Predicting immunization status at arrival in Tennessee stocker calves

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Abstract

This study aimed to test if externally observed physical characteristics of calves on arrival at a stocker facility could be used to predict calfhood immunization status based on observed antibody titer levels. Knowledge of highly correlated features could allow stocker operators to mitigate the risk of bovine respiratory disease through informed buying practices and targeted management strategies, thus lowering morbidity, mortality and treatment costs. Ear notches, blood and visual attributes were collected for 408 stocker calves at 4 farms in Tennessee. Each animal was tested for bovine viral diarrhea virus-persistently infected status and titer levels for 3 viral agents that are present in most respiratory disease vaccines. Multiple visual characteristics were predictive of likely vaccination for BRD-causing agents, including the presence of a prior ear tag (1.6 times), castrated males (1.26 times), polled cattle (4.8 times), body condition score (1.46 times per full score), and being declared vaccinated or preconditioned (2.2 times).

Additionally, we followed 60 calves through the stocker phase to ascertain how preconditioning affected downstream health events and performance. We found that preconditioned calves had lower BRD incidence, a higher occurrence of multiple detectible viral titers, and gained more weight over 56 days than did naïve cattle. Interestingly, many calves marketed as preconditioned did not have detectible viral titers on arrival. This work identifies informed, practical solutions and management decisions for stocker operations when purchasing calves. It also lays the groundwork for future work identifying ways to deliver precision management to stocker cattle.

Key words: bovine respiratory disease, stocker cattle, southeast, preconditioning

Introduction

Bovine respiratory disease (BRD) is the primary cause of mortality and morbidity in the cattle industry.[1–4] Southeastern stocker calves are considered high risk for BRD due to inconsistent weaning methodologies, extensive commingling at sale facilities, transportation stress, and low prevalence of immunization.[5] With over 50% of stocker cattle being marketed through auction facilities, commingling is a major driver of BRD incidence in the Southeast as lot sizes are typically small (< 50 calves), and management practices are highly variable.[6] BRD is a multifaceted disease driven by multiple agent, host, and environmental factors. Numerous vaccines are available that successfully reduce BRD prevalence.[7–9] Still, other factors, such as poor vaccine timing and improper administration, can inhibit the vaccine's efficacy. As such, vaccinating calves does not guarantee immunization for BRD.[5] Common viral agents associated with BRD include bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza type 3 virus (PI3), bovine herpesvirus (BHV-1), and bovine respiratory coronavirus (BRCV).[10]

Our study used the serological antibody titer presence of 3 crucial BRD viruses (BVDV, BRSV, and IBR) to identify likely immunized individuals. We chose these pathogens because, among the commercially available respiratory viral vaccines in the United States, the majority contain at least 2 of these 3 viral pathogens. We surmised animals possessing multiple detectible titers were likely to have acquired these from vaccination rather than natural infections. Titer abundances for BRD-causing pathogens in many studies have been shown to indicate protective immunity in many disease challenge studies,[11–13] though not universally.[14] Importantly, this assumes that vaccines were effectively delivered at the appropriate time following the disappearance of maternal antibodies.[15]

This study aimed to identify indicators of possible immunization status for BRD in calves at arrival at a stocker facility. Knowledge of immunization indicators could help producers make additional informed purchasing and calf management decisions. We developed proof-of-concept predictive models for likely BRD-pathogen vaccination status using these indicators. This work aims to help producers manage calves more precisely on arrival. We expect that precise on-arrival best management practices could reduce the prevalence of BRD, antibiotic usage, morbidity, mortality and associated costs of BRD.

Materials and methods

Study procedures

Animal Care and Use Committee approval was granted for this study (University of Tennessee IACUC Protocol 2864-0921). Private producers not associated with the university agreed to client consent forms for this observational study. For both studies, whole blood samples and ear notches were collected, and visual attributes were recorded from weaned calves on arrival at 4 Tennessee stocker operations (n = 408). All animals sampled for this study were purchased at auction facilities in Tennessee and immediately transported to stocker facilities. There were 7 different sampling dates over 8 months (September to April). Sampling and data collection occurred on the day following the sale and transport at 3 of our 4 locations. The fourth location performed intake processing within 7 days after purchase. For each animal, 12 visual attributes were collected: sex (heifer, steer or bull), castration status of males (freshly cut, cut and fully healed, single retained testicle, or intact), approximate frame score (small, medium, large),[16] hair coat score (1-slick to 5-full winter coat),[17] body conditioning score (1 to 9),[18,19] presence of a prior ear tag, approximate body weight was visually estimated, horn status (horned, dehorned, polled), coat color, *Bos indicus* influence (yes or no), and signs of illness on arrival. Based on sale information, we were also informed if calves had received vaccines before shipment. We refer to this parameter as "declared vaccinated." Following our data collection during intake processing, calves were handled and managed according to each individual producer's standard health management practices. To ensure there was no discrepancy in visual data collection, a single individual collected all visual data at each sampling location and visit throughout the study. We did not receive follow-up information on disease incidence from producers.

BVDV-PI testing

Ear notches (1 cm × 1 cm) were collected from each calf. Refrigerated samples were shipped to the Tennessee Department of Agriculture's Kord Animal Health Diagnostic Lab (Nashville, TN) for bovine viral diarrhea virus-persistently infected (BVDV-PI) testing within 1 day of collection, following Kord Animal Health Diagnostic Laboratory procedures. Ear notches were subjected to an antigen capture enzyme-linked immunosorbent assay (ACE) test and were analyzed in batches as directed by demand. Results were reported as positive or negative for individual animals. It is unlikely, but possible, that this test could identify acute BVDV infections.[20] The follow-up testing to confirm whether positive results were PI animals was outside this survey project's scope.

Blood collection and titer evaluation

Approximately 10 mL of whole blood was collected from each calf via the jugular vein prior to vaccination or antibiotic treatments. The whole blood was centrifuged at 2,000 × g for 10 minutes at 4 °C. After, the serum was aliquoted into 2 mL microcentrifuge tubes and stored at 4 °C until shipping. Refrigerated serum was sent to the Iowa State Veterinary Diagnostic lab (ISU VDL; Ames, IA) for virus-neutralizing tests (VNT). Titer detection limits were < 1:4 for BRSV and < 1:2 for BVDV and IBR. The antibody agent A51908 was used for the ELISA for all viruses.

We classified animals into 3 groups based on detected titer levels for each pathogen. We set detectible viral titer thresholds at < 1:32 for BVDV, < 1:4 for BRSV, and < 1:2 for IBR based on previous work.[21–25] The first group, considered naïve, had no detectible viral titers for the 3 tested pathogens. This led us to believe that they had never been exposed to a vaccine. A second group consisted of animals that carried a detectable titer for only 1 virus, consistent with natural exposure to a single viral pathogen. Those calves had to have titer values above the detection thresholds. Finally, we classified animals as likely immunized when they carried detectible titers for at least 2 of the 3 viruses. We assumed that at least 2 detectible titers would likely coincide with receiving a modern BRD vaccine.

Post-intake health and performance recording

A subset of 60 animals (30 naïve and 30 marketed as preconditioned) were purchased and managed at the Middle Tennessee Research Center (Spring Hill, TN). Upon intake, calves were processed and given a 2-dose vaccine for IBR, BVD Types I and II, PI3, BRSV and *Haemophilus somni* [1] (2 mL subcutaneous, booster at 3 weeks), a single-dose 7-way pinkeye vaccination [1] (2 mL subcutaneous), a single-dose *Pasteurella* vaccine[1] (2 mL subcutaneous), a single-dose of autogenous pink eye bacterin (2 mL subcutaneous), pour-on eprinomectin[2] (1 mL per 22 pounds), and a zeranol implant[2] (36 mg). These animals were closely monitored for post-arrival BRD symptoms. Additionally, weights were collected post-processing on days 14, 35 and 56 before daily feeding. Immediately after processing, calves were comingled and managed as a single unit. All 60 calves were co-housed on a ryegrass-based pasture for the entire time spent at the farm. All 60 steers were rotated on a total of 18 acres that were subdivided into 3 equal-sized paddocks. Calves were rotated weekly to aid in erosion and mud control throughout winter and early spring. Calves had access to free choice hay and were given supplemental feed through a total mixed ration (TMR) and a concentrated feed from an automated smart feeder (C-Lock Super SmartFeed). Calves were fed 40 pounds of corn silage as fed with access to a smart feeder, which allotted up to 5-pound 30% concentrate daily. Not all calves learned to use the smart feeder, and as a result, did not receive supplemental concentrate feed. This group of calves was on-farm from December 8, 2021, to their sale date of April 4, 2022. All 60 head were marketed together with an average weight of 834 lb.

Health observations were recorded based on the criteria detailed in Step et al.[26] Calves were treated for BRD when they had a clinical score of 1 or 2 and a rectal temperature of greater than 104 °F or if they had clinical severity scores of 3 or 4 regardless of rectal temperature. Calves received florfenicol[2] (subcutaneously 6 mL per 100 pounds of body weight) for their first treatment, tulathromycin[2] (subcutaneously 1.1 mL per 100 pounds of body weight) for their second treatment, and ceftiofur[2] (subcutaneously 1.5 mL per 100 pounds of body weight) for their third treatment. The 60 calves in this study were also included in the 408 animals used to identify indicators of likely immunization.

Statistical methods

Our study was purely observational and epidemiological, where we treated individual calves as study units. In all statistical analyses, we treated each stocker operation as a random variable to account for distinct buying practices that could confound the interpretation of results. We cleaned data and calculated descriptive statistics in R using various packages from the tidyverse.[27,28] We modeled likely immunized status (i.e., 2 detectible titers) as a binary dependent variable in univariate mixed effect logistic regression models to assess potential associations between visual calf factors and likely immunization. We analyzed all attributes described above in the units described. We also analyzed hair score as a binary "good" or "bad" based on season, where late spring and summer loads were classified as "bad" when they had hair scores of 5. Animals in fall and winter loads were set to "missing" for this binary trait. Using the significant calf variables from these univariate models ($P < 0.05$), we developed multiple variable logistic regression models to test multiple combinations of factors associated with the probability of likely immunization. We assessed overall model fits using the Akaike Information Criterion (AIC).[29] Using the best-fitting models, we performed 10-fold cross-validation to evaluate the predictive ability of each combination of visual factors. We used observed and predicted immunization statuses based on included visual characteristics to calculate correlation coefficients R^2, Root Mean Squared Errors (RMSE), and accuracy for each model we tested. Lastly, we evaluated whether model predictive abilities significantly differed with paired t-tests.

We evaluated how preconditioning affected calf weight gain and disease incidence for study 2. We used SAS 9.4 (SAS Inst., Inc., Cary, NC) to calculate summary statistics for the

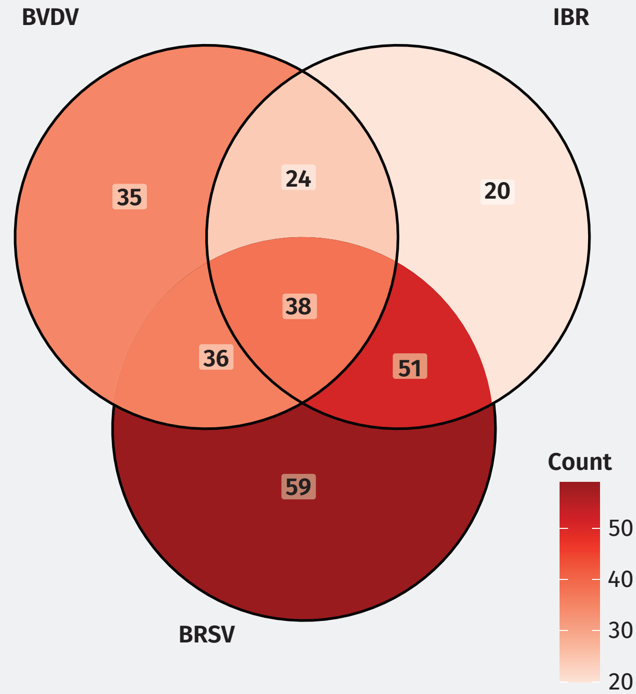
2 groups of cattle (preconditioned and naïve). We used frequency procedures to evaluate categorical data (tag, precondition status, horn status, etc.). We used Chi-squared statistics to assess if preconditioned animals were 1) less likely to be treated at least once for BRD and 2) if they performed better at regular checkpoints during the stocker phase (based on average daily gain, ADG) than their naïve contemporaries. Average daily gain values were calculated for specific gain periods based on weights taken on days 0, 14, 35 and 56. Animals were classified as "sick" for a period if they were treated for BRD between weighing dates (on days 0, 14, 35 and 56). We also performed Chi-squared tests to determine if BRD incidence differed for animals with detectible BVDV or IBR titers (no BRSV titers were detected in this subset of animals). We considered group differences significant when $P < 0.05$.

Results

Indicators of likely immunization

Of the sampled population, 37% (151/408) of calves were likely to have been vaccinated for viral agents implicated in BRD on arrival at their respective stocker facilities based on possessing at least 2 detectible viral titers. Only 9% of the sampled population had detectible titers for all 3 viruses. A single sampled calf was BVDV positive tissue-based ACE test. The rates of animals with detectible serological BVDV titers in this group were not statistically different from other processed groups. Thirty-five percent (143/408) of calves had no detectible titer for any of the 3 viruses. We observed that 28% of animals had detectible titers for a single virus: 5% for IBR, 15% for BRSV and 8% for BVDV (Figure 1).

Figure 1: Venn diagram of viral titer presence for 3 BRD pathogens (BVDV, BRSV, and IBR) in 408 weaned stocker calves in Tennessee. The overlaps of circles represent cattle that we considered immunized at arrival. Darker red colors represent higher numbers of animals per category.



Calves purchased in the fall and winter were universally negative for detectible BRSV titers, but calves purchased in the spring were nearly all positive (97.5%). This coincided with a significant increase ($P < 0.05$) in the proportion of animals that had detectible titers for multiple viral agents in the spring (Figure 2). Notably, spring-purchased calves were all observed at the same farm, though from multiple purchase lots.

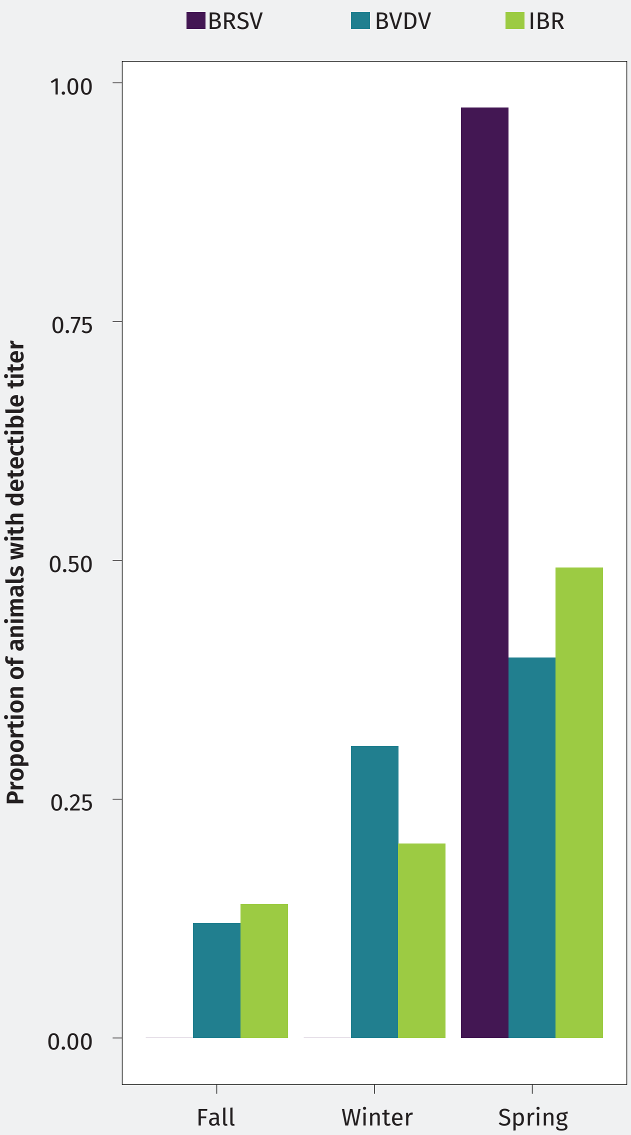
Seven percent of calves exhibited visual signs of illness on arrival. The top 3 clinical issues were eye lesions consistent with infectious bovine keratoconjunctivitis (4.4%), papilloma or other skin lesions (2.0%), and mucopurulent nasal discharge (1.5%). In general, the surveyed sample was consistent with the animal makeup of most Tennessee stocker operations. The sampled population was overwhelmingly Angus-influenced, 81.8% black-hided and 93.6% polled, with minimal *Bos indicus* influence. There was an even proportion of heifers, bulls and steers (35.5%, 33.8% and 30.6%, respectively). Most calves weighed between 300 and 700 pounds (86.6%), and the majority had moderate body condition scores. They ranged from 200 pounds to 1,000 pounds, with a mean of 477 and a median of 500. Detailed descriptive statistics for all visual characteristics collected are presented in Table 1.

Three individual visual calf factors were significantly associated with likely immunization status based on univariate logistic regression models that included farm as a random effect. Horned cattle were 20.4% as likely as polled cattle to be considered immunized ($P = 0.01$). Calves marketed as preconditioned were 2.2 times more likely than cattle with no health history ($P = 0.003$) to be immunized. Finally, calves that had a prior ear tag present were 1.6 times more likely than calves with no tag on arrival ($P = 0.03$) (Table 2) to show titers indicative of immunization. Other calf factors were also statistically significant (visual weight, frame score, visually ill on arrival). Correlations between visual calf factors and the presence of individual viral titers and likely immunization are shown in Figure 3. No other individual factors were significantly associated with likely vaccination.

We observed an interesting non-linear relationship between animal approximate body weight and the presence of multiple BRD-implicated viral titers (Figure 4). We identified higher occurrences of animals with multiple titers in light (< 400 lbs.) and heavy (> 600 lbs.) calves. Calves that weighed approximately 500 lbs. were considered vaccinated at the lowest rate of any weight class. Continuous weight and weight class (light, medium, heavy) were significantly associated with vaccination, but discrete 100-weight increments were not significant predictors. The class of 800+ lb. calves had only one animal that appeared likely vaccinated. Due to this non-linear relationship, we did not include approximate body weight as a factor in correlation analyses or linear models.

Logistic regression models that fit multiple calf factors simultaneously further demonstrated that sex, tag and seller-reported health records were the best indicators of likely immunization (Table 3). The accuracy of predictive models improved with additional calf variables, but the changes were not statistically significant (Table 3). When increasing the model complexity, we observed that upon adding the declared vaccinated factor, the presence of a prior ear tag was no longer statistically significant. This suggests that marketing calves as vaccinated is likely confounded with tag variable, overpowering it in multi-variable analysis. Increasing model complexities resulted in increases in the mean R^2 from 6% of the variation being explained by the data to 23.4% variation explained. RMSE and accuracy changes did not differ across

Figure 2: The proportion of calves sampled in each season had detectible titers of BRSV, BVDV or IBR on arrival. Based on observations of 408 calves. There was a significant ($P < 0.05$) interaction of titer presence by season, but calves being considered immunized were not deemed significant by season.



cross-validated models. The relatively low predictive ability of our models is likely rooted in the low number of farms and number of animals sampled.

Information about animals pre-sale was limited. Purchasers identified a limited sample of animals that were bought with knowledge of a previous vaccination. We did not have information on which vaccines were delivered or when they were administered. Animals declared vaccinated were more likely to have multiple detectible viral titers than animals purchased at regular sales ($P = 0.0029$). Despite this significant difference, only 53% (36/68) of those animals appeared to be vaccinated for BRD-associated viruses based on observed titer levels.

Comparing immune health, disease incidence and performance differences between preconditioned and naïve calves

A subset of 60 male weaned calves (average body weight 581 lb. [SD = 15.8]) were followed through the stocker phase. This allowed us to collect pre-sale information, detailed health and treatment records, and body weights on days 14, 35 and 56 post-processing on arrival. Thirty calves were purchased at designated preconditioned sales and had health and vaccination records. The remaining 30 calves were purchased at a weekly non-preconditioned sale. We refer to these 2 groups throughout as preconditioned and naïve, respectively.

These animals underwent the same onboarding sample collections and evaluation described by the larger stocker calf survey. Of the 60 calves, 28% (n = 17) had a detectible titer for BVDV, and 23% (n = 14) had detectible titers for IBR. None of the calves possessed a detectible titer for BRSV. None of the naïve calves had detectable titers for IBR, while 2 had titers for BVDV (Figure 5). Twelve of the 30 calves purchased as preconditioned possessed detectible titers for at least 2 BRD-associated pathogens. None of the calves in the naïve group were considered immunized by VNT. In this population, 52% (31/60) of calves were treated at least once for BRD. Of the preconditioned calves, 33% (10/30) were treated at least once for BRD, whereas 70% (21/30) of the non-preconditioned calves received at least one BRD treatment.

Illness occurrence peaked immediately before the 2-week mark, consistent with previous observations.[30] Preconditioned calves were less likely to be treated for BRD than naïve calves. Of the 30 calves that we followed through the stocker phase, only 13 were treated for BRD compared with 25 of the naïve animals. In addition to preconditioning status, the presence of a detectible titer was protective for individual animals. We observed a significantly lower incidence of BRD in calves that had detectible BVDV titers vs. not (23.5% vs. 68.2%) and IBR titers vs. not (21.4% vs. 60.9%). Calves without titers for BVDV were 2.7 (95% CI: 1.1, 6.6) times more likely to contract BRD than calves with titers, and calves without titers to IBR were 5.7 (95% CI: 1.4, 24) times more likely than calves with titers. Calves that had at least 2 detectible titers were 13.5% as likely to be treated for clinical BRD than animals without multiple titers ($P = 0.006$).

In addition to avoiding disease, the preconditioned calves gained 26.3 more pounds on average than the naïve group over the first 56 days ($P = 0.015$) of their stocker phase (Figure 6). Preconditioned calves averaged 3.09 lbs. (1.40 kg) gain/day, while naïve calves averaged only 2.62 lbs. (1.19 kg) gain/day. The magnitude of differences in ADG between preconditioned and naïve cattle was largest early in the stocker phase (Figure 7). During the first 2 weeks of the trial, preconditioned calves gained 1.25 lbs. more per day than their naïve counterparts. Despite this large effect size of preconditioning, the differences were not statistically significant ($P = 0.11$). This difference decreased to 0.4 lbs./day between days 14 and 35 ($P = 0.487$) and to 0.03 between days 35 and 56 ($P = 0.943$) However, there was a significant difference between the preconditioned and non-preconditioned classes of calves over the course of the study.

We also observed differences in the weight gains between calves that became sick during various time periods of the stocker phase, though none reached statistical significance (Figure 8). The ADG impact of contracting clinical BRD was consistent

Table 1: Descriptive statistics for the 14 visual characteristics collected on 408 Tennessee stocker calves.

Visual characteristic	Observation	Number of animals	Percent of population
Sex	Heifer	145	35.5%
	Bull	138	33.8%
	Steer	125	30.6%
Tag present	No	231	56.6%
	Yes	177	43.4%
Visual approximate body weight (lb.)	200	4	0.98%
	300	62	15.2%
	400	122	29.9%
	500	115	28.2%
	600	55	13.5%
	700	39	9.6%
	800	8	2%
	900 +	3	0.74%
Body condition score*	4-borderline thin	52	12.8%
	5-moderate	319	78.2%
	6-slightly fleshy	37	9.0%
Docility score†	1-Docile	220	53.9%
	2-Restless	124	30.4%
	3-Nervous	40	9.8%
	4-Flighty	24	5.9%
Castration (of 125 steers)	Cut and healed	116	92.8%
	Freshly cut	5	4.0%
	Stag	3	2.4%
	Cryptorchidism	1	0.8%
Frame score‡	Small	116	28.4%
	Medium	217	53.2%
	Large	75	18.4%
Horn status	Polled	382	93.6%
	Horned	26	6.4%
	Dehorned	0	0%
Bos indicus influence	No	404	99%
	Yes	4	0.98%
Coat color	Black	271	66.4%
	Black Baldy	63	15.4%
	Grey	27	6.6%
	Red	20	4.9%
	White	20	4.9%
	Red Baldy	7	1.7%

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Table 1 Con't:

Coat score§	1- Slick - 100% shed	73	17.9%
	2- Mostly - 75% shed	60	14.7%
	3- Halfway - 50% shed	135	33.1%
	4- Initial - 25% shed	48	11.8%
	5- Full coat - 0% shed	92	22.5%
Declared vaccinated	No	340	83.1%
	Yes	68	16.2%
Visually sick on arrival	Eye lesions	18	4.4%
	Skin issues	8	2%
	Nasal discharge	6	1.5%

* 1 to 9 scale from thin to fat.[18-19, 24]
† 1 to 9 scale from docile to aggressive.[18]
‡ USDA, U.S. standards for grades of feeder cattle in 2000.[20]
§ 1 to 5 scale from slick to full winter coat.[22]

Table 2: Odds ratios for the likelihood of calf being immunized for 8 visual calf observations. Based on 408 stocker calf observations. The odds ratio reported is the increased likelihood of vaccination if the calf has the state of the variable in parentheses (e.g. calves with health records are 2.2 times more likely to be vaccinated than calves without).

Calf variable (comparison)	Odds ratio of likely vaccinated	*P*-value
Health records (Yes)	2.20	0.003
Horns (Polled)	4.84	0.011
Sex (compared with in-tact bull)		
(Steer)	1.26	0.82
(Heifer)	0.367	0.422
Coat score For every increase in coat score by 1	Calf odds increase by 0.80	0.004
Prior tag (Yes)	1.56	0.030
Docility For every increase in docility score by 1	Calf odds increase by 0.86	0.224
Body condition score For every increase in condition score by 1	Calf odds increase by 1.46	0.071

during the first 2 measured gain periods (-0.61 and -0.58 lbs./day, respectively). No animals were treated for BRD between days 35 and 56. There was a significant weight gain difference regarding group on day 35 for healthy animals vs. sick animals.

Discussion

Our studies used data from 4 stocker operations across Tennessee to evaluate whether on-arrival visual indicators could be used to predict likely calfhood vaccination for viruses implicated in BRD infection. We used detectible viral titers as our indicator for likely vaccination. Assuming proper vaccine handling, delivery and timing, we would expect protective titers from a modified-live vaccine to persist for > 1 month.[13]

We found that animal sex, tag and being declared vaccinated on arrival were the factors most strongly associated with likely immunization (i.e., detectable titers for at least 2 of BVDV, BRSV and IBR). Sex (heifer, steer, bull), cattle with a tag present that were polled, castrated, had better body condition, possessed some declaration of vaccination, or were more docile had better odds of being immunized than cattle without those attributes. These factors are all likely indicators of elevated management that coincided with an increased likelihood of vaccination. Castrated males had significantly higher levels of likely vaccination compared with bull calves across the sample, serving as the major driver for this association with sex. Further, the presence of a previous tag also likely indicated a level of management protocols previously in an animal's lifetime.

Figure 3: A correlation plot depicting the relationships between visual calf factors, titer abundances and likely immunization status for 408 weaned Tennessee stocker calves. Point size is proportional to the absolute value of the magnitude of correlation. Positive and negative correlations are represented as a scale from blue (positive) to red (negative).

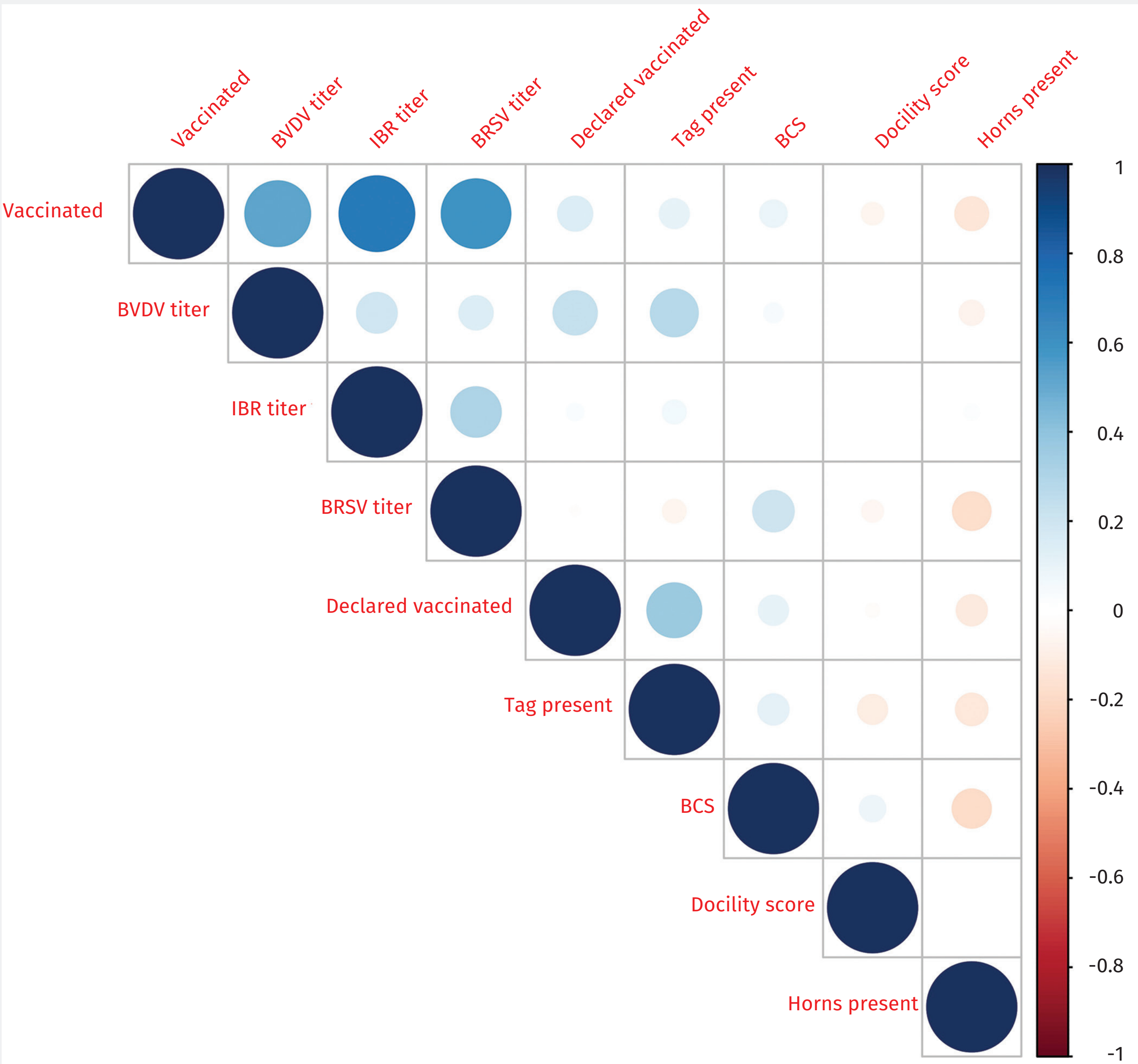


Figure 4: Bar plot representing the proportion of animals within each weight class that our study identified as having at least 2 BRD-associated viral titers present. The numbers on top of each bar represent the fraction of animals in each weight class with multiple viral titers, suggesting vaccination for BRD.

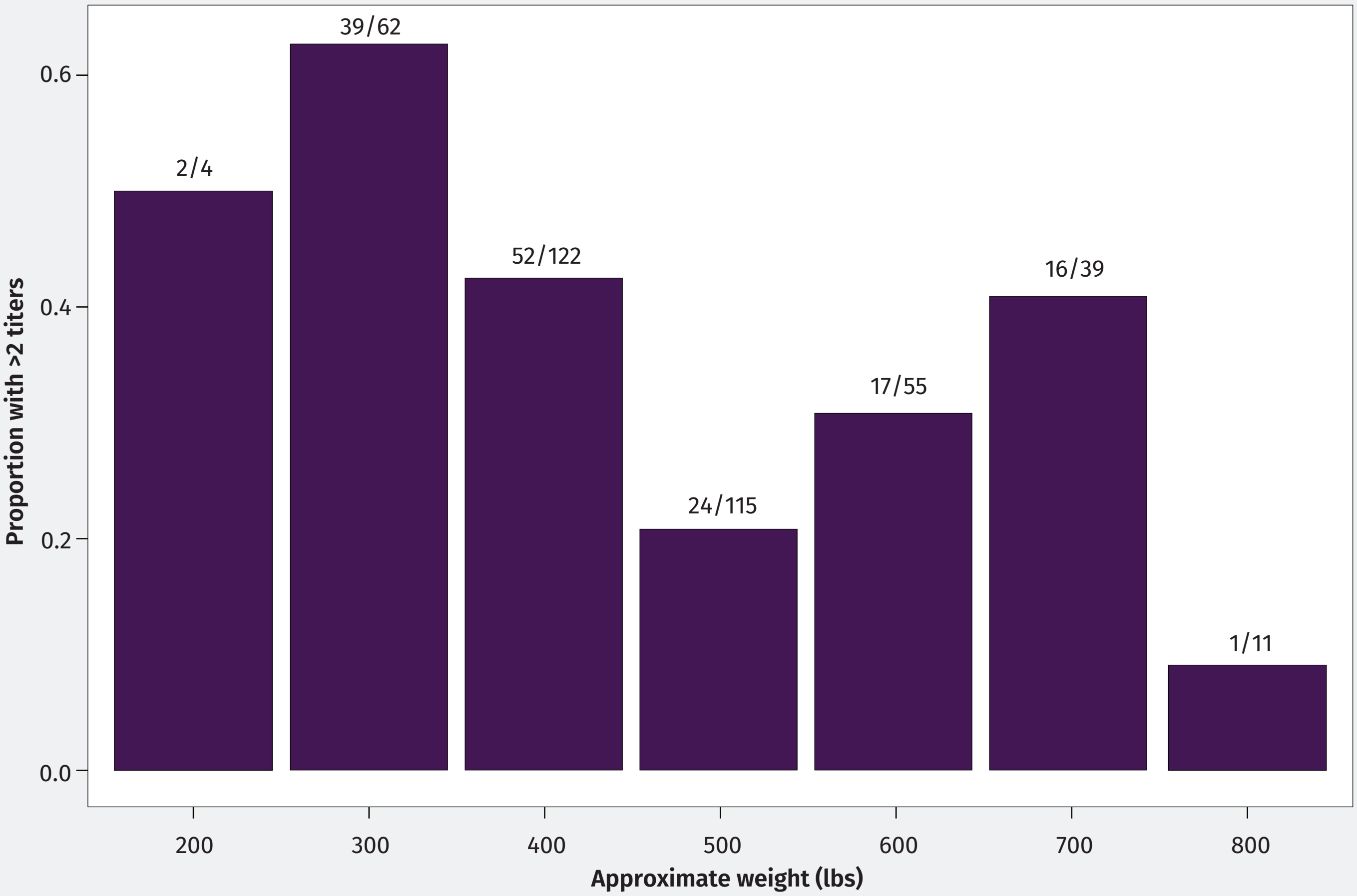


Table 3: Model construction for predicting immunization status from visual calf factors. Models were built from the least to the most complex, adding in significant variables stepwise. Lower AIC values indicate more favorable model fits.

Model evaluation		Model predictive ability				
Model	**Variables**	**AIC**	**Statistically significant variables**	**Mean (R2)***	**Mean (RMSE)†**	**Mean (ACC) ‡**
1	Sex + Tag	536	Sex: P = 0.048 Tag: P = 0.031	0.062	0.484	0.630
2	Sex + Tag + Horn status	540	Sex: P = 0.048 Tag: P = 0.035	0.125	0.480	0.625
3	Sex + Tag + Frame	537	Sex: P = 0.017 Tag: P = 0.029 Frame: P = 0.003	0.176	0.478	0.615
4	Sex + Tag + Frame + Health records	514	Sex: P = 0.005 Frame: P = < 0.001 Health rec: P = < 0.001	0.234	0.474	0.607

* R2: Measures the proportion of the variance that the independent variable (calf characteristics) explains the dependent variable (immunization status) in a regression model.

† RMSE - root mean square error: This is the prediction errors, standard deviation of the residuals.

‡ ACC - accuracy: The accuracy of the times the predictive model correctly predicts immunization status based on calf characteristics.

We observed an interesting relationship between approximate body weight and multiple-titer presence in calves. Very light calves and heavier calves had higher rates of being considered vaccinated. This is likely due to the presence of maternal titers for light calves and higher levels of management on heavier animals. The medium-sized calves that might be considered more conventional stockers had low rates of likely vaccination. The decreased likelihood of vaccination in larger calves (> 800 lbs.) likely stemmed from either being generally mismanaged or from the waning of titers from calfhood vaccinations. These weight relationships may have also been confounded with the origin of loads purchased at each stocker location. Larger surveys of multiple stocker operations will be needed to characterize this relationship more concretely between weight and likelihood of immunization.

Each of these attributes suggests a higher degree of management and vaccination is likely a concurrent practice. It is worth noting that vaccinations, even when delivered correctly, may still fail to generate a detectible titer at our sampling time. While viral titer is not a true gold standard for evaluating vaccination against BRD pathogens, it is the most straightforward method for evaluating the likelihood of immunization from a single touchpoint on producer stocker farms.

We developed a preliminary model that used visual indicator traits collected at intake to predict the immunization status of calves. That said, predictive abilities were generally low, likely due to our limited sample size and the scope of seasons and environments in which we were able to collect samples. Other factors could generate noise in predicting vaccination for BRD pathogens due to the fact that we use a retroactive survey of BRD vaccine-associated titers as our indicator of prior vaccination. Even in cases where vaccination occurs, generating a robust and protective immune response could be impaired by improper vaccination handling or administration.[31] Variability in the individual calf response to vaccination could also result in different levels of titers being detected at the time of intake at stocker operations.[31,32] Further, the type of vaccine (killed vs. modified-live) could also result in differential immune responses and post-vaccination titer abundances.

Figure 5: Histogram depicting titer levels on arrival for preconditioned (n = 30) and naïve (n = 30) calves based on viral neutralizing tests for BVDV, IBR and BRSV. Note that no animals showed detectable titers for BRSV.

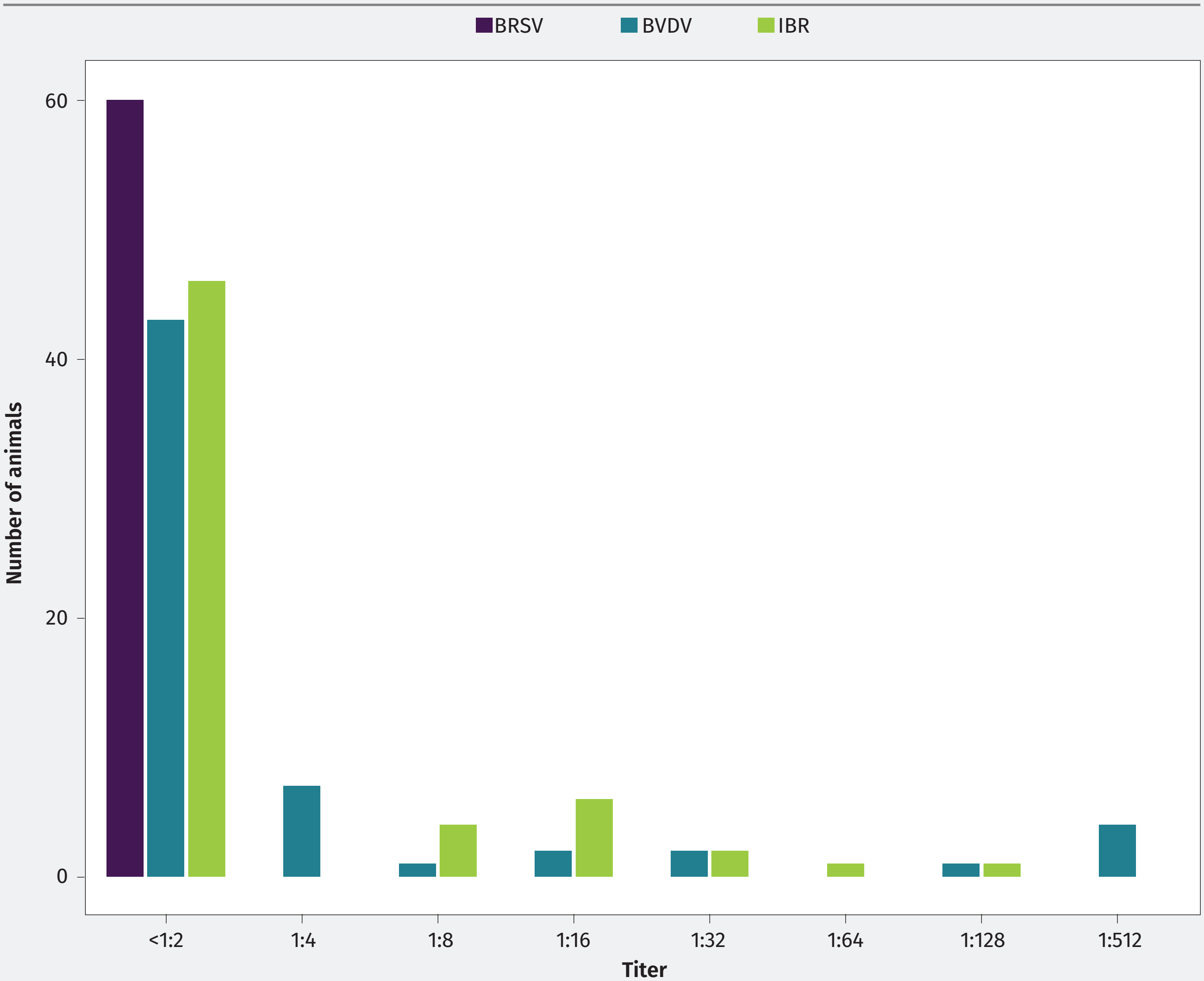
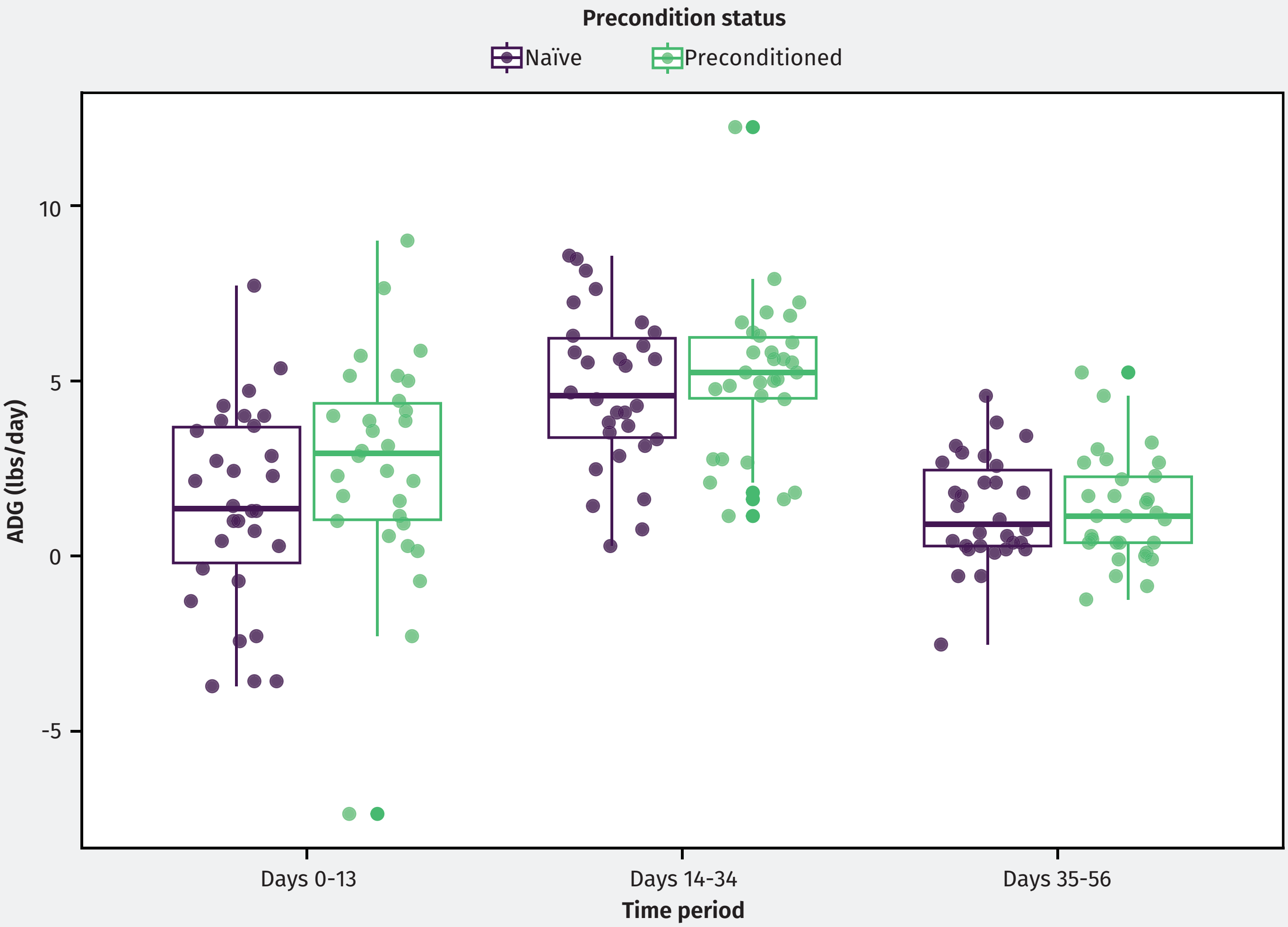


Figure 6: Average daily gain of preconditioned (n = 30) and naïve (n = 30) calves over the first 56 days of their stocker phase. Average and first and third quartiles are represented by the boxplots. Individual animal ADGs are represented by points. Both are colored by the precondition status.



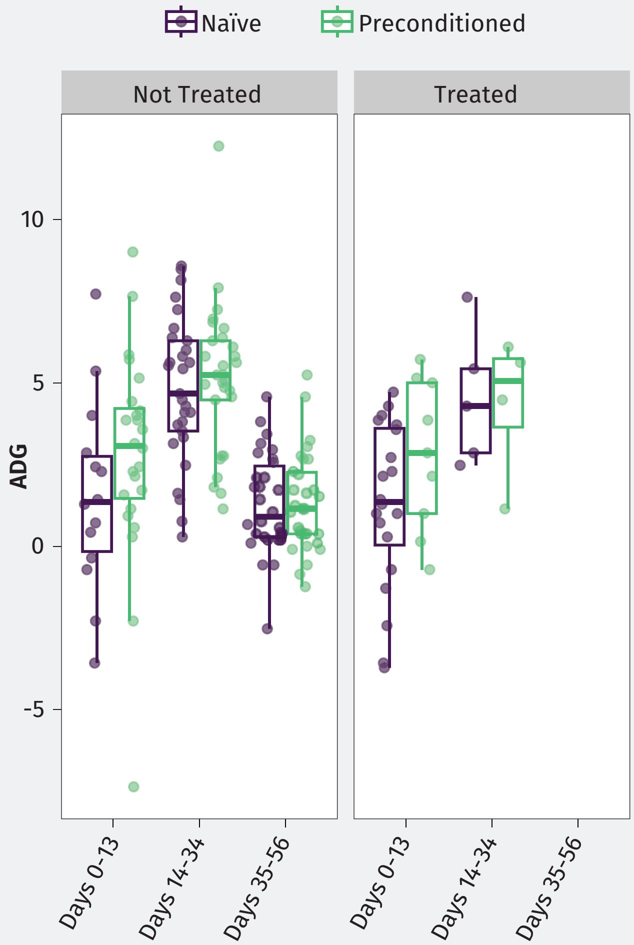
Finally, in some cases, calves that were properly immunized earlier in life may lack detectible titers at our time of observation, despite being primed for strong immune responses. Previous work has shown that immunity acquired very early in an animal's life can directly enhance their ability to mount a more robust immune response to pathogens despite a lack of detectible titers later in life.[33,34] Nevertheless, knowledge of these informative visual indicators can be important tools for helping producers make informed purchasing decisions and tailoring on-farm management strategies. For example, producers looking to buy lower-risk cattle might look for calves with an ear tag present that are also castrated.

We did not observe detectible titers for BRSV in the calves until sampling during the spring months. It is also possible that this occurrence was an artifact of the purchasing practices of a single stocker operator or variations in the origins of mixed lots of calves not known by our team. Future work should aim to sample over multiple years and across seasons to adequately represent the full range of pathogens and environmental stressors. This would allow for a more in-depth look at the seasonality of vaccination and pathogen loads. This seasonal variation could be a function of management differences for groups of available cattle over a year or due to seasonal changes in viruses.[35]

Since many of the cattle sampled in the last 2 groups in April had a positive titer for BRSV and a titer for IBR and/or BVD, we hypothesize the elevated BRSV titers were the result of vaccination, not necessarily active infection. The presence of a single viral titer to BVDV or IBR appeared to lend helpful immunity that allowed animals to avoid clinical illness. There can, of course, be a seasonal effect to viral shedding and exposure in the spring and fall months because of an influx of calves being marketed in the fall season, thus providing higher rates of comingling and stress.[23] This also can explain the higher presence of detectible titers in the spring groups. There is a limitation in assuming that a calf with 2 or more titers is truly immunized, as we do not have precise information on when the vaccines were given. Variations in vaccines given, the titer duration gradient, and the physiological responses of individual calves mean that a single blood draw may not paint a complete picture of the animal's health history.

Future surveys inspired by the work presented here could integrate more granular definitions of animal attributes that might improve the quality of our predictive model. For example, breaking binary characteristics into more informative classes (e.g., presence of horns needing to be tipped vs. very small horns vs. scurs). This, coupled with a more robust sampling of operations across geographical regions and seasons,

Figure 7: Average daily gain of preconditioned (n = 30) and naïve (n = 30) calves over 3 time periods following intake processing. Significance indicated by (*) noted at day 35 post-processing in healthy vs. sick animals.



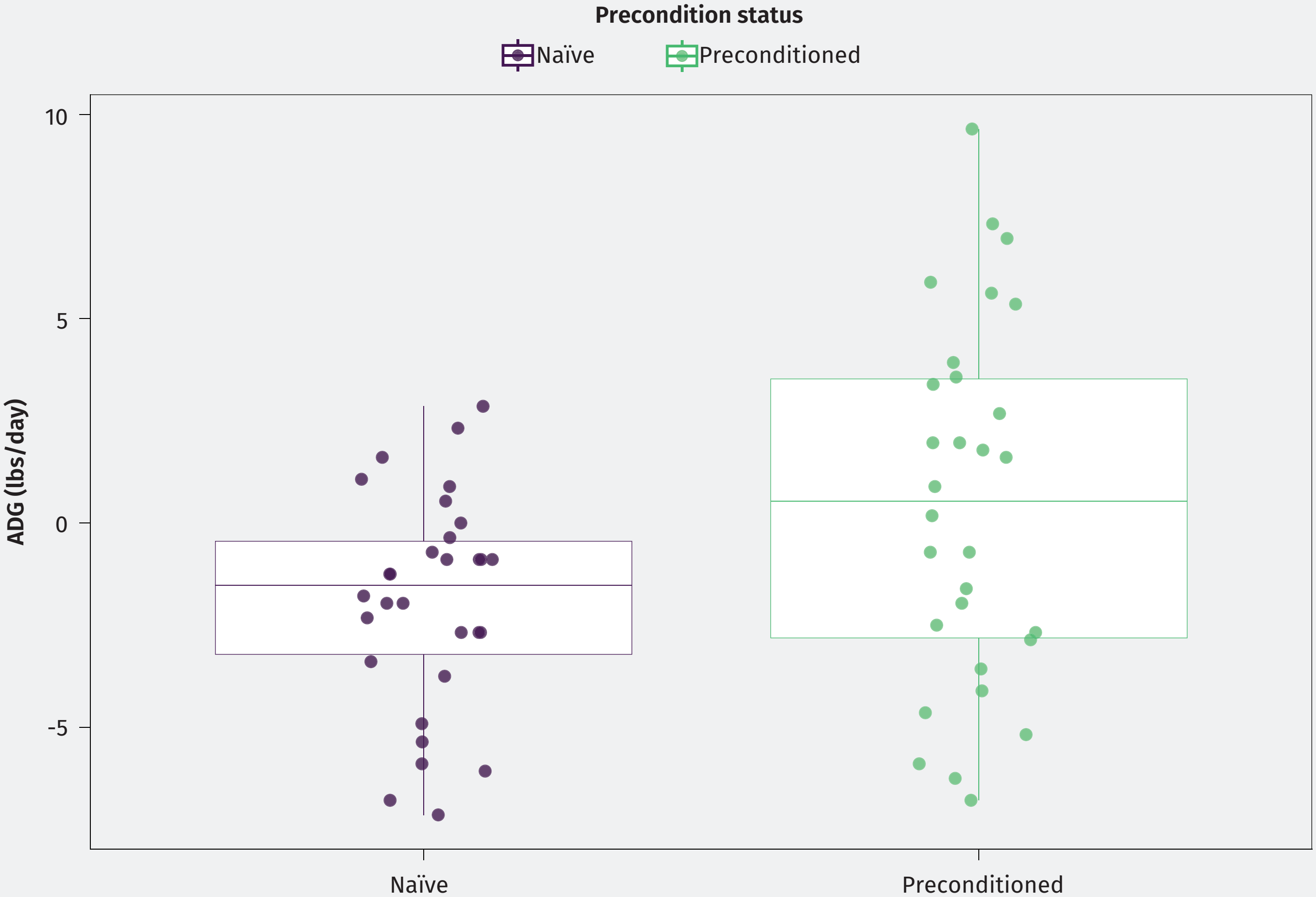
could help improve the robustness of predictions. Additionally, we did not have detailed information on the origins of groups of animals purchased at non-university-owned herds. This would have been valuable data that could have both informed our predictive models and helped discern seasonal variations in titer abundances versus those driven by sampling bias. In addition to more detailed and deeper sampling, future work would also benefit from follow-up testing of animal antibody responses following vaccination. It is possible that some of the animals marketed as previously vaccinated, yet lacked detectible titers may have retained some of the benefit of early vaccination not captured by our one-time observations. This would also help us better identify the other factors that impact vaccine efficacy in stocker calf populations.

Our second study followed 60 calves through a stocker phase. It demonstrated that preconditioned cattle experienced reduced BRD incidence, resulting in significantly more weight gain over 56 days compared with naïve cattle. These results underscore the positive impact of prior immunity and reduced weaning stress that has long been recognized as benefits of preconditioning.[26,36] Surprisingly, the number of calves without detectable titers at arrival time indicated that many calves marketed as preconditioned may not have been vaccinated. This is in direct contrast to what stocker producers perceive when paying premiums for preconditioned animals. There is an expectation that calves have had their vaccinations, been fully weaned, and trained to eat from a feed bunk/water trough.[37] While some of our data does suggest that these calves may have been weaned and prepared to eat out of a bunk, as preconditioned calves gained more weight and adapted better to the new environment, the lack of viral titers in many of these calves suggests they had not received BRD vaccines. Although we cannot definitively say whether cattle are vaccinated, this study underlines the possible need for greater transparency in verified buying programs. Even in cases where calves were not considered vaccinated by our definition, the presence of a single detectible titer significantly decreased the likelihood that they would be treated for BRD in the stocker period. This largely agrees with the findings in Step et al.[26] that suggested vaccination, in addition to preconditioning, may only provide marginal additional value.

While the 60-day weight period is short, we observed that some animals appeared to have time points with significant fluctuations in ADG. We learned this was likely caused by a time point where water consumption and feed intake occurred immediately before weights were taken. That said, ADG values over the course of the entire trial are the most informative of an animal's aggregate performance. While the difference in means between preconditioned and naïve groups were most prominent during the first gain period, large amounts of variation across groups prevented statistical significance at this time point. Variability reduced over time, and we observed a significant difference in gain at day 28 ($P = 0.026$) and day 42 ($P = 0.012$) time points (Figure 4). Throughout the trial, preconditioned calves gained an average of 22.7 lb. more than their naïve counterparts. However, by the end of the observational ADGs between intervals became nearly identical for the 2 groups. This increased similarity is likely due to naïve calves acclimating to a new environment, learning to eat out of a feed bunk and drink from a water trough, and largely recovering from early BRD and returning to normal feed intake. We also observed a significant weight gain difference between groups on day 35 for healthy animals vs. sick animals. This indicates that clinically ill calves likely went off feed for a period and thus experienced lowered ADG compared to healthy animals (Figure 5). We note that our studied population had heavier starting weights compared to typical stocker animals in Tennessee. Differences in gain over the stocker period would have likely been more pronounced with a lighter sample of calves. While we did not test comingling, pen density or infection rates, our data does outline a clear benefit to purchasing preconditioned calves, as they had lower rates of BRD and enhanced performance over the trial period. This helps contextualize the potential value of buying cattle with "low-risk" indicators.

Our findings from this survey of Tennessee stocker animals will not likely surprise cattle buyers. They are very attuned to how risk varies across the population. However, their use of this information might be of interest, as seasonality and cyclicity in the markets can substantially shift risk tolerance and buying practices. In situations where supply is plentiful, buyers are more likely to invest in preconditioned animals.[38] In markets where supply is tight, buyers may be more concerned with securing animals regardless of preconditioning status. Visual indicators of likely immunization identified by this study can help inform buying practices in environments where economic signals encourage lower-risk purchases.

Figure 8: Boxplots of average daily gain over 3 consecutive periods for calves broken out by their preconditioning status and whether or not they were treated for BRD during the first 56 days of the stocker phase. Average daily gain was calculated between weights taken at initial processing, day 14, day 35 and day 56. Animals were considered "treated" only if they received treatment for BRD during the specified ADG block.



Funding

United States Department of Agriculture National Institute of Food and Agriculture Multistate project NC1192: An integrated approach to control of Bovine Respiratory Disease. Any work or recommendations of this study is not reflective of the United States Department of Agriculture National Institute of Food and Agriculture, but is that of the authors.

End notes

[a] Express™ 5-HS Cattle Vaccine (Boehringer Ingelheim, Duluth, GA)

[b] Alpha-7/MB-1® Cattle Vaccine (Boehringer Ingelheim, Duluth, GA)

[c] Presponse® Hm Cattle Vaccine (Boehringer Ingelheim, Duluth, GA)

[d] Autogenous Bacterin (Newport Laboratories, Worthington, MN)

[e] Eprinex® Pour-On for Beef and Dairy Cattle (Boehringer Ingelheim, Duluth, GA)

[f] Ralgro® (Merck Animal Health, Rahway, NJ)

[g] Nuflor® (Merck Animal Health, Rahway, NJ)

[h] Draxxin® (Zoetis Inc., Parsippany, NJ)

[i] Excede® (Zoetis Inc., Parsippany, NJ)

Conflicts of interest

The author have no financial and/or non-financial conflicts of interest relative to this study.

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