Vaccine 30 (2012) 6559–6563

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Measuring bovine viral diarrhea virus vaccine response: Using a commercially available ELISA as a surrogate for serum neutralization assays

M.G. Gonda[a,*], X. Fang[a,1], G.A. Perry[a], C. Maltecca[b]

[a] Department of Animal Science, South Dakota State University, Box 2170, Brookings, SD, 57007, USA
[b] Department of Animal Science, North Carolina State University, Box 7621, Raleigh, NC, 27695, USA

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form 27 July 2012
Accepted 19 August 2012
Available online 1 September 2012

Keywords:
Bovine viral diarrhoea virus
Serum neutralization
ELISA
Vaccine response

ABSTRACT

Genetic selection in livestock offers the opportunity to improve bovine viral diarrhea virus (BVDV) vaccine response, but first we must define how vaccine response should be measured. For measuring humoral vaccine response, serum neutralization (SN) measures antibodies that can neutralize BVDV, but relative to enzyme-linked immunosorbent assay (ELISA) is time consuming, technically demanding, and expensive. The ELISA, however, measures total BVDV-specific antibodies, regardless of whether the antibodies can neutralize BVDV. Our objective was to test whether a commercially available BVDV antibody ELISA could be used as a surrogate (or indicator trait) for neutralizing antibodies as measured by SN. Angus and Angus-cross calves ($n = 193$) from two South Dakota research herds were vaccinated for BVDV-1 and BVDV-2. Sera and plasma samples ($n = 406$) were collected from these calves at the time of vaccination and post-vaccination (20–72 days post-vaccination). The BVDV-specific antibody concentration was measured on each serum and plasma sample by (1) a commercially available total antibody ELISA, (2) BVDV-1 SN, and (3) BVDV-2 SN. Correlation between the ELISA and SN tests was estimated with a Spearman correlation coefficient. Higher BVDV ELISA sample-to-positive (S/P) ratios were positively correlated with higher BVDV-1 ($\rho = 0.809$) and BVDV-2 ($\rho = 0.638$) SN titers ($P < 0.0001$), although the relationship was weaker when SN titers were <1:64. Higher BVDV-1 SN titers were also positively correlated with higher BVDV-2 SN titers ($\rho = 0.708$; $P < 0.0001$). The correlation between ELISA S/P ratios and SN titers was lower when calves were ≤2 months of age ($\rho = 0.344$–0.566). Our results suggest that increased ELISA S/P ratios were associated with higher SN titers. We conclude that this BVDV antibody ELISA can be used as a surrogate for BVDV-1 and -2 SN titers when investigating genetic determinants of vaccine response, as long as samples are collected at 2 months of age or older.

Published by Elsevier Ltd.

1. Introduction

Bovine viral diarrhea virus (BVDV) is one of several pathogens that are part of the Bovine Respiratory Disease Complex in cattle [1]. This pathogen has significant economic impacts on beef and dairy cattle herds throughout the world [2–4]. Clinical disease signs are highly variable, but most animals that become infected with BVDV eventually recover [5,6]. When BVDV infects pregnant cows, the virus can cause abortion, stillbirth, mummification, congenital defects, and calves persistently infected with BVDV. These persistently infected calves are particularly troubling because these calves shed BVDV in their secretions, which increases the risk that herd mates will become infected [5]. A BVDV infection also has immunosuppressive and synergistic effects, increasing the likelihood of secondary infections [7].

Vaccination is commonly employed by beef producers to reduce the incidence of BVDV. Despite widespread vaccination for BVDV, mortality and morbidity caused by this virus was reported to cost the US beef industry $93.52 per animal [2]. Response to vaccines varies among individuals; some individuals mount a strong, protective response to vaccination while others mount a weak response, or even are non-responders to the vaccine [8]. Human and livestock studies have found that vaccine response is heritable [9,10]; indeed, loci associated with vaccine response have been identified in humans and cattle [11,12]. These findings suggest that we can select for individuals that are better protected following vaccination, increasing overall herd health. However, before we can begin exploring opportunities for genetic selection, we need to define how to measure vaccine response.

For measuring the humoral response to BVDV, traditionally serum neutralization (SN) has been used. From a biological standpoint, SN is most relevant because only antibodies that can

* Corresponding author. Tel.: +1 605 688 5442; fax: +1 605 688 6170.
E-mail addresses: Michael.Gonda@sdstate.edu (M.G. Gonda), george_is_in@hotmail.com (X. Fang), George.Perry@sdstate.edu (G.A. Perry), christian_maltecca@ncsu.edu (C. Maltecca).
[1] Present address: Department of Animal Science, Texas A&M University, 2471 TAMU, College Station, TX, 77843, USA.

0264-410X/$ – see front matter. Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.vaccine.2012.08.047

neutralize BVDV are measured. Unfortunately, reproducibility of SN is low, partly because SN is a technically demanding procedure [13]. Furthermore, two species of BVDV are commonly found in the USA (BVDV-1 and BVDV-2) and SN would need to be completed separately for both species. An alternative to SN may be an enzyme-linked immunosorbent assay (ELISA). Several commercially available BVDV antibody ELISA kits are available that could be used to assay BVDV-specific serum antibody concentrations. These ELISAs are more reproducible than SN and are also less expensive. One drawback of the ELISA is that this technique measures all BVDV-specific antibodies, regardless of whether the antibodies can neutralize BVDV. If the antibody concentration measured by the ELISA is not correlated with SN titers, then results from the ELISA would not be biologically meaningful. However, if BVDV-specific ELISA and SN results were correlated, then a BVDV-specific ELISA may be a useful indicator trait for neutralizing antibody concentration.

Our hypothesis was that a commercially available BVDV antibody ELISA could be used as a surrogate for BVDV-1 and -2 SN in cattle. To test this hypothesis, 406 sera or plasma samples from 193 BVDV-vaccinated Angus and Angus cross calves were collected and tested for BVDV antibodies by (1) ELISA, (2) BVDV-1 SN, and (3) BVDV-2 SN. Our objective was to estimate the correlation between these three BVDV-specific antibody tests in these calves.

2. Materials and methods

2.1. Animals and sample collection

A total of 406 sera or plasma samples from 193 calves were sampled from two cow-calf research herds: (1) SDSU Cow-calf Teaching and Research Unit (Brookings, SD) and (2) SDSU Cow Camp (Miller, SD). All cows and calves were tested for persistent infection with BVDV and no persistently infected animals were found. At the SDSU Cow-Calf Teaching and Research Unit, sera or plasma samples ($n = 241$) from Angus and Angus–Simmental calves ($n = 97$) were collected at three time points. Time points for blood collection were chosen to evaluate the best time point for measuring humoral immune response to BVDV vaccination (results not reported here). Plasma was collected when calves were vaccinated intramuscularly for BVDV, while sera was collected at 41 days post-vaccination and 72 days post-vaccination. When one or more of the assays failed for a plasma or serum sample, then the sample was removed prior to data analysis. Thus, the number of plasma and sera samples used at each time point was less than the total number of calves. A total of 87 (plasma), 59 (serum), and 95 (serum) samples at time of vaccination, 41 days post-vaccination, and 72 days post-vaccination, respectively, were used for comparing the ELISA with BVDV-1 and BVDV-2 SN. The vaccine used on these calves (Bovi-Shield GOLD-5, Pfizer, Inc., New York, NY) included both modified live BVDV-1 and BVDV-2, infectious bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial virus. The same vaccine was used for cows and calves; efficacy of this vaccine has been demonstrated [14]. Calf age at time of first sampling ranged from 2.5 to 15 weeks (approximately <1–4 months). At the time of the third collection, calf age ranged from about 13 to 25 weeks (approximately 3–6 months).

At the SDSU Cow Camp, sera and plasma samples ($n = 165$) from crossbred calves ($n = 96$) were collected at two time points. Breed composition of the calves consisted of Angus, Chianina, and Maine Anjou crosses. Plasma ($n = 75$) was collected when the calves were vaccinated intranasally for BVDV. A serum sample ($n = 90$) was also collected 20 days post-vaccination. As for the previous herd, the number of samples per time point is less than the number of calves because not all calves were sampled at each time point. The BVDV vaccine used on these calves (Onset 5 IN, Intervet Inc., Millsboro, DE) included all of the modified-live viruses described for the vaccine above. The same vaccine was used for cows and calves. Although the manufacturer reported vaccine efficaciousness in product literature, we are not aware of any publications confirming this result. Calf age at time of first sampling ranged from about 2 to 9 weeks (approximately <1–2 months). At the time of the second collection, calf age ranged from about 5 to 12 weeks (approximately 1–3 months).

About 10 mL of blood was collected from the jugular vein into Vacutainer tubes (BD, Franklin Lakes, NJ) with EDTA (for plasma collection) or without an anticoagulant (for serum collection). Blood samples were centrifuged to collect plasma and sera samples. Plasma and sera were stored at −20 °C prior to use. The animal research described in this manuscript was in compliance with SDSU's Institutional Animal Care and Use Committee.

2.2. BVDV antibody ELISA

Total antibodies specific for BVDV in sera and plasma were measured with a commercially available ELISA (BVDV Ab ELISA, IDEXX Switzerland AG, Liebefeld-Bern, Switzerland; catalogue #99-44000) following the manufacturer's protocol. Sera, plasma, and controls were diluted 1:5 with sample diluent provided by the manufacturer. The optical densities (ODs) were measured with an absorbance microplate reader. Positive and negative BVDV controls, provided by the manufacturer of the kit, were included on all plates and samples and controls were assayed in duplicate. The ODs were averaged for each sample and control and converted to S/P ratios as follows.

$$\frac{S}{P} = \frac{\bar{S} - \bar{N}}{\bar{P} - \bar{N}}$$

where $\bar{S}$ = sample mean OD, $\bar{N}$ = negative control mean OD, and $\bar{P}$ = positive control mean OD. For each plate, the difference between the positive and negative control mean ODs was >0.150 and the negative control mean OD was <0.250. The intra-assay coefficient of variation (CV) and inter-assay CV for this ELISA was 8.05% and 8.01%, respectively.

2.3. BVDV-1 and BVDV-2 SN

Protective antibodies specific for either BVDV-1 or BVDV-2 were measured by SN. The SN tests were completed at the SDSU Animal Disease Research and Diagnostic Laboratory as previously described [15]. Briefly, after complement was deactivated by heating to 56 °C for 30 min, two-fold dilutions (1:8–1:8192) of serum or plasma were incubated with either (1) Singer strain, a cytopathic type 1a BVDV or (2) 125-C, a cytopathic type 2 BVDV for BVDV-1 SN and BVDV-2 SN, respectively (∼500 TCID50). Sera or plasma was cultured with bovine turbinate cells for five days. Presence or absence of a virus-induced cytopathic effect was observed and results were reported as the reciprocal of the highest dilution which resulted in absence of a cytopathic effect.

2.4. Statistical analysis

The correlation coefficients between BVDV-1 SN, BVDV-2 SN, and the ELISA were estimated by Spearman's rank correlation test with JMP 8.0 software (SAS Institute Inc., Cary, NC). The Spearman correlation coefficient was chosen because the SN titers were ordinal measurements [16]. After logarithmically transforming SN titers ($\log_{10}$), all applicable test assumptions were met. Correlation coefficients were estimated for the entire dataset, and for each herd, age quartiles, and biological sample type (plasma or serum)