA (WEISS et al., 2016).

The immune response to BoHV-1 infection (and vaccination as well) is balanced, including generation of cell-mediated immunity (CMI), especially cytotoxic T lymphocytes (CTLs) and antibodies. CTLs are important for virus clearing and recovery from the primary infection, whereas virus-neutralizing (VN) antibodies are involved in protection from reinfection (BABIUK et al., 1996). Modified live vaccines (MLV) are generally more immunogenic than inactivated ones since they are able to induce both humoral and cellular responses (BABIUK et al., 1996).

BoHV-1 vaccination of calves with passive immunity is somewhat problematic since maternal antibodies may affect vaccine efficacy, depending on the level of maternal antibodies and vaccine antigen as well (PETRINI et al., 2019). Usually, MLV are less affected by passive antibodies and are generally able to prime the immune system such a robust secondary

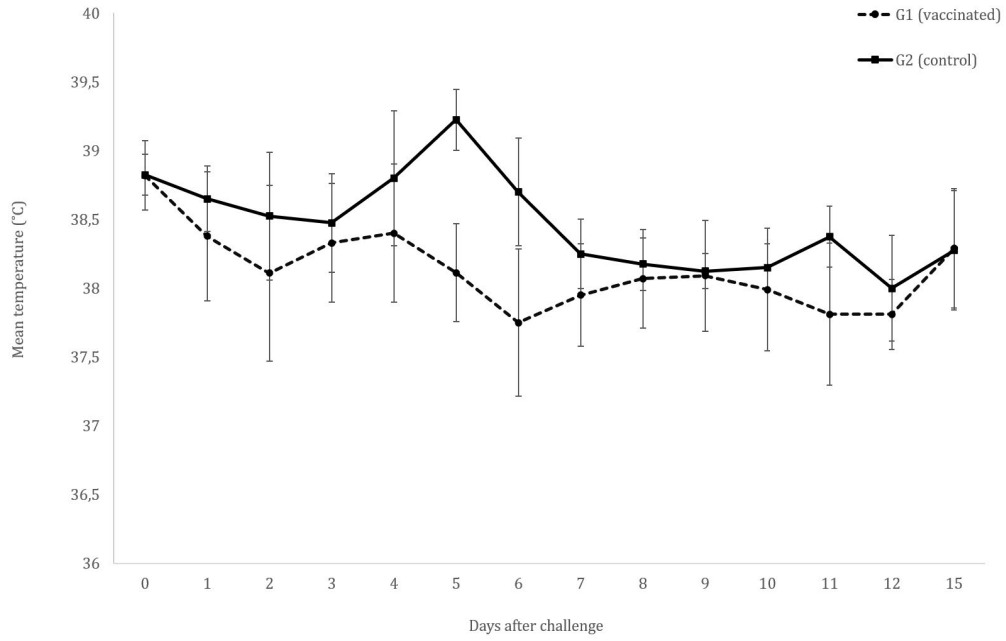

Figure 3 - Mean body temperature of calves in the vaccinated (G1) and control group (G2) following challenge.

response develops after a booster vaccination (HILL et al., 2019). In this line, IN immunization has arisen as an alternative for BoHV-1 vaccination in the presence of passive immunity (CHAMORRO et al. 2016; EARLEY et al., 2018; ELLIS et al., 2014; HILL et al., 2019). Maternally acquired antibodies consist predominantly of systemic IgG, which have little access to mucosal surfaces and, thus, would have little effect on the replication of locally administered vaccine virus (CHAMORRO et al. 2016; EARLEY et al., 2018).

As the main route of BoHV-1 infection is through the mucosal epithelium, the stimulation of immunity in these surfaces would be desirable to afford protection. Conventional BoHV-1 MLV vaccines (and inactivated as well) are administered parenterally and result predominantly in systemic rather than mucosal immunity. In contrast, IN immunization may confer both systemic and mucosal protection mediated by locally secreted antibodies (IgA, IgG), interferons and promptly recruited T CD4+ and T CD8+ lymphocytes (LEVINGS; ROTH, 2013; NEUTRA; KOZLOWSKI, 2006; LOEHR et al., 2000). Immunization at mucosal surfaces results in robust local antibody response (IgA, IgG) and, additionally, mucosal-stimulated B and T cells home to other mucosal surfaces conferring widespread mucosal immunity (NEUTRA, KOZLOWSKI, 2006). A temperature-sensitive BoHV-1 vaccine administered IN was shown to induce secretory IgA and a cell mediated response (FRERICHS et al., 1982). A recombinant BoHV-1 defective in gE was immunogenic and afforded clinical and virological protection in heifers upon intravaginal immunization and challenge (WEISS et al., 2010). Thus, in addition to overcome passive immunity, the strategy of IN immunization would also induce mucosal immunity.

In our study, instillation of BoHV-1gEΔ intranasally was followed by virus replication in low to moderate titers up to day 8 pi, not accompanied by local or respiratory signs, reflecting the usual phenotype of gE-defective BoHV-1 strains (KAASHOEK et al., 1996). Thus, the recombinant BoHV-1gEΔ proved to be safe for IN immunization of young calves in titers higher than those of most MLV vaccines ($10^{5\text{-}6.5}TCID_{50}$/mL). This recombinant has been previously shown to be safe for parenteral immunization in high virus titers ($10^{8}TCID_{50}$/mL) (WEISS et al., 2016). Unfortunately, we did not address whether the vaccine virus could be transmitted to susceptible contacts, a concern regarding MLV vaccines administered IN (CHASE et al., 2017).

Intranasal BoHV-1gEΔ immunization did not result in increase in neutralizing antibody titers

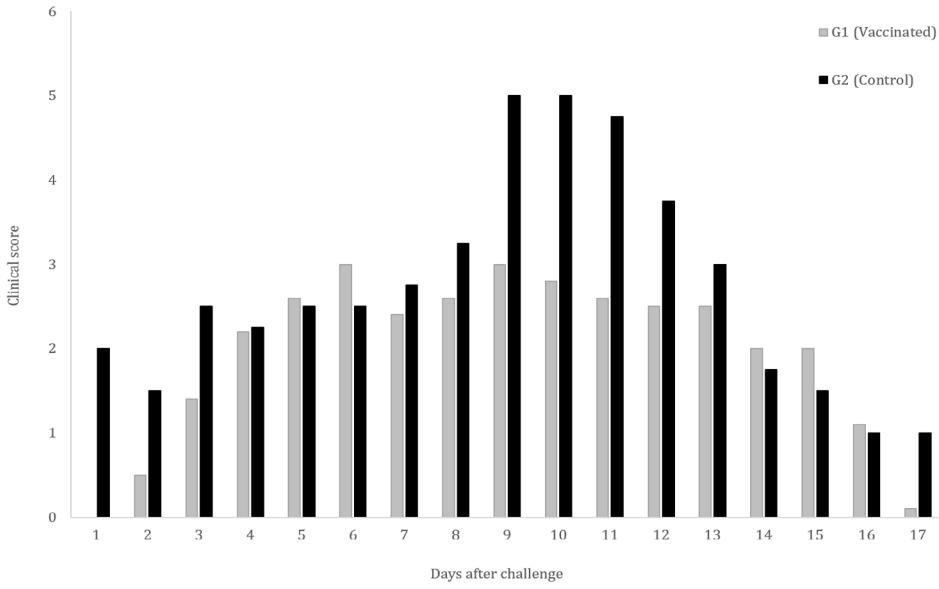

Figure 4 - Mean clinical score (nasal secretion, nasal lesion and dyspnea) of calves in the control group (G1) and vaccinated group (G2) post viral challenge.

as ascertained by VN test performed at days 30pi and 55pi (Figure 2). Rather, most vaccinated animals presented a gradual decrease in VN titers, similar to that observed in control animals and compatible with natural waning of passive antibodies. Passive immunity has been suggested to be more detrimental to humoral than to cellular response to vaccination (HILL et al., 2019). Thus, even unable to stimulate a raise in antibody titers, vaccination in colostrum-fed calves would be able to stimulate T-cell proliferation such that an anamnestic response will be observed upon a subsequent (HILL et al., 2019).

In fact, challenge exposure of vaccinated and control calves at day 55pi evidenced two distinct patterns of serological response. Whereas the control calves showed a kinetics of seroconversion typical of a primary response, most vaccinated animals showed an abrupt and strong increase in VN titers, typical of a secondary response (Figure 1). Thus; although, the vaccination did not result in an immediate increase in VN titers, it certainly sufficed to prime the immune system, as observed in previous studies (CHAMORRO et al., 2016; HILL et al., 2019; NEUTRA; KOZLOWSKI, 2006).

Upon challenge, vaccinated calves shed virus for a shorter period than did the controls ($P < 0.01$). In addition, the magnitude of virus shedding by vaccinated animals was lower than in controls from day 6 post-challenge (pc) onwards. It is conceivable that local CMI (CD4+ and CD8+ lymphocytes), interferons and antibody response (locally secreted IgA and IgG) primed by IN vaccination contributed for restriction of virus replication upon challenge. The prompt increase in VN titers after challenge may have also contributed for restricting virus replication. In this sense, in addition to clinical protection, vaccines should be able to reduce virus shedding as to reduce transmission to other animals (HILL et al., 2019).

The duration and magnitude of clinical signs were also reduced in the vaccinated group. Vaccinated calves developed a milder disease and recovered faster than the controls. The partial clinical protection was likely a consequence of the restricted virus replication in the nasal cavity by CMI and humoral mechanisms, as described above. Taken together with the reduced virus shedding (both in magnitude and duration), the attenuation of the clinical course would be greatly beneficial at herd level.

Considering the strength of challenge (amount of virus and challenge procedure) – very

unlikely to occur in natural conditions – it is likely that IN immunization with the recombinant BoHV-1gEΔ would confer adequate protection upon naturally occurring virus exposures. Thus, in addition to parenteral immunization with live or inactivated/adjuvanted virus (WEISS et al., 2016), the recombinant BoHV-1gEΔ may be suitable for intranasal immunization of calves in the presence of passive immunity.

In summary, our results are promising towards the use of the recombinant BoHV-1gEΔ as a vaccine strain. Additional studies may include: i. Investigation of the duration of immunity and the effects of booster immunization at different intervals; ii. Investigation of protection by a more realistic challenge (e.g. natural exposure to infected animals); iii. Evaluating protection in pregnant cows against reproductive failure/losses; iv. Investigation of immunogenicity upon intravaginal immunization, among others.

CONCLUSION

The recombinant BoHV-1gEΔ is safe and immunogenic for calves with passive antibodies upon intranasal immunization, as demonstrated by virological, clinical and serological evaluation by vaccination-challenge experiments.

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DECLARATION OF CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment was approved by an Institutional Animal Ethics Committee (UFSM, approval # 5740140421).

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

ACKERMANN, M.; ENGELS, M. Pro and contra IBR-eradication. **Veterinary Microbiology**, v.113, n.3–4, p.293–302, 2006. Available from: <https://doi.org/10.1016/j.vetmic.2005.11.043>. Accessed: Jul. 18, 2020. doi: 10.1016/j.vetmic.2005.11.043.

ANZILIERO, D. et al. A recombinant bovine herpesvirus 5 defective in thymidine kinase and glycoprotein E is attenuated and immunogenic for calves. **Pesquisa Veterinária Brasileira**, v.31, n.1, p.23–30, 2011. Available from: <https://doi.org/10.1590/S0100-736X2011000100004>. Accessed: Jul. 18, 2020. doi: 10.1590/S0100-736X2011000100004.

BABIUK, L. A.; DRUNEN LITTEL-VAN DEN HURK, S. VAN; TIKOO, S. K. Immunology of bovine herpesvirus 1 infection. **Veterinary Microbiology**, v.53, n.1–2, p.31–42, 1996. Avaliable from: <https://pubmed.ncbi.nlm.nih.gov/9010996/>. Accessed: Feb. 18, 2022. doi: 10.1016/s0378-1135(96)01232-1.

BOSCH, J. C. et al. An attenuated bovine herpesvirus 1 marker vaccine induces a better protection than two inactivated marker vaccines. **Veterinary Microbiology**, v.52, n.3–4, p.223–234, 1996. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0378113596000703>. Accessed: Feb. 18, 2022. doi: 10.1016/S0378-1135(96)00070-3.

CHAMORRO, M. F.; WOOLUMS, A.; WALZ, P. H. Vaccination of calves against common respiratory viruses in the face of maternally derived antibodies(IFOMA). **Animal Health Research Reviews**, v.17, n.2, p.79–84, 2016. Available from: <https://doi.org/10.1017/S1466252316000013>. Accessed: Oct. 2020. doi: 10.1017/S1466252316000013.

CHASE, C. C. L. et al. Bovine herpesvirus 1 modified live virus vaccines for cattle reproduction: Balancing protection with undesired effects. **Veterinary Microbiology**, v.206, p.69–77, 2017. Available from: <https://doi.org/10.1016/j.vetmic.2017.03.016>. Accessed: Jan. 18, 2021. doi: 10.1016/j.vetmic.2017.03.016.

EARLEY, B. et al. Effect of suckler cow vaccination against glycoprotein E (gE)-negative bovine herpesvirus type 1 (BoHV-1) on passive immunity and physiological response to subsequent bovine respiratory disease vaccination of their progeny. **Research in Veterinary Science**, v.118, p.43–51, 2018. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0034528817301935>. Acessed: Oct, 18, 2020. doi: 10.1016/j.rvsc.2018.01.005.

ELLIS, J. et al. Inhibition of priming for bovine respiratory syncytial virus-specific protective immune responses following parenteral vaccination of passively immune calves. **The Canadian Veterinary Journal = La revue veterinaire canadienne**, v.55, n.12, p.1180–5, 2014. Available from: </pmc/articles/PMC4231808/>. Accessed: Oct. 18, 2021.

EL-KHOLY, A. A. et al. Construction, characterization and immunogenicity of a glycoprotein E negative bovine herpesvirus-1.1 Egyptian strain "Abu-Hammad". **Journal of Virological Methods**, v.194, n.1–2, p.74–81, 2013. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S016609341300311X>. Accessed: Sept. 18, 2020.

ENGELENBURG, F. A. C. et al. A glycoprotein E deletion mutant of bovine herpesvirus 1 is avirulent in calves. **Journal**

of General Virology, v.75, n.9, p.2311–2318, 1994. Available from: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/0022-1317-75-9-2311#tab2>. Accessed: Feb. 18, 2022. doi: 10.1089/08828240260066305.

FLORES, E. F. et al. Efficacy of a deletion mutant bovine herpesvirus-1 (BHV-1) vaccine that allows serologic differentiation of vaccinated from naturally infected animals. **Journal of Veterinary Diagnostic Investigation**, v.5, n.4, p.534–540, 1993. Available from: <http://journals.sagepub.com/doi/10.1177/104063879300500406>. Accessed: Jan. 18, 2021. doi: 10.1177/104063879300500406.

FRANCO, A. C. et al. A Brazilian glycoprotein E-negative bovine herpesvirus type 1.2a (BHV-1.2a) mutant is attenuated for cattle and induces protection against wild-type virus challenge. **Pesquisa Veterinaria Brasileira**, v.22, n.4, p.135–140, 2002. Available from: <https://www.scielo.br/j/pvb/a/L5y3z9WpvjKKVmj9GQ4xXPb/?lang=en>. Accessed: Feb. 18, 2022. doi: 10.1590/S0100-736X2002000400002.

FRERICHS, G. et al. Safety and efficacy of live and inactivated infectious bovine rhinotracheitis vaccines. **Veterinary Record**, v.111, n.6, p.116–122, 1982. Available: <https://pubmed.ncbi.nlm.nih.gov/6289508/>. Accessed: Feb. 18, 2022. doi: 10.1136/vr.111.6.116.

HILL, K. et al. Immune memory induced by intranasal vaccination with a modified-live viral vaccine delivered to colostrum fed neonatal calves. **Vaccine**, v.37, n.51, p.7455–7462, 2019. Available from: <https://doi.org/10.1016/j.vaccine.2019.09.080>. Accessed: Jul. 18, 2021. doi: 10.1016/j.vaccine.2019.09.080.

ICTV. **Herpesviridae - International Committee on Taxonomy of Viruses (ICTV)**, 2019. Available from: <https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsdna-viruses-2011/w/dsdna_viruses/91/herpesviridae>. Accessed: Oct. 18, 2020.

JONES, C.; CHOWDHURY, S. Bovine herpesvirus type 1 (BHV-1) is an important cofactor in the bovine respiratory disease complex. **Veterinary Clinics: Food Animal Practice**, v.26, n.2, p.303–321, 2010. Available from: <https://doi.org/10.1016/J.CVFA.2010.04.007>. Accessed: Oct. 18, 2021. doi: 10.1016/J.CVFA.2010.04.007.

KAASHOEK, M. J.; RIJSEWIJK, F. A. M.; OIRSCHOT, J. T. VAN. Persistence of antibodies against bovine herpesvirus 1 and virus reactivation two to three years after infection. **Veterinary Microbiology**, v.53, n.1–2, p.103–110, 1996. Available from: <https://doi.org/10.1016/S0378-1135(96)01238-2>. Accessed: Jan. 18, 2021. doi: 10.1016/S0378-1135(96)01238-2.

LEVINGS, R. L.; ROTH, J. A. Immunity to bovine herpesvirus 1: I. Viral lifecycle and innate immunity. **Animal Health Research Reviews**, v.14, n.1, p.88–102, 2013. Available from: <https://doi.org/10.1017/S1466252313000042>. Accessed: Jan. 18, 2021. doi: 10.1017/S1466252313000042.

LOEHR, B. I. et al. Gene gun-mediated DNA immunization primes development of mucosal immunity against bovine herpesvirus 1 in cattle. **Journal of Virology**, v.74, n 13, p.6077–6086, 2000. Available from: </pmc/articles/PMC112106/>. Accessed: Feb. 18, 2022. doi: 10.1128/JVI.74.13.6077-6086.2000.

MUYLKENS, B. et al. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. **Veterinary Research**, v.38, n.2, p.181–209, 2007. Available from: <https://doi.org/10.1051/vetres:2006059>. Accessed: Oct. 18, 2021. doi: 10.1051/vetres:2006059.

NEUTRA, M. R.; KOZLOWSKI, P. A. Mucosal vaccines: the promise and the challenge. **Nature Reviews Immunology**, v.6, n.2, p.148–158, 2006. Available from: <https://doi.org/10.1038/nri1777>. Accessed: Oct. 18, 2021. doi: 10.1038/nri1777.

PETRINI, S. .; ISCARO, C. .; RIGHI, C. . Antibody responses to bovine alphaherpesvirus 1 (BoHV-1) in passively immunized Calves. **Viruses**, v.11, n.1, p.23, 2019. Available from: <https://doi.org/10.3390/v11010023>. Accessed: Aug. 2019. doi: 10.3390/v11010023.

RAAPERI, K.; ORRO, T.; VILTROP, A. Epidemiology and control of bovine herpesvirus 1 infection in Europe. **The Veterinary Journal**, v.201, n.3, p.249–256, 2014. Available from: <https://doi.org/10.1016/j.tvjl.2014.05.040>. Accessed: Jun. 2021. doi: 10.1016/j.tvjl.2014.05.040.

WEISS, M. . et al. Genital immunization of heifers with a glycoprotein E deleted, recombinant bovine herpesvirus 1 strain confers protection upon challenge with a virulent isolate. **Pesquisa Veterinária Brasileira**, v.30, n.1, p.42–50, 2010. Avaliable from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2010000100007&lng=en&nrm=iso&tlng=en>. Accessed: Oct. 2020. doi: 10.1590/S0100-736X2010000100007.

WEISS, M. et al. A glycoprotein E gene-deleted bovine herpesvirus 1 as a candidate vaccine strain. **Brazilian Journal of Medical and Biological Research**, v.48, n.9, p.843–851, 2015. Available from: <https://doi.org/10.1590/1414-431x20154243>. Accessed: Oct. 18, 2020. doi: 10.1590/1414-431x20154243.

WEISS, M. et al. Safety and immunogenicity of a glycoprotein E gene-deleted bovine herpesvirus 1 strain as a candidate vaccine strain. **Pesquisa Veterinária Brasileira**, v.36, n.11, p.1067–1074, 2016. Available from: <https://doi.org/10.1590/s0100-736x2016001100002>. Accessed: Oct. 18, 2020. doi: 10.1590/s0100-736x2016001100002.