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RESEARCH ARTICLE

Reactivity Induced by Inactivated Infectious Bovine Rhinotracheitis Vaccination and Its Related Factors in Holstein Calves Following A Modified Live Vaccine Schedule

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ABSTRACT

Infectious bovine rhinotracheitis (IBR) is a critical respiratory disease affecting calves worldwide; however, the optimal schedule for inactivated IBR vaccination remains unknown. This study investigated the reactivity induced by inactivated IBR vaccine and its related factors in Holstein calves following a modified live vaccine schedule. IBR reactivity in 94 calves was investigated. Calves were classified based on changes in IBR reactivity from pre-vaccination to 8 weeks post-vaccination. Factors associated with changes in IBR reactivity were evaluated. IBR reactivity levels decreased in 62.8% calves despite vaccination, whereas those of calves with low pre-vaccination IBR reactivity levels increased. Analysis of IBR reactivity according to birth weight revealed that calves born overweight had high IBR reactivity levels pre-vaccination, and vaccination failed to increase reactivity levels in 88.2% of these calves. Improvement in IBR reactivity was not necessarily associated with pre-vaccination IBR reactivity levels. The >20% decrease classifications showed lower IBR reactivity at pre-vaccination than the <20% decrease classifications. The classifications that showed better improvement in IBR reactivity had higher monocyte, monocyte-to-lymphocyte ratio, lactate dehydrogenase, and creatine kinase values at pre-vaccination. These results indicate that the schedule for inactivated IBR vaccination is ineffective in calves, birth weight may be related to passive immunity in IBR reactivity, and inactivated IBR vaccine-induced reactivity may be associated with the identified four blood parameters and passive immunity. Subsequent investigations are necessary to establish an appropriate timetable for inactivated IBR vaccination and uncover mechanisms underlying the relationship between the four blood parameters and inactivated IBR vaccination.

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INTRODUCTION

Infectious bovine rhinotracheitis (IBR), caused by bovine alphaherpesvirus 1 (BoHV-1), is a critical respiratory disease affecting calves worldwide. IBR manifests as pyrexia, nasal discharge and plaques, cough, dyspnea, anorexia, and lethargy (Nettleton and Russell, 2017). The virus acts as a major initiator of the bovine respiratory disease complex by altering the upper respiratory tract environment and exerting immunomodulatory effects (Filion et al., 1983, Ohmann and Babiuk, 1985). The virus mainly spreads via nasal exudates and cough droplets through direct or indirect contact (Iscaro et al., 2021). Many countries have struggled to overcome the impact of BoHV-1. Many European countries have implemented "test-and-slaughter" and "test-and-removal" strategies combined with vaccination as a response to BoHV-1, whereas the United States of America has employed a vaccination strategy (Fulton et al., 2015).

Calves are born with agammaglobulinemia; therefore, the high quality of colostrum plays an important role in calf health. Colostrum provides passive immunity acquired from IBR-vaccinated dams, which could protect calves and reduce the pathological severity of IBR (Pospíšil et al.,

1983). Since maternal immunity inhibits antibody production when calves are vaccinated against IBR, the modified live IBR vaccine should be administered at a specific age (3–4 months) to avoid maternal antibody interference (Petrini et al., 2019). However, the modified live IBR vaccine has potential disadvantages, including immunosuppression, abortion, and latency, which have led to developing inactivated vaccines (Nandi et al., 2009). Inactivated vaccines have shorter maternal antibody interference against pathogens than modified live vaccines (Chase et al., 2008). However, an ideal time schedule for administering the inactivated IBR vaccine in calves remains unknown.

The status of calves should be evaluated to ensure optimization of antibody concentration by vaccination. In human medicine, immunization following vaccination depends on birth weight (Batra et al., 2009). Although calves with heavier weights have lower risks of respiratory diseases than those with lighter weights (Sanderson et al., 2008), little information is available regarding the association between birth weight and immune responses following vaccination. Numerous studies have suggested that oxidative stress compromises immunity, whereas antioxidant supplementations improve immune responses to vaccination in calves (Mattioli et al., 2020, Cuervo et al., 2021, Nayak and Abuelo, 2021, Otomaru et al., 2021). Similarly, nutritional deficiency impairs immune responses to vaccination in calves (Griebel et al., 1987, McGill et al., 2019). Considering that helper T cells control immune responses associated with vaccination (De Brun et al., 2021), assessing the immunological status could help increase antibody concentration by vaccination.

Hematological and serum biochemical parameters aid in evaluating cattle health and inferring the immunological status. Respiratory diseases in cattle are associated with high aspartate transaminase and globulin levels and low albumin levels (Šoltésová et al., 2015, Çam et al., 2016). IBR-infected cattle have high white blood cell counts with high neutrophil and monocyte percentages but low lymphocyte percentages (Çam et al., 2016); however, the association between these parameters and improvement of antibody concentration by IBR vaccination remains unknown.

Therefore, we investigated the reactivity induced by inactivated IBR vaccine administered according to a modified live vaccine schedule and evaluate factors (passive immunity, birth weight, and hematological and serum biochemical parameters) associated with vaccine-induced reactivity to help establish an appropriate IBR vaccination strategy for calves.

MATERIALS AND METHODS

Animals*:* Calves included in this study were born and raised at a farm of the National Institute of Animal Science free of IBR in Republic of Korea. The vaccination schedule for calves was similar to that for the modified live vaccine since the schedule for inactivated IBR vaccine was not established. The calves were intramuscularly vaccinated with 5 mL of a comprehensive inactivated vaccine (Barvac Elite 4-HS, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri, USA) containing the IBR virus twice (at 14 and 16 weeks) (Figure 1). Biannually (spring and fall), all heifers and cows (aged >1 year) on the farm were intramuscularly vaccinated with 5 mL of an inactivated vaccine (Barvac Elite 4-HS, Boehringer Ingelheim Vetmedica, Inc.).

The experimental Holstein calves (n=94) were born between November 2017 and February 2019 and vaccinated between February 2018 and May 2019. The calves were clinically healthy and had no history of respiratory diseases from birth until the end of this study.

Blood Sampling and Analyses: Blood sampling was performed at 14 (pre-vaccination), 18 (2 weeks post-vaccination), 20, and 24 weeks of calf age (Figure 1). Blood was drawn from the jugular vein and collected using ethylenediaminetetraacetic acid and serum-separating tubes.

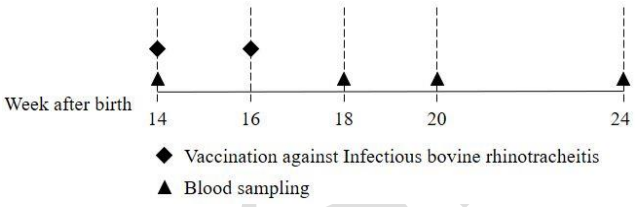

Fig. 1: Scheme of this study; Vaccination against infectious bovine rhinotracheitis and blood sampling. Calves aged 14 weeks were vaccinated first. The second vaccination was performed 2 weeks later (at 16 weeks). Blood sampling was performed before the first vaccination and 2, 4, and 8 weeks after the second vaccination.

Serum was separated by centrifuging the serum-separating tubes at 3,000 rpm (2,600 g) for 10 min and stored at –70°C pending analysis. Biochemical analysis of the serum was conducted on a single day using a biochemistry automated analyzer (Hitachi 7180, Hitachi Ltd., Tokyo, Japan) after calibration and quality control assessments with commercial enzyme assay kits (Fujifilm Wako Pure Chemical Ltd., Osaka, Japan).

The IDEXX IBR gB X3 Ab test (IDEXX Inc.) was used to detect antibodies against IBR. The enzyme-linked immunosorbent assay results were analyzed using a microplate photometer to measure optical density at a 450-nm wavelength. The testing procedure and the reactivity was calculated following the manufacturer's instructions.

Complete blood count results of 66 calves born after June 27, 2018 were obtained using a hematology analyzer (Procyte Dx® hematology analyzer, IDEXX Inc.). Pre-vaccination blood samples were analyzed for hematological and biochemical parameters.

Experimental Group Definition: The birth weight of Holstein calves was categorized as underweight (<36 kg, n=24), normal weight (≥36 kg and ≤45 kg, n=53), and overweight (>45 kg, n=17) based on the average weight addressed by Holstein Association USA, Inc. Calves were divided into decrease and increase classifications by subtracting the reactivity pre-vaccination from that at 8 weeks post-vaccination, as follows: >20% decrease (>20%D), <20% decrease (<20%D), <20% increase (<20%I), and >20 increase (>20%I).

Statistical Analyses: Statistical analyses were performed using the Statistical Package for the Social Sciences software (version 26.0; IBM Corp., Armonk, NY, USA).

The Shapiro–Wilk and Levene tests were performed for normality analysis and equality of variances for the independent t-test, one-way analysis of variance with the post-hoc Duncan multiple range test, and a linear mixed model with Bonferroni post-hoc analysis. The Kruskal–Wallis with the Mann–Whitney U with Bonferroni's method and Friedman tests with post-hoc Wilcoxon signed rank test were used for parameters not satisfying normality analysis or equality of variances. A linear mixed model with Bonferroni post-hoc analysis and the Friedman test with post-hoc Wilcoxon signed rank test were performed to evaluate repeated reactivity measurements. The chi-square test with the Mann–Whitney U with Bonferroni's method was performed to determine associations between changes in classifications of IBR reactivity and calf birth weight. Data are presented as mean±standard deviation. Results with *p*-values of <0.05 indicated statistical significance. Statistical significance for the Mann–Whitney U with Bonferroni's method was set at $p<0.017$ for three classifications and $p<0.0083$ for four classifications.

RESULTS

IBR Reactivity Before and After Vaccination: We confirmed that the newborn calves, heifers, and cows on the farm had high IBR antibody concentrations prior to this study. The average IBR reactivity value was 94.3 ± 0.1% for the heifers and cows, whereas that for newborn calves (1-day-old) was 93.4 ± 0.3% according to IBR virus gB X3 antibody testing (IDEXX Inc., Westbrook, Maine, USA). Overall, the average IBR reactivity in the 94 calves decreased from pre-vaccination (82.5±14.9) to 8 weeks post-vaccination (79.2±14.0) (Table 1). Regarding changes in IBR reactivity from pre-vaccination to 8 weeks post-vaccination, 62.8% (59/94) and 37.2% (35/94) of calves belonged to the decrease and increase classifications, respectively. The average IBR reactivity levels in the decrease classification were 90.8±4.4 at pre-vaccination and 72.0±12.9 at 8 weeks post-vaccination, whereas those in the increase classification were 68.5±16.0 at pre-vaccination and 91.3±3.8 at 8 weeks post-vaccination ($p<0.001$). A significant change in IBR reactivity began to appear at 4 weeks post-vaccination ($p<0.01$). The >40% and 40%–30% decrease classifications showed lower percentages (89.1 and 89.0±4.6, respectively) at pre-vaccination than the 30%–20% (91.2±4.3), 20%–10% (91.2±4.4), and <10% (91.3±4.7) decrease classifications. In the increase classifications, calves with low IBR reactivity at pre-vaccination showed greater improvements in IBR reactivity after vaccination.

We divided the decrease and increase classifications into detailed categories according to the extent of changes in IBR reactivity (Table 2). The >20%D (90.4±4.4) and <20%D (91.3±4.5) classifications had higher IBR reactivity levels than the <20%I classification (81.5±5.9) at pre-vaccination, while the <20%I classification had higher IBR reactivity levels than the >20%I classification (57.5±13.2) ($p<0.001$). The pre-vaccination IBR reactivity of the >20%D classification was lower than that of the <20%D classification; however, these results were not significant ($p>0.05$). The IBR reactivity of the >20%D and <20%D classifications gradually decreased from pre-vaccination to 8 weeks post-vaccination. The IBR reactivity levels of the <20%I classification decreased from pre-vaccination to 2 weeks post-vaccination but increased from 4 to 8 weeks post-vaccination. In the >20%I classification, IBR reactivity continually increased from pre-vaccination to 8 weeks post-vaccination. The IBR reactivity level at 8 weeks post-vaccination was the lowest in the >20%D classification (61.9±8.4), followed by the <20%D (81.7±8.1), <20%I (89.8±4.3), and >20%I (92.5±2.8) classifications ($p<0.001$).

Association Between IBR Reactivity and Calf Birth Weight: We analyzed IBR reactivity according to calf birth weight (Table 3). Calves showed different IBR reactivity at pre-vaccination ($p=0.023$). Calves that were underweight at birth showed lower IBR reactivity at pre-vaccination (76.9±16.9) than those with normal weight (82.7±15.2) and overweight (89.8±5.4) at birth ($p=0.023$). However, calves that were overweight at birth had lower IBR reactivity levels (74.7±12.5) at 8 weeks post-vaccination than those that were underweight (79.4±16.4) and with normal weight (80.5±13.3) at birth, although the differences were not significant ($p>0.05$). The association between IBR reactivity changes (decrease and increase) and calf birth weight was evaluated. Higher percentages of the calves that were underweight (54.2%) and with normal weight (37.7%) had increased IBR reactivity than those that were overweight (11.8%) ($p=0.022$).

Association of IBR Reactivity with Hematological and Serum Biochemical Parameters: We evaluated hematological and serum biochemical parameters according to changes in IBR reactivity (>20%D, <20%D, <20%I, and >20%I). Regarding hematological parameters, MCH, reticulocyte, monocyte, and monocyte-to-lymphocyte ratio values differed among the classifications ($p<0.05$) (Figure 2). However, improvement in IBR reactivity was not associated with MCH and reticulocyte values. The MCH value was the lowest in the >20%D classification, followed by that in the <20%I, <20%D, and >20%I classifications. The reticulocyte value reached its lowest point in the <20%I classification, and highest in the <20%D classification. Classifications with better improvement in IBR reactivity had significantly higher monocyte and monocyte-to-lymphocyte ratio values. The >20%I classification showed the highest monocyte (31.8±10.1%) and monocyte-to-lymphocyte ratio (0.82±0.33) values, followed by the <20%I (30.4±6.7% and 0.75±0.25), <20%D (26.0±6.5% and 0.61±0.23), and >20%D (25.0±8.1% and 0.56±0.24) classifications ($p<0.05$).

Regarding serum biochemical parameters, creatinine, LDH, and CK levels differed across the classifications ($p<0.05$) (Figure 3). However, improvement in IBR reactivity was not associated with creatinine levels; the <20%I classification had low creatinine levels, whereas the >20%D classification had high creatinine levels ($p<0.05$). LDH and CK levels were associated with improved IBR reactivity. Classifications showing better improvement in IBR reactivity had significantly higher LDH and CK levels. The >20%I classification showed the highest LDH and CK levels, followed by the <20%I, <20%D, and >20%D classifications ($p<0.05$).

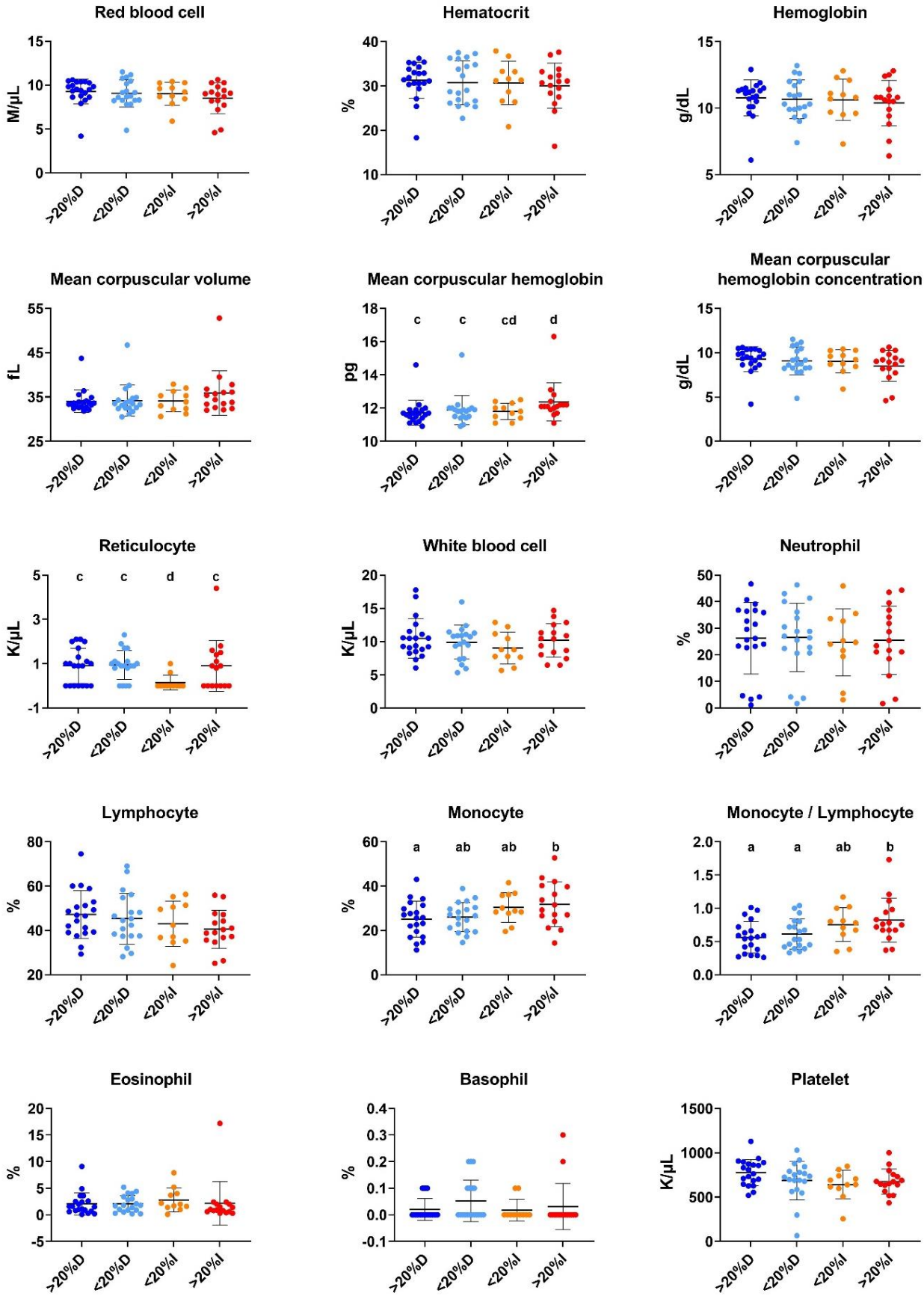

Fig 2: Pre-vaccination complete blood counts for the classifications; (n=66: >20%D, n=20; <20%D, n=19; <20%I, n=11; >20%I, n=16). The calves were divided according to changes in IBR reactivity, calculated by subtracting the IBR reactivity pre-vaccination from that at 8 weeks post-vaccination. The decrease classifications indicate calves with lower IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. The increase classifications indicate calves with higher IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. Abbreviations: >20%D, >20% decrease; <20%D, <20% decrease; <20%I, <20% increase; >20%I, >20% increase. [a–b]: Different letters indicate significant differences (p<0.05, Duncan test). [c–d]: Different letters indicate significant differences (p<0.0083, Mann–Whitney U test with Bonferroni's method)

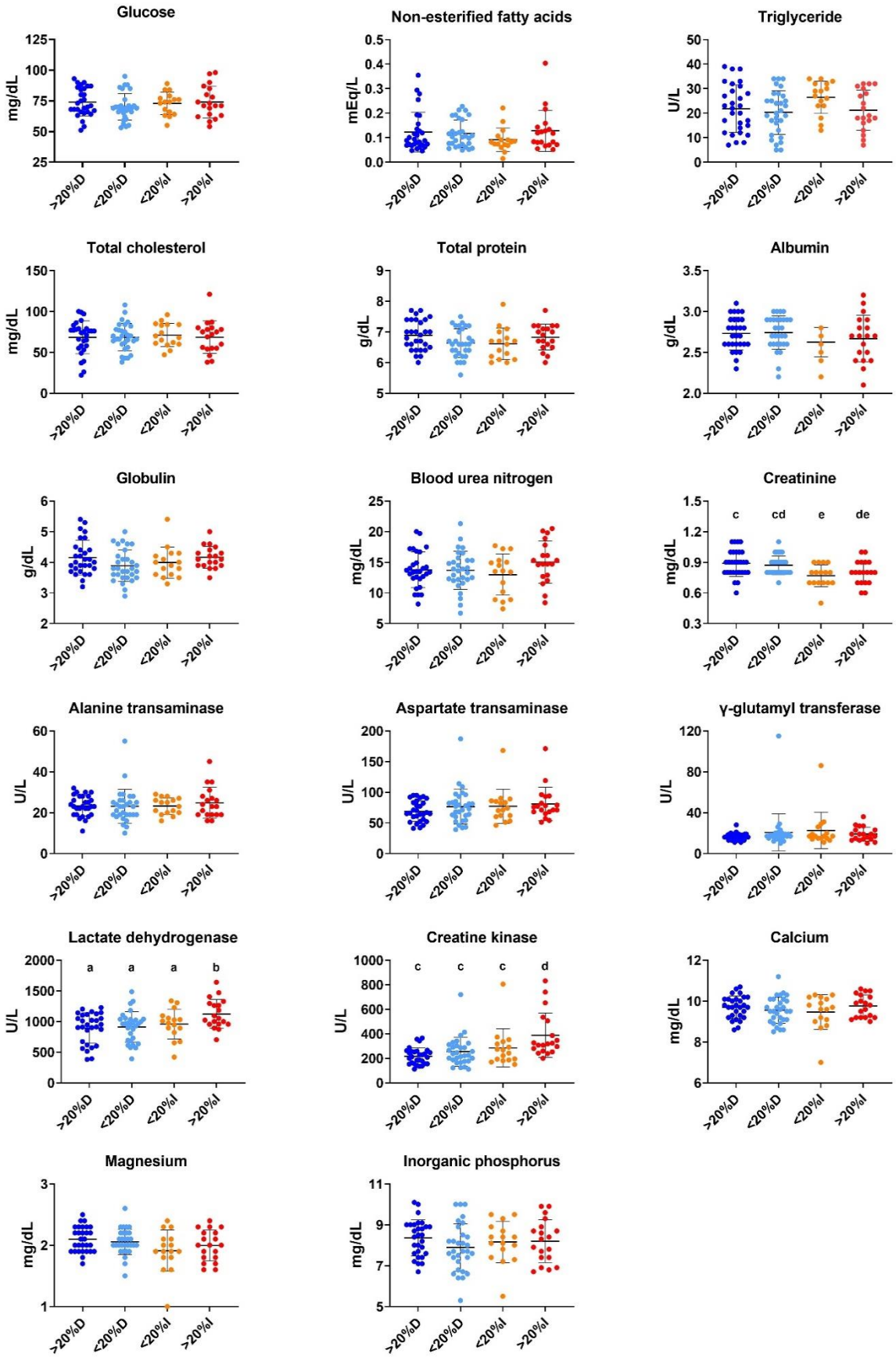

Fig. 3: Pre-vaccination serum biochemistry results for the classifications; The calves were divided according to changes in IBR reactivity, calculated by subtracting the IBR reactivity pre-vaccination from that at 8 weeks post-vaccination. The decrease classifications indicate calves with lower IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. The increase classifications indicate calves with higher IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. Abbreviations: >20%D, >20% decrease; <20%D, <20% decrease; <20%I, <20% increase; >20%I, >20% increase. [a–b]: Different letters indicate significant differences (p<0.05, Duncan test). [c–d]: Different letters indicate significant differences (p<0.0083, Mann–Whitney U test with Bonferroni's method).

Table 1: Changes in infectious bovine rhinotracheitis reactivity after inactivated IBR vaccination.

Variable	Number	Pre-vaccination	2 weeks post-vaccination	4 weeks post-vaccination	8 weeks post-vaccination
Total	94	82.5±14.9	83.6±9.8	82.0±10.7	79.2±14.0
Decrease	59 (62.8%)	90.8±4.4***	84.2±9.5	79.5±10.9**	72.0±12.9***
Increase	35 (37.2%)	68.5±16.0***	82.6±10.5	86.4±8.7**	91.3±3.8***
p-value		<0.001	0.452	0.002	<0.001
>40% decrease	1 (1.1%)	89.1	68.5	56.4	41.4
40%–30% decrease	10 (10.6%)	89.0±4.6	79.3±10.7	72.2±9.2	56.0±5.4
30%–20% decrease	18 (19.1%)	91.2±4.3	84.1±8.7	78.5±10.2	66.3±5.9
20%–10% decrease	15 (16.0%)	91.2±4.4	84.9±8.8	80.1±9.4	76.2±6.5
<10% decrease	15 (16.0%)	91.3±4.7	87.9±8.8	86.3±10.0	87.2±5.3
<10% increase	10 (11.6%)	84.7±4.4	82.1±7.8	81.9±11.1	88.6±4.9
10%–20% increase	6 (6.4%)	76.2±4.0	76.7±9.5	83.5±7.6	91.7±2.1
20%–30% increase	9 (9.6%)	66.5±5.5	78.0±13.5	86.7±7.1	91.0±3.4
30%–40% increase	5 (5.3%)	60.4±2.2	88.3±6.0	92.9±1.0	93.9±0.9
>40% increase	5 (5.3%)	38.4±8.4	93.2±1.4	93.4±0.7	93.8±0.7

Abbreviation: IBR, infectious bovine rhinotracheitis. Calves were divided by subtracting IBR reactivity before vaccination from IBR reactivity at 8 weeks post-vaccination. The decrease classifications indicate calves with lower IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. The increase classifications indicate calves with higher IBR reactivity at 8 weeks post-vaccination than at pre-vaccination. IBR reactivity data are presented as mean±standard deviation. The decrease and increase groups were analyzed by an independent *t*-test. **p<0.01; ***p<0.001.

Table 2: Changes in IBR reactivity for the classifications.

Variable	>20%D	<20%D	<20%I	>20%I	*p*-value
Total number	29	30	16	19	
IBR reactivity					
Pre-vaccination	90.4±4.4 [a]	91.3±4.5 [a]	81.5±5.9 [b]	57.5±13.2 [c]	<0.001
2 weeks post-vaccination	81.9±9.7	86.4±8.8	80.1±8.6	84.7±11.6	0.023
4 weeks post-vaccination	75.6±10.6 [a]	83.2±10.0 [abc]	82.5±9.7 [b]	89.9±5.9 [c]	<0.001
8 weeks post-vaccination	61.9±8.4 [a]	81.7±8.1 [b]	89.8±4.3 [c]	92.5±2.8 [c]	<0.001

Abbreviation: IBR, infectious bovine rhinotracheitis; >20%D, >20% decrease; <20%D, <20% decrease; <20%I, <20% increase; >20%I, >20% increase. IBR reactivity data are presented as mean±standard deviation. The >20% decrease, <20% decrease, <20% increase, and >20% increase classifications were analyzed using the Kruskal–Wallis test. [a-c]: Different letters in the same row indicate significant differences (p<0.0083, Mann–Whitney U test with Bonferroni method).

Table 3: Association between IBR reactivity and calf birth weight.

Variable	Underweight (<36 kg)	Normal weight (36–45 kg)	Overweight (>45 kg)	*p*-value
Total number	24	53	17	
IBR reactivity				
Pre-vaccination	76.9±16.9 [a]	82.7±15.2 [ab]	89.8±5.4 [b]	0.023
2 weeks post-vaccination	80.4±12.4	84.7±8.7	84.7±8.7	0.181
4 weeks post-vaccination	80.7±13.5	83.0±10.1	80.7±7.9	0.596
8 weeks post-vaccination	79.4±16.4	80.5±13.3	74.7±12.5	0.344
Decrease	11 [a] (45.8%)	33 [a] (62.3 %)	15 [b] (88.2%)	0.022
Increase	13 [a] (54.2%)	20 [a] (37.7%)	2 [b] (11.8%)	
>40% decrease	1 (4.2%)			
40%–30% decrease	4 (16.7%)	3 (5.7%)	3 (17.6%)	
30%–20% decrease	1 (4.2%)	13 (24.5%)	4 (23.5%)	
20%–10% decrease	4 (16.7%)	7 (13.2%)	4 (23.5%)	
<10% decrease	1 (4.2%)	10 (18.9%)	4 (23.5%)	
<10% increase	3 (12.5%)	6 (11.3%)	1 (5.9%)	
10%–20% increase	2 (8.3%)	3 (5.7%)	1 (5.9%)	
20%–30% increase	4 (16.7%)	5 (9.4%)		
30%–40% increase	2 (8.3%)	3 (5.7%)		
>40% increase	2 (8.3%)	3 (5.7%)		

Abbreviation: IBR, infectious bovine rhinotracheitis. IBR reactivity data are presented as mean±standard deviation. [a-b]: Different letters in the same row indicate significant differences (p<0.017, Mann–Whitney U test with Bonferroni's method). Cross-tabulation analysis between calf birth weight and changes (decrease and increase) was performed using the chi-square test.

DISCUSSION

In this study, IBR reactivity of >60% of the calves decreased despite vaccination. The percentage observed in the decrease classification was 1.68 times more than that in the increase classification, and the decrease classification had higher antibody levels than the increase classification at pre-vaccination. We speculated that passive immunity through colostrum could be impacting the active immunity through vaccination. This finding indicates that although 3 or 4 months may be a suitable age for modified live IBR vaccination (Petrini et al., 2019), 14 weeks of age may not be an appropriate time for administering the inactivated IBR vaccine to the dairy herd, during which cows and neonatal calves had high IBR antibody levels.

The results also revealed that calf birth weight was associated with improvement in IBR reactivity induced by IBR vaccination. Pre-vaccination IBR reactivity differed significantly based on calf birth weight, which could lead to different responses. Most overweight calves had decreased IBR reactivity levels owing to the high level of antibodies received via colostrum at pre-vaccination. It is unclear whether calves heavier at birth absorbed more IBR antibodies from colostrum or maintained IBR antibody levels better than those lighter at birth. Considering that calf birth weight is associated with dam-related diseases and lighter calves show higher risks of bovine respiratory disease (Pinchak et al., 2004, Sanderson et al., 2008, Ha et al., 2023), calf birth weight may need to be considered while formulating IBR vaccination schedules. Further

studies are required to elucidate why calves heavier at birth had higher IBR reactivity level than those that were lighter at birth. These results could assist in developing a precise individualized vaccination schedule for the inactivated IBR vaccine.

In this study, improvement in IBR reactivity was not inevitably associated with IBR antibodies at pre-vaccination. There may be other factors besides the level of passive immunity influencing IBR vaccine-induced reactivity. The >20%D classification had lower IBR reactivity level than the <20%D classification, supporting the notion that other factors, except for the antibody concentration at pre-vaccination, might affect improvement in IBR reactivity. Monocyte, monocyte-to-lymphocyte ratio, LDH, and CK values may provide insight into factors influencing improvement in IBR reactivity.

Improvement in IBR reactivity by IBR vaccination was better in calves with higher monocyte percentages and monocyte-to-lymphocyte ratios. After migrating into tissues, monocytes differentiate into macrophages, microglial cells, or dendritic cells that induce acquired immune responses by presenting antigens to T lymphocytes (Kaneko et al., 2008, Scott and Stockham, 2013). Monocyte-derived dendritic cells play a pivotal role in vaccination-elicited inflammation through antigen presentation and T-lymphocyte activation (Qu et al., 2014). Thus, monocytes may function as a factor influencing IBR reactivity improvement through IBR vaccination. Although the inactivated vaccine usually stimulates only humoral immunity (Jones and Chowdhury, 2010), the underlying reason remains unclear as lymphocytes comprise various sub-populations, including B lymphocytes, T lymphocytes, and null cells. T lymphocytes are subdivided into helper T lymphocytes and cytotoxic T lymphocytes (Thrall et al., 2012). Further studies are required to elucidate the association between the monocyte-to-lymphocyte ratio and the improvement of reactivity-induced inactivated IBR vaccination.

In this study, elevated pre-vaccination LDH levels were associated with improved responses to IBR vaccination. High LDH levels are associated with proinflammatory dendritic cells and macrophages (Manoharan et al., 2021). LDH is a critical enzyme that reversibly catalyzes the conversion of "pyruvate to lactate" or "lactate to pyruvate," regulates glycolysis, and increases with damage to most cells (Scott and Stockham, 2013, Rogatzki et al., 2015, Ha et al., 2022). The functions of antigen-presenting cells are suppressed by lactate. Lactate also inhibits the differentiation, maturation, and activation of dendritic cells and prevents macrophages from producing cytokines (Manoharan et al., 2021). As we did not measure lactate values, their effect is unclear. Elevated LDH levels might contribute to the increased antibody production in response to IBR vaccination by regulating lactate levels.

The calves that showed greater improvement in IBR reactivity at post-vaccination had elevated CK levels at pre-vaccination. However, the reason for this finding is unclear because no study to date has investigated the association between CK activity and response to vaccination. The levels of CK, mainly used as an indicator for mostly skeletal and occasionally cardiac muscle diseases, can increase due to bovine endometritis (Sattler and Fürll, 2004, Kaneko et al., 2008, Scott and Stockham, 2013). CK reversibly catalyzes the transfer of phosphate from "creatine phosphate to adenosine diphosphate" to form adenosine triphosphate (ATP) in the cytoplasm (Kaneko et al., 2008, Scott and Stockham, 2013). Brain-type CK regulates T-cell proliferation and activation by controlling the T-cell receptor signaling by supporting increased ATP levels and supports cellular ATP required for the phagocytosis of macrophages (Kazak and Cohen, 2020). This finding suggests that CK supports immune responses to inactivated IBR vaccination.

This study has some limitations despite presenting some new findings. First, we used the schedule for a modified live IBR vaccine for an inactivated IBR vaccination before determining the ideal time to administer the inactivated IBR vaccine. Furthermore, the inactivated vaccine used in this study was multivalent; thus, other pathogens might affect the IBR vaccine. This study was conducted on a farm where cows and neonatal calves had high IBR antibody levels, but the mechanisms of the identified four blood parameters influencing IBR reactivity improvement remain unclear. However, our findings suggest that an inactivated IBR vaccination following a modified live IBR vaccine may be inadequate, and other factors may influence IBR reactivity improvement by IBR vaccination as maternal antibodies do.

Conclusion: This is the first field study to suggest that the inactivated IBR vaccine should be administered following a schedule different from that for the modified live vaccine. In dairy herds with cows and neonatal calves with high IBR antibody levels, the inactivated IBR vaccine should be administered to calves older than 14 weeks. Although future studies need to elucidate how IBR reactivity through passive immunity is associated with calf birth weight and how reactivity induced by inactivated IBR vaccination is influenced by the values of monocytes, monocyte-to-lymphocyte ratio, LDH, and CK levels, as well as passive immunity, our findings could contribute to a better understanding of immune responses to IBR vaccination and may aid in establishing optimal IBR vaccination strategies in calves.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Republic of Korea (approval number: NIAS-2020127). All experimental procedures involving animals were conducted strictly according to relevant guidelines and regulations. All methods used for in vivo studies in cows were according to the Consolidated Standards of Reporting Trials guidelines.

Consent for publication: Not applicable

Availability of data and materials: The dataset analyzed in the current study is available from the first author upon reasonable request.

Competing interests: The authors declare no competing interests.

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