Integrating animal genomics with animal health: Genetics of vaccine response in cattle

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Introduction

Beef cattle breeders have successfully developed EPDs for a large number of traits, including average daily gain, carcass merit, stayability, calving ease, and feed efficiency. Despite these successes, a genetic prediction for animal health in beef cattle remains elusive. The reasons why a genetic prediction for animal health has not been developed are 1) limited recording and availability of phenotypic records on disease incidence to breed associations and 2) low heritability of disease traits. In spite of these challenges, several quantitative trait loci (QTL) associated with disease susceptibility have been mapped for pinkeye (Casas and Stone, 2006), Johne's disease (albeit in dairy breeds only) (Settles et al., 2009; Gonda et al., 2007), Bovine Respiratory Disease (Neibergs et al., 2011), and trypanosomiasis (Hanotte et al., 2003). Disease phenotypes in these studies have often been categorically defined: "healthy" or "non-infected" vs. "sick" or "infected". These QTL mapping studies have been valuable for unraveling the genetic architecture of disease susceptibility in cattle.

The main limitation with these studies is that the "healthy" or "non-infected" animals are assumed to be resistant to the disease. However, animals may be "non-infected" because they are 1) truly resistant to the disease, 2) not exposed to the pathogen that causes the disease, or 3) the diagnostic test result for infection was a false-negative, i.e., the animal was classified as non-infected when the animal was truly infected with the pathogen. Incorrectly classifying susceptible animals (i.e., infected) as resistant to disease (i.e., non-infected) results in lower heritabilities and decreased power for mapping disease loci.

An alternative phenotype for disease resistance is measurement of immune response to commercially available vaccines. When vaccinated, animals exhibit different immune responses; some animals mount a strong response to the vaccine, while other animals mount a weak response or do not respond to the vaccine at all. Can we increase the percentage of animals that mount a strong immune response to vaccination and thus would be better protected from disease? Traditionally, researchers have focused on improving vaccine response by developing more effective vaccines that protect a larger percentage of a herd. As a complementary approach, could we select for animals that respond more robustly to currently available vaccines? This approach would circumvent the limitation of incomplete disease exposure; all animals would be vaccinated with the same vaccine, thus ensuring uniform exposure. In addition to genetic selection for stronger vaccine response, identification of genes affecting vaccine response variation will also help vaccine manufacturers design more better vaccines.

My hypothesis was that vaccine response in cattle is a heritable trait and we can identify DNA markers associated with vaccine response. These DNA markers could then be used to develop a DNA test for vaccine response. Towards this goal, this paper reports completion of three objectives regarding Bovine Viral Diarrhea Virus (BVDV) vaccine response.

1. Comparison of three measures of the humoral response to BVDV vaccination: a) enzyme-linked immunosorbent assay (ELISA), b) serum neutralization (SN)-1, and c) SN-2.

2. Test whether sire of the calf was associated with BVDV vaccine response. If sire of the calf was associated with vaccine response, then this result would strongly suggest a genetic component to BVDV vaccine response variation in cattle.

3. Test whether a polymorphism in the leptin gene was associated with BVDV vaccine response. The leptin gene is involved in pathways that affect the immune response. This leptin polymorphism has also been associated with carcass and growth traits; if this polymorphism is associated with immune response, then producers could inadvertently be selecting for less immunity when selecting on this leptin polymorphism.

Materials & Methods

Experiment One: This experiment was designed to answer objective one. We collected 406 sera or plasma samples from 193 Angus or Angus-influenced calves that had been vaccinated for BVDV with either Bovi-Shield GOLD 5 (Pfizer, Inc.) or Onset 5 (Intervet). Sera and plasma samples were collected at the time of vaccination and 15-30 days post-vaccination. Calves were raised at the South Dakota State University (SDSU) Cow-calf Teaching and Research Unit (Brookings, SD) or the SDSU Cow Camp (Miller, SD). Calves had not previously been vaccinated for BVDV, although dams had been vaccinated.

Antibodies to BVDV were measured in sera and plasma by 1) BVDV-specific ELISA (Idexx Inc.), 2) SN-1, and 3) SN-2. The ELISA measures total BVDV antibodies regardless of whether antibodies can protect the calf from infection. The SN measures only BVDV-1 (SN-1) or BVDV-2 (SN-2) antibodies that can protect the calf from infection. The SN tests were completed by the Animal Disease & Research Diagnostic Laboratory at SDSU. The correlation between the ELISA, SN-1, and SN-2 was measured with a Spearman correlation coefficient.

Experiment Two: This experiment was designed to answer objectives two and three. Angus and Angus-influenced calves (n = 267) were sampled from three herds: 1) SDSU Cow-calf Teaching and Research Unit (Brookings, SD), 2) SDSU Cow Camp (Miller, SD), and 3) SDSU Antelope Research Station (Buffalo, SD). Calves were vaccinated with Pyramid-5, which includes BVDV-1 and BVDV-2, at 1-8 months of age. Calves had not previously been vaccinated for BVDV, although dams had been vaccinated. Blood samples were collected at the time of vaccination and 21-28 days post-vaccination.

The blood sample at time of vaccination was used for three purposes: 1) DNA isolation, 2) measurement of baseline BVDV antibodies present in blood at time of vaccination, and 3) identification of calves persistently infected (PI) with BVDV. Regarding purpose 2, calves would have absorbed BVDV antibodies from colostrum that could still be detected in the calves' blood. Regarding purpose 3, if a PI calf was identified, all of the calves in the same contemporary group would likely have been exposed to BVDV prior to vaccination and we would need to remove this group from the study. The post-vaccination blood sample was used to measure BVDV-specific antibodies circulating in blood post-vaccination. The BVDV antibodies were measured with a BVDV-specific ELISA (Idexx).

Vaccine response was measured by subtracting BVDV antibodies present at time of vaccination from BVDV antibodies present post-vaccination. At the SDSU Antelope Research Herd, sire of the calf was determined by parentage testing (GeneSeek, Lincoln, NE). At the other herds, dams were artificially inseminated and sire of the calf was determined by herd records. The leptin polymorphism was genotyped as described previously (Buchanan et al., 2002). Vaccine response was regressed on sire of the calf and leptin genotype, with covariate age at vaccination and fixed effects herd and gender also included in the linear model.

Results & Discussion

Objective 1: The ELISA was significantly, positively correlated ($P < 0.0001$) with SN-1 ($\rho = 0.809$) and SN-2 ($\rho = 0.638$).

As shown below (**Table 1**), ELISA S/P ratios are positively correlated with SN titers and can thus be used as an indicator trait for SN titers. The relationship between ELISA and SN is linear when titers are > 1:64. However, the relationship between ELISA and SN is not linear when titers are low. Still, low SN titers are indicative of low ELISA S/P ratios and we should be able to use the ELISA as an indicator trait for SN titers even at low antibody levels.

Table 1. Comparison of SN-1 and SN-2 titers with mean ELISA sample-to-positive (S/P) ratios.

SN Type 1		SN Type 2	
SN 1 Titers	μ ELISA S/P (± SD)	SN 2 Titers	μ ELISA S/P (± SD)
< 1:8	0.286 (± 0.271)	< 1:8	0.339 (± 0.298)
1:8	0.361 (± 0.263)	1:8	0.430 (± 0.253)
1:16	0.324 (± 0.166)	1:16	0.423 (± 0.263)
1:32	0.472 (± 0.208)	1:32	0.566 (± 0.333)
1:64	0.484 (± 0.242)	1:64	0.667 (± 0.430)
1:128	0.631 (± 0.254)	1:128	0.980 (± 0.536)
1:256	0.933 (± 0.475)	1:256	1.001 (± 0.592)
1:512	1.151 (± 0.454)	1:512	1.255 (± 0.577)
1:1024	1.388 (± 0.392)	1:1024	1.410 (± 0.506)
1:2048	1.563 (± 0.353)	1:2048	1.552 (± 0.465)
1:4096	1.797 (± 0.489)	1:4096	1.780 (± 0.411)
1:8192	2.084 (± 0.314)	1:8192	1.883 (± 0.793)

Objectives 2-3: No PI BVDV calves were found. Sire of the calf was significantly associated with vaccine response ($P < 0.05$). Because vaccine response was heritable for other vaccines in cattle (O'Neill et al., 2006) and in humans (Kimman et al., 2007), these results strongly suggest that BVDV vaccine response is heritable in cattle. Therefore, it should be possible to identify DNA markers associated with BVDV vaccine response which can be used for genetic selection. We chose not to estimate heritability for BVDV vaccine response with this data because the estimate would not be precise given the small number of calves in the study.

The leptin polymorphism was not associated with BVDV vaccine response ($P = 0.26$). Selecting for favorable leptin alleles should have no impact on humoral BVDV vaccine response.

Implications

This study is the first step towards development of a DNA test for vaccine response that producers could use to select for healthier cattle. With the development of dense single-nucleotide polymorphism genotyping panels (e.g., 850K SNP, 50K SNP), the number of DNA markers found to be associated with economically important traits should increase substantially throughout this decade. For animal health traits, the limiting factor will now be collection of a sufficient number of phenotypes on animal health that can be used for DNA testing. For this reason, development of a DNA test for vaccine response will be a long and laborious process. Additionally, many questions remain unanswered:

1. What is the genetic correlation between vaccine response and disease susceptibility?

2. How should vaccine response be measured? Should we focus on measures of the humoral (antibody) immune response, the cell-mediated immune response, or both?

3. What is the genetic correlation between vaccine response and other economically important traits?

4. Which DNA markers are associated with vaccine response? After discovery, these associations will need to be confirmed in an independent gene mapping population before use.

5. How can we best transition from discovery of DNA markers associated with animal health to implementation of a tool useful for producers for making selection decisions?

Acknowledgements

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