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Antibody titers to vaccination are not predictive of level of protection against a BVDV type 1b challenge in *Bos indicus* - *Bos taurus* steers

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ABSTRACT

Subclinical illness associated with infection is thought to reduce performance and increase production costs in feedlot cattle, but underlying components remain largely unidentified. Vaccination is frequently used in feedlot settings but producers lack metrics that evaluate the effectiveness of vaccination programs. The goal of this study was to determine if levels of serum neutralizing antibody titers were predictive of levels of vaccine protection in a commercial setting. During this four-year study, Angus-Nellore steers housed in a production feedlot setting were assigned to 1 of 3 vaccine treatments: killed vaccine (kV), modified live virus (MLV) vaccine, or no vaccine (control), and were challenged with a noncytopathic 1b field strain of bovine viral diarrhea virus. Rectal temperature and levels of circulating lymphocytes and platelets were monitored following challenge. While no animals were diagnosed as clinically ill with respiratory disease, indicators of disease (pyrexia, lymphopenia, and thrombocytopenia) were observed. The MLV treatment elicited higher antibody titers to the vaccination than the kV, and calves in the MLV treatment had higher mean titers at challenge. The year that elicited the highest antibody response to the vaccination and the year with the lowest frequency of phenotypic responses to the challenge were not concurrent. The MLV treatment had the highest proportion, 34.68%, of animals that were protected against the challenge regardless of the pre-challenge antibody titer and had the fewest number of lymphopenia cases in response to the challenge. Both vaccine treatments mitigated thrombocytopenia when compared to the control treatment, and the MLV treatment reduced lymphopenia; however, these symptoms were not completely eliminated in vaccinated animals. Pyrexia was present in 40.11% of the animals, but no difference in the frequency of cases between treatments was observed. Pre-challenge vaccination response was not indicative of the level of protection nor was anamnestic antibody response correlated with health status.

1. Introduction

Cattle infected with bovine viral diarrhea virus (BVDV) present with a variety of clinical and subclinical symptoms determined by the virulence of the BVDV strain and the immune status of the animal [1–4]. BVDV has been associated with respiratory disease, reproductive failure and gastrointestinal disease [5,6], and outbreaks reduce the productivity and economic viability of cattle populations [7–9]. BVDV infection results in immunosuppression, predisposing cattle to secondary infections that may lead to bovine respiratory disease (BRD) [3,5,6,10,11]. Some animals with acute BVDV infections have subclinical symptoms making it challenging to identify and to determine necessary protection for disease prevention [12,13].

High levels of BVDV immunity are associated with protection from disease, improved productivity, and economic benefits in pre-feedlot animals [6]. Both humoral and cell-mediated immune responses provide protection against respiratory infection [11,14,15], but detectable levels of humoral and/or cell-mediated immunity do not assure protection against infections [16–18]. While the presence of neutralizing antibodies is frequently used as a measurement of immune response to vaccination, the threshold of neutralizing antibodies required for protection against BVDV infection is unknown [15].

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The incomplete effectiveness of BVDV vaccines is likely due to the heterogeneity among different viral strains and the unique interaction of the virus with the host immune system [14,19], along with genetic and individual variation of the host [19,20]. Vaccination with either a killed or modified life virus (MLV) product is a major component of prevention programs for both persistent and acute BVDV infections [21,22]. It is desirable to achieve maximal response to vaccination at minimal physiological expense to avoid reduced performance [23].

Numerous vaccine and challenge studies have been performed with differences in immune responses observed, but few have been performed in production settings to evaluate the efficacy of vaccinations in a commercial operation [24–26]. This is information is important for feedlot producers as they design and evaluate vaccination programs. This study was conducted in commercial production setting over four years and utilized F_2 and F_3 Indicine-Taurine steers. The goals were to determine whether (1) differences existed in immune protection between cattle, with the same levels antibodies in sera, that were vaccinated with killed or MLV vaccines; (2) the level of serum neutralizing antibody levels following vaccination were predictive of level of protection; and (3) current industry metrics are adequate for identification of sick animals post-pathogen exposure.

2. Materials and methods

This project was reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee and the Texas A&M University Institutional Biosafety Committee.

2.1. Animal population

Angus-Nellore F_2 and F_3 yearling steers were used for the study. Calves were spring-born in 2009, 2010, 2011, and 2012 and were castrated prior to weaning. Steers were weaned at approximately 7 months of age and received 3 clostridial vaccinations with Clostri Shield 7 (Novartis Animal Health US, Inc., Greensboro, NC) at approximately 70 days of age, 3 weeks prior to weaning, and at weaning. Calves did not receive any vaccines against respiratory pathogens. Steers were tested for persistent BVDV infection at the Texas Veterinary Medical Diagnostics Laboratory (TVMDL; Amarillo, TX) prior to enrollment using an immunohistochemistry or antigen-capture ELISA assay on ear notch samples. All animals in this study tested negative for BVDV persistent infection and were sero-negative for antibodies against BVDV types la, 1b, and 2 prior to enrollment.

2.2. Vaccination treatments

Steers were classified by sire and randomly assigned to 1 of 3 vaccine treatments: killed vaccine (kV; n = 119), MLV vaccine (n = 124), or control (n = 116). Animals assigned to the kV treatment received Novartis Virashield® 6, according to label directions, receiving an initial dose on day -56 or -49, and a second dose 21 days later. Steers in the MLV treatment received a single dose of Novartis Arsenal® 4.1, according to label directions, on the same day the second dose was administered to the kV treatment group. Vaccine products were labeled for protection against BVDV 1 and 2, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, and parainfluenza 3, specific strains for kV and MLV vaccines (Table S1). Control treatment steers received no vaccination or sham injection. The MLV vaccinated steers were housed in isolation from the other treatment groups for 7–10 days following vaccination. Steers were assigned to 1 of 4 pens with treatment-sire groups balanced across pens.

2.3. Challenge

All steers were challenged, day 0, intranasally with 5 mL of 1×10^5 $TCID_{50}$ BVDV CA401186a (2.5 mL of inoculum per nasal passage) 25–35 days after booster vaccination. BVDV CA401186a was obtained from USDA-ARS National Animal Disease Center, Ames, IA [27]. The challenge strain CA0401186a was classified as a BVDV 1b based on 5′ UTR sequence comparisons [3]. When used as a challenge strain in BVDV-free dairy calves, significant reduction in platelet counts, lymphocyte counts, and pyrexia were observed, however severe acute BVDV was not observed [3].

2.4. Sample and data collection

Rectal temperatures were recorded on days 0, 3, 7, 10, 14, 28, and 42 post-challenge (PC).

Sera were prepared from whole blood collected on vaccination days, and days 0, 14, 28, and 42 PC and stored at −20 °C until used. Serum neutralizations against cytopathic BVDV strains 1a, 1b, and 2 (Table S1) were performed in duplicate on serially diluted (1:4 to 1:4096) sera by TVMDL (Amarillo, TX). The end titer was determined as base 2 log of the highest dilution that showed no cytopathic effect.

Whole blood was collected in EDTA vacutainers (Becton, Dickinson and Company, Franklinlakes, NJ) on days 0, 7, 14, 28, and 42 PC. Samples were shipped overnight and differential WBC counts, platelets, and red blood cell characteristics were determined on blood samples using a CELL-DYN 3700 blood analyzer (Abbott Laboratories, Abbott Park, IL) at the University of Arkansas Nutrition Laboratory (Fayetteville, AR).

Steers were observed for objective clinical assessment twice daily for the first 14 days PC, and then once daily through day 42 PC, by the same trained individual for all four years of challenge and once daily by the same licensed veterinarian. Objective clinical assessments were recorded on a 6-point scale for each symptom: cough, ocular and nasal secretion, depression, diarrhea, and anorexia [28]. Criteria for clinical diagnosis was a score >3 for a single symptom or a combined score $\geqslant$3 for two or more symptoms. Animals with rectal temperatures over 40.0 °C, regardless of clinical scores, were treated once with tulathromycin (Zoetis, Kalamazoo, MI) according to label directions.

2.5. Calculations and statistical analyses

Declines in lymphocytes and platelets were calculated as the difference between the lowest circulating lymphocyte/platelet count PC and day 0 count, divided by the count on day 0 PC and multiplied by 100 to generate the percent decline [3]. Anamnestic antibody response was defined as area under the curve (AUC) of the log base 2 titers from days 0 to 42 PC, using the trapezoidal summation method [29].

Disease phenotypes were analyzed as 2-level categorization (healthy and sick). Pyrexia defined as an elevated rectal temperature of 1 SD greater than day 0 PC temperature for 2 or more consecutive collection days within 14 days PC. Lymphopenia and thrombocytopenia, defined as >40% maximum decline. Clinical presentation was defined by presentation of pyrexia, lymphopenia, and thrombocytopenia. Subclinical presentation was lymphopenia and thrombocytopenia with no pyrexia.

Response to vaccination was analyzed as antibody titers in 4 categories (no, low, mid, and high). No titer included animals with no detectable titers post-vaccination (n = 153). Low titers were $\geqslant$2 and <4 (n = 87). Mid titers were $\geqslant$4 and <6 (n = 87). High titers were $\geqslant$6 (n = 32).

Statistical analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC). Mixed model analysis was used for daily rectal tem-

perature, maximum lymphocyte and platelet decline, BVDV 1b titer post-vaccination, and daily anamnestic antibody response differences with fixed effects of treatment, year and the interaction tested. Glimmix analysis was used for binary disease symptom phenotypes of: no disease symptoms, pyrexia, lymphopenia, thrombocytopenia, and clinical/subclinical presentation. Fixed effects of pre-challenge titers or BVDV 1b AUC, treatment, year, and appropriate interactions were fit into the model. Effects were considered significant at an alpha of 0.05.

3. Results

3.1. Response to vaccine treatments

Response to vaccination was measured by neutralizing antibodies on day 0 of the BVDV 1b challenge (25–35 days after booster vaccination). The steers in the MLV treatment had higher 1b mean titers for 3 out of the 4 years compared to the kV and control treatments (Table 1). Highest 1b mean titers for both the kV and MLV treatments were observed in 2010 (Table 1). The lowest mean titer for the kV was observed in 2012, while the lowest MLV treatment titer was observed in 2011 (Table 1). The peak type 2 titers were observed in 2012 for both MLV and kV treatments. BVDV 1a vaccine titers were highest in 2012 for the MLV treatment, while 2011 had the numeric peak in the kV treatment with no statistical significance between 2011 and 2012 (Table 1). No differences were observed in the control treatment for all BVDV genotypes as no detectable titers were measured in steers in the control treatment (Table 1). Year effects were observed in all BVDV antibody titers post-vaccination, however the year effects were different for the two vaccine treatments (Table 1). BVDV 1b antibody titers were higher for the MLV treatment than for the kV or control treatments, while the mean type 2 titers were higher in the kV treatment pre-challenge (Table 1).

The distributions of antibody titers between the two vaccine treatments were different (Fig. 1; Fig. S1). Responses to the vaccines showed a bell-shaped curve in response to the kV for all years, while in 2010 more animals in the MLV treatment exhibited high titers than no or low titers (Fig. 1). The kV treatment elicited more no and low titer responses than mid and high responses (Fig. 1a). Alternatively, the MLV treatment elicited mid and high titers with fewer no titer responses (Fig. 1b). The titers produced by the MLV treatment were evenly distributed with a titer of 3 at the peak of 2011 and 2013 challenge years and a peak titer of 4 in 2010 and 2012 (Fig. S1). The 2010 response to the kV was a bell curve similar to the response observed for the MLV treatment, however higher numbers of no responders were observed in 2011–2013 with the greatest proportion of the animals in those years in the no and low response groups (Fig. S1).

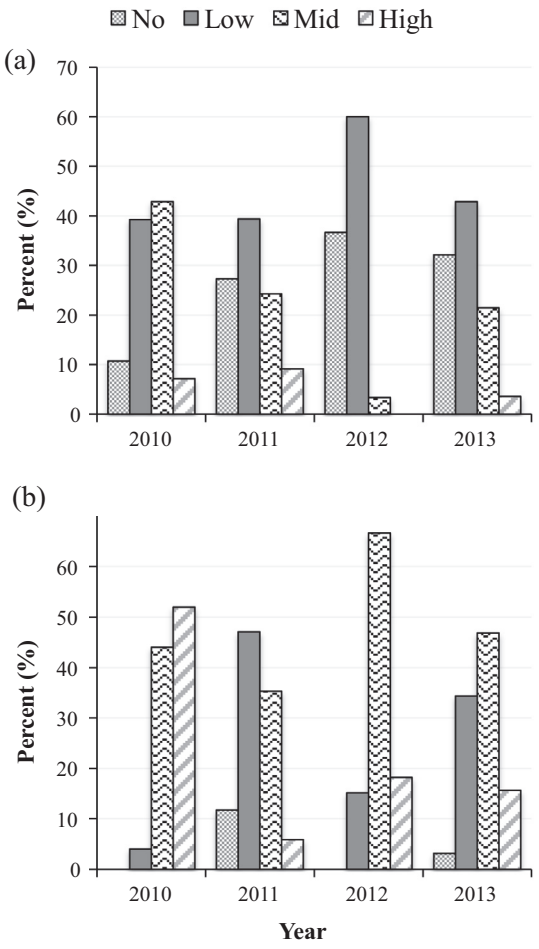

Fig. 1. Antibody titers by year. Distribution of BVDV 1b antibody titers in steers at day 0 (No: titer = 0; Low: 2 ⩽ Titer < 4; Mid: 4 ⩽ Titer < 6; and High: Titer ⩾ 6) to (a) Killed Vaccine (kV) and (b) MLV vaccine by year.

Table 1
BVDV treatment antibody titer means. BVDV 1b average neutralizing antibody titers by challenge year to the vaccination at day 0.

	N	BVDV 1b titer (SD)	BVDV 1a titer (SD)	BVDV 2 titer (SD)
kV				
2010	28	3.29 (1.67)^{a}	0.96 (1.53)^{a}	2.14 (1.74)^{a}
2011	33	2.73 (2.10)^{a,c}	2.85 (2.24)^{b,c}	3.27 (2.13)^{b}
2012	30	1.63 (1.35)^{b}	2.43 (1.57)^{b,d}	3.57 (2.16)^{b}
2013	28	2.29 (1.88)^{b,c}	1.14 (1.63)^{a}	2.18 (2.04)^{a}
MLV				
2010	25	5.64 (1.38)^{d}	2.32 (1.77)^{b,e}	1.20 (1.26)^{c}
2011	34	3.00 (1.48)^{a}	2.03 (1.34)^{d,e}	0.91 (1.26)^{c}
2012	33	4.45 (1.23)^{e}	3.33 (1.22)^{c}	2.52 (1.42)^{a}
2013	32	4.16 (1.53)^{e}	1.34 (1.62)^{a}	1.09 (1.44)^{c}
Control				
2010	23	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{d}
2011	33	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.06 (0.35)^{d}
2012	32	0.00 (0.00)^{f}	0.09 (0.53)^{f}	0.00 (0.00)^{d}
2013	28	0.00 (0.00)^{f}	0.07 (0.38)^{f}	0.00 (0.00)^{d}

Alphabetic superscripts indicate differences across years within a treatment and across treatments at $P < 0.05$ within a column (BVDV genotype).

3.2. Response to the BVDV 1b challenge

The maximum platelet decline observed was −93.6%, while maximum lymphocyte decline was −80.6%. Animals in the control treatment group had the greatest decline in circulating lymphocytes and platelets. Lymphocyte counts in animals receiving MLV treatment declined less ($P < 0.05$) than in animals receiving either the kV or control treatments (Fig. 2a). The average maximum platelet decline in the MLV treatment was less than in the control treatment ($P < 0.05$); the platelet decline in the kV treatment was intermediate to MLV and control, and not statistically different from either (Fig. 2b).

The highest individual recorded temperature in the first 14 days PC was 41.8 °C, in the control treatment. Mean day 0 temperatures were not different among treatments; steers receiving the MLV vaccine had lower mean rectal temperatures through day 7 PC compared to the other treatments ($P < 0.001$) (Fig. 2b). No differences were observed among treatments for mean rectal temperatures by day 10 PC. In addition to rectal temperature collections, animals were observed daily for additional clinical respiratory symptoms. No animal met the threshold for clinical illness or warranted additional rectal temperature measurements for therapeutic treatment based on objective clinical assessment.

When challenged, 20.89% of the animals did not present with pyrexia or subclinical signs. Lymphopenia was the most frequent sign of subclinical disease (55.15%) PC. Thrombocytopenia was observed in 40.67% of the animals and 40.11% of the animals presented with pyrexia PC. These responses varied by treatment and year (Table 2).

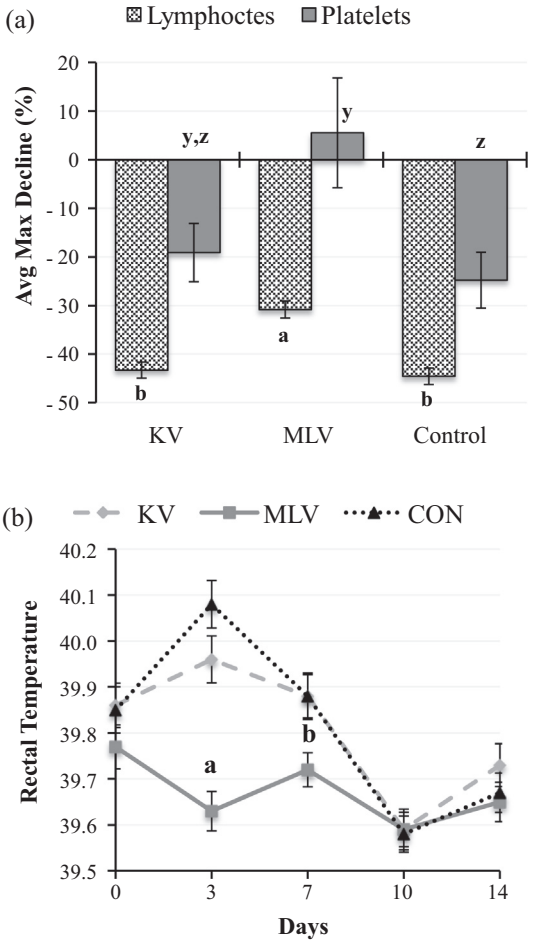

Fig. 2. Lymphocyte and platelet decline and rectal temperature response to BVDV challenge. (a) Greatest decline (%) in circulating lymphocytes and platelets by treatment (killed vaccine (kV), modified live virus (MLV) vaccine, or control (non-vaccinated)) post-challenge with BVDV 1b CA0401186. Data displayed as means ± SEM. Superscripts [a,b] represent significant differences ($P < 0.001$) between treatment groups for lymphocyte decline. Superscripts [y,z] represent significant differences ($P < 0.05$) between treatment groups for platelet decline. (b) Mean rectal temperature by treatment (killed vaccine (kV), modified live virus (MLV) vaccine, or control (CON; non-vaccinated)) across the first 14 days post-challenge. Significant treatment differences within day are denoted by [a]$P < 0.001$ between MLV and kV/Control treatments, and [b]$P < 0.05$ between MLV and kV/Control treatments.

3.3. Vaccine effects on response to BVDV 1b challenge

Vaccine treatment affected the response to the challenge for most phenotypes. The MLV treatment had the greatest proportion of animals (34.68%) without pyrexia and subclinical signs PC (Table 2). No difference was observed in protection from the kV treatment compared to the control treatment for prevention of lymphopenia or no symptoms (Table 2). The MLV treatment significantly reduced ($P < 0.05$) the number of animals that presented with lymphopenia to approximately half of the kV and control treatments (Table 2). Thrombocytopenia was observed in significantly fewer individuals ($P < 0.05$) receiving vaccination compared to controls (Table 2). No treatment differences were observed in the percentage of animals that presented with pyrexia.

3.4. Year effects of response phenotypes to BVDV 1b challenge

Year effects on response phenotypes were highly variable. The year effect trended similarly for lymphopenia and thrombocytopenia. Lymphopenia and thrombocytopenia were most prevalent in 2010 ($P < 0.05$); approximately 70% of the animals in that year presented with reduced cell counts in response to the challenge (Table 2). No differences were observed in lymphopenia and thrombocytopenia prevalence in 2011–2013 (Table 2). The greatest proportion of animals to present with no disease signs was in 2011, and that same year showed the lowest number of steers presenting with pyrexia in response to the challenge but not significantly different from 2010 levels (Table 2). The year with the highest proportion of pyrexia presentation was not the same year with the greatest proportion of steers with lymphopenia and thrombocytopenia (Table 2).

3.5. Titers and protection from disease

Detectable titers were found in 70.70% of the individuals without symptoms PC (Fig. 3a). The MLV treatment group included significantly more individuals with detectable titers and no disease signs ($P < 0.05$), accounting for 53% of the healthy animals (Fig. 3a). No difference in pyrexia frequency was observed between titer levels ($P > 0.05$) (Fig. S2a). Animals with no detectable titers presented with lymphopenia and thrombocytopenia at a higher frequency than animals with detectable levels (Fig. 3b and S2b). Lymphopenia was more frequent at high titers in the MLV treatment, but was more frequent at low titer in the kV treatment (Fig. 3b). When vaccine titers were in the low to mid range, the frequency of thrombocytopenia presented less frequently than when no titers were detectable (Fig. S2b). No treatment interaction was observed between antibody titers post-vaccination and pyrexia or thrombocytopenia ($P > 0.05$).

3.6. Clinical versus subclinical response

The frequency of lymphopenia and thrombocytopenia with pyrexia (clinical) and without pyrexia (subclinical) were compared (Fig. 4a). The subclinical presentation occurred at 1.24 times as often as clinical presentation. Subclinical disease symptoms occurred more frequently in steers with mid to high titers. No

Table 2
Disease symptoms by treatment. Percentage of animals with (1) no disease signs (2) clinical sign of pyrexia (2 or more consecutive time points of rectal temperature >1 SD from baseline temperature), (3) lymphopenia (>40% decline in lymphocyte counts), and (4) thrombocytopenia (>40% reduction in platelet counts) following BVDV 1b challenge by treatment and year.

	N	No disease signs	Pyrexia	Lymphopenia	Thrombocytopenia
Treatment					
kV	119	16.81[b]	40.34	64.71[b]	37.82[a]
MLV	124	34.68[a]	34.68	33.87[a]	31.45[a]
Control	116	10.34[b]	45.69	68.10[b]	53.45[b]
Year					
2010	76	10.53[a]	36.84[b,c]	71.05[a]	72.37[a]
2011	100	32.00[b]	25.00[c]	52.00[b]	29.00[b]
2012	95	20.00[a]	54.74[a]	52.63[b]	28.42[b]
2013	88	18.18[a]	44.32[a,b]	47.73[b]	39.77[b]

[a,b,c] Superscripts indicate differences between treatment or year within a column.

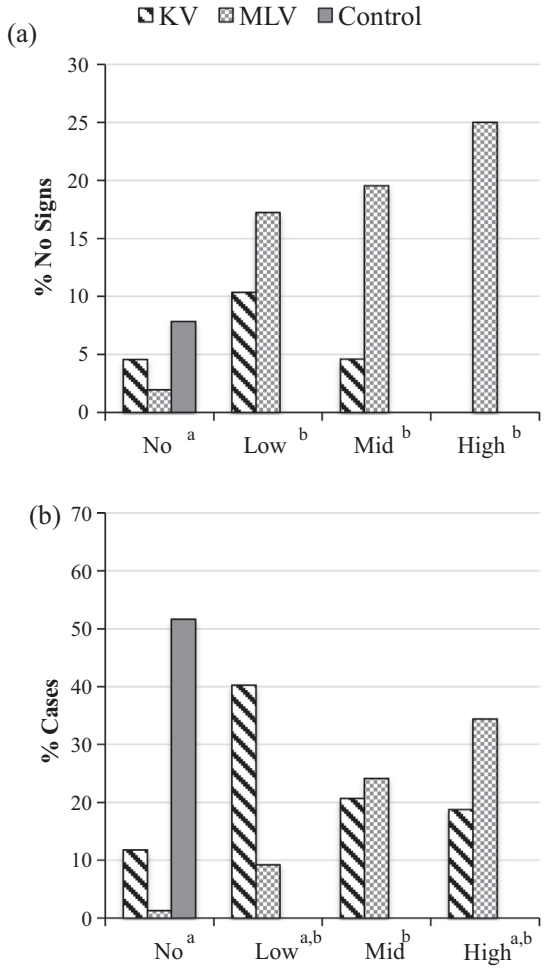

Fig. 3. Prevalence of symptoms of disease by vaccine titer. Proportion of animals with no, low, mid, or high BVDV 1b vaccine titers (day 0) that present with (a) No disease symptoms and (b) post BVDV 1b challenge. (No: titer = 0; Low: $2 \leqslant$ Titer < 4; Mid: $4 \leqslant$ Titer < 6; and High: Titer $\geqslant$ 6) [a,b,c]Alphabetic scripts represent significant ($P < 0.05$) differences between titers. (a) and (c) Had significant interactions for titers by treatment.

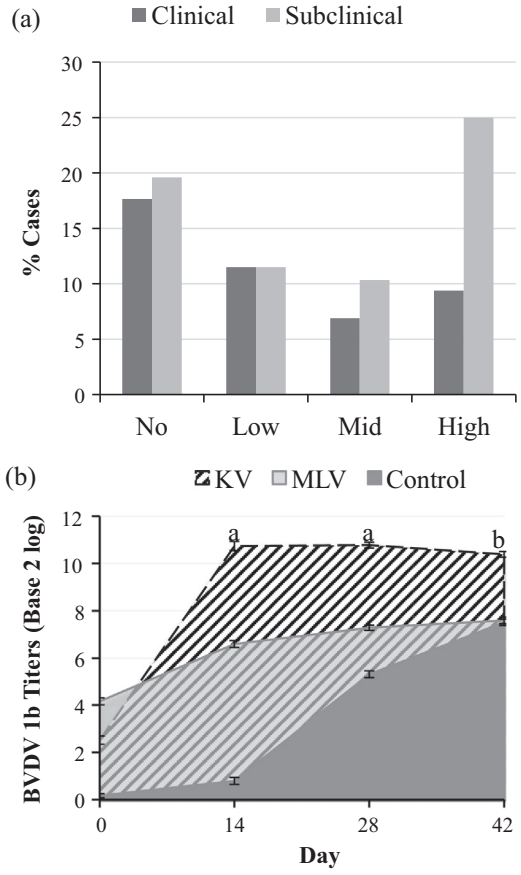

Fig. 4. Clinical versus subclinical disease by vaccine titer and anamnestic antibody response. (a) Frequency of animals that presented with clinical (pyrexia, lymphopenia, and thrombocytopenia) symptoms versus the subclinical (lymphopenia and thrombocytopenia) signs of disease across the four vaccine titer categories (no, low, mid, and high). (b) Mean antibody titer (base 2 log) for BVDV type 1b at days 0, 14, 28, and 42 with area under the curve (AUC) shaded for each vaccine treatment (killed vaccine (kV), modified live virus (MLV) vaccine, or control (non-vaccinated)). Means reported for each treatment at days 0, 14, 28 and 42 post-challenge with SEM. [a]Significant ($P < 0.05$) differences between kV, MLV, and control treatments. [b]Significant ($P < 0.05$) difference between kV and MLV/Control treatments.

differences in numbers of clinical versus subclinical cases were observed in steers with no to low titers (Fig. 4a).

3.7. Anamnestic antibody response to BVDV challenge

The greatest anamnestic antibody response was observed in the kV treatment group, by day 14 PC (Fig. 4b). Year differences in the anamnestic response to vaccination were similar to those described for response to vaccination (not shown). The MLV treatment had higher titers on day 0 in response to vaccination, but demonstrated lower anamnestic antibody response to the challenge than the kV treatment (Fig. 4b). By day 42, no difference was observed between the MLV and control treatment (Fig. 4b). Anamnestic antibody response PC was not different for healthy versus sick animals that presented with pyrexia, lymphopenia, or thrombocytopenia or the combination ($P > 0.05$) (Fig. S3).

4. Discussion

We observed that the MLV treatment elicited higher antibody titers compared to the kV treatment. The fact that animals in the kV treatment group developed higher anamnestic responses compared to the MLV treatment PC is probably related to greater replication of the virus in the kV groups and conversely greater protection against viral replication in the MLV groups. These results correlate with previous studies that found that MLV vaccines elicit more robust and longer lasting immune responses compared to killed vaccines, suggesting why the vaccine titers were higher for the MLV treatment [14,23,30]. Animals that were vaccinated with an MLV vaccine were better protected against the challenge, especially those steers in the no, low and mid titer category. Similar to our findings, vaccinated animals were reported to have protection even in the absence of titers from vaccination [28]. Conversely, calves with lower serum neutralizing antibody levels at entry to the feedlot have been reported to be at increased risk for BRD treatment, illness, and reduced net value to the owner [26]. Our results suggest that the immune response elicited by the MLV vaccine stimulated more than a humoral antibody response, as animals with no and low titers were better protected than the kV treatment animals at the same titers. Furthermore, protection against a BVDV challenge can occur in the absence of detectable post-vaccination antibody titers [8,31], and several studies have shown little correlation between increased antibody titers and disease prevention [32]. Our results correspond with others' suggesting that the type of immune response may be more important for protection against viral pathogens than increased titers of neutralizing antibodies [11,33,34]: both a primed T- and B-cell response may be required to offer optimal protection against the challenge.

Respiratory infection in feedlots is typically diagnosed through observed clinical assessment, and affected animals are treated based on elevated rectal temperature [35]. However, multiple studies have documented no observed objective clinical signs post BVDV challenge, although leukopenia and pyrexia were reported shortly following challenge [31,36–39]. These findings suggest that not all BVDV infections present with visible symptoms.

Rectal temperatures were recorded on pre-determined days rather than as a final clinical threshold following initial objective clinical assessment, as would be the case in a field protocol. Observations of pyrexia were observed in cattle with leukopenia similar to previous studies [16,22,37–39], but not all cattle with lymphocytopenia and thrombocytopenia experienced pyrexia. Based on our data, rectal temperature is not a reliable indicator of morbidity, and utilization of rectal temperature to identify sick cattle may miss a portion of sub-clinically ill cattle. The presence of subclinically ill, undiagnosed cattle in a population may have large financial impacts, which are difficult for operators to directly assess [4,40]. Assessment of disease presentation by leukopenia and thrombocytopenia may increase the detection of "sick" animals compared to traditional observational diagnoses.

Lymphocytopenia and thrombocytopenia are subclinical indicators of BVDV infection. Lymphopenia was observed in both vaccinated and unvaccinated calves post-challenge, with an average reduction of 39.4% in lymphocytes following the challenge, similar to other reports [1,3,12,34,37,41]. Large variations in circulating lymphocyte counts among steers were observed, including calves with lymphocytes elevated by 40% from baseline to calves with severe lymphopenia of 80% reductions, suggesting that individual variation exists within the population and that some calves develop a more robust immunity for protection against the BVDV challenge than others. Results from this study and others suggest that a protective response from vaccination should prevent leukopenia and thrombocytopenia during pathogen exposure [8,13].

Vaccination reduced the subclinical effects of the BVDV challenge. Calves that received MLV were less susceptible to lymphopenia and thrombocytopenia than non-vaccinated animals, similar to other studies [23,38,42]. The MLV treatment appeared to elicit a more robust immunological response to protect against lymphopenia compared to the kV treatment, although unmeasured. Vaccinated steers displayed less severe lymphocytopenia and thrombocytopenia following the challenge than unvaccinated steers, and this concept is likely important in production environments. The lower antibody titers observed in the anamnestic response indicated that the MLV treatment had greater protection from vaccination, which resulted in less viral replication PC. This suggests that the MLV vaccine likely stimulated both a humoral and cell-mediated immune response (not measured in this study). Titers following pathogen exposure cannot be used to gauge the level of protection within a population.

In conclusion, this study suggests that antibody titer may not be a reliable metric of protective immune response against a BVDV 1b challenge or of disease status. Reber et al. [32] similarly reported weak to no correlations between humoral and cellular immune responses, and Ridpath [31] previously suggested that level of antibody titers may not be a reliable indicator of level of protection. Based on these results the titer threshold for protection is variable and is dependent on the other immune components that have been primed by the vaccination. To find the protection titer threshold it may be critical to understand the type of vaccination and the humoral immune response stimulated, as well as animal-to-animal variation, i.e. genetics. More steers in this experiment had lymphocytopenia and thrombocytopenia than pyrexia, suggesting that rectal temperature does not identify all animals with infection. Additionally, rectal temperature used alone as a predictor for morbidity likely misses a substantial proportion of cattle with BVDV infection. These results show a benefit from BVDV vaccination even in the presence of low detectable titers, and that the MLV vaccine provided better protection against BVDV 1b challenge in a production setting. Undiagnosed, sub-clinically ill animals in commercial feedlots likely present significant health management obstacles as viral reservoirs and potential sources of reduced production efficiency. Vaccine efficacy for commercial use needs to be evaluated on the prevention of subclinical disease presentation and the stimulation of both humoral and cell-mediated arms of the immune response as antibody titers alone do not appear to be indicative of the level of protection offered by a vaccination.

Conflict of interest

All authors of this research declare no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.08.087.

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